STUDIES ON ANTI-BREAST CANCER AND ANTI-COLON CANCER ACTIVITIES IN BERGENIA LIGULATA (WALL.) ENGL.

THESIS SUBMITTED FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

IN

SCIENCE

BY

SAMHITA DE

INDEX NO. 42/19/Life Sc./26

REGISTRATION NO.: SLSBT1504219

DEPARTMENT OF LIFE SCIENCE AND BIOTECHNOLOGY

JADAVPUR UNIVERSITY

2023

CERTIFICATE FROM THE SUPERVISOR

This is to certify that the thesis entitled "**Studies on anti-breast cancer and anti-colon cancer activities in *Bergenia ligulata (Wall.) Engl.***" Submitted by Smt. **Samhita De** who got her name registered on**27.08.2019** for the award of Ph. D. (Science) degree of Jadavpur University, is absolutely based upon his own work under the supervision of **Mahadeb Pal** and that neither this thesis nor any part of it has been submitted for either any degree / diploma or any other academic award anywhere before.

23.11.23

(Signature of the supervisor with date and seal)

Mahadeb Pal, Ph.D.
Professor
Division of Molecular Medicine
BOSE INSTITUTE
P-1/12, C.I.T. SCHEME VIIM
Kolkata-700 054, India

Dedicated

To

Science and Humanity

ACKNOWLEDGEMENTS

It is a great pleasure and privilege for me to convey my profound gratitude with deep indebtedness and immense admiration to my supervisor Prof. Mahadeb Pal, who provided immeasurable guidance and support at every phase of the doctoral program. His effort and help have made my journey in this program more rewarding, both academically and personally. Prof. Pal's enthusiasm, dedication, integral view on research, for providing high-quality work was the real motivating force for me. Further, I would like to thank Prof. Kaushik Biswas, Prof. Atin Kumar Mondal, Prof. Anup Kumar Mishra for their support during the course of my stay in the program. I would also like to express my appreciation to Prof. Jayanta Mukhopadhyay, Dr. Kuldeep Jana, Prof. Tapan Kumar Dutta and Prof. Paramesh Chandra Seal for their continuous help and support. I would like to thank my Research Advisory Committee (RAC) members Dr. Nakul C. Maiti and Prof. Ratan Gachhui, for their technical suggestions, valuable insights, and advice during this study. I would like to thank my lab mates and colleagues, Suvranil Ghosh, Anirban Manna, Chirantan Majumdar, Hossainoor Rahaman Sareng, for their constant help and support. Last but not the least, I owe deep sense of gratitude to the most important people in my life: my parents, brother, and husband, for their selfless love, patience, and persistent inspiration during the course of this journey.

Abstract

INDEX No.: 42/19/Life Sc./26

Thesis title: **Studies on anti-breast cancer and anti-colon cancer activities in *Bergenia ligulata (Wall.) Engl.***

Submitted by: **Samhita De.**

Cancer is the prime cause of mortality worldwide. Globally, there were 19.9 million new cases of cancer, which took about 10 million lives in 2020. Colorectal cancer (CRC), the 2nd most diagnosed cancer, claimed about 0.9 million lives. Breast cancer, on the other hand, the most diagnosed cancer in women, with an estimated 2.3 million new cases globally, has claimed 69000 deaths. Overall, the incidence is 2 to 3 times higher in developed than the developing countries. Per current trends, both cancer incidences deaths are predicted to rise in the coming decades. Drug resistance, recurrence, general toxicity, and unaffordability to many have always been major concerns with current cancer therapy. These signify the importance of alternate treatment strategies that would overcome/reduce these limitations of the current treatments. An increasing number of in vitro and in vivo studies with phytochemicals highlighted their great potential as chemotherapy agents alone or in combination with existing therapies. *Bergenia ligulata* is known for its various medicinal properties in Indian traditional and folk medicine. In this study, an HPLC-purified fraction, with the highest activity against CRC and breast cancer cells was isolated from *Bergenia ligulata* rhizome. This fraction is termed PFBL (polyphenol-rich fraction of *Bergenia ligulata*). LC-MS analysis detected a group of eleven compounds in PFBL. The molecular basis of anti-breast and anti-CRC-specific activities of PFBL was investigated in both in vitro and in mice models. PFBL sensitized both CRC (HCT116 and HT29) and breast cancer (MCF7 and MDAMB231) cells at a concentration that is not toxic to normal cells, such as NKE. Notably, PFBL induced death in CRC and breast cancer cells by distinct mechanisms. The HCT116 cells died majorly by autophagy, as suggested by the alteration of the cellular level of several characteristic markers, such as upregulation of LC3-II levels and downregulation of mTORC1 activities. The major involvement of apoptosis was detected in the MCF7 cells, characterized by the dose-dependent increase of cleaved PARP1, caspase 7, 8, and 9, and other associated markers. Elevation of cellular reactive oxygen species (ROS) levels was evidenced by DCFDA staining. Abrogation of PFBL-induced anti-cancer activity by n-acetyl cysteine (NAC) pre-treatment, indicating the elevation of intracellular ROS levels as a key mediator of PFBL action. Surprisingly, shRNA-mediated downregulation of AMPK protected these cells from PFBL action, suggesting the role of AMPK in PFBL-induced death in both cell types. PFBL treatments regressed both CT26- and 4T1-induced solid tumors in BALB/c mice majorly by induction of autophagic and apoptotic cell death, respectively. Consistent with in vitro results, PFBL efficiently reduced lung metastasis of CF26 and 4T1 cells in pre-clinical (mice) models without affecting the healthy animals. Altogether, the present study highlighted PFBL as a potent anti-colon and anti-breast cancer agent that can be considered for clinical trials for future use in cancer treatment.

Samhita De.
23.11.23

23.11.23

Mahadeb Pal, Ph.D.
Professor
Division of Molecular Medicine
BOSE INSTITUTE
P-1/12, C.I.T. SCHEME VIIM
Kolkata-700 054, India

ABBREVIATIONS

4EBP1	Eukaryotic translation initiation factor 4E-binding protein1
5FU	5-Fluorouracil
AMPK	AMP-activated protein kinase
ATM	ATM serine/threonine kinase
ATP	Adenosine triphosphate
BC	Breast cancer
CALR	Calreticulin;
CaMKKβ	Ca2+/calmodulin activated protein kinases
CCND1	Cyclin D1
CRC	Colorectal cancer, colon cancer
DOX	Doxorubicin
EMT	Epithelial to mesenchymal transition
GSH	Reduced glutathione
LC3B	Microtubule-associated proteins 1A/1B light chain 3B;
LKB1	Liver Kinase B1; Serine/Threonine Kinase 11 - STK11
MAO-A	Monoamine oxidase-A
MCL-1	myeloid cell leukemia sequence 1
MLH1	MutL homolog 1
MMP2/9	Matrix metalloproteinase 2/9

MSH2/6	Mut S homolog 2/6
mTOR	Mammalian target of rapamycin
NAC	N-acetyl cysteine
NO	Nitric oxide;
PALB2	Partner and localizer of BRCA2
PARP1	Poly (ADP-ribose) polymerase1
PDCD4	Programmed cell death protein 4
PFBL	Polyphenol rich fraction of *Bergenia ligulata*
PHF2	PHD Finger Protein 2
PMS2	PMS1 homolog2, mismatch repair system component
PUMA	p53 upregulated modulator of apoptosis
Rnase A	Ribonuclease A
ROS	Reactive oxygen species
S6k	Ribosomal protein S6 kinase;
SCID	Severe combined immune deficient mice
SMAD-3	Mothers against decapentaplegic homolog 3
SOD1/2	Superoxide dismutase 1/2;
TGFβ-R1	Transforming growth factor β receptor type 1
TP53	Tumor protein 53
ULK1	Unc-51-like kinase 1

Table of Contents

2. Chapter II

MATERIALS AND METHODS

CHAPTER 1
GENERAL INTRODUCTION

1.1 Introduction:

Cancer can be a death sentence to many. This devastating disease claims millions of lives annually on a global scale. Metastasis is the prime cause of mortality in patients with colorectal and breast cancer worldwide. Colorectal cancer (CRC), held the third position in cancer occurrence and ranked second in mortality in 2020 (Sung et al., 2021). In the same year, breast cancer, emerged as the most frequently reported cancer globally among females (Sung et al., 2021). Limited treatment options are available for these deadly diseases which are very expensive and have many long-term side effects. Factors such as insufficient knowledge, limited awareness, economic hardship, suboptimal healthcare, and challenging living conditions contribute to cancer's prominence as a significant threat, especially in developing nations like India. Drug resistance, recurrence, and metastasis also enhance severity of the disease. An increasing number of in vitro and in vivo studies reporting notable anticancer activities in various plant extracts and compounds isolated from plants highlighted great potential in plant-based activities for their future use as chemotherapy agents alone or in combination with existing ones.

1.2 Colon cancer:

Globally, colon cancer or colorectal cancer (CRC) ranks as the third most frequently diagnosed cancer and the second leading cause of cancer-related deaths (ebecca L. Siegel, 2021; Siegel et al., 2021). In 2021, the United States alone reported 104,270 new cases and 52,980 deaths (Sung et al., 2021). In 2020, the global figures stood at 1.9 million new cases and 935,000 deaths, marking an increase from 2018 when 1.8 million new cases and 861,000 deaths were reported (ebecca L. Siegel, 2021; Siegel et al., 2021). Projections suggest that the global burden of CRC will surge by 60% to 2.2 million new cases and 1.1 million deaths by 2030 (Arnold et al., 2017; Ferlay et al., 2019a; Haque et al., 2021; Swetha et al., 2021). The rise in cases is attributed to factors like sedentary lifestyles, shifting dietary habits involving processed foods, tobacco use, and heavy alcohol consumption. In India, the estimated incidence of colon cancer in 2016 was 63,000, with notable regional variations (Choudhari et al., 2020; Dhillon et al., 2018).

The introduction of screening programs in the United States since 1990 has consistently reduced CRC incidence among individuals aged 50 and older (Ranjan et al., 2019; Siegel et al., 2020). Conversely, CRC incidence has exhibited a significant and steady rise (2% per year) among those under 50, leading to the identification of young-onset CRC (yCRC) (Long et al., 2021; Mauri et al., 2019; Muppala, 2020; Siegel et al., 2017). Although yCRC accounts for

only 10% of total CRC cases, it disproportionately affects those aged 40 to 49 (Afrin et al., 2020; Ernst J. Kuipers, 2015; Long et al., 2021; Murphy et al., 2017; Siegel et al., 2017; Venugopal and Stoffel, 2019; You et al., 2012; Zhao et al., 2018), with predictions indicating a doubling of yCRC cases among individuals younger than 35 by 2030, highlighting racial disparities (Holowatyj et al., 2016; Thanikachalam and Khan, 2019).

Current treatment options for CRC encompass laparoscopic surgery, resection, palliative care, neoadjuvant chemotherapy, and radiotherapy (Holowatyj et al., 2016; Thanikachalam and Khan, 2019). However, these treatments often come with undesirable side effects, variable efficacy, and substantial costs. The exploration of phytochemicals for cancer prevention and treatment has been a longstanding area of interest (Aiello et al., 2019; Ferlay et al., 2019b). Plants have played a role in traditional medicine for various ailments and offer an alternative approach to healthcare (Long et al., 2021; Siegel et al., 2017). More than 3,000 plant species exhibit anticancer properties, with around thirty plant-derived compounds undergoing preclinical testing (Arnold et al., 2017; Haque et al., 2021). Citrus fruits, vegetables, and medicinal plants have demonstrated promising preclinical anticancer activity (Arnold et al., 2017; Haque et al., 2021). Secondary metabolites isolated from plants have exhibited potential in reducing inflammation, promoting apoptosis, with antioxidant, anticarcinogenic, and antimetastatic properties (Aiello et al., 2019; Choudhari et al., 2020; Dhillon et al., 2018; Ferlay et al., 2019b). The appeal of phytochemicals lies in their relatively safer and cost-effective nature, and they are already widely consumed by humans. Although research is ongoing and shows promise, clinical approval has been granted to only a limited number of natural compounds, although the clinical studies of many is hindered by challenges such as low bioavailability (Arnold et al., 2017; Haque et al., 2021; Mauri et al., 2019; Muppala, 2020).

1.2.1 CRC Risk Factors:

Familial, hereditary, and lifestyle factors may carry distinct risk for development of CRC (Keum and Giovannucci, 2019). Genetic syndromes account for 20-30% of CRC cases and can be categorized into non-polyposis and polyposis types (as shown in Table 1.1). Lynch syndrome, which is an alternative term for non-polyposis syndrome, is an autosomal dominant disorder linked to a malfunction in DNA mismatch repair genes, including hMLH1, hMSH2, hMSH6, or hPMS2 (Lynch et al., 1993; Niessen et al., 2006). This genetic mutation leads to the presence of microsatellite instability (MSI) regions, which are also associated with approximately 15% of sporadic CRC cases. As expected, individuals with MSI regions are at an elevated risk for other cancers, such as endometrial carcinoma (Lynch et al., 1993).

Table 1.1 Genes involved in different CRC syndromes and associated clinical symptoms.

Syndrome	Genetic Defects	Clinical Manifestations	Ref.
Hereditary nonpolyposis cancer syndromes			
Lynch syndrome	*MLH1, MSH2, MSH6, MSH3,* and *PMS2*	Increased risk for CRC, (10–47%) depending on gene mutated; asymptomatic unless altered bowel habits, GI bleeding due to tumors/polyps occurs; increased risk for endometrial cancer; extracolonic manifestations are associated as Muir-Torre, Turcot.	(Lynch et al., 1993; Møller et al., 2018; Seppälä et al., 2021)
Muir-Torre syndrome (HNPCC + Sebaceous gland malignancies)	*MLH1, MSH2, MSH6,* and *PMS2*	Sebaceous skin tumor/keratoacanthoma and Lynch syndrome features.	(Grzybowski and Jablonska, 2009; Ponti et al., 2016)
Turcot syndrome type 1 (HNPCC with primary brain tumors)	*MMR,* MLH1, and PMS2	Features of Lynch syndrome + primary brain tumors.	(Haggar and Boushey, 2009; Hamilton et al., 1995; Kidambi et al., 2019; Rutz et al., 1991)
Hereditary polyposis colorectal cancers			
Familial adenomatous polyposis (FAP) syndrome	*APC*	More than colorectal adenomatous polyps; 100% cancer risk	(Galiatsatos and Foulkes, 2006; Kidambi et al., 2019)
Turcot syndrome type II (FAP with Primary Brain tumors)	*APC*	FAP syndrome + primary brain tumors, medulloblastoma, glioblastoma, astrocytoma.	(Haggar and Boushey, 2009; Hamilton et al., 1995; Kidambi et al., 2019; Rutz et al., 1991)
Gardner syndrome	*APC*	FAP syndrome+ extraintestinal manifestations of desmoid tumors; sebaceous cysts; osteomas of mandible, skull, fibromatosis, congenital hypertrophy of retinal pigment epithelium (CHRPE); adrenal adenomas.	(Coffin et al., 2007; Kiessling et al., 2019)

Adenomatous polyposis syndromes	*APC* and *MUTYH*	Increased number of colorectal adenomas (10–100 s), serrated polyposis, mixed polyps; duodenal adenomas are common; 43–33% increased risk of CRC; increased thyroid nodules, adrenal lesions, jawbone cysts.	(Curia et al., 2020; Kidambi et al., 2019; Sutcliffe et al., 2019; Vogt et al., 2009)
Juvenile polyposis coli	*BMPR1A* and *SMAD4*	Multiple hamartomatous polyps in the GI tract- mainly colorectum; rectal bleeding due to polyps is a common presenting symptom; anemia due to bleeding is common; extracolonic manifestations hereditary hemorrhagic Telangiectasia (HHT) telangiectasias of buccal mucosa and skin, epistaxis, and anemia, with AV malformations; colorectal cancer risk 38.7% increased.	(Brosens et al., 2007; Calva-Cerqueira et al., 2009; Gallione et al., 2004)
Peutz-Jeghers syndrome	*STK11*	Mucocutaneous pigmentation; hamartomatous polyps; 39% increased risk for CRC.	(Kopacova et al., 2009; Westerman et al., 1999)
Cowden syndrome (multiple hamartoma syndrome)	*PTEN*	Mucocutaneous lesions and macrocephaly; skin manifestations; uterine leiomyomas, ovarian cysts; multiple hamartomas on any organ; increased risk of breast, thyroid, renal, endometrial, and colorectal cancer; 9–16% risk of CRC.; increased risk for malignant melanomas; specific dysplastic gangliocytoma of the cerebellum; Lhermitte-Duclos disease is specific to Cowden disease.	(LLOYD and Dennis, 1963; Smerdel et al., 2020)

Abbreviations: MUTYH, mutY DNA glycosylase; STK11, serine/threonine kinase; 11SMAD4, mothers against decapentaplegic homolog 4; PTEN, phosphate and tensin homolog; BMPR1A, bone morphogenic protein receptor type 1A; MLH, MutL homolog; MSH, MutS homolog; MMR, mismatch repair; Ref., References;

Various risk factors, including familial, hereditary, and lifestyle-related elements, contribute independently to the development of CRC. Genetic syndromes, constituting 20-30% of CRC cases, can be categorized into non-polyposis and polyposis types (as depicted in Table 1.1). For instance, Familial Adenomatous Polyposis syndrome (FAP), characterized by the formation of multiple polyps in the gastrointestinal tract, is caused by a germline mutation in the adenomatous polyposis coli (APC) gene (Fodde et al., 2001; Lamlum et al., 1999; Miyaki et al., 1994). Individuals inheriting such polyposis syndromes face a significantly heightened risk of developing colon cancer, with a potential risk increase of up to 100% (Kanth et al., 2017). Moreover, these individuals are also susceptible to other gastrointestinal cancers and desmoid tumors. Other polyposis syndromes include MUTYH-associated polyposis (MAP), Peutz-Jeughers syndrome (STK11), Juvenile polyposis syndrome (SMAD4 and BMPR1A),

hyperplastic polyposis (HPP), familial CRC (FCC) syndrome X, and Cowden syndrome (PTEN), all of which elevate the risk of CRC development (Jasperson et al., 2010; Kidambi et al., 2019; Mokarram et al., 2017).

Chronic inflammatory bowel diseases, encompassing ulcerative colitis and Crohn's disease, pose an increased risk of CRC for affected individuals (Potack and Itzkowitz, 2008). Additionally, previous abdominopelvic radiation exposure constitutes a potent risk factor for CRC, particularly among survivors of childhood cancer (Armenian and Robison, 2013). Furthermore, individuals undergoing radiation therapy for prostate cancer face an elevated risk of developing rectal carcinoma, further substantiating the association between prior radiation therapy and CRC (Baxter et al., 2009). Cystic fibrosis also features a connection with CRC, as patients with this condition face a 5–10 times greater risk of CRC. Consequently, special management protocols for CRC screening, especially post-transplant, are implemented for these individuals (Hadjiliadis et al., 2018).

Lifestyle factors, including smoking, alcohol consumption, obesity, sedentary behaviours, and the presence of chronic diseases, collectively pose a substantial overall risk for the development of sporadic CRC (Botteri et al., 2008; Cai et al., 2014b; Kyrgiou et al., 2017). A westernized diet characterized by high consumption of processed foods and red meat, coupled with a deficiency in fruits, fibre, and leafy vegetables, can contribute to the onset of CRC (Thanikachalam and Khan, 2019; Wong et al., 2019). Conversely, increased consumption of vegetables, fruits, and dietary fibres serve as a protective measure against CRC. Research has explored the relationship between CRC risk and the dietary inflammatory index (DII) of food, with a higher DII indicative of a pro-inflammatory state and a subsequent increase in CRC risk (Shivappa et al., 2017). Numerous studies have delved into the use of anti-inflammatory foods and drugs for CRC chemoprevention and treatment, with findings suggesting that higher intake of calcium, magnesium, and potassium is associated with a reduced occurrence of CRC (Meng et al., 2019).

The risk of CRC is low in vegetarians compared to meat eaters with an HR ratio of 0.49 [95% confidence interval (CI): 0.36 to 0.66], and 0.73 [95% CI: 0.54 to 0.99] when not adjusted and adjusted (for sociodemographic and lifestyle factors, multimorbidity, and body mass index) respectively. When CRC was subcategorized, the HR of 0.69 [95% CI: 0.48 to 0.99] for the colon and 0.43 [95% CI: 0.22 to 0.82] for the proximal colon was ob-served in vegetarians, which is much less compared to meat eaters (Parra-Soto et al., 2022). Adherence to the

Mediterranean diet was found to be associated with a low risk of rectal cancer with RR of 0.82 [95% CI: 0.71 to 0.95] for rectal cancer, 0.94 [95% CI: 0.87 to 1.02] for proximal colon cancer, and 0.91 [95% CI: 0.79 to 1.04] for distal colon cancer (Zhong et al., 2020). The unhealthy diet pattern is associated with CRC-specific mortality with RR/HR of 1.52 [95% CI: 1.13 to 2.06] (Hoang et al., 2020). The high intake of dietary calcium and magnesium is negatively associated with CRC risk with HR of 0.76 [95% CI: 0.72 to 0.80] and 0.80 [95% CI: 0.73 to 0.87], respectively. The higher intake of dietary heme, however, was positively correlated to colon cancer incidence with HR of 1.01 (95% CI: 0.82 to 1.19) and rectal cancer incidence with HR of 1.04 [95% CI: 0.67 to 1.42) (Meng et al., 2019). The increase in DII score, and CRC are found to be positively associated with an overall increased risk of CRC by 40% with RR of 1.40 [95% CI: 1.26 to 1.55] (Shivappa et al., 2017). Smoking and CRC shows a positive association with ever smoker versus never smoker, the pooled RR was 1.18 [95% CI: 1.11 to 1.25], and the pooled risk estimate was 1.25 [95% CI: 1.14 to 1.37] (Botteri et al., 2008). Alcohol consumption is also associated with an increased risk for CRC mortality. In comparison, the pooled RR was 1.03 [95% CI: 0.93 to 1.15] for any, 0.97 for light drinkers who consume ≤ 12.5 g of ethanol/day, 1.04 [95% CI: 0.94 to 1.16] for moderate drinkers who consume 12.6–49.9 g ethanol/day), 1.04 [95% CI: 0.94 to 1.16] for heavy drinking men (who consume ≥ 50 g ethanol/day), which is higher than heavy drinking women [pooled RR = 0.79 (95% CI: 0.40 to 1.54)] (Cai et al., 2014a).

1.2.2 CRC Pathogenesis:

The pathogenesis of colon cancer can be broadly categorized into three primary pathways: chromosomal instability (CIN) or the classic adenoma-carcinoma sequence (Cho and Vogelstein, 1992a, b), the CpG island methylator phenotype (CIMP), and microsatellite instability (MSI) pathway (Tariq and Ghias, 2016). While these pathways are distinct, there is potential overlap between them, and they all involve the gradual accumulation of multiple mutations that eventually lead to the development of CRC (Hermsen et al., 2002).

The classic adenoma-carcinoma sequence, which accounts for 65–70% of sporadic cases, is commonly observed in left-sided CRCs (Baran et al., 2018). This pathway is characterized by the dysfunction or inactivation of the APC gene located on chromosome 5q21. APC plays a pivotal role as a "gatekeeper" in colonic neoplasia and has been implicated in familial adenomatous polyposis (FAP) syndrome. In individuals with inactivating mutations in both copies of the APC gene, the development of CRC is virtually inevitable (Gryfe et al., 1997; Levy et al., 1994). APC regulates cell growth and differentiation through the Wnt/β-

catenin signaling pathway. The Wnt pathway is a crucial cellular signaling system involved in various developmental events, including embryological development and tissue homeostasis, by governing cellular proliferation and differentiation. Dysregulation of the Wnt pathway can lead to cancer development. Suppression of the Wnt/β-catenin pathway results in reduced cellular proliferation and fewer intestinal stem cells (Dow et al., 2015). Activating mutations in the Wnt/β-catenin pathway are central to CRC pathogenesis, with over 90% of CRC cases carrying mutations within this pathway (Network, 2012). It has been observed that the loss or deletion of APC function contributes to CRC development, while restoring APC function can lead to the regression of adenomas by reducing Wnt activity (Dow et al., 2015).

In addition to APC, other mutations in the Wnt pathway, such as those in the CTNNB1 gene encoding β-catenin, can activate Wnt signaling. R-spondins (RSPOs) are another group of activators of the Wnt signal and associated with up to 10% of CRC mutations. Antagonism of RSPO3 with paclitaxel effectively targets Wnt signaling in CRC (Fischer et al., 2017). Increased expression of β-catenin in CRC cells is associated with a worse prognosis and advanced disease stage. Thus, the presence of CRC metastasis has been correlated with the combined β-catenin odds ratio within the nucleus (Chen et al., 2013).

In the absence of APC function, β-catenin translocates to the nucleus and, in conjunction with the DNA binding factor TCF, stimulates the uncontrolled overexpression of target genes like c-Myc and cyclin D1, promoting the growth of colonic epithelium (Dow et al., 2015). Subsequently, mutations in KRAS play a role in molecular pathogenesis by promoting adenoma formation (Grady and Markowitz, 2002). Finally, mutations in p53 facilitate the progression of CRC (Armaghany et al., 2012). While p53 and KRAS have significant roles in the adenoma-carcinoma pathway, mice with knockout APC genes develop carcinoma regardless of their KRAS and p53 status, and the reintroduction of APC restores normal cellular differentiation and crypt formation (Dow et al., 2015; Keum and Giovannucci, 2019).

The microsatellite instability pathway occurs due to the inactivation of DNA mismatch repair genes, which includes ATPases hMSH2, hMSH6, hMSH3, hMLH1, hPMS2, hPMS1, and hMLH3, as involved in Lynch syndrome (Lynch et al., 2006). The MSI pathway is involved in roughly 15% of CRCs, 3% of which are Lynch syndrome while the rest are sporadic, mainly caused by MLH1 hypermethylation. Finally, the CpG island methylator phenotype (CIMP) is involved in silencing genes by hypermethyla-tion of CpG islands on their

promoters (Toyota et al., 1999; Weisenberger et al., 2006). CIMP has been associated with older patients, female patients, and right-sided lesions with high MSI and BRAF mutations. CIMP is also associated with PI3K mutations but lacks KRAS and p53 mutations. A clearer insight and greater understanding of CIMP is required to better study the treatment and prevention of CRC (Advani et al., 2018).

1.2.3 CRC Chemoprevention

Chemoprevention aims to intervene, prevent, suppress, and reverse the initiation and progression of carcinogenesis. It further attempts to decrease the recurrence of cancer through the usage of drugs, vitamins, and nutritional supplements (Jänne and Mayer, 2000; Katona and Weiss, 2020). Various agents, including nonsteroidal anti-inflammatory drugs (NSAIDs), such as aspirin, and other agents, such as metformin, statins, minerals, and vitamins, have been previously studied for their chemopreventive benefits regarding CRC (Table 1.2). There is little doubt that a significant stride has been made into the unventured territories for the chemoprevention of CRC.

Table 1.2. Various drugs alone and in combination tested for their effects on clinical CRC chemoprevention studies.

Drugs	Study design	Mechanism	Main findings	References
Aspirin	Meta-analysis	COX-2 inhibition	There was a dose-dependent reduction in the risk of CR by aspirin. An aspirin dose of 75-100 mg/day reduced the risk by 10%, and 325 mg/day reduced the risk by 35% (Meta-analysis of 45 studies [RR= 0.73, 95% confidence interval (CI) 0.69-0.78])	(Bosetti et al., 2020; Cao et al., 2016; Chan et al., 2007; Rothwell et al., 2010)
Non-aspirin NSAIDS	Meta-analysis	COX-2 inhibition	Data from 23 studies suggested using higher doses of non-aspirin NSAIDs in the general population aged 40 years or older reduced CRC risk, specifically for white women, for distal colon cancer. (Pooled ODDs ratio was 0.74 (0.67-0.81), I2 = 75.9%, p < 0.001.)	(Tomić et al., 2019)
Sulindac+ DFMO	RCT	Sulindac inhibits COX-2 DFMO-irreversibly inhibits Ornithine decarboxylase (polyamine synthesis)	Significant reduction of recurrent adenomas (12 versus 41 %, risk ratio 0.30), advanced adenomas (0.7 versus 8.5 %, risk ratio 0.09), and multiple adenomas (0.7 versus13.2 %, risk ratio 0.06)	(Meyskens et al., 2008; Thomasset et al., 2009)
DFMO + Aspirin	RCT	Aspirin- COX-2 inhibition DFMO- Inhibits polyamine synthesis	After one year of treatment, in the DFMO+ aspirin arm vs. placebo, there was a significant reduction in rectal aberrant crypt foci (precursor of rectal carcinoma). (74% vs. 45%, P = 0.020).	(Sinicrope et al., 2019)

		Both combined may have a synergistic action.	No statistically significant reduction of colorectal adenomas was observed.	
Erlotinib + Sulindac	RCT	Erlotinib is an EGFR inhibitor; sulindac is a COX-2 inhibitor.	In 82 patients of familial adenomatous polyposis, Sulindac + Erlotinib was associated with a 69.4% decrease in those with an intact colorectum compared with placebo (95%CI,28.8%-109.2%; P=.009)	(Samadder et al., 2018)
Celecoxib	Meta-analysis	Selective COX 2 inhibitor, more specific for inflammation, with fewer GI side effects. Celecoxib has higher cardiovascular mortality.	3 RCTs (involving 4420 patients) and 3 post-trial studies (2,159) showed a significant reduction in the incidence of adenoma RR (0.67 [95% CI, 0.62-0.72] compared with placebo). There was an increased risk of cardiovascular mortality with twice dosing 400 mg celecoxib (RR 3.42 [95% CI, 1.56-7.46]). Once-a-day dosing did not show an increased CV risk. (1.01[95% CI, 0.70-1.46]).	(Veettil et al., 2019)
Clopidogrel	Case-control Study	Clopidogrel inhibits platelet aggregation via irreversible inhibition of the P2Y12 receptor	Clopidogrel decreased CRC risk in patients receiving treatment > 1 year. (0.65% AOR; 95% CI, 0.55–0.78). Dual antiplatelet therapy (Clopidogrel aspirin) had the same effect as either drug is taken as monotherapy.	(Rodríguez-Miguel et al., 2019)
Metformin	Meta-analysis	Activates AMPK, inhibits mTOR pathway.	Metformin users had a significantly lower incidence. CRC (RR 0.76, CI 0.69-0.84, $p < 0.001$) compared with non-metformin users. Further analysis on the overall survival of metastatic CRC patients revealed significantly higher survival rates in metformin users (HR 0.77, CI 0.68-0.87, $p < 0.001$).	(Ng et al., 2020)
UCDA	Cohort Study	Has antioxidant action. Prevents NF-κB and AP1 activity. Inhibits c-Myc	Chronic liver disease patients with UCDA have a reduced risk of colorectal cancer. UDCA use was associated with a reduced risk of CRC (hazard ratio, 0.60; 95% confidence interval [CI], 0.39-0.92).	(Huang et al., 2016)
Statin	Meta-analysis	*3-HMGCOA* reductase inhibitor decreases cholesterol synthesis. Antioxidant activity; shows pro-apoptotic effects on human CRC lines. Anti-inflammatory properties.	14 studies involving 130,994 patients. In terms of post-diagnosis statin uses, the pooled HR of all-cause mortality was 0.86 (95% CI, 0.76-0.98), and the pooled HR of CSM was 0.79 (95%CI, 0.70-0.89) (Cancer-Specific Mortality).	(Barbalata et al., 2020; Li et al., 2019b)
Menopausal hormone therapy	Nationwide Cohort Study (Norway)	Estrogens have been proposed to alter bile acid composition,	The current use of postmenopausal hormone therapy was associated with a decreased CRC risk. RR (for combined estrogen-progestin therapy) in oral	(Botteri et al., 2017)

(combined estrogen-progestin)		modulate colonic transit. Decrease production of mitogenic insulin-like growth factor.	formulations was 0.86 (95% CI 0.71 to 1.05)	
Bisphosphonates	Meta-analysis	Inhibits osteoclastic bone resorption, Anti-apoptotic effect.	Meta-analysis of 34 studies and 4,508,261 participants. There was a significant reduction in the risk of CRC. (RR = 0.89, 95% CI: 0.81–0.98)	(Li et al., 2020b)

Abbreviations: RCT, randomized control trial; RR, relative risk; HR, hazard ratio; OR, odds ratio, AOR, adjusted odds ratio; CI, confidence interval; DFMO, difluoromethylornithine; UCDA, ursodeoxycholic acid.

In CRC involving the APC/β-catenin pathway, cyclooxygenase-2 (COX-2) is often implicated in the early and later stages of the adenoma sequence, driving the formation into a carcinoma (Castellone et al., 2005; Eberhart et al., 1994; Oshima et al., 1996; Wang and DuBois, 2010). Furthermore, COX-2 overexpression produces vascular endothelial growth factor (VEGF), which promotes tumor angiogenesis (Masferrer et al., 2000; Seno et al., 2002). Hence by targeting COX-2, various studies have shown that NSAIDs, ranging from aspirin and sulindac to the more selective COX-2 inhibitors, such as celecoxib, have proven benefits in reducing disease risk (Arber et al., 2006; Nan et al., 2015). In the 1990s, the U.S. Preventive Services Task Force recommended aspirin to prevent non-high-risk CRC (Chubak et al., 2016; Dehmer et al., 2016; Katona and Weiss, 2020).

Other drugs, such as metformin, showed promising effects in reducing the risk of CRC development. Recent meta-analyses showed that metformin could reduce CRC risk by 22% (Liu et al., 2017). In an ongoing ASAMET trial for the tertiary prevention of stage I-III CRC, patients were administered low doses of aspirin combined with metformin for a potential synergistic chemo-preventive action (Petrera et al., 2018). Statins, a specific inhibitor of HMG-CoA reductase in the mevalonate synthesis pathway, have been recommended to lower serum lipid levels (Chan et al., 2003). Statins were shown to reduce CRC alone and in combination with NSAIDS (Suh et al., 2011; Teraoka et al., 2011). Further investigations on multiple agents such as antioxidants, minerals, such as selenium, and vitamins, including A, C, E, and β-carotene, were previously believed to have benefits in decreasing the risk of CRC, yet they have yielded mixed results (Katona and Weiss, 2020; Malila et al., 1999; Papaioannou et al., 2011). Studies on folate's use to lower CRC risks also yielded mixed results (Katona and Weiss, 2020). Fiber, alcohol, monounsaturated fatty acids, polyunsaturated fatty acids, omega-3, omega-6, niacin, thiamine, riboflavin, vitamin B6, vitamin B12, zinc, magnesium, selenium, vitamin A, vitamin C, vitamin D, vitamin E, folic acid, β-carotene, anthocyanin, flavonoids,

garlic, ginger, onions, thyme, oregano, saffron, turmeric, rosemary, eugenol, caffeine, and tea have all demonstrated anti-inflammatory benefits, and therefore reduce the risk of CRC development (Vanio and Bianchini, 2002; Yusof et al., 2012). A higher intake of dietary fiber, pertaining to whole grains, was associated with a lower CRC risk in men (He et al., 2019).

1.2.4 CRC Treatment

CRC incidence and mortality have been efficiently controlled by routine screening and removal of polyps through colonoscopy (Brenner et al., 2014). Surgery, chemotherapy, and immunotherapy are mainstay treatments for CRC; the stage of CRC progression in each patient determines an appropriate combination. The treatment of CRC depends upon the diagnosis through tumor/node/metastasis (TNM) staging of the lesion. Adjuvant chemotherapy with fluorouracil (5-FU) decreases death rates in patients with high-risk stage II colon cancer by 3-5% and 10-15% in stage III disease alone (Wells et al., 2017). MSI/MMR protein levels determined by IHC aid in deciding the adjuvant therapy (Argilés et al., 2020; Ribic et al., 2003; Sargent et al., 2010). Furthermore, after primary tumor resection, TNM or immunoscore can be considered to assess the tumor recurrence risk (Angell et al., 2020).

Single-agent therapy with 5-FU or therapy with multiple agents composed of 5-FU and oxaliplatin (FOLFOX), 5-FU and irinotecan (FOXFIRI) (IRI), or capecitabine and oxaliplatin (CAPOX), capecitabine (CAP), and irinotecan (CAPIRI) as first line chemotherapy is recommended based on the sensitivity and the stage of the disease. In many cases, single-agent chemotherapies yielded better results than combination therapy, given the associated systemic toxicity and unsatisfying responses (Falcone et al., 2007; Souglakos et al., 2006; Xie et al., 2020). A combination of 5-FU or CAP with oxaliplatin (OX) is recommended for stage III CRC for three to six months. Patients with intermediate-risk stage II CRC are recommended either 5-FU or CAP, which are added to OX, if the patients are high risk (stage II), for three months (Argilés et al., 2020). The International Duration Evaluation of Adjuvant Chemotherapy (IDEA) collaboration helped investigate whether three or six months of adjuvant chemotherapy was necessary, as cumulative toxicity develops from fluoropyrimidines/oxaliplatin in the form of peripheral sensory neuropathy. Results show that the overall disease-free survival was similar at 74.6% and 75.5% for three months and six months, respectively. After three months of treatment, the overall sensory peripheral neuropathy reduced from 34% to 11%. However, per ESMO guidelines, stage III CRC should still be treated with six months of FOLFOX or CAPOX if the patient falls within the high-risk

category. For patients who do not tolerate oxaliplatin, capecitabine or LVGFU2 can be acceptable alternatives (Argilés et al., 2020).

Various forms of supplemental targeted immunotherapies are considered to aid chemotherapy. Monoclonal antibodies are used to attack various potential genes, such as ERFR, VEGF, and PDL-1/PDL-1. Cetuximab, an anti-EGFR chimeric monoclonal antibody, and bevacizumab, an anti-VEGF chimeric monoclonal antibody, both of which prolong OS, were the first line targeted drugs approved by the United States Food and Drug Administration (FDA) in 2004 (Cunningham et al., 2004; Mendelsohn et al., 2015). An immune checkpoint blocker α-PD1/PDL-1 antibody, in combination with chemo- and radiation therapy, was approved by the FDA for MSI-H and dMMR classes of CRCs for sustained progression-free survival (Oliveira et al., 2019). Cetuximab yielded a positive outcome for CRC that did not respond to single-agent IRI or fluoropyrimidine therapy. Combining cetuximab with IRI, fluorocytidine, or OX delivered promising results (Jonker et al., 2007; Mendelsohn et al., 2015). EGFR (epidermal growth factor receptor) is overexpressed in various cancers to different extents, including 25-75% in CRC (Roskoski Jr, 2014). Cetuximab, once bound, results in the internalization and degradation of EGFR (Sunada et al., 1986). However, cetuximab was inactive in CRCs carrying the RAS (KRAS) mutation. Like EGFR, the VEGF level is also elevated in CRC, predicting a poor prognosis (Shibuya, 2011). Along with an elevated VEGF level, increased vascular endothelial growth factor receptor (VEGFR) activity is found in adenomas, as well as in the metastatic stage of CRC (Guba et al., 2004; Xie et al., 2020). While cetuximab is not suitable as a second line agent, bevacizumab is often an excellent choice.

1.2.5 Phenolic compounds with in-vivo anti-CRC activity:

Plants produce phenolic compounds as secondary metabolites, which typically feature multiple aromatic rings containing two or more hydroxyl groups. Phenolic compounds exhibit a vast array of chemical structures, with approximately 8000 different varieties. These compounds are classified into various classes based on their chemical structures. Examples include flavonoids (such as anthocyanidins, flavanols, flavanones, flavones, flavonols, and isoflavonoids) and non-flavonoids, encompassing phenolic acids (including hydroxycinnamic acids and hydroxybenzoic acids), coumarins, stilbenes, lignans, and tannins (de Paulo Farias et al., 2021; Tsimogiannis and Oreopoulou, 2019; Vuolo et al., 2019). Fruits and vegetables are rich sources of phenolic compounds, and many of these compounds are renowned for their

noteworthy pharmacological properties, which include antioxidant, anti-inflammatory, neuroprotective, and anticancer attributes (Darvesh et al., 2010; Kiruthiga et al., 2020).

While Western medicines have made substantial contributions to CRC chemoprevention and treatment, extracts from numerous plants and plant-derived products continue to be utilized. Throughout history, humanity has employed plants as traditional or ethnic medicines for various purposes, including the prevention and treatment of ailments like cancer. The primary factors contributing to their enduring popularity are the relatively minimal side effects, widespread availability, and cost-effectiveness when compared to synthetic drugs (Jain et al., 2021; Karimi et al., 2015; Samec et al., 2020). Over the past few decades, there has been steady progress in the identification of bioactive secondary metabolites in plants, including phenolic compounds, and a growing understanding of their mechanisms of action, which helps elucidate their health-promoting properties (Bishayee and Sethi, 2016; Huang et al., 2019b; Rajamanickam and Agarwal, 2008; VM, 2021). Phytochemicals with in vivo anti-CRC activity reported latey is listed in Table 1.3.

Table 1.3: Anti-CRC effects and mechanisms of action of phenolic compounds based on in vivo studies.

Phytocompound	Source	Animal Model studied	Dose and route of administration	Mode of action	Ref.
Flavonoids					
2,3,5,4'-tetrahydroxystilbene-2-O-β-D-glucoside	*Polygonum multiflorum* Thunb	AOM-induced colon carcinogenesis in male F144 rats	Oral administration, 30, 150, 250 mg/kg	Decreased the number of aberrant cryptic foci (ACF) by 47%-54%; suppressed tumor growth; down-regulated NF-κB in nucleus and cytoplasm; downregulated carcinoembryonic antigen (CEA).	(Lin et al., 2017)
4'-hydroxychalcone	Herb, teas, and spices	APC^{min} mice	Oral administration, 10 mg/kg	Reduced the incidences and size of adenomas; induced apoptosis; suppressed proliferation of polyps; downregulated Ki67; downregulated *c-Myc, Axin2* and *CD44* gene expression.	(Chen et al., 2021)
Aciculatin	*Chrysopogon aciculatus*	HCT116 induced tumor xenograft in	Intraperitoneal injection, 30 mg/kg	Suppressed tumor growth without losing weight; Upregulated the expression of p53	(Lai et al., 2012)

		severe combined immunodeficient (SCID) mice		and downregulated the expression of Ki67; induced apoptosis; arrested cells in sub G_1 phase.	
Apigenin	Parsley, wheat, onions, apples, and tea plants	Azoxymethane (AOM)-induced CF-1 mice and Min mice carrying mutant adenomatous polyposis coli (APC) gene	Oral administration of 0.1% dietary apigenin	Reduction of ODC activity and ACF formation.	(Au et al., 2006)
		Male BALB/c-nu mice	Intraperitoneal injection, 20 mg/kg	Upregulation of Fas-associated protein with death domain (FADD) expression and its phosphorylation; induction apoptosis of CRC cells.	(Wang et al., 2011)
		Male BALB/c-nu mice injected with SW480 cells	Route of administration not reported, Dose: 50 mg/kg	Elevation of transgelin and downregulation of MMP-9 expression via reducing Akt phosphorylation at Ser473 and Thr308.	(Chunhua et al., 2013)
		APCMin/+ mice	Oral gavage, 25 and 50 mg/kg	Reduction of the number of polyps; induction of p53 activity.	(Zhong et al., 2010)
		Nude BALB/c mice injected with HT-29 cells	Subcutaneous injection, 35 mg/kg	Induction of autophagy through inhibition mTOR/PI3K/Akt signaling pathway targeting; induction of apoptosis.	(Chen et al., 2019)
		Severe combined immunodeficient mice (SCID)	Oral gavage, 25 mg/kg	Suppression of prosurvival regulators Mcl-1, Akt, and ERK	(Shao et al., 2013)
		NEDD9 knock downed DLD1 cells mediated metastasis model in female athymic nude mice	Intraperitoneal injection, 20 mg/kg	Suppressed invasion, migration, and metastasis by downregulating overexpressed Neural precursor cells expressed developmentally downregulated 9 (NEDD9).	(Dai et al., 2016)
Baicalein	*Scutellaria baicalensis* Georgi	AMO and DSS induced colon tumor in male ICR mice	Oral administration, 1,5 and 10 mg/kg	Restored colon length; reduced tissue inflammation.	(Kim et al., 2013a)

		SW620 xenograft in BALB/c nude mice	Intraperitoneal injection, 50 mg/kg	Suppressed tumor growth by 55% without losing body weight.	(Chen et al., 2012b)
		CT-26 derived tumor in female BALB/c mice	Intraperitoneal injection, 20 and 40 mg/kg	Reduced tumor growth rate; downregulated TLR4 and p-IκBα protein expression; inhibited NF-κB pathway.	(Song et al., 2022)
		HT-29 cell-induced tumor xenograft in male nude mice	Oral administration, 10 mg/kg	Suppressed tumor growth by 29.33% compared to the control group; induced apoptosis; upregulated p53 and p21.	(Kim et al., 2012)
		DLD-1 tumor xenograft in BALB/c athymic nude mice	Intragastric administration, 20 mg/kg	Suppression of tumor growth; inhibition of ERK phosphorylation; downregulation of MMP-2 and MMP-9.	(Chai et al., 2017)
		HCT116 tumor xenograft in male BALB/c nude mice	Intraperitoneal injection, 100 mg/kg	Prevent tumor growth; decrease circMYH9, mir761 and HDGF.	(Zhang et al., 2021a)
Boeravinone B	*Boerhaavia diffusa*	DMH-induced CRC in Swiss albino Wistar rats	Intraperitoneal injection, 20 and 40 mg/kg	Decreased the number of tumor incidences; downregulated LPO; upregulated catalase, SOD and GSH; downregulated TNF-α, IL-1β, IL-6, COX-2, PGE2 and iNOS, and upregulated levels of IL-4 and IL-10; down regulated MPO; downregulated the expression of GDI2 mRNA.	(Zhou et al., 2022)
Chrysin	*Passiflora caerulea, Passiflora incarnata, Oroxylum indicum*	AOM-induced ACF in male F344 rats	Dietary administration, 0.001% and 0.01%	Reduction of mitotic index and elevation of increased apoptotic index in 'normal appearing' crypts. Modulation of cryptal cell proliferation activity and apoptosis in the early step of colon tumor development. Significant reduction in the frequency of ACF. 49% and 45% reduction in ACF with 0.001% and 0.01% chrysin	(Miyamoto et al., 2006)

				supplementation respectively.	
		Male albino rats injected with DMH+DSS	Oral administration, 125 and 250 mg/kg	Reduction in the level of CXCL1, AREG and MMP9 levels. Antioxidant activity against CYP2E1.	(Salama and Allam, 2021)
Curcumin	*Curcuma longa*	DSS-induced colitis in C57BL/6 mice	Oral consumption as dietary supplement, 0.6%	Reduced tumor incidences; inhibited nuclear translocation of β-catenin; downregulated TNF-α and interferon-γ; downregulated COX-2 and p53.	(Villegas et al., 2011)
		HCT116 tumor xenograft in female ICR SCID mice	Intragastric administration, 500 mg/kg	Suppressed tumor growth; inhibited proteasome; suppressed proliferation; induced apoptosis.	(Milacic et al., 2008)
		AOM-DSS induced CRC in male C57BL/6 mice	Oral gavage, 500 mg/kg	Reduced CRC tumor number from 8.25±3.24 (AOM-DSS treated group) to 5.25±2.65 (AOM-DSS + curcumin treatment group) and size of tumor from 45.86±29.86mm^2(AOM-DSS treated group) to 13.89±9.99 mm^2(AOM-DSS + curcumin treatment group); downregulated IL-1β, IL-6, Cox-2 and β-catenin; downregulated Axin2 by inhibiting Wnt/ β-catenin pathway.	(Hao et al., 2021)
Cyanidin	Blackberries (*Rubus fruticosus*)	Apc^{Min} mice	Dietary supplementation, 0.03%, 0.1% or 0.3%	Reduction of Adenoma counts.	(Cooke et al., 2006)
Daidzein	Soybeans and soy-based products, and nuts	Male albino rats injected with DMH+DSS	Oral administration, 5 and 10 mg/kg	Reduction in the level of CXCL1, AREG and MMP9 levels. Antioxidant activity against CYP2E1.	(Salama and Allam, 2021)
Delphinidin	Berries, pomegranates, eggplant, roselle, and wine	Male BALB/c nude mice xenograft with luciferase-transfected DLD-1 cells	Intraperitoneal injection, 100μM	Suppression of integrin/FAK nexus; elevation of miR-204-3p levels.	(Huang et al., 2019a)
Diosmetin	Chamomile, parsley, rosemary,	NCr nu/nu nude mice injected with	Oral administration, 50 and 100 mg/kg	Inhibition of anti-apoptotic Bcl-2; upregulation of proapoptotic Bax.	(Koosha et al., 2019)

	rooibos tea, green tea, and other plants fof the mint and citrus family (Lamiaceae)	HCT-116 cells			
EGCG	*Camellia sinensis* L. Ktze	SW837 xenograft in male BALB/c nude mice	Oral administration, 0.01% and 0.1%	Reduced tumor by 56% and 74% compared to control set by volume with a treatment concentration of 0.01% and 0.10% EGCG, respectively; Tumor growth suppression by preventing VEGFR-2 phosphorylation; Blocked phosphorylation of Akt and ERK.	(Shimizu et al., 2010)
		DMH-induced CRC in male Wistar rats	Oral administration, 50, 100 and 200 mg/kg	Lowered ACF formation; reduced tumor volume.	(Wang et al., 2020b)
Eriodictyol	*Eriodictyon californicum*	DMH-induced colon carcinogenesis in male albino Wistar rats	Intragastrical administration, 200 mg/kg	Suppressed the number of polyps, aberrant crypt foci (ACF) and lipid peroxidation levels; decreased AgNOR; upregulated antioxidant enzymes like catalase, SOD, GP_X, GST, GSH and GR.	(Mariyappan et al., 2017)
Euxanthone	*Polygala caudata*	HT-29 cells induced tumor in BALB/c nude mice	Intraperitoneal injection, 20 and 40 mg/kg	Suppressed tumor growth; induced apoptosis; upregulated Bax; downregulated Bcl2; induced caspase-3 cleavage; downregulated CIP2A (cancerous inhibitor of PP2A) expression and upregulated PP2A (protein phosphatase 2A).	(Wang et al., 2018b)
Fisetin	Strawberry, apple, persimmon, grapes, onion, and cucumber	AOM and DSS induced colitis-associated colorectal cancer (CAC) in male BALB/c mice	Oral administration, 20 mg/kg	Suppressed dysplastic lesions; Induced apoptosis in colonic tissue; downregulated Bcl-2 and STAT3.	(Kunchari Kalaimathi and Sudhandiran, 2016)

		FC1 mice, 3K1 mice, ApcMin/+ males, 3K1ApcMin /+ mice, B6 congenic strain, B6 FC13K1Apc Min/+ mice	Intraperitoneal injection, 1 mg/animal	Upregulation of AMPK phosphorylation; inhibition of PI3K/Akt/mTOR signaling.	(Khan et al., 2019a)
		Male athymic nude mice	Oral administration, 400 and 800 mg/kg	Induction of caspase-8 and cyt. c activity; inhibition of IGF1R and Akt; upregulation apoptosis.	(Jeng et al., 2018)
		Male BALB/c nude mice	Subcutaneous injection, 5 mg/kg	Suppression of oncoprotein securin in CT-26 tumor in a p53-independent fashion.	(Leu et al., 2016)
		BALB/c mice	Tail vein injection, 50 mg/kg	Inhibition of angiogenesis; inhibition of program cell death.	(Chen et al., 2015)
		HCT116 induced tumor xenograft in mice NOD/Shi-scid-IL2R gamma (null) (NOG)	Intraperitoneal injection, 30, 60 and 120 mg/kg	Suppressed tumor growth in a dose-dependent manner compared to control group.	(Li et al., 2022)
Formononetin	*Astragalus membranaceus*	Female Balb/c-nu/nu mice injected with HCT-116 cells	Intraperitoneal injection, 20 mg/kg	Inhibition of VEGF, MMP-2 and MMP-9 levels.	(Auyeung et al., 2012)
Furowanin A	*Millettia pachycarpa* Benth	HT-29 cell induced tumor xenograft in male athymic BALB/c nude mice	Intraperitoneal injection, 20 and 40 mg/kg	Supressed tumor growth, induced apoptosis and autophagy; upregulated cleaved caspase-3, LC3BII, Beclin and p27; downregulated Ki-67, pSTAT3, Mcl-1, p62, and cyclin D.	(Ma et al., 2019)
Genistein	*Genista tinctoria*	DMH induced colon cancer in Wistar rats	Oral administration, 2.5 mg/kg	Regulation of tumor microenvironment. Upregulation of enzymatic (SOD, CAT, GPx and GR) and nonenzymatic (Vitamin A, Vitamin C, Vitamin E and GSH) antioxidant levels. Activation of NRF2 and HO-1. Reduced	(Sekar et al., 2016)

				expression of CD133, CD44 and β-catenin.	
		AOM induced colon cancer in Sprague-Dawley rats	Dietary supplementation, 140 mg/kg	Suppression of the expression of cyclin-D1 and c-Myc. Decreased expression of Wnt signaling genes (Wnt5a, Sfrp1, Sfrp2, Sfrp5). Downregulation of Wnt/ β-catenin pathway.	(Zhang et al., 2013)
Genkwanin	Dried flower buds of *Daphne genkwa*	APC(Min/+) mice	Oral administration, 12.5 and 25 mg/kg	Induction of host defense; reduction of proinflammatory cytokine levels.	(Wang et al., 2015)
		AOM/DSS-induced C57BL/6J mice	Oral administration, 22.5 mg/kg	Inhibition of pro-inflammatory cytokines; inhibition of colon cancer growth by triggering tumor cell death.	(Yin et al., 2021)
Hesperidin	Citrus fruits	Azoxymethane-induced Swiss albino mice	Oral administration, 25 mg/kg	Inhibition of NF-kB and its effector components, iNOS and COX-2; reduction of the cellular oxidative indicators and improvement of antioxidant state.	(Saiprasad et al., 2013)
		Azoxymethane-induced Male Swiss albino mice	Oral administration, 25 mg/kg	Inhibiting the constitutively active Aurora-A driven PI3K/Akt/GSK-3 and mTOR; activation of autophagy.	(Saiprasad et al., 2014)
		Azoxymethane-induced male F344 rats	Oral administration, 1000 ppm	Inhibition of ACF formation; reduction of colonic mucosal ornithine decarboxylase activity, and polyamine levels in the blood.	(Tanaka et al., 1997)
		1,2-dimethylhydrazine (DMH)-induced colorectal cancer (CRC) in Albino rats	Oral administration, 25 mg/kg	Elevation of expression of Smad4 as well as activin A genes.	(El-Deek et al., 2022)
Hinokiflavone	*Selaginella tamariscina*, *Juniperus phoenicea*, and *Rhus succedanea*	CT-26 induced tumor in female BALB/c mice	Intraperitoneal injection, 25 and 50 mg/kg	Suppressed tumor growth and proliferation; induced apoptosis; downregulated Ki67 and MMP9.	(Zhou et al., 2019)

Icariside II	*Epimedi* H erba	SW620 induced tumor xenograft in nude BALB/c mice	Intraperitoneal injection, 25 mg/kg	Suppressed tumor growth; induced apoptosis.	(Shi et al., 2022)
Icaritin	*Epimedium sp.*	HT-29 induced tumor xenograft in male nude mice	Oral gavage, 10 mg/kg	Suppressed tumor growth and volume; knocking down AMPK increases sensitivity of the HT-29 tumors to icaritin.	(Zhou et al., 2017)
Isoangustone A	*Glycyrrhiza sp.*	SW480 tumor xenograft in male BALB/c nu/nu mice	Intraperitoneal injection, 10 mg/kg	Suppressed tumor growth; autophagic cell death; upregulated phosphorylation of AMPK and acetyl CoA carboxylase (ACC); upregulated LC3B-1 and II levels.	(Tang et al., 2021)
Isoliquiritigenin	*Glycyrrhiza glabra*	AOM/DSS induced colon carcinogenesis in male BALB/c mice	Intragastrical administration, 3, 15 and 75 mg/kg	Protected against colitis associated tumorigenesis; inhibited macrophage polarization; upregulated TNF-α, INF-γ and IL-12; downregulated TGF-β, IL-10 and IL-1ra; downregulated COX-2 mRNA.	(Zhao et al., 2014)
	Glycyrrhiza uralensis Fisher	AOM treated colon carcinogenesis in 344 rats	Oral administration, 100 ppm dietary supplementation	Suppressed ACF formation; Induced apoptosis.	(Takahashi et al., 2004)
Isorhamnetin	*Opuntia ficus-indica*	HT-29 RFP Cells Xenograft in immunosuppressed mice	Oral administration, dose not reported	Elevation of cleaved Caspase-9, Hdac11, and Bai1 proteins are highly expressed.	(Antunes-Ricardo et al., 2021)
		FVB/N mice treated with AOM/DSS	Oral administration, dietary supplement, dose not reported	Inhibition of nuclear translocation of β-catenin and c-Src stimulation caused by AOM/DSS; activation of C-terminal Src kinase (CSK).	(Saud et al., 2013)
Kaempferol	Apple, tea, broccoli, and grapefruit	DMH induced colorectal carcinogenesis in male Wistar rats	Oral administration, 200 mg/kg	Restoration of antioxidant enzymes, such as catalase, superoxide dismutase, and glutathione peroxidase.	(Nirmala and Ramanathan, 2011a)
		DMH-induced colon carcinoma in male	Oral administration, 200 mg/kg	Reduction in multiple plaque lesions and preneoplastic lesions	(Hassan et al., 2021)

		Sprague Dawley rats			
Luteolin	Celery, parsley, broccoli, onion leaves, carrots, peppers, cabbages, and tea	DMH induced carcinogenesis in male Wistar rats	Subcutaneous injection, 0.2 mg/kg	Reduced the number of tumor polyps and colon polyploids; COX-2 level in blood and colonic tissue decreases.	(Osman et al., 2015)
		AOM-induced CRC in male BALB/c mice	Oral administration, 1.2 mg/kg	Brings down the elevated levels of alkaline phosphatase and lactate dehydrogenase, suppresses the iNOS and COX-2 expression levels.	(Pandurangan et al., 2014b)
		AOM-induced CRC in male BALB/c mice	Oral administration, 1.2 mg/kg	Reduced the level of phase 1 enzymes (cytochrome b_5, cytochrome P_{450}, cytochrome b_5 RH, etc..) and enhanced the expression of phase11 enzymes (UDP-GT and GST) in colonic tissue; upregulates Nrf2 expression.	(Pandurangan et al., 2014a)
		CT-26 mediated lung metastasis	Oral administration, 10 and 50 mg/kg	Suppression of tumor lung nodules and nodule volume; inhibition of MMP-9 expression.	(Kim et al., 2013b)
Lysionotin	*Lysionotus pauciflorus* Maxim	HCT116 tumor xenograft in athymic nude mice	Intraperitoneal injection, 20 mg/kg	Suppressed tumor growth by induction of ferroptosis.	(Gao et al., 2022)
Magnolin	*Magnolia biondii*	HCT116 tumor xenograft in female BALB/c athymic nude mice	Intraperitoneal injection, 20 mg/kg	Suppressed tumor growth; downregulates LIF, p-STAT3 and Mcl-1.	(Yu et al., 2018a)
Morin	Old fustic (*Chloroph ora tinctoria*) and osage orange (*Maclura pomifera*)	Male athymic nude mice injected with HCT-116 cells	Intraperitoneal injection, 30 and 60 mg/kg	NF-κB signaling pathway inactivation.	(Chen and Zhang, 2019)
		Male albino Wistar rats injected with DMH	Intraperitoneal injection, 30 and 60 mg/kg	Modulation of tumor metabolism through via β-cateinin/c-myc signaling, glycolysis and glutaminolysis pathways.	(Sharma et al., 2017c)
		Pirc rats (F344/NTac-Apc am1137)	Dietary supplementation, 50 mg/kg	Restoration of the sensitivity to apoptosis by inhibiting the low molecular weight	(Lori et al., 2019)

				protein tyrosine phosphatase (LMW-PTP)	
		Male albino Wistar rats injected with DMH	Intragastric administration, 50 mg/kg	Reduction of ACF formation; reduction of fecal and mucosal biotransformation enzymes.	(Vennila and Nalini, 2009)
		Male albino Wistar rats injected with DMH	Intragastric administration, 50 mg/kg	Inhibition of NF-κB and inflammatory mediators; inhibition of pro-apoptotic pathway.	(Sharma et al., 2018)
		DMH-induced colon carcinogenesis in a male Wistar rats	Oral administration, 50 mg/kg	Reduction of lipid hydroperoxides products (LOOH), conjugated dienes (CD); elevation of antioxidants enzymes superoxide dismutase (SOD), catalase (CAT), glutathione S-transferase (GST), glutathione peroxidase (GPx), glutathione reductase (GR), and reduced glutathione (GSH) levels in the intestine, colon, and caecum.	(Sreedharan et al., 2009)
Myricetin	Tea, barriers, fruits, vegetables	DMH induced rat colon carcinogenesis.	Dietary supplementation, 50, 100 and 200 mg/kg myricetin	Restoration of antioxidant enzymes such as catalase, glutathione peroxidase and GSH.	(Nirmala and Ramanathan, 2011b)
		APC(Min/+) C57BL/6J mice	Oral gavage, 100 mg/kg	Promote apoptosis in adenomatous polyps. Lower IL-6 and PGE2, and downregulation of the phosphorylated p38 MAPK/Akt/mTOR signaling pathway	(Li et al., 2016)
		AOM/DSS-induced in BALB/c mice	Oral gavage, 40 and 100 mg/kg	Decrease the levels of inflammatory factors such as TNF-, IL-1, IL-6, NF-B, p-NF-B, COX-2, PCNA, and cyclin D1. Inhibition of the development of colorectal tumors and shrink colorectal polyps.	(Zhang et al., 2018a)
		AOM/DSS induced colitis in C57BL/6 mice	Oral administration, 100 mg/kg	Decrease in CSF/M-CSF, IL-6, and TNF-α in colonic mucosa and inhibition of the NF-κB/IL-6/STAT3 pathway	(Wang et al., 2018a)
Naringenin	Oranges, lemons,	AOM-induced rat model	Dietary supplement, 0.02%	reduce the number of high multiplicity aberrant crypt foci	(Leonardi et

	and grapefruit			(HMACF) by 51% and the proliferative index by 32%	al., 2010)
		(DSS)-induced murine colitis model	Oral administration, 50 mg/kg	inhibition of iNOS, ICAM-1, MCP-1, COX-2, TNF-α, and IL-6 transcript levels	(Dou et al., 2013)
Naringin	Oranges, lemons, and grapefruit	1,2-dimethylhydrazine (DMH) induced Female Wistar rats	Oral gavage, 10, 100, 200 mg/kg	Reduction of cell proliferation and tissue iron levels; upregulation of antioxidant mineral levels.	(Sequeto et al., 2014)
		AOM/DSS to induce Male C57BL/6 mice	Oral gavage, 50 and 100 mg/kg	Suppression of ER stress-induced autophagy in colorectal mucosal cells.	(Zhang et al., 2018b)
Nobiletin	Peel of various *Citrus* fruits	AOM-DSS-induced colon carcinogenesis in male CD-1 (ICR) mice	Oral, dietary supplement, 100 ppm	Reduction in tumor incidences and multiplicity.	(Miyamoto et al., 2008)
Orientin	*Ocimum sanctum*	DMH-induced CRC in male Wister rats	Intraperitoneal injection, 10 mg/ kg	Reduces NF-κB; reduces TNF-α and IL-6; downregulated Ki67 and PCNA; suppressed iNOS and COX-2.	(Thangaraj and Vaiyapuri, 2017)
		DMH-induced CRC in male Wister rats	Intraperitoneal injection, 10 mg/ kg	Suppressed crypt multiplicity; elevated the level of anti-oxidants; downregulated phase I enzymes and upregulated phase II enzymes to normal levels; reduced ACF.	(Thangaraj et al., 2018)
Oroxylin A	*Scutellaria baicalensis*	AOM-DSS induced CRC in C57BL/6 mice	Dietary supplementation, 50, 100 and 200 mg/kg	Suppressed tumor formation and colitis associated CRC; downregulates IL-6, IL-1β, p-STAT3, cyclin D, Bcl2, and upregulates Bax; induces apoptosis.	(Yang et al., 2013)
		HCT116 tumor xenograft in male athymic BALB/c nude mice and AOM-DSS induced colon carcinogenes	Oral administration, 150 and 300 mg/kg	Suppresses carcinogenesis and primary colon cancer progression; reduces triglyceride; downregulates HIF1α, Srebp1, FASN, ADRP and FABP7; upregulates CPT1.	(Ni et al., 2017)

		is in male C57BL/6 mice			
Pectolinarigenin	*Cirsium chanroenicum*	Murine CT26 CRC cells were introduced into BALB/C mice	Intraperitoneal injection, 25 and 50 mg/kg	Stat3 suppression; apoptotic death of cancer cells.	(Gan et al., 2019)
Peonidin	Sweet potato (*Ipomoea batatas*)	Azoxymethane-induced CF-1 mice	Dietary supplementation, 10 to 30%	Inhibition of cell cycle at the G1 phase; elevation of caspase-3	(Lim et al., 2013)
Petunidin	*Lycium ruthenicum*	Nude mice	Intraperitoneal injection, 25 and 50 mg/kg	Induction of ferroptosis via inhibiting SLC7A11	(Han et al., 2022)
Phloretin	*Manchurian apricot*	COLO 205 cells derived tumor in BALB/c nude mice	Route of administration not reported, 25 mg/kg	Inhibited tumor growth; upregulated p53, p21 and E-cadherin	(Lin et al., 2016)
Procyanidin	Cider apple (*Malus domestica*)	Azoxymethane-induced Wistar rats	Oral administration, 0.01%	Suppression of protein kinase C, down-regulation of polyamine production, and stimulation of caspase-3	(Gossé et al., 2005)
		Male C57/BL6 mice transfected with CT26 cells	Oral gavage, 30 mg/kg	Cellular oxidative stress reduction through Nrf2/ARE Signaling.	(Zhu et al., 2021)
Quercetin	Apples, nuts, cauliflower, cabbage, onions, grapes, berries, broccoli, citrus fruits, cherries, green tea, and coffee	AOM-induced colon tumor in C57BL/6J male mice	Dietary supplementation, 0.5%	Upregulated CB1-R; induced apoptosis; downregulated STAT3 and p-STAT3 protein levels in AOM treated mice; Downregulated Bax/Bcl2 ratio in AOM treated mice.	(Tutino et al., 2018)
		Subcutaneous DLD-1 human colon tumor fragment implant in male athymic nu/nu mice	Intraperitoneal injection, 30 mg/kg	Enhanced radiosensitivity by inhibiting ATM-mediated signaling pathway.	(Lin et al., 2012)
		AOM induced CRC in male weanling Sprague-Dawley rats	Dietary supplement, 4.5 g/kg	Inhibition of proliferation; apoptosis induction; reduction in number of crypts; suppression of COX1, COX2 and iNOS expression.	(Warren et al., 2009)

Rutin	Buckwheat, Mez, Labisia pumila, Sophora japonica L., Schum, Canna indica L., and Ruta graveolens L.	SW480 cell-induced tumor xenograft	Intraperitoneal injection, 20 mg/kg	Suppression of tumor growth, angiogenesis, and VEGF levels.	(Alonso-Castro et al., 2013)
Scutellarin	*Scutellaria barbata*	AOM/DSS-induced male C57BL/6 mice	Intraperitoneal injection, 25, 50 and 100 mg/kg	Inhibition of Wnt/β-catenin signal transduction.	(Zeng et al., 2021)
		RKO cells were subcutaneously implanted into female nude mice	Intraperitoneal injection, 50, 150 and 300 mg/kg	Inhibition of several crucial components involved in tumor growth apoptosis and metastasis.	(Xiong et al., 2020)
		AOM/DSS-induced mice	Intraperitoneal injection, 25, 50 and 100 mg/kg	Suppression of the function of the Hedgehog signaling cascade.	(Zeng et al., 2022)
Silibinin	*Silybum marianum*	Chorioallantoic membrane (CAM) assay with LoVo cell deposition on eight days old fertilized chicken egg	Rout of administration not reported, 9.64 µg/mL	Decrease in vascular density index (VDI); upregulation of Flt-1 (fms-like tyrosine kinase) gene	(Yang et al., 2005)
		AOM-induced CRC in male Wistar rats	Intragastric intonation, 300 mg/kg	Suppression of preneoplastic lesion formation; reduction in inflammatory markers like MMP7, IL-1β and TNF-α; activation of apoptosis; sub G0/G1 cell cycle arrest.	(Kauntz et al., 2012)
Tangeretin		HT-29 induced tumor xenograft in BALB/c nude mice	Rout of administration not reported, 5 mg/kg	Suppress tumor growth	(Bao et al., 2020)
Taxifolin	Olive oil, grapes, citrus fruits, and onion	HCT116 tumor xenograft in athymic male nude mice	Intraperitoneal injection, 15 and 25 mg/kg	Suppression of tumor growth; induce apoptosis; cyclin D inhibition; β-catenin degradation; inhibition of Akt phosphorylation.	(Razak et al., 2018)

Tricin	Rice bran, oats, barley, and wheat	Colon26-Luc cells induced colon tumor and lung metastasis model in BALB/c mice	Oral gavage, 19 and 37.5 mg/kg	Suppressed tumor growth affecting mice body weight; reduced metastasis incidences to 46.2% in tricin treated group in comparison with a control group with 69.2% metastasis incidences.	(Yue et al., 2020)
		AOM-DSS induced CRC in male Crj: CD-1 mice	Dietary supplement, 50 and 250 ppm	Restored colonic length; reduced number of incidences and multiplicity of adenomas and adenocarcinomas in AOM-DSS+ tricin-treated group; Downregulated PCNA; downregulated TNF-α.	(Oyama et al., 2009)
Troxerutin	Tea and coffee	DMH-induced colon carcinogenesis in male albino Wistar rats	Oral administration, 12.5, 25 and 50 mg/kg	Brings down the ACF formation and crypt multiplicity in a dose-dependent manner; reduces the phase 1 xenobiotic metabolizing enzymes (cytochrome P450, cytochromeb_5, cytochrome P4502E1, NADPH-cytochrome P450 reductase, and NADH-cytochrome b_5 reductase) and upregulates phase II xenobiotic metabolizing enzymes (GST, DTD and UDPGT).	(Vinothkumar et al., 2014)
Vitexin	Passionflower, bamboo leaves, pearl, and millet	HCT116 tumor xenograft in nude BALB/c mice	Oral administration, 25, 50 and 100 mg/kg	Suppressed tumor growth; increase phosphorylation of JNK; upregulates LC3 II and ApoL1.	(Bhardwaj et al., 2017)
		$HCT116^{DR}$-induced tumor xenograft in female athymic BALB/c nude mice	Oral administration, 25 and 50 mg/kg	Suppressed tumor growth; induce apoptosis; downregulate HSP90, HSP70, HSP27, Atg7, Beclin-1, LC3 II and Bcl2; upregulates Bax and PARP1, cleaves caspase-3 and caspase-9.	(Bhardwaj et al., 2018)
Wogonin	*Scutellaria baicalensis*, *Scutellaria radix*	the AOM/DSS-induced colitis related colon	Gastric intubation, 60 mg/kg	lowering the expression and secretion of IL-6 and IL-1β and decreasing cell proliferation and	(Yao et al., 2014)

		cancer in C57BL/6 mice		expression of NF-κB. Increase in NRF2 nuclear translocation	
		AOM-DSS-induced CRC animal model in C57BL/6 mice	Route of administration not reported, 50 and 100 mg/kg	Reduction in tumor multiplicity. Revert colon length to normal.	(Feng et al., 2018a)
		SW480 induced tumor xenograft in BALB/c nude mice	Intraperitoneal injection, 2 mM	Downregulation of YAP-1 and IRF3. Upregulation of p-YAP.	(You et al., 2022)
Xanthohumol	*Humulus lupulus*	AOM-induced colorectal carcinogenesis in male Sprague-Dawley rats	Oral gavage, 5 mg/kg	Suppressed tumor growth; induced apoptosis; suppressed COX2 and iNOS	(Liu et al., 2020)
Zapotin	Tropical fruit zapote blanco (*Casimiroa edulis*)	AOM/DSS-induced female CF-1 mice	Intragastric administration, 5 and 10 mg/kg	Induction of cell cycle arrest and Apoptosis.	(Murillo et al., 2007)
Phenolic acids					
Caffeic acid	Coffee, wine tea	CT-26 induced lung metastasis in BALB/c mice	Oral administration, 0.1 and 0.5 g/kg	Inhibition of MEK1 and TOPK. Inhibition of ERK and activation of p90RSK. Suppression of TAP induced activation of AP1, NF-κB and ERK signaling. Suppression of TAP, EGF and H-Ras induced neoplastic transformation. Inhibition of lung metastasis.	(Kang et al., 2011b)
		HCT116 induced tumor xenograft in NSG mice	Intraperitoneal injection, 10 mg/kg	Inhibition of CSC growth and self-renewal by inhibition of PI3K/Akt signaling.	(Park et al., 2020)
		HCT116 tumor xenograft in BALB/c AnN Foxn-1 nude mice	Oral administration of CAPE 50 nmol/kg	Inhibition of PI3K/Akt pathway. Inhibition of mTOR by AMPK activation. Inhibition of MMP-9. Suppression of cyclin D1, Cdk4, cyclin E, c-Myc, and N-cadherin expression, and upregulation of p21. Suppression of tumor biomarker PCNA and FASN expression.	(Chiang et al., 2014)

		HT-29 induced tumor xenograft in BALB/c nude mice	Intragastric administration, CAPE (10 mg/kg); CAPE-pNO2 5, (10 and 20 mg/kg)	Upregulation of p53, p27, p21, cytochrome c (cyt. c), and cleaved caspase-3. Downregulation of procaspase-3, Cdk2, and c-Myc. Inhibition of tumor growth and VEGF expression.	(Tang et al., 2017)
		HCT116 induced tumor xenograft in nude mice	Oral administration, 0.2 and 2 mg/kg	Suppression of tumor growth. Inhibition of autophagic cell death. Cell cycle arrest in S phase.	(Chen et al., 2020)
Chlorogenic acid	Apple, betel, coffee beans, kiwi, grapes, eggplant, pear, plum, potato, and tea	Methylazoxy methanol (MAM) acetate-induced carcinogenes is hamsters	Oral administration, 0.025% dietary supplement	Reduction of colon tumor incidences both in male and female Syrian golden hamsters. Correlation of anticancer activity to its the antioxidative effect/ inhibition of activity of microsomal enzyme.	(Tanaka et al., 1990)
		AOM-induced ACF in colon of male F344 rats	Oral administration, 0.025% dietary supplement	Reduction of ACF (initiation and growth of ACF) per rat colon in a time-dependent manner (supplementation with diet).	(Morishita et al., 1997)
Ellagic acid		Azoxymethane (AOM) induced colon tumors in rats	Oral administration, 250, 2500 and 5000 ppm	Inhibition of incidence of adenocarcinomas in the small intestine (no noticeable change in colon tumor incidence)	(Rao et al., 1991)
		1,2-dimethyl hydrazine (DMH) induced colon cancer in rats	Oral administration, 60 mg/kg	Lower frequency of aberrant crypt foci (ACF) and lipid peroxidation; increased activity of antioxidant enzymes (such as superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, and glutathione-s-transferase); restoration of antioxidant levels such as vitamin C, vitamin-E and glutathione in colon tissue.	(Umesalma and Sudhandiran, 2010a)
		DMH-induced colon cancer in Wistar albino rats	Oral administration, 60 mg/kg	Inhibition of NF-kB and its target genes iNOS, COX-2, TNF-α and IL-6; restoration of levels of different tumor markers such as 5'-nucleotidase (5'-	(Umesalma and Sudhandiran, 2010b)

				ND), gamma-glutamyl transpeptidase (γ-GT), carcinoembryonic antigen (CEA), alpha-fetoprotein (AFP) and lactate dehydrogenase (LDH).	
		DMH-induced colon cancer in rats	Oral administration, 60 mg/kg	Inhibition of DMH induced (PI3K)-p58 activation; downregulation of p-Akt, Bcl-2; upregulation of Bax.	(Umesalma and Sudhandiran, 2011)
		DMH-induced colorectal cancer in rats	Oral administration, 60 mg/kg	Inhibition of aberrant crypt foci (ACF) formation; increased activity of antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), and glutathione reductase (GR); inhibition of ornithine decarboxylase (ODC) expression through inhibition of c-MYC.	(Kumar et al., 2012)
		DMH-induced colon cancer in male Laca mice	Oral administration, 10 mg/kg	Restoration of colon membrane alterations due to cisplatin-induced toxicity in DMH-induced colon cancer.	(Goyal et al., 2022)
Ferulic acid	Rice, wheat, pineapple, grains, and peanuts	AOM-induced colon cancer in male Fischer 344 rats	Oral administration, 250 ppm and 500 ppm	Reduced number and size of AOM-induced adenomas; increased activity of phase II detoxifying enzymes GST and quinone reductase (QR).	(Kawabata et al., 2000)
		AOM induced colon carcinogenesis in F344 rats	Dietary supplement of 3-(4'-geranyloxy-3-methoxyphenyl)-2propenoate (geranylated derivative of ferulic acid) 0.1% and 0.2%	Significant decrease in the number of ACF	(Han et al., 2001)
Gallic acid	Barriers and pomegranates	DMH-induced colon cancer in male Wister rats	Oral administration, 50 mg/kg	Reduction of lipid peroxidation (LPO) products such as thiobarbituric acid relative substances (TBARS), lipid hydroperoxides (LOOH), conjugated dienes (CD), and	(Giftson et al., 2010)

				elevated levels of antioxidants such as SOD, CAT, reduced glutathione (GSH), GR and GPx. Reduction of ascorbic acid and tocopherol levels.	
		SW480 induced tumor xenograft in NOD scid gamma NSG mice	Intraperitoneal injection, 200 mg/kg	Antitumor activity is mediated by interaction with G-Quadruplexes	(Sanchez-Martin et al., 2022)
		DMH-induced colon cancer in male albino Wister rats	Oral administration, 50 mg/kg	Elevation of xenobiotic metabolizing enzymes such as cytochrome p450, cytochrome b5, glutathione-S-transferase, DT-diaphorase and gamma-glutamyl transpeptidase.	(Senapathy et al., 2011)
Geraniin	*Phyllanthus amarus*	SW480 induced tumor xenograft in NUDE mice	Oral administration, 10, 20 and 40 mg/kg	Suppressed tumor growth; induced apoptosis; inhibited phosphorylation of PI3K and Akt in a dose-dependent manner.	(Zhou et al., 2020)
p-Coumaric acid	Mushrooms, apples, pears, barriers, oranges, and beans	DMH-induced colon carcinogenesis in male albino Wistar rats	Intragastric intubation, 100 mg/kg	Reduces aberrant cryptic foci (ACF), dysplastic ACF (DACF), mucin-depleted foci (MDF) and β-catenin accumulated crypts (BCAC).	(Sharma et al., 2017b)
Syringic acid	Olives, dates, pumpkins, grapes, and palms	Dextran sulfate sodium (DSS) induced mice	Oral administration, 25 mg/kg	Decrease level of inflammatory cytokines (such as nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2)), proinflammatory cytokines (like TNF-α, IL-1β, IL-6); reduced activation and accumulation of p-STAT-3 by decreasing expression of p65-NF-κB.	(Fang et al., 2019)
		DMH induced colorectal cancer in male rats	Oral administration, 50 mg/kg	Reduced tumor incidences, tumor volume and average number of tumors.	(Mihanfar et al., 2021)
Lignans					

Arctigenin	*Arctium lappa, Forsythia suspensa.*	CT-26 cells derived lung metastasis model in BALB/c mice	Oral gavage, 50 mg/kg	Less number of lung nodules; induced apoptosis in lung tissue; inhibited EMT in lung tissue; induced cleavage of caspase-3, caspase-9, and PARP; downregulated Bcl-2 and Bcl-xL; upregulated Bax.	(Han et al., 2016)
Daurinol	*Haplophyllum dauricum*	HCT116-induced tumor xenograft in athymic BALB/c (*Slc/nu*) nude mice	Oral administration, 5 and 10 mg/kg	Suppressed tumor growth; compared to etoposide it is less toxic; significantly upregulated p-Chk1(Ser345)/Chk1.	(Kang et al., 2011a)
Dehydrodiisoeugenol	*Myristica fragrans* Houtt	HCT116, zsw480, and patient-derived xenograft in female NOD/ SCID mice	Intraperitoneal injection, 40 mg/kg	Suppressed tumor growth by inducing ER stress; upregulated BiP, PERK, and IRE1α.	(Li et al., 2021)
Gomisin A	*Schisandra chinesis*	CT-26 cell induced lung metastasis in female BALB/c mice	Intraperitoneal injection, 50 mg/kg	Suppressed lung metastasis; reduced the number of lung nodules; increased phosphorylation of AMPK and p38 in lung tissue.	(Kee et al., 2018)
Honokiol	*Magnolia grandiflora*	SW620 cell induced tumor xenograft in female athymic BALB/c nude mice nu/nu	Intragastric administration, 50 mg/kg	Inhibited proliferation of CRC; upregulated TGF-β1 and p53 by upregulating BMP7.	(Li et al., 2020a)
Justicidin A	*Justicia procumbens*	HT-29 tumor xenograft in NOD-SCID mice	Oral administration, 6.2 mg/kg	Suppressed tumor growth; induced autophagy in tumor tissue; induced apoptosis.	(Won et al., 2015)
Magnolol	*Magnolia officinalis*	CT-26 and HT-29 induced tumor in BALB/c and Cg-Foxn1^{nu}/Crl Narl nude mice respectively	Rout of administration not reported, 50 and 100 mg/kg	Inhibits tumor growth; upregulation of Fas, Fas-L, cleaved caspase-3, cleaved caspase-9 and cleaved caspase-8; downregulated NF-κB, PKCδ, ERK, Akt, C-FLIP, and MCL-1; induces apoptosis; inhibition of PKCδ-NF-κB signaling.	(Su et al., 2020)

		HCT116 tumor xenograft in female BLB/c nude mice	Intraperitoneal injection, 5 mg/kg	Suppressed tumor growth without showing any toxicity.	(Kang et al., 2012)
Schisandrin B	*Schisandra chinensis, Schisandra propinqua, and Schisandra rubriflora*	AOM-DSS-induced CRC in C57BL/6 mice	Oral administration, 3.7-30 mg/kg	Suppression of SIRT1.	(Pu et al., 2021)
Secoisolariciresinol	*Fitzroya cupressoides* and *Crossosoma bigelovii*	HCT116 mediated tumor xenograft in male BALB/c nude mice	Rout of administration not reported, 50, 100 and 200 mg/kg	Tumor growth inhibition; Suppression of tumor growth; pyroptosis induction; downregulated Ki-67; upregulated expression of N-GSDMD	(Chen et al., 2022)
		DSS-induced colitis in mice	Dietary supplementation, 200 mg/kg	Tumor growth suppression; reduction in inflammatory cytokines (IL-1β, IL-18, TNF-α); inhibited NLRP1	(Wang et al., 2020c)
Sesaminol	*Sesamum indicum*	Ethanol-induced CRC in male C57BL/6NCr mice	Oral administration, 2.5 mg/mice	Reduction in ethanol induced colonic lesions; downregulation of iNOS and CYP2E1; lower TNF-α, IL-6, MCP-1 and NF-κB levels; increase in cell adhesion by upregulation of ZO-1, occluding and cladulin-1.	(Ohira et al., 2022)
Tracheloside	*Carthamus tinctorious* L. (safflower)	CT-26 induced lung metastasis in BALB/c mice	Oral administration, 25 and 50 mg/kg	Prevention of lung metastasis. Induction of apoptosis. Upregulation of E-cadherin RNA and downregulation of N-cadherin, vimentin, snail and twist RNA.	(Shin et al., 2021)
Vitexin	*Vitex negudo*	HCT116 tumor xenograft in female nu/nu mice	Intraperitoneal injection, 40 mg/kg	Tumor growth suppression; tumor volume reduction by 75%; upregulation of PUMA and p53; induction of PUMA mediated apoptosis.	(Chen et al., 2018)
Stilbenes					
Piceatannol	Red and white grapes	AOM plus DSS induced colon tumor in C57BL/6J mice	Oral administration, 5 and 12.5 mg/kg	Decrease tumor number and size; decrease Ki67 and COX-2 positive cell number; downregulation of	(Kimura, 2022)

				MCP1 and PD1 levels in the colon.	
Polydatin	*Picea sitchensis*	Caco-2 tumor xenograft in C57BL/6 mice	Subcutaneously into the tumor, 150 mg/kg	Suppressed tumor growth; upregulates miR-382; downregulates PD-L1.	(Jin et al., 2020)
Pterostilbene	Blueberries and cranberries	AOM induced colonic ACF preneoplastic lesions and adenomas in male ICR mice	Oral administration, 50 or 250 ppm	Reduction of ACF and adenoma formation; induction of apoptosis; downregulation of iNOS and COX-2; inhibition of Wnt/β-catenin signaling through suppressing phosphorylation of GSK3β; Inhibition of VEGF, cyclin D and MMPs in mouse colon; inhibition of AOM induced Ras, PI3K/Akt and EGFR signaling.	(Chiou et al., 2010)
		AOM-induced colon tumors in F344 rats	Oral administration, 40 ppm	Reduction of the proliferation of non-metastatic adenomas; downregulation of IL-1β, IL-4, TNF-α, PCNA, β-catenin and cyclin D and phosphor-NF-κB/p65.	(Paul et al., 2010)
		AOM-induced colon tumor in male BALB/c mice	Oral administration, 50 or 250 ppm	Reduction of NF-kB through inhibition of PKC-β phosphorylation; downregulation of iNOS, COX-2 and aldose reductase; blockade of inflammation and oxidative stress by Nrf2 signaling mediated upregulation of HO-1 and GR.	(Chiou et al., 2011)
		CL187 transplantation model in BALB/c nude mice	Intraperitoneal injection, 25, 50, 100 and 200 mg/kg	Inhibition of topoisomerase 1 (Top1) mediated DNA damage repair pathway through inhibiting the Top1 enzyme.	(Zhang et al., 2021b)
		AOM induced colonic ACF preneoplastic lesions in F344 rats	Oral administration, 40 ppm	Inhibition of AOM induced ACF as well as multiple clusters of aberrant crypt formation; blockade of cell proliferation and iNOS.	(Suh et al., 2007)
Resveratrol	Grapes, blueberries, raspberries	GFP containing LoVo cell-mediated	Intragastric administration, 50, 100 and 150 mg/kg	Metastasis inhibition; decrease in tumor size in a dose-dependent manner.	(Ji et al., 2015)

	, mulberries, and peanuts	metastasis model in mice			
Miscellaneous compounds					
Oleuropein	Olives *(Olea europaea)*	AOM-induced CRC in female A/J mice	Dietary supplementation, 125 mg/kg	Prevents pre-neoplastic lesions; sub downs tumor incidences from 57% to 14%; prevents AOM-induced DNA damage.	(Seppor ta et al., 2016)
Thymol	*Thymus vulgaris* L.	HCT116 induced tumor xenograft and lung metastasis model in BALB/c nude mice	Intraperitoneal injection, 75 and 150 mg/kg	Induction of apoptosis. Reverse EMT marker expression (upregulation of E-cadherin and downregulation of N-cadherin). Suppression of lung metastasis by inhibiting Wnt/β-catenin pathway.	(Zeng et al., 2020)
Verbascoside	Genus, *Cistanche*	HCT116 induced tumor xenograft in BALB/c nude mice	Tail vein injection, 20, 40, and 80 mg/kg	Upregulation of HIPK2, P53, Bax, and downregulation of Bcl2.	(Zhou et al., 2014a)

Abbreviation: ACF, aberrant crypt foci; AFP, α-fetoprotein; AOM, azoxymethane; APC, adenomatous polyposis coli; BAX, B-cell lymphoma 2 associated x protein; BCL-2, B-cell lymphoma 2; BID, BH3 interacting-domain death agonist; CAT, catalase; CEA, carcinoembryonic antigen; CIP2A, cancerous inhibitor of PP2A; c-MYC, cellular myelocytomatosis oncogene; COX-2, cyclooxy-genase-2; DMH, 1,2-dimethylhydrazin; DNMT, DNA methyltransferase; EGCG, (-)epigallocatechin gallate; EGF-β, epidermal growth factor-β; EGFR, epidermal growth factor receptor; ERK, extracellular-signal-regulated kinase; GPx, glutathione peroxidase; GR, glutathione reductase; GSK-3β, glycogen synthase kinase-3β; IGF2, insulin like growth factor 2; IGFBP3, insulin like growth factor binding protein 3; IL-6, interleukin 6; iNOS, inducible nitric oxide synthase; KRAS, Kirsten rat sarcoma viral oncogene homolog; LC3b, light chain 3B of microtubule-associated proteins 1A/1B; LDH, lactate dehydrogenase; MAM, methyl azoxymethane; MAPK, mitogen-activated protein kinase; MMP, matrix metalloproteinase; mTOR, mammalian target of rapamycin; NF-κβ, nuclear factor-κβ; Nrf-2, nuclear factor erythroid -2 related factor; ODC, ornithine decarboxylase; PCNA, proliferating cell nuclear antigen; PI3K, phosphoinositide 3-kinase; PP2A, protein phosphatase 2A; PTEN, phosphatase and TENsin homolog deleted on chromosome 10; SIRT, Sirtuin 1; SOD, superoxide dismutase; STAT3, signal transducer and activator of transcription 3; TNF-α, tumor necrosis factor-α; VEGF, vascular endothelial growth factor; γ-GT, γ-glutamyl transpeptidase. (Source: De.et al, 2023 (De et al., 2023))

1.3 Breast cancer:

Breast cancer (BC) has the highest occurrences and is the prime cause of cancer-related deaths in females. Globally, there were 2,261,419 new cases of BC, with 684,996 reported deaths in 2020 (Sung et al., 2021). It has been forecasted that there will be 3.19 million new

cases with 1.04 million deaths by 2040 (Arnold et al., 2022). With the advancement in screening techniques, reported BC incidences are almost twice as in transitioned countries (55.9 per 100,000) than transitioning countries (29.7 per 100,000), with the related mortality rate higher in transitioning countries (15.0 per 100,000) than the transitioned countries (12.8 per 100,000) (Sung et al., 2021). Lifestyle modifications, late marriage and late first childbirth, night shift work, and hormonal replacement therapy are associated with BC development in developed nations. In developing nations, the primary factors contributing to BC's elevated incidence and mortality rates are inadequate awareness of the disease, inadequate screening initiatives, delayed diagnosis, and a lack of sufficient medical facilities (da Costa Vieira et al., 2017; Shulman et al., 2010; Tfayli et al., 2010). Even the availability of numerous BC treatment options, including surgical procedures, radiotherapy, chemotherapy, and immunotherapy could not limit the high incidence and mortality rates (Brand et al., 2000; Nounou et al., 2015; Sharma et al., 2010). Early detection can offer significant advantages for treatment with extending life expectancy, but reoccurrence and chances of death are high primarily due to the insidious nature of metastasis. The emergence of drug resistance represents another formidable obstacle in BC treatment. Chemotherapeutic drugs often entail enduring side effects with a lasting impact on patients' well-being. Therefore, search for drugs with lesser side effects without compromising the efficacy is an urgent need. Some natural compounds were reported to have potent anticancer and anti-metastatic potential as well as to enhance the efficacy and reduce toxicity of conventional chemotherapeutic agents when employed in combination therapies.

1.3.1 Breast Cancer risk factors:

Breast cancer (BC) risk factors encompass a spectrum of genetic, hormonal, and lifestyle elements (Anderson et al., 2014; Anothaisintawee et al., 2013; Barnard et al., 2015; Harbeck et al., 2019; Howell et al., 2014; Kamińska et al., 2015; McTiernan, 2003; Ozsoy et al., 2017; Patterson et al., 2010; Rock and Demark-Wahnefried, 2002; Singletary, 2003; Sun et al., 2017; Yang et al., 2007). Mutations in the BCA1 and BCA2 genes play a pivotal role, significantly heightening the susceptibility to BC (Mehrgou and Akouchekian, 2016). Alterations in genes such as PALB2 (Antoniou et al., 2014), ATM (Concannon, 2002), CHEK2 (Cybulski et al., 2011), CDH1 (Pharoah et al., 2001), STK11 (Nakanishi et al., 2005), PTEN (Hobert and Eng, 2009; Tan et al., 2012), TP53 (Børresen-Dale, 2003) and NF1 (Madanikia et al., 2012) also act as a risk factor for BC (Allison, 2012; Cancer, 2001; Colditz et al., 2012; Crivellari et al., 2007; Hulka, 1996; Kungu et al., 2002; Polyak, 2007). These genetic

alterations tend to cluster within specific populations and families, emphasizing their hereditary nature (Allison, 2012; Polyak, 2007; Walsh et al., 2006). Furthermore, BC risk diverges between estrogen receptor-positive (ER+) and progesterone receptor-positive (PR+) cancers, with hormone replacement therapy and elevated estrogen levels linked to heightened risk (Vogel, 2008). While age remains a paramount risk determinant, given that most diagnoses occur in women aged 50 or older, BC can manifest in younger women and, albeit rarely, in men (Crivellari et al., 2007). Family history emerges as another critical factor, as having a first-degree relative (mother, sister, daughter) afflicted with BC amplifies one's own risk (Colditz et al., 2012; Collins et al., 2006). Additionally, a personal history of BC or specific non-cancerous breast conditions can further elevate susceptibility. Lifestyle choices wield considerable influence, with sedentary habits, excessive alcohol consumption, obesity, and diets replete with processed foods all contributing to an augmented risk profile (Anderson et al., 2014; Anothaisintawee et al., 2013; Barnard et al., 2015; Harbeck et al., 2019; Howell et al., 2014; Kamińska et al., 2015; Kungu et al., 2002; McTiernan, 2003; Ozsoy et al., 2017; Patterson et al., 2010; Rock and Demark-Wahnefried, 2002; Singletary, 2003; Sun et al., 2017; Yang et al., 2007). Lastly, previous chest radiation, especially during adolescence, emerges as a noteworthy risk factor, predisposing individuals to the development of BC in later life (Anderson et al., 2014; Anothaisintawee et al., 2013; Barnard et al., 2015; Harbeck et al., 2019; Howell et al., 2014; Kamińska et al., 2015; McTiernan, 2003; Ozsoy et al., 2017; Patterson et al., 2010; Rock and Demark-Wahnefried, 2002; Singletary, 2003; Sun et al., 2017; Yang et al., 2007). These multifaceted risk factors collectively underscore the importance of proactive measures and early detection in BC prevention and management.

1.3.2 Breast Cancer pathogenesis:

Each BC case is unique itself. Based on the expression profile of hormone and growth factor receptors, BC is categorized as basal-like, ERBB2+, normal breast-like, Luminal subtype A, Luminal subtype B, and Luminal subtype C (Sørlie et al., 2001). The BC negative for expression of ER (estrogen receptor), PR (progesterone receptor), and HER2 (human epidermal growth factor receptor) are called triple-negative breast cancer (TNBC) (Kashyap et al., 2022; Tan and Swain, 2008). Mutations in tumor suppressor genes, such as tumor suppressor protein p53 (TP53) (Berns et al., 2000; Bertheau et al., 2008; Brigham et al., 2012; Chen et al., 2012a; Silwal-Pandit et al., 2014), and the activation of oncogenes, such as HER2/neu (Krishnamurti and Silverman, 2014), are pivotal in the transformation of normal

breast cells into cancerous ones, instigating unchecked cell proliferation. Additionally, the presence of chronic inflammation within breast tissue can significantly foster the initiation of tumors. Inflammatory mediators and intricate signaling pathways, exemplified by NF-kB, have been directly implicated in the underlying mechanisms of BC pathogenesis (Kumar et al., 2022). Furthermore, epigenetic modifications, encompassing DNA methylation and histone alterations, have the capacity to reshape gene expression patterns, further contributing to the development of BC (Dworkin et al., 2009). Notably, the tumor microenvironment, comprised of immune cells, fibroblasts, and extracellular matrix constituents, assumes a pivotal role in the progression and metastasis of BC (Soysal et al., 2015). This intricate interplay of genetic, epigenetic, and microenvironmental factors underscores the multifaceted nature of BC pathogenesis (Feng et al., 2018b).

1.3.3 Breast Cancer chemoprevention:

Among all recorded BC cases, over 70% are characterized by the status of estrogen receptors (Aouad et al., 2022; Pan et al., 2017). Despite the administration of adjuvant endocrine therapy for estrogen receptor-positive BC, the risk of recurrence remains notably high, particularly in the early stages (Marcinkowski et al., 2017; Pan et al., 2017). Selective estrogen receptor modulators (SERMs), exemplified by tamoxifen and raloxifene, have garnered attention for their ability to diminish BC risk, particularly in individuals at high risk (Fisher et al., 1998). These medications function by obstructing estrogen receptors within breast tissue, impeding the hormone's influence on cancer development. Furthermore, aromatase inhibitors such as exemestane and anastrozole have demonstrated their effectiveness in diminishing BC risk, particularly in postmenopausal women (Goss et al., 2011). These inhibitors operate by suppressing estrogen production, a hormone associated with BC progression. Intriguingly, research has suggested that the consistent use of aspirin and nonsteroidal anti-inflammatory drugs (NSAIDs) may correlate with a reduced risk of breast cancer, attributable to their anti-inflammatory properties(Gierach et al., 2008). Sufficient vitamin D levels have been associated with reduced breast cancer risk, suggesting that vitamin D supplementation could be considered as a potential preventive strategy against the disease (Mohr et al., 2014)

1.3.4 Breast Cancer treatment:

Numerous treatment options are available for BC, encompassing surgical procedures, radiotherapy, chemotherapy, and immunotherapy (Brand et al., 2000; Nounou et al., 2015; Sharma et al., 2010). The surgical removal of axillary lymph nodes (ALND, axillary lymph node dissection) serves a dual purpose: it aids in assessing the extent of cancer cell dissemination and can also be a therapeutic intervention, but is not necessary and could not be a treatment option for all patients (Sener, 2020). Radiation therapy is employed in the treatment of all breast cancer subtypes; however, its significance is particularly notable in the case of TNBC (He et al., 2018). Radiation therapy resistance may manifest in advanced invasive breast cancer (Delaney et al., 2005). Cancer stem cells, in conjunction with a hypoxic tumor microenvironment characterized by low oxygen levels, contribute to enhanced cell proliferation and resistance to apoptosis, potentially playing a role in resistance to radiation therapy (Gilreath et al., 2021; Qi et al., 2017). Various chemotherapy protocols have been employed in BC management, such as anthracycline-based options such as doxorubicin and taxane-based treatments like paclitaxel (Gradishar et al., 2018). The selection of these regimens is typically customized to align with the specific subtype and stage of BC, ensuring a more targeted therapeutic approach (Gu et al., 2022). Alkylating agents (such as cyclophosphamide, thiotepa, and L-phenylalanine mustard), antimetabolites [like 5-fluorouracil (5FU) and methotrexate], vinca alkaloids (including vincristine and vinblastine), and antitumor agents (such as doxorubicin, mitomycin, and others) are effective drugs against metastatic BC (Henderson, 1991). The chemotherapy regimen consisting of taxanes, anthracyclines, and cyclophosphamide can lead to the development of resistance in BC patients (Coley, 2008). Approaches, such as adjuvent bevacizumab, have shown promise in the context of HER2-negative BC treatment, offering novel strategies for combating this challenging disease (Slamon et al., 2011) with a 5 years survival in patients with metastatic BC is less than 30% (Slamon et al., 2011).

1.3.5 Phenolic compounds with in vivo anti-breast cancer activities:

Extensive research has shed light on the promising therapeutic potential of phytochemicals, compounds derived from plants, in BC treatment (Avtanski and Poretsky, 2018; Bishayee and Sethi, 2016; Ham et al., 2015; Losada-Echeberría et al., 2017; Rabi and Bishayee, 2009; Reuben et al., 2012; Sinha et al., 2012). These group of compounds mainly dietary phytochemicals, nutritional herbs, and other natural compounds, are often regarded as safer with being more readily accessible. Plants naturally produce phenolic compounds as

secondary metabolites, characterized by their multiple aromatic rings containing two or more hydroxyl groups. These phenolic compounds encompass an extensive variety, with approximately 8000 chemical structures (Vuolo et al., 2019). They are categorized into distinct classes based on their chemical structures, including flavonoids (such as anthocyanidins, flavanols, flavanones, flavones, flavonols, and isoflavonoids) and non-flavonoids, which encompass phenolic acids (like hydroxycinnamic acids and hydroxybenzoic acids), coumarins, stilbenes, lignans, and tannins (Tsimogiannis and Oreopoulou, 2019). Fruits and vegetables serve as significant sources of these phenolic compounds. Many phenolic compounds are recognized for their pharmacological properties, which include antioxidative, anti-diabetic, anti-inflammatory, neuroprotective, and anticancer effects (Darvesh et al., 2010; de Paulo Farias et al., 2021; Kiruthiga et al., 2020; Vuolo et al., 2019). Although Western medications have demonstrated substantial efficacy in the prevention and treatment of BC, extracts derived from various plants and plant-based products continue to be utilized. Throughout history, humans have harnessed the therapeutic potential of plants as traditional or ethnical remedies for addressing diverse health concerns, including cancer. The predominant factors contributing to their widespread acceptance include their presumed minimal side effects, ready accessibility, and affordability when compared to synthetic drugs (Jain et al., 2021; Karimi et al., 2015). Polyphenolic phytochemicals with in vivo anti-breast cancer activity are listed in Table 1.4.

Table 1.4: Anti-breast cancer effects and mechanisms of action of phenolic compounds based on in vivo studies.

Phyto-compounds	Source	Animal Model Studied	Dose and Route of Administration	Mode of Action	Ref.
Flavonoids					
Apigenin	Parsley, wheat, onions, apples, and tea plants	Her2/neu-positive BT-474 xenograft tumors in nude mice	Intraperitoneal injection, 50mg/kg body weight	Induced apoptosis; suppressed tumor growth; downregulated the expression of Her2/neu; reduced the level of VEGF.	(Khan et al., 2019b)
		MD-AMB-231 tumor xenograft in BALB/c nude mice	Cells were treated with 20μM apigenin before injecting into the mice	Suppressed cancer stem cells like properties; suppressed YAP/TAZ transcription	(Li et al., 2018b)
Baicalein	*Scutellaria baicalensis* Georgi	MD-AMB-231 tumor xenograft in	Intra gastric gavage, 50 and 100 mg/kg body weight	Suppressed expression of vimentin and snail; upregulated N-cadherin; suppressed metastasis.	(Ma et al., 2016)

		BALB/c nude mice		Downregulated SATB1, WNT and β-catenin.	
		MD-AMB-231 tumor xenograft in BALB/c nude mice	Intraperitoneal injection; 10 mg/kg body weight	Upregulated the expression of lncRNA PAX8-AS1-N; suppressed tumor growth; induced apoptosis.	(Yu et al., 2018b)
		MD-AMB-231 and MCF7 tumor xenograft in BALB/c nude mice	Intragastric gavage; 100mg/kg body weight	Suppressed PI3K/AKT pathway; induced apoptotic and autophagic cell death;	(Yan et al., 2018)
Chrysin	*Passiflora caerulea, Passiflora incarnata, Oroxylum indicum*	EAC induced tumor xenograft in Swiss Albino mice	Intravenous injection, 10mg/kg body weight	Suppressed tumor growth by induction of apoptosis.	(Ghosh et al., 2023)
		MD-AMB-231 induced tumor xenograft in BALB/c nude mice	Intravenous injection, 10mg/kg body weight	Enhanced p53 mediated apoptosis; downregulated MMPs and NFκB by enhanced GPER (G protein coupled estrogen receptor) expression;	(Kim and Jung, 2020)
		4T1 induced solid tumor and lung metastasis in BALB/c mice	Oral administration, 250mg/kg body body weight	Suppressed tumor growth and metastatic potential;	(Lirdprapamongkol et al., 2013)
Curcumin	*Curcuma longa*	BALB-neuT mice transgenic for the neu oncogene	-	Suppressed tumor;	(Masuelli et al., 2013)
		DMBA to induce breast tumors in SENCAR mice	Dietary supplement, 2mg/kg body weight	Suppressed breast tumor;	(Siddiqui et al., 2013)
		MD-AMB-231 induced tumor xenograft in BALB/c nude mice	Intraperitoneal injection, 50 and 200 mg/kg body weight	Induced apoptosis; suppressed tumor growth;	(Lv et al., 2014)
		MD-AMB-231 induced tumor xenograft in	Dietary supplement, 6% (25mg/diet)	Suppressed tumor growth and angiogenesis; induced apoptosis; suppressed the expression of PECAM-1,	(Bimonte et al., 2015)

		Foxn1nu/nu mice		cyclin D1, and p65; suppressed NFκB activation;	
		MD-AMB-231 induced tumor xenograft in athymic mice	Intraperitoneal injection, 300 mg/kg body weight	suppressed tumor growth and proliferation; prevented angiogenesis;	(Carvalho Ferreira et al., 2015)
Cyanidin-3-glucoside	Mulberries, blackberries (*Rubus fruticosus*)	BT474 induced tumor xenograft in nude mice	Intraperitoneal injection, 6 mg/kg body weight	Suppressed tumor growth;	(Liu et al., 2014)
		MD-AMB 453 induced tumor xenograft in nude mice	-	Induced apoptosis; suppressed tumor growth;	(Cho et al., 2017)
Delphinidin-3-glucoside	Berries, pomegranates, eggplant, roselle, and wine	MD-AMB-231-Luc-GFP induced tumor xenograft in athymic mice	Oral administration, 40 mg/kg body weight	Suppressed Akt activation; prevented Akt mediated expression and binding of IRF1 to HOTAIR promoter; downregulated HOTAIR expression; suppressed tumor growth;	(Yang et al., 2016)
EGCG	*Camellia sinensis* L. Ktze	MD-AMB-231 induced tumor xenograft in athymic nude mice (NCr-nu/nu)	Oral administration, 1 mg/animal	Induced apoptosis; Suppressed proliferation;	(Thangapazham et al., 2007)
		MD-AMB-231 induced tumor xenograft in athymic nude mice	Subcutaneous injection, 50 mg/kg body weight	Suppressed tumor growth; induced apoptosis; inhibited proteasome;	(Landis-Piwowar et al., 2007)
		MD-AMB-231 induced tumor xenograft in CD1 athymic nude mice	Intraperitoneal injection, 25 mg/kg body weight	Suppressed tumor growth;	(Somers-Edgar et al., 2008)
		4T1induced tumor in BALB/c mice	Intraperitoneal injection, 5 to 20 mg/ kg body weight	Suppressed tumor growth; EGCG downregulated activity and transcription of hexokinase (HK), phosphofructokinase (PFK), lactic dehydrogenase (LDH), and pyruvate kinase (PK); suppressed expression of hypoxia-inducible factor 1α	(Wei et al., 2018)

				(HIF1α), glucose; transporter 1 (GLUT1), and vascular endothelial growth factor (VEGF);	
		MCF7 induced tumor xenograft in CB-17 severe combined immunodeficient (SCID) mice	Oral administration, 100 mg/kg body weight	Suppressed tumor growth; suppressed miR-25 expression;	(Zan et al., 2019)
Eriodictyol	*Eriodictyon californicum*	4T1 induced tumor in BALB/c mice	Intraperitoneal injection, 60 mg/kg body weight	Suppressed tumor growth; inhibited metastasis;	(Debnath et al., 2022)
Fisetin	Strawberry, apple, persimmon, grapes, onion, and cucumber	MD-AMB-231 induced tumor xenograft in nude mice	Intraperitoneal injection, 100 mg/kg body weight	Suppressed primary tumor growth and lung metastasis; upregulated PTEN suppressed PI3K-Akt-GSK-3β pathway;	(Li et al., 2018a)
		4T1 induced tumor in BALB/c mice	Intraperitoneal injection, 223 mg/kg body weight	Inhibited tumor growth; induced apoptosis;	(Sun et al., 2018a)
Formononetin	*Astragalus membranaceus*	MD-AMB-231-luc induced tumor xenograft in BALB/c nude mice	Intraperitoneal injection, 10 and 20 mg/kg body weight	Suppressed lung metastasis; increased survival rates;	(Zhou et al., 2014b)
		MD-AMB-231-induced tumor xenograft in BALB/c nude mice	Intragastric administration, 100 mg/kg body weight	Inhibited tumor growth; suppressed phosphorylation of PI3K, Akt, STAT3, and MMP-2/9; inhibited FGF2/FGFR2 signaling pathways that in turn suppressed angiogenesis;	(Wu et al., 2015)
Genistein	*Genista tinctoria*	MD-AMB-231 induced tumor xenograft in athymic nude mice	Dietary supplement, 750 µg genistein/g	Suppressed tumor growth.	(Vantyghem et al., 2005)
		F3II cells induced tumor xenograft in syngeneic mice	Intraperitoneal injection, 10 mg/kg body weight	Suppressed tumor growth; prevented angiogenesis;	(Farina et al., 2006)

Hesperetin	Citrus fruits	Aromatase overexpressing MCF7 tumor xenograft in athymic mice.	Dietary administration, 1000ppm and 5000ppm.	Suppressed tumor growth and expression of estrogen receptor gene pS2 at RNA level in tumor tissue; acted as aromatase inhibitor; Downregulated Cyclin D1, CDK4 and Bcl-x(L) at protein level.	(Ye et al., 2012)
Hinokiflavone	*Selaginella tamariscina*, *Juniperus phoenicea*, and *Rhus succedanea*	MD-AMB-231 tumor xenograft in BALB/c nude mice	Intraperitoneal injection, 20 and 40 mg/kg body weight	Suppressed tumor growth; prevented migration; downregulated MMP2;	(Huang et al., 2020)
Icaritin	*Epimedium* sp.	MCF7 induced tumor xenograft in NU/NU nude mice	Tail vein injection; 35 mg/kg body weight	Suppressed tumor growth;	(Wang et al., 2020a)
Isoliquiritigenin	*Glycyrrhiza glabra, Glycyrrhiza uralensis* Fisher	MD-AMB-231 induced tumor xenograft in nude mice	Intraperitoneal injection, 25 and 50 mg/kg body weight	Suppressed tumor growth; downregulated VEGF expression;	(Wang et al., 2013)
		MD-AMB-231 induced tumor xenograft in NOD/SCID mice	Oral gavage, 50 mg/kg body weight	Suppressed tumor growth;	(Wang et al., 2014)
Kaempferol	Apple, tea, broccoli, and grapefruit	MCF7 induced tumor xenograft in BALB/c nude mice	Subcutaneous injection, 100 mg/kg body weight	Suppressed 17β-estradiol and triclosn induced tumor growth;	(Kim et al., 2016)
Luteolin	Celery, parsley, broccoli, onion leaves, carrots, peppers, cabbages, and tea	4T1induced tumor in BALB/c mice	Intravenous injection, 100 mg/kg body weight	Reduced DOX induced toxicity;	(Du et al., 2008)
		MD-AMB-231 induced tumor xenograft in BALB/c nude mice	Dietary supplement, 0.01% and 0.05%	Suppressed tumor growth;	(Lee et al., 2012a)
		MD-AMB-231 induced tumor xenograft in	Tail vein injection, 20 and 40 mg/kg body weight	Suppressed tumor growth;	(Sun et al., 2015)

		BALB/c nude mice			
		MD-AMB-231 induced tumor xenograft in BALB/c nude mice	Intraperitoneal injection, 30 mg/kg body weight	Suppressed tumor growth; induced apoptosis; inhibited proliferation;	(Jeon et al., 2015)
		T47-D induced tumor xenograft in nude mice	Intraperitoneal injection, 20 mg/kg body weight	Suppressed tumor growth; suppessed VEGF expression; reduced angiogenesis;	(Cook et al., 2015)
		MD-AMB-231 induced lung metastasis in athymic mice.	Intraperitoneal injection,10, 20 and 40 mg/kg body weight	Suppressed lung metastasis; suppressed VEGF;	(Cook et al., 2016)
		MD-AMB-231 induced lung metastasis in nude mice.	Intraperitoneal injection, 100 mg/kg body weight	Suppressed lung metastasis; reversed EMT; suppressed β-catenin expression;	
		4t1 induced tumor in BALB/c mice.	Intraperitoneal injection, 20 and 40 mg/kg body weight	Suppressed tumor growth; downregulated SGK1, and phosphorylation of FOXO3a along with upregulated BNIP3; upregulated of BAX, Bim, Beclin1, and LC3II;	(Wu et al., 2023a)
Morin	Old fustic (*Chloroph ora tinctoria*) and osage orange (*Maclura pomifera*)	MD-AMB-231 induced tumor xenograft in athymic nude mice	Intraperitoneal injection, 10 mg/kg body weight	Suppressed tumor growth; prevented EMT; inhibited Akt activation;	(Jin et al., 2014)
		MD-AMB-231 induced lung metastasis in athymic nude mice	Intraperitoneal injection, 10 mg/kg body weight	Suppressed lung metastasis; inhibited EMT by suppressing VCAM-1 and N-cadherin;	(Lee et al., 2016)
Myricetin	Tea, barriers, fruits, vegetables	4T1 induced lung metastasis in BALB/c mice	Intraperitoneal injection, 25 and 50 mg/kg body weight	Suppressed lung metastasis; reduced no of lung nodules; suppressed MMP2 and MMP9;	(Ci et al., 2018)
Naringenin	Oranges, lemons, and grapefruit	T47-D induced tumor xenograft in	Oral administration, 50 mg/kg body weight	Suppressed tumor growth and proliferation by FKBP4/NR3C1/NRF2 signaling pathway;	(Xiong et al., 2022)

		BALB/c nude mice			
Naringin	Oranges, lemons, and grapefruit	MD-AMB-231 induced tumor xenograft in severe combined immunodeficiency (SCID) hairless mice	Intraperitoneal injection, 100 mg/kg body weight	Suppressed tumor growh; upregulated p21; downregulated survivin and β-catenin	(Li et al., 2013)
Nobiletin	Peel of various *Citrus* fruits	MD-AMB-231 induced tumor xenograft in athymic nude mice	Intraperitoneal injection, 10 mg/kg body weight	Suppressed tumor growth; inhibited NFκB signaling;	(Kim et al., 2022)
		MCF7 induced tumor xenograft and liver metastasis in BALB/c nude mice	Oral administration, 30 and 60 mg/kg body weight	Suppressed tumor growth; downregulated phosphorylation of ERK, PI3K and STAT3; suppressed liver metastasis;	(Wu et al., 2023b)
Oroxylin A	*Scutellaria baicalensis*	MD-AMB-231 induced tumor xenograft in BALB/c athymic nude mice	Intravenous injection, 50 and 100 mg/kg body weight	Suppressed tumor growth; downregulated HIF1α; upregulated SIRT3 and SOD;	(Wei et al., 2015)
		4T1 induced tumor in BALB/c mice	Oral administration, 80 mg/kg body weight	Suppressed tumor growth; prevented CAF activation by suppressing ACTN1 expression; downregulated phosphorylation of FAK and STAT3;	(Cao et al., 2020)
Phloretin	*Manchurian apricot*	MD-AMB-231 induced tumor xenograft in BALB/c nude mice	Intraperitoneal injection, 10 and 50 mg/kg body weight	Suppressed tumor growth; upregulated p53, p21 and E-cadherin protein level; downregulated N-cadherin and vimentin;	(Wu et al., 2018)
Quercetin	Apples, nuts, cauliflower, cabbage, onions, grapes, berries, broccoli, citrus	MCF7 induced tumor xenograft in BALB/c nude mice	Intraperitoneal injection, 50, 100 and 200 mg/kg body weight	Suppressed tumor growth; induced apoptosis;	(Hashemzaei et al., 2017)
		MCF7 induced	Intravenous injection, 15	Suppressed tumor growth and improved survival;	(Sun et al., 2023)

	fruits, cherries, green tea, and coffee	tumor xenograft in BALB/c nude mice	mg/kg body weight		
		EAC induced tumor in Swiss albino mice	Intraperitoneal injection, 50 mg/kg body weight	Suppressed tumor growth; inhibited angiogenesis; activated vitamin D3 receptor;	(Sannappa Gowda et al., 2023)
		7,12-dimethylbenz(a)anthracene (DMBA) induced mammary carcinoma in Sprague-Dawley rats, chick embryo angiogenesis assay (CEA),	Intratumoral injection, 15 mg/kg body weight	Suppressed tumo growth; downregulated of vimentin, N-cadherin, Snail, Slug, Twist, MMP-2, MMP-9, p-EGFR, VEGFR-2, p-PI3K, Akt and p-GSK3β in protein level; upregulated E-cadherin; suppressed EMT;	(Balakrishnan et al., 2016)
Rutin	Buckwheat, Mez, Labisia pumila, *Sophora japonica* L., Schum, *Canna indica* L., and *Ruta graveolens* L.	EAC induced tumor in Swiss albino mice	Oral administration, 20 mg/kg body weight	Suppressed tumor growth; induced apoptosis;	(Saleh et al., 2019)
		MD-AMB-231 induced tumor xenograft in BALB/c nude mice	Subcutaneous injection, 30 mg/kg body weight	Suppressed tumor growth; downregulated phosphorylation of EGFR and FAK;	(Lee et al., 2023)
Scutellarin	*Scutellaria barbata*	MCF7 induced tumor xenograft in BALB/c nude mice	Intraperitoneal injection, 200 mg/kg body weight	Suppressed tumor growth; downregulated YAP expression while increasing its phosphorylation;	(Hou et al., 2017)
		MD-AMB-231 induced tumor xenograft in BALB/c nude mice	Intragastric administration; 1 and 10 mg/kg body weight	Suppressed Inhibited TNFα-TNFR2-ERK1/2-EZH2 signaling; blocked TNFα-induced vascular endothelial barrier breakdown; prevented metastasis;	(Mei et al., 2022)
		4T1 induced tumor in BALB/c mice	Intragastric administration; 10 mg/kg body weight	Suppressed tumor growth and lung and liver metastasis;	(Mei et al., 2023)
Silibinin	*Silybum marianum*	4T1 induced tumor in	Intratumoral injection, 1 and 2 mg/kg	Suppressed tumor growth; induced apoptosis;	(Firouzi et al., 2022)

		BALB/c mice			
		4T1 induced metastasis in BALB/c mice	Intraperitoneal injection, 30 mg/kg body weight	Suppressed lung metastasis;	(Xu et al., 2013)
Taxifolin	Olive oil, grapes, citrus fruits, and onion	4T1 induced tumor metastasis in BALB/c mice	Oral gavage, 100 mg/kg body weight	Suppressed primary tumor growth; prevented lung metastasis;	(Li et al., 2019a)
		7,12-dimethylbenz(a)anthracene (DMBA) induced mammary carcinoma in Sprague Dawley rats	Gastric ingestion; 10, 20, and 40 mg/kg body weight	Suppressed tumor growth, downregulated the RNA and protein level of AhR, CYP1A1, and CYP1B1;	(Haque and Pattanayak, 2017)
Tricin	Rice bran, oats, barley, and wheat	Tricin pretreated MD-AMB-468 induced tumor xenograft in MF1 athymic nude mice	Subcutaneous injection of tricin pre-treated cells, 0.11, 1.1 and 11 μM	Suppressed tumor growth at a cell treatment dose of 11μM;	(Cai et al., 2004)
Wogonin	*Scutellaria baicalensis*, *Scutellaria radix*	MD-AMB-231 and 4T1 induced tumor in BALB/c nude mice	Intraperitoneal injection, 40 mg/kg body weight	Suppressed tumor growth and induced senescence; upregulated g P16, γ-H2AX, GLB1 and H3K9Me3; downregulated H3K9Ac and TXNRD2; encouraged macrophages M1 polarization;	(Yang et al., 2020)
		MD-AMB-231 and T47D induced tumor xenograft in nude BALB/cAnNCrjBgi-nu mice	Oral gavage, 0.1, 1.0 and 10 mg/kg body weight	Suppressed tumor growth;	(Chung et al., 2008)
Xanthohumol	*Humulus lupulus*	4T1 induced tumor in BALB/c nude mice	Intragastric administration, 100 and 200 mg/kg body weight	Suppressed tumor growth; induced caspase 3 cleavage; downregulated surviving; induced apoptosis; suppressed Notch1 and Ki67 expression;	(Sun et al., 2018b)

		KPL-3C induced tumor xenograft in BALB/c nude mice	Intraperitoneal injection, 0.3 and 1.0 mg/kg body weight	Suppressed tumor growth; downregulate phosphorylation of Akt and MAPK; suppressed BIG3-PHB2 complex formation;	(Yoshimaru et al., 2014)
Phenolic acids					
Caffeic acid	Coffee, wine tea	Ehrlich ascites carcinoma (EAC) cells induced tumor in albino mice	Intraperitoneal injection, 2.25 mg/kg body weight	Suppressed tumor growth; induced caspase 3 cleavage indicating induction of apoptosis; inhibited VEGF;	(Motawi et al., 2016)
		MHCC97H cells induced tumor xenograft in BALB/c nude mice	Intraperitoneal injection, 10 mg/kg body weight	Suppressed tumor growth; downregulated CD44, EpCAM, Oct-4, Lin-28B, and SMAD2; upregulated miR-148a; suppressed cancer stem cells (CSCs)-like properties;	(Li et al., 2015)
		MCF7 and MD-AMB231 induced tumor xenograft in Ncr-nu-nu mice	Dietary supplementation, 10, 50, and 250 nmol/mouse	Suppressed tumor growth and volume;	(Wu et al., 2011)
Chlorogenic acid	Apple, betel, coffee beans, kiwi, grapes, eggplant, pear, plum, potato, and tea	MCF7 induced tumor xenograft in BALB/c nude mice	Intraperitoneal injection, 10 mg/kg body weight	Suppressed tumor growth; downregulated LRP6, vimentin, N-cadherin, ZEB1, MMP9, and MMP2; upregulated E-cadherin and ZO-1 expression; inhibited EMT; prevented metastasis;	(Xue et al., 2023)
Ellagic acid		MD-AMB-231 induced tumor xenograft in BALB/c nude mice	Intraperitoneal injection, 50 and 100 mg/kg body weight	Suppressed tumor growth; decreased phosphorylation of VEGFR2, Akt, and JNK; downregulated CD31;	(Wang et al., 2012)
		MDA-MB-231 or BT-549 induced tumor xenograft in NOD/SCID mice	Oral gavage, 50 mg/kg body weight	Suppressed tumor growth by targeting ACTAN4; decreased CSC population by inhibiting CD44+CD24−/low phenotypes; suppressed metastatic potential;	(Wang et al., 2017)

		EAC induced tumor in Swiss albino mice	Intraperitoneal injection, 50 and mg/kg body weight	Suppressed tumor growth; induced apoptosis;	(Kaur et al., 2021)
Ferulic acid	Rice, wheat, pineapple, grains, and peanuts	MD-AMB-231 induced tumor xenograft in BALB/c nude mice	Oral gavage, 100 mg/kg body weight	Suppressed tumor growth; induced caspase 3 activation and apoptosis; reduced Ki67; suppressed EMT;	(Zhang et al., 2016)
Gallic acid	Barriers and pomegranates	MD-AMB-231 and T47D induced tumor xenograft in BALB/c nude mice	Oral gavage, 1-9 mg/kg body weight	Suppressed tumor growth; upregulated transcript level of CYP1A1, miR-212 and miR-132;	(Hanieh et al., 2022)
Lignans					
Arctigenin	*Arctium lappa, Forsythia suspensa.*	MD-AMB-231 induced tumor xenograft in BALB/c nude mice	Intraperitoneal injection, 4 and mg/kg body weight	Suppressed tumor growth; induced apoptosis by upregulating P-p38 and downregulating Bcl2; induced nuclear translocation of ATF;	(Hsieh et al., 2014)
		MD-AMB-231 induced tumor xenograft in nude immunodeficient (nu/nu) mice	Intraperitoneal injection, 15 mg/kg body weight	Suppressed tumor growth; induced phosphorylation of STAT3; transcriptionally downregulated Cyclin D1 and Mcl1;	(Feng et al., 2017)
		4T1 induced tumor in BALB/c mice	Intragastrica administration, 50 mg/kg body weight	Suppressed tumor growth; suppressed proliferation by downregulating Ki67;	(Shi et al., 2020)
Honokiol	*Magnolia grandiflora*	MD-AMB-231 induced tumor xenograft in athymic nude mice	Intraperitoneal injection, 3 mg/kg body weight	Suppressed tumor growth; upregulated P-AMPK, LKB1, P-ACC and downregulated Ki67, P-S6K;	(Nagalingam et al., 2012)
		MD-AMB-231 induced tumor xenograft in athymic nude mice	Intraperitoneal injection, 3 mg/kg body weight	Suppressed tumor growth; downregulated Vimentin and Fibronectin; upregulated CK18;	(Avtanski et al., 2014)

		MD-AMB-231 induced tumor xenograft in nude mice	Intraperitoneal injection, 3 mg/kg body weight	Suppressed tumor growth; downregulated MTA1, Wnt1, β-catenin and cyclin D1;	(Avtanski et al., 2015)
Magnolol	*Magnolia officinalis*	MD-AMB-231 and MCF7 induced tumor xenograft in nude immunodeficient (nu/nu) mice	Intraperitoneal injection, 40 mg/kg body weight	Suppressed tumor growth; suppressed invasiveness; downregulated MMP9;	(Liu et al., 2013)
Vitexin	*Vitex negudo*	MCF7 induced tumor xenograft in athymic BALB/c mice	Intraperitoneal injection, 2 mg/kg body weight	Suppressed tumor growth and proliferation; prevented ER-phagy; inhibited FAM134B-BiP complex;	(Chipurupalli et al., 2022)
Stilbene					
Polydatin	*Picea sitchensis*	4T1 induced tumor in BALB/c nude mice	Intraperitoneal injection, 100 mg/kg body weight	Suppressed tumor growtg; induced apoptosis; downregulated VEGF, Ki67, HIF1α, HK2, P-PI3K and P-AKT;	(Zhang et al., 2019)
		MD-AMB-231 induced tumor xenograft in BALB/c nude mice	Intraperitoneal injection, 25 mg/kg body weight	Suppressed tumor growth; downregulated Nrf2;	(Li et al., 2023)
Pterostilbene	Blueberries and cranberries	MD-AMB-231 induced tumor xenograft in 01B74-Athymic NCr-nu/nu nude mice	Oral gavage; 56 mg/kg body weight	Suppressed tumor growth;	(Pan et al., 2014)
		MD-AMB-231 induced tumor xenograft in NOD/SCID mice	Intraperitoneal injection, 10 mg/kg body weight	Suppressed tumor growth; suppressed EMT markers like vimentin, Src, Slug, Twist and ZEB1;	(Su et al., 2015)
Resveratrol	Grapes, blueberries, raspberries	MD-AMB-231 induced tumor xenograft in	Intraperitoneal injection, 25 mg/kg body weight	Suppressed tumor growth; induced apoptosis;	(Garvin et al., 2006)

	, mulberries, and peanuts	athymic nude mice			
		Intravenous injection of 4T1 in BALB/c mice	Oral administration, 200 mg/kg body weight	Suppressed lung metastasis; downregulated MMP9 activity;	(Lee et al., 2012b)
		MD-AMB-231 induced tumor xenograft in athymic nude mice	Intraperitoneal injection, 100 mg/kg body weight	Suppressed lung metastasis;	(Sun et al., 2019)

1.4 *Bergenia ligulate*:

Bergenia ligulata, recognized by its alternate names such as Indian rhubarb or Pashanbheda, is an enduring herb that originates from the Himalayan region, with a notable presence in countries like India, Nepal, and Bhutan. It belongs to the Saxifragaceae plant family and holds significant importance in the domains of traditional medicine, particularly in Ayurvedic and traditional Tibetan medicinal practices. This plant can be commonly encountered in regions such as Afghanistan, Pakistan, Central Asia, and East Asia. It typically thrives in the damp and shaded environments of rocky cliffs and crevices. This plant has been reported for its anti-viral (Rajbhandari et al., 2001; Rajbhandari et al., 2003), antilithiatic (Aggarwal et al., 2014; Ballabh et al., 2008; Bashir and Gilani, 2009; Garimella et al., 2001; Joshi et al., 2005; Sadat et al., 2015), anti-microbial (Agnihotri et al., 2015), anti-oxidant (Agnihotri et al., 2015; Sadat et al., 2015), anti-inflammatory (Singh et al., 2021) and anti-cancer (Faheem et al., 2022; Ghosh et al., 2021) properties.

1.4.1 Identifying characteristics of *Bergenia ligulata* (Wall.) Engl.:

Bergenia ligulata is primarily a perennial herb characterized by short, wide (approximately 2.5 cm), fleshy, and creeping stems that typically reach a height of 32-35 cm. It produces oval to round leaves measuring 5-15 cm in size. The leaves are arranged alternately and may be either stipule-free or have an adnate stipule, small hairy featuring on both the upper and lower surfaces. The flowers exhibit a color spectrum that includes white, purple, or pink. They have a diameter of around 3.2 cm and are organized in pentamerous clusters within a cymose panicle. The petals are arranged in an imbricate or valvate fashion, and the ovary is

united, typically composed of 3-5 carpels with axile placentation. The number of styles matches that of the carpels along with capitates or subcapitate stigmas. The plant produces a significant quantity of ovules and seed. It has rhizome as it is a legume (Gurav and Gurav, 2014; Singh et al., 2017). The rhizome is firm and takes on a barrel-shaped or cylindrical form, with a length of 1-3 cm and width of 1-2 cm. Its surface is covered with small roots, ridges, furrows, wrinkles, and root scars appeared to be brawn in colour containing aromatic odour and tasted dry, puckering, or rough sensation in the mouth (Srivastava and Rawat, 2008).

1.4.2 Phytochemical content of *Bergenia ligulata*:

Bergenia ligulata is a rich source for various medically important bio-active compounds of different phytochemical classes including phenolics, flavonoids, alkaloids, terpenoids, saponins, catechol, etc. The compounds reported in in this plant include bergenin, 11/4-O-galloyl bergenin, 11-O-P-hydroxy-benzoyl bergenin, arbutin, gallic acid, protocatechuic acid, chlorogenic acid, (+)-catechin-7-O-β-D-glucopyranoside, methyl gallate, leucocyanidin, eriodictyol-7-O-β-D- glucopyranoside, 4-hydroxy benzoic acid, 6-O-P-hydroxy-benzoyl arbutin, 6-O-protocatechuoyl arbutin, syringic acid, ferulic acid, (+)-afzelechin, paashanolactone, caryophyllene, 1,8-cineole, β-eudesmol, β-sitosterol, (+)-(6S)-parasorbic acid, 3-methyl-2-buten-1-ol, phytol, tannic acid, isovalaric acid, stigmasterol, avicularin, terpinen-4-ol, quercetin, (z)-asarone, reynoutrin, and sitoinoside I (Roychoudhury et al., 2022). The therapeutic significance of *Bergenia ligulata* is listed in table 1.5.

Table: 1.5: Therapeutic significance of *Bergenia ligulata:*

Therapeutic property	Part of plant used/ active component used	Treatment dose/ IC50	Experimental system used	Therapeutic effectiveness	Ref.
Antiviral	Rhizome, methanolic extract	54 and 56 µg/ml	In-vitro antiviral assay using Madine-darby canine kidney (MDCK) cells and Vero cells	Effective against influenza and Herpes simplex virus respectively;	(Rajbhandari et al., 2001)
	Rhizome, methanolic-	10 and 70 µg/ml	In-vitro antiviral assay using Madine-	Effective against influenza and Herpes simplex virus respectively;	(Rajbhandari et

	aqueous extract		darby canine kidney (MDCK) cells and Vero cells		al., 2001)
Antimicrobial	Leaf, methanolic extract	300 to 550 µg/ml	In-vitro anti-microbial assay	Effective against Escherichia coli, Pseudomonas corrugate, Bacillus subtilis, Streptomyces griseobrunneus, Streptomyces sampsonii;	(Agnihotri et al., 2015)
Antidiabetic	Rhizome, ethanolic extract, ethyl acetate fraction, (+)-afzelechin	0.13 mM	In-vitro cell free assays	Inhibited the activity of α-glucosidase;	(Saijyo et al., 2008)
Antilithiatic	Seed, aqueous extract	0.1g/mL	In-vitro cell free system	Inhibited calcium and phosphate precipitation;	(Garimella et al., 2001)
	Rhizome, methanolic-aqueous extract	Intraperitoneal injection, 5 and 10 mg/kg body weight	In-vivo, Wistar rats	Inhibited calcium oxalate stone formation; prevented the action of oxidative stress;	(Bashir and Gilani, 2009)
	Rhizome, ethanolic extract; bergenin	Oral administration, 10 mg/kg body weight	In-vivo, Wistar rats	Suppress oxidative stress; downregulated lactate dehydrogenase (LDH) activity which indirectly suppressed the calcium oxalate crystal formation; downregulated ALP (Alkaline phosphatase) activity;	(Aggarwal et al., 2014)
	Rhizome, aqueous extract; DCM fraction	Oral gavage, 185 mg/kg body weight	Ethylene glycol induced urolithiasis in Wistar rats	Suppressed amyloid deposition in Kupffer cells; suppressed calcium oxalate deposition; reduced the number of calcium oxalate crystals;	(Sharma et al., 2017a)
	Rhizome, ethanolic extract	200 µg/ml	In-vitro calcium oxalate crystallization assays using rat renal epithelial cell lines NRK-52E and CRL-1571	Reduced calcium oxalate crystal number and size;	(Singh et al., 2021)
Antioxidant	Aerial part, Ethanolic extract; 11-O-galloylbergenin	7.45 and 5.39 µg/ml	In-vitro cell free assay	Showed antioxidant potentials; inhibited urease activity;	(Sadat et al., 2015)

Anti-inflammatory	Rhizome, aqueous and ethanolic extract	Oral administration, 1 mg/kg body weight	Carrageenan induced inflammation model Wistar rats	Suppressed inflammation; downregulated superoxide dismutase (SOD);	(Sajad et al., 2010)
Anticancer	Rhizome, ethyl acetate fraction	Intraperitoneal injection; 30 mg/kg body weight	PC3 induced tumor xenograft in NOD-SCID mice	Suppressed tumor growth; induced apoptosis; inhibited NRF2 antioxidant response; monoamine oxidase A (MAO-A) induced reactive oxygen species (ROS) generation;	(Ghosh et al., 2021)
	Whole plant, aqueous extract	10 µg/ml	In-vitro assays using MCF7, HCT116 and A549 cells	Induced P53 mediated apoptosis; suppressed cancer cell growth, g2/m cell cycle arrest; induced ROS production;	(Faheem et al., 2022)

1.5 Aims and objectives of the present study:

Objective 1: Isolation and purification of solvent fraction from *Bergenia ligulata* most active against colon cancer and breast cancer by cell-based assays.

Objective 2: Chemical/molecular characterization of the active fraction by mass spectrometry.

Objective 3: Identification of underlying mechanism of action of the most active fraction.

Objective 4: Validation of potential anticancer activity in preclinical models.

References:

Advani, S.M., Advani, P., DeSantis, S.M., Brown, D., VonVille, H.M., Lam, M., Loree, J.M., Sarshekeh, A.M., Bressler, J., Lopez, D.S., 2018. Clinical, pathological, and molecular characteristics of CpG island methylator phenotype in colorectal cancer: a systematic review and meta-analysis. Translational oncology 11, 1188-1201.

Afrin, S., Giampieri, F., Gasparrini, M., Forbes-Hernández, T.Y., Cianciosi, D., Reboredo-Rodriguez, P., Zhang, J., Manna, P.P., Daglia, M., Atanasov, A.G., 2020. Dietary phytochemicals in colorectal cancer prevention and treatment: A focus on the molecular mechanisms involved. Biotechnology advances 38, 107322.

Aggarwal, D., Kaushal, R., Kaur, T., Bijarnia, R.K., Puri, S., Singla, S.K., 2014. The most potent antilithiatic agent ameliorating renal dysfunction and oxidative stress from Bergenia ligulata rhizome. Journal of ethnopharmacology 158, 85-93.

Agnihotri, V., Sati, P., Jantwal, A., Pandey, A., 2015. Antimicrobial and antioxidant phytochemicals in leaf extracts of Bergenia ligulata: a Himalayan herb of medicinal value. Natural Product Research 29, 1074-1077.
Aiello, P., Sharghi, M., Mansourkhani, S.M., Ardekan, A.P., Jouybari, L., Daraei, N., Peiro, K., Mohamadian, S., Rezaei, M., Heidari, M., 2019. Medicinal plants in the prevention and treatment of colon cancer. Oxidative Medicine and Cellular Longevity 2019.
Allison, K.H., 2012. Molecular pathology of breast cancer: what a pathologist needs to know. American journal of clinical pathology 138, 770-780.
Alonso-Castro, A.J., Domínguez, F., García-Carrancá, A., 2013. Rutin exerts antitumor effects on nude mice bearing SW480 tumor. Archives of medical research 44, 346-351.
Anderson, W.F., Rosenberg, P.S., Prat, A., Perou, C.M., Sherman, M.E., 2014. How many etiological subtypes of breast cancer: two, three, four, or more? Journal of the National Cancer Institute 106, dju165.
Angell, H.K., Bruni, D., Barrett, J.C., Herbst, R., Galon, J., 2020. The immunoscore: colon cancer and beyond. Clinical cancer research 26, 332-339.
Anothaisintawee, T., Wiratkapun, C., Lerdsitthichai, P., Kasamesup, V., Wongwaisayawan, S., Srinakarin, J., Hirunpat, S., Woodtichartpreecha, P., Boonlikit, S., Teerawattananon, Y., 2013. Risk factors of breast cancer: a systematic review and meta-analysis. Asia Pacific Journal of Public Health 25, 368-387.
Antoniou, A.C., Casadei, S., Heikkinen, T., Barrowdale, D., Pylkäs, K., Roberts, J., Lee, A., Subramanian, D., De Leeneer, K., Fostira, F., 2014. Breast-cancer risk in families with mutations in PALB2. New England Journal of Medicine 371, 497-506.
Antunes-Ricardo, M., Guardado-Félix, D., Rocha-Pizaña, M., Garza-Martínez, J., Acevedo-Pacheco, L., Gutiérrez-Uribe, J., Villela-Castrejón, J., López-Pacheco, F., Serna-Saldívar, S., 2021. Opuntia ficus-indica Extract and Isorhamnetin-3-O-Glucosyl-Rhamnoside Diminish Tumor Growth of Colon Cancer Cells Xenografted in Immune-Suppressed Mice through the Activation of Apoptosis Intrinsic Pathway. Plant Foods for Human Nutrition 76, 434-441.
Aouad, P., Zhang, Y., De Martino, F., Stibolt, C., Ali, S., Ambrosini, G., Mani, S.A., Maggs, K., Quinn, H.M., Sflomos, G., 2022. Epithelial-mesenchymal plasticity determines estrogen receptor positive breast cancer dormancy and epithelial reconversion drives recurrence. Nature communications 13, 4975.
Arber, N., Eagle, C.J., Spicak, J., Rácz, I., Dite, P., Hajer, J., Zavoral, M., Lechuga, M.J., Gerletti, P., Tang, J., 2006. Celecoxib for the prevention of colorectal adenomatous polyps. New England Journal of Medicine 355, 885-895.
Argilés, G., Tabernero, J., Labianca, R., Hochhauser, D., Salazar, R., Iveson, T., Laurent-Puig, P., Quirke, P., Yoshino, T., Taieb, J., 2020. Localised colon cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Annals of Oncology 31, 1291-1305.
Armaghany, T., Wilson, J.D., Chu, Q., Mills, G., 2012. Genetic alterations in colorectal cancer. Gastrointestinal cancer research: GCR 5, 19.
Armenian, S.H., Robison, L.L., 2013. Childhood cancer survivorship: an update on evolving paradigms for understanding pathogenesis and screening for therapy-related late effects. Current opinion in pediatrics 25, 16.
Arnold, M., Morgan, E., Rumgay, H., Mafra, A., Singh, D., Laversanne, M., Vignat, J., Gralow, J.R., Cardoso, F., Siesling, S., 2022. Current and future burden of breast cancer: Global statistics for 2020 and 2040. The Breast 66, 15-23.
Arnold, M., Sierra, M.S., Laversanne, M., Soerjomataram, I., Jemal, A., Bray, F., 2017. Global patterns and trends in colorectal cancer incidence and mortality. Gut 66, 683-691.
Au, A., Li, B., Wang, W., Roy, H., Koehler, K., Birt, D., 2006. Effect of dietary apigenin on colonic ornithine decarboxylase activity, aberrant crypt foci formation, and tumorigenesis in different experimental models. Nutrition and cancer 54, 243-251.
Auyeung, K.K.-W., Law, P.-C., Ko, J.K.-S., 2012. Novel anti-angiogenic effects of formononetin in human colon cancer cells and tumor xenograft. Oncology reports 28, 2188-2194.
Avtanski, D., Poretsky, L., 2018. Phyto-polyphenols as potential inhibitors of breast cancer metastasis. Molecular Medicine 24, 1-17.

Avtanski, D.B., Nagalingam, A., Bonner, M.Y., Arbiser, J.L., Saxena, N.K., Sharma, D., 2014. Honokiol inhibits epithelial–mesenchymal transition in breast cancer cells by targeting signal transducer and activator of transcription 3/Zeb1/E-cadherin axis. Molecular oncology 8, 565-580.
Avtanski, D.B., Nagalingam, A., Kuppusamy, P., Bonner, M.Y., Arbiser, J.L., Saxena, N.K., Sharma, D., 2015. Honokiol abrogates leptin-induced tumor progression by inhibiting Wnt1-MTA1-β-catenin signaling axis in a microRNA-34a dependent manner. Oncotarget 6, 16396.
Balakrishnan, S., Bhat, F., Raja Singh, P., Mukherjee, S., Elumalai, P., Das, S., Patra, C., Arunakaran, J., 2016. Gold nanoparticle–conjugated quercetin inhibits epithelial–mesenchymal transition, angiogenesis and invasiveness via EGFR/VEGFR-2-mediated pathway in breast cancer. Cell proliferation 49, 678-697.
Ballabh, B., Chaurasia, O., Ahmed, Z., Singh, S.B., 2008. Traditional medicinal plants of cold desert Ladakh—used against kidney and urinary disorders. Journal of ethnopharmacology 118, 331-339.
Bao, H., Zheng, N., Li, Z., Zhi, Y., 2020. Synergistic effect of tangeretin and atorvastatin for colon cancer combination therapy: targeted delivery of these dual drugs using RGD Peptide decorated nanocarriers. Drug design, development and therapy 14, 3057.
Baran, B., Ozupek, N.M., Tetik, N.Y., Acar, E., Bekcioglu, O., Baskin, Y., 2018. Difference between left-sided and right-sided colorectal cancer: a focused review of literature. Gastroenterology research 11, 264.
Barbalata, C.I., Tefas, L.R., Achim, M., Tomuta, I., Porfire, A.S., 2020. Statins in risk-reduction and treatment of cancer. World Journal of Clinical Oncology 11, 573.
Barnard, M.E., Boeke, C.E., Tamimi, R.M., 2015. Established breast cancer risk factors and risk of intrinsic tumor subtypes. Biochimica et Biophysica Acta (BBA)-Reviews on Cancer 1856, 73-85.
Bashir, S., Gilani, A.H., 2009. Antiurolithic effect of Bergenia ligulata rhizome: an explanation of the underlying mechanisms. Journal of ethnopharmacology 122, 106-116.
Baxter, N.N., Goldwasser, M.A., Paszat, L.F., Saskin, R., Urbach, D.R., Rabeneck, L., 2009. Association of colonoscopy and death from colorectal cancer. Annals of internal medicine 150, 1-8.
Berns, E.M., Foekens, J.A., Vossen, R., Look, M.P., Devilee, P., Henzen-Logmans, S.C., van Staveren, I.L., van Putten, W.L., Inganäs, M., Gelder, M.E.M.-v., 2000. Complete sequencing of TP53 predicts poor response to systemic therapy of advanced breast cancer. Cancer Research 60, 2155-2162.
Bertheau, P., Espié, M., Turpin, E., Lehmann, J., Plassa, L.-F., Varna, M., Janin, A., de Thé, H., 2008. TP53 status and response to chemotherapy in breast cancer. Pathobiology 75, 132-139.
Bhardwaj, M., Cho, H.J., Paul, S., Jakhar, R., Khan, I., Lee, S.-J., Kim, B.-Y., Krishnan, M., Khaket, T.P., Lee, H.G., 2018. Vitexin induces apoptosis by suppressing autophagy in multi-drug resistant colorectal cancer cells. Oncotarget 9, 3278.
Bhardwaj, M., Paul, S., Jakhar, R., Khan, I., Kang, J.I., Kim, H.M., Yun, J.W., Lee, S.-J., Cho, H.J., Lee, H.G., 2017. Vitexin confers HSF-1 mediated autophagic cell death by activating JNK and ApoL1 in colorectal carcinoma cells. Oncotarget 8, 112426.
Bimonte, S., Barbieri, A., Palma, G., Rea, D., Luciano, A., D'Aiuto, M., Arra, C., Izzo, F., 2015. Dissecting the role of curcumin in tumour growth and angiogenesis in mouse model of human breast cancer. BioMed research international 2015.
Bishayee, A., Sethi, G., 2016. Bioactive natural products in cancer prevention and therapy: Progress and promise, Seminars in cancer biology. Elsevier, pp. 1-3.
Børresen-Dale, A.L., 2003. TP53 and breast cancer. Human mutation 21, 292-300.
Bosetti, C., Santucci, C., Gallus, S., Martinetti, M., La Vecchia, C., 2020. Aspirin and the risk of colorectal and other digestive tract cancers: an updated meta-analysis through 2019. Annals of Oncology 31, 558-568.
Botteri, E., Iodice, S., Bagnardi, V., Raimondi, S., Lowenfels, A.B., Maisonneuve, P., 2008. Smoking and colorectal cancer: a meta-analysis. Jama 300, 2765-2778.
Botteri, E., Støer, N.C., Sakshaug, S., Graff-Iversen, S., Vangen, S., Hofvind, S., De Lange, T., Bagnardi, V., Ursin, G., Weiderpass, E., 2017. Menopausal hormone therapy and colorectal cancer: a linkage between nationwide registries in Norway. BMJ open 7, e017639.
Brand, T.C., Sawyer, M.M., King, T.A., Bolton, J.S., Fuhrman, G.M., 2000. Understanding patterns of failure in breast cancer treatment argues for a more thorough investigation of axillary lymph nodes in node negative patients. The American journal of surgery 180, 424-427.

Brenner, H., Chang–Claude, J., Jansen, L., Knebel, P., Stock, C., Hoffmeister, M., 2014. Reduced risk of colorectal cancer up to 10 years after screening, surveillance, or diagnostic colonoscopy. Gastroenterology 146, 709-717.
Brigham, Hospital, W.s., 13, H.M.S.C.L.P.P.J.K.R., 25, G.d.a.B.C.o.M.C.C.J.D.L.A., Ilya, I.f.S.B.R.S.K.R.B.B.B.B.R.E.T.L.J.T.V.Z.W.S., 2012. Comprehensive molecular portraits of human breast tumours. Nature 490, 61-70.
Brosens, L.A., Van Hattem, A., Hylind, L.M., Iacobuzio-Donahue, C., Romans, K.E., Axilbund, J., Cruz-Correa, M., Tersmette, A.C., Offerhaus, G.J.A., Giardiello, F.M., 2007. Risk of colorectal cancer in juvenile polyposis. Gut 56, 965-967.
Cai, H., Hudson, E.A., Mann, P., Verschoyle, R.D., Greaves, P., Manson, M.M., Steward, W.P., Gescher, A.J., 2004. Growth-inhibitory and cell cycle-arresting properties of the rice bran constituent tricin in human-derived breast cancer cells in vitro and in nude mice in vivo. British Journal of Cancer 91, 1364-1371.
Cai, S., Li, Y., Ding, Y., Chen, K., Jin, M., 2014a. Alcohol drinking and the risk of colorectal cancer death. European Journal of Cancer Prevention 23, 532-539.
Cai, S., Li, Y., Ding, Y., Chen, K., Jin, M., 2014b. Alcohol drinking and the risk of colorectal cancer death: a meta-analysis. European Journal of Cancer Prevention 23, 532-539.
Calva-Cerqueira, D., Chinnathambi, S., Pechman, B., Bair, J., Larsen-Haidle, J., Howe, J., 2009. The rate of germline mutations and large deletions of SMAD4 and BMPR1A in juvenile polyposis. Clinical genetics 75, 79-85.
Cancer, C.G.o.H.F.i.B., 2001. Familial breast cancer: collaborative reanalysis of individual data from 52 epidemiological studies including 58 209 women with breast cancer and 101 986 women without the disease. The Lancet 358, 1389-1399.
Cao, Y., Cao, W., Qiu, Y., Zhou, Y., Guo, Q., Gao, Y., Lu, N., 2020. Oroxylin A suppresses ACTN1 expression to inactivate cancer-associated fibroblasts and restrain breast cancer metastasis. Pharmacological Research 159, 104981.
Cao, Y., Nishihara, R., Qian, Z.R., Song, M., Mima, K., Inamura, K., Nowak, J.A., Drew, D.A., Lochhead, P., Nosho, K., 2016. Regular aspirin use associates with lower risk of colorectal cancers with low numbers of tumor-infiltrating lymphocytes. Gastroenterology 151, 879-892. e874.
Carvalho Ferreira, L., S Arbab, A., Victorasso Jardim-Perassi, B., Ferraz Borin, T., RS Varma, N., Iskander, A., Shankar, A., M Ali, M., Aparecida Pires de Campos Zuccari, D., 2015. Effect of curcumin on pro-angiogenic factors in the xenograft model of breast cancer. Anti-Cancer Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Anti-Cancer Agents) 15, 1285-1296.
Castellone, M.D., Teramoto, H., Williams, B.O., Druey, K.M., Gutkind, J.S., 2005. Prostaglandin E2 promotes colon cancer cell growth through a Gs-axin-ß-catenin signaling axis. Science 310, 1504-1510.
Chai, Y., Xu, J., Yan, B., 2017. The anti-metastatic effect of baicalein on colorectal cancer. Oncology Reports 37, 2317-2323.
Chan, A.T., Ogino, S., Fuchs, C.S., 2007. Aspirin and the risk of colorectal cancer in relation to the expression of COX-2. New England Journal of Medicine 356, 2131-2142.
Chan, K.K., Oza, A.M., Siu, L.L., 2003. The statins as anticancer agents. Clinical cancer research 9, 10-19.
Chen, C., Kuo, Y.-H., Lin, C.-C., Chao, C.-Y., Pai, M.-H., Chiang, E.-P.I., Tang, F.-Y., 2020. Decyl caffeic acid inhibits the proliferation of colorectal cancer cells in an autophagy-dependent manner in vitro and in vivo. PloS one 15, e0232832.
Chen, J., Zhong, J., Liu, Y., Huang, Y., Luo, F., Zhou, Y., Pan, X., Cao, S., Zhang, L., Zhang, Y., 2018. Purified vitexin compound 1, a new neolignan isolated compound, promotes PUMA-dependent apoptosis in colorectal cancer. Cancer medicine 7, 6158-6169.
Chen, M.-B., Zhu, Y.-Q., Xu, J.-Y., Wang, L.-Q., Liu, C.-Y., Ji, Z.-Y., Lu, P.-H., 2012a. Value of TP53 status for predicting response to neoadjuvant chemotherapy in breast cancer: a meta-analysis. PloS one 7, e39655.
Chen, Q., Lei, J., Zhou, J., Ma, S., Huang, Q., Ge, B., 2021. Chemopreventive effect of 4'-hydroxychalcone on intestinal tumorigenesis in Apc Min mice Corrigendum in/10.3892/ol. 2021.12741. Oncology letters 21, 1-1.
Chen, R., Zhang, L., 2019. Morin inhibits colorectal tumor growth through inhibition of NF-κB signaling pathway. Immunopharmacology and Immunotoxicology 41, 622-629.

Chen, T., Wang, Z., Zhong, J., Zhang, L., Zhang, H., Zhang, D., Xu, X., Zhong, X., Wang, J., Li, H., 2022. Secoisolariciresinol diglucoside induces pyroptosis by activating caspase-1 to cleave GSDMD in colorectal cancer cells. Drug Development Research.
Chen, W.-C., Kuo, T.-H., Tzeng, Y.-S., Tsai, Y.-C., 2012b. Baicalin induces apoptosis in SW620 human colorectal carcinoma cells in vitro and suppresses tumor growth in vivo. Molecules 17, 3844-3857.
Chen, X., Xu, H., Yu, X., Wang, X., Zhu, X., Xu, X., 2019. Apigenin inhibits in vitro and in vivo tumorigenesis in cisplatin-resistant colon cancer cells by inducing autophagy, programmed cell death and targeting m-TOR/PI3K/Akt signalling pathway. J. buon 24, 488-493.
Chen, Y., Wu, Q., Song, L., He, T., Li, Y., Li, L., Su, W., Liu, L., Qian, Z., Gong, C., 2015. Polymeric micelles encapsulating fisetin improve the therapeutic effect in colon cancer. ACS applied materials & interfaces 7, 534-542.
Chen, Z., He, X., Jia, M., Liu, Y., Qu, D., Wu, D., Wu, P., Ni, C., Zhang, Z., Ye, J., 2013. β-catenin overexpression in the nucleus predicts progress disease and unfavourable survival in colorectal cancer: a meta-analysis. PLoS One 8, e63854.
Chiang, E.-P.I., Tsai, S.-Y., Kuo, Y.-H., Pai, M.-H., Chiu, H.-L., Rodriguez, R.L., Tang, F.-Y., 2014. Caffeic acid derivatives inhibit the growth of colon cancer: involvement of the PI3-K/Akt and AMPK signaling pathways. PloS one 9, e99631.
Chiou, Y.-S., Tsai, M.-L., Nagabhushanam, K., Wang, Y.-J., Wu, C.-H., Ho, C.-T., Pan, M.-H., 2011. Pterostilbene is more potent than resveratrol in preventing azoxymethane (AOM)-induced colon tumorigenesis via activation of the NF-E2-related factor 2 (Nrf2)-mediated antioxidant signaling pathway. Journal of agricultural and food chemistry 59, 2725-2733.
Chiou, Y.-S., Tsai, M.-L., Wang, Y.-J., Cheng, A.-C., Lai, W.-M., Badmaev, V., Ho, C.-T., Pan, M.-H., 2010. Pterostilbene inhibits colorectal aberrant crypt foci (ACF) and colon carcinogenesis via suppression of multiple signal transduction pathways in azoxymethane-treated mice. Journal of agricultural and food chemistry 58, 8833-8841.
Chipurupalli, S., Ganesan, R., Martini, G., Mele, L., Reggio, A., Esposito, M., Kannan, E., Namasivayam, V., Grumati, P., Desiderio, V., 2022. Cancer cells adapt FAM134B/BiP mediated ER-phagy to survive hypoxic stress. Cell Death & Disease 13, 357.
Cho, E., Chung, E.Y., Jang, H.-Y., Hong, O.-Y., Chae, H.S., Jeong, Y.-J., Kim, S.-Y., Kim, B.-S., Yoo, D.J., Kim, J.-S., 2017. Anti-cancer effect of cyanidin-3-glucoside from mulberry via caspase-3 cleavage and DNA fragmentation in vitro and in vivo. Anti-Cancer Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Anti-Cancer Agents) 17, 1519-1525.
Cho, K.R., Vogelstein, B., 1992a. Genetic alterations in the adenoma–carcinoma sequence. Cancer 70, 1727-1731.
Cho, K.R., Vogelstein, B., 1992b. Suppressor gene alterations in the colorectal adenoma-carcinoma sequence. Journal of Cellular Biochemistry 50, 137-141.
Choudhari, A.S., Mandave, P.C., Deshpande, M., Ranjekar, P., Prakash, O., 2020. Phytochemicals in cancer treatment: From preclinical studies to clinical practice. Frontiers in pharmacology 10, 1614.
Chubak, J., Whitlock, E.P., Williams, S.B., Kamineni, A., Burda, B.U., Buist, D.S., Anderson, M.L., 2016. Aspirin for the prevention of cancer incidence and mortality: systematic evidence reviews for the US Preventive Services Task Force. Annals of internal medicine 164, 814-825.
Chung, H., Jung, Y.m., Shin, D.H., Lee, J.Y., Oh, M.Y., Kim, H.J., Jang, K.S., Jeon, S.J., Son, K.H., Kong, G., 2008. Anticancer effects of wogonin in both estrogen receptor-positive and-negative human breast cancer cell lines in vitro and in nude mice xenografts. International journal of cancer 122, 816-822.
Chunhua, L., Donglan, L., Xiuqiong, F., Lihua, Z., Qin, F., Yawei, L., Liang, Z., Ge, W., Linlin, J., Ping, Z., 2013. Apigenin up-regulates transgelin and inhibits invasion and migration of colorectal cancer through decreased phosphorylation of AKT. The Journal of nutritional biochemistry 24, 1766-1775.
Ci, Y., Zhang, Y., Liu, Y., Lu, S., Cao, J., Li, H., Zhang, J., Huang, Z., Zhu, X., Gao, J., 2018. Myricetin suppresses breast cancer metastasis through down-regulating the activity of matrix metalloproteinase (MMP)-2/9. Phytotherapy Research 32, 1373-1381.

Coffin, C.M., Hornick, J.L., Zhou, H., Fletcher, C.D., 2007. Gardner fibroma: a clinicopathologic and immunohistochemical analysis of 45 patients with 57 fibromas. The American journal of surgical pathology 31, 410-416.
Colditz, G.A., Kaphingst, K.A., Hankinson, S.E., Rosner, B., 2012. Family history and risk of breast cancer: nurses' health study. Breast cancer research and treatment 133, 1097-1104.
Coley, H.M., 2008. Mechanisms and strategies to overcome chemotherapy resistance in metastatic breast cancer. Cancer treatment reviews 34, 378-390.
Collins, L.C., Baer, H.J., Tamimi, R.M., Connolly, J.L., Colditz, G.A., Schnitt, S.J., 2006. The influence of family history on breast cancer risk in women with biopsy-confirmed benign breast disease: results from the Nurses' Health Study. Cancer 107, 1240-1247.
Concannon, P., 2002. ATM heterozygosity and cancer risk. nature genetics 32, 89-90.
Cook, M.T., Liang, Y., Besch-Williford, C., Goyette, S., Mafuvadze, B., Hyder, S.M., 2015. Luteolin inhibits progestin-dependent angiogenesis, stem cell-like characteristics, and growth of human breast cancer xenografts. Springerplus 4, 1-16.
Cook, M.T., Liang, Y., Besch-Williford, C., Hyder, S.M., 2016. Luteolin inhibits lung metastasis, cell migration, and viability of triple-negative breast cancer cells. Breast Cancer: Targets and Therapy, 9-19.
Cooke, D., Schwarz, M., Boocock, D., Winterhalter, P., Steward, W.P., Gescher, A.J., Marczylo, T.H., 2006. Effect of cyanidin-3-glucoside and an anthocyanin mixture from bilberry on adenoma development in the ApcMin mouse model of intestinal carcinogenesis—Relationship with tissue anthocyanin levels. International journal of cancer 119, 2213-2220.
Crivellari, D., Aapro, M., Leonard, R., von Minckwitz, G., Brain, E., Goldhirsch, A., Veronesi, A., Muss, H., 2007. Breast cancer in the elderly. Journal of clinical oncology 25, 1882-1890.
Cunningham, D., Humblet, Y., Siena, S., Khayat, D., Bleiberg, H., Santoro, A., Bets, D., Mueser, M., Harstrick, A., Verslype, C., 2004. Cetuximab monotherapy and cetuximab plus irinotecan in irinotecan-refractory metastatic colorectal cancer. New England journal of medicine 351, 337-345.
Curia, M.C., Catalano, T., Aceto, G.M., 2020. MUTYH: Not just polyposis. World Journal of Clinical Oncology 11, 428.
Cybulski, C., Wokołorczyk, D., Jakubowska, A., Huzarski, T., Byrski, T., Gronwald, J., Masojć, B., Dębniak, T., Górski, B., Blecharz, P., 2011. Risk of breast cancer in women with a CHEK2 mutation with and without a family history of breast cancer. Journal of Clinical Oncology 29, 3747-3752.
da Costa Vieira, R.A., Biller, G., Uemura, G., Ruiz, C.A., Curado, M.P., 2017. Breast cancer screening in developing countries. Clinics 72, 244-253.
Dai, J., Van Wie, P.G., Fai, L.Y., Kim, D., Wang, L., Poyil, P., Luo, J., Zhang, Z., 2016. Downregulation of NEDD9 by apigenin suppresses migration, invasion, and metastasis of colorectal cancer cells. Toxicology and applied pharmacology 311, 106-112.
Darvesh, A.S., Carroll, R.T., Bishayee, A., Geldenhuys, W.J., Van der Schyf, C.J., 2010. Oxidative stress and Alzheimer's disease: dietary polyphenols as potential therapeutic agents. Expert review of neurotherapeutics 10, 729-745.
de Paulo Farias, D., de Araujo, F.F., Neri-Numa, I.A., Pastore, G.M., 2021. Antidiabetic potential of dietary polyphenols: A mechanistic review. Food Research International 145, 110383.
De, S., Paul, S., Manna, A., Majumder, C., Pal, K., Casarcia, N., Mondal, A., Banerjee, S., Nelson, V.K., Ghosh, S., 2023. Phenolic phytochemicals for prevention and treatment of colorectal cancer: A critical evaluation of in vivo studies. Cancers 15, 993.
Debnath, S., Sarkar, A., Mukherjee, D.D., Ray, S., Mahata, B., Mahata, T., Parida, P.K., Das, T., Mukhopadhyay, R., Ghosh, Z., 2022. Eriodictyol mediated selective targeting of the TNFR1/FADD/TRADD axis in cancer cells induce apoptosis and inhibit tumor progression and metastasis. Translational Oncology 21, 101433.
Dehmer, S.P., Maciosek, M.V., Flottemesch, T.J., LaFrance, A.B., Whitlock, E.P., 2016. Aspirin for the primary prevention of cardiovascular disease and colorectal cancer: a decision analysis for the US Preventive Services Task Force. Annals of internal medicine 164, 777-786.
Delaney, G., Jacob, S., Featherstone, C., Barton, M., 2005. The role of radiotherapy in cancer treatment: estimating optimal utilization from a review of evidence-based clinical guidelines. Cancer: Interdisciplinary International Journal of the American Cancer Society 104, 1129-1137.

Dhillon, P.K., Mathur, P., Nandakumar, A., Fitzmaurice, C., Kumar, G.A., Mehrotra, R., Shukla, D., Rath, G., Gupta, P.C., Swaminathan, R., 2018. The burden of cancers and their variations across the states of India: the Global Burden of Disease Study 1990–2016. The Lancet Oncology 19, 1289-1306.
Dou, W., Zhang, J., Sun, A., Zhang, E., Ding, L., Mukherjee, S., Wei, X., Chou, G., Wang, Z.-T., Mani, S., 2013. Protective effect of naringenin against experimental colitis via suppression of Toll-like receptor 4/NF-κB signalling. British Journal of Nutrition 110, 599-608.
Dow, L.E., O'Rourke, K.P., Simon, J., Tschaharganeh, D.F., van Es, J.H., Clevers, H., Lowe, S.W., 2015. Apc restoration promotes cellular differentiation and reestablishes crypt homeostasis in colorectal cancer. Cell 161, 1539-1552.
Du, G.-J., Song, Z.-H., Lin, H.-H., Han, X.-f., Zhang, S., Yang, Y.-m., 2008. Luteolin as a glycolysis inhibitor offers superior efficacy and lesser toxicity of doxorubicin in breast cancer cells. Biochemical and biophysical research communications 372, 497-502.
Dworkin, A.M., Huang, T.H.-M., Toland, A.E., 2009. Epigenetic alterations in the breast: Implications for breast cancer detection, prognosis and treatment, Seminars in cancer biology. Elsevier, pp. 165-171.
ebecca L. Siegel, M.K.D.M., MPH; Hannah E. Fuchs, BS; Ahmedin Jemal, 2021. Cancer Statistics, 2021. CA Cancer J Clin.
Eberhart, C.E., Coffey, R.J., Radhika, A., Giardiello, F.M., Ferrenbach, S., Dubois, R.N., 1994. Up-regulation of cyclooxygenase 2 gene expression in human colorectal adenomas and adenocarcinomas. Gastroenterology 107, 1183-1188.
El-Deek, S.E., Abd-Elghaffar, S.K., Hna, R.S., Mohamed, H.G., El-Deek, H.E., 2022. Effect of hesperidin against induced colon cancer in rats: impact of Smad4 and activin a signaling pathway. Nutrition and Cancer 74, 697-714.
Ernst J. Kuipers, W.M.G., David Lieberman, Thomas Seufferlein, Joseph J. Sung, Petra G. Boelens, Cornelis J. H. van de Velde & Toshiaki Watanabe 2015. Colorectal cancer. Nature Reviews Disease Primers.
Faheem, M.M., Bhagat, M., Sharma, P., Anand, R., 2022. Induction of p53 mediated mitochondrial apoptosis and cell cycle arrest in human breast cancer cells by plant mediated synthesis of silver nanoparticles from Bergenia ligulata (Whole plant). International Journal of Pharmaceutics 619, 121710.
Falcone, A., Ricci, S., Brunetti, I., Pfanner, E., Allegrini, G., Barbara, C., Crinò, L., Benedetti, G., Evangelista, W., Fanchini, L., 2007. Phase III trial of infusional fluorouracil, leucovorin, oxaliplatin, and irinotecan (FOLFOXIRI) compared with infusional fluorouracil, leucovorin, and irinotecan (FOLFIRI) as first-line treatment for metastatic colorectal cancer: the Gruppo Oncologico Nord Ovest. Journal of clinical oncology 25, 1670-1676.
Fang, W., Zhu, S., Niu, Z., Yin, Y., 2019. The protective effect of syringic acid on dextran sulfate sodium-induced experimental colitis in BALB/c mice. Drug Development Research 80, 731-740.
Farina, H.G., Pomies, M., Alonso, D.F., Gomez, D.E., 2006. Antitumor and antiangiogenic activity of soy isoflavone genistein in mouse models of melanoma and breast cancer. Oncology reports 16, 885-891.
Feng, Q., Wang, H., Pang, J., Ji, L., Han, J., Wang, Y., Qi, X., Liu, Z., Lu, L., 2018a. Prevention of wogonin on colorectal cancer tumorigenesis by regulating p53 nuclear translocation. Frontiers in pharmacology 9, 1356.
Feng, T., Cao, W., Shen, W., Zhang, L., Gu, X., Guo, Y., Tsai, H.-i., Liu, X., Li, J., Zhang, J., 2017. Arctigenin inhibits STAT3 and exhibits anticancer potential in human triple-negative breast cancer therapy. Oncotarget 8, 329.
Feng, Y., Spezia, M., Huang, S., Yuan, C., Zeng, Z., Zhang, L., Ji, X., Liu, W., Huang, B., Luo, W., 2018b. Breast cancer development and progression: Risk factors, cancer stem cells, signaling pathways, genomics, and molecular pathogenesis. Genes & diseases 5, 77-106.
Ferlay, J., Colombet, M., Soerjomataram, I., Mathers, C., Parkin, D., Piñeros, M., Znaor, A., Bray, F., 2019a. Estimating the global cancer incidence and mortality in 2018: GLOBOCAN sources and methods. International journal of cancer 144, 1941-1953.
Ferlay, J., Colombet, M., Soerjomataram, I., Mathers, C., Parkin, D.M., Piñeros, M., Znaor, A., Bray, F., 2019b. Estimating the global cancer incidence and mortality in 2018: GLOBOCAN sources and methods. International journal of cancer 144, 1941-1953.

Firouzi, J., Sotoodehnejadnematalahi, F., Shokouhifar, A., Rahimi, M., Sodeifi, N., Sahranavardfar, P., Azimi, M., Janzamin, E., Safa, M., Ebrahimi, M., 2022. Silibinin exhibits anti-tumor effects in a breast cancer stem cell model by targeting stemness and induction of differentiation and apoptosis. BioImpacts: BI 12, 415.
Fischer, M.M., Yeung, V.P., Cattaruzza, F., Hussein, R., Yen, W.-C., Murriel, C., Evans, J.W., O'Young, G., Brunner, A.L., Wang, M., 2017. RSPO3 antagonism inhibits growth and tumorigenicity in colorectal tumors harboring common Wnt pathway mutations. Scientific reports 7, 1-9.
Fisher, B., Costantino, J.P., Wickerham, D.L., Redmond, C.K., Kavanah, M., Cronin, W.M., Vogel, V., Robidoux, A., Dimitrov, N., Atkins, J., 1998. Tamoxifen for prevention of breast cancer: report of the National Surgical Adjuvant Breast and Bowel Project P-1 Study. JNCI: Journal of the National Cancer Institute 90, 1371-1388.
Fodde, R., Smits, R., Clevers, H., 2001. APC, signal transduction and genetic instability in colorectal cancer. Nature reviews cancer 1, 55-67.
Galiatsatos, P., Foulkes, W.D., 2006. Familial adenomatous polyposis. Official journal of the American College of Gastroenterology| ACG 101, 385-398.
Gallione, C.J., Repetto, G.M., Legius, E., Rustgi, A.K., Schelley, S.L., Tejpar, S., Mitchell, G., Drouin, É., Westermann, C.J., Marchuk, D.A., 2004. A combined syndrome of juvenile polyposis and hereditary haemorrhagic telangiectasia associated with mutations in MADH4 (SMAD4). The Lancet 363, 852-859.
Gan, C., Li, Y., Yu, Y., Yu, X., Liu, H., Zhang, Q., Yin, W., Yu, L., Ye, T., 2019. Natural product pectolinarigenin exhibits potent anti-metastatic activity in colorectal carcinoma cells in vitro and in vivo. Bioorganic & Medicinal Chemistry 27, 115089.
Gao, Z., Jiang, J., Hou, L., Ji, F., 2022. Lysionotin Induces Ferroptosis to Suppress Development of Colorectal Cancer via Promoting Nrf2 Degradation. Oxidative Medicine and Cellular Longevity 2022.
Garimella, T., Jolly, C., Narayanan, S., 2001. In vitro studies on antilithiatic activity of seeds of Dolichos biflorus Linn. and rhizomes of Bergenia ligulata Wall. Phytotherapy research 15, 351-355.
Garvin, S., Öllinger, K., Dabrosin, C., 2006. Resveratrol induces apoptosis and inhibits angiogenesis in human breast cancer xenografts in vivo. Cancer letters 231, 113-122.
Ghosh, N., Kundu, M., Ghosh, S., Das, A.K., De, S., Das, J., Sil, P.C., 2023. pH-responsive and targeted delivery of chrysin via folic acid-functionalized mesoporous silica nanocarrier for breast cancer therapy. International Journal of Pharmaceutics 631, 122555.
Ghosh, S., Dutta, N., Banerjee, P., Gajbhiye, R.L., Sareng, H.R., Kapse, P., Pal, S., Burdelya, L., Mandal, N.C., Ravichandiran, V., 2021. Induction of monoamine oxidase A-mediated oxidative stress and impairment of NRF2-antioxidant defence response by polyphenol-rich fraction of Bergenia ligulata sensitizes prostate cancer cells in vitro and in vivo. Free Radical Biology and Medicine 172, 136-151.
Gierach, G.L., Lacey, J.V., Schatzkin, A., Leitzmann, M.F., Richesson, D., Hollenbeck, A.R., Brinton, L.A., 2008. Nonsteroidal anti-inflammatory drugs and breast cancer risk in the National Institutes of Health–AARP Diet and Health Study. Breast Cancer Research 10, 1-13.
Giftson, J.S., Jayanthi, S., Nalini, N., 2010. Chemopreventive efficacy of gallic acid, an antioxidant and anticarcinogenic polyphenol, against 1, 2-dimethyl hydrazine induced rat colon carcinogenesis. Investigational new drugs 28, 251-259.
Gilreath, C., Boerma, M., Qin, Z., Hudson, M.K., Wang, S., 2021. The hypoxic microenvironment of breast cancer cells promotes resistance in radiation therapy. Frontiers in Oncology 10, 629422.
Goss, P.E., Ingle, J.N., Alés-Martínez, J.E., Cheung, A.M., Chlebowski, R.T., Wactawski-Wende, J., McTiernan, A., Robbins, J., Johnson, K.C., Martin, L.W., 2011. Exemestane for breast-cancer prevention in postmenopausal women. New England Journal of Medicine 364, 2381-2391.
Gossé, F., Guyot, S., Roussi, S., Lobstein, A., Fischer, B., Seiler, N., Raul, F., 2005. Chemopreventive properties of apple procyanidins on human colon cancer-derived metastatic SW620 cells and in a rat model of colon carcinogenesis. Carcinogenesis 26, 1291-1295.
Goyal, Y., Koul, A., Ranawat, P., 2022. Ellagic acid modulates cisplatin toxicity in DMH induced colorectal cancer: Studies on membrane alterations. Biochemistry and Biophysics Reports 31, 101319.
Gradishar, W.J., Anderson, B.O., Balassanian, R., Blair, S.L., Burstein, H.J., Cyr, A., Elias, A.D., Farrar, W.B., Forero, A., Giordano, S.H., 2018. Breast cancer, version 4.2017, NCCN clinical practice guidelines in oncology. Journal of the National Comprehensive Cancer Network 16, 310-320.

Grady, W.M., Markowitz, S.D., 2002. Genetic and epigenetic alterations in colon cancer. Annual review of genomics and human genetics 3, 101-128.
Gryfe, R., Bapat, B., Gallinger, S., Swallow, C., Redston, M., Couture, J., 1997. Molecular biology of colorectal cancer. Current problems in cancer 21, 233-299.
Grzybowski, A., Jablonska, S., 2009. Muir–Torre Syndrome—Is It Really a New Syndrome? The American journal of dermatopathology 31, 799-802.
Gu, J., Tong, T., He, C., Xu, M., Yang, X., Tian, J., Jiang, T., Wang, K., 2022. Deep learning radiomics of ultrasonography can predict response to neoadjuvant chemotherapy in breast cancer at an early stage of treatment: a prospective study. European radiology, 1-11.
Guba, M., Seeliger, H., Kleespies, A., Jauch, K.-W., Bruns, C., 2004. Vascular endothelial growth factor in colorectal cancer. International journal of colorectal disease 19, 510-517.
Gurav, S., Gurav, N., 2014. A Comprehensive review: Bergenia ligulata Wall-A controversial clinical candidate. Int. J. Pharm. Sci. Rev. Res 5, 1630-1642.
Hadjiliadis, D., Khoruts, A., Zauber, A.G., Hempstead, S.E., Maisonneuve, P., Lowenfels, A.B., Braid, A.L., Cullina, J., Daggett, A., Fink, A., 2018. Cystic fibrosis colorectal cancer screening consensus recommendations. Gastroenterology 154, 736-745. e714.
Haggar, F.A., Boushey, R.P., 2009. Colorectal cancer epidemiology: incidence, mortality, survival, and risk factors. Clinics in colon and rectal surgery 22, 191-197.
Ham, S.L., Nasrollahi, S., Shah, K.N., Soltisz, A., Paruchuri, S., Yun, Y.H., Luker, G.D., Bishayee, A., Tavana, H., 2015. Phytochemicals potently inhibit migration of metastatic breast cancer cells. Integrative Biology 7, 792-800.
Hamilton, S.R., Liu, B., Parsons, R.E., Papadopoulos, N., Jen, J., Powell, S.M., Krush, A.J., Berk, T., Cohen, Z., Tetu, B., 1995. The molecular basis of Turcot's syndrome. New England Journal of Medicine 332, 839-847.
Han, B.S., Park, C.B., Takasuka, N., Naito, A., Sekine, K., Nomura, E., Taniguchi, H., Tsuno, T., Tsuda, H., 2001. A Ferulic Acid Derivative, Ethyl 3-(4′-Geranyloxy-3-methoxyphenyl)-2-propenoate, as a New Candidate Chemopreventive Agent for Colon Carcinogenesis in the Rat. Japanese journal of cancer research 92, 404-409.
Han, L., Yan, Y., Fan, M., Gao, S., Zhang, L., Xiong, X., Li, R., Xiao, X., Wang, X., Ni, L., 2022. Pt3R5G inhibits colon cancer cell proliferation through inducing ferroptosis by down-regulating SLC7A11. Life Sciences 306, 120859.
Han, Y.-H., Kee, J.-Y., Kim, D.-S., Mun, J.-g., Jeong, M.-Y., Park, S.-H., Choi, B.-M., Park, S.-J., Kim, H.-J., Um, J.-Y., 2016. Arctigenin inhibits lung metastasis of colorectal cancer by regulating cell viability and metastatic phenotypes. Molecules 21, 1135.
Hanieh, H., Ibrahim, H.-I.M., Mohammed, M., Alwassil, O.I., Abukhalil, M.H., Farhan, M., 2022. Activation of aryl hydrocarbon receptor signaling by gallic acid suppresses progression of human breast cancer in vitro and in vivo. Phytomedicine 96, 153817.
Hao, J., Dai, X., Gao, J., Li, Y., Hou, Z., Chang, Z., Wang, Y., 2021. Curcumin suppresses colorectal tumorigenesis via the Wnt/β-catenin signaling pathway by downregulating Axin2. Oncology Letters 21, 1-1.
Haque, A., Brazeau, D., Amin, A.R., 2021. Perspectives on natural compounds in chemoprevention and treatment of cancer: an update with new promising compounds. European Journal of Cancer 149, 165-183.
Haque, M.W., Pattanayak, S.P., 2017. Taxifolin inhibits 7, 12-dimethylbenz (a) anthracene-induced breast carcinogenesis by regulating AhR/CYP1A1 signaling pathway. Pharmacognosy magazine 13, S749.
Harbeck, N., Penault-Llorca, F., Cortes, J., Gnant, M., Houssami, N., Poortmans, P., Ruddy, K., Tsang, J., Cardoso, F., 2019. Breast cancer. Nature Reviews Disease Primers 5, 66.
Hashemzaei, M., Delarami Far, A., Yari, A., Heravi, R.E., Tabrizian, K., Taghdisi, S.M., Sadegh, S.E., Tsarouhas, K., Kouretas, D., Tzanakakis, G., 2017. Anticancer and apoptosis-inducing effects of quercetin in vitro and in vivo. Oncology reports 38, 819-828.
Hassan, E.S., Hassanein, N.M., Ahmed, H.M.S., 2021. Probing the chemoprevention potential of the antidepressant fluoxetine combined with epigallocatechin gallate or kaempferol in rats with induced early stage colon carcinogenesis. Journal of Pharmacological Sciences 145, 29-41.

He, M.Y., Rancoule, C., Rehailia-Blanchard, A., Espenel, S., Trone, J.-C., Bernichon, E., Guillaume, E., Vallard, A., Magné, N., 2018. Radiotherapy in triple-negative breast cancer: Current situation and upcoming strategies. Critical reviews in oncology/hematology 131, 96-101.
He, X., Wu, K., Zhang, X., Nishihara, R., Cao, Y., Fuchs, C.S., Giovannucci, E.L., Ogino, S., Chan, A.T., Song, M., 2019. Dietary intake of fiber, whole grains and risk of colorectal cancer: An updated analysis according to food sources, tumor location and molecular subtypes in two large US cohorts. International journal of cancer 145, 3040-3051.
Henderson, I., 1991. Chemotherapy for metastatic disease. Breast diseases, 604-665.
Hermsen, M., Postma, C., Baak, J., Weiss, M., Rapallo, A., Sciutto, A., Roemen, G., Arends, J.W., Williams, R., Giaretti, W., 2002. Colorectal adenoma to carcinoma progression follows multiple pathways of chromosomal instability. Gastroenterology 123, 1109-1119.
Hoang, T., Kim, H., Kim, J., 2020. Dietary intake in association with all-cause mortality and colorectal cancer mortality among colorectal cancer survivors: A systematic review and meta-analysis of prospective studies. Cancers 12, 3391.
Hobert, J.A., Eng, C., 2009. PTEN hamartoma tumor syndrome: an overview. Genetics in Medicine 11, 687-694.
Holowatyj, A.N., Ruterbusch, J.J., Rozek, L.S., Cote, M.L., Stoffel, E.M., 2016. Racial/ethnic disparities in survival among patients with young-onset colorectal cancer. Journal of Clinical Oncology 34, 2148.
Hou, L., Chen, L., Fang, L., 2017. Scutellarin inhibits proliferation, invasion, and tumorigenicity in human breast cancer cells by regulating HIPPO-YAP signaling pathway. Medical Science Monitor: International Medical Journal of Experimental and Clinical Research 23, 5130.
Howell, A., Anderson, A.S., Clarke, R.B., Duffy, S.W., Evans, D.G., Garcia-Closas, M., Gescher, A.J., Key, T.J., Saxton, J.M., Harvie, M.N., 2014. Risk determination and prevention of breast cancer. Breast Cancer Research 16, 1-19.
Hsieh, C.-J., Kuo, P.-L., Hsu, Y.-C., Huang, Y.-F., Tsai, E.-M., Hsu, Y.-L., 2014. Arctigenin, a dietary phytoestrogen, induces apoptosis of estrogen receptor-negative breast cancer cells through the ROS/p38 MAPK pathway and epigenetic regulation. Free Radical Biology and Medicine 67, 159-170.
Huang, C.-C., Hung, C.-H., Hung, T.-W., Lin, Y.-C., Wang, C.-J., Kao, S.-H., 2019a. Dietary delphinidin inhibits human colorectal cancer metastasis associating with upregulation of miR-204-3p and suppression of the integrin/FAK axis. Scientific reports 9, 1-11.
Huang, W.-K., Hsu, H.-C., Liu, J.-R., Yang, T.-S., Chen, J.-S., Chang, J.W.-C., Lin, Y.-C., Yu, K.-H., Kuo, C.-F., See, L.-C., 2016. The association of ursodeoxycholic acid use with colorectal cancer risk: A nationwide cohort study. Medicine 95.
Huang, W., Liu, C., Liu, F., Liu, Z., Lai, G., Yi, J., 2020. Hinokiflavone induces apoptosis and inhibits migration of breast cancer cells via EMT signalling pathway. Cell biochemistry and function 38, 249-256.
Huang, X.-m., Yang, Z.-j., Xie, Q., Zhang, Z.-k., Zhang, H., Ma, J.-y., 2019b. Natural products for treating colorectal cancer: A mechanistic review. Biomedicine & Pharmacotherapy 117, 109142.
Hulka, B.S., 1996. Epidemiology of susceptibility to breast cancer. Progress in clinical and biological research 395, 159-174.
Jain, A., Madu, C.O., Lu, Y., 2021. Phytochemicals in chemoprevention: a cost-effective complementary approach. Journal of Cancer 12, 3686.
Jasperson, K.W., Tuohy, T.M., Neklason, D.W., Burt, R.W., 2010. Hereditary and familial colon cancer. Gastroenterology 138, 2044-2058.
Jeng, L.B., Kumar Velmurugan, B., Chen, M.C., Hsu, H.H., Ho, T.J., Day, C.H., Lin, Y.M., Padma, V.V., Tu, C.C., Huang, C.Y., 2018. Fisetin mediated apoptotic cell death in parental and Oxaliplatin/irinotecan resistant colorectal cancer cells in vitro and in vivo. Journal of Cellular Physiology 233, 7134-7142.
Jänne, P.A., Mayer, R.J., 2000. Chemoprevention of colorectal cancer. New England Journal of Medicine 342, 1960-1968.
Jeon, Y.W., Ahn, Y.E., Chung, W.S., Choi, H.J., Suh, Y.J., 2015. Synergistic effect between celecoxib and luteolin is dependent on estrogen receptor in human breast cancer cells. Tumor Biology 36, 6349-6359.

Ji, Q., Liu, X., Han, Z., Zhou, L., Sui, H., Yan, L., Jiang, H., Ren, J., Cai, J., Li, Q., 2015. Resveratrol suppresses epithelial-to-mesenchymal transition in colorectal cancer through TGF-β1/Smads signaling pathway mediated Snail/E-cadherin expression. BMC cancer 15, 1-12.
Jin, H., Lee, W.S., Eun, S.Y., Jung, J.H., Park, H.-S., Kim, G., Choi, Y.H., Ryu, C.H., Jung, J.M., Hong, S.C., 2014. Morin, a flavonoid from Moraceae, suppresses growth and invasion of the highly metastatic breast cancer cell line MDA-MB-231 partly through suppression of the Akt pathway. International journal of oncology 45, 1629-1637.
Jin, Y., Zhan, X., Zhang, B., Chen, Y., Liu, C., Yu, L., 2020. Polydatin exerts an antitumor effect through regulating the miR-382/PD-L1 axis in colorectal cancer. Cancer Biotherapy & Radiopharmaceuticals 35, 83-91.
Jonker, D.J., O'Callaghan, C.J., Karapetis, C.S., Zalcberg, J.R., Tu, D., Au, H.-J., Berry, S.R., Krahn, M., Price, T., Simes, R.J., 2007. Cetuximab for the treatment of colorectal cancer. New England Journal of Medicine 357, 2040-2048.
Joshi, V.S., Parekh, B.B., Joshi, M.J., Vaidya, A.D., 2005. Inhibition of the growth of urinary calcium hydrogen phosphate dihydrate crystals with aqueous extracts of Tribulus terrestris and Bergenia ligulata. Urological research 33, 80-86.
Kamińska, M., Ciszewski, T., Łopacka-Szatan, K., Miotła, P., Starosławska, E., 2015. Breast cancer risk factors. Menopause Review/Przegląd Menopauzalny 14, 196-202.
Kang, K., Oh, S.H., Yun, J.H., Jho, E.H., Kang, J.-H., Batsuren, D., Tunsag, J., Park, K.H., Kim, M., Nho, C.W., 2011a. A novel topoisomerase inhibitor, daurinol, suppresses growth of HCT116 cells with low hematological toxicity compared to etoposide. Neoplasia 13, 1043-IN1030.
Kang, N.J., Lee, K.W., Kim, B.H., Bode, A.M., Lee, H.-J., Heo, Y.-S., Boardman, L., Limburg, P., Lee, H.J., Dong, Z., 2011b. Coffee phenolic phytochemicals suppress colon cancer metastasis by targeting MEK and TOPK. Carcinogenesis 32, 921-928.
Kang, Y.-J., Park, H.J., Chung, H.-J., Min, H.-Y., Park, E.J., Lee, M.A., Shin, Y., Lee, S.K., 2012. Wnt/β-catenin signaling mediates the antitumor activity of magnolol in colorectal cancer cells. Molecular pharmacology 82, 168-177.
Kanth, P., Grimmett, J., Champine, M., Burt, R., Samadder, J.N., 2017. Hereditary colorectal polyposis and cancer syndromes: a primer on diagnosis and management. Official journal of the American College of Gastroenterology| ACG 112, 1509-1525.
Karimi, A., Majlesi, M., Rafieian-Kopaei, M., 2015. Herbal versus synthetic drugs; beliefs and facts. Journal of nephropharmacology 4, 27.
Kashyap, D., Pal, D., Sharma, R., Garg, V.K., Goel, N., Koundal, D., Zaguia, A., Koundal, S., Belay, A., 2022. Global increase in breast cancer incidence: risk factors and preventive measures. BioMed research international 2022.
Katona, B.W., Weiss, J.M., 2020. Chemoprevention of colorectal cancer. Gastroenterology 158, 368-388.
Kauntz, H., Bousserouel, S., Gosse, F., Marescaux, J., Raul, F., 2012. Silibinin, a natural flavonoid, modulates the early expression of chemoprevention biomarkers in a preclinical model of colon carcinogenesis. International journal of oncology 41, 849-854.
Kaur, H., Ghosh, S., Kumar, P., Basu, B., Nagpal, K., 2021. Ellagic acid-loaded, tween 80-coated, chitosan nanoparticles as a promising therapeutic approach against breast cancer: In-vitro and in-vivo study. Life Sciences 284, 119927.
Kawabata, K., Yamamoto, T., Hara, A., Shimizu, M., Yamada, Y., Matsunaga, K., Tanaka, T., Mori, H., 2000. Modifying effects of ferulic acid on azoxymethane-induced colon carcinogenesis in F344 rats. Cancer letters 157, 15-21.
Kee, J.-Y., Han, Y.-H., Mun, J.-G., Park, S.-H., Jeon, H.D., Hong, S.-H., 2018. Gomisin a suppresses colorectal lung metastasis by inducing AMPK/P38-mediated apoptosis and decreasing metastatic abilities of colorectal cancer cells. Frontiers in pharmacology 9, 986.
Keum, N., Giovannucci, E., 2019. Global burden of colorectal cancer: emerging trends, risk factors and prevention strategies. Nature reviews Gastroenterology & hepatology 16, 713-732.
Khan, N., Jajeh, F., Eberhardt, E.L., Miller, D.D., Albrecht, D.M., Van Doorn, R., Hruby, M.D., Maresh, M.E., Clipson, L., Mukhtar, H., 2019a. Fisetin and 5-fluorouracil: Effective combination for PIK3CA-mutant colorectal cancer. International journal of cancer 145, 3022-3032.

Khan, T., Ali, M., Khan, A., Nisar, P., Jan, S.A., Afridi, S., Shinwari, Z.K., 2019b. Anticancer plants: A review of the active phytochemicals, applications in animal models, and regulatory aspects. Biomolecules 10, 47.
Kidambi, T.D., Kohli, D.R., Samadder, N.J., Singh, A., 2019. Hereditary polyposis syndromes. Current treatment options in gastroenterology 17, 650-665.
Kiessling, P., Dowling, E., Huang, Y., Ho, M.L., Balakrishnan, K., Weigel, B.J., Highsmith, W.E., Niu, Z., Schimmenti, L.A., 2019. Identification of aggressive Gardner syndrome phenotype associated with a de novo APC variant, c. 4666dup. Molecular Case Studies 5, a003640.
Kim, D.H., Hossain, M.A., Kang, Y.J., Jang, J.Y., Lee, Y.J., Im, E., Yoon, J.-H., Kim, H.S., Chung, H.Y., Kim, N.D., 2013a. Baicalein, an active component of Scutellaria baicalensis Georgi, induces apoptosis in human colon cancer cells and prevents AOM/DSS-induced colon cancer in mice. International journal of oncology 43, 1652-1658.
Kim, E., Kim, Y.-J., Ji, Z., Kang, J.M., Wirianto, M., Paudel, K.R., Smith, J.A., Ono, K., Kim, J.-A., Eckel-Mahan, K., 2022. ROR activation by Nobiletin enhances antitumor efficacy via suppression of IκB/NF-κB signaling in triple-negative breast cancer. Cell death & disease 13, 374.
Kim, H.Y., Jung, S.K., Byun, S., Son, J.E., Oh, M.H., Lee, J., Kang, M.J., Heo, Y.S., Lee, K.W., Lee, H.J., 2013b. Raf and PI3K are the molecular targets for the anti-metastatic effect of Luteolin. Phytotherapy Research 27, 1481-1488.
Kim, K.M., Jung, J., 2020. Upregulation of G protein-coupled estrogen receptor by Chrysin-nanoparticles inhibits tumor proliferation and metastasis in triple negative breast Cancer Xenograft model. Frontiers in endocrinology 11, 560605.
Kim, S.-H., Hwang, K.-A., Choi, K.-C., 2016. Treatment with kaempferol suppresses breast cancer cell growth caused by estrogen and triclosan in cellular and xenograft breast cancer models. The Journal of nutritional biochemistry 28, 70-82.
Kim, S.-J., Kim, H.-J., Kim, H.-R., Lee, S.-H., Cho, S.-D., Choi, C.-S., Nam, J.-S., Jung, J.-Y., 2012. Antitumor actions of baicalein and wogonin in HT-29 human colorectal cancer cells. Molecular medicine reports 6, 1443-1449.
Kimura, Y., 2022. Long-term oral administration of piceatannol (3, 5, 3′, 4′-tetrahydroxystilbene) attenuates colon tumor growth induced by azoxymethane plus dextran sulfate sodium in C57BL/6J mice. Nutrition and Cancer 74, 2184-2195.
Kiruthiga, C., Devi, K.P., Nabavi, S.M., Bishayee, A., 2020. Autophagy: a potential therapeutic target of polyphenols in hepatocellular carcinoma. Cancers 12, 562.
Koosha, S., Mohamed, Z., Sinniah, A., Alshawsh, M.A., 2019. Evaluation of anti-tumorigenic effects of diosmetin against human colon cancer xenografts in athymic nude mice. Molecules 24, 2522.
Kopacova, M., Tacheci, I., Rejchrt, S., Bures, J., 2009. Peutz-Jeghers syndrome: diagnostic and therapeutic approach. World journal of gastroenterology: WJG 15, 5397.
Krishnamurti, U., Silverman, J.F., 2014. HER2 in breast cancer: a review and update. Advances in anatomic pathology 21, 100-107.
Kumar, H., Kumar, R.M., Bhattacharjee, D., Somanna, P., Jain, V., 2022. Role of Nrf2 signaling cascade in breast cancer: Strategies and treatment. Frontiers in Pharmacology 13, 720076.
Kumar, K.N., Raja, S.B., Vidhya, N., Devaraj, S.N., 2012. Ellagic acid modulates antioxidant status, ornithine decarboxylase expression, and aberrant crypt foci progression in 1, 2-dimethylhydrazine-instigated colon preneoplastic lesions in rats. Journal of agricultural and food chemistry 60, 3665-3672.
Kunchari Kalaimathi, S., Sudhandiran, G., 2016. Fisetin ameolirates the azoxymethane and dextran sodium sulfate induced colitis associated colorectal cancer. Int J Pharm Clin Res 8, 551-560.
Kungu, A., Hamajima, N., Hirose, K., 2002. Alcohol, tobacco and breast cancer--collaborative reanalysis of individual data from 53 epidemiological studies, including 58,515 women with breast cancer and 95,067 women without the disease.
Kyrgiou, M., Kalliala, I., Markozannes, G., Gunter, M.J., Paraskevaidis, E., Gabra, H., Martin-Hirsch, P., Tsilidis, K.K., 2017. Adiposity and cancer at major anatomical sites: umbrella review of the literature. Bmj 356.
Lai, C.-Y., Tsai, A.-C., Chen, M.-C., Chang, L.-H., Sun, H.-L., Chang, Y.-L., Chen, C.-C., Teng, C.-M., Pan, S.-L., 2012. Aciculatin induces p53-dependent apoptosis via MDM2 depletion in human cancer cells in vitro and in vivo.

Lamlum, H., Ilyas, M., Rowan, A., Clark, S., Johnson, V., Bell, J., Frayling, I., Efstathiou, J., Pack, K., Payne, S., 1999. The type of somatic mutation at APC in familial adenomatous polyposis is determined by the site of the germline mutation: a new facet to Knudson's' two-hit'hypothesis. Nature medicine 5, 1071-1075.
Landis-Piwowar, K.R., Huo, C., Chen, D., Milacic, V., Shi, G., Chan, T.H., Dou, Q.P., 2007. A novel prodrug of the green tea polyphenol (−)-epigallocatechin-3-gallate as a potential anticancer agent. Cancer research 67, 4303-4310.
Lee, E.-J., Oh, S.-Y., Sung, M.-K., 2012a. Luteolin exerts anti-tumor activity through the suppression of epidermal growth factor receptor-mediated pathway in MDA-MB-231 ER-negative breast cancer cells. Food and chemical toxicology 50, 4136-4143.
Lee, H.S., Ha, A.W., Kim, W.K., 2012b. Effect of resveratrol on the metastasis of 4T1 mouse breast cancer cells in vitro and in vivo. Nutrition research and practice 6, 294-300.
Lee, J., Jin, H., Lee, W.S., Nagappan, A., Choi, Y.H., Kim, G.S., Jung, J., Ryu, C.H., Shin, S.C., Hong, S.C., 2016. Morin, a flavonoid from moraceae, inhibits cancer cell adhesion to endothelial cells and EMT by downregulating VCAM1 and ncadherin. Asian Pacific Journal of Cancer Prevention 17, 3071-3075.
Lee, J., Sim, W., Kim, J., Choi, C., Jeon, J., 2023. Discovering the anti-cancer phytochemical rutin against breast cancer through the methodical platform based on traditional medicinal knowledge. BMB reports, 5915-5915.
Leonardi, T., Vanamala, J., Taddeo, S.S., Davidson, L.A., Murphy, M.E., Patil, B.S., Wang, N., Carroll, R.J., Chapkin, R.S., Lupton, J.R., 2010. Apigenin and naringenin suppress colon carcinogenesis through the aberrant crypt stage in azoxymethane-treated rats. Experimental biology and medicine 235, 710-717.
Leu, J.D., Wang, B.S., Chiu, S.J., Chan, C.Y., Chen, C.C., Chen, F.D., Avirmed, S., Lee, Y.J., 2016. Combining fisetin and ionizing radiation suppresses the growth of mammalian colorectal cancers in xenograft tumor models. Oncology letters 12, 4975-4982.
Levy, D.B., Smith, K.J., Beazer-Barclay, Y., Hamilton, S.R., Vogelstein, B., Kinzler, K.W., 1994. Inactivation of both APC alleles in human and mouse tumors. Cancer research 54, 5953-5958.
Li, C., Zhang, K., Pan, G., Ji, H., Li, C., Wang, X., Hu, X., Liu, R., Deng, L., Wang, Y., 2021. Dehydrodiisoeugenol inhibits colorectal cancer growth by endoplasmic reticulum stress-induced autophagic pathways. Journal of Experimental & Clinical Cancer Research 40, 1-15.
Li, H., Yang, B., Huang, J., Xiang, T., Yin, X., Wan, J., Luo, F., Zhang, L., Li, H., Ren, G., 2013. Naringin inhibits growth potential of human triple-negative breast cancer cells by targeting β-catenin signaling pathway. Toxicology letters 220, 219-228.
Li, J., Gong, X., Jiang, R., Lin, D., Zhou, T., Zhang, A., Li, H., Zhang, X., Wan, J., Kuang, G., 2018a. Fisetin inhibited growth and metastasis of triple-negative breast cancer by reversing epithelial-to-mesenchymal transition via PTEN/Akt/GSK3β signal pathway. Frontiers in Pharmacology 9, 772.
Li, J., Hu, L., Zhou, T., Gong, X., Jiang, R., Li, H., Kuang, G., Wan, J., Li, H., 2019a. Taxifolin inhibits breast cancer cells proliferation, migration and invasion by promoting mesenchymal to epithelial transition via β-catenin signaling. Life Sciences 232, 116617.
Li, J., Zhang, J., Zhu, Y., Afolabi, L.O., Chen, L., Feng, X., 2023. Natural Compounds, Optimal Combination of Brusatol and Polydatin Promote Anti-Tumor Effect in Breast Cancer by Targeting Nrf2 Signaling Pathway. International Journal of Molecular Sciences 24, 8265.
Li, L., Wang, M., Yang, H., Li, Y., Huang, X., Guo, J., Liu, Z., 2022. Fisetin Inhibits Trypsin Activity and Suppresses the Growth of Colorectal Cancer in Vitro and in Vivo. Natural Product Communications 17, 1934578X221115511.
Li, Q., Ma, Y., Liu, X.L., Mu, L., He, B.C., Wu, K., Sun, W.J., 2020a. Anti-proliferative effect of honokiol on SW620 cells through upregulating BMP7 expression via the TGF-β1/p53 signaling pathway. Oncology Reports 44, 2093-2107.
Li, Y.-W., Xu, J., Zhu, G.-Y., Huang, Z.-J., Lu, Y., Li, X.-Q., Wang, N., Zhang, F.-X., 2018b. Apigenin suppresses the stem cell-like properties of triple-negative breast cancer cells by inhibiting YAP/TAZ activity. Cell Death Discovery 4, 105.
Li, Y.-Y., Gao, L.-J., Zhang, Y.-X., Liu, S.-J., Cheng, S., Liu, Y.-P., Jia, C.-X., 2020b. Bisphosphonates and risk of cancers: a systematic review and meta-analysis. British Journal of Cancer 123, 1570-1581.

Li, Y., Cui, S.-X., Sun, S.-Y., Shi, W.-N., Song, Z.-Y., Wang, S.-Q., Yu, X.-F., Gao, Z.-H., Qu, X.-J., 2016. Chemoprevention of intestinal tumorigenesis by the natural dietary flavonoid myricetin in APCMin/+ mice. Oncotarget 7, 60446.
Li, Y., He, X., Ding, Y.e., Chen, H., Sun, L., 2019b. Statin uses and mortality in colorectal cancer patients: An updated systematic review and meta-analysis. Cancer medicine 8, 3305-3313.
Li, Y., Jiang, F., Chen, L., Yang, Y., Cao, S., Ye, Y., Wang, X., Mu, J., Li, Z., Li, L., 2015. Blockage of TGFβ-SMAD2 by demethylation-activated miR-148a is involved in caffeic acid-induced inhibition of cancer stem cell-like properties in vitro and in vivo. FEBS Open Bio 5, 466-475.
Lim, S., Xu, J., Kim, J., Chen, T.Y., Su, X., Standard, J., Carey, E., Griffin, J., Herndon, B., Katz, B., 2013. Role of anthocyanin-enriched purple-fleshed sweet potato p40 in colorectal cancer prevention. Molecular nutrition & food research 57, 1908-1917.
Lin, C.-L., Jeng, J.-H., Wu, C.-C., Hsieh, S.-L., Huang, G.-C., Leung, W., Lee, C.-T., Chen, C.-Y., Lee, C.-H., 2017. Chemopreventive potential of 2, 3, 5, 4′-tetrahydroxystilbene-2-O-β-D-glucoside on The formation of aberrant crypt foci in azoxymethane-induced colorectal cancer in rats. BioMed research international 2017.
Lin, C., Yu, Y., Zhao, H.-g., Yang, A., Yan, H., Cui, Y., 2012. Combination of quercetin with radiotherapy enhances tumor radiosensitivity in vitro and in vivo. Radiotherapy and oncology 104, 395-400.
Lin, S.-T., Tu, S.-H., Yang, P.-S., Hsu, S.-P., Lee, W.-H., Ho, C.-T., Wu, C.-H., Lai, Y.-H., Chen, M.-Y., Chen, L.-C., 2016. Apple polyphenol phloretin inhibits colorectal cancer cell growth via inhibition of the type 2 glucose transporter and activation of p53-mediated signaling. Journal of agricultural and food chemistry 64, 6826-6837.
Lirdprapamongkol, K., Sakurai, H., Abdelhamed, S., Yokoyama, S., Maruyama, T., Athikomkulchai, S., Viriyaroj, A., Awale, S., Yagita, H., Ruchirawat, S., 2013. A flavonoid chrysin suppresses hypoxic survival and metastatic growth of mouse breast cancer cells. Oncology reports 30, 2357-2364.
Liu, F., Yan, L., Wang, Z., Lu, Y., Chu, Y., Li, X., Liu, Y., Rui, D., Nie, S., Xiang, H., 2017. Metformin therapy and risk of colorectal adenomas and colorectal cancer in type 2 diabetes mellitus patients: A systematic review and meta-analysis. Oncotarget 8, 16017.
Liu, H., Zhang, L., Li, G., Gao, Z., 2020. Xanthohumol protects against Azoxymethane-induced colorectal cancer in Sprague-Dawley rats. Environmental toxicology 35, 136-144.
Liu, W., Xu, J., Liu, Y., Yu, X., Tang, X., Wang, Z., Li, X., 2014. Anthocyanins potentiate the activity of trastuzumab in human epidermal growth factor receptor 2-positive breast cancer cells in vitro and in vivo. Molecular Medicine Reports 10, 1921-1926.
Liu, Y., Cao, W., Zhang, B., Liu, Y.-q., Wang, Z.-y., Wu, Y.-p., Yu, X.-j., Zhang, X.-d., Ming, P.-h., Zhou, G.-b., 2013. The natural compound magnolol inhibits invasion and exhibits potential in human breast cancer therapy. Scientific Reports 3, 3098.
LLOYD, K.M., Dennis, M., 1963. Cowden's disease: a possible new symptom complex with multiple system involvement. Annals of internal medicine 58, 136-142.
Long, J., Guan, P., Hu, X., Yang, L., He, L., Lin, Q., Luo, F., Li, J., He, X., Du, Z., 2021. Natural polyphenols as targeted modulators in colon cancer: molecular mechanisms and Applications. Frontiers in Immunology 12, 635484.
Lori, G., Paoli, P., Femia, A.P., Pranzini, E., Caselli, A., Tortora, K., Romagnoli, A., Raugei, G., Caderni, G., 2019. Morin-dependent inhibition of low molecular weight protein tyrosine phosphatase (LMW-PTP) restores sensitivity to apoptosis during colon carcinogenesis: Studies in vitro and in vivo, in an Apc-driven model of colon cancer. Molecular Carcinogenesis 58, 686-698.
Losada-Echeberría, M., Herranz-López, M., Micol, V., Barrajón-Catalán, E., 2017. Polyphenols as promising drugs against main breast cancer signatures. Antioxidants 6, 88.
Lv, Z.-D., Liu, X.-P., Zhao, W.-J., Dong, Q., Li, F.-N., Wang, H.-B., Kong, B., 2014. Curcumin induces apoptosis in breast cancer cells and inhibits tumor growth in vitro and in vivo. International journal of clinical and experimental pathology 7, 2818.
Lynch, H.T., Boland, C.R., Gong, G., Shaw, T.G., Lynch, P.M., Fodde, R., Lynch, J.F., de la Chapelle, A., 2006. Phenotypic and genotypic heterogeneity in the Lynch syndrome: diagnostic, surveillance and management implications. European journal of human genetics 14, 390-402.

Lynch, H.T., Smyrk, T.C., Watson, P., Lanspa, S.J., Lynch, J.F., Lynch, P.M., Cavalieri, R.J., Boland, C.R., 1993. Genetics, natural history, tumor spectrum, and pathology of hereditary nonpolyposis colorectal cancer: an updated review. Gastroenterology 104, 1535-1549.
Ma, X., Yan, W., Dai, Z., Gao, X., Ma, Y., Xu, Q., Jiang, J., Zhang, S., 2016. Baicalein suppresses metastasis of breast cancer cells by inhibiting EMT via downregulation of SATB1 and Wnt/β-catenin pathway. Drug Design, Development and Therapy, 1419-1441.
Ma, Z., Bao, X., Gu, J., 2019. Furowanin A-induced autophagy alleviates apoptosis and promotes cell cycle arrest via inactivation STAT3/Mcl-1 axis in colorectal cancer. Life sciences 218, 47-57.
Madanikia, S.A., Bergner, A., Ye, X., Blakeley, J.O.N., 2012. Increased risk of breast cancer in women with NF1. American journal of medical genetics Part A 158, 3056-3060.
Malila, N., Virtamo, J., Virtanen, M., Albanes, D., Tangrea, J.A., Huttunen, J.K., 1999. The effect of α-tocopherol and β-carotene supplementation on colorectal adenomas in middle-aged male smokers. Cancer Epidemiology and Prevention Biomarkers 8, 489-493.
Marcinkowski, E.F., Ottesen, R., Niland, J., Vito, C., 2017. Acceptance of adjuvant chemotherapy recommendations in early-stage hormone-positive breast cancer. Journal of Surgical Research 214, 79-85.
Mariyappan, P., Kalaiyarasu, T., Manju, V., 2017. Effect of eriodictyol on preneoplastic lesions, oxidative stress and bacterial enzymes in 1, 2-dimethyl hydrazine-induced colon carcinogenesis. Toxicology Research 6, 678-692.
Masferrer, J.L., Leahy, K.M., Koki, A.T., Zweifel, B.S., Settle, S.L., Woerner, B.M., Edwards, D.A., Flickinger, A.G., Moore, R.J., Seibert, K., 2000. Antiangiogenic and antitumor activities of cyclooxygenase-2 inhibitors. Cancer research 60, 1306-1311.
Masuelli, L., Benvenuto, M., Fantini, M., Marzocchella, L., Sacchetti, P., DI STEFANO, E., Tresoldi, I., Izzi, V., Bernardini, R., Palumbo, C., 2013. Curcumin induces apoptosis in breast cancer cell lines and delays the growth of mammary tumors in neu transgenic mice. Journal of BIOLOGICAL REGULATORS & Homeostatic Agents 27, 105-119.
Mauri, G., Sartore-Bianchi, A., Russo, A.G., Marsoni, S., Bardelli, A., Siena, S., 2019. Early-onset colorectal cancer in young individuals. Molecular oncology 13, 109-131.
McTiernan, A., 2003. Behavioral risk factors in breast cancer: can risk be modified? The oncologist 8, 326-334.
Mehrgou, A., Akouchekian, M., 2016. The importance of BRCA1 and BRCA2 genes mutations in breast cancer development. Medical journal of the Islamic Republic of Iran 30, 369.
Mei, X.-y., Zhang, J.-n., Jia, W.-y., Lu, B., Wang, M.-n., Zhang, T.-y., Ji, L.-l., 2022. Scutellarin suppresses triple-negative breast cancer metastasis by inhibiting TNFα-induced vascular endothelial barrier breakdown. Acta Pharmacologica Sinica 43, 2666-2677.
Mei, X., Ouyang, H., Zhang, H., Jia, W., Lu, B., Zhang, J., Ji, L., 2023. Scutellarin suppresses the metastasis of triple-negative breast cancer via targeting TNFα/TNFR2-RUNX1-triggered G-CSF expression in endothelial cells. Biochemical Pharmacology 217, 115808.
Mendelsohn, J., Prewett, M., Rockwell, P., Goldstein, N.I., 2015. CCR 20th anniversary commentary: a chimeric antibody, C225, inhibits EGFR activation and tumor growth. AACR.
Meng, Y., Sun, J., Yu, J., Wang, C., Su, J., 2019. Dietary intakes of calcium, iron, magnesium, and potassium elements and the risk of colorectal cancer: a meta-analysis. Biological trace element research 189, 325-335.
Meyskens, F.L., McLaren, C.E., Pelot, D., Fujikawa-Brooks, S., Carpenter, P.M., Hawk, E., Kelloff, G., Lawson, M.J., Kidao, J., McCracken, J., 2008. Difluoromethylornithine plus sulindac for the prevention of sporadic colorectal adenomas: a randomized placebo-controlled, double-blind trial. Cancer prevention research 1, 32-38.
Mihanfar, A., Darband, S.G., Sadighparvar, S., Kaviani, M., Mirza-Aghazadeh-Attari, M., Yousefi, B., Majidinia, M., 2021. In vitro and in vivo anticancer effects of syringic acid on colorectal cancer: Possible mechanistic view. Chemico-Biological Interactions 337, 109337.
Milacic, V., Banerjee, S., Landis-Piwowar, K.R., Sarkar, F.H., Majumdar, A.P., Dou, Q.P., 2008. Curcumin inhibits the proteasome activity in human colon cancer cells in vitro and in vivo. Cancer research 68, 7283-7292.

Miyaki, M., Konishi, M., Kikuchi-Yanoshita, R., Enomoto, M., Igari, T., Tanaka, K., Muraoka, M., Takahashi, H., Amada, Y., Fukayama, M., 1994. Characteristics of somatic mutation of the adenomatous polyposis coli gene in colorectal tumors. Cancer research 54, 3011-3020.
Miyamoto, S., Kohno, H., Suzuki, R., Sugie, S., Murakami, A., Ohigashi, H., Tanaka, T., 2006. Preventive effects of chrysin on the development of azoxymethane-induced colonic aberrant crypt foci in rats. Oncology reports 15, 1169-1173.
Miyamoto, S., Yasui, Y., Tanaka, T., Ohigashi, H., Murakami, A., 2008. Suppressive effects of nobiletin on hyperleptinemia and colitis-related colon carcinogenesis in male ICR mice. Carcinogenesis 29, 1057-1063.
Mohr, S.B., Gorham, E.D., Kim, J., Hofflich, H., Garland, C.F., 2014. Meta-analysis of vitamin D sufficiency for improving survival of patients with breast cancer. Anticancer research 34, 1163-1166.
Mokarram, P., Albokashy, M., Zarghooni, M., Moosavi, M.A., Sepehri, Z., Chen, Q.M., Hudecki, A., Sargazi, A., Alizadeh, J., Moghadam, A.R., 2017. New frontiers in the treatment of colorectal cancer: Autophagy and the unfolded protein response as promising targets. Autophagy 13, 781-819.
Møller, P., Seppälä, T.T., Bernstein, I., Holinski-Feder, E., Sala, P., Evans, D.G., Lindblom, A., Macrae, F., Blanco, I., Sijmons, R.H., 2018. Cancer risk and survival in path_MMR carriers by gene and gender up to 75 years of age: a report from the Prospective Lynch Syndrome Database. Gut 67, 1306-1316.
Morishita, Y., Yoshimi, N., Kawabata, K., Matsunaga, K., Sugie, S., Tanaka, T., Mori, H., 1997. Regressive effects of various chemopreventive agents on azoxymethane-induced aberrant crypt foci in the rat colon. Japanese journal of cancer research 88, 815-820.
Motawi, T.K., Abdelazim, S.A., Darwish, H.A., Elbaz, E.M., Shouman, S.A., 2016. Could caffeic acid phenethyl ester expand the antitumor effect of tamoxifen in breast carcinoma? Nutrition and Cancer 68, 435-445.
Muppala, S., 2020. Phytochemicals Targeting Colorectal Cancer Growth and Metastasis. Critical Reviews™ in Oncogenesis 25.
Murillo, G., Hirschelman, W.H., Ito, A., Moriarty, R.M., Kinghorn, A.D., Pezzuto, J.M., Mehta, R.G., 2007. Zapotin, a phytochemical present in a Mexican fruit, prevents colon carcinogenesis. Nutrition and cancer 57, 28-37.
Murphy, C.C., Lund, J.L., Sandler, R.S., 2017. Young-onset colorectal cancer: earlier diagnoses or increasing disease burden? Gastroenterology 152, 1809.
Nagalingam, A., Arbiser, J.L., Bonner, M.Y., Saxena, N.K., Sharma, D., 2012. Honokiol activates AMP-activated protein kinase in breast cancer cells via an LKB1-dependent pathway and inhibits breast carcinogenesis. Breast cancer research 14, 1-16.
Nakanishi, C., Yamaguchi, T., Iijima, T., Saji, S., Toi, M., Mori, T., Miyaki, M., 2005. Germline mutation of the LKB1/STK11 gene with loss of the normal allele in an aggressive breast cancer of Peutz-Jeghers syndrome. Oncology 67, 476-479.
Nan, H., Hutter, C.M., Lin, Y., Jacobs, E.J., Ulrich, C.M., White, E., Baron, J.A., Berndt, S.I., Brenner, H., Butterbach, K., 2015. Association of aspirin and NSAID use with risk of colorectal cancer according to genetic variants. Jama 313, 1133-1142.
Network, C.G.A., 2012. Comprehensive molecular characterization of human colon and rectal cancer. Nature 487, 330.
Ng, C.-A.W., Jiang, A.A., Toh, E.M.S., Ng, C.H., Ong, Z.H., Peng, S., Tham, H.Y., Sundar, R., Chong, C.S., Khoo, C.M., 2020. Metformin and colorectal cancer: a systematic review, meta-analysis and meta-regression. International Journal of Colorectal Disease, 1-12.
Ni, T., He, Z., Dai, Y., Yao, J., Guo, Q., Wei, L., 2017. Oroxylin A suppresses the development and growth of colorectal cancer through reprogram of HIF1α-modulated fatty acid metabolism. Cell Death & Disease 8, e2865-e2865.
Niessen, R.C., Berends, M.J., Wu, Y., Sijmons, R.H., Hollema, H., Ligtenberg, M.J., de Walle, H.E., de Vries, E.G., Karrenbeld, A., Buys, C.H., 2006. Identification of mismatch repair gene mutations in young patients with colorectal cancer and in patients with multiple tumours associated with hereditary non-polyposis colorectal cancer. Gut 55, 1781-1788.
Nirmala, P., Ramanathan, M., 2011a. Effect of kaempferol on lipid peroxidation and antioxidant status in 1, 2-dimethyl hydrazine induced colorectal carcinoma in rats. European journal of pharmacology 654, 75-79.

Nirmala, P., Ramanathan, M., 2011b. Effect of myricetin on 1, 2 dimethylhydrazine induced rat colon carcinogenesis. Journal of experimental therapeutics & oncology 9.
Nounou, M.I., ElAmrawy, F., Ahmed, N., Abdelraouf, K., Goda, S., Syed-Sha-Qhattal, H., 2015. Breast cancer: conventional diagnosis and treatment modalities and recent patents and technologies. Breast cancer: basic and clinical research 9, BCBCR. S29420.
Ohira, H., Oikawa, D., Kurokawa, Y., Aoki, Y., Omura, A., Kiyomoto, K., Nakagawa, W., Mamoto, R., Fujioka, Y., Nakayama, T., 2022. Suppression of colonic oxidative stress caused by chronic ethanol administration and attenuation of ethanol-induced colitis and gut leakiness by oral administration of sesaminol in mice. Food & Function 13, 9285-9298.
Oliveira, A.F., Bretes, L., Furtado, I., 2019. Review of PD-1/PD-L1 inhibitors in metastatic dMMR/MSI-H colorectal cancer. Frontiers in oncology 9, 396.
Oshima, M., Dinchuk, J.E., Kargman, S.L., Oshima, H., Hancock, B., Kwong, E., Trzaskos, J.M., Evans, J.F., Taketo, M.M., 1996. Suppression of intestinal polyposis in ApcΔ716 knockout mice by inhibition of cyclooxygenase 2 (COX-2). Cell 87, 803-809.
Osman, N.H., Said, U.Z., El-Waseef, A.M., Ahmed, E.S., 2015. Luteolin supplementation adjacent to aspirin treatment reduced dimethylhydrazine-induced experimental colon carcinogenesis in rats. Tumor Biology 36, 1179-1190.
Oyama, T., Yasui, Y., Sugie, S., Koketsu, M., Watanabe, K., Tanaka, T., 2009. Dietary tricin suppresses inflammation-related colon carcinogenesis in male Crj: CD-1 mice. Cancer Prevention Research 2, 1031-1038.
Ozsoy, A., Barça, N., Dolek, B.A., Aktaş, H., Elverici, E., Araz, L., Ozkaraoğlu, O., 2017. The relationship between breast cancer and risk factors: a single-center study. European journal of breast health 13, 145.
Pan, C., Hu, Y., Li, J., Wang, Z., Huang, J., Zhang, S., Ding, L., 2014. Estrogen receptor-α36 is involved in pterostilbene-induced apoptosis and anti-proliferation in in vitro and in vivo breast cancer. PLoS One 9, e104459.
Pan, H., Gray, R., Braybrooke, J., Davies, C., Taylor, C., McGale, P., Peto, R., Pritchard, K.I., Bergh, J., Dowsett, M., 2017. 20-year risks of breast-cancer recurrence after stopping endocrine therapy at 5 years. New England Journal of Medicine 377, 1836-1846.
Pandurangan, A.K., Ananda Sadagopan, S.K., Dharmalingam, P., Ganapasam, S., 2014a. Luteolin, a bioflavonoid inhibits Azoxymethane-induced colorectal cancer through activation of Nrf2 signaling. Toxicology mechanisms and methods 24, 13-20.
Pandurangan, A.K., Kumar, S.A.S., Dharmalingam, P., Ganapasam, S., 2014b. Luteolin, a bioflavonoid inhibits azoxymethane-induced colon carcinogenesis: Involvement of iNOS and COX-2. Pharmacognosy magazine 10, S306.
Papaioannou, D., Cooper, K., Carroll, C., Hind, D., Squires, H., Tappenden, P., Logan, R., 2011. Antioxidants in the chemoprevention of colorectal cancer and colorectal adenomas in the general population: a systematic review and meta-analysis. Colorectal disease 13, 1085-1099.
Park, S.-R., Kim, S.-R., Hong, I.-S., Lee, H.-Y., 2020. A novel therapeutic approach for colorectal cancer stem cells: blocking the PI3K/Akt signaling axis with caffeic acid. Frontiers in Cell and Developmental Biology 8, 585987.
Parra-Soto, S., Ahumada, D., Petermann-Rocha, F., Boonpoor, J., Gallegos, J.L., Anderson, J., Sharp, L., Malcomson, F.C., Livingstone, K.M., Mathers, J.C., 2022. Association of meat, vegetarian, pescatarian and fish-poultry diets with risk of 19 cancer sites and all cancer: findings from the UK Biobank prospective cohort study and meta-analysis. BMC medicine 20, 1-16.
Patterson, R.E., Cadmus, L.A., Emond, J.A., Pierce, J.P., 2010. Physical activity, diet, adiposity and female breast cancer prognosis: a review of the epidemiologic literature. Maturitas 66, 5-15.
Paul, S., DeCastro, A.J., Lee, H.J., Smolarek, A.K., So, J.Y., Simi, B., Wang, C.X., Zhou, R., Rimando, A.M., Suh, N., 2010. Dietary intake of pterostilbene, a constituent of blueberries, inhibits the β-catenin/p65 downstream signaling pathway and colon carcinogenesis in rats. Carcinogenesis 31, 1272-1278.
Petrera, M., Paleari, L., Clavarezza, M., Puntoni, M., Caviglia, S., Briata, I.M., Oppezzi, M., Mislej, E.M., Stabuc, B., Gnant, M., 2018. The ASAMET trial: a randomized, phase II, double-blind, placebo-controlled, multicenter, 2× 2 factorial biomarker study of tertiary prevention with low-dose aspirin and metformin in stage I-III colorectal cancer patients. BMC cancer 18, 1-9.

Pharoah, P.D., Guilford, P., Caldas, C., Consortium, I.G.C.L., 2001. Incidence of gastric cancer and breast cancer in CDH1 (E-cadherin) mutation carriers from hereditary diffuse gastric cancer families. Gastroenterology 121, 1348-1353.
Polyak, K., 2007. Breast cancer: origins and evolution. The Journal of clinical investigation 117, 3155-3163.
Ponti, G., Manfredini, M., Tomasi, A., Pellacani, G., 2016. Muir–Torre Syndrome and founder mismatch repair gene mutations: A long gone historical genetic challenge. Gene 589, 127-132.
Potack, J., Itzkowitz, S.H., 2008. Colorectal cancer in inflammatory bowel disease. Gut and liver 2, 61.
Pu, Z., Zhang, W., Wang, M., Xu, M., Xie, H., Zhao, J., 2021. Schisandrin B Attenuates colitis-associated colorectal cancer through SIRT1 linked SMURF2 signaling. The American Journal of Chinese Medicine 49, 1773-1789.
Qi, X.S., Pajonk, F., McCloskey, S., Low, D.A., Kupelian, P., Steinberg, M., Sheng, K., 2017. Radioresistance of the breast tumor is highly correlated to its level of cancer stem cell and its clinical implication for breast irradiation. Radiotherapy and Oncology 124, 455-461.
Rabi, T., Bishayee, A., 2009. Terpenoids and breast cancer chemoprevention. Breast cancer research and treatment 115, 223-239.
Rajamanickam, S., Agarwal, R., 2008. Natural products and colon cancer: current status and future prospects. Drug development research 69, 460-471.
Rajbhandari, M., Wegner, U., Jülich, M., Schoepke, T., Mentel, R., 2001. Screening of Nepalese medicinal plants for antiviral activity. Journal of ethnopharmacology 74, 251-255.
Rajbhandari, M., Wegner, U., Schoepke, T., Lindequist, U., Mentel, R., 2003. Inhibitory effect of Bergenia ligulata on influenza virus A. Die Pharmazie-An International Journal of Pharmaceutical Sciences 58, 268-271.
Ranjan, A., Ramachandran, S., Gupta, N., Kaushik, I., Wright, S., Srivastava, S., Das, H., Srivastava, S., Prasad, S., Srivastava, S.K., 2019. Role of phytochemicals in cancer prevention. International journal of molecular sciences 20, 4981.
Rao, C.V., Tokumo, K., Rigotty, J., Zang, E., Kelloff, G., Reddy, B.S., 1991. Chemoprevention of colon carcinogenesis by dietary administration of piroxicam, α-difluoromethylornithine, 16α-fluoro-5-androsten-17-one, and ellagic acid individually and in combination. Cancer research 51, 4528-4534.
Razak, S., Afsar, T., Ullah, A., Almajwal, A., Alkholief, M., Alshamsan, A., Jahan, S., 2018. Taxifolin, a natural flavonoid interacts with cell cycle regulators causes cell cycle arrest and causes tumor regression by activating Wnt/β-catenin signaling pathway. BMC cancer 18, 1-18.
Reuben, S.C., Gopalan, A., Petit, D.M., Bishayee, A., 2012. Modulation of angiogenesis by dietary phytoconstituents in the prevention and intervention of breast cancer. Molecular nutrition & food research 56, 14-29.
Ribic, C.M., Sargent, D.J., Moore, M.J., Thibodeau, S.N., French, A.J., Goldberg, R.M., Hamilton, S.R., Laurent-Puig, P., Gryfe, R., Shepherd, L.E., 2003. Tumor microsatellite-instability status as a predictor of benefit from fluorouracil-based adjuvant chemotherapy for colon cancer. New England Journal of Medicine 349, 247-257.
Rock, C.L., Demark-Wahnefried, W., 2002. Can lifestyle modification increase survival in women diagnosed with breast cancer? The Journal of nutrition 132, 3504S-3509S.
Rodríguez-Miguel, A., García-Rodríguez, L.A., Gil, M., Montoya, H., Rodríguez-Martín, S., de Abajo, F.J., 2019. Clopidogrel and low-dose aspirin, alone or together, reduce risk of colorectal cancer. Clinical Gastroenterology and Hepatology 17, 2024-2033. e2022.
Roskoski Jr, R., 2014. The ErbB/HER family of protein-tyrosine kinases and cancer. Pharmacological research 79, 34-74.
Rothwell, P.M., Wilson, M., Elwin, C.-E., Norrving, B., Algra, A., Warlow, C.P., Meade, T.W., 2010. Long-term effect of aspirin on colorectal cancer incidence and mortality: 20-year follow-up of five randomised trials. The Lancet 376, 1741-1750.
Roychoudhury, S., Das, D., Das, S., Jha, N.K., Pal, M., Kolesarova, A., Kesari, K.K., Kalita, J.C., Slama, P., 2022. Clinical Potential of Himalayan Herb Bergenia ligulata: An Evidence-Based Study. Molecules 27, 7039.
Rutz, H.P., de Tribolet, N., Calmes, J.M., Chapuis, G., 1991. Long-time survival of a patient with glioblastoma and Turcot's syndrome: case report. Journal of neurosurgery 74, 813-815.

Sadat, A., Uddin, G., Alam, M., Ahmad, A., Siddiqui, B.S., 2015. Structure activity relationship of bergenin, p-hydroxybenzoyl bergenin, 11-O-galloylbergenin as potent antioxidant and urease inhibitor isolated from Bergenia ligulata. Natural Product Research 29, 2291-2294.
Saijyo, J., Suzuki, Y., Okuno, Y., Yamaki, H., Suzuki, T., Miyazawa, M., 2008. α-Glucosidase inhibitor from Bergenia ligulata. Journal of Oleo Science 57, 431-435.
Saiprasad, G., Chitra, P., Manikandan, R., Sudhandiran, G., 2013. Hesperidin alleviates oxidative stress and downregulates the expressions of proliferative and inflammatory markers in azoxymethane-induced experimental colon carcinogenesis in mice. Inflammation Research 62, 425-440.
Saiprasad, G., Chitra, P., Manikandan, R., Sudhandiran, G., 2014. Hesperidin induces apoptosis and triggers autophagic markers through inhibition of Aurora-A mediated phosphoinositide-3-kinase/Akt/mammalian target of rapamycin and glycogen synthase kinase-3 beta signalling cascades in experimental colon carcinogenesis. European journal of cancer 50, 2489-2507.
Sajad, T., Zargar, A., Ahmad, T., Bader, G., Naime, M., Ali, S., 2010. Antibacterial and anti-inflammatory potential Bergenia ligulata. Am. J. Biomed. Sci 2, 313-321.
Salama, A.A., Allam, R.M., 2021. Promising targets of chrysin and daidzein in colorectal cancer: Amphiregulin, CXCL1, and MMP-9. European Journal of Pharmacology 892, 173763.
Saleh, A., ElFayoumi, H.M., Youns, M., Barakat, W., 2019. Rutin and orlistat produce antitumor effects via antioxidant and apoptotic actions. Naunyn-Schmiedeberg's Archives of Pharmacology 392, 165-175.
Samadder, N.J., Kuwada, S.K., Boucher, K.M., Byrne, K., Kanth, P., Samowitz, W., Jones, D., Tavtigian, S.V., Westover, M., Berry, T., 2018. Association of sulindac and erlotinib vs placebo with colorectal neoplasia in familial adenomatous polyposis: secondary analysis of a randomized clinical trial. JAMA oncology 4, 671-677.
Samec, M., Liskova, A., Koklesova, L., Samuel, S.M., Zhai, K., Buhrmann, C., Varghese, E., Abotaleb, M., Qaradakhi, T., Zulli, A., 2020. Flavonoids against the Warburg phenotype—concepts of predictive, preventive and personalised medicine to cut the Gordian knot of cancer cell metabolism. Epma Journal 11, 377-398.
Sanchez-Martin, V., Plaza-Calonge, M.d.C., Soriano-Lerma, A., Ortiz-Gonzalez, M., Linde-Rodriguez, A., Perez-Carrasco, V., Ramirez-Macias, I., Cuadros, M., Gutierrez-Fernandez, J., Murciano-Calles, J., 2022. Gallic Acid: A Natural Phenolic Compound Exerting Antitumoral Activities in Colorectal Cancer via Interaction with G-Quadruplexes. Cancers 14, 2648.
Sannappa Gowda, N.G., Shiragannavar, V.D., Puttahanumantharayappa, L.D., Shivakumar, A.T., Dallavalasa, S., Basavaraju, C.G., Bhat, S.S., Prasad, S.K., Vamadevaiah, R.M., Madhunapantula, S.V., 2023. Quercetin activates vitamin D receptor and ameliorates breast cancer induced hepatic inflammation and fibrosis. Frontiers in Nutrition 10, 582.
Sargent, D.J., Marsoni, S., Monges, G., Thibodeau, S.N., Labianca, R., Hamilton, S.R., French, A.J., Kabat, B., Foster, N.R., Torri, V., 2010. Defective mismatch repair as a predictive marker for lack of efficacy of fluorouracil-based adjuvant therapy in colon cancer. Journal of Clinical Oncology 28, 3219.
Saud, S.M., Young, M.R., Jones-Hall, Y.L., Ileva, L., Evbuomwan, M.O., Wise, J., Colburn, N.H., Kim, Y.S., Bobe, G., 2013. Chemopreventive Activity of Plant Flavonoid Isorhamnetin in Colorectal Cancer Is Mediated by Oncogenic Src and β-CateninIsorhamnetin-Induced CSK Inhibits Colorectal Cancer. Cancer research 73, 5473-5484.
Sekar, V., Anandasadagopan, S.K., Ganapasam, S., 2016. Genistein regulates tumor microenvironment and exhibits anticancer effect in dimethyl hydrazine-induced experimental colon carcinogenesis. Biofactors 42, 623-637.
Senapathy, J.G., Jayanthi, S., Viswanathan, P., Umadevi, P., Nalini, N., 2011. Effect of gallic acid on xenobiotic metabolizing enzymes in 1, 2-dimethyl hydrazine induced colon carcinogenesis in Wistar rats–a chemopreventive approach. Food and chemical toxicology 49, 887-892.
Sener, S.F., 2020. Advances in axillary surgery for breast cancer 2019. Journal of Surgical Oncology 121, 20-24.
Seno, H., Oshima, M., Ishikawa, T.-o., Oshima, H., Takaku, K., Chiba, T., Narumiya, S., Taketo, M.M., 2002. Cyclooxygenase 2-and prostaglandin E2 receptor EP2-dependent angiogenesis in ApcΔ716 mouse intestinal polyps. Cancer research 62, 506-511.
Seppälä, T., Latchford, A., Negoi, I., Sampaio Soares, A., Jimenez-Rodriguez, R., Sánchez-Guillén, L., Evans, D., Ryan, N., Crosbie, E., Dominguez-Valentin, M., 2021. European guidelines from the EHTG

and ESCP for Lynch syndrome: an updated third edition of the Mallorca guidelines based on gene and gender. British Journal of Surgery 108, 484-498.
Sepporta, M.V., Fuccelli, R., Rosignoli, P., Ricci, G., Servili, M., Fabiani, R., 2016. Oleuropein prevents Azoxymethane-induced Colon crypt dysplasia and leukocytes DNA damage in a/J mice. Journal of medicinal food 19, 983-989.
Sequetto, P.L., Oliveira, T.T., Maldonado, I.R., Augusto, L.E.F., Mello, V.J., Pizziolo, V.R., Almeida, M.R., Silva, M.E., Novaes, R.D., 2014. Naringin accelerates the regression of pre-neoplastic lesions and the colorectal structural reorganization in a murine model of chemical carcinogenesis. Food and Chemical Toxicology 64, 200-209.
Shao, H., Jing, K., Mahmoud, E., Huang, H., Fang, X., Yu, C., 2013. Apigenin sensitizes colon cancer cells to antitumor activity of ABT-263. Molecular cancer therapeutics 12, 2640-2650.
Sharma, G.N., Dave, R., Sanadya, J., Sharma, P., Sharma, K., 2010. Various types and management of breast cancer: an overview. Journal of advanced pharmaceutical technology & research 1, 109.
Sharma, I., Khan, W., Parveen, R., Alam, M.J., Ahmad, I., Ansari, M.H.R., Ahmad, S., 2017a. Antiurolithiasis activity of bioactivity guided fraction of Bergenia ligulata against ethylene glycol induced renal calculi in rat. BioMed Research International 2017.
Sharma, S.H., Chellappan, D.R., Chinnaswamy, P., Nagarajan, S., 2017b. Protective effect of p-coumaric acid against 1, 2 dimethylhydrazine induced colonic preneoplastic lesions in experimental rats. Biomedicine & Pharmacotherapy 94, 577-588.
Sharma, S.H., Kumar, J.S., Chellappan, D.R., Nagarajan, S., 2018. Molecular chemoprevention by morin–A plant flavonoid that targets nuclear factor kappa B in experimental colon cancer. Biomedicine & Pharmacotherapy 100, 367-373.
Sharma, S.H., Thulasingam, S., Chellappan, D.R., Chinnaswamy, P., Nagarajan, S., 2017c. Morin and Esculetin supplementation modulates c-myc induced energy metabolism and attenuates neoplastic changes in rats challenged with the procarcinogen 1, 2-dimethylhydrazine. European Journal of Pharmacology 796, 20-31.
Shi, C.-J., Li, S.-Y., Shen, C.-H., Pan, F.-F., Deng, L.-Q., Fu, W.-M., Wang, J.-Y., Zhang, J.-F., 2022. Icariside II suppressed tumorigenesis by epigenetically regulating the circβ-catenin-Wnt/β-catenin axis in colorectal cancer. Bioorganic Chemistry 124, 105800.
Shi, H., Zhao, L., Guo, X., Fang, R., Zhang, H., Dong, G., Fu, J., Yan, F., Zhang, J., Ning, Z., 2020. Arctigenin attenuates breast cancer progression through decreasing GM-CSF/TSLP/STAT3/β-Catenin signaling. International Journal of Molecular Sciences 21, 6357.
Shibuya, M., 2011. Vascular endothelial growth factor (VEGF) and its receptor (VEGFR) signaling in angiogenesis: a crucial target for anti-and pro-angiogenic therapies. Genes & cancer 2, 1097-1105.
Shimizu, M., Shirakami, Y., Sakai, H., Yasuda, Y., Kubota, M., Adachi, S., Tsurumi, H., Hara, Y., Moriwaki, H., 2010. (−)-Epigallocatechin gallate inhibits growth and activation of the VEGF/VEGFR axis in human colorectal cancer cells. Chemico-biological interactions 185, 247-252.
Shin, M.-K., Jeon, Y.-D., Hong, S.-H., Kang, S.-H., Kee, J.-Y., Jin, J.-S., 2021. In vivo and In vitro effects of Tracheloside on colorectal cancer cell proliferation and metastasis. Antioxidants 10, 513.
Shivappa, N., Godos, J., Hébert, J.R., Wirth, M.D., Piuri, G., Speciani, A.F., Grosso, G., 2017. Dietary inflammatory index and colorectal cancer risk—a meta-analysis. Nutrients 9, 1043.
Shulman, L.N., Willett, W., Sievers, A., Knaul, F.M., 2010. Breast cancer in developing countries: opportunities for improved survival. Journal of oncology 2010.
Siddiqui, R.A., Harvey, K.A., Walker, C., Altenburg, J., Xu, Z., Terry, C., Camarillo, I., Jones-Hall, Y., Mariash, C., 2013. Characterization of synergistic anti-cancer effects of docosahexaenoic acid and curcumin on DMBA-induced mammary tumorigenesis in mice. BMC cancer 13, 1-16.
Siegel, R.L., Miller, K.D., Fedewa, S.A., Ahnen, D.J., Meester, R.G., Barzi, A., Jemal, A., 2017. Colorectal cancer statistics, 2017. CA: a cancer journal for clinicians 67, 177-193.
Siegel, R.L., Miller, K.D., Fuchs, H.E., Jemal, A., 2021. Cancer statistics, 2021. Ca Cancer J Clin 71, 7-33.
Siegel, R.L., Miller, K.D., Goding Sauer, A., Fedewa, S.A., Butterly, L.F., Anderson, J.C., Cercek, A., Smith, R.A., Jemal, A., 2020. Colorectal cancer statistics, 2020. CA: a cancer journal for clinicians 70, 145-164.

Silwal-Pandit, L., Vollan, H.K.M., Chin, S.-F., Rueda, O.M., McKinney, S., Osako, T., Quigley, D.A., Kristensen, V.N., Aparicio, S., Børresen-Dale, A.-L., 2014. TP53 mutation spectrum in breast cancer is subtype specific and has distinct prognostic relevance. Clinical Cancer Research 20, 3569-3580.
Singh, A., Tandon, S., Nandi, S.P., Kaur, T., Tandon, C., 2021. Downregulation of inflammatory mediators by ethanolic extract of Bergenia ligulata (Wall.) in oxalate injured renal epithelial cells. Journal of Ethnopharmacology 275, 114104.
Singh, M., Pandey, N., Agnihotri, V., Singh, K., Pandey, A., 2017. Antioxidant, antimicrobial activity and bioactive compounds of Bergenia ciliata Sternb.: A valuable medicinal herb of Sikkim Himalaya. Journal of Traditional and Complementary Medicine 7, 152-157.
Singletary, S.E., 2003. Rating the risk factors for breast cancer. Annals of surgery 237, 474.
Sinha, D., Biswas, J., Sung, B., B Aggarwal, B., Bishayee, A., 2012. Chemopreventive and chemotherapeutic potential of curcumin in breast cancer. Current drug targets 13, 1799-1819.
Sinicrope, F.A., Velamala, P.R., Song, L.M.W.K., Viggiano, T.R., Bruining, D.H., Rajan, E., Gostout, C.J., Kraichely, R.E., Buttar, N.S., Schroeder, K.W., 2019. Efficacy of difluoromethylornithine and aspirin for treatment of adenomas and aberrant crypt foci in patients with prior advanced colorectal neoplasms. Cancer Prevention Research 12, 821-830.
Slamon, D., Eiermann, W., Robert, N., Pienkowski, T., Martin, M., Press, M., Mackey, J., Glaspy, J., Chan, A., Pawlicki, M., 2011. Adjuvant trastuzumab in HER2-positive breast cancer. New England journal of medicine 365, 1273-1283.
Smerdel, M.P., Skytte, A.-B., Jelsig, A.M., Ebbehøj, E., Stochholm, K., 2020. Revised Danish guidelines for the cancer surveillance of patients with Cowden Syndrome. European journal of medical genetics 63, 103873.
Somers-Edgar, T.J., Scandlyn, M.J., Stuart, E.C., Le Nedelec, M.J., Valentine, S.P., Rosengren, R.J., 2008. The combination of epigallocatechin gallate and curcumin suppresses ERα-breast cancer cell growth in vitro and in vivo. International journal of cancer 122, 1966-1971.
Song, L., Zhu, S., Liu, C., Zhang, Q., Liang, X., 2022. Baicalin triggers apoptosis, inhibits migration, and enhances anti-tumor immunity in colorectal cancer via TLR4/NF-κB signaling pathway. Journal of Food Biochemistry 46, e13703.
Sørlie, T., Perou, C.M., Tibshirani, R., Aas, T., Geisler, S., Johnsen, H., Hastie, T., Eisen, M.B., Van De Rijn, M., Jeffrey, S.S., 2001. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. Proceedings of the National Academy of Sciences 98, 10869-10874.
Souglakos, J., Androulakis, N., Syrigos, K., Polyzos, A., Ziras, N., Athanasiadis, A., Kakolyris, S., Tsousis, S., Kouroussis, C., Vamvakas, L., 2006. FOLFOXIRI (folinic acid, 5-fluorouracil, oxaliplatin and irinotecan) vs FOLFIRI (folinic acid, 5-fluorouracil and irinotecan) as first-line treatment in metastatic colorectal cancer (MCC): a multicentre randomised phase III trial from the Hellenic Oncology Research Group (HORG). British journal of cancer 94, 798-805.
Soysal, S.D., Tzankov, A., Muenst, S.E., 2015. Role of the tumor microenvironment in breast cancer. Pathobiology 82, 142-152.
Sreedharan, V., Venkatachalam, K.K., Namasivayam, N., 2009. Effect of morin on tissue lipid peroxidation and antioxidant status in 1, 2-dimethylhydrazine induced experimental colon carcinogenesis. Investigational new drugs 27, 21-30.
Srivastava, S., Rawat, A.K.S., 2008. Botanical and phytochemical comparison of three Bergenia species.
Su, C.-M., Lee, W.-H., Wu, A.T., Lin, Y.-K., Wang, L.-S., Wu, C.-H., Yeh, C.-T., 2015. Pterostilbene inhibits triple-negative breast cancer metastasis via inducing microRNA-205 expression and negatively modulates epithelial-to-mesenchymal transition. The Journal of nutritional biochemistry 26, 675-685.
Su, C.-M., Weng, Y.-S., Kuan, L.-Y., Chen, J.-H., Hsu, F.-T., 2020. Suppression of PKCδ/NF-κB Signaling and Apoptosis Induction through Extrinsic/Intrinsic Pathways Are Associated with Magnolol-Inhibited Tumor Progression in Colorectal Cancer In Vitro and In Vivo. International Journal of Molecular Sciences 21, 3527.
Suh, N., Paul, S., Hao, X., Simi, B., Xiao, H., Rimando, A.M., Reddy, B.S., 2007. Pterostilbene, an active constituent of blueberries, suppresses aberrant crypt foci formation in the azoxymethane-induced colon carcinogenesis model in rats. Clinical Cancer Research 13, 350-355.

Suh, N., Reddy, B.S., DeCastro, A., Paul, S., Lee, H.J., Smolarek, A.K., So, J.Y., Simi, B., Wang, C.X., Janakiram, N.B., 2011. Combination of atorvastatin with sulindac or naproxen profoundly inhibits colonic adenocarcinomas by suppressing the p65/β-catenin/cyclin D1 signaling pathway in rats. Cancer Prevention Research 4, 1895-1902.
Sun, D.-W., Zhang, H.-D., Mao, L., Mao, C.-F., Chen, W., Cui, M., Ma, R., Cao, H.-X., Jing, C.-W., Wang, Z., 2015. Luteolin inhibits breast cancer development and progression in vitro and in vivo by suppressing notch signaling and regulating MiRNAs. Cellular Physiology and Biochemistry 37, 1693-1711.
Sun, J., Li, M., Lin, K., Liu, Z., Wang, Z., Wang, W., Zhao, Y., Zhen, Y., Zhang, S., 2023. Delivery of quercetin for breast cancer and targeting potentiation via hyaluronic nano-micelles. International Journal of Biological Macromolecules 242, 124736.
Sun, X., Ma, X., Li, Q., Yang, Y., Xu, X., Sun, J., Yu, M., Cao, K., Yang, L., Yang, G., 2018a. Anti-cancer effects of fisetin on mammary carcinoma cells via regulation of the PI3K/Akt/mTOR pathway: In vitro and in vivo studies. International Journal of Molecular Medicine 42, 811-820.
Sun, Y.-S., Zhao, Z., Yang, Z.-N., Xu, F., Lu, H.-J., Zhu, Z.-Y., Shi, W., Jiang, J., Yao, P.-P., Zhu, H.-P., 2017. Risk factors and preventions of breast cancer. International journal of biological sciences 13, 1387.
Sun, Y., Zhou, Q.-M., Lu, Y.-Y., Zhang, H., Chen, Q.-L., Zhao, M., Su, S.-B., 2019. Resveratrol inhibits the migration and metastasis of MDA-MB-231 human breast cancer by reversing TGF-β1-induced epithelial-mesenchymal transition. Molecules 24, 1131.
Sun, Z., Zhou, C., Liu, F., Zhang, W., Chen, J., Pan, Y., Ma, L., Liu, Q., Du, Y., Yang, J., 2018b. Inhibition of breast cancer cell survival by Xanthohumol via modulation of the Notch signaling pathway in vivo and in vitro. Oncology letters 15, 908-916.
Sunada, H., Magun, B.E., Mendelsohn, J., MacLeod, C.L., 1986. Monoclonal antibody against epidermal growth factor receptor is internalized without stimulating receptor phosphorylation. Proceedings of the National Academy of Sciences 83, 3825-3829.
Sung, H., Ferlay, J., Siegel, R.L., Laversanne, M., Soerjomataram, I., Jemal, A., Bray, F., 2021. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: a cancer journal for clinicians 71, 209-249.
Sutcliffe, E.G., Thompson, A.B., Stettner, A.R., Marshall, M.L., Roberts, M.E., Susswein, L.R., Wang, Y., Klein, R.T., Hruska, K.S., Solomon, B.D., 2019. Multi-gene panel testing confirms phenotypic variability in MUTYH-Associated Polyposis. Familial cancer 18, 203-209.
Swetha, M., Keerthana, C., Rayginia, T.P., Anto, R.J., 2021. Cancer chemoprevention: A strategic approach using phytochemicals. Frontiers in Pharmacology 12.
Takahashi, T., Takasuka, N., Iigo, M., Baba, M., Nishino, H., Tsuda, H., Okuyama, T., 2004. Isoliquiritigenin, a flavonoid from licorice, reduces prostaglandin E2 and nitric oxide, causes apoptosis, and suppresses aberrant crypt foci development. Cancer Science 95, 448-453.
Tan, A.R., Swain, S.M., 2008. Therapeutic strategies for triple-negative breast cancer. The Cancer Journal 14, 343-351.
Tan, M.-H., Mester, J.L., Ngeow, J., Rybicki, L.A., Orloff, M.S., Eng, C., 2012. Lifetime cancer risks in individuals with germline PTEN mutations. Clinical Cancer Research 18, 400-407.
Tanaka, T., Makita, H., Kawabata, K., Mori, H., Kakumoto, M., Satoh, K., Hara, A., Sumida, T., Tanaka, T., Ogawa, H., 1997. Chemoprevention of azoxymethane-induced rat colon carcinogenesis by the naturally occurring flavonoids, diosmin and hesperidin. Carcinogenesis 18, 957-965.
Tanaka, T., Nishikawa, A., Shima, H., Sugie, S., Shinoda, T., Yoshimi, N., Iwata, H., Mori, H., 1990. Inhibitory effects of chlorogenic acid, reserpine, polyprenoic acid (E-5166), or coffee on hepatocarcinogenesis in rats and hamsters, Antimutagenesis and anticarcinogenesis mechanisms II. Springer, pp. 429-440.
Tang, H., Yao, X., Yao, C., Zhao, X., Zuo, H., Li, Z., 2017. Anti-colon cancer effect of caffeic acid p-nitro-phenethyl ester in vitro and in vivo and detection of its metabolites. Scientific reports 7, 1-11.
Tang, S., Cai, S., Ji, S., Yan, X., Zhang, W., Qiao, X., Zhang, H., Ye, M., Yu, S., 2021. Isoangustone A induces autophagic cell death in colorectal cancer cells by activating AMPK signaling. Fitoterapia 152, 104935.
Tariq, K., Ghias, K., 2016. Colorectal cancer carcinogenesis: a review of mechanisms. Cancer biology & medicine 13, 120.

Teraoka, N., Mutoh, M., Takasu, S., Ueno, T., Yamamoto, M., Sugimura, T., Wakabayashi, K., 2011. Inhibition of intestinal polyp formation by pitavastatin, a HMG-CoA reductase inhibitor. Cancer prevention research 4, 445-453.
Tfayli, A., Temraz, S., Abou Mrad, R., Shamseddine, A., 2010. Breast cancer in low-and middle-income countries: an emerging and challenging epidemic. Journal of oncology 2010.
Thangapazham, R.L., Singh, A.K., Sharma, A., Warren, J., Gaddipati, J.P., Maheshwari, R.K., 2007. Green tea polyphenols and its constituent epigallocatechin gallate inhibits proliferation of human breast cancer cells in vitro and in vivo. Cancer letters 245, 232-241.
Thangaraj, K., Natesan, K., Palani, M., Vaiyapuri, M., 2018. Orientin, a flavanoid, mitigates 1, 2 dimethylhydrazine-induced colorectal lesions in Wistar rats fed a high-fat diet. Toxicology Reports 5, 977-987.
Thangaraj, K., Vaiyapuri, M., 2017. Orientin, a C-glycosyl dietary flavone, suppresses colonic cell proliferation and mitigates NF-κB mediated inflammatory response in 1, 2-dimethylhydrazine induced colorectal carcinogenesis. Biomedicine & Pharmacotherapy 96, 1253-1266.
Thanikachalam, K., Khan, G., 2019. Colorectal cancer and nutrition. Nutrients 11, 164.
Thomasset, S., Berry, D.P., Cai, H., West, K., Marczylo, T.H., Marsden, D., Brown, K., Dennison, A., Garcea, G., Miller, A., 2009. Pilot study of oral anthocyanins for colorectal cancer chemoprevention. Cancer prevention research 2, 625-633.
Tomić, T., Domínguez-López, S., Barrios-Rodríguez, R., 2019. Non-aspirin non-steroidal anti-inflammatory drugs in prevention of colorectal cancer in people aged 40 or older: a systematic review and meta-analysis. Cancer epidemiology 58, 52-62.
Toyota, M., Ahuja, N., Ohe-Toyota, M., Herman, J.G., Baylin, S.B., Issa, J.-P.J., 1999. CpG island methylator phenotype in colorectal cancer. Proceedings of the National Academy of Sciences 96, 8681-8686.
Tsimogiannis, D., Oreopoulou, V., 2019. Classification of phenolic compounds in plants, Polyphenols in plants. Elsevier, pp. 263-284.
Tutino, V., De Nunzio, V., Tafaro, A., Bianco, G., Gigante, I., Scavo, M.P., D'ALESSANDRO, R., Refolo, M.G., Messa, C., Caruso, M.G., 2018. Cannabinoid receptor-1 up-regulation in azoxymethane (AOM)-treated mice after dietary treatment with quercetin. Anticancer Research 38, 4485-4491.
Umesalma, S., Sudhandiran, G., 2010a. Chemomodulation of the antioxidative enzymes and peroxidative damage in the colon of 1, 2-dimethyl hydrazine-induced rats by ellagicacid. Phytotherapy Research 24, S114-S119.
Umesalma, S., Sudhandiran, G., 2010b. Differential inhibitory effects of the polyphenol ellagic acid on inflammatory mediators NF-κB, iNOS, COX-2, TNF-α, and IL-6 in 1, 2-dimethylhydrazine-induced rat colon carcinogenesis. Basic & clinical pharmacology & toxicology 107, 650-655.
Umesalma, S., Sudhandiran, G., 2011. Ellagic acid prevents rat colon carcinogenesis induced by 1, 2 dimethyl hydrazine through inhibition of AKT-phosphoinositide-3 kinase pathway. European journal of pharmacology 660, 249-258.
Vanio, H., Bianchini, F., 2002. IARC handbooks of cancer prevention. Volume.
Vantyghem, S.A., Wilson, S.M., Postenka, C.O., Al-Katib, W., Tuck, A.B., Chambers, A.F., 2005. Dietary genistein reduces metastasis in a postsurgical orthotopic breast cancer model. Cancer Research 65, 3396-3403.
Veettil, S.K., Nathisuwan, S., Ching, S.M., Jinatongthai, P., Lim, K.G., Kew, S.T., Chaiyakunapruk, N., 2019. Efficacy and safety of celecoxib on the incidence of recurrent colorectal adenomas: a systematic review and meta-analysis. Cancer management and research 11, 561.
Vennila, S., Nalini, N., 2009. Modifying effects of morin on the development of aberrant crypt foci and bacterial enzymes in experimental colon cancer. Food and chemical toxicology 47, 309-315.
Venugopal, A., Stoffel, E.M., 2019. Colorectal cancer in young adults. Current treatment options in gastroenterology 17, 89-98.
Villegas, I., Sánchez-Fidalgo, S., de la Lastra, C.A., 2011. Chemopreventive effect of dietary curcumin on inflammation-induced colorectal carcinogenesis in mice. Molecular nutrition & food research 55, 259-267.
Vinothkumar, R., Kumar, R.V., Sudha, M., Viswanathan, P., Balasubramanian, T., Nalini, N., 2014. Modulatory effect of troxerutin on biotransforming enzymes and preneoplasic lesions induced by 1, 2-dimethylhydrazine in rat colon carcinogenesis. Experimental and Molecular Pathology 96, 15-26.

VM, A.A.Z.S.D., 2021. International Natural Product Sciences Taskforce Supuran CT Nat. Rev. Drug Discovery 20, 200-216.
Vogel, V.G., 2008. Epidemiology, genetics, and risk evaluation of postmenopausal women at risk of breast cancer. Menopause 15, 782-789.
Vogt, S., Jones, N., Christian, D., Engel, C., Nielsen, M., Kaufmann, A., Steinke, V., Vasen, H.F., Propping, P., Sampson, J.R., 2009. Expanded extracolonic tumor spectrum in MUTYH-associated polyposis. Gastroenterology 137, 1976-1985. e1910.
Vuolo, M.M., Lima, V.S., Junior, M.R.M., 2019. Phenolic compounds: Structure, classification, and antioxidant power, Bioactive compounds. Elsevier, pp. 33-50.
Walsh, T., Casadei, S., Coats, K.H., Swisher, E., Stray, S.M., Higgins, J., Roach, K.C., Mandell, J., Lee, M.K., Ciernikova, S., 2006. Spectrum of mutations in BRCA1, BRCA2, CHEK2, and TP53 in families at high risk of breast cancer. Jama 295, 1379-1388.
Wang, D., DuBois, R.N., 2010. The role of COX-2 in intestinal inflammation and colorectal cancer. Oncogene 29, 781-788.
Wang, F., Song, Z.-Y., Qu, X.-J., Li, F., Zhang, L., Li, W.-B., Cui, S.-X., 2018a. M10, a novel derivative of Myricetin, prevents ulcerative colitis and colorectal tumor through attenuating robust endoplasmic reticulum stress. Carcinogenesis 39, 889-899.
Wang, N., Wang, Q., Tang, H., Zhang, F., Zheng, Y., Wang, S., Zhang, J., Wang, Z., Xie, X., 2017. Direct inhibition of ACTN4 by ellagic acid limits breast cancer metastasis via regulation of β-catenin stabilization in cancer stem cells. Journal of Experimental & Clinical Cancer Research 36, 1-19.
Wang, N., Wang, Z.-Y., Mo, S.-L., Loo, T.Y., Wang, D.-M., Luo, H.-B., Yang, D.-P., Chen, Y.-L., Shen, J.-G., Chen, J.-P., 2012. Ellagic acid, a phenolic compound, exerts anti-angiogenesis effects via VEGFR-2 signaling pathway in breast cancer. Breast cancer research and treatment 134, 943-955.
Wang, N., Wang, Z., Peng, C., You, J., Shen, J., Han, S., Chen, J., 2014. Dietary compound isoliquiritigenin targets GRP78 to chemosensitize breast cancer stem cells via β-catenin/ABCG2 signaling. Carcinogenesis 35, 2544-2554.
Wang, N., Zhou, F., Guo, J., Zhu, H., Luo, S., Cao, J., 2018b. Euxanthone suppresses tumor growth and metastasis in colorectal cancer via targeting CIP2A/PP2A pathway. Life sciences 209, 498-506.
Wang, Q.R., Yao, X.Q., Wen, G., Fan, Q., Li, Y.-J., Fu, X.Q., Li, C.K., Sun, X.G., 2011. Apigenin suppresses the growth of colorectal cancer xenografts via phosphorylation and up-regulated FADD expression. Oncology Letters 2, 43-47.
Wang, X., Song, Z.-J., He, X., Zhang, R.-Q., Zhang, C.-F., Li, F., Wang, C.-Z., Yuan, C.-S., 2015. Antitumor and immunomodulatory activity of genkwanin on colorectal cancer in the APCMin/+ mice. International immunopharmacology 29, 701-707.
Wang, Y., Huang, T., Li, H., Fu, J., Ao, H., Lu, L., Han, M., Guo, Y., Yue, F., Wang, X., 2020a. Hydrous icaritin nanorods with excellent stability improves the in vitro and in vivo activity against breast cancer. Drug Delivery 27, 228-237.
Wang, Y., Jin, H.-Y., Fang, M.-Z., Wang, X.-F., Chen, H., Huang, S.-L., Kong, D.-S., Li, M., Zhang, X., Sun, Y., 2020b. Epigallocatechin gallate inhibits dimethylhydrazine-induced colorectal cancer in rats. World journal of gastroenterology 26, 2064.
Wang, Z., Chen, T., Yang, C., Bao, T., Yang, X., He, F., Zhang, Y., Zhu, L., Chen, H., Rong, S., 2020c. Secoisolariciresinol diglucoside suppresses Dextran sulfate sodium salt-induced colitis through inhibiting NLRP1 inflammasome. International Immunopharmacology 78, 105931.
Wang, Z., Wang, N., Han, S., Wang, D., Mo, S., Yu, L., Huang, H., Tsui, K., Shen, J., Chen, J., 2013. Dietary compound isoliquiritigenin inhibits breast cancer neoangiogenesis via VEGF/VEGFR-2 signaling pathway. PloS one 8, e68566.
Warren, C.A., Paulhill, K.J., Davidson, L.A., Lupton, J.R., Taddeo, S.S., Hong, M.Y., Carroll, R.J., Chapkin, R.S., Turner, N.D., 2009. Quercetin may suppress rat aberrant crypt foci formation by suppressing inflammatory mediators that influence proliferation and apoptosis. The Journal of nutrition 139, 101-105.
Wei, L., Zhou, Y., Qiao, C., Ni, T., Li, Z., You, Q., Guo, Q., Lu, N., 2015. Oroxylin A inhibits glycolysis-dependent proliferation of human breast cancer via promoting SIRT3-mediated SOD2 transcription and HIF1α destabilization. Cell death & disease 6, e1714-e1714.

Wei, R., Mao, L., Xu, P., Zheng, X., Hackman, R.M., Mackenzie, G.G., Wang, Y., 2018. Suppressing glucose metabolism with epigallocatechin-3-gallate (EGCG) reduces breast cancer cell growth in preclinical models. Food & function 9, 5682-5696.
Weisenberger, D.J., Siegmund, K.D., Campan, M., Young, J., Long, T.I., Faasse, M.A., Kang, G.H., Widschwendter, M., Weener, D., Buchanan, D., 2006. CpG island methylator phenotype underlies sporadic microsatellite instability and is tightly associated with BRAF mutation in colorectal cancer. Nature genetics 38, 787-793.
Wells, K.O., Hawkins, A.T., Krishnamurthy, D.M., Dharmarajan, S., Glasgow, S.C., Hunt, S.R., Mutch, M.G., Wise, P., Silviera, M.L., 2017. Omission of adjuvant chemotherapy is associated with increased mortality in patients with T3N0 colon cancer with inadequate lymph node harvest. Diseases of the Colon & Rectum 60, 15-21.
Westerman, A.M., Entius, M.M., De Baar, E., Boor, P.P., Koole, R., Van Velthuysen, M.L.F., Offerhaus, G.J.A., Lindhout, D., De Rooij, F.W., Wilson, J.P., 1999. Peutz-Jeghers syndrome: 78-year follow-up of the original family. The Lancet 353, 1211-1215.
Won, S.J., Yen, C.H., Liu, H.S., Wu, S.Y., Lan, S.H., Jiang-Shieh, Y.F., Lin, C.N., Su, C.L., 2015. Justicidin A-induced autophagy flux enhances apoptosis of human colorectal cancer cells via class III PI3K and Atg5 pathway. Journal of Cellular Physiology 230, 930-946.
Wong, M.C., Ding, H., Wang, J., Chan, P.S., Huang, J., 2019. Prevalence and risk factors of colorectal cancer in Asia. Intestinal research 17, 317.
Wu, J., Omene, C., Karkoszka, J., Bosland, M., Eckard, J., Klein, C.B., Frenkel, K., 2011. Caffeic acid phenethyl ester (CAPE), derived from a honeybee product propolis, exhibits a diversity of anti-tumor effects in pre-clinical models of human breast cancer. Cancer letters 308, 43-53.
Wu, K.-H., Ho, C.-T., Chen, Z.-F., Chen, L.-C., Whang-Peng, J., Lin, T.-N., Ho, Y.-S., 2018. The apple polyphenol phloretin inhibits breast cancer cell migration and proliferation via inhibition of signals by type 2 glucose transporter. Journal of food and drug analysis 26, 221-231.
Wu, L., Lin, Y., Gao, S., Wang, Y., Pan, H., Wang, Z., Pozzolini, M., Yang, F., Zhang, H., Yang, Y., 2023a. Luteolin inhibits triple-negative breast cancer by inducing apoptosis and autophagy through SGK1-FOXO3a-BNIP3 signaling. Frontiers in Pharmacology 14, 1200843.
Wu, X.Y., Xu, H., Wu, Z.F., Chen, C., Liu, J.Y., Wu, G.N., Yao, X.Q., Liu, F.K., Li, G., Shen, L., 2015. Formononetin, a novel FGFR2 inhibitor, potently inhibits angiogenesis and tumor growth in preclinical models. Oncotarget 6, 44563.
Wu, Y., Li, Q., Lv, L.-l., Chen, J.-x., Ying, H.-f., Ruan, M., Zhu, W.-h., Xu, J.-y., Zhang, C.-y., Zhang, K.-y., 2023b. Nobiletin inhibits breast cancer cell migration and invasion by suppressing the IL-6-induced ERK-STAT and JNK-c-JUN pathways. Phytomedicine 110, 154610.
Xie, Y.-H., Chen, Y.-X., Fang, J.-Y., 2020. Comprehensive review of targeted therapy for colorectal cancer. Signal transduction and targeted therapy 5, 1-30.
Xiong, H., Chen, Z., Lin, B., Xie, B., Liu, X., Chen, C., Li, Z., Jia, Y., Wu, Z., Yang, M., 2022. Naringenin regulates FKBP4/NR3C1/NRF2 axis in autophagy and proliferation of breast cancer and differentiation and maturation of dendritic cell. Frontiers in immunology 12, 745111.
Xiong, L.-L., Du, R.-L., Xue, L.-L., Jiang, Y., Huang, J., Chen, L., Liu, J., Wang, T.-H., 2020. Anti-colorectal cancer effects of scutellarin revealed by genomic and proteomic analysis. Chinese medicine 15, 1-15.
Xu, P., Yin, Q., Shen, J., Chen, L., Yu, H., Zhang, Z., Li, Y., 2013. Synergistic inhibition of breast cancer metastasis by silibinin-loaded lipid nanoparticles containing TPGS. International journal of pharmaceutics 454, 21-30.
Xue, W., Hao, J., Zhang, Q., Jin, R., Luo, Z., Yang, X., Liu, Y., Lu, Q., Ouyang, Y., Guo, H., 2023. Chlorogenic acid inhibits epithelial-mesenchymal transition and invasion of breast cancer by down-regulating LRP6. Journal of Pharmacology and Experimental Therapeutics 384, 254-264.
Yan, W., Ma, X., Zhao, X., Zhang, S., 2018. Baicalein induces apoptosis and autophagy of breast cancer cells via inhibiting PI3K/AKT pathway in vivo and vitro. Drug design, development and therapy, 3961-3972.
Yang, D., Guo, Q., Liang, Y., Zhao, Y., Tian, X., Ye, Y., Tian, J., Wu, T., Lu, N., 2020. Wogonin induces cellular senescence in breast cancer via suppressing TXNRD2 expression. Archives of Toxicology 94, 3433-3447.

Yang, S.-H., Lin, J.-K., Huang, C.-J., Chen, W.-S., Li, S.-Y., Chiu, J.-H., 2005. Silibinin inhibits angiogenesis via Flt-1, but not KDR, receptor up-regulation1. Journal of Surgical Research 128, 140-146.
Yang, X., Luo, E., Liu, X., Han, B., Yu, X., Peng, X., 2016. Delphinidin-3-glucoside suppresses breast carcinogenesis by inactivating the Akt/HOTAIR signaling pathway. BMC cancer 16, 1-8.
Yang, X., Zhang, F., Wang, Y., Cai, M., Wang, Q., Guo, Q., Li, Z., Hu, R., 2013. Oroxylin A inhibits colitis-associated carcinogenesis through modulating the IL-6/STAT3 signaling pathway. Inflammatory bowel diseases 19, 1990-2000.
Yang, X.R., Sherman, M.E., Rimm, D.L., Lissowska, J., Brinton, L.A., Peplonska, B., Hewitt, S.M., Anderson, W.F., Szeszenia-Dabrowska, N., Bardin-Mikolajczak, A., 2007. Differences in risk factors for breast cancer molecular subtypes in a population-based study. Cancer Epidemiology Biomarkers & Prevention 16, 439-443.
Yao, J., Zhao, L., Zhao, Q., Zhao, Y., Sun, Y., Zhang, Y., Miao, H., You, Q., Hu, R., Guo, Q., 2014. NF-κB and Nrf2 signaling pathways contribute to wogonin-mediated inhibition of inflammation-associated colorectal carcinogenesis. Cell death & disease 5, e1283-e1283.
Ye, L., Chan, F.L., Chen, S., Leung, L.K., 2012. The citrus flavonone hesperetin inhibits growth of aromatase-expressing MCF-7 tumor in ovariectomized athymic mice. The Journal of nutritional biochemistry 23, 1230-1237.
Yin, H.-F., Yin, C.-M., Ouyang, T., Sun, S.-D., Chen, W.-G., Yang, X.-L., He, X., Zhang, C.-F., 2021. Self-Nanoemulsifying Drug Delivery System of Genkwanin: A Novel Approach for Anti-Colitis-Associated Colorectal Cancer. Drug Design, Development and Therapy 15, 557.
Yoshimaru, T., Komatsu, M., Tashiro, E., Imoto, M., Osada, H., Miyoshi, Y., Honda, J., Sasa, M., Katagiri, T., 2014. Xanthohumol suppresses oestrogen-signalling in breast cancer through the inhibition of BIG3-PHB2 interactions. Scientific reports 4, 7355.
You, W., Di, A., Zhang, L., Zhao, G., 2022. Effects of wogonin on the growth and metastasis of colon cancer through the Hippo signaling pathway. Bioengineered 13, 2586-2597.
You, Y.N., Xing, Y., Feig, B.W., Chang, G.J., Cormier, J.N., 2012. Young-onset colorectal cancer: is it time to pay attention? Archives of internal medicine 172, 287-289.
Yu, H., Yin, S., Zhou, S., Shao, Y., Sun, J., Pang, X., Han, L., Zhang, Y., Gao, X., Jin, C., 2018a. Magnolin promotes autophagy and cell cycle arrest via blocking LIF/Stat3/Mcl-1 axis in human colorectal cancers. Cell death & disease 9, 1-13.
Yu, X., Cao, Y., Tang, L., Yang, Y., Chen, F., Xia, J., 2018b. Baicalein inhibits breast cancer growth via activating a novel isoform of the long noncoding RNA PAX8-AS1-N. Journal of cellular biochemistry 119, 6842-6856.
Yue, G.G.-L., Gao, S., Lee, J.K.-M., Chan, Y.-Y., Wong, E.C.-W., Zheng, T., Li, X.-X., Shaw, P.-C., Simmonds, M.S., Lau, C.B.-S., 2020. A natural flavone tricin from grains can alleviate tumor growth and lung metastasis in colorectal tumor mice. Molecules 25, 3730.
Yusof, A.S., Isa, Z.M., Shah, S.A., 2012. Dietary patterns and risk of colorectal cancer: a systematic review of cohort studies (2000-2011). Asian Pacific Journal of Cancer Prevention 13, 4713-4717.
Zan, L., Chen, Q., Zhang, L., Li, X., 2019. Epigallocatechin gallate (EGCG) suppresses growth and tumorigenicity in breast cancer cells by downregulation of miR-25. Bioengineered 10, 374-382.
Zeng, Q., Che, Y., Zhang, Y., Chen, M., Guo, Q., Zhang, W., 2020. Thymol Isolated from Thymus vulgaris L. inhibits colorectal cancer cell growth and metastasis by suppressing the Wnt/β-catenin pathway. Drug Design, Development and Therapy 14, 2535.
Zeng, S., Chen, L., Sun, Q., Zhao, H., Yang, H., Ren, S., Liu, M., Meng, X., Xu, H., 2021. Scutellarin ameliorates colitis-associated colorectal cancer by suppressing Wnt/β-catenin signaling cascade. European Journal of Pharmacology 906, 174253.
Zeng, S., Tan, L., Sun, Q., Chen, L., Zhao, H., Liu, M., Yang, H., Ren, S., Ming, T., Tang, S., 2022. Suppression of colitis-associated colorectal cancer by scutellarin through inhibiting Hedgehog signaling pathway activity. Phytomedicine 98, 153972.
Zhang, M.-J., Su, H., Yan, J.-Y., Li, N., Song, Z.-Y., Wang, H.-J., Huo, L.-G., Wang, F., Ji, W.-S., Qu, X.-J., 2018a. Chemopreventive effect of Myricetin, a natural occurring compound, on colonic chronic inflammation and inflammation-driven tumorigenesis in mice. Biomedicine & Pharmacotherapy 97, 1131-1137.

Zhang, T., Zhu, X., Wu, H., Jiang, K., Zhao, G., Shaukat, A., Deng, G., Qiu, C., 2019. Targeting the ROS/PI3K/AKT/HIF-1α/HK2 axis of breast cancer cells: Combined administration of Polydatin and 2-Deoxy-d-glucose. Journal of Cellular and Molecular Medicine 23, 3711-3723.
Zhang, W., Liu, Q., Luo, L., Song, J., Han, K., Liu, R., Gong, Y., Guo, X., 2021a. Use Chou's 5-steps rule to study how Baicalin suppresses the malignant phenotypes and induces the apoptosis of colorectal cancer cells. Archives of Biochemistry and Biophysics 705, 108919.
Zhang, X., Lin, D., Jiang, R., Li, H., Wan, J., Li, H., 2016. Ferulic acid exerts antitumor activity and inhibits metastasis in breast cancer cells by regulating epithelial to mesenchymal transition. Oncology reports 36, 271-278.
Zhang, Y.-S., Wang, F., Cui, S.-X., Qu, X.-J., 2018b. Natural dietary compound naringin prevents azoxymethane/dextran sodium sulfate-induced chronic colorectal inflammation and carcinogenesis in mice. Cancer Biology & Therapy 19, 735-744.
Zhang, Y., Li, Q., Zhou, D., Chen, H., 2013. Genistein, a soya isoflavone, prevents azoxymethane-induced up-regulation of WNT/β-catenin signalling and reduces colon pre-neoplasia in rats. British journal of nutrition 109, 33-42.
Zhang, Y., Li, Y., Sun, C., Chen, X., Han, L., Wang, T., Liu, J., Chen, X., Zhao, D., 2021b. Effect of pterostilbene, a natural derivative of resveratrol, in the treatment of colorectal cancer through Top1/Tdp1-mediated DNA repair pathway. Cancers 13, 4002.
Zhao, H., Zhang, X., Chen, X., Li, Y., Ke, Z., Tang, T., Chai, H., Guo, A.M., Chen, H., Yang, J., 2014. Isoliquiritigenin, a flavonoid from licorice, blocks M2 macrophage polarization in colitis-associated tumorigenesis through downregulating PGE2 and IL-6. Toxicology and applied pharmacology 279, 311-321.
Zhao, Y., Hu, X., Zuo, X., Wang, M., 2018. Chemopreventive effects of some popular phytochemicals on human colon cancer: A review. Food & function 9, 4548-4568.
Zhong, Y., Krisanapun, C., Lee, S.-H., Nualsanit, T., Sams, C., Peungvicha, P., Baek, S.J., 2010. Molecular targets of apigenin in colorectal cancer cells: involvement of p21, NAG-1 and p53. European Journal of Cancer 46, 3365-3374.
Zhong, Y., Zhu, Y., Li, Q., Wang, F., Ge, X., Zhou, G., Miao, L., 2020. Association between Mediterranean diet adherence and colorectal cancer: a dose-response meta-analysis. The American Journal of Clinical Nutrition 111, 1214-1225.
Zhou, C., Gu, J., Zhang, G., Dong, D., Yang, Q., Chen, M.-B., Xu, D., 2017. AMPK-autophagy inhibition sensitizes icaritin-induced anti-colorectal cancer cell activity. Oncotarget 8, 14736.
Zhou, C., Ou, W., Xu, Q., Lin, L., Xu, F., Chen, R., Miao, H., 2022. Chemoprotective effect of boeravinone B against 1, 2-dimethyl hydrazine induced colorectal cancer in rats via suppression of oxidative stress and inflammatory reaction. Journal of gastrointestinal oncology 13, 1832-1841.
Zhou, J., Zhao, R., Ye, T., Yang, S., Li, Y., Yang, F., Wang, G., Xie, Y., Li, Q., 2019. Antitumor activity in colorectal cancer induced by hinokiflavone. Journal of Gastroenterology and Hepatology 34, 1571-1580.
Zhou, L.-a., Liu, T.-b., Lü, H.-n., 2020. Geraniin inhibits proliferation and induces apoptosis through inhibition of phosphatidylinositol 3-kinase/Akt pathway in human colorectal cancer in vitro and in vivo. Anti-Cancer Drugs 31, 575-582.
Zhou, L., Feng, Y., Jin, Y., Liu, X., Sui, H., Chai, N., Chen, X., Liu, N., Ji, Q., Wang, Y., 2014a. Verbascoside promotes apoptosis by regulating HIPK2–p53 signaling in human colorectal cancer. BMC cancer 14, 1-11.
Zhou, R., Xu, L., Ye, M., Liao, M., Du, H., Chen, H., 2014b. Formononetin inhibits migration and invasion of MDA-MB-231 and 4T1 breast cancer cells by suppressing MMP-2 and MMP-9 through PI3K/AKT signaling pathways. Hormone and metabolic research 46, 753-760.
Zhu, X., Tian, X., Yang, M., Yu, Y., Zhou, Y., Gao, Y., Zhang, L., Li, Z., Xiao, Y., Moses, R.E., 2021. Procyanidin B2 promotes intestinal injury repair and attenuates colitis-associated tumorigenesis via suppression of oxidative stress in mice. Antioxidants & Redox Signaling 35, 75-92.

CHAPTER 2

MATERIALS AND METHODS

2. Materials and methods:

2.1. Cell culture:

Human cell lines [colon cancer cells (HCT116 and HT29), breast cancer cells (MCF7 and MDAMB231), normal/immortalized cells (NKE)] and mouse cells [(colon cancer cells (CT26), breast cancer cells (4T1)] and were purchased from ATCC. The cells were cultured in Gibco DMEM (#12800-058), or RPMI (#31800-014) media supplemented with 10% FBS (Gibco, heat-inactivated, US origin), sodium bicarbonate (3.7 g/l), Penicillin streptomycin (Pen-Strep) (50 µg/ml), Amphotericin B (2.5 µg/ml), Gentamycin (50 µg/ml) and non-essential amino acids within a humified incubator at 37°C supplemented with 5% CO_2 (Ghosh et al., 2021).

2.2. Antibodies:

The antibodies were purchased either from CST (cell signaling technology), ABclonal, Abcam, or Santa Cruz biotech. Antibodies against PARP1 (#9532), Bax (#5023), Bid (#2002), pS6K (#9205), S6K (#9202), pAKT (#9271), AKT (#9272), p4EBP1 (#2855), 4EBP1 (#9644), E-cadherin (#3195), N-cadherin (#13116), Twist-1 (#46702), Vimentin (#5741), Caspase 3 (#9662), Ki-67 (#12202), Caspase 3 (#622704), Bcl2 (#658701), Survivin (#614701), VEGF (A12303), PARP1 (#46D11), LC3II (#2775), P-AMPK (#2535), AMPK (#2532), Cyclin D1 (#sc450), CDK4 (#sc260), β-actin (#ab49900), anti-rabbit secondary antibody (#sc2004), and anti-mouse secondary antibody (#sc2005) were used.

2.3. Preparation of Polyphenol-rich fraction of *Bergenia ligulata* (PFBL):

Dried rhizomes of *Bergenia ligulata* were powdered and soaked in methanol for two weeks. The methanolic extract was then vacuum dried and subjected to column chromatographic separation/elution using silica gel column by using hexane, dichloromethane, ethyl acetate, and methanol, respectively. Based on preliminary data ethyl acetated fraction was found to be most cytotoxic, and thus this fraction was subjected to further separation. Reverse phase preparative HPLC with C18 column (column bed volume= 50 ml; flow rate at 10 ml/min) using a gradient of water and methanol (Ghosh et al., 2021) was used to purify the ethyl acetate fraction. All the peaks were collected (as fraction 10 ml) using mobile phase applied 100% water to 100% methanol among which the peak 4 was most was found to be most toxic against breast- and colon cancer cells. The peak 4 was further purified in analytical HPLC using a gradient of 100% water to 100% methanol as mobile phase with a flow rate of 1 ml/min to

collect 1ml fractions. Here peak 7 was found to be most toxic against breast and colon cancer cells and this peak 7 was named polyphenol-rich fraction of *Bergenia ligulate* (PFBL).

2.4. Phytochemical analysis:

The abundance of different types of phytochemicals, mainly phenolics, flavonoids, alkaloids, and tannins in PFBL, were determined qualitatively and quantitatively using standard protocols (Mukemre et al., 2020; Silva et al., 2020; Sina et al., 2021).

2.5. HPLC and LCMS:

Crude ethyl acetate fraction was purified using Waters 2998 HPLC using reverse phase C18 column (bed volume, 50 ml; sample loading volume, 500 µl (10 mg/ml); mobile phage, a gradient of 100% water to 100% methanol in 60 minutes at a flow rate of 10 ml/min at 25°C) at described earlier (Ghosh et al 2021).

The most active fraction was characterised using Waters Xevo G2-XS QTof LCMS instrument in Bose Institute central facility applying a gradient of 95% water to 95% acetonitrile with a flow rate of 40µl/min using Waters 1.7 µm ACQUITY UPLC® BEH C18 column (1.0 x100mm column).

2.6. Cell viability (MTT) assay:

Cells grown in complete DMEM media in a 48-well plate to ~70% confluency were treated with different concentrations of fractions/drugs for 24 h. The growth media was removed and the cells were incubated with MTT [3-(4, 5-dimethylthiazol-2, 5-diphenyl tetrazolium bromide)] 0.5 mg/ml solution for 4 h at 37°C in the dark. Next, after the complete removal of MTT solution, 200µl of DMSO was added to each well to suspend the crystals. The dissolved solutions were transferred to a 96-well plate to measure the OD 570 nm in a spectrophotometer (Ghosh et al., 2021).

2.7. Clonogenic assay:

Cells seeded in a 6-well plate (500 cells/well) for overnight were treated with DMSO (vehicle) or different concentrations of PFBL. After 24 h, cells in the wells incubated in complete fresh media devoid of PFBL for 10 days. The cells were fixed with 3.7% formaldehyde solution, followed by staining with crystal violet (0.05% crystal violet in 20% methanol) solution for 2 h. Wells were then washed with PBS several times until the background was clear, dried, and imaged with Bio-Rad Gel-Doc (Ghosh et al., 2021).

2.8. Scratch assay:

Cells grown to ~70% confluency in a plate/well were treated with vehicle/DMSO or different concentrations of PFBL as desired. Next, a scratch was made vertically through the cells with a 200 µl pipette tip, washed with PBS, followed by incubation in the growth media to image the plates at 0 h, 24 h, 48 h and 72 h, respectively using a phase-contrast microscope. The scratch areas were measured and plotted (Ghosh et al., 2021).

2.9. Cell cycle analysis:

Cell cycle analysis was performed using a protocol supplied by BD Sciences. After desired treatment, the cells were trypsinised, harvested and washed twice with ice-cold PBS. The cells collected after centrifugation at 1000xg for 3 min at 4°C were suspended in 80% ethanol, and stored at −20°C overnight. Next day, cells were pelleted down for rehydration in 500 µl ice-cold PBS for 2 h at 4°C followed by the RNase A treatment for 2 h at 37°C. Next, cells were incubated with PI for 30 min at room temperature in the dark, and cell cycle distribution was measured using BD FACS Verse (Ghosh et al., 2021).

2.10. Apoptosis assay:

Apoptosis was analysed using a protocol provided by BD sciences. After treatment, the cells were trypsinised, collected, and washed twice with PBS. The cells were then processed using manufacturer protocol by suspending the cells in 100 µl 1X binding buffer and staining the cells with FITC-ANNEXIN-V solution for 45 min followed by PI staining for 25 min. All the incubations are performed at room temperature in the dark. Then the samples were analysed using BD FACS Verse for apoptosis (Ghosh et al., 2021).

2.11. JC-1 staining:

After treatment, the cells were washed twice with PBS and incubated with JC1 (final concentration of 3 µM) containing serum-free media for 30 min at 37°C in a CO_2 incubator in the dark. After incubation, cells were washed twice with PBS, and images were captured (Ghosh et al., 2021).

2.12. Measurement of cellular ROS using the DCFDA method:

DCFDA (2',7'-dichlorodihydrofluorescein diacetate also called H2DCFDA) staining is a process for measuring intercellular ROS production. After treating the cells with DMSO or different concentrations of PFBL, the cells were washed twice with PBS and incubated in the

dark at 37°C in serum-free media containing DCFDA (final concentration 10 µM) for 60 min. Then, after discarding the media the cells were washed twice with PBS and imaged using a fluorescent microscope (Ghosh et al., 2021).

2.13. DAPI staining:

After treatment, the media was removed, and the cells were washed twice with PBS followed by fixing the cells using 3.7% formaldehyde for 15 min. Then, the cells were incubated with DAPI in the dark for 30 followed by washing with PBS twice. The images were captured using a fluorescent microscope (Ghosh et al., 2021).

2.14. Transfection:

The cells were grown in a 35 mm dish to a confluency of 70%. DNA and Lipofectamine were used in a ratio of 2:1. The required amounts of plasmid DNA and Lipofectamine (of twice the volume of plasmid DNA) were incubated in 500 µl of Opti-MEM aliquoted in two microcentrifuge tubes for 5 min. Next, the Lipofectamine mixture was added to the DNA mixture dropwise, mixed well, and incubated for 15 min. After that, the DNA-lipofectamine mixture was dropwise added to the plate/cells depleted of growth media. Next, 1 ml complete growth media was added to the plate to cover the cells well before incubation, the growth media was replaced with fresh media after 8 h. Following 48 h, cells were harvested and processed for expression levels of desired genes per objective (Dalby et al., 2004).

2.15. Whole cell lysate preparation and Western blotting:

After harvesting and washing with ice-cold PBS, the cells were resuspended in lysis buffer (40 mM HEPES buffer pH 7.4 with 1% Triton X-100, 300 mM NaCl, 1 mM PMSF, 15 µg/ml leupeptin, 15 µg/ml aprotinin 25 mM NaF and 25 mM Na_3V0_4) and incubated in ice for 30 min. The lysate was then sonicated to collect the clear supernatant by centrifugation at 13000 rpm for 15 min at 4°C (whole cell extract). Using Bio-Rad Bradford reagent, the protein concentration was measured. The required amount of protein (20 to 100 µg of lysate, depending upon the abundance of the desired protein in a lysate) was separated by denaturing SDS PAGE. After separation, the proteins in the gel were transferred to the PVDF membrane by wet transfer method to incubate the membrane for 60 min at room temperature in blocking buffer supplemented with 5% BSA. The membrane was then incubated with blocking buffer (30 min) followed primary antibody (1:1000 dilution in 5% BSA) overnight at 4°C. Next, the membrane was washed three times with PBST (PBS supplemented with 0.25% tween 20) and incubated

with HRP-tagged secondary antibody for 1 h. The membrane was washed three times with PBST and developed using Bio-Rad detection reagent before imaged with Bio-Rad Chemi-Doc (Ghosh et al., 2021).

2.16. shRNA transduction:

The second generation of lentiviral transfection system has been used to generate viral particles containing shRNA constructs. The plasmid constructs-(i) the scrambled shRNA in pLKO.1 vector was as a gift from David Sabatini (addgene # 1864) (Jacinto et al., 2004), (ii) the transfer plasmid (pLKO.1-TRC) cloning vector, as a gift from David Root (Addgene # 10878) (Moffat et al., 2006), (ii) the packaging plasmid (psPAX2), was a gift from Didier Trono, Addgene # 12260) and (iv) the envelope plasmid (pMD2.G), was a gift from Didier Trono, Addgene # 12259). To knockdown a gene, the gene-specific shRNA was designed and cloned into pLKO.1-TRC cloning vector as per Addgene protocol (https://www.addgene.org/protocols/plko). Lentivirus was produced in HEK293T cells by seeding 0.4×10^6 cells in a 25 cm flask. After 24 h, cells were transfected with 4 µg of pLKO.1 construct (containing scrambled shRNA or target shRNA), 3 µg of psPAX2, and 1 µg of pMD2.G using 32 µg of PEI (Polysciences). Supernatant/media containing lentiviral particles were harvested 48 h and 72 h post-transfection. The dead cells and cell debris were removed by centrifugation at 3000 rpm for 5 minutes. Followed by passage through a syringe filter (0.45 µm pore size). Cells (0.2×10^6 cells in a 35 mm dish) were transduced by adding 2 ml of the lentiviral particle-containing medium. After two days of post-infection, cells were harvested and checked for the expression of target protein using western blotting (Moffat et al., 2006; Sarbassov et al., 2005).

2.17. Tumor xenograft model:

BALB/c male or female mice (from 4 to 6 weeks old, with body weight in the range from 20 to 25 grams) were procured acclimatized before injected with CT26 or 4T1 cells ($2x10^6$ cells/mice) by subcutaneous injection. For solid breast tumor, the 4T1 cells were injected subcutaneously in the mammary gland. After observation of palpable tumors (around two weeks), the animals were randomly segregated into four groups and treated with the vehicle or PFBL or 5FU or combination of 5FU and PFBL for CT26 tumors. The 4T1 tumor groups were treated with the vehicle, or PFBL, or DOX, or DOX and PFBL combined (N=5). The animals were treated every alternate day for two weeks and the tumor volume was measured on every treatment day before treatment. After completion of the experiment, the animals were sacrificed, and tumors were excised. The weight of each tumor was recorded (Ghosh et al.,

2021). The animal studies were performed following the institutional ethical committee guidelines and prior permission with Ref. no. IAEC/BI/002/2021.

2.18. In vivo metastasis model:

For each type of lung metastasis model, BALB/c male or female mice (from 4 to 6 weeks old, with body weight in the range from 20 to 25 grams, 30 in number) were procured, acclimatize. The CT26 or 4T1 cells ($2x10^5$ cells/mice) were injected by tail vein injection and randomly segregated into three groups (N=10) one without treatment and the other two groups treated with different concentrations of PFBL for 4 weeks. After completion of the experiment, the mice were euthanized, and the lungs were collected (O'Reilly et al., 1994; Walser et al., 2006). The animal studies were performed following the institutional ethical committee guidelines and prior permission with Ref. no. IAEC/BI/002/2021.

2.19. Hematoxylin and Eosin (H&E) staining and immunohistochemistry (IHC):

Following standard protocol, the collected tumors, lungs, spleens, and other organs of mice were harvested and stored in 4% formalin to embed in paraffin blocks. Paraffin-embedded tissues were sectioned and fixed on lysine-coated slides. The tissue sections were deparaffinized, processed, and stained with hematoxylin and eosin (H and E). The slides with the tissue sections were stained with the desired primary antibodies, followed by incubation with biotinylated secondary antibodies and developed using DAB substrate (VECTOR labs) (Ghosh et al., 2021).

2.20. In vivo toxicity study:

The toxicity assessment involved measuring various parameters, including body weight, spleen size, blood cell counts (RBC and WBC), and serum levels of different enzymes like alkaline phosphatase (ALP), glutamic-oxaloacetic transaminase (SGOT), and glutamic pyruvic transaminase (SGPT) (El Chediak et al. 2018; Gitnick 1981; and Jasper et al. 2012). 30 healthy BALB/c male or female mice (from 4 to 6 weeks old, with body weight in the range from 20 to 25 grams) were procured and randomly divided into six experimental groups (N=5). The groups were treated every alternate day for 30 days either with the vehicle or PFBL, 5FU, 5FU+PFBL, DOX, or a combination of DOX and PFBL by IP injection. Starting from day zero to day 30, the body weight of each mouse was taken on every other day. After the completion of the treatment (30th days), the mice were sacrificed. The blood samples from each mice/group were collected and used for RBC and WBC count by using RBC and WBC diluting fluid (Stanbo Reagents, Kolkata) following manufacturer protocols. The blood serum level of SGOT, SGPT and ALP was measured using SGOT (AST) test kit, MBK SGPT assay kit, and

MBK alkaline phosphatase assay kit obtained from ARKRAY Health care. The spleens were also isolated from mice and measured. The animal studies were performed following the institutional ethical committee guidelines and prior permission with Ref. no. IAEC/BI/002/2021.

2.21. Statistical analysis:

All the graphs included were plotted using GraphPad prism 5.03 software. The statistical analysis was done using IBM SPSS 25.0, and based on the type of requirements, one-way ANOVA or two-way ANOVA was performed to determine the statistical significance.

References:

Dalby, B., Cates, S., Harris, A., Ohki, E.C., Tilkins, M.L., Price, P.J., Ciccarone, V.C., 2004. Advanced transfection with Lipofectamine 2000 reagent: primary neurons, siRNA, and high-throughput applications. Methods 33, 95-103.

El Chediak, A., Haydar, A.A., Hakim, A., Massih, S.A., Hilal, L., Mukherji, D., Temraz, S., Shamseddine, A., 2018. Increase in spleen volume as a predictor of oxaliplatin toxicity. Therapeutics and clinical risk management, 653-657.

Ghosh, S., Dutta, N., Banerjee, P., Gajbhiye, R.L., Sareng, H.R., Kapse, P., Pal, S., Burdelya, L., Mandal, N.C., Ravichandiran, V., 2021. Induction of monoamine oxidase A-mediated oxidative stress and impairment of NRF2-antioxidant defence response by polyphenol-rich fraction of Bergenia ligulata sensitizes prostate cancer cells in vitro and in vivo. Free Radical Biology and Medicine 172, 136-151.

Gitnick, G., 1981. Assessment of liver function. Surgical Clinics of North America 61, 197-207.

Jacinto, E., Loewith, R., Schmidt, A., Lin, S., Rüegg, M.A., Hall, A., Hall, M.N., 2004. Mammalian TOR complex 2 controls the actin cytoskeleton and is rapamycin insensitive. Nature cell biology 6, 1122-1128.

Jasper, R., Locatelli, G.O., Pilati, C., Locatelli, C., 2012. Evaluation of biochemical, hematological and oxidative parameters in mice exposed to the herbicide glyphosate-Roundup®. Interdisciplinary toxicology 5, 133.

Moffat, J., Grueneberg, D.A., Yang, X., Kim, S.Y., Kloepfer, A.M., Hinkle, G., Piqani, B., Eisenhaure, T.M., Luo, B., Grenier, J.K., 2006. A lentiviral RNAi library for human and mouse genes applied to an arrayed viral high-content screen. Cell 124, 1283-1298.

Mukemre, M., Konczak, I., Uzun, Y., Dalar, A., 2020. Phytochemical profile and biological activities of Anatolian Plantain (Plantago anatolica). Food Bioscience 36, 100658.

O'Reilly, M.S., Holmgren, L., Shing, Y., Chen, C., Rosenthal, R.A., Moses, M., Lane, W.S., Cao, Y., Sage, E.H., Folkman, J., 1994. Angiostatin: a novel angiogenesis inhibitor that mediates the suppression of metastases by a Lewis lung carcinoma. cell 79, 315-328.

Sarbassov, D.D., Guertin, D.A., Ali, S.M., Sabatini, D.M., 2005. Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex. Science 307, 1098-1101.

Silva, L.S.B., Perasoli, F.B., Carvalho, K.V., Vieira, K.M., Lopes, M.T.P., de Souza, G.H.B., Dos Santos, O.D.H., Freitas, K.M., 2020. Melaleuca leucadendron (L.) L. flower extract exhibits antioxidant and photoprotective activities in human keratinocytes exposed to ultraviolet B radiation. Free Radical Biology and Medicine 159, 54-65.

Sina, H., Dramane, G., Tchekounou, P., Assogba, M.F., Chabi-Sika, K., Boya, B., Socohou, A., Adjanohoun, A., Baba-Moussa, L., 2021. Phytochemical composition and in vitro biological activities of Morinda citrifolia fruit juice. Saudi journal of biological sciences 28, 1331-1335.

Walser, T.C., Rifat, S., Ma, X., Kundu, N., Ward, C., Goloubeva, O., Johnson, M.G., Medina, J.C., Collins, T.L., Fulton, A.M., 2006. Antagonism of CXCR3 inhibits lung metastasis in a murine model of metastatic breast cancer. Cancer research 66, 7701-7707.

CHAPTER 3

PURIFICATION OF ANTI-COLON AND ANTI-BREAST CANCER ACTIVITIES FROM BERGENIA LIGULATA AND ITS TOXICITY STUDY IN HEALTHY MICE

3.1 INTRODUCTION:

Dietary supplements with fruits and vegetables have been known for their medicinal properties since ancient times (Greenwald et al., 2001). Eighty percent of world's population more or less rely on herbal medicines for their health benefits (Ekor, 2014). In fact, close to 50% of current anticancer medications are of natural origin. Many plants in India are reported to carry unique medicinal properties with poorly understood molecular bases of their actions (Dhar et al., 1968). *Bergenia ligulata* was selected for this study based on its known anti-inflammatory, antimicrobial, antioxidant, and anticancer properties, with the molecular basis of actions yet to be understood (Agnihotri et al., 2015; Nazir et al., 2011; Rajkumar et al., 2011; Sajad et al., 2010). Many compounds identified in the extracts, such as bergenin, afzelechin, gallic acid, tannic acid, spirolactone, and β-sitosterol, were reported to carry potential health benefits based on their anti-oxidant and anti-inflammatory properties (Sadat et al., 2015; Sajad et al., 2010).This herb predominantly occurs in the temperate region of the Himalayas (Ruby et al., 2012).

3.2 RESULTS:

3.2.1 Purification of active fraction from *Bergenia ligulata* that is most toxic to colon and breast cancer cells:

Bergenia ligulata rhizomes, obtained from Yucca Enterprise, Mumbai, were shade-dried, powdered and soaked in methanol followed by step wise extraction with n-hexane, dichloromethane, ethyl acetate, and methanol as shown in [Fig. 3.1]. After extraction, each fraction was filtered through 0.22 μm syringe filters (Merck-Millipore) and vacuum dried. These extracts were stored at 4°C in a light-protected environment until tested for their toxicity studies against the human colorectal cancer (HCT116) and breast cancer (MCF7) cells [Fig. 3.2]. The ethyl acetate fraction (EtOAc) showed highest toxicity among all the collected solvent fractions (Fig 3.2) against HCT116 and MCF7 cells but no toxicity was observed against normal NKE cells. The ethyl acetate fraction was further resolved in reverse phase preparative HPLC using C-18 column (50 ml column bed volume) where a gradient of 100% water to 100% methanol was used as mobile phase represented by the elution profile (chromatogram) [Fig. 3.3].

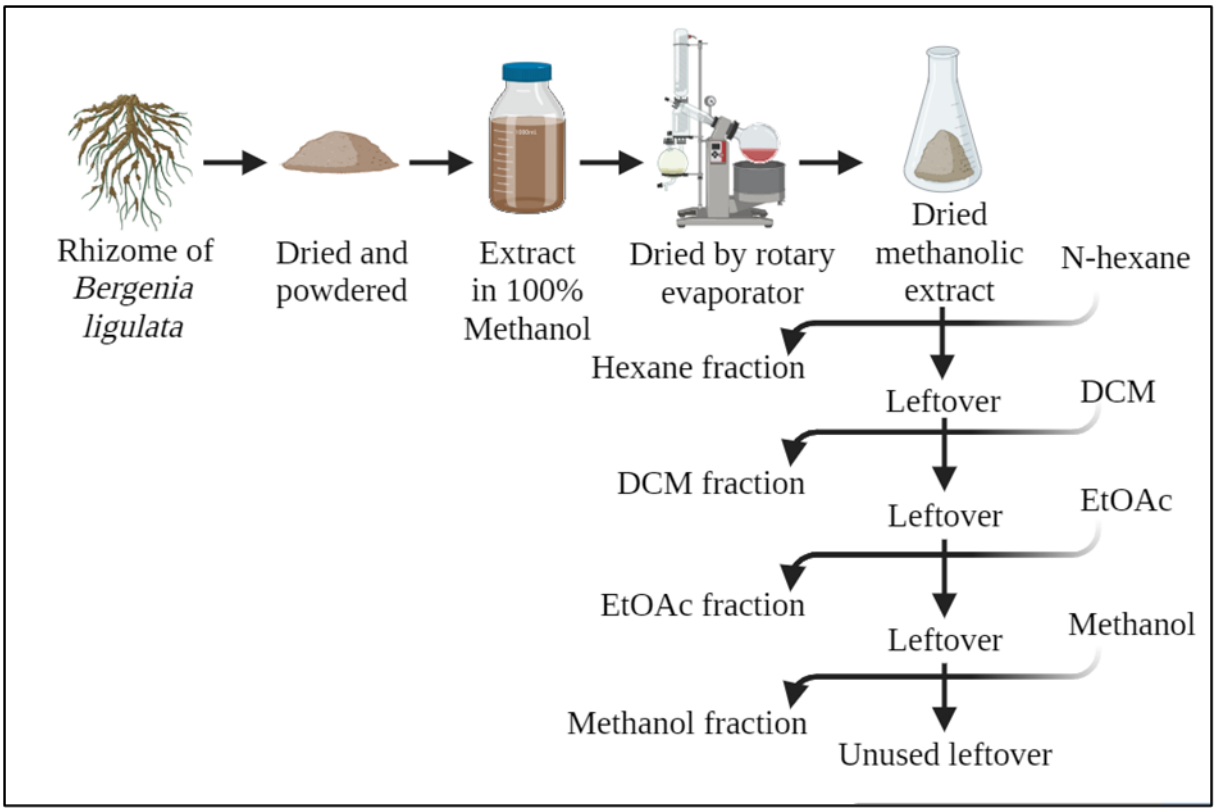

Figure 3.1: Schematic workflow of activity-guided fractionation of anti-cancer activity from *Bergenia ligulata* rhizome extract.

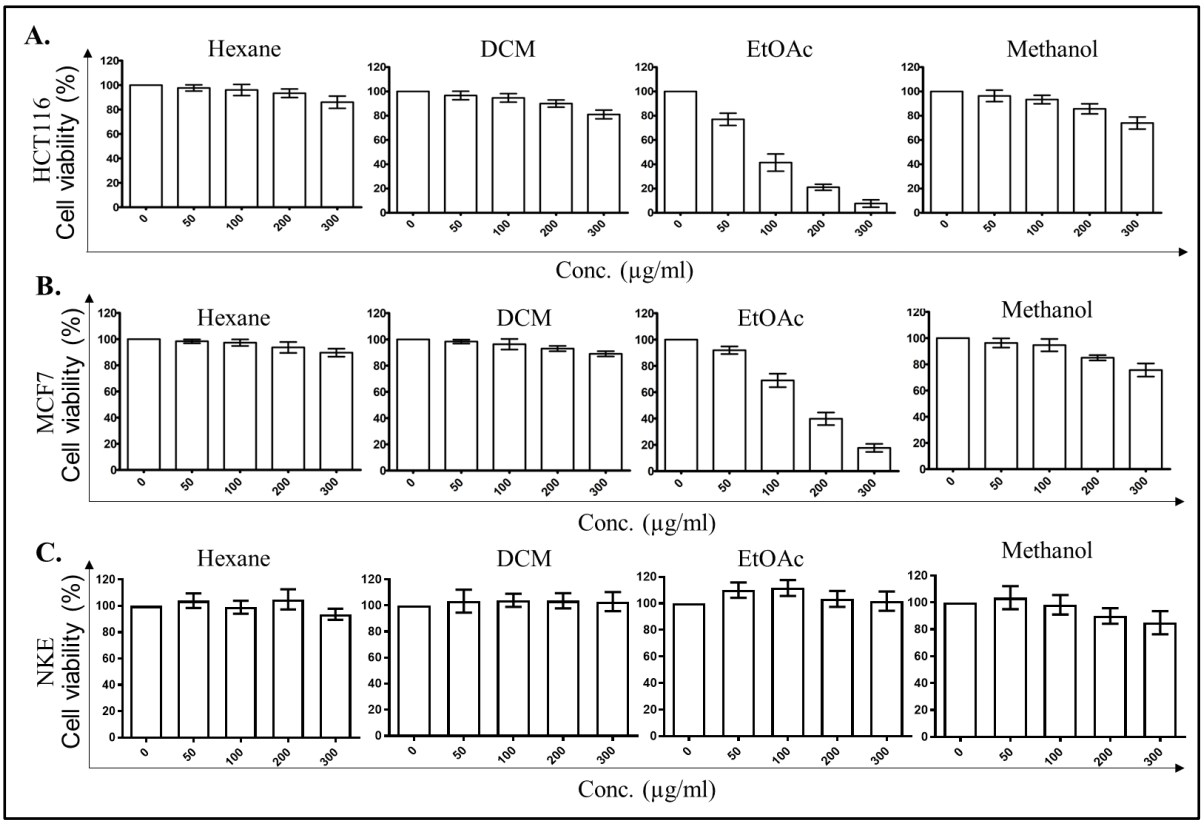

Figure 3.2. Toxicity analysis of different solvent fractions of *Bergenia ligulata* rhizome against HCT116 and MCF7 cells. Increasing concentrations (from vehicle 0 to 300 µg/ml) of

n-Hexane, dichloromethane (DCM), ethylacetate (EtOAc) and methanolic fractions were dissolved in DMSO and tested by MTT assay for their cytotoxicity against the indicated cells. The duration of treatments was 24 h, as described in Materials and Methods (Chapter 2).

Among all the collected peaks, as shown in [Fig 3.3], peak 4 was further separated using a smaller reverse phase analytical C18 column [Fig. 3.5] using the gradient of 100% water to 100% methanol. Here, peak 7 [Fig 3.5] showed the most cytotoxic effects against colon and breast cancer cells [Fig. 3.7]. Notably, the Peak 7 showed little toxicity towards the NKE cells [Fig 3.7]. Peak 7 was then collected in sufficient amount to perform in vitro and in vivo experiments. This peak 7 collected was marked as PFBL (polyphenol-rich fraction of *Bergenia ligulata*). The analytical HPLC profile for ethyl acetate fraction of Bergenia ligulata, peak 4 and peak 7 (as discussed earlier) in Fig. 3.4, Fig. 3.5 and Fig. 3.6 respectively.

Ethyl acetate fraction preparative HPLC profile

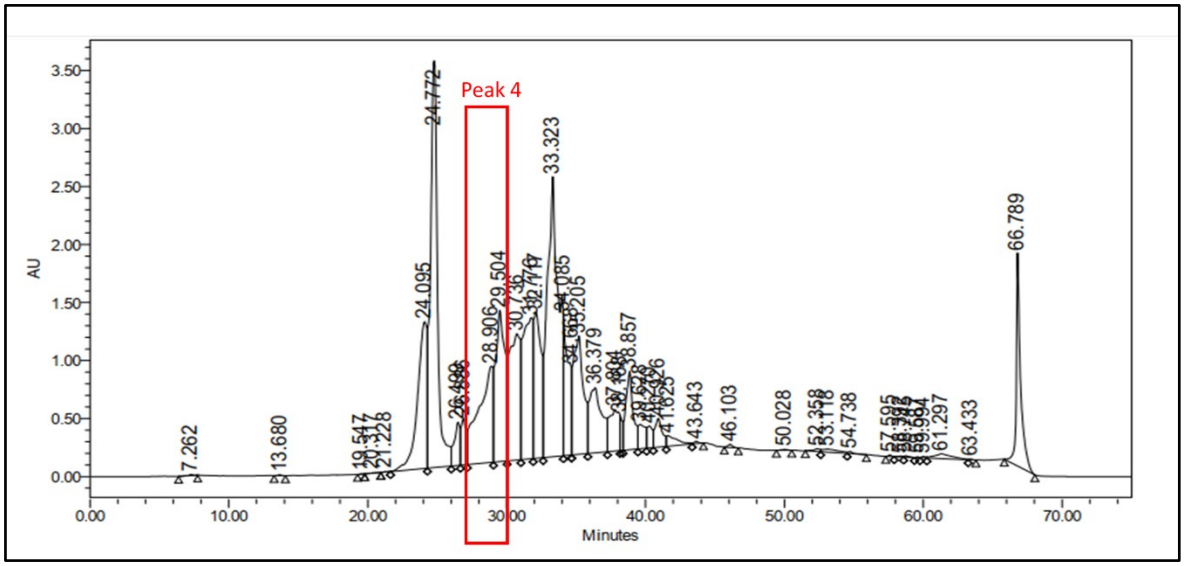

Figure 3.3: Preparative HPLC elution profile of ethyl acetate fraction of *Bergenia ligulate*. The mobile phase applied was from 100% water to 100% methanol with a flow rate of 10 ml/min. Each fraction volume collected 10 ml. Peak 4 indicated by red rectangle.

Ethyl acetate fraction analytical HPLC profile

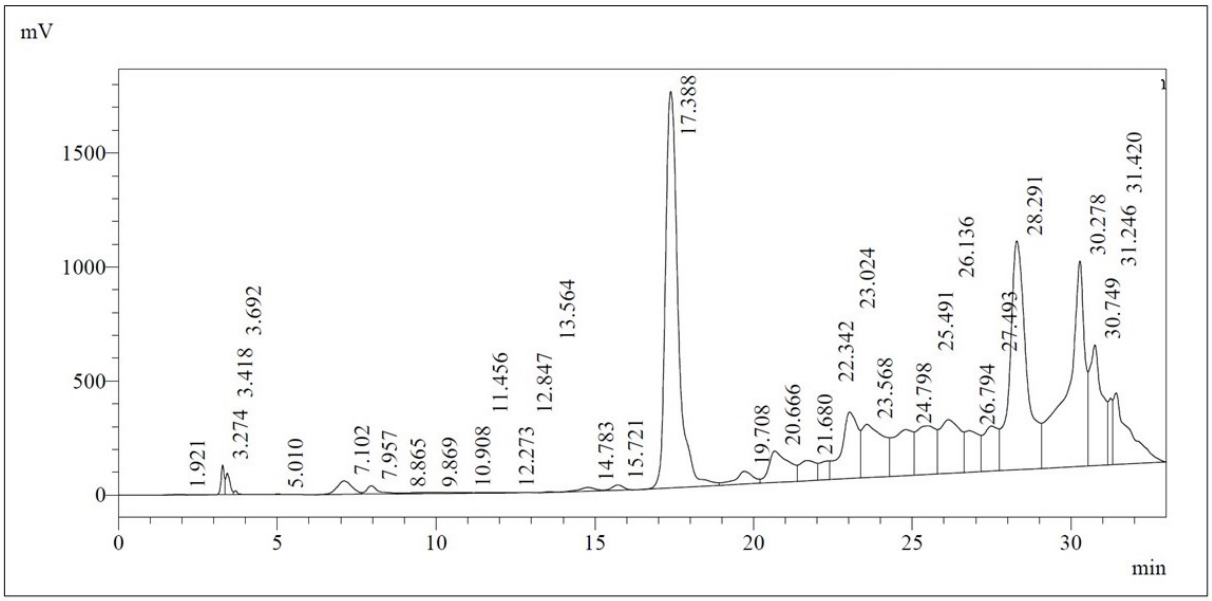

Figure 3.4: Analytical HPLC elution profile of ethyl acetate fraction of *Bergenia ligulata*. The mobile phase applied was from 100% water to 100% methanol with a flow rate of 1 ml/min.

Peak 4 analytical HPLC profile

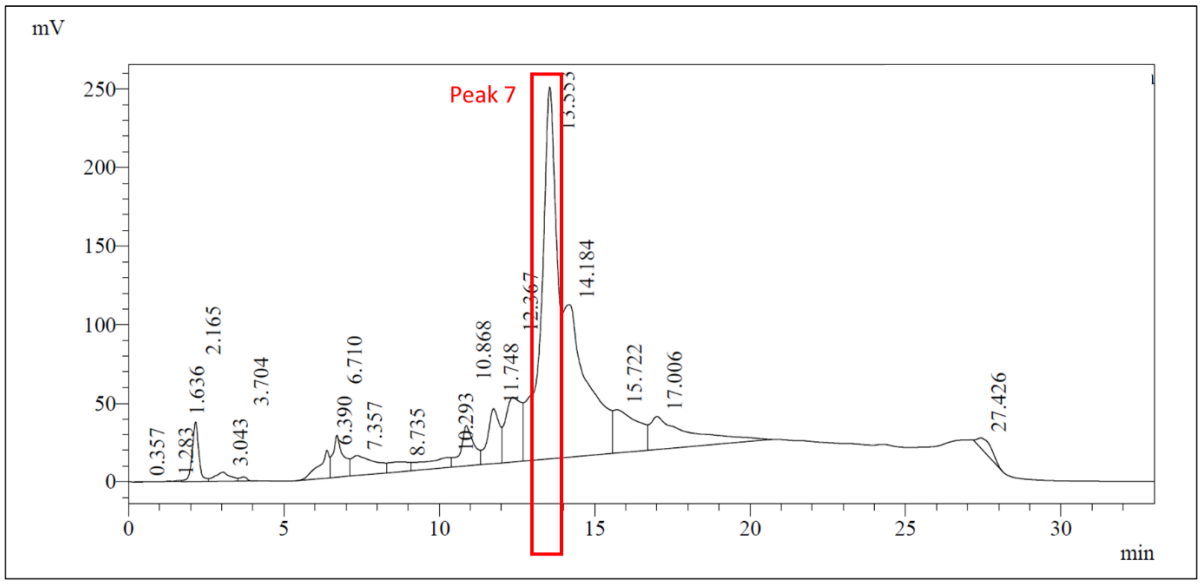

Figure 3.5: Analytical HPLC profile of peak 4 of ethyl acetate fraction of *Bergenia ligulata*. The mobile phase applied was from 100% water to 100% methanol with a flow rate of 1 ml/min. Each fraction volume collected 1 ml. Peak 7 indicated by red rectangle.

Peak 7 analytical HPLC profile

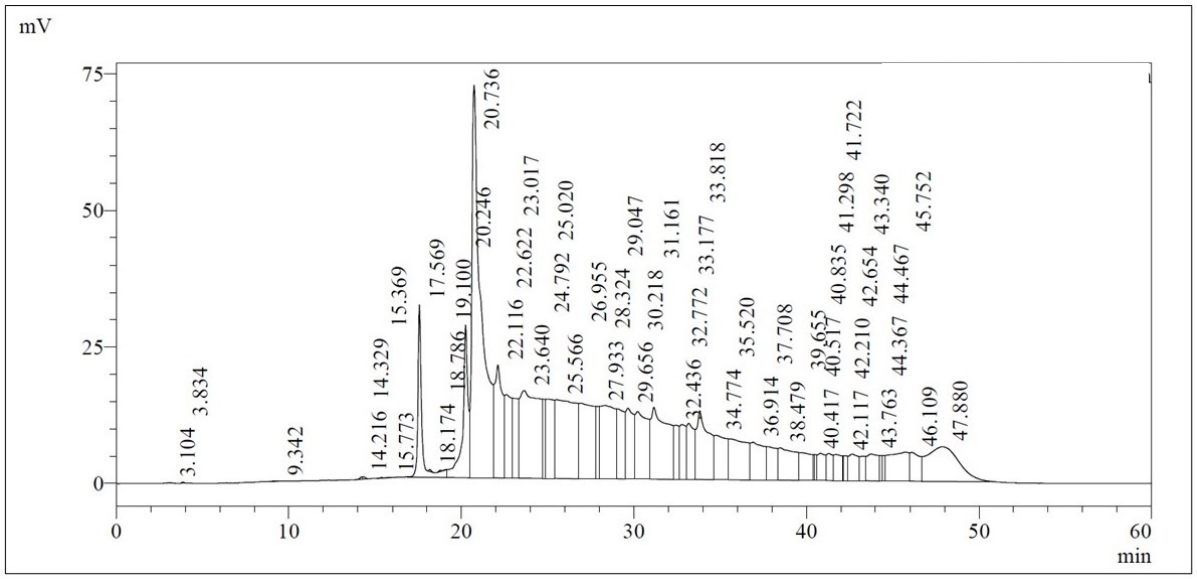

Figure 3.6: Analytical HPLC profile of peak 7 (PFBL) collected from HPLC of peak 4 of ethyl acetate fraction of Bergenia ligulata. The mobile phase applied was from 100% water to 100% methanol with a flow rate of 1 ml/min.

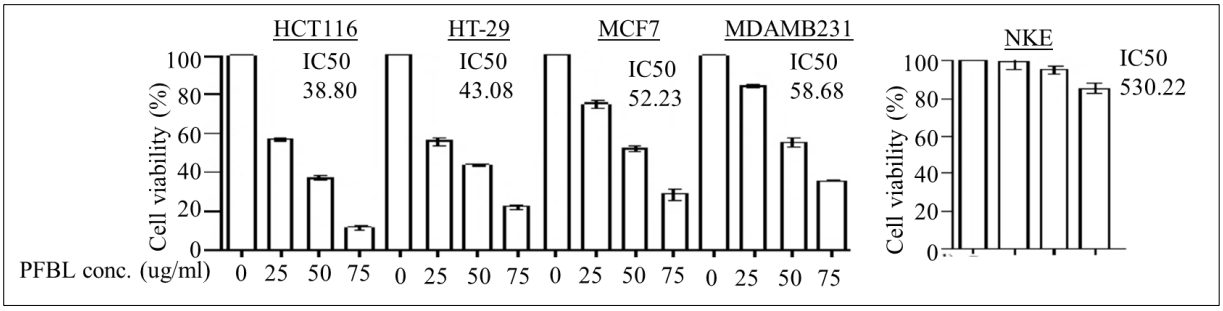

Figure 3.7: PFBL sensitizes different colon and breast cancer cells with little toxicity to NKE cells. The cells were treated with PFBL concentrations for 24 h as indicated by MTT assay as describe in Materials and Methods.

3.2.2 Phytochemical analysis of polyphenol-rich fraction of Bergenia ligulata (PFBL):

The phytochemical analysis of PFBL was carried out using standard methods as described in Chapter 2. Phytochemical analysis of PFBL indicated the presence of maximum amounts of flavonoids along with some amounts of alkaloids, reducing sugars, anthraquinones, proteins, saponins, lignin, tannins and glycosides as indicated in Table 3.1. Steroids and amino acids were not detected (Table 3.1). Based on quantitative analysis per gram of PFBL, 103±0.9

mg gallic acid equivalent, 38.23±2.22 mg catechin equivalent, 16.45±2.90 mg pilocarpine equivalent, and 10.23±1.03 tannic acid equivalent was present as mentioned in Table 3.2.

Table: 3.1: Qualitative analysis of the constituents in PFBL

Chemical groups	Qualitative Tests	Colour change	Presence or Absence
Alkaloids	Dragendorff's reagent	Cream colour	+
	Wegner's reagent	Orange brown	-
	Mayer's reagent	Reddish brown	+
Reducing sugars	Fehling's reagent	Red like brick	+
	Benedict's reagent	Red like brick	+
Steroids	Salkowaski test	Reddish blue green fluorescence	–
Anthraquinones	Bontrager's test	Upper red lower pink	+
Proteins	Lugol's reagent	Faint yellow	+
	Millon's reagent	White	+
Saponins	1% Lead acetate solution	White	+
Amino acids	Ninhydrin solution	Violet color	–
Lignin	Phloroglucinol + HCl	Red	+
Tannins	10% NH_4OH solution	Yellow fluorescence	+
	10% lead acetate solution	White	+
	5% $FeCl_3$ solution	Blackish blue	+
Flavonoids	Shinoda test	Crimson red	+++
Glycosides	10% NaOH solution	White	+

Table: 3.2 Quantitative phytochemical analysis of PFBL

Quantitative test	Value
Total phenol (mg GAE/g)	103 ±.09
Total flavonoid (mg CE/g)	38.23±2.22
Total alkaloid (mg of PE/g)	16.45±2.90
Total tannin (mg of TAE/g)	10.23±.1.03

3.2.3 Analysis of PFBL by LCMS:

PFBL was characterised using Waters Xevo G2-XS QT of LCMS instrument in Bose Institute central facility applying a gradient of 95% water to 95% acetonitrile with a flow rate of 40 µl/min using Waters 1.7 µm ACQUITY UPLC® BEH C18 column (1.0 x 100mm column). PFBL was resolved in 11 major peaks. The highest peak corresponded to molecular mass of 274.2460 Da, which is close to the molecular weight of Afzelechin (247.26) [Fig. 3.8B]. Based on a comparison of the LCMS profile with the metabolite database and *Bergenia ligulata* compound list the probable compounds present in the PFBL are Afzelechin (274.26), 4/11-O-galloyl bergenin (480.3), Catechin (290.26), Arbutin (272.25), Quercetin (302.236), Z-asarone (208.275), Dihydroergotamine (678.56), Ferulic acid (194), Aloe emodin (270.24), Chlorogenic acid (354), and Phytol (286.23). The details of the identified process such as their mass, retention time, and relative abundance of the compounds are listed in Table 3.3.

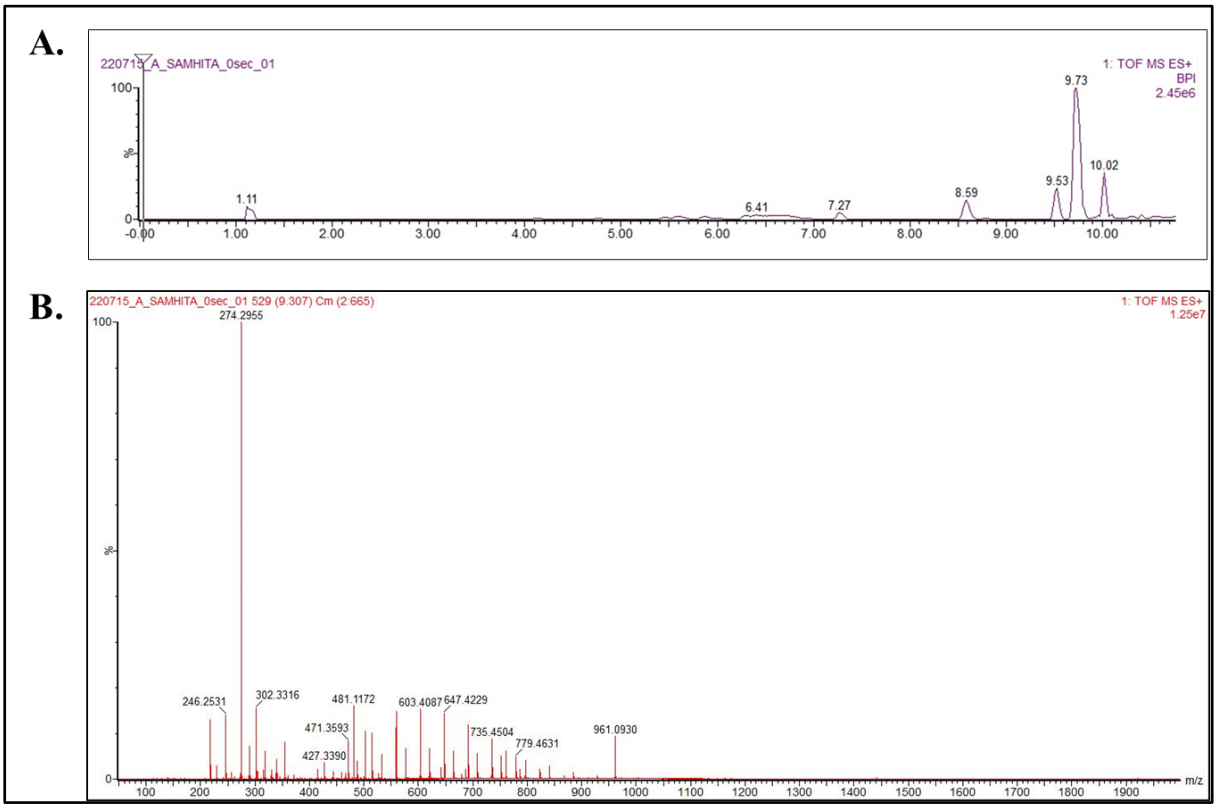

Figure 3.8: Elution profile of PFBL in LCMS. [A] LCMS chromatogram of Peak 7, and [B] the corresponding mass spectra.

Table 3.3: List of compounds identified in PFBL by LCMS analysis.

m/z	Neutral mass (Da)	Retention time (min)	Chromatographic peak width (min)	Maximum Abundance	Name of compounds
355.0970133	354	1.1097	0.0858	24178.4958	Chlorogenic acid
481.1199693	480	6.406666667	0.777866667	108672.3718	4/11-O- galloyl bergenin
961.0941501	960	6.589683333	0.560516667	179680.7949	4/11-O- galloyl bergenin
340.2885942	678.5626356	7.270316667	0.18875	40144.13683	Dihydroergotamine
218.2076712	194	8.591583333	0.2002	29473.88284	Ferulic acid
246.2533721	208.257	9.52965	0.1487	38909.31405	(Z)-asarone
274.2941379	274.26	9.729833333	0.18875	210245.0084	Afzelechin
274.3605766	272.25	9.729833333	0.18875	29259.71431	Arbutin
274.2114728	270.24	9.76415	0.085783333	27657.79759	Aloe emodin
318.3307841	286.23	9.76415	0.137266667	21535.52474	Phytol
290.2936901	290.26	9.798466667	0.1201	24075.78057	Catechin
302.3326412	302.236	10.02153333	0.13155	51304.23125	Quercetin

3.2.4 PFBL was well tolerated in healthy mice:

PFBL was checked for toxicity in preclinical model alone or in combination with anti-cancer drugs used in the clinic, such as DOX and 5FU. The body weight, spleen size, blood cell count (RBC and WBC) and serum level of different enzymes (such as alkaline phosphatase (ALP), glutamic-oxaloacetic transaminase (SGOT) and glutamic pyruvic transaminase (SGPT)) were measured as toxicity parameters taking control group results as standard for toxicity assessment (El Chediak et al., 2018; Gitnick, 1981; Jasper et al., 2012). Thirty BALB/c mice were divided into 6 groups (N=5) and treated with the vehicle (DMSO), PFBL (100 mg/kg body weight), 5FU (10 mg/kg body weight), 5FU (10 mg/kg body weight) + PFBL (100 mg/ kg body weight), DOX (5 mg/kg body weight), and DOX (5 mg/kg body weight) + PFBL (100 mg/kg body weight), by intraperitoneal injection in every alternate day for 30 days. The body weights of the mice were recorded every time before injection during the treatment period. In the control and PFBL-treated groups, the animals gained close to 10% and 20% of their body weights, while in the 5FU and DOX-treated groups, the animals drastically lost close to 20% and 30% of their body weights, respectively [Fig. 3.9]. When combined with 5FU and DOX, PFBL could restore the loss of body weight induced by the anticancer drugs induced by the anticancer drug in both groups [Fig. 3.9A]. There is no enlargement in spleen size in the PFB-treated group compared to the control group. The 5FU-treated group showed the most enlarged spleens, while the DOX-treated group showed relatively less increase in spleen size. The spleens in mice treated with the combination of PFBL with either 5FU or Dox showed slight or little enlargement [Fig. 3.9B]. The ALP, SGOT, and SGPT levels were also tested in the blood specimens isolated from these animals [Fig. 3.9 C-E] where in PFBL treated group there

was no significant change in compare to control group, but serum level of these enzymes enhanced in 5FU and DOX treated groups. The RBC and WBC counts were decreased in 5FU and DOX treated groups where no change was observed in PFBL treated group in compare to control set [Fig. 3.9 F-G]. PFBL was found to be nontoxic to or well tolerated by these animals. Notably, PFBL when used in combination dramatically ameliorated the toxic effect of DOX and 5FU in mice [Fig. 3.9].

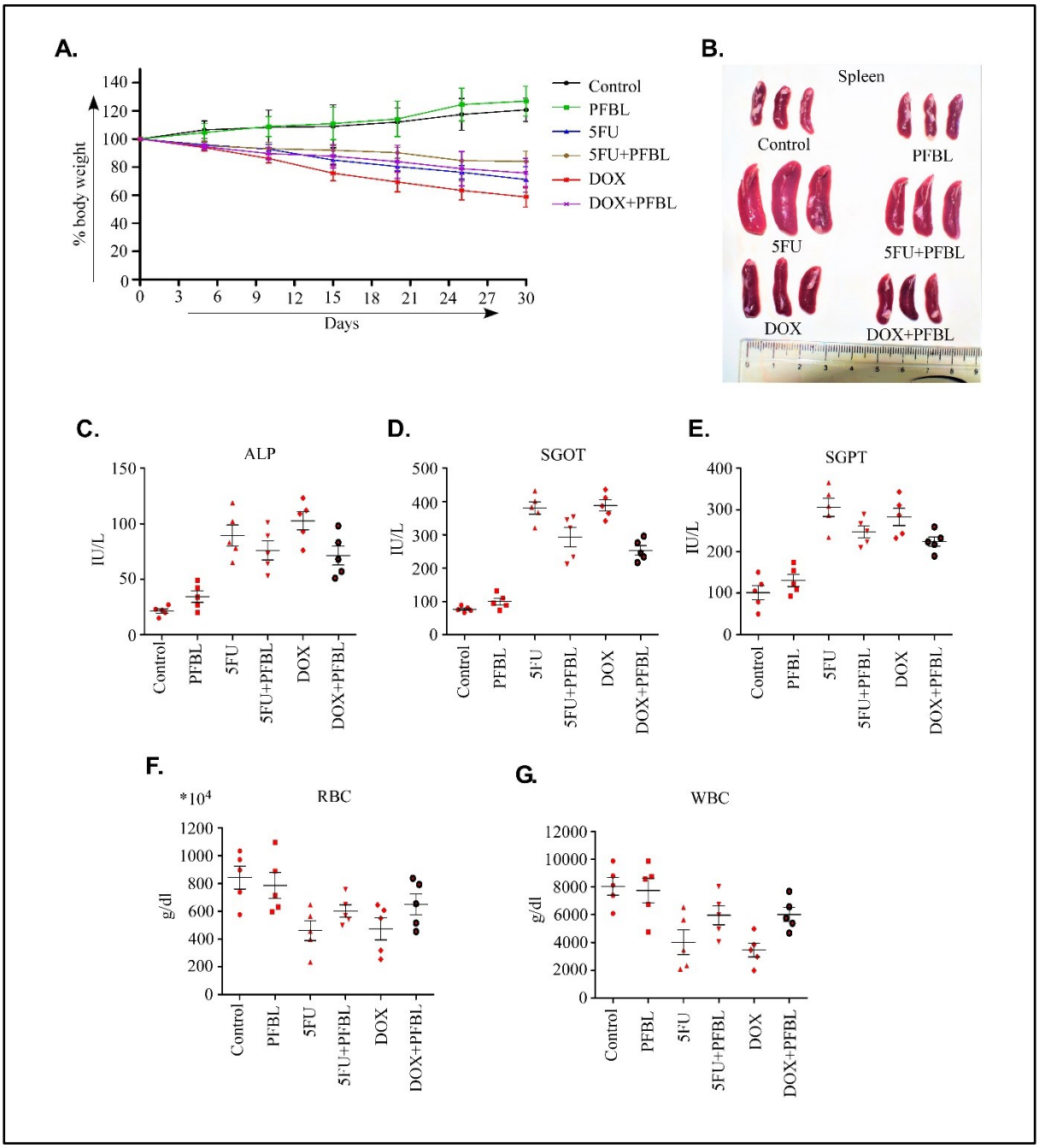

Figure 3.9: PFBL is well tolerated and reduced 5FU and DOX-induced toxicity in healthy mice. BALB/c mice were treated with the vehicle, or PFBL (100 mg/kg body weight) or 5FU or DOX or their combinations for 30 days (n=5). [A] the line graph representing the change in

the body weight of mice during the treatment period. [B] Representative images of the spleens isolated from different experimental groups of mice shown in panel A. The blood drawn from different experimental groups of mice was used to test [C] serum alkaline phosphatase (ALP) level, [D] serum glutamic-oxaloacetic transaminase (SGOT) level, [E] serum glutamic pyruvic transaminase (SGPT) level, and the number of [F] RBC and [G] WBC counts were plotted by dot plot.

3.3 DISCUSSION:

Bergenia ligulate has been reported for its anti-cancer and anti-inflammatory activities. Among different solvent fractions tested, it was ultimately revealed that the EtOAc fraction was the most toxic to these cells against the HCT116 and MCF7 cells compared to that of n-hexane, DCM, and methanolic fractions. The most active EtOAc fraction was further fractionated using reverse-phase HPLC. Phytochemical analysis revealed the presence of alkaloids, reducing sugars, anthraquinones, proteins, saponins, lignin, tannins, flavonoids, and glycosides, where flavonoids were highly prominent in the active fraction with phenol content estimated at 103±0.9 mg GAE/gm. Therefore, the activity was termed polyphenol-rich fraction (PFBL) based on its phytochemical composition. Decrease in body weight, enlargement of spleen, drop in blood cell count and liver toxicity (increases serum ALP, SGOT, SGPT levels) are used as a measure of toxicity assessment (El Chediak et al., 2018; Gitnick, 1981; Jasper et al., 2012) in animals, and treatment with chemotherapeutic drugs often causes these side effects in cancer patients. No significant change was observed in the spleen size of PFBL treated group in comparison with control group suggested PFBL as nontoxic and safe to use in healthy animals [Fig. 3.9]. The serum ALP, SGOT, and SGPT levels also found to be normal in these experimental animals. Moreover, PFBL could reduce the toxicity induced by DOX and 5FU. The LCMS analysis identified twelve major compounds in PFBL which are probably responsible for the PFBL action. Some compounds, such as catechin, quercetin, and ferulic acid, are known for their anti-oxidant and anti-inflammatory activities.

In brief, PFBL was found to be toxic against breast and colon cancer cells without being toxic to healthy animals. 11 major compounds have been identified in PFBL using LCMS analysis, possibly responsible for PFBL action. The molecular basis for PFBL action has been examined further.

References:

Agnihotri, V., Sati, P., Jantwal, A., Pandey, A., 2015. Antimicrobial and antioxidant phytochemicals in leaf extracts of Bergenia ligulata: a Himalayan herb of medicinal value. Natural Product Research 29, 1074-1077.
Dhar, M., Dhar, M., Dhawan, B., Mehrotra, B., Ray, C., 1968. Screening of Indian plants for biological activity: Part I.
Ekor, M., 2014. The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. Frontiers in pharmacology 4, 177.
El Chediak, A., Haydar, A.A., Hakim, A., Massih, S.A., Hilal, L., Mukherji, D., Temraz, S., Shamseddine, A., 2018. Increase in spleen volume as a predictor of oxaliplatin toxicity. Therapeutics and clinical risk management, 653-657.
Ghosh, S., Dutta, N., Banerjee, P., Gajbhiye, R.L., Sareng, H.R., Kapse, P., Pal, S., Burdelya, L., Mandal, N.C., Ravichandiran, V., 2021. Induction of monoamine oxidase A-mediated oxidative stress and impairment of NRF2-antioxidant defence response by polyphenol-rich fraction of Bergenia ligulata sensitizes prostate cancer cells in vitro and in vivo. Free Radical Biology and Medicine 172, 136-151.
Gitnick, G., 1981. Assessment of liver function. Surgical Clinics of North America 61, 197-207.
Greenwald, P., Clifford, C., Milner, J., 2001. Diet and cancer prevention. European journal of cancer 37, 948-965.
Jasper, R., Locatelli, G.O., Pilati, C., Locatelli, C., 2012. Evaluation of biochemical, hematological and oxidative parameters in mice exposed to the herbicide glyphosate-Roundup®. Interdisciplinary toxicology 5, 133.
Nazir, N., Koul, S., Qurishi, M.A., Najar, M.H., Zargar, M.I., 2011. Evaluation of antioxidant and antimicrobial activities of Bergenin and its derivatives obtained by chemoenzymatic synthesis. European journal of medicinal chemistry 46, 2415-2420.
Rajkumar, V., Guha, G., Kumar, R.A., 2011. Anti-neoplastic activities of Bergenia ciliata rhizome. Journal of Pharmacy Research 4, 443-445.
Ruby, K., Dwivedi, J., Chauhan, R., 2012. Pashanbheda a golden herb of Himalaya: a review. International Journal of Pharmacy Review & Research 2, 97-105.
Sajad, T., Zargar, A., Ahmad, T., Bader, G., Naime, M., Ali, S., 2010. Antibacterial and anti-inflammatory potential Bergenia ligulata. Am. J. Biomed. Sci 2, 313-321.
Sadat, A., Uddin, G., Alam, M., Ahmad, A., Siddiqui, B.S., 2015. Structure activity relationship of bergenin, p-hydroxybenzoyl bergenin, 11-O-galloylbergenin as potent antioxidant and urease inhibitor isolated from Bergenia ligulata. Natural Product Research 29, 2291-2294.

CHAPTER 4

STUDY OF ANTI-CANCER ACTIVITY OF POLY PHENOL RICH FRACTION OF BERGENIA LIGULATA RHIZOME AGAINST COLON CANCER

4.1 INTRODUCTION:

Colorectal cancer (CRC), the 2nd most diagnosed after lung cancer, shared 9.4% of the new cases, with ~1.9 million claiming about 0.9 million lives (Sung et al., 2021). Although the execution of an effective screening program has reduced the number of deaths, data suggest the global CRC burden to rise by 60% by 2030. Limitations of the existing treatment strategies underscored the necessity for searching for new agents that could be used alone or in combination with the existing chemotherapeutics regimens. Metastasis is the prime cause of mortality in patients with CRC. Almost 20% of CRC patients were simultaneously detected with metastasis (Elmore, 2007). Early detection can help in cure and can minimize the risk for mortality but there is always a high risk for reoccurrence even after surgery and chemotherapy. Most existing treatment options being non-specific, cannot distinguish between normal and cancer cells and thus are toxic to the host. Therefore, the search for anticancer agents that can specifically sensitize the cancer cells without affecting the normal cells, along with anti-metastatic activity, is of an urgent need. Notably, an increasing number of in-vitro and in vivo studies reporting significant anti-cancer and anti-metastatic activities in various extracts and compounds isolated from plants highlighted great potential for their use as chemotherapy agents alone or in combination with existing therapy regimens. *Bergenia ligulata* locally known as "Pashanbhed" is known for its various medicinal properties, including anti-inflammatory and anti-cancer activities in Indian traditional and folk medicine.

Reactive Oxygen Species (ROS) such as superoxide anion ($O_2^{\cdot -}$), hydroxyl radical ($OH^{\cdot}$), and hydrogen peroxide (H_2O_2) are highly reactive molecules (Halliwell, 2011; Winterbourn, 2015). Cells carry an anti-oxidant defense system to neutralize their ROS as necessary (Halliwell, 2011). However, a certain level of ROS is required for normal cellular functions (Covarrubias et al., 2008). A relatively higher level of ROS in a cell resulting from a defective antioxidant defense system can lead to death through damage to the cellular micro- and macromolecules (Halliwell, 2011; Redza-Dutordoir and Averill-Bates, 2016).

Cells execute the death pathways, such as apoptosis and autophagy, in a regulated way to maintain homeostasis. Apoptosis can be induced by extrinsic or intrinsic signals or both. These two pathways can interplay or antagonize each other or may act separately depending upon the upstream effector molecules activated and their interactions (Xi et al., 2022). Both play crucial roles in removing unnecessary or damaged cells upon activation of different cascades (Mariño et al., 2014). ROS is known to induce the different apoptotic cell death

pathways, such as the mitochondrial pathway, death receptor-mediated pathway, and endoplasmic reticulum stress pathway (Redza-Dutordoir and Averill-Bates, 2016).

Autophagy is primarily a self-engulfment process for cell survival by digesting and recycling dysfunctional macromolecules to maintain cellular homeostasis (Baehrecke, 2005; Codogno and Meijer, 2005; Gozuacik and Kimchi, 2004; Levine and Yuan, 2005; Marino and López-Otín, 2004). Besides cell survival, autophagy can lead to cell death depending on the cellular signals (Kanzawa et al., 2003; Kanzawa et al., 2005) (Reef et al., 2006).

AMP-activated protein kinase (AMPK), a member of the serine/threonine protein kinase family, is a prime sensor and effector of cellular energy metabolism (Hardie, 2007). Increased concentration of AMP molecules in the cell activates AMPK by binding to AMPKγ subunit (Carling et al., 2011; Garcia and Shaw, 2017; Hardie et al., 2011; Hardie et al., 2012; Xiao et al., 2013). ROS can control the AMPK activity either by alteration of concentrations of adenine nucleotides (Auciello et al., 2014) or directly in an AMP/ATP ratio-independent manner (Emerling et al., 2009). Evidence suggest that AMPK can activate autophagy in response to several stressors such as glucose starvation (Herrero-Martín et al., 2009; Liang et al., 2007; Matsui et al., 2007; Meley et al., 2006; Vingtdeux et al., 2010). AMPK induces autophagy by inhibiting mTOR (mammalian target of rapamycin) in a TSC2 and Raptor-dependent manner (mTORC1) (Gwinn et al., 2008; Inoki et al., 2003).

Bergenia ligulata has been used in Indian traditional folk medicine for treating kidney stones for centuries (Gurav and Gurav, 2014). The roots and rhizomes of the plant are believed to be used by Himalayan tribes to cure common general illnesses and different kidney-related abnormalities (Ruby et al., 2012). The major bioactive phytochemicals present in *B. ligulata* are bergenin (Pandey et al., 2017), afzelechin (Reddy et al., 1999), β-sitosterol (Das et al., 2022), catechin, leucocyanidin, gallic acid, and tannic acid (Dix and Srivastava, 1989). *B. ligulata* showed oxidative stress-induced anti-prostate cancer activity and apoptotic cell death in vitro and in vivo (Ghosh et al., 2021). In this chapter, the anti-CRC activity of HPLC purified polyphenol-rich fraction of *B. ligulata* (PFBL) rhizome is presented. The underlying molecular mechanism of PFBL action was investigated and confirmed using flow cytometry and western blots and validated in an in vivo mouse solid tumor model. To investigate the in vivo anti-metastatic properties of PFBL, a lung metastasis mouse model was used.

4.2 RESULTS:

4.2.1 Polyphenol-rich fraction of *Bergenia ligulata* (PFBL) is toxic to colon cancer cells:

The sensitivity of PFBL was tested by treating colon cancer cells (HCT116 and HT29) with an increasing concentration PFBL (25 µg/ml, 50 µg/ml, and 75 µg/ml) by MTT assay as described in Chapter 2, materials and methods. The IC50 values were also calculated. Results revealed that the HCT116 cells are more susceptible to PFBL (with an IC_{50} value of 38.8 µg/ml) in comparison to HT29 cells (with an IC_{50} value of 50.08 µg/ml) [Fig. 4.1A]. The morphology of HCT116 cells gradually changed with increasing doses of PFBL [Fig. 4.1B]. The colony-forming ability of HCT116 cells was gradually diminished with increasing PFBL concentration; at a PFBL concentration of 10 µg/ml, there was a 50% loss of the clones [Fig. 4.1C-D]. In the wound healing assay, PFBL dose-dependently inhibited the migration potential of HCT116 cells [Fig. 4.1E-F].

4.2.2 PFBL treatment induces G0/G1 cell cycle arrest of HCT116 cells:

PFBL treatment arrests HCT116 cells in the G0/G1 phase as indicated by dose-dependent increase of population in the G0/G1 phase of the cell cycle after 24 h of treatment estimated by flow cytometry after propidium iodide (PI) staining [Fig. 4.2A-B]. Overall, there was ~40% increase in the G0/G1 cell population of the cells. Western blots were performed to understand the mechanism of cell death in HCT116 cells after treatment with 40 µg/ml PFBL for different time points (12 h, 24 h, 36 h, and 48 h). There was a significant decrease in the CDK4 and Cyclin D1 protein levels [Fig. 4.2F-G].

4.2.3 PFBL and induces apoptotic death of HCT116 cells:

To investigate the relative involvement of apoptosis in colon cancer cell death, the HCT116 cells were treated with 25 µg/ml, 50 µg/ml, 75 µg/ml, and 100 µg/ml of PFBL. After 24 h of treatment, cells were stained with Annexin-V-FITC, followed by both flow cytometry and confocal microscopy. The results revealed that cells were directed to apoptosis in a dose-dependent manner as suggested by 2.5%, 5%, 10%, and 15% apoptotic cell populations in response to treatment with 25 µg/ml, 50 µg/ml, 75 µg/ml, and 100 µg/ml of PFBL, respectively in flow cytometry analysis [Fig. 4.2C-E].

To understand the initiation path of the apoptotic signal, JC1 staining was performed after 24 h PFBL treatment. A shift of JC1 staining from red to green was observed in a dose-dependent manner under a fluorescent microscope, indicating a change in mitochondrial membrane potential [Fig. 4.3A]. This suggested that PFBL drastically damaged the mitochondrial membrane permeability and interfered with the mitochondrial redox potential.

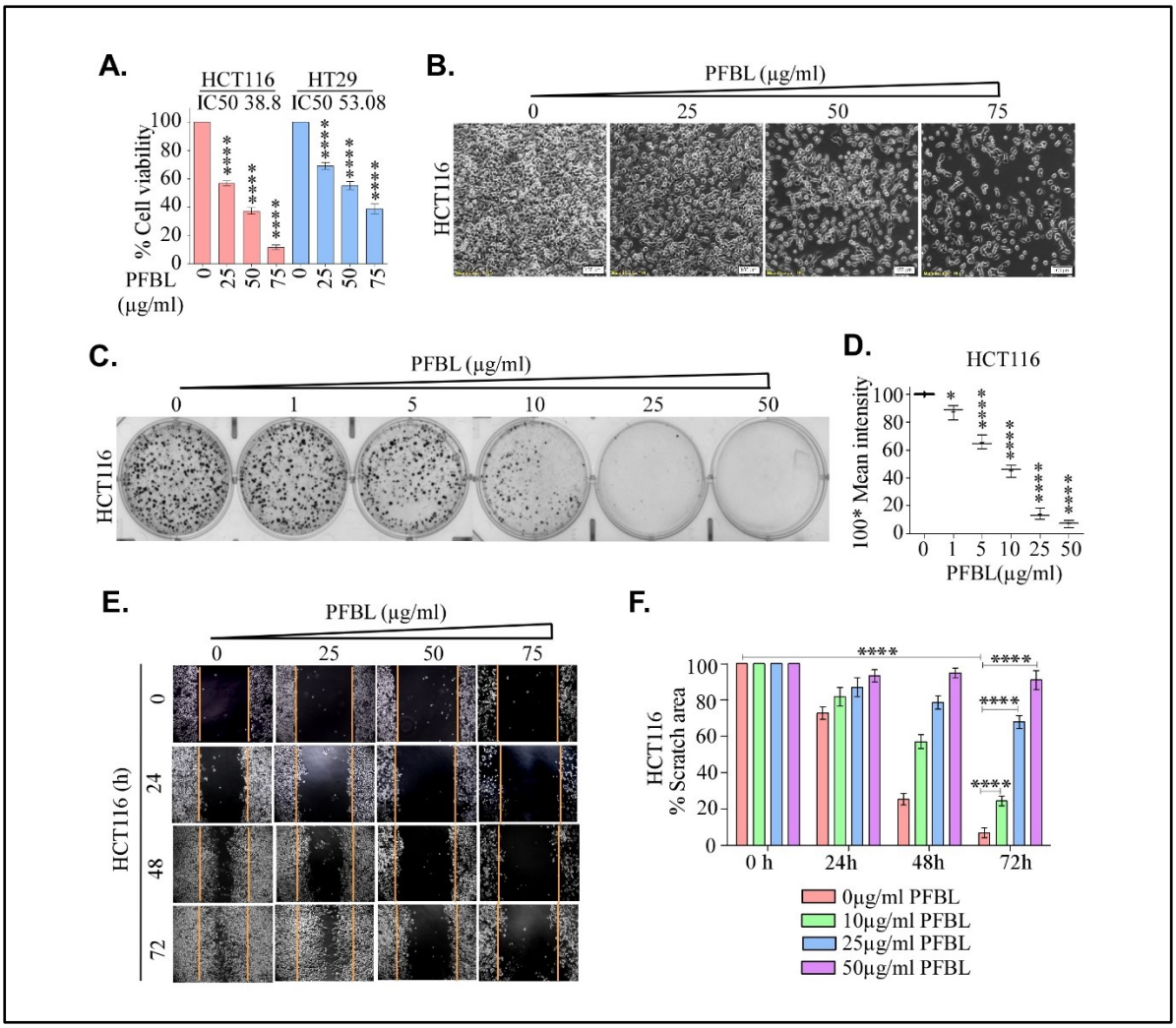

Figure 4.1: PFBL sensitizes colon cancer cells. [A] Bar graph representing the dose-dependent sensitivity of the cells (HCT116 and HT29) determined by MTT assay after 24 h treatment. The IC_{50} values were shown. **[B]** Phase contrast images of cells treated with increasing concentrations of PFBL for 24 h as indicated. **[C-D]** Colony-forming ability of cells counted after 24 h treatment. Cells were stained with crystal violet after fixing with formaldehyde **[E]** Phase contrast images showing the effect of the treatment on cellular migration potential by wound healing assay. **[F]** Bar graph representing the estimation of wound healing assay.

Western blots were performed to better understand the molecular pathway of cell death in HCT116 cells after treatment with 40 µg/ml PFBL for different time points (12 h, 24 h, 36 h, and 48 h). The results suggested no significant changes in the markers of apoptotic signalling pathways, such as cleavage in the PARP1, caspase 7, caspase 8, and caspase 9 molecules. The alterations in the levels of Bcl2, Bid, Survivin, and Bax were observed [Figure 4.2F-G]. To verify the weightage of caspase-dependent apoptotic death, cells were pre-treated for 3 h with pan-caspase inhibitor Z-VAD-FMK before treatment with 40 µg/ml PFBL for 24 h. The effect of Z-VAD-FMK pre-treatment was analyzed by FACS (after dual staining with Annexin-V-

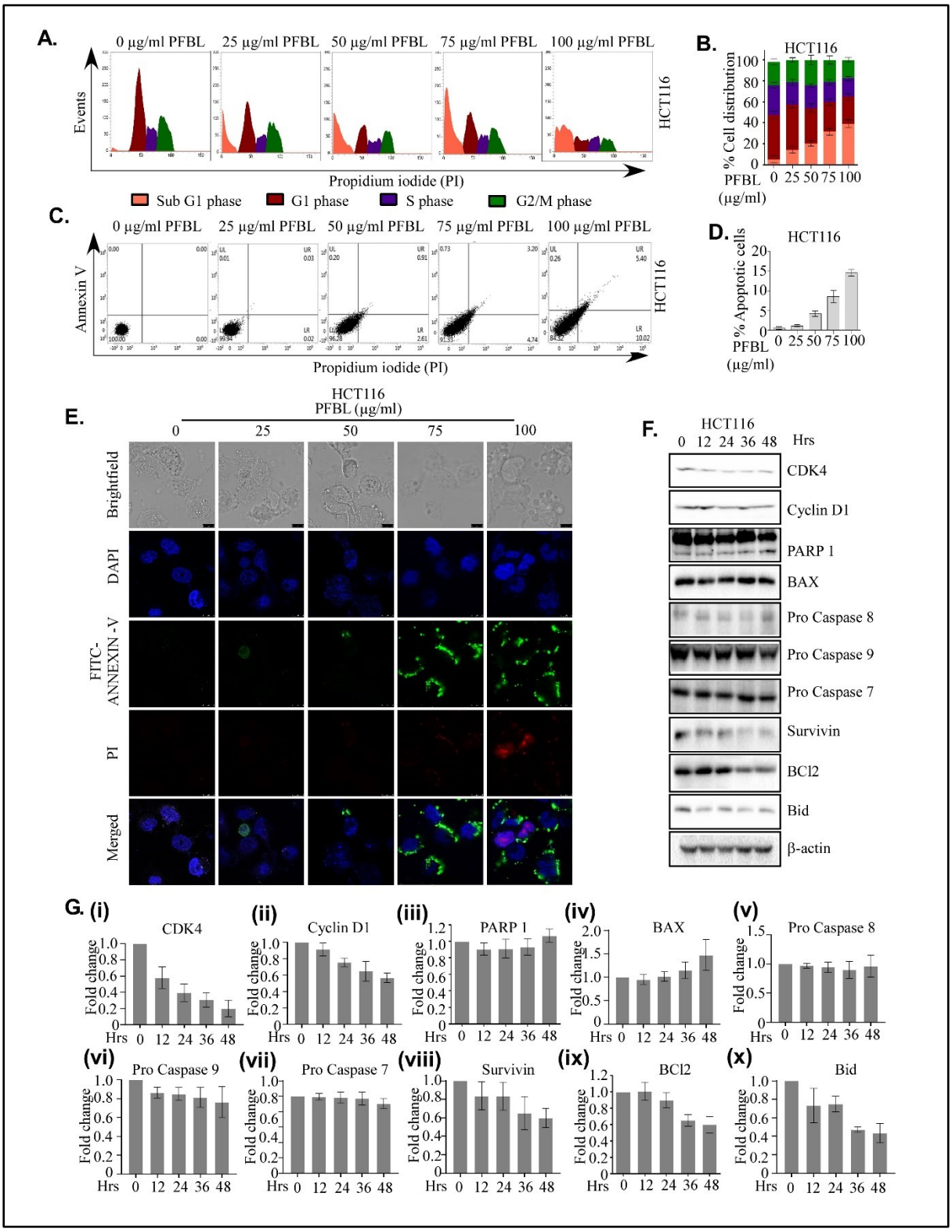

Figure 4.2: PFBL arrests cells at G0/G1 phase and induces apoptosis. [A] Histograms showing the effect of different doses of PFBL treatments on cell cycle phases generated by FACS analysis. **[B]** Bar diagram representing the distribution of cells in different cell cycle phases upon treatment as shown. **[C]** FACS assay representing the status of FITC-Annexin-V and/or PI positive cell population upon treatment as shown. The quantitation of the assay is shown in a bar graph **[D]**. **[E]** Confocal images captured after exposure of cells to increasing

concentrations of PFBL for 24 h followed by staining with FITC-Annexin-V/PI/DAPI. **[F]** Immunoblots of lysates of cells pre-treated with PFBL (Concentration?!) for different durations with different cellular apoptotic markers. The β-actin level was determined as an internal loading control. The densitometric quantitation of bands relative to β-actin is shown by bar graphs **[G]**.

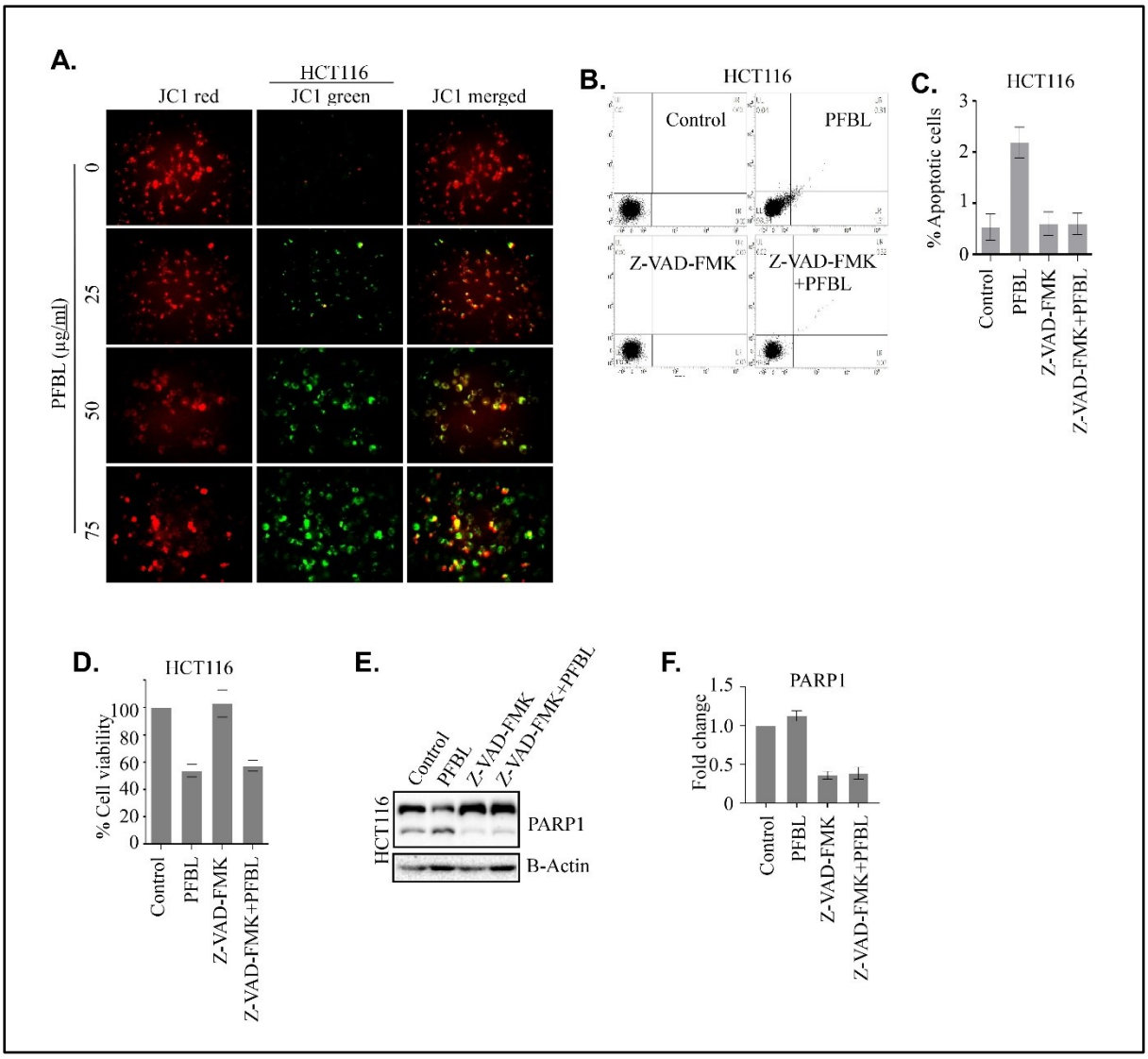

Figure 4.3: PFBL induces apoptosis by mitochondrial dysfunction in HCT116 cells. [A] Fluorescence microscopy images representing the change in mitochondrial permeability (from red to green shift) upon treatment with increasing concentrations of PFBL. **[B]** FITC-Annexin-V and/or PI positive population of PFBL (Concentration 40µg/ml) treated cells pre-treated with or without pan-caspase inhibitor Z-VAD-FMK for 3 h assayed by flow cytometry. Quantitation of the apoptotic cell population shown by a bar graph **[C]**. **[D]** Bar graph representing the viability (MTT assay) of cells upon 24 h treatment with PFBL. Cells pre-treated with pan-caspase inhibitor Z-VAD-FMK for 3 h are indicated. **[E]** Immunoblots of lysates off PFBL treated cells. Cells that were pre-treated or not with Z-VAD-FMK (for 3 h) are indicated. The β-actin level was determined as a loading control. **[F]** Bar graph representing the fold change of PARP1 level relative to β-actin level measured by densitometric quantitation of bands in the immunoblot shown in panel [E].

-FITC and PI), MTT assay, and western blotting [Fig. 4.3B-F]. Results revealed that pre-treatment with Z-VAD-FMK diminished the PFBL-induced apoptotic cell population and PARP1 cleavage and provided only ~3% protection from cell death, indicating the share of apoptosis in the killing mechanism [Fig. 4.3B-F].

PFBL at 40 μg/ml (IC50=38.8 μg/ml) [Fig. 4.1], killed less than 3% of HCT116 cells after 24 h of treatment by apoptosis per reversion by Z-VAD-FMK pre-treatment, indicating that this is not the major death mechanism that PFBL induces to kill these cells.

4.2.4 PFBL induces autophagic death of HCT116 cells:

We already found very minimal involvement of apoptosis in PFBL-induced death of HCT116 cells [Fig. 4.3]. So, we tested for the involvement of autophagy in these cells' death. Autophagy can be activated in the cell by different stimuli that can ultimately lead to autophagosome formation, and the cleavage of LC3II proteins that occur after insertion in autophagosome can be used as an autophagy marker. For this, LC3II-GFP plasmid was transfected in HCT116 cells, followed by treatment with increasing concentrations of PFBL. Next, the presence of LC3II-GFP puncta formation was monitored by confocal microscopy [Fig. 4.4A]. The percentage of cells with LC3II-GFP puncta increased with increasing concentration of PFBL. Consistently, there was a gradual increase in the LC3BII level detected by western blotting [Fig. 4.4B-C]. To check the involvement of the mTOR pathway in PFBL-induced autophagy, different upstream and downstream protein levels of this pathway were checked. The phosphorylation level of 4EBP1, S6K, and AKT was downregulated, whereas the phosphorylation level of AMPK (at T172) was upregulated [Fig. 4.4B-C] indicating mTOR inhibition. To verify the degree of involvement of autophagy in PFBL-induced death, cells were pre-treated with autophagy inhibitor chloroquine for 3 h before treatment with PFBL for 24 h analyzed by MTT assay [Fig. 4.4D]. Chloroquine pre-treatment provided major protection from toxicity induced by PFBL, indicating the significant role of autophagy in PFBL-induced death of HCT116 calls [Fig. 4.4D].

4.2.5 PFBL-induced cell death is dependent on ROS production in HCT116 cells:

PFBL treatment increases the production of reactive oxygen species (ROS) that is visualized by a fluorescence microscope and FACS upon DCFDA staining [Fig. 4.5A-B]. The PFBL-mediated ROS production in HCT116 cells was ameliorated upon pre-treatment with quenching agent N-acetylcysteine (NAC) [Fig. 4.5A-B]. Consistently, NAC pre-treatment

significantly restored cell viability in PFBL-treated cells as measured by MTT assay [Fig. 4.5C]. Pre-treatment with NAC not only increased cell survival but also suppressed autophagy and apoptosis as indicated by decreased LC3BII and decreased PARP1 level upon combined NAC and PFBL treatment compared with only PFBL treatment detected by western blotting [Fig. 4.5D-E]. NAC pre-treatment also reduced the phosphorylation of AMPK close to the untreated level. This data shows that PFBL induced cellular ROS production is the major cause of cell death.

4.2.6 AMPK as a key mediator of PFBL action:

We have already observed a dose-dependent increase of phosphorylation at T172 residue (activatory phosphorylation) of AMPK upon PFBL treatment [Figure 4.4B]. The involvement of AMPK in PFBL-induced cell death was further investigated by shRNA-mediated knockdown of AMPK. Cell viability of PFBL-treated cells increased in AMPK downregulated samples measured by MTT assay [Fig. 4.6A]. Consistently, western blot results revealed that PFBL action was diminished in AMPK down-regulated cells [Fig. 4.6B-C]. AMPK knockdown restored cellular CDK4 and Cyclin D1 protein levels that were downregulated upon PFBL treatment, indicating the involvement of AMPK in PFBL-induced G0/G1 cell cycle arrest. AMPK downregulation also reduced the cellular levels of cleaved-PARP1 and LC3II [Fig. 4.6B-C].

4.2.7 PFBL reverses epithelial to mesenchymal transition in HCT116 cells:

Epithelial-to-mesenchymal transition (EMT) is a crucial step in cancer metastasis. Cancer cells become more aggressive, migratory, and invasive due to enhanced mesenchymal properties. In metastatic cancer cells, E-cadherin expression decreases while the N-cadherin expression goes up. In our earlier study, we found that PFBL inhibited colony forming ability and migration potential of HCT116 cells in a dose-dependent manner [Fig. 4.1C-F]. So, we tested the metastatic potential of HCT116 cells after treatment with PFBL for different time points by monitoring different epithelial and mesenchymal markers by immunoblots. Results suggested that PFBL decreased mesenchymal markers such as N-cadherin, Snail, Twist, and Vimentin with simultaneous upregulation of the expression of epithelial markers such as E-cadherin in a time-dependent manner, as shown [Fig. 4.7A-B]. These results indicated that PFBL suppresses metastatic potentials of the HCT116 cells by suppressing the EMT.

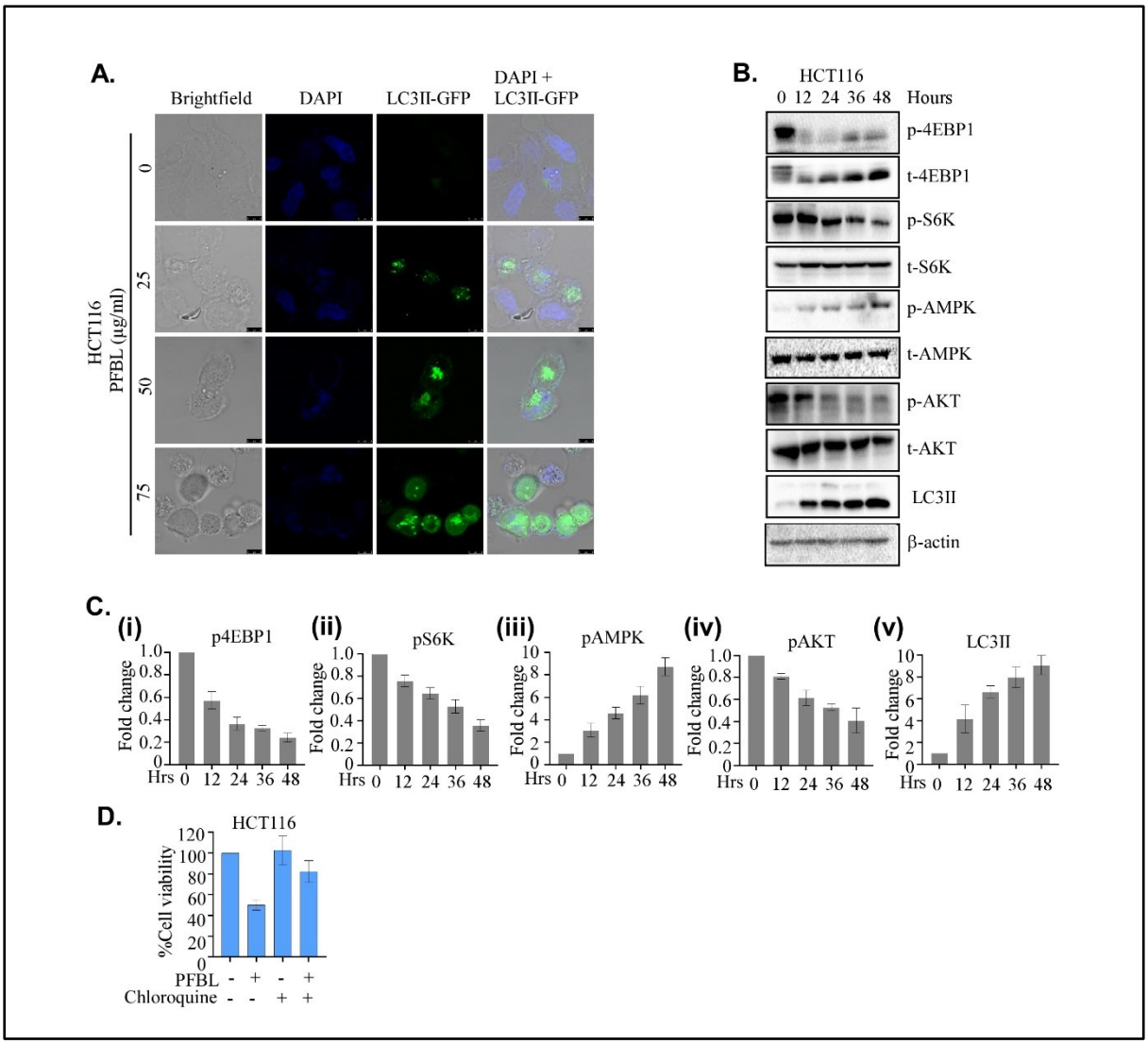

Figure 4.4: PFBL induces death in HCT116 cells by autophagy: [A] Confocal images of LC3II-GFP transfected cells treated with increasing concentrations of PFBL for 24 h. Cells stained with DAPI are also indicated. **[B]** Immunoblots of lysates of cells treated with 40 µg/ml PFBL for different duration. The β-actin level was detected in the same blot as the loading control as indicated. The fold change of signals in the blots was estimated by densitometric quantitation of bands compared to the β-actin level **[C]. [D]** Cell viability (MTT) assay indicating the role of autophagy (as chloroquine-mediated protection) in PFB- induced death.

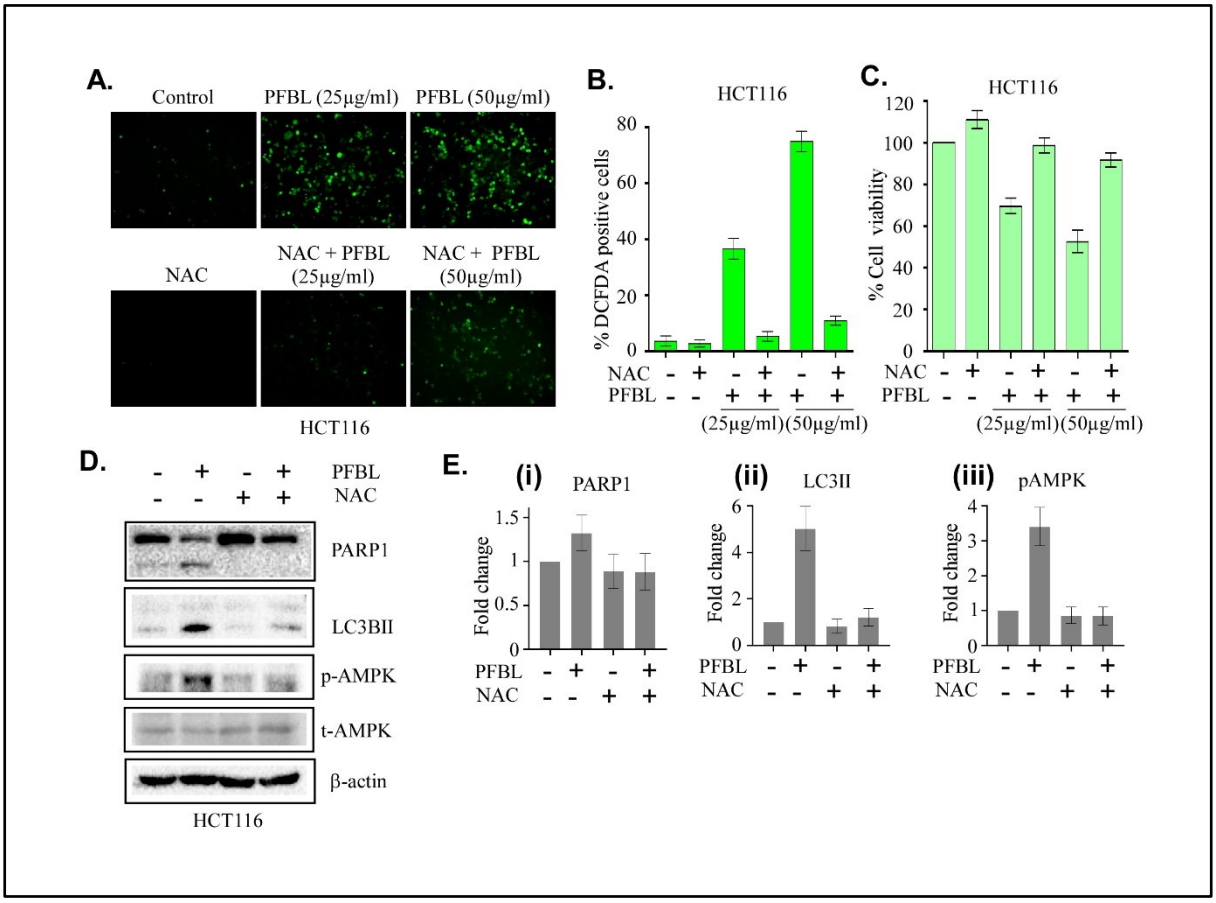

Figure 4.5: PFBL-induces ROS generation is responsible for the death of HCT116 cells. **[A]** Fluorescence microscopy images of HCT116 cells treated for 24 h with the indicated concentration of PFBL with or without NAC (concentration 10 mg/ml) pre-treatment for 4 h. The cells were stained with DCFDA before imaging. The DCFDA-positive and viable cell population in the assay are shown in a bar graph in panels **[B]** and **[C],** respectively, as indicated. **[D]** Immunoblots of lysates of PFBL-treated cells with or without pre-treatment with NAC for 4 h with cell markers. The β-actin level was detected as a loading control. The fold change of signals in the blots was estimated by densitometric quantitation of indicated bands compared to the β-actin level, as shown in panel **[E].**

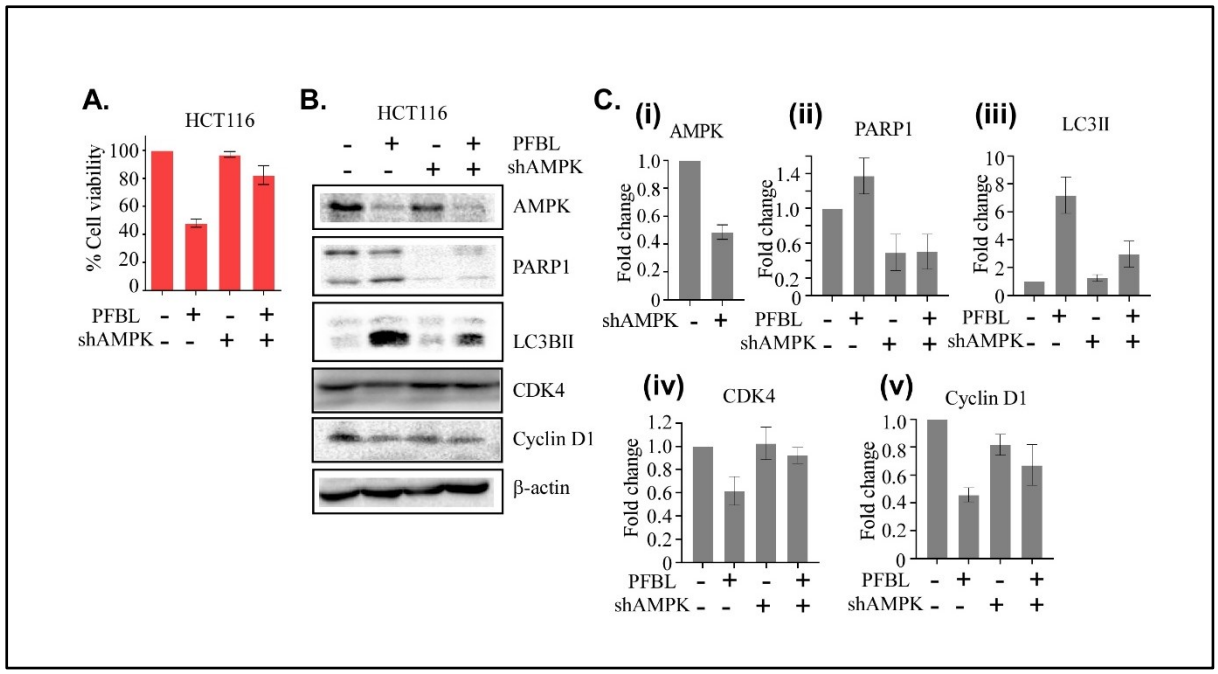

Figure 4.6: shRNA-mediated downregulation of AMPK diminishes the effect of PFBL. [A] Bar graph representing the effect of AMPK downregulation in PFBL-induced cell death determined by MTT assay. **[B]** Immunoblots representing the effect of AMPK downregulation in PFBL treatment on apoptotic and autophagic markers. The β-actin level was detected as a loading control. The fold change of signals in the blots was estimated by densitometric quantitation of indicated bands compared to the β-actin level, as shown in panel **[C].**

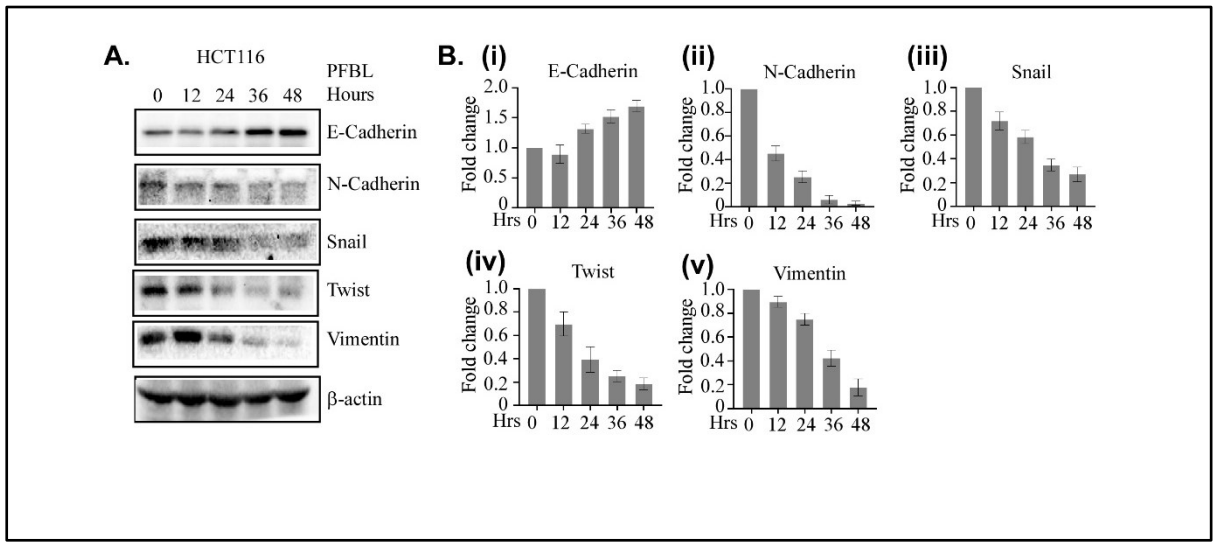

Figure 4.7: PFBL inhibits epithelial to mesenchymal transition in HCT116 cells. [A] Immunoblots of lysates of cells treated with 40 µg/ml PFBL for 24 h for different cellular markers as indicated. The β-actin level was detected as a loading control. **[B]** The fold change of signals in the blots was estimated by densitometric quantitation of indicated bands compared to the β-actin level as indicated.

4.2.8 In vivo antitumor activity of PFBL:

The results of an efficient anticancer activity of PFBL against the HCT116 cells led us to test its potential for anti-CRC activity in vivo. So, we investigated the anti-tumor effect of PFBL on the growth of mouse tumor xenograft, i.e., mouse colon cancer cell line CT26 derived subcutaneous solid tumor harbored in BALB/c mice, alone as well as in combination with 5FU. 5FU is used in the current anti-CRC treatment regimen in the clinic. Mice with palpable tumors (1 week after injection of $1x10^6$ CT26 cells) were randomly divided into four groups (n=5). For CT26 tumor-bearing mice, each group received either vehicle or PFBL or 5FU or a combination of 5FU and PFBL. The treatment was repeated every alternative day for 15 days, and the tumor size was measured on every treatment day. After completion of the treatment period, the mice were sacrificed, and tumors were excised and measured. Results indicated that PFBL, 5FU, and combination treatment regressed tumors by 80%, 60%, and 90%, respectively [Fig. 4.8A]. The change in animal body weight throughout the treatment period was recorded and plotted [Fig. 4.8B]. The change in the tumor weight was also prominent [Fig.4.8C]. The H & E staining of tumor tissue sections distinctly indicated the clearer regions in treated samples compared to denser regions in vehicle-treated tumors [Fig 4.8D]. Immunoblot with tumor tissue lysate indicated a similar mechanism of cell death as observed in the case of HCT116 cells treated with PFBL. There is no significant change in PARP1 cleavage band and cleaved caspase3 level in PFBL-treated samples compared to the control group [Fig. 4.8E-F] where is in samples treated with 5-FU alone or in combination with PFBL, there is ~3 fold increase in PARP1 cleaved band and ~2 fold and ~1.5 fold increase in cleaved caspase 3 levels, respectively. There is ~10-fold and ~6-fold increase in LC3II protein level in PFBL alone and PFBL and 5FU combined treated samples, respectively, where there is no significant increase in LC3II levels in 5FU treated samples. In addition, PFBL treatment alone or in combination with 5FU increased E-cadherin while decreasing the N-cadherin levels [Fig.4.8E-F]. This data indicated that PFBL induced CT26 tumor cell death by autophagy and reverses EMT in tumor cells in vivo.

4.2.9 In-vivo anti-metastatic activity of PFBL:

After observing the in vitro and in vivo anti-tumor activity of PFBL (Fig 4.8), we investigated whether PFBL can act as a potent anti-metastatic agent of CRC in vivo. For this, we tested PFBL in a CT26-induced lung metastasis model in BALB/c mice. We injected $1x10^5$ CT26 cells through the tail-vain in 30 mice and randomly segregated the mice into 3 groups (N=10) followed by 4 weeks of treatment with vehicle or 25 mg or 50 mg PFBL/kg body weight of mice on every alternative day. After four weeks, the mice were euthanized, and the

lungs were isolated. For both the lung metastasis model, the lungs in the 25 mg/kg PFBL and 50 mg/kg PFBL treatment groups were smaller than the control group, which has almost doubled the size of swelled lungs [Fig. 4.9A]. The number of visible lung nodules was counted and plotted by dot plot [Fig. 4.9B]. For CT26 lung metastasis, for the control group, 25 mg/kg PFBL and 50 mg/kg PFBL group, the average number of lung nodules were 58, 37, and 20, respectively. The H & E staining of lung tissue sections with lung nodules more dense regions in vehicle-treated compared to the PFBL treatment groups. The size of the dense regions decreased with increasing drug doses [Fig 4.8C]. Therefore, PFBL successfully suppressed lung metastasis of CT26 cells in vivo.

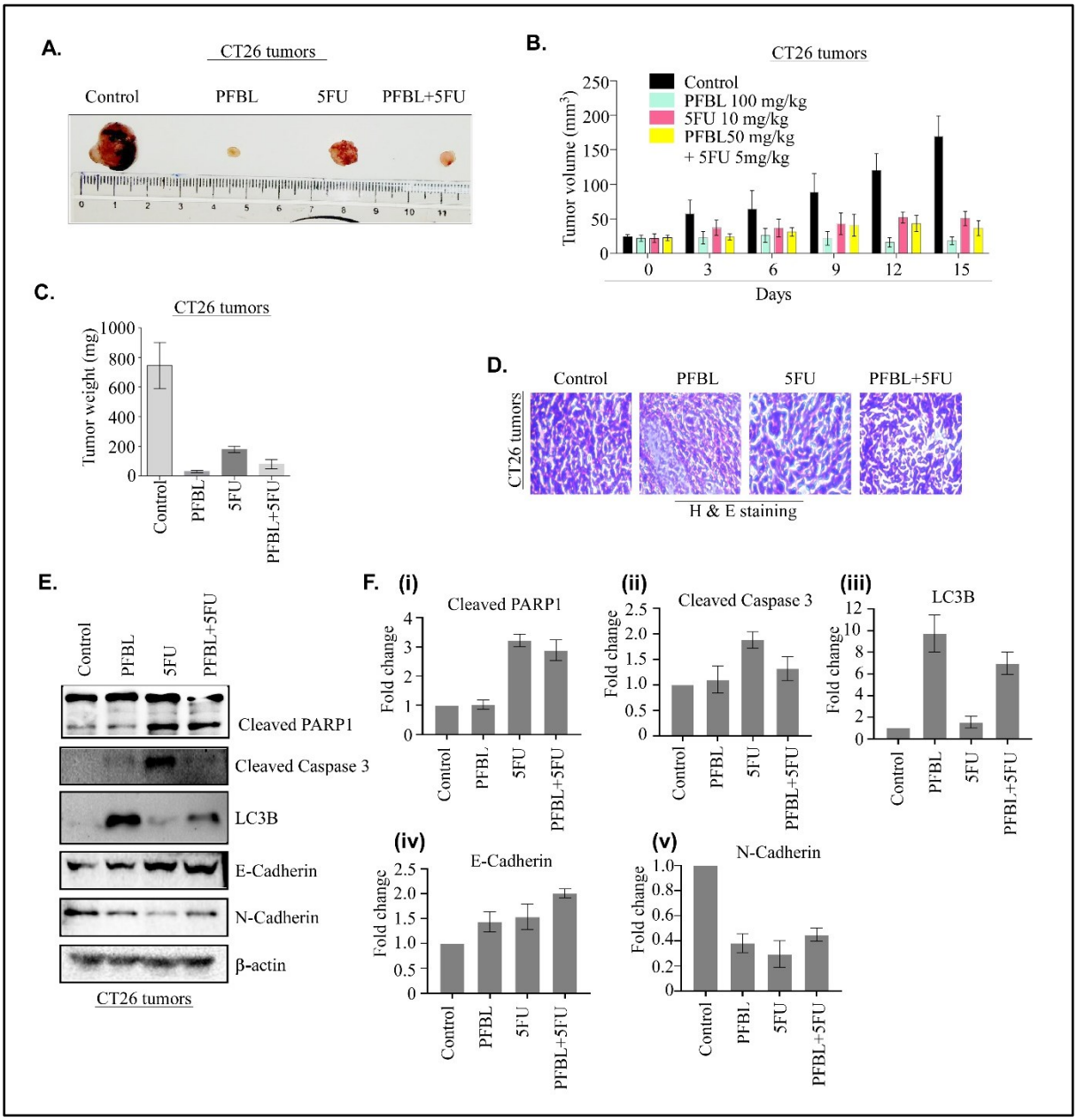

Figure 4.8: PFBL suppresses CT26 cell-induced solid tumor in BALB/c mouse model. [A] Representative image of tumors excised from BALB/c mice after treatment with the vehicle or PFBL alone or in combination with 5FU as indicated. **[B]** Bar graphs representing the changes in tumor volumes as measured during the treatment period. **[C]** Bar graphs representing the weights of the tumors after excision from the mice. **[D]** Representative images of haematoxylin and eosin-stained tumor sections. **[E]** Immunoblots of whole cell lysate extracted from tumor samples representing different apoptotic, autophagic, and EMT markers. The β-actin level was detected as a loading control. The fold change of signals in the blots was estimated by densitometric quantitation of indicated bands compared to the β-actin level, as shown in panel **[F].**

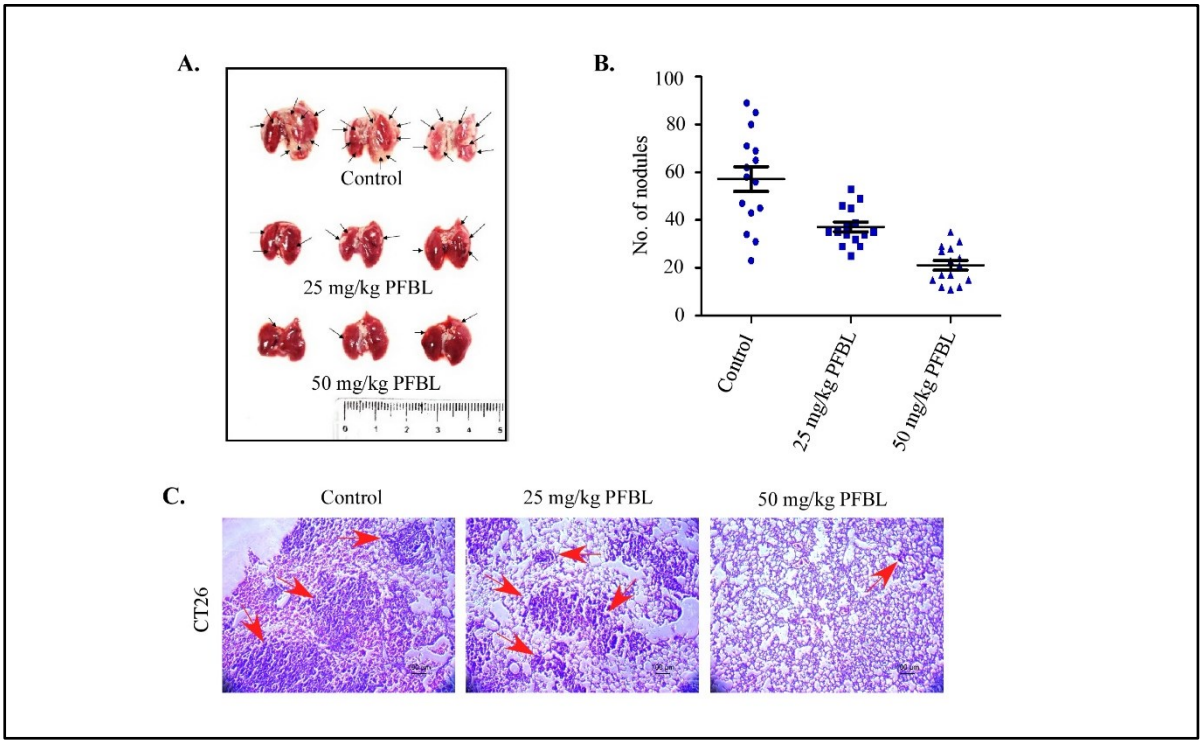

Figure 4.9: PFBL suppresses lung metastasis of CT26 cells in BALB/c mice model. [A] Representative images of lung nodules after treatment with vehicle or different concentrations of PFBL where the black arrows indicate the lung nodules. **[B]** Scatter plot representing the number of lung nodules in each experimental group after treatment with vehicle or different concentrations of PFBL as indicated. **[C]** Representative images of haematoxylin and eosin-stained lung sections of each experimental group where a red arrow indicates the lung nodules.

4.3 DISCUSSION:

The study revealed and analyzed the anti-CRC activity of PFBL. PFBL kills human HCT116 cells and CT26 tumor cells mainly through the induction of autophagy [Fig. 4.4, 4.8]. Over 40% of cells died by autophagy upon exposure to 40 µg/ml PFBL (IC50=38.8 µg/ml), which was mostly reversed by pre-treatment of cells with autophagy inhibitor chloroquine [Fig. 4.4D].

PFBL also induces cell death by apoptosis. However, compared to autophagy, the apoptotic cell population was increased relatively to a smaller extent with increasing doses of PFBL treatment of HCT116 cells [Fig. 4.2]. There were less than 5% apoptotic HCT116 cells when exposed to 50 µg/ml PFBL (IC_{50} 38.8) for 24 h, which was mostly reversed by pre-treatment with pan-caspase inhibitor Z-VAD-FMK.

While upregulation of apoptosis was always correlated with cancer cell death, autophagy was shown to play dual roles in cancer cells. Regulated autophagy was implicated in cancer cell survival under different stressful conditions, although probable uncontrolled/excessive autophagy led to cancer cell death (Dutta et al., 2022; Kocaturk et al., 2019).

Consistent with the in vitro results, autophagy as a key mediator in PFBL activity was revealed in in vivo analysis; there was major evidence of upregulation of autophagy markers in CRC death in vivo in contrast to the apoptosis markers [Fig. 4.8]. In contrast, apoptotic cell death was evidenced in the 5FU-induced death was revealed [Fig. 4.8E]. The major role of autophagy in the death of CRC exposed to phytochemicals was reported (Dutta a et al., 2022).

PFBL function in this pathway involves the increase of AMPK phosphorylation with subsequent inhibition of the mTOR pathway [Fig. 4.4]. The inhibition of mTORC1 was reflected by the inhibition of phosphorylation of its downstream targets S6K and 4EBP1 [Fig.4.4]. An energy sensor, AMPK, has been implicated in autophagy activation by inhibiting the mTOR pathway in response to various cellular stressors (Gwinn et al., 2008; Herrero-Martín et al., 2009; Inoki et al., 2003; Liang et al., 2007; Matsui et al., 2007; Meley et al., 2006; Vingtdeux et al., 2010). mTOR protein kinase has been implicated in various cellular aspects such as cell growth, proliferation, and autophagy. In particular, the correlation of mTOR complexes with tumor growth and metastasis underscored the significance of mTOR inhibition in cancer therapy (Hua et al., 2019). Cellular mTOR exists in two forms, mTORC1 and mTORC2. The mTORC1, through sensing growth factor signaling, plays a critical role in cellular autophagy (Dossou and Basu, 2019). Here, mTORC1 activity was monitored through monitoring its downstream targets 4EBP1 and S6K (Roux and Topisirovic, 2018). PFBL showed inhibitory activity against mTORC2, as evidenced by its inhibitory effect on AKT [Fig. 4.4].

In FACS analysis with PI staining, the G0/G1 cell population increased with increasing concentration of PFBL. PFBL treatment also downregulates CDK4 and cyclin D1 levels along

with increased phosphorylation of AMPK, indicating AMPK-mediated G0/G1 cell cycle arrest [Fig. 4.2] that was further confirmed by the effect of AMPK downregulation using shRNA [Fig. 4.6].

We found that the basis of the anti-tumor activity of PFBL is the elevation of intracellular ROS levels in HCT116 cells because pre-treatment of cells with NAC abrogates its function. Pre-treatment of cells with NAC inhibited the AMPK phosphorylation and cell death induced by PFBL treatment (Fig. 4.5]. ROS can upregulate AMPK activity (Auciello et al., 2014). This data indicates that PFBL-mediated ROS production is responsible for the AMPK activation. The most cancer chemotherapeutic agents at least partly rely on the upregulation of intracellular ROS as a mechanism of their function (Marullo et al., 2013).

In the PFBL-treated cells, the levels of N-cadherin, Snail, Twist, and Vimentin went down, while that of E-cadherin was upregulated. This data indicates that PFBL suppresses EMT and, thus, the metastatic potential of CRC cells. Reports linking AMPK activation with abrogation of EMT, and cancer cell metastasis are available (Dong et al., 2023; Han et al., 2018). The antimetastatic function of natural compounds with the activation of AMPK was reported (Peng et al., 2023).

Altogether, data indicates that PFBL kills colon cancer cells majorly by autophagy, and the observed anticancer activity is caused by the elevation of intracellular ROS levels. PFBL treatment activates AMPK which is a key mediator of PFBL mediated autophagic death of HCT116 cells. PFBL treatment also suppressed EMT in HCT116 cells. Similar to the in vitro results, PFBL regressed CT26 induced solid tumor by majorly induction of autophagy and suppressed lung metastasis in mice model.

References:

Auciello, F.R., Ross, F.A., Ikematsu, N., Hardie, D.G., 2014. Oxidative stress activates AMPK in cultured cells primarily by increasing cellular AMP and/or ADP. FEBS letters 588, 3361-3366.
Baehrecke, E.H., 2005. Autophagy: dual roles in life and death? Nature reviews Molecular cell biology 6, 505-510.
Carling, D., Mayer, F.V., Sanders, M.J., Gamblin, S.J., 2011. AMP-activated protein kinase: nature's energy sensor. Nature chemical biology 7, 512-518.
Codogno, P., Meijer, A.J., 2005. Autophagy and signaling: their role in cell survival and cell death. Cell Death & Differentiation 12, 1509-1518.
Covarrubias, L., Hernández-García, D., Schnabel, D., Salas-Vidal, E., Castro-Obregón, S., 2008. Function of reactive oxygen species during animal development: passive or active? Developmental biology 320, 1-11.

Das, C., Kumari, B., Singh, M.P., Singh, S., 2022. A Literary Review and Therapeutic Action of Pashanbheda (Bergenia ligulata Wall) described by Shamhita in Ashmari Roga. Journal of Ayurveda and Integrated Medical Sciences 7, 105-114.
Dix, B., Srivastava, S., 1989. Tannin constituents of Bergenia ligulata roots. Ind. J. Nat. Prod 5, 24-25.
Dong, Y., Hu, H., Zhang, X., Zhang, Y., Sun, X., Wang, H., Kan, W., Tan, M.-j., Shi, H., Zang, Y., 2023. Phosphorylation of PHF2 by AMPK releases the repressive H3K9me2 and inhibits cancer metastasis. Signal Transduction and Targeted Therapy 8, 95.
Dossou, A.S., Basu, A., 2019. The emerging roles of mTORC1 in macromanaging autophagy. Cancers 11, 1422.
Dutta, N., Pemmaraju, D.B., Ghosh, S., Ali, A., Mondal, A., Majumder, C., Nelson, V.K., Mandal, S.C., Misra, A.K., Rengan, A.K., 2022. Alkaloid-rich fraction of Ervatamia coronaria sensitizes colorectal cancer through modulating AMPK and mTOR signalling pathways. Journal of Ethnopharmacology 283, 114666.
Elmore, S., 2007. Apoptosis: a review of programmed cell death. Toxicologic pathology 35, 495-516.
Emerling, B.M., Weinberg, F., Snyder, C., Burgess, Z., Mutlu, G.M., Viollet, B., Budinger, G.S., Chandel, N.S., 2009. Hypoxic activation of AMPK is dependent on mitochondrial ROS but independent of an increase in AMP/ATP ratio. Free Radical Biology and Medicine 46, 1386-1391.
Garcia, D., Shaw, R.J., 2017. AMPK: mechanisms of cellular energy sensing and restoration of metabolic balance. Molecular cell 66, 789-800.
Ghosh, S., Dutta, N., Banerjee, P., Gajbhiye, R.L., Sareng, H.R., Kapse, P., Pal, S., Burdelya, L., Mandal, N.C., Ravichandiran, V., 2021. Induction of monoamine oxidase A-mediated oxidative stress and impairment of NRF2-antioxidant defence response by polyphenol-rich fraction of Bergenia ligulata sensitizes prostate cancer cells in vitro and in vivo. Free Radical Biology and Medicine 172, 136-151.
Gozuacik, D., Kimchi, A., 2004. Autophagy as a cell death and tumor suppressor mechanism. Oncogene 23, 2891-2906.
Gurav, S., Gurav, N., 2014. A Comprehensive review: Bergenia ligulata Wall-A controversial clinical candidate. Int J Pharm Sci Rev Res 5, 1630-1642.
Gwinn, D.M., Shackelford, D.B., Egan, D.F., Mihaylova, M.M., Mery, A., Vasquez, D.S., Turk, B.E., Shaw, R.J., 2008. AMPK phosphorylation of raptor mediates a metabolic checkpoint. Molecular cell 30, 214-226.
Halliwell, B., 2011. Free radicals and antioxidants–quo vadis? Trends in pharmacological sciences 32, 125-130.
Han, Y.-H., Kee, J.-Y., Hong, S.-H., 2018. Rosmarinic acid activates AMPK to inhibit metastasis of colorectal cancer. Frontiers in pharmacology 9, 68.
Hardie, D.G., 2007. AMP-activated/SNF1 protein kinases: conserved guardians of cellular energy. Nature reviews Molecular cell biology 8, 774-785.
Hardie, D.G., Carling, D., Gamblin, S.J., 2011. AMP-activated protein kinase: also regulated by ADP? Trends in biochemical sciences 36, 470-477.
Hardie, D.G., Ross, F.A., Hawley, S.A., 2012. AMPK: a nutrient and energy sensor that maintains energy homeostasis. Nature reviews Molecular cell biology 13, 251-262.
Herrero-Martín, G., Høyer-Hansen, M., García-García, C., Fumarola, C., Farkas, T., López-Rivas, A., Jäättelä, M., 2009. TAK1 activates AMPK-dependent cytoprotective autophagy in TRAIL-treated epithelial cells. The EMBO journal 28, 677-685.
Hua, H., Kong, Q., Zhang, H., Wang, J., Luo, T., Jiang, Y., 2019. Targeting mTOR for cancer therapy. Journal of hematology & oncology 12, 1-19.
Inoki, K., Zhu, T., Guan, K.-L., 2003. TSC2 mediates cellular energy response to control cell growth and survival. Cell 115, 577-590.
Kanzawa, T., Kondo, Y., Ito, H., Kondo, S., Germano, I., 2003. Induction of autophagic cell death in malignant glioma cells by arsenic trioxide. Cancer research 63, 2103-2108.
Kanzawa, T., Zhang, L., Xiao, L., Germano, I.M., Kondo, Y., Kondo, S., 2005. Arsenic trioxide induces autophagic cell death in malignant glioma cells by upregulation of mitochondrial cell death protein BNIP3. Oncogene 24, 980-991.
Kocaturk, N.M., Akkoc, Y., Kig, C., Bayraktar, O., Gozuacik, D., Kutlu, O., 2019. Autophagy as a molecular target for cancer treatment. European Journal of Pharmaceutical Sciences 134, 116-137.

Levine, B., Yuan, J., 2005. Autophagy in cell death: an innocent convict? The Journal of clinical investigation 115, 2679-2688.
Liang, J., Shao, S.H., Xu, Z.-X., Hennessy, B., Ding, Z., Larrea, M., Kondo, S., Dumont, D.J., Gutterman, J.U., Walker, C.L., 2007. The energy sensing LKB1–AMPK pathway regulates p27kip1 phosphorylation mediating the decision to enter autophagy or apoptosis. Nature cell biology 9, 218-224.
Marino, G., López-Otín, C., 2004. Autophagy: molecular mechanisms, physiological functions and relevance in human pathology. Cellular and Molecular Life Sciences CMLS 61, 1439-1454.
Mariño, G., Niso-Santano, M., Baehrecke, E.H., Kroemer, G., 2014. Self-consumption: the interplay of autophagy and apoptosis. Nature reviews Molecular cell biology 15, 81-94.
Marullo, R., Werner, E., Degtyareva, N., Moore, B., Altavilla, G., Ramalingam, S.S., Doetsch, P.W., 2013. Cisplatin induces a mitochondrial-ROS response that contributes to cytotoxicity depending on mitochondrial redox status and bioenergetic functions. PloS one 8, e81162.
Matsui, Y., Takagi, H., Qu, X., Abdellatif, M., Sakoda, H., Asano, T., Levine, B., Sadoshima, J., 2007. Distinct roles of autophagy in the heart during ischemia and reperfusion: roles of AMP-activated protein kinase and Beclin 1 in mediating autophagy. Circulation research 100, 914-922.
Meley, D., Bauvy, C., Houben-Weerts, J.H., Dubbelhuis, P.F., Helmond, M.T., Codogno, P., Meijer, A.J., 2006. AMP-activated protein kinase and the regulation of autophagic proteolysis. Journal of biological chemistry 281, 34870-34879.
Pandey, R., Kumar, B., Meena, B., Srivastava, M., Mishra, T., Tiwari, V., Pal, M., Nair, N.K., Upreti, D.K., Rana, T.S., 2017. Major bioactive phenolics in Bergenia species from the Indian Himalayan region: Method development, validation and quantitative estimation using UHPLC-QqQLIT-MS/MS. PloS one 12, e0180950.
Peng, B., Zhang, S.-Y., Chan, K.I., Zhong, Z.-F., Wang, Y.-T., 2023. Novel Anti-Cancer Products Targeting AMPK: Natural Herbal Medicine against Breast Cancer. Molecules 28, 740.
Reddy, U.D.C., Chawla, A.S., Deepak, M., Singh, D., Handa, S.S., 1999. High pressure liquid chromatographic determination of bergenin and (+)-afzelechin from different parts of Paashaanbhed (Bergenia ligulata yeo). Phytochemical Analysis: An International Journal of Plant Chemical and Biochemical Techniques 10, 44-47.
Redza-Dutordoir, M., Averill-Bates, D.A., 2016. Activation of apoptosis signalling pathways by reactive oxygen species. Biochimica et Biophysica Acta (BBA)-Molecular Cell Research 1863, 2977-2992.
Reef, S., Zalckvar, E., Shifman, O., Bialik, S., Sabanay, H., Oren, M., Kimchi, A., 2006. A short mitochondrial form of p19ARF induces autophagy and caspase-independent cell death. Molecular cell 22, 463-475.
Roux, P.P., Topisirovic, I., 2018. Signaling pathways involved in the regulation of mRNA translation. Molecular and cellular biology.
Ruby, K., Dwivedi, J., Chauhan, R., 2012. Pashanbheda a golden herb of Himalaya: a review. International Journal of Pharmacy Review & Research 2, 97-105.
Sung, H., Ferlay, J., Siegel, R.L., Laversanne, M., Soerjomataram, I., Jemal, A., Bray, F., 2021. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: a cancer journal for clinicians 71, 209-249.
Vingtdeux, V., Giliberto, L., Zhao, H., Chandakkar, P., Wu, Q., Simon, J.E., Janle, E.M., Lobo, J., Ferruzzi, M.G., Davies, P., 2010. AMP-activated protein kinase signaling activation by resveratrol modulates amyloid-β peptide metabolism. Journal of Biological Chemistry 285, 9100-9113.
Winterbourn, C.C., 2015. Are free radicals involved in thiol-based redox signaling? Free Radical Biology and Medicine 80, 164-170.
Xiao, B., Sanders, M.J., Carmena, D., Bright, N.J., Haire, L.F., Underwood, E., Patel, B.R., Heath, R.B., Xi, H., Wang, S., Wang, B., Hong, X., Liu, X., Li, M., Shen, R., Dong, Q., 2022. The role of interaction between autophagy and apoptosis in tumorigenesis. Oncology Reports 48, 1-16.
Walker, P.A., Hallen, S., 2013. Structural basis of AMPK regulation by small molecule activators. Nature communications 4, 1-10.

CHAPTER 5

STUDY OF ANTI-CANCER ACTIVITY OF POLY PHENOL RICH FRACTION OF BERGENIA LIGULATA RHIZOME AGAINST BREAST CANCER

5.1 INTRODUCTION:

Breast cancer is the major cause of cancer-related deaths in women. Per GLOBOCAN 2020, breast cancer has surpassed lung cancer as the most diagnosed cancer in women, with an estimated 2.3 million new cases globally with 69000 deaths. Overall, the cancer incidence was 2-3 fold higher in the developed countries than the developing countries. The death rate of female breast cancer was considerably higher in the developing countries than the developed ones. A 47% rise in global cancer burden has been predicted from 2020 to 2040. A 95% increase in cancer cases in nations with low HDI (Human Development Index), typically face challenges related to healthcare access, economic development, and other social indicators, which may contribute to the higher relative increase in cancer cases. Medium HDI countries are typically in a transitional phase in terms of economic and social development and may be undergoing rapid changes that affect cancer incidences, expected to experience a substantial relative increase in cancer cases with a 64% rise (Sung et al., 2021). According to a recent survey, about 0.3 million women will be newly diagnosed with breast cancer, with 43170 deaths in 2023 in United States (Siegel et al., 2023). Current treatment options include surgical removal of the tumor tissue followed by chemotherapy and or radiation therapy, endocrine therapy, immunotherapy, and adjuvant therapy (Waks and Winer, 2019). Sometimes, chemotherapy/radiation therapy precedes surgical removal of the tumor tissue. Although early detection usually results in a better prognosis. Metastasis is the prime cause of mortality and reoccurrence in patients with breast cancer worldwide (Hagemeister Jr et al., 1980). The development of drug resistance is another major barrier to breast cancer treatment. Nonspeficity of action or general toxicity of chemotherapeutics and radiation is a major issue with current treatment regimens with long term side effects. The high cost of the current treatment makes them unaffordable to many economically weaker sections in society. These limitations of the current treatments underscored the importance of finding alternate treatment strategies that will minimize the side effects by targeting only the cancer cells and making them affordable to all.

Notably, various studies, in vitro and in vivo, reported significant anti-cancer and anti-metastatic activities in various plant extracts, highlighting great potential for their use as chemotherapy agents alone or in combination with existing therapy regimens. *Bergenia ligulata* locally known as “Pashanbhed”, is known for its various medicinal properties, including anti-inflammatory and anti-cancer activities in Indian traditional and folk medicine.

Reactive Oxygen Species (ROS) such as superoxide anion ($O_2^{\cdot -}$), hydroxyl radical ($OH^{\cdot}$), and hydrogen peroxide (H_2O_2) are highly reactive molecules (Halliwell, 2011; Winterbourn, 2015). To cope with these ROS, cells carry an anti-oxidant defense system (Halliwell, 2011). However, a low level of ROS is required for normal cellular processes such as cellular growth, survival, and proliferation (Covarrubias et al., 2008; Shin and Cheong, 2019). A relatively higher level of ROS with defective antioxidant defense system can result in the death of cells (Halliwell, 2011; Redza-Dutordoir and Averill-Bates, 2016).

AMP-activated protein kinase (AMPK), is a prime sensor and effector of cellular energy metabolism to maintain the cellular homeostasis (Hardie, 2007). Increased concentration of cellular AMP level activates AMPK (Carling et al., 2011; Garcia and Shaw, 2017; Hardie et al., 2011; Hardie et al., 2012; Xiao et al., 2013). ROS was implicated in the control of the AMPK activity in MCF7 cells (Jeon et al., 2021) either by alteration of concentrations of adenine nucleotides (Auciello et al., 2014), or by AMP/ATP ratio independent manner (Emerling et al., 2009). ROS-mediated AMPK activation can induce apoptosis in MCF7 breast cancer cells (Kim et al., 2018). AMPK was reported can be activate autophagy in response to stressors, such as glucose starvation as well (Herrero-Martín et al., 2009; Liang et al., 2007; Matsui et al., 2007; Meley et al., 2006; Vingtdeux et al., 2010). AMPK induces autophagy through inhibiting mTOR (mammalian target of rapamycin) in a TSC2 (Inoki et al., 2003) and Raptor (Gwinn et al., 2008) dependent manner.

In this chapter, the anti-breast cancer activity of polyphenol-rich fraction of *B. ligulata* (PFBL) rhizome is presented.

The underlying molecular mechanism of PFBL action was investigated and confirmed using flow cytometry and western blots and validated in an in vivo mouse solid tumor model. To investigate the in vivo anti-metastatic properties of PFBL, a lung metastasis mouse model was used.

RESULTS:

5.2.1 Polyphenol-rich fraction of Bergenia ligulata (PFBL) is toxic to breast cancer cells:

The sensitivity of PFBL was tested by treating estrogen-dependent less metastatic breast cancer cell line MCF7 and hormone-independent MDAMB231 cell line with an increasing concentration of the fraction (25 μg/ml, 50 μg/ml, and 75 μg/ml) by MTT assay as described in materials and methods [Fig. 5.1A-F]. The IC50 values were also calculated. Results revealed that the MCF7 cells are more susceptible to PFBL (with an IC_{50} value of 52.23 μg/ml) in comparison to MDAMB231 cells (with an IC_{50} value of 58.68 μg/ml) [Fig. 5.1A]. The morphology of MCF7 cells gradually changed with increasing doses of PFBL. The colony-forming ability of MCF7 cells was gradually diminished with increasing PFBL concentration. At 25 μg/ml of the fraction, there was more than 50% loss of the clones [Fig. 5.1C-D]. In the wound healing assay, PFBL dose-dependently inhibited the migration potential of MCF7 cells [Fig. 5.1E-F].

5.2.2 PFBL treatment induces G0/G1 cell cycle arrest of MCF7 cells:

PFBL treatment arrests MCF7 cells in the G0/G1 phase as indicated by the dose-dependent increase of population in the G0/G1 phase of the cell cycle estimated by flow cytometry after propidium iodide (PI) staining [Fig. 4.2A-B]. Overall, there was a ~40% increase in the G0/G1 cell population of the cells. Western blots were performed to understand the mechanism of cell death in MCF7 cells after treatment with 50 μg/ml PFBL for different time points (12 h, 24 h, 36 h, and 48 h). There was a significant decrease in the CDK4 and Cyclin D1 protein levels [Fig. 5.2F-G].

5.2.3 PFBL induced apoptotic death of MCF7 cells:

Based on the occurrence of cells arrested in the G0/G1 phase, relative involvement of apoptosis in MCF7 cells were analyzed after exposing them to different concentration of PFBL (25 μg/ml, 50 μg/ml, 75 μg/ml, and 100 μg/ml). After 24 h of treatment, cells were stained with Annexin-V-FITC, followed by flow cytometry. The results revealed that cells were directed to apoptosis in a dose-dependent manner as suggested by 34%, 58%, 73%, and 88% apoptotic cell populations in response to treatment with 25 μg/ml, 50 μg/ml, 75 μg/ml, and 100 μg/ml of PFBL, respectively [Fig. 5.2C-E].

To understand the initiation path of the apoptotic signal, JC1 staining was performed after 24 h PFBL treatment. A shift of JC1 staining from red to green was observed in a dose-dependent manner under a fluorescent microscope, indicating a change in mitochondrial

membrane potential [Fig. 5.3A]. This suggested that PFBL drastically damaged the mitochondrial membrane permeability and interfered with the mitochondrial redox potential.

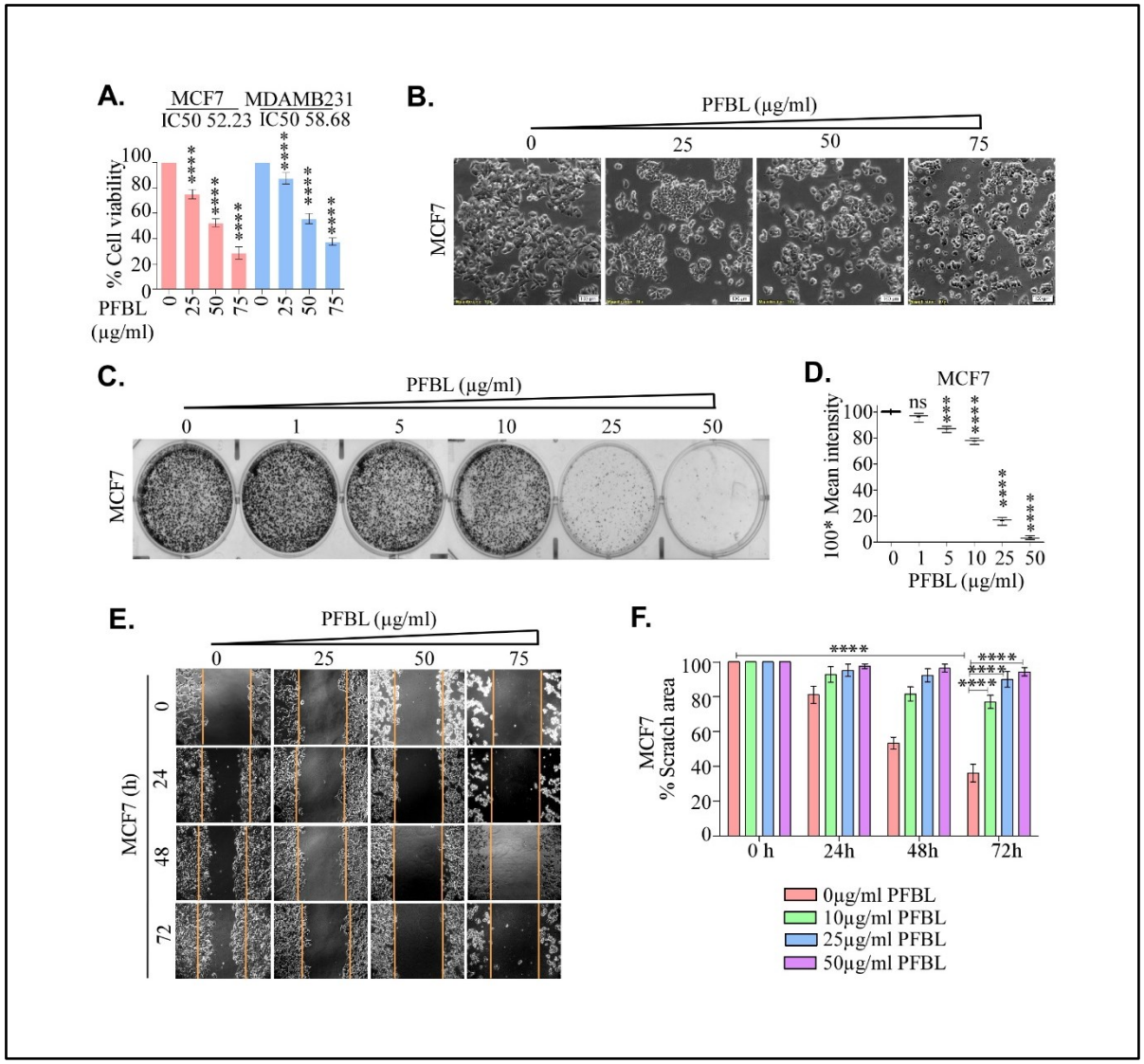

Figure 5.1: PFBL sensitizes breast cancer cells. [A] Bar graph representing the dose-dependent sensitivity of the cells (MCF7 and MDAMB231) determined by MTT assay after 24 h treatment. The IC_{50} values were shown. **[B]** Phase contrast images of cells treated with increasing concentrations of PFBL for 24 h, as indicated. **[C-D]** Colony-forming ability of cells counted after 24 h treatment. Cells were stained with crystal violet after fixing with formaldehyde **[E].** Phase contrast images showing the effect of the treatment on cellular migration potential estimated by wound healing assay. The quantification of the effect is represented by bar graph **[F].**

Western blots were performed to better understand the molecular pathway of cell death in MCF7 cells after treatment with 50 µg/ml PFBL for different time points (12 h, 24 h, 36 h, and 48 h). The results suggested significant changes in the markers of apoptotic signaling

pathways, such as cleavage in the PARP1, pro-caspase 7, pro-caspase 8, and pro-caspase 9 molecules. There was a decrease in pro-caspase 8 and pro-caspase 9 levels. The alterations in the levels of Bcl2, Bid, Survivin, and Bax were observed [Figure 5.2F-G]. To verify the weightage of caspase-dependent apoptotic death, cells were pre-treated for 3 h with pan-caspase inhibitor Z-VAD-FMK before treatment with 50 μg/ml PFBL for 24 h. The effect of Z-VAD-FMK pre-treatment was analyzed by FACS (after dual staining with Annexin-V-FITC and PI), MTT assay, and western blotting [Fig. 5.3B-F]. Results revealed that pre-treatment with Z-VAD-FMK reduced the apoptotic cell population and PARP1 cleavage and provided about 40% protection from cell death, indicating the share of apoptosis in the killing mechanism [Fig. 5.3B-F] and indicating that caspase-dependent apoptosis is the major death mechanism.

5.2.4 PFBL induces autophagic death of MCF7 cells:

We already found the crucial invlvement of apoptosis in PFBL-induced death of MCF7 cells [Fig. 5.3]. There was still ~10% cell death even after pre-treatment withpan-caspase inhibitor before exposure to PFBL. So, we tested for the involvement of autophagy in these cells' death. Autophagy can be activated in the cell by different stimuli that can ultimately lead to autophagosome formation, and the cleavage of LC3II proteins that occur after insertion in autophagosomes can be used as an autophagy marker. For this, LC3II-GFP plasmid was transfected in MCF7 cells, followed by treatment with increasing concentrations of PFBL. Next, the presence of LC3II-GFP puncta formation was monitored by confocal microscopy [Fig. 5.4A]. The percentage of cells with LC3II-GFP puncta increased with increasing concentration of PFBL. Consistently, there was a gradual increase in the LC3BII level detected by western blotting using 50 μg/ml PFBL for different time points (12 h, 24 h, 36 h, and 48 h) [Fig. 5.4B-C]. To check the involvement of the mTOR pathway in PFBL-induced autophagy, different upstream and downstream protein levels of this pathway were checked. The phosphorylation level of 4EBP1, S6K, and AKT was downregulated, whereas the phosphorylation level of AMPK (at T172) was upregulated [Fig. 5.4B-C] indicating mTOR inhibition. To verify the degree of involvement of autophagy in PFBL-induced death, cells were pre-treated with autophagy inhibitor chloroquine for 3 h before treatment with PFBL for 24 h analyzed by MTT assay [Fig. 5.4D]. Chloroquine pre-treatment provided 10% to 15% protection from toxicity induced by PFBL, indicating the involvement of autophagy in PFBL-induced death of MCF7 calls [Fig. 5.4D].

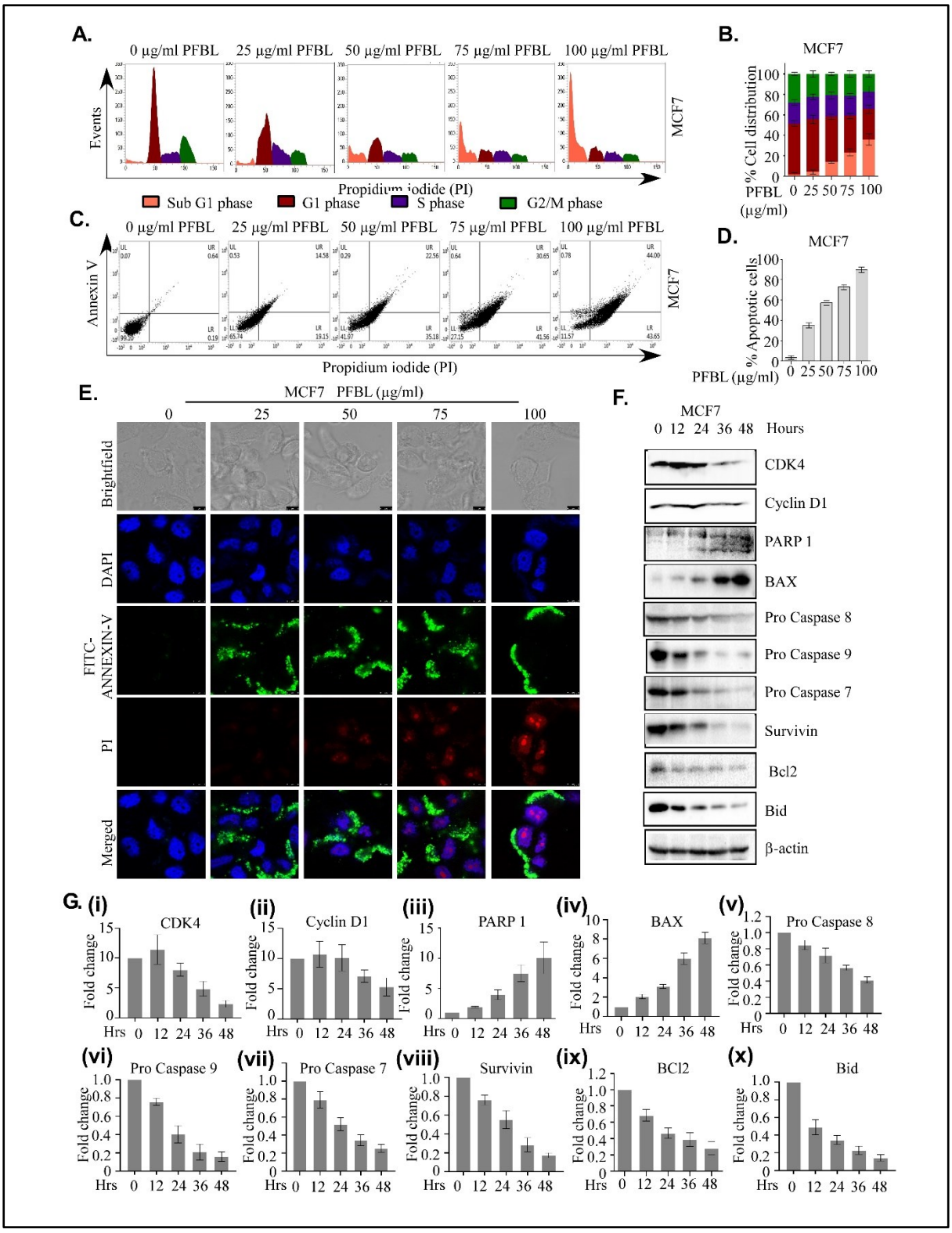

Figure 5.2: PFBL arrests cells at G0/G1 phase and induces apoptosis. [A] Histograms showing the effect of different PFBL treatments (with the indicated concentration for 24 h) on cell cycle phases generated by FACS analysis. **[B]** Bar diagram representing the distribution of cells in different cell cycle phases upon treatment as shown. **[C]** FACS assay representing the status of FITC-Annexin-V and/or PI positive cell population upon treatment (PFBL at 50 µg/ml) as shown. The quantitation of the assay is shown in a bar graph **[D]**. **[E]** Confocal

images captured after exposure of cells to increasing concentrations of PFBL for 24 h followed by staining with FITC-Annexin-V/PI/DAPI. **[F]** Immunoblots of lysates off cells pre-treated with PFBL for different duration with different cellular apoptotic markers. β-actin level was determined as an internal loading control. Densitometric quantitation of bands relative to β-actin is shown by bar graphs **[G]**.

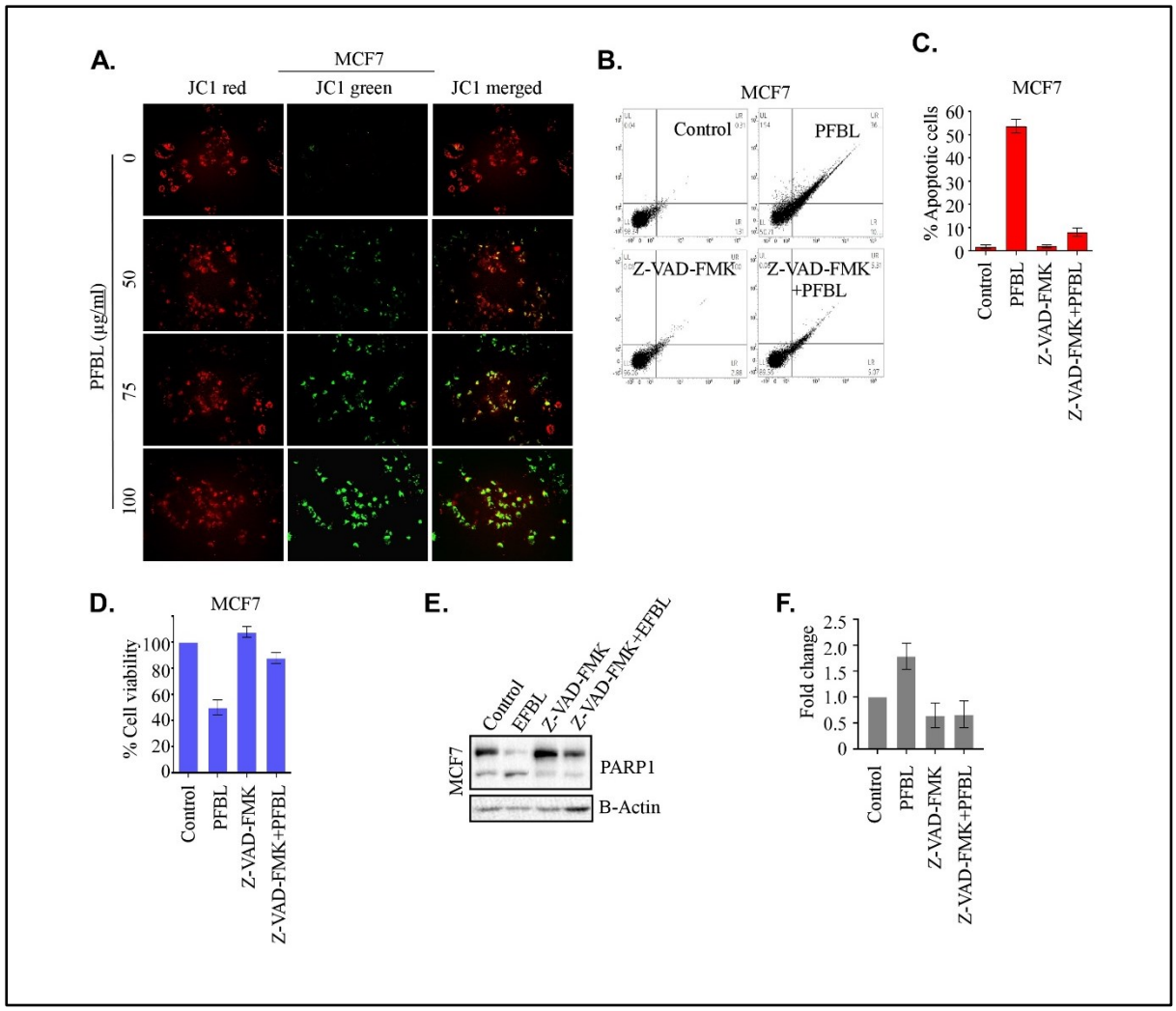

Figure 5.3: PFBL induces apoptosis by mitochondrial dysfunction in MCF7 cells. [A] Fluorescence microscopy images representing the change in mitochondrial permeability (from red to green shift) upon treatment with increasing concentrations of PFBL (24 h). **[B]** FITC-Annexin-V and/or PI positive population of PFBL treated cells pre-treated with or without pan-caspase inhibitor Z-VAD-FMK for 3 h assayed by flow cytometry. Quantitation of the apoptotic cell population is shown by a bar graph **[C]**. **[D]** Bar graph representing the viability (MTT assay) of cells upon treatment with PFBL. Cells pre-treated for 3 h with pan-caspase inhibitor Z-VAD-FMK as indicated. **[E]** Immunoblots of lysates off PFBL treated cells. Cells that were pre-treated or not with Z-VAD-FMK for 3 h are indicated. The β-actin level was determined as a loading control. **[F]** Bar graph representing the fold change of PARP1 level relative to β-actin level measured by densitometric quantitation of bands in the immunoblot shown in panel [E].

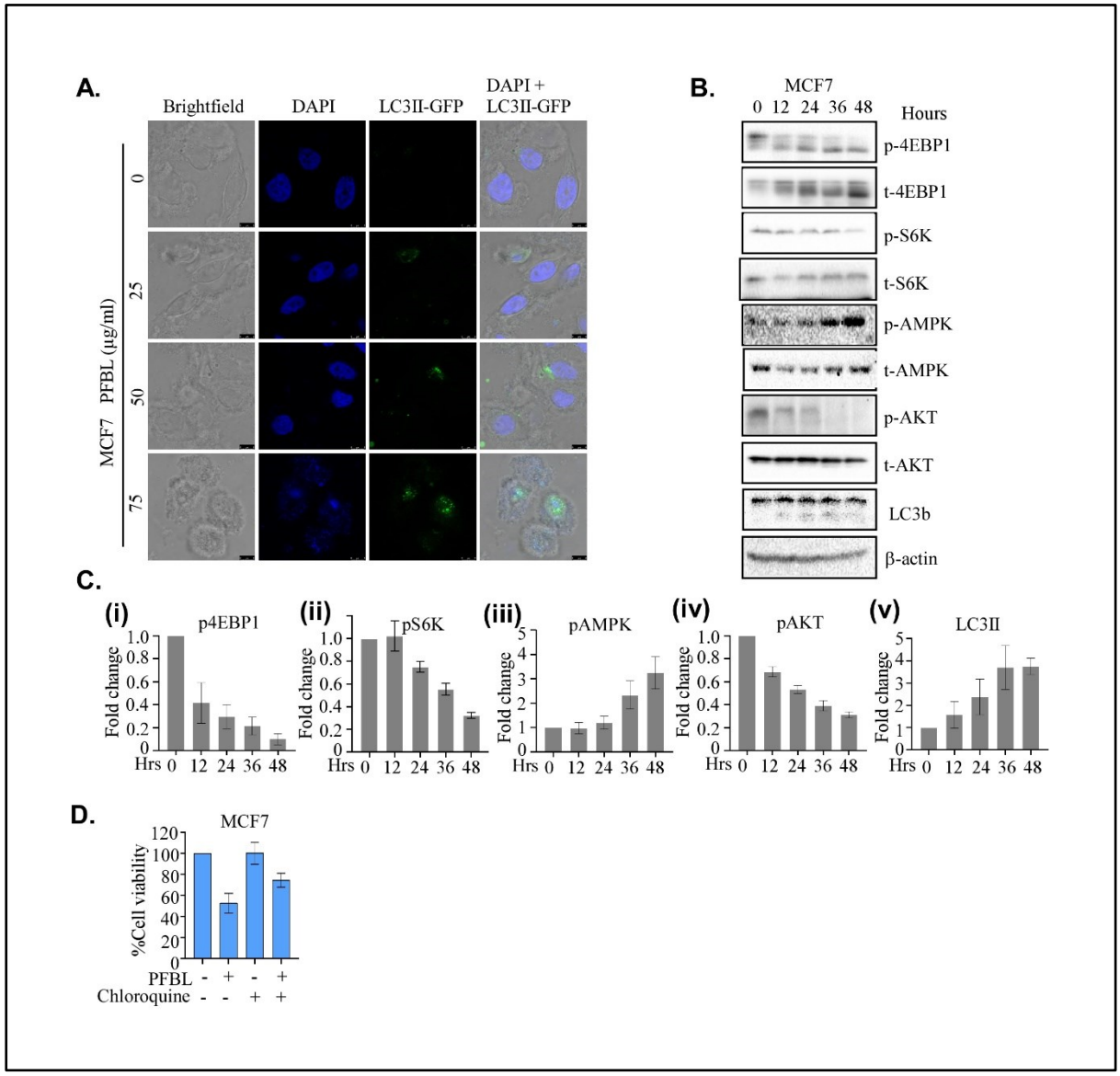

Figure 5.4: PFBL induces death in MCF7 cells by autophagy: [A] Confocal images of LC3II-GFP transfected cells treated with increasing concentrations of PFBL for 24 h. Cells stained with DAPI are also indicated. **[B]** Immunoblots of lysates of cells treated with PFBL for different durations with 50 µg/ml. The β-actin level was detected in the same blot as the loading control as indicated. The fold change of signals in the blots was estimated by densitometric quantitation of bands compared to the β-actin level **[C]. [D]** Cell viability (MTT) assay indicating the role of autophagy (as chloroquine-mediated protection) in PFBL-induced death.

5.2.5 PFBL-induced cell death is dependent on ROS production in MCF7 cells:

PFBL treatment increases the production of reactive oxygen species (ROS) that is visualized by a fluorescence microscope and FACS upon DCFDA staining [Fig. 5.5A-B]. The PFBL-mediated ROS production in MCF7 cells was ameliorated upon pre-treatment with quenching agent N-acetylcysteine (NAC) [Fig. 5.5A-B]. Consistently, NAC pre-treatment

significantly restored cell viability in PFBL-treated cells as measured by MTT assay [Fig. 5.5C]. Pre-treatment with NAC not only increased cell survival but also suppressed autophagy and apoptosis as indicated by decreased LC3BII and decreased PARP1 level upon combined NAC and PFBL treatment compared with only PFBL treatment detected by western blotting [Fig. 5.5D-E]. NAC pre-treatment also reduced the phosphorylation of AMPK close to the untreated level. This data shows that PFBL induced cellular ROS production is the major cause of cell death. PFBL induces the production of reactive oxygen species (ROS) as detected by DCFDA staining under fluorescence microscope. The amount of ROS production is measured by FACS. The PFBL-mediated ROS production in breast cancer cells was ameliorate upon pre-treatment with NAC. Pre-treatment with NAC decreases PARP1 cleavage.

5.2.6 AMPK as a key mediator of PFBL action:

We have already observed a dose-dependent increase of phosphorylation at T172 residue (activatory phosphorylation) of AMPK upon PFBL treatment [Figure 5.4B]. The involvement of AMPK in PFBL-induced cell death was further investigated by shRNA-mediated knockdown of AMPK. Cell viability of PFBL-treated cells increased in AMPK downregulated samples measured by MTT assay [Fig. 5.6A]. Consistently, western blot results revealed that PFBL action was diminished in AMPK down-regulated cells [Fig. 5.6B-C]. AMPK knockdown restored cellular CDK4 and Cyclin D1 protein levels that were downregulated upon PFBL treatment, indicating the involvement of AMPK in PFBL-induced G0/G1 cell cycle arrest. AMPK downregulation also reduced the cellular levels of cleaved-PARP1 and LC3II [Fig. 5.6B-C].

5.2.7 PFBL reverses epithelial to mesenchymal transition in MCF7 cells:

Epithelial-to-mesenchymal transition (EMT) is a crucial step in cancer metastasis. Cancer cells become more aggressive, migratory, and invasive due to enhanced mesenchymal properties. In metastatic cancer cells, E-cadherin expression decreases while the N-cadherin expression increases. In our earlier study, we found that PFBL inhibited colony forming ability and migration potential of MCF7 cells in a dose-dependent manner [Fig. 5.1C-F]. So, we tested the metastatic potential of MCF7 cells after treatment with 50 μg/ml PFBL for different time points (12 h, 24 h, 36 h, and 48 h) by monitoring different epithelial and mesenchymal markers by immunoblots. Results suggested that PFBL decreased mesenchymal markers such as N-cadherin, Snail, Twist, and Vimentin with simultaneous upregulation of the expression of epithelial markers such as E-cadherin in a time-dependent manner, as shown [Fig. 5.7A-B]. These results indicated that PFBL suppresses metastatic potentials of the MCF7 cells by suppressing the EMT.

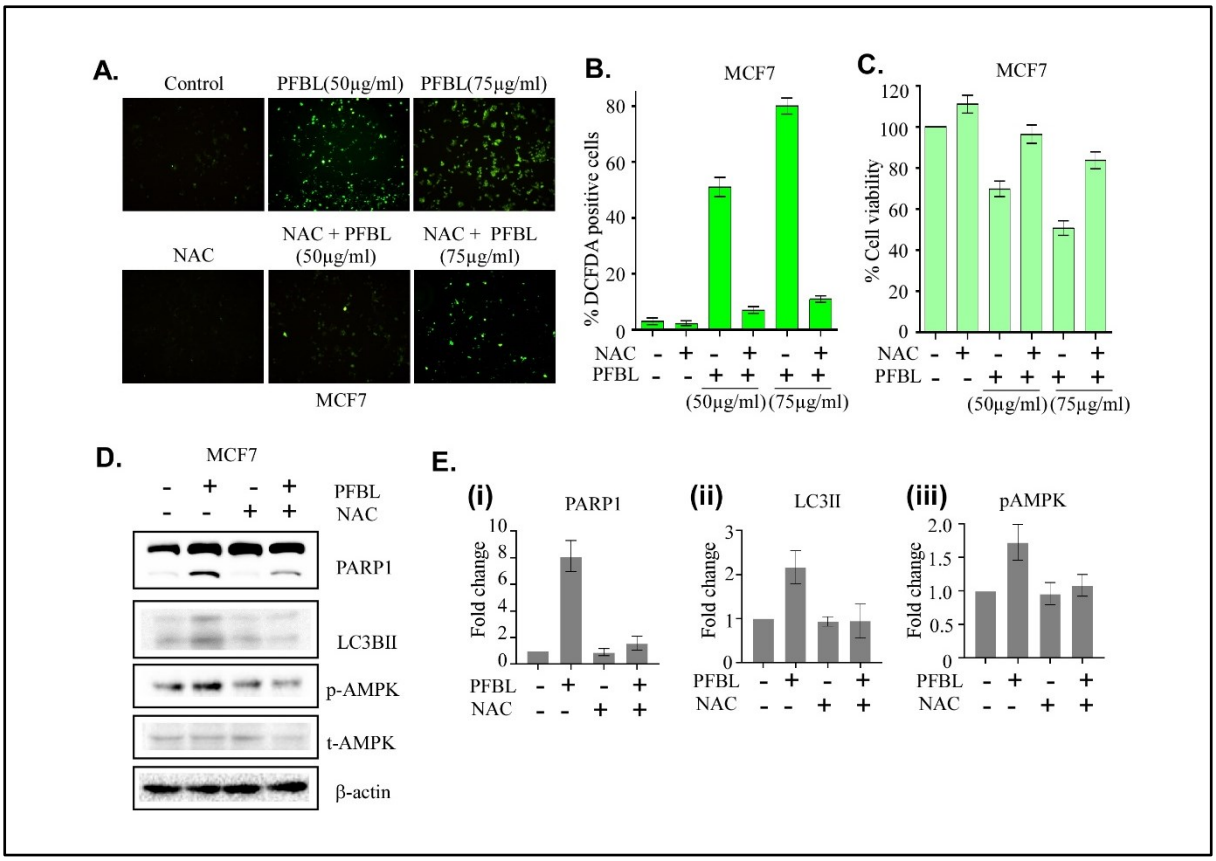

Figure 5.5: PFBL induces ROS generation is responsible for the death of MCF7 cell. [A] Fluorescence microscopy images of MCF7 cells treated for 24 h with PFBL with or without NAC pre-treatment for 4 h. The cells were stained with DCFDA before imaging. The DCFDA positive and viable cell population in the assay are shown in bar graphs in panels **[B]** and **[C],** respectively, as indicated. **[D]** Immunoblots of lysates of PFBL-treated cells with or without pre-treatment with NAC for 4 h with cell markers. The β-actin level was detected as a loading control. The fold change of signals in the blots was estimated by densitometric quantitation of indicated bands compared to the β-actin level, as shown in panel **[E].**

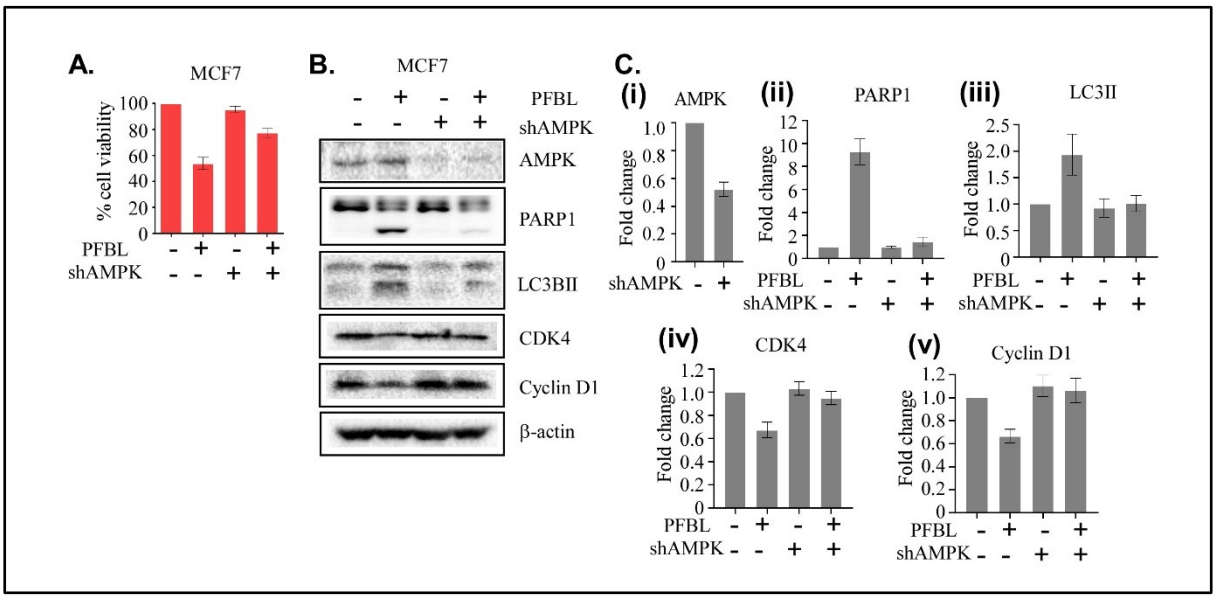

Figure 5.6: shRNA-mediated down regulation of AMPK diminishes the effect of PFBL. [A] Bar graph representing the effect of AMPK downregulation in PFBL-induced cell death determined by MTT assay. **[B]** Immunoblots representing the effect of AMPK downregulation in PFBL treatment on apoptotic and autophagy pathways. The β-actin level was detected as a loading control. The fold change of signals in the blots was estimated by densitometric quantitation of indicated bands compared to the β-actin level, as shown in panel **[C].**

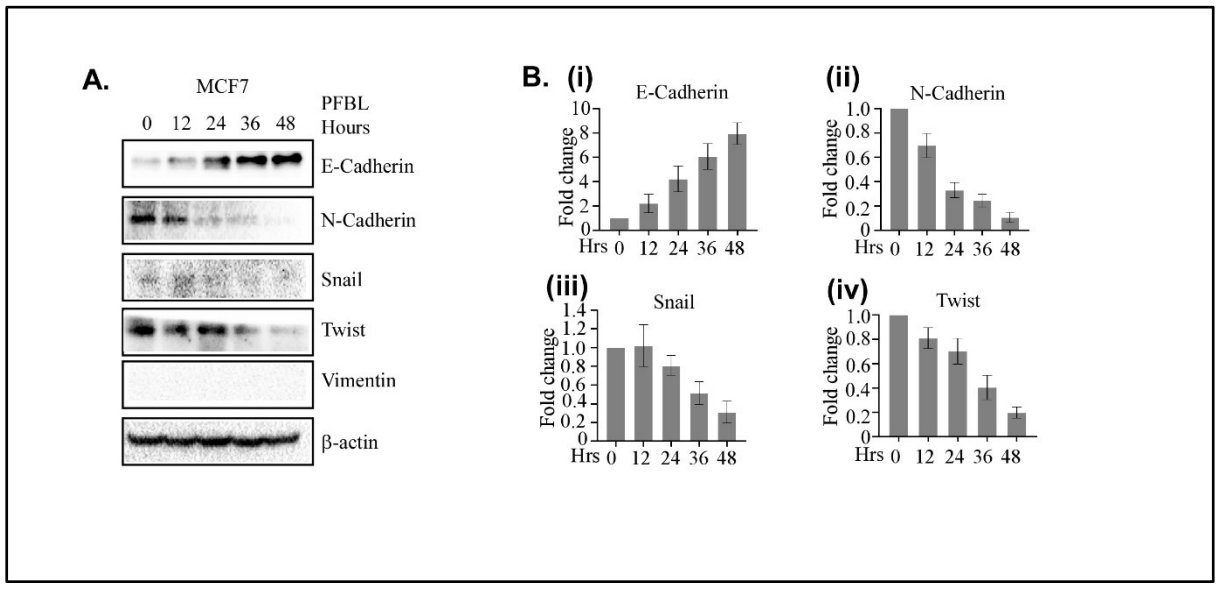

Figure 5.7: PFBL inhibits epithelial to mesenchymal transition in MCF7 cells. [A] Immunoblots of lysates of cells treated with PFBL for 24 h for different cellular markers as indicated. The β-actin level was detected as a loading control. **[B]** The fold change of signals in the blots was estimated by densitometric quantitation of indicated bands compared to the β-actin level as indicated.

5.2.8 In vivo antitumor activity of PFBL:

The results of an efficient anticancer activity of PFBL against the MCF7 cells led us to test its potential for anti-breast cancer activity in vivo. So, we investigated the anti-tumor effect of PFBL on the growth of mouse tumor xenograft, i.e., mouse breast cancer cell line 4T1 derived subcutaneous solid tumor harbored in BALB/c mice, alone as well as in combination with DOX. DOX is used in the current breast cancer treatment regimen in the clinic. Mice with palpable tumors (1 week after injection of $1x10^6$ 4T1 cells) were randomly divided into four groups (n=5). For 4T1 tumor-bearing mice, each group received either vehicle or PFBL or DOX or a combination of DOX and PFBL. The treatment was repeated every alternative day for 15 days, and the tumor size was measured on every treatment day. After completion of the treatment period, the mice were sacrificed, and tumors were excised and measured. Results indicated that PFBL, DOX, and combination treatment regressed 4T1 tumors by 88%, 80%,

and 90%, respectively [Fig. 5.8A-C]. The change in animal body weight throughout the treatment period was recorded and plotted [Fig. 5.8B]. The change in the tumor weight was also prominent [Fig.5.8C]. The H & E staining of tumor tissue sections distinctly indicated the clearer regions in treated samples compared to denser regions in vehicle-treated tumors [Fig 5.8D]. Immunoblot with tumor tissue lysate indicated a similar mechanism of cell death as observed in the case of MCF7 cells treated with PFBL. There is a significant change in the PARP1 cleavage band. In protein level in samples treated with PFBL, DOX alone, or in DOX and PFBL, there was about 6-fold, 7-fold, and 8-fold increase in PARP1 cleaved band, respectively. For cleaved caspase 3 levels also increased by about 2-fold, 2.5-fold and 3.5-fold, respectively. There was about 2-fold increase in LC3II protein level in PFBL alone and PFBL and DOX combined treated lysates. In addition, PFBL treatment alone or in combination with DOX increased E-cadherin while decreasing the N-cadherin levels [Fig.5.8E-F]. This data indicated that PFBL induced 4T1 tumor cell death by both apoptosis and autophagy. PFBL also reversed EMT in tumor cells in vivo.

5.2.9 In vivo anti-metastatic activity of PFBL:

After observing the in vitro and in vivo anti-tumor activity of PFBL (Fig 4.8), we investigated whether PFBL can act as a potent anti-metastatic agent for breast cancer treatment in vivo. For this, we tested PFBL in a 4T1-induced lung metastasis model in BALB/c mice. We injected $1x10^5$ 4T1 cells through the tail-vain in 30 mice and randomly segregated the mice into 3 groups (n=10) followed by 4 weeks of treatment with vehicle or 25 mg or 50 mg PFBL/kg body weight of mice on every alternative day. After four weeks, the mice were euthanized, and the lungs were isolated. For both the lung metastasis model, the lungs in the 25 mg/kg PFBL and 50 mg/kg PFBL treatment groups were smaller than the control group, which has almost doubled the size of swelled lungs [Fig. 5.9A]. The number of visible lung nodules was counted and plotted by dot plot [Fig. 5.9B]. For 4T1 lung metastasis, for the control group, 25 mg/kg PFBL and 50 mg/kg PFBL group, the average number of lung nodules were 67, 18, and 11 respectively. The H & E staining of lung tissue sections with lung nodules more dense regions in vehicle-treated compared to the PFBL treatment groups. The size of the dense regions decreased with increasing drug doses [Fig 5.9C]. Therefore, PFBL successfully suppressed lung metastasis of 4T1 cells in vivo.

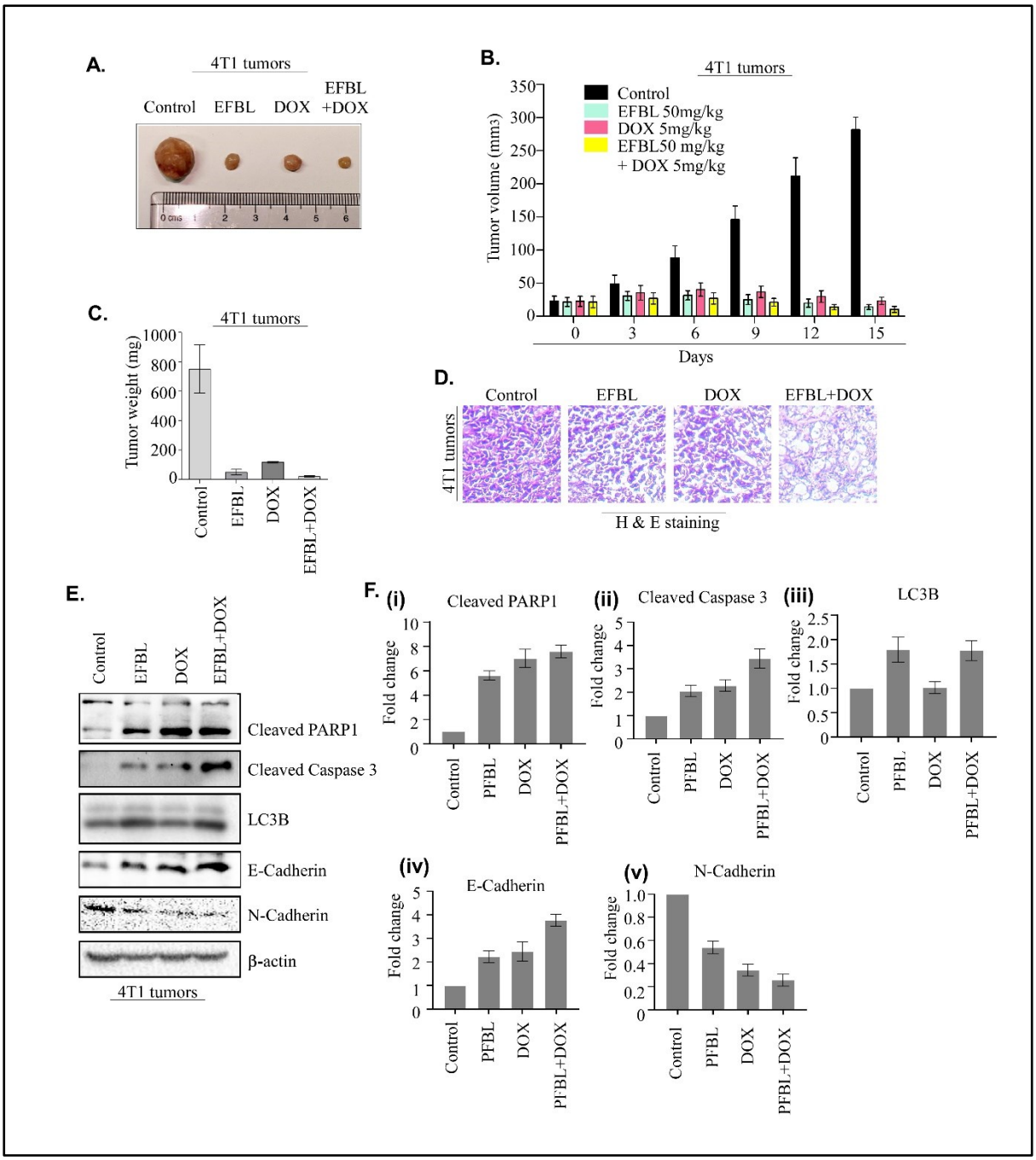

Figure 5.8: PFBL suppresses 4T1 cell induced solid tumor in BALB/c mouse model. [A] Representative image of tumors excised from BALB/c mice after treatment with vehicle or PFBL alone or in combination with DOX as indicated. **[B]** Bar graph representing the changes in tumor volumes as measured during the treatment period. **[C]** Bar graph representing the weights of the tumors after excision from the mice. **[D]** Representative images of haematoxylin and eosin-stained paraffin-embedded tumor sections. **[E]** Immunoblots of whole cell lysate extracted from tumor samples representing different apoptotic, autophagic, and EMT markers. The β-actin level was detected as a loading control. The fold change of signals in the blots was estimated by densitometric quantitation of indicated bands compared to the β-actin level as shown in panel **[F].**

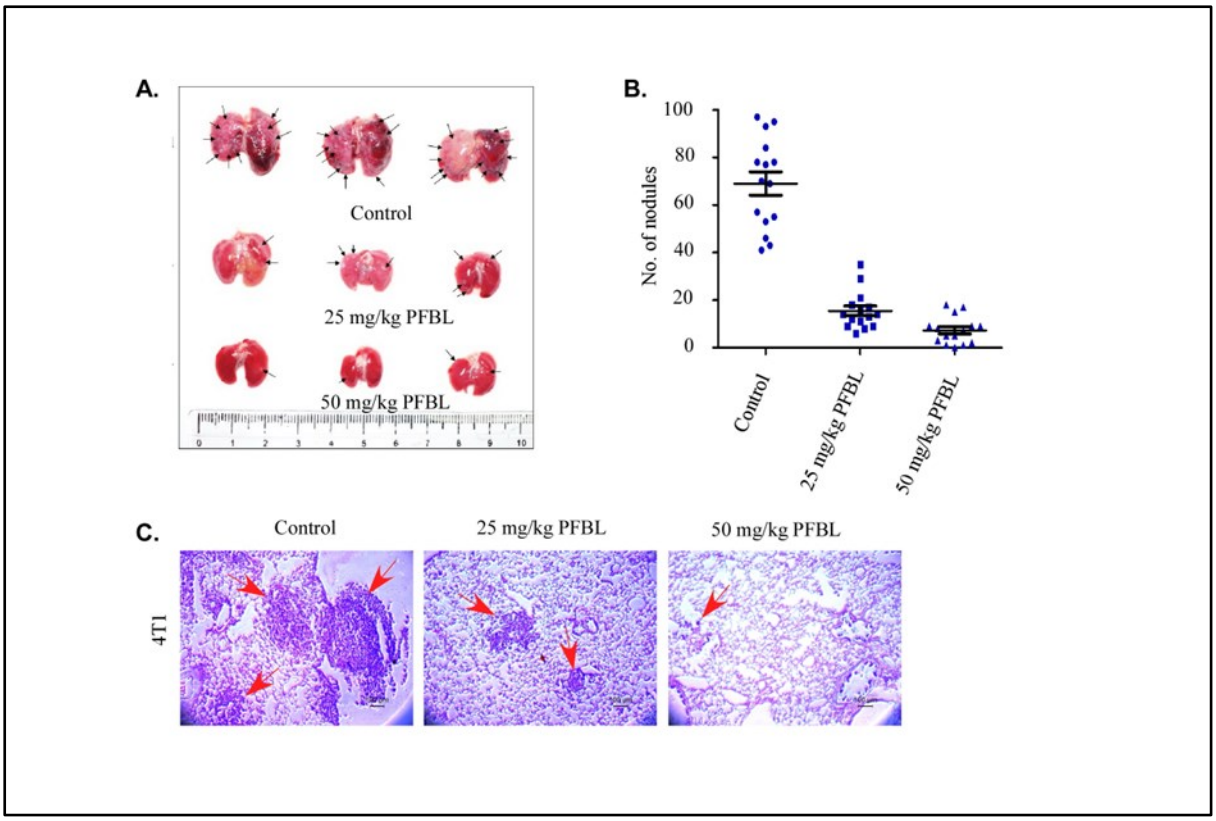

Figure 5.9: PFBL suppresses lung metastasis of 4T1 cells in BALB/c mice model. [A] Representative images of lung nodules after treatment with the vehicle or different concentrations of PFBL where the black arrows indicate the lung nodules. **[B]** Scatter plot representing the number of lung nodules in each experimental group after treatment with vehicle or different concentrations of PFBL as indicated. **[C]** Representative images of haematoxylin and eosin stained paraffin-embedded lung sections of each experimental group where a red arrow indicates the lung nodules.

5.3 DISCUSSION:

Our study analyzed the anti-breast cancer activity of PFBL. Results suggested that PFBL kills human MCF7 cells and 4T1-induced tumor cells mainly through the induction of apoptosis [Fig. 5.2, 5.8]. About 40% of MCF7 cells died of apoptosis, which was reversed by pre-treatment cells with Z-VAD-FMK. Almost 10% of cells died by autophagy upon exposure to 50 µg/ml PFBL (IC50=52.23 µg/ml), which was mostly reversed by pre-treatment of cells with autophagy inhibitor chloroquine [Fig. 5.4D].

PFBL induces MCF7 cell death mainly by apoptosis. However, compared to apoptosis, the autophagic population (as denoted by LC3B-GFP puncta formation and western data) was also increased although relatively to a smaller extent, with increasing doses of PFBL treatment of MCF7 cells [Fig. 5.4]. There were almost 55% apoptotic MCF7 cells when exposed to 50

μg/ml PFBL (IC_{50} 52.23) for 24 h, which was mostly reversed by pre-treatment with pan-caspase inhibitor Z-VAD-FMK (Fig. 5.3).

While upregulation of apoptosis was always correlated with cancer cell death, autophagy was shown to play dual roles in cancer cells. Regulated autophagy was implicated in cancer cell survival under different stressful conditions, although uncontrolled/excessive autophagy supposedly led to cancer cell death (Muhammad et al., 2017; Stellrecht et al., 2014; Wong et al., 2017).

Consistent with the in vitro results, the major role of apoptosis in PFBL activity was revealed in in vivo analysis; there was major evidence of upregulation of apoptotic markers in 4T1 solid tumor in vivo in contrast to the autophagic markers [Fig. 5.8]. Evidence of apoptosis in the DOX-induced death was revealed [Fig. 5.8E]. The major role of apoptosis in the death of breast cancer exposed to phytochemicals was reported elsewhere (Ahn et al., 2020; Ren et al., 2018; Stellrecht et al., 2014).

PFBL function in this pathway involves the increase of AMPK phosphorylation with subsequent inhibition of the mTOR pathway [Fig. 5.4]. The inhibition of mTORC1 was reflected by the inhibition of phosphorylation of its downstream targets S6K and 4EBP1 [Fig.5.4]. An energy sensor, AMPK, has been implicated in apoptosis and autophagy activation by inhibiting the mTOR pathway in response to various cellular stressors (Ahn et al., 2020; Ren et al., 2018; Stellrecht et al., 2014). mTOR protein kinase has been implicated in various cellular aspects such as cell growth, proliferation, apoptosis, and autophagy. In particular, the correlation of mTOR complexes with tumor growth and metastasis underscored the significance of mTOR inhibition in cancer therapy (Hua et al., 2019). Cellular mTOR exists in two forms, mTORC1 and mTORC2. The mTORC1, through sensing growth factor signalling, plays a critical role in cellular autophagy (Dossou and Basu, 2019). Here, mTORC1 activity was monitored through monitoring its downstream targets 4EBP1 and S6K (Roux and Topisirovic, 2018). PFBL also showed an inhibitory activity against mTORC2, as evidenced by its inhibitory effect on AKT [Fig. 5.4].

In FACS analysis with PI staining, the G0/G1 cell population increased with increasing concentration of PFBL. PFBL treatment also downregulates CDK4 and cyclin D1 levels along with increased phosphorylation of AMPK, indicating AMPK-mediated G0/G1 cell cycle arrest [Fig. 5.2] that was further confirmed by the effect of AMPK downregulation using shRNA

[Fig. 5.6]. It has already been reported that AMPK can arrest cells at G0/G1 phase by downregulating CDK4 and cyclin D1 (Wang et al., 2018).

Based on the present study the anti-tumor activity of PFBL is mediated by the elevation of intracellular ROS levels in MCF7 cells which is diminished by pre-treatment of cells with NAC. Pre-treatment of cells with NAC inhibited the AMPK phosphorylation and cell death induced by PFBL treatment [Fig. 5.5]. ROS can upregulate AMPK activity (Auciello et al., 2014). This data indicates that PFBL-mediated ROS production is responsible for the AMPK activation. The most cancer chemotherapeutic agents at least partly rely on the upregulation of intracellular ROS as a mechanism of their function (Marullo et al., 2013). NAC pre-treatment also diminishes the PFBL-induced PARP1 cleavage and inhibits the LC3B [Fig. 5.5]. It is already reported that ROS can activate both apoptotic and autophagic pathways simultaneously (Redza-Dutordoir and Averill-Bates, 2016) (Mariño et al., 2014). This data confirms the role of PFBL-induced ROS production in apoptotic and autophagic death of MCF7 cells.

In the PFBL-treated cells, the levels of N-cadherin, Snail, Twist, and Vimentin went down, while that of E-cadherin was upregulated [Fig 5.7, 5.8]. This data indicates that PFBL suppresses EMT and, thus, the metastatic potential of breast cancer cells. Reports linking AMPK activation with abrogation of EMT, and cancer cell metastasis are available (Dong et al., 2023). The antimetastatic function of natural compounds with the activation of AMPK was reported (Peng et al., 2023).

For the first time, we showed that a polyphenol rich fraction from Bergenia ligulata has potent anti-breast cancer and anti-metastatic properties in preclinical models. PFBL treatment suppresses alone or in combination with DOX, the 4T1 induced solid tumor developed in BALB/c mice model. PFBL also induced PARP1 cleavage in tumor tissue. LC3II levels are high in tumor tissue treated with PFBL. The N-cadherin level was reduced and E-cadherin level was increased in mice tumor tissue upon PFBL treatment. Further, the number of 4T1-associated lung nodules and lung size was decreased with increasing concentrations of PFBL, indicating PFBL has anti-metastatic properties.

References:

Ahn, J., Kim, H., Yang, K.M., 2020. ω-hydroxyundec-9-enoic acid induction of breast cancer cells apoptosis through generation of mitochondrial ROS and phosphorylation of AMPK. Archives of Pharmacal Research 43, 735-743.

Auciello, F.R., Ross, F.A., Ikematsu, N., Hardie, D.G., 2014. Oxidative stress activates AMPK in cultured cells primarily by increasing cellular AMP and/or ADP. FEBS letters 588, 3361-3366.

Carling, D., Mayer, F.V., Sanders, M.J., Gamblin, S.J., 2011. AMP-activated protein kinase: nature's energy sensor. Nature chemical biology 7, 512-518.
Covarrubias, L., Hernández-García, D., Schnabel, D., Salas-Vidal, E., Castro-Obregón, S., 2008. Function of reactive oxygen species during animal development: passive or active? Developmental biology 320, 1-11.
Dong, Y., Hu, H., Zhang, X., Zhang, Y., Sun, X., Wang, H., Kan, W., Tan, M.-j., Shi, H., Zang, Y., 2023. Phosphorylation of PHF2 by AMPK releases the repressive H3K9me2 and inhibits cancer metastasis. Signal Transduction and Targeted Therapy 8, 95.
Dossou, A.S., Basu, A., 2019. The emerging roles of mTORC1 in macromanaging autophagy. Cancers 11, 1422.
Emerling, B.M., Weinberg, F., Snyder, C., Burgess, Z., Mutlu, G.M., Viollet, B., Budinger, G.S., Chandel, N.S., 2009. Hypoxic activation of AMPK is dependent on mitochondrial ROS but independent of an increase in AMP/ATP ratio. Free Radical Biology and Medicine 46, 1386-1391.
Garcia, D., Shaw, R.J., 2017. AMPK: mechanisms of cellular energy sensing and restoration of metabolic balance. Molecular cell 66, 789-800.
Gwinn, D.M., Shackelford, D.B., Egan, D.F., Mihaylova, M.M., Mery, A., Vasquez, D.S., Turk, B.E., Shaw, R.J., 2008. AMPK phosphorylation of raptor mediates a metabolic checkpoint. Molecular cell 30, 214-226.
Hagemeister Jr, F.B., Buzdar, A.U., Luna, M.A., Blumenschein, G.R., 1980. Causes of death in breast cancer a clinicopathologic study. Cancer 46, 162-167.
Halliwell, B., 2011. Free radicals and antioxidants–quo vadis? Trends in pharmacological sciences 32, 125-130.
Hardie, D.G., 2007. AMP-activated/SNF1 protein kinases: conserved guardians of cellular energy. Nature reviews Molecular cell biology 8, 774-785.
Hardie, D.G., Carling, D., Gamblin, S.J., 2011. AMP-activated protein kinase: also regulated by ADP? Trends in biochemical sciences 36, 470-477.
Hardie, D.G., Ross, F.A., Hawley, S.A., 2012. AMPK: a nutrient and energy sensor that maintains energy homeostasis. Nature reviews Molecular cell biology 13, 251-262.
Herrero-Martín, G., Høyer-Hansen, M., García-García, C., Fumarola, C., Farkas, T., López-Rivas, A., Jäättelä, M., 2009. TAK1 activates AMPK-dependent cytoprotective autophagy in TRAIL-treated epithelial cells. The EMBO journal 28, 677-685.
Hua, H., Kong, Q., Zhang, H., Wang, J., Luo, T., Jiang, Y., 2019. Targeting mTOR for cancer therapy. Journal of hematology & oncology 12, 1-19.
Inoki, K., Zhu, T., Guan, K.-L., 2003. TSC2 mediates cellular energy response to control cell growth and survival. Cell 115, 577-590.
Jeon, H., Jin, Y., Myung, C.-S., Heo, K.-S., 2021. Ginsenoside-Rg2 exerts anti-cancer effects through ROS-mediated AMPK activation associated mitochondrial damage and oxidation in MCF-7 cells. Archives of Pharmacal Research 44, 702-712.
Kim, M.Y., Bo, H.H., Choi, E.O., Kwon, D.H., Kim, H.J., Im Ahn, K., Ji, S.Y., Jeong, J.-W., Park, S.-H., Hong, S.-H., 2018. Induction of apoptosis by Citrus unshiu peel in human breast cancer MCF-7 cells: involvement of ROS-dependent activation of AMPK. Biological and Pharmaceutical Bulletin 41, 713-721.
Liang, J., Shao, S.H., Xu, Z.-X., Hennessy, B., Ding, Z., Larrea, M., Kondo, S., Dumont, D.J., Gutterman, J.U., Walker, C.L., 2007. The energy sensing LKB1–AMPK pathway regulates p27kip1 phosphorylation mediating the decision to enter autophagy or apoptosis. Nature cell biology 9, 218-224.
Mariño, G., Niso-Santano, M., Baehrecke, E.H., Kroemer, G., 2014. Self-consumption: the interplay of autophagy and apoptosis. Nature reviews Molecular cell biology 15, 81-94.
Marullo, R., Werner, E., Degtyareva, N., Moore, B., Altavilla, G., Ramalingam, S.S., Doetsch, P.W., 2013. Cisplatin induces a mitochondrial-ROS response that contributes to cytotoxicity depending on mitochondrial redox status and bioenergetic functions. PloS one 8, e81162.
Matsui, Y., Takagi, H., Qu, X., Abdellatif, M., Sakoda, H., Asano, T., Levine, B., Sadoshima, J., 2007. Distinct roles of autophagy in the heart during ischemia and reperfusion: roles of AMP-activated protein kinase and Beclin 1 in mediating autophagy. Circulation research 100, 914-922.

Meley, D., Bauvy, C., Houben-Weerts, J.H., Dubbelhuis, P.F., Helmond, M.T., Codogno, P., Meijer, A.J., 2006. AMP-activated protein kinase and the regulation of autophagic proteolysis. Journal of biological chemistry 281, 34870-34879.
Muhammad, N., Steele, R., Isbell, T.S., Philips, N., Ray, R.B., 2017. Bitter melon extract inhibits breast cancer growth in preclinical model by inducing autophagic cell death. Oncotarget 8, 66226.
Peng, B., Zhang, S.-Y., Chan, K.I., Zhong, Z.-F., Wang, Y.-T., 2023. Novel Anti-Cancer Products Targeting AMPK: Natural Herbal Medicine against Breast Cancer. Molecules 28, 740.
Redza-Dutordoir, M., Averill-Bates, D.A., 2016. Activation of apoptosis signalling pathways by reactive oxygen species. Biochimica et Biophysica Acta (BBA)-Molecular Cell Research 1863, 2977-2992.
Ren, P., Ren, X., Cheng, L., Xu, L., 2018. Frankincense, pine needle and geranium essential oils suppress tumor progression through the regulation of the AMPK/mTOR pathway in breast cancer. Oncology Reports 39, 129-137.
Roux, P.P., Topisirovic, I., 2018. Signaling pathways involved in the regulation of mRNA translation. Molecular and cellular biology.
Shin, M.-K., Cheong, J.-H., 2019. Mitochondria-centric bioenergetic characteristics in cancer stem-like cells. Archives of pharmacal research 42, 113-127.
Siegel, R.L., Miller, K.D., Wagle, N.S., Jemal, A., 2023. Cancer statistics, 2023. Ca Cancer J Clin 73, 17-48.
Stellrecht, C.M., Vangapandu, H.V., Le, X.-F., Mao, W., Shentu, S., 2014. ATP directed agent, 8-chloro-adenosine, induces AMP activated protein kinase activity, leading to autophagic cell death in breast cancer cells. Journal of hematology & oncology 7, 1-13.
Sung, H., Ferlay, J., Siegel, R.L., Laversanne, M., Soerjomataram, I., Jemal, A., Bray, F., 2021. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: a cancer journal for clinicians 71, 209-249.
Vingtdeux, V., Giliberto, L., Zhao, H., Chandakkar, P., Wu, Q., Simon, J.E., Janle, E.M., Lobo, J., Ferruzzi, M.G., Davies, P., 2010. AMP-activated protein kinase signaling activation by resveratrol modulates amyloid-β peptide metabolism. Journal of Biological Chemistry 285, 9100-9113.
Waks, A.G., Winer, E.P., 2019. Breast cancer treatment: a review. Jama 321, 288-300.
Wang, Y., Xu, W., Yan, Z., Zhao, W., Mi, J., Li, J., Yan, H., 2018. Metformin induces autophagy and G0/G1 phase cell cycle arrest in myeloma by targeting the AMPK/mTORC1 and mTORC2 pathways. Journal of Experimental & Clinical Cancer Research 37, 1-12.
Winterbourn, C.C., 2015. Are free radicals involved in thiol-based redox signaling? Free Radical Biology and Medicine 80, 164-170.
Wong, V.K.W., Zeng, W., Chen, J., Yao, X.J., Leung, E.L.H., Wang, Q.Q., Chiu, P., Ko, B.C., Law, B.Y.K., 2017. Tetrandrine, an activator of autophagy, induces autophagic cell death via PKC-α inhibition and mTOR-dependent mechanisms. Frontiers in pharmacology 8, 351.
Xiao, B., Sanders, M.J., Carmena, D., Bright, N.J., Haire, L.F., Underwood, E., Patel, B.R., Heath, R.B., Walker, P.A., Hallen, S., 2013. Structural basis of AMPK regulation by small molecule activators. Nature communications 4, 1-10.

THESIS DISCUSSION

DISCUSSION

Cancer, a term that strikes fear into the hearts of millions, is a complex and devastating disease that has plagued humanity for centuries. Its intricate web of causes, diverse manifestations, and elusive cures make it one of the most formidable challenges in the field of medicine. Cancer is characterized by the uncontrolled division and growth of abnormal cells. The malignant or neoplastic cells can infiltrate and destroy surrounding healthy tissues, leading to severe health complications and if left unchecked, death.

In the present study, a polyphenol-rich fraction (PFBL) was isolated after HPLC purification from *Bergenia ligulata* rhizome extract with the highest activity against human colon cancer and breast cancer cells without an adverse effect on the immortalized normal (NKE) cells [Fig. 3.7]. Surprisingly, the activity was cell-type specific; in colon cancer, PFBL primely induced autophagic death [Fig. 4.4] while the breast cancer cells died majorly by apoptosis [Fig. 5.3]. In contrast to autophagy, the predominant death of breast cancer cells by apoptosis may be explained by a mutation in their Beclin1 gene, a key player in the autophagy death pathway. Although the Beclin1 level is consistently abundant in normal breast epithelial cells, it is diminished in human breast epithelial carcinoma cell lines and tissues. Approximately 40% to 75% of human breast and ovarian cancer cells exhibit mono-allelic deletion in the Beclin1 gene (Aita et al., 1999; Liang et al., 1999). This result may explain the death of breast cancer cells predominantly by apoptosis. However, PFBL induced arrest in both the colon and breast cancer cells at the G0/G1 phase [Fig. 4.2, 5.2]. In addition, PFBL-induced AMPK activation served as a major criterion in G0/G1 cell cycle arrest, initiating autophagy and apoptosis [Figs. 4.6, 5.6].

Epithelial-mesenchymal transition (EMT) is a phenomenon where cells lose their epithelial characteristics, such as cell-cell adhesion, and gain mesenchymal traits, enabling them to invade surrounding tissues and facilitate metastasis. PFBL has demonstrated the ability to halt this transformation of CRC and breast cancer cells, as revealed by reduced expression of key EMT markers, such as N-cadherin, Snail, Twist, and Vimentin, while promoting the expression of E-cadherin, a protein that facilitates cellular adhesion [Fig 4.7, 5.7]. The anti-metastatic potential of PFBL has been demonstrated against the colon and breast cancer cells in the preclinical (mice) model [Fig. 4.9, 5.9]. Therefore, PFBL emerges as a promising agent in reversing a crucial process in cancer progression. These findings signified the potential of

PFBL in hindering the spread of both of colon- and breast cancer. [Fig. 4.8, 5.8], without affecting the surrounding normal cells [Fig. 3.9].

The constitution of PFBL was defined by LC-MS analysis of the fraction. An LC-MS analysis identified the presence of eleven compounds which include Afzelechin (274.26 D), 4/11-O-galloyl bergenin (480.3 D), Catechin (290.26 D), Arbutin (272.25 D), Quercetin (302.236 D), Z-asarone (208.275 D), Dihydroergotamine (678.56 D), Ferulic acid (194 D), Aloe emodin (270.24 D), Chlorogenic acid (354 D), and Phytol (286.23 D) (Table 3.3). Earlier, the presence of Bergenin (Pandey et al., 2017), Afzelechin (Reddy et al., 1999), β-sitosterol (Das et al., 2022), Catechin, Leucocyanidin, Gallic acid, and Tannic acid (Dix and Srivastava, 1989) were reported as major bioactive compounds in *Bergenia ligulata*. This Himalayan herb is used in Indian traditional/folk medicine for treating kidney stones (Gurav and Gurav, 2014). The roots and rhizome of the plant are used by the Himalayan tribes to treat their common minor illnesses and kidney-related abnormalities (Ruby et al., 2012).

Stressful conditions, such as inflammation, fat storage, pooled blood, benign or malignant growths, and overproduction of cells, can cause the spleen to enlarge (De Porto et al., 2010). Therefore, the spleen size was measured as a toxicity marker after treating the healthy mice with PFBL and 5FU or DOX as positive controls. Notably, little changes were observed in the spleen sizes of animals treated with PFBL, although, as expected, enlargement in spleen size was observed in the 5FU and DOX-treated animals. Surprisingly, supplementation of DOX and 5FU with PFBL led to amelioration of the toxicity (that is when PFBL was used in synergy with 5FU and DOX) in those animals as revealed by the reduction of their spleen enlargement caused by these anticancer drugs [Fig. 3.9]. Therefore, PFBL was well-tolerated, safe, and ameliorative of the toxicity associated with current anticancer drugs 5FU and DOX in the preclinical (mice) model.

PFBL-induced elevation of ROS production was the central cause of its toxicity to the cancer cells as NAC pre-treatment protected the cancer cells [Fig. 4.5 and Fig 5.5]. The result indicated the defective antioxidant response pathway in these cancer cells. In contrast, the resistance of the normal cells to PFBL-induced ROS production underlies their fully functional antioxidant defence pathways, inducible under PFBL-induced oxidative stress. Healthy cells uphold oxidative balance by regulating reactive oxygen species (ROS) generation through modulating their metabolic and signaling pathways (Snezhkina et al., 2019). Compromised antioxidant defence systems make the cancer cells susceptible to enhanced oxidative stress

(Perillo et al., 2020). ROS can be produced in mitochondria, peroxisome, or endoplasmic reticulum upon exposure to different stimuli, including anti-cancer therapies (Perillo et al., 2020). Earlier, a polyphenol-rich fraction derived from *Bergenia ligulata* rhizome demonstrated selective toxicity against the PC3 cells, both in vitro and in PC3-tumor xenografts, without adverse effects on the host/mice. This fraction worked through producing elevated levels of reactive oxygen species (ROS) in PC3 cells. It was found that this activity enhanced the catalytic activity of MAO-A (Monoamine Oxidase A) and inhibited the defective NRF2-mediated antioxidant response by degradation of NRF2 upon phosphorylation by GSK3β in PC3 cells. The normal cells were protected due to their fully functional NRF2 antioxidant pathway. In addition, in normal cells, the elevation of ROS by PFBL treatment was neutralized by rapid enhancement of the catalytic activity of catalase and SOD enzymes (Ghosh et al., 2021).

AMP-activated protein kinase (AMPK) is a serine/threonine protein kinase family member and a prime sensor of cellular energy metabolism (Hardie, 2007). AMPK is a homotrimer of catalytic α and regulatory β and γ subunits. A conventional serine/threonine kinase domain is present at the N-terminal of the catalytic subunit. Phosphorylation of Thr172 in the activation loop by upstream kinases, including LKB1:STRAD:MO25 complex and Ca^{2+}/calmodulin activated protein kinases (CaMKKβ) increases the activity of the kinase (Hawley et al., 2003; Hawley et al., 1996; Hawley et al., 2005; Hurley et al., 2005; Shaw et al., 2004; Woods et al., 2005; Woods et al., 2003). AMPK can be activated either by AMP, ADP, or Ca^{2+} -mediated canonical pathway, or in an AMP, ADP, and Ca^{2+} -independent manner, by ROS and DNA damage induced PI-3-like kinase or ATM-mediated non-canonical pathway. AMP, ADP, and ATP bind to the AMPKγ subunit with equal affinity, but ATP binding does not activate the enzyme (Xiao et al., 2011). LKB1 complex provides maximum basal level phosphorylation at Thr172 and binding of AMP to AMPK-γ subunit to enhance phosphorylation and prevent dephosphorylation of AMPK (Davies et al., 1995; Hawley et al., 1995). The N-terminal myristylation of the β subunit is essential to exert an effect on AMPK phosphorylation by AMP and ADP (Oakhill et al., 2010; Oakhill et al., 2011). CaMKKβ-mediated AMPK activation requires enhanced cellular levels of Ca^{2+}, independent of AMP or ADP levels (Fogarty et al., 2010). Notably, higher levels of cellular ROS can activate AMPK in 3 ways, either by inhibition of mitochondrial ATPase (Hawley et al., 2010), or by oxidation/glutathionylation of conserved cysteine residues of AMPKα (Zmijewski et al., 2010) or by ATM (Ditch and Paull, 2012; Siebel et al., 2013) in an LKB1 dependent manner (Sapkota

et al., 2002). A *Bergenia ligulata* fraction, active against prostate cancer, was reported to cause DNA damage (Ghosh et al., 2021), suggesting involvement of ATM in AMPK activation following PFBL treatment.

AMPK induces different cellular fate, such as autophagy, in response to stress, such as glucose starvation (Herrero-Martín et al., 2009; Liang et al., 2007; Matsui et al., 2007; Meley et al., 2006; Vingtdeux et al., 2010). AMPK was demonstrated to induce autophagy through inhibition of mTORC1 (mammalian target of rapamycin) in a TSC2 (Inoki et al., 2003) and Raptor (Gwinn et al., 2008) dependent manner or by directly phosphorylating ULK1 (Egan et al., 2011; Kim et al., 2011). mTOR can form two different complexes: mTORC1 and mTORC2. The mTORC1 complex modulates its downstream targets, such as 4EBP1 and ribosomal S6 kinase. One of the primary functions of mTORC1 is to suppress autophagy by hindering the activity of the autophagy initiator complex made up of ULK1, Atg13, Atg101, and FIP200 (Hosokawa et al., 2009; Jung et al., 2009). Under well-nourished conditions, mTORC1 phosphorylates ULK1 at Ser638 and Ser758, suppressing its kinase activity and preventing autophagy initiation (Shang et al., 2011). So, AMPK-mediated inhibition of mTORC1 can directly initiate autophagy through the activation of ULK1.

The involvement of AMPK in apoptosis remains a subject of debate. Its role in a cell's fate, whether promoting survival or cell death, depends on factors such as the nature of the stress, how long the signaling cascade remains active, and the cell type (Villanueva-Paz et al., 2016). AMPK activation can also induce apoptosis by activating the JNK pathway (Meisse et al., 2002). p53 and p27 activation and mTORC1 inhibition were also demonstrated to induce cellular apoptosis (Faivre et al., 2006). AMPK induces Bcl2-family member Bim-mediated apoptosis. Overstimulation of glutamate receptors, known as excitotoxicity, can disrupt cellular ion balances and lead to necrosis or apoptosis. Excitotoxic necrosis occurs due to rapid and irreversible ATP depletion, while the ability to restore cellular energy levels is believed to be essential for initiating excitotoxic apoptosis. A brief decline in cellular energy levels and the concurrent activation of AMPK are crucial for initiating excitotoxic apoptosis. The proapoptotic protein Bim is activated in various excitotoxicity scenarios, where it mediates excitotoxic apoptosis and prevents delayed disruptions in calcium levels, mitochondrial function, and the translocation of apoptosis-inducing factors. Activation of Bim relies on the activation of AMPK, and prolonged AMPK activation alone can induce Bim gene expression and trigger cell death by apoptosis (Concannon et al., 2010). In breast cancer cells, AMPK activation leads to apoptosis induction (Li et al., 2015). The caloric restriction that results in

AMPK activation was shown to induce apoptosis through the reduction of the anti-apoptotic Bcl2 family protein MCL-1 in the Eμ-Myc lymphoma cells that lack p53 or Noxa, Puma, or Bim genes (Meynet et al., 2013).

PFBL arrested the cancer cells at the G0/G1 phase in an AMPK-dependent manner. AMPK has been observed to trigger p53 activation in response to metabolic stress through phosphorylation of human MDMX on Ser342 in vitro and ex vivo. This phosphorylation enhances the interaction between MDMX and 14-3-3 proteins, which substantially stabilizes p53 by impeding the ubiquitylation of p53. Notably, MDM2, another crucial negative regulator of p53, is not phosphorylated by AMPK. The AMPK-induced association of MDMX with 14-3-3 proteins in the pathway of p53 activation was impaired in mouse embryo fibroblasts that carry S341A, S367A, and S402A mutations in the MDMX protein. Interaction of MDMX and p53 was impaired in cells devoid of AMPK. AMPK was shown to block cell cycle progression by two distinct mechanisms. Stabilization of p53 by blocking its proteasomal degradation by AMPK-mediated phosphorylation of E3-ubiquitin ligase MDMX on Ser342 (He et al., 2014). p53 can also be stabilized by AMPK-mediated phosphorylation at Ser15 in response to glucose deprivation to induce reversible cell cycle arrest at the G1/S phase (Bode and Dong, 2004; Jones et al., 2005; Motoshima et al., 2006). Stimulation of the LKB1/AMPK signaling pathway can also lead to cell cycle arrest at the G1 phase (Liang et al., 2014).

PFBL effectively reduced the size of solid tumors in both colon and breast cancer cell models in BALB/c mice, when used alone and in combination with conventional chemotherapy drugs. PFBL treatment triggered autophagic cell death in CT26 colon tumor cells and apoptotic cell death in 4T1 breast cancer cells in vivo [Fig. 4.8, 5.8], corroborating the findings observed in in vitro experiments [Fig. 4.4, 5.2],

PFBL reduced N-cadherin, Snail, Twist, and Vimentin levels and increased E-cadherin expression in vitro and in-vivo lung metastasis models in BALB/c mice [Fig. 4.9, 5.9]. Previous research has demonstrated the link between AMPK activation and the suppression of EMT, as well as the metastasis of cancer cells. Rosmarinic acid, a polyphenol presents in herbs such as rosemary and mint, downregulated the expression of critical EMT markers, including N-cadherin, Snail, Twist, and Vimentin, with simultaneous upregulation of E-cadherin expression in an AMPK dependent manner (Han et al., 2018). Metformin-induced AMPK activation led to activatory phosphorylation of PHF2 (an H3K9me2 demethylase), resulting in the expression of epigenetically silenced epithelial genes and lass metastatic phenotype (Dong et al., 2023).

In this study, a polyphenol-rich fraction (PFBL) from Bergenia ligulata rhizome was purified with potent toxicity against the colon and breast cancer cells with little or no adverse effect on the normal cells. PFBL was found to be safe in animals. When administered alone or in combination, it ameliorated the adverse side effects of standard anti-cancer drugs such as 5FU and DOX in preclinical models. The PFBL induced death in these cancer cells by a distinct mechanism. The toxicity of cancer cells was mediated by AMPK activation induced by the elevation of intracellular ROS levels. Notably, PFBL inhibited the EMT/metastasis in colon and breast cancer cells in preclinical/mouse models as well. The LC-MS analysis identified 11 compounds in the PFBL. It will be interesting to understand if these compounds, alone or in combination, are responsible for the observed anticancer activities. Overall, this study highlighted the significance of PFBL for consideration for a clinical trial on a priority basis to verify its potential as a novel anticancer therapeutic agent.

References:

Aita, V.M., Liang, X.H., Murty, V., Pincus, D.L., Yu, W., Cayanis, E., Kalachikov, S., Gilliam, T.C., Levine, B., 1999. Cloning and genomic organization of beclin 1, a candidate tumor suppressor gene on chromosome 17q21. Genomics 59, 59-65.

Bode, A.M., Dong, Z., 2004. Post-translational modification of p53 in tumorigenesis. Nature Reviews Cancer 4, 793-805.

Concannon, C.G., Tuffy, L.P., Weisová, P., Bonner, H.P., Dávila, D., Bonner, C., Devocelle, M.C., Strasser, A., Ward, M.W., Prehn, J.H., 2010. AMP kinase–mediated activation of the BH3-only protein Bim couples energy depletion to stress-induced apoptosis. Journal of Cell Biology 189, 83-94.

Das, C., Kumari, B., Singh, M.P., Singh, S., 2022. A Literary Review and Therapeutic Action of Pashanbheda (Bergenia ligulata Wall) described by Shamhita in Ashmari Roga. Journal of Ayurveda and Integrated Medical Sciences 7, 105-114.

Davies, S.P., Helps, N.R., Cohen, P.T., Hardie, D.G., 1995. 5′-AMP inhibits dephosphorylation, as well as promoting phosphorylation, of the AMP-activated protein kinase. Studies using bacterially expressed human protein phosphatase-2Cα and native bovine protein phosphatase-2AC. FEBS letters 377, 421-425.

De Porto, A., Lammers, A., Bennink, R., Ten Berge, I., Speelman, P., Hoekstra, J., 2010. Assessment of splenic function. European journal of clinical microbiology & infectious diseases 29, 1465-1473.

Ditch, S., Paull, T.T., 2012. The ATM protein kinase and cellular redox signaling: beyond the DNA damage response. Trends in biochemical sciences 37, 15-22.

Dix, B., Srivastava, S., 1989. Tannin constituents of Bergenia ligulata roots. Ind. J. Nat. Prod 5, 24-25.

Dong, Y., Hu, H., Zhang, X., Zhang, Y., Sun, X., Wang, H., Kan, W., Tan, M.-j., Shi, H., Zang, Y., 2023. Phosphorylation of PHF2 by AMPK releases the repressive H3K9me2 and inhibits cancer metastasis. Signal Transduction and Targeted Therapy 8, 95.

Egan, D., Kim, J., Shaw, R.J., Guan, K.-L., 2011. The autophagy initiating kinase ULK1 is regulated via opposing phosphorylation by AMPK and mTOR. Autophagy 7, 643-644.

Faivre, S., Kroemer, G., Raymond, E., 2006. Current development of mTOR inhibitors as anticancer agents. Nature reviews Drug discovery 5, 671-688.

Fogarty, S., Hawley, S.A., Green, K.A., Saner, N., Mustard, K.J., Hardie, D.G., 2010. Calmodulin-dependent protein kinase kinase-β activates AMPK without forming a stable complex: synergistic effects of Ca2+ and AMP. Biochemical Journal 426, 109-118.
Ghosh, S., Dutta, N., Banerjee, P., Gajbhiye, R.L., Sareng, H.R., Kapse, P., Pal, S., Burdelya, L., Mandal, N.C., Ravichandiran, V., 2021. Induction of monoamine oxidase A-mediated oxidative stress and impairment of NRF2-antioxidant defence response by polyphenol-rich fraction of Bergenia ligulata sensitizes prostate cancer cells in vitro and in vivo. Free Radical Biology and Medicine 172, 136-151.
Gurav, S., Gurav, N., 2014. A Comprehensive review: Bergenia ligulata Wall-A controversial clinical candidate. Int J Pharm Sci Rev Res 5, 1630-1642.
Gwinn, D.M., Shackelford, D.B., Egan, D.F., Mihaylova, M.M., Mery, A., Vasquez, D.S., Turk, B.E., Shaw, R.J., 2008. AMPK phosphorylation of raptor mediates a metabolic checkpoint. Molecular cell 30, 214-226.
Han, Y.-H., Kee, J.-Y., Hong, S.-H., 2018. Rosmarinic acid activates AMPK to inhibit metastasis of colorectal cancer. Frontiers in pharmacology 9, 68.
Hardie, D.G., 2007. AMP-activated/SNF1 protein kinases: conserved guardians of cellular energy. Nature reviews Molecular cell biology 8, 774-785.
Hawley, S.A., Boudeau, J., Reid, J.L., Mustard, K.J., Udd, L., Mäkelä, T.P., Alessi, D.R., Hardie, D.G., 2003. Complexes between the LKB1 tumor suppressor, STRADα/β and MO25α/β are upstream kinases in the AMP-activated protein kinase cascade. Journal of biology 2, 1-16.
Hawley, S.A., Davison, M., Woods, A., Davies, S.P., Beri, R.K., Carling, D., Hardie, D.G., 1996. Characterization of the AMP-activated protein kinase kinase from rat liver and identification of threonine 172 as the major site at which it phosphorylates AMP-activated protein kinase. Journal of Biological Chemistry 271, 27879-27887.
Hawley, S.A., Pan, D.A., Mustard, K.J., Ross, L., Bain, J., Edelman, A.M., Frenguelli, B.G., Hardie, D.G., 2005. Calmodulin-dependent protein kinase kinase-β is an alternative upstream kinase for AMP-activated protein kinase. Cell metabolism 2, 9-19.
Hawley, S.A., Ross, F.A., Chevtzoff, C., Green, K.A., Evans, A., Fogarty, S., Towler, M.C., Brown, L.J., Ogunbayo, O.A., Evans, A.M., 2010. Use of cells expressing γ subunit variants to identify diverse mechanisms of AMPK activation. Cell metabolism 11, 554-565.
Hawley, S.A., Selbert, M.A., Goldstein, E.G., Edelman, A.M., Carling, D., Hardie, D.G., 1995. 5′-AMP activates the AMP-activated protein kinase cascade, and Ca2+/calmodulin activates the calmodulin-dependent protein kinase I cascade, via three independent mechanisms. Journal of Biological Chemistry 270, 27186-27191.
He, G., Zhang, Y.-W., Lee, J.-H., Zeng, S.X., Wang, Y.V., Luo, Z., Dong, X.C., Viollet, B., Wahl, G.M., Lu, H., 2014. AMP-activated protein kinase induces p53 by phosphorylating MDMX and inhibiting its activity. Molecular and cellular biology 34, 148-157.
Herrero-Martín, G., Høyer-Hansen, M., García-García, C., Fumarola, C., Farkas, T., López-Rivas, A., Jäättelä, M., 2009. TAK1 activates AMPK-dependent cytoprotective autophagy in TRAIL-treated epithelial cells. The EMBO journal 28, 677-685.
Hosokawa, N., Hara, T., Kaizuka, T., Kishi, C., Takamura, A., Miura, Y., Iemura, S.-i., Natsume, T., Takehana, K., Yamada, N., 2009. Nutrient-dependent mTORC1 association with the ULK1–Atg13–FIP200 complex required for autophagy. Molecular biology of the cell 20, 1981-1991.
Hurley, R.L., Anderson, K.A., Franzone, J.M., Kemp, B.E., Means, A.R., Witters, L.A., 2005. The Ca2+/calmodulin-dependent protein kinase kinases are AMP-activated protein kinase kinases. Journal of Biological Chemistry 280, 29060-29066.
Inoki, K., Zhu, T., Guan, K.-L., 2003. TSC2 mediates cellular energy response to control cell growth and survival. Cell 115, 577-590.
Jones, R.G., Plas, D.R., Kubek, S., Buzzai, M., Mu, J., Xu, Y., Birnbaum, M.J., Thompson, C.B., 2005. AMP-activated protein kinase induces a p53-dependent metabolic checkpoint. Molecular cell 18, 283-293.
Jung, C.H., Jun, C.B., Ro, S.-H., Kim, Y.-M., Otto, N.M., Cao, J., Kundu, M., Kim, D.-H., 2009. ULK-Atg13-FIP200 complexes mediate mTOR signaling to the autophagy machinery. Molecular biology of the cell 20, 1992-2003.
Kim, J., Kundu, M., Viollet, B., Guan, K.-L., 2011. AMPK and mTOR regulate autophagy through direct phosphorylation of Ulk1. Nature cell biology 13, 132-141.

Li, X., Wang, L., Zhou, X.E., Ke, J., De Waal, P.W., Gu, X., Tan, M., Wang, D., Wu, D., Xu, H.E., 2015. Structural basis of AMPK regulation by adenine nucleotides and glycogen. Cell research 25, 50-66.
Liang, J., Shao, S.H., Xu, Z.-X., Hennessy, B., Ding, Z., Larrea, M., Kondo, S., Dumont, D.J., Gutterman, J.U., Walker, C.L., 2007. The energy sensing LKB1–AMPK pathway regulates p27kip1 phosphorylation mediating the decision to enter autophagy or apoptosis. Nature cell biology 9, 218-224.
Liang, X., Wang, P., Gao, Q., Tao, X., 2014. Exogenous activation of LKB1/AMPK signaling induces G1 arrest in cells with endogenous LKB1 expression. Molecular Medicine Reports 9, 1019-1024.
Liang, X.H., Jackson, S., Seaman, M., Brown, K., Kempkes, B., Hibshoosh, H., Levine, B., 1999. Induction of autophagy and inhibition of tumorigenesis by beclin 1. Nature 402, 672-676.
Matsui, Y., Takagi, H., Qu, X., Abdellatif, M., Sakoda, H., Asano, T., Levine, B., Sadoshima, J., 2007. Distinct roles of autophagy in the heart during ischemia and reperfusion: roles of AMP-activated protein kinase and Beclin 1 in mediating autophagy. Circulation research 100, 914-922.
Meisse, D., Van de Casteele, M., Beauloye, C., Hainault, I., Kefas, B.A., Rider, M.H., Foufelle, F., Hue, L., 2002. Sustained activation of AMP-activated protein kinase induces c-Jun N-terminal kinase activation and apoptosis in liver cells. FEBS letters 526, 38-42.
Meley, D., Bauvy, C., Houben-Weerts, J.H., Dubbelhuis, P.F., Helmond, M.T., Codogno, P., Meijer, A.J., 2006. AMP-activated protein kinase and the regulation of autophagic proteolysis. Journal of biological chemistry 281, 34870-34879.
Meynet, O., Zunino, B., Happo, L., Pradelli, L.A., Chiche, J., Jacquin, M.A., Mondragón, L., Tanti, J.-F., Taillan, B., Garnier, G., 2013. Caloric restriction modulates Mcl-1 expression and sensitizes lymphomas to BH3 mimetic in mice. Blood, The Journal of the American Society of Hematology 122, 2402-2411.
Motoshima, H., Goldstein, B.J., Igata, M., Araki, E., 2006. AMPK and cell proliferation–AMPK as a therapeutic target for atherosclerosis and cancer. The Journal of physiology 574, 63-71.
Oakhill, J.S., Chen, Z.-P., Scott, J.W., Steel, R., Castelli, L.A., Ling, N., Macaulay, S.L., Kemp, B.E., 2010. β-Subunit myristoylation is the gatekeeper for initiating metabolic stress sensing by AMP-activated protein kinase (AMPK). Proceedings of the National Academy of Sciences 107, 19237-19241.
Oakhill, J.S., Steel, R., Chen, Z.-P., Scott, J.W., Ling, N., Tam, S., Kemp, B.E., 2011. AMPK is a direct adenylate charge-regulated protein kinase. Science 332, 1433-1435.
Pandey, R., Kumar, B., Meena, B., Srivastava, M., Mishra, T., Tiwari, V., Pal, M., Nair, N.K., Upreti, D.K., Rana, T.S., 2017. Major bioactive phenolics in Bergenia species from the Indian Himalayan region: Method development, validation and quantitative estimation using UHPLC-QqQLIT-MS/MS. PloS one 12, e0180950.
Perillo, B., Di Donato, M., Pezone, A., Di Zazzo, E., Giovannelli, P., Galasso, G., Castoria, G., Migliaccio, A., 2020. ROS in cancer therapy: The bright side of the moon. Experimental & molecular medicine 52, 192-203.
Reddy, U.D.C., Chawla, A.S., Deepak, M., Singh, D., Handa, S.S., 1999. High pressure liquid chromatographic determination of bergenin and (+)-afzelechin from different parts of Paashaanbhed (Bergenia ligulata yeo). Phytochemical Analysis: An International Journal of Plant Chemical and Biochemical Techniques 10, 44-47.
Ruby, K., Dwivedi, J., Chauhan, R., 2012. Pashanbheda a golden herb of Himalaya: a review. International Journal of Pharmacy Review & Research 2, 97-105.
Sapkota, G.P., Deak, M., Kieloch, A., Morrice, N., Goodarzi, A.A., Smythe, C., Shiloh, Y., Lees-Miller, S.P., Alessi, D.R., 2002. Ionizing radiation induces ataxia telangiectasia mutated kinase (ATM)-mediated phosphorylation of LKB1/STK11 at Thr-366. Biochemical Journal 368, 507-516.
Shang, L., Chen, S., Du, F., Li, S., Zhao, L., Wang, X., 2011. Nutrient starvation elicits an acute autophagic response mediated by Ulk1 dephosphorylation and its subsequent dissociation from AMPK. Proceedings of the National Academy of Sciences 108, 4788-4793.
Shaw, R.J., Kosmatka, M., Bardeesy, N., Hurley, R.L., Witters, L.A., DePinho, R.A., Cantley, L.C., 2004. The tumor suppressor LKB1 kinase directly activates AMP-activated kinase and regulates apoptosis in response to energy stress. Proceedings of the National Academy of Sciences 101, 3329-3335.

Siebel, A., Cubillos-Rojas, M., Santos, R.C., Schneider, T., Bonan, C.D., Bartrons, R., Ventura, F., Rodrigues de Oliveira, J., Rosa, J.L., 2013. Contribution of S6K1/MAPK signaling pathways in the response to oxidative stress: activation of RSK and MSK by hydrogen peroxide. PLoS One 8, e75523.
Snezhkina, A.V., Kudryavtseva, A.V., Kardymon, O.L., Savvateeva, M.V., Melnikova, N.V., Krasnov, G.S., Dmitriev, A.A., 2019. ROS generation and antioxidant defense systems in normal and malignant cells. Oxidative medicine and cellular longevity 2019.
Villanueva-Paz, M., Cotán, D., Garrido-Maraver, J., Oropesa-Ávila, M., de la Mata, M., Delgado-Pavón, A., de Lavera, I., Alcocer-Gómez, E., Álvarez-Córdoba, M., Sánchez-Alcázar, J.A., 2016. AMPK regulation of cell growth, apoptosis, autophagy, and bioenergetics. AMP-activated protein kinase, 45-71.
Vingtdeux, V., Giliberto, L., Zhao, H., Chandakkar, P., Wu, Q., Simon, J.E., Janle, E.M., Lobo, J., Ferruzzi, M.G., Davies, P., 2010. AMP-activated protein kinase signaling activation by resveratrol modulates amyloid-β peptide metabolism. Journal of Biological Chemistry 285, 9100-9113.
Woods, A., Dickerson, K., Heath, R., Hong, S.-P., Momcilovic, M., Johnstone, S.R., Carlson, M., Carling, D., 2005. Ca2+/calmodulin-dependent protein kinase kinase-β acts upstream of AMP-activated protein kinase in mammalian cells. Cell metabolism 2, 21-33.
Woods, A., Johnstone, S.R., Dickerson, K., Leiper, F.C., Fryer, L.G., Neumann, D., Schlattner, U., Wallimann, T., Carlson, M., Carling, D., 2003. LKB1 is the upstream kinase in the AMP-activated protein kinase cascade. Current biology 13, 2004-2008.
Xiao, B., Sanders, M.J., Underwood, E., Heath, R., Mayer, F.V., Carmena, D., Jing, C., Walker, P.A., Eccleston, J.F., Haire, L.F., 2011. Structure of mammalian AMPK and its regulation by ADP. Nature 472, 230-233.
Zmijewski, J.W., Banerjee, S., Bae, H., Friggeri, A., Lazarowski, E.R., Abraham, E., 2010. Exposure to hydrogen peroxide induces oxidation and activation of AMP-activated protein kinase. Journal of Biological Chemistry 285, 33154-33164.

SUMMARY

This study analysed a potent anti-colon and breast cancer activity of a polyphenol-rich fraction of Bergenia ligulata (PFBL). PFBL is well tolerated in the normal cells and healthy mice. PFBL reversed metastasis by preventing the epithelial-mesenchymal transition of these cancer cells. PFBL-induced ROS-mediated AMPK activation resulted in autophagic and apoptotic death of colon and breast cancer cells, respectively, along with G_0/G_1 cell cycle arrest. PFBL suppressed CT26 and 4T1 solid tumors in BALB/c mice by induction of autophagic and apoptotic effects in healthy animals. PFBL also ameliorated the 5FU and DOX-induced toxicity in mice when used in combination. PFBL suppressed colon and breast cancer cell metastasis as revealed in the reduction of lung colonization of CT26 and 4T1 cells injected in BALB/c mice at a dose non-toxic to the healthy animals.

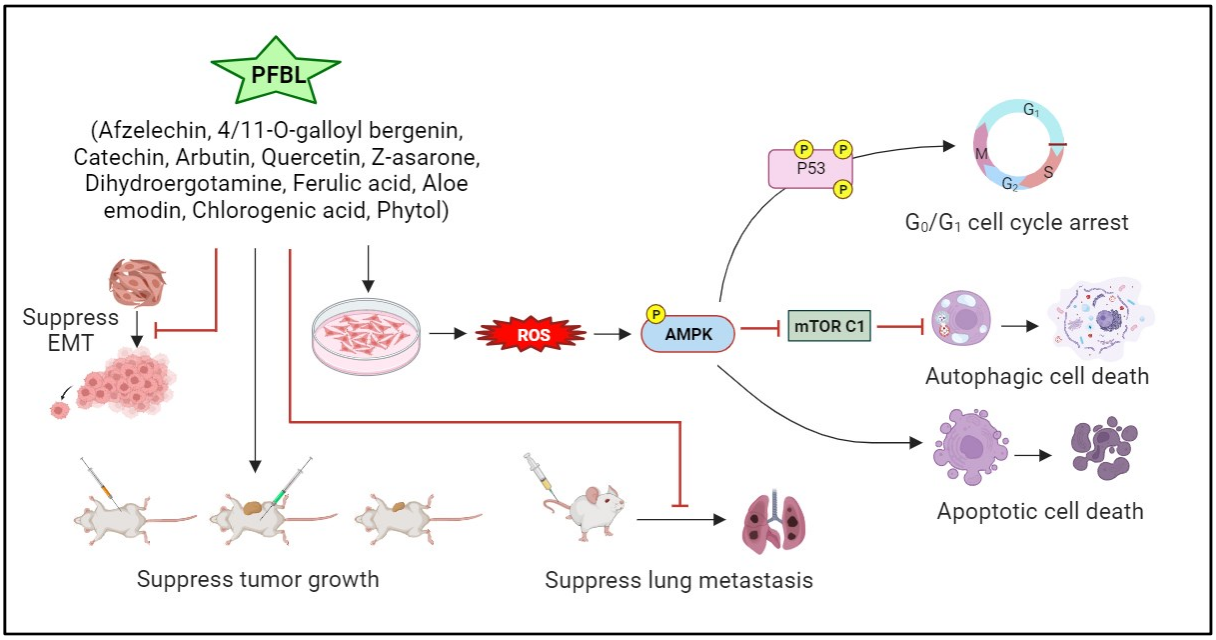

Figure 6.1: A graphical representation of PFBL action. PFBL-induced ROS generation in cancer cells activates the energy sensor AMPK. PFBL blocks cell cycle at G_0/G_1 phase in an AMPK dependent manner. AMPK induces autophagic death of colon and breast cancer cells by inhibiting mTORC1 pathway. AMPK also induces apoptotic death of breast cancer cells. PFBL regresses solid tumor including colonization of CT26 and 4T1 cells in the lung in BALB/c mice.

Publications:

1. **De, S**., Paul, S., Manna, A., Majumder, C., Pal, K., Casarcia, N., Mondal, A., Banerjee, S., Nelson, V.K., Ghosh, S., Hazra J., Bhattacharjee A., Mandal S.C., Pal M., Bishayee A.. 2023. Phenolic phytochemicals for prevention and treatment of colorectal cancer: A critical evaluation of in vivo studies. Cancers 15, 993.
2. Ghosh, N., Kundu, M., Ghosh, S., Das, A.K., **De, S**., Das, J., Sil, P.C., 2023. pH-responsive and targeted delivery of chrysin via folic acid-functionalized mesoporous silica nanocarrier for breast cancer therapy. International Journal of Pharmaceutics 631, 122555.
3. Ghosh, N., **De, S**., Sil, P.C., 2023. Mononuclear Methylimidazole-Oxidovanadium (IV) Complex Inhibits Triple Negative Breast Carcinoma and Metastasis through ROS- and ER Stress-Mediated Mitochondrial Dysfunction. Cellular Signalling (under communication).

Presentations:

1. Symposium on " Current Trends in Biology for Human Diseases and Medicine" organised by Society of Biological Chemists (I), KOLKATA CHAPTER, 19-21st March 2021. (Oral presentation).
2. 4th Indo Oncology Summit. 11-13th November 2022. (Poster presentation)
3. 91st annual meeting of Society of Biological Chemists, India. 8-11th December 2022. (Poster presentation)

cancers

MDPI

Review

Phenolic Phytochemicals for Prevention and Treatment of Colorectal Cancer: A Critical Evaluation of In Vivo Studies

Samhita De [1,†], Sourav Paul [2,†], Anirban Manna [1,‡], Chirantan Majumder [1,‡], Koustav Pal [3], Nicolette Casarcia [4], Arijit Mondal [5], Sabyasachi Banerjee [6], Vinod Kumar Nelson [7], Suvranil Ghosh [1], Joyita Hazra [8], Ashish Bhattacharjee [2], Subhash Chandra Mandal [9], Mahadeb Pal [1,*] and Anupam Bishayee [4,*]

1 Division of Molecular Medicine, Bose Institute, Kolkata 700 054, India
2 Department of Biotechnology, National Institute of Technology, Durgapur 713 209, India
3 Jawaharlal Institute Post Graduate Medical Education and Research, Puducherry 605 006, India
4 College of Osteopathic Medicine, Lake Erie College of Osteopathic Medicine, Bradenton, FL 34211, USA
5 Department of Pharmaceutical Chemistry, M.R. College of Pharmaceutical Sciences and Research, Balisha 743 234, India
6 Department of Pharmaceutical Chemistry, Gupta College of Technological Sciences, Asansol 713 301, India
7 Department of Pharmacology, Raghavendra Institute of Pharmaceutical Education and Research, Anantapur 515 721, India
8 Department of Biotechnology, Indian Institute of Technology, Chennai 600 036, India
9 Department of Pharmaceutical Technology, Jadavpur University, Kolkata 700 032, India
* Correspondence: mahadeb@jcbose.ac.in or palmahadeb@gmail.com (M.P.); abishayee@lecom.edu or abishayee@gmail.com (A.B.)
† These authors contributed equally to this work.
‡ These authors contributed equally to this work.

Simple Summary: Colorectal cancer (CRC) is a significant cause of death worldwide. The inefficacy of the current treatment regimens is reflected in the frequent recurrence and emergence of a drug-resistant form of CRC. Numerous published reports from independent investigators around the globe have shown the great potential of natural products as a source of anti-CRC drug-leads with novel functions. Here, we have reviewed the literature on phenolic phytochemicals carrying anti-CRC activity in various in vivo models and analyzed their molecular basis of action to understand the implications of these findings in the future treatment and prevention of CRC.

Abstract: Colorectal cancer (CRC) is the third most diagnosed and second leading cause of cancer-related death worldwide. Limitations with existing treatment regimens have demanded the search for better treatment options. Different phytochemicals with promising anti-CRC activities have been reported, with the molecular mechanism of actions still emerging. This review aims to summarize recent progress on the study of natural phenolic compounds in ameliorating CRC using in vivo models. This review followed the guidelines of the Preferred Reporting Items for Systematic Reporting and Meta-Analysis. Information on the relevant topic was gathered by searching the PubMed, Scopus, ScienceDirect, and Web of Science databases using keywords, such as "colorectal cancer" AND "phenolic compounds", "colorectal cancer" AND "polyphenol", "colorectal cancer" AND "phenolic acids", "colorectal cancer" AND "flavonoids", "colorectal cancer" AND "stilbene", and "colorectal cancer" AND "lignan" from the reputed peer-reviewed journals published over the last 20 years. Publications that incorporated in vivo experimental designs and produced statistically significant results were considered for this review. Many of these polyphenols demonstrate anti-CRC activities by inhibiting key cellular factors. This inhibition has been demonstrated by antiapoptotic effects, antiproliferative effects, or by upregulating factors responsible for cell cycle arrest or cell death in various in vivo CRC models. Numerous studies from independent laboratories have highlighted different plant phenolic compounds for their anti-CRC activities. While promising anti-CRC activity in many of these agents has created interest in this area, in-depth mechanistic and well-designed clinical studies are needed to support the therapeutic use of these compounds for the prevention and treatment of CRC.

check for updates

Citation: De, S.; Paul, S.; Manna, A.; Majumder, C.; Pal, K.; Casarcia, N.; Mondal, A.; Banerjee, S.; Nelson, V.K.; Ghosh, S.; et al. Phenolic Phytochemicals for Prevention and Treatment of Colorectal Cancer: A Critical Evaluation of In Vivo Studies. *Cancers* **2023**, *15*, 993. https://doi.org/10.3390/cancers15030993

Academic Editor: Antonio V. Sterpetti

Received: 30 December 2022
Revised: 30 January 2023
Accepted: 30 January 2023
Published: 3 February 2023

Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

Keywords: colorectal cancer; phenolic compounds; prevention; treatment; molecular mechanisms; in vivo

1. Introduction

The diagnosis of colorectal cancer (CRC) is a death sentence to many. CRC is the third most diagnosed and second leading cause of cancer mortality worldwide [1]. In the United States alone, there were 149,500 new cases and 52,980 deaths in 2021, with an estimated 151,030 new cases for 2022 [1]. Globally, there were 1.9 million new cases and 935,000 deaths in 2020 [2]. These numbers have risen since 2018, as at that time statistics were noted to be 1.8 million new cases and 861,000 deaths [3]. Analyses predicted the global CRC burden to rise by 60% to 2.2 million new cases and 1.1 million deaths by 2030 [3–6]. Rising cases are attributed to a more sedentary lifestyle and altered dietary habits, such as consuming processed foods, tobacco usage, and heavy alcohol consumption. India's incidence of colon cancer in 2016 was estimated to be 63,000, with a sizeable interstate variation [7,8].

Since the implementation of a screening program in the United States in 1990, CRC incidence has consistently decreased in the population of those older than 50 years [9,10]. In contrast, CRC incidence has shown a significant and steady increase (2% per year) in the population of those less than 50 years of age, which is called young-onset CRC (yCRC) [9,11,12]. While yCRC comprises only 10% of total CRC incidence, 75% of yCRC incidence affects the population of those between 40 and 49 years of age [9,11–15]. A study undertaken between 1975 and 2010 predicted that yCRC would double by 2030 in the U.S. population of those younger than 35, indicating racial disparity [9,11–15].

Current treatment options available for colorectal cancer include laparoscopic surgery, resection, palliative, neoadjuvant chemotherapy, and radiotherapy [15–22]. Chemotherapy causes undesirable side effects. In addition to being frequently ineffective, current treatments are expensive.

Utilizing phytochemicals for cancer treatment and prevention has been a matter of serious discussion for decades [3,23]. Plants have been used to treat many diseases in traditional medicine and have been a forefront in alternative approach. Over 3000 plant species have anticancer activities, with thirty plant-derived compounds undergoing preclinical testing [5]. Anticancer activity in citrus fruits, allium vegetables, and medicinal plants has demonstrated preclinical success [5,8]. Secondary plant metabolites have been shown to decrease inflammation and increase apoptosis in addition to possessing antioxidant, anticarcinogenic, and antimetastatic properties [8,23,24]. The attraction to phytochemicals arises from relatively safer and cost-efficient natural products, and their consumption by humans is widespread [5]. While research is being conducted, often with promising results, only a limited number of natural compounds have been approved for clinical use, while the clinical application of many is hindered due to low bioavailability [5,23].

Numerous literature reviews and studies on natural compounds in CRC were dissected and sorted thoroughly for relevant and vital information. It was noted that very few articles reviewed CRC and the therapeutic prospects with polyphenols [25,26]. There is no review literature explaining all classes of phenolic compounds and their signaling pathways in contrast with CRC. We have also noted that few previous reviews have focused on using plant extracts and fractions rich in phenols and pure phenolic compounds [25,26]. Some have examined flavonoids and their effects on CRC [27–36], yet no such reviews consider other classes of phenolic compounds and their effects on CRC. In contrast, numerous reviews were dedicated to discussing the deadly disease of CRC, but did not examine natural products for its treatment. A few reviews that included CRC studied general nutrition and dietary effects, but the literature examined dietary products, such as calcium, fiber, processed meats, or medicinal plants, rather than plant phenolic compounds [37–41]. Furthermore, a review was noted to include the effects of phytochemicals on CRC, but only mentioned specific biochemical properties and pathways of cancer development [42]. In

view of the aforementioned limitations, our present review is up-to-date and offers the most recent information compared to previously published works. In this review, we first evaluated pertinent literature to present the characteristics of CRC and identify common risk factors and current treatment options. Then, we evaluated various in vivo studies on different phenolic phytochemicals to understand the potential of these natural agents for CRC prevention and treatment. We hope these phenolic phytocompounds spark interest in conducting new studies to eventually aid in decreasing the prevalence and lowering the risk of CRC.

2. Risk Factors

Familial, hereditary, and lifestyle factors are independent risk factors for developing CRC [43]. Genetic syndromes comprise 20–30% of CRC cases and can be divided into non-polyposis and polyposis types (Table 1). Lynch syndrome, an alternate term for the non-polyposis syndrome, is an autosomal dominant disease associated with a defect in DNA mismatch repair genes, such as *hMLH1*, *hMSH2*, *hMSH6*, or *hPMS2* [44,45]. This mutation results in microsatellite instability (MSI) regions, which is also associated with ~15% of sporadic CRC cases. As expected, individuals with MSI regions carry an increased risk for other cancers, such as endometrial carcinoma [44].

Table 1. Genes involved in different CRC syndromes and associated clinical symptoms.

Syndrome	Genetic Defects	Clinical Manifestations	References
Hereditary nonpolyposis cancer syndromes			
Lynch syndrome	*MLH1*, *MSH2*, *MSH6*, *MSH3*, and *PMS2*	Increased risk for CRC, (10–47%) depending on gene mutated; asymptomatic unless altered bowel habits, GI bleeding due to tumors/polyps occurs; increased risk for endometrial cancer; extracolonic manifestations are associated as Muir-Torre, Turcot.	[44,46,47]
Muir-Torre syndrome (HNPCC + Sebaceous gland malignancies)	*MLH1*, *MSH2*, *MSH6*, and *PMS2*	Sebaceous skin tumor/keratoacanthoma and Lynch syndrome features.	[48,49]
Turcot syndrome type 1 (HNPCC with primary brain tumors)	*MMR*, *MLH1*, and *PMS2*	Features of Lynch syndrome + primary brain tumors.	[50–53]
Hereditary polyposis colorectal cancers			
Familial adenomatous polyposis (FAP) syndrome	*APC*	More than colorectal adenomatous polyps; 100% cancer risk	[50,54]
Turcot syndrome type II (FAP with Primary Brain tumors)	*APC*	FAP syndrome + primary brain tumors, medulloblastoma, glioblastoma, astrocytoma.	[50–53]
Gardner syndrome	*APC*	FAP syndrome+ extraintestinal manifestations of desmoid tumors; sebaceous cysts; osteomas of mandible, skull, fibromatosis, congenital hypertrophy of retinal pigment epithelium (CHRPE); adrenal adenomas.	[55,56]
Adenomatous polyposis syndromes	*APC* and *MUTYH*	Increased number of colorectal adenomas (10–100 s), serrated polyposis, mixed polyps; duodenal adenomas are common; 43–33% increased risk of CRC; increased thyroid nodules, adrenal lesions, jawbone cysts.	[50,57–59]
Juvenile polyposis coli	*BMPR1A* and *SMAD4*	Multiple hamartomatous polyps in the GI tract- mainly colorectum; rectal bleeding due to polyps is a common presenting symptom; anemia due to bleeding is common; extracolonic manifestations hereditary hemorrhagic Telangiectasia (HHT) telangiectasias of buccal mucosa and skin, epistaxis, and anemia, with AV malformations; colorectal cancer risk 38.7% increased.	[60–62]
Peutz-Jeghers syndrome	*STK11*	Mucocutaneous pigmentation; hamartomatous polyps; 39% increased risk for CRC.	[63,64]
Cowden syndrome (multiple hamartomasyndrome)	*PTEN*	Mucocutaneous lesions and macrocephaly; skin manifestations; uterine leiomyomas, ovarian cysts; multiple hamartomas on any organ; increased risk of breast, thyroid, renal, endometrial, and colorectal cancer; 9–16% risk of CRC.; increased risk for malignant melanomas; specific dysplastic gangliocytoma of the cerebellum; Lhermitte-Duclos disease is specific to Cowden disease.	[65,66]

Abbreviations: MUTYH, mutY DNA glycosylase; STK11, serine/threonine kinase; 11SMAD4, mothers against decapentaplegic homolog 4; PTEN, phosphate and tensin homolog; BMPR1A, bone morphogenic protein receptor type 1A; MLH, MutL homolog; MSH, MutS homolog; MMR, mismatch repair.

Familial adenomatous polyposis syndrome (FAP), which is characterized by multiple polyp formations in the gastrointestinal tract, is caused by a germline mutation in the adenomatous polyposis coli (APC) gene [67–69]. Inheriting a polyposis syndrome can increase an individual's risk of developing colon cancer up to 100% [70]. Furthermore, these patients carry the risk of developing other gastrointestinal cancers and desmoid tumors. MUTYH-associated polyposis (MAP), Peutz-Jeughers syndrome (*STK11*), Juvenile polyposis syndrome (*SMAD4* and *BMPR1A*), hyperplastic polyposis (HPP), familial CRC (FCC) syndrome X, and Cowden syndrome (*PTEN*) are other polyposis syndromes that predispose individuals to an increased risk of developing CRC [50,71,72].

Chronic inflammatory bowel diseases, which encompass both ulcerative colitis and Crohn's disease, predispose individuals to CRC [73]. Additionally, previous abdominopelvic radiation is a potent risk factor for CRC, especially for childhood cancer survivors [74]. Furthermore, individuals receiving prostate cancer-related radiation therapy are at a higher risk of developing rectal carcinoma, supporting previous radiation therapy as a risk factor for CRC [75]. Cystic fibrosis is also implicated in CRC, as there is a 5–10 times greater risk of acquiring CRC in these patients. As a result, they have a separate management for CRC screening, especially post-transplant [76].

Lifestyle patterns, such as smoking, consumption of alcohol, obesity, sedentary lifestyles, and chronic diseases, pose a potent overall risk of developing sporadic CRC [77–79]. A westernized diet, rich in processed foods and red meat and deficient in fruits, fiber, and leafy vegetables, can contribute to CRC development [16,80]. Conversely, consuming more vegetables, fruits, and fiber is protective against CRC. A meta-analysis has elucidated the risk of CRC with food's dietary inflammatory index (DII). A higher DII correlating with a pro-inflammatory state increases CRC risk [81]. Numerous studies have explored the opposite end of the spectrum, examining anti-inflammatory foods and drugs for CRC chemoprevention and treatment. This is supported by a case-control meta-analysis where a higher intake of calcium, magnesium, and potassium lowered the occurrence of CRC [82].

The risk of CRC is low in vegetarians compared to meat eaters with an HR ratio of 0.49 [95% confidence interval (CI): 0.36 to 0.66], and 0.73 [95% CI: 0.54 to 0.99] when not adjusted and adjusted (for sociodemographic and lifestyle factors, multimorbidity, and body mass index) respectively. When CRC was subcategorized, the HR of 0.69 [95% CI: 0.48 to 0.99] for the colon and 0.43 [95% CI: 0.22 to 0.82] for the proximal colon was observed in vegetarians, which is much less compared to meat eaters [83]. Adherence to the Mediterranean diet was found to be associated with a low risk of rectal cancer with RR of 0.82 [95% CI: 0.71 to 0.95] for rectal cancer, 0.94 [95% CI: 0.87 to 1.02] for proximal colon cancer, and 0.91 [95% CI: 0.79 to 1.04] for distal colon cancer [84]. The unhealthy diet pattern is associated with CRC-specific mortality with RR/HR of 1.52 [95% CI: 1.13 to 2.06] [85]. The high intake of dietary calcium and magnesium is negatively associated with CRC risk with HR of 0.76 [95% CI: 0.72 to 0.80] and 0.80 [95% CI: 0.73 to 0.87], respectively. The higher intake of dietary heme, however, was positively correlated to colon cancer incidence with HR of 1.01 (95% CI: 0.82 to 1.19) and rectal cancer incidence with HR of 1.04 [95% CI: 0.67 to 1.42] [82]. The increase in DII score, and CRC are found to be positively associated with an overall increased risk of CRC by 40% with RR of 1.40 [95% CI: 1.26 to 1.55] [81]. Smoking and CRC shows a positive association with ever smoker versus never smoker, the pooled RR was 1.18 [95% CI: 1.11 to 1.25], and the pooled risk estimate was 1.25 [95% CI: 1.14 to 1.37] [77]. Alcohol consumption is also associated with an increased risk for CRC mortality. In comparison, the pooled RR was 1.03 [95% CI: 0.93 to 1.15] for any, 0.97 for light drinkers who consume $\leq$12.5 g of ethanol/day, 1.04 [95% CI: 0.94 to 1.16] for moderate drinkers who consume 12.6–49.9 g ethanol/day), 1.04 [95% CI: 0.94 to 1.16] for heavy drinking men (who consume $\geq$50 g ethanol/day), which is higher than heavy drinking women [pooled RR = 0.79 (95% CI: 0.40 to 1.54)] [78].

3. Pathogenesis

Overall, the pathogenesis of colon cancer involves three main pathways: the chromosomal instability (CIN)/classic adenoma-carcinoma sequence [86,87], the CpG island methy-

lator phenotype (CIMP), and the microsatellite instability (MSI) pathway [88]. While these are separate pathways, there is potential overlap within them. Moreover, they involve the stepwise accumulation of multiple mutations, eventually leading to CRC development [89].

The classic adenoma-carcinoma sequence accounts for 65–70% of sporadic diseases commonly observed as left-sided CRCs [90]. This mechanism involves a dysfunctional/inactivated APC gene located on chromosome 5q21. APC, a "gatekeeper" of colonic neoplasia, has been implicated in familial adenomatous polyposis (FAP) syndrome. The onset of CRC is inevitable in a population with an inactivating mutation in both copies of the APC gene [91,92]. APC controls cell growth and differentiation through the Wnt/β-catenin signaling pathway. The Wnt pathway is an essential cellular signaling system by which several developmental events for embryological and tissue homeostasis occur, involving cellular proliferation and differentiation. Deregulation of the Wnt pathway can lead to the development of cancer. When the Wnt/β-catenin pathway is suppressed, there is a lower rate of cellular proliferation and fewer intestinal stem cells [93]. Activating mutations of Wnt/β-catenin leads to the pathogenesis of CRC. Over 90% of CRC cases carry mutations within this pathway [94]. It has been found that APC deletion/loss of function leads to CRC development, while restoring APC function can regress adenomas by reducing Wnt activity [93].

Apart from APC, there are other Wnt activating mutations, such as mutations in the CTNNB1 gene encoding β-catenin. R-spondins are another module of Wnt signal activators, which are associated with up to 10% of CRC mutations. Antagonism of RSPO3 with paclitaxel effectively targeted Wnt signaling in CRC [95]. A higher expression of ß-catenin in CRC cells is associated with a worse prognosis and advanced stage of the disease. Because of this, CRC metastasis was determined by the combined β-catenin odds ratio in the nucleus [96].

In the absence of APC function, β-catenin translocate to the nucleus. In cooperation with the DNA binding factor TCF, it promotes the growth of colonic epithelium via uncontrolled overexpression of its targets c-Myc and cyclin D1 [93]. Next, a mutation in KRAS contributes to molecular pathogenesis by promoting adenoma formation [97]. Finally, a mutation in p53 facilitates the progression of CRC [98]. Although important roles of p53 and KRAS were implied in the adenoma-carcinoma pathway, mouse knockout of APC develops carcinoma irrespective of its KRAS and p53 status, and re-introduction of APC restores cellular differentiation and normal crypt formation [43,93].

The microsatellite instability pathway occurs due to the inactivation of DNA mismatch repair genes, which includes ATPases hMSH2, hMSH6, hMSH3, hMLH1, hPMS2, hPMS1, and hMLH3, as involved in Lynch syndrome [99]. The MSI pathway is involved in roughly 15% of CRCs, 3% of which are Lynch syndrome while the rest are sporadic, mainly caused by MLH1 hypermethylation. Finally, the CpG island methylator phenotype (CIMP) is involved in silencing genes by hypermethylation of CpG islands on their promoters [100,101]. CIMP has been associated with older patients, female patients, and right-sided lesions with high MSI and BRAF mutations. CIMP is also associated with PI3K mutations but lacks KRAS and p53 mutations. A clearer insight and greater understanding of CIMP is required to better study the treatment and prevention of CRC [102].

4. Chemoprevention

Chemoprevention aims to intervene, prevent, suppress, and reverse the initiation and progression of carcinogenesis. It further attempts to decrease the recurrence of cancer through the usage of drugs, vitamins, and nutritional supplements [66]. Various agents, including nonsteroidal anti-inflammatory drugs (NSAIDs), such as aspirin, and other agents, such as metformin, statins, minerals, and vitamins, have been previously studied for their chemopreventive benefits regarding CRC (Table 2). There is little doubt that a significant stride has been made into the unventured territories for the chemoprevention of CRC.

Table 2. Various drugs alone and in combination tested for their effects on clinical CRC chemoprevention studies.

Drugs	Study Design	Mechanism	Main Findings	References
Aspirin	Meta-analysis	COX-2 inhibition	There was a dose-dependent reduction in the risk of CR by aspirin. An aspirin dose of 75–100 mg/day reduced the risk by 10%, and 325 mg/day reduced the risk by 35% (Meta-analysis of 45 studies [RR = 0.73, 95% confidence interval (CI) 0.69–0.78])	[103–106]
Non-aspirin NSAIDS	Meta-analysis	COX-2 inhibition	Data from 23 studies suggested using higher doses of non-aspirin NSAIDs in the general population aged 40 years or older reduced CRC risk, specifically for white women, for distal colon cancer. (Pooled ODDs ratio was 0.74 (0.67–0.81), I2 = 75.9%, $p < 0.001$.)	[107]
Sulindac+ DFMO	RCT	Sulindac inhibits COX-2 DFMO- irreversibly inhibits Ornithine decarboxylase (polyamine synthesis)	Significant reduction of recurrent adenomas (12 vs. 41%, risk ratio 0.30), advanced adenomas (0.7 vs. 8.5%, risk ratio 0.09), and multiple adenomas (0.7 vs. 13.2%, risk ratio 0.06)	[108,109]
DFMO + Aspirin	RCT	Aspirin inhibits COX-2 DFMO inhibits polyamine synthesis Both combined may have a synergistic action.	After one year of treatment, in the DFMO + aspirin arm vs. placebo, there was a significant reduction in rectal aberrant crypt foci (precursor of rectal carcinoma). (74% vs. 45%, $p = 0.020$). No statistically significant reduction of colorectal adenomas was observed.	[110]
Erlotinib + Sulindac	RCT	Erlotinib is an EGFR inhibitor; sulindac is a COX-2 inhibitor.	In 82 patients of familial adenomatous polyposis, Sulindac + Erlotinib was associated with a 69.4% decrease in those with an intact colorectum compared with placebo (95% CI, 28.8−109.2%; $p = 0.009$)	[111]
Celecoxib	Meta-analysis	Selective COX-2 inhibitor, more specific for inflammation, with fewer GI side effects. Celecoxib has higher cardiovascular mortality	3 RCTs (involving 4420 patients) and 3 post-trial studies (2159) showed a significant reduction in the incidence of adenoma RR (0.67 [95% CI, 0.62–0.72] compared with placebo). There was an increased risk of cardiovascular mortality with twice dosing 400 mg celecoxib (RR 3.42 [95% CI, 1.56–7.46]). Once-a-day dosing did not show an increased CV risk. (1.01 [95% CI, 0.70–1.46]).	[112]
Clopidogrel	Case-control Study	Clopidogrel inhibits platelet aggregation via irreversible inhibition of the P2Y12 receptor	Clopidogrel decreased CRC risk in patients receiving treatment >1 year. (0.65% AOR; 95% CI, 0.55–0.78). Dual antiplatelet therapy (Clopidogrel aspirin) had the same effect as either drug is taken as monotherapy.	[113]
Metformin	Meta-analysis	Activates AMPK, inhibits mTOR pathway	Metformin users had a significantly lower incidence. CRC (RR 0.76, CI 0.69–0.84, $p < 0.001$) compared with non-metformin users. Further analysis on the overall survival of metastatic CRC patients revealed significantly higher survival rates in metformin users (HR 0.77, CI 0.68–0.87, $p < 0.001$).	[114]
UCDA	Cohort Study	Has antioxidant action. Prevents NF-κB and AP1 activity. Inhibits c-Myc	Chronic liver disease patients with UCDA have a reduced risk of colorectal cancer. UDCA use was associated with a reduced risk of CRC (hazard ratio, 0.60; 95% confidence interval [CI], 0.39–0.92).	[115]
Statin	Meta-analysis	*3-HMGCOA* reductase inhibitor decreases cholesterol synthesis. Antioxidant activity; shows pro-apoptotic effects on human CRC lines. Anti-inflammatory properties	14 studies involving 130,994 patients. In terms of post-diagnosis statin uses, the pooled HR of all-cause mortality was 0.86 (95% CI, 0.76–0.98), and the pooled HR of CSM was 0.79 (95%CI, 0.70–0.89) (Cancer-Specific Mortality).	[116,117]

Table 2. *Cont.*

Drugs	Study Design	Mechanism	Main Findings	References
Menopausal hormone therapy (combined estrogen-progestin)	Nationwide Cohort Study (Norway)	Estrogens have been proposed to alter bile acid composition, modulate colonic transit. Decrease production of mitogenic insulin-like growth factor	The current use of postmenopausal hormone therapy was associated with a decreased CRC risk. RR (for combined estrogen-progestin therapy) in oral formulations was 0.86 (95% CI 0.71 to 1.05)	[118]
Bisphosphonates	Meta-analysis	Inhibits osteoclastic bone resorption, Anti-apoptotic effect	Meta-analysis of 34 studies and 4,508,261 participants. There was a significant reduction in the risk of CRC. (RR = 0.89, 95% CI: 0.81–0.98)	[119]

Abbreviations: RCT, randomized control trial; RR, relative risk; HR, hazard ratio; OR, odds ratio, AOR, adjusted odds ratio; CI, confidence interval; DFMO, difluoromethylornithine; UCDA, ursodeoxycholic acid.

In CRC involving the APC/β-catenin pathway, cyclooxygenase-2 (COX-2) is often implicated in the early and later stages of the adenoma sequence, driving the formation into a carcinoma [120–123]. Furthermore, COX-2 overexpression produces vascular endothelial growth factor (VEGF), which promotes tumor angiogenesis [124,125]. Hence, by targeting COX-2, various studies have shown that NSAIDs, ranging from aspirin and sulindac to the more selective COX-2 inhibitors, such as celecoxib, have proven benefits in reducing disease risk [126,127]. In the 1990s, the U.S. Preventive Services Task Force recommended aspirin to prevent non-high-risk CRC [128–130].

Other drugs, such as metformin, showed promising effects in reducing the risk of CRC development. Recent meta-analyses showed that metformin could reduce CRC risk by 22% [131]. In an ongoing ASAMET trial for the tertiary prevention of stage I–III CRC, patients were administered low doses of aspirin combined with metformin for a potential synergistic chemo-preventive action [132]. Statins, a specific inhibitor of HMG-CoA reductase in the mevalonate synthesis pathway, have been recommended to lower serum lipid levels [133]. Statins were shown to reduce CRC alone and in combination with NSAIDS [134,135]. Further investigations on multiple agents, such as antioxidants, minerals, such as selenium, and vitamins, including A, C, E, and β-carotene, were previously believed to have benefits in decreasing the risk of CRC, yet they have yielded mixed results [130,136,137]. Studies on folate's use to lower CRC risks also yielded mixed results [130]. Fiber, alcohol, monounsaturated fatty acids, polyunsaturated fatty acids, omega-3, omega-6, niacin, thiamine, riboflavin, vitamin B6, vitamin B12, zinc, magnesium, selenium, vitamin A, vitamin C, vitamin D, vitamin E, folic acid, β-carotene, anthocyanin, flavonoids, garlic, ginger, onions, thyme, oregano, saffron, turmeric, rosemary, eugenol, caffeine, and tea have all demonstrated anti-inflammatory benefits, and therefore reduce the risk of CRC development [138,139]. A higher intake of dietary fiber, pertaining to whole grains, was associated with a lower CRC risk in men [140].

5. Treatment

CRC incidence and mortality have been efficiently controlled by the routine screening and removal of polyps through colonoscopy [141]. Surgery, chemotherapy, and immunotherapy are mainstay treatments for CRC; the stage of CRC progression in each patient determines an appropriate combination. The treatment of CRC depends upon the diagnosis through tumor/node/metastasis (TNM) staging of the lesion. Adjuvant chemotherapy with fluorouracil (5-FU) decreases death rates in patients with high-risk stage II colon cancer by 3–5% and 10–15% in stage III disease alone [142]. MSI/MMR protein levels determined by IHC aid in deciding the adjuvant therapy [143–145]. Furthermore, after primary tumor resection, TNM or immunoscore can be considered to assess the tumor recurrence risk [146].

Single-agent therapy with 5-FU or therapy with multiple agents composed of 5-FU and oxaliplatin (FOLFOX), 5-FU and irinotecan (FOXFIRI) (IRI), or capecitabine and oxaliplatin (CAPOX), capecitabine (CAP), and irinotecan (CAPIRI) as first line chemotherapy is recommended based on the sensitivity and the stage of the disease. In many cases, single-agent chemotherapies yielded better results than combination therapy, given the associated systemic toxicity and unsatisfying responses [147–149]. A combination of 5-FU or CAP with oxaliplatin (OX) is recommended for stage III CRC for three to six months. Patients with intermediate-risk stage II CRC are recommended either 5-FU or CAP, which are added to OX, if the patients are high risk (stage II), for three months [145]. The International Duration Evaluation of Adjuvant Chemotherapy (IDEA) collaboration helped investigate whether three or six months of adjuvant chemotherapy was necessary, as cumulative toxicity develops from fluoropyrimidines/oxaliplatin in the form of peripheral sensory neuropathy. Results show that the overall disease-free survival was similar at 74.6% and 75.5% for three months and six months, respectively. After three months of treatment, the overall sensory peripheral neuropathy reduced from 34% to 11%. However, per ESMO guidelines, stage III CRC should still be treated with six months of FOLFOX or CAPOX if

the patient falls within the high-risk category. For patients who do not tolerate oxaliplatin, capecitabine, or LVGFU2 can be acceptable alternatives [145].

Various forms of supplemental targeted immunotherapies are considered to aid chemotherapy. Monoclonal antibodies are used to attack various potential genes, such as ERFR, VEGF, and PDL-1/PDL-1. Cetuximab, an anti-EGFR chimeric monoclonal antibody, and bevacizumab, an anti-VEGF chimeric monoclonal antibody, both of which prolong OS, were the first line targeted drugs approved by the United States Food and Drug Administration (FDA) in 2004 [150,151]. An immune checkpoint blocker α-PD1/PDL-1 antibody, in combination with chemo- and radiation therapy, was approved by the FDA for MSI-H and dMMR classes of CRCs for sustained progression-free survival [152]. Cetuximab yielded a positive outcome for CRC that did not respond to single-agent IRI or fluoropyrimidine therapy. Combining cetuximab with IRI, fluorocytidine, or OX delivered promising results [151,153]. EGFR (epidermal growth factor receptor) is overexpressed in various cancers to different extents, including 25–75% in CRC [154]. Cetuximab, once bound, results in the internalization and degradation of EGFR [111]. However, cetuximab was inactive in CRCs carrying the RAS (KRAS) mutation. Like EGFR, the VEGF level is also elevated in CRC, predicting a poor prognosis [155]. Along with an elevated VEGF level, increased vascular endothelial growth factor receptor (VEGFR) activity is found in adenomas, as well as in the metastatic stage of CRC [147,156]. While cetuximab is not suitable as a second line agent, bevacizumab is often an excellent choice.

6. Literature Search Methodology

We have followed the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines [157] for this work. Four scholarly databases, namely PubMed, Scopus, ScienceDirect, and Web of Science, were utilized to screen the literature for the last 20 years (2002 to 2022 November) by searching the title, abstract, and key words section with the key words “colorectal cancer” AND “phenolic compounds”, “colorectal cancer” AND “polyphenol”, “colorectal cancer” AND “phenolic acids”, “colorectal cancer” AND “flavonoids”, “colorectal cancer” AND “stilbene”, and “colorectal cancer” AND “lignan.” All search results were gathered, and duplicate files were removed. Next, literature was scanned based on title and abstract. Selected articles were then searched for retrieval. After reading the full articles, preclinical studies (in vivo animal models) with polyphenols were selected and incorporated. The methodology for literature search and study selection is depicted in Figure 1.

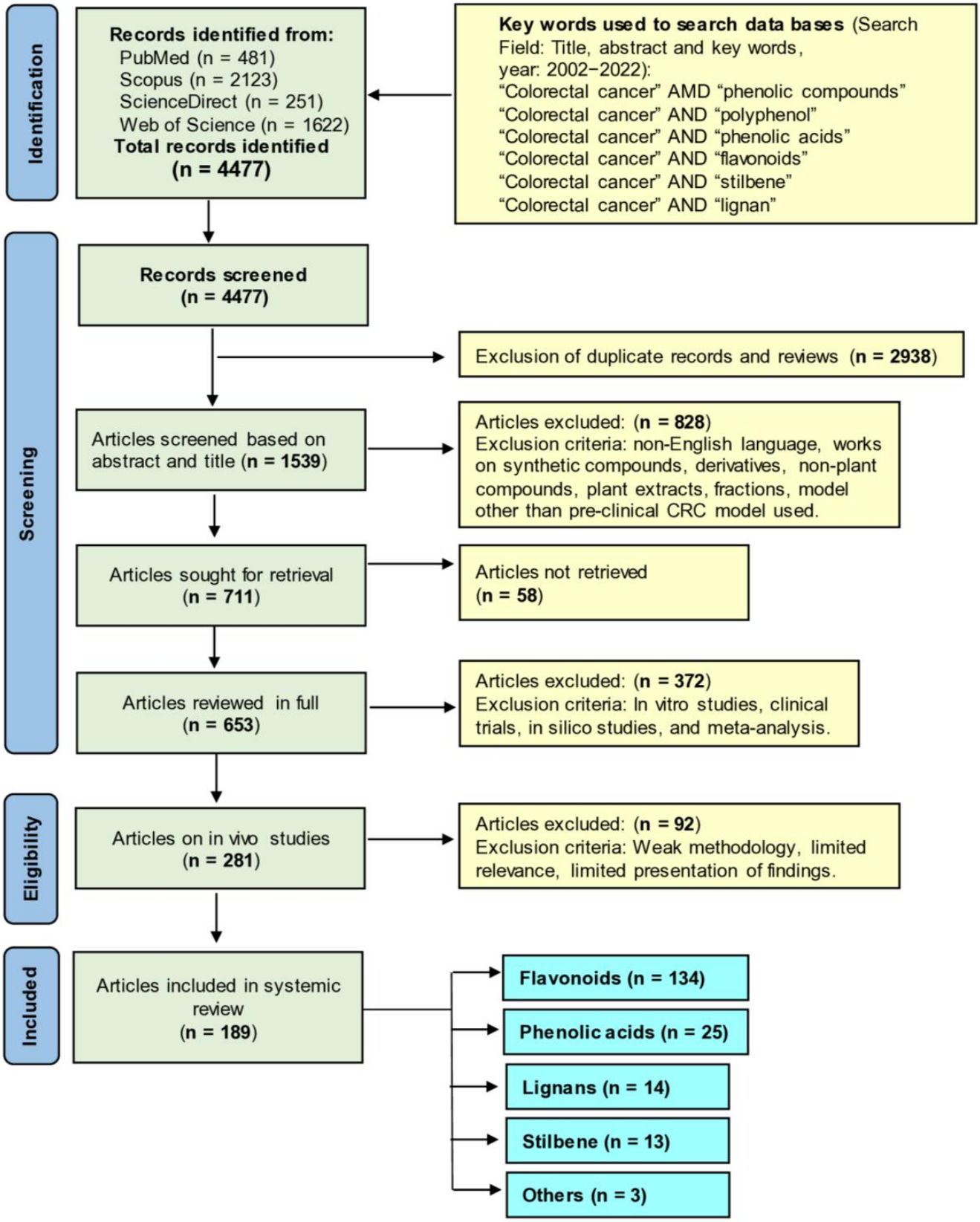

Figure 1. The PRISMA flow chart summarizing the literature search. Here "n" represents the number of articles.

7. Phenolic Compounds with In Vivo Anti-CRC Activities

Plants synthesize phenolic compounds as secondary metabolites and carry multiple aromatic rings with two or more hydroxyl groups. Phenolic compounds carry a wide (~8000 different) variety of chemical structures. Based on chemical structures, phenolic compounds are divided into different classes, such as flavonoids (e.g., anthocyanidins, flavanols, flavanones, flavones, flavonols, and isoflavoniods) and non-flavonoids, including phenolic acids (e.g., hydroxycinnamic acids and hydroxybenzoic acids), coumarins, stilbenes, lignans, and tannins [158–160]. Significant sources of phenolic compounds are fruits and vegetables. Various phenolic compounds are known for their interesting pharmacological properties, including antioxidant, anti-inflammatory, neuroprotective, and anticancer properties [161,162].

While western medicines have significant effects on CRC chemoprevention and treatment, extracts of numerous plants and plant products are still currently in use, as humanity has used plants for thousands of years as traditional or ethnic medicines for the prevention and treatment of various ailments, including cancer. The primary reasons for their popularity are fewer side effects, easy availability, and low cost compared to synthetic drugs [163–165]. Over the last several decades, steady progress has been achieved in identifying the bioactive secondary metabolites of plants, such as phenolic compounds, and understanding their mode of action to explain their health benefits [166–169]. In the following sections, we aim to summarize the anti-CRC effects of phenolic compounds based on animal studies. Table 3 describes the in vivo CRC activity of the compounds as revealed by our literature search as depicted in Figure 1. We have selected 16 relatively well-studied compounds to describe their anti-CRC activities in a greater detail in the following sections. The chemical structures of various classes of phenolic compounds with in vivo anti-CRC activities are presented in Figures 2–5.

Table 3. Anti-CRC effects and mechanisms of action of phenolic compounds based on in vivo studies.

Phytocompound	Source	Animal Model Studied	Dose and Route of Administration	Mode of Action	Reference
Flavonoids					
2,3,5,4′-tetrahydroxystilbene-2-O-β-D-glucoside	*Polygonum multiflorum* Thunb	AOM-induced colon carcinogenesis in male F144 rats	Oral administration, 30, 150, 250 mg/kg	Decreased the number ofACF by 47–54%; suppressed tumor growth; downregulated NF-κB in nucleus and cytoplasm; downregulated CEA	[170]
4′-hydroxychalcone	Herb, teas, and spices	$APC^{Min/+}$ mice	Oral administration, 10 mg/kg	Reduced the incidences and size of adenomas; induced apoptosis; suppressed proliferation of polyps; downregulated Ki-67; downregulated *c-Myc*, *Axin2* and *CD44* gene expression	[171]
Aciculatin	*Chrysopogon aciculatus*	HCT116 induced tumor xenograft SCID mice	Intraperitoneal injection, 30 mg/kg	Suppressed tumor growth without losing weight; upregulated the expression of p53 and downregulated the expression of Ki-67; induced apoptosis; arrested cells in sub G_1 phase	[172]
Apigenin	Parsley, wheat, onions, apples, and tea plants	AOM-induced CF-1 mice and Min mice carrying mutant APC gene	Oral administration of 0.1% dietary apigenin	Reduced ACF formation and ODC activity	[173]
		Male BALB/c-nu mice	Intraperitoneal injection, 20 mg/kg	Induced apoptosis of CRC cells; upregulated FADD expression and its phosphorylation	[174]
		Male BALB/c-nu mice injected with SW480 cells	Route of administration not reported, 50 mg/kg	Elevated transgelin and downregulation of MMP-9 expression via reducing Akt phosphorylation at Ser473 and Thr308	[175]
		$APC^{Min/+}$ mice	Oral gavage, 25 and 50 mg/kg	Reduced the number of polyps; induced of p53 activity	[176]
		Nude BALB/c mice injected with HT-29 cells	Subcutaneous injection, 35 mg/kg	Induced apoptosis; induced autophagy through inhibition mTOR/PI3K/Akt signaling pathway	[177]
		SCID mice	Oral gavage, 25 mg/kg	Suppressed prosurvival regulators Mcl-1, Akt, and ERK	[178]
		NEDD9 knock downed DLD1 cells mediated metastasis model in female athymic nude mice	Intraperitoneal injection, 20 mg/kg	Suppressed invasion, migration, and metastasis by downregulating overexpressed Neural precursor cells expressed NEDD9	[179]

Table 3. *Cont.*

Phytocompound	Source	Animal Model Studied	Dose and Route of Administration	Mode of Action	Reference
Baicalein	*Scutellaria baicalensis* Georgi	AMO and DSS induced colon tumor in male ICR mice	Oral administration, 1,5 and 10 mg/kg	Restored colon length; reduced tissue inflammation.	[180]
		SW620 xenograft in BALB/c nude mice	Intraperitoneal injection, 50 mg/kg	Suppressed tumor growth by 55% without losing body weight	[181]
		CT-26 derived tumor in female BALB/c mice	Intraperitoneal injection, 20 and 40 mg/kg	Reduced tumor growth rate; downregulated TLR4 and p-IκBα protein expression; inhibited NF-κB	[182]
		HT-29 cell-induced tumor xenograft in male nude mice	Oral administration, 10 mg/kg	Suppressed tumor growth by 29.33% compared to the control group; induced apoptosis; upregulated p53 and p21	[183]
		DLD-1 tumor xenograft in BALB/c athymic nude mice	Intragastric administration, 20 mg/kg	Suppression of tumor growth; inhibition of ERK phosphorylation; downregulation of MMP-2 and MMP-9	[184]
		HCT116 tumor xenograft in NSG immunodeficient mice	Intraperitoneal injection, 50 mg/kg	Suppressed tumorigenesis; inhibited colon cancer growth; induced apoptosis and senescence	[185]
		HCT116 tumor xenograft in athymic BALB/c nude mice	Intraperitoneal injection, 80 mg/kg	Suppressed tumor growth; induced senescence; upregulated DEPP; activated Ras/Raf/MEK/ERK pathway	[186]
		HT-29 tumor xenograft in nude mice	Intraperitoneal injection, 50 and 100 mg/kg	Suppressed tumor growth	[187]
		HCT116 tumor xenograft in athymic BALB/c nude mice	Intraperitoneal injection, 100 and 200 mg/kg	Suppressed tumor growth; induced apoptosis; suppressed cancer stem cells; inhibited EMT and cyclin D1	[188]
		$APC^{Min/+}$ mice	Oral administration, 30 mg/kg	Reduced tumor numbers; suppressed IL-1β, IL-2, IL-6, and IL-10	[189]
		HCT116 tumor xenograft in male BALB/c nude mice	Intraperitoneal injection, 100 mg/kg	Suppressed tumor growth; decreased circMYH9, mir761 and HDGF	[190]

Table 3. *Cont.*

Phytocompound	Source	Animal Model Studied	Dose and Route of Administration	Mode of Action	Reference
Boeravinone B	*Boerhaavia diffusa*	DMH-induced CRC in Swiss albino Wistar rats	Intraperitoneal injection, 20 and 40 mg/kg	Decreased the number of tumor incidences; downregulated LPO; upregulated catalase, SOD and GSH; downregulated TNF-α, IL-1β, IL-6, COX-2, PGE2 and iNOS; upregulated levels of IL-4 and IL-10; down regulated MPO; downregulated the expression of GDI2 mRNA	[191]
Chrysin	*Passiflora caerulea, Passiflora incarnata, Oroxylum indicum*	AOM-induced ACF in male F344 rats	Dietary administration, 0.001% and 0.01%	Reduced mitotic index and increased apoptotic index; reduced the frequency of ACF	[192]
		Male albino rats injected with DMH + DSS	Oral administration, 125 and 250 mg/kg	Reduced the level of CXCL1, AREG and MMP-9	[193]
Curcumin	*Curcuma longa*	DSS-induced colitis in C57BL/6 mice	Oral consumption as dietary supplement, 0.6%	Reduced tumor incidences; inhibited nuclear translocation of β-catenin; downregulated TNF-α and interferon-γ; downregulated COX-2 and p53	[194]
		HCT116 tumor xenograft in female ICR SCID mice	Intragastric administration, 500 mg/kg	Suppressed tumor growth; inhibited proteasome; suppressed proliferation; induced apoptosis	[195]
		AOM-DSS induced CRC in male C57BL/6 mice	Oral gavage, 500 mg/kg	Reduced CRC tumor number; downregulated IL-1β, IL-6, COX-2 and β-catenin; suppressed Axin2 by inhibiting Wnt/β-catenin pathway	[196]
		AOM-induced colonic preneoplastic lesion in C57BL/KsJ-db/db obese mice	Dietary supplement, 0.2% and 2.0%	Inhibited colonic premalignant lesion	[197]
		HCT116 tumor xenograft in athymic nu/nu nude mice	Oral administration, 1 g/kg	Enhanced the efficacy of radiation therapy; suppressed NF-κB activity and expression	[198]
		Colo205 and LoVo tumor xenografts in athymic nu/nu mice	Tail vein injection, 40 mg/kg	Inhibited tumor growth; suppressed angiogenesis	[199]
		AOM-induced colon carcinogenesis in $Il10^{-/-}$ mice	Oral administration, 1%	Reduced colon tumors	[200]
		AOM/DSS-induced colitis in C5757BL/6 mice	Oral administration, 25 mg/kg	Suppressed colitis-associated colon cancer and reduced tumor number	[201]

Table 3. *Cont.*

Phytocompound	Source	Animal Model Studied	Dose and Route of Administration	Mode of Action	Reference
Cyanidin	Blackberries (*Rubus fruticosus*)	$Apc^{Min/+}$ mice	Dietary supplementation, 0.03%, 0.1% or 0.3%	Reduced adenoma counts	[202]
Daidzein	Soybeans and soy-based products, and nuts	Male albino rats injected with DMH + DSS	Oral administration, 5 and 10 mg/kg	Reduced the level of CXCL1, AREG and MMP-9	[193]
Delphinidin	Berries, pomegranates, eggplant, roselle, and wine	Male BALB/c nude mice xenograft with luciferase-transfected DLD-1 cells	Intraperitoneal injection, 100 μM	Suppressed integrin/FAK nexus; elevated miR-204–3p levels	[203]
Diosmetin	Chamomile, parsley, rosemary, rooibos tea, green tea, and other plants fof the mint and citrus family (Lamiaceae)	NCr nu/nu nude mice injected with HCT-116 cells	Oral administration, 50 and 100 mg/kg	Downregulated Bcl-2; upregulated Bax	[204]
EGCG	*Camellia sinensis* L. Ktze	SW837 xenograft in male BALB/c nude mice	Oral administration, 0.01% and 0.1%	Reduced tumor growth; inhibited phosphorylation of VEGFR-2, Akt and ERK	[205]
		AOM-induced colonic premalignant lesions C57BL/KsJ-db/db mice	Oral administration, 0.01% and 0.1%	Decreased p-IGF-IR, p-GSK-3β, β-catenin, COX-2 and cyclin D1 in colonic mucosa; reduced IGF-I, insulin, triglyceride, cholesterol and leptin in serum	[206]
		AOM-induced colonic carcinogenesis in ICR mice	Oral administration, 0.25% and 0.5%	Inhibited large ACF formation; inhibited iNOS and COX-2	[207]
		HCT116-SDCSCs tumor xenograft in athymic nude mice	Cells were pretreated, 100 μM	Suppressed tumor formation; downregulated Notch1, Bmi1, Suz12, and Ezh1; upregulated miR-34a, miR-145 and miR-200c	[208]
		DMH-induced colon carcinogenesis in Wister rats	Oral administration, 0.2%	Inhibited ACF and induced apoptosis	[209]
		DMH-induced CRC in male Wistar rats	Oral administration, 50, 100 and 200 mg/kg	Lowered ACF formation; reduced tumor volume	[210]

Table 3. *Cont.*

Phytocompound	Source	Animal Model Studied	Dose and Route of Administration	Mode of Action	Reference
Eriodictyol	*Eriodictyon californicum*	DMH-induced colon carcinogenesis in male albino Wistar rats	Intragastrical administration, 200 mg/kg	Suppressed the number of polyps, ACF and lipid peroxidation levels; upregulated catalase, SOD, GP_X, GST, GSH and GR	[211]
Euxanthone	*Polygala caudata*	HT-29 cells induced tumor in BALB/c nude mice	Intraperitoneal injection, 20 and 40 mg/kg	Suppressed tumor growth; induced apoptosis; upregulated Bax; downregulated Bcl-2; induced caspase-3 cleavage; downregulated CIP2A expression and upregulated PP2A	[212]
Fisetin	Strawberry, apple, persimmon, grapes, onion, and cucumber	AOM and DSS induced CAC in male BALB/c mice	Oral administration, 20 mg/kg	Suppressed dysplastic lesions; induced apoptosis in colonic tissue; downregulated Bcl-2 and STAT3	[213]
		FC1 mice, 3K1 mice, $Apc^{Min/+}$ males, $3K1Apc^{Min/+}$ mice, B6 congenic strain, B6 $FC13K1Apc^{Min/+}$ mice	Intraperitoneal injection, 1 mg/animal	Upregulated AMPK phosphorylation; suppressed PI3K/Akt/mTOR signaling	[214]
		Male athymic nude mice	Oral administration, 400 and 800 mg/kg	Induced apoptosis, caspase-8 and cyt.; inhibited IGF1R and Akt	[215]
		CT-26 tumor in BALB/c nude mice	Subcutaneous injection, 5 mg/kg	Suppressed oncoprotein securin in p53-independent fashion	[216]
		BALB/c mice	Tail vein injection, 50 mg/kg	Inhibited programmed cell death and angiogenesis	[217]
		HCT116 tumor xenograft in mice NOD/Shi-scid-IL2R gamma (null) (NOG)	Intraperitoneal injection, 30, 60 and 120 mg/kg	Suppressed tumor growth in a dose-dependent manner	[218]
Flavone	Fruits and vegetables	DMM-induced colon carcinogenesis in C57BL/6J mice	Subcutaneous injection, 15 and 400 mg/kg	Suppressed ACF formation and multiplicity	[219]
Formononetin	*Astragalus membranaceus*	Female BALB/c-nu/nu mice injected with HCT-116 cells	Intraperitoneal injection, 20 mg/kg	Decreased VEGF, MMP-2 and MMP-9 levels	[220]
Furowanin A	*Millettia pachycarpa* Benth	HT-29 tumor xenograft in male athymic BALB/c nude mice	Intraperitoneal injection, 20 and 40 mg/kg	Suppressed tumor growth, induced apoptosis and autophagy; upregulated cleaved caspase-3, LC3BII, Beclin and p27; downregulated Ki-67, pSTAT3, Mcl-1, p62, and cyclin D	[221]

Table 3. *Cont.*

Phytocompound	Source	Animal Model Studied	Dose and Route of Administration	Mode of Action	Reference
Genistein	*Genista tinctoria*	DMH-induced colon cancer in Wistar rats	Oral administration, 2.5 mg/kg	Regulated tumor microenvironment; upregulated SOD, CAT, GPx, GR, vitamin A, vitamin C, vitamin E and GSH; activated NRF2 and HO-1; reduced expression of CD133, CD44 and β-catenin	[222]
		AOM-induced colon cancer in Sprague-Dawley rats	Dietary supplementation, 140 mg/kg	Suppressed the expression of cyclin-D1 and c-Myc; decreased expression of Wnt5a, Sfrp1, Sfrp2, and Sfrp5; downregulated Wnt/ β-catenin pathway	[223]
		HCT116 tumor xenograft in athymic BALB/c mice	Oral administration, 75 mg/kg	Didn't inhibit tumor growth; suppress metastasis; downregulated MMP-2 and EGFR3	[224]
Genkwanin	Dried flower buds of *Daphne genkwa*	$APC^{Min/+}$ mice	Oral administration, 12.5 and 25 mg/kg	Inducted host defense; reduced proinflammatory cytokine levels	[225]
		AOM/DSS-induced C57BL/6J mice	Oral administration, 22.5 mg/kg	Suppressed colon cancer growth by triggering tumor cell death; inhibited of pro-inflammatory cytokines	[226]
Hesperidin	Citrus fruits	AOM-induced Swiss albino mice	Oral administration, 25 mg/kg	Inhibited NF-κB, iNOS and COX-2; reduced cellular oxidative indicators and improved antioxidant status	[227]
		AOM-induced male Swiss albino mice	Oral administration, 25 mg/kg	Inhibited the constitutively active Aurora-A driven PI3K/Akt/GSK-3 and mTOR; activated autophagy	[228]
		AOM-induced male F344 rats	Oral administration, 1000 ppm	Inhibited ACF formation; reduced colonic mucosal ODC activity and polyamine levels in the blood	[229]
		DMH-induced CRC in albino rats	Oral administration, 25 mg/kg	Elevated the expression of Smad4 and activin A	[230]
Hinokiflavone	*Selaginella tamariscina*, *Juniperus phoenicea*, and *Rhus succedanea*	CT-26 tumor in female BALB/c mice	Intraperitoneal injection, 25 and 50 mg/kg	Suppressed tumor growth and proliferation; induced apoptosis; downregulated Ki-67 and MMP-9	[231]
Icariside II	*Epimedi* Herba	SW620 tumor xenograft in nude BALB/c mice	Intraperitoneal injection, 25 mg/kg	Suppressed tumor growth; induced apoptosis	[232]
Icaritin	*Epimedium* sp.	HT-29 tumor xenograft in male nude mice	Oral gavage, 10 mg/kg	Suppressed tumor growth and volume	[233]

Table 3. *Cont.*

Phytocompound	Source	Animal Model Studied	Dose and Route of Administration	Mode of Action	Reference
Isoangustone A	*Glycyrrhiza* sp.	SW480 tumor xenograft in male BALB/c nu/nu mice	Intraperitoneal injection, 10 mg/kg	Suppressed tumor growth; induced autophagic cell death; upregulated phosphorylation of AMPK, ACC and LC3B-1 and II levels	[234]
Isoliquiritigenin	*Glycyrrhiza glabra*	AOM/DSS-induced colon carcinogenesis in male BALB/c mice	Intragastrical administration, 3, 15 and 75 mg/kg	Suppressed tumorigenesis; inhibited macrophage polarization; upregulated TNF-α, INF-γ and IL-12; downregulated TGF-β, IL-10 and IL-1 and COX-2	[235]
	Glycyrrhiza uralensis Fisher	AOM-treated colon carcinogenesis in 344 rats	Oral administration, 100 ppm dietary supplementation	Suppressed ACF formation; induced apoptosis	[236]
Isorhamnetin	*Opuntia ficus-indica*	HT-29 RFP xenograft in immunosuppressed mice	Oral administration, dose not reported	Elevated cleaved caspase-9, Hdac11, and Bai1 proteins	[237]
		FVB/N mice treated with AOM/DSS	Oral administration, dietary supplement, dose not reported	Inhibited nuclear translocation of β-catenin and c-Src stimulation; activated CSK	[238]
Kaempferol	Apple, tea, broccoli, and grapefruit	DMH-induced colorectal carcinogenesis in male Wistar rats	Oral administration, 200 mg/kg	Restored CAT, SOD, and GPx	[239]
		DMH-induced colon carcinoma in male Sprague Dawley rats	Oral administration, 200 mg/kg	Reduced multiple plaque lesions and preneoplastic lesions	[240]
		DMH-induced colitis in Sprague-Dawley albino rats	Oral administration, 200 mg/kg	Reduced multiplicity of the ACF; downregulated COX-2 and PCNA	[241]

Table 3. *Cont.*

Phytocompound	Source	Animal Model Studied	Dose and Route of Administration	Mode of Action	Reference
Luteolin	Celery, parsley, broccoli, onion leaves, carrots, peppers, cabbages, and tea	DMH-induced carcinogenesis in male Wistar rats	Subcutaneous injection, 0.2 mg/kg	Reduced the number of tumor polyps and colon polyploids; decreased COX-2 level in blood and colonic tissue	[242]
		AOM-induced CRC in male BALB/c mice	Oral administration, 1.2 mg/kg	Reduced the levels of alkaline phosphatase and lactate dehydrogenase; suppressed iNOS and COX-2	[243]
		AOM-induced CRC in male BALB/c mice	Oral administration, 1.2 mg/kg	Reduced cytochrome b_5, cytochrome P450 and cytochrome b_5; enhanced the expression of UDP-GT and GST in colonic tissue; upregulated Nrf2	[244]
		CT-26 mediated lung metastasis	Oral administration, 10 and 50 mg/kg	Suppressed lung nodules and nodule volume; inhibited MMP-9 expression	[245]
		AOM-induced colon carcinogenesis in BALB/c mice	Oral administration, 1.2 mg/kg	Inhibited MMP-2 and MMP-9; downregulated γ-glutamyl transferase, 5′ nucleotidase, cathepsin D, and carcinoembryonic antigen	[246]
		HT-29 tumor xenograft in BALB/c nude mice	Intragastric administration, 100 mg/kg	Suppressed CRC metastasis; upregulated miR-384; downregulated pleiotrophin expression	[247]
		HT-29 tumor xenograft in BALB/c nude mice	Intraperitoneal injection, 50 mg/kg	Inhibited tumor growth; induced apoptosis	[248]
Lysionotin	*Lysionotus pauciflorus* Maxim	HCT116 tumor xenograft in athymic nude mice	Intraperitoneal injection, 20 mg/kg	Suppressed tumor growth; induced ferroptosis	[249]
Magnolin	*Magnolia biondii*	HCT116 tumor xenograft in female BALB/c athymic nude mice	Intraperitoneal injection, 20 mg/kg	Suppressed tumor growth; downregulated LIF, STAT3 and Mcl-1	[250]

Table 3. *Cont.*

Phytocompound	Source	Animal Model Studied	Dose and Route of Administration	Mode of Action	Reference
Morin	Old fustic (*Chlorophora tinctoria*) and osage orange (*Maclura pomifera*)	Male athymic nude mice injected with HCT-116 cells	Intraperitoneal injection, 30 and 60 mg/kg	Inactivated NF-κB signaling	[251]
		Male albino Wistar rats injected with DMH	Intraperitoneal injection, 30 and 60 mg/kg	Modulated tumor metabolism via β-cateinin/c-myc signaling, glycolysis and glutaminolysis pathways	[252]
		Pirc rats (F344/NTac-Apc am1137)	Dietary supplementation, 50 mg/kg	Restored the sensitivity to apoptosis by inhibiting LMW-PTP	[253]
		Male albino Wistar rats injected with DMH	Intragastric administration, 50 mg/kg	Reduced ACF formation; suppressed fecal and mucosal biotransformation enzymes	[254]
		Male albino Wistar rats injected with DMH	Intragastric administration, 50 mg/kg	Inhibited NF-κB and inflammatory mediators; suppressed proapoptotic pathway	[255]
		DMH-induced colon carcinogenesis in a male Wistar rats	Oral administration, 50 mg/kg	Reduced lipid hydroperoxides and CD; increased superoxide SOD, CAT, GST, GPx, GR; decreased GSH	[256]
Myricetin	Tea, barriers, fruits, vegetables	DMH-induced rat colon carcinogenesis	Dietary supplementation, 50, 100 and 200 mg/kg myricetin	Restored CAT, GPx and GSH	[257]
		$APC^{Min/+}$ C57BL/6J mice	Oral gavage, 100 mg/kg	Promoted apoptosis in adenomatous polyps; lowered IL-6 and PGE2; downregulated p38 MAPK/Akt/mTOR signaling pathway	[258]
		AOM/DSS-induced in BALB/c mice	Oral gavage, 40 and 100 mg/kg	Inhibited the development of colorectal tumors and colorectal polyps; decreased the levels of TNF-, IL-1, IL-6, NF-κB, p-NF-κB, COX-2, PCNA, and cyclin D1	[259]
		AOM/DSS-induced colitis in C57BL/6 mice	Oral administration, 100 mg/kg	Decreased CSF/M-CSF, IL-6, and TNF-α in colonic mucosa; inhibited NF-κB/IL-6/STAT3 pathway	[260]

Table 3. *Cont.*

Phytocompound	Source	Animal Model Studied	Dose and Route of Administration	Mode of Action	Reference
Naringenin	Oranges, lemons, and grapefruit	AOM-induced colon carcinogenesis in rats	Dietary supplement, 0.02%	Reduce the number of HMACF by 51% and the proliferative index by 32%	[261]
		DSS-induced murine colitis model	Oral administration, 50 mg/kg	Decreased iNOS, ICAM-1, MCP-1, COX-2, TNF-α, and IL-6 transcript levels	[262]
		HT-29 tumor xenograft in athymic NIH Swiss nude mice	Oral administration, 40 mg/kg	Suppressed tumor growth; inhibited COX-1	[263]
Naringin	Oranges, lemons, and grapefruit	DMH-induced female Wistar rats	Oral gavage, 10, 100, 200 mg/kg	Reduced cell proliferation and tissue iron levels; upregulated antioxidant mineral levels	[264]
		AOM/DSS-induce Male C57BL/ 6 mice	Oral gavage, 50 and 100 mg/kg	Suppressed ER stress-induced autophagy in colorectal mucosal cells	[265]
		AOM-induced ACF in Sprague Dawley rats	Oral administration, 200 mg/kg	Reduced total number of ACF; suppressed proliferation; induced apoptosis; downregulated COX-2 and iNOS	[266]
Nobiletin	Peel of various *Citrus* fruits	AOM-DSS-induced colon carcinogenesis in male CD-1 mice	Oral, dietary supplement, 100 ppm	Reduced tumor incidences and multiplicity	[267]
Orientin	*Ocimum sanctum*	DMH-induced CRC in male Wister rats	Intraperitoneal injection, 10 mg/kg	Reduced NF-κB, TNF-α and IL-6; downregulated Ki-67 and PCNA; suppressed iNOS and COX-2	[268]
		DMH-induced CRC in male Wister rats	Intraperitoneal injection, 10 mg/kg	Suppressed ACF and crypt multiplicity; elevated the level of antioxidants; downregulated phase I enzymes and upregulated phase II enzymes	[269]
Oroxylin A	*Scutellaria baicalensis*	AOM-DSS induced CRC in C57BL/6 mice	Dietary supplementation, 50, 100 and 200 mg/kg	Suppressed tumor formation and colitis associated CRC; induced apoptosis; downregulated IL-6, IL-1β, p-STAT3, cyclin D, and Bcl-2; upregulated Bax	[270]
		HCT116 tumor xenograft in male athymic BALB/c nude mice and AOM-DSS induced colon carcinogenesis in male C57BL/ 6 mice	Oral administration, 150 and 300 mg/kg	Suppressed carcinogenesis and primary colon cancer progression; reduced triglyceride; downregulated HIF1α, Srebp1, FASN, ADRP and FABP7; upregulated CPT1	[271]
Pectolinarigenin	*Cirsium chanroenicum*	Murine CT26 CRC cells were introduced into BALB/C mice	Intraperitoneal injection, 25 and 50 mg/kg	Induced apoptotic death of cancer cells; suppression STAT3	[272]

Table 3. *Cont.*

Phytocompound	Source	Animal Model Studied	Dose and Route of Administration	Mode of Action	Reference
Peonidin	Sweet potato (*Ipomoea batatas*)	AOM-induced CF-1 mice	Dietary supplementation, 10 to 30%	Blocked cell cycle at the G1 phase; activated caspase-3	[273]
Petunidin	*Lycium ruthenicum*	Nude mice	Intraperitoneal injection, 25 and 50 mg/kg	Induced ferroptosis via inhibiting SLC7A11	[274]
Phloretin	*Manchurian apricot*	COLO 205 cells derived tumor in BALB/c nude mice	Route of administration not reported, 25 mg/kg	Inhibited tumor growth; upregulated p53, p21 and E-cadherin	[275]
Polyphenon E		AOM-induced colon carcinogenesis in F344 rats	Oral administration, 0.24%	Induced apoptosis; decreased eicosanoid, prostaglandin E2, and interleukin B4 in plasma; decreased nuclear β-catenin and increased expression of RXRα,β and γ in adenocarcinomas	[276]
Procyanidin	Cider apple (*Malus domestica*)	AOM-induced Wistar rats	Oral administration, 0.01%	Suppressed protein kinase; down-regulated of polyamine production; stimulated caspase-3	[277]
		Male C57/BL6 mice transfected with CT26 cells	Oral gavage, 30 mg/kg	Reduced cellular oxidative stress through modulation of Nrf2/ARE signaling	[278]
Quercetin	Apples, nuts, cauliflower, cabbage, onions, grapes, berries, broccoli, citrus fruits, cherries, green tea, and coffee	AOM-induced colon tumor in C57BL/6J male mice	Dietary supplementation, 0.5%	Induced apoptosis; upregulated CB1-R; downregulated STAT3 and p-STAT3; downregulated Bax/Bcl-2 ratio	[279]
		Subcutaneous DLD-1 human colon tumor fragment implant in male athymic nu/nu mice	Intraperitoneal injection, 30 mg/kg	Enhanced radiosensitivity by inhibiting ATM-mediated signaling pathway	[280]
		AOM-induced CRC in male weanling Sprague-Dawley rats	Dietary supplement, 4.5 g/kg	Reduced the number of crypts; inhibited proliferation; induced apoptosis; suppressed COX-1, COX-2 and iNOS	[281]
		AOM/DSS induced colon carcinogenesis in C57BL/6J mice	Dietary supplementation, 30 mg/kg	Reduced number and size of colon tumors; suppressed inflammation; downregulated LOP, NO, SOD, G6PD, and GSH	[282]
		CT-26 lung tumor metastasis in BALB/c mice	Intraperitoneal injection, 50 mg/kg	Suppressed lung metastasis; induced apoptosis	[283]
		HT-29 tumor xenograft in BALB/c nude mice	Subcutaneous injection, 10 mg/kg	Enhanced radiosensitivity; inhibited Notch-1 signaling	[284]

Table 3. *Cont.*

Phytocompound	Source	Animal Model Studied	Dose and Route of Administration	Mode of Action	Reference
Rutin	Buckwheat, Mez, Labisia pumila, *Sophora japonica* L., Schum, *Canna indica* L., and *Ruta graveolens* L.	SW480 cell-induced tumor xenograft	Intraperitoneal injection, 20 mg/kg	Suppressed tumor growth; decreased angiogenesis and VEGF levels	[285]
Scutellarin	*Scutellaria barbata*	AOM/DSS-induced male C57BL/6 mice	Intraperitoneal injection, 25, 50 and 100 mg/kg	Inhibited Wnt/β-catenin signal transduction	[286]
		RKO cells were subcutaneously implanted into female nude mice	Intraperitoneal injection, 50, 150 and 300 mg/kg	Suppressed tumor growth and metastasis	[287]
		AOM/DSS-induced mice	Intraperitoneal injection, 25, 50 and 100 mg/kg	Suppressed the Hedgehog signaling cascade	[288]
Silibinin	*Silybum marianum*	LoVo cell deposition on eight days old fertilized chicken egg	Route of administration not reported, 9.64 μg/mL	Decreased in VDI; upregulated *Flt-1* gene	[289]
		AOM-induced CRC in male Wistar rats	Intragastric intonation, 300 mg/kg	Suppressed preneoplastic lesion formation; activated apoptosis; registered sub G0/G1 cell cycle arrest; reduced MMP-7, IL-1β and TNF-α	[290]
Tangeretin	Peel of citrus fruits	HT-29 induced tumor xenograft in BALB/c nude mice	Route of administration not reported, 5 mg/kg	Suppressed tumor growth	[291]
Taxifolin	Olive oil, grapes, citrus fruits, and onion	HCT116 tumor xenograft in athymic male nude mice	Intraperitoneal injection, 15 and 25 mg/kg	Suppressed tumor growth; induced apoptosis; inhibited cyclin D; degraded β-catenin; inhibited of Akt phosphorylation	[292]
Tricin	Rice bran, oats, barley, and wheat	Colon26-Luc colon tumor and lung metastasis model in BALB/c mice	Oral gavage, 19 and 37.5 mg/kg	Suppressed tumor growth; reduced metastasis incidence	[293]
		AOM-DSS induced CRC in male Crj: CD-1 mice	Dietary supplement, 50 and 250 ppm	Restored colonic length; reduced number of incidences and multiplicity of adenomas and adenocarcinomas; downregulated PCNA and TNF-α	[294]

Table 3. *Cont.*

Phytocompound	Source	Animal Model Studied	Dose and Route of Administration	Mode of Action	Reference
Troxerutin	Tea and coffee	DMH-induced colon carcinogenesis in male albino Wistar rats	Oral administration, 12.5, 25 and 50 mg/kg	Lowered ACF formation and crypt multiplicity; reduced cytochrome P450, cytochrome b_5, cytochrome P4502E1, NADPH-cytochrome P450 reductase, and NADH-cytochrome b_5 reductase and upregulates phase GST, DTD and UDPGT	[295]
Vitexin	Passionflower, bamboo leaves, pearl, and millet	HCT116 tumor xenograft in nude BALB/c mice	Oral administration, 25, 50 and 100 mg/kg	Suppressed tumor growth; increased phosphorylation of JNK; upregulated LC3 II and ApoL1	[296]
		$HCT116^{DR}$ tumor xenograft in female athymic BALB/c nude mice	Oral administration, 25 and 50 mg/kg	Suppressed tumor growth; induced apoptosis; downregulated HSP90, HSP70, HSP27, Atg7, Beclin-1, LC3 II and Bcl-2; upregulated Bax and PARP1; cleaved caspase-3 and caspase-9	[297]
Wogonin	*Scutellaria baicalensis*, *Scutellaria radix*	AOM/DSS-induced colitis related colon cancer in C57BL/6 mice	Gastric intubation, 60 mg/kg	Decreased cell proliferation; lowered the expression and secretion of IL-6 and IL-1β and expression of NF-κB; increased Nrf2 nuclear translocation	[298]
		AOM-DSS-induced CRC animal model in C57BL/6 mice	Route of administration not reported, 50 and 100 mg/kg	Reduced tumor multiplicity; reverted colon length to normal	[299]
		SW480 induced tumor xenograft in BALB/c nude mice	Intraperitoneal injection, 2 mM	Downregulated of YAP-1 and IRF3; upregulated p-YAP	[300]
Xanthohumol	*Humulus lupulus*	AOM-induced colorectal carcinogenesis in male Sprague-Dawley rats	Oral gavage, 5 mg/kg	Suppressed tumor growth; induced apoptosis; suppressed COX-2 and iNOS	[301]
Zapotin	Tropical fruit zapote blanco (*Casimiroa edulis*)	AOM/DSS-induced female CF-1 mice	Intragastric administration, 5 and 10 mg/kg	Induced cell cycle arrest and apoptosis	[302]

Table 3. *Cont.*

Phytocompound	Source	Animal Model Studied	Dose and Route of Administration	Mode of Action	Reference
Phenolic acids					
Caffeic acid	Coffee, wine tea	CT-26 lung metastasis in BALB/c mice	Oral administration, 0.1 and 0.5 g/kg	Inhibited lung metastasis; suppressed MEK1, TOPK, and TAP-induced activation of AP1, NF-κB and ERK signaling; inhibited TAP, EGF and H-Ras induced neoplastic transformation	[303]
		HCT116 tumor xenograft in NSG mice	Intraperitoneal injection, 10 mg/kg	Inhibited CSC growth and self-renewal by inhibition of PI3K/Akt signaling	[304]
		HCT116 tumor xenograft in BALB/c AnN Foxn-1 nude mice	Oral administration, 50 nmol/kg	Inhibited PI3K/Akt/mTOR pathway; suppressed MMP-9, cyclin D1, Cdk4, cyclin E, PCNA, FASN c-Myc, and N-cadherin expression; upregulated p21	[305]
		HT-29 tumor xenograft in BALB/c nude mice	Intragastric administration, CAPE (10 mg/kg); CAPE-pNO2 5, (10 and 20 mg/kg)	Inhibited tumor growth and VEGF expression; upregulated p53, p27, p21, cyt. c, and cleaved caspase-3; downregulated procaspase-3, Cdk2, and c-Myc;	[306]
		HCT116 tumor xenograft in nude mice	Oral administration, 0.2 and 2 mg/kg	Suppressed tumor growth; displayed cell cycle arrest in S phase and autophagic cell death	[307]
Chlorogenic acid	Apple, betel, coffee beans, kiwi, grapes, eggplant, pear, plum, potato, and tea	MAM acetate-induced carcinogenesis hamsters	Oral administration, 0.025% dietary supplement	Reduced colon tumor incidences; registered antioxidative effect; inhibited the activity of microsomal enzyme	[308]
		AOM-induced ACF in colon of male F344 rats	Oral administration, 0.025% dietary supplement	Reduced ACF formation and growth	[309]

Table 3. *Cont.*

Phytocompound	Source	Animal Model Studied	Dose and Route of Administration	Mode of Action	Reference
Ellagic acid		AOM-induced colon tumors in rats	Oral administration, 250, 2500 and 5000 ppm	Inhibited the incidence of adenocarcinomas in the small intestine	[310]
		DMH-induced colon cancer in rats	Oral administration, 60 mg/kg	Lowered the frequency of ACF and lipid peroxidation; increased the activity of CAT, SOD, GPx, GR and GST; restored the levels of vitamin C, vitamin E and GSH	[311]
		DMH-induced colon cancer in Wistar albino rats	Oral administration, 60 mg/kg	Inhibited NF-κB, iNOS, COX-2, TNF-α and IL-6; restored the levels 5′-ND, γ-GT, CEA, AFP and LDH	[312]
		DMH-induced colon cancer in rats	Oral administration, 60 mg/kg	Inhibited PI3K-p58 activation; downregulated Akt and Bcl-2; upregulated Bax	[313]
		DMH-induced colorectal cancer in rats	Oral administration, 60 mg/kg	Inhibited ACF formation; increased the activity of CAT, SOD, GPx, and GR; inhibited ODC expression through inhibition of c-MYC	[314]
		DMH-induced colon cancer in male Laca mice	Oral administration, 10 mg/kg	Restored colon membrane alterations	[315]
Ferulic acid	Rice, wheat, pineapple, grains, and peanuts	AOM-induced colon cancer in male Fischer 344 rats	Oral administration, 250 ppm and 500 ppm	Reduced number and size of adenomas; increased the activity of GST and QR	[316]
		AOM-induced colon carcinogenesis in F344 rats	Dietary supplement of 3-(4′-geranyloxy-3-methoxyphenyl)-2propenoate (geranylated derivative of ferulic acid) 0.1% and 0.2%	Decreased the number of ACF	[317]

Table 3. *Cont.*

Phytocompound	Source	Animal Model Studied	Dose and Route of Administration	Mode of Action	Reference
Gallic acid	Barriers and pomegranates	DMH-induced colon cancer in male Wister rats	Oral administration, 50 mg/kg	Reduced lipid peroxidation, LOOH, CD, SOD, CAT, GSH, GR and GPx; reduced ascorbic acid and tocopherol levels	[318]
		SW480 induced tumor xenograft in NOD SCID gamma NSG mice	Intraperitoneal injection, 200 mg/kg	Exerted antitumor activity mediated by interaction with G-quadruplexes	[319]
		DSS-induced acute colitis in C57BL/6 mice	Oral administration, 5 and 25 mg/kg	Suppressed acute colitis; inhibited phosphorylation of STAT3	[320]
		HCT116 and HT-29 tumor xenografts in BALB/c nude mice	Intraperitoneal injection, 80 mg/kg	Suppressed p-SRC, p-EGFR, p-Akt and p-STAT3	[321]
		Ulcerative colitis in rats	Oral administration, 10 mg/kg	Suppressed colon cancer; induced ferroptosis	[322]
		DMH-induced colon cancer in male albino Wister rats	Oral administration, 50 mg/kg	Elevated the activity of cytochrome P450, cytochrome b5, GST, DT-diaphorase and γ-GT	[323]
Geraniin	*Phyllanthus amarus*	SW480 tumor xenograft in nude mice	Oral administration, 10, 20 and 40 mg/kg	Suppressed tumor growth; induced apoptosis; inhibited phosphorylation of PI3K and Akt	[324]
p-Coumaric acid	Mushrooms, apples, pears, barriers, oranges, and beans	DMH-induced colon carcinogenesis in male albino Wistar rats	Intragastric intubation, 100 mg/kg	Reduced ACF, DACF, MDF and BCAC	[325]
Syringic acid	Olives, dates, pumpkins, grapes, and palms	DSS-induced mice	Oral administration, 25 mg/kg	Decreased the level of iNOS, COX-2, TNF-α, IL-1β and IL-6; reduced activation and accumulation of p-STAT-3 by decreasing expression of p65-NF-κB	[326]
		DMH-induced colorectal cancer in male rats	Oral administration, 50 mg/kg	Reduced tumor incidences, tumor volume and average number of tumors	[327]
Lignans					
Arctigenin	*Arctium lappa, Forsythia suspensa.*	CT-26 cells derived lung metastasis model in BALB/c mice	Oral gavage, 50 mg/kg	Reduced the number of lung nodules; induced apoptosis in lung tissue; inhibited EMT in lung tissue; induced cleavage of caspase-3, caspase-9, and PARP; downregulated Bcl-2 and Bcl-xL; upregulated Bax	[328]

Table 3. *Cont.*

Phytocompound	Source	Animal Model Studied	Dose and Route of Administration	Mode of Action	Reference
Daurinol	*Haplophyllum dauricum*	HCT116 tumor xenograft in athymic BALB/c (*Slc*/*nu*) nude mice	Oral administration, 5 and 10 mg/kg	Suppressed tumor growth; upregulated p-Chk1(Ser345)/Chk1	[329]
Dehydrodiisoeugenol	*Myristica fragrans* Houtt	HCT116, zsw480, and patient-derived xenograft in female NOD/SCID mice	Intraperitoneal injection, 40 mg/kg	Suppressed tumor growth by inducing ER stress; upregulated BiP, PERK, and IRE1α	[330]
Gomisin A	*Schisandra chinesis*	CT-26 induced lung metastasis in female BALB/c mice	Intraperitoneal injection, 50 mg/kg	Suppressed lung metastasis; reduced the number of lung nodules; increased phosphorylation of AMPK and p38 in lung tissue	[331]
Honokiol	*Magnolia grandiflora*	SW620 tumor xenograft in female athymic BALB/c nude mice nu/nu	Intragastric administration, 50 mg/kg	Inhibited proliferation of CRC; upregulated TGF-β1 and p53 by upregulating BMP7	[332]
Justicidin A	*Justicia procumbens*	HT-29 tumor xenograft in NOD-SCID mice	Oral administration, 6.2 mg/kg	Suppressed tumor growth; induced autophagy in tumor tissue; induced apoptosis	[333]
Magnolol	*Magnolia officinalis*	CT-26 and HT-29 tumor in BALB/c and Cg-Foxn1^{nu}/CrlNarl nude mice respectively	Route of administration not reported, 50 and 100 mg/kg	Inhibited tumor growth; induced apoptosis; upregulation of Fas, Fas-L, cleaved caspase-3, cleaved caspase-9 and cleaved caspase-8; downregulated NF-κB, PKCδ, ERK, Akt, C-FLIP, and MCL-1; inhibition of PKCδ-NF-κB signaling	[334]
		HCT116 tumor xenograft in female BLB/c nude mice	Intraperitoneal injection, 5 mg/kg	Suppressed tumor growth without showing any toxicity	[335]
Schisandrin B	*Schisandra chinensis, Schisandra propinqua, and Schisandra rubriflora*	AOM-DSS-induced CRC in C57BL/6 mice	Oral administration, 3.7–30 mg/kg	Suppressed SIRT1	[336]
Secoisolariciresinol	*Fitzroya cupressoides* and *Crossosoma bigelovii*	HCT116 tumor xenograft in male BALB/c nude mice	Route of administration not reported, 50, 100 and 200 mg/kg	Inhibited tumor growth; induced pyroptosis; downregulated Ki-67; upregulated N-GSDMD	[337]
		DSS-induced colitis in mice	Dietary supplementation, 200 mg/kg	Suppressed tumor growth; reduced IL-1β, IL-18, TNF-α and NLRP1	[338]

Table 3. *Cont.*

Phytocompound	Source	Animal Model Studied	Dose and Route of Administration	Mode of Action	Reference
Sesaminol	*Sesamum indicum*	Ethanol-induced CRC in male C57BL/6NCr mice	Oral administration, 2.5 mg/mice	Reduced colonic lesions; downregulated iNOS and CYP2E1; lowered TNF-α, IL-6, MCP-1 and NF-κB levels; increased cell adhesion by upregulation of ZO-1, occludin and cladulin-1	[339]
Tracheloside	*Carthamus tinctorious* L. (safflower)	CT-26 lung metastasis in BALB/c mice	Oral administration, 25 and 50 mg/kg	Suppressed lung metastasis; induced apoptosis; upregulated E-cadherin RNA; downregulated N-cadherin, vimentin, snail and twist RNA	[340]
Vitexin	*Vitex negudo*	HCT116 tumor xenograft in female nu/nu mice	Intraperitoneal injection, 40 mg/kg	Inhibited tumor growth and lowered tumor volume; upregulated PUMA and p53; induced PUMA-mediated apoptosis	[341]
Stilbenes					
Piceatannol	Red and white grapes	AOM/DSS-induced colon tumor in C57BL/6J mice	Oral administration, 5 and 12.5 mg/kg	Decreased tumor number and size; decreased Ki-67- and COX-2-positive cell number; downregulated MCP1 and PD1	[342]
Polydatin	*Picea sitchensis*	Caco-2 tumor xenograft in C57BL/6 mice	Subcutaneously into the tumor, 150 mg/kg	Suppressed tumor growth; upregulated miR-382; downregulated PD-L1	[343]

Table 3. *Cont.*

Phytocompound	Source	Animal Model Studied	Dose and Route of Administration	Mode of Action	Reference
Pterostilbene	Blueberries and cranberries	AOM-induced colonic ACF preneoplastic lesions and adenomas in male ICR mice	Oral administration, 50 or 250 ppm	Reduced ACF and adenoma formation; induced apoptosis; downregulated iNOS and COX-2; inhibited Wnt/β-catenin signaling through suppressing phosphorylation of GSK3β; inhibited VEGF, cyclin D, MMPs, Ras, PI3K/Akt and EGFR	[344]
		AOM-induced colon tumors in F344 rats	Oral administration, 40 ppm	Reduced the proliferation of non-metastatic adenomas; downregulated IL-1β, IL-4, TNF-α, PCNA, β-catenin and cyclin D and p-NF-κB/p65	[345]
		AOM-induced colon tumor in male BALB/c mice	Oral administration, 50 or 250 ppm	Reduced NF-κB through inhibition of PKC-β phosphorylation; downregulated iNOS, COX-2 and aldose reductase; upregulated HO-1, GR and Nrf2 signaling	[346]
		CL187 transplantation model in BALB/c nude mice	Intraperitoneal injection, 25, 50, 100 and 200 mg/kg	Inhibited Top1-mediated DNA damage repair pathway	[347]
		AOM-induced colonic ACF preneoplastic lesions in F344 rats	Oral administration, 40 ppm	Inhibited ACF formation; blocked cell proliferation and iNOS	[348]
Resveratrol	Grapes, blueberries, raspberries, mulberries, and peanuts	LoVo cell-mediated metastasis model in mice	Intragastric administration, 50, 100 and 150 mg/kg	Inhibited metastasis; decreased tumor size; suppressed TGF-β1/Smad pathway; downregulated Snail and vimentin; upregulated E-cadherin expression	[349]
		$APC^{CKO}/Kras^{mut}$ mice	Dietary supplementation, 150 ppm and 300 ppm	Suppressed tumor formation; reduced tumor size; downregulated Kras expression	[350]
		DSS-induced colon carcinogenesis in rats	Oral administration, 60 mg/kg	Reduced ACF and MDF	[351]
		HCT116 tumor xenograft in ICR SCID mice	Oral administration, 150 mg/kg	Suppressed tumor growth; induced apoptosis; inhibited NF-κB	[352]
		COLO250-luc tumor xenograft in athymic mice	Injection in tumor, 6 μg/implant	Suppressed tumor growth	[353]

Table 3. *Cont.*

Phytocompound	Source	Animal Model Studied	Dose and Route of Administration	Mode of Action	Reference
		HT-29 tumor xenograft in BALB/c nu/nu mice	Intragastric administration, 480, 960 and 1920 mg/kg	Suppressed VEGF-mediated angiogenesis	[354]
Miscellaneous compounds					
Oleuropein	Olives (*Olea europaea*)	AOM-induced CRC in female A/J mice	Dietary supplementation, 125 mg/kg	Suppressed preneoplastic lesions; lowered tumor incidences; prevented DNA damage	[355]
Thymol	*Thymus vulgaris* L.	HCT116 tumor xenograft and lung metastasis model in BALB/c nude mice	Intraperitoneal injection, 75 and 150 mg/kg	Induced apoptosis; upregulated E-cadherin; downregulated N-cadherin; suppressed lung metastasis by inhibiting Wnt/β-catenin pathway	[356]
Verbascoside	Genus, *Cistanche*	HCT116 tumor xenograft in BALB/c nude mice	Tail vein injection, 20, 40, and 80 mg/kg	Upregulated HIPK2, p53 and Bax; downregulation Bcl-2	[357]

Abbreviation: ACC, acetyl CoA carboxylase; ACF, aberrant crypt foci; AFP, α-fetoprotein; AOM, azoxymethane; APC, adenomatous polyposis coli; BAX, B-cell lymphoma 2 associated x protein; BCAC, β-catenin accumulated crypts; BCL-2, B-cell lymphoma 2; BID, BH3 interacting-domain death agonist; CAC, colitis-associated colorectal cancer; CAT, catalase; CEA, carcinoembryonic antigen; CD, conjugated dienes; CIP2A, cancerous inhibitor of PP2A; c-MYC, cellular myelocytomatosis oncogene; COX-2, cyclooxygenase-2; CRC, colorectal cancer; CSK, C-terminal Src kinase; DACF, dysplastic aberrant crypt foci; DMH, 1,2-dimethylhydrazine; DNMT, DNA methyltransferase; EGCG, (-) epigallocatechin gallate; EGF-β, epidermal growth factor-β; EGFR, epidermal growth factor receptor; ERK, extracellular-signal-regulated kinase; FADD, Fas-associated protein with death domain; Flt-1, fms-like tyrosine kinase-1; GPx, glutathione peroxidase; GR, glutathione reductase; GSK-3β, glycogen synthase kinase-3β; GSH, glutathione; GST, glutathione S-transferase; γ-GT, γ-glutamyl transpeptidase; HMACF, high multiplicity aberrant crypt foci; IGF2, insulin like growth factor 2; IGFBP3, insulin like growth factor binding protein 3; IL-6, interleukin 6; iNOS, inducible nitric oxide synthase; KRAS, Kirsten rat sarcoma viral oncogene homolog; LC3b, light chain 3B of microtubule-associated proteins 1A/1B; LDH, lactate dehydrogenase; LMW-PTP, low molecular weight protein tyro-sine phosphatase; MAM, methyl azoxymethane; MAPK, mitogen-activated protein kinase; MDF, mucin-depleted foci; MMP, matrix metalloproteinase; mTOR, mammalian target of rapamycin; 5′-ND, 5′-nucleotidase; NEDD9, developmentally downregulated 9; NF-κβ, nuclear factor-κβ; Nrf-2, nuclear factor erythroid -2 related factor; ODC, ornithine decarboxylase; PCNA, proliferating cell nuclear antigen; PI3K, phosphoinositide 3-kinase; PP2A, protein phosphatase 2A; PTEN, phosphatase and TENsin homolog deleted on chromosome 10; QR, quinone reductase; SCID, severe combined immunodeficient; SIRT, Sirtuin 1; SOD, superoxide dismutase; STAT3, signal transducer and activator of transcription 3; TNF-α, tumor necrosis factor-α; Top1, topoisomerase 1; VDI, vascular density index; VEGF, vascular endothelial growth factor; γ-GT, γ-glutamyl transpeptidase.

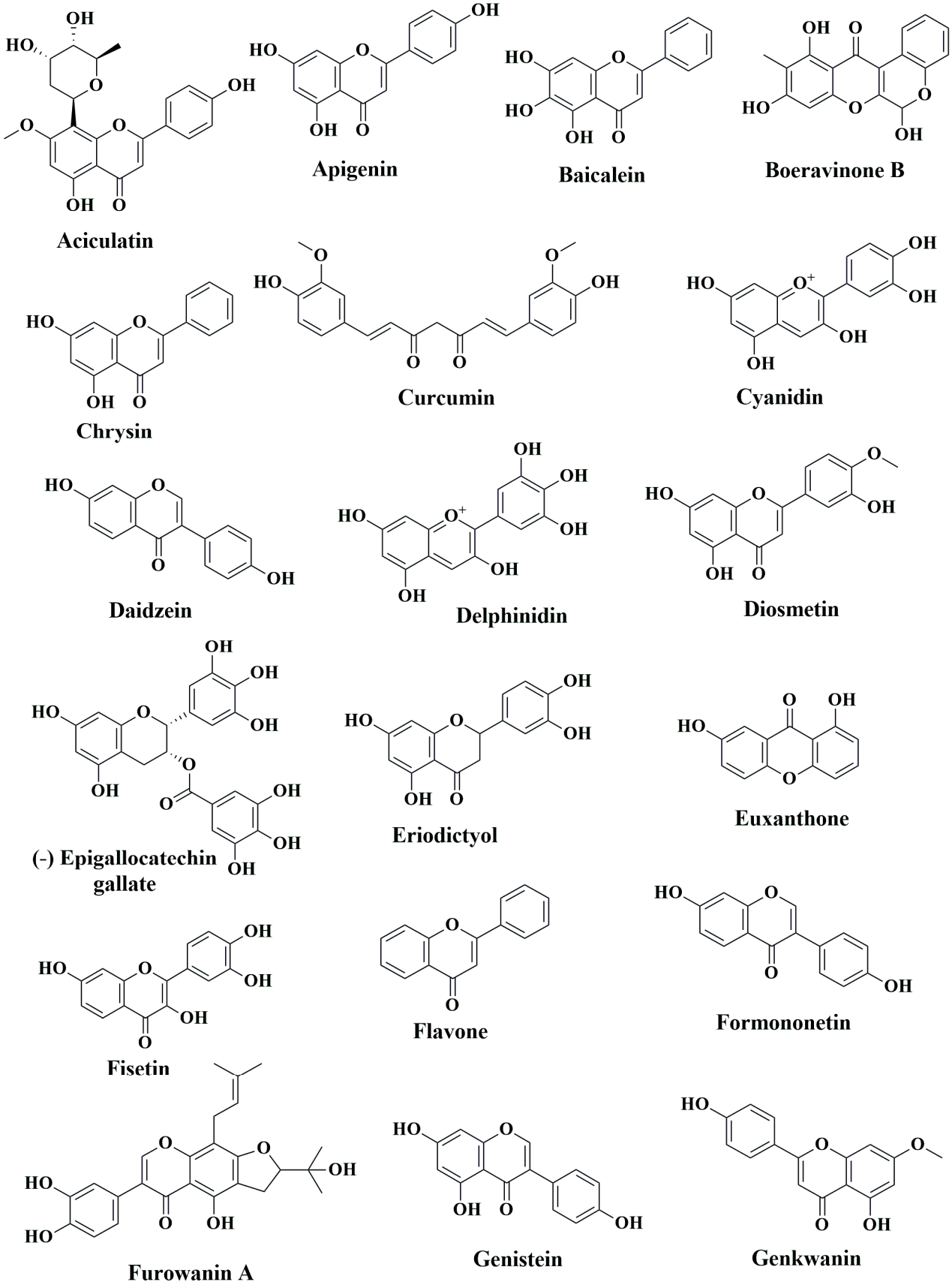

Figure 2. *Cont.*

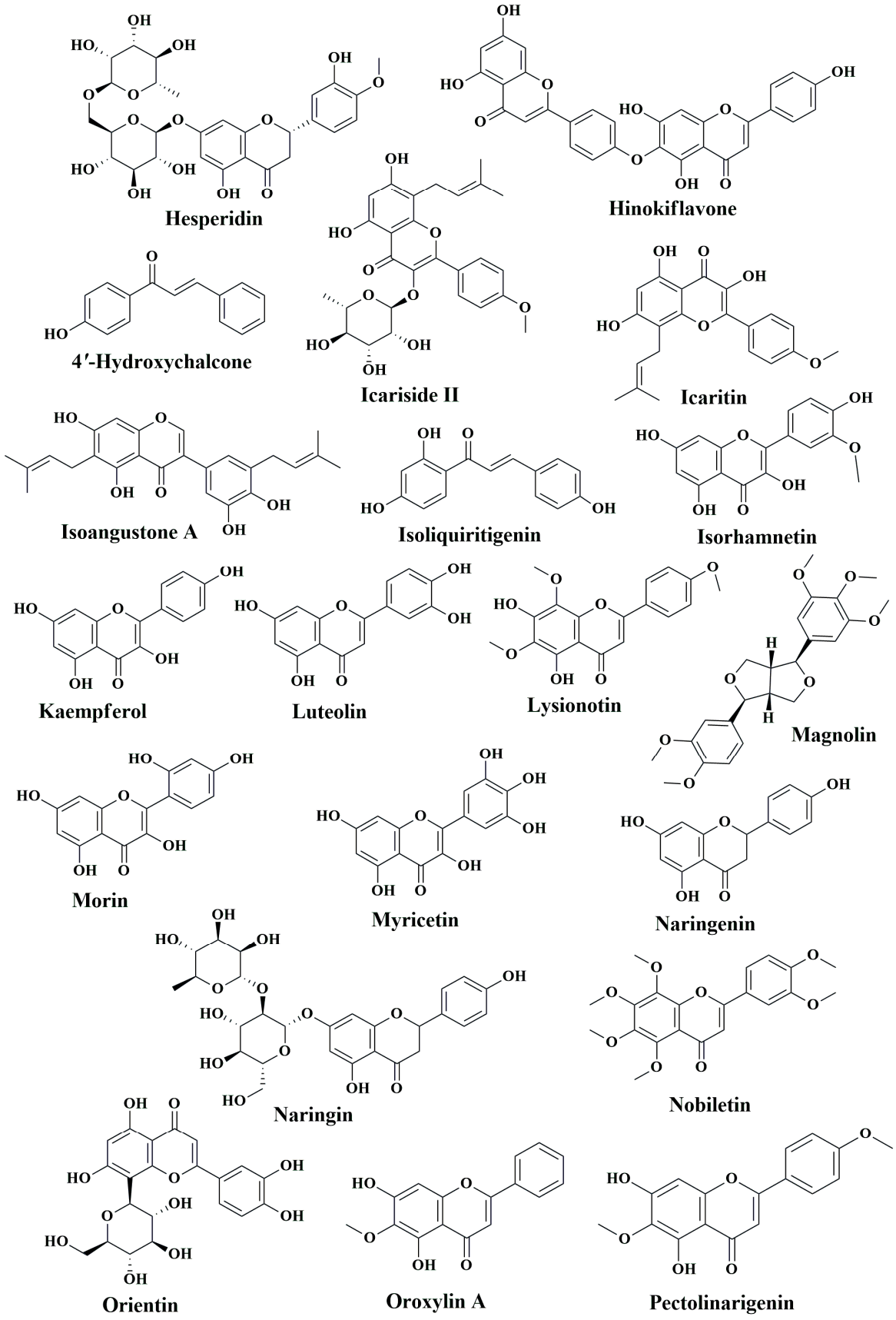

Figure 2. *Cont.*

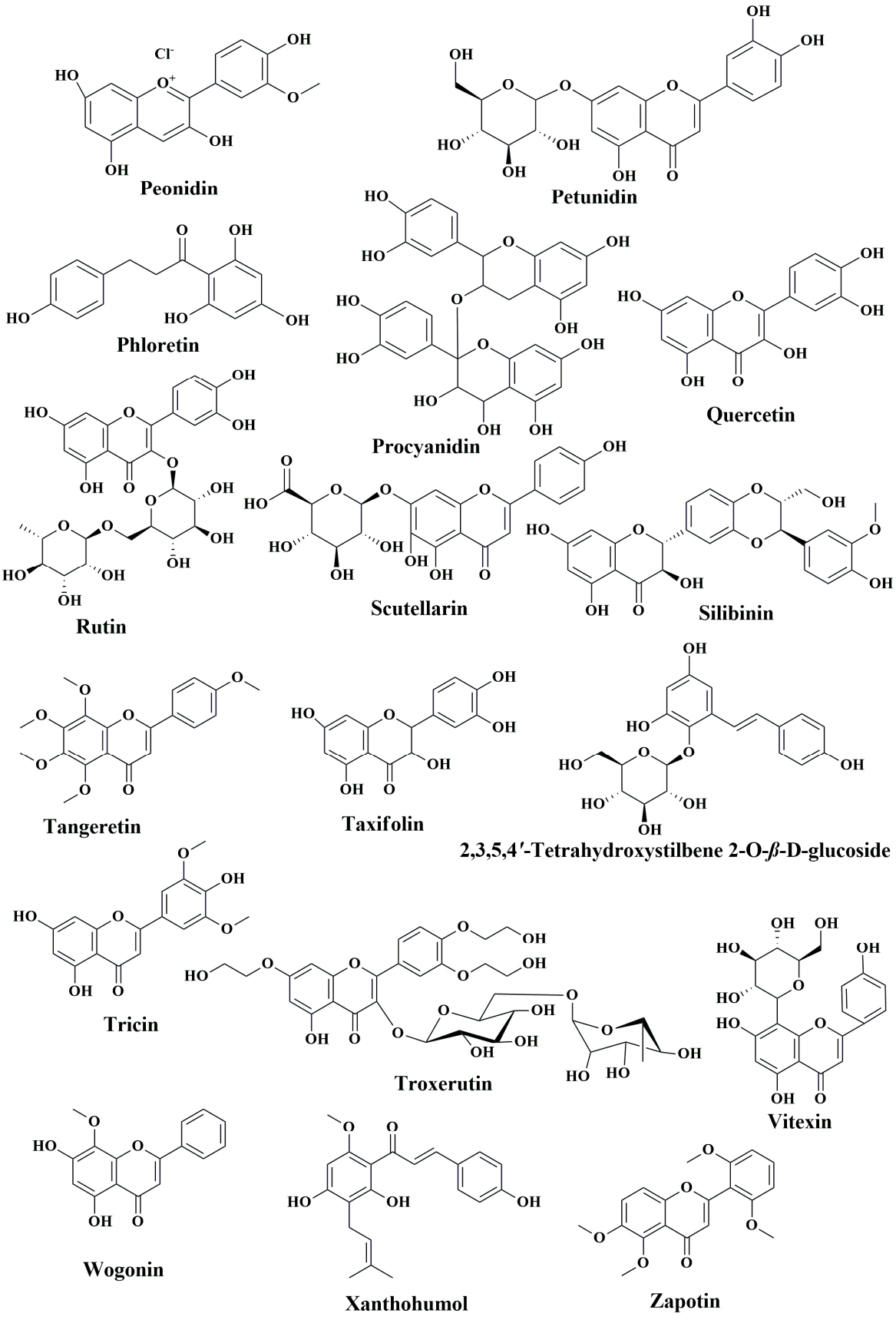

Figure 2. Chemical structures of flavonoids with in vivo anti-CRC activities.

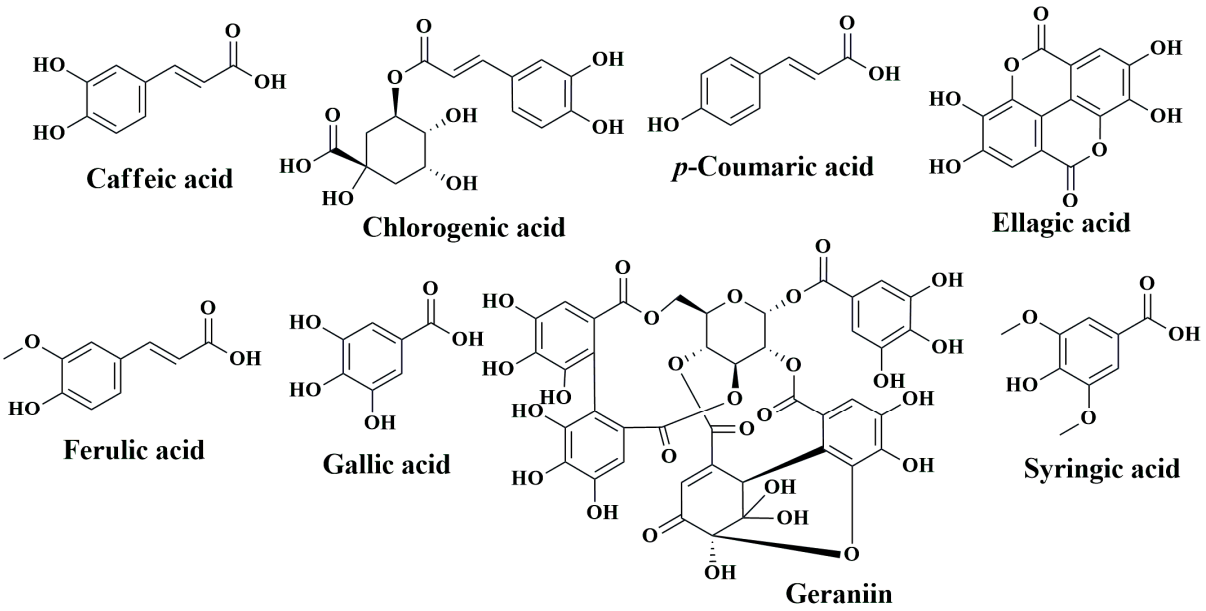

Figure 3. Chemical structures of phenolic acids with in vivo anti-CRC activities.

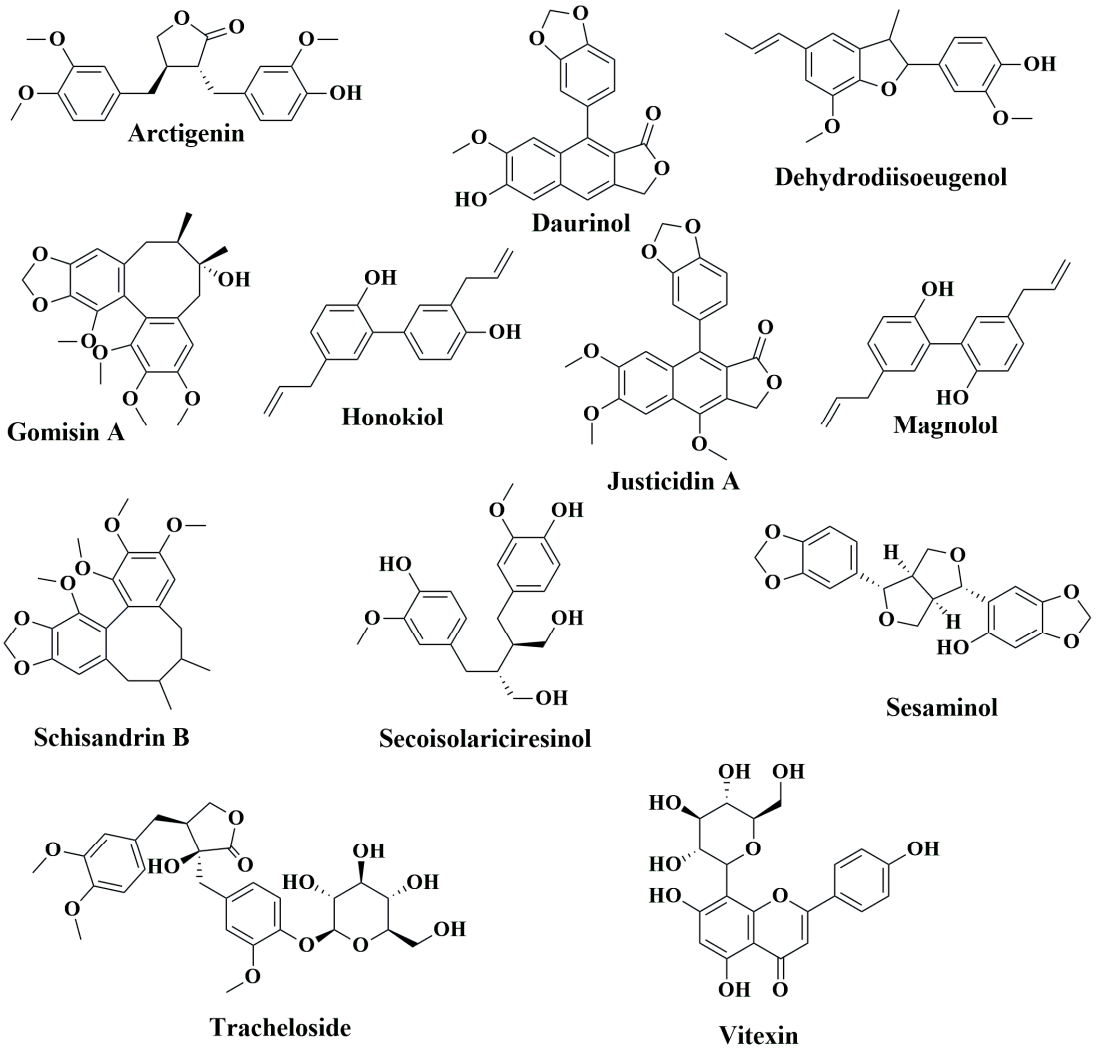

Figure 4. Chemical structures of lignans with in vivo anti-CRC activities.

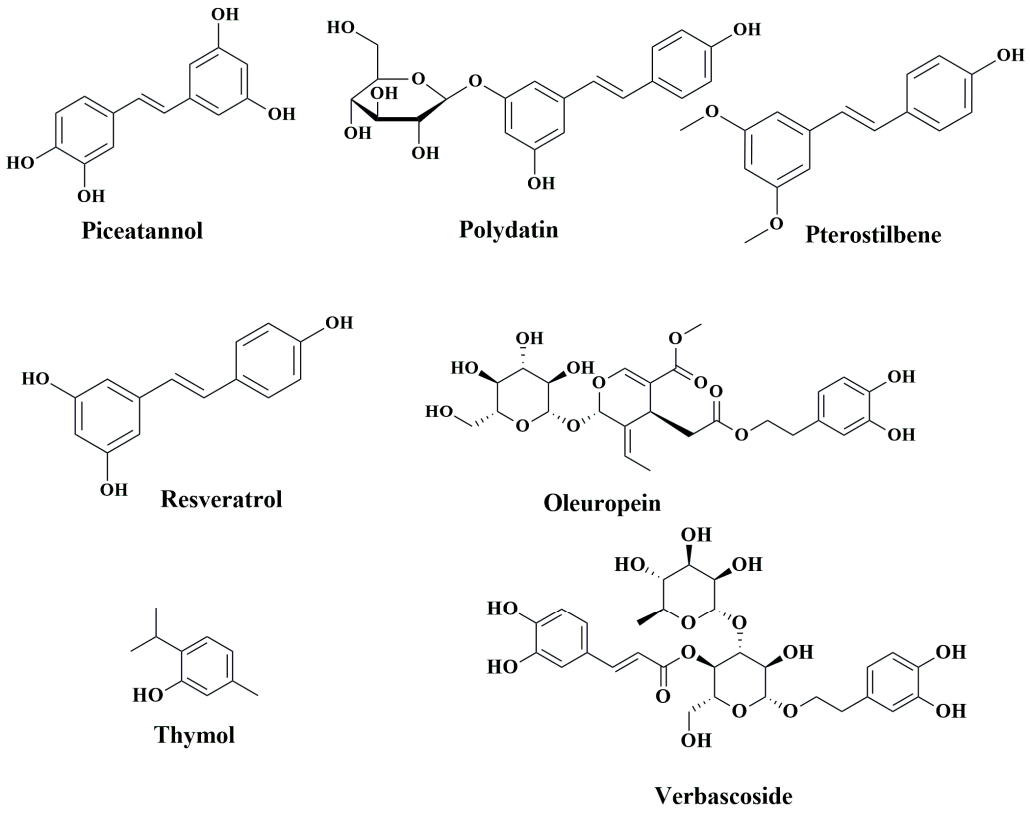

Figure 5. Chemical structures of stilbenes and miscellaneous compounds with in vivo anti-CRC activities.

7.1. Flavonoids

7.1.1. Baicalin

Baicalin (molecular weight: 446.4 g/mol), conjointly called baicalein 7-O-glucuronide and 7-D-glucuronic acid-5, 6-dihydroxyflavone or known by its chemical name, 5, 6 dihydroxy-4-oxo-2phenyl-chromen-7-yl) oxy-3, 4, 5-trihydeoxytetrahydropyran-2-carboxylic acid, is a glycosyloxyflavone. It is a key component of a variety of traditional medicine preparations, consisting of Sho-Saiko-To, Yangkun pills, Kushen decoction, and Shuanghuanglian injections. Scutellariae radix, Scutellaria planipes, Scutellaria rehderiana, and Scutellaria scandens are only a few of the Scutellaria species that contain the compound baicalin, which is extensively distributed throughout the genus [358].

Baicalein suppressed AOM/DSS-induced colon tumors in mice and induced apoptotic cell death. Baicalein suppressed inflammation by PARP1-mediated NF-κB inhibition [180]. A dose of 50 mg/kg baicalin suppressed the growth of highly metastatic SW620 tumor xenograft in BALB/c nude mice [181]. Baicalin inhibited the TLR4/NF-κB signaling and significantly suppressed CT-26 tumor growth, migration, and invasion. Anti-tumor immunity was also enhanced by an increase in $CD4^+$ and $CD8^+$ T cells in CT-26 tumors [182]. Baicalein treatment induced apoptosis in a p53-mediated Akt-dependent manner and suppressed HT-29 tumor xenograft [183]. In another study, baicalein suppressed MMP-2 and MMP-9 and inhibited DLD1 tumor growth and metastatic effects by inhibiting phosphorylation of ERK [184].

Dou et al. [185] showed that baicalein and baicalin can significantly inhibit the growth of HCT116 tumor xenograft by inducing tumor cell apoptosis and senescence through inhibiting the telomerase reverse transcriptase. It has also been hypothesized that the control of colon cancer cell apoptosis and senescence is caused by the MAPK, ERK, and p38 signaling pathways. Wang et al. [186] verified that baicalin application increased the expression of DEPP and triggered its downstream target Ras/Raf/MEK/ERK and p16INK4A/Rb pathways by serving as an antioxidant, resulting in senescence in colon carcinoma cells in HCT116 tumor model in BALB/c athymic nude mice. It was revealed

that baicalin inhibited the HT-29 xenograft tumor in nude mice by suppressing c-Myc as the driver of miRNAs responsible for oncogenic development (oncomiRs). These findings demonstrated an association of c-Myc in baicalin-mediated inhibition of colon cancer growth [187]. In orthotopically transplanted tumors of CRC cells in BALB/c nude mice, baicalin administration lowered the levels of marker proteins for cell cycle, EMT, and stemness [188].

Wang et al. [189] observed that the baicalein therapy dramatically decreased tumor numbers in the small intestine and colon of $Apc^{Min/+}$ mice. Furthermore, reduced levels of inflammatory cytokines, such IL-1, IL-2, IL-6, G-CSF, and GM-CSF B, in this mouse tumor model suggested that baicalein's anti-CRC action was mediated by reducing gut inflammation. Baicalin treatment suppressed HCT116 tumor xenograft growth by downregulation of CircMYH9 and HDGF, and upregulation of miR-761 [190].

7.1.2. Curcumin

Curcumin, with the chemical name (1E, 6E)-1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione(diferuloylmethane), is a hydrophobic polyphenol derived from the roots of a well-known Indian spice, turmeric (Curcuma longa). Consumption of turmeric is believed to provide protection from numerous ailments, including CRC [359–362]. Anti-CRC activities of curcumin were demonstrated by several independent groups. Curcumin reduced putative precursor colonic lesions, e.g., aberrant crypt foci (ACF), through suppressing the levels of proinflammatory cytokines, such as TNF-α and IL-6, and proinflammatory mediators, such as COX-2, in obese and diabetic (db/db) mice [197]. Adiponectin plays an important anti-inflammatory role in the gut [363–365]. Curcumin increased the adiponectin level in both AOM-treated and untreated C57BL/KsJ-db/db (db/db) mice [197]. Leptin levels are directly proportional to body fat. High serum leptin levels can cause inflammation-mediated CRC [366,367]. Curcumin was able to reduce the body fat content along with serum leptin levels, and thus reduce the severity of CRC. This study also observed AMPK activation and COX-2 inhibition in those animals [197].

Curcumin reduced DSS-induced ACF and β-catenin accumulation. Due to its anti-inflammatory properties, curcumin suppressed pro-inflammatory cytokines and COX-2 and iNOS in DSS-induced colonic tissue [194]. Curcumin suppressed the growth of HCT116 tumor xenograft in ICR SCID mice. Curcumin treatment led to proteasome inhibition and induction of apoptosis which, in turn, suppressed the HCT116 tumor growth [195]. In another study, curcumin inhibited AOM/DSS-induced tumorigenesis in mice. Curcumin also downregulated Axin2 and exerted its anticancer activity by Axin2 mediated inhibition of the Wnt/β-catenin pathway [196].

Curcumin was found to inhibit HCT116-induced xenografts in male nude mice, along with suppressing NF-κB regulated genes, including Bcl-2, c-FLIP, IAP1, and survivin. It further cleaved procaspase-3 and procaspase-9. Curcumin pretreatment sensitized the tumor xenograft to γ-radiation and suppressed NF-κB activity by inhibiting the binding of NF-κB to its response element on its target genes, thus minimizing invasion, migration, and angiogenesis. Curcumin ameliorated the γ-radiation mediated increase of cellular proinflammatory mediator COX-2 and c-Myc in a HCT116 xenograft tumor model [198,199].

Furthermore, curcumin was found to modulate gut microbiome habitat in AOM-injected IL10-/-mice and was implicated in the function of anti-inflammation and the maintenance of gut homeostasis. The aberrant cytoplasmic and nuclear localization of β-catenin in AOM-treated wild-type and AOM/Il-10-/-mice was significantly reduced by curcumin treatment [200].

Curcumin enhanced the anti-CRC activity of capecitabine in HCT116 tumor xenografts in male athymic nu/nu mice through the induction of apoptosis and inhibition of angiogenesis, invasion, and metastatic factors, such as VEGF, ICAM-1, and MMP-9, and CXCR4. Inhibition of COX-2 and cell cycle progression mediators, cyclin D1 and c-Myc, was also observed in the curcumin-treated animals. The anti-CRC effects of liposomal curcumin alone and combined with oxaliplatin were tested on CRC xenografts induced by Colo205

and LoVo cells in athymic nu/nu mice. The combination therapy showed efficient tumor growth inhibition by apoptosis (PARP-1 cleavage). Liposomal curcumin also inhibited angiogenesis in consistence with the inhibition of VEGF, CD31, and IL-8 expression [201]. Phytosomal curcumin was tested for its ameliorative effects on an AOM/DSS model of colitis-associated CRC alone and in combination with 5-FU in in vivo. Curcumin, alone and in combination, functioned through modulating Wnt/β-catenin signaling and E-cadherin activities. Curcumin administered by oral gavage and in combination with 5-FU significantly inhibited GSK3 α/β and cyclin D1 expression. Curcumin was shown to reduce oxidative stress induced ACF and colon injuries induced by AOM/DSS by upregulating endogenous antioxidative enzymes, such as superoxide dismutase (SOD), catalase (CAT), thiolase, and inducing autophagy by upregulating beclin1 [200].

7.1.3. Catechins

Catechins are a group of polyphenols abundantly present in tea, cocoa, berries, grapes, and apples. Catechins have a myriad of health benefits, and their anticancer properties have been extensively studied [368,369]. Kim et al. [370] examined the effects of green tea polyphenol (GTP) dosage on DSS-induced acute colitis and DMH and DSS-induced colon cancer developed in male ICR mice. GTP contained 70% of total catechins, half of which were EGCG and 3% being caffeine. This study showed that a specific dosage of GTP was effective in ameliorating the carcinogenic effect of DSS/DMH. The basis of this activity was implicated in the antioxidant properties of GTP. If the dosage was higher or lower than the effective dose, GTP was ineffective. This is potentially due to a loss of, or insufficient, antioxidant properties. Depending on the treatment conditions, GTFP can exhibit antioxidant or pro-oxidant properties [371].

The anticancer effect of EGCG was also tested on azoxymethane (AOM)-induced male C57BL/KsJ-db/db (db/db) mice. EGCG caused a significant reduction in the levels of IGF-IR, phospho-IGF-IR, phospho-GSK-3β, β-catenin, COX-2, and cyclin D1. There was also an increase in serum IGFBP3 and a decrease in serum IGF-I, insulin, triglyceride, cholesterol, and leptin in the treated mice [206].

Zhong et al. [207] investigated the acetylated-EGCG activity against protumorigenic inflammatory mediators in AOM-mediated colitis-induced CRC in a male mouse model. Acetylated-EGCG inhibited the expression of pro-tumorigenic inflammatory mediators, such as inducible nitric oxide synthase (iNOS) and COX-2. iNOS is one of the enzymes that remain in ACF and causes the continuous formation of nitric oxide (NO), leading to the promotion of tumorigenesis [372–374]. Furthermore, COX-2 converts arachidonate to prostaglandin E2. A sustained overexpression of prostaglandin E2 in the tissues may lead to epithelial cell cancers, including CRC [207,375,376].

EGCG showed the antistemness and chemosensitizing effects on xenograft tumors of HCT116 spheroid-derived cancer stem cells in male nude mice. EGCG inhibited CRC stemness and sensitized 5-FU-resistant HCT116 cells. EGCG suppressed stemness markers, such as Notch-1, and upregulated the expression of tumor suppressive miRNAs, including miR34a, miR200c, and miR-145 [208].

Another study demonstrated the effects of green tea catechins alone and in combination with curcumin on DMH-induced colon cancer in male Wistar rats [209]. A 32-week-long dietary treatment with curcumin, green tea catechins, and their combination showed a significant reduction in the number of colorectal aberrant cryptic foci in these animals. Notably, the combinatorial treatment had a greater effect than that with either of the compounds acting alone. A significant decrease in the proliferation index and an increase in the apoptotic index were reported in the groups treated with a combination of the compounds, compared to the mock-treated group or those receiving only DMH [209].

The anticancer effect of polyphenol E (PPE) was tested on AOM-treated F344 rats. PPE is a standardized GTP mixture containing 65% EGCG and other catechins. After AOM treatment, the animals were given a 20% high-fat diet, with or without 0.24% PPE for 34 weeks. PPE treatment resulted in a significant reduction in tumor size and the

number of tumors in these animals. PPE was shown to decrease nuclear β-catenin levels, induce apoptosis, and increase the levels of RXR-α, RXR-β and RXR-γ expression in adenocarcinomas. This was accompanied by the lowering of proinflammatory eicosanoids, prostaglandin E2, and leukotriene B4 in the plasma [276].

7.1.4. Fisetin

Fisetin is a hydroxy flavone under the subgroup of flavonoid found in various fruits and vegetables, such as strawberry, apple, persimmon, grapes, onion, and cucumber. In an AOM/DSS-induced colitis associated CRC model in BALB/c mice, fisetin suppressed dysplastic lesions through inducing apoptosis in the colonic tissue along with downregulation of Bcl-2 and STAT3, and upregulation of cleaved-caspase-3 and BAX. Fisetin treatment restored the level of enzymatic (SOD, CAT, GPx, and GR) and non-enzymatic (vitamin E, and vitamin C) antioxidants in DMH-induced colonic tissue back to normal [213].

Fisetin treatment resulted in activation of AMPKα and inhibition of PI3K/Akt/mTOR signaling pathway along with decreased expression of PI3K, reduced Akt phosphorylation in PIK3CA mutants. In $FC^{1}3K^{1}Apc^{Min/+}$ mice, fisetin decreased the occurrence of colonic tumor incidences. In combination with 5-FU, fisetin reduced the overall colonic tumor incidences [214].

Fisetin inhibited growth of LoVo tumor xenograft in athymic nude mouse model. Mechanistic study revealed that fisetin acted by inducing apoptosis in tumor tissue through activation of caspase-8 and increased cyt. c expression. In the tumor tissue of treated animals, inhibition of IGF1R and Akt activation was observed [215].

Although CT-26 tumor growth was suppressed upon the intratumoral injection of fisetin, HCT116 tumors were not sensitive to the similar treatment where a combination of radiation with fisetin was more effective. Fisetin suppressed the oncoprotein securin in CT-26 tumor in a p53-independent fashion, but securin null HCT116 tumors are more sensitive to fisetin treatment [216].

Fisetin suppressed HCT116 induced tumor growth in NOD/Shi-scid-IL2R gamma (null) (NOG) mice in a dose-dependent manner compared to control group [218]. Another study showed that due to poor water solubility, the fisetin micelles, composed of poly(ethylene glycol)-poly(ε-caprolactone), i.e., MPEG-PCL, are more efficient antitumor agents over free fisetin as tested in CT-26 tumor model. MPEG-PCL showed enhanced inhibition of angiogenesis through inducing apoptotic cell death [217].

7.1.5. Genistein

Genistein, a naturally occurring isoflavone, was first isolated from Genista tinctoria. Its anticancer properties have been extensively studied [377]. Sekar et al. [222] examined genistein's role in regulating the tumor microenvironment in DMH-induced colon cancer in Wistar rats. This study revealed that genistein could regulate enzymatic (SOD, CAT, GPx, and GR) and non-enzymatic (vitamin E, vitamin C, vitamin A, and GSH) antioxidants in DMH-induced colonic tissue environments. It was found that the loss of mucin secretion in DMH-induced animals was restored by genistein. There was also a reduction of mast cell population and collagen deposition in genistein-treated animals compared to mock-treated animals. Argyrophilic nuclear organizer region and proliferating cell nuclear antigen, two prognostic markers, were decreased by genistein in DMH-treated rats. Genistein activated NRF2 and its downstream target, heme oxygenase-1, and alleviated DMH-induced oxidative stress. Higher expression of colonic stem cell markers, such as CD133, CD44, and β-catenin, was found to be reduced by genistein in DMH-treated animals [222].

It was shown that oral administration of genistein to mice carrying orthotopically implanted human CRC did not inhibit tumor growth. However, it did show inhibition of distant metastasis formation at a dose non-toxic to the animals. Subsequent biochemical analyses showed genistein-mediated downregulation of matrix metalloproteinase-2 (MMP-2) and FMS-related tyrosine kinase 4, also known as vascular endothelial growth factor receptor 3, suggesting its inhibitory role against neoangiogenesis in mouse tumors [224].

Chen et al. [378] conducted a study in which clinical signatures of the anti-CRC activity of genistein were tested in clinical samples of plasma, tumor tissue samples, and standard tissue samples isolated from patients. The expression of miR-95, serum glucocorticoid kinase 1 (SGK1), Bcl-2, and Erk1 was highly elevated in samples of CRC compared to the normal samples. Furthermore, genistein could sensitize CRC SW620 cells to apoptosis with increased LDH content in a concentration-dependent manner, accompanied by downregulation of endogenous miR-95, SGK1, and Erk1 activities [378].

Zhang et al. [223] studied the effect of genistein on AOM-induced colon carcinogenesis in male Sprague Dawley rats. The animals were given a control diet, soya protein isolate (SPI), and a genistein diet orally, starting from gestation to 13 weeks of age. Pre-AOM treatment analysis was performed by taking samples at seven weeks of age, and the remaining rats were AOM-treated at this time for six weeks for analysis. Compared to the control group, AOM injections did not cause aberrant nuclear accumulation of β-catenin in SPI and genistein-treated groups. Moreover, SPI and genistein suppressed the expression of cyclin-D1 and c-Myc. In addition, the expression of Wnt signaling genes (Wnt5a, Sfrp1, Sfrp2, Sfrp5) was decreased to a level comparable to that of pre-AOM treatment by SPI and genistein. Furthermore, the rats fed SPI and genistein had lower numbers of total aberrant crypts, which correlated with the reduction in Wnt/β-catenin signaling. Genistein also lowered the number of ACF [223].

The first clinical study to assess the safety and tolerability of genistein in combination with chemotherapy for the treatment of metastatic CRC was conducted by Pivota et al. [379]. Patients diagnosed with metastatic CRC but not previously treated were administered FOLFOX or FOLFOX-bevacizumab. Genistein (60 mg/day) was given orally for seven days every two weeks. Treatment was started four days before chemotherapy and continued through days one through three of infusion chemotherapy. In this trial, thirteen patients received combinatorial treatment. Treatment with genistein alone resulted in mild side effects, such as headaches, nausea, and hot flashes, with one subject experiencing grade 3 hypertension. There was no increase in chemotherapy-related adverse events when genistein was added to FOLFLOX. The best overall response rate for the genistein supplementation of the chemotherapy regimen was 61.5%. The median progression-free survival of the same study was 11.5 months [379].

7.1.6. Kaempferol

Kaempferol, a dietary flavanol found in many plants, including apple, tea, broccoli, and grapefruit, has been demonstrated to carry antitumor effects based on preclinical studies [380]. Nirmala et al. [239] demonstrated the beneficial effects of orally administered kaempferol with intravenous irinotecan in 1,2-dimethyl hydrazine (DMH)-induced colorectal carcinoma in male Wistar rats. In the kaempferol-fed animal groups, levels of DMH-induced erythrocyte lysate levels and decreased liver thiobarbituric acid reactive substances. Levels of several antioxidant enzymes, such as catalase, superoxide dismutase, and glutathione peroxidase, were recovered, and the most successful effects were achieved at a dose of 200 mg/kg body weight of kaempferol (which is comparable to irinotecan).

The combined effect of fluoxetine, an antidepressant drug, and kaempferol in alleviation of DMH-induced colon carcinoma in male Sprague Dawley rats was also analyzed. Compared to fluoxetine and kaempferol alone, combined treatment of these two agents caused greater reduction in multiple plaque lesions and preneoplastic lesions in the colonic tissues. This combinatorial treatment also reduced tissue concentration of malondialdehyde and NO. Both serum and tissue β-catenin levels were significantly decreased by the combinatorial treatment. There was also a significant increase in serum and tissue caspase-3 levels. PCNA and COX-2 positive cells in the colon of animals receiving the combinatorial treatment were lower when compared to fluoxetine and kaempferol treatments alone [240].

Hassanein et al. [241] studied the effect of sulindac in combination with either EGCG or kaempferol in DMH-induced colon carcinogenesis in male Sprague Dawley rats. The combinations of EGCG and kaempferol with sulindac, a nonsteroidal anti-inflammatory

drug, caused great enhancement of sulindac's antioxidant, anti-inflammatory, antiproliferative, and apoptotic activities. Sulindac combined with both compounds caused a decrease in thiobarbituric acid-reactive substance, tissue NO, and both serum and tissue β-catenin. Downregulation of PCNA and COX-2 and a decrease in the number of ACF caused by DMH administration were also noted [241].

7.1.7. Luteolin

Luteolin (3′,4′,5,7-tetrahydroxyflavone) was discovered in different fruits, vegetables, and medicinal herbs. Plants rich in luteolin are used for treating various ailments, such as hypertension, inflammation, and cancer in Chinese traditional medicine [381,382]. The anti-CRC activity, as well as the anti-angiogenic, anti-invasive, and antimetastatic effects of luteolin were studied using AOM-induced colitis models of male BALB/c mice. Upregulation of γ-glutamyl transferase (GGT), found in a number of human neoplasms, facilitates neoplastic progression and metastasis [246,383]. GGT and 5′-nucleotidase (5′ND) were inhibited in AOM-treated mice by luteolin. Furthermore, luteolin reduced other tumor markers in AOM-treated animals, such as cathepsin-D and carcinoembryonic antigen (CEA), which are correlated with poor prognosis [246]. Luteolin inhibited invasion and metastasis by reducing the expression of MMP-2 and MMP-9 along with enhancing expression of tissue inhibitor metalloproteinases 2 (TIMP-2) [246]. Mast cells were associated with enhanced angiogenesis and tumor malignancy [384]. It was found that luteolin also decreased giant mast cell and total mast cell populations in AOM-treated mice, compared to AOM-induced control animals [246].

Luteolin reduced the number and size polyps of DSS-treated mice. Upon luteolin treatment, DSS-induced oxidative stress, level of carcinoembryonic antigen and COX-2 were decreased in colonic tissue [242]. In another study, luteolin was shown to suppress AOM-induced CRC by downregulating iNOS and COX-2 expression level [243]. Luteolin also suppressed AOM-induced CRC by activating Nrf2/Keap1 pathway [244].

Luteolin inhibited HT29 xenograft's growth in nude mice by an activity consistent with modulation of miR-384/pleiotrophin axis [247]. miR384 expression was found to be downregulated in the majority (83%) of CRC biopsy samples, correlating with the invasiveness and migratory abilities of CRC [385]. Pleiotrophin plays a major role in angiogenesis through upregulation of VEGF in CRC [386]. Luteolin treatment of HT-29 cell-induced xenograft tumor developed in female nude BALB/c mice efficiently suppressed the migration of CRC cells from the spleen to the liver and metastasis through upregulation of miR-384/pleiotrophin axis. Luteolin upregulated the expression of miR-384, which, by targeting pleiotrophin expression, inhibited the expression of MMP-2, MMP-3, MMP-9, MMP-16, as well as invasion and metastasis of CRC [247]. Luteolin, in synergy with adenovirus CD55-TRAIL, inhibited HT-29 xenografts in female BALB/c nude mice through increasing the apoptotic activity [248].

In another study, luteolin showed antimetastatic activity against CT-26 lung metastasis by downregulating MMP-2 and MMP-9. MEK and Akt phosphorylation was suppressed by the inhibition of Raf and PI3K by luteolin [245].

7.1.8. Myricetin

Myricetin (3,3′,4′,5,5′,7-hexahydroxyflavone), a naturally occurring flavonoid pigment, is typically present in fruits, herbs, and nuts. The presence of three hydroxyl groups at 3-′, 4-′, and 5′-carbon positions makes myricetin unique from other flavanols [387]. Studies by Nirmala and Ramachandran [257] showed the efficacy of myricetin on 1,2-dimethylhydrazine-induced rat colon carcinogenesis. They demonstrated that myricetin administration reduced the incidence of tumor-bearing rats and tumors in total. Furthermore, myricetin supplementation dramatically decreased intestinal tumorigenesis developed in adenomatous polyposis coli multiple intestinal neoplasia ($APC^{Min/+}$) mice. Additionally, myricetin treatment improved the antioxidant enzymes, including catalase, glutathione peroxidase, and GSH, in a dose-dependent manner [257].

Li et al. [258] assessed the effectiveness of myricetin against intestinal tumorigenesis in adenomatous polyposis coli multiple intestinal neoplasia ($APC^{Min/+}$) mice. Promoting apoptosis in adenomatous polyps, myricetin-fed $APC^{Min/+}$ mice grew fewer, smaller polyps and did not appear to experience negative side effects. By modifying the GSK-3 and Wnt/-catenin pathways, lowering the levels of the proinflammatory cytokines IL-6 and PGE2, and downregulating the phosphorylated p38 MAPK/Akt/mTOR signaling pathway, myricetin prevents the growth of intestinal tumors [258].

AOM/DSS-induced mice were used by Zhang et al. [259] to test myricetin's effectiveness against chronic colonic inflammation and inflammation-driven carcinogenesis. Myricetin significantly decreased the levels of inflammatory factors, such as TNF-, IL-1, IL-6, NF-B, p-NF-B, COX-2, PCNA, and cyclin D1, to inhibit the development of colorectal tumors and shrink colorectal polyps [259].

M10, a new derivative of myricetin, was tested by Wang et al. [205] to show that M10 inhibits robust endoplasmic reticulum (ER) stress-induced autophagy in inflamed colonic mucosal cells of AOM/DSS-induced mice model. The decreased levels of proinflammatory mediators, such CSF/M-CSF, IL-6, and TNF-α, in colonic mucosa and the prevention of the NF-κB/IL-6/STAT3 pathway, were shown to be associated with the antitumor activity [260].

7.1.9. Naringenin

Naringenin, a flavonoid found mostly in citrus fruits and vegetables with no taste or color, carries antioxidant, anti-inflammatory, antiviral, antimicrobial, and antitumor properties [388]. In addition, naringenin was found to reduce the number of high multiplicity aberrant crypt foci (HMACF) by 51% and the proliferative index by 32% in an AOM-induced rat model. Here, naringenin was implied to prevent CRC through decreasing proliferation and increasing apoptosis of luminal surface colonocytes [261].

Naringenin inhibited a dextran sulfate sodium (DSS)-induced murine colitis model. The inhibitory action was correlated with the inhibition of iNOS, ICAM-1, MCP-1, COX-2, TNF-α, and IL-6 transcript levels. The decrease in TNF-α and IL-6 levels was consistent with the suppression of TLR4 mRNA and protein in the colon mucosa. LPS-induced nuclear translocation of p65/RelA was also inhibited by naringenin in RAW264.7 cells, suggesting its action through TLR4 inhibition [262].

6-C-(E-phenylethenyl)-naringenin (6CEPN) inhibited anchorage independent growth of CRC cells, as well as in a CRC-induced xenograft in a dose-dependent manner through the inhibition of COX-1, an underlying cause of malignant character of CRC cells [263].

Naringin was shown to reduce tumor size and growth of AMO or DSS-induced CRC model in C57BL/6 mice by suppressing ER stress-induced autophagy in colorectal mucosal cells [265]. Another study showed naringin-mediated inhibition of tumor cell proliferation and AOM-induced CRC through inducing apoptosis in an AOM-injected Sprague-Dawley rat model [266,389].

7.1.10. Quercetin

Quercetin (3,4,5,7-pentahydroxyflavone), a polyphenolic flavonoid, was isolated from vegetables, fruits, grain, seeds, and tea [282,390]. Quercetin was shown to carry various pharmacological properties, including anticancer properties. It was further found to be effective against AOM/DSS-mediated colitis induced CRC and showed a decrease in mucin-depleted foci and aberrant crypt foci development [391]. In addition, quercetin treatment was shown to efficiently reduce AOM/DSS-induced inflammation, a major cause of colon carcinogenesis [282,392,393]. In another study, quercetin was found to restore leukocyte levels lost by AOM/DSS treatment. It was also noted that quercetin efficiently downregulated various oxidative stress-related markers, such as lipid peroxide (LPO), NO, SOD, glucose-6-phosphate (G6PD), and glutathione (GSH), explaining its role in neutralizing inflammation. The metabolic profiling of sera demonstrated the effect of quercetin through the downregulation of biomarkers that are upregulated in AOM/DSS-treated mice [282].

In a metastatic cancer model induced in BALB/c mice by CT-26 cells, quercetin was shown to be effective through inducing the intrinsic pathway of apoptosis, along with upregulating the p-38 MAPK pathway. Notably, quercetin function was correlated with modulation of the EMT markers, such as downregulation of N-cadherin, snail, MMP-2, and MMP-9, while E-cadherin, TIMP-1, and TIMP-2 were upregulated [283].

Quercetin augmented radio-sensitization of CRC cells observed in HT-29 tumor xenografts through induction of apoptosis. Combining quercetin with a low dosage of 5Gy radiation effectively suppressed CRC cell proliferation with little toxicity towards normal colonic epithelial cells, CCD-18Co. The combinational therapy was found to target cancer stem cells, as suggested by the reduction of cancer stemness factors, such as DCLK-1, CD24, Lgr5, CD29, and CD44, and the colonosphere formation. The proportion of CD133+ cells also decreased in DLD-1 and HT-29 cells under combinatorial treatments [284].

Li et al. [284] further observed that the combinational therapy of ionizing radiation and quercetin targets the notch-signaling pathway through the downregulation of γ-secretase. The combinational therapy of ionizing radiation and quercetin effectively reduced the expression of γ-secretase complex components nicastrin, PEN2, APH1, presenilin-1, and presenilin-2, which suppressed notch cleavage and thus notch signaling. The combination therapy also inhibited the expression of Jagged-1 and cleaved Notch-1 protein levels [284].

Quercetin induced antiproliferative activity and proapoptotic effects are mediated by the upregulation of cannabinoid receptor-1 (CB1-R) in AOM-treated mice. The downregulation of STAT3 and pSTAT3 was also observed [279].

When radiation therapy was used with quercetin treatment, it suppressed the tumor size of the DLD1 tumor xenograft in athymic nude mice, indicating that quercetin enhanced the radiosensitivity of DLD1 tumors [280].

7.1.11. Rutin

Rutin, a glycosidic derivative of quercetin, is also known as quercetin-3-O-rutinoside or vitamin-P. It is known to carry antimicrobial, antifungal, anti-inflammatory, anticancer, and antiallergic properties, with poor solubility in water [394]. Rutin naturally occurs in various plants, including buckwheat, Mez, Labisia pumila, *Sophora japonica* L., Schum, *Canna indica* L., and *Ruta graveolens* L. [395,396]. In a dose-dependent manner, rutin suppressed SW480 cell-induced tumor growth in a tumor xenograft model without affecting the organ or body weight. In the same model, rutin was shown to enhance mean survival time by 50 days and suppressed angiogenesis through decreasing the serum VEGF level [285].

7.1.12. Tangeretin

Fruits and vegetables contain a wide variety of flavonoids. Citrus fruit flavonoids exhibit various biological effects, such as anticancer and antitumor properties. For example, tangeretin, a polymethoxylated (5,6,7,8,4′-pentamethoxyflavone) flavone, is predominant in the peel of citrus fruits and is thought to operate as a natural resistance factor against pathogenic fungus. In addition, tangeretin has been demonstrated to have several biological properties, including the capacity to suppress cancer cell growth [397].

Bao et al. [291] sought to create a nano-system that included tangeretin (TAGE) and atorvastatin (ATST) and was embellished with RGD (cyclized arginine-glycine-aspartic acid sequences) to treat colon cancer. To assess the anticancer effects of these two drugs on colon cancer cells and in female BALB/c mice harboring cancer models, these researchers produced ATST and TAGE combination nanosystems; RGD-ATST/TAGE CNPs. Results indicated that the RGD-decorated nano-system was more hazardous to HT-29 cells than the undecorated nano-system and that the weight ratio of ATST to TAGE, at which the highest synergism was seen, was 1:1. The integrated nano-systems had a high in vivo biodistribution in the tumor site and effectively reduced in vivo tumor development without significantly harming the treated mice's primary organs and tissues [291].

7.1.13. Wogonin

The medicinal plant *Scutellaria baicalensis* and the traditional Chinese medicine of Huang-Qin (Scutellaria radix) include a significant active monoflavonoid called wogonin (5,7-dihydroxy-8-methoxyflavone). Wogonin has many therapeutic possibilities, including anti-inflammatory and anticancer effects. It has also been observed to inhibit the development of several types of cancer cells with excellent specificity between normal cells and cancer cells [398,399].

To study wogonin's role in colitis-associated colorectal cancer (CAC), Yao et al. [298] developed the AOM/DSS-induced C57BL/6 mice paradigm. They discovered that wogonin markedly reduced the prevalence of tumors and prevented the growth of colorectal adenomas by lowering the expression and secretion of IL-6 and IL-1β, as well as decreasing the cell proliferation and expression of NF-κB in adenomas and adjacent tissues. Further, it increased Nrf2 nuclear translocation in those same tissues [298].

Feng et al. [299] evaluated wogonin's anti-colon cancer effect in an AOM-DSS-induced CRC animal model. They discovered that wogonin decreased tumor abundance and kept colon length within normal range without adversely affecting other organs. In addition, wogonin administration inhibited the SW480 cell-induced xenograft growth in BALB/c mice. Another study, by You et al. [300], further examined the effects of wogonin in mice with colon cancer. Treatment with wogonin abrogated the survival and metastasis properties of colon cancer cells in vivo. A detailed analysis revealed that wogonin-mediated upregulation of p-YAP1 level was responsible for the observed anti-colon cancer effect. This suggested the involvement of the Hippo signaling pathway in the process.

7.2. Phenolic Acids

7.2.1. Caffeic Acid

Caffeic acid (3,4-dihydroxycinnamic acid) is a nonflavonoid catechol with potent antioxidant properties. It is found in almost all plants as an intermediate in the lignin biosynthesis pathway. The prime source of caffeic acid is coffee. Caffeic acid possesses various pharmacological properties, such as antioxidant, anti-inflammatory, anticancer, and neuroprotective effects [400]. Caffeic acid, by direct interaction, inhibited MEK1 and TOPK activity in an ATP non-competitive manner. Kang et al. [303] conducted experiments using caffeic acid on a mouse tumor model. It demonstrated action by inhibition of ERK and p90RSK activation. Caffeic acid suppressed the TPA-induced activation of AP1, NF-κB, and ERK signaling, and thus neoplastic transformation induced by TPA, EGF, and H-Ras. Through inhibition of ERK functions, caffeic acid inhibited lung metastasis of CT-26 cells. This study also indicated the usefulness of caffeic acid in reducing ERK activity in patient tumor samples.

Caffeic acid effectively inhibited cancer stem cells (CSC) and reduced radiation-induced sphere formation of CD133+ and CD44+ CSC in two patient-derived tumor xenograft (PDTX) models of human CRC in immune-suppressed mice. In vivo, the radiation-induced elevation of PI3K/Akt signaling pathway was also suppressed by caffeic acid. In caffeic acid-treated xenograft samples, the abundance of CD133+ and CD44+ subpopulations of CSC cells were decreased. In addition, CD44+ and CD133+ cells of CRC lost their ability for self-renewal, migration, and CSC-like properties due to caffeic acid in a PDTX xenograft model. Inhibition of PI3K/Akt signaling was described as a significant mode of action caffeic acid in inhibiting CSC proliferation [304].

Both caffeic acid phenethyl ester (CAPE) and caffeic acid phenylpropyl ester (CAPPE) could inhibit HCT116 cell-induced tumor xenograft in immune-compromised mice through inhibition of PI3K/Akt and inactivation of mTORC1 by AMPK activation. Treatment with CAPE and CAPPE reduced the MMP-9 level at a non-hepatotoxic concentration. In addition, CAPE and CAPPE suppressed expression of cyclin D1, Cdk4, cyclin E, c-Myc, and N-cadherin, and upregulated p21 in vivo. Expression of tumor biomarkers, such as PCNA and FASN, was also suppressed by CAPE and CAPPE in tumor tissue [305].

CAPE and caffeic acid p-nitro-phenethyl ester (CAPE-pNO2) upregulated the levels of p53, p27, p21, cytochrome c (cyt. C), and cleaved caspase-3, but downregulated procaspase-

3, Cdk2, and c-Myc in HT-29 tumor xenograft in mice. There was a dose-dependent inhibition of tumor growth and VEGF expression by these compounds, with no visible toxicity to normal cells [306].

Consumption of decyl caffeic acid inhibited tumor growth in mice with a HCT116-induced tumor xenograft. The mechanism of action involved the induction of cell cycle arrest at the S phase as well as autophagy [307].

7.2.2. Gallic Acid

Gallic acid (3,4,5-trihydoxy benzoic acid) is a naturally occurring polyhydroxy phenolic acid found as an active compound in various fruits, nuts, food compounds, vegetables, and numerous plants, such as green chicory, grapes, blackberries, raspberries, blueberries, and strawberries. Gallic acid is well known for its antimicrobial, antioxidant, anti-inflammatory, and anticancer potential [401,402]. In a dose-dependent manner, gallic acid was shown to inhibit DSS-induced colitis in mice through the inhibition of STAT3 phosphorylation [320]. This inhibitory mechanism includes reduced proinflammatory mediators Th1, TNF-α, and IL-6, and chemokines, such as KC and MCP-1 [320].

In another study, the inhibitory effects of gallic acid were tested in HCT116 and HT29 cells and tumor xenografts in BALB/c mice. The function of pro-oncogenic factors, such as Src, STAT3, EGFR, and Akt, along with key players in the apoptosis pathway were analyzed. The results demonstrated inhibition of STAT3 and Akt by inhibiting Src and EGFR functions. Furthermore, net enhancement of the cleaved caspase-3 and caspase-9 suggested the involvement of apoptosis as the mechanism behind cell death [321].

Gallic acid was shown to ameliorate ulcerative colitis-associated CRC induced in rats by TNBS treatment by modulating ferroptosis, an iron-dependent process of cellular necrosis [322]. Gene expression profiling interactive analysis (GEPIA) and bioinformatics analysis identified significant involvement of ferroptosis-related genes in CRC prognosis. This analysis indicated that eight ferroptosis-related genes are involved in cell survival. This docking study suggested that gallic acid could induce ferroptosis by modulating some of these genes [322].

7.3. Stilbenes

Resveratrol

Resveratrol (3,5,4′-trihydroxystilbene), a stilbenoid that can be found in peanuts, skin of red grapes, and blueberries, has been studied for its potential anticancer properties [403,404]. Saud et al. [350] used a mouse model with a knocked-out APC locus, and Kras activated specifically in the distant colon to study the effect of resveratrol on sporadic CRC. The mice received a diet supplemented with resveratrol (150 or 300 ppm) before the appearance of tumors. This resulted in a 60% inhibition of tumor production and loss of Kras expression in 40% of mice that developed tumors. Oral administration of resveratrol for tumor bearing mice resulted in complete tumor remission in 33% of mice and a decrease in tumor size in 97% of the remaining mice. Upregulation of miR-96, a negative regulator of Kras expression, in non-tumoral and tumoral colonic tissues suggested that resveratrol exerted its anti-CRC effects by downregulating Kras expression [350]. Alfaras et al. [351] examined the effects of oral administration of trans-resveratrol on DMH-induced precancerous colonic lesions in male Sprague-Dawley rats. This resulted in the reduction of aberrant cryptic foci by 52% and mucin depleted foci by 45% in the colon. In colonic contents, dihydroresveratrol was the most abundant compound detected, followed by trans-resveratrol and its derivatives [351]. Synergistic effects of resveratrol and curcumin on CRC were studied by Majumdar et al. [352].

One study analyzed the effects of resveratrol and its PLGA-chitosan based nanoformulation in animal models (both xenograft and orthotopic) of colon cancer. Both the compound and its nanoformulation caused an appreciable decrease in tumor growth and hemoglobin percentages of tumor mass, signifying reduced angiogenesis with nanoformulation exhibiting more bioavailability and functional efficacy than [353]. Resveratrol combined with ginkgetin, a phytochemical obtained from Ginkgo biloba, exhibited a synergistic effect in suppressing VEGF-induced endothelial cell proliferation, migration, invasion, and tube formation in HT29

cell-induced xenografts in mice. When administered together, these two compounds demonstrated a synergistic antitumor effect with 5-FU, causing a reduction in micro vessel density of the tumors. Furthermore, the combinatorial treatment relieved the 5-FU-induced inflammatory response by lowering the expression of COX-2 and inflammatory cytokines [354]. Resveratrol also suppressed TGF-β1/Smad signaling, downregulated Snail and vimentin, and upregulated E-cadherin expression, which in turn inhibited EMT [349].

8. Phenolics in Clinical Trials for CRC Treatment

Many of the compounds discussed here, such as curcumin, resveratrol, EGCG, genistein, and fisetin, entered into different phases of clinical trials. Curcumin, the most studied phytochemical in both preclinical and clinical studies, has been tested for its effectiveness as an anti-inflammatory agent as well as its potential in prevention, management and therapy of different cancer types, including CRC [405]. The anticancer potential of resveratrol has been documented through studying its efficacy, safety, and pharmacokinetics in more than 244 clinical trials, with additional clinical trials currently being carried out by independent groups [406,407]. Although the clinical utility of resveratrol is well documented, the rapid metabolism and poor bioavailability have limited its therapeutic use [406,408]. Clinical trials on green tea extract containing EGCG as the major active component were conducted, demonstrating the good tolerance of the agent with no significant advantage of its inclusion between the placebo and the treated groups [409]. The efficacy of flavonoid fisetin supplementation on the inflammatory status and MMP levels was tested in small groups of CRC patients, while several markers were measured to assess its therapeutic efficacy, treatment with this polyphenol primarily resulted in the significant changes in IL-8 concentrations compared to the placebo group [410]. The safety and tolerability of genistein in combination with a chemotherapy agent in metastatic CRC were studied in a clinical trial with a small group of patients receiving FOLFOX or FOLFOX-bevacizumab. The results demonstrated the safety and tolerability of the treatment with notable efficacy [379]. While the results of these studies are encouraging, additional studies are needed to assess the long-term use of these phytochemicals in the clinic.

9. Conclusions and Future Perspectives

CRC is the third most diagnosed and second leading cause of cancer-related death worldwide. According to recent statistics, CRC claims close to a million lives, which is about half of the population it affects globally every year. Although the CRC death rate has declined due to routine screening and early detection, CRC incidence is predicted to be doubled by the end of this decade due to various reasons, demanding an urgent need to overcome the limitations of current treatment strategies, including the development of alternative therapy regimens. This review aims to present a detailed account of the recent advances in studies on various phenolic phytochemicals with anti-CRC activities demonstrated in animal experiments with the underlying molecular basis of their actions (summarized in Table 3).

As discussed here, the phytochemicals were reported to act through inhibiting hallmarks of various CRC attributes, such as the potential of cell growth and proliferation, self-renewal, invasion, migration, and angiogenesis through inducing apoptosis, ferroptosis, and autophagy-mediated cell death pathways (Figure 6). These activities involved the modulation of various pathways, such as the levels of proinflammatory cytokines and chemokines (IL-1, IL-6, ICAM-I, TNF, COX-2, iNOS, KC, and MCP1), oxidative stress markers and pathways (SOD, catalase, thiolase, glutathione peroxidase, GSH and Keap1/NRF2/GSK-3β/HO-1), cell cycle regulators (cyclin D1, cyclin E, and CDK 4/6), apoptotic/autophagy regulators (p21, p53, caspase-3, caspase-9, Bax, Bcl-2, Bak, and Beclin1), proliferative signaling pathways regulators (PI3K/Akt/mTOR/AMPK, Wnt/β-catenin, MAPK-p38, ERK, MEK, and c-Myc), regulators of invasion, migration, metastasis, and angiogenesis (Notch1, STAT-3, VEGF, CD31, MMP-2, MMP-3, MMP-9, MMP-16, EGFR, Twist1, Vimentin, FMS-related tyrosine kinase 4, endothelial growth receptor-3, Snail, N-cadherin, E-cadherin, TIMP-1, and TIMP-2), stemness

(CD133, CD44, ALDH1, CD29, DCLK-1, and LGR5) and expression of tumor suppressive miRNAs (miR34a, miR200c, and miR145). The downregulation of COX-2 levels can be achieved upon treatment with EGCG [206], curcumin [194,197], kaempferol [239], luteolin [242,243], myricetin [259], naringenin [262], piceatannol [342], pterostilbene [344], syringic acid [326], boeravinone B [191], hesperidin [227], isoliquiritigenin [235], orientin [268], quercetin [281], and xanthohumol [301]. Caffeic acid suppressed TPA-induced activation of AP1 and NF-κB signaling [303]. Many phytophenols can induce an antioxidant response, such as EGCG, gallic acid, boeravinone B, eriodyctyol, luteolin, and morin. Caffeic acid phenethyl ester and caffeic acid phenylpropyl ester-induced mTOR inhibition through the activation of AMPK [305]. Isoangustone A upregulated AMPK phosphorylation in vivo [234]. Pterostilbene inhibited EGFR in an AOM-induced colonic adenomas in mice [344].

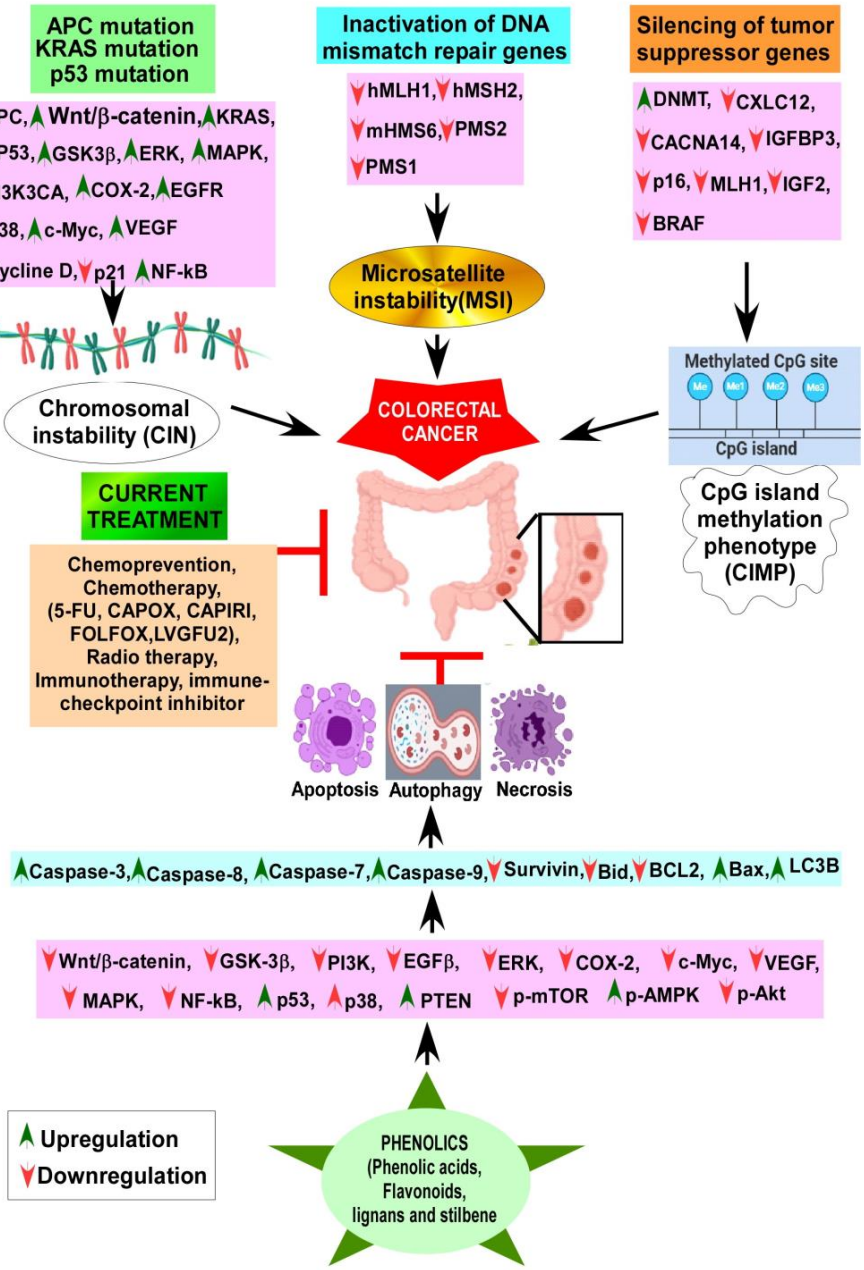

Figure 6. Genetic and molecular basis of colorectal cancer along with the current treatment strategies where potentials of phenolic compounds were indicated. CIN, MSI and CIMP are the prime factors in CRC development. Besides the currently available chemotherapeutic treatment strategies, different polyphenols are reported to induce CRC cell death by apoptosis and/or autophagy and/or necrosis.

There is increasing evidence in favor of the idea that diet can influence the intestinal microbiome and thus the risk of CRC. Diets rich in fruits and vegetables can be associated with gut microbiome rich in Prevotella compared with Bacteroides associated with good colonic health while the consumption of diet with low plant-based food rich in processed food led to the opposite effects [411,412]. Diets rich in plant-based nutraceuticals could regulate host immune and inflammatory behavior and thus gut homeostasis through modulating the composition and functionality of the gut microbiome [413]. Therefore, CRC incidence and progression can be reduced by modulating gut microbiome by careful choice of diet and phytochemicals which could be a promising and efficient way to reduce the burden of CRC [413]. Gut microbiota can digest dietary phytochemicals by their unique ability to produce short chain fatty acids, such as butyric acid, with anti-inflammatory and antineoplastic activity [414]. Phenolic phytochemicals have served us as an important source of novel drugs/leads. While the studies discussed here provided encouraging results, several issues are needed to be considered to get a step closer to the end users, such as:

1. Apparently, the functions of many phytochemicals are limited by their poor solubility, absorption, and bioavailability. Encapsulation by nano-formulation as well as chemical derivatization of the compound could resolve this issue.
2. Some cases reproducing the activity observed in preclinical animal models into the clinic/human could be challenging due to several factors. Success in this endeavor requires careful optimization in administered doses to assess functional synergy, if any, with anti-CRC regimens used in the clinic. Once positive results are obtained in the preclinical settings, testing the validity of the finding, such as safety and efficacy, in clinical trials with appropriate controls will be important to move further.
3. It is reasonable to think that a phenolic compound showing very weak and toxic activity can yield desirable effect when combined with another phytochemical. Therefore, a careful combination of selected polyphenols can yield unique anti-CRC activity. It is important to clearly determine the maximum tolerable dose of a phytochemical to better understand its therapeutic efficacy alone or in combination with another phytochemical or drug.
4. Once a phenolic compound with unique anti-CRC activity is identified, it would be important to develop strategies to synthesize the compound in the laboratory, given the very low abundance of a secondary metabolite in the plants. A detailed understanding of the pharmacophore responsible for the observed function should be helpful for chemical synthesis or semi-synthesis, and cellular target identification of the compound. Given the structural complexity of the plant secondary metabolites, it is often a major challenge for natural product chemists and medicinal chemists to solve. Ideally, the simultaneous engagement of experts from interdisciplinary areas, such as ethnopharmacology, molecular biology, biochemistry, natural product chemistry, medicinal chemistry, bioinformatics, and pharmacology, will be necessary to achieve progress in real-time in harvesting the full potential of natural products as the source of novel drug leads.

Author Contributions: Conceptualization, M.P.; methodology, S.D. and S.P.; investigation, S.D., S.P., A.B. (Ashish Bhattacharjee) and S.C.M.; data curation, S.D. and S.P.; writing—original draft preparation, S.D., S.P., K.P., A.M. (Anirban Manna), J.H., V.K.N., C.M., N.C. and A.B. (Anupam Bishayee); writing—review and editing, M.P., K.P., N.C. and A.B. (Anupam Bishayee); visualization, S.G., A.M. (Arijit Mondal) and S.B.; supervision, M.P. and A.B. (Anupam Bishayee); project administration, M.P. and A.B. (Anupam Bishayee), funding acquisition, M.P. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Science and Engineering Research Board, Department of Biotechnology (DBT), and Department of Science and Technology (DST) to M.P. S.D. is a University Grant Commission (UGC) Senior Research Fellow (SRF), A.M. (Anirban Manna) is a DBT SRF, and C.M. is a DST Inspire Fellow. S.G. was an SRF of the Council of Scientific and Industrial Research.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the writing of the manuscript.

Abbreviations

5-FU	5-fluorouracil
5′ND	5-nucleotidase
6CEPN	6-C-(E-phenylethyl)-naringenin
ACF	aberrant crypt foci
AOM	azoxymethane
APC	adenomatous polyposis coli
ATST	atorvastatin
BAX	B-cell lymphoma 2-associated x protein
BCL-2	B-cell lymphoma 2
BID	BH3 interacting-domain death agonist
BRAF-B	rapidly accelerated fibrosarcoma/murine sarcoma viral oncogene homolog B
CAC	colitis-associated colorectal cancer
CACNA14	voltage-dependent P/Q type calcium channel subunit alpha1A
CAP	capecitabine
CAPE,	caffeic acid phenethyl ester
CAPPE	caffeic acid phenylpropyl ester
CAPE-pNO2	caffeic acid p-nitro-phenylethyl ester
CAPIRI	capecitabine and irinotecan
CAPOX	capecitabine and oxaliplatin
CEA	carcinoembryonic antigen
CIMP	CpG island methylation phenotype
CIN	chromosomal instability
CLXC12	C–X–C chemokine 12
COX-2	cyclooxygenase-2
CRC	colorectal cancer
CSC	cancer stem cell
cyt. c	cytochrome c
DII	dietary inflammatory index
Dkk	Dickkopf
DMH	dimethyl hydrazine
DNMT	DNA methyltransferase
DSS	dextran sulphate sodium
Dvl	Discevelled
EGF-β	epidermal growth factor-β
EGFR	epidermal growth factor receptor
ER	endoplasmic reticulum
ERK	extracellular signal-regulated kinase
FAP	familial adenomatous polyposis syndrome
FDA	Federal Drug Administration
FOLFOX	5-FU and oxaliplatin
FOXFIRI	5-FU and irinotecan
FZD	Frizzled receptor
G6PD	glucose-6-phosphate dehydrogenase
GEPIA	gene expression profiling interactive analysis
GGT	γ-glutamyl transferase
GSH	glutathione
GSK-3β	glycogen synthase kinase-3β
GTP	green tea polyphenol
HMACF	high multiplicity aberrant crypt foci
hMLH1	human MutL homolog 1
HPP	hyperplastic polyposis
IDEA	International Duration Evaluation of Adjuvant Chemotherapy

IGF-2	insulin like growth factor-2
IGFBP3	insulin like growth factor-binding protein 3
iNOS	inducible nitric oxide synthase
IRI	irinotecan
KRAS	Kirsten rat sarcoma viral oncogene homolog
LC3B	light chain 3B of microtubule-associated proteins 1A/1B
LPO	lipid peroxide
MAP	MUTYG- associated polyposis
MAPK	mitogen-activated protein kinase
MMP	matrix metalloproteinase
MSI	microsatellite instability
mTOR	mammalian target of rapamycin
NF-κβ	nuclear factor-κβ
NO	nitric oxide
NSAID	nonsteroidal anti-inflammatory drug
oncomiRs	oncogenic miRNAs
OPE	orange peel extract
OX	oxaliplatin
PDTX	patient-derived tumor xenograft
PI3K	phosphoinositide 3-kinase
PPE	polyphenol E
PTEN	phosphatase and tensin homolog deleted on chromosome 10
SEER	surveillance epidemiology and end results
SGK1	serum glucocorticoid kinase 1
Skip	Ski-interacting protein
SOD	superoxide dismutase
SPI	soya protein isolate
TAGE	tangeretin
TIMP	tissue inhibitor metalloproteinase
TNM	tumor/node/metastasis
TRPV1	transient receptor potential vanilloid 1
VEGF	vascular endothelial growth factor
VEGFR	vascular endothelial growth factor receptor
YAP	yes-associated protein
yCRC	young-onset colorectal cancer

References

1. Siegel, R.L.; Miller, K.D.; Fuchs, H.E.; Jemal, A. Cancer statistics, 2021. *CA Cancer J. Clin.* **2021**, *71*, 7–33. [CrossRef] [PubMed]
2. Sung, H.; Ferlay, J.; Siegel, R.L.; Laversanne, M.; Soerjomataram, I.; Jemal, A.; Bray, F. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J. Clin.* **2021**, *71*, 209–249. [CrossRef] [PubMed]
3. Ferlay, J.; Colombet, M.; Soerjomataram, I.; Mathers, C.; Parkin, D.; Piñeros, M.; Znaor, A.; Bray, F. Estimating the global cancer incidence and mortality in 2018: GLOBOCAN sources and methods. *Int. J. Cancer* **2019**, *144*, 1941–1953. [CrossRef] [PubMed]
4. Arnold, M.; Sierra, M.S.; Laversanne, M.; Soerjomataram, I.; Jemal, A.; Bray, F. Global patterns and trends in colorectal cancer incidence and mortality. *Gut* **2017**, *66*, 683–691. [CrossRef] [PubMed]
5. Haque, A.; Brazeau, D.; Amin, A.R. Perspectives on natural compounds in chemoprevention and treatment of cancer: An update with new promising compounds. *Eur. J. Cancer* **2021**, *149*, 165–183. [CrossRef]
6. Swetha, M.; Keerthana, C.; Rayginia, T.P.; Anto, R.J. Cancer chemoprevention: A strategic approach using phytochemicals. *Front. Pharmacol.* **2021**, *12*, 809308.
7. Dhillon, P.K.; Mathur, P.; Nandakumar, A.; Fitzmaurice, C.; Kumar, G.A.; Mehrotra, R.; Shukla, D.; Rath, G.; Gupta, P.C.; Swaminathan, R. The burden of cancers and their variations across the states of India: The Global Burden of Disease Study 1990–2016. *Lancet Oncol.* **2018**, *19*, 1289–1306. [CrossRef]
8. Choudhari, A.S.; Mandave, P.C.; Deshpande, M.; Ranjekar, P.; Prakash, O. Phytochemicals in cancer treatment: From preclinical studies to clinical practice. *Front. Pharmacol.* **2020**, *10*, 1614. [CrossRef]
9. Siegel, R.L.; Miller, K.D.; Goding Sauer, A.; Fedewa, S.A.; Butterly, L.F.; Anderson, J.C.; Cercek, A.; Smith, R.A.; Jemal, A. Colorectal cancer statistics, 2020. *CA Cancer J. Clin.* **2020**, *70*, 145–164. [CrossRef]

10. Ranjan, A.; Ramachandran, S.; Gupta, N.; Kaushik, I.; Wright, S.; Srivastava, S.; Das, H.; Srivastava, S.; Prasad, S.; Srivastava, S.K. Role of phytochemicals in cancer prevention. *Int. J. Mol. Sci.* **2019**, *20*, 4981. [CrossRef]
11. Siegel, R.L.; Miller, K.D.; Fedewa, S.A.; Ahnen, D.J.; Meester, R.G.; Barzi, A.; Jemal, A. Colorectal cancer statistics, 2017. *CA Cancer J. Clin.* **2017**, *67*, 177–193. [CrossRef] [PubMed]
12. Mauri, G.; Sartore-Bianchi, A.; Russo, A.G.; Marsoni, S.; Bardelli, A.; Siena, S. Early-onset colorectal cancer in young individuals. *Mol. Oncol.* **2019**, *13*, 109–131. [CrossRef] [PubMed]
13. Murphy, C.C.; Lund, J.L.; Sandler, R.S. Young-onset colorectal cancer: Earlier diagnoses or increasing disease burden? *Gastroenterology* **2017**, *152*, 1809–1812.e1803. [CrossRef] [PubMed]
14. You, Y.N.; Xing, Y.; Feig, B.W.; Chang, G.J.; Cormier, J.N. Young-onset colorectal cancer: Is it time to pay attention? *Arch. Intern. Med.* **2012**, *172*, 287–289. [CrossRef] [PubMed]
15. Holowatyj, A.N.; Ruterbusch, J.J.; Rozek, L.S.; Cote, M.L.; Stoffel, E.M. Racial/ethnic disparities in survival among patients with young-onset colorectal cancer. *J. Clin. Oncol.* **2016**, *34*, 2148. [CrossRef] [PubMed]
16. Thanikachalam, K.; Khan, G. Colorectal cancer and nutrition. *Nutrients* **2019**, *11*, 164. [CrossRef]
17. Tanaka, S.; Oka, S.; Chayama, K. Colorectal endoscopic submucosal dissection: Present status and future perspective, including its differentiation from endoscopic mucosal resection. *J. Gastroenterol.* **2008**, *43*, 641–651. [CrossRef]
18. Chakedis, J.; Schmidt, C.R. Surgical treatment of metastatic colorectal cancer. *Surg. Oncol. Clin.* **2018**, *27*, 377–399. [CrossRef]
19. Mojtahedi, Z.; Koo, J.S.; Yoo, J.; Kim, P.; Kang, H.-T.; Hwang, J.; Joo, M.K.; Shen, J.J. Palliative care and life-sustaining/local procedures in colorectal Cancer in the United States hospitals: A ten-year perspective. *Cancer Manag. Res.* **2021**, *13*, 7569–7577. [CrossRef]
20. Townsend, A.; Price, T.; Karapetis, C. Selective internal radiation therapy for liver metastases from colorectal cancer. *Cochrane Database Syst. Rev.* **2009**, CD007045. [CrossRef]
21. Kanani, A.; Veen, T.; Søreide, K. Neoadjuvant immunotherapy in primary and metastatic colorectal cancer. *Br. J. Surg.* **2021**, *108*, 1417–1425. [CrossRef]
22. Loughrey, M.B. Neoadjuvant immunotherapy and colorectal cancer treatment: Implications for the primary role of surgery. *Color. Dis. Off. J. Assoc. Coloproctol. Great Br. Irel.* **2022**, *24*, 1460–1461. [CrossRef]
23. Aiello, P.; Sharghi, M.; Mansourkhani, S.M.; Ardekan, A.P.; Jouybari, L.; Daraei, N.; Peiro, K.; Mohamadian, S.; Rezaei, M.; Heidari, M. Medicinal plants in the prevention and treatment of colon cancer. *Oxidative Med. Cell. Longev.* **2019**, *2019*, 2075614. [CrossRef] [PubMed]
24. Muppala, S. Phytochemicals Targeting Colorectal Cancer Growth and Metastasis. *Crit. Rev. Oncog.* **2020**, *25*, 141–149. [CrossRef] [PubMed]
25. Sain, A.; Sahu, S.; Naskar, D. Potential of Olive oil and its phenolic compounds as therapeutic intervention against colorectal cancer: A comprehensive review. *Br. J. Nutr.* **2021**, *128*, 1257–1273. [CrossRef] [PubMed]
26. Cueva, C.; Silva, M.; Pinillos, I.; Bartolomé, B.; Moreno-Arribas, M.V. Interplay between dietary polyphenols and oral and gut microbiota in the development of colorectal cancer. *Nutrients* **2020**, *12*, 625. [CrossRef]
27. Andrews, L. Dietary flavonoids for the prevention of colorectal cancer. *Clin. J. Oncol. Nurs.* **2013**, *17*, 671–673. [CrossRef]
28. Woo, H.D.; Kim, J. Dietary flavonoid intake and risk of stomach and colorectal cancer. *World J. Gastroenterol.* **2013**, *19*, 1011. [CrossRef]
29. Kocic, B.; Kitic, D.; Brankovic, S. Dietary flavonoid intake and colorectal cancer risk: Evidence from human population studies. *J. BUON* **2013**, *18*, 34–43.
30. Jin, H.; Leng, Q.; Li, C. Dietary flavonoid for preventing colorectal neoplasms. *Cochrane Database Syst. Rev.* **2012**, CD009350. [CrossRef]
31. Han, S.; Cao, Y.; Guo, T.; Lin, Q.; Luo, F. Targeting lncRNA/Wnt axis by flavonoids: A promising therapeutic approach for colorectal cancer. *Phytother. Res.* **2022**, *36*, 4024–4040. [CrossRef] [PubMed]
32. Pereira-Wilson, C. Can dietary flavonoids be useful in the personalized treatment of colorectal cancer? *World J. Gastrointest. Oncol.* **2022**, *14*, 1115. [CrossRef] [PubMed]
33. Fernández, J.; Silván, B.; Entrialgo-Cadierno, R.; Villar, C.J.; Capasso, R.; Uranga, J.A.; Lombó, F.; Abalo, R. Antiproliferative and palliative activity of flavonoids in colorectal cancer. *Biomed. Pharmacother.* **2021**, *143*, 112241. [CrossRef] [PubMed]
34. Afshari, K.; Haddadi, N.S.; Haj-Mirzaian, A.; Farzaei, M.H.; Rohani, M.M.; Akramian, F.; Naseri, R.; Sureda, A.; Ghanaatian, N.; Abdolghaffari, A.H. Natural flavonoids for the prevention of colon cancer: A comprehensive review of preclinical and clinical studies. *J. Cell. Physiol.* **2019**, *234*, 21519–21546. [CrossRef]
35. Li, Y.; Zhang, T.; Chen, G.Y. Flavonoids and colorectal cancer prevention. *Antioxidants* **2018**, *7*, 187. [CrossRef]
36. Koosha, S.; Alshawsh, M.A.; Looi, C.Y.; Seyedan, A.; Mohamed, Z. An association map on the effect of flavonoids on the signaling pathways in colorectal cancer. *Int. J. Med. Sci.* **2016**, *13*, 374. [CrossRef]
37. Potter, J.D. Nutrition and colorectal cancer. *Cancer Causes Control* **1996**, *7*, 127–146. [CrossRef]
38. Martinez, M.E.; Willett, W.C. Calcium, vitamin D, and colorectal cancer: A review of the epidemiologic evidence. *Cancer Epidemiol. Biomark. Prev.* **1998**, *7*, 163–168.
39. Terry, P.; Giovannucci, E.; Michels, K.B.; Bergkvist, L.; Hansen, H.; Holmberg, L.; Wolk, A. Fruit, vegetables, dietary fiber, and risk of colorectal cancer. *J. Natl. Cancer Inst.* **2001**, *93*, 525–533. [CrossRef]

40. Santarelli, R.L.; Pierre, F.; Corpet, D.E. Processed meat and colorectal cancer: A review of epidemiologic and experimental evidence. *Nutr. Cancer* **2008**, *60*, 131–144.
41. Benarba, B.; Pandiella, A. Colorectal cancer and medicinal plants: Principle findings from recent studies. *Biomed. Pharmacother.* **2018**, *107*, 408–423. [CrossRef] [PubMed]
42. La Vecchia, S.; Sebastián, C. Metabolic pathways regulating colorectal cancer initiation and progression. *Semin. Cell Dev.Biol.* **2020**, *98*, 63–70. [CrossRef] [PubMed]
43. Keum, N.; Giovannucci, E. Global burden of colorectal cancer: Emerging trends, risk factors and prevention strategies. *Nat. Rev. Gastroenterol. Hepatol.* **2019**, *16*, 713–732. [CrossRef] [PubMed]
44. Lynch, H.T.; Smyrk, T.C.; Watson, P.; Lanspa, S.J.; Lynch, J.F.; Lynch, P.M.; Cavalieri, R.J.; Boland, C.R. Genetics, natural history, tumor spectrum, and pathology of hereditary nonpolyposis colorectal cancer: An updated review. *Gastroenterology* **1993**, *104*, 1535–1549. [CrossRef] [PubMed]
45. Niessen, R.C.; Berends, M.J.; Wu, Y.; Sijmons, R.H.; Hollema, H.; Ligtenberg, M.J.; de Walle, H.E.; de Vries, E.G.; Karrenbeld, A.; Buys, C.H. Identification of mismatch repair gene mutations in young patients with colorectal cancer and in patients with multiple tumours associated with hereditary non-polyposis colorectal cancer. *Gut* **2006**, *55*, 1781–1788. [CrossRef] [PubMed]
46. Seppälä, T.; Latchford, A.; Negoi, I.; Sampaio Soares, A.; Jimenez-Rodriguez, R.; Sánchez-Guillén, L.; Evans, D.; Ryan, N.; Crosbie, E.; Dominguez-Valentin, M. European guidelines from the EHTG and ESCP for Lynch syndrome: An updated third edition of the Mallorca guidelines based on gene and gender. *Br. J. Surg.* **2021**, *108*, 484–498. [CrossRef]
47. Møller, P.; Seppälä, T.T.; Bernstein, I.; Holinski-Feder, E.; Sala, P.; Evans, D.G.; Lindblom, A.; Macrae, F.; Blanco, I.; Sijmons, R.H. Cancer risk and survival in path_MMR carriers by gene and gender up to 75 years of age: A report from the Prospective Lynch Syndrome Database. *Gut* **2018**, *67*, 1306–1316. [CrossRef]
48. Ponti, G.; Manfredini, M.; Tomasi, A.; Pellacani, G. Muir–Torre Syndrome and founder mismatch repair gene mutations: A long gone historical genetic challenge. *Gene* **2016**, *589*, 127–132. [CrossRef]
49. Grzybowski, A.; Jablonska, S. Muir–Torre Syndrome—Is It Really a New Syndrome? *Am. J. Dermatopathol.* **2009**, *31*, 799–802. [CrossRef]
50. Kidambi, T.D.; Kohli, D.R.; Samadder, N.J.; Singh, A. Hereditary polyposis syndromes. *Curr. Treat. Options Gastroenterol.* **2019**, *17*, 650–665. [CrossRef]
51. Hamilton, S.R.; Liu, B.; Parsons, R.E.; Papadopoulos, N.; Jen, J.; Powell, S.M.; Krush, A.J.; Berk, T.; Cohen, Z.; Tetu, B. The molecular basis of Turcot's syndrome. *N. Engl. J. Med.* **1995**, *332*, 839–847. [CrossRef]
52. Rutz, H.P.; de Tribolet, N.; Calmes, J.M.; Chapuis, G. Long-time survival of a patient with glioblastoma and Turcot's syndrome: Case report. *J. Neurosurg.* **1991**, *74*, 813–815. [CrossRef] [PubMed]
53. Haggar, F.A.; Boushey, R.P. Colorectal cancer epidemiology: Incidence, mortality, survival, and risk factors. *Clin. Colon Rectal Surg.* **2009**, *22*, 191–197. [CrossRef] [PubMed]
54. Galiatsatos, P.; Foulkes, W.D. Familial adenomatous polyposis. *Off. J. Am. Coll. Gastroenterol. ACG* **2006**, *101*, 385–398. [CrossRef] [PubMed]
55. Coffin, C.M.; Hornick, J.L.; Zhou, H.; Fletcher, C.D. Gardner fibroma: A clinicopathologic and immunohistochemical analysis of 45 patients with 57 fibromas. *Am. J. Surg. Pathol.* **2007**, *31*, 410–416. [CrossRef]
56. Kiessling, P.; Dowling, E.; Huang, Y.; Ho, M.L.; Balakrishnan, K.; Weigel, B.J.; Highsmith, W.E.; Niu, Z.; Schimmenti, L.A. Identification of aggressive Gardner syndrome phenotype associated with a de novo APC variant, c. 4666dup. *Mol. Case Stud.* **2019**, *5*, a003640. [CrossRef] [PubMed]
57. Sutcliffe, E.G.; Thompson, A.B.; Stettner, A.R.; Marshall, M.L.; Roberts, M.E.; Susswein, L.R.; Wang, Y.; Klein, R.T.; Hruska, K.S.; Solomon, B.D. Multi-gene panel testing confirms phenotypic variability in MUTYH-Associated Polyposis. *Fam. Cancer* **2019**, *18*, 203–209. [CrossRef] [PubMed]
58. Curia, M.C.; Catalano, T.; Aceto, G.M. MUTYH: Not just polyposis. *World J. Clin. Oncol.* **2020**, *11*, 428. [CrossRef]
59. Vogt, S.; Jones, N.; Christian, D.; Engel, C.; Nielsen, M.; Kaufmann, A.; Steinke, V.; Vasen, H.F.; Propping, P.; Sampson, J.R. Expanded extracolonic tumor spectrum in MUTYH-associated polyposis. *Gastroenterology* **2009**, *137*, 1976–1985.e1910. [CrossRef]
60. Calva-Cerqueira, D.; Chinnathambi, S.; Pechman, B.; Bair, J.; Larsen-Haidle, J.; Howe, J. The rate of germline mutations and large deletions of SMAD4 and BMPR1A in juvenile polyposis. *Clin. Genet.* **2009**, *75*, 79–85. [CrossRef]
61. Gallione, C.J.; Repetto, G.M.; Legius, E.; Rustgi, A.K.; Schelley, S.L.; Tejpar, S.; Mitchell, G.; Drouin, É.; Westermann, C.J.; Marchuk, D.A. A combined syndrome of juvenile polyposis and hereditary haemorrhagic telangiectasia associated with mutations in MADH4 (SMAD4). *Lancet* **2004**, *363*, 852–859. [CrossRef] [PubMed]
62. Brosens, L.A.; Van Hattem, A.; Hylind, L.M.; Iacobuzio-Donahue, C.; Romans, K.E.; Axilbund, J.; Cruz-Correa, M.; Tersmette, A.C.; Offerhaus, G.J.A.; Giardiello, F.M. Risk of colorectal cancer in juvenile polyposis. *Gut* **2007**, *56*, 965–967. [CrossRef]
63. Kopacova, M.; Tacheci, I.; Rejchrt, S.; Bures, J. Peutz-Jeghers syndrome: Diagnostic and therapeutic approach. *World J. Gastroenterol.* **2009**, *15*, 5397. [CrossRef] [PubMed]
64. Westerman, A.M.; Entius, M.M.; De Baar, E.; Boor, P.P.; Koole, R.; Van Velthuysen, M.L.F.; Offerhaus, G.J.A.; Lindhout, D.; De Rooij, F.W.; Wilson, J.P. Peutz-Jeghers syndrome: 78-year follow-up of the original family. *Lancet* **1999**, *353*, 1211–1215. [CrossRef]
65. Smerdel, M.P.; Skytte, A.-B.; Jelsig, A.M.; Ebbehøj, E.; Stochholm, K. Revised Danish guidelines for the cancer surveillance of patients with Cowden Syndrome. *Eur. J. Med. Genet.* **2020**, *63*, 103873. [CrossRef] [PubMed]

66. Lloyd, K.M.; Dennis, M. Cowden's disease: A possible new symptom complex with multiple system involvement. *Ann. Intern. Med.* **1963**, *58*, 136–142. [CrossRef]
67. Miyaki, M.; Konishi, M.; Kikuchi-Yanoshita, R.; Enomoto, M.; Igari, T.; Tanaka, K.; Muraoka, M.; Takahashi, H.; Amada, Y.; Fukayama, M. Characteristics of somatic mutation of the adenomatous polyposis coli gene in colorectal tumors. *Cancer Res.* **1994**, *54*, 3011–3020.
68. Lamlum, H.; Ilyas, M.; Rowan, A.; Clark, S.; Johnson, V.; Bell, J.; Frayling, I.; Efstathiou, J.; Pack, K.; Payne, S. The type of somatic mutation at APC in familial adenomatous polyposis is determined by the site of the germline mutation: A new facet to Knudson's' two-hit'hypothesis. *Nat. Med.* **1999**, *5*, 1071–1075. [CrossRef]
69. Fodde, R.; Smits, R.; Clevers, H. APC, signal transduction and genetic instability in colorectal cancer. *Nat. Rev. Cancer* **2001**, *1*, 55–67. [CrossRef]
70. Kanth, P.; Grimmett, J.; Champine, M.; Burt, R.; Samadder, J.N. Hereditary colorectal polyposis and cancer syndromes: A primer on diagnosis and management. *Off. J. Am. Coll. Gastroenterol. ACG* **2017**, *112*, 1509–1525. [CrossRef]
71. Mokarram, P.; Albokashy, M.; Zarghooni, M.; Moosavi, M.A.; Sepehri, Z.; Chen, Q.M.; Hudecki, A.; Sargazi, A.; Alizadeh, J.; Moghadam, A.R. New frontiers in the treatment of colorectal cancer: Autophagy and the unfolded protein response as promising targets. *Autophagy* **2017**, *13*, 781–819. [CrossRef] [PubMed]
72. Jasperson, K.W.; Tuohy, T.M.; Neklason, D.W.; Burt, R.W. Hereditary and familial colon cancer. *Gastroenterology* **2010**, *138*, 2044–2058. [CrossRef]
73. Potack, J.; Itzkowitz, S.H. Colorectal cancer in inflammatory bowel disease. *Gut Liver* **2008**, *2*, 61. [CrossRef] [PubMed]
74. Armenian, S.H.; Robison, L.L. Childhood cancer survivorship: An update on evolving paradigms for understanding pathogenesis and screening for therapy-related late effects. *Curr. Opin. Pediatr.* **2013**, *25*, 16. [CrossRef] [PubMed]
75. Baxter, N.N.; Goldwasser, M.A.; Paszat, L.F.; Saskin, R.; Urbach, D.R.; Rabeneck, L. Association of colonoscopy and death from colorectal cancer. *Ann. Intern. Med.* **2009**, *150*, 1–8. [CrossRef]
76. Hadjiliadis, D.; Khoruts, A.; Zauber, A.G.; Hempstead, S.E.; Maisonneuve, P.; Lowenfels, A.B.; Braid, A.L.; Cullina, J.; Daggett, A.; Fink, A. Cystic fibrosis colorectal cancer screening consensus recommendations. *Gastroenterology* **2018**, *154*, 736–745.e714. [CrossRef]
77. Botteri, E.; Iodice, S.; Bagnardi, V.; Raimondi, S.; Lowenfels, A.B.; Maisonneuve, P. Smoking and colorectal cancer: A meta-analysis. *JAMA* **2008**, *300*, 2765–2778. [CrossRef]
78. Cai, S.; Li, Y.; Ding, Y.; Chen, K.; Jin, M. Alcohol drinking and the risk of colorectal cancer death: A meta-analysis. *Eur. J. Cancer Prev.* **2014**, *23*, 532–539. [CrossRef]
79. Kyrgiou, M.; Kalliala, I.; Markozannes, G.; Gunter, M.J.; Paraskevaidis, E.; Gabra, H.; Martin-Hirsch, P.; Tsilidis, K.K. Adiposity and cancer at major anatomical sites: Umbrella review of the literature. *BMJ* **2017**, *356*, j477. [CrossRef]
80. Wong, M.C.; Ding, H.; Wang, J.; Chan, P.S.; Huang, J. Prevalence and risk factors of colorectal cancer in Asia. *Intest. Res.* **2019**, *17*, 317. [CrossRef]
81. Shivappa, N.; Godos, J.; Hébert, J.R.; Wirth, M.D.; Piuri, G.; Speciani, A.F.; Grosso, G. Dietary inflammatory index and colorectal cancer risk—A meta-analysis. *Nutrients* **2017**, *9*, 1043. [CrossRef] [PubMed]
82. Meng, Y.; Sun, J.; Yu, J.; Wang, C.; Su, J. Dietary intakes of calcium, iron, magnesium, and potassium elements and the risk of colorectal cancer: A meta-analysis. *Biol. Trace Elem. Res.* **2019**, *189*, 325–335. [CrossRef] [PubMed]
83. Parra-Soto, S.; Ahumada, D.; Petermann-Rocha, F.; Boonpoor, J.; Gallegos, J.L.; Anderson, J.; Sharp, L.; Malcomson, F.C.; Livingstone, K.M.; Mathers, J.C. Association of meat, vegetarian, pescatarian and fish-poultry diets with risk of 19 cancer sites and all cancer: Findings from the UK Biobank prospective cohort study and meta-analysis. *BMC Med.* **2022**, *20*, 1–16. [CrossRef]
84. Zhong, Y.; Zhu, Y.; Li, Q.; Wang, F.; Ge, X.; Zhou, G.; Miao, L. Association between Mediterranean diet adherence and colorectal cancer: A dose-response meta-analysis. *Am. J. Clin. Nutr.* **2020**, *111*, 1214–1225. [CrossRef] [PubMed]
85. Hoang, T.; Kim, H.; Kim, J. Dietary intake in association with all-cause mortality and colorectal cancer mortality among colorectal cancer survivors: A systematic review and meta-analysis of prospective studies. *Cancers* **2020**, *12*, 3391. [CrossRef]
86. Cho, K.R.; Vogelstein, B. Genetic alterations in the adenoma–carcinoma sequence. *Cancer* **1992**, *70*, 1727–1731. [CrossRef]
87. Cho, K.R.; Vogelstein, B. Suppressor gene alterations in the colorectal adenoma-carcinoma sequence. *J. Cell. Biochem.* **1992**, *50*, 137–141. [CrossRef]
88. Tariq, K.; Ghias, K. Colorectal cancer carcinogenesis: A review of mechanisms. *Cancer Biol. Med.* **2016**, *13*, 120. [CrossRef]
89. Hermsen, M.; Postma, C.; Baak, J.; Weiss, M.; Rapallo, A.; Sciutto, A.; Roemen, G.; Arends, J.W.; Williams, R.; Giaretti, W. Colorectal adenoma to carcinoma progression follows multiple pathways of chromosomal instability. *Gastroenterology* **2002**, *123*, 1109–1119. [CrossRef]
90. Baran, B.; Ozupek, N.M.; Tetik, N.Y.; Acar, E.; Bekcioglu, O.; Baskin, Y. Difference between left-sided and right-sided colorectal cancer: A focused review of literature. *Gastroenterol. Res.* **2018**, *11*, 264. [CrossRef]
91. Gryfe, R.; Bapat, B.; Gallinger, S.; Swallow, C.; Redston, M.; Couture, J. Molecular biology of colorectal cancer. *Curr. Probl. Cancer* **1997**, *21*, 233–299. [CrossRef] [PubMed]
92. Levy, D.B.; Smith, K.J.; Beazer-Barclay, Y.; Hamilton, S.R.; Vogelstein, B.; Kinzler, K.W. Inactivation of both APC alleles in human and mouse tumors. *Cancer Res.* **1994**, *54*, 5953–5958. [PubMed]
93. Dow, L.E.; O'Rourke, K.P.; Simon, J.; Tschaharganeh, D.F.; van Es, J.H.; Clevers, H.; Lowe, S.W. Apc restoration promotes cellular differentiation and reestablishes crypt homeostasis in colorectal cancer. *Cell* **2015**, *161*, 1539–1552. [CrossRef] [PubMed]

94. Cancer Genome Atlas Network. Comprehensive molecular characterization of human colon and rectal cancer. *Nature* **2012**, *487*, 330. [CrossRef]
95. Fischer, M.M.; Yeung, V.P.; Cattaruzza, F.; Hussein, R.; Yen, W.-C.; Murriel, C.; Evans, J.W.; O'Young, G.; Brunner, A.L.; Wang, M. RSPO3 antagonism inhibits growth and tumorigenicity in colorectal tumors harboring common Wnt pathway mutations. *Sci. Rep.* **2017**, *7*, 1–9. [CrossRef]
96. Chen, Z.; He, X.; Jia, M.; Liu, Y.; Qu, D.; Wu, D.; Wu, P.; Ni, C.; Zhang, Z.; Ye, J. β-catenin overexpression in the nucleus predicts progress disease and unfavourable survival in colorectal cancer: A meta-analysis. *PLoS ONE* **2013**, *8*, e63854. [CrossRef]
97. Grady, W.M.; Markowitz, S.D. Genetic and epigenetic alterations in colon cancer. *Annu. Rev. Genom. Hum. Genet.* **2002**, *3*, 101–128. [CrossRef]
98. Armaghany, T.; Wilson, J.D.; Chu, Q.; Mills, G. Genetic alterations in colorectal cancer. *Gastrointest. Cancer Res.* **2012**, *5*, 19.
99. Lynch, H.T.; Boland, C.R.; Gong, G.; Shaw, T.G.; Lynch, P.M.; Fodde, R.; Lynch, J.F.; de la Chapelle, A. Phenotypic and genotypic heterogeneity in the Lynch syndrome: Diagnostic, surveillance and management implications. *Eur. J. Hum. Genet.* **2006**, *14*, 390–402. [CrossRef]
100. Toyota, M.; Ahuja, N.; Ohe-Toyota, M.; Herman, J.G.; Baylin, S.B.; Issa, J.-P.J. CpG island methylator phenotype in colorectal cancer. *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 8681–8686. [CrossRef]
101. Weisenberger, D.J.; Siegmund, K.D.; Campan, M.; Young, J.; Long, T.I.; Faasse, M.A.; Kang, G.H.; Widschwendter, M.; Weener, D.; Buchanan, D. CpG island methylator phenotype underlies sporadic microsatellite instability and is tightly associated with BRAF mutation in colorectal cancer. *Nat. Genet.* **2006**, *38*, 787–793. [CrossRef]
102. Advani, S.M.; Advani, P.; DeSantis, S.M.; Brown, D.; VonVille, H.M.; Lam, M.; Loree, J.M.; Sarshekeh, A.M.; Bressler, J.; Lopez, D.S. Clinical, pathological, and molecular characteristics of CpG island methylator phenotype in colorectal cancer: A systematic review and meta-analysis. *Transl. Oncol.* **2018**, *11*, 1188–1201. [CrossRef]
103. Bosetti, C.; Santucci, C.; Gallus, S.; Martinetti, M.; La Vecchia, C. Aspirin and the risk of colorectal and other digestive tract cancers: An updated meta-analysis through 2019. *Ann. Oncol.* **2020**, *31*, 558–568. [CrossRef] [PubMed]
104. Cao, Y.; Nishihara, R.; Qian, Z.R.; Song, M.; Mima, K.; Inamura, K.; Nowak, J.A.; Drew, D.A.; Lochhead, P.; Nosho, K. Regular aspirin use associates with lower risk of colorectal cancers with low numbers of tumor-infiltrating lymphocytes. *Gastroenterology* **2016**, *151*, 879–892.e874. [CrossRef] [PubMed]
105. Chan, A.T.; Ogino, S.; Fuchs, C.S. Aspirin and the risk of colorectal cancer in relation to the expression of COX-2. *N. Engl. J. Med.* **2007**, *356*, 2131–2142. [CrossRef] [PubMed]
106. Rothwell, P.M.; Wilson, M.; Elwin, C.-E.; Norrving, B.; Algra, A.; Warlow, C.P.; Meade, T.W. Long-term effect of aspirin on colorectal cancer incidence and mortality: 20-year follow-up of five randomised trials. *Lancet* **2010**, *376*, 1741–1750. [CrossRef] [PubMed]
107. Tomić, T.; Domínguez-López, S.; Barrios-Rodríguez, R. Non-aspirin non-steroidal anti-inflammatory drugs in prevention of colorectal cancer in people aged 40 or older: A systematic review and meta-analysis. *Cancer Epidemiol.* **2019**, *58*, 52–62. [CrossRef]
108. Meyskens, F.L.; McLaren, C.E.; Pelot, D.; Fujikawa-Brooks, S.; Carpenter, P.M.; Hawk, E.; Kelloff, G.; Lawson, M.J.; Kidao, J.; McCracken, J. Difluoromethylornithine plus sulindac for the prevention of sporadic colorectal adenomas: A randomized placebo-controlled, double-blind trial. *Cancer Prev. Res.* **2008**, *1*, 32–38. [CrossRef]
109. Thomasset, S.; Berry, D.P.; Cai, H.; West, K.; Marczylo, T.H.; Marsden, D.; Brown, K.; Dennison, A.; Garcea, G.; Miller, A. Pilot study of oral anthocyanins for colorectal cancer chemoprevention. *Cancer Prev. Res.* **2009**, *2*, 625–633. [CrossRef]
110. Sinicrope, F.A.; Velamala, P.R.; Song, L.M.W.K.; Viggiano, T.R.; Bruining, D.H.; Rajan, E.; Gostout, C.J.; Kraichely, R.E.; Buttar, N.S.; Schroeder, K.W. Efficacy of difluoromethylornithine and aspirin for treatment of adenomas and aberrant crypt foci in patients with prior advanced colorectal neoplasms. *Cancer Prev. Res.* **2019**, *12*, 821–830. [CrossRef]
111. Samadder, N.J.; Kuwada, S.K.; Boucher, K.M.; Byrne, K.; Kanth, P.; Samowitz, W.; Jones, D.; Tavtigian, S.V.; Westover, M.; Berry, T. Association of sulindac and erlotinib vs placebo with colorectal neoplasia in familial adenomatous polyposis: Secondary analysis of a randomized clinical trial. *JAMA Oncol.* **2018**, *4*, 671–677. [CrossRef] [PubMed]
112. Veettil, S.K.; Nathisuwan, S.; Ching, S.M.; Jinatongthai, P.; Lim, K.G.; Kew, S.T.; Chaiyakunapruk, N. Efficacy and safety of celecoxib on the incidence of recurrent colorectal adenomas: A systematic review and meta-analysis. *Cancer Manag. Res.* **2019**, *11*, 561. [CrossRef] [PubMed]
113. Rodríguez-Miguel, A.; García-Rodríguez, L.A.; Gil, M.; Montoya, H.; Rodríguez-Martín, S.; de Abajo, F.J. Clopidogrel and low-dose aspirin, alone or together, reduce risk of colorectal cancer. *Clin. Gastroenterol. Hepatol.* **2019**, *17*, 2024–2033.e2022. [CrossRef] [PubMed]
114. Ng, C.-A.W.; Jiang, A.A.; Toh, E.M.S.; Ng, C.H.; Ong, Z.H.; Peng, S.; Tham, H.Y.; Sundar, R.; Chong, C.S.; Khoo, C.M. Metformin and colorectal cancer: A systematic review, meta-analysis and meta-regression. *Int. J. Color. Dis.* **2020**, *35*, 1501–1512. [CrossRef]
115. Huang, W.-K.; Hsu, H.-C.; Liu, J.-R.; Yang, T.-S.; Chen, J.-S.; Chang, J.W.-C.; Lin, Y.-C.; Yu, K.-H.; Kuo, C.-F.; See, L.-C. The association of ursodeoxycholic acid use with colorectal cancer risk: A nationwide cohort study. *Medicine* **2016**, *95*, e2980. [CrossRef]
116. Li, Y.; He, X.; Ding, Y.e.; Chen, H.; Sun, L. Statin uses and mortality in colorectal cancer patients: An updated systematic review and meta-analysis. *Cancer Med.* **2019**, *8*, 3305–3313. [CrossRef]
117. Barbalata, C.I.; Tefas, L.R.; Achim, M.; Tomuta, I.; Porfire, A.S. Statins in risk-reduction and treatment of cancer. *World J. Clin. Oncol.* **2020**, *11*, 573. [CrossRef]

118. Botteri, E.; Støer, N.C.; Sakshaug, S.; Graff-Iversen, S.; Vangen, S.; Hofvind, S.; De Lange, T.; Bagnardi, V.; Ursin, G.; Weiderpass, E. Menopausal hormone therapy and colorectal cancer: A linkage between nationwide registries in Norway. *BMJ Open* **2017**, *7*, e017639. [CrossRef]
119. Li, Y.-Y.; Gao, L.-J.; Zhang, Y.-X.; Liu, S.-J.; Cheng, S.; Liu, Y.-P.; Jia, C.-X. Bisphosphonates and risk of cancers: A systematic review and meta-analysis. *Br. J. Cancer* **2020**, *123*, 1570–1581. [CrossRef]
120. Eberhart, C.E.; Coffey, R.J.; Radhika, A.; Giardiello, F.M.; Ferrenbach, S.; Dubois, R.N. Up-regulation of cyclooxygenase 2 gene expression in human colorectal adenomas and adenocarcinomas. *Gastroenterology* **1994**, *107*, 1183–1188. [CrossRef]
121. Oshima, M.; Dinchuk, J.E.; Kargman, S.L.; Oshima, H.; Hancock, B.; Kwong, E.; Trzaskos, J.M.; Evans, J.F.; Taketo, M.M. Suppression of intestinal polyposis in ApcΔ716 knockout mice by inhibition of cyclooxygenase 2 (COX-2). *Cell* **1996**, *87*, 803–809. [CrossRef] [PubMed]
122. Castellone, M.D.; Teramoto, H.; Williams, B.O.; Druey, K.M.; Gutkind, J.S. Prostaglandin E2 promotes colon cancer cell growth through a Gs-axin-ß-catenin signaling axis. *Science* **2005**, *310*, 1504–1510. [CrossRef] [PubMed]
123. Wang, D.; DuBois, R.N. The role of COX-2 in intestinal inflammation and colorectal cancer. *Oncogene* **2010**, *29*, 781–788. [CrossRef]
124. Masferrer, J.L.; Leahy, K.M.; Koki, A.T.; Zweifel, B.S.; Settle, S.L.; Woerner, B.M.; Edwards, D.A.; Flickinger, A.G.; Moore, R.J.; Seibert, K. Antiangiogenic and antitumor activities of cyclooxygenase-2 inhibitors. *Cancer Res.* **2000**, *60*, 1306–1311. [PubMed]
125. Seno, H.; Oshima, M.; Ishikawa, T.-O.; Oshima, H.; Takaku, K.; Chiba, T.; Narumiya, S.; Taketo, M.M. Cyclooxygenase 2-and prostaglandin E2 receptor EP2-dependent angiogenesis in ApcΔ716 mouse intestinal polyps. *Cancer Res.* **2002**, *62*, 506–511. [PubMed]
126. Nan, H.; Hutter, C.M.; Lin, Y.; Jacobs, E.J.; Ulrich, C.M.; White, E.; Baron, J.A.; Berndt, S.I.; Brenner, H.; Butterbach, K. Association of aspirin and NSAID use with risk of colorectal cancer according to genetic variants. *JAMA* **2015**, *313*, 1133–1142. [CrossRef]
127. Arber, N.; Eagle, C.J.; Spicak, J.; Rácz, I.; Dite, P.; Hajer, J.; Zavoral, M.; Lechuga, M.J.; Gerletti, P.; Tang, J. Celecoxib for the prevention of colorectal adenomatous polyps. *N. Engl. J. Med.* **2006**, *355*, 885–895. [CrossRef]
128. Dehmer, S.P.; Maciosek, M.V.; Flottemesch, T.J.; LaFrance, A.B.; Whitlock, E.P. Aspirin for the primary prevention of cardiovascular disease and colorectal cancer: A decision analysis for the US Preventive Services Task Force. *Ann. Intern. Med.* **2016**, *164*, 777–786. [CrossRef]
129. Chubak, J.; Whitlock, E.P.; Williams, S.B.; Kamineni, A.; Burda, B.U.; Buist, D.S.; Anderson, M.L. Aspirin for the prevention of cancer incidence and mortality: Systematic evidence reviews for the US Preventive Services Task Force. *Ann. Intern. Med.* **2016**, *164*, 814–825. [CrossRef]
130. Katona, B.W.; Weiss, J.M. Chemoprevention of colorectal cancer. *Gastroenterology* **2020**, *158*, 368–388. [CrossRef]
131. Liu, F.; Yan, L.; Wang, Z.; Lu, Y.; Chu, Y.; Li, X.; Liu, Y.; Rui, D.; Nie, S.; Xiang, H. Metformin therapy and risk of colorectal adenomas and colorectal cancer in type 2 diabetes mellitus patients: A systematic review and meta-analysis. *Oncotarget* **2017**, *8*, 16017. [CrossRef] [PubMed]
132. Petrera, M.; Paleari, L.; Clavarezza, M.; Puntoni, M.; Caviglia, S.; Briata, I.M.; Oppezzi, M.; Mislej, E.M.; Stabuc, B.; Gnant, M. The ASAMET trial: A randomized, phase II, double-blind, placebo-controlled, multicenter, 2× 2 factorial biomarker study of tertiary prevention with low-dose aspirin and metformin in stage I-III colorectal cancer patients. *BMC Cancer* **2018**, *18*, 1–9. [CrossRef]
133. Chan, K.K.; Oza, A.M.; Siu, L.L. The statins as anticancer agents. *Clin. Cancer Res.* **2003**, *9*, 10–19. [PubMed]
134. Teraoka, N.; Mutoh, M.; Takasu, S.; Ueno, T.; Yamamoto, M.; Sugimura, T.; Wakabayashi, K. Inhibition of intestinal polyp formation by pitavastatin, a HMG-CoA reductase inhibitor. *Cancer Prev. Res.* **2011**, *4*, 445–453. [CrossRef] [PubMed]
135. Suh, N.; Reddy, B.S.; DeCastro, A.; Paul, S.; Lee, H.J.; Smolarek, A.K.; So, J.Y.; Simi, B.; Wang, C.X.; Janakiram, N.B. Combination of atorvastatin with sulindac or naproxen profoundly inhibits colonic adenocarcinomas by suppressing the p65/β-catenin/cyclin D1 signaling pathway in rats. *Cancer Prev. Res.* **2011**, *4*, 1895–1902. [CrossRef]
136. Malila, N.; Virtamo, J.; Virtanen, M.; Albanes, D.; Tangrea, J.A.; Huttunen, J.K. The effect of α-tocopherol and β-carotene supplementation on colorectal adenomas in middle-aged male smokers. *Cancer Epidemiol. Prev. Biomark.* **1999**, *8*, 489–493.
137. Papaioannou, D.; Cooper, K.; Carroll, C.; Hind, D.; Squires, H.; Tappenden, P.; Logan, R. Antioxidants in the chemoprevention of colorectal cancer and colorectal adenomas in the general population: A systematic review and meta-analysis. *Color. Dis.* **2011**, *13*, 1085–1099. [CrossRef]
138. Yusof, A.S.; Isa, Z.M.; Shah, S.A. Dietary patterns and risk of colorectal cancer: A systematic review of cohort studies (2000–2011). *Asian Pac. J. Cancer Prev.* **2012**, *13*, 4713–4717. [CrossRef]
139. Vanio, H.; Bianchini, F. Weight Control and Physical Activity. In *IARC Handbooks of Cancer Prevention*; IARC Press: Lyon, France, 2002.
140. He, X.; Wu, K.; Zhang, X.; Nishihara, R.; Cao, Y.; Fuchs, C.S.; Giovannucci, E.L.; Ogino, S.; Chan, A.T.; Song, M. Dietary intake of fiber, whole grains and risk of colorectal cancer: An updated analysis according to food sources, tumor location and molecular subtypes in two large US cohorts. *Int. J. Cancer* **2019**, *145*, 3040–3051. [CrossRef]
141. Brenner, H.; Chang–Claude, J.; Jansen, L.; Knebel, P.; Stock, C.; Hoffmeister, M. Reduced risk of colorectal cancer up to 10 years after screening, surveillance, or diagnostic colonoscopy. *Gastroenterology* **2014**, *146*, 709–717. [CrossRef]
142. Wells, K.O.; Hawkins, A.T.; Krishnamurthy, D.M.; Dharmarajan, S.; Glasgow, S.C.; Hunt, S.R.; Mutch, M.G.; Wise, P.; Silviera, M.L. Omission of adjuvant chemotherapy is associated with increased mortality in patients with T3N0 colon cancer with inadequate lymph node harvest. *Dis. Colon Rectum* **2017**, *60*, 15–21. [CrossRef] [PubMed]

143. Ribic, C.M.; Sargent, D.J.; Moore, M.J.; Thibodeau, S.N.; French, A.J.; Goldberg, R.M.; Hamilton, S.R.; Laurent-Puig, P.; Gryfe, R.; Shepherd, L.E. Tumor microsatellite-instability status as a predictor of benefit from fluorouracil-based adjuvant chemotherapy for colon cancer. *N. Engl. J. Med.* **2003**, *349*, 247–257. [CrossRef] [PubMed]
144. Sargent, D.J.; Marsoni, S.; Monges, G.; Thibodeau, S.N.; Labianca, R.; Hamilton, S.R.; French, A.J.; Kabat, B.; Foster, N.R.; Torri, V. Defective mismatch repair as a predictive marker for lack of efficacy of fluorouracil-based adjuvant therapy in colon cancer. *J. Clin. Oncol.* **2010**, *28*, 3219. [CrossRef]
145. Argilés, G.; Tabernero, J.; Labianca, R.; Hochhauser, D.; Salazar, R.; Iveson, T.; Laurent-Puig, P.; Quirke, P.; Yoshino, T.; Taieb, J. Localised colon cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann. Oncol.* **2020**, *31*, 1291–1305. [CrossRef] [PubMed]
146. Angell, H.K.; Bruni, D.; Barrett, J.C.; Herbst, R.; Galon, J. The immunoscore: Colon cancer and beyond. *Clin. Cancer Res.* **2020**, *26*, 332–339. [CrossRef] [PubMed]
147. Xie, Y.-H.; Chen, Y.-X.; Fang, J.-Y. Comprehensive review of targeted therapy for colorectal cancer. *Signal Transduct. Target. Ther.* **2020**, *5*, 1–30.
148. Falcone, A.; Ricci, S.; Brunetti, I.; Pfanner, E.; Allegrini, G.; Barbara, C.; Crinò, L.; Benedetti, G.; Evangelista, W.; Fanchini, L. Phase III trial of infusional fluorouracil, leucovorin, oxaliplatin, and irinotecan (FOLFOXIRI) compared with infusional fluorouracil, leucovorin, and irinotecan (FOLFIRI) as first-line treatment for metastatic colorectal cancer: The Gruppo Oncologico Nord Ovest. *J. Clin. Oncol.* **2007**, *25*, 1670–1676.
149. Souglakos, J.; Androulakis, N.; Syrigos, K.; Polyzos, A.; Ziras, N.; Athanasiadis, A.; Kakolyris, S.; Tsousis, S.; Kouroussis, C.; Vamvakas, L. FOLFOXIRI (folinic acid, 5-fluorouracil, oxaliplatin and irinotecan) vs FOLFIRI (folinic acid, 5-fluorouracil and irinotecan) as first-line treatment in metastatic colorectal cancer (MCC): A multicentre randomised phase III trial from the Hellenic Oncology Research Group (HORG). *Br. J. Cancer* **2006**, *94*, 798–805.
150. Cunningham, D.; Humblet, Y.; Siena, S.; Khayat, D.; Bleiberg, H.; Santoro, A.; Bets, D.; Mueser, M.; Harstrick, A.; Verslype, C. Cetuximab monotherapy and cetuximab plus irinotecan in irinotecan-refractory metastatic colorectal cancer. *N. Engl. J. Med.* **2004**, *351*, 337–345. [CrossRef]
151. Mendelsohn, J.; Prewett, M.; Rockwell, P.; Goldstein, N.I. CCR 20th anniversary commentary: A chimeric antibody, C225, inhibits EGFR activation and tumor growth. *Clin. Cancer Res.* **2015**, *21*, 227–229. [CrossRef]
152. Oliveira, A.F.; Bretes, L.; Furtado, I. Review of PD-1/PD-L1 inhibitors in metastatic dMMR/MSI-H colorectal cancer. *Front. Oncol.* **2019**, *9*, 396. [CrossRef] [PubMed]
153. Jonker, D.J.; O'Callaghan, C.J.; Karapetis, C.S.; Zalcberg, J.R.; Tu, D.; Au, H.-J.; Berry, S.R.; Krahn, M.; Price, T.; Simes, R.J. Cetuximab for the treatment of colorectal cancer. *N. Engl. J. Med.* **2007**, *357*, 2040–2048. [CrossRef] [PubMed]
154. Roskoski, R., Jr. The ErbB/HER family of protein-tyrosine kinases and cancer. *Pharmacol. Res.* **2014**, *79*, 34–74. [CrossRef]
155. Shibuya, M. Vascular endothelial growth factor (VEGF) and its receptor (VEGFR) signaling in angiogenesis: A crucial target for anti-and pro-angiogenic therapies. *Genes Cancer* **2011**, *2*, 1097–1105. [CrossRef]
156. Guba, M.; Seeliger, H.; Kleespies, A.; Jauch, K.-W.; Bruns, C. Vascular endothelial growth factor in colorectal cancer. *Int. J. Color. Dis.* **2004**, *19*, 510–517. [CrossRef] [PubMed]
157. Page, M.J.; McKenzie, J.E.; Bossuyt, P.M.; Boutron, I.; Hoffmann, T.C.; Mulrow, C.D.; Shamseer, L.; Tetzlaff, J.M.; Akl, E.A.; Brennan, S.E. The PRISMA 2020 statement: An updated guideline for reporting systematic reviews. *Syst. Rev.* **2021**, *88*, 105906.
158. De Paulo Farias, D.; de Araujo, F.F.; Neri-Numa, I.A.; Pastore, G.M. Antidiabetic potential of dietary polyphenols: A mechanistic review. *Food Res. Int.* **2021**, *145*, 110383. [CrossRef]
159. Tsimogiannis, D.; Oreopoulou, V. Classification of phenolic compounds in plants. *Polyphen. Plants (Second Ed.)* **2019**, 263–284. [CrossRef]
160. Vuolo, M.M.; Lima, V.S.; Junior, M.R.M. Phenolic compounds: Structure, classification, and antioxidant power. *Bioact. Compd.* **2019**, 33–50. [CrossRef]
161. Kiruthiga, C.; Devi, K.P.; Nabavi, S.M.; Bishayee, A. Autophagy: A potential therapeutic target of polyphenols in hepatocellular carcinoma. *Cancers* **2020**, *12*, 562. [CrossRef]
162. Darvesh, A.S.; Carroll, R.T.; Bishayee, A.; Geldenhuys, W.J.; Van der Schyf, C.J. Oxidative stress and Alzheimer's disease: Dietary polyphenols as potential therapeutic agents. *Expert Rev. Neurother.* **2010**, *10*, 729–745. [CrossRef]
163. Karimi, A.; Majlesi, M.; Rafieian-Kopaei, M. Herbal versus synthetic drugs; beliefs and facts. *J. Nephropharmacol.* **2015**, *4*, 27. [PubMed]
164. Samec, M.; Liskova, A.; Koklesova, L.; Samuel, S.M.; Zhai, K.; Buhrmann, C.; Varghese, E.; Abotaleb, M.; Qaradakhi, T.; Zulli, A. Flavonoids against the Warburg phenotype—Concepts of predictive, preventive and personalised medicine to cut the Gordian knot of cancer cell metabolism. *Epma J.* **2020**, *11*, 377–398. [CrossRef] [PubMed]
165. Jain, A.; Madu, C.O.; Lu, Y. Phytochemicals in chemoprevention: A cost-effective complementary approach. *J. Cancer* **2021**, *12*, 3686. [CrossRef] [PubMed]
166. Rajamanickam, S.; Agarwal, R. Natural products and colon cancer: Current status and future prospects. *Drug Dev. Res.* **2008**, *69*, 460–471. [CrossRef]
167. Bishayee, A.; Sethi, G. Bioactive natural products in cancer prevention and therapy: Progress and promise. *Semin. Cancer Biol.* **2016**, *40*, 1–3. [CrossRef]

168. Huang, X.-M.; Yang, Z.-J.; Xie, Q.; Zhang, Z.-K.; Zhang, H.; Ma, J.-Y. Natural products for treating colorectal cancer: A mechanistic review. *Biomed. Pharmacother.* **2019**, *117*, 109142. [CrossRef]
169. Atanasov, A.G.; Zotchev, S.B.; Dirsch, V.M.; Supuran, C.T. Natural products in drug discovery: Advances and opportunities. *Nat. Rev. Drug Discov.* **2021**, *20*, 200–216. [CrossRef]
170. Lin, C.-L.; Jeng, J.-H.; Wu, C.-C.; Hsieh, S.-L.; Huang, G.-C.; Leung, W.; Lee, C.-T.; Chen, C.-Y.; Lee, C.-H. Chemopreventive potential of 2, 3, 5, 4′-tetrahydroxystilbene-2-O-β-D-glucoside on The formation of aberrant crypt foci in azoxymethane-induced colorectal cancer in rats. *BioMed Res. Int.* **2017**, *2017*, 3634915. [CrossRef]
171. Chen, Q.; Lei, J.; Zhou, J.; Ma, S.; Huang, Q.; Ge, B. Chemopreventive effect of 4′-hydroxychalcone on intestinal tumorigenesis in Apc Min mice Corrigendum in/10.3892/ol. 2021.12741. *Oncol. Lett.* **2021**, *21*, 213. [CrossRef]
172. Lai, C.-Y.; Tsai, A.-C.; Chen, M.-C.; Chang, L.-H.; Sun, H.-L.; Chang, Y.-L.; Chen, C.-C.; Teng, C.-M.; Pan, S.-L. Aciculatin induces p53-dependent apoptosis via MDM2 depletion in human cancer cells in vitro and in vivo. *PLoS ONE* **2012**, *7*, e42192. [CrossRef]
173. Au, A.; Li, B.; Wang, W.; Roy, H.; Koehler, K.; Birt, D. Effect of dietary apigenin on colonic ornithine decarboxylase activity, aberrant crypt foci formation, and tumorigenesis in different experimental models. *Nutr. Cancer* **2006**, *54*, 243–251. [CrossRef]
174. Wang, Q.R.; Yao, X.Q.; Wen, G.; Fan, Q.; Li, Y.-J.; Fu, X.Q.; Li, C.K.; Sun, X.G. Apigenin suppresses the growth of colorectal cancer xenografts via phosphorylation and up-regulated FADD expression. *Oncol. Lett.* **2011**, *2*, 43–47. [CrossRef] [PubMed]
175. Chunhua, L.; Donglan, L.; Xiuqiong, F.; Lihua, Z.; Qin, F.; Yawei, L.; Liang, Z.; Ge, W.; Linlin, J.; Ping, Z. Apigenin up-regulates transgelin and inhibits invasion and migration of colorectal cancer through decreased phosphorylation of AKT. *J. Nutr. Biochem.* **2013**, *24*, 1766–1775. [CrossRef]
176. Zhong, Y.; Krisanapun, C.; Lee, S.-H.; Nualsanit, T.; Sams, C.; Peungvicha, P.; Baek, S.J. Molecular targets of apigenin in colorectal cancer cells: Involvement of p21, NAG-1 and p53. *Eur. J. Cancer* **2010**, *46*, 3365–3374. [CrossRef]
177. Chen, X.; Xu, H.; Yu, X.; Wang, X.; Zhu, X.; Xu, X. Apigenin inhibits in vitro and in vivo tumorigenesis in cisplatin-resistant colon cancer cells by inducing autophagy, programmed cell death and targeting m-TOR/PI3K/Akt signalling pathway. *J. Buon* **2019**, *24*, 488–493. [PubMed]
178. Shao, H.; Jing, K.; Mahmoud, E.; Huang, H.; Fang, X.; Yu, C. Apigenin sensitizes colon cancer cells to antitumor activity of ABT-263. *Mol. Cancer Ther.* **2013**, *12*, 2640–2650. [CrossRef] [PubMed]
179. Dai, J.; Van Wie, P.G.; Fai, L.Y.; Kim, D.; Wang, L.; Poyil, P.; Luo, J.; Zhang, Z. Downregulation of NEDD9 by apigenin suppresses migration, invasion, and metastasis of colorectal cancer cells. *Toxicol. Appl. Pharmacol.* **2016**, *311*, 106–112. [CrossRef]
180. Kim, D.H.; Hossain, M.A.; Kang, Y.J.; Jang, J.Y.; Lee, Y.J.; Im, E.; Yoon, J.-H.; Kim, H.S.; Chung, H.Y.; Kim, N.D. Baicalein, an active component of Scutellaria baicalensis Georgi, induces apoptosis in human colon cancer cells and prevents AOM/DSS-induced colon cancer in mice. *Int. J. Oncol.* **2013**, *43*, 1652–1658. [CrossRef]
181. Chen, W.-C.; Kuo, T.-H.; Tzeng, Y.-S.; Tsai, Y.-C. Baicalin induces apoptosis in SW620 human colorectal carcinoma cells in vitro and suppresses tumor growth in vivo. *Molecules* **2012**, *17*, 3844–3857. [CrossRef]
182. Song, L.; Zhu, S.; Liu, C.; Zhang, Q.; Liang, X. Baicalin triggers apoptosis, inhibits migration, and enhances anti-tumor immunity in colorectal cancer via TLR4/NF-κB signaling pathway. *J. Food Biochem.* **2022**, *46*, e13703. [CrossRef]
183. Kim, S.-J.; Kim, H.-J.; Kim, H.-R.; Lee, S.-H.; Cho, S.-D.; Choi, C.-S.; Nam, J.-S.; Jung, J.-Y. Antitumor actions of baicalein and wogonin in HT-29 human colorectal cancer cells. *Mol. Med. Rep.* **2012**, *6*, 1443–1449. [CrossRef] [PubMed]
184. Chai, Y.; Xu, J.; Yan, B. The anti-metastatic effect of baicalein on colorectal cancer. *Oncol. Rep.* **2017**, *37*, 2317–2323. [CrossRef] [PubMed]
185. Dou, J.; Wang, Z.; Ma, L.; Peng, B.; Mao, K.; Li, C.; Su, M.; Zhou, C.; Peng, G. Baicalein and baicalin inhibit colon cancer using two distinct fashions of apoptosis and senescence. *Oncotarget* **2018**, *9*, 20089. [CrossRef] [PubMed]
186. Wang, Z.; Ma, L.; Su, M.; Zhou, Y.; Mao, K.; Li, C.; Peng, G.; Zhou, C.; Shen, B.; Dou, J. Baicalin induces cellular senescence in human colon cancer cells via upregulation of DEPP and the activation of Ras/Raf/MEK/ERK signaling. *Cell Death Dis.* **2018**, *9*, 217. [CrossRef] [PubMed]
187. Tao, Y.; Zhan, S.; Wang, Y.; Zhou, G.; Liang, H.; Chen, X.; Shen, H. Baicalin, the major component of traditional Chinese medicine Scutellaria baicalensis induces colon cancer cell apoptosis through inhibition of oncomiRNAs. *Sci. Rep.* **2018**, *8*, 14477. [CrossRef] [PubMed]
188. Yang, B.; Bai, H.; Sa, Y.; Zhu, P.; Liu, P. Inhibiting EMT, stemness and cell cycle involved in baicalin-induced growth inhibition and apoptosis in colorectal cancer cells. *J. Cancer* **2020**, *11*, 2303. [CrossRef]
189. Wang, C.-Z.; Zhang, C.-F.; Luo, Y.; Yao, H.; Yu, C.; Chen, L.; Yuan, J.; Huang, W.-H.; Wan, J.-Y.; Zeng, J. Baicalein, an enteric microbial metabolite, suppresses gut inflammation and cancer progression in Apc Min/+ mice. *Clin. Transl. Oncol.* **2020**, *22*, 1013–1022. [CrossRef] [PubMed]
190. Zhang, W.; Liu, Q.; Luo, L.; Song, J.; Han, K.; Liu, R.; Gong, Y.; Guo, X. Use Chou's 5-steps rule to study how Baicalin suppresses the malignant phenotypes and induces the apoptosis of colorectal cancer cells. *Arch. Biochem. Biophys.* **2021**, *705*, 108919. [CrossRef]
191. Zhou, C.; Ou, W.; Xu, Q.; Lin, L.; Xu, F.; Chen, R.; Miao, H. Chemoprotective effect of boeravinone B against 1, 2-dimethyl hydrazine induced colorectal cancer in rats via suppression of oxidative stress and inflammatory reaction. *J. Gastrointest. Oncol.* **2022**, *13*, 1832–1841. [CrossRef]
192. Miyamoto, S.; Kohno, H.; Suzuki, R.; Sugie, S.; Murakami, A.; Ohigashi, H.; Tanaka, T. Preventive effects of chrysin on the development of azoxymethane-induced colonic aberrant crypt foci in rats. *Oncol. Rep.* **2006**, *15*, 1169–1173. [CrossRef]

193. Salama, A.A.; Allam, R.M. Promising targets of chrysin and daidzein in colorectal cancer: Amphiregulin, CXCL1, and MMP-9. *Eur. J. Pharmacol.* **2021**, *892*, 173763. [CrossRef] [PubMed]
194. Villegas, I.; Sánchez-Fidalgo, S.; de la Lastra, C.A. Chemopreventive effect of dietary curcumin on inflammation-induced colorectal carcinogenesis in mice. *Mol. Nutr. Food Res.* **2011**, *55*, 259–267. [CrossRef]
195. Milacic, V.; Banerjee, S.; Landis-Piwowar, K.R.; Sarkar, F.H.; Majumdar, A.P.; Dou, Q.P. Curcumin inhibits the proteasome activity in human colon cancer cells in vitro and in vivo. *Cancer Res.* **2008**, *68*, 7283–7292. [CrossRef] [PubMed]
196. Hao, J.; Dai, X.; Gao, J.; Li, Y.; Hou, Z.; Chang, Z.; Wang, Y. Curcumin suppresses colorectal tumorigenesis via the Wnt/β-catenin signaling pathway by downregulating Axin2. *Oncol. Lett.* **2021**, *21*, 186. [CrossRef]
197. Kubota, M.; Shimizu, M.; Sakai, H.; Yasuda, Y.; Terakura, D.; Baba, A.; Ohno, T.; Tsurumi, H.; Tanaka, T.; Moriwaki, H. Preventive effects of curcumin on the development of azoxymethane-induced colonic preneoplastic lesions in male C57BL/KsJ-db/db obese mice. *Nutr. Cancer* **2012**, *64*, 72–79. [CrossRef] [PubMed]
198. Kunnumakkara, A.B.; Diagaradjane, P.; Guha, S.; Deorukhkar, A.; Shentu, S.; Aggarwal, B.B.; Krishnan, S. Curcumin sensitizes human colorectal cancer xenografts in nude mice to γ-radiation by targeting nuclear factor-κB–regulated gene products. *Clin. Cancer Res.* **2008**, *14*, 2128–2136. [CrossRef]
199. Li, L.; Ahmed, B.; Mehta, K.; Kurzrock, R. Liposomal curcumin with and without oxaliplatin: Effects on cell growth, apoptosis, and angiogenesis in colorectal cancer. *Mol. Cancer Ther.* **2007**, *6*, 1276–1282. [CrossRef]
200. McFadden, R.-M.T.; Larmonier, C.B.; Shehab, K.W.; Midura-Kiela, M.; Ramalingam, R.; Harrison, C.A.; Besselsen, D.G.; Chase, J.H.; Caporaso, J.G.; Jobin, C. The role of curcumin in modulating colonic microbiota during colitis and colon cancer prevention. *Inflamm. Bowel Dis.* **2015**, *21*, 2483–2494. [CrossRef]
201. Marjaneh, R.M.; Rahmani, F.; Hassanian, S.M.; Rezaei, N.; Hashemzehi, M.; Bahrami, A.; Ariakia, F.; Fiuji, H.; Sahebkar, A.; Avan, A. Phytosomal curcumin inhibits tumor growth in colitis-associated colorectal cancer. *J. Cell. Physiol.* **2018**, *233*, 6785–6798. [CrossRef]
202. Cooke, D.; Schwarz, M.; Boocock, D.; Winterhalter, P.; Steward, W.P.; Gescher, A.J.; Marczylo, T.H. Effect of cyanidin-3-glucoside and an anthocyanin mixture from bilberry on adenoma development in the ApcMin mouse model of intestinal carcinogenesis—Relationship with tissue anthocyanin levels. *Int. J. Cancer* **2006**, *119*, 2213–2220. [CrossRef]
203. Huang, C.-C.; Hung, C.-H.; Hung, T.-W.; Lin, Y.-C.; Wang, C.-J.; Kao, S.-H. Dietary delphinidin inhibits human colorectal cancer metastasis associating with upregulation of miR-204-3p and suppression of the integrin/FAK axis. *Sci. Rep.* **2019**, *9*, 18954. [CrossRef] [PubMed]
204. Koosha, S.; Mohamed, Z.; Sinniah, A.; Alshawsh, M.A. Evaluation of anti-tumorigenic effects of diosmetin against human colon cancer xenografts in athymic nude mice. *Molecules* **2019**, *24*, 2522. [CrossRef] [PubMed]
205. Shimizu, M.; Shirakami, Y.; Sakai, H.; Yasuda, Y.; Kubota, M.; Adachi, S.; Tsurumi, H.; Hara, Y.; Moriwaki, H. (−)-Epigallocatechin gallate inhibits growth and activation of the VEGF/VEGFR axis in human colorectal cancer cells. *Chem.-Biol. Interact.* **2010**, *185*, 247–252. [CrossRef] [PubMed]
206. Shimizu, M.; Shirakami, Y.; Sakai, H.; Adachi, S.; Hata, K.; Hirose, Y.; Tsurumi, H.; Tanaka, T.; Moriwaki, H. (−)-Epigallocatechin gallate suppresses azoxymethane-induced colonic premalignant lesions in male C57BL/KsJ-db/db mice. *Cancer Prev. Res.* **2008**, *1*, 298–304. [CrossRef]
207. Zhong, Y.; Chiou, Y.-S.; Pan, M.-H.; Ho, C.-T.; Shahidi, F. Protective effects of epigallocatechin gallate (EGCG) derivatives on azoxymethane-induced colonic carcinogenesis in mice. *J. Funct. Foods* **2012**, *4*, 323–330. [CrossRef]
208. Toden, S.; Tran, H.-M.; Tovar-Camargo, O.A.; Okugawa, Y.; Goel, A. Epigallocatechin-3-gallate targets cancer stem-like cells and enhances 5-fluorouracil chemosensitivity in colorectal cancer. *Oncotarget* **2016**, *7*, 16158. [CrossRef]
209. Xu, G.; Ren, G.; Xu, X.; Yuan, H.; Wang, Z.; Kang, L.; Yu, W.; Tian, K. Combination of curcumin and green tea catechins prevents dimethylhydrazine-induced colon carcinogenesis. *Food Chem. Toxicol.* **2010**, *48*, 390–395. [CrossRef]
210. Wang, Y.; Jin, H.-Y.; Fang, M.-Z.; Wang, X.-F.; Chen, H.; Huang, S.-L.; Kong, D.-S.; Li, M.; Zhang, X.; Sun, Y. Epigallocatechin gallate inhibits dimethylhydrazine-induced colorectal cancer in rats. *World J. Gastroenterol.* **2020**, *26*, 2064. [CrossRef]
211. Mariyappan, P.; Kalaiyarasu, T.; Manju, V. Effect of eriodictyol on preneoplastic lesions, oxidative stress and bacterial enzymes in 1, 2-dimethyl hydrazine-induced colon carcinogenesis. *Toxicol. Res.* **2017**, *6*, 678–692. [CrossRef]
212. Wang, N.; Zhou, F.; Guo, J.; Zhu, H.; Luo, S.; Cao, J. Euxanthone suppresses tumor growth and metastasis in colorectal cancer via targeting CIP2A/PP2A pathway. *Life Sci.* **2018**, *209*, 498–506. [CrossRef]
213. Kunchari Kalaimathi, S.; Sudhandiran, G. Fisetin ameolirates the azoxymethane and dextran sodium sulfate induced colitis associated colorectal cancer. *Int. J. Pharm. Clin. Res.* **2016**, *8*, 551–560.
214. Khan, N.; Jajeh, F.; Eberhardt, E.L.; Miller, D.D.; Albrecht, D.M.; Van Doorn, R.; Hruby, M.D.; Maresh, M.E.; Clipson, L.; Mukhtar, H. Fisetin and 5-fluorouracil: Effective combination for PIK3CA-mutant colorectal cancer. *Int. J. Cancer* **2019**, *145*, 3022–3032. [CrossRef]
215. Jeng, L.B.; Kumar Velmurugan, B.; Chen, M.C.; Hsu, H.H.; Ho, T.J.; Day, C.H.; Lin, Y.M.; Padma, V.V.; Tu, C.C.; Huang, C.Y. Fisetin mediated apoptotic cell death in parental and Oxaliplatin/irinotecan resistant colorectal cancer cells in vitro and in vivo. *J. Cell. Physiol.* **2018**, *233*, 7134–7142. [CrossRef] [PubMed]
216. Leu, J.D.; Wang, B.S.; Chiu, S.J.; Chan, C.Y.; Chen, C.C.; Chen, F.D.; Avirmed, S.; Lee, Y.J. Combining fisetin and ionizing radiation suppresses the growth of mammalian colorectal cancers in xenograft tumor models. *Oncol. Lett.* **2016**, *12*, 4975–4982. [CrossRef]

217. Chen, Y.; Wu, Q.; Song, L.; He, T.; Li, Y.; Li, L.; Su, W.; Liu, L.; Qian, Z.; Gong, C. Polymeric micelles encapsulating fisetin improve the therapeutic effect in colon cancer. *ACS Appl. Mater. Interfaces* **2015**, *7*, 534–542. [CrossRef]
218. Li, L.; Wang, M.; Yang, H.; Li, Y.; Huang, X.; Guo, J.; Liu, Z. Fisetin Inhibits Trypsin Activity and Suppresses the Growth of Colorectal Cancer in Vitro and in Vivo. *Nat. Prod. Commun.* **2022**, *17*, 1934578X221115511. [CrossRef]
219. Winkelmann, I.; Diehl, D.; Oesterle, D.; Daniel, H.; Wenzel, U. The suppression of aberrant crypt multiplicity in colonic tissue of 1, 2-dimethylhydrazine-treated C57BL/6J mice by dietary flavone is associated with an increased expression of Krebs cycle enzymes. *Carcinogenesis* **2007**, *28*, 1446–1454. [CrossRef]
220. Auyeung, K.K.-W.; Law, P.-C.; Ko, J.K.-S. Novel anti-angiogenic effects of formononetin in human colon cancer cells and tumor xenograft. *Oncol. Rep.* **2012**, *28*, 2188–2194. [CrossRef]
221. Ma, Z.; Bao, X.; Gu, J. Furowanin A-induced autophagy alleviates apoptosis and promotes cell cycle arrest via inactivation STAT3/Mcl-1 axis in colorectal cancer. *Life Sci.* **2019**, *218*, 47–57. [CrossRef]
222. Sekar, V.; Anandasadagopan, S.K.; Ganapasam, S. Genistein regulates tumor microenvironment and exhibits anticancer effect in dimethyl hydrazine-induced experimental colon carcinogenesis. *Biofactors* **2016**, *42*, 623–637. [CrossRef]
223. Zhang, Y.; Li, Q.; Zhou, D.; Chen, H. Genistein, a soya isoflavone, prevents azoxymethane-induced up-regulation of WNT/β-catenin signalling and reduces colon pre-neoplasia in rats. *Br. J. Nutr.* **2013**, *109*, 33–42. [CrossRef] [PubMed]
224. Xiao, X.; Liu, Z.; Wang, R.; Wang, J.; Zhang, S.; Cai, X.; Wu, K.; Bergan, R.C.; Xu, L.; Fan, D. Genistein suppresses FLT4 and inhibits human colorectal cancer metastasis. *Oncotarget* **2015**, *6*, 3225. [CrossRef] [PubMed]
225. Wang, X.; Song, Z.-J.; He, X.; Zhang, R.-Q.; Zhang, C.-F.; Li, F.; Wang, C.-Z.; Yuan, C.-S. Antitumor and immunomodulatory activity of genkwanin on colorectal cancer in the APCMin/+ mice. *Int. Immunopharmacol.* **2015**, *29*, 701–707. [CrossRef] [PubMed]
226. Yin, H.-F.; Yin, C.-M.; Ouyang, T.; Sun, S.-D.; Chen, W.-G.; Yang, X.-L.; He, X.; Zhang, C.-F. Self-Nanoemulsifying Drug Delivery System of Genkwanin: A Novel Approach for Anti-Colitis-Associated Colorectal Cancer. *Drug Des. Dev. Ther.* **2021**, *15*, 557. [CrossRef] [PubMed]
227. Saiprasad, G.; Chitra, P.; Manikandan, R.; Sudhandiran, G. Hesperidin alleviates oxidative stress and downregulates the expressions of proliferative and inflammatory markers in azoxymethane-induced experimental colon carcinogenesis in mice. *Inflamm. Res.* **2013**, *62*, 425–440. [CrossRef]
228. Saiprasad, G.; Chitra, P.; Manikandan, R.; Sudhandiran, G. Hesperidin induces apoptosis and triggers autophagic markers through inhibition of Aurora-A mediated phosphoinositide-3-kinase/Akt/mammalian target of rapamycin and glycogen synthase kinase-3 beta signalling cascades in experimental colon carcinogenesis. *Eur. J. Cancer* **2014**, *50*, 2489–2507. [CrossRef]
229. Tanaka, T.; Makita, H.; Kawabata, K.; Mori, H.; Kakumoto, M.; Satoh, K.; Hara, A.; Sumida, T.; Tanaka, T.; Ogawa, H. Chemoprevention of azoxymethane-induced rat colon carcinogenesis by the naturally occurring flavonoids, diosmin and hesperidin. *Carcinogenesis* **1997**, *18*, 957–965. [CrossRef]
230. El-Deek, S.E.; Abd-Elghaffar, S.K.; Hna, R.S.; Mohamed, H.G.; El-Deek, H.E. Effect of hesperidin against induced colon cancer in rats: Impact of Smad4 and activin a signaling pathway. *Nutr. Cancer* **2022**, *74*, 697–714. [CrossRef]
231. Zhou, J.; Zhao, R.; Ye, T.; Yang, S.; Li, Y.; Yang, F.; Wang, G.; Xie, Y.; Li, Q. Antitumor activity in colorectal cancer induced by hinokiflavone. *J. Gastroenterol. Hepatol.* **2019**, *34*, 1571–1580. [CrossRef]
232. Shi, C.-J.; Li, S.-Y.; Shen, C.-H.; Pan, F.-F.; Deng, L.-Q.; Fu, W.-M.; Wang, J.-Y.; Zhang, J.-F. Icariside II suppressed tumorigenesis by epigenetically regulating the circβ-catenin-Wnt/β-catenin axis in colorectal cancer. *Bioorganic Chem.* **2022**, *124*, 105800. [CrossRef]
233. Zhou, C.; Gu, J.; Zhang, G.; Dong, D.; Yang, Q.; Chen, M.-B.; Xu, D. AMPK-autophagy inhibition sensitizes icaritin-induced anti-colorectal cancer cell activity. *Oncotarget* **2017**, *8*, 14736. [CrossRef] [PubMed]
234. Tang, S.; Cai, S.; Ji, S.; Yan, X.; Zhang, W.; Qiao, X.; Zhang, H.; Ye, M.; Yu, S. Isoangustone A induces autophagic cell death in colorectal cancer cells by activating AMPK signaling. *Fitoterapia* **2021**, *152*, 104935. [CrossRef] [PubMed]
235. Zhao, H.; Zhang, X.; Chen, X.; Li, Y.; Ke, Z.; Tang, T.; Chai, H.; Guo, A.M.; Chen, H.; Yang, J. Isoliquiritigenin, a flavonoid from licorice, blocks M2 macrophage polarization in colitis-associated tumorigenesis through downregulating PGE2 and IL-6. *Toxicol. Appl. Pharmacol.* **2014**, *279*, 311–321. [CrossRef] [PubMed]
236. Takahashi, T.; Takasuka, N.; Iigo, M.; Baba, M.; Nishino, H.; Tsuda, H.; Okuyama, T. Isoliquiritigenin, a flavonoid from licorice, reduces prostaglandin E2 and nitric oxide, causes apoptosis, and suppresses aberrant crypt foci development. *Cancer Sci.* **2004**, *95*, 448–453. [CrossRef] [PubMed]
237. Antunes-Ricardo, M.; Guardado-Félix, D.; Rocha-Pizaña, M.; Garza-Martínez, J.; Acevedo-Pacheco, L.; Gutiérrez-Uribe, J.; Villela-Castrejón, J.; López-Pacheco, F.; Serna-Saldívar, S. Opuntia ficus-indica Extract and Isorhamnetin-3-O-Glucosyl-Rhamnoside Diminish Tumor Growth of Colon Cancer Cells Xenografted in Immune-Suppressed Mice through the Activation of Apoptosis Intrinsic Pathway. *Plant Foods Hum. Nutr.* **2021**, *76*, 434–441. [CrossRef]
238. Saud, S.M.; Young, M.R.; Jones-Hall, Y.L.; Ileva, L.; Evbuomwan, M.O.; Wise, J.; Colburn, N.H.; Kim, Y.S.; Bobe, G. Chemopreventive Activity of Plant Flavonoid Isorhamnetin in Colorectal Cancer Is Mediated by Oncogenic Src and β-CateninIsorhamnetin-Induced CSK Inhibits Colorectal Cancer. *Cancer Res.* **2013**, *73*, 5473–5484. [CrossRef]
239. Nirmala, P.; Ramanathan, M. Effect of kaempferol on lipid peroxidation and antioxidant status in 1, 2-dimethyl hydrazine induced colorectal carcinoma in rats. *Eur. J. Pharmacol.* **2011**, *654*, 75–79. [CrossRef]
240. Hassan, E.S.; Hassanein, N.M.; Ahmed, H.M.S. Probing the chemoprevention potential of the antidepressant fluoxetine combined with epigallocatechin gallate or kaempferol in rats with induced early stage colon carcinogenesis. *J. Pharmacol. Sci.* **2021**, *145*, 29–41. [CrossRef]

241. Hassanein, N.M.; Hassan, E.S.; Hegab, A.M.; Elahl, H.M.S. Chemopreventive effect of sulindac in combination with epigallocatechin gallate or kaempferol against 1, 2-dimethyl hydrazine-induced preneoplastic lesions in rats: A comparative study. *J. Biochem. Mol. Toxicol.* **2018**, *32*, e22198. [CrossRef]
242. Osman, N.H.; Said, U.Z.; El-Waseef, A.M.; Ahmed, E.S. Luteolin supplementation adjacent to aspirin treatment reduced dimethylhydrazine-induced experimental colon carcinogenesis in rats. *Tumor Biol.* **2015**, *36*, 1179–1190. [CrossRef]
243. Pandurangan, A.K.; Kumar, S.A.S.; Dharmalingam, P.; Ganapasam, S. Luteolin, a bioflavonoid inhibits azoxymethane-induced colon carcinogenesis: Involvement of iNOS and COX-2. *Pharmacogn. Mag.* **2014**, *10*, S306. [PubMed]
244. Pandurangan, A.K.; Ananda Sadagopan, S.K.; Dharmalingam, P.; Ganapasam, S. Luteolin, a bioflavonoid inhibits Azoxymethane-induced colorectal cancer through activation of Nrf2 signaling. *Toxicol. Mech. Methods* **2014**, *24*, 13–20. [CrossRef] [PubMed]
245. Kim, H.Y.; Jung, S.K.; Byun, S.; Son, J.E.; Oh, M.H.; Lee, J.; Kang, M.J.; Heo, Y.S.; Lee, K.W.; Lee, H.J. Raf and PI3K are the molecular targets for the anti-metastatic effect of Luteolin. *Phytother. Res.* **2013**, *27*, 1481–1488. [CrossRef] [PubMed]
246. Pandurangan, A.; Dharmalingam, P.; Sadagopan, S.; Ganapasam, S. Luteolin inhibits matrix metalloproteinase 9 and 2 in azoxymethane-induced colon carcinogenesis. *Hum. Exp. Toxicol.* **2014**, *33*, 1176–1185. [CrossRef]
247. Yao, Y.; Rao, C.; Zheng, G.; Wang, S. Luteolin suppresses colorectal cancer cell metastasis via regulation of the miR-384/pleiotrophin axis. *Oncol. Rep.* **2019**, *42*, 131–141. [CrossRef]
248. Xiao, B.; Qin, Y.; Ying, C.; Ma, B.; Wang, B.; Long, F.; Wang, R.; Fang, L.; Wang, Y. Combination of oncolytic adenovirus and luteolin exerts synergistic antitumor effects in colorectal cancer cells and a mouse model. *Mol. Med. Rep.* **2017**, *16*, 9375–9382. [CrossRef] [PubMed]
249. Gao, Z.; Jiang, J.; Hou, L.; Ji, F. Lysionotin Induces Ferroptosis to Suppress Development of Colorectal Cancer via Promoting Nrf2 Degradation. *Oxidative Med. Cell. Longev.* **2022**, *2022*, 1366957. [CrossRef]
250. Yu, H.; Yin, S.; Zhou, S.; Shao, Y.; Sun, J.; Pang, X.; Han, L.; Zhang, Y.; Gao, X.; Jin, C. Magnolin promotes autophagy and cell cycle arrest via blocking LIF/Stat3/Mcl-1 axis in human colorectal cancers. *Cell Death Dis.* **2018**, *9*, 702. [CrossRef]
251. Chen, R.; Zhang, L. Morin inhibits colorectal tumor growth through inhibition of NF-κB signaling pathway. *Immunopharmacol. Immunotoxicol.* **2019**, *41*, 622–629. [CrossRef]
252. Sharma, S.H.; Thulasingam, S.; Chellappan, D.R.; Chinnaswamy, P.; Nagarajan, S. Morin and Esculetin supplementation modulates c-myc induced energy metabolism and attenuates neoplastic changes in rats challenged with the procarcinogen 1, 2-dimethylhydrazine. *Eur. J. Pharmacol.* **2017**, *796*, 20–31. [CrossRef]
253. Lori, G.; Paoli, P.; Femia, A.P.; Pranzini, E.; Caselli, A.; Tortora, K.; Romagnoli, A.; Raugei, G.; Caderni, G. Morin-dependent inhibition of low molecular weight protein tyrosine phosphatase (LMW-PTP) restores sensitivity to apoptosis during colon carcinogenesis: Studies in vitro and in vivo, in an Apc-driven model of colon cancer. *Mol. Carcinog.* **2019**, *58*, 686–698. [CrossRef] [PubMed]
254. Vennila, S.; Nalini, N. Modifying effects of morin on the development of aberrant crypt foci and bacterial enzymes in experimental colon cancer. *Food Chem. Toxicol.* **2009**, *47*, 309–315.
255. Sharma, S.H.; Kumar, J.S.; Chellappan, D.R.; Nagarajan, S. Molecular chemoprevention by morin–A plant flavonoid that targets nuclear factor kappa B in experimental colon cancer. *Biomed. Pharmacother.* **2018**, *100*, 367–373. [CrossRef]
256. Sreedharan, V.; Venkatachalam, K.K.; Namasivayam, N. Effect of morin on tissue lipid peroxidation and antioxidant status in 1, 2-dimethylhydrazine induced experimental colon carcinogenesis. *Investig. New Drugs* **2009**, *27*, 21–30. [CrossRef] [PubMed]
257. Nirmala, P.; Ramanathan, M. Effect of myricetin on 1, 2 dimethylhydrazine induced rat colon carcinogenesis. *J. Exp. Ther. Oncol.* **2011**, *9*, 101–108.
258. Li, Y.; Cui, S.-X.; Sun, S.-Y.; Shi, W.-N.; Song, Z.-Y.; Wang, S.-Q.; Yu, X.-F.; Gao, Z.-H.; Qu, X.-J. Chemoprevention of intestinal tumorigenesis by the natural dietary flavonoid myricetin in APCMin/+ mice. *Oncotarget* **2016**, *7*, 60446. [CrossRef]
259. Zhang, M.-J.; Su, H.; Yan, J.-Y.; Li, N.; Song, Z.-Y.; Wang, H.-J.; Huo, L.-G.; Wang, F.; Ji, W.-S.; Qu, X.-J. Chemopreventive effect of Myricetin, a natural occurring compound, on colonic chronic inflammation and inflammation-driven tumorigenesis in mice. *Biomed. Pharmacother.* **2018**, *97*, 1131–1137. [CrossRef]
260. Wang, F.; Song, Z.-Y.; Qu, X.-J.; Li, F.; Zhang, L.; Li, W.-B.; Cui, S.-X. M10, a novel derivative of Myricetin, prevents ulcerative colitis and colorectal tumor through attenuating robust endoplasmic reticulum stress. *Carcinogenesis* **2018**, *39*, 889–899. [CrossRef]
261. Leonardi, T.; Vanamala, J.; Taddeo, S.S.; Davidson, L.A.; Murphy, M.E.; Patil, B.S.; Wang, N.; Carroll, R.J.; Chapkin, R.S.; Lupton, J.R. Apigenin and naringenin suppress colon carcinogenesis through the aberrant crypt stage in azoxymethane-treated rats. *Exp. Biol. Med.* **2010**, *235*, 710–717. [CrossRef]
262. Dou, W.; Zhang, J.; Sun, A.; Zhang, E.; Ding, L.; Mukherjee, S.; Wei, X.; Chou, G.; Wang, Z.-T.; Mani, S. Protective effect of naringenin against experimental colitis via suppression of Toll-like receptor 4/NF-κB signalling. *Br. J. Nutr.* **2013**, *110*, 599–608. [CrossRef]
263. Li, H.; Zhu, F.; Chen, H.; Cheng, K.W.; Zykova, T.; Oi, N.; Lubet, R.A.; Bode, A.M.; Wang, M.; Dong, Z. 6-C-(E-phenylethenyl)-Naringenin Suppresses Colorectal Cancer Growth by Inhibiting Cyclooxygenase-16CEPN Suppresses Colon Cancer by Inhibiting COX-1. *Cancer Res.* **2014**, *74*, 243–252. [CrossRef]
264. Sequetto, P.L.; Oliveira, T.T.; Maldonado, I.R.; Augusto, L.E.F.; Mello, V.J.; Pizziolo, V.R.; Almeida, M.R.; Silva, M.E.; Novaes, R.D. Naringin accelerates the regression of pre-neoplastic lesions and the colorectal structural reorganization in a murine model of chemical carcinogenesis. *Food Chem. Toxicol.* **2014**, *64*, 200–209. [CrossRef]

265. Zhang, Y.-S.; Wang, F.; Cui, S.-X.; Qu, X.-J. Natural dietary compound naringin prevents azoxymethane/dextran sodium sulfate-induced chronic colorectal inflammation and carcinogenesis in mice. *Cancer Biol. Ther.* **2018**, *19*, 735–744. [CrossRef] [PubMed]
266. Vanamala, J.; Leonardi, T.; Patil, B.S.; Taddeo, S.S.; Murphy, M.E.; Pike, L.M.; Chapkin, R.S.; Lupton, J.R.; Turner, N.D. Suppression of colon carcinogenesis by bioactive compounds in grapefruit. *Carcinogenesis* **2006**, *27*, 1257–1265. [CrossRef] [PubMed]
267. Miyamoto, S.; Yasui, Y.; Tanaka, T.; Ohigashi, H.; Murakami, A. Suppressive effects of nobiletin on hyperleptinemia and colitis-related colon carcinogenesis in male ICR mice. *Carcinogenesis* **2008**, *29*, 1057–1063. [CrossRef] [PubMed]
268. Thangaraj, K.; Vaiyapuri, M. Orientin, a C-glycosyl dietary flavone, suppresses colonic cell proliferation and mitigates NF-κB mediated inflammatory response in 1, 2-dimethylhydrazine induced colorectal carcinogenesis. *Biomed. Pharmacother.* **2017**, *96*, 1253–1266. [CrossRef]
269. Thangaraj, K.; Natesan, K.; Palani, M.; Vaiyapuri, M. Orientin, a flavanoid, mitigates 1, 2 dimethylhydrazine-induced colorectal lesions in Wistar rats fed a high-fat diet. *Toxicol. Rep.* **2018**, *5*, 977–987. [CrossRef]
270. Yang, X.; Zhang, F.; Wang, Y.; Cai, M.; Wang, Q.; Guo, Q.; Li, Z.; Hu, R. Oroxylin A inhibits colitis-associated carcinogenesis through modulating the IL-6/STAT3 signaling pathway. *Inflamm. Bowel Dis.* **2013**, *19*, 1990–2000. [CrossRef]
271. Ni, T.; He, Z.; Dai, Y.; Yao, J.; Guo, Q.; Wei, L. Oroxylin A suppresses the development and growth of colorectal cancer through reprogram of HIF1α-modulated fatty acid metabolism. *Cell Death Dis.* **2017**, *8*, e2865. [CrossRef]
272. Gan, C.; Li, Y.; Yu, Y.; Yu, X.; Liu, H.; Zhang, Q.; Yin, W.; Yu, L.; Ye, T. Natural product pectolinarigenin exhibits potent anti-metastatic activity in colorectal carcinoma cells in vitro and in vivo. *Bioorganic Med. Chem.* **2019**, *27*, 115089. [CrossRef]
273. Lim, S.; Xu, J.; Kim, J.; Chen, T.Y.; Su, X.; Standard, J.; Carey, E.; Griffin, J.; Herndon, B.; Katz, B. Role of anthocyanin-enriched purple-fleshed sweet potato p40 in colorectal cancer prevention. *Mol. Nutr. Food Res.* **2013**, *57*, 1908–1917. [CrossRef] [PubMed]
274. Han, L.; Yan, Y.; Fan, M.; Gao, S.; Zhang, L.; Xiong, X.; Li, R.; Xiao, X.; Wang, X.; Ni, L. Pt3R5G inhibits colon cancer cell proliferation through inducing ferroptosis by down-regulating SLC7A11. *Life Sci.* **2022**, *306*, 120859. [CrossRef]
275. Lin, S.-T.; Tu, S.-H.; Yang, P.-S.; Hsu, S.-P.; Lee, W.-H.; Ho, C.-T.; Wu, C.-H.; Lai, Y.-H.; Chen, M.-Y.; Chen, L.-C. Apple polyphenol phloretin inhibits colorectal cancer cell growth via inhibition of the type 2 glucose transporter and activation of p53-mediated signaling. *J. Agric. Food Chem.* **2016**, *64*, 6826–6837. [CrossRef]
276. Hao, X.; Xiao, H.; Ju, J.; Lee, M.-J.; Lambert, J.D.; Yang, C.S. Green tea polyphenols inhibit colorectal tumorigenesis in azoxymethane-treated F344 rats. *Nutr. Cancer* **2017**, *69*, 623–631. [CrossRef] [PubMed]
277. Gossé, F.; Guyot, S.; Roussi, S.; Lobstein, A.; Fischer, B.; Seiler, N.; Raul, F. Chemopreventive properties of apple procyanidins on human colon cancer-derived metastatic SW620 cells and in a rat model of colon carcinogenesis. *Carcinogenesis* **2005**, *26*, 1291–1295. [CrossRef] [PubMed]
278. Zhu, X.; Tian, X.; Yang, M.; Yu, Y.; Zhou, Y.; Gao, Y.; Zhang, L.; Li, Z.; Xiao, Y.; Moses, R.E. Procyanidin B2 promotes intestinal injury repair and attenuates colitis-associated tumorigenesis via suppression of oxidative stress in mice. *Antioxid. Redox Signal.* **2021**, *35*, 75–92. [CrossRef]
279. Tutino, V.; De Nunzio, V.; Tafaro, A.; Bianco, G.; Gigante, I.; Scavo, M.P.; D'ALESSANDRO, R.; Refolo, M.G.; Messa, C.; Caruso, M.G. Cannabinoid receptor-1 up-regulation in azoxymethane (AOM)-treated mice after dietary treatment with quercetin. *Anticancer Res.* **2018**, *38*, 4485–4491. [CrossRef]
280. Lin, C.; Yu, Y.; Zhao, H.-g.; Yang, A.; Yan, H.; Cui, Y. Combination of quercetin with radiotherapy enhances tumor radiosensitivity in vitro and in vivo. *Radiother. Oncol.* **2012**, *104*, 395–400. [CrossRef]
281. Warren, C.A.; Paulhill, K.J.; Davidson, L.A.; Lupton, J.R.; Taddeo, S.S.; Hong, M.Y.; Carroll, R.J.; Chapkin, R.S.; Turner, N.D. Quercetin may suppress rat aberrant crypt foci formation by suppressing inflammatory mediators that influence proliferation and apoptosis. *J. Nutr.* **2009**, *139*, 101–105. [CrossRef]
282. Lin, R.; Piao, M.; Song, Y.; Liu, C. Quercetin suppresses AOM/DSS-induced colon carcinogenesis through its anti-inflammation effects in mice. *J. Immunol. Res.* **2020**, *2020*, 9242601. [CrossRef]
283. Kee, J.-Y.; Han, Y.-H.; Kim, D.-S.; Mun, J.-G.; Park, J.; Jeong, M.-Y.; Um, J.-Y.; Hong, S.-H. Inhibitory effect of quercetin on colorectal lung metastasis through inducing apoptosis, and suppression of metastatic ability. *Phytomedicine* **2016**, *23*, 1680–1690. [CrossRef] [PubMed]
284. Li, Y.; Wang, Z.; Jin, J.; Zhu, S.-X.; He, G.-Q.; Li, S.-H.; Wang, J.; Cai, Y. Quercetin pretreatment enhances the radiosensitivity of colon cancer cells by targeting Notch-1 pathway. *Biochem. Biophys. Res. Commun.* **2020**, *523*, 947–953. [CrossRef]
285. Alonso-Castro, A.J.; Domínguez, F.; García-Carrancá, A. Rutin exerts antitumor effects on nude mice bearing SW480 tumor. *Arch. Med. Res.* **2013**, *44*, 346–351. [CrossRef] [PubMed]
286. Zeng, S.; Chen, L.; Sun, Q.; Zhao, H.; Yang, H.; Ren, S.; Liu, M.; Meng, X.; Xu, H. Scutellarin ameliorates colitis-associated colorectal cancer by suppressing Wnt/β-catenin signaling cascade. *Eur. J. Pharmacol.* **2021**, *906*, 174253. [CrossRef]
287. Xiong, L.-L.; Du, R.-L.; Xue, L.-L.; Jiang, Y.; Huang, J.; Chen, L.; Liu, J.; Wang, T.-H. Anti-colorectal cancer effects of scutellarin revealed by genomic and proteomic analysis. *Chin. Med.* **2020**, *15*, 1–5. [CrossRef] [PubMed]
288. Zeng, S.; Tan, L.; Sun, Q.; Chen, L.; Zhao, H.; Liu, M.; Yang, H.; Ren, S.; Ming, T.; Tang, S. Suppression of colitis-associated colorectal cancer by scutellarin through inhibiting Hedgehog signaling pathway activity. *Phytomedicine* **2022**, *98*, 153972. [CrossRef]
289. Yang, S.-H.; Lin, J.-K.; Huang, C.-J.; Chen, W.-S.; Li, S.-Y.; Chiu, J.-H. Silibinin inhibits angiogenesis via Flt-1, but not KDR, receptor up-regulation1. *J. Surg. Res.* **2005**, *128*, 140–146. [CrossRef]

290. Kauntz, H.; Bousserouel, S.; Gosse, F.; Marescaux, J.; Raul, F. Silibinin, a natural flavonoid, modulates the early expression of chemoprevention biomarkers in a preclinical model of colon carcinogenesis. *Int. J. Oncol.* **2012**, *41*, 849–854. [CrossRef]
291. Bao, H.; Zheng, N.; Li, Z.; Zhi, Y. Synergistic effect of tangeretin and atorvastatin for colon cancer combination therapy: Targeted delivery of these dual drugs using RGD Peptide decorated nanocarriers. *Drug Des. Dev. Ther.* **2020**, *14*, 3057. [CrossRef]
292. Razak, S.; Afsar, T.; Ullah, A.; Almajwal, A.; Alkholief, M.; Alshamsan, A.; Jahan, S. Taxifolin, a natural flavonoid interacts with cell cycle regulators causes cell cycle arrest and causes tumor regression by activating Wnt/β-catenin signaling pathway. *BMC Cancer* **2018**, *18*, 1–18. [CrossRef]
293. Yue, G.G.-L.; Gao, S.; Lee, J.K.-M.; Chan, Y.-Y.; Wong, E.C.-W.; Zheng, T.; Li, X.-X.; Shaw, P.-C.; Simmonds, M.S.; Lau, C.B.-S. A natural flavone tricin from grains can alleviate tumor growth and lung metastasis in colorectal tumor mice. *Molecules* **2020**, *25*, 3730. [CrossRef]
294. Oyama, T.; Yasui, Y.; Sugie, S.; Koketsu, M.; Watanabe, K.; Tanaka, T. Dietary tricin suppresses inflammation-related colon carcinogenesis in male Crj: CD-1 mice. *Cancer Prev. Res.* **2009**, *2*, 1031–1038. [CrossRef]
295. Vinothkumar, R.; Kumar, R.V.; Sudha, M.; Viswanathan, P.; Balasubramanian, T.; Nalini, N. Modulatory effect of troxerutin on biotransforming enzymes and preneoplasic lesions induced by 1, 2-dimethylhydrazine in rat colon carcinogenesis. *Exp. Mol. Pathol.* **2014**, *96*, 15–26. [CrossRef]
296. Bhardwaj, M.; Paul, S.; Jakhar, R.; Khan, I.; Kang, J.I.; Kim, H.M.; Yun, J.W.; Lee, S.-J.; Cho, H.J.; Lee, H.G. Vitexin confers HSF-1 mediated autophagic cell death by activating JNK and ApoL1 in colorectal carcinoma cells. *Oncotarget* **2017**, *8*, 112426. [CrossRef]
297. Bhardwaj, M.; Cho, H.J.; Paul, S.; Jakhar, R.; Khan, I.; Lee, S.-J.; Kim, B.-Y.; Krishnan, M.; Khaket, T.P.; Lee, H.G. Vitexin induces apoptosis by suppressing autophagy in multi-drug resistant colorectal cancer cells. *Oncotarget* **2018**, *9*, 3278. [CrossRef]
298. Yao, J.; Zhao, L.; Zhao, Q.; Zhao, Y.; Sun, Y.; Zhang, Y.; Miao, H.; You, Q.; Hu, R.; Guo, Q. NF-κB and Nrf2 signaling pathways contribute to wogonin-mediated inhibition of inflammation-associated colorectal carcinogenesis. *Cell Death Dis.* **2014**, *5*, e1283. [CrossRef]
299. Feng, Q.; Wang, H.; Pang, J.; Ji, L.; Han, J.; Wang, Y.; Qi, X.; Liu, Z.; Lu, L. Prevention of wogonin on colorectal cancer tumorigenesis by regulating p53 nuclear translocation. *Front. Pharmacol.* **2018**, *9*, 1356. [CrossRef]
300. You, W.; Di, A.; Zhang, L.; Zhao, G. Effects of wogonin on the growth and metastasis of colon cancer through the Hippo signaling pathway. *Bioengineered* **2022**, *13*, 2586–2597. [CrossRef]
301. Liu, H.; Zhang, L.; Li, G.; Gao, Z. Xanthohumol protects against Azoxymethane-induced colorectal cancer in Sprague-Dawley rats. *Environ. Toxicol.* **2020**, *35*, 136–144. [CrossRef]
302. Murillo, G.; Hirschelman, W.H.; Ito, A.; Moriarty, R.M.; Kinghorn, A.D.; Pezzuto, J.M.; Mehta, R.G. Zapotin, a phytochemical present in a Mexican fruit, prevents colon carcinogenesis. *Nutr. Cancer* **2007**, *57*, 28–37. [CrossRef]
303. Kang, N.J.; Lee, K.W.; Kim, B.H.; Bode, A.M.; Lee, H.-J.; Heo, Y.-S.; Boardman, L.; Limburg, P.; Lee, H.J.; Dong, Z. Coffee phenolic phytochemicals suppress colon cancer metastasis by targeting MEK and TOPK. *Carcinogenesis* **2011**, *32*, 921–928. [CrossRef]
304. Park, S.-R.; Kim, S.-R.; Hong, I.-S.; Lee, H.-Y. A novel therapeutic approach for colorectal cancer stem cells: Blocking the PI3K/Akt signaling axis with caffeic acid. *Front. Cell Dev. Biol.* **2020**, *8*, 585987. [CrossRef]
305. Chiang, E.-P.I.; Tsai, S.-Y.; Kuo, Y.-H.; Pai, M.-H.; Chiu, H.-L.; Rodriguez, R.L.; Tang, F.-Y. Caffeic acid derivatives inhibit the growth of colon cancer: Involvement of the PI3-K/Akt and AMPK signaling pathways. *PLoS ONE* **2014**, *9*, e99631. [CrossRef]
306. Tang, H.; Yao, X.; Yao, C.; Zhao, X.; Zuo, H.; Li, Z. Anti-colon cancer effect of caffeic acid p-nitro-phenethyl ester in vitro and in vivo and detection of its metabolites. *Sci. Rep.* **2017**, *7*, 1–11. [CrossRef]
307. Chen, C.; Kuo, Y.-H.; Lin, C.-C.; Chao, C.-Y.; Pai, M.-H.; Chiang, E.-P.I.; Tang, F.-Y. Decyl caffeic acid inhibits the proliferation of colorectal cancer cells in an autophagy-dependent manner in vitro and in vivo. *PLoS ONE* **2020**, *15*, e0232832. [CrossRef]
308. Tanaka, T.; Nishikawa, A.; Shima, H.; Sugie, S.; Shinoda, T.; Yoshimi, N.; Iwata, H.; Mori, H. Inhibitory effects of chlorogenic acid, reserpine, polyprenoic acid (E-5166), or coffee on hepatocarcinogenesis in rats and hamsters. *Basic Life Sci.* **1990**, *52*, 429–440.
309. Morishita, Y.; Yoshimi, N.; Kawabata, K.; Matsunaga, K.; Sugie, S.; Tanaka, T.; Mori, H. Regressive effects of various chemopreventive agents on azoxymethane-induced aberrant crypt foci in the rat colon. *Jpn. J. Cancer Res.* **1997**, *88*, 815–820. [CrossRef]
310. Rao, C.V.; Tokumo, K.; Rigotty, J.; Zang, E.; Kelloff, G.; Reddy, B.S. Chemoprevention of colon carcinogenesis by dietary administration of piroxicam, α-difluoromethylornithine, 16α-fluoro-5-androsten-17-one, and ellagic acid individually and in combination. *Cancer Res.* **1991**, *51*, 4528–4534.
311. Umesalma, S.; Sudhandiran, G. Chemomodulation of the antioxidative enzymes and peroxidative damage in the colon of 1, 2-dimethyl hydrazine-induced rats by ellagicacid. *Phytother. Res.* **2010**, *24*, S114–S119. [CrossRef]
312. Umesalma, S.; Sudhandiran, G. Differential inhibitory effects of the polyphenol ellagic acid on inflammatory mediators NF-κB, iNOS, COX-2, TNF-α, and IL-6 in 1, 2-dimethylhydrazine-induced rat colon carcinogenesis. *Basic Clin. Pharmacol. Toxicol.* **2010**, *107*, 650–655. [CrossRef]
313. Umesalma, S.; Sudhandiran, G. Ellagic acid prevents rat colon carcinogenesis induced by 1, 2 dimethyl hydrazine through inhibition of AKT-phosphoinositide-3 kinase pathway. *Eur. J. Pharmacol.* **2011**, *660*, 249–258. [CrossRef]
314. Kumar, K.N.; Raja, S.B.; Vidhya, N.; Devaraj, S.N. Ellagic acid modulates antioxidant status, ornithine decarboxylase expression, and aberrant crypt foci progression in 1, 2-dimethylhydrazine-instigated colon preneoplastic lesions in rats. *J. Agric. Food Chem.* **2012**, *60*, 3665–3672. [CrossRef]
315. Goyal, Y.; Koul, A.; Ranawat, P. Ellagic acid modulates cisplatin toxicity in DMH induced colorectal cancer: Studies on membrane alterations. *Biochem. Biophys. Rep.* **2022**, *31*, 101319. [CrossRef]

316. Kawabata, K.; Yamamoto, T.; Hara, A.; Shimizu, M.; Yamada, Y.; Matsunaga, K.; Tanaka, T.; Mori, H. Modifying effects of ferulic acid on azoxymethane-induced colon carcinogenesis in F344 rats. *Cancer Lett.* **2000**, *157*, 15–21. [CrossRef]
317. Han, B.S.; Park, C.B.; Takasuka, N.; Naito, A.; Sekine, K.; Nomura, E.; Taniguchi, H.; Tsuno, T.; Tsuda, H. A Ferulic Acid Derivative, Ethyl 3-(4′-Geranyloxy-3-methoxyphenyl)-2-propenoate, as a New Candidate Chemopreventive Agent for Colon Carcinogenesis in the Rat. *Jpn. J. Cancer Res.* **2001**, *92*, 404–409. [CrossRef]
318. Giftson, J.S.; Jayanthi, S.; Nalini, N. Chemopreventive efficacy of gallic acid, an antioxidant and anticarcinogenic polyphenol, against 1, 2-dimethyl hydrazine induced rat colon carcinogenesis. *Investig. New Drugs* **2010**, *28*, 251–259. [CrossRef]
319. Sanchez-Martin, V.; Plaza-Calonge, M.d.C.; Soriano-Lerma, A.; Ortiz-Gonzalez, M.; Linde-Rodriguez, A.; Perez-Carrasco, V.; Ramirez-Macias, I.; Cuadros, M.; Gutierrez-Fernandez, J.; Murciano-Calles, J. Gallic Acid: A Natural Phenolic Compound Exerting Antitumoral Activities in Colorectal Cancer via Interaction with G-Quadruplexes. *Cancers* **2022**, *14*, 2648. [CrossRef]
320. Jeong, J.H.; Kim, E.Y.; Choi, H.J.; Chung, T.W.; Kim, K.J.; Kim, S.Y.; Ha, K.T. Gallic acid inhibits STAT3 phosphorylation and alleviates DDS-induced colitis via regulating cytokine production. *J. Physiol. Pathol. Korean Med.* **2016**, *30*, 338–346. [CrossRef]
321. Lin, X.; Wang, G.; Liu, P.; Han, L.; Wang, T.; Chen, K.; Gao, Y. Gallic acid suppresses colon cancer proliferation by inhibiting SRC and EGFR phosphorylation. *Exp. Ther. Med.* **2021**, *21*, 1–11. [CrossRef]
322. Hong, Z.; Tang, P.; Liu, B.; Ran, C.; Yuan, C.; Zhang, Y.; Lu, Y.; Duan, X.; Yang, Y.; Wu, H. Ferroptosis-related genes for overall survival prediction in patients with colorectal cancer can be inhibited by gallic acid. *Int. J. Biol. Sci.* **2021**, *17*, 942. [CrossRef]
323. Senapathy, J.G.; Jayanthi, S.; Viswanathan, P.; Umadevi, P.; Nalini, N. Effect of gallic acid on xenobiotic metabolizing enzymes in 1, 2-dimethyl hydrazine induced colon carcinogenesis in Wistar rats–a chemopreventive approach. *Food Chem. Toxicol.* **2011**, *49*, 887–892. [CrossRef] [PubMed]
324. Zhou, L.-A.; Liu, T.-B.; Lü, H.-N. Geraniin inhibits proliferation and induces apoptosis through inhibition of phosphatidylinositol 3-kinase/Akt pathway in human colorectal cancer in vitro and in vivo. *Anti-Cancer Drugs* **2020**, *31*, 575–582. [CrossRef] [PubMed]
325. Sharma, S.H.; Chellappan, D.R.; Chinnaswamy, P.; Nagarajan, S. Protective effect of p-coumaric acid against 1, 2 dimethylhydrazine induced colonic preneoplastic lesions in experimental rats. *Biomed. Pharmacother.* **2017**, *94*, 577–588. [CrossRef] [PubMed]
326. Fang, W.; Zhu, S.; Niu, Z.; Yin, Y. The protective effect of syringic acid on dextran sulfate sodium-induced experimental colitis in BALB/c mice. *Drug Dev. Res.* **2019**, *80*, 731–740. [CrossRef]
327. Mihanfar, A.; Darband, S.G.; Sadighparvar, S.; Kaviani, M.; Mirza-Aghazadeh-Attari, M.; Yousefi, B.; Majidinia, M. In vitro and in vivo anticancer effects of syringic acid on colorectal cancer: Possible mechanistic view. *Chem.-Biol. Interact.* **2021**, *337*, 109337. [CrossRef]
328. Han, Y.-H.; Kee, J.-Y.; Kim, D.-S.; Mun, J.-g.; Jeong, M.-Y.; Park, S.-H.; Choi, B.-M.; Park, S.-J.; Kim, H.-J.; Um, J.-Y. Arctigenin inhibits lung metastasis of colorectal cancer by regulating cell viability and metastatic phenotypes. *Molecules* **2016**, *21*, 1135. [CrossRef]
329. Kang, K.; Oh, S.H.; Yun, J.H.; Jho, E.H.; Kang, J.-H.; Batsuren, D.; Tunsag, J.; Park, K.H.; Kim, M.; Nho, C.W. A novel topoisomerase inhibitor, daurinol, suppresses growth of HCT116 cells with low hematological toxicity compared to etoposide. *Neoplasia* **2011**, *13*, 1043–1057. [CrossRef]
330. Li, C.; Zhang, K.; Pan, G.; Ji, H.; Li, C.; Wang, X.; Hu, X.; Liu, R.; Deng, L.; Wang, Y. Dehydrodiisoeugenol inhibits colorectal cancer growth by endoplasmic reticulum stress-induced autophagic pathways. *J. Exp. Clin. Cancer Res.* **2021**, *40*, 1–15. [CrossRef]
331. Kee, J.-Y.; Han, Y.-H.; Mun, J.-G.; Park, S.-H.; Jeon, H.D.; Hong, S.-H. Gomisin a suppresses colorectal lung metastasis by inducing AMPK/P38-mediated apoptosis and decreasing metastatic abilities of colorectal cancer cells. *Front. Pharmacol.* **2018**, *9*, 986. [CrossRef]
332. Li, Q.; Ma, Y.; Liu, X.L.; Mu, L.; He, B.C.; Wu, K.; Sun, W.J. Anti-proliferative effect of honokiol on SW620 cells through upregulating BMP7 expression via the TGF-β1/p53 signaling pathway. *Oncol. Rep.* **2020**, *44*, 2093–2107. [CrossRef]
333. Won, S.J.; Yen, C.H.; Liu, H.S.; Wu, S.Y.; Lan, S.H.; Jiang-Shieh, Y.F.; Lin, C.N.; Su, C.L. Justicidin A-induced autophagy flux enhances apoptosis of human colorectal cancer cells via class III PI3K and Atg5 pathway. *J. Cell. Physiol.* **2015**, *230*, 930–946. [CrossRef] [PubMed]
334. Su, C.-M.; Weng, Y.-S.; Kuan, L.-Y.; Chen, J.-H.; Hsu, F.-T. Suppression of PKCδ/NF-κB Signaling and Apoptosis Induction through Extrinsic/Intrinsic Pathways Are Associated with Magnolol-Inhibited Tumor Progression in Colorectal Cancer In Vitro and In Vivo. *Int. J. Mol. Sci.* **2020**, *21*, 3527. [CrossRef] [PubMed]
335. Kang, Y.-J.; Park, H.J.; Chung, H.-J.; Min, H.-Y.; Park, E.J.; Lee, M.A.; Shin, Y.; Lee, S.K. Wnt/β-catenin signaling mediates the antitumor activity of magnolol in colorectal cancer cells. *Mol. Pharmacol.* **2012**, *82*, 168–177. [CrossRef]
336. Pu, Z.; Zhang, W.; Wang, M.; Xu, M.; Xie, H.; Zhao, J. Schisandrin B Attenuates colitis-associated colorectal cancer through SIRT1 linked SMURF2 signaling. *Am. J. Chin. Med.* **2021**, *49*, 1773–1789. [CrossRef] [PubMed]
337. Chen, T.; Wang, Z.; Zhong, J.; Zhang, L.; Zhang, H.; Zhang, D.; Xu, X.; Zhong, X.; Wang, J.; Li, H. Secoisolariciresinol diglucoside induces pyroptosis by activating caspase-1 to cleave GSDMD in colorectal cancer cells. *Drug Dev. Res.* **2022**, *83*, 1152–1166. [CrossRef]
338. Wang, Z.; Chen, T.; Yang, C.; Bao, T.; Yang, X.; He, F.; Zhang, Y.; Zhu, L.; Chen, H.; Rong, S. Secoisolariciresinol diglucoside suppresses Dextran sulfate sodium salt-induced colitis through inhibiting NLRP1 inflammasome. *Int. Immunopharmacol.* **2020**, *78*, 105931. [CrossRef]

339. Ohira, H.; Oikawa, D.; Kurokawa, Y.; Aoki, Y.; Omura, A.; Kiyomoto, K.; Nakagawa, W.; Mamoto, R.; Fujioka, Y.; Nakayama, T. Suppression of colonic oxidative stress caused by chronic ethanol administration and attenuation of ethanol-induced colitis and gut leakiness by oral administration of sesaminol in mice. *Food Funct.* **2022**, *13*, 9285–9298. [CrossRef]
340. Shin, M.-K.; Jeon, Y.-D.; Hong, S.-H.; Kang, S.-H.; Kee, J.-Y.; Jin, J.-S. In vivo and In vitro effects of Tracheloside on colorectal cancer cell proliferation and metastasis. *Antioxidants* **2021**, *10*, 513. [CrossRef] [PubMed]
341. Chen, J.; Zhong, J.; Liu, Y.; Huang, Y.; Luo, F.; Zhou, Y.; Pan, X.; Cao, S.; Zhang, L.; Zhang, Y. Purified vitexin compound 1, a new neolignan isolated compound, promotes PUMA-dependent apoptosis in colorectal cancer. *Cancer Med.* **2018**, *7*, 6158–6169. [CrossRef]
342. Kimura, Y. Long-term oral administration of piceatannol (3, 5, 3′, 4′-tetrahydroxystilbene) attenuates colon tumor growth induced by azoxymethane plus dextran sulfate sodium in C57BL/6J mice. *Nutr. Cancer* **2022**, *74*, 2184–2195. [CrossRef]
343. Jin, Y.; Zhan, X.; Zhang, B.; Chen, Y.; Liu, C.; Yu, L. Polydatin exerts an antitumor effect through regulating the miR-382/PD-L1 axis in colorectal cancer. *Cancer Biother. Radiopharm.* **2020**, *35*, 83–91. [CrossRef] [PubMed]
344. Chiou, Y.-S.; Tsai, M.-L.; Wang, Y.-J.; Cheng, A.-C.; Lai, W.-M.; Badmaev, V.; Ho, C.-T.; Pan, M.-H. Pterostilbene inhibits colorectal aberrant crypt foci (ACF) and colon carcinogenesis via suppression of multiple signal transduction pathways in azoxymethane-treated mice. *J. Agric. Food Chem.* **2010**, *58*, 8833–8841. [CrossRef]
345. Paul, S.; DeCastro, A.J.; Lee, H.J.; Smolarek, A.K.; So, J.Y.; Simi, B.; Wang, C.X.; Zhou, R.; Rimando, A.M.; Suh, N. Dietary intake of pterostilbene, a constituent of blueberries, inhibits the β-catenin/p65 downstream signaling pathway and colon carcinogenesis in rats. *Carcinogenesis* **2010**, *31*, 1272–1278. [CrossRef] [PubMed]
346. Chiou, Y.-S.; Tsai, M.-L.; Nagabhushanam, K.; Wang, Y.-J.; Wu, C.-H.; Ho, C.-T.; Pan, M.-H. Pterostilbene is more potent than resveratrol in preventing azoxymethane (AOM)-induced colon tumorigenesis via activation of the NF-E2-related factor 2 (Nrf2)-mediated antioxidant signaling pathway. *J. Agric. Food Chem.* **2011**, *59*, 2725–2733. [CrossRef] [PubMed]
347. Zhang, Y.; Li, Y.; Sun, C.; Chen, X.; Han, L.; Wang, T.; Liu, J.; Chen, X.; Zhao, D. Effect of pterostilbene, a natural derivative of resveratrol, in the treatment of colorectal cancer through Top1/Tdp1-mediated DNA repair pathway. *Cancers* **2021**, *13*, 4002. [CrossRef]
348. Suh, N.; Paul, S.; Hao, X.; Simi, B.; Xiao, H.; Rimando, A.M.; Reddy, B.S. Pterostilbene, an active constituent of blueberries, suppresses aberrant crypt foci formation in the azoxymethane-induced colon carcinogenesis model in rats. *Clin. Cancer Res.* **2007**, *13*, 350–355. [CrossRef]
349. Ji, Q.; Liu, X.; Han, Z.; Zhou, L.; Sui, H.; Yan, L.; Jiang, H.; Ren, J.; Cai, J.; Li, Q. Resveratrol suppresses epithelial-to-mesenchymal transition in colorectal cancer through TGF-β1/Smads signaling pathway mediated Snail/E-cadherin expression. *BMC Cancer* **2015**, *15*, 1–12. [CrossRef]
350. Saud, S.M.; Li, W.; Morris, N.L.; Matter, M.S.; Colburn, N.H.; Kim, Y.S.; Young, M.R. Resveratrol prevents tumorigenesis in mouse model of Kras activated sporadic colorectal cancer by suppressing oncogenic Kras expression. *Carcinogenesis* **2014**, *35*, 2778–2786. [CrossRef]
351. Alfaras, I.; Juan, M.E.; Planas, J.M. trans-Resveratrol reduces precancerous colonic lesions in dimethylhydrazine-treated rats. *J. Agric. Food Chem.* **2010**, *58*, 8104–8110. [CrossRef]
352. Majumdar, A.P.; Banerjee, S.; Nautiyal, J.; Patel, B.B.; Patel, V.; Du, J.; Yu, Y.; Elliott, A.A.; Levi, E.; Sarkar, F.H. Curcumin synergizes with resveratrol to inhibit colon cancer. *Nutr. Cancer* **2009**, *61*, 544–553. [CrossRef]
353. Sudha, T.; El-Far, A.H.; Mousa, D.S.; Mousa, S.A. Resveratrol and its nanoformulation attenuate growth and the angiogenesis of xenograft and orthotopic colon cancer models. *Molecules* **2020**, *25*, 1412. [CrossRef] [PubMed]
354. Hu, W.-H.; Chan, G.K.-L.; Duan, R.; Wang, H.-Y.; Kong, X.-P.; Dong, T.T.-X.; Tsim, K.W.-K. Synergy of ginkgetin and resveratrol in suppressing VEGF-induced angiogenesis: A therapy in treating colorectal cancer. *Cancers* **2019**, *11*, 1828. [CrossRef] [PubMed]
355. Sepporta, M.V.; Fuccelli, R.; Rosignoli, P.; Ricci, G.; Servili, M.; Fabiani, R. Oleuropein prevents Azoxymethane-induced Colon crypt dysplasia and leukocytes DNA damage in a/J mice. *J. Med. Food* **2016**, *19*, 983–989. [CrossRef] [PubMed]
356. Zeng, Q.; Che, Y.; Zhang, Y.; Chen, M.; Guo, Q.; Zhang, W. Thymol Isolated from Thymus vulgaris L. inhibits colorectal cancer cell growth and metastasis by suppressing the Wnt/β-catenin pathway. *Drug Des. Dev. Ther.* **2020**, *14*, 2535. [CrossRef] [PubMed]
357. Zhou, L.; Feng, Y.; Jin, Y.; Liu, X.; Sui, H.; Chai, N.; Chen, X.; Liu, N.; Ji, Q.; Wang, Y. Verbascoside promotes apoptosis by regulating HIPK2–p53 signaling in human colorectal cancer. *BMC Cancer* **2014**, *14*, 1–11. [CrossRef] [PubMed]
358. Singh, S.; Meena, A.; Luqman, S. Baicalin mediated regulation of key signaling pathways in cancer. *Pharmacol. Res.* **2021**, *164*, 105387. [CrossRef]
359. Patel, V.B.; Misra, S.; Patel, B.B.; Majumdar, A.P. Colorectal cancer: Chemopreventive role of curcumin and resveratrol. *Nutr. Cancer* **2010**, *62*, 958–967. [CrossRef]
360. Prasad, S.; Aggarwal, B.B. Turmeric, the golden spice. In *Herbal Medicine: Biomolecular and Clinical Aspects*, 2nd ed.; CRC Press/Taylor & Francis: Boca Raton, FL, USA, 2011; pp. 263–288.
361. Wang, Y.; Bu, C.; Wu, K.; Wang, R.; Wang, J. Curcumin protects the pancreas from acute pancreatitis via the mitogen-activated protein kinase signaling pathway. *Mol. Med. Rep.* **2019**, *20*, 3027–3034. [CrossRef]
362. Mishra, S.; Palanivelu, K. Thread Rating. *Ann. Indian Acad. Neurol.* **2008**, *11*, 13–19. [CrossRef]
363. Zorena, K.; Jachimowicz-Duda, O.; Ślęzak, D.; Robakowska, M.; Mrugacz, M. Adipokines and obesity. Potential link to metabolic disorders and chronic complications. *Int. J. Mol. Sci.* **2020**, *21*, 3570. [CrossRef]

364. Achari, A.E.; Jain, S.K. Adiponectin, a therapeutic target for obesity, diabetes, and endothelial dysfunction. *Int. J. Mol. Sci.* **2017**, *18*, 1321. [CrossRef] [PubMed]
365. Weidinger, C.; Ziegler, J.F.; Letizia, M.; Schmidt, F.; Siegmund, B. Adipokines and their role in intestinal inflammation. *Front. Immunol.* **2018**, *9*, 1974. [CrossRef] [PubMed]
366. Kelesidis, T.; Kelesidis, I.; Chou, S.; Mantzoros, C.S. Narrative review: The role of leptin in human physiology: Emerging clinical applications. *Ann. Intern. Med.* **2010**, *152*, 93–100. [CrossRef] [PubMed]
367. Iikuni, N.; Kwan Lam, Q.L.; Lu, L.; Matarese, G.; Cava, A.L. Leptin and inflammation. *Curr. Immunol. Rev.* **2008**, *4*, 70–79. [CrossRef] [PubMed]
368. Aggarwal, V.; Tuli, H.S.; Tania, M.; Srivastava, S.; Ritzer, E.E.; Pandey, A.; Aggarwal, D.; Barwal, T.S.; Jain, A.; Kaur, G. Molecular mechanisms of action of epigallocatechin gallate in cancer: Recent trends and advancement. *Semin. Cancer Biol.* **2022**, *80*, 256–275. [CrossRef]
369. Musial, C.; Kuban-Jankowska, A.; Gorska-Ponikowska, M. Beneficial properties of green tea catechins. *Int. J. Mol. Sci.* **2020**, *21*, 1744. [CrossRef]
370. Kim, J.S.; Kim, J.-M.; Jeong-Ja, O.; Jeon, B.S. Inhibition of inducible nitric oxide synthase expression and cell death by (−)-epigallocatechin-3-gallate, a green tea catechin, in the 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine mouse model of Parkinson's disease. *J. Clin. Neurosci.* **2010**, *17*, 1165–1168. [CrossRef]
371. Kim, M.; Murakami, A.; Miyamoto, S.; Tanaka, T.; Ohigashi, H. The modifying effects of green tea polyphenols on acute colitis and inflammation-associated colon carcinogenesis in male ICR mice. *Biofactors* **2010**, *36*, 43–51. [CrossRef]
372. De Oliveira, G.A.; Cheng, R.Y.; Ridnour, L.A.; Basudhar, D.; Somasundaram, V.; McVicar, D.W.; Monteiro, H.P.; Wink, D.A. Inducible nitric oxide synthase in the carcinogenesis of gastrointestinal cancers. *Antioxid. Redox Signal.* **2017**, *26*, 1059–1077. [CrossRef]
373. Cianchi, F.; Cortesini, C.; Fantappiè, O.; Messerini, L.; Schiavone, N.; Vannacci, A.; Nistri, S.; Sardi, I.; Baroni, G.; Marzocca, C. Inducible nitric oxide synthase expression in human colorectal cancer: Correlation with tumor angiogenesis. *Am. J. Pathol.* **2003**, *162*, 793–801. [CrossRef]
374. Mandal, P. Molecular signature of nitric oxide on major cancer hallmarks of colorectal carcinoma. *Inflammopharmacology* **2018**, *26*, 331–336. [CrossRef] [PubMed]
375. Sheng, J.; Sun, H.; Yu, F.-B.; Li, B.; Zhang, Y.; Zhu, Y.-T. The role of cyclooxygenase-2 in colorectal cancer. *Int. J. Med. Sci.* **2020**, *17*, 1095. [CrossRef] [PubMed]
376. Greenhough, A.; Smartt, H.J.; Moore, A.E.; Roberts, H.R.; Williams, A.C.; Paraskeva, C.; Kaidi, A. The COX-2/PGE 2 pathway: Key roles in the hallmarks of cancer and adaptation to the tumour microenvironment. *Carcinogenesis* **2009**, *30*, 377–386. [CrossRef]
377. Tuli, H.S.; Tuorkey, M.J.; Thakral, F.; Sak, K.; Kumar, M.; Sharma, A.K.; Sharma, U.; Jain, A.; Aggarwal, V.; Bishayee, A. Molecular mechanisms of action of genistein in cancer: Recent advances. *Front. Pharmacol.* **2019**, *10*, 1336. [CrossRef] [PubMed]
378. Chen, X.; Gu, J.; Wu, Y.; Liang, P.; Shen, M.; Xi, J.; Qin, J. Clinical characteristics of colorectal cancer patients and anti-neoplasm activity of genistein. *Biomed. Pharmacother.* **2020**, *124*, 109835. [CrossRef]
379. Pintova, S.; Dharmupari, S.; Moshier, E.; Zubizarreta, N.; Ang, C.; Holcombe, R.F. Genistein combined with FOLFOX or FOLFOX–Bevacizumab for the treatment of metastatic colorectal cancer: Phase I/II pilot study. *Cancer Chemother. Pharmacol.* **2019**, *84*, 591–598. [CrossRef] [PubMed]
380. Felice, M.R.; Maugeri, A.; De Sarro, G.; Navarra, M.; Barreca, D. Molecular Pathways Involved in the Anti-Cancer Activity of Flavonols: A Focus on Myricetin and Kaempferol. *Int. J. Mol. Sci.* **2022**, *23*, 4411. [CrossRef]
381. Lin, Y.; Shi, R.; Wang, X.; Shen, H.-M. Luteolin, a flavonoid with potential for cancer prevention and therapy. *Curr. Cancer Drug Targets* **2008**, *8*, 634–646. [CrossRef]
382. Ganai, S.A.; Sheikh, F.A.; Baba, Z.A.; Mir, M.A.; Mantoo, M.A.; Yatoo, M.A. Anticancer activity of the plant flavonoid luteolin against preclinical models of various cancers and insights on different signalling mechanisms modulated. *Phytother. Res.* **2021**, *35*, 3509–3532. [CrossRef]
383. Xiao, Y.; Yang, H.; Lu, J.; Li, D.; Xu, C.; Risch, H.A. Serum gamma-glutamyltransferase and the overall survival of metastatic pancreatic cancer. *BMC Cancer* **2019**, *19*, 1–7. [CrossRef]
384. Ribatti, D.; Crivellato, E. Mast cells, angiogenesis, and tumour growth. *Biochim. Biophys. Acta BBA Mol. Basis Dis.* **2012**, *1822*, 2–8. [CrossRef] [PubMed]
385. Wang, Y.-X.; Chen, Y.-R.; Liu, S.-S.; Ye, Y.-P.; Jiao, H.-L.; Wang, S.-Y.; Xiao, Z.-Y.; Wei, W.-T.; Qiu, J.-F.; Liang, L. MiR-384 inhibits human colorectal cancer metastasis by targeting KRAS and CDC42. *Oncotarget* **2016**, *7*, 84826. [CrossRef] [PubMed]
386. Kong, Y.; Bai, P.-S.; Nan, K.-J.; Sun, H.; Chen, N.-Z.; Qi, X.-G. Pleiotrophin is a potential colorectal cancer prognostic factor that promotes VEGF expression and induces angiogenesis in colorectal cancer. *Int. J. Color. Dis.* **2012**, *27*, 287–298. [CrossRef]
387. Zhu, M.-L.; Zhang, P.-M.; Jiang, M.; Yu, S.-W.; Wang, L. Myricetin induces apoptosis and autophagy by inhibiting PI3K/Akt/mTOR signalling in human colon cancer cells. *BMC Complement. Med. Ther.* **2020**, *20*, 1–9. [CrossRef]
388. Rauf, A.; Shariati, M.A.; Imran, M.; Bashir, K.; Khan, S.A.; Mitra, S.; Emran, T.B.; Badalova, K.; Uddin, M.; Mubarak, M.S. Comprehensive review on naringenin and naringin polyphenols as a potent anticancer agent. *Environ. Sci. Pollut. Res.* **2022**, *29*, 31025–31041. [CrossRef] [PubMed]
389. Ghanbari-Movahed, M.; Jackson, G.; Farzaei, M.H.; Bishayee, A. A Systematic Review of the Preventive and Therapeutic Effects of Naringin against Human Malignancies. *Front. Pharmacol.* **2021**, *12*, 250. [CrossRef] [PubMed]

390. Gao, R.; Wang, L.; Yang, Y.; Ni, J.; Zhao, L.; Dong, S.; Guo, M. Simultaneous determination of oleanolic acid, ursolic acid, quercetin and apigenin in Swertia mussotii Franch by capillary zone electrophoresis with running buffer modifier. *Biomed. Chromatogr.* **2015**, *29*, 402–409. [CrossRef] [PubMed]
391. Khan, F.; Niaz, K.; Maqbool, F.; Ismail Hassan, F.; Abdollahi, M.; Nagulapalli Venkata, K.C.; Nabavi, S.M.; Bishayee, A. Molecular targets underlying the anticancer effects of quercetin: An update. *Nutrients* **2016**, *8*, 529. [CrossRef]
392. Terzić, J.; Grivennikov, S.; Karin, E.; Karin, M. Inflammation and colon cancer. *Gastroenterology* **2010**, *138*, 2101–2114.e2105. [CrossRef]
393. Long, A.G.; Lundsmith, E.T.; Hamilton, K.E. Inflammation and colorectal cancer. *Curr. Color. Cancer Rep.* **2017**, *13*, 341–351. [CrossRef]
394. Nouri, Z.; Fakhri, S.; Nouri, K.; Wallace, C.E.; Farzaei, M.H.; Bishayee, A. Targeting multiple signaling pathways in cancer: The rutin therapeutic approach. *Cancers* **2020**, *12*, 2276. [CrossRef] [PubMed]
395. Chua, L.S. A review on plant-based rutin extraction methods and its pharmacological activities. *J. Ethnopharmacol.* **2013**, *150*, 805–817. [CrossRef] [PubMed]
396. Negahdari, R.; Bohlouli, S.; Sharifi, S.; Maleki Dizaj, S.; Rahbar Saadat, Y.; Khezri, K.; Jafari, S.; Ahmadian, E.; Gorbani Jahandizi, N.; Raeesi, S. Therapeutic benefits of rutin and its nanoformulations. *Phytother. Res.* **2021**, *35*, 1719–1738. [CrossRef]
397. Pan, M.-H.; Chen, W.-J.; Lin-Shiau, S.-Y.; Ho, C.-T.; Lin, J.-K. Tangeretin induces cell-cycle G1 arrest through inhibiting cyclin-dependent kinases 2 and 4 activities as well as elevating Cdk inhibitors p21 and p27 in human colorectal carcinoma cells. *Carcinogenesis* **2002**, *23*, 1677–1684. [CrossRef]
398. He, L.; Lu, N.; Dai, Q.; Zhao, Y.; Zhao, L.; Wang, H.; Li, Z.; You, Q.; Guo, Q. Wogonin induced G1 cell cycle arrest by regulating Wnt/β-catenin signaling pathway and inactivating CDK8 in human colorectal cancer carcinoma cells. *Toxicology* **2013**, *312*, 36–47. [CrossRef]
399. Tan, H.; Li, X.; Yang, W.-H.; Kang, Y. A flavone, Wogonin from Scutellaria baicalensis inhibits the proliferation of human colorectal cancer cells by inducing of autophagy, apoptosis and G2/M cell cycle arrest via modulating the PI3K/AKT and STAT3 signalling pathways. *J. BUON* **2019**, *24*, 1143–1149. [PubMed]
400. Alam, M.; Ahmed, S.; Elasbali, A.M.; Adnan, M.; Alam, S.; Hassan, M.I.; Pasupuleti, V.R. Therapeutic implications of caffeic acid in cancer and neurological diseases. *Front. Oncol.* **2022**, *12*, 860508. [CrossRef]
401. Banerjee, N.; Kim, H.; Krenek, K.; Talcott, S.T.; Mertens-Talcott, S.U. Mango polyphenolics suppressed tumor growth in breast cancer xenografts in mice: Role of the PI3K/AKT pathway and associated microRNAs. *Nutr. Res.* **2015**, *35*, 744–751. [CrossRef]
402. Boghossian, S.; Hawash, A. Chemoprevention in colorectal cancer-where we stand and what we have learned from twenty year's experience. *Surgeon* **2012**, *10*, 43–52. [CrossRef]
403. Ko, J.-H.; Sethi, G.; Um, J.-Y.; Shanmugam, M.K.; Arfuso, F.; Kumar, A.P.; Bishayee, A.; Ahn, K.S. The role of resveratrol in cancer therapy. *Int. J. Mol. Sci.* **2017**, *18*, 2589. [CrossRef]
404. Bishayee, A. Cancer Prevention and Treatment with Resveratrol: From Rodent Studies to Clinical TrialsResveratrol and Cancer: In vivo and Clinical Studies. *Cancer Prev. Res.* **2009**, *2*, 409–418. [CrossRef] [PubMed]
405. Arslan, A.K.K.; Uzunhisarcıklı, E.; Yerer, M.B.; Bishayee, A. The golden spice curcumin in cancer: A perspective on finalized clinical trials during the last 10 years. *J. Cancer Res. Ther.* **2022**, *18*, 19–26.
406. Singh, A.P.; Singh, R.; Verma, S.S.; Rai, V.; Kaschula, C.H.; Maiti, P.; Gupta, S.C. Health benefits of resveratrol: Evidence from clinical studies. *Med. Res. Rev.* **2019**, *39*, 1851–1891. [CrossRef] [PubMed]
407. Wu, X.-Y.; Zhai, J.; Huan, X.-K.; Xu, W.-W.; Tian, J.; Farhood, B. A Systematic Review of the Therapeutic Potential of Resveratrol During Colorectal Cancer Chemotherapy. *Mini Rev. Med. Chem.* **2022**. [CrossRef]
408. Ma, Z.; Wang, N.; He, H.; Tang, X. Pharmaceutical strategies of improving oral systemic bioavailability of curcumin for clinical application. *J. Control. Release* **2019**, *316*, 359–380. [CrossRef]
409. Seufferlein, T.; Ettrich, T.J.; Menzler, S.; Messmann, H.; Kleber, G.; Zipprich, A.; Frank-Gleich, S.; Algül, H.; Metter, K.; Odemar, F. Green tea extract to prevent colorectal adenomas, results of a randomized, placebo-controlled clinical trial. *Off. J. Am. Coll. Gastroenterol. ACG* **2022**, *117*, 884–894. [CrossRef]
410. Farsad-Naeimi, A.; Alizadeh, M.; Esfahani, A.; Aminabad, E.D. Effect of fisetin supplementation on inflammatory factors and matrix metalloproteinase enzymes in colorectal cancer patients. *Food Funct.* **2018**, *9*, 2025–2031. [CrossRef]
411. Ganesan, K.; Jayachandran, M.; Xu, B. Diet-derived phytochemicals targeting colon cancer stem cells and microbiota in colorectal cancer. *Int. J. Mol. Sci.* **2020**, *21*, 3976. [CrossRef]
412. Greiner, A.K.; Papineni, R.V.; Umar, S. Chemoprevention in gastrointestinal physiology and disease. Natural products and microbiome. *Am. J. Physiol.-Gastrointest. Liver Physiol.* **2014**, *307*, G1–G15. [CrossRef]
413. Dacrema, M.; Ali, A.; Ullah, H.; Khan, A.; Di Minno, A.; Xiao, J.; Martins, A.M.C.; Daglia, M. Spice-Derived Bioactive Compounds Confer Colorectal Cancer Prevention via Modulation of Gut Microbiota. *Cancers* **2022**, *14*, 5682. [CrossRef]
414. O'keefe, S.J. Diet, microorganisms and their metabolites, and colon cancer. *Nat. Rev. Gastroenterol. Hepatol.* **2016**, *13*, 691–706. [CrossRef] [PubMed]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.