Role of Sonneratia Apetala Against Ehrlich Ascites Carcinoma Through In-vitro and In-vivo Approaches

Thesis Submitted for the Partial Fulfillment of the Degree of Master of Pharmaceutical Technology Faculty of Engineering & Technology
Jadavpur University
2024

BY

Agnipravo Naskar
Exam Roll No- M4PHP24009
Reg.No- 163688 of 2022-23
Class Roll No- 002211402047

Under the guidance of
Prof. Pallab Kanti Haldar
Professor

Deparment of Pharmaceutical Technology
Jadavpur University
Kolkata- 700032
India

CERTIFICATE

This is to certify that the thesis entitled **"ROLE OF *SONNERATIA APETALA* AGAINST EHRLICH ASCITES CARCINOMA CELL THROUGH *IN-VITRO* AND *IN-VIVO* APPROACHES"** submitted by **Agnipravo Naskar,** with registration in the year 2022-2023 for the partial fulfillment of degree of Master of Pharmaceutical Technology, Jadavpur University, is absolutely based upon her own research project work under my supervision. Her thesis has not been submitted before for any degree/diploma or any other academic award elsewhere.

Supervised by:

Prof. Pallab Kanti Haldar
M.Pharm, Ph.D, FIC
Professor
Deparment of Pharmaceutical Technology
Jadavpur University

Forwarded by

Prof. Amalesh Samanta
Head of the Department
Professor
Deparment of Pharmaceutical Technology
Jadavpur University

Prof. Dipak Laha

Dean
Faculty of Engineering &Tenchnology
Jadavpur University
Kolkata- 700 032

ACKNOWLEDGEMENT

I am grateful to the almighty to bless me with such an excellent opportunity to fulfill my dreams of pursuing my degree in the best possible way. I want to express my gratitude towards my respected guide Prof. Pallab Kanti Haldar, for his valuable advice, untiring support and motivation. I am equally grateful to our head of the department Prof. Amalesh Samanta, for allowing me to carry out this project smoothly and providing all the possible facilities. Funds from AICTE (GPAT 2022) have also proved to be of much help. I want to thank my family for having faith in me and encouraging me throughout my career. Last but not the least, this project would have been impossible without the support, advice and help of my seniors Ms. Suparna Ghosh, Mr. Barun Dasgupta, Mr. Sandipan Jana, Mr. Srijan Gayen, Mrs. Soma Chowdhury.I also thankful my labmates Ms. Monihar Pervin, and Ms. Anirbita Ghosh.

Date:

Agnipravo Naskar

Department of Pharmaceutical Technology
Jadavpur University
Kolkata- 700032

Dedicated to my Family

&

Mentor

PREFACE

The present thesis entitled **"Role of *Sonneratia apetala* Buch.-Ham. Against Ehrlich Ascites Carcinoma through *in-vitro* and *in-vivo* approaches"** comprises the work done by the author in Division of Pharmacology, Department of Pharmaceutical Technology, Jadavpur University, Kolkata, for the degree of Master of Pharmaceutical Technology. The immense wealth of the plant kingdom has become a target forthe search of new drugs and lead compounds by drug companies. Their easy availability, low toxicity, lesser or almost minimal side effects has prompted us to apply medicinal plants in therapeutic management of different diseases. The traditional uses need scientific background for proper value and so they are currently an important part of research. Thus the thesis covered the abovementioned study in a logical sequence with other factors related to the study. In conclusion, the detailed study has been linked up in a manner to justify the relation of the work to establish the pharmacological action of the *Sonneratia apetala*, especially anti-cancer activity.

Date: **Agnipravo Naskar**

CONTENT

CHAPTER 1: INTRODUCTION

1. BACKGROUND OF THE STUDY

Despite significant advancements in diagnosis and treatment, cancer remains a formidable threat to our society (Kotnis et al., 2005). This is the second most common disease after cardiovascular disorders for maximum deaths in the world (Jemal et al., 2007). Cancer is a multi-gene, multi-step disease originating from a single abnormal cell with an altered DNA sequence. It is characterized by uncontrolled cellular growth, local tissue invasion and distant metastatis (Dashora et al., 2010). The four characteristics of cancer cells that distinguish them from normal cells are uncontrolled proliferation, de-differentiation and loss of function, invasiveness, and metastasis. A normal cell turns into cancer cell because of one or more mutations in its DNA, which can be acquired or inherited. These altered cells divide uncontrollably to form lumps or masses of tissue called tumors. Another hallmark of the disease is resistance to apoptosis (programmed cell death, either by inactivation of pro-apoptotic factors or by activation of anti-apoptotic factors).

A tumor that is not capable of indefinite growth and does not invade the healthy surrounding tissue extensively is benign. A tumor that continues to grow and becomes progressively invasive is malignant; the term cancer refers specifically to a malignant tumor. Beyond uncontrolled growth, malignant tumors exhibit metastasis. During this process, small clusters of cancerous cells—originating from the initial or primary tumor—are released. These cells travel through blood vessels and lymphatics, reaching other sites in the body where they continue to proliferate, forming secondary tumors. Chemotherapy is now considered as the most effective method of cancer treatment. Unfortunately, currently available cancer chemotherapeutic agents insidiously affect the host cells, especially bone marrow, epithelial tissues, gonads and the reticula-endothelial system (Mascarenhas et al., 1994). A major challenge for medical oncology is to develop therapeutic modalities that will prevent toxicity induced by anticancer treatments without impairing their pharmacological effects (Das et al., 2012).

Natural products are considered to have exceptional value in the development of effective anticancer drugs with minimal host cell toxicity and high antioxidant and eradication potential (Gupta et al., 2007).

Researchers have recently focused on the use of Ehrlich's ascites carcinoma (EAC) cells in the investigation of plants effect to cure cancer. The Ehrlich tumor was initially described as a spontaneous murine mammary adenocarcinoma. It is a rapidly growing carcinoma with very

aggressive behavior and can grow in almost all strains of mice. In the ascitic form, it has been used as a transplantable tumor model to investigate the antitumor effects of several substances (Segura et al., 2000).

1.1. Statistics:

There are an estimated 14.1 million cancer cases around the world in 2024, out of these 7.4 million cases are in men and 6.7 million in women. The numbers of deaths are 8.2 million in 2024. (American Cancer Society, 2024; Global Cancer Statistics, 2024.) This number is expected to be increased to 24 million by 2035 (Torre et al., 2012). According to the statistics the deaths occurred for different cancers are:

i. Lung cancer (1.6 million deaths)
ii. Liver cancer (7,45,500 deaths)
iii. Stomach cancer (7,23,100 deaths)
iv. Colorectal cancer (6,93,900 deaths)
v. Breast cancer (5,21,900 deaths)
vi. Esophageal cancer (4,00,200 deaths)
vii. Cervical cancer (2,65,700 deaths)
viii. Non-Hodgkin lymphoma cancer (1,99,700 deaths)
ix. Urinary bladder cancer (1,65,100 deaths)
x. Cancer of the lip and oral cavity (1,45,400 deaths)
xi. Nasopharyngeal cancer (50,800 deaths)

1.2. Classification of Cancers by Cellular Origin and Tissue Type:

Cancers are classified by the type of cell that the tumor cells resemble and are therefore presumed to be the origin of the tumor (WHO classifications of Tumors Editorial Board, 2020). These types include:

i. **Carcinoma:** Cancers derived from epithelial cells. This group includes most of the common cancers, particularly in the aged person and nearly all those developing in the breast, prostate, lung, pancreas and colon.
ii. **Sarcoma:** Cancers arising from connective tissues (i.e. bone, cartilage, fat, nerve), each of which develops from cells originating in mesenchyma outside the bone marrow.

iii. **Lymphoma and leukemia:** These two classes of cancers arise from hematopoietic (blood-forming) cells that leave the marrow and tend to mature in the lymph nodesand blood, respectively. Leukemia is the most common type of cancer in childrenaccounting for about 30%.

iv. **Germ cell tumor:** Cancers derived from pluripotent cells, most often presenting in the testicle or the ovary (seminoma and dysgerminoma, respectively).

v. **Blastoma:** Cancers derived from immature "precursor" cells or embryonic tissue. Blastomas are more common in children than in older adults.

1.3. Etiology of Cancer:

Cancer is ultimately the result of cells that uncontrollably grow and do not die. Normal cells in the body follow an orderly path of growth, division and death. Programmed cell death is called apoptosis and when this process breaks down, cancer begins to form. Unlike regular cells, cancer cells do not experience programmatic death and instead continue to grow and divide. This leads to a mass of abnormal cells that grows out of control. It is nearly impossible to prove what caused a cancer in any individual, because most cancers have multiple possible causes. The great majority of cancers (90 - 95% of cases), are due to environmental factors. The remaining 5 - 10% is due to inherited genetics.

1.3.1. Environmental factors:

In the context of cancer research, ‘environmental’ encompasses various factors, including lifestyle, economic conditions, and behavioral choices—not limited solely to pollution. Common environmental factors that contribute to cancer death include tobacco (25 - 30%), diet and obesity (30 - 35%), infections (15 - 20%), ionizing and non-ionizing radiation (10%), stress, lack of physical activity and environmental pollutants (National Cancer Institute, 2022).

1.3.2. Hereditary and genetic factors:

Some childhood cancers may be influenced by genetic factors. Additionally, it is possible for various forms of cancer to occur more than once within a family. Less than 0.3% of the populations are carriers of a genetic mutation that has a large effect on cancer risk and these causes less than 3 - 10% of all cancer. Cells can experience uncontrolled growth if there are mutations to DNA and therefore, alterations to the genes involved in cell division. Four key types of gene are responsible for the cell division process: oncogenes give signal to the cells when to divide, tumor suppressor genes instruct cells when not to divide, suicide genes control

apoptosis and instruct the cell to kill itself if something goes wrong and DNA-repair genes instruct a cell to repair damaged DNA (Larsen and Minna, 2011).

Cancer occurs when a cell's gene mutations make the cell unable to correct DNA damage and unable to commit suicide. Similarly, cancer is a result of mutations that inhibit oncogene and tumor suppressor gene function, leading to uncontrollable cell growth. Certain inherited mutations in the genes BRCAl (Breast cancer 1) and BRCA2 (Breast cancer 2) with more than 75% risk of breast cancer and ovarian cancer (Chen et al., 2011).

1.3.3. The six hallmarks of cancer: DNA mutations result in defects in the regulatory path of a cell, which disrupt normal cell proliferation behavior (Flora et al., 1996). Individual cell behavior is not autonomous and it usually relies on external signals from surrounding cells in the tissue or microenvironment (Luca et al., 2003), which causes different types specific changes of normal cells. The common changes occur in cell physiology that results in cancer can be described as follows:

i. **Immortality:** Continuous cell division and limitless replication: Normal cells have a certain lifespan. Cancer cells manipulate the cell to keep dividing indefinitely by producing proteins that enable them to do so (Cheng et al., 2004).

ii. **Produce 'Go' signals (growth factors from oncogenes):** Most cells wait for a 'Go' signal before dividing but cancer cells don't bother waiting, they produce their own chemical message and continue dividing (Liou et al., 2011).

iii. **Override 'Stop' signal (anti-growth signals from tumor suppressor genes):** Even if neighboring cells produce a 'Stop' signal, cancer cells override these signals and continue dividing (Sluis et al., 1994).

iv. **Resistance to cell death (apoptosis):** Normal cells sometimes react to stress by triggering a 'Self Destruct' button and killing itself, but cancer cells sneak past these signals and continue divide.

v. **Angiogenesis:** Cancer cells make sure they can keep growing by stimulating the developing of new blood vessels to keep their nutrient supply lines open.

vi. **Metastasis:** The final stage in tumor progression is the migration and spread of cancers to different sites from where they originated.

Table 1.1:Tumor Suppressor Genes, Protein Functions, and Associated Neoplasms

Tumor suppressor gene	Protein function	Neoplasm(s)
APC (adenomatous polyposis coli)	Cell adhesion	Colon
BRCA1 (Breast cancer 1)	Transcription factor	Ovary and breast
BRCA 2 (Breast cancer 2)	DNA repair	Ovary and breast
CDK4 (cyclin-dependent kinase 4)	Cyclin D Kinase	Melanoma
NF1 (neurofibromin 1)	GTPase	Neuroblastoma
P53 (tumor protein p53)	Transcription factor	Lung, colon, breast
Rb (retinoblastoma protein)	Cell cycle check point	Retinoblastoma
WT1 (Wilms tumor 1)	Transcription factor	Childhood kidney

1.3.4. Carcinogens:

Carcinogens are a class of substances that are directly responsible for damaging DNA, promoting or aiding cancer. Tobacco, asbestos, arsenic, radiation such as gamma and x-rays, the sun and compounds in car exhaust fumes are all examples of carcinogens. When our bodies are exposed to carcinogens, free radicals are formed that try to steal electrons from other molecules in the body. These free radicals damage cells and affect their ability to function normally (Agboola et al., 2024).

1.3.5. Infections:

A virus that can cause cancer is called an oncovirus. These include human papilloma virus (cervical carcinoma), Epstein-Barr virus (B-cell lymphoproliferative disease and nasopharyngeal carcinoma), Kaposi's sarcoma herpes virus (Kaposi's sarcoma and primary effusion lymphomas), hepatitis B and hepatitis C viruses (hepatocellular carcinoma) and human T-cell leukemia virus-1 (T-cell leukemias). Bacterial infection may also increase the risk of cancer, as seen in Helicobacter pylori-induced gastric carcinoma. Epstein-Barr virus and HIV, the virus that causes AIDS, have been linked to an increased risk of developing certain childhood cancers, such as Hodgkin and non-Hodgkin lymphoma. Possibly, the virus alters a cell in some way. That cell then reproduces an altered cell and eventually, these alterations become a cancer cell that reproduces more cancer cells. (Raja et al., 2023).

1.3.6. Hormones:

Some hormones play a role in the development of cancer by promoting cell proliferation.Insulin-like growth factors and their binding proteins play a key role in cancer cell proliferation, differentiation and apoptosis, suggesting possible involvement in carcinogenesis. Hormones are important agents in breast, endometrium, prostate, ovary, testis, and thyroid and bone cancer. For example, the daughters of women who have breast cancer have significantly higher levels of estrogen and progesterone than the daughters of women without breast cancer. These higher hormone levels may explain why these women have higher risk of breast cancer, even in the absence of a breast-cancer gene (Boguszewski et al., 2016).

1.3.7. Environmental exposures:

Research has investigated a potential direct link between childhood cancers and exposure to pesticides, fertilizers, and power lines. Some forms of high-dose chemotherapy and radiation. In some cases, children who have been exposed to these agents may develop a second malignancy later in life. These strong anticancer agents can alter cells and/or the immune system. A second malignancy is a cancer that appears as a result from treatment of a different cancer (Newby et al., 2005).

1.4. Pathophysiology:

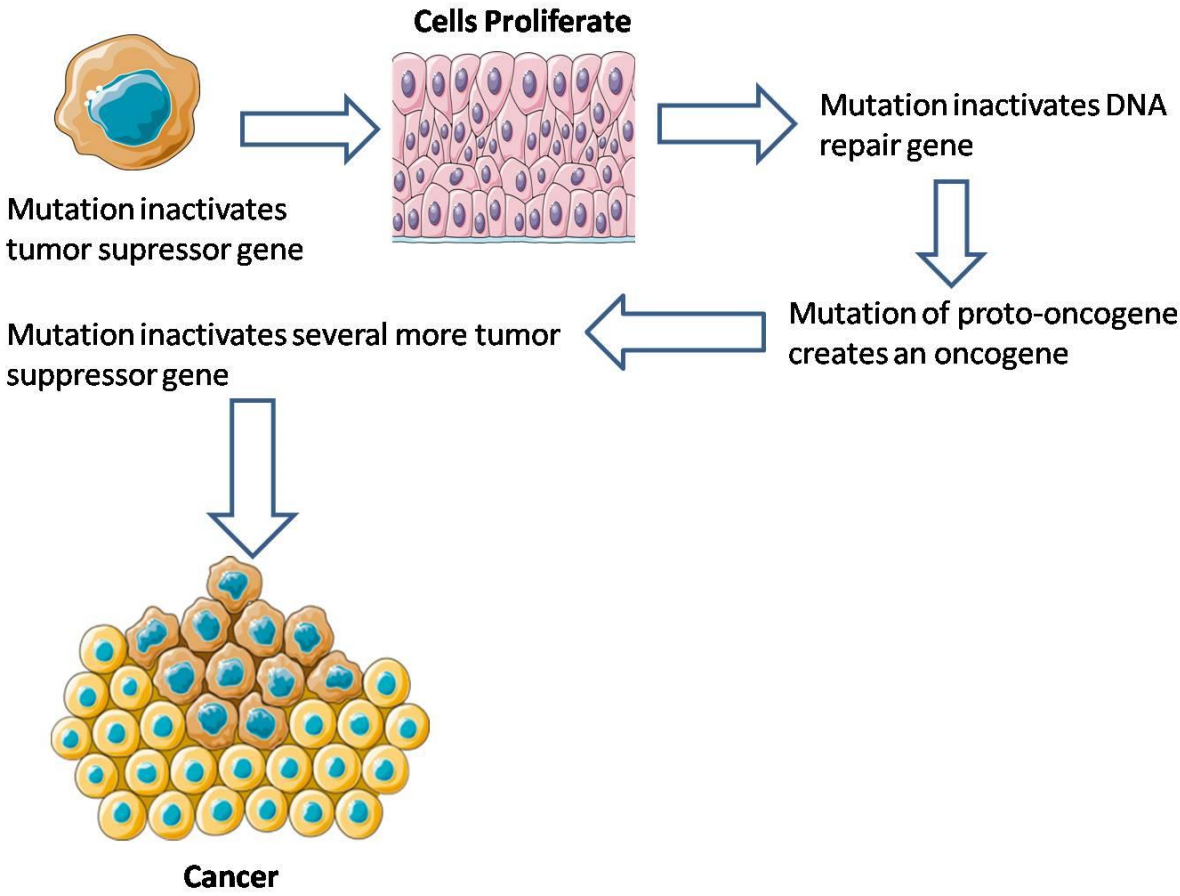


Figure 1.1: Causes of cancers (mutations)

Cancer, characterized by uncontrolled growth of abnormal cells, arises due to changes in the DNA within cells. Here's a brief overview of its pathophysiology:

Cellular Changes:

Normally, cells produce signals to regulate their division. If these signals are faulty or missing, cells may grow excessively and form a tumor.

Mutation in the genetic apparatus of cells plays a central role. This includes disturbances in gene expression, activation of oncogenes (genes promoting tumor growth), and inactivation of tumor suppressor genes. Damage to the genetic apparatus, along with anti-tumor gene inactivation, is essential for malignant tumor development.

1.4.1. Metastasis:

Cancer cells can break away from the original tumor and spread through the blood or lymph system to distant locations, forming additional tumors. Metastasis is a critical step in cancer

progression.In summary, cancer results from DNA changes that disrupt normal cell growth and lead to uncontrolled cell division and tumor formation.(Popper H et al., 2016).

1.5. The cell cycle and cancer:

The connection between the cell cycle and cancer is obvious: cell cycle machinery controls cell proliferation, and cancer is a disease of inappropriate cell proliferation. Fundamentally, all cancers permit the existence of too many cells. However, this excess cell number is linked in a vicious cycle with a reduction in sensitivity to signals that normally instruct a cell to adhere, differentiate, or die. This combination of altered properties increases the difficulty of deciphering which changes are primarily responsible for causing cancer. The hallmark of cancer is deranged growth control (Pardee et al., 1978). Checkpoints are defective in cancer cells (Hartwell and Kastan, 1994). Control mechanisms are usually lost by mutation, for example many cancers lose or have mutated p53 genes, or have alterations in some components of the Rb pathway. In addition, carcinogenic viruses such as SV40 produce proteins, such as T-antigen, which bypass G1/S control and transform cells, mainly by eliminating p53 and pRb (Sherr et.al., 1996). Defective checkpoint mechanisms in cancers, as well as defective DNA repair, produce further chromosomal aberrations and genomic instability (Nojima et.al., 1997). Thus checkpoint controls limit the appearance of genetic variants and the progression of cancer. The first genetic alterations shown to contribute to cancer development were gain-of-function mutations. These mutations define a set of oncogenes that are mutant versions of normal cellular proto-oncogenes. The products of proto-oncogenes function in signal transduction pathways that promote cell proliferation. However, transformation by individual oncogenes can be redundant (mutation of one of several genes will lead to transformation) or can be cell type-specific (mutations will transform some cells but have no effect on others). This suggests that multiple, distinct pathways of genetic alteration lead to cancer. Cancer is caused by alterations in oncogenes, tumor-suppressor genes and microRNA genes. A single genetic change is rarely sufficient for the development of a malignant tumor. Most evidence points to a multistep process of sequential alterations in several, often many oncogenes, tumor-suppressor genes, or microRNA genes in cancer cells. Historically, transformation events in cancer have been defined as initiation events (contributing to the early stages of neoplastic transition) or progression events (referring to subsequent transformative processes). Ontogenesis encodes proteins that control cell proliferation, apoptosis, or both. These oncogenes can become activated through structural

alterations resulting from mutations or gene fusions (Konopka et al., 1985), by being juxtaposed to enhancer elements (Tsujimoto et al., 1985), or through amplification. Translocations and mutations may occur as initiating events (Finger et al., 1986), while amplification typically occurs during tumor progression (Carlo et al., 2008).

1.5.1. Cancer Cell Cycle Phases:

To understand cancer mechanisms we have to first understand the cell cycle and how it is related to cancer.

The term cell cycle refers to the sequence of events that take place within a cell as it tools up for division. The phases of the cell cycle are:

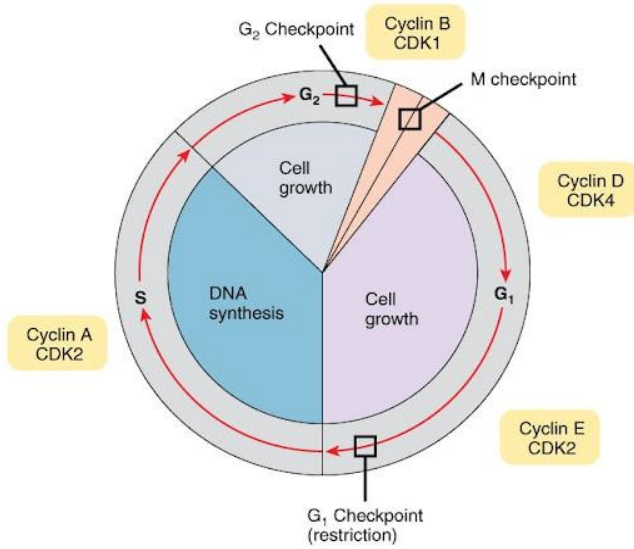


Figure 1.2: G_1, S, G_2 and M phase

M is the phase of mitosis.

S is the phase of DNA synthesis.

G, is the gap between the mitosis that gave rise to the cell and the S phase; during G1, the cell is preparing for DNA synthesis.

G2 is the gap between S phase and the mitosis that will give rise to two daughter cells; during G2, the cell is preparing for the mitotic division into two daughter cells.

Finally, there is a fifth state, G_0 (also known as quiescence) into which the cell may reversibly exit from G1, if it is deprived of the appropriate growth-promoting signals (Garrett et al., 2001).

Growth factor action stimulates a quiescent cell, often referred to as being in the G_0 (G-nought) phase to divide, i.e. to start on G phase.

1.5.2. Cell cycle checkpoint:

Cell cycle checkpoint is a mechanism that maintains the observed order of events of each cell cycle (Hartwell and Weinert, 1989). Major function of these checkpoints is to see that the integrity of the genome remains intact throughout the cell cycle. Cell division requires the controlled timing of two critical events of the cell cycle: S phase (DNA replication) and M phase (mitosis). Entry into each of these phases is carefully regulated, and there are thus two 'check points' (restriction points) in the cycle: one at the start of S and one at the start of M. DNA damage results in the cycle being stopped at one or other of these. The integrity of the check points is critical for the maintenance of genetic stability and failure of the check points to stop the cycle when it is appropriate to do so is a hallmark of cancer. The first of these occurs at the G1/S phase transition and is a major sensor of DNA damage. The cell may also arrest later in S phase due to incomplete DNA replication or again, damage to the DNA. Next is the G2/M checkpoint, which monitors the fidelity of DNA replication and like the G1/S checkpoint is an important sensor of DNA damage.

1.5.3.Positive regulator of cell cycle:

The cycle is initiated when a growth factor acts on a quiescent cell, provoking it to divide. One of the main actions of a growth factor is to stimulate production of the cell cycle regulators, which are coded for by the delayed response genes. The main components of the control system that determine progress through the cycle are two families of proteins: cyclins and cyclin-dependent kinases (CDKs) (Raleigh et al., 2000).

The heart of the regulatory apparatus during the cell cycle progression is a family of enzymes, called the cyclin dependent kinases. The active forms of CDKs are a complex of at least two proteins, a kinase and a cyclin. They often contain other proteins with poorly understood functions. These complexes undergo change in the kinase and cyclin components that are believed to drive the cell from one stage of the cell cycle to another (Pardee et al., 1989). According to this paradigm, the cell cycle is determined by the constellation of proteins that are activated or inactivated by phosphorylation, a result of the activity of the CDKs during that stage. In mammalian cells, a succession of kinase Subunits (CDK4, CDK6, CDK2, and CDC2) is

expressed along with a succession of cyclins (cyclin D, E, A, and B), as the cells progress from G1 to mitosis (Xiong et al., 1991) CDK4 and CDK6 complexed with one of several D-type cyclins functions early in the G1 phase, probably in response to growth factors. CDK2 that complexed with cyclin E, cyclin A, or both is essential for the Gl to S transition and DNA replication, respectively. CDC2 that complexed with cyclin A and cyclin B is essential for mitosis. Each cdk is inactive until it binds to a cyclin, the binding enabling the cdk to phosphorylate the proteins necessary for a particular step in the cycle. It is the cyclin that determines which proteins are phosphorylated. After the phosphorylation event has taken place, the cyclin is degraded by the ubiquitin/ protease system. (Sherr et al., 1993)

In quiescent Go cells, cyclin D is present in low concentration, and an important regulatory protein-the Rb protein is hypophosphorylated. HypophosphorylatedRb holds the cell cycle in check at check point 1 by inhibiting the expression of several proteins critical for cell cycle progression. The Rb protein accomplishes this by binding to the E2F transcription factors, which control the expression of the genes that code for cyclins E and A, for DNA polymerase, for thymidine kinase, for dihydrofolate reductase, etc. all essential for DNA replication during S phase. During G1, the concentration of cyclin D increases and the cyclin D/ck complex phosphorylates and activates the necessary proteins. In mid-G1, the cyclin D/edk complex phosphorylates the Rb protein, releasing transcription factor ELF; this then activates the genes for the components specified above that are essential for the next phase-DNA synthesis-namely cyclins E and A, DNA polymerase and so on (Morgan et al., 1995)

Cyclin E/cdk and cyclin A/edk regulate progress through S phase, phosphorylating and thus activating proteins/enzymes involved in DNA synthesis. (Hwang et al., 1998)

Cyclin A/edk and cyclin B/ck complexes are active during G2 phase and are necessary for entry into M phase, i.e. for passing check point 2. The presence of cyclin B/cdk complexes in the nucleus is required for mitosis to commence (Zhan et al., 1999).

1.5.4.Negative regulator of cell cycle:

There are two families of inhibitors:

• The CIP family (cd inhibitory proteins, also termed KIP or kinase inhibitory proteins)-p21, p27 and p57.

• The Ink family (inhibitors of kinases)-p16, p19 and p15.

The p53 gene has been called the 'guardian of the genome. It codes for a protein transcription factor-the p53 protein. In normal healthy cells, the steady-state concentration of the p53 protein is low. But when there is DNA damage, the protein accumulates and activates the transcription of several genes, one of which codes for p21. Protein p21 inactivates cyclin/edk complexes, thus preventing Rb phosphorylation, which means that the cycle is arrested at check point 1. This allows for DNA repair. If the repair is successful, the cycle proceeds past check point 1 into S phase. If the repair is unsuccessful, the p53 gene triggers apoptosis-cell suicide.

1.6. Current treatment available to treat cancer:

Many treatment options for cancer exist, with the primary ones including surgery, chemotherapy, radiation therapy, hormonal therapy, targeted therapy and palliative care. Which treatments are used depends on the type, location and grade of the cancer as well as the person's health and wishes. The treatment intent may be curative or not curative. Some of the treatments are described below:

1.6.1. Chemotherapy:

Chemotherapy is the treatment of cancer with one or more cytotoxic anti-neoplastic drugs (chemotherapeutic agents) as part of a standardized regimen. The term encompasses any of a large variety of different anticancer drugs, which are divided into broad categories such as alkylating agents and antimetabolites. Traditional chemotherapeutic agents act by killing cells that divide rapidly, one of the main properties of most cancer cells (Krishna et al., 2009).

1.6.2. Radiation:

Radiation therapy involves the use of ionizing radiation in an attempt to either cure or improve the symptoms of cancer. It works by damaging the DNA of cancerous tissue leading to cellular death (Jaffray et al., 2015).

1.6.3. Surgery:

Surgery is the primary method of treatment of most isolated solid cancers and may play a role in palliation and prolongation of survival (Wang et al., 2019).

1.6.4. Hormonal therapy:

Many hormonal antitumor agents are functional agonist or antagonist of the steroid hormone family. Adrenocorticoids, antiandrogen, estrogen, antiestrogen, progestins, aromatase inhibitor, gonadotropin-releasing hormone agonists, somatostatin analogues etc (Mahadik et al., 2022).

1.6.5. Biological therapy:

• Immunotherapy: Cytokines, Cellular therapy, Tumor vaccine.

• Hematopoietic growth factors (Capitini et al., 2009).

1.7. Nature as a source of anticancer drugs:

Natural products from plant, animal and minerals have been the basis of the treatment of various human diseases. Current estimate explains that about 80% of people in developing countries still depend on traditional medicine based largely on species of plants and animals for their primary health care. The use of herbal medicine is becoming popular due to the high toxicity and side effects of allopathic medicines. The popularity of herbal medicine has been increasing day by day. There are many traditional systems of medicine in the world, each with different associated philosophies and cultural origins. Some of these, such as Tibetan traditional medicine, remain relatively localized in their country of origin; while others such as Ayurvedic and Chinese traditional medicines are increasingly used in many different areas of the world. The various indigenous systems such as Siddha, Ayurveda, Unani and Allopathy used several plant species to treat different diseases (Rabe and Staden, 1997). In India around 20,000 medicinal plant species have been recorded. India has one of the richest traditions of plant based therapy in the world. There are estimates, to be around 25,000 effective plant based formulations, used in folk medicine and known to the rural communities in India (Kamboj et al., 2000). In the last century, roughly 121 pharmaceutical products were formulated based on the traditional knowledge obtained from various sources (Verma and Singh, 2008). The use of traditional medicine has been increasing in developed countries also, mainly due to the failure of modern medicine to provide effective treatment for chronic diseases and emergence of multi-drug resistant bacteria and parasites. The adverse effects of chemical drugs, questioning of the approaches and assumptions of allopathic medicine, their increasing costs and greater public access to information on traditional medicine has also led to an increase in interest in alternative treatments. Plants have a long history of use in the treatment of cancer and it is significant that over 60% of currently used anti-cancer agents have come from natural sources. Naturally occurring drugs that are part of the war against cancer include vinca alkaloids (vincristine, vinblastine, vindesine and vinorelbine), taxanes (paclitaxel, docetaxel), podophyllotoxin and its derivative (etoposide, teniposide), camptothecin and its derivatives (topothecan, irinothecan), anthracyclines (doxorubicin, daunorubicin, epirubicin, idarubicin) and others. The approved anti-

cancer agents is doxorubicin (2002), estradiol (2004), cholorophyll and 1- aspartic acid and taxol nanoparticles (2005) (Butler et al., 2005). Three new drugs also introduced in 2007 originate from microbial sources for the treatment of cancer is marine alkaloid trabectedin, epothilone derivative ixabepilone and temsirolimus (Bailly et al., 2009). Nature is an attractive source of new therapeutic candidate compounds as a tremendous chemical diversity is found in millions of species of plants, animals, marine organisms and microorganisms as potential anti-cancer agent (Newman et al., 2003).

1.7.1. Plants used for cancer treatment:

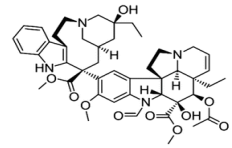
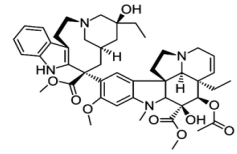
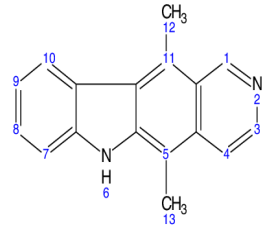
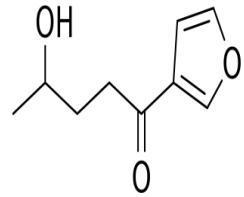
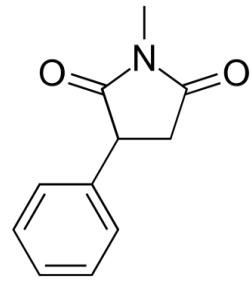
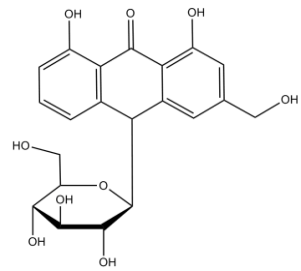
India is the largest producer of medicinal plants and is rightly called the "Botanical garden of the World". The history of plant as source of anti-cancer agents started in the 1950s with the discovery and development of the vinca alkaloids (vinblastine and vincristine) and the isolation of the cytotoxic podophyllotoxins. Vinca alkaloid was responsible for an increase in the cure rates for Hodgkin's disease and some forms of leukemia (Devita et al.,1970). Vincristine inhibits microtubule assembly, inducing tubulin self-association into coiled spiral aggregates (Noble RL.,1990). Etoposide is an epipodophyllotoxin, derived from the mandrake plant *Podophyllum peltatum* and the wild chervil *Podophyllum emodi* (Stahelin et al.,1973). It has also significant activity against small-cell lung carcinoma (Harvey A et al.,1999). Etoposide is a topoisomerase II inhibitor, stabilizing enzyme-DNA cleavable complexes leading to DNA breaks (Liu et al., 1989). The paclitaxel and docetaxel has been shown antitumor activity against breast, ovarian and other tumor types in the clinical trial. Paclitaxel stabilizes microtubules and leading to mitotic arrest (Wani et al., 1971). In addition, the camptothecin derivatives irinotecan and topotecan have shown significant antitumor activity against colorectal and ovarian cancer respectively (Creemers et al.,1996). These compounds were initially obtained from the bark and wood of *Nyssacea Camptotheca accuminata* and act by inhibiting topoisomerase I (Liu et al., 2000). The taxanes and the camptothecins are presently approved for human use in various countries. Rohitukine the plant alkaloid, isolated from the leaves and stems of *Dysoxy lumbinectariferum* (Maliaceae) (Harmon et al., 1979). Synthetic flavone derived from rohitukine, Flavopiridol representing the first cyclin-dependent kinase inhibitor to enter the clinical trial (Losiewicz et al., 1994). The mechanism of action involves interfering with the phosphorylation of cyclin-dependent kinases and arrest cell-cycle progression at growth phase G1 or G2 (Worland et al., 1993). Homoharringtonine an alkaloid isolated from the Chinese tree

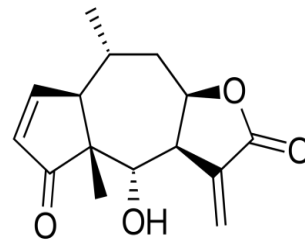
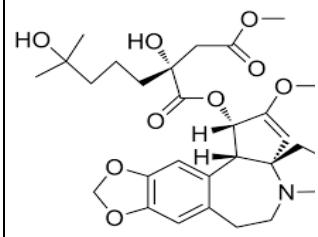
Cephalotaxus harringtonia (Cephalotaxaca) (Powell et al., 1970). The mechanism of action is the inhibition of protein synthesis and blocking cell-cycle progression (Zhou et al., 1995). It has shown efficacy against various leukemias (Kantarjian et al., 1996), A lung-cancer-specific antineoplastic agent 4-Ipomeanol is isolated from the sweet potato *Ipomoea batata* (Convolvulaceae) (Rowinsky et al., 1993). The mechanism of action is converted into DNA-binding metabolites upon metabolic activation by cytochrome P450 enzymes that are present in cells of the lung (Rehm et al., 1993). DNA topoisomerase I inhibitor B-lapachone, that induces cell-cycle delay at Gl or S (synthesis) phase before inducing either apoptotic or necrotic cell death in a variety of human carcinoma cells, including ovary, colon, lung, prostate and breast (Li el al., 1999). Various types of anti-cancer plant are Zedoary (*Curcuma zedoaria*), Rodent Tuber (*Typhoniumflagelliforme*), God's Crown (*Phaleriamacrocarpa*), Madagaskar Periwinkle (*Catharanthusrosens*), Artocarpus Integer (*Selaginellacorymbosa*), Bamboo Grass (*Loathatreum Gracies*) , handsome (*Taraxacummongolicum*), fruit makasar (*Bruccajavanica*), Garlic (*Allium sativum)*, Echo China (*Smilax china*), Sunflower (*Helianthus annus*), Leunca (*Solanum nigrum*), Job's Tears (*CoixLachryma-Jobi*), Bamboo Rope (*Asparagus cochinchinensis*) and others.

1.8. Dietary source of anti-cancer agents:

Natural dietary agents including fruits, vegetables and spices have drawn a great deal of attention from both the scientific community and the general public owing to their demonstrated ability to suppress cancers.

Table 1.2: Anticancer Bioactive Compounds from Various Plant Sources

Plant Name	Family	Anticancer bioactive compound	Structure	Reference
Catharanthus roseus	Apocynaceae	vinblastine and vincristine	 A.Vincristine  B.Vinblastine	Devita et.al.,1970
Podophyllum Peltatum	Berberidaceae	ellipticine		Prabhadevi et.al.,2012
Ipomoea batatas	Convolvulaceae	4-ipomeanol		Rowinsky et. al.,1993
Penstemon deutus	Scrophulariaceae	Phensuximide		Jolad et.al.,1976
Aloe Vera	Liliaceae	Aloin		Majumder et.al.,2019

Helenium autumnale	Compositae	Helenalin		Li et.al.,1999
Cephalotaxusharringtonia	Cephalotaxaca	Homoharringtonine		Powell et.al.,1970

Natural products discovered from medicinal plants have played an important role in the treatment of cancer. Natural products or natural product derivatives comprised 14 of the top 35 drugs in 2000 based on worldwide sales (Butlet et al., 2004). Two plant derived natural products, paclitaxel and camptothecin were estimated to account for nearly one-third of the global anticancer market or about $3 billion of $9 billion in total annually in 2002 (Oberlines and Kroll, 2004). There are more than 270,000 higher plants existing on this planet. But only a small portion has been explored phytochemical. It is anticipated that plants can provide potential bioactive compounds for the development of new leads to combat cancer diseases.

1.9. Mangrove as a source of anticancer agents:

i. Sundarban: The Sundarban, a World Heritage Site recognised by UNESCO, is the biggest single tract of productive mangrove forest ecosystems in the world, measuring over 10,000 km^2 and 60% of which is found in Bangladesh and 40% in India (Pramanik et. al., 2019; Chanda et. al., 2016). One of the world's delicate and complex ecosystems is said to be the Sundarbans. It has a wide variety of flora, including 13 types of orchids, 165 algae, and 334 plant species that are separated into 245 groups and 75 families (Prain et al., 1903). Furthermore, it has a diverse range of wildlife. 210 species of white fish, 24 species of prawns, 14 species of crabs, 43 species of mollusks, 49 species of mammals, 59 species of reptiles, 8 species of amphibians, and almost 260 species of birds include them. Many rare and endangered species of animals may be discovered in this area, including the Javan rhino, the Indian python, the Royal Bengal tiger (Panthera tigris L.),

wild buffalo, hog deer, and barking deer. The estuary crocodile, the deer, and the Irrawaddy dolphin (Banglapedia et al., 2021; Gopal and Chauhan, 2006). Nearly all of the Sundarbans' vegetation and wildlife are at risk by climate change, particularly due to the rise in sea level (Gopal and Chauhan, 2006; Titumir et al., 2020). Modification silt and elevated salinity seriously harm diversity. Furthermore, plants and animals lose their sources of food and shelter as a result of forestry (Gopal and Chauhan, 2006). Many researchers have already carried out ethnobotanical research on diverse ethnic and tribal people living in distinct places across Bangladesh. The Rakhine tribal groups in Patuakhali and Barguna District (Islam et. al., 2020); the Pangkhua ethnic community in BilaichariUpazilla of Rangamati District (Faruque et al., 2019); the Chakma, Marma, and Tanchayanga indigenous communities in the Bandarban District (Faruque et. al., 2018); the Chakma, Marma, Tanchanga, Tripura, Pankua, Murang, and Lushi indigenous and tribal people living in Rangamati District (Kadir et. al., 2012); the Garos and Hajongs communities in the Garo Hills of Durgapur sub-district under Netrakona District (Khan et. al., 2015); and the Garo, Koch and Hajong tribal communities and native people living in Madhupur forest (Islam et. al., 2014). A few ethnobotanical studies have also been carried out by a number of researchers in the Sundarbans (Ahmed et. al., 2016; Mollik et. al., 2009) and the surrounding areas (Ray et. al., 2021; Ray and Mondal, 2018; Dulla and Jahan, 2017). Nevertheless, none of the research examined the medical benefits of mangrove plants or the plants consumed by the forest community, who live near the Sundarbans. Therefore, the Sundarban forest peoples' ethnobotanical survey has remained completely untouched.

ii. Reason to choose sundarban plants: Mangrove plants have gained attention in anticancer research due to their unique properties and rich phytochemical content. Here are some reasons why mangroves are chosen for such studies:

- Biodiversity and Adaptation:
  - Mangroves thrive in harsh coastal environments, adapting to high salinity, tidal fluctuations, and nutrient limitations.
  - Their ability to produce diverse secondary metabolites makes them valuable sources of bioactive compounds (Singh et al., 2017)

- Rich Phytochemical Diversity:
    - Mangroves contain various bioactive molecules, including flavonoids, terpenoids, alkaloids, and polyphenols.
    - These compounds exhibit potential anticancer properties. (Cerri et al., 2022)
- Ethnobotanical Significance:
    - Traditional coastal communities have used mangrove plants for various ailments, including cancer.
    - Ethnopharmacological knowledge guides researchers toward promising candidates (Heinrich et al., 2009)
- Sustainability and Availability:
    - Mangroves are abundant and renewable resources.
    - Their sustainable utilization benefits both local communities and global health (Mahmud et al., 2014).

iii. Some example of mangrove plants with anticancer activity: Mangrove plants have many anticancer properties. Some of them are mentioned in the table 1.3.

Table 1.3: Anticancer Potential of Mangrove Plants: Bioactive Compounds and Research Insights

Plants	Bioactive compounds	Potential anticancer activity	Parts used	References
Rhizophora	Phenolic compounds, flavonoids, terpenoids	Cytotoxic activity against various cancer cell lines	Bark and leaves	Kerry et. al., 2018
Bruguieragymnorrhiza	Tannins, saponins, flavonoids	Activity against HepG2 cell line	Bark and leaves	Lanka et al., 2023
Aegicerascorniculatum	Triterpene, saponins	Novel compounds with cytotoxic properties	Leaves	Sheela et al., 2018
Excoecariaagallocha	Diterpenoids, triterpenoids	Isolated compounds showing anticancer effects	Steam bark	Cerri et al., 2022
Sonneratia paracaseolaris	Flavonoids, alkaloids	Extracts tested for anticancer efficacy	leaves	Das et al., 2015

Xylocarpus genus	Limonoids, flavonoids	Compounds with growth inhibitory effects on cancer cells	Leaves	Kerry et al., 2018
Ceriopstagal	Triterpenoids, steroids	Antiproliferative activity against cancer cell lines	Bark	Lanka et al., 2023
Bruguieracylindrica	Flavonoids, catechins	Inhibition of cancer cell proliferation	Leaves	Sheela et al., 2018

References:

American Cancer Society.(2024). Global Cancer Statistics, 2024. Retrieved from https://pressroom.cancer.org/GlobalCancerStatistics 2024

National Cancer Institute. (2022). The Genetics of Cancer. Retrieved from https://www.cancer.gov/about-cancer/causes-prevention/genetics.

Vasto, S., Carruba, G., Lio, D., Colonna-Romano, G., Di Bona, D., Candore, G., Caruso, C., 2009. Inflammation, ageing and cancer. Mechanisms of Ageing and Development 130, 40-45

Larsen, J.E., Minna, J.D., 2011. Molecular biology of lung cancer: clinical implications. Clinics in Chest Medicine 32, 703-740

Chen, Z., Zhang, Y., Yang, J., Jin, M., Wang, X.W., Shen, Z.Q., Qiu, Z., Zhao, G., Wang, J., Li, J.W., 2011. Estrogen promotes benzo[alpyrene-induced lung carcinogenesis through oxidative stress damage and cytochrome c-mediated caspase-3 activation pathways in female mice. Cancer Letters 308, 14-22.

Agboola, O., Emmanuel, A.O., Sabina, C. E., Anejo-Okopi, J., Naomi, A. I., Ene, U. I., &Olowoyo, J.O. (2024). Occurrence of Carcinogens and their Potential Effects on Human Health–A Review. *UMYU Scientifica*, *3*(1), 129-143.

Raja, K., Arumugam, S., Ahmed, S. S., &Veerabathiran, R. (2023). Evolution of viruses: tumor complications. In Oncogenic Viruses (pp. 197-222). Academic Press.

Boguszewski, C. L., da Silva Boguszewski, M. C., &Kopchick, J. J. (2016).Growth hormone, insulin-like growth factor system and carcinogenesis. EndokrynologiaPolska, 67(4), 414-426.

Popper, H. H. (2016). Progression and metastasis of lung cancer. Cancer and Metastasis Reviews, 35, 75-91.

Krishna, I. V., Vanaja, G. R., Kumar, N., & Suman, G. (2009). Cytotoxic studies of anti-neoplastic drugs on human lymphocytes–In vitro studies. Cancer Biomarkers, 5(6), 261-272.

Jaffray, D. A., &Gospodarowicz, M. K. (2015). Radiation therapy for cancer. Cancer: disease control priorities, 3, 239-248.

Wang, S., Liu, Y., Feng, Y., Zhang, J., Swinnen, J., Li, Y., & Ni, Y. (2019). A review on curability of cancers: more efforts for novel therapeutic options are needed. Cancers, 11(11), 1782.

Mahadik, N., Bhattacharya, D., Padmanabhan, A., Sakhare, K., Narayan, K. P., & Banerjee, R. (2022). Targeting steroid hormone receptors for anti-cancer therapy—A review on small molecules and nanotherapeutic approaches. Wiley Interdisciplinary Reviews: Nanomedicine and Nanobiotechnology, 14(2), e1755.

Bailly C. Ready for a comeback of natural products in oncology.BiochemPharmacol. 2009;77: 1447-57.

Bertino JR. Irinotecan for colorectal cancer. Semin Oncol. 1997; 24: S18-$23.

Brand KA and Hermfisse U. Aerobic glycolysis by proliferating cells: a protective strategy against reactive oxygen species.The FASEB journal. 1997; 11(5): 388-95.

Bromberg N, Juliana LD, Caio VR, Marcelly VP, Nelson D, Helena BN, Marcela H, GiselleZJ. Growth inhibition and pro-apoptotic activity of violacein in Ehrlich ascites tumour. Chem Biol Interact. 2010; 186: 43-52.

Butler MS. Natural products to drugs: natural product derived compounds in clinical trials.
Nat Prod Rep. 2005; 22: 162-95.

Butlet MS.The role of natural product chemistry in drug discovery. J. Nat. Prod. 2004; 67: 2141-53.

Carlo M. Ontogenesis and Cancer.New England Journal of Medicine.2008; 358: 502-11.

Cragg G, Suffness M. Metabolism of plant-derived anticancer agents. PharmacolTher. 1988; 37: 425-32.

Creemers GJ, Bolis G, Gore M. Topotecan, an active drug in the second-line treatment ofepithelial ovarian cancer. J Clin Oncol.1996; 14: 3056-61.

Das S, Bhattacharya S, Pramanik G, Haldar PK. Antitumour effect of Diospyros cordifoliabark on Ehrlich ascites carcinoma-bearing Swiss albino mice. Nat Prod Res. 2012;26(17): 1631-3.

Dashora NV, Sodde J, Bhagat KS, Prabhu RL."Antitumor activity of dendrophthoe falcateagainst chrlich ascites carcinoma in swiss albino mice".Pharm. Crops.2010; 2: 1-7.

De Vita VT Jr, Serpick AA, Carbone PO.Combination chemotherapy in the treatment ofadvanced Hodgkin's disease.Ann Intern Med. 1970; 73: 881-95.

Finger LR, Harvey RC, Moore RCA, Showe L.C, Croce CM.A common mechanism of chromosomal translocation in T- and B-cell neoplasia.Science.1986; 234: 982-5.

Gupta M, Mazumder UK, Haldar PK, Kandar CC. Anticancer activity of Indigoferaaspalathoides and Wedeliacalendulaceae in Swiss albino mice. Iran J Pharm Res.

2007; 6: 141-5.

Gupta M, Mazumder UK, Rath N, Mukhopadhyay DK. Antitumour activity of methanolic extract of Cassia fistula L. seed against Ehrlich ascites carcinoma.JEthnopharmacol. 2000; 72: 151-6.

Harmon AD, Weiss U, Silverton JV. The structure of rohutukine, the main alkaloid ofAmoorarohituka (syn. Aphanamixispolystachya) (Maliaceae).Tetrahydron. 1979;20: 721-4.

Hartwell LH and Weinert TA. Checkpoints: controls that ensure the order of cell cycleevents. Science. 1989; 246(4930): 629-34.

Hartwell LH and Kastan MB.Cell cycle control and cancer.Science.1994; 266: 1821-8.

Harvey AL. Medicines from nature: are natural products still relevant to drug discovery.
Trends Pharmacol Sci. 1999; 20: 196-8.

Hwang A and Muschel RJ. Radiation and the G2 phase of the cell cycle. Radiat. Res. 1998;
150 (Suppl.): S52-$59.

Jemal A, Siegel R, Ward E, Murray T, Xu J, Thun MJ.Cancer statistics.CA Cancer J Clin.
2007; 57: 43-66.

Kamboj VP. Herbal medicine.Current Science. 2000; 78(1): 35-9.

Kantarjian HM, O'Brien S, Anderlini P. Treatment of myelogenous leukemia: current statusand investigational options. Blood.1996; 87: 3069-81.

Kelland LR. Flavopiridol, the first cyclin-dependent kinase inhibitor to enter the clinic:current status. Ex Opin Inv Drugs.2000; 9: 2903-11.

Копорка J3, Watanabe SM, Singer JW, Collins SJ, Witte ON. Cell lines and clinical isolates derived from Phl-positive chronic myelogenous leukemia patients express c-abl proteins with a common structural alteration. Proc. Natl. Acad. Sci. 1985; 82: 1810-4.

Kotnis A, Sarin R, Mulherkar R. Genotype, phenotype and cancer: Role of low penetrance genes and environment in tumor susceptibility. J. Biosci. 2005; 30: 93-102.

Li YZ, Li CJ, Pinto AV. Release of mitochondrial cytochrome c in both apoptosis and necrosis induced by B-lapachone in human carcinoma cells. Mol Med. 1999; 4: 232-9.

Liu LF, Desai SD, Li TK. Mechanism of action of camptothecin.Ann New York Acad Sci.
2000; 922: 1-10.

Liu LF. DNA topoisomerase poisons as antitumor drugs. Annu Rev Biochem.1989; 58: 351-75.

López-Lázaro M. A new view of carcinogenesis and an alternative approach to cancertherapy.Molecular medicine. 2010; 16 (3-4): 144-53.

Losiewicz MD, Carlson BA, Kaur G. Potent inhibition of cdc2 kinase activity by the flavonoid L86-8275.BiochemBiophys Res Commun.1994; 201: 589-95.

Mascarenhas M. Structure-activity characterization, a quick method to screen mushrooms for the presence of antitumour glucans.Mushroom Res. 1994; 3: 77-80.

Morgan DO. Principles of CDK regulation.Nature.1995; 374: 131-4.

Newman DJ, Cragg GM, Snader KM. Natural products as sources of new drugs over the period 1981-2002. J. Nat. Prod. 2003; 66: 1022-37.

Noble RL, The discovery of the vinca alkaloids - chemotherapeutic agents against cancer.
Biochem Cell Biol. 1990; 68: 1344-51.

Nojima H. Cell cycle checkpoints, chromosome stability and the progression of cancer. Hum Cell. 1997; 10: 221-30.

Oberlines NH and Kroll DJ. Camptothecins and taxol: historic achievement in natural products research. J. Nat. Prod. 2004; 67: 129-35.

Pardee AB. Gl events and regulation of cell proliferation.Science.1989; 246: 603-8.

Pardee AB, Dubrow R, Hamlin JL, Kletzien R. Animal Annu Rev Biochem.1978; 47: 715-50.

Powell RG, Weisleder D, Smith CR Jr. Structures of harringtonine, isoharringtonine, and homoharringtonine. Tetrahydron Lett. 1970; 11: 815-8.

Rabe T and Van Staden J. Antibacterial activity of South African plants used for medicinal purposes. J. Ethnopharmacol. 1997; 56(1): 81-7.

Raleigh JM.and O'Connell MJ. The G2 DNA damage checkpoint targets both Weel and Cdc25. J. Cell Sci. 2000; 113: 1727-36.

Rehm S and Devor DE.Acute effects of 4-ipomeanol on experimental lung tumors with bronchiolar or alveolar cell features in Syrian hamsters or C3H/HeNCr mice.J Cancer Res Clin Oncol.1993; 120: 41-50.

Rowinsky EK, Noe DA, Ettinger DS. Phasel and pharmacological study of the pulmonary cytotoxin 4-ipomeanol on a single dose schedule in lung cancer patients: hepatotoxicity is dose limiting in humans. Cancer Res. 1993; 53: 1794-1801.

Segura JA, Barbero LG, Marquez J. Ehrlich ascites tumour unbalances splenic cellpopulations and reducedresponsiveness of T cells to Staphylococcus aureus enterotoxin B stimulation. Immunomol Lett. 2000; 74.

Sherr CJ. Mammalian G1 cyclins.Cell.1993; 73: 1059-65.

Sherr C.J. Cancer cell cycles.Science.1996; 274: 1672-7.

Stähelin H. Actvity of a new glycosidic lignan derivative (VP-16-213) related to podophyllotoxin in experimental tumors. Eur J Cancer. 1973; 9: 215-21.

Tsujimoto Y, Gorham J, Cossman J, Jaffe E, Croce CM. The t (14;18) chromosome translocations involved in B-cell neoplasms result from mistakes in DJ joining.Science. 1985; 229: 1390-3.

Torre AL, Bray F, Siegel RL, Ferlay J, Tieulent JL, Jemal A. Global cancer statistics. CA:Cancer J. Clin. 2012; 65(2): 87-108.

Wani MC, Taylor HL, Wall ME.Plant antitumor agents. VI. The isolation and structure of taxol, a novel antileukemic and antitumor agent from Taxus brevifolia. J Am Chem Soc. 1971; 93: 2325-7.

Weinberg, Robert A. "The Biology of Cancer." Garland Science. 2014; 231.

Worland PJ, Kaur G, Stetler-Stevenson M. Alteration of the phosphorylation state of p32cdc2 kinase by the flavone L86-8275 in breast carcinoma cells. BiochemPharmacol. 1993;46: 1831-6.

Xiong Y, Connolly T, Futcher B, Beach D. Human D-type cyclin. Cell.1991; 65: 691-9.

Zhan Q, Antinore MJ, Wang XW, Carrier F, Smith ML, Harris CC, Fornace AJ. Association with Cdc2 and inhibition of Cdc2/cyclinB1 kinase activity by the P53-regulated protein Gadd45.Oncogene.1999; 18: 2892-900.

Zhou DC, Zittoun R, Marie JP. Homoharringtonine: an effective new natural product in cancer chemotherapy. Bull Cancer. 1995; 82: 987-95.

Zumrutdal ME, Mehmet 0, Mehmet T, Mehmet EK, Kenan D, Atilla A, Isik DK, Ibrahim HK, Omer C, Fatih K. Effect of Lawsoniainermis treatment on mice with sarcoma.Afr J Biotechnol. 2008; 7(16): 2781-6.

Chanda, A., Mukhopadhyay, A., Ghosh, T., Akhand, A., Mondal, P., Ghosh, S.,Mukherjee, S., Wolf, J., L´az´ar, A.N., Rahman, M.M., Salehin, M., Chowdhury, S.M.,Hazra, S., 2016. Blue carbon stock of the Bangladesh sundarban mangroves: whatcould Be the scenario after a century? Wetlands 36, 1033–1045. https://doi.org/10.1007/s13157-016-0819-7.

Pramanik, A., Sengupta, S., Bhattacharyya, M., 2019. In: Das, S., Dash, H.R.B.T. (Eds.),Chapter 5 - Microbial Diversity and Community Analysis of the SundarbansMangrove, a World Heritage Site. Academic Press, pp. 65–76. https://doi.org/10.1016/B978-0-12-814849-5.00005-8. M.D. in the G.E.

Prain, D., 1903. Flora of the Sundribuns, Calcutta, Printed at the Government CentralPrinting Office. Retrieved from the Library of Congress.
https://hdl.loc.gov/loc.gdc/gdclccn.agr03001093v2n4

Banglapedia, 2021b. Sundarbans, the Banglapedia - Natl. Encycl. Bangladesh. https://en.banglapedia.org/index.php/Sundarbans,_The.

Gopal, B., Chauhan, M., 2006.Biodiversity and its conservation in the sundarbanmangrove ecosystem.Aquat. Sci. 68, 338–354. https://doi.org/10.1007/s00027-006-0868-8.

Titumir, R.A.M., Afrin, T., Islam, M.S., 2020. In: Saito, O., Subramanian, S.M.,Hashimoto, S., Takeuchi, K. (Eds.), Traditional Knowledge, Institutions and HumanSociality in Sustainable Use and Conservation of Biodiversity of the Sundarbans ofBangladesh BT - Managing Socio-Ecological Production Landscapes and Seascapesfor Sustainable Communities in Asia: Mapping and Navigating Stakeholders, Policyand Action. Springer Singapore, Singapore, pp. 67–92. https://doi.org/10.1007/978-981-15-1133-2_5.

Islam, M.T., Sharifi-Rad, J., Martorell, M., Ali, E.S., Asghar, M.N., Deeba, F., Firoz, C.K.,Mubarak, M.S., 2020. Chemical profile and therapeutic potentials of Xylocarpusmoluccensis (Lam.) M. Roem.: a literature-based review. J. Ethnopharmacol. 259,112958. https://doi.org/10.1016/j.jep.2020.112958.

Faruque, M.O., Feng, G., Khan, M.N.A., Barlow, J.W., Ankhi, U.R., Hu, S.,Kamaruzzaman, M., Uddin, S.B., Hu, X., 2019. Qualitative and quantitativeethnobotanical study of the Pangkhua community in BilaichariUpazilla, Rangamatidistrict, Bangladesh. J. Ethnobiol. Ethnomed.15, 8.https://doi.org/10.1186/s13002-019-0287-2.

Faruque, M.O., Uddin, S.B., Barlow, J.W., Hu, S., Dong, S., Cai, Q., Li, X., Hu, X., 2018.Quantitative ethnobotany of medicinal plants used by indigenous communities inthe bandarban district of Bangladesh. Front. Pharmacol.9, 40.

Kadir, M.F., Bin Sayeed, M.S., Mia, M.M.K., 2012. Ethnopharmacological survey ofmedicinal plants used by indigenous and tribal people in Rangamati, Bangladesh.J.Ethnopharmacol. 144, 627–637. https://doi.org/10.1016/j.jep.2012.10.003.

Khan, M.A., Islam, M.K., Siraj, M.A., Saha, S., Barman, A.K., Awang, K., Rahman, M.M.,Shilpi, J.A., Jahan, R., Islam, E., Rahmatullah, M., 2015. Ethnomedicinal survey ofvarious communities residing in Garo Hills of Durgapur, Bangladesh. J. Ethnobiol.Ethnomed. 11, 44.https://doi.org/10.1186/s13002-015-0033-3.

Islam, M.K., Saha, S., Mahmud, I., Mohamad, K., Awang, K., Jamal Uddin, S.,Rahman, M.M., Shilpi, J.A., 2014. An ethnobotanical study of medicinal plants used by tribal and native people of Madhupur forest area, Bangladesh. J. Ethnopharmacol.

151, 921–930.https://doi.org/10.1016/j.jep.2013.11.056.

Ahmed, F., Rahman, A., Mubassara, S., Hossain, G., 2016.Ethnobotany and antibacterialpotentiality of Blumealacera L. from Sundarban Mangrove forest of Bangladesh.Jahangirnagar Univ. J. Biol. Sci. 3 https://doi.org/10.3329/jujbs.v3i2.28282.

Ahmed, F., Rahman, A., Mubassara, S., Hossain, G., 2016.Ethnobotany and antibacterialpotentiality of Blumealacera L. from Sundarban Mangrove forest of Bangladesh.Jahangirnagar Univ. J. Biol. Sci. 3 https://doi.org/10.3329/jujbs.v3i2.28282.

Mollik, M.A.H., Faruque, M.R., Badruddaza, M., Chowdhury, A., Rahman, M.S., 2009.Medicinal plants from Sundarbans used for the prevention of cardiovasculardiseases: a pragmatic randomized ethnobotanical survey in Khulna division ofBangladesh. Eur. J. Integr. Med. 1, 231–232. https://doi.org/10.1016/j. eujim.2009.08.018.

Ray, T., Limon, S.H., Tuhin, S.H., Sharmin, A., 2021.Indigenous knowledge of plant usesby the community of Batiaghata, Khulna, Bangladesh. J. Med. Plants Res. 15,260–268

Ray, T., Mondal, K., 2018. Ethnobotanical study of munshiganj in shyamnagarUpazilla,Satkhira, Bangladesh. Am. J. Sustain. Agric. 12, 22–31.

Dulla, O., Jahan, F.I., 2017. Ethnopharmacological survey on traditional medicinalplants at kalaroaUpazila, Satkhira district, Khulna division, Bangladesh. J. Intercult.Ethnopharmacol. 6, 316–325. https://doi.org/10.5455/jice.20170719010256.

Singh, M., Kumar, A., Singh, R., & Pandey, K. D. (2017). Endophytic bacteria: a new source of bioactive compounds. 3 Biotech, 7, 1-14.

Heinrich, M., Edwards, S., Moerman, D. E., & Leonti, M. (2009). Ethnopharmacological field studies: a critical assessment of their conceptual basis and methods. Journal of ethnopharmacology, 124(1), 1-17.

Cerri F, Giustra M, Anadol Y, Tomaino G, Galli P, Labra M, Campone L, Colombo M. Natural Products from Mangroves: An Overview of the Anticancer Potential of *Avicennia marina*. Pharmaceutics. 2022 Dec 14;14(12):2793. doi: 10.3390/pharmaceutics14122793. PMID: 36559286; PMCID: PMC9787599.

Rout P. Bioprospecting of underutilized mangrove fruits used by coastal communities in the Odisha coast, India: a review. Food Sci Biotechnol. 2021 Dec 9;31(2):139-153. doi: 10.1007/s10068-021-01013-8. PMID: 35186345; PMCID: PMC8817954.

Kerry, R.G., Pradhan, P., Das, G., Gouda, S., Swamy, M.K., Patra, J.K. (2018). Anticancer Potential of Mangrove Plants: Neglected Plant Species of the Marine Ecosystem. In: Akhtar, M., Swamy, M. (eds) Anticancer plants: Properties and Application. Springer, Singapore.https://doi.org/10.1007/978-981-10-8548-2_13

Lanka, S., Katta, A., &Pandrangi, S. (2023). Bioprospecting mangrove plants fornovelanticancer compound. Environment and Ecology 4(4A), 2467-2473
https://doi.org/10.60151/envec/LLRY6733

Sheela Devi, A., Joseph, J., &Bhuvaneshwari, V. (2019).Identification of anticancer activity of phytoconstituents from mangrove. International Journal of Research in Pharmaceutical Sciences, 10(2), 791-794.
https://doi.org/10.26452/ijrps.v10i2.254

Anadol, Y. (2022). Natural Products from Mangroves: An Overview of the Anticancer Potential of Avicennia marina. Pharmaceutics.

Patra, J. K., Das, G., Gouda, S., &Mohanta, Y. K. (2015). Mangrove plants: A potential source for anticancer drugs. Indian Journal of Geo-Marine Sciences, 44(5), 666-672. Retrieved from http://nopr.niscpr.res.in/handle/123456789/34788

CHAPTER 2: LITERATURE REVIEW

2. Litterature Review of *Soneratia Apetala:*

Figure 2.1: Fruits of *Soneratia Apetala*

Figure 2.2: Leaves of *Sonneratia Apetala*

2.1. Taxonomical classification:

i. **Plant Name**- *Soneratia Apetala* Buch-Ham.

ii. **Common Name**- Keora

iii. **Vernicular Name**- Gujarati: Motitavar; Marathi: Chipi, Kandal; Oriya: Keruan; Tamil: Maramamaram; Telugu: Peddakalinga (Indian Names of Plants, 2011, https://sites.google.com/site/indiannamesofplants/)

2.1.1. Kingdom: Plantae

Subkingdom: Viridiplantae

Phylum: Phylum

Class: Magnoliopsida

Family: Lythraceae

Genus: Sonneratia

Species: *Sonneratia apetala*

2.2. Morphology of the plant:

With natural populations in Bangladesh, India (Andaman and Nicobar Islands, Andhra Pradesh, West Bengal), and Myanmar, *S. apetala* is a small to medium-sized tree that may grow to a height of 15 metres. Growing gregariously and producing nearly pure patches on newly formed alluvial regions along river banks and estuaries, it is an important component of mangrove forests. Its leaves are ediable, its wood is relatively durable, and its blooms are a significant source of honey. It is regarded as a prime plantation species along recently elevated coastal areas because of its pioneering character on freshly accreted land and its widespread utilisation.

2.3. Ethnomedicinal Evidence:

Soneratia apetala used in asthma, fevers, ulcer, swelling, sprains, bleeding, haemorrhage, piles, heart troubles (bark), antioxident, antibacterial, antifungal, astringent (Fruit) activity, diarrhoea (Fruit), gastrointestinal disorders (fruit), cancers, cardiovascular diseases, neurodegenerative diseases and diabetes (seeds) anti-inflamatory activity (Bark) (Nasrin S et.al.,2017). Also the plant used many where.

Rationale for selection of the plant:

We have good grounds to choose this plant for our study because of its many ethnomedicinal applications as well as the fact that it grows in the mangrove. Additionally, since some portions of this plant are edible, using it medicinally is probably not going to have as many negative consequences.

Figure 2.3: Whole plant of *Sonneratia apetala*

Figure 2.4: *Soneratia apetala* fruit

Table 2.1: Pharmacological evidence of the *Sonneratia Apetala*

Sl. No	Docoment Title	Year	Ethnopharamacological Use	Pharmacological Use	Journal name,DOI
1.	The Extract of Sonneratia apetala leaves and branches ameliorates hyperuricemia in Mice by regulating Renal uric Acid transporters and suppressing the activation of the JAK/STAT signaling Pathway	2021	Antidiabetic, antibacterial, and antioxidant capacities (Leaves, Branches)	Hyperuricemia (Leaves, Branches)	Frontiers in Pharmacology https://doi.org/10.3389/fphar.2021.698219
2.	A very detailed look at the environmental and other uses of this species.	2017	Asthma, fevers, ulcer, swelling, sprains, bleeding,haemorrhage,piles, heart troubles, (Bark) antioxident, antibacterial, antifungal,astringent(Fruit) activity, diarrhoea (Fruit) gastrointestinal disorders(Fruit) cancers, cardiovascular diseases, neurodegenerative diseases and diabetes (Seeds)antiinflamatory activity(Bark)		Publisher- Lambert Academic Publishing https://www.researchgate.net/publication/328162117_A_Monograph_on_Sonneratia_apetala_Buch-Ham
3.	Marine halophyte derived polyphenols inhibit glioma cell growth through mitogen-activated protein kinase signaling pathway	2023	Microbial infection	Anticancer (Leaf)	Biomedicine & Pharmacotherapy https://doi.org/10.1016/j.biopha.2023.114288
4.	Interspecific variations in leaf	2023		Leaf litter decompositio	Journal of Environmental

	litter decomposition and nutrient release from tropical mangroves			n	Management https://doi.org/10.1016/j.jenvman.2022.116902
5.	Standardised Sonneratiaapetala Buch.-Ham. fruit extract inhibits human neutrophil elastase and attenuates elastase-induced lung injury in mice	2023		COPD, Asthma	Frontiers in Pharmacology https://doi.org/10.3389/fphar.2022.1011216
6.	Phytochemical Profi ling and Bioactivity of A Mangrove Plant, Sonneratiaapetala, from Odisha Coast of India	2014		Anticancer (Leaves,Bark)	Chinese Journal of Integrative Medicine 10.1007/s11655-014-1854-y
7.	Standardised Sonneratiaapetala Buch.-Ham. fruit extract inhibits human neutrophil elastase and attenuates elastase-induced lung injury in mice	2022		Lung injury(Fruit)	Frontiers in Pharmacology https://doi.org/10.3389/fphar.2022.1011216
8.	Structural characterization of ellagitannin-rich fractions fromleaves of threeSonneratiaspecies, and their antioxidant activityandα-amylase inhibitory effect and mechanism			Inhibit α amylase and antioxidant	INTERNATIONAL JOURNAL OF FOOD https://doi.org/10.1080/10942912.2019.1675693
9.	Bioprospecting of	2022		Asthma,	Food Science and

	underutilized mangrove fruits used by coastal communities in the Odisha coast, India: a review			febrifuge, ulcers, swellings, sprains, bleeding, and hemorrhages (Fruit)	Biotechnology https://doi.org/10.1007/s10068-021-01013-8
10.	Therapeutic Potential and Ethnopharmacology of Dominant Mangroves of Bhitarkanika National Park, Odisha, India	2023		Hepatitis (Leaf) Leprosy (Leaf,Fruit)	**Chemistry & Biodiversity https://doi.org/10.1002/cbdv.202100857**
11.	Compounds from the fruits of mangrove Sonneratiaapetala: Isolation, molecular docking and antiaging effects using a Caenorhabditis elegans model			Antiaging	Bioorganic Chemistry doi:10.1016/j.bioorg.2020.103813

References-

Nasrin, Shamima& Hossain, Mahmood &AlamPhd, Md. (2017).A Monograph on SonneratiaapetalaBuch.-Ham.

Dahdouh-Guebas F. (Ed.) (2024). World Mangroves database. *Sonneratiaapetala* Banks. Accessed through: World Register of Marine Species at: https://www.marinespecies.org/aphia.php?p=taxdetails&id=235108 on 2024-05-29

cabicompendium.50590, CABI Compendium, doi:10.1079/cabicompendium.50590, CABI International, Sonneratiaapetala, (2022)

CHAPTER 3: PLAN OF WORK

3. PLAN OF WORK:

Objective: Evaluation of anticancer activity of indigenous plant extract.

Brief outline of the work to be performed:

- Literature survey and plant selection.
- Collection and identification of the selected plant.
- Extraction by using hydroalcoholic as solvent.
- Acute toxicity study by LD50 method as per OECD guidelines.
- Phytochemical Screening.
- Determination of Total Phenolic and Total Flavonoid Content.
- Evaluation of free radical scavenging activity of plant extract by in-vitro DPPH radical scavenging activity.
- In-vitro cytotoxic effect of the plant extract against Ehrlich Ascites Carcinoma cells. The in-vitro study involves:
    - MTT Assay
- In-vivo cytotoxic effects of the plant extract against Ehrlich Ascites Carcinoma cells. The in vivo study involves:
    - Determination of tumor volume and packed cell volume.
    - Tumor cell count.
    - Percentage increase in life span.
    - Determination of hematological parameters.
    - Estimation of biochemical parameters.
    - Determination of tissue antioxidant parameters.

CHAPTER 4: EXTRACTION AND PHYTOCHEMICAL SCREENING

4.1. COLLECTION AND EXTRACTION:

Dried *Sonneratia apetala* fruits weighing 500g were gathered from Sunderban regions of West Bengal, India on the November month of 2022. The entire dried plant was then ground into a powder using a mechanical grinder, and the resulting 85g of powder was extracted using a soxhlet apparatus and methanol:water (70:30) for 72hrs during 3 days (6.87% w/w, yield). Under low pressure, the solvent was totally eliminated in a rotating vacuum evaporator. For later usage, the concentrated extracts were kept in centrifuge tubes.

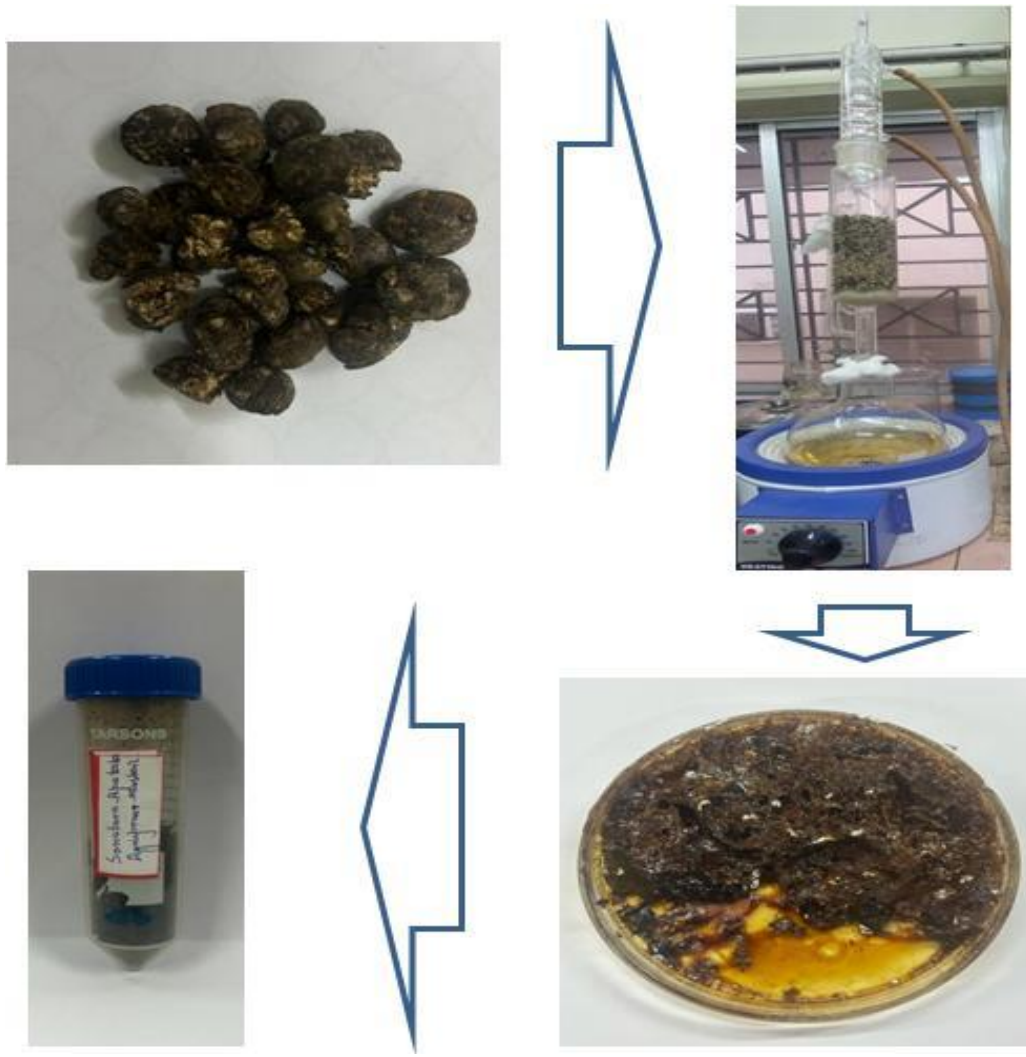


Figure 4.1: Process of Extraction

4.2. PHYTOCHEMICAL SCREENING: During the phytochemical screening process, I conducted a qualitative analysis to identify the various phytochemicals in the extract. Phytochemicals are natural compounds produced by plants that have bioactive properties (Ugochukwa et al., 2013). There are numerous phytochemical compounds with high antioxidant activity, such as vitamins, alkaloids, flavonoids, saponins, tannins, steroids, and other metabolites (Gracelin et al., 2013). It is therefore essential to determine the phytoconstituents present in a plant material or its extract.

4.2.1. Test for Steroid:

- **Liebermann- Burchard Test** (Zhou et al., 2004)

10 mg of extract was dissolved in 1ml of chloroform. 1ml of Acetic Anhydride was added following the addition of 2ml of concentrated sulphuric acid. Formation of reddish violet or pinkish colour indicated the presence of steroids.

- **Salkowski Test** (Bosila et al., 2005)

1ml of concentrated sulphuric acid was added to 10 mg of extract dissolved in 1ml of chloroform. A reddish blue colour exhibited by chloroform layer and green fluorescence by acid layer indicated the presence of steroid.

4.2.2. Test for Alkaloids

- **Mayer's test** (Raffauf et al., 1962)

1.2 ml of extract was taken in a test tube. 0.2 ml of dilute hydrochloric acid and 0.1 ml of Mayer's reagent were added. Formation of yellowish buff coloured precipitate gives positive test for alkaloids.

- Dragendroff's test (Swant et al., 2013)

0.1 ml of dilute hydrochloric acid and 0.1 ml of Dragendroff's reagent were added in 2ml solution of extract in a test tube. Development of orange brown coloured precipitate suggested the presence of alkaloids.

- **Wagner's test** (Wagner et al., 2022)

2 ml of extract solution was treated with dilute hydrochloric acid and 0.1 ml Wagner's reagent. Formation of reddish brown indicated the positive response for alkaloids.

4.2.3. Test for Flavonoids

- **Alkaline reagent test** (Ugochukwu SC et al., 2013)

2ml of extracts was treated with few drops of 20% sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute hydrochloric acid, indicates the presence of flavonoids.

- **Shinoda's test** (Palanisamy P et al., 2012)

Small quantity of the extract was dissolved in alcohol. Two to three piece of magnesium followed by concentrated hydrochloric acid was added and heated. Appearance of magenta colour demonstrates presence of flavonoids.

4.2.4. Test for Saponins (Shinha et al., 1985)

1ml solution of the extract was diluted with distilled water to 20 ml and shake in a graduated cylinder for 15 mins. Development of srable foam suggested the presence of saponins.

1 ml extract was treated with 1% lead acetate solution. Formation of white precipitate indicated the presence of saponins.

4.2.5. Test for Tannins (Segelman et al., 1969)

5 ml of extract solution was allowed to react with 1 ml 5% ferric chloride solution. Greenish black coloration indicated the presence of tannins.

5 ml of extract was treated with 1ml of 10% aqueous potassium dichromate solution. Formation of yellowish brown precipitate suggested the presence of tannins.

4.2.6. Test for Glycoside

- **Legal's test** (Salwaan et al., 2012)

The extract was dissolved in pyridine and sodium nitroprusside solution added to make it alkaline. The formation of pink red to red colour shows the presence of glycosides.

- **Brontrager's test** (Attah et al., 2019)

A few ml of dilute sulphuric acid added to 1 ml of the extract solution. Boiled, filtered and extracted the filtrate with chloroform. The chloroform layer was treated with 1ml of ammonia. The formation of red colour shows the presence of anthraquinone glycosides.

4.2.7. Test for Protein

- **Biuret Test** (Kumar et al,2012)

The extract was treated with 1 ml 10% sodium hydroxide solution and heated. A drop of 0.7% copper sulphate solution to the above mixture was added. The formation of purplish violet colour indicates the presence of proteins.

- **Millon Test** (Narasimhan et al., 2012)

3 ml test solutions were mixed with 5 ml Million's reagent separately. White precipitate was formed which on heating tuned to brick red. It indicates the presence of proteins.

4.2.8. Test for Carbohydrate

- **Benedict's test** (Bhandary et al., 2012)

Test solution was mixed with few drops of Benedict's reagent (alkaline solution containing cupric citrate complex) boiled in water bath, observed for the formation of reddish brown precipitate to show a positive result for the formation of carbohydrate.

- **Molishtest** (Salwaan et al., 2012)

To 2 ml of the extract, added 1ml of α-naphthol solution, and concentrated sulphuric acid through the sides of test tubes. Purple or reddish violet colour at the junction of the two liquid reveals the presence of carbohydrates.

4.2.9. Test for Triterpenoid

- **Salkowski test** (Nayak et al., 2011)

The test extract was treated with few drops of concentrated sulphuric acid. Formation of yellow colour at the lower layer suggested the presence of triterpenoids.

Results:

Table 4.1: Result of Phytochemical Screening

S. NO.	PHYTOCONSTITUENT	PRESENCE/ ABSENCE
1.	STEROID	-
2.	ALKALOID	+
3.	FLAVONOID	+
4.	SAPONIN	-
5.	TANNIN	+
6.	GLYCOSIDE	+
7.	PROTEIN	-
8.	CARBOHYDRATE	+
9.	TRITERPENOID	+

'+' indicates the presence of a particular phytoconstituent while **'-'** indicates the absence of the phytoconstituent.

In this plant *sonneratia apetala* the alkaloid, flavonoids, tannin, glycoside, carbohydrate and triterpenoid are present and the steroid, saponin and protein are absent.

References:

Bhandary S.K., Kumari S.N., Bhat V.S, Sharmila K.P., Bekal M.P. Preliminary phytochemical screening of various extracts of punicagranatum peel, whole fruit and seeds. Nitte University Journal of Health Science, 2012, vol 2, No.4.

Gracelin D.H.S., De britto A.J. and Kumar P.B.J.R. Qualitative and quantitative analysis of phytochemicals in five pterisspecies.International Journal of Pharmacy and Pharmaceutical Sciences, 2013, vol 5, Suppl 1.

Kumar A., Jha K.K., Kumar D., Agrawal A. and Gupta A. Preliminary phytochemical analysis of leaf and bark (mixture) extract of ficusinfectoria plant. The Pharma Journal,2012, vol 1, No.5.

Palanisamy P., Jayakar B., Kumuthavalli M.V., Kumar Y. and Srinath K.R. Preliminary phytochemical evaluation of whole plant extract of Dipteracanthus prostrates nees. International Research Journal of Pharmacy, 2012, 3(1).

Raffauf RF. A simple field test for alkaloid-containing plants. *Economic Botany.*1962; 16: 171-2.

Sawant, R. S., & Godghate, A. G. (2013). Qualitative phytochemical screening of rhizomes of Curcuma longa Linn. International Journal of Science, Environment and Technology, 2(4), 634-641.

Wagner, S., Klöckner, P., & Reemtsma, T. (2022). Aging of tire and road wear particles in terrestrial and freshwater environments–a review on processes, testing, analysis and impact. Chemosphere, 288, 132467.

Attah Alfa, A., Ayodeji, O. A., Donatus Teru, G. A., & Bajeh Tijani, K. (2019). Studies on the phytochemical compounds in the Ethanolic leaf extract (ELE), ethanolic bark extract (EBE) and ethanolic root extract (ERE) of Bridelia ferruginea Benth (euphorbiaceae). Asian Journal of Biochemistry, Genetics and Molecular Biology, 2(4), 1-8.

Narasimhan, R., & Mohan, A. (2012). Phytochemical screening of Sesamum indicum seed extract. World journal of pharmacy and pharmaceutical sciences, 1(4), 1298-1308.

Salwaan C, Singh A, Mittal A and Singh P. Investigation of the pharmacognostical, phytochemical and antioxidant studies of plant *withaniacoagulans*Dunal. *Journal of Pharmacognosy and Phytochemistry.*2012; 1: 3.

Segelman AB, Fransworth NR, Quimby MW. Biological and phytochemical evaluation of plants.3 False-negetive saponin test results induced by the presence of tannins.*Lloydia.*1969; 32(1): 52-8.

Ugochukwu SC, Arukwe UI, Ifeanyi O. Preliminary phytochemical screening of different solvent extracts of stem bark and roots of *Dennetiatripetala*G.Baker. *Asian Journal of Plant Science and Research.*2013; 3(3): 10-3.

Zhou H, Li YS, Tong XT, Liu HQ, Jiang SH, Zhu DY. Serratane-type Triterpenoids from *HuperziaSerata. Natural Product Research.*2004; 18: 453-9.

CHAPTER 5: IN-VITRO ANTIOXIDANT, TOTAL PHENOLIC AND TOTAL FLAVONOID CONTENT DETERMINATION

Oxidative Stress: An Overview

It is well known that oxidation plays a crucial role in both the human body and our food. The metabolism of oxidative substances is vital for the survival of our cells. The production of free radicals and other reactive oxygen species, which cause oxidative damage, is a direct result of this process. Free radicals are molecules that have an unpaired electron in their outer shell, and they are formed when oxygen is metabolized in our bodies. This is why free radicals can easily react with proteins, lipids, carbohydrates, and DNA, as they are highly reactive. They steal electrons from stable molecules nearby, initiating a chain reaction The life of a cell culminates in its eventual death. Free radicals, which can be derived from nitrogen (RNS) or oxygen (ROS), play a critical role in this process. Oxygen-derived free radicals include superoxide, hydroxyl, hydroperoxyl, peroxyl and alkoxyl, while non-radicals like oxygen and hydrogen peroxide also contribute. Nitrogen-derived oxidants such as dinitrogen trioxide, nitrogen dioxide, peroxy nitrate, and nitric oxide are also significant factors according to Badarinath et al. (2010). These free radicals, produced during various bodily processes, are associated with illnesses like heart disease, atherosclerosis, and cancer (AL-Dabbas et al, 2006). The antioxidant molecules created in vivo can scavenge these free radicals, but endogenous antioxidants cannot eliminate them entirely. Dietary antioxidants play a crucial role in fighting against an excess of free radicals (Wang et al, 2010). While synthetic antioxidants like butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) have been widely used in industrial processes due to their effectiveness, their usage in pharmaceuticals, cosmetics, and food products has been decreasing. This is because of reports linking them to potential carcinogenic and other harmful effects. Consequently, there is a growing interest in natural antioxidants present in fruits, vegetables, nuts, seeds, grains, tree bark, roots, spices, and herbs (Peiyuan et al, 2010).

The current study's objective was to assess the methanol extract of *Sonneratia apetala* was antioxidant or free radical scavenging activity using a variety of antioxidant test techniques.

5.1. Materials and Method

Chemicals:

1, 1- Diphenyl-2-picryl-hydrazyl(DPPH) was obtained from Sigma Chemicals, USA. Nitroblue tetrazolium (NBT), phenazine methosulphate (PMS), reduced nicotinamide adenine dinucleotide (NADH), napthyl ethylene diamine dihydrochloride, sodium nitroprusside, ascorbic acid, trichoroacetic acid (TCA), ethyldiamine tetra acetic acid (EDTA), sodium hydroxide (NaOH), hydrogen peroxide (H_2O_2), thiobarbituric acid (TBA). All reagent used were of high analytical grade.

5.2. DPPH radical scavenging activity

The method was performed as described by Cotelle et al. (1996), with some modifications to measure the DPPH radical scavenging activity. A total of 2.8ml of the test solution or standard ascorbic acid (dissolved in methanol) were mixed with 0.2ml of DPPH (100µM in methanol) in different concentration and the mixture was left to incubate for 30 minutes at 37ºC. We made sure to protect the reaction mixture containers and DPPH from light throughout the process. Using a spectrophotometer, the absorbance was then measured at 517 nm after the incubation period.

5.2.1. Percentage of inhibition calculation

The percentage inhibition was calculated from the following formula:

$$\% \text{ inhibition} = \frac{(\text{absorbance of control}) - (\text{absorbance of test sample})}{\text{Absorbance of control}} \times 100$$

5.2.2. Statistical Analysis

All the values are given as mean ± SEM. The IC50 (50% inhibitory concentration) values were calculated from the graphs plotted between concentrations versus percentage inhibition using GraphPad Prism software 8.0.2

5.2.3. Results

The outcomes of the in-vitro antioxidant tests demonstrated that the methanol extract of *Sonneratia apetala* exhibited quite excellent antioxidant activity.

The sample showed a gradual decrease in DPPH activity as the concentration increased, as indicated by the DPPH test. The IC50 values for ascorbic acid and HASA were found to be 31.65±3.5 µg/ml and 138.6±4.21 µg/ml, respectively, based on the DPPH graph.

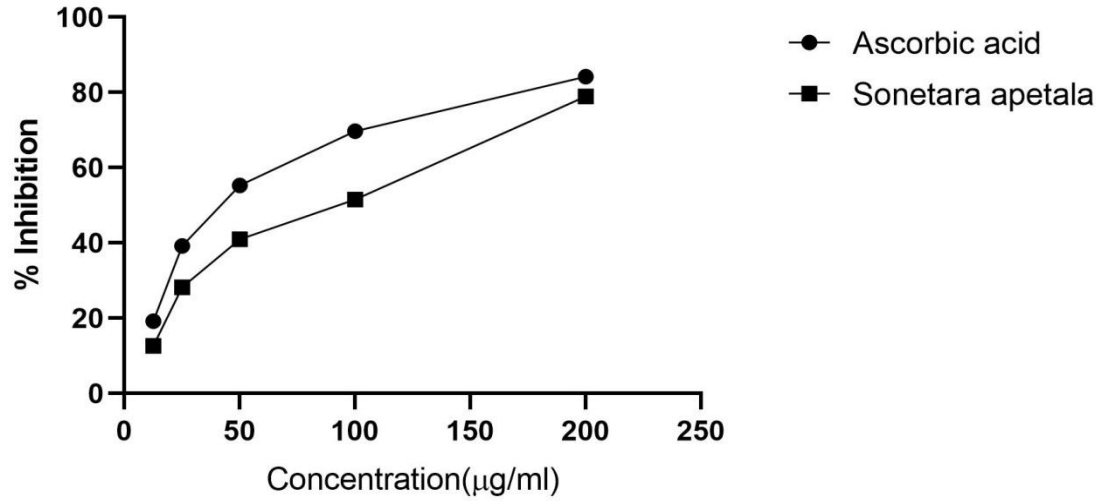


Figure 5.1: 1,1-diphenyl-2-picrylhydrazil(DPPH) scavenging activity of extract and ascorbic acid. The data represent the percentage of DPPH inhibiton. Each point represents the values obtained from the experiments (mean±SEM)

5.3. Total Phenolic Content

The Folin-Ciocalteu (FC) reagent was used to measure the total phenolic content with some modification of Lin and Tang (2007). In a conical flask, 1ml (1mg) of extract, 45ml of distilled water, and 1ml FC were mixed together and agitated for 30 minutes. After that, the mixture was agitated for two hours at room temperature with 3ml of Na2CO3 (2%) added. At 760nm, the absorbance was measured using distilled water as a blank. The following formula was used to get the total phenolic content.

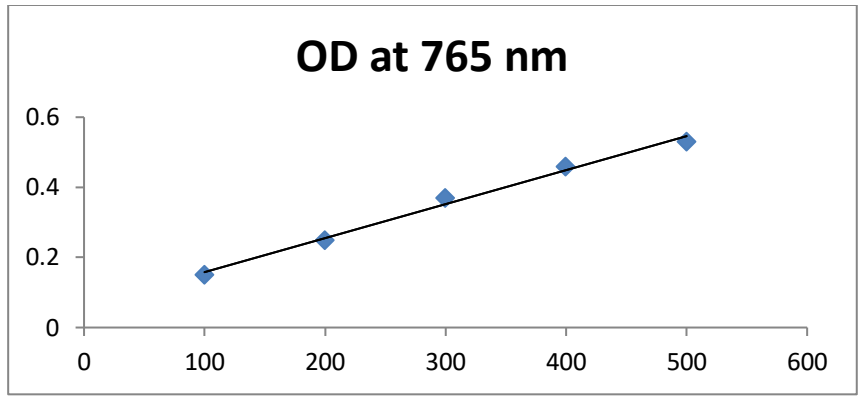


Figure 5.2: Total Phenolic Content

5.3.1. Result

Total phenolic content of HASA (Hydroalcoholic extract of *Sonneratia apetala*) was determined during the assay method was found to be 62 GAE (Galic acid equivalent),mg/gm shown in fig 5.2.

5.4. Total Flavonoid Content

This technique (Meda et.al, 2005) was used to calculate the total flavonoid content with some modification. The extract solution (0.1 mg/mL) was combined with 2ml of 2% aluminium trichloride ($AlCl_3$) in methanol. Spectrophotometer measurements for absorption at 415nm were taken. Next 10 minutes in comparison to a blank sample made up of 2 ml of extract solution with 2ml of methanol and no $AlCl_3$. Equipped with a standard curve and quercetin (25–200 µg/2 ml methanol) as the standard, the total flavonoid concentration was ascertained. The expression for the total flavonoid content is µg of quercetin equivalents (QE)/mg of extract.

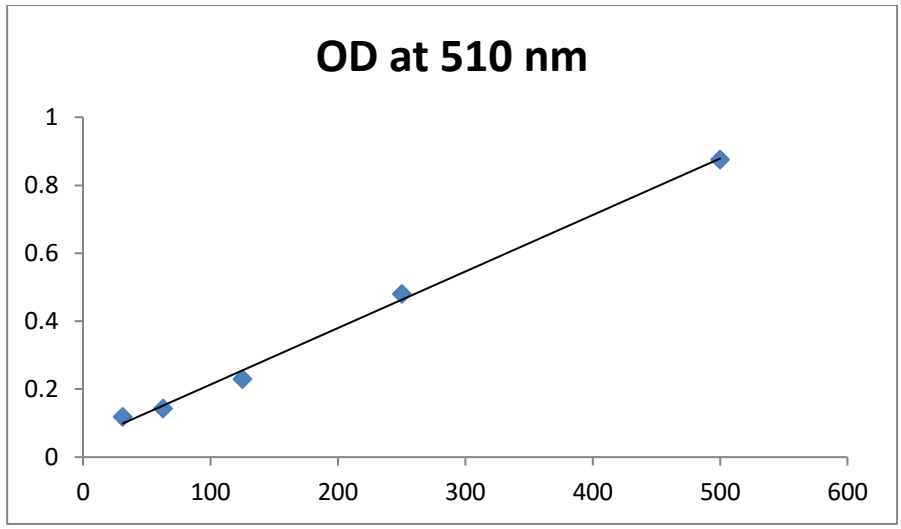


Figure 5.3: Total Flavonoid Content

5.4.1. Result

Flavonoids are large class of benzo-pyrone derivatives, ubiquitous in plants exhibit antioxidant activity. The flavonoid content of different concentrations of fruits extract is shown in Fig 5.3. Total flavonoid content of HASA (Hydroalcoholic extract of *Sonneratia apetala*) is 101 mg/gm of quercetin equivalents (QE)/mg of extract.

5.2.4. Discussion:

Approximately 6% of the oxygen we breathe is converted into harmful oxygen-derived free radicals through our body's normal processes. These radicals have the potential to cause damage to various cells and substances in our body. In some situations, it is necessary to introduce external antioxidants to combat these free radicals. Therefore, it is important to test the antioxidant capacity of any component, molecule, or extract to ensure its effectiveness. One solution to this problem is to use dietary supplements containing natural antioxidants as an alternative to traditional medications. By accepting an electron or hydrogen radical, a stable diamagnetic molecule can be formed from DPPH, a stable free radical. When the DPPH radical interacts with certain reducing agents, electrons combine and the solution changes color based on the number of electrons absorbed (Dash et al, 2005). This reaction is often used to assess the antioxidant activity of an extract or compound by measuring the decrease in absorbance at 517 nm, indicating a decrease in DPPH radicals.

5.5. Conclusion:

In short, the hydroalcoholic extract from the fruit of *Sonneratia apetala* plant shows potent antioxidant properties, likely explaining its use as a traditional remedy. However, the specific components responsible for these antioxidant effects are still unknown. Further research is needed to isolate and characterize these antioxidant compounds in the plant extract. Before considering clinical use, the antioxidant activity of this extract in living organisms must be studied. With its impressive antioxidant capabilities, there is potential for the extract to also possess anticancer properties. Previous research has indicated that scavenging free radicals could be beneficial in this regard.

REFERENCES:

Al-Dabbas MM, Suganuma T, Kiahara K, Hou DX, Fujii M, Journal of Ethnopharmacology, 206, vol 108, 287.

Antovich M, Prenzler PD, Patsalides E, Mcdonald S and Robards K. Methods for testing antioxidant activity.The Analyst, 2002, vol 127, 183-198.

Badarinath AV, Rao KM, Chetty CMS, Ramkanth S, Rajan TVS and Gnanaprakash K. A Review on In-vitro Antioxidant Methods: Comparisions, Correlations and Considerations. International Journal of PharmTech Research, 2010, vol2, 1276-1285.

Bala A, Kar B, Naskar S, Haldar PK and Majumder UK.Antioxidant activity of Cleome gyynandra by different in vitro free radical scavenging models. Journal of Interacademicia, 2009, 13(4), 430-436.

Battu GR, Ethadi SR, Veda PG, Swathi Pk, Chandrika K, Rao VA, et al. Evaluation of antioxidant and anti-inflammatory activity of Euphorbia heyneanaSpreng. Asian Pacific Journal of Tropical Biomedicine, 2011, 191-194.

Cotelle A, Bernier JL, Catteau JP, Pommery J, Wallet JC and GaydouEM.Antioxidant properties of hydroxyl-flavones.Free Radical Biology and Medicine, 1996, vol 20, 35-43.

Dolai N, Karmakar I, Kumar PBS, Kar B, Bala A and Haldar PK. Free radical scavenging activity of Castanopsisindica in mediating hepatoprotective activity of carbon tetrachloride intoxicated rats. Asi Pacific Jourcal of Tropical Biomedicine, 2012, 242-251.

Dash N, Nath LK, Bhise S, Bhuyan N. Antioxidant and antimicrobial activities if Heracleum nepalense D Don root. Tropical J Pharm Res. 2005; 4: 341-7.

Moncada A, Palmer PMJ, et al. Nitric Oxide: Physioology Pathophysiology and Pharmacology, 1991, 43, 109-142.

Naskar S, Islam A, Mazumder UK, Saha P, Haldar PK and Gupta M.In vitro and in vivo antioxidant potential of hydromethanolic extract of Phoenix dactylifera fruits. Journal of Scientific Research, 2010, vol 2, 144-157.

Peiyuan L, Lini H, Wei S, Rumeli L, Chaocheng D, Liangquan L, Yongkun D, Nana G, Chengsheng L and Chungling H. Free radical-scavenging capacity, antioxidant activity and phenolic content of Pouzolziazeylanica. Journal of Serbian Chemical Society, 2011, 76(5), 709-717.

Lin JY, Tang CY (2007) Determination of total phenolic and flavonoid contents in selected fruits and vegetables, as well as their stimulatory effects on mouse splenocyte proliferation. Food Antioxidant Studies Chemistry, 2007, vol101, 140-147.

A. Meda, C. E. Lamien, M. Romito, J. Millogo, and O. G. Nacoulma, Food Chemistry 91, 571 (2005).

CHAPTER 6: ACUTE TOXICITY STUDY

6.1. INTRODUCTION

Prior to start an in-vivo experiment using animal models it is necessary to evaluate the toxic characteristics of the test substance. Acute oral toxicity is determined by oral administration of a single dose of the test substance or multiple doses given within 24 h where adverse effect occurring within a short term (2-24 h). Acute oral toxicity study provides the information about the unwanted adverse effects arising due to exposure of external chemical substances. LD_{50} i.e., the dose which kills 50% of the test animals of a particular species is determined from acute oral toxicity. [Bruce R.D et al., 1985., Dixon W.J et al., 1965]

The safety of a drug or test substance is determined by its therapeutic index, greater the therapeutic index safer the test substance and vice versa. The objective of acute toxicity study is to establish the therapeutic index, therapeutic index is determined by the ratio between the pharmacologically effective dose (ED_{50}) and the median lethal dose (LD_{50}). [Choi, S.C et al., 1990, OECD Test Guideline 425, Ghosh MN et al.,2015].

In-vivo acute oral toxicity study was followed as per the standard protocol by Organization for Economic Co-operation and Development (OECD) test no.425, which is popularly known as up-and-down method. LD_{50} for this study was evaluated by using 10 animals of one species and strain against the hydroalcoholic extract of *Sonneratia Apetala* in Swiss albino mice [OECD Test Guideline 425].

6.2. PRINCIPLE

According to OECD guidelines, principle of the main test involves a single administration of dose in increased manner at a minimum of 48-hour intervals. The dose for the next animal is increased by the order of 3.2 times of initial dose, if animals remain alive. If there are signs of toxicity or lethalitythen next dose is decreased by a similar progression of dose. Animals are observed for 48 hours prior to make any decision on whatever and how much to dose the next animal. According to the guidelines dosing must be stopped when one of these criteria is pleased. The limit test was performed in maximum of five animals and the test dose used in this study was up to 2000 mg/kg or exceptionally 5000mg/kg (OECD Test Guideline 425, Kasture S et al., 2007).

6.3. EXPERIMENTAL ANIMALS

Adult female Swiss albino mice weighing 22-25g were grouped and housed in a clean polypropylene cage, maximum of four animals were kept in each cage. Animals were maintained

under standard laboratory conditions (room temperature 25°C, relative humidity 55-60% with illumination (dark/light) cycle 12/12h each day. Animals were fed with standard pellet diet procured from Kalyani feed milling plant, West Bengal.

6.4. TEST SAMPLE

Hydroalcoholic extract of *Sonneratia Apetala* (HASA), was solubilized in distilled water as per desired concentrations and sonicated until dissolved properly prior to administration.

6.5. TREATMENT SCHEDULE

The mice were fasted overnight. Then test samples were administered to the animals by oral gavage in a single dose. After administration of the test samples food was withdrawn for two hours.

6.6. OBSERVATIONS

All the animals were carefully observed after 30 minutes of dosing and the observation was continued first for 24h (first 4 hours were very crucial for observation) and then for further 24h, i.e., total 48h for morbidity or mortality. Additional observations for signs of toxicity included alteration of eye and mucosal membrane, respiratory, blood circulation, autonomic and central nervous systems and changes in behavioral pattern. Attentions were given any abnormalities like convulsions, salivations, diarrhea, lethargy, sleep and coma (OECD Test Guideline 425, Ghosh MN et al., 2008).

6.7. RESULTS AND CONCLUSION

The oral LD_{50} value of the hydroalcoholic extract of *Sonneratia Apetala* in Swiss albino mice was found to be 2000 mg/kg body weight. Based on this we selected 200 mg/kg body weight (low dose) and 400 mg/kg body weight (high dose) of HASA for our animal experiments.

References:

Acute Oral Toxicity (OECD Test Guideline 425) Statistical Programme (AOT425StatPgm).Version:
1.0,200[http//www.oecd.org/oecd/pages/home/displaygenerai/0,3380,EN-document5
24-nod i rectorate-no-24 -677 5-8.

Bruce R.D. (1985). An Up-and -Down Procedure for Acute Toxicity Testing.
Fundam. Appl. Tox., 5, 151-157.

Choi, S.C. (1990). Interval estimation of the LDs based on an up-and-down
experiment. Biometrics 46, 485 -492.

Dixon W.J.The Up-and-Down Method for Small Samples (1965). J. Amer.
Statist. Assoc. 60, 967-978.

Ghosh MN. Fundamentals of experimental pharmacology. Kolkata: Hilton &
Co.; 2008.

Kasture S. A handbook of experiments in pre-clinical pharmacology. Nashik,
India: Career Publications; 2007.

CHAPTER 7: IN-VITRO ANTICANCER STUDIES

7.IN-VITRO ANTICANCER STUDIES:

Using 5-FU as a reference standard, the in-vitro cytotoxicity of *Sonneratia Apetala* against EAC cells was assessed using the MTT test method. The in vivo anticancer activity of the plant was then assessed on Swiss albino mice.

7.1. MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) Assay:

7.1.1. Principle: This test measures the activity of a specific enzyme in cells by using a yellow dye called MTT. The dye is converted to a purple product in metabolically active cells, providing information about cell viability.

7.1.2. Method: 0.03 ml of EAC cells were added to a 96-well microplate with 10ml of PBS contaning 3×10^6cells. Each well was given 0.2ml of RPMI medium with penicillin and streptomycin. Then, 0.1 ml of various extract concentrations (50, 100, 150, 200, and 300mg/ml) in PBS were added. The control group received 0.1ml of PBS instead of the extract. The microplate was then placed in a CO2 incubator at 37°C for 24 hours with 5% CO_2. Each well was given 0.02ml of MTT solution (5 mg/ml) during the 24-hour incubation. After incubating for 4 hours at 37℃, the solution was removed and 0.02ml of DMSO was added to dissolve the purple formazan. The absorbance was then recorded at 570 nm to determine the IC50 value and inhibition percentage. (Tran et al., 2011; Van et al., 2011) Each value was measured triplicate. From the following formula the percentage inhibition calculated-

Percentage of inhibition =[(Absorbance of control – Absorbance of sample) /Absorbance of control]× 100

7.1.3. Statistical Analysis

Each value is shown as mean ± SEM. The IC50 (50% inhibitory concentration) values based on the concentration versus percentage inhibition plots.

7.1.4. Results and Discussion

The MTT experiment showed that after 24 hrs, the IC50 value was 22.92±3.92 This indicates that HASA is toxic to EAC cells and the longer the exposure time, the greater the toxicity to the cells.

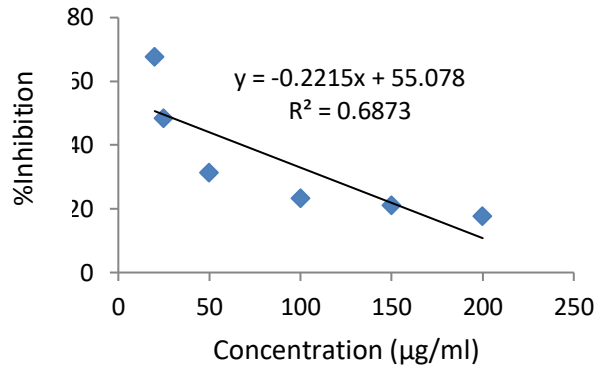


Figure 7.1: MTT assay of HASA. Each point represents mean ± SEM obtained from three individual experiments. The IC_{50} value at 24 hr was 22.92±3.92

7.1.5. Conclusion:

HASA has shown significant cytotoxic effects in laboratory tests, which have sparked interest in further studying its potential as an anticancer agent. As a result, the next phase of research involves studying its effects on cancer in Swiss albino mice.

References:

Tran SL, Puhar A, Ngo-Camus M, Ramarao N. Trypan Blue Dye Enters Viable Cells Incubated with the Pore-Forming Toxin HlyII of Bacillus cereus. Plos One.2011; 6: 22876.

Van Meerloo J, Kaspers GJ, Cloos J. Cell sensitivity assays: the MTT assay. Methods in Molecular Biology.2011; 731: 237-245.

CHAPTER 8: IN-VIVO ANTICANCER STUDIES

8. IN-VIVO ANTICANCER STUDIES:

The main goal of the study was to test the anticancer properties of a hydroalcoholic extract from *Sonneratia apetala* on Ehrlich Ascites Carcinoma (EAC) in Swiss albino mice.

8.1. Materials and method:

8.2. Chemicals:

Chemicals used for the study were Sodium Chloride (NaCl), Trypan blue, 5-fluorouracil (MERCK Limited, Mumbai, India). Trichloroacetic acid (TCA), Thiobarbituric acid (TBA), phenazine methosulphate (PMS), reduced nicotinamide adenine dinucleotide (NADH), nitroblue tetrazolium (NBT), dithionitro benzene (DTNB), Phosphate buffered saline (PBS), Glacial acetic acid, tris buffer, ethylenediamine tetraacetic acid (EDTA), glutathione (GSH) were obtained from Sigma chemicals. All other chemicals or reagents used for the experiment were of highest analytical grade.

8.3. Animals:

Healthy female Swiss albino mice of about 8 weeks of age with an average body weight of 23-25 g were used for the experiment. The mice were grouped and housed in poly acrylic cages (38 cm × 23 cm x 10 cm) with not more than six animals per cage (n=6). The animals were maintained under standard laboratory conditions (temperature 25-30 °C and 55-60% relative humidity with dark/light cycle 12/12 h) and were allowed free access to standard dry pellet diet and water ad libitum. The mice were acclimatized to laboratory conditions for 7 days before commencement of the experiment. All animal experiments performed according to CPCSEA (Approval number-JU/IAEC-24/53).

8.4. Transplantation of tumor:

The EAC cells were previously maintained in the animal house facility of Jadavpur University. Every ten days, 2×10^6 EAC cells were injected into each Swiss albino mouse to maintain the cells in the body. When the tumour cells were in the exponential growth phase (days 7-8), ascitic fluid was collected from a mouse bearing an EAC tumor. Each mice received 0.1 ml of a solution containing 2×10^6 tumour cells intraperitoneally.

8.5. Treatment schedule:

The groups are divided into 5 groups.

Group I: Normal saline control (Received 5 ml/kg, i.p.).

Group II: EAC control (Received 2×10^6 cells/mouse, i.p.).

Group III: Low dose + HASA (Received 200 mg/kg b.w., by oral gavage).

Group IV: High dose + HASA (Received 400 mg/kg b.w., by oral gavage).

Group V: 5-FU (Received 20mg/kg, i.p.).

30 female Swiss albino mice were divided into five groups (n=6).with six mice in each group. Except for Group I, all mice in each group were injected with EAC cells (2×10^6 cells/mouse i.p.). This was designated as day "0." Group II served as the EAC control, while Group I served as the standard saline control (5 ml/kg i.p.). Groups III and IV received hydroalcoholic extract *of Sonneratia apetala* (HASA) for nine consecutive days, starting 24 hours after EAC transplantation. The doses of HASA administered were 200 and 400 mg/kg b.w, orally. (Bala et al., 2010).

8.6. Tumor parameters:

The researchers utilized the animals' peritoneal cavities to extract the ascetic fluid and then measured its volume using a graduated tube. The packed cell volume was determined by centrifuging the ascetic fluid at 3000 rpm and measuring the volume of the resulting precipitate. (Yogesh et al., 2017).

8.7. Mean survival time:

The mean survival time of a particular group was estimated as (first death + Last death) ÷ 2 (Liobera et al., 2000).

8.8. Percentage increase in life span:

According to a study conducted on mice, researchers calculated the effect of HASA on life span by analyzing the difference in survival rates between the treatment group and the control group. This calculation is represented by the formula: ILS (%) = [(treatment group's average survival time ÷ control group's average survival time) - 1] x 100 (Sur et al., 1994).

8.9. Tumor cell count:

With a WBC pipette, I collected the ascetic fluid and mixed it 20 times with WBC fluid. Then, I used Neubauer's counting chamber to count the cells in each of the 64 (4 × 16) small squares using a drop of the diluted cell suspension. (Fuster et al., 2018).

8.10. Viable/ nonviable tumor cell count:

The scientists used the trypan blue test to see if the cell was alive or not. They used trypan blue dye with 0.4% concentration in normal saline to color the cells. When the dye was absorbed by the cells, they were no longer viable, but the cells that didn't absorb the dye remained alive (O'Brien et al., 2007). These viable and nonviable cells were counted according to the following formula:

Cell count = Number of cells × dilution factor / area × thickness of liquid film.

8.11. Hematological parameters:

The blood was collected by heart puncture from each group of mice. The blood was then taken into RBC and WBC pipettes, mixed with diluting fluids, and counted in a Neubauer counting chamber. The level of haemoglobin was determined using Sahil's hemoglobinometer. The blood components, such as RBC, WBC, and haemoglobin, were analyzed in different animal groups including treated, normal, and EAC control groups. (Hashem et al., 2020).

8.12. Biochemical parameters:

To determine the serum parameters, the blood samples were taken in EDTA tubes from each group and allowed to clot for 15 minutes before being centrifuged at 5,000 rpm for 100 min. The serum was then collected and analyzed usingadiagnostic reagent kit and a spectrophotometer to measure total protein, SGPT (Serum Glutamic Pyruvic Transaminase) and SGOT (Serum glutamic-oxaloacetic transaminase) (Ranji et al., 2019).

8.13. Preparation of liver and kidney homogenates:

The kidneys and liver that were dissected from the animals of each group and separated and homogenized in 10ml phosphate buffer (20 mM, pH7.4) before being centrifuged for 30 minutes at 4^{o} C at 12,000 rpm. The following tests, which are detailed below, were conducted using the supernatants that were collected to evaluate the antioxidant parameters. (Kou et al., 2017).

8.14. SOD (superoxide dismutase) assay:

The study was performed according to the Kakkar et al.,1984 to measurethe SOD activity. They mixed 0.1ml of liver and kidney tissue homogenate with 186 mM PMS, 780 mM NADH (Nicotinamide adenine dinucleotide), 100 mM phosphate buffer saline (pH 7.4), and 300 mM NBT(Nitro blue tetrazolium). The mixture was then incubated at 30^{o} C for 90 minutes. To stop the reaction, 1 ml of glacial acetic acid was added, and the absorbance of the chromogen produced was measured at 560 nm. The enzyme concentration required to decrease chromogen production by 50% in one minute is defined as one unit of SOD activity.

8.15. GSH (Glutathione) assay:

The GSH level was measured using the method Ellman et al., 1959. Approximately 0.1ml was determined using this method. Tissue homogenate and EDTA(0.02 M) were combined and placed in an ice bath for 10 min. After adding distilled water and 50% TCA, the mixture was once again placed in an ice bath for 10 min. The mixture was then centrifuged for 15 minutes at 3000 g. Triss buffer (pH 8.9) and DTNB were added to 1 ml of supernatant, and the absorbance at 412 nm was measured. A standard graph was created using various concentrations of a standard GSH solution (1mg/ml). With the help of the standard graph, GSH contents in the liver homogenates of the experimental animals were calculated.

8.16. Lipid peroxidation:

The study was performed according to the method of Ohkawa et al., 1979. Thiobarbituric acid reactive substances (TBARS) are utilized to quantify the level of lipid peroxidation in tissue homogenates. To do this, 0.5ml of each tissue homogenate was mixed with 10% w/v TCA and PBS (0.02 M, pH 7.4) and then left at room temperature for 30 minutes. The mixture was later centrifuged for 10 minutes at 3000 g, resulting in a pink color. When 1 ml of the supernatant was mixed with 1% w/v TBA and heated at 95^{o} C for 1 hour, a pink hue was produced. The absorbance of the sample at 535 nm was measured using a blank without tissue homogenate, but with all other chemicals. Since malondialdehyde (MDA) accounts for 99% of TBARS, the extinction coefficient of MDA (1.56 × 105 M-1 cm-1).

8.17. Histopathology Method:

For histological analysis, liver tissues were fixed in 10% formalin. After cutting the thin slices, they were stained with eosin and haematoxylin and examined under a light microscope (Olympus CX21i) in 10x zoom. (Uddandrao et al., 2018).

8.18. Statistical Analysis:

The data presented in this study represents the average standard error from three separate research projects. ANOVA was utilized to analyze the data from each experiment, while Dunnett's group means comparison was conducted with GraphPad Prism version 8.0.2.

8.19. Results:

8.19.1. Tumor Parameters:

When the EAC control group was compared to the group treated with HASA, there was a significant reduction in tumor volume, packed cell volume, and the number of EAC cells. Values are summarized in a table 8.1.

8.19.2. Life Span:

In comparison to the EAC control group to the standard, low dose HASA and high dose HASA group there was a substantial increase in both the mean survival time and the percentage of life span. The values are summarized in table 8.1.

8.19.3. Hematological parameters:

In contrast to the EAC control group to the standard, low dose HASA and high dose HASA group, the treated group's WBC count was found to be lower, but its haemoglobin and red blood cell counts were much higher and eventually returned to normal (Table 8.2).

8.19.4. Serum Parameters:

During the analysis of various blood parameters, it was found that HASA group had a higher total protein content compared to the EAC control group. Additionally, levels of SGOT (serum glutamic oxaloacetic transaminase) and SGPT (serum glutamic pyruvic transaminase) readings were significantly restored to normal levels in the HASA treated groups when compared to the EAC control group. Values are summarized in a table 8.1.

8.19.5. Antioxidant Parameters:

After comparing the groups treated with the extract to the EAC group, it was found that the level of malondialdehyde decreased, indicating a decrease in lipid peroxidation in the HASA-treated groups. It was also observed that both enzymatic and non-enzymatic antioxidants, like reduced glutathione and superoxide dismutase, had returned to their normal levels comparing to the EAC group. Values are summarized in a table 8.2 and 8.4

Table 8.1: Table shows the effect of HASA on various parameters such as tumor volume, packed cell volume, cell count, % of viability, % of nonviable cells, Mean survival time and the increase in life span. Values are represented as mean ± SEM. EAC control vs Treated group $^{*}p<0.05$.

Groups	Tumor Volume (ml)	Packed Cell Volume (ml)	Cell count (x 10^7/ml)		Viable %	Non-viable %	MST (Day)	ILS %
			Viable	Non-Viable				
EAC Control	1.83±0.07	1.61±0.06	7.72±0.27	0.40±0.01	95.07	4.93	20	0
EAC +200 mg/kg HASA	1.08±0.0*	0.74±0.04*	5.70±0.1*	3.30±0.0*	63.33	36.66	30	50
EAC +400 mg/kg HASA	0.85±0.0*	0.66±0.04*	3.98±0.0*	4.18±0.0*	48.77	51.22	36.5	82.5
EAC+5-FU(5mg/kg)	0.67±0.0*	0.38±0.02*	2.67±0.0*	5.43±0.0*	32.96	67.04	40	100

Table 8.2: Effects of HASA extract on hematological parameters. Values are represented as mean ± SEM. $^{a}p<0.05$ when compared to normal, $^{b}p<0.05$ when compared with EAC control group.

Parameters	Normal	EAC Control	EAC + 200mg/kg HASA	EAC + 400mg/kg HASA	EAC + 20mg/kg 5-FU
RBC	6.36 ± 0.13	3.15 ± 0.06[a]	3.74 ± 0.07[b]	5.01 ± 0.03[b]	6.3 ± 0.05[b]
WBC	3.49 ± 0.03	30.63 ± 0.04[a]	19.21±0.05[b]	16.19 ± 0.09[b]	14.67 ± 0.04[b]
Hb	10.9 ± 0.07	5.2 ± 0.11[a]	7.1 ± 0.11[b]	9.8 ± 0.08[b]	10.2 ± 0.05[b]

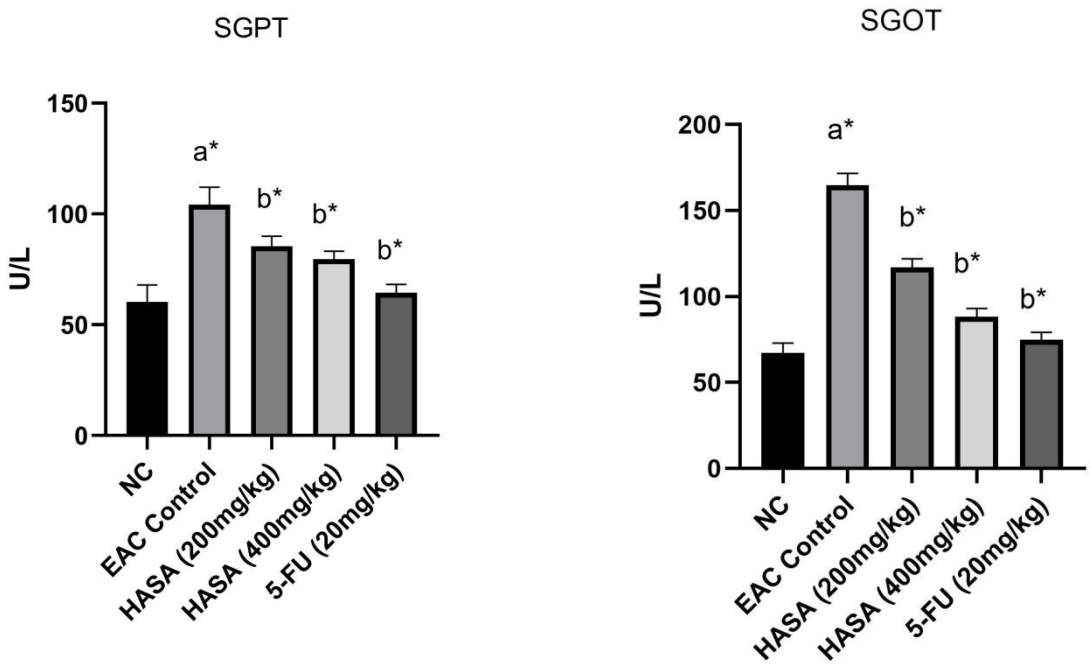
Table 8.3: Effects of HASA extract on serum parameters. Values are represented as mean ± SEM. [#]p<0.05 when compared to Normal and [*]p<0.05 when compared to EAC Control.

Parameters	Normal	EAC Control	200mg/kg HASA	400mg/kg HASA	20mg/kg 5-FU
Total protein (g/dl)	6.5 ± 0.22	2 ± 0.33[#]	3.7 ± 0.32[*]	4.4 ± 0.31[*]	5.7 ± 0.33[*]
SGOT (U/L)	74.89 ± 2.28	154.71 ± 2.86[#]	120.89 ±2.10[*]	84.89 ± 2.02[*]	80.29 ± 1.83[*]
SGPT (U/L)	67.5 ± 3.11	101.31 ± 3.20[#]	91.31 ± 1.84[*]	85 ± 1.46[*]	60.9 ± 1.53[*]

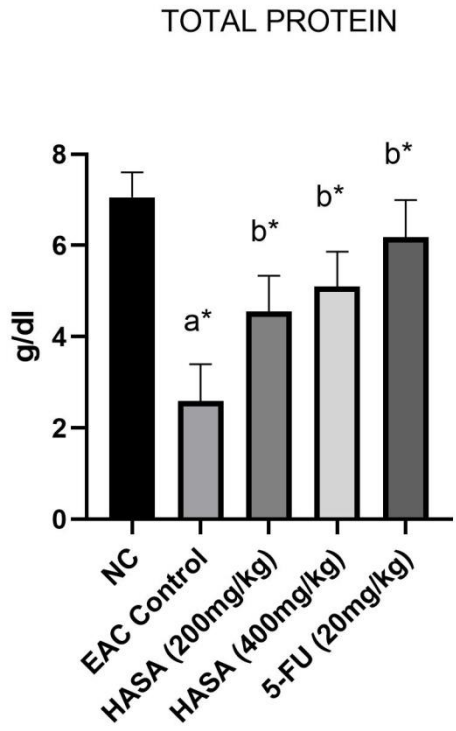
Table 8.4: Effects of HASA on different antioxidant parameters. The values are represented as mean ± SEM. a*,a# p<0.05 when EAC control compared to normal livers, and kidney, b*, b# p<0.05 when EAC control compared to EAC livers and kidneys.

Groups		Lipid peroxidation (nM MDA/mg protein)	Superoxide Dismutase (mU/min/mg protein)	Reduced Glutathione (ng/GSH/mg protein)
Normal	Liver	18.03 ± 2.22	94.45 ± 1.53	726.64 ± 17.03
	Kidney	30.82 ± 2.20	100.85 ± 1.80	764.03 ± 11.56
EAC	Liver	281.67 ± 10.79[a*]	28.41 ± 0.90[a*]	320.72 ± 2.25[a*]
	Kidney	352.94 ± 14.03[a#]	40.05 ± 1.89[a#]	301.42 ± 4.96[a#]
200mg/kg	Liver	89.52 ± 5.32[b*]	55.84 ± 1.52[b*]	350.43 ± 9.63[b*]

HASA	Kidney	$115.18 \pm 8.28^{b\#}$	$55.06 \pm 1.60^{b\#}$	$371.10 \pm 9.71^{b\#}$
400mg/kg HASA	Liver	$82.68 \pm 3.60^{b*}$	$71.45 \pm 1.26^{b*}$	$498.87 \pm 16.22^{b*}$
	Kidney	$77.54 \pm 4.50^{b\#}$	$73.25 \pm 1.58^{b\#}$	$450.98 \pm 10.16^{b\#}$
5-FU	Liver	$32.5 \pm 4.78^{b*}$	$86.03 \pm 1.26^{b*}$	$620.83 \pm 19.77^{b*}$
	Kidney	$40.22 \pm 2.39^{b\#}$	$83.65 \pm 1.70^{b\#}$	$620.45 \pm 15.20^{b\#}$



(A) **(B)**



(C)

Figure 8.1: Effects of different concentration of HASA on serum biochemical parameters. Values are represented as mean ± SEM. a* $p<0.05$ EAC control compare to normal and b* $p<0.05$ when treated groups compared to EAC control. (A) Represent the value of SGPT. (B) Represent the value of SGOT. (C) Represent the value of Total Protein.

LIVER

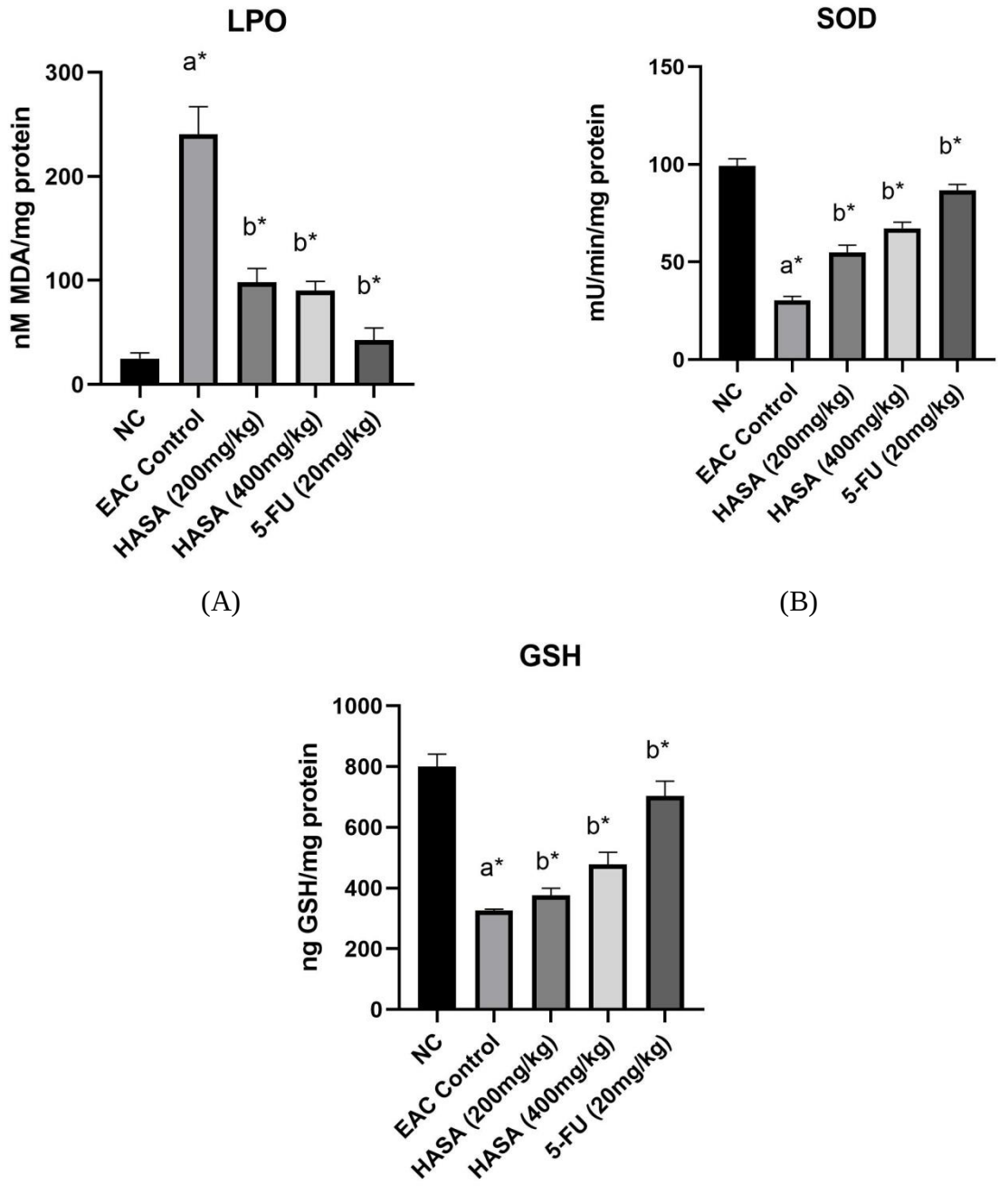
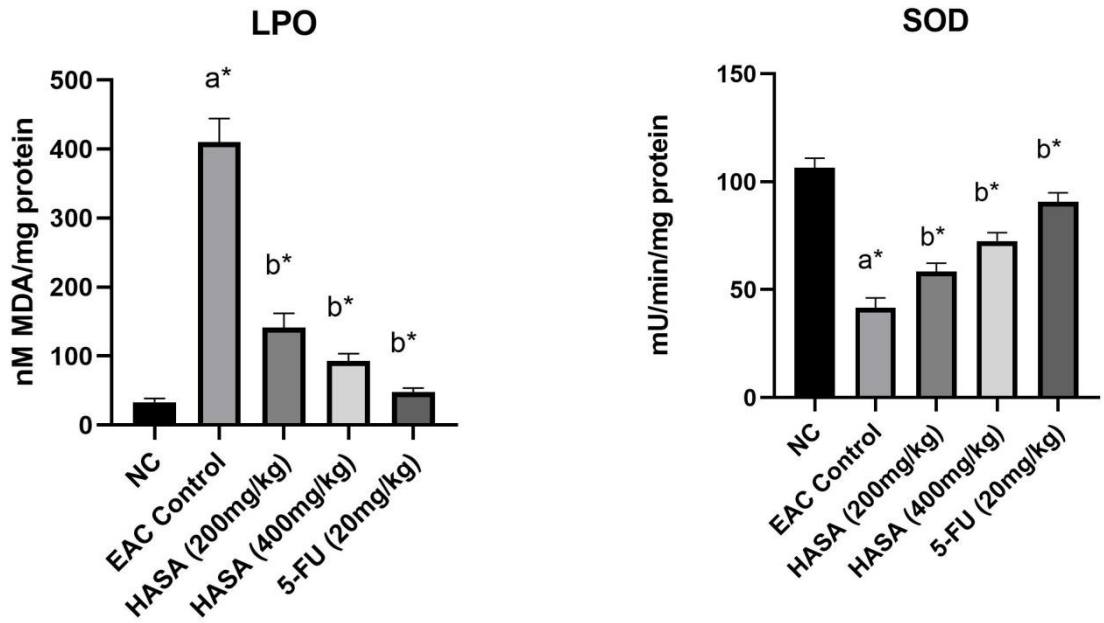


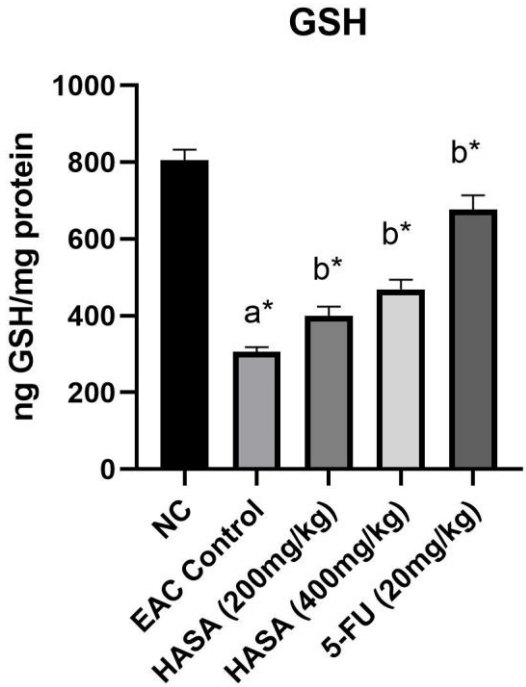
Figure 8.2: (A) Effects of different concentration of HASA on lipid peroxidation. (B) Effects of different concentration of HASA on SOD. (C) Effects of different concentration of HASA on GSH. Values are represented as mean ± SEM. a* $p<0.05$ EAC control compared to normal liver and b* $p<0.05$ when treated groups compared to EAC liver.

KIDNEY



(A)

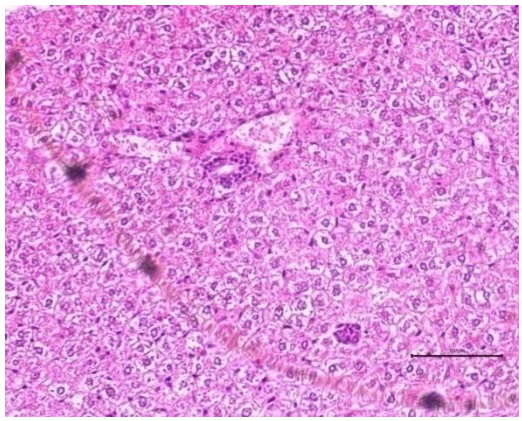
(B)



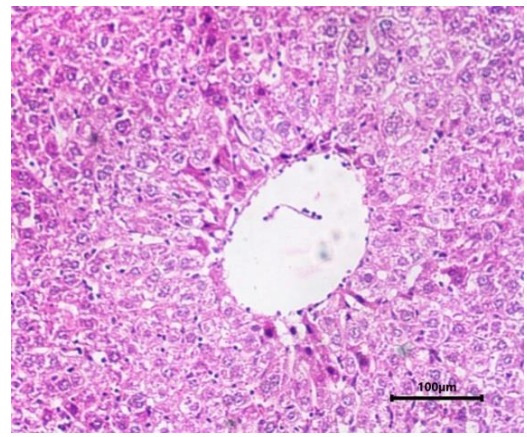
(C)

Figure 8.3: (A) Effects of different concentration of HASA on lipid peroxidation. (B) Effects of different concentration of HASA on SOD. (C) Effects of different concentration of HASA on GSH. Values are represented as mean ± SEM. a* $p<0.05$ EAC control compared to normal kidney and b* $p<0.05$ when treated groups compared to EAC kidney.

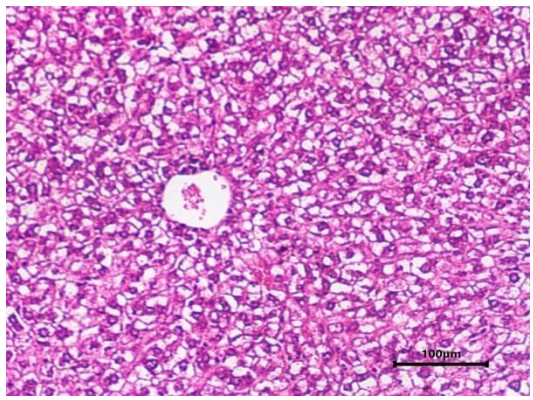
8.19. Histopathology:



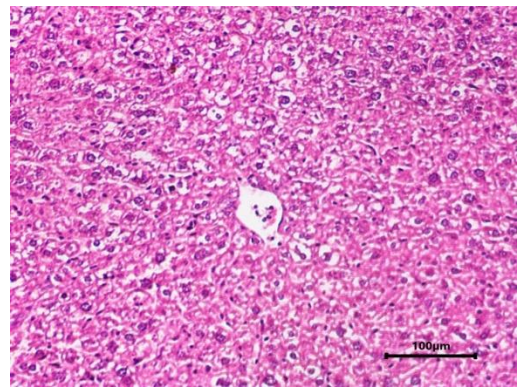
A. Normal Control Liver



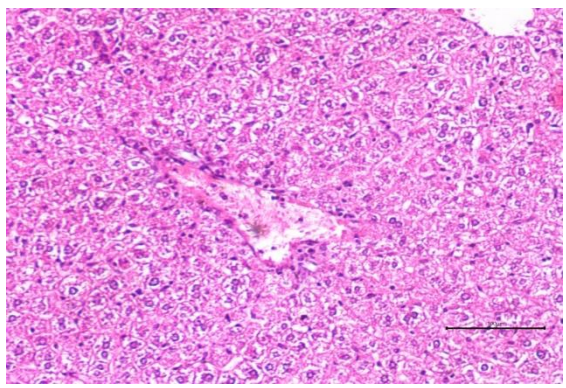
B. Disease Control Liver



C. 200mg/kg HASA Liver



D. 400mg/kg HASA Liver



E. 5-FU 20mg/kg Liver

Figure 8.8: Histopathology of experimental mice. A) Normal liver tissue showing prominent sinusoids. B) Predominantly acute inflammation in EAC control. C) Less acute inflammation in HASA low dose treated group. D) Regeneration of hepatocytes in HASA high dose treated group. E) Regeneration of functioning sinusoids in 5FU treated group.

8.20. Discussion:

In this study, we used the EAC cell line to investigate the anticancer properties of HASA. EAC cells are aggressive murine mammary adenocarcinoma cells that grow rapidly and can impact various strains of mice. When the Ehrlich ascitic tumor is implanted, it triggers a localized inflammatory response, increasing vascular permeability and leading to the formation of edema, cell migration, and a gradual accumulation of ascitic fluid (Dolai et al., 2012; Bala et al., 2010). Since the ascetic fluid provides tumour cells with a direct supply of sustenance, it is necessary for the formation of tumours (Shimizu et al., 2004). When compared to the tumour control group, HASA therapy was able to lower tumour volume, packed cell volume, and viable cell count, most likely as a result of its effectiveness in suppressing the peritoneal ascetic fluid. These findings suggest that HASA may have a direct cytotoxic impact on tumour cells or that it may have an indirect local effect that includes reduction of vascular permeability and stimulation of macrophages (Bala et al., 2010). According to Gupta et al. (2004), a reliable way to measure the effectiveness of an anticancer drug is by observing the extended lifespan of mice that have been treated with it. In a study comparing mice treated with HASA to those treated with a tumor, the HASA-treated group lived longer, indicating that HASA could be a promising option for cancer treatment. The most common challenges faced in cancer treatment are myelosuppression and anemia, as noted by Price and Greenfield (1958). When there is a breakdown of haemoglobin or a decrease in red blood cells, it can lead to anaemia. Treatment with HASA significantly improved haemoglobin and red blood cell levels compared to the group with tumours. After receiving HASA therapy, the white blood cell count, which had increased in the group with tumours, returned to nearly normal levels. Researchers have been studying serum enzymes as possible early indicators of cancer and as a way to monitor the progression and regression of illness (Kathiriya et al., 2010). Hepatotoxicity can occur when a harmful agent directly damages the liver or through toxic byproducts. In certain conditions, these substances may even lead to cancer (Dolai et al., 2012). Through our study, we found that the control group treated with EAC had higher levels of liver enzymes linked to hepatocellular damage, such as SGOT, SGPT while their total protein levels were lower. However, these biochemical markers returned to normal levels in the group treated with HASA. Imbalance between reactive oxygen metabolites and antioxidant defense mechanisms can cause "oxidative stress," disrupting various cellular processes and triggering pathological conditions (Bandyopadhyay et al.,1999 and Adesegun et

al., 2009). Yagi et al.,1991 found that oxidative stress can harm macromolecules such as lipids, leading to lipid peroxidation in living organisms. EAC-bearing mice showed significantly higher levels of lipid peroxides in the liver and kidney, but in the group treated with HASA, these levels were close to normal. This indicates that the extract can reduce the production of free radicals, thereby decreasing oxidative stress. Glutathione (GSH) is a potent inhibitor of neoplastic growth and a vital part of the body's natural antioxidant defense system. It plays a crucial role in protection and is present in high concentrations in the liver (Haldar et al.,2010). Mice with cancer had lower levels of a substance called reduced glutathione (GSH), possibly due to the disease's increased production of harmful molecules known as free radicals. However, when treated with HASA, the GSH levels in the liver of these mice were seen to rise compared to those in the control group with tumors. The body's natural defense system against free radicals includes enzymes, which work together to neutralize damaging substances like hydrogen peroxide and superoxide. The study also showed that the activity of SOD was reduced as tumors developed. Similar results were seen in mice with EAC tumors in this research. Treatment with HASA significantly increased the SOD levels indicating antioxidant and free radical scavenging activity of the extract.

References:

Adesegun SA, Fajana A, Orabueze C I and Coker HAB. Evaluation of Antioxidant Properties of Phaulopsisfascisepala C.B.CI.(Acanthaceae).eCAM, 2009, vol6, no2, 227-231.

Aebi H. Catalase, in: Packer L, ed. Methods in Enzymatic Analysis, New York: Academic Press, 1974, 673-84.

Bala A, Kar B, Haldar PK, Mazumder UK and Bera S. Evaluation of anticancer activity of Cleome gynandra on Ehrlich's ascites carcinoma treated mice. Journal of Ethnopharmacology, 2010, 129, 131-134.

Yogesh, N. (2017). Evaluation of in Vivo Anticancer Activity of Ethanolic Extract of Leaf of Plumeria Acutifolia on Daltons Ascites Lymphoma Induced Cancer in Mice (Doctoral dissertation, JKK Nattraja College of Pharmacy, Komarapalayam).

Llobera, J., Esteva, M., Rifa, J., Benito, E., Terrasa, J., Rojas, C., ...& Avella, A. (2000). Terminal cancer: duration and prediction of survival time. European journal of cancer, 36(16), 2036-2043.

Fuster, O., Andino, B., Pardo, A., &Laiz, B. (2018).Continuous ambulatory peritoneal dialysis, ascitic and pleural body fluids evaluation with the Mindray BC-6800 hematology analyzer.Journal of Clinical Laboratory Analysis, 32(2), e22240.

O'Brien, C. A., Pollett, A., Gallinger, S., & Dick, J. E. (2007).A human colon cancer cell capable of initiating tumour growth in immunodeficient mice. Nature, 445(7123), 106-110.

Hashem, M. A., Mahmoud, E. A., & Abd-Allah, N. A. (2020).Alterations in hematological and biochemical parameters and DNA status in mice bearing Ehrlich ascites carcinoma cells and treated with cisplatin and cyclophosphamide. Comparative Clinical Pathology, 29, 517-524.

Ranji, P., Agah, S., Heydari, Z., Rahmati-Yamchi, M., & Alizadeh, A. M. (2019).Effects of Lactobacillus acidophilus and Bifidobacterium bifidum probiotics on the serum biochemical parameters, and the vitamin D and leptin receptor genes on mice colon cancer.Iranian Journal of Basic Medical Sciences, 22(6), 631.

Kou, C. H., Han, J., Han, X. L., Zhuang, H. J., & Zhao, Z. M. (2017).Preparation and characterization of the Adriamycin-loaded amphiphilic chitosan nanoparticles and their application in the treatment of liver cancer. Oncology letters, 14(6), 7833-7841.

Bandyopadhyay U, Das D, and Banerjee RK. Reactive oxygen species: Oxidative damage and pathogenesis. Current science, 1999, vol77, 658.

Dolai N, Karmakar I, Kumar RBS, Kar B, Bala A, Haldar PK. Antioxidant activity of *Anthocephaluscadamba*attenuates the viability of Ehrlich Ascites Carcinoma cells in Mice. Journal of Ethnopharmacology 2012, 142, 865-870.

EIIman GL. Tissue sulfhydryl groups. Arch BiochemBiophys, 1959, 82, 70-77.

Gupta M, Mazumder UK, Kumar S, R., Sivakumar T, and Vamsi MLM.Antitumour activity and antioxidant status of Caesapiniabonducell against Ehrlich ascites carcinoma in Swiss albino mice. Journal of Pharmacological Science, 2004, vol94, 177-184.

Haldar PK, Kar B, Bala A, Bhattacharya S, Mazumder UK, Antitumor activity of *Sansevieriaroxburghiana*rhizome against Ehrlich's ascites carcinoma in mice. *Pharmaceutical Biology.*2010; 48: 1337-43.

Kakkar P, Das B, Vishwanath PN. A modified spectrophotometric assay of superoxide dismutase.*Indian Journal of Biochemistry and Biophysics.*1984; 2: 130-32.

Kathiriya K, Das EP, Kumar KB. Mathai Evaluation of antitumor and antioxidant activity of *Oxalis corniculataLinn.*Against Ehrlich Ascites Carcinoma on mice.*Iranian Journal Cancer Preview.*2010; 3: 157- 65.

Louzada S, Adega F and Chaves R. Defining the sister rat mammary tumor cell lines HH-16 cl.2/1 and HH-16.cl.4 as an in vitro cell model for Erbb2. PloS one 2012, 7(1), 29923.

Ohkawa H, Onishi N and Yagi K. Assay for lipid peroxidation in animal tissue by thiobarbituric acid reaction.Analytical Biochemistry, 1979, 95, 351-58.

Uddandrao, V.V.S., Parim, B., Nivedha, P.R. et al. Anticancer activity of pomegranate extract: effect on hematological and antioxidant profile against ehrlich-ascites-carcinoma in Swiss albino mice. Orient Pharm Exp Med 19, 243–250 (2019). https://doi.org/10.1007/s13596-018-0348-4

Prince VE and Greenfield RE. Anemia incancer, in: Greenstein, J.P., Haddow, A., (eds.) Advances in cancer research. Vol 5. New York: Academic Press. 1958, pp. 199-200.

Shimizu M, Azuma C, Taniguchi T and Murayama T. Expression of cytosolic phospholipase A2α in murine C12 cells, a variant of L929 cells, induces arachidonic acid release in response to phorbol myristate acetate and Ca2+ ionophores, but not to tumor necrosis factor-α. Journal of Pharmacological Science, 2004, vol96, 324-322.

Sur P, Bag SP, Khanam JA. Chloroacetohydraxamic acid as antitumor agent against Erlich Ascites Carcinoma in mice.*Neoplasma.*1997; 44: 197-201.

Yagi K. Lipid peroxides and human diseases. Chemistry and Physics of Lipids, 1991, vol45, 337-51.

CHAPTER 9: CONCLUSION

9. Conclusion:

This thesis is groundbreaking in the realm of scientific research on the methanol extract of the *Sonneratia apetala* plant. It delves into the topic of cancer, the utilization of natural products for cancer treatment, as well as an in-depth analysis and review of the plant being studied. The subsequent chapter details the processes of collection, extraction, and chemical characterization. Following that, there is a section dedicated to investigating acute toxicity. Subsequent chapters explore antioxidant studies, in-vivo anticancer activity, and in-vitro cytotoxicity. It appears that the hydroalcoholic extract of *Sonneratia apetala* fruits shows potential anticancer properties according to both laboratory and animal studies. The compounds mentioned in an earlier section of the paper, found in the plant, could be contributing to these effects. Alkaloids and flavonoids are two types of compounds known for their healing properties against various diseases, including cancer. Therefore, the effectiveness of the extract in fighting cancer might be attributed to the presence of flavonoids, alkaloids, or both. Research on antioxidants has shown that the extract possesses strong free radical scavenging abilities, indicating potential benefits in fighting cancer. Free radicals, known for causing mutations that can lead to cancer, can be effectively countered by the extract's antioxidant properties, reducing the risk of harmful effects.

Nonetheless, it is not enough to simply speculate that the extract's ability to combat cancer is linked to phytoconstituents. To pinpoint the specific molecule or compounds in the extract that are truly responsible for its anticancer properties, extensive isolation and characterization methods need to be employed. EAC cells were utilized as the basis for both the laboratory and animal studies on the extract's anticancer effects. To truly confirm it’s potential in fighting cancer, more well as other cancer models both in laboratory and animal studies, are essential.

9.1. Further scope:

It might include:

- Working on human specific cell lines and apoptotic studies.
- Isolation of phytoconstituents separately through LC-MS, HPLC and using other techniques related to find out active molecule.
- Find out molecular structure and functional groups/rings responsible for its activity.
- Formulation aspects.


Thank You