Phytochemical and Pharmacological Investigation of Important Volatile Principles from Traditionally Used Medicinal Plant(s) to be Useful for the Treatment of Different Ailments

Thesis submitted by

Anindita Kundu

Doctor of Philosophy (Pharmacy)

Department of Pharmaceutical Technology

Faculty Council of Engineering & Technology

Jadavpur University

Kolkata, India

2024

JADAVPUR UNIVERSITY

KOLKATA-700032, INDIA

INDEX NO -262/17/Ph

1. Title of the thesis : Phytochemical and Pharmacological Investigation of Important Volatile Principles from Traditionally Used Medicinal Plant(s) to be Useful for the Treatment of Different Ailments.

2.Name, Designation & Institution of the Supervisors:

Prof. (Dr.) Subhash C. Mandal
Professor,
Department of Pharmaceutical Technology,
Jadavpur University,
Kolkata-700032, INDIA
Email: scmandal1963@gmail.com

Prof. (Dr.) Amalesh Samanta
Professor,
Department of Pharmaceutical Technology,
Jadavpur University,
Kolkata-700032, INDIA
Email: amalesh.samanta@jadavpuruniversity.in

3. List of publication: 02

- **Anindita Kundu**, Vivekananda Mandal, Partha Pratim Maiti, Md. Harun Al Rashid, Subhash C. Mandal**.** *In-vitro*-Scientific evaluation of anti-inflammatory potential of leaf extracts from *Vitex negundo*: as a promising future drug candidate. *International Journal of Green Pharmacy*, 14(1):57-65, (2020).

- Partha Pratim Maiti, Nilanjan Ghosh, **Anindita Kundu**, Subhasis Panda, Biplab De, Subhash C. Mandal. Evaluation of anti-inflammatory and antinociceptive activity of methanol extract of *Calotropis gigantea* root. *International Journal of Green Pharmacy*. 11(3):198-205, (2017).

- **Anindita Kundu**, Nilanjan Ghosh, Vivekananda Mandal, Subhash C Mandal.. In search for the phytochemical composition and comparative evaluation of *In-vitro* antioxidant, antibacterial and anticancer potentials of methanol leaf extract and leaf essential oil of *Vitex negundo* grown in eastern part of India. (communicated in *Revista Brasileira de Farmacognosia*).

List of book chapter: 03

- **Anindita Kundu,** Vivekananda Mandal, Sujata Wangkheirakpam, Subhash C. Mandal. Mechanistic aspects of Neurodegeneration in Alzheimer's Disease and role of phytochemicals as restorative agents. Phytomedicine and Alzheimer's Disorder. 1st Edition. eBook ISBN9780429318429. **2020**: 12 Pages.

- Md. Harun Al Rashid, **Anindita Kundu**, Vivekananda Mandal, Phurpa Wangchuk, and Subhash C. Mandal. Preclinical and Clinical Trials of Indian Medicinal Plants in Disease Control. Herbal Medicine in India. ISBN: 978-981-13-7248-3 (eBook). **2019**: 119-142. https://doi.org/10.1007/978-981-13-7248-3_9.

- Nilanjan Ghosh, Rituparna C. Ghosh, **Anindita Kundu**, and Subhash C. Mandal. Herb and Drug Interaction. Natural Products and Drug Discovery. ISBN: 978-0-08-102081-4. **2018**:467-484.

4. List of patents: Nil

5. List of presentations in conference: 02

- **Anindita Kundu,** Subhash C Mandal. A comparative assessment of anti-inflammatory potential of methanolic extract and essential oil of *V.negundo* leaves: Promising candidates to be drugs of future. National conference on Emerging Trends In Modern Health Sciences (28-29th February. 2020) at Kolkata, West Bengal, (Poster Presentation).

- **Anindita Kundu,** Subhash C Mandal. A comparative study of phytochemical profiling of essential oil and methanolic extract: A holistic approach for management of various ailments. International conference on Current Scenario in Pharmaceutical Technology and Healthcare: A Move Towards Patient-Centric Approach (9-10th March. 2018) at Kolkata, West Bengal, (Poster Presentation).

"Statement of Originality"

I Anindita Kundu registered on 11/08/2017 do hereby declare that this thesis entitled "Phytochemical and Pharmacological Investigation of Important Volatile Principles from Traditionally Used Medicinal Plant(s) to be Useful for the Treatment of Different Ailments." contains literature survey and original research work done by the undersigned candidate as part of Doctoral studies.

All information in this thesis have been obtained and presented in accordance with existing academic rules and ethical conduct. I declare that, as required by these rules and conduct, I have fully cited and referred all materials and results that are not original to this work.

I also declare that I have checked this thesis as per the "Policy on Anti Plagiarism, Jadavpur University, 2019", and the level of similarity as checked by iThenticate software is 3%.

Anindita Kundu

Signature of Candidate:

Date: 15/05/2024

15/5/24

Certified by Supervisor:

(Signature with date, seal)

Prof. (Dr.) Subhash C Mandal
Professor
Dept. of Pharmaceutical Technology
Jadavpur University
Kolkata-700 032, India

15/5/24

Certified by Supervisor: Empowered by DC JU

(Signature with date, seal)

Prof. (Dr.) Amalesh Samanta
HOD
Dept. of Pharmaceutical Technology
Jadavpur University
Kolkata-700032, India

CERTIFICATE FROM THE SUPERVISORS

This is to certify that the thesis entitled **"Phytochemical and Pharmacological Investigation of Important Volatile Principles from Traditionally Used Medicinal Plant(s) to be Useful for the Treatment of Different Ailments"** submitted by **Smt. Anindita Kundu** who got her name registered on **11/08/2017** for the award of Ph.D. (Pharmacy) degree of Jadavpur University is absolutely based upon her thesis nor any part of the thesis has been submitted for any degree/diploma or any other academic award anywhere before.

15/5/24

Signature of the Supervisor

and date with Office Seal

Prof. (Dr.) Subhash C Mandal
Professor
Dept. of Pharmaceutical Technology
Jadavpur University
Kolkata-700 032, India

15/5/24.

Signature of the Supervisor

and date with Office Seal

Empowered by Jadavpur University

Prof. (Dr.) Amalesh Samanta
HOD
Dept. of Pharmaceutical Technology
Jadavpur University
Kolkata-700032, India

ACKNOWLEDGEMENTS

This project work was carried out at the Division of Pharmacognosy, Department of Pharmaceutical Technology, Faculty of Engineering and Technology, Jadavpur University. I want to show my sincere gratitude to all who have directly or indirectly contributed their ideas and energies in successful completion of my project.

I would like to express my sincere gratitude to my supervisor Prof. S. C. Mandal, Pharmacognosy and Phytotherapy Research Laboratory, Division of Pharmacognosy, Department of Pharmaceutical Technology, Jadavpur University, for leading this project from start to goal. I am indebted to him for enlightening me on the finer skill of dealing with problems. I attribute the level of my Ph.D. degree to his encouragement and without him this thesis would have not been completed. Besides my supervisor, I would like to express my sincere gratitude to Prof. Mahadeb Pal, Division of Molecular Medicine, Bose Institute, Kolkata, India. His helpful nature, clear leadership and provision of fearless work environment will be cherished in all walks of my life. I would like to convey my sincere gratitude to my Ph.D. Research Committee members, Prof. Sanmoy Karmakar, Prof. Amalesh Samanta, Prof. Pallab Kanti Haldar, Associate Prof. Saikat Dewanjee, Prof. Achintya Saha for sharing their insightful comments and valuable advices on my research work. I am also thankful to Dr. Vivekananda Mandal for support my research work in many ways.

Let me also thank to my seniors Dr. Partha Pratim Maiti, Dr. Sayani Majumder, Dr. Harun Al Rashid for their sincere helps in these years. My thanks also go to my juniors, especially Siddheswar Maiti, Debapratim Chakraborty, Camelia Biswas, Arunava Sen, Milan Tirtha Mete, Sourav Paul, Sanheeta Chakraborty for their support during my research work.

I consider myself blessed for my parents who are the most important pillar of my life and my greatest strength. Their unconditional love, support, effort always helps me to achieve my goals and stay strong through all my hardship. I wish to express my gratitude to my grandparents who provided me all the possibilities for education, always encouraged and believed in me and most importantly imbibed the values that made me the person what I am today. My special and deepest gratitude to my paternal grandfather, Late Dr. K. C. Kundu for his love, encouragement and inspiration to go further and do well and which eventually drove me to pursue a Ph.D. I dedicate my thesis to him. I consider myself fortunate for getting constant support and encouragement from my husband all through my candidature. I would also like to thank my relatives who have been a source of support and encouragement and helped me to achieve my goal and complete my dissertation work successfully.

I would like to convey my gratitude to Botanical Survey of India, for identifying the plant specimen for my research work. My gratitude also goes to Department of Biotechnology, Govt. of India for providing me the financial assistance during my research work.

Place: Jadavpur University, Kolkata.

Date: 15/05/2024

Anindita Kundu

Anindita Kundu

Dedicated

to my

Grandfather

Content

Page No.

Preface

Natural products are rich in bioactive constituents that serve as a reservoir of the resources of medicines. In recent times, the use of natural products are increasing progressively over the world and potentiating their use in the traditional healthcare system. Since the early time of civilization, natural products, especially the herbal medicines have been used to maintain and promote the health status and today, the medicinal herbs contribute to formulate the modern medicines as well. The plant derived medicines have gained popularity because of higher efficiency and level of safety, less abusiveness like side effects or adverse effects than the synthetic drugs. Most of the people are not knowledgeable enough about the traditional medicine and ecology indicating the non-toxicity of the natural products. So, the scientific study is required to justify their traditional uses and to establish the plants as the possible drug candidates in near future.

Vitex negundo (Linn.) is commonly used in folk medicines and widely distributed in India, Pakistan, Afghanistan, Sri Lanka, Thailand, Malaysia, eastern Africa and Madagascar especially in moist places. The plant is also commercially cultivated in some regions of Asia, Europe, North America and West Indies. However, all parts of *V. negundo* are used to treat different ailments, but leaves are strongly effective for the medicinal uses.

This research work aimed to explore the comparative potential of the organic extract and essential oil of *Vitex negundo* leaves. A comparative study of the bioactive compounds that were obtained from the methanol extract and essential oil of *V. negundo* leaves was also accomplished. The leaf extracts were investigated to explore the composition by using Gas chromatography mass spectrometry. And the result revealed the presence of several volatile organic compounds such as isocaryophyllene, caryophyllene, β-myrcene, phytol, oleic acid, 2-myristynoyl-glycinamide, camphor, γ-sitosterol, β-sitosterol, asarone and so on. The leaf

extracts were also evaluated for their antioxidant potentials by using different assay methods. And the research findings showed the stronger antioxidant potential of methanol extract than the leaf essential oil. The leaf extracts also quantitively evaluated for the bioactive compounds such as tocopherol, β-sitosterol and polyphenols. Result demonstrated the presence of higher amount of tocopherol, β-sitosterol and polyphenols in methanol extract than the essential oil. The antibacterial properties, anti-inflammatory and the anticancer potentials were also studied by using different appropriate *in-vitro* methods. For all the cases, the methanol extract exhibited the higher inhibitory potentials than the leaf essential oil. The different parts of the plant have been using traditionally to manage the various ailments. But the comparative potential of leaf organic extract and leaf essential oil was not studied before. Here, as the anticancer and antibacterial activities were checked on the human prostate cancer cells and the UTI pathogens respectively. So, the research findings also correlate the antibacterial, anti-inflammatory and anticancer potentials and suggest that the significant reduction of UTI pathogens could be helpful to lower the risk factors of subsequent development of prostate cancer. Therefore, the research findings justify its traditional use, which develops a future substantial value of this plant into the scientific discipline

List of abbreviations

AA - Arachidonic acid

ABTS - 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)

ADT- Androgen deprivation therapy

ALT - Alanine transaminase

AP-1 - Activator protein 1

AR - Androgen receptor

AST - Aspartate aminotransferase

ATCC - American type culture collection

CAT - Catalase

CCND1 - Cyclin D1

COX - Cyclooxygenases

DMEM - Dulbecco's modified eagle medium

DMSO - Dimethyl sulfoxide

DPPH - 2,2-diphenylpicrylhydrazyl

DRG -Dorsal root ganglion

EID - Emerging infectious disease

ELISA - Enzyme-linked immunosorbent assay

EO - Essential oil

ERK - Extracellular-signal-regulated protein kinase

ETV3 - ETS variant transcription factor 3

GC-MS - Gas chromatography-mass spectrometry

GPx - Glutathione peroxidase

GRB2 - Growth factor receptor bound protein 2

GSH - Glutathione

H_2O_2 - Hydrogen peroxide

HO-1 - Heme oxygenase-1

HPLC - High Performance Liquid Chromatography

ICAM - Intercellular adhesion molecule

IGF-1 - Insulin-like growth factor-1

IL - Interleukin

iNOS - Inducible nitric oxide synthase

JNK - Jun N-terminal kinase

LDH - Lactate dehydrogenase

LPO - Lipid hydroperoxide

LPS - Lipopolysaccharide

MAE - Microwave assisted extraction

MTT - 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide

NF-κB - Nuclear factor kappa B

NLRP3 - Nucleotide-binding domain (NOD)-like receptor protein 3

OA - Oleic acid

PBS - Phosphate-buffered solution

PC-3 - Prostate cancer cell line

PCa - Prostate cancer

PPAR-γ - Peroxisome proliferator- activated receptor-γ

PRDX2 - Peroxiredoxin 2

PSA - Prostate-specific antigen

ROS - Reactive oxygen species

STAT3 - Signal transducer and activator of transcription 3

TFC - Total flavonoid content

TNF - Tumor necrosis factor

TP53 - Tumor protein 53

TPC - Total phenolic content

TRAIL - TNF-related apoptosis-inducing ligand

TRPA1- Transient receptor potential ankyrin 1,

TRPV1 - Transient receptor potential cation channel subfamily V member 1

UTI - Urinary tract infection

VCAM-1 - Vascular cell adhesion molecule-1

VEGF - Vascular endothelial growth factor

VOC - Volatile organic component

ZFHX3 - Zinc finger homeobox 3

List of tables

List of figures

Chapter 1

Introduction

1. Introduction

Natural products are rich in bioactive constituents that serve as a reservoir of the resources of medicines. In recent times, the use of natural products are increasing progressively over the world and potentiating their use in the traditional healthcare system. Since the early time of civilization, natural products, especially the herbal medicines have been used to maintain and promote the health status and today, the medicinal herbs contribute to formulate the modern medicines as well. The plant derived medicines have gained popularity because of higher efficiency and level of safety, less abusiveness like side effects or adverse effects than the synthetic drugs. Most of the people are not knowledgeable enough about the uses of traditional medicine and ecology indicating the non-toxicity of the natural products (Rates, 2001).

Several ethnic communities across the globe have well known regarding the benefits of a specific herb from their progenitor's belief system and practices or through the knowledge of the aboriginal peoples (Heinrich et al., 2004). That system later contributed to the exploration of a new drug molecule in the treatment of various ailments. Although, some specific limitations should be considered before choosing a particular plant for the pharmacological investigations because different ethnic communities have their different understandings about the health promotion, disease prevention, chronic disease management and proactive approach for healthcare and wellbeing (Elisabetsky and Posey, 1986). Investigation for the promising potential herbs should be accomplished by the chemotaxonomic approach. The plants belong in the same group also possessing the similar bioactive compounds structurally and functionally as well. This helps to give an idea when the unknown compound is selected for its biological examination and whose chemical structure resembles the known chemical compound (Heinrich et al., 2004).

Phytoconstituents obtained from the various plant foods or medicinal plants have got identification for having the crucial role in management of various disease states (Neergheen et al., 2010). In the next step, the isolation and characterization of chemical compounds take place. In modern methods of drug discovery the chromatographic techniques have been widely employed to standardize the plant derived drugs intending for identifying and quantifying the presented biomarkers (Balunas and Kinghorn, 2005). Plant materials are subjected for the extraction and biological testing respectively and then starting the process for finding out and characterizing the bioactives accountable for exhibiting the desired pharmacological activities. Medicinal plants are the large resources for the bioactive phyto-compounds that have the power to combat several illnesses.

The American Society of Pharmacognosy described that pharmacognosy deals with the investigation of the physical, chemical, biochemical and biological properties of the crude drugs and searching for new drug compounds from the origin of nature (Balunas and Kinghorn, 2005). If any new molecule is not identified in the process of drug discovery, then the previously identified compound accompanied by the new biological property could be considered as the major drug lead compound.

Ethnopharmacology deals with the developmental role of conventional medicine. Out of total prescribed medicines, 25% of prescriptions are composed with the plant medicines, 121 plant derived bioactives are used presently and 252 compounds are counted as fundamental and crucial by the whole world. According to the World Health Organisation (WHO) large numbers of synthetic drug molecules come from natural sources (Rates, 2001). WHO also declared that three-quarters of peoples in the world depend on the traditionally used medicinal plants for health promotion or to manage the different diseases. In fact, WHO represents phytotherapy in the health programs and recommends fundamental processes for

the confirmation of drugs of plant sources in advanced countries (Vulto & Smet, 1998). Plants are being used in the therapies by different fashions such as in home remedies, pharmaceutical formulations like tinctures, fluid extracts, powders, pills and capsules etc (Rates, 2001).

Herbal medicines are also known as traditional medicines or natural medicines and mostly utilized by various civilizations and cultures as well since the primitive age has been initially explored by continuous investigation of clinical restorations and experiences of the native people. Although, the use of folklore medicinal plants may depend on the geographic locations due to having the difference in environments that influence the potential of the active constituents (Gilani and Rahman, 2005).

1.1. Prostate cancer

Prostate cancer (PCa) has been regarded as the second most common non-cutaneous solid cancer behind the prevalence of lung cancer and fifth leading cause of male cancer mortality worldwide. Since the last decades the cases of prostate cancer have been rising substantially (Crawford, 2003). The disease generally develops in elderly men who have crossed 55 years. Above 65 years of age are 65% more susceptible to prostate carcinoma, among these 25% of people are over 75 years (Fitzpatrick, 2008). The risk factors of PCa include the inheritance of the malignant genes, leading unhealthy lifestyle and moreover the consuming improper diet rich in red or processed meat, sugar, fatty dairy products, refined grains, and fewer fruits and vegetables. It has already been explored that western dietary patterns play a crucial role in increasing the incidences of carcinoma in western world but in addition a disproportion between the risk factors and protective factors also contribute to the development of PCa in the Southeast and East Asian countries which are the least affected countries in the world (Torricelli et al., 2011). Nowadays, the management of PCa comprises active surveillance at

its early phases and afterwards the surgery and androgen deprivation therapy (ADT). Androgen plays a key role for building up the structure and proper functioning of the prostate gland and the hormone is also involved in initiation and propagation of PCa (Huggins and Hodges, 1972). At the initial stage of treatment the ADT is remarkably effective but within two years it may gradually develop androgen independent to castration resistant propagation of PCa which implies a poor improvement and creates an urge for chemotherapy or any other adjunct treatment (Nurgali et al., 2018) . After achieving the significant success rate, the PCa therapy can hamper the viability of normal cells, or may show non-specificity in the field of treatments that brought about a great impact on the patients receiving PCa therapies. These limitations created an urgent demand for designing drug-targeted therapies. Medicinal plants have gained attention since the primitive era on account of their therapeutic potential against several diseases and cancer as well. Research results encourage the uses of different plant extracts with their novel protective and preventive actions towards the aggressiveness of fatal illness as PCa (Cragg and Newman, 2013).

1.1.1. Epidemiology

PCa is the second most common malignancy and the sixth leading cause of carcinoma related death across the world. In 2018, 1.276 million new incidences and 0.359 million death cases were estimated. PCa is found as fourth frequently detected carcinoma next to lung, breast, and colon carcinoma, and eighth most cause of death of advanced aged men and women as well. Continuation of this trend will lead to increase the PCa incidences of 2.2938 million in 2040. PCa is very commonly diagnosed over the 50% (105 of 185) of countries in the world and mortality rate observed in some countries as follows: Southern Africa (26.4%), Caribbean (25.4%) and middle Africa (22.4%). However, prostate cancer also very commonly affects the male populations in the UK and USA (Bray et al., 2018).

1.1.2. Etiology

Possible reasons of PCa are not yet completely understood. Some factors like old age, race, ethnicity, and geographic location have an impact on development of PCa. For instance, African-American, African-Caribbean is much more vulnerable in the field of forming the disease than Asian-American or Hispanic/Latino male humans. The advanced countries including North America, North-Western Europe, Australia, New Zealand have an aggressiveness to grow up the PCa. The autopsy data disclosed that the maximum cases of PCa were seen in the African population and whereas the minimum cases were observed in the Asian population (Jalloh et al., 2013). Markedly, the levels of prostate-specific antigen (PSA) were found to be in higher amounts in African black men as compared to the white men (Vijayakumar et al., 1998). Genome-wide association study (GWAS), candidate gene association reports and results of family history investigation stated the connection of numerous biomarkers with prostate malignancy. Chromosomes 8q24, 12q24, and 1q24-25 were identified for increasing the risk for PCa (Cheng et al., 2008). In the case of African-American men, the major causes for PCa death are ZFHX3, TP53 gene mutation, focal deletion in ETV3 gene and MYC gene amplification. Truncating mutations in KMT2D gene and CCND1 gene amplification had been detected for developing primary and localized PCa in African-American men (Koga et al., 2020). Poor nutrition and stress also positively contribute to rising disease risks (Chan and Giovannucci, 2001).

Research data indicates that the obese persons are more prone to form PCa. Irregular secretion of different hormones is closely related to obesity, like increased levels of estradiol, insulin, free insulin-like growth factor-1 (IGF-1), leptin and decreased levels of free testosterone, adiponectin promote the invasiveness of PCa (Buschemeyer and Freedland, 2007). Meta-analysis reported the possibilities of forming PCa from genetic inheritance is 5-

10% (Watkins Bruner et al., 2003). PCa is highly linked with the familial background such as one man has the high chance to get PCa if his father or brother is the patient of PCa. The risk of PCa can be increased with the mutations of breast cancer susceptibility gene 1 (BRCA1) and breast cancer susceptibility gene 2 (BRCA2) as well (Castro and Eeles, 2012). Genetic predisposition for PCa is connected with some of the genes such as DNA damage repair genes like CHEK2, ATM, PALB2, and RAD51D, DNA mismatch repair genes like MSH2, MSH6, MLH1, and PMS2 and tumor suppressor gene like RNASEL (Xiang et al., 2003). PCa can pass onto offspring when mutations occur in ELAC2 gene which promote proliferation via TGF-β signaling cascades and HOXB13 gene having role in maturation of prostate glands (Noda et al., 2006). Deletion mutation in the region of Xq26.3-q27.3 of the X chromosome retaining the androgen receptor (AR) gene has been referred for getting hereditary PCa (Xu et al., 1998). Any change in AR gene expression profile includes AR gene amplification, over expression of androgen, various AR splice variants and co-regulators linked with the AR therapy resistance or castration resistant prostate cancer (CRPC). In addition, WNT signal transductions also have a regulatory role mostly in the last phase of PCa like CRPC (Murillo-Garzón and Kypta, 2017). Deregulation of WNT signaling pathways can cause the growth and progression of PCa. The two types of WNT signaling pathways such as canonical (β-catenin dependent) and non-canonical (ß-catenin-independent) WNT signaling pathways revealed their participation in pathogenesis of PCa. Canonical WNT pathway exerts its activity through enabling some specific genes implicating ß-catenin-TCF/LEF1 transcription complexes (Komiya and Habas, 2008). On the other hand, the non-canonical WNT pathway is related with the late phase of CRPC controlling genes that influenced the cell adhesion and migration, cell survival, and angiogenesis (van Amerongen, 2012). Up-regulated LEF1 gene, ß-catenin-TCF-LEF1 transcription complex correlated with CRPC and in contrast, up-regulation of ETS-regulating gene (ERG) is associated with

progression and metastasis of PCa (Wu et al., 2013). Over expressed AR enhances the activities of WNT- β-catenin target genes played a major role in metastasis of PCa and that is because of activation of the β-catenin-TCF-LEF1 transcription complex dependant gene expressions (Lee et al., 2015). Combinedly, genomic alteration and the WNT pathways contribute to invasiveness of PCa cells. Apart from this, this WNT signaling pathway is also involved in immune evasion of PCa cells. Various reports revealed the involvement of tumor microenvironment (that produces WNT ligands) in forming the PCa and other different cancers. The WNT family members are released by stromal cells and display a crucial role in tumor growth, development, and drug resistance of PCa (Murillo-Garzón and Kypta, 2017).

Chronic inflammation leads to develop 20% of cancers or more than that (De Marzo et al., 2007) and several investigations proved that 80% of specimens of prostate biopsy carry the molecular signature for inflammation (Sfanos and De Marzo, 2012). Particularly, altered gut microbiome structure has relation with PCa. If the bacterial infection in the prostate persists for a long time, then it can cause inactivation of tumor suppressor, a homeobox protein NKX3.1 which cooperates with DNA repair mechanism. The inflammatory cells generate ROS and concurrently impaired the enzymatic activities for repairing the DNA damage. Chronic inflammation is closely connected with the AR signal instigating DNA damage by employing DNA topoisomerase 2β and inactivating the DNA damage repair genes (de Bono et al., 2020).

1.2. Inflammation

Balanced inflammatory response is an evolutionary conserved and protective mechanism of the mammalian body to defend against various insults like stress, injury, infection and to clear the localized deposition of unwanted metabolites and dead or damaged cells (Medzhitov, 2010). With removal of the noxious stimuli, the host immune system

simultaneously triggers the wound healing process. Acute inflammation is the first response against the detrimental substances and is noticed by frequent movement of plasma and innate immune system cells, such as movement of neutrophils and macrophages from the bloodstream to the damaged cells. Chronic inflammation is the response against inflammasomes and persists for a longer period of time and causes gradual alteration in cells located at the reaction site. Also it can cause the concomitant demolition and restoration of the wounded tissue. Apart from the stimulating factors, some of the common signs of inflammatory responses are high blood flow, increased level of cellular metabolism, vasodilatation, secretion of soluble mediators, extravasations of fluids and cellular influx (Ferrero-Miliani et al., 2007). The inflammatory substances activate the arachidonic acid pathway that subsequently release several chemical mediators including cytokines, serotonin, histamine, prostaglandin and leukotrienes which increases the vascular permeability and promotes leukocytes immigration in local area of inflammatory reactions (Dassoler et al., 2004). Mounting evidence indicates that the inflammation process is associated with a complex system rather than the only involvement at the molecular state. A wide variety of inflammations have been detected which are triggered by diverse molecules and controlled by regulatory systems. The pervading nature of inflammation creates a great impact to develop numerous diseases such as obesity, diabetes, arthritis cancer, Meniere's disease, stroke, asthma, autoimmune encephalomyelitis, glomerulonephritis, myasthenia gravis, inflammatory bowel disease, aging, hepatitis, retinopathy and cardiovascular diseases. Impaired regulation of inflammatory reactions is accountable for progression of several chronic diseases. Altered regulatory processes and failure of self-limiting function of immune cells can cause chronic inflammation. The cellular level of inflammatory responses is linked with the canonical pathway which commences with identification of pathogenic pattern, and commonly known as pathogen associated molecular pattern (PAMP) or even any residue of

injurious tissue, habitually called as damage associated molecular pattern (DAMP) by the pattern recognition receptors (PRRs). Generally, the different classes of receptors such as toll like receptors (TLRs), NOD like receptors (NLRs), c-type lectin like receptors (CLRs), and triggering receptors protein expressed on myeloid cells (TREM) are involved in this process (Sancho and Reis e Sousa, 2012). Ligand-receptor interaction enables the signaling pathways, in turn triggers the transcription factors including nuclear factor kappa B (NF-κB), signal transducers and activators of transcription 3 (STAT3), activator protein 1 (AP1) and related target genes that include different cytokines like tumor necrosis factor α (TNF-α), IL-8 and IL-6 (Tabas and Glass, 2013). Inflammatory process is associated with release of several cytokines, chemokines (TNF-α, IL-1β, COX-2, IFN-γ) and immune cell mobilization. However, the positive feedback loop enhances the reactions of immune cells.

Molecular level defense response depends upon the activity of resident immune cells including eosinophils, monocytes, natural killer cells, macrophages that exist in tissues which remain suppressed in normal state. At the first stage of the defense mechanism, the neutrophils get activated and subsequently instigate the migration of monocytes and leukocytes. After reaching the damaged area the monocyte further differentiates into macrophages and destroys the pathogens and gets ready to release several cytokines and chemokines including TNF-α, IL-1β and IL-8. When the leukocytes reach the local site of action, the mast cells produce the pro-inflammatory mediators such as histamine, and matrix remodeling enzymes like proteases. The migration process includes the activities of various surface proteins like E-selectin on the leukocytes with various adhesion molecules like intercellular adhesion molecule (ICAM) on the endothelium. After emigrating the site leukocytes generate reactive oxygen species (ROS) and proteases that eventually help to kill the pathogens. Due to having the non-specific cytotoxic properties, these molecules can cause harm to the host tissues which are reconditioned at the resolution phase. Finally the resolution

phase is accomplished only after reinstallation of structure of the normal tissue and vasculature (Pal et al., 2014; Tabas and Glass, 2013). Resolution stage includes elimination of pathogens, cell debris, removal of immune cells, pro-inflammatory cytokines, up-regulated IL-10 which is produced by regulatory T cells (T-reg) and nuclear receptors. Also IL-13 contributes in the resolution phase by generating the anti-inflammatory cytokines such as IL-10 and TGF-β (Nathan and Ding, 2010). 5- and 15-Lipoxygenase signaling cascades involved in the resolution phase by releasing some mediators like lipoxins, and resolvins. Heme oxygenase 1 (HO-1) lowers the inflammatory effects by eradicating pro-inflammatory molecule heme and producing the CO and bile pigments like biliverdin and bilirubin (Durante, 2011). This CO inhibits the lipopolysaccharides (LPS) stimulated TNF-α synthesis. Apart from this, CO also involves generating IL-10 from murine macrophages mediating through heme oxygenase 1 (Sheikh et al., 2011). Then IL-10 suppresses the synthesis of pro-inflammatory cytokines by stopping the activity of NF-κB principally mediated through STAT3. Innate inflammatory reaction is closely associated with inflammasomes that has a potential role in synthesis of IL-1β and IL-18 against the pathogenic attack and wounded tissue which gradually leads to developing a chronic disease (Schroder and Tschopp, 2010). Several mechanistic actions help to enable the resolution process. However, different disorders prevent activation of the resolution pathway and eventually develop a chronic inflammation that subsequently increases the risk factors for different diseases. ROS play a major role to activate the NF-κB pathway which is mostly related to the chronic inflammatory disease conditions. Generally, ROS is generated from the activated macrophages and electron transport chain of mitochondria. ROS could be worked as the signaling molecule or an important factor for inflammatory and anti-inflammatory reactions. Excessive ROS production induces the activation of NOX2 (NADPH oxidase 2) that are closely related to some inflammatory target proteins including matrix metalloproteinase-9 (MMP-9), vascular cell adhesion molecule-1 (VCAM-1), and cyclooxygenase-2 (COX-2)

(El-Benna et al., 2009). Superoxide free radicals and manganese superoxide dismutase (SOD) interacts in mitochondrial matrix and produces hydrogen peroxide that initiates the NF-κB signaling pathway and activates the pro-inflammatory cytokines, and inflammasomes in cytosol (Mittal et al., 2014).

1.3. Bacterial pathogenesis

In 1928, penicillin discovery changed the global scenario by helping mankind enormously in the field of medicine and it was believed that the penicillin could easily cure the bacterial infections (Lewis, 2013). But from 1950, the society was suffered with emerging and reemerging infectious diseases (EIDs) that significantly hampered the community health status. For instance, in 2010, the physicians were struggling to control a peculiar clinical condition which was accompanied with unbearable inflammation like transient ischemic attacks or venous thromboembolisms. At that time, it was diagnosed that the patients having either haematologic malignancies or autoimmune diseases (Grankvist et al., 2014) but not even the presence of any microbe. None of the patients was responded even after getting treated with the several antibiotic regimens. Finally, the presence of *Neoehrlichia mikurensis* was observed and it was closely related to the *Ehrlichia* spp., and the human ehrlichiosis. Then the infection was rapidly responded with the treatment of doxycycline. Basically, EID disseminated quickly in mankind in terms of events or geographical locations. After that EIDs were considered as the serious microbiologic threat for the public health (Lederberg et al., 1992). Since the last 20 years, health care system have been dealt with the problem of multidrug-resistance pathogens including vancomycin-resistant enterococci, extended-spectrum β-lactamase *E. coli*, methicillin-resistant *Staphylococcus aureus*, multidrug-resistant or extensively resistant tuberculosis and carbapenemase-encoding Gram negative bacteria. The rapid transmission demanded significant attention to control those emerging pathogens. In 2001, after the anthrax attack, the research laboratories were also paid an attention in

development of bioterrorism and virulent bacterial strains. In some cases, the atypical syndromes because of invasive bacteria made the populations vulnerable; for instance the lethal bacillary angiomatosis caused by *Bartonella henselae* (Barras and Greub, 2014).

As per the research data of last 30 years or more, the lists of immune-compromised patients have been increased. However, the advancement of medical science increased the survival rates of the patients having carcinoma, chronic diseases like diabetes, renal impairment, HIV, aging, autoimmune diseases or transplant therapies. Furthermore, the invasive bacterial diseases like endocarditis or sepsis caused by non-diphtheria *Corynebacterium* spp. were also included (Funke et al., 1997). Those pathogens are the usual inhabitant of mucosal layer and skin and can disturb the treatment by developing the multidrug resistance (Funke et al., 1997; Miguel-Martinez et al., 1996). The patient got infected from their environment and ultimately could develop septicaemia in splenectomized (Lion et al., 1996).

Among the offensive infections the urinary tract infections (UTIs) are the most common and could become serious if untreated for a time. Any age group of peoples including women, children and old age could get affected by a wide spectrum of bacterial strains including Gram-negative and Gram-positive bacteria or fungi (Flores-Mireles et al., 2015). Mostly they reside in the catheters, urinary tract and responsible for complicated or uncomplicated UTIs. *S. aureus, Enterococcus* spp., *K. pneumoniae, Candida* spp., *S. saprophyticus, P. mirabilis, P. aeruginosa, E. faecalis,* and *S. aureus , P. aeruginosa, Candida* spp reported for the reason for complicated and uncomplicated UTIs respectively. All the strains have the diverse sets of virulence factors and invasive potentials. Among these strains *P. mirabilis* invades the host cells in urinary tract by forming a colony, stones, fimbriae, adhesions, biofilm, protease, toxins and acquisiting the iron and zinc. *E. coli* attaches with the host epithelial cell by using the pili, forms the colony and affects badly the urinary system.

1.4. Plant profile

1.4.1. Taxonomical classification

Kingdom: Plantae

Order: Lamilales

Family: Verbenaceae

Genus: Vitex

Species: *Vitex negundo* L.

1.4.2. Habitat of *Vitex negundo*

Vitex negundo grows in humid areas or wetland and in mixed forests of several countries such as India, Pakistan, Afghanistan, Sri Lanka, Thailand, Malaysia, eastern Africa and Madagascar. The plant is also commercially cultivated in some regions of Asia, Europe, North America and West Indies. *Vitex negundo* is considered as a source of edible substances and wood as well. The aromatic plant is also widely found in parts of India, mostly 1500 m elevated areas from sea-level, in the outer Himalayan region (Khare, 2004).

Figure 1.1. Herbarium of *Vitex negundo* plant with inflorescence

1.4.3. Morphology of *Vitex negundo*

Vitex negundo is a woody, aromatic deciduous shrub having quadrangular, densely whitish, tomentose branchlets. *Vitex negundo* is commonly called the five-leaved chaste tree or chinese chaste tree or horseshoe vitex or nisinda or monk's pepper. The most salient characteristic of this plant is the specific arrangement of five pointed leaves that form a group at the central point and has a closest resemblance to plam. Height of the *Vitex negundo* is usually 2-5 m and leaves are lanceolate, acute and glabrous, 4-10 cm prolonged, pointed at two ends and possesses hair at the underneath of leaves. Colour of the flowers are bluish purple carried on axillary or terminal panicles upto 30 cm extended (Figure1.1.). Marginal leaflets possess a long petiole while the lateral leaflets bear the short petiole. Fruits are juicy, pulpy and globular in shape, becoming black in colour after ripening. It has four seeds in a round shape and has a diameter of 4 mm (Meena et al., 2011).

1.4.4. Phytochemical studies of *Vitex negundo*

Different parts of *Vitex negundo* contain a wide variety of phytoconstituents which are frequently used as bioactive constituents to prepare the pharmaceutical formulations. It was reported that the plant *Vitex negundo* is the source of ample amounts of secondary metabolites including steroids, triterpenoids, carbohydrates, alkaloids, phenolic compounds, saponins, xantho proteins, tannins, flavonoids (Sahayaraj et al., 2008).

The phytoconstituents have been identified from the leaf extracts of *Vitex negundo* are mentioned as follows: i.e. hydroxy-3,6,7,3′,4′-pentamethoxyflavone, 6′-p-hydroxybenzoyl mussaenosidic acid, 2′-p-hydroxybenzoyl mussaenosidic acid, protocatechuic acid, oleanolic acid, flavonoids, 5, 3′-dihydroxy-7,8,4′-trimethoxyflavanone, 5,3′-dihydroxy-6,7,4′-trimethoxyflavanone; viridiflorol, β-caryophyllene, sabinene, 4-terpineol, gamma-terpinene, caryophyllene oxide, 1-oceten-3-ol, globulol, angusid, casticin, vitamin-C, nishindine, gluco-

nonitol, phydroxybenzoic acid, sitosterol, betulinic acid [3β-hydroxylup-20-(29)-en-28-oic acid], ursolic acid [2β-hydroxyurs-12-en-28-oic acid], n-hentriacontanol, β-sitosterol, p-hydroxybenzoic acid and some of the compounds have been isolated from the seed are as follows: 3β -acetoxyolean-12-en-27-oic acid, 2α, 3α-dihydroxyoleana-5,12-dien-28-oic acid, 2β,3α diacetoxyoleana-5,12-dien-28-oic acid, 2α, 3β-diacetoxy-18-hydroxyoleana-5,12-dien-28-oic acid, vitedoin-A, vitedoin-B, a phenylnaphthalene-type lignan alkaloid, vitedoamine-A, β-sitosterol, p-hydroxybenzoic acid, 5-oxyisophthalic acid, n-tritriacontane, n-hentriacontane, n-pentatriacontane, n-nonacosane, 6-hydroxy-4-(4-hydroxy-3- methoxy-phenyl)- 3-hydroxymethyl-7-methoxy-3, 4-dihydro-2-naphthaldehyde (Basri et al., 2014).

Essential oil of fresh leaves, flowers, dried fruits of *Vitex negundo* contain the following phytochemicals such as - δ-guaiene, guaia-3,7-dienecaryophyllene epoxide, ethylhexadecenoate, α-selinene, germacren-4-ol, caryophyllene epoxide, (E)-nerolidol, β-selinene, α-cedrene, germacrene D, hexadecanoic acid, p-cymene and valencen and root extracts possess the 2β, 3α-diacetoxyoleana-5,12-dien-28-oic acid, 2α,3α-dihydroxyoleana-5,12-dien-28-oic acid, 2α,3β - diacetoxy-18-hydroxyoleana-5,12-dien-28-oic acid, vitexin and isovitexin, negundin-A, negundin-B, (+)- diasyringaresinol, (+)-lyoniresinol, vitrofolal-E and vitrofolal-F, acetyl oleanolic acid, sitosterol (Basri et al., 2014).

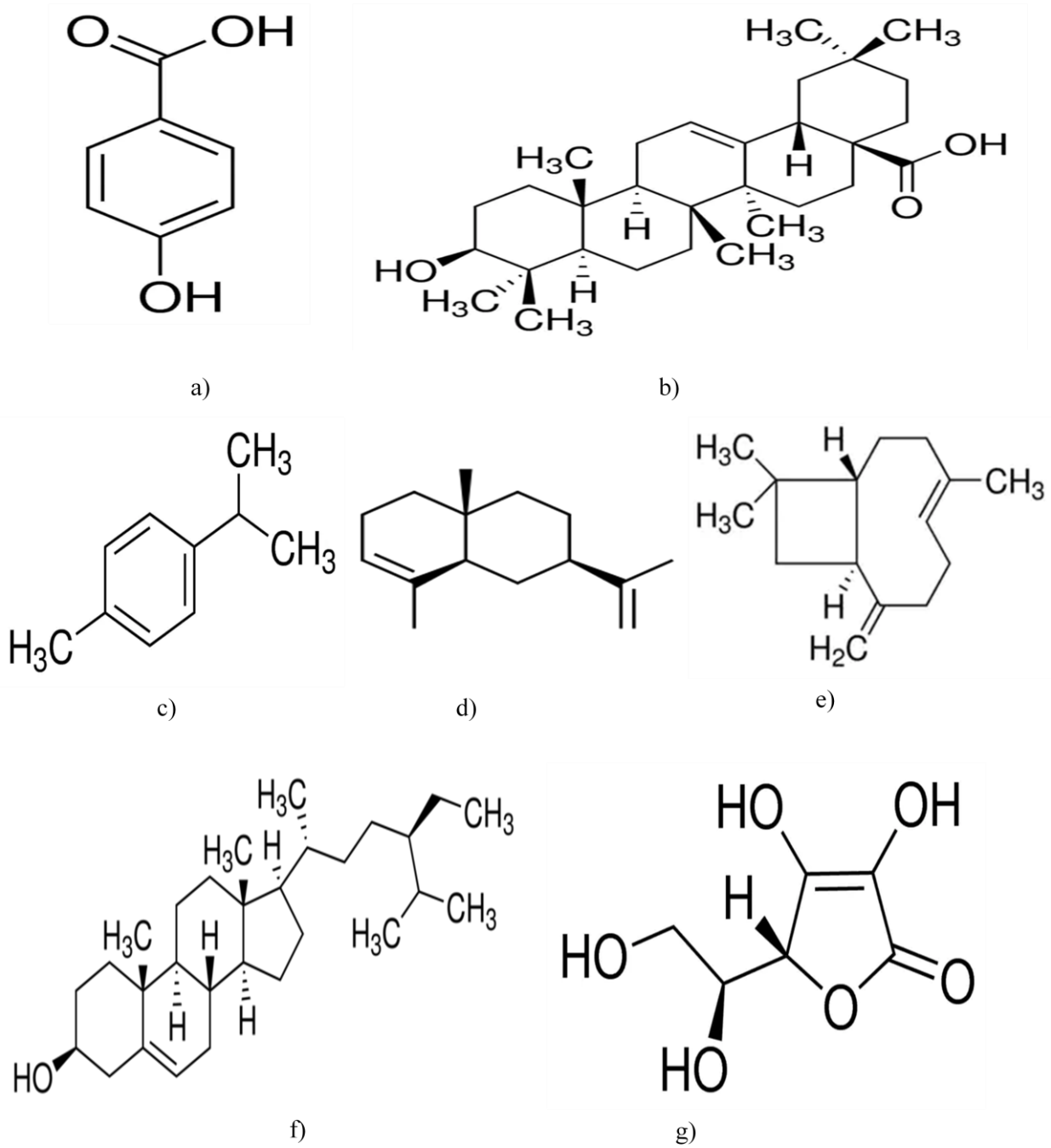


Figure 1.2. Chemical structure of some bioactive compounds such as a) 4-hydroxybenzoic acid, b) Oleanolic acid, c) P-Cymene, d) *α*-Selinene, e) *β*-Caryophyllene, f) *β*-sitosterol, g) Ascorbic acid which were isolated from the *Vitex negundo* leaves.

1.4.5. Taxonomical classification

Kingdom: Plantae

Order: Malvales

Family: Malvaceae

Genus: *Hibiscus*

Species: *Hibiscus rosa-sinensis* L.

1.4.6. Habitat of *Hibiscus rosa-sinensis*

The stunning flowering plant Hibiscus rosa-sinensis, often known as the "China rose" or "Queen of tropics," is mostly found in south-east China as well as several islands in the Pacific and Indian Oceans (Missoum, 2018).

Figure 1.3. Photograph of *Hibiscus rosa-sinensis*

1.4.7. Morphology of *Hibiscus rosa-sinensis*

Hibiscus rosa-sinensis is a big shrub with an uneven structure. It can be wide and dispersing or upright. The tree reaches a maximum height of 4.7 meters. Every kind of hibiscus flower includes stamen stalks, which can produce pollen. Hibiscus blossoms come in a huge red variation that may reach a length of 15cm. Depending on the cultivated kinds, the petals can have a single or double structure, and they can be smooth or scalloped. The pollan-producing anther is visible halfway up the column, and at apex of the column are five rounded stigma lobes. The Hibiscus plant has ovate-shaped leaves that are grouped alternately on the branches and grow to a length of 5 to 15 cm (Figure 1.2.). The leaves are broader at the base than at the tip. The leaves have serrated borders with lighter areas, and they can be either dark green or variegated. The red hibiscus fruit is a dry, five-segmented capsule that can hold up to three kidney-shaped, 2.5 cm. long seeds (Khristi and Patel, 2016).

1.4.8. Phytochemical studies of *Hibiscus rosa-sinensis*

Different parts of *H. rosa-sinensis* contain a wide variety of bioactive compounds. Research reports revealed that the leaves, flowers, stem and roots are rich in saponins, flavonoids, phlobatannins, terpenoids, riboflavin, thiamine and niacin. Furthermore, the flavonoids, phytosterols, glucosides, tannins, terpenoids and phenolic compounds were also recognised and having significant contribution in management of the various state of disorders. Flowers of *H. rosa-sinensis* contain moisture, nitrogen, fat, crude fibre, calcium, phosphorus, and iron which are having some nutrition values. GC-MS report of methanol flower extract revealed the presence of ethanimidic acid, propanal, 2,3dihydroxy propanamide, n-ethyl-ethylenediamine, o-methylisourea hydrogen sulfate, ethene, ethoxy-hexadecanoic acid, methyl ester, 2-butanamine, (s)-1,3,5-triazine-2,4,6-triamine, n-formylβ-alanine, (z)6, (z)9-pentadecadien-1-ol, butanedial, and methanecarbothiolic acid. Apart from these compounds the flowers also possess flavones including cyanidin-3, 5-diglucoside, 7-diglucoside, quercetin-3, kaempferol-3-xylosylglucoside. In addition, the leaves of *H. rosa-sinensis* was reported for the natural sources of phytoconstituents including ß-sitosterol, malvalic acids, cyanin, cyanidin, chlorides, quercetin, carotene, gentisic acid, mucilage, and catalase. Flavonoids, phenolics are the most important phytoconstituents which are having direct

connection with antioxidant effect. Moreover, the other identified phytochemicals including propanal, 2,3-dihydroxy, ethylenediamine, O-methylisourea hydrogen sulfate, 2-butanamine, (z)6,(z) 9-pentadecadien-1-ol and 1propanol were delineated for their antioxidant and antimicrobial properties (Jadhav et al., 2009; Missoum, 2018).

1.4.9. Taxonomical classification

Kingdom: Plantae

Order: Lamiales

Family: Acanthaceae

Genus: *Adhatoda*

Species: *Adhatoda vasica* L.

1.4.10. Habitat of *Adhatoda vasica*

The herb *Adhatoda vasica* intrinsically grows in filthy or barren areas of India and the subcontinent of India (Duraipandiyan et al., 2015). It is widely found in Sri Lanka, Nepal, Pakistan, Indonesia, Malaysia, China and Panama.

Figure 1.4. Photograph of *Adhatoda vasica*

1.4.11. Morphology of *Adhatoda vasica*

Adhatoda vasica is the dense and evergreen shrub having a height of 1 m to 2.5 m. The plant contains an herbaceous woody stem and the flower is dense and large in size. The length of the leaf is from 10 cm to 15 cm and the width is about 4 cm and fruit is clavate shaped (Figure 1.3.) (Sarkera et al., 2011).

1.4.12. Phytochemical studies of *Adhatoda vasica*

Different parts of *Adhatoda vasica* possess several phytoconstituents which are having significant therapeutic benefits. The leaves of *A. vasica* contain phenols, tannins, alkaloids, anthraquinone, saponins, flavonoids, steroids, alkanes, reducing sugars, essential oil and crystalline acid. Vasicine was found in the leaves of this plant. Besides, vasicine the other alkaloids such as vasicinone, deoxy-vasicinone, vasicinol, adhatodine, adhatonine, adhvasinone, anisotine and hydroxy-peganine were also presented in the leaves. Among all alkaloids the vasicine and vasicinone are the major compounds and responsible for showing the broncho-dilatory and respiratory stimulant activities (Balachandrana et al., 2017).

1.4.13. Taxonomical classification

Kingdom: Plantae

Order: Brassicales

Family: Caricaceae

Genus: *Carica*

Species: *Carica papaya* L.

Figure 1.5. Photograph of *Carica papaya*

1.4.14. Habitat of *Carica papaya*

Among the caricaceae family belonging plants the *Carica papaya* is one of the most valuable plants. The fruit crops mostly grow in the tropical and subtropical regions of different countries including India, Malaysia, Indonesia, Philippines, Sri Lanka and Oman. This plant is also commercially cultivated in various Asian countries. In some places the papaya is considered as a garden plant as well (Wadekar et al., 2021).

1.4.15. Morphology of *Carica papaya*

The plant is evergreen, mostly unbranched and 5 to 10 meters tall (Figure 1.4.). Usually, leaves are spirally organized at the top of the trunk. The leaves are oval shaped and having the diameter of 20 to 28 inches. White coloured latex is presented inside in every part of the

plant. Flowers of this plant is pale white in colour and floral dimorphism is evident. But both of the flowers are joined to the petals. Female flowers are having ovaries at their structure and all the five petals are lightly wrapped at their base point. Each part of this plant is having the medicinal significance and also have been used all over the world as the folklore medicine (Wadekar et al., 2021).

1.4.16. Phytochemical studies of *Carica papaya*

Various parts of this plants possess a wide variety of phytochemicals including volatile and non-volatile oils which are having some significant medicinal values. Tannin, saponin, alkaloids, flavonoids, and glycosides were found out from the extracts of *Carica papaya* leaves. Among the flavonoid compounds seven flavonoids including quercetin, kaempferol, kaempferol 3- rutinoside, quercetin3-(2G-rham nosylrutinoside), quercetin 3-rutinoside, kaempferol 3-(2G-rhamnosylrutinoside), myricetin 3-rhamnoside were identified. The leaf is also the natural source of phenolic compounds like kaempferol, protocatechuic acid, quercetin, 5,7-dimethoxy coumarin, caffeic acid, p-coumaric acid, and chlorogenic acid. Research findings also claimed that the n-hexane extract and methanol extract of leaves contain the different quantities of phytochemicals like anthraquinone, glycoside, tannin, saponin, flavonoid, steroid and resin. Futhurmore, the most important compounds including protocatechuic acid, chlorogenic acid, caffeic acid, quercetin, kaempferol, quercetin 3-O-α-1C4-rhamnopyranoside, quercetin-3-O-glucopyranuroside, quercetin-3-O-rutinoside, p-coumaric acid were obtained from the leaves of the *C. papaya* (Wadekar et al., 2021).

1.4.17. Assessment report on *Hibiscus rosa-sinensis, Adhatoda vasica* and *Carica papaya* leaves

Radical scavenging activities of *Hibiscus rosa-sinensis, Adhatoda vasica* and *Carica papaya* leaves extracts have been executed by using three sets of antioxidant tests (displayed in Table 1.1).

Table 1.1: Radical scavenging activities (IC_{50} values) of methanol extract and essential oil of *Hibiscus rosa-sinensis, Adhatoda vasica* and *Carica papaya* leaves.

		Assay methods		
Plant Name	**Plant Extract**	**DPPH radical (AAE µg/mL)**	**ABTS radical (AAE µg/mL)**	**H_2O_2 radical (AAE µg/mL)**
Hibiscus rosa-sinensis	Methanol extract	175.73 ± 2.63	64.58 ± 1.97	89.78±1.36
	Essential oil	1320.25±1.01	975.56±2.75	596.33±5.68
Adhatoda vasica	Methanol extract	125.23±2.57	25.85±1.05	67.33 ± 2.14
	Essential oil	1339±4.57	976.78±2.25	695.68±3.36
Carica papaya	Methanol extract	178.58±3.75	78.36±1.56	80.47±0.83
	Essential oil	375.57± 4.45	320.56±3.36	125.56±3.57

Results in table are expressed as mean (±) standard deviation of triplicate experiments. IC_{50} (µg/mL) values of methanol extract and essential oil were compared to ascorbic acid.

Anti-inflammatory activities of *Hibiscus rosa-sinensis, Adhatoda vasica* and *Carica papaya* leaves extracts have been executed by using COX-1 and COX-2 assay methods (shown in Table 1.2).

Table 1.2: Anti-inflammatory activities of methanol extract, essential oil of *Hibiscus rosa-sinensis, Adhatoda vasica* and *Carica papaya* leaves.

Plant Name	Plant Extract		% of COX-1 inhibition				% of COX-2 inhibition			
		Control	12.5(µg/ml)	25(µg/ml)	50(µg/ml)	Indomethacin (30mM)	12.5(µg/ml)	25(µg/m)	50(µg/ml)	Indomethacin (30mM)
Hibiscus rosa-sinensis	Methanol extract	0.99±0.01	1.25±0.05	2.79±0.12	1.78±0.14	94.44±0.91	1.1±0.08	2.14±0.06	1.79±0.18	61.14±0.75
	Essential oil	1.0±0.04	1.77±0.18	1.19±0.06	1.83±0.19	96.2±0.75	1.01±0.03	1.22±0.07	1.18±0.08	59.37±0.97
Adhatoda vasica	Methanol extract	0.97±0.04	1.18±0.03	1.52±0.16	2.02±0.23	95.05±0.87	2.17±0.1	1.92±0.28	1.32±0.08	58.76±0.56
	Essential oil	0.96±0.03	0.99±0.03	0.97±0.02	0.97±0.03	93.7±0.72	1.16±0.03	0.99±0.03	1.83±0.12	59.22±0.7
Carica papaya	Methanol extract	0.97±0.05	1.03±0.09	2.73±0.04	1.06±0.09	93.91±0.74	2.07±0.14	1.96±0.19	1.65±0.06	59.42±2.22
	Essential oil	1.0±0.03	1.87±0.12	1.0±0.04	0.98±0.05	96.07±0.49	1.11±0.07	1.04±0.07	1.79±0.17	59.96±1.03

Concentration dependant anti-inflammatory activities of a) methanol extract and b) essential oil against COX assay. The values are expressed as the percentage in comparison with the untreated (control) group and each value in table is represented as mean ± SD (n = 3).

To investigate the inhibitory effects on cell viability of human prostate cancer cell lines, the MTT assay was carried out by exposing the cultured PC3 cells to different doses (12.5 µg/ml, 25 µg/ml, 50 µg/ml, 75 µg/ml and 100 µg/ml) of the methanol extract and essential oil for 24 h and 48 h respectively (displayed in Table 1.3).

Table 1.3: Anticancer activities of methanol extract, essential oil of *Hibiscus rosa-sinensis, Adhatoda vasica* and *Carica papaya* leaves.

Plant Name	**Plant Extract**	**Control**	**12.5 (µg/ml)**	**25 (µg/ml)**	**50(µg/ml)**	**75(µg/ml)**	**100(µg/ml)**
Hibiscus rosa-sinensis	Methanol extract	98.3±0.5	98.63±0.25	96.43±2.19	97.53±1.16	97.7±2.09	95.1±2.29
	Essential oil	98.33±0.29	96.87±1.39	95.23±4.09	97.43±0.91	98.27±0.23	94.9±2.78
Adhatoda vasica	Methanol extract	96.9±0.85	97.13±1.85	95.63±1.02	94.87±2.71	96.57±1.86	98.1±0.61
	Essential oil	99.2±0.17	96.97±1.72	98.6±0.87	98.67±0.57	97.3±1.31	97.57±1.0
Carica papaya	Methanol extract	96.83±0.38	96.93±0.12	94.53±1.93	96.2±2.27	94.13±2.92	95.2±1.95
	Essential oil	98.1±0.36	98.0±0.89	97.43±1.02	96.0±1.32	96.7±1.18	96.7±1.21

Concentration-dependent cell viability of (a) methanol extract and (b) essential oil against PC3 cell line at 24 h. The values are expressed as the percentage in comparison with the untreated (control) cell group and each value in table is represented as mean ± SD (n = 3)

Plant Name	**Plant Extract**	**Control**	**12.5 (µg/ml)**	**25 (µg/ml)**	**50(µg/ml)**	**75(µg/ml)**	**100(µg/ml)**
Hibiscus rosa-sinensis	Methanol extract	97.3±1.0	97.23±0.8	95.97±0.96	96.23±0.55	96.47±0.45	95.47±0.31
	Essential oil	96.3±1.41	95.7±0.79	95.47±0.47	95.87±1.39	93.7±3.24	93.93±0.67
Adhatoda vasica	Methanol extract	95.1±1.06	96.5±1.61	94.2±1.71	95.63±0.7	94.93±0.57	95.23±0.83
	Essential oil	97.67±1.07	95.6±1.35	96.23±2.25	97.03±0.55	96.67±0.38	96.33±0.67
Carica papaya	Methanol extract	95.1±0.46	95.4±0.87	95.23±0.9	93.83±1.03	93.97±0.15	94.2±0.26
	Essential oil	96.7±0.44	94.57±0.9	95.0±1.32	94.13±1.69	94.57±1.1	94.13±1.38

Concentration-dependent cell viability of (a) methanol extract and (b) essential oil against PC3 cell line at 48 h. The values are expressed as the percentage in comparison with the untreated (control) cell group and each value in table is represented as mean ± SD (n = 3)

We investigated the comparative antioxidant, anti-inflammatory and anticancer potentials of bioactive molecules of the methanol extract and essential oil of *Hibiscus rosa-sinensis*, *Adhatoda vasica* and *Carica papaya* leaves. Among the extracts, the methanol leaf extract of *Hibiscus rosa-sinensis* showed only antioxidant potential. No significant anti-inflammatory and anticancer (PC3 cell lines models) activities were found. The leaf oil of *Hibiscus rosa-sinensis* did not show antioxidant potential. It also did not show any significant anti-inflammatory and anticancer (against PC3 cell lines) potentials. The methanol leaf extracts of *Adhatoda vasica* and *Carica papaya* showed antioxidant properties but no significant anti-inflammatory and anticancer (against PC3 cell lines) activities were revealed. On the other hand, the leaf oil of *Adhatoda vasica* did not express its antioxidant, anti-inflammatory and anticancer properties. Whereas, the leaf oil of *Carica papaya* expressed its antioxidant property but no anti-inflammatory and anticancer (against PC3 cell lines) properties were detected. As none of these three plants showed antioxidant, anti-inflammatory and anticancer properties consistently, we did not consider these plants for further analysis.

1.5. Present research envisage

In the present study, we investigated the chemical composition of the methanol extract and essential oil of *Vitex negundo* leaves and also evaluated the comparative antioxidant, antibacterial, anti-inflammatory and anticancer potentials of the bioactive molecules. We estimated the total phenolics and flavonoid contents of the leaf extracts. The methanol extract and the essential oil of *Vitex negundo* leaves had been evaluated for the β-sitosterol, polyphenol and tocopherol contents. The leaf extracts are also employed to measure its potential against the urinary tract infection pathogens and anti-inflammatory potentials by employing the RAW 264.7 cell line models. Besides, the *in-vitro* anticancer activities were assessed against the PC3 cell line models.

Chapter 2

Review of Literature

2. Review of Literature

2.1. Herbal management of prostate cancer

Several natural compounds are documented for playing the role in management of prostate cancer. The anti-cancer potential of some important phyto-constituents are reported below.

2.1.1. Curcumin

Curcumin is an active component of *Curcuma longa* and possesses significant biological potentials against cancer, inflammation, oxidation, neuronal damage, radiation damage (Amalraj et al., 2017). This yellow pigment exhibits its action by intervening with AR signaling, NF-κB-, AP-1-, PI3k/Akt/mTOR-, and Wnt/β-catenin/TGF-β/MYC pathways (Abd. Wahab et al., 2020). By interfering with these signaling pathways, curcumin can efficiently prevent the growth of PCa cells. By attenuating the activity of epidermal growth factor receptor (EGFR) tyrosine kinase, curcuminoid induces an apoptotic cell death in LNCaP and PC3 cells (Dorai et al., 2000). Curcumin triggers apoptotic pathways by lowering the expression of Bcl2 and increase expression of caspase-3 and caspase-8 through enabling TNF and suppression of NF-κB, protein kinase D (PKD), β-catenin on DU145 cells (Sundram et al., 2012). Curcumin revealed the AR-dependent anticancer effects on PCa cells via inhibiting the Wnt/β-catenin pathway, arresting cell cycle at G2 phase (Teiten et al., 2011). It destroys the PCa cells via blocking the glyoxalase system, reducing the level of ATP and Glutathione (GSH) and enhancing the level of methylglyoxal (Santel et al., 2008). Moreover, curcumin and α-tomatine combinedly can stop the growth of tumors on PC3-xenograft models (Huang et al., 2015). The compound prevents the cancer-associated fibroblast formation (CAFs) that promotes tumorigenesis, invasiveness, metastasis, and EMT in prostate malignancy. It reduces the CAFs dependant ROS production, suppresses the expression of IL-6 and CXC chemokine receptor-4 (CXCR-4) receptors of PC3 cells via

repressing the MAO-A/mTOR/HIF-1α signaling pathway (Du et al., 2015). The curcumin derivatives displayed the inhibitory effects on testosterone and DHT-stimulated AR activity (Schmidt and Figg, 2016). Recent investigation revealed the remarkable anticancer activity of curcumin-loaded lipid nanoparticles on PC3, DU145 cell lines that are docetaxel-resistant (Tanaudommongkon et al., 2020). The *in-vivo* and *ex-vivo* study demonstrated that lipid polymer hybrid nanoparticles (aptamer-PLGA-PEG) of curcumin and cabazitaxel exhibited enhanced drug delivery and anti-proliferative effect on PCa cell (Chen et al., 2020). Curcumin in combination with metformin showed more potent cytotoxicity and apoptosis against the PCa cells (Eslami et al., 2021).

2.1.2. Lycopene

Lycopene is one of the naturally occurring carotenoids found in some fruits and vegetables. This pigment is adequately presented in tomato *Lycopersicon esculentum* or *Solanum lycopersicum*. Antioxidant properties of this phytochemical decrease the possibilities of cardiovascular problems and skin injury (Cámara et al., 2013). Due to have the chemopreventive property, lycopene can show cytotoxicity against the PCa cell mediating mitochondrial apoptotic pathway. Lycopene suppressed the growth of many cancer cells including PCa by stopping the activities of HMG-CoA reductase and Ras. Apoptotic process is coupled with the cell cycle arrest and deactivation of NF-κB, JNK and Akt pathway (Palozza et al., 2010). Lycopene was documented for blocking the migration and invasiveness of PCa via inhibiting the function of integrins (Gioti and Tenta, 2015). Apoptosis is instigated because of repressing the expression of Akt mediator and enhancing the expression of miR let-7f1 (microRNA Lethal-7) (Mirahmadi et al., 2020). Data of the clinical tests disclosed that lycopene can effectively decrease the PCa carcinoma (from 24% to 36%) by stopping the transformation of high-grade prostate intraepithelial neoplasia to PCa (Mohanty et al., 2005). Lycopene showed the anti-inflammatory potential by lowering the

levels of IL-1, IL-6, IL-8, TNF-α in DU145, PC3, and LNCaP cells and tumor burden mice model as well (Jiang et al., 2019). Furthermore, lycopene has an impact on metabolism and signaling of androgen receptors expressed on an ex-vivo and animal model of PCa (Applegate et al., 2019).

2.1.3. Ellagic acid

Ellagic acid is presented in many fruits and vegetables such as in *Rubus* spp. (Rosaceae), *Vaccinium* spp. (Ericaceae) and highlighted for its antioxidant and anticancer effects. It enhances the possibilities of microtubule assembling while decreasing the polymerization of tubulin by cabazitaxel. Ellagic acid shows the blocking effect for drug efflux on castration-resistant PCa/22Rv1 cells while unable to alter the activity of docetaxel in the living system (Eskra et al., 2019). It also stops the tumor development through enabling the caspase 3 induced apoptosis process in the TRAM tumor model. Ellagic acid reduces the growth of LNCaP cells, enhances the levels of proteins are involved in cell cycle, increases the expression of p21Waf, P27Kip, cdk2, and cyclin E associated by suppression of cyclin D1 and Cdk1 activities in Sprague-Dawley (SD) rats (Naiki-Ito et al., 2015). In association with luteolin and punicic acid, ellagic acid resists tumorigenesis, metastasis and angiogenesis in PCa cells. Furthermore, ellagic acid was recorded for in reduction of CXCR-4, p-Akt/PI3K activities, blocking effects on tube formation, IL-8, VEGF, and angiogenesis in HMVEC cells (Wang et al., 2014).

2.1.4. Quercetin

Quercetin is the penta-hydroxylated flavonoid available in several fruits like berries, onions, apples and vegetables like tomatoes and tea as well. This phyto-compound is enriched with numerous pharmacological activities including anti-inflammatory, antioxidant, immunomodulatory, and anticancer activities. It retards cell proliferation, invasion,

migration, and tumorigenesis inside the body by reducing the expression of IncRNA MALAT1 and blocking the signal for PI3K/Akt activation (Lu et al., 2020b). Quercetin revert the docetaxel resistance via influencing AR and PI3K/Akt activations *in-vivo* and *ex-vivo*. This compound promotes apoptosis and lowers P-glycoprotein (P-gp), TWIST-1, PSA concentration *in-vivo* and increases efficiency of docetaxel by inverting the chemo-resistance (Lu et al., 2020a). In presence of resveratrol, quercetin can regulate methylation in promoter region, IGF1, Bcl-2, PTEN signaling and along with reduces the activations of EGFR, EGR3, and IL-6 whereas increases the activations of IGFBP7, NKX3.1 to decrease the tumor overload through apoptotic process (Singh et al., 2020). Combinedly, quercetin and metformin can instigate the caspase-dependent apoptosis signaling and lowers the cell invasion and cellular activities of Bcl-2, VEGF, and Akt/PI3K in *in-vitro* and *in-vivo* tumor models (Sun et al., 2018). Quercetin heightens the medicinal potency of paclitaxel, enhances the relevant factors to restrict the G2/M phase, increases possibilities for apoptosis, ER stress, triggers ROS production, blocks the cell migration and proliferation in *in-vitro* and *in-vivo* (Zhang et al., 2020). It destroys cells through mediating apoptosis via mitochondrial/ROS pathway along with down-regulating the activities of tyrosine-protein kinase-met (c-met) and PI3K/Akt pathway and reverting the doxorubicin resistance *in-vitro* (Shu et al., 2017). Nanomicellar formulation of quercetin can significantly kill the prostate carcinoma cells *ex-vivo* and *in-vivo* through mediating apoptosis (Zhao et al., 2016). It also inhibits the overload of tumor through blocking angiogenesis and metastasis by increasing the expression of thrombospondin-1 called as anti-angiogenesis factor. Quercetin suppresses the EGFR and PI3k/Akt pathway, PCNA expression, imentin, N-cadherin, cyclin D1, mRNA concentrations in snail slug (Firdous et al., 2014). The compound is reported for exhibiting the inhibitory action towards tumor growth via apoptosis by blocking the proliferative actions of IGFIR, AR, and Akt (Sharmila et al., 2014).

2.1.5. Resveratrol

Resveratrol (RES) is a naturally occurring polyphenolic substance obtained from the dried stem of *Vitis vinifera* (Tian and Liu, 2020). Trans-isomers of RES possess a wide range of bioactivities including anti-inflammatory, antioxidant, anti-aging, anticancer, immunomodulatory, neuro-protective, and cardio-protective activities. It has proved that RES can initiate the apoptotic pathway for LNCaP, DU145, and PC3 cells (Jasinski et al., 2013). RES stimulated enhanced apoptotic cell death in PCa cells in addition with doxorubicin, taxol, methotrexate, cytarabine, actinomycin D by blocking the survivin and encouraging programmed cell death (Gupta et al., 2011). RES also blocks the activities ofcyclooxygenase–2 (COX–2) and NF-κB in prostate carcinoma cells and increases the stimulation for apoptosis (Athar et al., 2009). *In-vivo* and *ex-vivo* experiments revealed that RES can stop PCa through suppressing the anti-apoptotic SphK1/S1P, pro-survival signaling (Brizuela et al., 2010). Clinical reports revealed that RES effectively reduces the serum concentration of androgen on benign prostate hyperplasia individuals but it doesn't produce any effect on growth of prostate tumor (Kjaer et al., 2015). In association with quercetin the resveratrol potentially inhibits the growth of tumor in TRAMP model via reducing the oxidative stress and enhancing the apoptotic signals (Singh et al., 2020). RES shows anti- proliferative and anti-apoptotic effects on PCa cells through blocking the AR, AR-V7, and Akt signaling pathway. RES inhibits invasiveness and migration of tumor by increasing the expression of eleven translocation-1 (TET1) accompanying TIMP2/3 status and suppressing the expression of MMP2/9, TNF-receptor associated factor 6 (TRAF6)/NF-κB/SLUG axis in prostate carcinoma cell lines (Wang et al., 2020). Moreover, RES decreases the prostate fibrosis-mediated inflammation (Vicari et al., 2020). RES inhibits the release of hepatocyte growth factor (HGF) from stromal cells and in turn represses the invasion and EMT in PCa cells (Hsieh and Wu, 2020).

2.2. Herbal management of inflammation

Several natural compounds are reported for exhibiting their anti-inflammatory properties. The anti-inflammatory potential of some important phyto-constituents are reported below.

2.2.1. Curcumin

Curcumin is the most common polyphenol and possesses the strong pain relieving property. Research findings in both the clinical and preclinical reports suggest the function of curcumin against inflammation. It was documented curcumin successfully manages the inflammatory disease like chronic anterior uveitis (CAU) (Lal et al., 1999). Later, it was revealed that the medicinal property of curcumin to mitigate idiopathic inflammatory orbital pseudotumor (Lal et al., 2000). Curcumin exerts its anti-inflammatory potential via blocking the arachidonic acid (AA) pathway, cyclooxygenase (COX), (Ammon et al., 1993) cytokines interleukin (IL) and tumor necrosis factor (TNF) (Kang et al., 1999) nuclear factor kappa B (NF-κB) (Bremner, 2005). It plays a role in stabilizing the lysosomal membrane (Srivastava et al., 1995). Oxidative stress and inflammation are closely associated with each other because the ROS is liberated by inflammatory cells. ROS and RNS are involved in up-regulation of pro-inflammatory genes and induces various chronic disorders such as Alzheimer's disease (AD), Parkinson's disease, multiple sclerosis, epilepsy, cerebral injury, cardiovascular disease, metabolic syndrome, cancer, allergy, asthma, bronchitis, colitis, arthritis, renal ischemia, psoriasis, diabetes, obesity, depression, fatigue, and acquired immune deficiency syndrome (AIDS) (Biswas, 2016; Panahi et al., 2016). TNF-α is one of the important factors for mediating the inflammation which is modulated through the function of the transcription factor, nuclear factor (NF)-κB. Apart from the activator namely TNF-α, the NF-κB may get activation signal by inflammatory cytokines, gram-negative bacteria, different viruses, environmental pollutants, chemical, physical, mechanical, and psychological stress, high

glucose, fatty acids, ultraviolet radiation, cigarette smoke and other related factors for developing various diseases. Curcumin shows the potential to inhibit the functions of NF-κB and NF-κB-dependent genes and thereby reduces the chances to form inflammations (Panahi et al., 2016).

2.2.2. Flavonoids

Flavonoids are polyphenolic compounds largely available in different parts of plants such as fruits, flowers, bark, roots, stems, grains. Among the 4000 of different flavonoids, the four groups are considered as major flavonoids including flavones, flavanones, catechins, anthocyanins (Cook, 1996). Flavonoids are traditionally used for their various medicinal values including in alleviate the inflammatory responses. Ternatin is a tetra-methoxy flavone and demonstrates the anti-inflammatory potential on rat carrageenan-induced pleurisy models (Souza et al., 1992). Anthocyane flavonoid is found in the juice of *Aronia melanocarpa* and is accountable for its anti-inflammatory activity on *in-vivo* experimental models like histamine-induced or serotonin-induced rat hind paw tests (Borissova et al., 1994). Quercetin is the one of the most important flavonoids obtained from onions, apples, broccoli, and berries. Quercetin and rutin can exert the anti-inflammatory effects in *in-vivo* rat models (Sanchez de et al., 2002). Flavonoids produce the anti-inflammatory effects by inhibiting the generation of eicosanoids and degranulation of neutrophil and other various ways are mentioned as follows, i.e. quercetin exhibit their anti-inflammatory activities via blocking the functions of COX and LOX (Kim et al., 1998), anthocyanins and their aglycone, cyanidin restrict the functions of COX-1 and COX-2 (Wang et al., 1999), anthocyanidins resist the lipopolysaccharide-induced activation of COX-2 via triggering the mitogen-activated protein kinase (MAPK) pathways, catechin dimers block the biosynthesis of prostaglandin (Damas et al., 1985).

2.2.3. Parthenolide

Parthenolide is the sesquiterpene lactone isolated from the traditionally used medicinal plant like *Tanacetum parthenium* (Li-Weber et al., 2002). Sesquiterpene lactone is responsible for the anti-inflammatory property and the Mexican Indians utilize it to alleviate the skin and various organ infections (Heinrich et al., 1998). Parthenolide possesses a remarkable anti-inflammatory potential inside the body and since ancient times it has been employed in treatment of fevers, migraine, and arthritis. Feverfew shows the significant analgesic action and highlights for its efficacy in management of severe migraine attack and also minimizes the distinct symptoms of migraine like nausea, vomiting, noise sensitivity and light sensitivity. Parthenolide exhibits its anti-inflammatory activity through inhibiting the inflammatory gene expression like nitric oxide (NO) synthase, intracellular adhesion molecule-1 and pro-inflammatory cytokines such as TNF-α, IL-1, IL-4, IL-8 and IL-12 (Li-Weber et al., 2002). Along with this, the parthenolide resists the activation of NF-κB which plays a crucial role in inflammatory reactions and immune response (Hehner et al., 1999).

2.2.4. Lyprinol

Lyprinol is the lipid extract found in the New Zealand green-lipped mussel (NZGLM) and shows the efficacy to treat arthritis. The oil extract is the mixture of some components such as triglycerides, sterol esters, sterols, polar lipids and free fatty acids (Sinclair et al., 2000). Lyprinol expresses its anti-inflammatory effect on *in-vivo* rat models including adjuvant induced polyarthritis model and collagen (II)-induced arthritis (Halpern, 2000). Pretreatment of lyprinol shows the remarkable reduction of the distress of inflammatory bowel disease (IBD) in mice models. As compared to the fish oil (EPA/DHA) the stabilized lipid extract of NZGLM can produce more beneficial effects due to the presence of an ample amount of omega 3 fatty acids in its extract (Tenikoff et al., 2004). The reports of both controlled and

randomized clinical trials revealed that the lyprinol can cause significant improvements in various inflammatory disease conditions such as osteoarthritis, rheumatoid arthritis, and asthma in the human body (Cho et al., 2003). Combinedly, lyprinol with EPA and DHA in a form of dietary supplement is used in treating rheumatoid arthritis (Gruenwald et al., 2004). Lyprinol exhibits its anti-inflammatory effects by lowering the pro-inflammatory LTB4 in monocytes, through blocking the 5- lipoxygenase (5-LOX) and COX arachidonate oxygenation pathways and by decreasing the concentration of TXB2, PGE2, and IL-1 (Dugas, 2000).

2.2.5. 1,8-Cineole

1,8-Cineole is the monoterpene oxide and the major component of essential oil of medicinal plants such as eucalyptus, sage, rosemary, psidium etc. The compound is used as a percutaneous penetrating agent and along with the management of several diseases including bronchitis, sinusitis and rheumatism (Santos and Rao, 2000, 2002). Additionally, it has the potential to combat with the inflammatory reactions by employing different *in-viv*o models like carrageenan-induced paw oedema, cotton pellet-induced granuloma, and the acetic acid induced increase in peritoneal capillary permeability. 1,8-cineol can successfully inhibit the inflammatory reactions associated with asthma (Juergens et al., 2003). It also possesses the steroid-saving activity which indicates the anti-inflammatory effect along with its mucolytic properties for respiratory diseases like asthma. Some researchers reported the inhibitory activity of 1,8-cineole towards the inflammatory mediators like leukotriene B4 (LTB4) and PGE2 produced by the patients who had bronchial asthma (Juergens et al., 1998a). Furthermore, the 1,8-cineole resists the generation of TNF-α, IL-1β, LTB4 and thromboxane B2 (TXB2) in a concentration-dependent manner (Juergens et al., 1998b).

2.3. Herbal management of bacterial infection

Several natural compounds are reported for exhibiting their anti-bacterial properties. The anti-bacterial potential of some important phyto-constituents are reported below.

2.3.1. Coumarins

Phenolic compounds like flavonoids, coumarin derivatives are documented for the wide range of pharmacological effects such as antioxidant, antimicrobial, and anti-inflammatory effects (Stefanachi et al., 2018). Several coumarins are reported for preventing the harmful effects of different bacterial pathogens including *Staphylococcus aureus*. Among the coumarin derivatives the osthenol showed the significant antibacterial potential against the *Bacillus cereus* and *S. aureus* strains with a MIC value from 62.5 to 125µg/mL. Research findings also suggested that the addition hydrophobic molecules in C8 position and OH group in C7 position could enhance the antibacterial potential of coumarin derivatives (Souza et al., 2005). Courmarin derivatives can inhibit the activities of *S. aureus,* including methicillin-resistant *Staphylococcus aureus* strains (MRSA) by binding to the B subunit of DNA gyrase enzyme (Feng et al., 2020; Hu et al., 2020).

2.3.2. Essential oils

Essential oil (EO) is comprised of the mixture of volatile components which are mostly terpenoid class of compounds. Usually, the EO are found in glandular trichomes, secretory ducts, and oil cells of plants and recognized for exhibiting the antimicrobial, sedative, anti-inflammatory, analgesic, local anaesthetic, spasmolytic activities. Apart from these, the EO is also used as a flavoring agent in food, cosmetics and agronomic industries. For having the strong preventive actions on microbial growth, the EOs are extensively used as food or cosmetic preservatives (Bakkali et al., 2008). EO exerts the antibacterial effects by interfering

with the cell wall integrity, membrane permeability, activity of proton pump and by decreasing the membrane potential, reducing the amount of ATP, blocking the bacterial metabolism, altering the layers of fatty acid in bacterial cytoplasmic membrane and denaturing the cellular components (Chouhan et al., 2017; Li et al., 2019; Wang et al., 2020; Zhang et al., 2016). Moreover, EOs can hamper the synthesis and secretion of bacterial toxins which may cause of the attenuation of bacterial virulence factors (Szabó et al., 2010) and additionally they can fight against the bacterial resistance if they used mutually in combination with the antibiotics (Langeveld et al., 2014). *Mentha piperita* is reported for the significant antibacterial activity against *Staphylococcus epidermidis* and *Staphylococcus aureus*, and including methicillin-resistant strains (Silva et al., 2019). Mostly, the EOs are the natural source of 1,8-cineole, terpinen-4-ol, thymol, carvone and carvacrol which have received a great importance for their antibacterial, antiviral and antifungal potentials (Jirovetz et al., 2005; Kotan et al., 2007; Sarkic and Stappen, 2018).

2.3.3. Flavonoids

Flavonoids are the secondary metabolites have received a great attention for their role in prevention of several bacterial strains. Flavonoids can exhibit their antibacterial potential either alone or with synergistic combinations. The virulence factors can be reduce by quorum quenching activity and the susceptibility for antimicrobials can be increased by blocking the activity of the efflux pump (Górniak et al., 2019; Xie et al., 2014). Furthermore, they can effectively inhibit the potential of the Gram-positive and Gram-negative bacteria by damaging the phospholipid bilayers of bacterial cell membrane, blocking the synthesis of ATP, respiratory chain and nucleic acid (Górniak et al., 2019). It was found that the hydroxylated structures of flavonoid compounds possessed better antibacterial effects whether the methylation in -OH groups reduced the antibacterial potential of semi-synthetic

flavonoids (Xie et al., 2014). Flavonoids successfully cured some skin infections by inhibiting the activities of Gram-positive bacteria, including *Staphylococcus epidermidis* or *Staphylococcus aureus* (Górniak et al., 2019). Flavonoids such as kaempferol, catechin, ononin, quercetin and quercetin, diosmetin, naringenin, baicalein, puerarin, linarin are reported for their wound healing properties and alleviation of the atopic dermatitis conditions respectively (Wu et al., 2021). It was reported that the green and yellow leaves of Walnut possessed the antibacterial activity against *Enterococcus faecalis*, *Listeria monocytogenes*, and methicillin-resistant *Staphylococcus aureus* (de Lima et al., 2014). Moreover, the flavonoid rich plant such as *Equisetum arvense*, *Urtica dioica*, *Rosa nutkana* demonstrated significant antibacterial potential against *Staphylococcus aureus*, *Micrococcus luteus*, and *Pseudomonas aeruginosa* (Gendron et al., 2021; Pallag et al., 2018).

2.4. Literature on pharmacological studies of *Vitex negundo*

The different parts of *Vitex negundo* have different therapeutic values. Pharmacological investigations revealed some important bioactivities which are reported below.

2.4.1. Antioxidant activity

Antioxidants combat oxidative stress by scavenging the free radicals and provide a therapeutic potential for several health issues like heart diseases, cancer, aging, gastric problems etc (Sen et al., 2010). *Vitex negundo* is the natural source of antioxidants. Vitedoin A has been identified as a potent antioxidant that showed greater antioxidant property rather than other natural antioxidants like vitamin E and L-cysteine (Ono et al., 2004). Plant extracts displayed its antioxidant potential by reducing the concentrations of superoxide dismutase, catalase and glutathione peroxidase in Freund's adjuvant induced arthritic-rat. The antioxidant potential is associated with presence of a wide range of polyphenols like

flavonoids, phenolic acids, tannins, phenolic diterpenes vitamin C and carotene and reduction of lipid peroxidation (Preethi, 2010).

2.4.2. Anti-inflammatory activity

Several research reports suggested the anti-inflammatory potential of this plant (Telang et al., 1999). Some significant anti-inflammatory activities were established on acute and sub-acute inflammation models. It was reported the anti-inflammatory potential of leaf extracts of *Vitex negundo* on carrageenan-induced rat paw edema and formaldehyde-induced rat paw edema models. The anti-inflammatory effects were produced through some possible ways including resisting the prostaglandin synthesis, inhibiting the histaminic activities and oxidative stress (Dharmasiri et al., 2003).

2.4.3. Anti-cancer activity

Histo-morphological analysis showed that the specific dose of *Vitex negundo* extract was non-toxic for stomach tissue whereas the dose became harmful for the other organ tissues like heart, liver and lung tissues (Tandon and Gupta, 2004). Research report revealed the anticancer properties of leaf extracts of *Vitex negundo* against COLO-320 tumour cells (Smit et al., 1995). Later, it was declared that the chloroform extract of leaves has its cytotoxic potential against the human cancer model cell lines (Díaz et al., 2003).

2.4.4. Antimicrobial activity

Antimicrobial activities of *Vitex negundo* are potentiated due to possessing the natural antioxidants in its structure (Salazar-Aranda et al., 2011). *Vitex negundo* exhibited the remarkable antimicrobial property because of having some polyphenolic compounds such as flavonoids, terpenoids, alkaloids, tannins, saponins in plant extracts and essential oils. The appearance of flavonoids, terpenoids and tannins in the plant of *Vitex negundo* has been

reported (Panda et al., 2009). The plant extracts showed the antibacterial activities against *Escherichia coli*, *Klebsiella aerogenes*, *Proteus vulgaris* and *Pseudomonas aerogenes* pathogens (Perumal Samy et al., 1998), and antifungal effects against *Alternaria alternata*, *Curvularia lunata*, *Trichophyton entagrophytes*, *Cryptococcus neoformans*, *Aspergillus niger*, *Candida albicans* (Aswar et al., 2009). Apart from the antimicrobial property, the plant also possessed the larvicidal, repellent and pesticidal properties. The larvicidal activities exerted on the following larvas including *Anopheles subpictus*, *Culex tritaeniorhynchus*, *Culex quinquefasciatus*, *Anopheles stephensi*, *Plutella xylostella*, *Cnaphalocrocis medinalis*, *Plasmodium falciparum* (Kamaraj et al., 2008) and the insecticidal activities were exhibited on *Callosobruchus maculatus*, *Phthorimaea operculella*, *Sitotroga cerealella*, *Aphis citricola*, *Aphis gossypii*, *Myzus persicae* (Paneru and Shivakoti, 2001). Research findings also suggested the importance of this plant as the bioinsecticides and biopesticides in Integrated Pest Management (IPM) (Rosell et al., 2008).

2.4.5. Enzyme-inhibitory activity

Root extracts of *Vitex negundo* have the power to inhibit the activities of some enzymes including lipoxygenase, butyryl-cholinesterase, α-chymotrypsin (Arif Lodhi et al., 2008), xanthine-oxidase and tyrosinase (Azhar-Ul-Haq et al., 2004). It was reported that the methanol extract of *Vitex negundo*'s leaves possesses the lignins that can suppress the activity of tyrosinase (Coulter et al., 2006). Research findings revealed the inhibitory potential of aqueous extract of the plant aerial parts of *Vitex negundo* towards HIV type 1 reverse transcriptase enzyme (Tandon and Gupta, 2005).

2.4.6. Effect on reproductive potential

The seed extract of *Vitex negundo* contains an adequate amount of flavonoid content which can influence the late phase of spermatogenesis of dogs (Bhargava, 1989) and also play the

modulatory role with the male reproductive system of rats (Das et al., 2004). Though this plant shows anti-androgenic activity, interestingly, some research data also justify its conventional use as aphrodisiac agent (Khare, 2004). Ethanolic extract of this plant demonstrates the estrogen-like activity and thus gets employed in hormone replacement therapy (Hu et al., 2007).

2.4.7. Drug potentiating ability

The extracts of *Vitex negundo* can heighten the effects of anti-inflammatory agents like ibuprofen and phenylbutazone, analgesic drugs like meperidine, aspirin, morphine and pethidine, sedatives and hypnotics such as pentobarbitone, diazepam and chlorpromazine, anticonvulsive drugs like diphenylhydantoin, valproic acid (Tandon and Gupta, 2005).

2.4.8. Anticonvulsant activity

The petroleum ether and butanol extracts of *Vitex negundo* leaves served better protective effects from electroshock seizures than the root extract of the plant. Petroleum ether extract of root efficiently prevented the leptazole induced convulsion while methanol extract of leaves significantly inhibited the strychnine and leptazole induced convulsions (Tandon and Gupta, 2005). In contrast, the ethanol extract of leaves didn't show any anticonvulsant effect. However, it enhanced the activities of other anticonvulsant agents and helped to minimize the dose and associated adverse effects (Mahalakshm et al., 2010).

2.4.9. Hepatoprotective activity

Vitex negundo showed the hepatoprotective property by reducing the levels of serum bilirubin, aspartate, aminotransferase, alanine aminotransferase, alkaline phosphates and total Protein (TP). Leaf extract of the plant prevented the d-galactosamine (Yang et al., 1987) or carbon tetrachloride induced liver injury (Tandon et al., 2008).

2.4.10. Other activities

Apart from these above mentioned properties the plant *Vitex negundo* also displayed the laxative property, antihistaminic property, antidiabetic property, CNS depressant property and antivenom property (Alam and Gomes, 2003).

2.5. Pharmacological activities of some important bioactive compounds

Some compounds have been mostly identified from the essential oils of different parts of the various plants and reported for their significant pharmacological activities. For instance, phytol is an example of diterpene which is commonly found in essential oil and have been reported for the antimicrobial, anti-inflammatory, anticancer and antioxidant activities (Swamy et al., 2017). In addition, γ-sitosterol, 2-myristynoyl-glycinamide, squalene, 1-methylethyl ester hexanoic acid, 4-methyl-2-heptanol, thunbergol were detected from the different plant essential oils and document for the antibacterial (Chelliah et al., 2017), antimicrobial (Kumari et al., 2012), antibacterial, antioxidant, antitumor, anticancer (Kumari et al., 2012), antibacterial, antifungal (Legault and Pichette, 2010), fragrance (Sales-Campos et al., 2013), antibacterial, antioxidant (Salem et al., 2014) activities respectively. Apart from these, maltol and lactose were identified from the organic extract of the plants and reported as flavor enhancer, antioxidant (Sales-Campos et al., 2013; Serra, 2015) and energy source of humans (Hepworth, 1984). Few more pharmacological properties of the some important phyto-constituents are discussed below.

2.5.1 Caryophyllene

We investigated the effects of β-caryophyllene oxide (CPO), a sesquiterpene isolated from essential oils of medicinal plants such as guava (*Psidium guajava*), oregano (*Origanum vulgare*), cinnamon (*Cinnamomum* spp.) clove (*Eugenia caryophyllata*), and black pepper

(*Piper nigrum*) (Park et al., 2011). Essential oil of the biblical balm of Gilead (*Commiphora gileadensis*) contains beta-caryophyllene (Amiel et al., 2012). Essential oil of *Salvia verticillata* contains a substantial amount of caryophyllene oxide (Karakaya et al., 2020).

β-caryophyllene was able to produce a significant protective effect on gastric mucosal lesions of rats (Tambe et al., 1996). The gastro-protective effect was recorded against the absolute ethanol and 0.6 N HCL induced gastric ulcer. Tambe et al. (1996) mentioned that in his earlier study he noticed the anti-inflammatory activity of β-caryophyllene which was elicited by attenuating the secretion of COX-2 and later he observed that β-caryophyllene possessed a role to reduce the release of gastric acid and pepsin.

The rhizome oil of *Zingiber nimmonii* contains a high amount of caryophyllene and its isomers including α-caryophyllene, β-caryophyllene and isocaryophyllene (Sabulal et al., 2006). The volatile oil produced significant antibacterial effects against *Bacillus subtilis*, *Pseudomonas aeruginosa* and antifungal activities *Candida glabrata, Candida. albicans* and *Aspergillus niger*. Caryophyllene and its derivatives were identified as the main components of the volatile oil and might be accountable for eliciting these pharmacological responses.

β-caryophyllene boosted up the anti-carcinogenic effects of α-humulene, iso-caryophyllene on MCF-7 cell lines (Legault and Pichette, 2010). Concurrent use of paclitaxel and β-caryophyllene fortified the anticancer effect of paclitaxel ten times on DLD-1 cells. The compound also developed in drug (calcein) deposition by various procedures. These observations indicated the transition of paclitaxel via the membrane and which would lead to potentiate the anticancer activities of the test molecule.

Some researchers represented the function of (E)-β-caryophyllene as an antibacterial compound which prevented the growth of a plant pathogen namely *Pseudomonas syringae* pv. *tomato* DC3000 (Huang et al., 2012). By initiating the defense signaling cascades; (E)-β-

caryophyllene served a protective effect to the floral tissues and floral volatile compounds and also may have an involvement in welcoming of pollinators.

β-Caryophyllene obtained from the essential oil of *Aquilaria crassna* and played a selective chemopreventive role in colorectal (HCT-116, HT-29) and pancreatic carcinoma (PANC-1) (Dahham et al., 2015). Additionally, β-Caryophyllene revealed a strong preventive effect against the oxidative stress and also showed the antibacterial and antifungal activities against the *Staphylococcus aureus* and *Trichoderma reesei* respectively. Being an apoptotic inducer, β-Caryophyllene interfered with the fragmentation pathway, integrity of mitochondrial cell membrane which eventually stopped propagation, movement, invasiveness and moreover the aggregation of cancer cells. Furthermore, α-caryophyllene and caryophyllene have been reported for their anticancer and anti-inflammatory activities (Elfalleh et al., 2019; Tungmunnithum et al., 2018). Essential oil of *Salvia verticillata* contains a substantial amount of caryophyllene oxide (Karakaya et al., 2020). The compound revealed significant anti-cholinesterase, antioxidant and anticancer activities. The potent cytotoxic effects were exhibited against the prostate cancer cell line (PC-3) and human glioblastoma cell line U-87 MG.

2.5.2. Myrcene

Myrcene, a monoterpene isolated from lemon grass oil (*Cymbopogon citratus*) (De-Oliveira et al., 1997). β-myrcene was also highlighted for having the potential anti-ulcer property by utilizing the antioxidant defense system (Bonamin et al., 2014). By activating of some antioxidants and enzymatic components including nitric oxide (NO), sulfhydryl groups (SH), glutathione content (GSH), glutathione peroxidase (GPx), glutathione reductase (Gr), β-myrcene created a protective barrier for the gastric mucosal layers. Apart from that, β-myrcene showed the effect against the bacterial growth of *Helicobacter pylori*.

β-myrcene played a pivotal role in restoration of cerebral ischemia-reperfusion damage (Burcu et al., 2016) in mice models. By enhancing the activity of the components (GSH, GPx, CAT, SOD) for antioxidant defense system and inhibiting the lipid peroxidation (thio-barbituric acid reactive substances level), β-myrcene assisted in retrieval of injured cardiac tissue.

Publication trends revealed the therapeutic potential of myrcene in management of renal inflammation and oxidative damage on adrenalectomized rat model. Inflammatory response was mitigated by repressing the expression of pro-inflammatory cytokines (IL-1, IL-6, and TNF-α), anti-inflammatory cytokines (IL-4 and IL-10), immunomodulatory biomarkers (IFNγ and NF-κB), biomarker for oxidative stress (malondialdehyde) and increasing the levels of antioxidant enzymes (CAT, GSH, and SOD). Furthermore, down-regulation of COX-2, iNOS, KIM-1 and renal functional parameters (UREA, LDH, total protein and creatinine) helped to alleviate the renal impairment (Tungmunnithum et al., 2018).

2.5.3. Farnesene

Farnesene was identified from the essential oil of *Cedrelopsis grevei* leaves and highlighted for the significant cytotoxic property against the human breast cancer cell lines (MCF-7) (Afoulous et al., 2013). Whereas, the other components of essential oil might be responsible for the antioxidant and antimalarial activities.

Farnesene and its isomers increased the cell viability in rat cerebral cortex cells (Turkez et al., 2014). The H_2O_2 induced neurotoxic effect was prevented by reversing the excessive production of intracellular LDH, and increasing the level of TAC and which assisted to inhibit any kind of oxidative injury in DNA structure. The *in-vitro* experiment suggested the compound farnesene and its isomer (α-farnesene, β-farnesene and mixture-farnesene) may

contribute to the management of Parkinson and Alzheimer's diseases. Moreover, α-farnesene was also reported as a flavoring agent (Russo, 2011).

Among the 171 components of essential oil the (E,E)-α-farnesene, (E)-ß-farnesene, and (E,E)- farnesol showed antiviral activity towards the SARS−CoV−2 infection (da Silva et al., 2020). The molecular docking analysis was executed the interaction of essential oil constituents with SARS CoV-2 major protease (SARS-CoV-2 Mpro), SARS-CoV-2 endoribonuclease (SARS-CoV-2Nsp15/NendoU), SARS-CoV-2 ADP-ribose-1.-phosphatase (SARS-CoV-2 ADRP), SARS-CoV-2 RNA-dependent RNA polymerase (SARS-CoV-2 RdRp), the attaching domain of the SARS-CoV-2 spike protein (SARS-CoV-2 rS), and human angiotensin-converting enzyme (hACE2). (E,E)-α-farnesene, (E)-ß-farnesene, and (E,E)- farnesol were considered as the most perfect docking ligands for the specific viral proteins (Turkez et al., 2014).

2.5.4. Oleic acid

Oleic acid was found out as the major component of sunflower oil and olive oil and proved the impact on hypertensive disorder of female volunteers. The research study showed the significant effect of OA in lowering the plasma lipids, lipoprotein profile and high blood pressure of the tested representatives who were treated with dietary sunflower oil and olive oil (Ruiz-Gutierrez et al., 1996).

Oleic acid (OA) has been studied for opposing the failure of TNF-α inhibited insulin secretion in type II diabetic mice model. OA exerted its effect by enhancing the insulin production in INS-1 cells and reversed the prohibitory effect of TNF-α by moving the peroxisome proliferator- activated receptor-γ (PPAR-γ) to the nucleus which supposed to be granted for the anti-inflammatory property of the unsaturated fatty acid.

Research findings demonstrated the potential of OA in attenuation of cellular growth and viability on gastric carcinoma HGC-27 and breast carcinoma MDA-MB-231 cell lines mediating the AMP-activated protein kinase (AMPK) signaling pathway (Li et al., 2014).

Oleic acid (OA) possesses remarkable anti-inflammatory and anticancer properties which was beneficial for balancing the progressive neurological damage at the early stage of Alzheimer's disease (AD). Results showed that Aβ25–35-induced cellular toxicity was reduced when the pheochromocytoma12 (PC12) cells were previously treated with OA. OA exhibited cyto-protective effects by improving the rate of cell viability, inhibiting the generation of uncontrolled levels of intracellular reactive oxygen species (ROS), resisting the up-regulation of pro-apoptotic factors, specifically caspase-3. Along with the above mentioned fact, the OA significantly decreased Aβ25–35-mediated phosphorylation of p38 mitogen-activated protein kinase (MAPK), extracellular signal-regulated kinase (ERK1/2), and c-Jun-N-terminal kinase (JNK). Pretreatment of OA helped to bring down elevated levels of cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) when PC12 cells were affected by Aβ25–35-stimulation. In succession, the I-κB degeneration was prevented and followed by retardation of nuclear factor-kappa B (NF-κB) translocation. Based upon the research evidence, the OA was nominated as a key player in the treatment of Alzheimer's disease (Kim et al., 2015; Sales-Campos et al., 2013). Huang et al. (2018) claimed a function of oleic acid other than the anti-inflammatory, anti-apototic and antioxidant properties (Huang et al., 2018). Here, 0.5 mM of oleic acid caused of the liver steatosis and the investigation demonstrated the role of diterpene lactone namely ginkgolide C in modulation of metabolic pathway for lipogenesis and depletion of the lipid retention by promoting lipolysis via sirt1/AMP activated protein kinase **(**AMPK) pathway in oleic acid-stimulated HepG2 cell lines. The change of fatty acid composition has been regarded as an indicator of altered lipid metabolism during human tumorigenesis, but the details are still unclear. It was

previously demonstrated a monounsaturated fatty acid (MUFA) named oleic acid (OA) was involved in renal cell carcinoma (RCC) cell growth, as an extracellular signaling molecule to regulate 786-O cell proliferation via the integrin-linked kinase (ILK) pathway. In this study, it was further observed the effects of OA on cell invasion of RCC and the potential mechanism by which OA worked was determined. The transwell invasion assay showed OA increased cell invasion of RCC in a dose-dependent manner. Western blotting results indicated ILK, COX-2, and MMP-9 proteins were involved for their high expressions and these effects were reversed when down-regulating the expression of ILK by special siRNA. The MMPs inhibitor GM6001 could weaken the abilities of OA on RCC cell invasion. These results suggested MUFA indeed affected cell invasion of RCC, which was dependent on the regulation of ILK pathway.

Oleic acid was discovered as the major constituent of the seed oil of *Camellia japonica* and detected by the most extensively used technique namely GC-MS (gas chromatography/mass spectrometry) screening method. *Camellia japonica* seed oil corroborated its ability to alleviate asthma (by using ovalbumin asthma murine model) where the maximum creditworthiness went to the oleic acid as the main component of composition of the seed oil. It significantly prevented the asthmatic condition by decreasing the eosinophil level in bronchioalveolar lavage fluid and repressing the production of Th2 (T-helper type-2 cell) mediated transcription factor like GATA-3 coupling with the various inflammatory cytokine level such as interleukin-4 (IL-4), interleukin-5 (IL-5), interleukin-13 (IL-13), and tumour necrosis factor-alpha (TNF-α). Evidently, the concentration-dependent modulation of immune cell transcription factor (T-bet) was observed and as well as down-regulation of bioactive cytokine IL-12p40 restricted the activation of the dependent subunits like IL-6. Also by suppressing the responses of the leukocytes, eosinophil in bronchioalveolar lavage

fluid and immunoglobulin E (IgE) in serum; the seed oil was certified as a good anti-asthmatic candidate (Lee et al., 2019).

It has been authenticated that seed oil of *Moringa oleifera* contains an ample amount of oleic acid (OA) was detected and quantified (72.27%) by the GC-MS (gas chromatography/mass spectrometry) analysis. Along with the oleic acid the elaidic acid (10.64%), palmitic acid (6.36%) and stearic acid (5.09%) also present in seed oil. *Moringa oleifera* seed oil (MOSO) and oleic acid played a contributory role to reduce the phenol-induced ear edema of mice by repressing the oxidative damage of phenolic compounds. In addition, MOSO didn't antagonize with the arachidonic acid (AA) induced ear edema whereas oleic acid did respond which stipulated OA as a leukotriene B4 receptor inhibitor. MOSO and OA successfully reversed the 12-O-tetradecanoylphorbol-13-acetate (TPA) induced ear edema by stopped enabling the protein kinase C (PKC) pathway was followed by phospholipase A2 (PLA2) activation, elevated levels of AA, prostaglandins and leukotrienes. However, the mifepristone (glucocorticoid antagonist) pretreated TPA model opposed the anti-inflammatory potential of MOSO and OA and that evidence confirmed the involvement of glucocorticoid receptors in management of anti-inflammatory effect. As compared to the conventional anti-inflammatory drug like dexamethasone; the MOSO had gained an attraction as a better anti-inflammatory agent for prolonged use because it was devoid of skin atrophy and lowering the relative weight of lymphoid organs (Cretella et al., 2020).

Bioactivities of OA have been assessed for antioxidant properties which could be able to combat the cadmium induced damage in cardiac and hepatic tissue. The observations revealed that OA assisted in balancing of cadmium-induced alterations of the hepatic enzymes (alanine aminotransferase and aspartate aminotransferase), reduction in excessive production of free radicals (superoxide anion free radical) and oxidative enzymes (xanthine

oxidase, xanthine dehydrogenase) as well as inflammatory cytokine levels (IL-1β, IL- 6, IL-10) and tumor necrosis factor (TNF-α) and thereby used to treat the liver and heart injury (Bhattacharjee et al., 2020).

Giulitti et al. (2021) showed in his study the contribution of OA to stop tumor progression through the autophagocytosis process under the *in-vitro* conditions using liver cancer cell lines such as Hep3B and Huh7.5 cells (Giulitti et al., 2021).

2.5.5. Citral

Citral is the naturally occurring acyclic monoterpene that was identified as one of the essential components of lemongrass oil and evaluated for understanding of the involvement in the inflammatory process. Citral expressed its anti-inflammatory property by overpowering the LPS-stimulated COX-2 mRNA and followed by subsequent protein expression using the proximal 3′-untranslated region of COX-2 gene. 200 μM of citral also showed the anti-inflammatory potential by enabling the PPARα and γ receptors in U937 model cell lines (Katsukawa et al., 2010). Observation of this study didn't support the previous report of Lin et al.(2008); where citral was unable to inhibit the protein expression of COX-2 using the RAW264.7 macrophage model cell lines (Lin et al., 2008). Mismatch of these two results could be justified by considering the uses of different experimental conditions like LPS concentration, duration of incubation with citral and different cell line models.

The antibacterial efficacy of the nanoemulsion of citral has been documented (Wen-Chien et al., 2017; Martins et al., 2017) . The study was investigated against the six bacterial strains including *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Enterococcus faecalis, Salmonella typhimurium, and Listeria monocytogenes* following disk diffusion assay method. The nanoemulsion (at ultrasonic power 18 W, duration of ultrasonication 120

seconds, so, ratio 0.4-0.6 associated with HLB value < 12) revealed the significant antibacterial effects against *L. monocytogenes* and *S. aureus*. Citral was discovered as the main constituent of essential oils of two plants like *Cymbopogon citratus* and *Cymbopogon giganteus*. Research group reported of the anti-proliferative effects of citral by proving the strong scavenging efficacy (DPPH, ABTS assay method), anti-inflammmatory potential (lipoxygenase inhibition assay) and anticancer activities against the human prostate cancer cell lines (LNCaP, PC-3)and human glioblastoma cell lines (SF-767, SF-763) (Bayala et al., 2018). Among those two types of essential oils, the leaf oil of *Cymbopogon citratus* showed higher anti-inflammatory and anti-proliferative potential because of having the citral as composition in larger quantities than the *Cymbopogon giganteus* leaf oil.

Citral was highlighted for the prevention of UVB-induced skin cancer. By decreasing the aberrant productions of inflammatory cytokines (IL-1ß, IL-4, IL-10, IL-23, TNF-alpha, IFN) and modifying the levels of relevant biomarkers of oxidative stress (total radical-trapping antioxidant parameter of skin, glutathione, catalase activity and malondialdehyde), citral proved its anticancer property (Kremer et al., 2019).

2.5.6. Camphor

Camphor is obtained from the wood of the camphor laurel tree (*Cinnamomum camphora*) (Xu, 2005) *Cinnamomum camphora* was identified as a natural source for camphor oil (Rahman et al., 2016). It was demonstrated that the camphor possessed a remarkable anticancer property at a particular dose (300 mg/kg body weight) by augmenting the activity of glutathione S-transferase. The *in-vivo* experiment revealed the significant increment of glutathione level (with a value of $p < 0.05$) in liver and established the effectiveness against cellular death by altering the levels of mutagenic or carcinogenic chemicals (Banerjee et al., 1995).

The camphor compound 714-X (mixture of camphor, nitrogen, ammonium salts, sodium chloride, ethanol) exhibited the anti-carcinogenic and immune boosting effect (Kaegi, 1998). The clinical report was written after getting confirmation regarding the safety and benefits of the Candian cancer patients. The report claimed that camphor having a special binding affinity with cancer cells and the ammonium salt took the responsibility for activation of the specific kinins which were accountable for inhibition of the unusual cellular growth.

Camphor was considered as a topical analgesic agent (Xu, 2005; Lee and Shibamoto, 2000). Pain relieving activity was exhibited by enabling the TRPV1 receptor and enhancing the DRG neuronal current. Additionally, the suppressive effect of camphor on TRPA1 receptor was assumed for contribution in anti-nociceptive action.

A research group obtained camphor from the leaf oil of *Ocimum kilimandscharicum* and documented for having the significant radical scavenging potential, anticancer and anti-inflammatory properties (de Lima et al., 2014). Essential oil showed cytotoxic activity against the human ovarian cancer cell lines. Camphor was considered as the major component of the leaf oil and showed the significant inhibition against inflammatory responses on carrageenan induced pleurisy and total leucocytes migration mice model.

Camphor oil was reported as an effective antibacterial agent against two bacterial strains such as *Streptococcus mutants* and *Enterococcus faecalis* (Rahman et al., 2016). It was stated the admixture of racemic camphor and *Lavandula latifolia* essential oil displayed synergistic antibacterial effects against two pathogens including *Staphylococcus aureus* and *Listeria monocytogenes* (Karaca et al., 2021). Camphor was recognized as an important constituent of *Lavandula latifolia* essential oil and camphor also highlighted for having a great impact in prevention of tested bacterial strains.

2.5.7. β-sitosterol

Research report revealed the promising cardio-protective potential of β-sitosterol by preventing the signal transduction pathways which promote inflammatory responses in human aortic endothelial cells (HAEC) (Loizou et al., 2010). β-sitosterol stopped the requirements for initial phase of atherogenesis by suppressing the expression of vascular adhesion molecule-1 (VCAM-1) and intracellular adhesion molecule-1 (ICAM-1) in TNF-alpha induced HAEC cell lines; including by lowering propagation of adhesiveness of the U937 cells on TNF-alpha induced HAEC cell lines and decreasing the possibility of nuclear factor-kB p65 phosphorylation. Additionally, it was also exhibited antimicrobial, anti-nociceptive and anticancer activities (Bin Sayeed et al., 2016).

It has been proved that n-butanol extract of *Moringa oleifera* seeds contain a substantial amount of β-sitosterol which was detected by thin layer chromatography (TLC) and documented for its anti-inflammatory activity in the airways by employing the ovalbumin-induced guinea pig asthma model. β-sitosterol efficiently exerted the activities on the sensitized and challenged animals by increasing the tidal volume, lowering the rate of respiration and cell counting, specifically eosinophils and neutrophils in bronchial fluid and blood as well. In addition, β-sitosterol exhibited its protective mechanism by inhibiting the production of histamine and excessive synthesis of Th2 (T-helper type-2 cell) mediated cytokines such as TNFα, IL-4, IL-5 (without affecting the release of IL-6) in localized region.

β-sitosterol was found out from the leaf extract of the medicinal plant *Nyctanthes arbortristis* and separated out by thin layer chromatography (TLC). And β-sitosterol was reported for its *in-vivo* analgesic activity (by hot plate test and acetic acid-induced writhing test) and anti-

inflammatory activity (carrageenan-induced hind paw edema model) in a concentration-dependant manner (Nirmal et al., 2012).

Afterwards, Yin et al. (2018) proved the hepato-protective function of β-sitosterol (Sit) and its esterified derivatives including 2-naphthoyl beta-sitosterol ester (Sit-N), 2-hydroxybenzoyl beta-sitosterol ester (Sit-S), furan-2-carbonyl beta-sitosterol ester (Sit-F), (2E,4E)-hexa-2,4-dienoyl beta-sitosterol ester (Sit-Sr). Out of all derivatives only the 2-naphthoyl Sit ester (Sit-N) showed a great impact in management of liver damage in an acute phase by reducing the level of aspartate aminotransferase (AST) and alanine transaminase (ALT) in serum. Apart from that the Sit-N proficiently displayed a remarkable reduction of inflammatory cytokine levels such as tumor necrosis factor (TNF-α), interleukin-1b (IL-1β) and interleukin-6 (IL-6) and also took a responsibility to improve the pharmacological activities of some antioxidant enzymes like superoxide dismutase (SOD), glutathione (GSH), catalase (CAT), and decreased the expression of malondialdehyde (MDA). Furthermore the study reported that Sit-N suppressed the lipopolysaccharide/D-galactosamine (LPS/GalN) induced liver injury by blocking the expression of TLR4 signaling pathway and NF-kB as transcription factor and by increasing antioxidant potential through enabling the transcription factor like Nrf2 (Yin et al., 2018).

2.5.8. Asarone

The drug α-asarone is one of the active principle components of *Acorus calamus* (Manikandan, and Devi 2005). V. oil of dried rhizome of *Acori tatarinowii* contains α-asarone & β-Asarone (Lam et al., 2017). α-asarone, a bioactive compound found in Acorus plant species, has been shown to exhibit neuroprotective, anti-oxidative, anti-inflammatory,

and cognitive-enhancing effects (Jo et al., 2018). *Acorus tatarinowii* is source of beta-asarone (Xiao et al., 2020).

α-asarone possessed a strong antioxidant property which could fight against the environmental noise-stress-induced oxidative injury in the rat brain (Manikandan and Devi, 2005). Excessive loud noise in the surrounding area could cause production of oxygen free radicals (OFR) which may ultimately develop various neurodegenerative disorders. α-asarone combated the stress related deformities in several lobes of the brain by restoring the levels of SOD and LPO, CAT, GPx, GSH, Vitamins C and E and protein thiols.

Two synthesized nitro derivatives of β-Asarone were reported for the better anticancer activities than β-Asarone (Shenvi et al., 2014). The compound 1(1-[2, 4, 5-trimethoxy phenyl]- 2-nitropropene) showed two times stronger anticancer potential against SW-982, HeLa, PC-3 and IMR-32 cell lines and whereas the compound 2 (1-[2, 4, 5-trimethoxy phenyl]-1- nitropropene) exerted five to ten times greater cytotoxic activities on the SW-982, HeLa, PC-3 and IMR-32 cancer cells.

α-asarone and β-asarone were isolated from the essential oil of the rhizome of *Acori Tatarinowii* and acquired recognition as a neuro-protective agent which inhibited the oxidative damage in astrocyte cells in rat models (Lam et al., 2017). The cytoprotective effects were exhibited by decreasing the production of ROS, encouraging the Nrf2-ARE self-defense system, triggering the Akt phosphorylation and successively activating the antioxidant enzyme system.

β-asarone demonstrated the anti-proliferative effect on human glioma U251 cell lines (Wang et al., 2018). The cellular proliferation was blocked by increasing activities of P53, LC3-II/I,

Beclin-1, AMPK and pAMPK and suppressing the expressions of P62, Bcl-2, mTOR and pmTOR. The study suggested that β-asarone could initiate the autophagy process by enabling the P53 pathway which played a key role in treatment of cancer.

It was explored that the α-asarone plays a beneficial role in retrieval of the spinal cord injury cases of rats (Jo et al., 2018). α-asarone counteracted against impaired locomotor system, decreased the neuroinflammatory responses and promoted the angiogenesis in the spinal cord. Moreover, it remarkably lowered the levels of TNF-α, IL-1β, IL-6, monocyte chemoattractant protein 1 (MCP-1), macrophage inflammatory protein 2 (MIP-2), and inducible nitric oxide synthase (iNOS) levels but elevated the levels of IL-4, IL-10, and arginase 1. Results of immunohistochemistry also revealed the decreased reactive gliosis, neuroinflammation and enhanced expression of M2 macrophage and angiogenesis. These observations indicated that α-asarone possessed a wound healing property in preservation of neuronal structures and functions against the injurious spinal cord (Sethi et al., 2018).

β-asarone represented a cardio-protective function counteracted with myocardial ischemia-reperfusion injury by preventing the inflammatory reactions and NLRP3 inflammasome dependent pyroptosis (Xiao et al., 2020). The study revealed that the β-asarone ameliorated the myocardial lesions, decreased the level of a specific biomarker namely serum cTNT in rat's heart. On the other hand, the compound reduced the granulocyte infiltration, swelling of the tissue, myeloperoxidase (MPO) activity, IL-1β level and improved the function of the left ventricle associated with better ejection fraction and fractional shortening.

Research findings established that the α-asarone played a significant role against anti-inflammatory responses and analgesic activities on mice models (Saldanha et al., 2020). Inflammation management was associated with the suppression of paw oedema,

polymorphonuclear leukocytes (PMN) involvement, iNOS activation and TNF-α generation. And the pain relieving property was exerted with interacting the adenosinergic and opioidergic systems and indicated the central and peripheral analgesic actions of α-asarone.

β-asarone reported for prevention of colon carcinoma on *in-vitro* (HCT116 cell lines) and *in-vivo* (HCT116 tumor cells) models by enabling the immune system and multiple regulatory systems as well (Chen et al., 2021). Different gene expressions require β-asarone in arresting cell cycle, cell division, cell proliferation and apoptosis. β-asarone suppressed the growth of HCT116 tumors of mice. Moreover, the compound participated in the antitumor immune response system by activating granulocyte colony-stimulating factor (G-CSF) and enhancing the counting of macrophage cells in the spleen. It was shown that the innate immune system was also actively involved in preventing liver metastasis.

Chapter 3

Objective and Plan of study

3. Objective and Plan of study

3.1. Objective

The objective of this research work to explore the bioactive compounds which are presented in methanol extract and essential oil of *Vitex negundo* leaves and investigated for the comparative bioactive potential by evaluating the *in-vitro* antioxidant activity, antibacterial activity, anti-inflammatory activity and anticancer activity.

3.2. Plan of study

The study was planned as delineated below:

3.2.1 Preparation of plant leaves extracts

Preparation of plant extract by microwave assisted extraction (MAE).

Preparation of essential oil by microwave assisted hydro-diffusion.

3.2.2. Identification of the bioactive phytoconstituents

The bioactive phytoconstituents of methanol extract and fresh leaf oil were investigated by Gas chromatography-mass spectrometry (GC-MS).

The identification and quantitative estimation of bioactive phytoconstituents that were presented in methanol extract and fresh leaf oil were examined by using High Performance Liquid Chromatography (HPLC).

3.2.3. *In-vitro* antioxidant studies

The antioxidant properties of methanol extract and essential oil were evaluated by using DPPH free radical scavenging activity method, ABTS radical scavenging activity method and Hydrogen peroxide (H_2O_2) scavenging activity method.

3.2.4. *In-vitro* antibacterial study

The antibacterial properties of methanol extract and essential oil were evaluated by using Agar disk diffusion assay method.

3.2.5. *In-vitro* anti-inflammatory study

The anti-inflammatory potential of methanol extract and essential oil were evaluated by estimating of the levels of IL-1β, IL-6, and IL-10 using ELISA kits (Abcam ELISA Kit, Cambridge, MA, USA) employing the RAW 264.7 cell lines.

3.2.6. *In-vitro* anticancer study

The anticancer properties of methanol extract and essential oil were evaluated by using the MTT assay method against the PC3 cell lines and the IC_{50} values were determined.

Chapter 4

Materials and Methods

4. Materials and Methods

4.1. Chemicals and reagents

Methanol was purchased from Spectrochem Pvt. Ltd., Mumbai, India; gallic acid, quercetin, DPPH, ABTS, hydrogen peroxide, tocopherol from HiMedia Laboratories Pvt. Ltd., Mumbai, India, and Sigma-Aldrich. Folin-denis reagent was purchased from Sigma Aldrich Co. (St. Louis, MO, USA). Dulbecco's Modified Eagle's Medium (DMEM) was procured from Gibco, Grand Island, NY 14072, USA1-716-774-6700. All the chemicals and reagents used in this research were of high analytical grade and obtained from Hi-Media Research Laboratories Pvt. Ltd., Mumbai, India.

4.2. Collection of plant materials

Fresh leaves of *Vitex negundo* were collected in the month of March 2018 from the nursery of the Maulana Azad College (Kolkata; West-Bengal the state of India). A voucher specimen was (TR-5, C160686, dated: 25.05.2018) certified by the plant taxonomist after depositing the specimen in Botanical Survey of India, Central National Herbarium, Botanical Garden, Howrah, West-Bengal, India. Then, the leaves were shade dried for 15 days and subjected for size reduction by a cutting mill. To acquire a uniform plant matrix, the small sections of leaves were passed through a 60 mesh size sieve and then kept in ziplock pouches for the experiment.

4.3. Preparation of plant extract by microwave assisted extraction (MAE)

The experiment was performed using a microwave extractor coupled with a high efficiency condensing unit. 1 g of dried and finely powdered leaf was precisely weighed and imbibed to methanol for 15 min. The extraction was conducted with an intermittent way of irradiation

for 1min and followed by cooling for 1 min. During the process of extraction the powdered sample was immersed in methanol for 27 min and exposed to microwave radiation for 6 min. The inbuilt condensing unit makes the extraction more effective and robust. After extraction the extract was passed through No.1 Whatman filter paper and then the solvent was evaporated by rotary vacuum evaporator was centrifuged at 82 rpm; 43°C. Afterwards, the dried residue was collected and reconstituted with methanol and stored for further qualitative and quantitative analysis (Kala et al., 2017).

4.4. Preparation of essential oil by microwave assisted hydro-diffusion and gravity

60 g of freshly collected leaves were cut into small pieces and precisely weighed and then subjected into the microwave extractor. The experiment was performed without adding any solvent. The extraction was conducted with an intermittent way of irradiation for 2 min with a high microwave power (60%=510W) and followed by natural cooling of the apparatus. For second cycle, the microwave power was reduced to 40% (340W) and for the remaining 4 cycles the microwave power was reduced to 20% (170W). After completing the 6 cycles, oil was deposited and finally collected by passing through the anhydrous sodium sulphate. Anhydrous sodium sulphate was used for removal the water. Afterwards, the oil was kept at 4°C for further qualitative and quantitative analysis (Chouhan et al., 2019).

4.5. Estimation of total phenolic content (TPC)

A preliminary test for total phenolic content was executed using the Folin-Ciocalteu method with three replicates of the sample (Chouhan et al., 2019). Briefly, aliquot of sample (mg/mL) was added in 4 mL of sodium carbonate solution (75 g/L) and 5 mL of 10% Folin- Ciocalteu reagent. Equation of the calibration curve was prepared ($y = 0.136x + 0.001$, $R^2 = 0.999$) by using the gallic acid (with a range of 10 to 80 μg/mL) as the standard and whereas the

methanol was used as a blank. Finally, the results were expressed as gallic acid equivalents in milligram per gram (mg GAE/g) of dried extract with the absorbance were measured at 765 nm using a UV-Vis spectrophotometer.

4.6. Estimation of total flavonoid content (TFC)

The total flavonoid content was quantitatively determined using the aluminium chloride assay method with slight modifications (Chouhan et al., 2019). In brief, leaf extract (mg/mL) was diluted with 300 µL 5% $NaNO_2$ solution, 300 µL of 10% $AlCl_3$ solution, 4 mL distilled water and allowed to incubate for 6 min. Thereafter, 2 mL of 1M NaOH solution was added to the mixture and checked the absorbance at 510 nm using a colorimeter. The total flavonoid content was calculated on the basis of calibration curve ($y = 0.097x + 1.489$, $R^2 = 0.994$) obtained by using quercetin (ranging from 10 to 80 µg/mL) as the standard solution and result was expressed in mg of quercetin equivalents (QE)/mg of plant extract.

4.7. Antioxidant assay

4.7.1. Free radical scavenging activity

The free radical scavenging activity of extract was assessed by the DPPH free radical scavenging method (Chouhan et al., 2019). 0.2 mL of the sample (100 µg/mL) was mixed with 2 mL of 0.1 mM 2,2-diphenyl- 1-picrylhydrazyl (DPPH) stock solution in methanol and left to incubate for 30 min in a dark place. Ascorbic acid was used as positive control and prepared in the same procedure. After the incubation period the absorbance was recorded at 517 nm against the methanol as a blank solution. The percentage of inhibition of DPPH was reported in comparison with the control solution (containing all of the reagents and methanol instead of the test sample). The antioxidant activity of the plant extract was calculated using the formula:

$$I\% = \frac{(Acontrol - Asample)}{Acontrol} \times 100$$

In the above-mentioned equation, A control is the absorbance of the control solution, and A sample is the absorbance of a sample solution.

4.7.2. ABTS radical scavenging activity

The ABTS radical scavenging activity assay was executed with little modifications of the method (Ondua et al., 2019). 7.4 mM of ABTS was mixed with 2.6 mM of potassium persulphate in an equal proportion. Then the prepared stock solution was kept in a dark place at room temperature for 12-16h. Thereafter, 1ml. of ABTS stock solution was mixed with different concentrations (20-100µg/ml) of 1ml of sample solutions (methanol extract and essential oil) and incubated in a dark place for 2hours. Finally, the absorbances of reaction mixtures were checked at 734 nm with the UV-Visible spectrophotometer. Ascorbic acid was used as positive control and produced in the same manner. Potassium persulphate was used as the blank solution. This assay was carried out in triplicate to establish the reliability and the inhibitory potential of ABTS was determined using the equation is given below:

$$I\% = \frac{(Acontrol - Asample)}{Acontrol} \times 100$$

In the aforementioned equation, A control is the absorbance of the control solution, and A sample is the absorbance of a sample solution.

4.7.3. Hydrogen peroxide (H_2O_2) scavenging activity

Hydrogen peroxide antiradical activity was determined with a little modification of method which was reported earlier (Rashid et al., 2018). Hydrogen peroxide (2 mmol) was dissolved into 50 mmol phosphate buffer solution at pH 7.4 and absorbance was measured at 230 nm with the UV-Visible spectrophotometer. A range of concentrations (20-100µg/ml) of samples

were prepared and then 0.4 ml of 50 mmol phosphate buffer (pH 7.4) was added in each of the concentration. Thereafter, the 0.6 ml of hydrogen peroxide solution was added and then the reaction mixture was transferred into the tube and allowed to vortex for 15 min. Ascorbic acid was used as the positive control and prepared in the same fashion. 50 mmol phosphate buffer solution was used as a control solution. The experiment was performed in triplicate to establish the reproducibility of the results. The scavenging potential of Hydrogen peroxide was calculated using the equation as follows:

$$\% \text{ Inhibition} = (1 - Ae/Ao) \times 100$$

Where, Ao is the absorbance of the control solution, and Ae is absorbance of sample solution.

4.8. Chromatographic analysis

4.8.1. GC-MS analysis

The components of leaf extracts were analyzed by GC equipped with a mass spectrometer detector and performed with a few modifications of method which was described earlier (Tohidi et al., 2017). GC-MS analysis was carried out using an Agilent 19091S-433UI gas chromatograph coupled with a HP-5ms 5% phenylmethylsiloxane capillary column (30 m × 0.25 mm i.d.; film thickness 0.25 μm). Aliquot of 1μL was injected into the GC injector port with the split ratio of 1:40. Initially, the oven temperature was set at 60°C for 5 mins. Afterwards, the temperature was increased to 240 °C with a successive increment rate of 3°C/min and followed by at 270 °C with a ramp of 5°C/min and finally kept constant for 8.5 min. Helium gas was considered as carrier gas and maintained a constant flow rate of 1 mL/min with the ionization voltage of 70 eV. The volatile organic components (VOC) were determined by matching their retention indices (RI, HP-5) with the database of NBS75K library. The name, structure and molecular weight of the identified VOC were comprehended

with conductance of the Standard and Technology (NIST) covering an extensive range of patterns stored in the library.

4.8.2. Analysis of β-sitosterol and polyphenols

Individual polyphenolic compounds (aspartic acid, gallic acid, dihydroxy benzoic acid, catechin, chlorogenic acid, vanillic acid, rutin, trans-cinnamic acid, ferulic acid, quercetin, apigenin, kaempferol, tannic acid, and ellagic acid) and phytosterol (β-sitosterol) were quantitively determined using high-performance liquid chromatography (HPLC) equipped with ultraviolet (UV) detector. β-sitosterol was qualitatively and quantitatively analyzed using HPLC system compatible with the Xbridge C18 Column (4.6 mm × 50 mm, 3.5 μm i.d.) at 25°C. The mobile phase was composed of a ratio of acetonitrile and water (95:5 v/v). The chromatographic separation was accomplished in an isocratic mode at the flow rate of 1 ml/min. Finally, the chromatogram was registered at 210 nm (Zhah et al., 2010); against the standard compound which was purchased from Sigma-Aldrich. The qualitative and quantitative estimation of polyphenols was performed using the HPLC system. The experiment was conducted with a slight modification of method as depicted before (Wang et al., 2019). The separation of test samples was performed using column symmetry C18 (4.6 mm × 150 mm, 5 μm i.d.) at 30°C. The mobile phase consisted of 0.17% acetic acid (A) and acetonitrile (B) with a flow rate of 1 ml/min in a gradient manner. Eventually, the chromatogram was recorded at 320 nm and quantified with comparison to external standards.

4.8.3. Analysis of tocopherol

The tocopherol composition of *Vitex negundo* extract was evaluated by High-performance liquid chromatography (Alliance model 2695) with a UV-Vis detector (2487). Tocopherol was separated by using C18 column (4.6 mm × 150 mm × 5 μm particle size), maintained at 30°C. Tocopherol was eluted out using a mixture of mobile phase i.e. methanol and water

(98:2 v/v) at a constant flow rate of 0.5mL/min and total run time of 15 min. 10 µL of sample (100µg/mL) was injected into the injector port and detection was carried out at 292 nm. The compound was detected and quantified with comparison of the peak area and time of the authentic standard (tocopherol) purchased from the Sigma Aldrich dissolved in methanol and diluted in the same manner (Ge et al., 2002).

4.9. *In-vitro* antibacterial assay

4.9.1. Test microorganisms

To determine the antibacterial activities the four bacterial strains including two gram positive (*Staphylococcus aureus* MTCC 9542, *Enterococcus faecalis* MTCC 389) and two gram negative (*Escherichia coli* MTCC 9541, *Pseudomonas aeruginosa* MTCC 8077) were used to assess the effectiveness of the leaf extracts.

4.9.2. Preparation of McFarland standard

McFarland number 0.5 standard consisted of 9.95 ml of 1% H_2SO_4 dissolved in distilled water and 0.05 ml of 1% of $BaCl_2$ dissolved in distilled water and used to evaluate the bacterial density. The mixture was preserved into an airtight container for further requirement.

4.9.3. Disk diffusion assay

The *in-vitro* antibacterial activity of leaf extracts were performed using agar disk diffusion method with little modifications as previously described (De Zoysa et al., 2019). Different concentrations (crude extract, 10-fold dilution, and 100-fold dilution) of leaf extracts were dissolved in 10% of DMSO. The density of bacterial cell suspension was set in accordance with 0.5 McFarland standard (1.0×10^8 CFU/ml) by employing a turbidometer. Then the bacterial strains were separately introduced onto the Mueller-Hinton agar plates and allowed

to be dried for 5 mins. Filter paper disks (Whatman No. 1, diameter = 6 mm) were dipped into the 10µL of leaf extracts and placed on the prepared Mueller-Hinton agar plates. To assess the test validity Chloramphenicol disk (30µg), DMSO-soaked filter paper disk were considered as positive control and negative control respectively and the plates were incubated for 18-24 h at 37°C. Thereafter, the sensitivities of bacterial strains to the plant extracts were evaluated by measuring the growth inhibition zones observed around the disk using a Vernier caliper. To establish the reproducibility of results the assay was performed in triplicate.

4.9.4. Minimum inhibitory concentration (MIC)

The minimum inhibitory concentrations were estimated by the microplate dilution method with a little modification of as previously reported (De Zoysa et al., 2019). Four fold serial dilutions of leaf extracts were prepared by dissolving in 10% of DMSO. Mueller-Hinton broth was used to develop the bacterial inoculums and where the density of bacterial cell suspension was adjusted in accordance with 0.5 McFarland turbidity standard (1.0×10^8 CFU/ml). Then 150µL volume of the previously diluted leaf extracts were withdrawn and added to each well of the 96-well micro-titer plate and followed by the addition of 50 µL of bacterial suspension to all the wells omitting the negative controls. For this experiment, the Chloramphenicol was used as positive control and 10% DMSO was the negative control where the leaf extracts were also presented without the presence of a bacterial cell. The 96-well plates were transferred to the incubator for 24 h at 37°C and absorbance was measured at 630 nm.

4.10. *In-vitro* anti-inflammatory assay

4.10.1. Cell line and culture conditions

Murine RAW264.7 macrophage cell line was purchased from American Type Culture Collection. The cells were cultured in DMEM with 10% heat-inactivated fetal bovine serum

and 1% penicillin-streptomycin, and supplemented in a humidified atmosphere at 37°C in 5% CO2. Plate the cells onto 96-well plate at a density of 2×10^4 and incubated for 24 h. For this experiment, the cells were pretreated with lipopolysaccharide (LPS).

4.10.2. Cell viability assay of RAW macrophages

The cell viability of RAW 264.7 cell was assessed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay which was earlier narrated and was used with slight modification (Nelson et al., 2016). About 70% confluent cells which were treated with different concentrations (6.25 μg/ml, 12.5 μg/ml, 25 μg/ml, 50 μg/ml, and 75 μg/ml) of plant extracts and incubated for 24 h. Thereafter, the culture medium was replaced by the fresh culture medium and MTT solution (0.5 mg/ml) was added. Subsequently, the plate was kept incubated for 3 h. In next step, the MTT solution was removed and 100 μl dimethyl sulfoxide was incorporated into each well to solubilize the formed formazan crystals. Finally, the absorbance was measured at 540 nm using a micro-plate reader. Cell viability of the test samples was expressed as percentage compared to the control solution.

4.10.3. LPS stimulation and assessment of inflammatory cytokines production (IL-1β, IL-6, and IL-10 level)

To investigate the inhibitory effect of the leaf extracts on cytokine levels, the RAW 264.7 cells were pretreated with LPS (1 μg/mL). LPS-induced RAW 264.7 cells (2×10^4) were seeded into 96 well-plates, and after 24 h incubation period the cells were again treated with the various concentrations (12.5 μg/ml, 25 μg/ml, and 50 μg/ml) of the methanol extract and essential oil; and then incubated for 24 h at 37°C, 5% CO_2. Supernatants were separated and stored at −20°C. Cells were thawed and used for the estimation of the levels of IL-1β, IL-6, and IL-10 using ELISA kits (Abcam ELISA Kit, Cambridge, MA, USA). These three experiments were carried out in accordance with the clear instructions that were provided by

the manufacturers. Eventually, the absorbance was checked at 450 nm using a micro-plate reader. Ibuprophane (1 µg/ml) was employed as standard and LPS was employed as the control (Kim et al., 2018).

4.11. *In-vitro* anticancer assay

4.11.1. Cell lines and cell culture conditions

The prostate cancer cell line (PC-3) and normal lung tissue cells (WI-38) were procured from American Type Culture Collection (ATCC) and cultured in the laboratory in DMEM supplemented with 10% FBS (Invitrogen), 1% L-Glutamine, 0.1 mM non-essential amino acid, and 100 U/ml penicillin/streptomycin at 37°C; in a humidified atmosphere with 5% of CO_2 (Nelson et al., 2016). The culture medium was replaced with the fresh medium at every 48 to 72h. At the time cells reached density, the culture medium was removed from the 75cm^2 flask and washed with phosphate-buffered saline (PBS). Afterwards, for removal of adhesion-proteins, the 1.5 mL of Trypsin-EDTA was added and placed into the incubator at 37°C in a humidified atmosphere with 5% of CO_2 for 5 mins. Subsequently, 3mL of culture media was given to terminate the action of trypsin. Then cells were transferred to a new flask for their sub-culture growth. Finally, cells were quantified by using a haemocytometer.

4.11.2. Cell survival assay

The effect of plant extract on cellular proliferation was evaluated with cell viability assay using the tetrazolium salt (3-[4,5-dimethylthiazol-2-yl]-2,5diphenyl tetrazolium bromide) (MTT) which was reduced by the mitochondrial succinate dehydrogenase and form the formazan crystal (Nelson et al., 2016). Cultured cells were grown until they reached 70% of confluence and then treated with the several concentrations (6.25 µg/ml, 12.5 µg/ml, 25 µg/ml, 50 µg/ml, and 75 µg/ml) of leaf extracts or DMSO as vehicle in triplicate. After 24 h, 48 h of incubation period the cell viability was assessed by adding MTT solution (5 mg/mL

in PBS buffer) into each well and incubated at 37°C for 3 h. Then, the cell culture media was removed from each well and newly formed formazan complexes were solublized in 200 µL DMSO. Finally, the absorbance was checked at 595 nm using micro-plate reader and values of cell viability in different concentration of samples were quantified and plotted on a graph. Different time points (24 h and 48 h) were evaluated during the experiment.

4.12. Statistical analysis

All the experiments were executed in triplicate. The results were expressed as means ± SD (n = 3) and the statistical analyses were performed using one-way analysis of variance (ANOVA), followed by Bonferroni's multiple comparison test was performed using GraphPad Prism (version 5.04) software.

Chapter 5

Results

5. Results

5.1. Estimation of TPC and TFC

Phenolics and flavonoids are the two vital indicators predominantly employed to represent the plant-based bioactivities in leaf extracts. In the current study, the TPC was quantified by the Folin-Ciocalteu assay method. The amounts of total phenolic contents in methanol extract and essential oil were found to be 1.6892 ± 0.11 mg GAE/gm and 1.6625 ± 0.13 mg GAE/gm respectively (displayed in Table 5.1). The results revealed the presence of a higher quantity of phenolic content in methanol extract than the essential oil.

The TFC in *Vitex* leaf was determined by aluminium chloride assay method and the amounts of TFC in methanol extract and essential oil were obtained to be 1.07 ± 0.07 mg QE/gm and 0.505 ± 0.06 mg QE/gm respectively (displayed in Table 5.1). Here, the results also exhibited the higher TFC value in methanol extract than the essential oil.

Table 5.1: Estimation of total phenolic and total flavonoid contents in methanol extract and essential oil of *Vitex negundo* leaves.

Plant Extract	Total phenolics (mg GAE /gm)	Total flavonoids (mg QE /gm)
Methanol extract	1.6892 mg/gm ± 0.11	1.07 mg/gm ± 0.07
Essential oil	1.6625 mg/mL ± 0.13	0.505 mg/mL± 0.06

The results are expressed as mg of gallic acid and quercetin equivalent/gm of methanol extract and essential oil of *Vitex negundo* leaves. Results in table are expressed as mean (±) standard deviation of triplicate experiments.

5.2. Antioxidant assay

Radical scavenging activities of *Vitex* leaf extracts have been currently executed by using three sets of antioxidant tests. In the first set of experiments, the antioxidant activities of methanol extract and essential oil were evaluated by DPPH radical scavenging assay. The IC_{50} value of methanol extract was found to be 89 µg/mL, while the essential oil showed 50% of inhibition at the dose of 300 µg/mL (shown in Table 5.2).

In the second set of experiments, the antioxidant activities of methanol extract and essential oil were performed by using ABTS assay method. Here, the results showed that the methanol extract exhibited the 50% inhibition at the dose of 10µg/mL, whereas the IC_{50} value of essential oil was found to be 100 µg/mL (shown in Table 5.2).

In the third set of experiments, the activities of methanol extract and essential oil were carried out by using the H_2O_2 assay method. The IC_{50} values of methanol extract and essential oil were found to be 57 µg/mL and 75 µg/mL respectively (shown in Table 5.2).

The obtained result from three sets of chemical assays exhibited higher antioxidant potential of methanol extract than the essential oil. With DPPH assay, the antiradical potential of the natural antioxidants was estimated. And where the methanol extract showed an effective antioxidant activity (IC_{50} – 90 µg/mL) while the essential oil showed a moderate antioxidant activity (IC_{50} – 300 µg/mL). The ABTS assay is widely applied to measure the effectiveness of the total antioxidant activity of substances. However, the methanol extract showed a significant antioxidant power with a low concentration (IC_{50} – 10 µg/mL) whereas the essential oil exhibited a moderate antioxidant activity (IC_{50} – 100 µg/mL). With hydrogen peroxide assay, the scavenging ability of leaf extracts to hydroxyl radicals was determined. Here, the methanol extract showed an effective scavenging activity with a concentration of 58

µg/mL (IC_{50} -58 µg/mL) while essential oil exhibited the same effectiveness with a slight higher concentration of 76µg/mL (IC_{50} -76 µg/mL).

Table 5.2: Radical scavenging activities (IC_{50} values) of methanol extract and essential oil of *Vitex negundo* leaves.

	Assay methods		
Plant Extract	**DPPH radical (AAE µg/mL)**	**ABTS radical (AAE µg/mL)**	**H_2O_2 radical (AAE µg/mL)**
Methanol extract	89 ± 0.57	10 ±1.15	57 ± 1
Essential oil	300 ± 1.53	100 ± 2	75 ± 0.58

Results in table are expressed as mean (±) standard deviation of triplicate experiments. IC_{50} (µg/mL) values of methanol extract and essential oil were compared to ascorbic acid.

5.3. Chromatographic analysis

5.3.1. GC-MS analysis

The qualitative analysis of crude extract and fresh leaf oil were investigated by GC-MS. The identified phytochemical constitutes of methanol extract and essential oil are summarized in (Figure 5.1 and Table 5.3, Table 5.4 respectively) following tables along with their names, molecular formulas, molecular weight and Chemical Abstracts Service (CAS) numbers. Total 15 volatile organic compounds were identified from methanol extract and 6 volatile organic compounds were identified from essential oil. In methanol extract, the recognized major bioactive volatile organic compounds were isocaryophyllene, caryophyllene, β-myrcene, 1-methylethyl ester hexanoic acid, 3-methyl-decanoic acid, α-farnesene, thunbergol, (isomer 1) cis-trans citral, phytol, oleic acid, (o-nitrophenyl)-hydrazone valeraldehyde, 2-myristynoyl-glycinamide, squalene, maltol, lactose. In essential oil, the detected vital bioactive volatile

organic compounds were 4-methyl-2-heptanol, 2-myristynoyl-glycinamide, camphor, γ-sitosterol, β-sitosterol, asarone.

Table 5.3: The identified volatile components of methanol extract of *Vitex negundo* leaf are enlisted below:

Compound name	Mole formula	Mole weight	(CAS) number
α-caryophyllene	$C_{15}H_{24}$	204.35	006753-98-6
Caryophyllene	$C_{15}H_{24}$	204.35	000087-44-5
β-myrcene	$C_{10}H_{16}$	136.23	000123-35-3
1-methylethyl ester hexanoic acid	$C_9H_{18}O_2$	158.24	002311-46-8
3-methyl-decanoic acid	$C_{11}H_{22}O_2$	186.29	060308-82-9
α-farnesene	$C_{15}H_{24}$	204.35	000502-61-4
Thunbergol	$C_{20}H_{34}O$	290.5	025269-17-4
(Isomer 1) cis-trans citral	$C_{10}H_{16}O$	152.23	000000-00-0
Phytol	$C_{20}H_{40}O$	296.539	000150-86-7
Oleic acid	$C_{18}H_{34}O_2$	282.5	000112-80-1
(o-nitrophenyl)-hydrazone valeraldehyde	$C_{11}H_{15}N_3O_2$	221.26	005977-70-8
2-myristynoyl-glycinamide	$C_{16}H_{28}N_2O_2$	280.41	000000-00-0
Squalene	$C_{30}H_{50}$	410.730	007683-64-9
Maltol	$C_6H_6O_3$	126.11	000118-71-8
Lactose	$C_{12}H_{22}O_{11}$	342.3	000063-42-3

The volatile organic compounds were recognized by employing of the NBS75K Library and HP-5ms capillary column

Table 5.4: The identified chemical compositions of essential oil in *Vitex negundo* leaf are listed below:

Compound name	Mole formula	Mole weight	(CAS) number
4-methyl-2-heptanol	$C_8H_{18}O$	130.23	056298-90-9
2-myristynoyl-glycinamide	$C_{16}H_{28}N_2O_2$	280.41	000000-00-0
Camphor	$C_{10}H_{16}O$	152.23	000076-22-2
γ-sitosterol	$C_{29}H_{50}O$	414.7	000083-47-6
β-sitosterol	$C_{29}H_{50}O$	414.7	000083-46-5
Asarone	$C_{12}H_{16}O_3$	208.25	002883-98-9

The volatile organic compounds were recognized by employing of the NBS75K Library and HP-5ms capillary column.

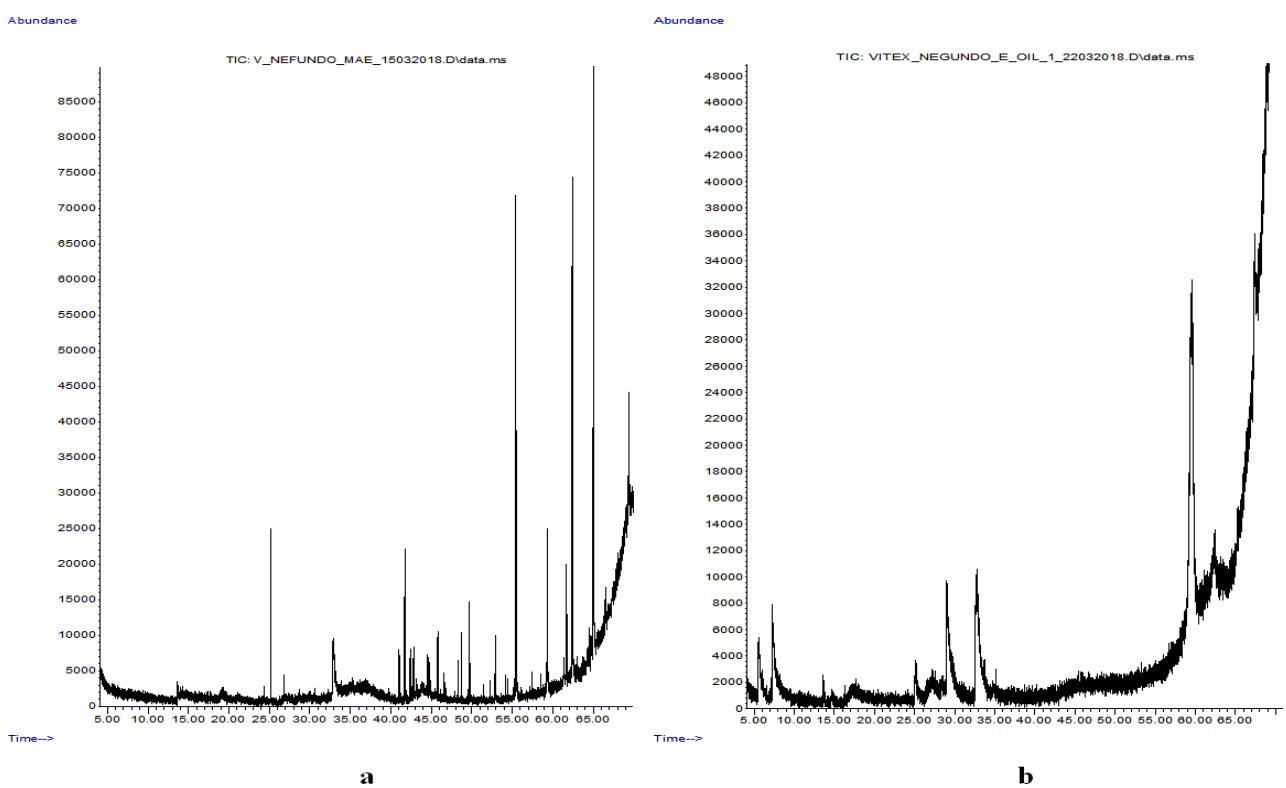


Figure 5.1. Chromatograms of volatile organic compounds were obtained from the (a) methanol extract (b) essential oil of *Vitex negundo* leaf.

5.3.2. Analysis of β-sitosterol

In the present study, β-sitosterol was identified and quantified by an optimized HPLC method. Figure 5.2.a) displayed the chromatogram of β-sitosterol methanol extract of *Vitex* leaves. The amount of β-sitosterol was obtained to be 425.45 μg/gm in methanol extract.

Figure 5.2.b) clearly demonstrated the chromatogram of β-sitosterol and the amount was found to be 23.42 μg/gm in essential oil. This experiment revealed that the methanol extract possessed much higher quantity of β-sitosterol than the essential oil.

Table 5.5 represented the quantifications of β-sitosterol were obtained from methanol extract and essential oil of *Vitex* leaves and figure 5.2.c) showed the chromatogram of β-sitosterol of standard compound.

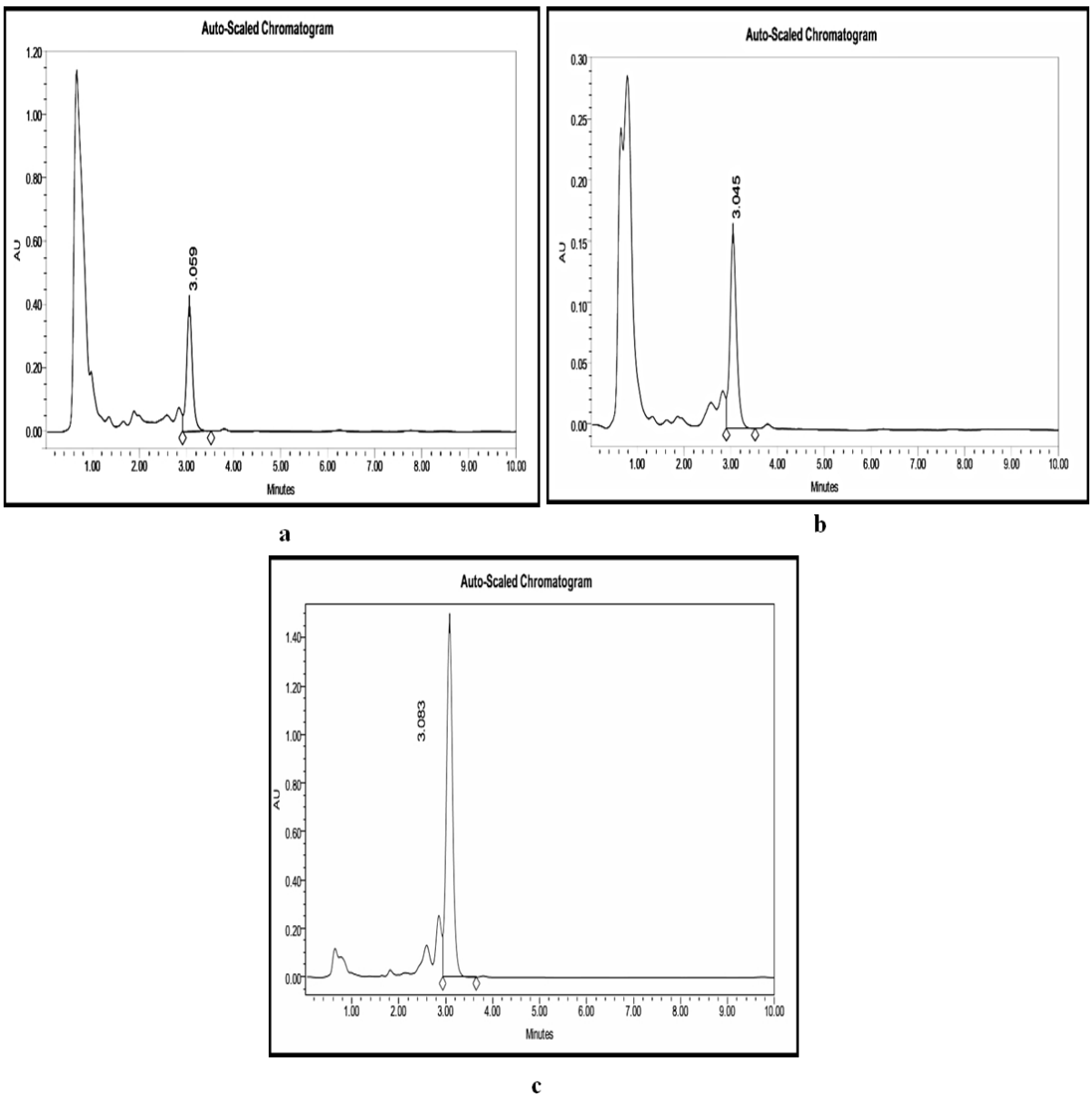


Figure 5.2: RP-HPLC chromatogram of β-sitosterol was found in a) methanol extract b) essential oil and c) standard compound of *Vitex negundo* leaf.

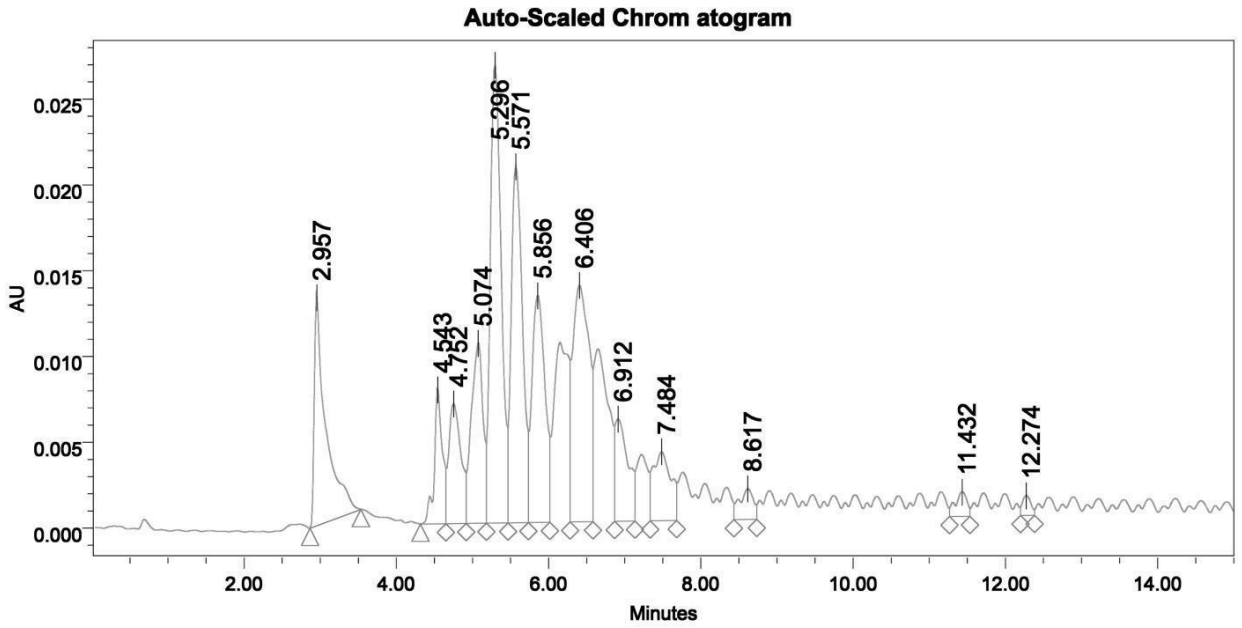
Table 5.5: Quantification of β-sitosterol in methanol extract and essential oil of *Vitex negundo* leaf.

SL NO	Peak Name	Retention time	Area	Height	Amount (µg/gm)
1	β- Sitosterol (methanol extract)	3.059	3478289	398596	425.45
2	β- Sitosterol (E.Oil)	3.045	1564544	160783	23.42

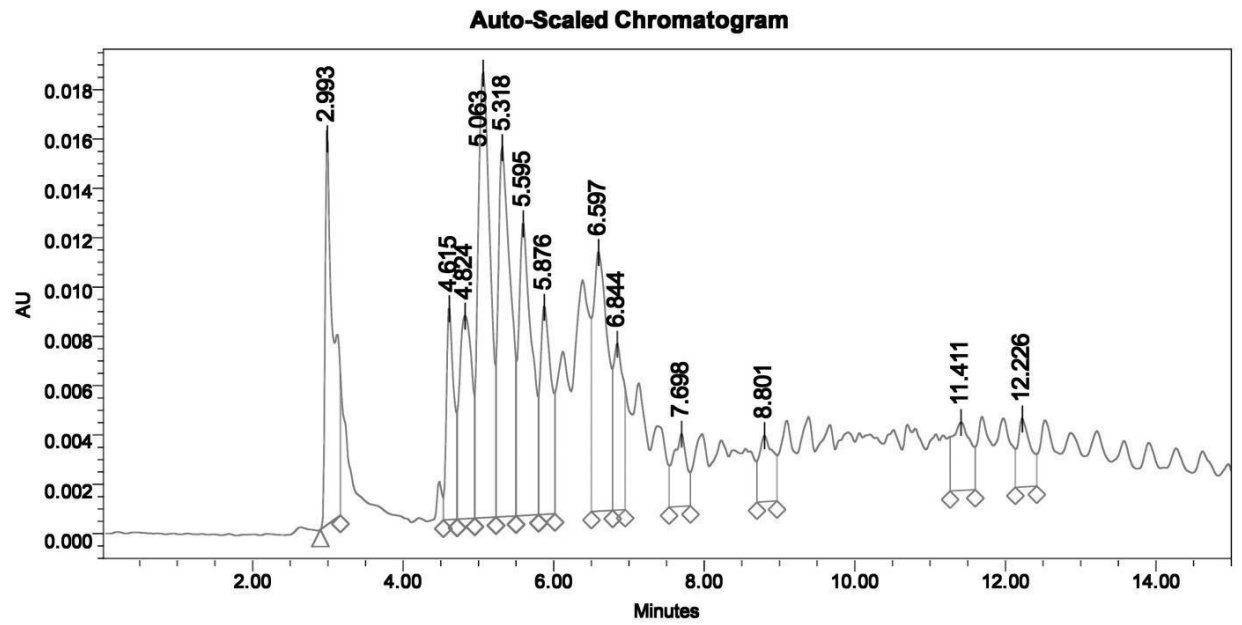
5.3.3. Analysis of polyphenols

An array of polyphenols was detected by HPLC; separation associated with UV detection. The HPLC fingerprints showed the appearance of being a collage of thirteen compounds such as aspartic acid, gallic acid, dihydroxy benzoic acid, catechin, chlorogenic acid, vanillic acid, rutin, transcinnamic acid, ferulic acid, quercetin, apegenin, kaempferol, tannic acid with different elution times.

Figure 5.3.a) demonstrated the chromatogram for polyphenols analysis of methanol extract of *Vitex* leaves and Table 6 revealed the amounts of polyphenols were presented in methanol extract. Among the above mentioned compounds, the rutin (480.39 µg/gm) and vanillic acid (976.86 µg/gm) were extensively found out in methanol extract. Figure 5.3.b) demonstrated the chromatogram for polyphenols analysis of essential oil and Table 5.6. evidently disclosed the amounts of polyphenols were presented in essential oil. As compared to essential oil, the methanol extract possessed a higher amount of individual polyphenolic compound and which leads to exhibit the potential of the methanol extract. Two major polyphenols such as vanillic acid (81.87 µg/gm) and trans-cinnamic acid (55.35 µg/gm) were detected in essential oil.



a



b

Figure 5.3: HPLC chromatograms of polyphenols were detected in a) methanol extract and b) essential oil of *Vitex negundo* leaf.

Table 5.6: Quantification of polyphenols in methanol extract of *Vitex negundo* leaf.

SL NO	Peak Name	Retention time	Area	Height	Amount (µg/gm)
1	Aspartic acid	2.957	142228	13685	7.93
2	Gallic acid	4.543	57473	7994	10.04
3	Dihydroxy benzoic acid	4.752	78588	6999	87.43
4	Catechin	5.074	113245	10590	82.77
5	Chlorogenic acid	5.296	266070	26762	28.20
6	Vanillic acid	5.571	213456	20861	976.86
7	Rutin	5.856	158276	13253	480.39
8	Transcinnamic acid	6.406	208049	13806	66.66
9	Ferulic acid	6.912	70162	5965	7.02
10	Quercetin	7.484	64826	4013	95.43
11	Apegenin	8.617	22583	1775	6.96
12	Kaempferol	11.432	14965	1420	0.896
13	Tannic acid	12.274	8812	1149	1.785

Table 5.7: Quantification of polyphenols in essential oil of *Vitex negundo* leaf.

SL NO	Peak Name	Retention time	Area	Height	Amount (µg/gm)
1	Aspartic acid	2.993	121392	15916	0.829
2	Gallic acid	4.615	59887	8584	1.22
3	Dihydroxy benzoic acid	4.824	94935	8254	1.29
4	Catechin	5.063	205650	18078	18.39
5	Chlorogenic acid	5.318	171837	15009	2.23
6	Vanillic acid	5.595	146223	11837	81.87
7	Rutin	5.876	85217	8479	0.32
8	Transcinnamic acid	6.597	141925	10490	55.35
9	Ferulic acid	6.844	59645	6734	0.73
10	Quercetin	7.698	36503	2926	6.57
11	Apegenin	8.801	34787	2685	1.31
12	Kaempferol	11.411	45572	2761	0.33
13	Tannic acid	12.226	33111	2788	0.82

5.3.4. Analysis of tocopherol

Figure 5.4. revealed the presence of tocopherol in methanol extract and essential oil of *Vitex negundo* leaves. The contents of tocopherol in methanol extract and essential oil were obtained to be 414.87 µg/gm and 52.66 µg/lit respectively (shown in table 5.8). Such results indicated the presence of higher amounts of tocopherol in methanol extract and lower content of tocopherol was recorded in the essential oil.

Table 5.8: Estimation of tocopherol in methanol extract and essential oil of *Vitex negundo* leaves.

Plant Extract	Tocopherol (µg/gm) /(µg/lit)
Methanol extract	414.87 ± 1.52
Essential oil	52.66 ± 2.17

The results are expressed as µg of α-tocopherol equivalent/gm of methanol extract and essential oil of *Vitex negundo* leaves. Results in table are expressed as mean (±) standard deviation of triplicate experiments.

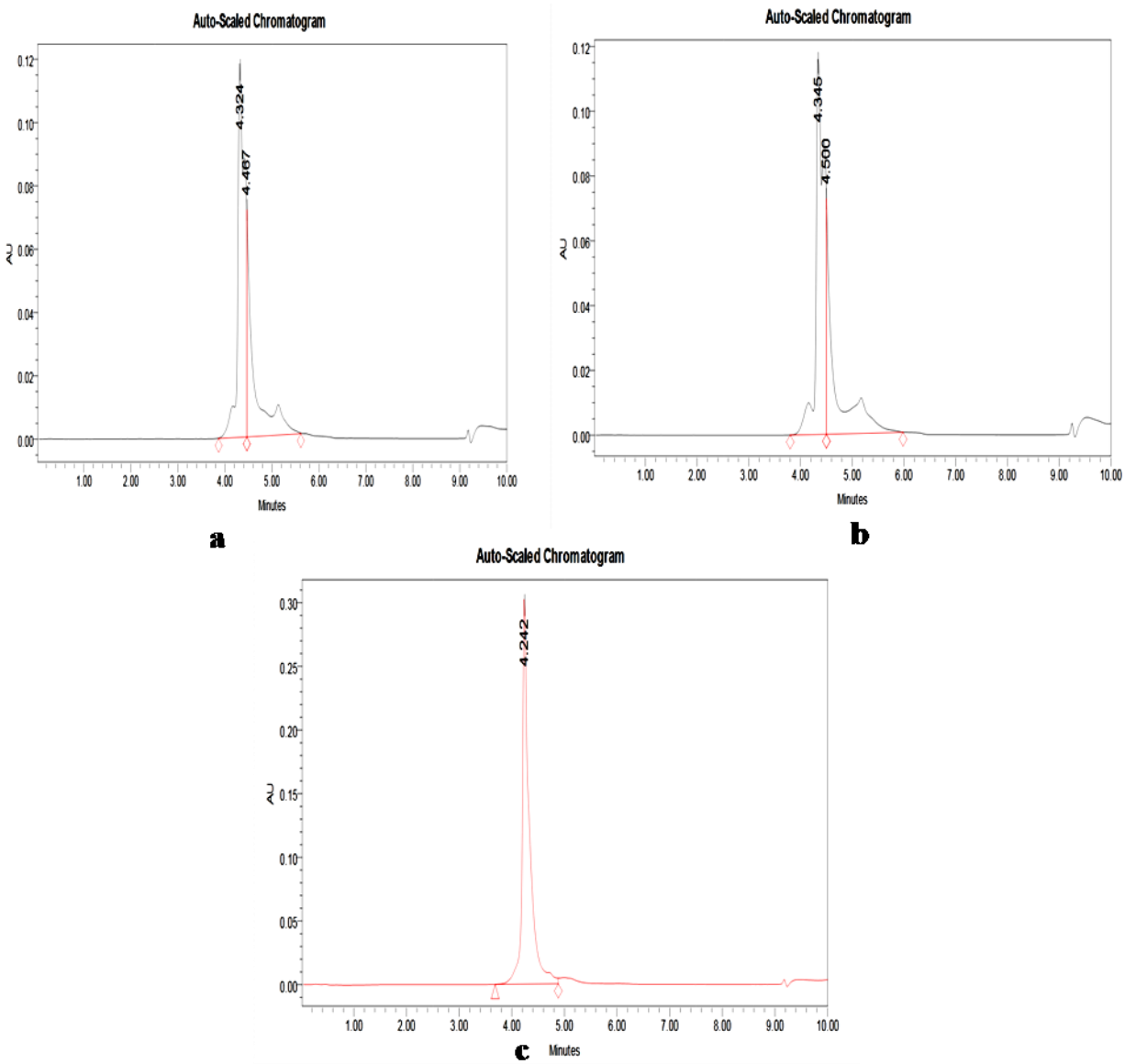


Figure 5.4: Reversed phase-high-performance liquid chromatography of tocopherol was detected in (a) methanol extract (b) essential oil and (c) standard compound of *Vitex negundo* leaf.

5.4. *In-vitro* antibacterial activity

5.4.1. Effects of methanol extracts and essential oil on bacteria

In current research work, the leaf extracts of *Vitex negundo* exhibited antibacterial activities against the four test bacteria commonly cause for urinary tract infection (UTI). Both of the extracts showed the growth inhibition zone with a range of 17-27 mm (displayed in Table

5.9.). *In vitro,* antibacterial assay showed that the methanol extract of *Vitex* leaf formed an inhibition zone of 17 ± 0.58 mm, 19 ± 1.0 mm, 23 ± 0.46 mm, 21 ± 0.40 mm against *Staphylococcus aureus*, *Enterococcus faecalis* and *Escherichia coli*, *Pseudomonas aeruginosa* strains respectively. Among these four pathogenic strains the *Escherichia coli* revealed the maximum zone of inhibition.

On the other hand, essential oil of *vitex* leaf also revealed remarkable antibacterial activities with an inhibition zone of 23 ± 0.95 mm, 27 ± 0.82 mm, 22 ± 0.76 mm, 21 ± 0.85 mm against *Staphylococus aureus*, *Enterococcus faecalis* and *Escherichia coli*, *Pseudomonas aeruginosa* respectively. Here, the essential oil showed the maximum potential antibacterial activity against the *Enterococcus faecalis*.

The determination of MIC values (minimum inhibitory concentration) of leaf extracts were performed only after getting the significant zone of inhibition. The obtained MIC potentials of leaf extracts were lesser than the MIC potentials of the used reference standard (chloramphenicol). The moderate MIC values of methanol extract were found to be 6.75 mg/mL, 4.16 mg/mL, 1.66 mg/mL and 4.20 mg/mL, against the above mentioned test organisms. In case of essential oil, the MIC obtained values were 9.89 mg/mL, 8.79 mg/mL, 9.90 mg/mL and 12.72 mg/mL respectively (displayed in Table 5.9).

The present study revealed that the methanol extract exhibited the more potential antibacterial activities against gram negative bacteria such as *Escherichia coli* with a value of $p < 0.0001$ and whereas the essential oil showed its potential towards the gram positive bacteria such as *Enterococcus faecalis* with a value of $p < 0.0001$. Table 5.9 represents the zone of inhibitions and the obtained MIC values of leaf extracts and chloramphenicol against the four selected pathogenic strains.

Table 5.9: Antibacterial activity of methanol extract, essential oil of *V. negundo* leaves and Chloramphenicol were assessed against Gram's positive (*S. aureus, E. faecalis*) and Gram's negative (*E. coli, P. aeruginosa*) bacterial strains.

Bacteria	Methanol extract		Essential oil		Chloramphenicol	
	Inhibition zone diameter (mm)	MIC Value (mg/mL)	Inhibition zone diameter (mm)	MIC Value (mg/mL)	Inhibition zone diameter (mm)	MIC Value (mg/mL)
Staphylococcus aureus	17 ± 0.58	6.75	23 ± 0.95	9.89	33 ± 0.40	1
Enterococcus faecalis	19 ± 1.0	4.16	27 ± 0.82	8.79	29 ± 0.25	1
Escherichia coli	23 ± 0.46	1.66	22 ± 0.76	9.90	36 ± 0.35	1
Pseudomonas aeruginosa	21 ± 0.40	4.20	21 ± 0.85	12.72	31 ± 0.80	1

The results of inhibition zone diameter are expressed as mm and MIC values are expressed as mg/mL. Results in table are expressed as mean (±) standard deviation of three independant experiments. ***$p < 0.0001$ statistically significant in comparison with respective controls.

5.5. *In-vitro* anti-inflammatory activity

5.5.1. Effects of methanol extracts and essential oil on viability of RAW macrophage cell lines

In order to assess the effect of cell viability on Raw 264.7 macrophage cell lines, the MTT assay was carried out at 24 h with various concentrations (6.25 µg/ml, 12.5 µg/ml, 25 µg/ml, 50 µg/ml, 75 µg/ml) of methanol extract and essential oil; and ibuprofen (1 µg/ml).

Figure 5.5.a) showed the percentage of cell viability of methanol extract on RAW 264.7 macrophages. The results exhibited a slight reduction (16.3% - 24.4%) in cell viability with gradual increment of the concentrations.

Figure 5.5.b) represented the no obvious cytotoxicity of essential oil on RAW 264.7 cells. The figure also displayed the little reduction (13% - 24.4%) in cell viability at different concentrations up to 75 µg/ml. In this experiment, the Figure 5.5.a) and 5.5.b) demonstrated almost similar cytotoxic effects on RAW 264.7 cells.

Hence, the concentrations were used on or under 75µg/ml, producing negative effect on cell viability and considered as the appropriate concentrations for evaluating the anti-inflammatory activity on RAW 264.7 macrophages (Figure 5.5.a) and 5.5.b)). The concentration of ibuprofen (1 µg/ml) was used also demonstrating a non-toxic effect on cell viability and thus that particular dose warrants for assessing the anti-inflammatory activity on RAW 264.7 macrophages.

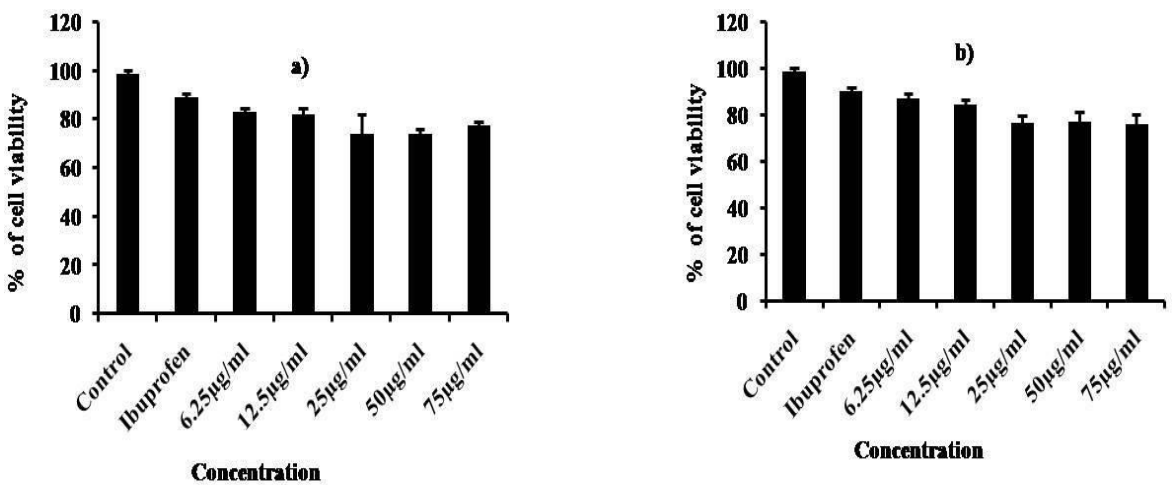


Figure 5.5: Concentration dependant cell viability of a) methanol extract and b) essential oil against RAW macrophage cell line at 24 hours. Ibuprofen (1 µg/mL) is showing no cytotoxicity to RAW macrophage cells. The values are expressed as the percentage in comparison with the untreated (control) cell group and each bar in the graph is represented as mean ± SD (n = 3).

5.5.2. Effects of methanol extracts and essential oil on LPS induced inflammatory cytokine production

To investigate the anti-inflammatory activity of *Vitex negundo* leaf extracts, the ELISA based assay was performed on RAW 264.7 macrophage cells.

The strong inhibitory effects of leaf extracts were found out against two types of pro-inflammatory cytokines including IL-1β, IL-6 and reinforcing effects towards the anti-inflammatory cytokine such as IL-10. Figure 5.6.a), 5.6.b), 5.6.c) demonstrated the ability of methanol extract to restrict the *in-vitro* release of pro-inflammatory cytokines and enhance the release of anti-inflammatory cytokine using lipo-polysaccharide (LPS)-stimulated RAW264.7 cells against a range of three different (12.5 µg/ml, 25 µg/ml, 50 µg/ml) effective doses. Based upon the effectiveness of methanol extract, the 50 µg/ml dose has been considered as a strong significant dose with a value of $p < 0.001$ for all the biomarkers; when the effective dose was compared with LPS induced inflammatory groups. Interestingly, somewhere the 50 µg/ml dose exhibited the significant difference and even the stronger anti-inflammatory activity, when the 50 µg/ml dose was compared with the positive drug ibuprofen (1µg/ml).

Figure 5.7.a), 5.7.b), 5.7.c) represented the potential of essential oil towards the *in-vitro* anti-inflammatory activity. Afterwards, the anti-inflammatory assay of essential oil revealed almost the similar effects on different cytokines; as produced by the methanol extract. The three graphs Figure 5.7.a), 5.7.b), 5.7.c) displayed the dose dependant anti-inflammatory property of essential oil, where the 50 µg/ml dose was again proved as the most effective dose (for all biomarkers; except IL-10) with a value of $p < 0.001$. Just in one exception, the leaf oil exhibited the maximum activity on IL-10 at the 50 µg/ml dose with a statistical significance $p < 0.01$. Result also showed that the bio-activities of essential oil was directly

proportional with the effective doses and the 50 µg/ml dose exhibited a significant difference in comparison with the positive drug ibuprofen (1 µg/ml).

In case of extract, interleukin-6 exhibited evidently maximum rate of inhibition whereas in case of essential oil interleukin-6 again showed the maximum degree of preventive effects. In comparison with essential oil the methanol extract exhibited distinctly higher inhibitory activity on RAW cells. Interleukin-6 (IL-6) is conventionally known as a major secreted factor and generally secrets in the feedback reaction of cell damage or infection.

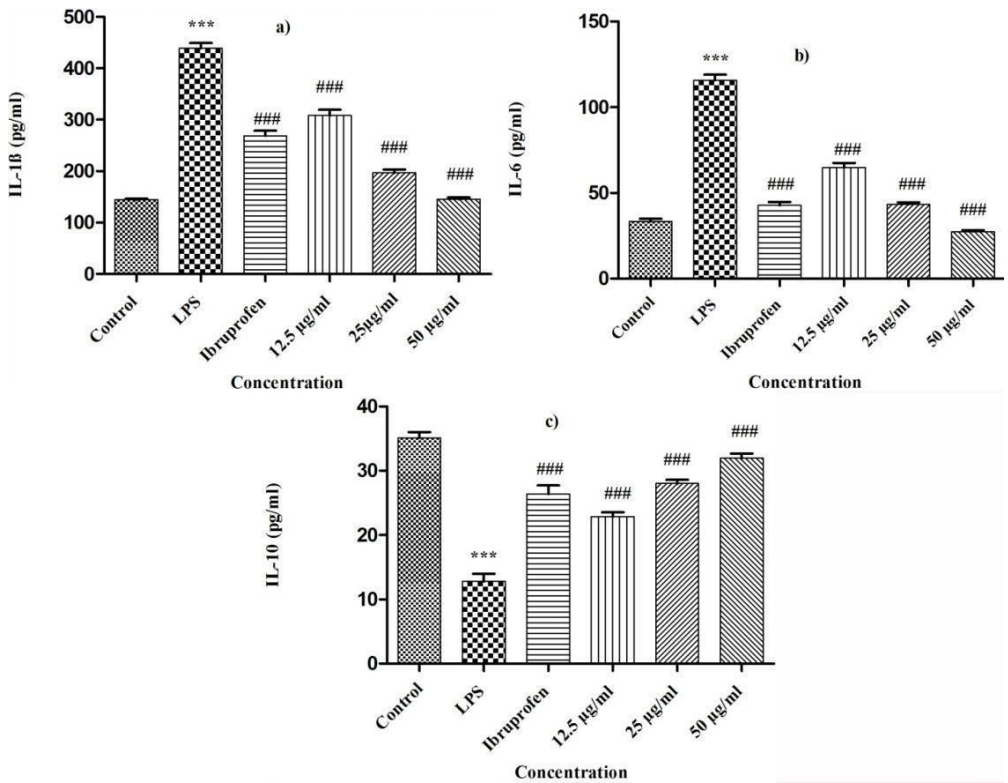


Figure 5.6. Concentration dependent anti-inflammatory effects of methanol extract of *V. negundo* on RAW 264.7 macrophages. The RAW 264.7 cells were treated with leaf extract (12.5 µg/ml, 25 µg/ml and 50 µg/ml) in presence of LPS (1µg/ml). The concentrations of a) IL-1β, b) IL-6, c) IL-10 were measured in culture media using the booklets were provided with the commercial ELISA kits. Results are represented as the mean ± SD of at least triplicates of each experiment. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$, statistically significant in comparison with control; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, and $^{\#\#\#}p < 0.001$, statistically significant in comparison with LPS-induced inflammatory groups by one-way ANOVA and followed by bonferroni's multiple comparison test.

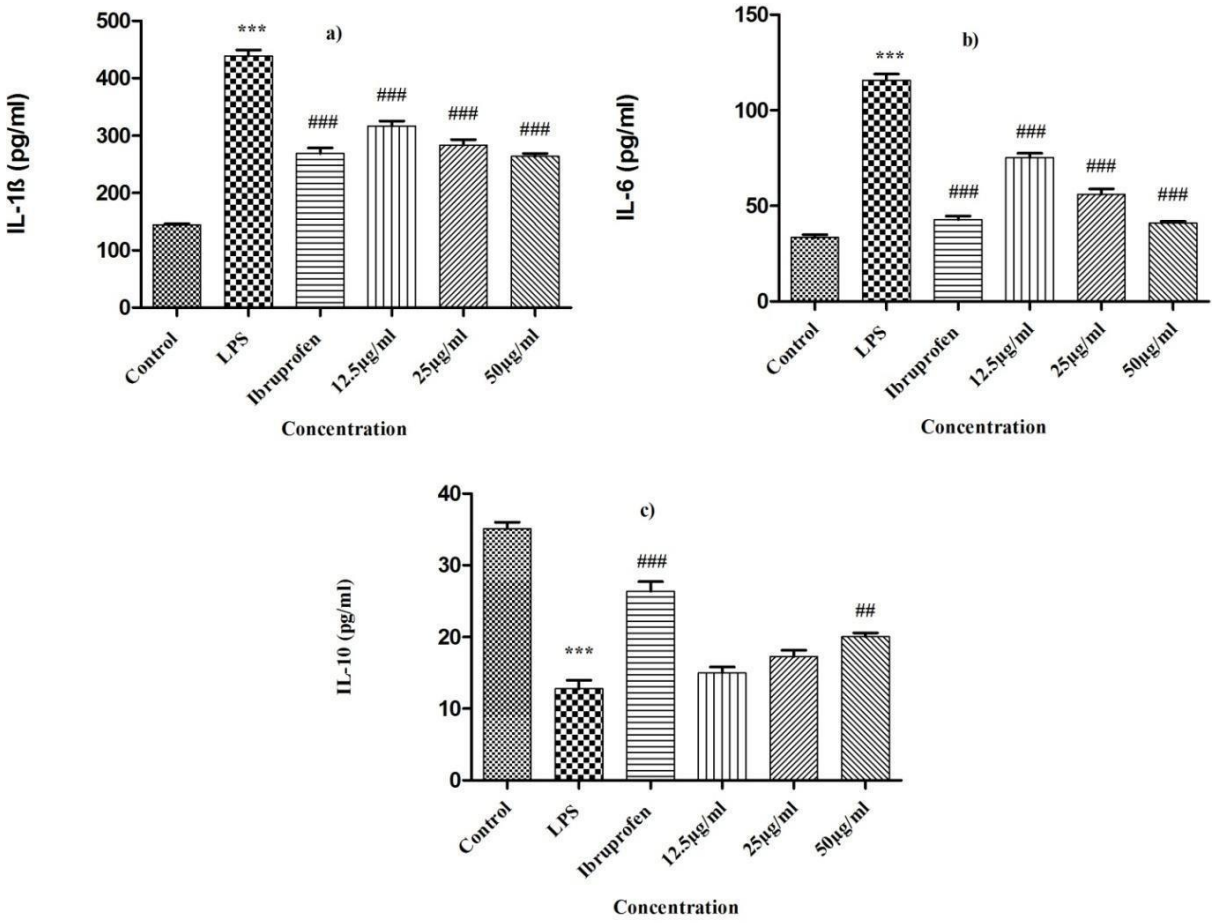


Figure 5.7. Concentration dependant anti-inflammatory effects of essential oil of *V. negundo* on RAW 264.7 macrophages. The RAW 264.7 cells were treated with leaf oil (12.5 µg/ml, 25 µg/ml and 50 µg/ml) in presence of LPS (1 µg/ml). The concentrations of a) IL-1β, b) IL-6, c) IL-10 were measured in culture media using the booklets were provided with the commercial ELISA kits. Results are represented as the mean ± SD of at least triplicates of each experiment. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$, statistically significant in comparison with control; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, and $^{\#\#\#}p < 0.001$, statistically significant in comparison with LPS-induced inflammatory groups by one-way ANOVA and followed by bonferroni's multiple comparison test.

5.6. *In-vitro* anticancer activity

5.6.1. Effects of methanol extracts and essential oil on viability of human prostate cancer (PC3) cell lines

To investigate the inhibitory effects on cell viability of human prostate cancer cell lines; the MTT assay was carried out by exposing the cultured PC3 cells to different doses (6.25 µg/ml, 12.5 µg/ml, 25 µg/ml, 50 µg/ml, and 75 µg/ml) of the methanol extract and essential oil for 24 h and 48 h respectively.

Figure 5.10.a) and 5.10.b) demonstrate the significant preventive effects of methanol extract and essential oil on viability of PC3 cells in a concentration dependant manner at 24 h. The 100 µg/ml dose was determined as the strong significant dose with a value of $p < 0.0001$ for both methanol extract and essential oil respectively.

Figure 5.12.a) and 5.12.b) clearly indicate the dose dependant significant cytotoxic effects of methanol extract and essential oil against PC3 cell lines at 48 h. Here, the 100 µg/ml concentration was again considered as the strong effective dose with a value of $p < 0.0001$ for both methanol extract and essential oil respectively.

The IC_{50} value of methanol extract was found to be 90 µg/ml and 70 µg/ml at 24 h and 48 h respectively. In case of essential oil the IC_{50} value was observed to be 95 µg/ml at 48 h.

The dose dependant structural deformities in PC3 cell lines are represented in Figure 11 and Figure 13. Here, the result exhibited the stronger anti-proliferative effects of methanol extract as compared to the essential oil. The remarkable cytotoxic effects of leaf extracts were characterized by the irreversible morphological changes of cells and dissociation from the surface of the plate at higher doses. However, these concentrations showed the positive effects on cell viability of WI38 cell lines and recommended as favorable doses for assessing the cytotoxic activity on PC3 cell (Figure 5.8 and Figure 5.9).

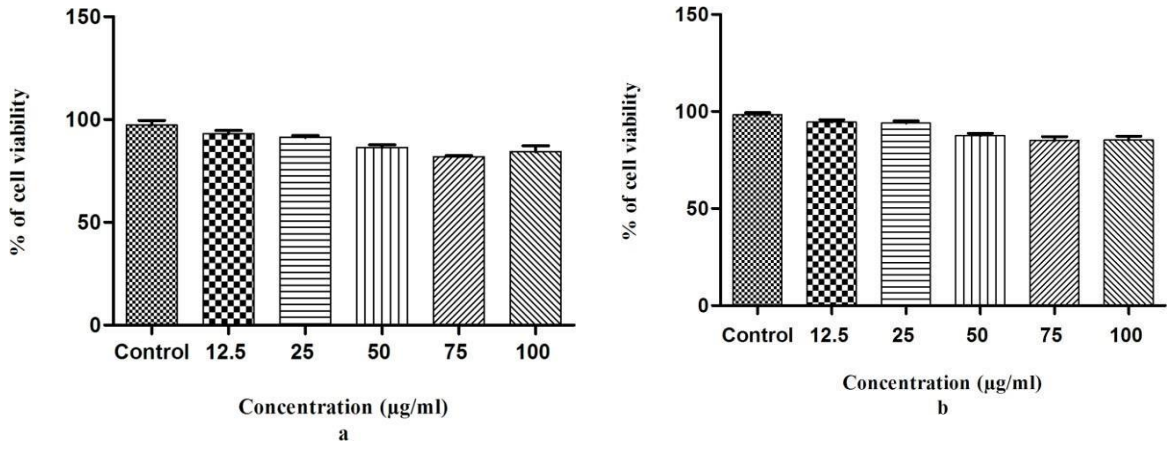


Figure 5.8. Concentration-dependent cell viability of (a) methanol extract and (b) essential oil against WI38 cell line at 24 h. The values are expressed as the percentage in comparison with the untreated (control) cell group and each bar in the graph is represented as mean ± SD (n = 3).

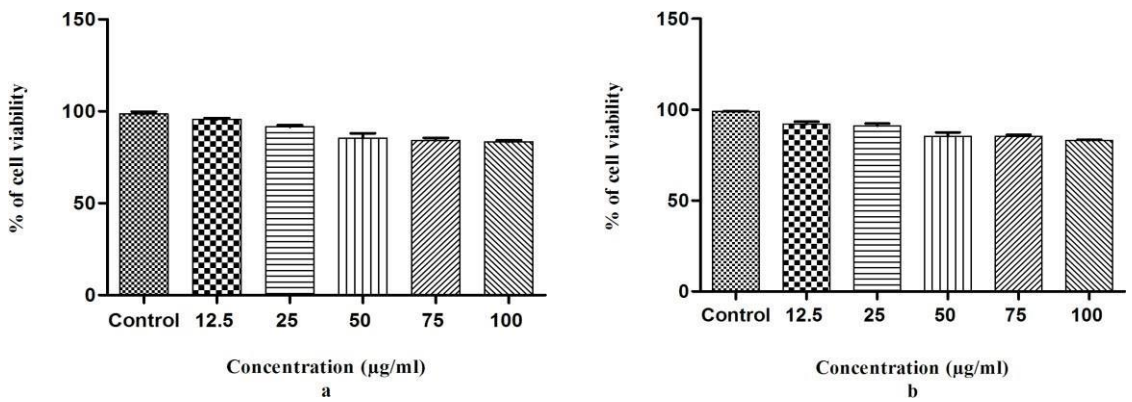


Figure 5.9. Concentration-dependent cell viability of (a) methanol extract and (b) essential oil against WI38 cell line at 48 h. The values are expressed as the percentage in comparison with the untreated (control) cell group and each bar in the graph is represented as mean ± SD (n = 3)

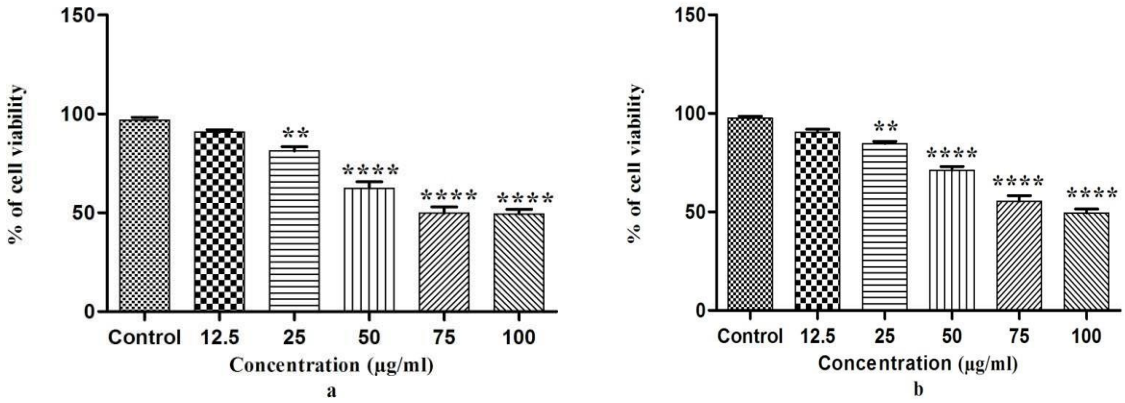


Figure 5.10. Concentration-dependent cell viability of (a) methanol extract and (b) essential oil against PC3 cell line at 24 h. The PC3 cells were treated with leaf extracts (12.5 µg/ml, 25 µg/ml, 50 µg/ml, 75 µg/ml, 100 µg/ml) for 24 h. Results are represented as the mean ± SD of at least triplicates of each experiment. *p < 0.05, **p < 0.01, ***p < 0.001 and ****p < 0.0001, statistically significant in comparison with control groups by one-way ANOVA and followed by Bonferroni's multiple comparison test.

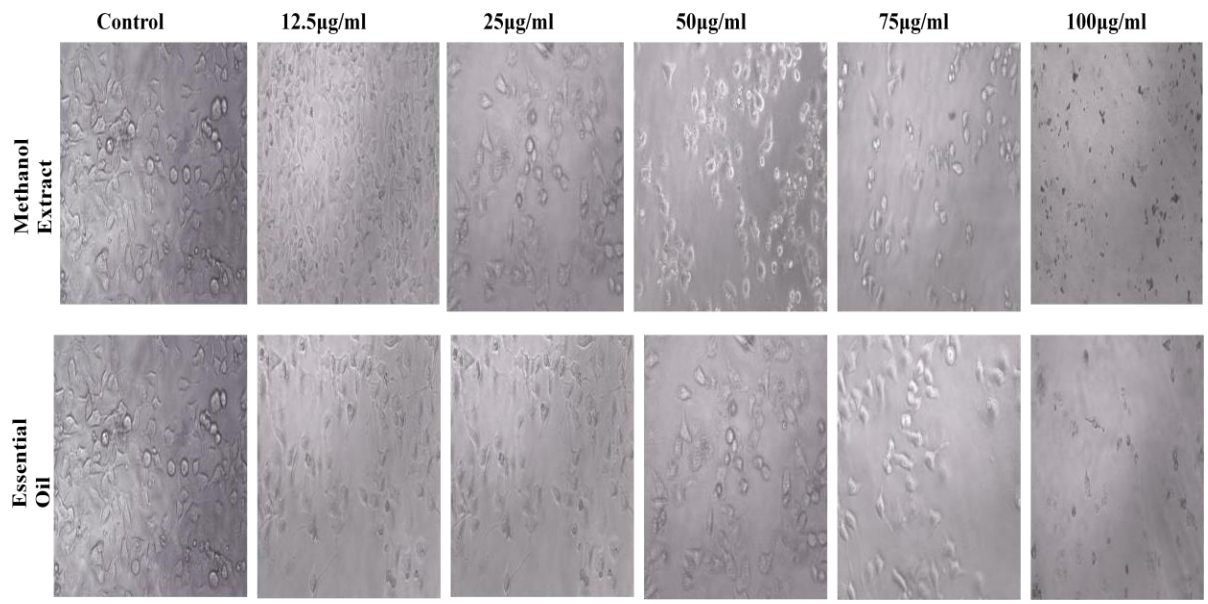


Figure 5.11. Concentration-dependent finite deformation effects in cell morphology of PC3. After treating with the 12.5 µg/ml, 25 µg/ml, 50 µg/ml, 75 µg/ml, 100µg/ml of methanol extract and essential oil for 24 h.

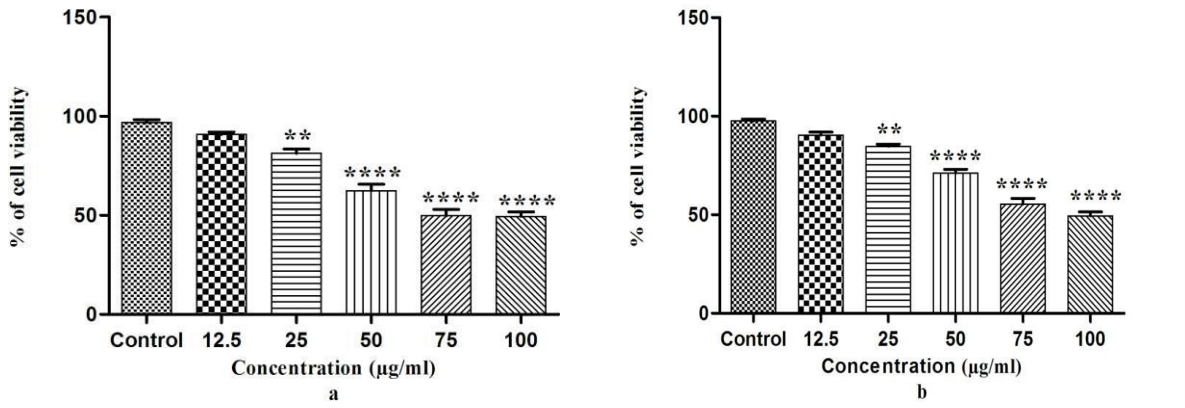


Figure 5.12. Concentration-dependent cell viability of (a) methanol extract and (b) essential oil against PC3 cell line at 48 h. The PC3 cells were treated with leaf extracts (12.5 µg/ml, 25 µg/ml, 50 µg/ml, 75 µg/ml, 100 µg/ml) for 48 h. Results are represented as the mean ± SD of at least triplicates of each experiment. *p < 0.05, **p < 0.01, ***p < 0.001 and ****p < 0.0001, statistically significant in comparison with control groups by one-way ANOVA and followed by Bonferroni's multiple comparison test.

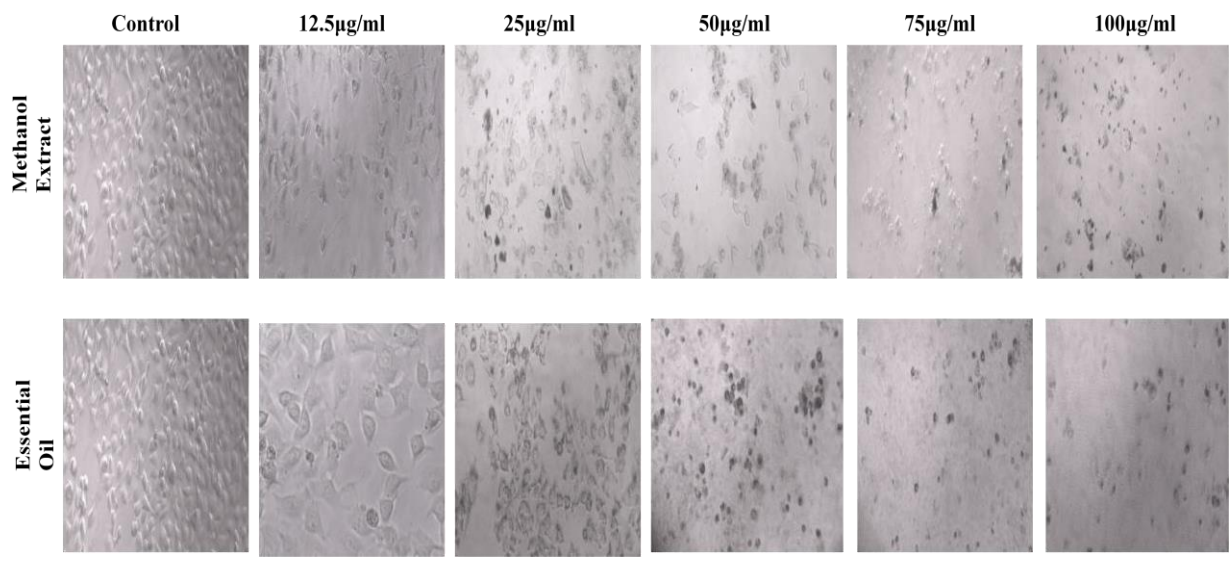


Figure 5.13. Concentration-dependent finite deformation effects in cell morphology of PC3. After treating with the 12.5 µg/ml, 25 µg/ml, 50 µg/ml, 75 µg/ml, 100 µg/ml of methanol extract and essential oil for 48 h.

Chapter 6

Discussion

6. Discussion

In this study, we followed the microwave-assisted extraction conditions which were previously optimized before commencement of the research work. It was observed that this research work demonstrated these highly sensitive extraction conditions as certain and robust for bringing out the higher amounts of phenolic compounds from the plant cells without damaging their bioactivities and using a minimum laboratory set up cost as well. To get the maximum yield of essential leaf oil, we also maintained the extraction conditions which were also optimized before commencement of the study. Furthermore, we have checked the use of different polar solvents as solvents for successful extractions of phenolics and flavonoid compounds. And ultimately methanol was selected to make successful the extraction procedure. Phenolics and flavonoids both are the most important phyto-constituents that are found in our test samples and which are accounted for their role in scavenging potential or chelating ability. These phytochemicals are widely known as promising source of natural antioxidants and responsible for exhibiting the anticancer, antibacterial, anti-inflammatory activities and which leads to project those compounds as an excellent drug candidate for pharmaceutical field.

Plant essential oils are predominantly characterized as the high volatile secondary metabolites of plants, sporadic resistance problems; and the essential oils mainly consist of terpenes, aromatic compounds and their derivatives. In recent research trends, the essential oils have received widespread recognition as the antibacterial, antifungal, antioxidant, insecticidal and flavoring agents. It was mentioned in chapter-2 (literature review) that the essential oil plays a significant role in prevention of cell proliferation and inflammatory diseases. Some of the important components of essential oil such as β-sitosterol, oleic acid, isocaryophyllene,

caryophyllene, phytol, citral have been cited for their promising anti-inflammatory and chemo-preventive properties.

Presence of monounsaturated fatty acid like oleic acid facilitates to improve the body immune system by successfully eliminating some of pathogens, potentiating the role of macrophages, lymphocytes and neutrophils. In addition, it has a contributory role to minimize the inflammatory response which ultimately helps to cure or manage various diseases.

In-vitro antioxidant assays were predominantly executed to build up a direct relationship between the degree of scavenging activity and therapeutic applications. To our knowledge, the rate of electron transfer of ABTS is much quicker than DPPH; and this kinetic reaction makes a subtle difference in mechanism of actions of DPPH and ABTS at antioxidant activity level. DPPH radical reaction is worked on the use of very crowded radical whereas the ABTS is applicable for low crowded radical. The DPPH radical gets chemically involved with polyphenols (catechins, proanthocyanidins), and the highly reactive ABTS gets chemically involved with a broader range of antioxidants (Mareček et al., 2017). In addition, the use of hydrogen peroxide assay is most significant for the evaluation of radical scavenging abilities of phenolic and non-phenolic compounds. Here, the leaf extracts demonstrated a remarkable antioxidant potential against the highly reactive species like hydroxyl radical, singlet oxygen, non-radical reactive oxygen species. Consequently, the leaf extracts may play an important role in mitigating cellular stress which in turn plays a profound role in arresting a wide range of onset diseases like cancer and inflammatory disease.

β-sitosterol is one of the important phytosterols which was identified from our plant extracts. β-sitosterol is one of the remarkable component of functional foods and having a crucial role in management of inflammatory diseases. Many of the scientific investigations previously proved a significant role of β-sitosterol in attenuating topical inflammation and exhibiting its

anti-inflmmatory property by interfering with arachidonic acid cascade. Furthermore, due to enhancement of antioxidant defense system, reduction in serum cholesterol levels, effectively blocking the expression of vascular adhesion molecules (VCAM-1 and ICAM-1); makes β-sitosterol a well proven cardio-protective agent.

Our study revealed that the leaf extracts of *Vitex negundo* contain a wide array of polyphenols (aspartic acid, gallic acid, dihydroxy benzoic acid, catechin, chlorogenic acid, vanillic acid, rutin, trans-cinnamic acid, ferulic acid, quercetin, apigenin, kaempferol, and tannic acid) was detected by HPLC separation with the distinct elution times. The qualitative and quantitative estimation of polyphenols were obtained from the plant extracts would be compared against of the chromatograms of each standard compound. Apigenin, catechin, rutin are three important phenolic compounds and they are registered to represent for significant anti-inflammatory property by modulating the activation of NLRP3 inflammasome. Anti-inflammatory effect of polyphenols has been already documented and action may be exerted notably through regulating the various transcription factors (AP-1, NF-κB, and protooncogenes), second messengers (cGMP, cAMP, protein kinases, and calcium), enzymes and compounds (iNOS, COX-2), cytokines (IL-1β, TNF-α), neuropeptides, proteases which are directly involved in inflammatory reactions. Rutin is known as a glycoside of quercetin and widely distributed in plant materials. It is responsible to reduce the inflammation by lowering the production of pro-inflammatory cytokines, modulating the antioxidant enzyme activities and activation of the mitogen-activated protein kinase cascade. Research evidence suggested that the vanillic acid exerts its anti-inflammatory property by inhibiting oxidative stress, pro-inflammatory cytokine generation, and NFκB activation in rodent inflammation models. Trans-cinnamic acid belongs to the group of styrene and recorded for exerting a wide range of therapeutic activities including antioxidant, anti-inflammatory and anticancer properties. Predominantly, chronic diseases linked with

excessive generation of ROS which leads to a wide spectrum of oxidative stress reactions. Many of signalling molecules and peroxiredoxin 2 (PRDX2) are released after protein oxidations which eventually initiates the inflammatory reactions. Phenolics are mostly appreciated as radical scavenger and received attention for the treatment of several diseases including cancer, and neurodegenerative diseases or for use in anti-aging products. Furthermore, the flavonoids are pharmacologically potent bioactive compounds which are associated with analgesic, anti-inflammatory and antioxidant properties. Hence, phenolics and flavonoids provide therapeutic synergism which indicates towards the plant-based bioactivity by deactivating the pro-oxidative enzyme promote health status and quality of life (Kundu et al., 2020).

Presence of ample amount of tocopherol in our test samples suggested that the plant is one of the most important natural sources of antioxidants. Furthermore, β-carotene, α-tocopherol and ascorbic acid synergistically could prevent the cell proliferation by inhibiting phosphorylation of c-Jun N-terminal kinase (JNK), extracellular-signal-regulated protein kinase (ERK), activation of mitogen-activated protein kinase signaling cascades; and also by stopping up-regulation of total p53 and Bax proteins, phosphorylation of p53 and proliferating cellular nuclear antigen proteins in the lungs of mammals. Besides providing the antioxidant effects towards the cells it also reduces the chance of pre-neoplastic lung injury as well and thus imparted to be a more beneficial chemotherapeutic approach for carcinogenesis. In addition, the role of tocopherol on depleting the level of pro-inflammatory mediators by modulating the cyclooxygenase pathway has also been well documented (Rhimi et al., 2018).

In this investigation, the *Vitex negundo* showed a significant antibacterial potential. The chemical constituent of methanol extract and essential oil contain monoterpene hydrocarbons

and which seems to exhibit its antibacterial activity by disrupting the cytoplasmic cell membrane and by encouraging efflux of the cellular potassium ions of microorganisms.

It has been shown in our research work that the methanol extract and essential oil of leaves of *Vitex negundo* were found to be the richest source of polyphenols. Some of the phenolic acids such as gallic, caffeic, and ferulic acids are responsible for their strong growth suppressive activity towards the gram positive (*S. aureus* and *E. faecalis*) and gram negative bacteria (*E. coli* and *P. aeruginosa*); and its due to presence and positioning of their hydroxyl group.

Polyphenols have been extensively investigated for their several potential clinical significances including antioxidant, antimicrobial, anti-inflammatory, anticancer properties. As compared to other polyphenols, the flavan-3-ols, flavonols, and tannins have been recognized for their broad spectrum and powerful antimicrobial activity. Several research investigations suggested different modes of actions of polyphenols as antimicrobial agents. By counterbalancing bacterial toxins, preventing the formation of biofilm, and decreasing the ability of the host receptor binding site; they exhibit their antimicrobial efficacy. Furthermore, by promoting the effect of other antibiotics and by acting as reactive species scavengers, lipoxygenase inhibitor; polyphenols could reduce the detrimental impact on cellular components. From the standpoint of a qualitative research approach, our findings of present work are in line with prior empirical work. The comparative analysis of antibacterial potential of complex mixture of numerous bioactive compounds of *Vitex* leaf extracts was validated and the work tends to give an indication to the trial drugs as a primordial antibacterial agent in near future.

Here, the research findings also exhibited the significant successful inhibition of IL-6, IL-1β and enhanced expression of IL-10 cytokines. IL-6 plays a contributory role to promote tumorigenesis and followed by modulating related factors are responsible to form cancer by

triggering relevant cell-signaling pathways including JAK/STAT3, Ras/ERK, and PI3 K/Akt which induce cell proliferation, invasion, metastasis, and angiogenesis and most evidently the metabolism. Thus, the IL-6 dominated cell-signaling might be playing a counter-regulatory role independently or in adjuvant therapy for the treatment of chronic diseases. Interleukin-1β is widely known as a prototypic pro-inflammatory cytokine and having a crucial role to produce acute and chronic inflammation which ultimately may responsible for autoimmune disorders. Though IL-1β plays a key role to maintain the homeostatic balance (such as in the regulation of feeding, sleep, and temperature) but aberrant production of IL-1β initiates a various types of inflammatory diseases including rheumatoid arthritis, neuropathic pain, inflammatory bowel disease, osteoarthritis, vascular disease, multiple sclerosis, and Alzheimer's disease. In current study the plant extracts showed the evidence of blocking effect against IL-1β which suggests the blockade of IL-1β receptor as a potential therapeutic strategy for the management of different inflammatory diseases. Interleukin 10 (IL-10) is commonly appreciated as a potent anti-inflammatory cytokine and serves a key role in regulating host immune response towards the pathogens such as viruses, bacteria, fungi and protozoa. Inadequacy or aberrant expression of IL-10 may cause to develop several microbial infections and lead to progression of inflammatory bowel disease and numerous autoimmune diseases. In this experiment, the plant extracts exhibited the up-regulated expression against IL-10. By coordinating the response of pro-inflammatory and anti-inflammatory cytokines, IL-10 maintains the immunomodulatory balance in living system and can be considered as a therapeutic opportunity (Kundu et al., 2020).

A significant correlation exists between polyphenols consumption and reduced risk of prostate cancer. Here, exposure to polyphenols may regulate the signaling cascades by increasing or decreasing some levels of proteins, producing pro-oxidant or antioxidant effects, stopping progression of cell cycle and initiating the apoptosis pathway.

For instance; apigenin has been found to be growth inhibitor of prostate cancer cells by pulling down the potential of mitochondrial membrane and subsequently by producing the cytochrome c into cytoplasm, reducing the levels of anti-apoptotic proteins Bcl-2 and Bcl-2-extra-large (Bcl-XL) proteins and elevating the level of Bax. Additionally, apigenin plays a cardinal role in prevention of prostate cancer by involving TNF-related apoptosis-inducing ligand (TRAIL) and death receptor 5 (DR5) (Costea et al., 2019). Ellagic acid is a well known natural phenolic compound and our research findings showed that ellagic acid is adequately presentd in our research sample and that plays a pivotal role in management of prostate cancer (Kundu et al., 2020). By impairing the activity of heme oxygenase system, blocking the activity of vascular endothelial growth factor (VEGF) and osteoprotegerin (OPG), suppressing the expression of cyclin D1, downregulating the levels of prostate-specific antigen (PSA), ellagic acid slows down the progression of metastasis and may induce the cell cycle arrest. One of the major metabolites of anthocyanin is protocatechuic acid and which is potentially employed for apoptotic cell death in prostate cancer. Through enhanced caspase-3 activity, decreased VEGF activity, impared the mitochondrial membrane potential and reduced the levels of pro-inflammatory cytokines (IL-6, IL-8), protocatechuic acid destroys the prostate cancer cells (Semaming et al., 2015). Here, as the anticancer and antibacterial activities were checked on the human prostate cancer cells and the UTI pathogens respectively. So, the research findings also correlate the antibacterial, anti-inflammatory and anticancer potentials and suggest that the significant reduction of UTI pathogens could be helpful to lower the risk factors of subsequent development of prostate cancer. Therefore, the research findings justify its traditional use, which develops a future substantial value of this plant into the scientific discipline.

Chapter 7

Conclusion

7. Conclusion

This research study also comes up with proofs on the worth of *Vitex negundo* leaves as an excellent source of volatile organic compounds. The results of this scientific study indicate that the methanol extract and essential oil of the plant extracts contain substantial amount of antioxidants (β-sitosterol, polyphenols and α-tocopherol). Combinedly, β-sitosterol and polyphenols can enable the free radical scavenging activities which suggests that the plant is a natural source of antioxidants. The methanol extract and essential oil are coupled with strong antibacterial activities against UTI causing pathogens including *Staphylococcus aureus*, *Enterococcus faecalis* and *Escherichia coli*, *Pseudomonas aeruginosa*. The data also revealed that the methanol extract and essential oil of the plant extracts possess significant anti-inflammatory properties. Both of the leaf extracts demonstrated the significant anti-inflammatory potentials by preventing the release of IL-1β, IL-6 and enhancing the release of IL-10. The key role of obtained phyto-constituents is in alleviation from chronic disorder like cancer and inflammation. However, for all these cases, the methanol extract exhibited the higher inhibitory potentials than the leaf essential oil. Our results vividly demonstrated that the methanol extract is also more active against prostate carcinoma cell lines as compared to the essential oil and that suggested the potential use of these extracts as a holistic approach to manage the life-threatening diseases including prostate cancer, risk factors for developing prostate cancer, bacterial infections and inflammation which are emerging issues in global health. Hence, this research work is contemplated as a preliminary analysis that is empowering the diverse therapeutic applications and novelty of this endemic species. Therefore, further research should be aimed to investigate the mechanism of action of these extracts towards its specific targets. Here, only four types of bacterial strains were employed for this study but use of more similar bacterial strains should increase the importance of this study. Moreover, isolation of the bioactive compounds and the *in-vivo* experiments data could help to reach the study to the more advanced stage.

References

8. References

A. J. Sinclair, K. J. Murphy D. Marine lipids: Overview 'News insights and lipid composition of Lyprinol(TM). Allerg Immunol 2000;32:261–71.

Abd. Wahab NA, H. Lajis N, Abas F, Othman I, Naidu R. Mechanism of Anti-Cancer Activity of Curcumin on Androgen-Dependent and Androgen-Independent Prostate Cancer. Nutrients 2020;12:679.

Afoulous S, Ferhout H, Raoelison EG, Valentin A, Moukarzel B, Couderc F, et al. Chemical composition and anticancer, antiinflammatory, antioxidant and antimalarial activities of leaves essential oil of Cedrelopsis grevei. Food Chem Toxicol 2013;56:352–62.

Alam M., Gomes A. Snake venom neutralization by Indian medicinal plants (*Vitex negundo* and *Emblica officinalis*) root extracts. J Ethnopharmacol 2003;86:75–80.

Amalraj A, Pius A, Gopi Sreerag, Gopi Sreeraj. Biological activities of curcuminoids, other biomolecules from turmeric and their derivatives – A review. J Tradit Complement Med 2017;7:205–33.

van Amerongen R. Alternative Wnt Pathways and Receptors. Cold Spring Harb Perspect Biol 2012;4:a007914–a007914.

Amiel E, Ofir R, Dudai N, Soloway E, Rabinsky T, Rachmilevitch S. β -Caryophyllene, a Compound Isolated from the Biblical Balm of Gilead (*Commiphora gileadensis*), Is a Selective Apoptosis Inducer for Tumor Cell Lines. Evidence-Based Complement Altern Med 2012;2012:1–8.

Ammon HPT, Safayhi H, Mack T, Sabieraj J. Mechanism of antiinflammatory actions of curcumine and boswellic acids. J Ethnopharmacol 1993;38:105–12.

Applegate C, Rowles J, Erdman J. Can Lycopene Impact the Androgen Axis in Prostate Cancer?: A Systematic Review of Cell Culture and Animal Studies. Nutrients 2019;11:633.

Arif Lodhi M, Azhar-ul-Haq, Iqbal Choudhary M, Malik A, Ahmad S. α-Chymotrypsin inhibition studies on the lignans from *Vitex negundo* Linn. J Enzyme Inhib Med Chem 2008;23:400–5.

Aswar, P.B., Khadabadi, S.S., Kuchekar, B.S., Rajurkar, R.M., Saboo, S.S. and Javarkar RD. In Vitro Evaluation of Anti-Bacterial and Anti-Fungal Activity of *Vitex nigundo* (Verbenaceae). Ethnobot Leafl 2009;13:962–7.

Athar M, Back JH, Kopelovich L, Bickers DR, Kim AL. Multiple molecular targets of resveratrol: Anti-carcinogenic mechanisms. Arch Biochem Biophys 2009;486:95–102.

Azhar-Ul-Haq, Malik A, Anis I, Khan SB, Ahmed E, Ahmed Z, et al. Enzymes Inhibiting Lignans from Vitex negundo. Chem Pharm Bull (Tokyo) 2004;52:1269–72.

Babu AKM; USN; MMR; MMP; R. A review of the important chemical constituents and medicinal uses of *Vitex* genus. Asian J Tradit Med 2011;6:54–60.

Bakkali F, Averbeck S, Averbeck D, Idaomar M. Biological effects of essential oils – A review. Food Chem Toxicol 2008;46:446–75.

Balachandrana C, Arun Y, Sangeetha YB, Duraipandiyan V, Awale S, Emi N, et al. *In vitro* and *in vivo* anticancer activity of 2-acetyl-benzylamine isolated from *Adhatoda vasica* L. leaves. Biomed Pharmacother 2017;93:796–06.

Balunas MJ, Kinghorn AD. Drug discovery from medicinal plants. Life Sci 2005;78:431–41.

Banerjee S, Welsch CW, Rao AR. Modulatory influence of camphor on the activities of hepatic carcinogen metabolizing enzymes and the levels of hepatic and extrahepatic reduced glutathione in mice. Cancer Lett 1995;88:163–9.

Barras V, Greub G. History of biological warfare and bioterrorism. Clin Microbiol Infect 2014;20:497–502.

Basri F, Sharma HP, Firdaus S, Jain P, Ranjan A. A review of ethnomedicinal plant-Vitex negundo Linn. Int J Adv Res 2014;2:882–94.

Bayala B, Bassole IHN, Maqdasy S, Baron S, Simpore J, Lobaccaro J-MA. Cymbopogon citratus and Cymbopogon giganteus essential oils have cytotoxic effects on tumor cell cultures. Identification of citral as a new putative anti-proliferative molecule. Biochimie 2018;153:162–70.

Bhargava SK. Antiandrogenic effects of a flavonoid-rich fraction of *Vitex negundo* seeds: A histological and biochemical study in dogs. J Ethnopharmacol 1989;27:327–39.

Bhattacharjee B, Pal PK, Chattopadhyay A, Bandyopadhyay D. Oleic acid protects against cadmium induced cardiac and hepatic tissue injury in male Wistar rats: A mechanistic study. Life Sci 2020;244:117324.

Biswas SK. Does the Interdependence between Oxidative Stress and Inflammation Explain the Antioxidant Paradox? Oxid Med Cell Longev 2016;2016:1–9.

Bonamin F, Moraes TM, dos Santos RC, Kushima H, Faria FM, Silva MA, et al. The effect of a minor constituent of essential oil from Citrus aurantium: The role of β-myrcene in preventing peptic ulcer disease. Chem Biol Interact 2014;212:11–9.

de Bono J, Mateo J, Fizazi K, Saad F, Shore N, Sandhu S, et al. Olaparib for Metastatic Castration-Resistant Prostate Cancer. N Engl J Med 2020;382:2091–102.

Borissova P, Valcheva S, Belcheva A. Antiinflammatory effect of flavonoids in the natural juice from Aronia melanocarpa, rutin and rutin-magnesium complex on an experimental model of inflammation induced by histamine and serotonin. Acta Physiol Pharmacol Bulg 1994;20:25–30.

Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2018;68:394–424.

Brizuela L, Dayon A, Doumerc N, Ader I, Golzio M, Izard J, et al. The sphingosine kinaseα1 survival pathway is a molecular target for the tumor-suppressive tea and wine polyphenols in prostate cancer. FASEB J 2010;24:3882–94.

Burcu GB, Osman C, Aslı C, Namik OM, Neşe BT. The protective cardiac effects of B-myrcene after global cerebral ıschemia/reperfusion in C57BL/J6 mouse. Acta Cir Bras 2016;31:456–62.

Buschemeyer WC, Freedland SJ. Obesity and Prostate Cancer: Epidemiology and Clinical Implications. Eur Urol 2007;52:331–43.

Cámara M, de Cortes Sánchez-Mata M, Fernández-Ruiz V, Cámara RM, Manzoor S, Caceres JO. Lycopene, 2013, p. 383–426.

Camele I, Elshafie HS, Caputo L, De Feo V. Anti-quorum Sensing and Antimicrobial Effect of Mediterranean Plant Essential Oils Against Phytopathogenic Bacteria. Front Microbiol 2019;10.

Castro E, Eeles R. The role of BRCA1 and BRCA2 in prostate cancer. Asian J Androl 2012;14:409–14.

Chan JM, Giovannucci EL. Diet: Dairy Products, Calcium, and Vitamin D and Risk of Prostate Cancer. Epidemiol Rev 2001;23:87–92.

Chelliah R, Ramakrishnan S, Antony U. Nutritional quality of Moringa oleifera for its bioactivity and antibacterial properties. Int Food Res J 2017;24:825.

Chen M, Zhuang Y-W, Wu C-E, Peng H-Y, Qian J, Zhou J-Y. β-asarone suppresses HCT116 colon cancer cell proliferation and liver metastasis in part by activating the innate immune system. Oncol Lett 2021;21:435.

Chen Y, Deng Y, Zhu C, Xiang C. Anti prostate cancer therapy: Aptamer-functionalized, curcumin and cabazitaxel co-delivered, tumor targeted lipid-polymer hybrid nanoparticles. Biomed Pharmacother 2020;127:110181.

Cheng I, Plummer SJ, Jorgenson E, Liu X, Rybicki BA, Casey G, et al. 8q24 and prostate cancer: association with advanced disease and meta-analysis. Eur J Hum Genet 2008;16:496–505.

Cho SH, Jung YB, Seong SC, Park H Bin, Byun KY, Lee DC, et al. Clinical efficacy and safety of Lyprinol, a patented extract from New Zealand green-lipped mussel (Perna Canaliculus) in patients with osteoarthritis of the hip and knee: a multicenter 2-month clinical trial. Eur Ann Allergy Clin Immunol 2003;35:212–6.

Chouhan KBS, Tandey R, Sen KK, Mehta R, Mandal V. Extraction of phenolic principles: Value addition through effective sample pretreatment and operational improvement. J Food Meas Charact 2019;13:177–86.

Chouhan S, Sharma K, Guleria S. Antimicrobial Activity of Some Essential Oils—Present Status and Future Perspectives. Medicines 2017;4:58.

COOK N. Flavonoids?Chemistry, metabolism, cardioprotective effects, and dietary sources. J Eur Ceram Soc 1996:66–76.

Costea T, Nagy P, Ganea C, Szöllősi J, Mocanu M-M. Molecular Mechanisms and Bioavailability of Polyphenols in Prostate Cancer. Int J Mol Sci 2019;20:1062.

Coulter ID, Hardy ML, Morton SC, Hilton LG, Tu W, Valentine D, et al. Antioxidants vitamin C and vitamin E for the prevention and treatment of cancer. J Gen Intern Med 2006;21:735–44.

Cragg GM, Newman DJ. Natural products: A continuing source of novel drug leads. Biochim Biophys Acta - Gen Subj 2013;1830:3670–95.

Crawford ED. Epidemiology of prostate cancer. Urology 2003;62:3–12.

Cretella ABM, Soley B da S, Pawloski PL, Ruziska RM, Scharf DR, Ascari J, et al. Expanding the anti-inflammatory potential of Moringa oleifera: topical effect of seed oil on skin inflammation and hyperproliferation. J Ethnopharmacol 2020;254:112708.

Daglia M. Polyphenols as antimicrobial agents. Curr Opin Biotechnol 2012;23:174–81.

Dahham S, Tabana Y, Iqbal M, Ahamed M, Ezzat M, Majid Aman, et al. The Anticancer, Antioxidant and Antimicrobial Properties of the Sesquiterpene β-Caryophyllene from the Essential Oil of Aquilaria crassna. Molecules 2015;20:11808–29.

Damas J, Bourdon V, Remacle-Volon G LJ. Proinflammatory flavonoids which are inhibitors of prostaglandin biosynthesis. Prostaglandins Leukot Med 1985;19:11–24.

Das S, Parveen S, Kundra CP, Pereira BMJ. Reproduction in male rats is vulnerable to treatment with the flavonoid-rich seed extracts of *Vitex negundo*. Phyther Res 2004;18:8–13.

Dassoler, M.; Schwanz, M.; Busseto, F.; Moreira, E.A.; Gutierrez L. Perfil fitoquímico e ensaio farmacológico de Averrhoa carambola L. (Oxalidaceae). J Bras Fitom 2004;2:4–8.

De-Oliveira ACA., F. Ribeiro-Pinto L, Paumgartten FJ. In vitro inhibition of CYP2B1 monooxygenase by β-myrcene and other monoterpenoid compounds. Toxicol Lett 1997;92:39–46.

Dharmasiri M., Jayakody JRA., Galhena G, Liyanage SS., Ratnasooriya W. Anti-inflammatory and analgesic activities of mature fresh leaves of *Vitex negundo*. J Ethnopharmacol 2003;87:199–206.

Díaz F, Chávez D, Lee D, Mi Q, Chai H-B, Tan GT, et al. Cytotoxic Flavone Analogues of Vitexicarpin, a Constituent of the Leaves of *Vitex negundo*. J Nat Prod 2003;66:865–7.

Diederich. Anti-proliferative potential of curcumin in androgen-dependent prostate cancer cells occurs through modulation of the Wingless signaling pathway. Int J Oncol 2011;38.

Dorai T, Gehani N, Katz A. Therapeutic potential of curcumin in human prostate cancer. II. Curcumin inhibits tyrosine kinase activity of epidermal growth factor receptor and depletes the protein. Mol Urol 2000;4:1–6.

Du Y, Long Q, Zhang L, Shi Y, Liu X, Li X, et al. Curcumin inhibits cancer-associated fibroblast-driven prostate cancer invasion through MAOA/mTOR/HIF-1α signaling. Int J Oncol 2015;47:2064–72.

Dugas B. Lyprinol inhibits LTB4 production by human monocytes. Allerg Immunol (Paris) 2000;32:284–9.

Duraipandiyan V, Al-Dhabi NA, Balachandran C, Ignacimuthu S, Sankar C, Balakrishna K. Antimicrobial, Antioxidant, and Cytotoxic Properties of Vasicine Acetate Synthesized from Vasicine Isolated from *Adhatoda vasica* L. Biomed Res Int 2015;2015:7Pages.

Durante W. Protective role of heme oxygenase-1 against inflammation in atherosclerosis. Front Biosci 2011;16:2372.

El-Benna J, Dang PM-C, Gougerot-Pocidalo M-A, Marie J-C, Braut-Boucher F. p47phox, the phagocyte NADPH oxidase/NOX2 organizer: structure, phosphorylation and implication in diseases. Exp Mol Med 2009;41:217.

Elfalleh W, Kirkan B, Sarikurkcu C. Antioxidant potential and phenolic composition of extracts from Stachys tmolea: An endemic plant from Turkey. Ind Crops Prod 2019;127:212–6.

Elisabetsky, E. & Posey D. Ethnopharmacological research and natural resources of humid tropics: the case o f Kayapo Indians and its implications for medical science. An Do 10 Simp Do Trop Umido 1986;2:85–93.

Eskra JN, Schlicht MJ, Bosland MC. Effects of Black Raspberries and Their Ellagic Acid and Anthocyanin Constituents on Taxane Chemotherapy of Castration-Resistant Prostate Cancer Cells. Sci Rep 2019;9:4367.

Eslami SS, Jafari D, Montazeri H, Sadeghizadeh M, Tarighi P. Combination of Curcumin and Metformin Inhibits Cell Growth and Induces Apoptosis without Affecting the Cell Cycle in LNCaP Prostate Cancer Cell Line. Nutr Cancer 2021;73:1026–39.

Fahmidabinti Abd Rahman, Vishnu Priya, Gayathri R GR V. In vitro Antibacterial Activity of Camphor oil against Oral Microbes. Int J Pharm Sci Rev Res, 2016;39:119–21.

Farhadi F, Khameneh B, Iranshahi M, Iranshahy M. Antibacterial activity of flavonoids and their structure–activity relationship: An update review. Phyther Res 2019;33:13–40.

Fauziya Basri, H.P. Sharma, Sazya Firdaus PJ and AR. A review of ethnomedicinal plant-Vitex negundo Linn. Int J Adv Res 2014;2:882–94.

Feng D, Zhang A, Yang Y, Yang P. Coumarin-containing hybrids and their antibacterial activities. Arch Pharm (Weinheim) 2020;353:1900380.

Ferrero-Miliani L, Nielsen OH, Andersen PS, Girardin SE. Chronic inflammation: importance of NOD2 and NALP3 in interleukin-1β generation. Clin Exp Immunol 2007;147:227–35.

Firdous AB, Sharmila G, Balakrishnan S, RajaSingh P, Suganya S, Srinivasan N, et al. Quercetin, a natural dietary flavonoid, acts as a chemopreventive agent against prostate cancer in an in vivo model by inhibiting the EGFR signaling pathway. Food Funct 2014;5:2632–45.

Fitzpatrick JM. Management of localized prostate cancer in senior adults: the crucial role of comorbidity. BJU Int 2008;101:16–22.

Flores-Mireles AL, Walker JN, Caparon M, Hultgren SJ. Urinary tract infections: epidemiology, mechanisms of infection and treatment options. Nat Rev Microbiol 2015;13:269–84.

Funke G, von Graevenitz A, Clarridge JE, Bernard KA. Clinical microbiology of coryneform bacteria. Clin Microbiol Rev 1997;10:125–59.

Gadisa E, Weldearegay G, Desta K, Tsegaye G, Hailu S, Jote K, et al. Combined antibacterial effect of essential oils from three most commonly used Ethiopian traditional medicinal plants on multidrug resistant bacteria. BMC Complement Altern Med 2019;19:24.

Ge Y, Yan H, Hui B, Ni Y, Wang S, Cai T. Extraction of Natural Vitamin E from Wheat Germ by Supercritical Carbon Dioxide. J Agric Food Chem 2002;50:685–9.

Gendron F, Nilson S, Ziffle V, Johnny S, Louie D, Diamente P. Antimicrobial Effectiveness on Selected Bacterial Species and Alkaloid and Saponin Content of <i>Rosa nutkana</i> C. Presl (Nootka Rose) and <i>Urtica dioica</i> L. (Stinging Nettle) Extracts. Am J Plant Sci 2021;12:720–33.

Gilani AH, Atta-ur-Rahman. Trends in ethnopharmacology. J Ethnopharmacol 2005;100:43–9.

Gioti K, Tenta R. Bioactive Natural Products against Prostate Cancer: Mechanism of Action and Autophagic/Apoptotic Molecular Pathways. Planta Med 2015;81:543–62.

Giulitti F, Petrungaro S, Mandatori S, Tomaipitinca L, de Franchis V, D'Amore A, et al. Anti-tumor Effect of Oleic Acid in Hepatocellular Carcinoma Cell Lines via Autophagy Reduction. Front Cell Dev Biol 2021;9.

Górniak I, Bartoszewski R, Króliczewski J. Comprehensive review of antimicrobial activities of plant flavonoids. Phytochem Rev 2019;18:241–72.

Grankvist A, Andersson P-O, Mattsson M, Sender M, Vaht K, Hoper L, et al. Infections With the Tick-Borne Bacterium "Candidatus Neoehrlichia mikurensis" Mimic Noninfectious Conditions in Patients With B Cell Malignancies or Autoimmune Diseases. Clin Infect Dis 2014;58:1716–22.

Gruenwald J, Graubaum H-J, Hansen K, Grube B. Efficacy and tolerability of a combination of Lyprinol® and high concentrations of EPA and DHA in inflammatory rheumatoid disorders. Adv Ther 2004;21:197–201.

Gullón B, Eibes G, Moreira MT, Dávila I, Labidi J, Gullón P. Antioxidant and antimicrobial activities of extracts obtained from the refining of autohydrolysis liquors of vine shoots. Ind Crops Prod 2017;107:105–13.

Gupta SC, Kannappan R, Reuter S, Kim JH, Aggarwal BB. Chemosensitization of tumors by resveratrol. Ann N Y Acad Sci 2011;1215:150–60.

Rashid MHA , Bharadwaj P V, Majumder S, Mandal V, Pal M, Mandal SC, et al. Antioxidant and anticancer activity of extract and fractions obtained from Diospyros melanoxylon Roxb. Leaves and correlation with their polyphenolic profiles. Int J Pharm Pharm Sci 2018;10:6–16.

Halpern GM. Anti-inflammatory effects of a stabilized lipid extract of Perna canaliculus (Lyprinol). Allerg Immunol (Paris) 2000;32:272–8.

Hehner SP, Hofmann TG, Dröge W, Schmitz ML. The antiinflammatory sesquiterpene lactone parthenolide inhibits NF-kappa B by targeting the I kappa B kinase complex. J Immunol 1999;163:5617–23.

Heinrich M, Barnes J, Gibbons S, & Williamson EM. Fundamentals of pharmacognosy and phytotherapy. 2004.

Heinrich M, Ankli A, Frei B, Weimann C, Sticher O. Medicinal plants in Mexico: healers' consensus and cultural importance. Soc Sci Med 1998;47:1859–71.

Heinrich PB & M. Natural products and their role as inhibitors of the pro-inflammatory transcription factor NF- κB. Phytochem Rev 2005;4:27–37.

Hepworth JD. Pyrans and fused pyrans:(iii) Synthesis and applications. Compr Heterocycles Chem Katritzky, AR, Rees, CW, Eds; Elsevier Oxford, UK 1984;3:737–883.

Hsieh T, Wu JM. Resveratrol Suppresses Prostate Cancer Epithelial Cell Scatter/Invasion by Targeting Inhibition of Hepatocyte Growth Factor (HGF) Secretion by Prostate Stromal Cells and Upregulation of E-cadherin by Prostate Cancer Epithelial Cells. Int J Mol Sci 2020;21:1760.

Hu C-F, Zhang P-L, Sui Y-F, Lv J-S, Ansari MF, Battini N, et al. Ethylenic conjugated coumarin thiazolidinediones as new efficient antimicrobial modulators against clinical methicillin-resistant Staphylococcus aureus. Bioorg Chem 2020;94:103434.

Hu Y, Zhang QY, Hou TT, Xin HL, Zheng HC, Rahman K, et al. Estrogen-like activities in *Vitex* species from China determined by a cell based proliferation assay. Pharmazie 2007;62:872–5.

Huang H, Chen X, Li D, He Y, Li Y, Du Z, et al. Combination of α-Tomatine and Curcumin Inhibits Growth and Induces Apoptosis in Human Prostate Cancer Cells. PLoS One 2015;10:e0144293.

Huang M, Sanchez-Moreiras AM, Abel C, Sohrabi R, Lee S, Gershenzon J, et al. The major volatile organic compound emitted from Arabidopsis thaliana flowers, the sesquiterpene (E)-β-caryophyllene, is a defense against a bacterial pathogen. New Phytol 2012;193:997–1008.

Huang W-C, Chen Y-L, Liu H-C, Wu S-J, Liou C-J. Ginkgolide C reduced oleic acid-induced lipid accumulation in HepG2 cells. Saudi Pharm J 2018;26:1178–84.

Huggins C, Hodges C V. Studies on Prostatic Cancer: I. The Effect of Castration, Of Estrogen and of Androgen Injection on Serum Phosphatases in Metastatic Carcinoma of the Prostate. CA Cancer J Clin 1972;22:232–40.

Jadhav VM, Thorat RM, Kadam VJ, Sathe NS. *Hibiscus rosa sinensis* Linn – ‘‘Rudrapuspa’’: A Review. J Pharm Res 2009;2:1168-73.

Jalloh M, Friebel TM, Sira Thiam F, Niang L, Sy C, Siby T, et al. Evaluation of 4,672 routine prostate biopsies performed in six African countries. J Africain Du Cancer / African J Cancer 2013;5:144–54.

Jasinski, M., Jasinska, L., Ogrodowczyk M. Resveratrol in prostate diseases - a short review. Cent Eur J Urol 2013;66:144–149.

Jiang L-N, Liu Y-B, Li B-H. Lycopene exerts anti-inflammatory effect to inhibit prostate cancer progression. Asian J Androl 2019;21:80.

Jirovetz L, Buchbauer G, Denkova Z, Stoyanova A, Murgov I, Schmidt E, et al. Antimicrobial testinas and gas chromatoaraphic analvsis of pure oxvaenated monoterpenes 1.8-cineole, α-terpineol, terpinen-4-ol and camphor as well as target comoounds in essential oils of pine (*Pinus pinaster*), rosemary (*Rosmarinus officinalis*), tea tree. Sci Pharm 2005;73:27–39.

Jo M-J, Kumar H, Joshi HP, Choi H, Ko W-K, Kim JM, et al. Oral Administration of α-Asarone Promotes Functional Recovery in Rats With Spinal Cord Injury. Front Pharmacol 2018;9.

Juergens U., Dethlefsen U, Steinkamp G, Gillissen A, Repges R, Vetter H. Anti-inflammatory activity of 1.8-cineol (eucalyptol) in bronchial asthma: a double-blind placebo-controlled trial. Respir Med 2003;97:250–6.

Juergens UR, Stöber M, Schmidt-Schilling L, Kleuver T, Vetter H. Antiinflammatory effects of euclyptol (1.8-cineole) in bronchial asthma: inhibition of arachidonic acid metabolism in human blood monocytes ex vivo. Eur J Med Res 1998a;3:407–12.

Juergens UR, Stöber M, Vetter H. Inhibition of cytokine production and arachidonic acid metabolism by eucalyptol (1.8-cineole) in human blood monocytes in vitro. Eur J Med Res 1998b;3:508–10.

Kaegi E. Unconventional therapies for cancer: 6. 714-X. Can Med Assoc 1998;158:1621–4.

Kala HK, Mehta R, Sen KK, Tandey R, Mandal V. Strategizing method optimization of microwave-assisted extraction of plant phenolics by developing standard working principles for universal robust optimization. Anal Methods 2017;9:2089–103.

Kamaraj C, Rahuman AA, Bagavan A. Antifeedant and larvicidal effects of plant extracts against Spodoptera litura (F.), Aedes aegypti L. and Culex quinquefasciatus Say. Parasitol Res 2008;103:325–31.

Kang BY, Chung SW, Chung W-J, Im S-Y, Hwang SY, Kim TS. Inhibition of interleukin-12 production in lipopolysaccharide-activated macrophages by curcumin. Eur J Pharmacol 1999;384:191–5.

Karaca N, Şener G, Demirci B, Demirci F. Synergistic antibacterial combination of Lavandula latifolia Medik. essential oil with camphor. Zeitschrift Für Naturforsch C 2021;76:169–73.

Karakaya S, Yilmaz SV, Özdemir Ö, Koca M, Pınar NM, Demirci B, et al. A caryophyllene oxide and other potential anticholinesterase and anticancer agent in Salvia verticillata subsp. amasiaca (Freyn & Bornm.) Bornm. (Lamiaceae). J Essent Oil Res 2020;32:512–25.

Katsukawa M, Nakata R, Takizawa Y, Hori K, Takahashi S, Inoue H. Citral, a component of lemongrass oil, activates PPARα and γ and suppresses COX-2 expression. Biochim Biophys Acta - Mol Cell Biol Lipids 2010;1801:1214–20.

Khare CP. Encyclopedia of Indian medicinal plants : rational Western therapy, Ayurvedic and other traditional usage, botany 2004:474–6.

Kim H, Youn K, Yun E-Y, Hwang J-S, Jeong W-S, Ho C-T, et al. Oleic acid ameliorates Aβ-induced inflammation by downregulation of COX-2 and iNOS via NFκB signaling pathway. J Funct Foods 2015;14:1–11.

Khristi V, Patel VH. Therapeutic potential of *Hibiscus rosa sinensis*: A Review. Int J Nutr Diet 2016;4:105-23.

Kim HP, Mani I, Iversen L, Ziboh VA. Effects of naturally-occurring flavonoids and biflavonoids on epidermal cyclooxygenase and lipoxygenase from guinea-pigs. Prostaglandins, Leukot Essent Fat Acids 1998;58:17–24.

Kim S, Kundu A, Chun R, Han SH, Pandey AK, Yoo S, et al. Direct Synthesis of 2-Acyl Acridines Using Aldimines and Anthranils: Evaluation of Cytotoxicity and Anti-Inflammatory Activity. Asian J Org Chem 2018;7:2069–75.

Kjaer TN, Ornstrup MJ, Poulsen MM, Jørgensen JOL, Hougaard DM, Cohen AS, et al. Resveratrol reduces the levels of circulating androgen precursors but has no effect on, testosterone, dihydrotestosterone, PSA levels or prostate volume. A 4-month randomised trial in middle-aged men. Prostate 2015;75:1255–63.

Knox KL. Nosocomial Endocarditis Caused by Corynebacterium amycolatum and Other Nondiphtheriae Corynebacteria. Emerg Infect Dis 2002;8:97–9.

Koga Y, Song H, Chalmers ZR, Newberg J, Kim E, Carrot-Zhang J, et al. Genomic Profiling of Prostate Cancers from Men with African and European Ancestry. Clin Cancer Res 2020;26:4651–60.

Komiya Y, Habas R. Wnt signal transduction pathways. Organogenesis 2008;4:68–75.

Kotan R, Kordali S, Cakir A. Screening of Antibacterial Activities of Twenty-One Oxygenated Monoterpenes. Zeitschrift Für Naturforsch C 2007;62:507–13.

Kremer JL, Melo GP, Marinello PC, Bordini HP, Rossaneis AC, Sábio LR, et al. Citral prevents UVB-induced skin carcinogenesis in hairless mice. J Photochem Photobiol B Biol 2019;198:111565.

Kumari N, Dwarakanath BS, Das A, Bhatt AN. Role of interleukin-6 in cancer progression and therapeutic resistance. Tumor Biol 2016;37:11553–72.

Kundu A, Mandal V, Maiti PP, Rashid MH Al, Mandal SC. In-vitro-Scientific evaluation of anti-inflammatory potential of leaf extracts from Vitex negundo: as a promising future drug candidate. Int J Green Pharm 2020;14.

Lal B, Kapoor AK, Agrawal PK, Asthana OP, Srimal RC. Role of curcumin in idiopathic inflammatory orbital pseudotumours. Phyther Res 2000;14:443–7.

Lal B, Kapoor AK, Asthana OP, Agrawal PK, Prasad R, Kumar P, et al. Efficacy of Curcumin in the Management of Chronic Anterior Uveitis. Phyther Res 1999;13:318–22.

Lam KYC, Yao P, Wang H, Duan R, Dong TTX, Tsim KWK. Asarone from Acori Tatarinowii Rhizome prevents oxidative stress-induced cell injury in cultured astrocytes: A signaling triggered by Akt activation. PLoS One 2017;12:e0179077.

Langeveld WT, Veldhuizen EJA, Burt SA. Synergy between essential oil components and antibiotics: a review. Crit Rev Microbiol 2014;40:76–94.

Lederberg J, Shope RE, Jr. SCO. Emerging Infections: Microbial Threats to Health in the United States. Washington, D.C.: National Academies Press; 1992.

Lee E, Ha S, Logan SK. Divergent Androgen Receptor and Beta-Catenin Signaling in Prostate Cancer Cells. PLoS One 2015;10:e0141589.

Lee K-G, Shibamoto T. Antioxidant Properties of Aroma Compounds Isolated from Soybeans and Mung Beans. J Agric Food Chem 2000;48:4290–3.

Lee S-Y, Bae C-S, Seo N-S, Na C-S, Yoo HY, Oh D-S, et al. Camellia japonica oil suppressed asthma occurrence via GATA-3 & IL-4 pathway and its effective and major component is oleic acid. Phytomedicine 2019;57:84–94.

Legault J, Pichette A. Potentiating effect of β-caryophyllene on anticancer activity of α-humulene, isocaryophyllene and paclitaxel. J Pharm Pharmacol 2010;59:1643–7.

Lewis K. Platforms for antibiotic discovery. Nat Rev Drug Discov 2013;12:371–87.

Li-Weber M, Giaisi M, Treiber M, Krammer P. The anti-inflammatory sesquiterpene lactone parthenolide suppresses IL-4 gene expression in peripheral blood T cells. Eur J Immunol 2002;32:3587–97.

Li S, Zhou T, Li C, Dai Z, Che D, Yao Y, et al. High Metastaticgastric and Breast Cancer Cells Consume Oleic Acid in an AMPK Dependent Manner. PLoS One 2014;9:e97330.

Li Z-H, Cai M, Liu Y-S, Sun P-L, Luo S-L. Antibacterial Activity and Mechanisms of Essential Oil from Citrus medica L. var. sarcodactylis. Molecules 2019;24:1577.

De Lima VT, Vieira MC, Kassuya CAL, Cardoso CAL, Alves JM, Foglio MA, et al. Chemical composition and free radical-scavenging, anticancer and anti-inflammatory activities of the essential oil from Ocimum kilimandscharicum. Phytomedicine 2014;21:1298–302.

Lin C-T, Chen C-J, Lin T-Y, Tung JC, Wang S-Y. Anti-inflammation activity of fruit essential oil from *Cinnamomum insularimontanum* Hayata. Bioresour Technol 2008;99:8783–7.

Lion C, Escande F, Burdin JC. Capnocytophaga canimorsus infections in human: Review of the literature and cases report. Eur J Epidemiol 1996;12:521–33.

Loizou S, Lekakis I, Chrousos GP, Moutsatsou P. β-Sitosterol exhibits anti-inflammatory activity in human aortic endothelial cells. Mol Nutr Food Res 2010;54:551–8.

Lu X, Chen D, Yang F, Xing N. Quercetin Inhibits Epithelial-to-Mesenchymal Transition (EMT) Process and Promotes Apoptosis in Prostate Cancer via Downregulating lncRNA MALAT1. Cancer Manag Res 2020a;Volume 12:1741–50.

Lu X, Yang F, Chen Dexi, Zhao Q, Chen Dong, Ping H, et al. Quercetin reverses docetaxel resistance in prostate cancer via androgen receptor and PI3K/Akt signaling pathways. Int J Biol Sci 2020b;16:1121–34.

Mahalakshm R, Rajesh P, Ramesh N, Balasubram V, Kannan VR. Hepatoprotective Activity on *Vitex negundo* Linn. (Verbenaceae) by using Wistar Albino Rats in Ibuprofen Induced Model. Int J Pharmacol 2010;6:658–63.

Manikandan S, Devi R. Antioxidant property of α-asarone against noise-stress-induced changes in different regions of rat brain. Pharmacol Res 2005;52:467–74.

Mareček V, Mikyška A, Hampel D, Čejka P, Neuwirthová J, Malachová A, et al. ABTS and DPPH methods as a tool for studying antioxidant capacity of spring barley and malt. J Cereal Sci 2017;73:40–5.

Martins HB, Selis N das N, Souza CLS e, Nascimento FS, Carvalho SP de, Gusmão LD, et al. Anti-Inflammatory Activity of the Essential Oil Citral in Experimental Infection with Staphylococcus aureus in a Model Air Pouch. Evidence-Based Complement Altern Med 2017;2017:1–10.

De Marzo AM, Platz EA, Sutcliffe S, Xu J, Grönberg H, Drake CG, et al. Inflammation in prostate carcinogenesis. Nat Rev Cancer 2007;7:256–69.

Medzhitov R. Inflammation 2010: New Adventures of an Old Flame. Cell 2010;140:771–6.

Miguel-Martinez I, Fernández-Fuertes F, Ramos-Macías A, Bosch-Benitez JM, Martín-Sánchez AM. Sepsis due to Multiply ResistantCorynebacterium amycolatum. Eur J Clin Microbiol Infect Dis 1996;15:617–8.

Mirahmadi M, Azimi-Hashemi S, Saburi E, Kamali H, Pishbin M, Hadizadeh F. Potential inhibitory effect of lycopene on prostate cancer. Biomed Pharmacother 2020;129:110459.

Missoum A. An update review on *Hibiscus rosa sinensis* phytochemistry and medicinal uses. J Ayu Her Med 2018;4:135-46.

Mittal M, Siddiqui MR, Tran K, Reddy SP, Malik AB. Reactive Oxygen Species in Inflammation and Tissue Injury. Antioxid Redox Signal 2014;20:1126–67.

Mohanty NK, Saxena S, Singh UP, Goyal NK, Arora RP. Lycopene as a chemopreventive agent in the treatment of high-grade prostate intraepithelial neoplasia. Urol Oncol Semin Orig Investig 2005;23:383–5.

Murillo-Garzón V, Kypta R. WNT signalling in prostate cancer. Nat Rev Urol 2017;14:683–96.

Muthukumarasamy S, Mohan VR, others. GC-MS determination of bioactive components of Canscora perfoliata Lam.(Gentianaceae). J Appl Pharm Sci 2012;2:210–4.

Naiki-Ito A, Chewonarin T, Tang M, Pitchakarn P, Kuno T, Ogawa K, et al. Ellagic acid, a component of pomegranate fruit juice, suppresses androgen-dependent prostate carcinogenesis via induction of apoptosis. Prostate 2015;75:151–60.

Nathan C, Ding A. Nonresolving Inflammation. Cell 2010;140:871–82.

Neergheen VS, Bahorun T, Taylor EW, Jen L-S, Aruoma OI. Targeting specific cell signaling transduction pathways by dietary and medicinal phytochemicals in cancer chemoprevention. Toxicology 2010;278:229–41.

Nelson VK, Ali A, Dutta N, Ghosh S, Jana M, Ganguli A, et al. Azadiradione ameliorates polyglutamine expansion disease in Drosophila by potentiating DNA binding activity of heat shock factor 1. Oncotarget 2016;7:78281.

Nirmal SA, Pal SC, Mandal SC, Patil AN. Analgesic and anti-inflammatory activity of β-sitosterol isolated from Nyctanthes arbortristis leaves. Inflammopharmacology 2012;20:219–24.

Noda D, Itoh S, Watanabe Y, Inamitsu M, Dennler S, Itoh F, et al. ELAC2, a putative prostate cancer susceptibility gene product, potentiates TGF-β/Smad-induced growth arrest of prostate cells. Oncogene 2006;25:5591–600.

Nurgali K, Jagoe RT, Abalo R. Editorial: Adverse Effects of Cancer Chemotherapy: Anything New to Improve Tolerance and Reduce Sequelae? Front Pharmacol 2018;9.

Ondua M, Njoya EM, Abdalla MA, McGaw LJ. Anti-inflammatory and antioxidant properties of leaf extracts of eleven South African medicinal plants used traditionally to treat inflammation. J Ethnopharmacol 2019;234:27–35.

Ono M, Nishida Y, Masuoka C, Li J-C, Okawa M, Ikeda T, et al. Lignan Derivatives and a Norditerpene from the Seeds of Vitex negundo. J Nat Prod 2004;67:2073–5.

Osonga FJ, Akgul Ali, Miller RM, Eshun GB, Yazgan I, Akgul Ayfer, et al. Antimicrobial Activity of a New Class of Phosphorylated and Modified Flavonoids. ACS Omega 2019;4:12865–71.

Pal S, Bhattacharjee A, Ali A, Mandal NC, Mandal SC, Pal M. Chronic inflammation and cancer: potential chemoprevention through nuclear factor kappa B and p53 mutual antagonism. J Inflamm 2014;11:23.

Pallag A, Filip GA, Olteanu D, Clichici S, Baldea I, Jurca T, et al. Equisetum arvense L. Extract Induces Antibacterial Activity and Modulates Oxidative Stress, Inflammation, and Apoptosis in Endothelial Vascular Cells Exposed to Hyperosmotic Stress. Oxid Med Cell Longev 2018;2018:1–14.

Palozza P, Colangelo M, Simone R, Catalano A, Boninsegna A, Lanza P, et al. Lycopene induces cell growth inhibition by altering mevalonate pathway and Ras signaling in cancer cell lines. Carcinogenesis 2010;31:1813–21.

Panahi Y, Hosseini MS, Khalili N, Naimi E, Simental-Mendía LE, Majeed M, et al. Effects of curcumin on serum cytokine concentrations in subjects with metabolic syndrome: A post-hoc analysis of a randomized controlled trial. Biomed Pharmacother 2016;82:578–82.

Panda SK, Thatoi HN, Dutt and SK. Antibacterial activity and phytochemical screening of leaf and bark extracts of Vitex negundo l. from similipal biosphere reserve, Orissa. J Med Plants Res 2009;3:294–300.

Paneru, R.B. and Shivakoti GP. Use of botanicals for the management of pulse beetle (*Callosobruchus maculatus* F.) in lentil. Nepal Agric Res J 2001;4:27–30.

Park K-R, Nam D, Yun H-M, Lee S-G, Jang H-J, Sethi G, et al. β-Caryophyllene oxide inhibits growth and induces apoptosis through the suppression of PI3K/AKT/mTOR/S6K1 pathways and ROS-mediated MAPKs activation. Cancer Lett 2011;312:178–88.

Perumal Samy R, Ignacimuthu S, Sen A. Screening of 34 Indian medicinal plants for antibacterial properties. J Ethnopharmacol 1998;62:173–81.

R. Preethi VVD and ML. Antimicrobial and Antioxidant Efficacy of Some Medicinal Plants Against Food Borne Pathogen. Adv Biol Res (Rennes) 2010;4:122–5.

Rashid MHA, P.v.p. DB, Majumder S, Mandal V, Pal M, Mandal SC, et al. antioxidant and anticancer activity of extract and fractions obtained from *Diospyros melanoxylon* roxb. leaves and correlation with their polyphenolic profiles. Int J Pharm Pharm Sci 2018;10:6.

Rates SM. Plants as source of drugs. Toxicon 2001;39:603–13.

Ravi, K. Sahayaraj and C. Preliminary phytochemistry of ipomea carnea jacq. and *Vitex negundo* linn. leaves. Int J Chem Sci 2008;6:1–6.

Rhimi W, Ben Salem I, Iatta R, Chaabane H, Saidi M, Boulila A, et al. *Dittrichia viscosa* L. leaves lipid extract: An unexploited source of essential fatty acids and tocopherols with antifungal and anti-inflammatory properties. Ind Crops Prod 2018;113:196–201.

Rosell G, Quero C, Coll J, Guerrero A. Biorational insecticides in pest management. J Pestic Sci 2008;33:103–21.

RS Telang, S Chatterjee CV. Study on analgesic and anti-inflammatory activities of Vitex negundo Linn. Indian J Pharmacol 1999;31:363–6.

Ruiz-Gutirrez V, Muriana FJG, Guerrero A, Cert AM, Villar J. Plasma lipids, erythrocyte membrane lipids and blood pressure of hypertensive women after ingestion of dietary oleic acid from two different sources. J Hypertens 1996;14:1483–90.

Russo EB. Taming THC: potential cannabis synergy and phytocannabinoid-terpenoid entourage effects. Br J Pharmacol 2011;163:1344–64.

Sabulal B, Dan M, J AJ, Kurup R, Pradeep NS, Valsamma RK, et al. Caryophyllene-rich rhizome oil of *Zingiber nimmonii* from South India: Chemical characterization and antimicrobial activity. Phytochemistry 2006;67:2469–73.

Saikat Sen RC, Sridhar C, Reddy YSR, D B. Free radicals, antioxidants, Diseases and phytomedicines: current status and future prospect. Int J Pharm Sci Rev Res 2010;3:91–100.

Salazar-Aranda R, Pérez-López LA, López-Arroyo J, Alanís-Garza BA, Waksman de Torres N. Antimicrobial and Antioxidant Activities of Plants from Northeast of Mexico. Evidence-Based Complement Altern Med 2011;2011:1–6.

Saldanha AA, Vieira L, de Oliveira FM, Lopes D de O, Ribeiro RIM de A, Thomé RG, et al. Anti-inflammatory and central and peripheral anti-nociceptive activities of α-asarone through the inhibition of TNF-α production, leukocyte recruitment and iNOS expression, and participation of the adenosinergic and opioidergic systems. Inflammopharmacology 2020;28:1039–52.

Salem MZM, Ali HM, Basalah MO. Essential oils from wood, bark, and needles of *Pinus roxburghii* Sarg. from Alexandria, Egypt: Antibacterial and antioxidant activities. BioResources 2014;9:7454–66.

Sales-Campos H, Reis de Souza P, Crema Peghini B, Santana da Silva J, Ribeiro Cardoso C. An Overview of the Modulatory Effects of Oleic Acid in Health and Disease. Mini Rev Med Chem 2013;13:201–10.

Sanchez de MF, Galvez J, Romero JA ZA. Effect of quercitrin on acute and chronic experimental colitis in the rat. Life Sci 2002;70:3097–108.

Sancho D, Reis e Sousa C. Signaling by Myeloid C-Type Lectin Receptors in Immunity and Homeostasis. Annu Rev Immunol 2012;30:491–529.

Santel T, Pflug G, Hemdan NYA, Schäfer A, Hollenbach M, Buchold M, et al. Curcumin Inhibits Glyoxalase 1—A Possible Link to Its Anti-Inflammatory and Anti-Tumor Activity. PLoS One 2008;3:e3508.

Santos FA, Rao VSN. Possible role of mast cells in cineole-induced scratching behavior in mice. Food Chem Toxicol 2002;40:1453–7.

Santos FA, Rao VSN. Antiinflammatory and antinociceptive effects of 1,8-cineole a terpenoid oxide present in many plant essential oils. Phyther Res 2000;14:240–4.

Sarkera AK, Chowdhury JU, Bhuiyan HR. Chemical Composition and Antimicrobial Activity of Essential Oil Collected from *Adhatoda vasica* Leaves. Bangladesh J Sci Ind Res 2011;46:191-94.

Sarkic A, Stappen I. Essential Oils and Their Single Compounds in Cosmetics—A Critical Review. Cosmetics 2018;5:11.

Bin Sayeed M, Karim S, Sharmin T, Morshed M. Critical Analysis on Characterization, Systemic Effect, and Therapeutic Potential of Beta-Sitosterol: A Plant-Derived Orphan Phytosterol. Medicines 2016;3:29.

Schmidt KT, Figg WD. The potential role of curcumin in prostate cancer: the importance of optimizing pharmacokinetics in clinical studies. Transl Cancer Res 2016;5:S1107–10.

Schroder K, Tschopp J. The Inflammasomes. Cell 2010;140:821–32.

Semaming Y, Pannengpetch P, Chattipakorn SC, Chattipakorn N. Pharmacological Properties of Protocatechuic Acid and Its Potential Roles as Complementary Medicine. Evidence-Based Complement Altern Med 2015;2015:1–11.

Serra S. Recent Developments in the Synthesis of the Flavors and Fragrances of Terpenoid Origin, 2015, p. 201–26.

Sethi G, Sankaranarayanan S, Sukhija M. Low lactose in the nutritional management of diarrhea: Case reports from India. Clin Epidemiol Glob Heal 2018;6:160–2.

Sfanos KS, De Marzo AM. Prostate cancer and inflammation: the evidence. Histopathology 2012;60:199–215.

Sharmila G, Bhat FA, Arunkumar R, Elumalai P, Raja Singh P, Senthilkumar K, et al. Chemopreventive effect of quercetin, a natural dietary flavonoid on prostate cancer in in vivo model. Clin Nutr 2014;33:718–26.

Sheikh SZ, Hegazi RA, Kobayashi T, Onyiah JC, Russo SM, Matsuoka K, et al. An Anti-Inflammatory Role for Carbon Monoxide and Heme Oxygenase-1 in Chronic Th2-Mediated Murine Colitis. J Immunol 2011;186:5506–13.

Shenvi S, Diwakar L, Reddy GC. Nitro Derivatives of Naturally Occurring β -Asarone and Their Anticancer Activity. Int J Med Chem 2014;2014:1–5.

Shu Y, Xie B, Liang Z, Chen J. Quercetin reverses the doxorubicin resistance of prostate cancer cells by downregulating the expression of c‑met. Oncol Lett 2017.

da Silva JKR, Figueiredo PLB, Byler KG, Setzer WN. Essential Oils as Antiviral Agents, Potential of Essential Oils to Treat SARS-CoV-2 Infection: An In-Silico Investigation. Int J Mol Sci 2020;21:3426.

Silva JM, Pereira CV, Mano F, Silva E, Castro VIB, Sá-Nogueira I, et al. Therapeutic Role of Deep Eutectic Solvents Based on Menthol and Saturated Fatty Acids on Wound Healing. ACS Appl Bio Mater 2019;2:4346–55.

Singh Chouhan KB, Tandey R, Sen KK, Mehta R, Mandal V. A unique model of gravity assisted solvent free microwave based extraction of essential oil from mentha leaves ensuring biorefinery of leftover waste biomass for extraction of nutraceuticals: Towards cleaner and greener technology. J Clean Prod 2019;225:587–98.

Singh CK, Chhabra G, Ndiaye MA, Siddiqui IA, Panackal JE, Mintie CA, et al. Quercetin–Resveratrol Combination for Prostate Cancer Management in TRAMP Mice. Cancers (Basel) 2020;12:2141.

Smit HF, Woerdenbag HJ, Singh RH, Meulenbeld GJ, Labadie RP, Zwaving JH. Āyurvedic herbal drugs with possible cytostatic activity. J Ethnopharmacol 1995;47:75–84.

Soetan KO, Oyekunle MA, AOO and FMA. Evaluation of the antimicrobial activity of saponins extract of Sorghum Bicolor L. Moench. African J Biotechnol 2006;23:2405–7.

Souza MF, Rao VS, Silveira ER. Anti-anaphylactic and anti-inflammatory effects of ternatin, a flavonoid isolated from Egletes viscosa Less. Brazilian J Med Biol Res = Rev Bras Pesqui Medicas e Biol 1992;25:1029–32.

Souza SM de, Monache FD, Smânia A. Antibacterial Activity of Coumarins. Zeitschrift Für Naturforsch C 2005;60:693–700.

Srivastava KC, Bordia A, Verma SK. Curcumin, a major component of food spice turmeric (*Curcuma longa*) inhibits aggregation and alters eicosanoid metabolism in human blood platelets. Prostaglandins, Leukot Essent Fat Acids 1995;52:223–7.

Stefanachi A, Leonetti F, Pisani L, Catto M, Carotti A. Coumarin: A Natural, Privileged and Versatile Scaffold for Bioactive Compounds. Molecules 2018;23:250.

Sun S, Gong F, Liu P, Miao Q. Metformin combined with quercetin synergistically repressed prostate cancer cells via inhibition of VEGF/PI3K/Akt signaling pathway. Gene 2018;664:50–7.

Sundram V, Chauhan SC, Ebeling M, Jaggi M. Curcumin Attenuates β-catenin Signaling in Prostate Cancer Cells through Activation of Protein Kinase D1. PLoS One 2012;7:e35368.

Swamy MK, Arumugam G, Kaur R, Ghasemzadeh A, Yusoff MM, Sinniah UR. GC-MS Based Metabolite Profiling, Antioxidant and Antimicrobial Properties of Different Solvent Extracts of Malaysian Plectranthus amboinicus Leaves. Evidence-Based Complement Altern Med 2017;2017:1–10.

Szabó MÁ, Varga GZ, Hohmann J, Schelz Z, Szegedi E, Amaral L, et al. Inhibition of quorum-sensing signals by essential oils. Phyther Res 2010;24:782–6.

Tabas I, Glass CK. Anti-Inflammatory Therapy in Chronic Disease: Challenges and Opportunities. Science (80-) 2013;339:166–72.

Tanaudommongkon I, Tanaudommongkon A, Prathipati P, Nguyen JT, Keller ET, Dong X. Curcumin Nanoparticles and Their Cytotoxicity in Docetaxel-Resistant Castration-Resistant Prostate Cancer Cells. Biomedicines 2020;8:253.

Tandon VR, Gupta RK. An experimental evaluation of anti-convulsant activity of *Vitex-negundo*. Indian J Physiol Pharmacol 2005;49:199–205.

Tandon VR, Khajuria V, Kapoor B, Kour D, Gupta S. Hepatoprotective activity of *Vitex negundo* leaf extract against anti-tubercular drugs induced hepatotoxicity. Fitoterapia 2008;79:533–8.

Tariq S, Wani S, Rasool W, Shafi K, Bhat MA, Prabhakar A, et al. A comprehensive review of the antibacterial, antifungal and antiviral potential of essential oils and their

chemical constituents against drug-resistant microbial pathogens. Microb Pathog 2019;134:103580.

Tenikoff D, Murphy K, Le M, Butler R, Howarth G, Howe P. Lyprinol(TM): a potential preventive treatment for experimental inflammatory bowel disease (IBD). Asia Pac J Clin Nutr 2004;13:S94.

Tian B, Liu J. Resveratrol: a review of plant sources, synthesis, stability, modification and food application. J Sci Food Agric 2020;100:1392–404.

Tohidi B, Rahimmalek M, Arzani A. Essential oil composition, total phenolic, flavonoid contents, and antioxidant activity of Thymus species collected from different regions of Iran. Food Chem 2017;220:153–61.

Torricelli P, Ricci P, Provenzano B, Lentini A, Tabolacci C. Synergic effect of α-tocopherol and naringenin in transglutaminase-induced differentiation of human prostate cancer cells. Amino Acids 2011;41:1207–14.

Tungmunnithum D, Thongboonyou A, Pholboon A, Yangsabai A. Flavonoids and Other Phenolic Compounds from Medicinal Plants for Pharmaceutical and Medical Aspects: An Overview. Medicines 2018;5:93.

Turkez H, Sozio P, Geyikoglu F, Tatar A, Hacimuftuoglu A, Di Stefano A. Neuroprotective Effects of Farnesene Against Hydrogen Peroxide-Induced Neurotoxicity In vitro. Cell Mol Neurobiol 2014;34:101–11.

Vicari E, Arancio A, Catania VE, Vicari BO, Sidoti G, Castiglione R, et al. Resveratrol reduces inflammation-related Prostate Fibrosis. Int J Med Sci 2020;17:1864–70.

Vijayakumar MDS, Winter MS, Sause MDK, Gallagher MDW, Michalski MDMJ, Roach MDJ M, et al. Prostate-Specific Antigen Levels are Higher in African-American Than in White Patients in a Multicenter Registration Study: Results of RTOG 94-12. Int J Radiat Oncol 1998;40:17–25.

Vishal Tandon RG. Histomorphological changes induced by *Vitex negundo* in albino rats. Indian J Pharmacol 2004;36:176–7.

Vulto AG & Smet PAGM. Meyler's side effects o f drugs: an encyclopedia o f adverse reactions and interactions. 1998.

Wadekar AB, Nimbalwar MG, Panchale WA, Gudalwar BR, Manwar JV, Bakal RL. Morphology, phytochemistry and pharmacological aspects of Carica papaya. An Review. GSC Biol Pharm Sci 2021;14:234–48.

Wang H, Nair MG, Strasburg GM, Chang Y-C, Booren AM, Gray JI, et al. Antioxidant and Antiinflammatory Activities of Anthocyanins and Their Aglycon, Cyanidin, from Tart Cherries. J Nat Prod 1999;62:294–6.

Wang J, Vanga SK, Raghavan V. High-intensity ultrasound processing of kiwifruit juice: Effects on the ascorbic acid, total phenolics, flavonoids and antioxidant capacity. LWT 2019;107:299–307.

Wang K, Chen Z, Shi J, Feng Y, Yu M, Sun Y, et al. Resveratrol inhibits the tumor migration and invasion by upregulating TET1 and reducing TIMP2/3 methylation in prostate carcinoma cells. Prostate 2020;80:977–85.

Wang L, Li W, Lin M, Garcia M, Mulholland D, Lilly M, et al. Luteolin, ellagic acid and punicic acid are natural products that inhibit prostate cancer metastasis. Carcinogenesis 2014;35:2321–30.

Wang N, Zhang Q, Luo L, Ning B, Fang Y. β-asarone inhibited cell growth and promoted autophagy via P53/Bcl-2/Bclin-1 and P53/AMPK/mTOR pathways in Human Glioma U251 cells. J Cell Physiol 2018;233:2434–43.

Wang X, Shen Y, Thakur K, Han J, Zhang J-G, Hu F, et al. Antibacterial Activity and Mechanism of Ginger Essential Oil against Escherichia coli and Staphylococcus aureus. Molecules 2020;25:3955.

Watkins Bruner D, Moore D, Parlanti A, Dorgan J, Engstrom P. Relative risk of prostate cancer for men with affected relatives: Systematic review and meta-analysis. Int J Cancer 2003;107:797–803.

Wen-Chien Lu, Da-Wei Huang, Chiun C.R. Wang, Ching-Hua Yeh, Jen-Chieh Tsai, Yu-Ting Huang P-HL. Preparation, characterization, and antimicrobial activity of nanoemulsions incorporating citral essential oil. J Food Drug a n a l y s i S 2017;xxx.

Wu L, Zhao JC, Kim J, Jin H-J, Wang C-Y, Yu J. ERG Is a Critical Regulator of Wnt/LEF1 Signaling in Prostate Cancer. Cancer Res 2013;73:6068–79.

Wu S, Pang Y, He Y, Zhang X, Peng L, Guo J, et al. A comprehensive review of natural products against atopic dermatitis: Flavonoids, alkaloids, terpenes, glycosides and other compounds. Biomed Pharmacother 2021;140:111741.

Xiang Y, Wang Z, Murakami J, Plummer S, Klein EA, Carpten JD, et al. Effects of RNase L mutations associated with prostate cancer on apoptosis induced by 2',5'-oligoadenylates. Cancer Res 2003;63:6795–801.

Xiao B, Huang X, Wang Q, Wu Y. Beta-Asarone Alleviates Myocardial Ischemia–Reperfusion Injury by Inhibiting Inflammatory Response and NLRP3 Inflammasome Mediated Pyroptosis. Biol Pharm Bull 2020;43:1046–51.

Xie Y, Yang W, Tang F, Chen X, Ren L. Antibacterial Activities of Flavonoids: Structure-Activity Relationship and Mechanism. Curr Med Chem 2014;22:132–49.

Xu H. Camphor Activates and Strongly Desensitizes the Transient Receptor Potential Vanilloid Subtype 1 Channel in a Vanilloid-Independent Mechanism. J Neurosci 2005;25:8924–37.

Xu J, Meyers D, Freije D, Isaacs S, Wiley K, Nusskern D, et al. Evidence for a prostate cancer susceptibility locus on the X chromosome. Nat Genet 1998;20:175–9.

Yang L-L, Yen K-Y, Kiso Y, Hikino H. Antihepatotoxic actions of formosan plant drugs. J Ethnopharmacol 1987;19:103–10.

Yin Y, Liu X, Liu J, Cai E, Zhu H, Li H, et al. Beta-sitosterol and its derivatives repress lipopolysaccharide/d-galactosamine-induced acute hepatic injury by inhibiting the oxidation and inflammation in mice. Bioorg Med Chem Lett 2018;28:1525–33.

Yukihiro Tambe, Hitomi Tsujiuchi, Gisho Honda, Yasumasa Ikeshiro and ST. Gastric Cytoprotection of the Non-Steroidal Anti-Inflammatory Sesquiterpene,β - Caryophyllene. Planta Med 1996;62:469—470.

Zhang X, Huang J, Yu C, Xiang L, Li L, Shi D, et al. Quercetin Enhanced Paclitaxel Therapeutic Effects Towards PC-3 Prostate Cancer Through ER Stress Induction and ROS Production. Onco Targets Ther 2020;Volume 13:513–23.

Zhang Y, Liu X, Wang Y, Jiang P, Quek S. Antibacterial activity and mechanism of cinnamon essential oil against Escherichia coli and Staphylococcus aureus. Food Control 2016;59:282–9.

Zhao J, Liu J, Wei T, Ma X, Cheng Q, Huo S, et al. Quercetin-loaded nanomicelles to circumvent human castration-resistant prostate cancer in vitro and in vivo. Nanoscale 2016;8:5126–38.

De Zoysa MHN, Rathnayake H, Hewawasam RP, Gaya Bandara Wijayaratne WMD. Determination of In Vitro Antimicrobial Activity of Five Sri Lankan Medicinal Plants against Selected Human Pathogenic Bacteria. Int J Microbiol 2019;2019:1–8.

Annexure

9. Annexure

Annexure I: Identification certificate of plant specimen

भारत सरकार
GOVERNMENT OF INDIA
पर्यावरण, वन और जलवायु परिवर्तन मंत्रालय
MINISTRY OF ENVIRONMENT, FOREST & CLIMATE CHANGE
फैक्स/ Fax: (033)26686226
दूरभाष/ Phone: (033)26683235/3364
इमेल/ E-mail: calherbarium@yahoo.co.in

सत्यमेव जयते

भारतीय वनस्पति सर्वेक्षण
BOTANICAL SURVEY OF INDIA
केंद्रीय राष्ट्रीय पादपालय
CENTRAL NATIONAL HERBARIUM
हावड़ा / HOWRAH – 711 103

संख्या / No.: CNH/Tech.II/2018/45 दिनांक / Date:07-06-2018

To,
Ms. Anindita Kundu
Department of Pharmaceutical Technology
Jadavpur University
Kolkata–700032
West Bengal

Sub.: Identification of one plant specimen – reg.

Dear Ms. Kundu,

Please refer to your letter no. JU/PHAM/245/18 dated 24th May 2018 along with one plant specimen for identification. The specimen has been identified by the concerned expert as:

Sl. No.	Specimen No.	Scientific Name	Family
1	JU-2	**Vitex negundo** L.	Lamiaceae

The receipt of ₹ 50/- (Rupees Fifty only) Receipt No. TR-5, C-160686 dated 25-05-2018 is enclosed herewith.

Your specimen is returned herewith.

Yours sincerely

7.6.2018

V.P. PRASAD
Scientist 'D'

वानिक 'डी' Sc
केंद्री। राष्ट्रीय पा य
Central National Herbarium
भारतीय वनस्पति सर्वेक्षण
Botanical Survey of India
हावरा / Howrah-711103

Botanical Survey of India, Central National Herbarium, P.O. – Botanic Garden, Howrah – 711 103, West Bengal, India.

Annexure II: Publication

ORIGINAL ARTICLE

In-vitro-Scientific evaluation of anti-inflammatory potential of leaf extracts from *Vitex negundo*: as a promising future drug candidate

Anindita Kundu[1], Vivekananda Mandal[2], Partha Pratim Maiti[3], Md. Harun Al Rashid[4], Subhash C. Mandal[1]

[1]Department of Pharmaceutical Technology, Pharmacognosy and Phytotherapy Research Laboratory, Division of Pharmacognosy, Jadavpur University, Kolkata, West Bengal, India, [2]Institute of Pharmacy, Guru Ghasidas Central University, Bilaspur, Chhattisgarh, India, [3]Department of Pharmaceutical Technology, University of North Bengal, Darjeeling, West Bengal, India, [4]Samsi Rural Hospital, Ratua-1, Malda, West Bengal, India

Abstract

Introduction: *Vitex negundo* (Linn.) is commonly used in folk medicines and widely distributed in India, especially in moist places. However, all parts of *V. negundo* are used to treat different pathophysiology, but leaves are strongly effective for medicinal uses. The aim of this study is to compare the anti-inflammatory potential of the methanolic extract and essential oil of *V. negundo* leaves. A comparative study of the bioactive compounds that were obtained from the methanolic extract and essential oil of *V. negundo* leaves was accomplished. **Materials and Methods:** First, the methanolic extract and essential oil were evaluated for their anti-inflammatory activities employing the RAW 264.7 cells. Subsequently, the identification and quantification of the β-sitosterol of methanolic extract and essential oil of leaves were evaluated using high-performance liquid chromatography (HPLC). The quantitative evaluations of polyphenolics were executed using HPLC. **Results:** The dose-dependent anti-inflammatory activities of the methanolic extract and essential oil were validated. Moreover, it was observed that the 50 µg/ml dose was found to be significant ($P < 0.001$) against the pro-inflammatory cytokines such as interleukin-1β (IL-1β) and IL-6. In case of anti-inflammatory cytokine such as IL-10, the 50 µg/ml dose was found to produce significant effects where the statistical significance was $P < 0.001$ and $P < 0.01$ for the methanolic extract and essential oil, respectively. Adequate amounts of β-sitosterol and polyphenols were found out in the methanolic extract and essential oil of leaves of *V. negundo*. **Conclusion:** The research findings suggest the significant anti-inflammatory properties of the methanolic extract and essential oil, but the methanolic extract showed a stronger effect. Furthermore, the essential oil of *V. negundo* could be used for the development of an ideal pharmaceutical formulation for effective delivery to people. Here, results justify its traditional use, which develops a future substantial value of this plant into the scientific discipline.

Key words: Anti-inflammatory activity, polyphenols, *Vitex negundo*, β-sitosterol

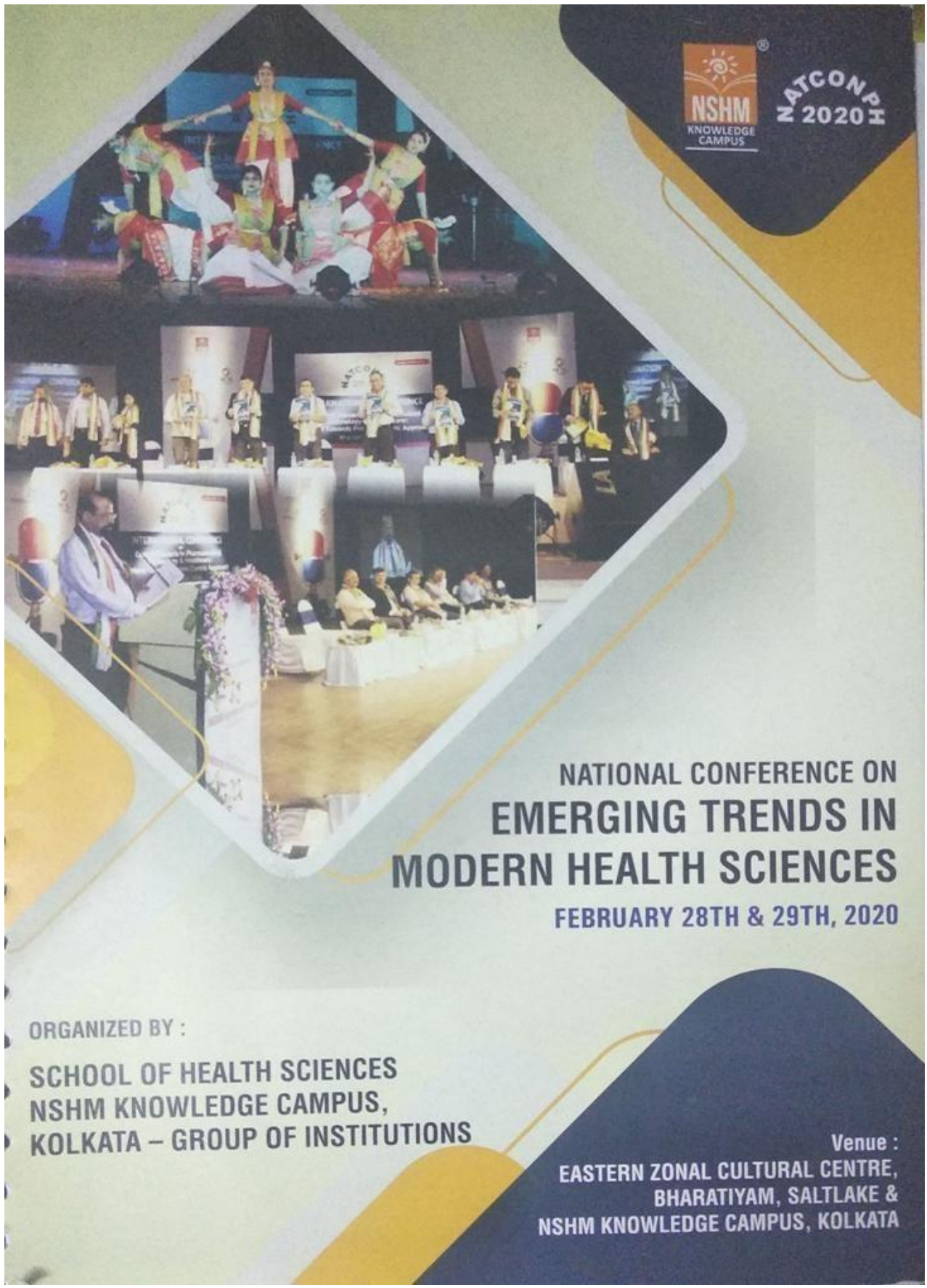
INTRODUCTION

Inflammation is extensively comprehended as a multifactorial and prolonged developmental process, where the inflammatory cytokines trigger the upregulation of inflammatory reactions by activating the stimulating factors and signaling cascades. Abundant production of inflammatory mediators prostaglandin E2, chemokines (e.g., chemokine C-C motif ligand 2 and chemokine CXC motif ligand 8), and pro-inflammatory mediators such as tumor

Address for correspondence:
Subhash C. Mandal, Department of Pharmaceutical Technology, Pharmacognosy and Phytotherapy Research Laboratory, Division of Pharmacognosy, Jadavpur University, Kolkata - 700032, West Bengal, India.
Mobile: +91-9433098372.
E-mail: scmandal1963@gmail.com

Received: 25-10-2019
Revised: 11-11-2019
Accepted: 18-11-2019

Annexure III: Conference certificates


NSHM
KNOWLEDGE CAMPUS
NATCONPH 2020
NATIONAL CONFERENCE ON
EMERGING TRENDS IN
MODERN HEALTH SCIENCES
FEBRUARY 28TH & 29TH, 2020
ORGANIZED BY :
SCHOOL OF HEALTH SCIENCES
NSHM KNOWLEDGE CAMPUS,
KOLKATA – GROUP OF INSTITUTIONS
Venue :
EASTERN ZONAL CULTURAL CENTRE,
BHARATIYAM, SALTLAKE &
NSHM KNOWLEDGE CAMPUS, KOLKATA

used as redox-free-radical initiator. %grafting (%G), %grafting efficiency (%GE) and %conversion (%Cn) were calculated to evaluate the synthetic conditions. The grafted copolymer was then characterized by FTIR, solid-sate 13C NMR, DSC, TGA, elemental analysis, viscosity, XRD and water-absorption capacity. The acute oral toxicity and biodegradability study were also studied. A significant yield of grafted copolymer with %grafting of 644.1 was shown with the synthetic condition including 12 g of monomer, 400 mg CAN and 3 min microwave irradiation. The FTIR, NMR, elemental analysis and viscosity study corroborate the formation of carboxymethyl okra copolymer. The toxicity study and biodegradability study also exhibited the bio-compatibility and biodegradable nature of the copolymer, which might make the copolymer suitable for fabrication into drug delivery systems as smart semi-synthetic biopolymer.

POSTER NO.: PP109

IMPROVED BIO-DISTRIBUTION STUDY OF ROPINIROLE HYDROCHLORIDE LOADED CONTROLLED RELEASE MICROSPHERES

[1]Koyel Kar*, [2]Nripendra Nath Bala, [3]Rabindra Nath Pal
[1]Department of Pharmaceutical Chemistry, BCDA College of Pharmacy and Technology, Hridaypur-700127, Dist- North 24 Parganas, West Bengal, India.
[2]Department of Pharmaceutics, BCDA College of Pharmacy and Technology, Hridaypur- 700127, Dist- North 24 Parganas, West Bengal, India.
[3]Department of Pharmaceutics, Calcutta Institute of Pharmaceutical Technology and Allied Health Sciences, Uluberia- 711316, Dist- Howrah, West Bengal, India.

In the present study, the improved bio-distribution of ropinirole hydrochloride loaded controlled release microspheres were evaluated for the treatment of Parkinson's disease. All animal experiments were approved and performed in BCDA College of Pharmacy and Technology in accordance with the guidelines of Institutional Animal Ethics Committee (CPCSEA Registration No: 04/IAEC (2)/S/BCDA/2017). The study was executed for selective batches. Rabbit was chosen as a model for the study. The collected blood plasma was subjected to HPLC analysis to evaluate pharmacokinetic and correlation of in vitro-in vivo characteristics. The Cmax of F12 batch was 271.5 ng/ml and the corresponding Tmax was 5 h. The bioavailability of F12 was significantly increased compared to the marketed drug. The elimination was also less rapid with the micro particulate formulation. The in-vivo drug release denoted that F12 could increase the bio-distribution in rabbits effectively confirming in-vitro-in-vivo correlation. Assessment of AUC manifested that the relative bio-distribution was significantly increased for F12. Thus, the formulated microsphere seems to be a potential candidate as controlled release dosage form for the treatment of Parkinson's disease.

POSTER NO.: PP110

A COMPARATIVE ASSESSMENT OF ANTI-INFLAMMATORY POTENTIAL OF METHANOLIC EXTRACT AND ESSENTIAL OIL OF V. NEGUNDO LEAVES: PROMISING CANDIDATES TO BE DRUGS OF FUTURE

Anindita Kundu1,[2]*, Subhash C. Mandal[1]
[1]Department of Pharmaceutical Technology, Pharmacognosy and Phytotherapy Research Laboratory, Division of Pharmacognosy, Jadavpur University, Kolkata, West Bengal, India
[2]School of Pharmacy, Sister Nivedita University, Kolkata, West Bengal, India

This research work intends to compare the anti-inflammatory potential of the methanolic extract and essential oil of V. negundo leaves. A comparative study of the contents of bioactive compounds which were acquired from the methanolic extract and essential oil of V. negundo leaves was executed. In this study, the methanolic extract and essential oil were assessed for their anti-inflammatory activities employing the RAW 264.7 cells. Afterwards, the identifications and quantifications of the β-sitosterol and polyphenolics of methanolic extract and essential oil of leaves were carried out using high- performance liquid chromatography. The dose-dependent anti-inflammatory activities of the methanolic extract and essential oil were affirmed. It was observed that the 50 µg/ml dose was found to be significant ($P < 0.001$) against the pro-inflammatory cytokines such as interleukin-1β (IL-1β) and IL-6. In case of anti-inflammatory cytokine such as IL-10, the 50 µg/ml dose was found to produce significant effects where the statistical significance was $P < 0.001$ and $P < 0.01$ for the methanolic extract and essential oil, respectively. Ample amounts of β-sitosterol and polyphenols were presented in the methanolic extract and essential oil of leaves of V. negundo. The research outcomes revealed the significant anti-inflammatory properties of the methanolic extract and essential oil. Besides, the essential oil of V. negundo could be used for the development of an ideal pharmaceutical formulation for effective delivery to people.

****

NSHM KNOWLEDGE CAMPUS

INTERNATIONAL CONFERENCE
ON
CURRENT SCENARIO IN PHARMACEUTICAL TECHNOLOGY & HEALTHCARE:
A MOVE TOWARDS PATIENT-CENTRIC APPROACH

ATICONPH 2018

KOLKATA – MARCH 9 & 10, 2018

ORGANIZED BY :

NSHM COLLEGE OF PHARMACEUTICAL TECHNOLOGY & DEPARTMENT OF HEALTHCARE MANAGEMENT
NSHM KNOWLEDGE CAMPUS, KOLKATA – GROUP OF INSTITUTIONS

CERTIFICATE

This is to certify that Anindita Kundu *has presented Poster / Oral paper in the International Conference on "Current Scenario in Pharmaceutical Technology & Healthcare : A Move Towards Patient-Centric Approach" organized by* **NSHM** *College of Pharmaceutical Technology & Department of Healthcare Management,* **NSHM** *Knowledge Campus, Kolkata-Group of Institutions on 9th and 10th March 2018 at Science City Auditorium and Institute campus, Kolkata.*

Conference Chairman


NSHM
INTERNATIONAL CONFERENCE
ON
CURRENT SCENARIO IN PHARMACEUTICAL TECHNOLOGY &
HEALTHCARE: A MOVE TOWARDS PATIENT- CENTRIC APPROACH
VENUE :
SCIENCE CITY AUDITORIUM, KOLKATA &
NSHM KNOWLEDGE CAMPUS, KOLKATA
KOLKATA
MARCH 09 -10
2018
ORGANIZED BY :
NSHM COLLEGE OF PHARMACEUTICAL TECHNOLOGY &
DEPARTMENT OF HEALTHCARE MANAGEMENT
NSHM KNOWLEDGE CAMPUS, KOLKATA – GROUP OF INSTITUTIONS

P241

MEDICINAL PLANTS: USED IN THE TREATMENT OF SKIN DISEASES

Sanchita Ghosh*

NSHM Knowledge Campus, Kolkata – Group of Institutions, 124, BL Saha Road, Kolkata 700053, India.

Email: sanchitaghosh2095@gmail.com

Skin diseases are numerous and a frequently occurring health problem affecting all ages from the neonates to the elderly. Maintaining healthy skin is an important factor for a healthy body. Many people may develop skin diseases that affect the skin, including cancer, herpes and cellulitis. Numerous individuals may create skin maladies that influence the skin, including tumor, herpes and cellulitis. Some wild plants and their parts are often used to treat these ailments. Natural treatment is cheap and claimed to be safe. It is also suitable raw material for production of new synthetic agents. A total of 57 medicinal plants representing 34 families are reported for their therapeutic use against skin ailments. The predominant families are Euphorbiaceae and Fabaceae. Most preferred species for the management of skin ailments are Andrographis paniculata (Burm. f.) Wall. ex. Nees., Annona squamosa L., Azadirachta indica A. Juss., Calophyllum inophyllum L., Cissampelos pareira L., Lantana camara L, Ocimum sanctum L. In most of the skin treatments with medicinal plants, the herbal preparations are administered topically. Further scientific research is required to evaluate biochemical constituent as well as the pharmacologically useful alkaloids, tannins, resins and any other beneficial plant product which is available from the local flora for the enhanced posterity of mankind.

P242

MEDICINAL PLANTS: THEIR USE IN ANTICANCER TREATMENT

Arun Prakash Karak*

NSHM Knowledge Campus, Kolkata – Group of Institutions, 124, BL Saha Road, Kolkata 700053, India.

Email Id: Arunprksh84@gmail.com

Cancer is severely affecting the health of more and more people worldwide and there is an ever increasing demand to discover new therapies to treat this life-threatening disease. Compounds derived from natural source have drawn interest of many researchers as they are considered to have less toxic side effects compared to their synthetic counterparts. The huge library of structurally diverse natural compounds and their equally diverse bioactivity potential has been exploited to find lead compounds that on further molecular modification could produce semi-synthetic compounds of improved therapeutic potential. Application of newer tools like high-throughput screening, combinatorial chemistry, computational chemistry and bioinformatics are able to complement the drug discovery process. Further, drug delivery technologies like nanoparticles have been employed to enhance anticancer activities of plant-derived drugs by controlling the release of the compound and investigating new methods for administration.

P243

A COMPARATIVE STUDY OF PHYTOCHEMICAL PROFILING OF ESSENTIAL OIL AND METHANOLIC EXTRACT: A HOLISTIC APPROACH FOR MANAGEMENT OF VARIOUS AILMENTS

Anindita Kundu, Subhash C. Mandal

Pharmacognosy and Phytotherapy Research Laboratory, Division of Pharmacognosy, Department of Pharmaceutical Technology, Jadavpur University, Kolkata, India 700032

E.mail address of presenting author- akundupharmtech@gmail.com

The aim of this study is to investigate and evaluate the bioactive compounds which are presented in *Vitex negundo* a traditionally used medicinal plant. Leaves of *Vitex negundo* were subjected to microwave-assisted extraction and also for the solvent free microwave-assisted hydro-diffuion method respectively. In comparison with other conventional extraction techniques, the microwave assisted extraction is most effective, sophisticated and promising approach to obtain the highest yields of extractives (33.3%) and essential oil (13 mL/75 gm of fresh plant). The quantitative estimation of total phenolic content (TPC) and the total flavonoid content (TFC) were determined by using Folin-Ciocalteau assay and Aluminium Chloride assay respectively. Results showed the presence of significant amount of TPC of methanolic extract (1.6892mg/gm) and essential oil (1.6625mg/gm); and TFC of methanolic extract (1.07mg/gm) and essential oil (0.515mg/gm) respectively. In-vitro antioxidant assay was determined by using DPPH radical scavenging activity. IC50 value of extract and essential oil was found to be 90µg/mL and 300µg/mL respectively. Presence of ample amounts of phenolic and flavonoid compounds could be responsible for its antioxidant activity. This plant can be developed as a drug candidate in the future for the treatment and management of different ailments.

Anindita kundu
15/05/2024