EVALUATION OF THE ANTICANCER POTENTIAL OF STEM BARK EXTRACTS/FRACTION(S) OF *Prosopis africana* AND *Tamarindus indica* AGAINST MCF-7 CELL LINE

BY

SAIDU, Aishatu

MTech/SLS/2018/8022

DEPARTMENT OF BIOCHEMISTRY

FEDERAL UNIVERSITY OF TECHNOLOGY, MINNA.

JUNE, 2023

EVALUATION OF THE ANTICANCER POTENTIAL OF STEM BARK EXTRACTS/FRACTION(S) OF *Prosopis africana* AND *Tamarindus indica* AGAINST MCF-7 CELL LINE

BY

SAIDU, Aishatu
MTech/SLS/2018/8022

THESIS SUBMITTED TO THE POSTGRADUATE SCHOOL, FEDERAL UNIVERSITY OF TECHNOLOGY, MINNA, NIGERIA IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF TECHNOLOGY IN BIOCHEMISTRY

JUNE, 2023.

ABSTRACT

Breast cancer is the leading cause of cancer deaths among women globally. Current drugs and therapies for cancer treatments are accompanied with various side effects causing limitations on the dose of drug administered over time which results to high incidence rate of cancer cases globally. This study was designed to evaluate the anticancer potentials of stembark extracts/fraction(s) of *P. africana* and *T. indica* against MCF-7 cell lines. The quantitative phytochemical compositions and the partitioning of the crude methanol extract of *P. africana* and *T. indica* were determined using standard methods. Preliminary cytotoxic screening of the more active extract fractions was carried out using Brine Shrimps lethality test. Whereas, the cytotoxic activity of the most active fraction was evaluated using CellTiter-Glo® 2.0 assay kit against MCF-7 cell lines at concentrations of 50, 100, 250, 500 and 1000µg/ml for 24hrs and 48hrs respectively. Adenosine triphosphate (ATP) assay was carried out to determine the number of surviving cells after treatment. The quantitative phytochemical compositions of the crude methanol extract of *P. africana* revealed the presence of Phenols (848.78±0.29), Saponins (236.27±0.68), Tannins (560.23±0.77), Flavonoids (65.65±0.06) and Alkaloids (105.72±0.04); whereas, *T. indica* revealed the presence of Phenols (744.38±0.17), Saponins (357.03±0.03), Tannins (47.94±0.01), Flavonoids (174.68±0.15) and Alkaloids (359.62±0.48). The acute oral toxicity test of the crude extracts shows negative results on all the tested rats and the lethal dose (LD_{50}) was recorded to be greater than 5000mg/kg. The cytotoxic effects of *P. africana* crude extract on brine shrimps exhibited higher activity with lethal concentration (LC_{50}) 31.62ppm than *T. indica* crude extracts with LC_{50} 109.78ppm. The most active fraction (aqueous fraction) of *P. africana* with LC_{50} of 9.20µg/ml exhibited significantly higher cytotoxic activity than other fractions with an LC_{50} of 49.24µg/ml (ethyl acetate) and 1156.04µg/ml (n-hexane). The aqueous fraction of *P. africana* at 1000µg/ml exhibited 88% cytotoxicity against MCF-7 cell lines when exposed for 24hrs with an inhibitory concentration (IC_{50}) of 235.87µg/ml and 91% after 48hrs of exposure with an IC_{50} of 52.04µg/ml. The Adenosine triphosphate (ATP) assay result shows a dose and time dependent activity against MCF-7 cell lines. The results of this study revealed that the aqueous fraction of *P. africana* significantly reduced the proliferation of MCF-7 cell lines and may have a potential therapeutic agent for treatment of breast cancer.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

ABBREVIATIONS

Abbreviations	**Meaning**
WHO	World Health Organization
LD_{50}	Lethal dose
DALYs	Disability-Adjusted Life Years
ASIR	Age-standardized incidence rates
HDI	Human Development Index
MIR	Mortality-to-incidence ratio
CK	Cytokines CSCs
Cancer stem cells bCSCs cancer stem cells	Breast
BRCA2/BRCA1	Breast cancer gene 2 & gene 1
HRT	Hormonal replacement therapy
SSRI	Selective serotonin reuptake inhibitors
BMI	Body Mass Index
SFN	Sulforaphane

DDT	Dichlorodiphenyltrichloroethane PCB Polychlorinated biphenyl
PAH	Polycyclic aromatic hydrocarbons
LCIS	Lobular carcinoma in situ
ILC	Infiltrating lobular carcinoma
ER	Estrogen receptor
EMT	Epithelial-mesenchymal transition
ROS	Reactive oxygen species
VEGF	Vascular endothelial growth factor
EGCG	Epigallocatechin-3-gallate
ECG	Epicatechin-3-gallate
EC	Epicatechin
EGC	Epigallocatechin
MMP-2	Matrix metalloproteinase-2
FBS	Foetal bovine serum
DMEM	Dulbecco's Modified Eagle's medium
PBS	Phosphate buffer saline
BSLA	Brine Shrimp Lethality Assay

LIST OF APPENDICES

Appendix **Title** **Pages**

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

The word "cancer" refers to a group of diseases that are known for their unchecked cellular growth, invasion of healthy cells, and dissemination to distant organs via blood and lymphatic vessels (Elnour *et al*., 2017; Lewandowska *et al.,* 2019). The basic processes that underlie the growth of cancer include the change of healthy cells into precancerous lesions that later evolve into malignant tumors in a multistage process (Idikio, 2011). These biological modifications are as a result of the interaction of a person's genetic makeup with outside factors such as physical, chemical and biological carcinogens (Yadav *et al*., 2018). Molecular insights show that the ability of cancerous cell to proliferate in an uncontrolled manner, escape apoptosis, invade neighboring tissues and distant organs is as a results of oncogenes, tumor suppressor genes, and repair genes (Idikio, 2011). These processes occur in a multistep manner and often take place over many years; this is a key factor that cancer manifests at older age when sufficient carcinogenic mutations have accumulated and cellular repair mechanisms have been suppressed (Idikio, 2011).

The World Health Organization (WHO) described cancer as one of the leading causes of morbidity and mortality globally (WHO, 2018). Although it is difficult to assess the validity of individual aetiological factors, it can be concluded that interaction of several risk factors plays the major role in cancer development. Such as biological factors (poor diet, lack of physical activity, mutagenic or carcinogenic compounds in food formed as a result of poor food storage and food processing, infection, etc.), chemical factors (Tobacco smoking, alcohol, etc.), and physical factors (exposure to electromagnetic field,

ultraviolet radiation, ionizing radiation, etc.) (Lewandowska *et al*., 2019). The International Agency for Research on Cancer's; Global cancer incidence, mortality and prevalence (GLOBOCAN) 2018, estimates cancer incidence and mortality to be the leading cause of death throughout the world in the twenty-first century (Bray *et al*., 2018). In 2018, there were an estimated 18.1 million new cancer cases and 9.6 million cancer deaths globally (Bray *et al*., 2018; WHO, 2018). Lung cancer and female breast cancer were the most commonly diagnosed malignancy (each

11.6% of total overall cases), followed by cancer of colon and rectum (10.2%), prostate (7.1%), stomach (5.7%), and liver (4.7%) (Bray *et al*., 2018; WHO, 2018). The increasing cancer burden is due to several factors, including population growth and ageing as well as the changing prevalence of certain causes of cancer, linked to social and economic development (WHO, 2018). Worldwide, breast cancer, which affects predominantly women, is the most common malignancy and the second leading cause of cancer death mostly in less developed countries, although in rare occasions breast cancer can affect men or children (Ghoncheh *et al*., 2019). Increased incidence of cancer in recent years and its impact on different physical, mental, and social dimensions of human life have turned it to a major problem of the century (Poorkiani *et al*., 2019). Breast cancer mainly involves the inner layer of milk glands or lobules and ducts (tiny tubes that carry the milk).

The primary risk factors of breast cancer include age, hormonal imbalance, race, economic status, and iodine deficiency in diet (Zillich *et al*., 2022). The incidence of breast cancer is almost 1-in-8 women, requiring complete tissue removal, chemotherapy, radiotherapy, and hormone therapy (Heravi *et al*., 2016). Till today, despite considerable efforts, cancer still remains an aggressive killer worldwide.

1.2 Statement of the Research Problem

Breast cancer is the leading cause of death throughout the world (Bray *et al*., 2018; WHO, 2018). Breast cancer, which affects predominantly women, is the most common malignancy and the second leading cause of cancer death mostly in less developed countries (Ghoncheh *et al*., 2019). The incidence of breast cancer is almost 1-in-8 women (Heravi *et al*., 2016). Breast cancer has present an important effect on society and life quality of women (such as premature death and reduced productivity). Whereas, conventional therapies for the treatment and management of breast cancer have several side effects due to their lack of specificity (affecting both tumor and normal cells), causing systemic toxicity and increased risk of other forms of cancers (Nguyen *et al.,* 2019). Although extensive research efforts have been made to understand breast cancer and find new ways to combat it, nonetheless, the available treatments mainly include; surgery, radiotherapy, immunotherapy and systemic therapies (chemotherapy, hormonal therapy and targeted therapies), but these techniques adversely affect the healthy cells. These therapies cause undesirable side effects that may vary from short term to life threatening conditions which leads to a low therapeutic index and consequently puts limitations on the dose of the drug to be administered over time (Alexander, 2019). However, the increased resistance of cancerous cells to anticancer agents or drugs has presented a great challenge worldwide, in form of unsatisfactory outcomes and increase rate of cancer deaths. Furthermore, due to the prohibitive cost of anticancer therapies and high level of poverty in Nigeria, patients without health insurance or full government support has resulted to increased cancer cases and more cancer deaths (Chun, 2019; Braakhuis *et al.*, 2019). These necessitate a need to fold back on exploring plant source remedies.

1.3. Aim and Objectives

1.3.1. Aim of the study

The aim of this study was to evaluate the anticancer potential of the stembark extracts/fraction(s) of *Prosopis africana* and *Tamarindus indica* using MCF-7 cell lines.

1.3.2. Objectives

The objectives of this study were to determine the;

i. phytochemical composition of methanol extracts of *P. africana* and *T. indica* (stem bark) ii. lethal dose (LD_{50}) of methanol extracts of *P. africana* and *T. indica* crude extracts

iii. IC_{50} of the methanol extracts of *P. africana and T. indica* on brine shrimps and subsequently solvent partitioning on the more active extract.

iv. IC_{50} of the solvent fractions of the more active extract on brine shrimps and the antioxidant activity of the most active fraction using DPPH scavenging assay

v. cytotoxic effect of the most active fraction on MCF-7 cell line.

1.4 Justification for the Study

Herbal medicine as an imperative branch of complementary and alternative medicine has increasingly grown in the last decade for cancer-related goals, making them a reliable means to increase efficacy and reduce toxicity associated with chemotherapy in different malignancies (Darvishi *et al.,* 2017). Many experiments have demonstrated that natural

products can effectively regulate proliferation, differentiation and expression of breast cancer cells *in vitro* and *in vivo* (Barretina *et al*., 2022). Natural products have served as an infinite reservoir of various diversified chemical compounds, driving pharmaceutical industries for many years (Mishra and Tiwari, 2011; Henciya *et al*., 2017). In the discipline of cancer therapeutics, natural products hold a great potential (Jiang *et al.,* 2019). In accordance to World Health Organization, about 80% of population depends upon plantderived traditional medicines (Jiang *et al.,* 2019). *Prosopis africana* commonly known as

African mesquite (iron tree) is the only genus indigenous to Africa, (Chukwunomso, *et al.,* 2020). The species belonging to the *Prosopis* genus have been traditionally used for the treatment of asthma, birth/postpartum pains, conjunctivitis, diabetes, diarrhea, expectorant, fever, flu, lactation, liver infection, malaria, pains, rheumatism, scabies, skin inflammations, stomach ache (Abarca *et al.,* 2007; Cattaneo *et al.,* 2014; Ahmed *et al.,* 2015; Umair *et al.,* 2017; Jahromi *et al.,* 2018; Younis *et al.,* 2018).Whereas *Tamarindus indica* (*T. indica*) commonly referred to “Saamia” in Hausa or “date of India”. It is one of

the fruit tree species that is used as traditional medicine extensively (Hussein *et al.,* 2017).

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Cancer

Cancers are a group of diseases characterized by uncontrolled growth and spread of abnormal cells. Cancer develops when gene changes as a result of carcinogens that make one cell or a few cells to begin to grow and multiply beyond the rate of normal cell division (Ferlay, *et al*., 2018). These may cause a growth called tumor. Carcinogens are class of substances that are directly responsible for damaging DNA by producing free radicals.

These free radicals damage cells and affect their ability to function normally (Ferlay, *et al*., 2018). A primary tumor is the name given to the point of cancer origin. Cancer can spread to other parts of the body which is known as a secondary tumor or a metastasis, if the spread of cancer cells at the metastasis stage is not controlled, it can result to death (Sudha and Shankarrao, 2019). Cancer, in general, is a large group of diseases that can affect any organ of the body (WHO, 2018).

2.2 Classification of Cancer

Classification of cancer is highly complicated due to the incidence of a wide variety of cancers arising in different tissues of the human body. They can be categorized either on the basis of their primary site of origin or based on their tissue types (Guruvayoorappan *et al*., 2020).

2.2.1 Carcinoma

Carcinoma, malignancies of epithelial tissues that covers surfaces of the organ, glands, or body structures. It starts in the tissue or the skin, which covers the glands and internal organ surface. It forms a solid tumor. For example, Breast cancer, prostate cancer, colorectal cancer,

lung cancer (Guruvayoorappan *et al*., 2020). Carcomas constitutes approximately 80–90% of all cancer cases and generally affects the organs or glands capable of secretion such as breast that produce milk. It can be either adenocarcinoma; which develops in an organ or gland, and squamous cell carcinoma, which originates in the

squamous epithelium.

2.2.2 Sarcoma

Sarcoma is another type of cancer which originates in supportive and connective tissues such as nerves, tendons, joints, fat, blood vessels, bone, lymph vessels, muscles, or cartilage. Example includes; osteosarcoma, chondrosarcoma, leiomyosarcoma.

2.2.3 Myeloma

Myeloma also called multiple myeloma is cancer that originates in the plasma cells of bone marrow. The plasma cells produce some of the proteins found in blood. Plasma cells are white blood cells that make antibodies that protect us from infection. In myeloma, the cells grow too much, crowding out normal cells in the bone marrow that make red blood cells, platelets, and other white blood cells (Gupta *et al*., 2013).

2.2.4 Leukemia

Leukemias ("liquid cancers" or "blood cancers") are cancers of the bone marrow (the site of blood cell production). The word leukemia means "white blood" in Greek. The disease is often associated with the overproduction of immature white blood cells. These immature white blood cells do not perform as well as they should, therefore the patient is often prone to

infection. Leukemia also affects red blood cells and can cause poor blood clotting and fatigue due to anemia. Examples of leukemia include:

- Myelogenous or granulocytic leukemia (malignancy of the myeloid and granulocytic white blood cell).

- Lymphatic, lymphocytic, or lymphoblastic leukemia (malignancy of the lymphoid and lymphocytic blood cell)

- Polycythemia vera or erythremia (malignancy of various blood cell products, but with red cells predominating)

2.2.5 Lymphoma

Lymphomas, also known as the "solid cancers", develop in the nodes or glands of the lymphatic system which are involved in the purification of bodily fluids and production of lymphocytes. Lymphatic system refer to a network of vessels, nodes, and organs (specifically the spleen, tonsils, and thymus) that purify bodily fluids and produce infection-fighting white blood cells, or lymphocytes. Lymphomas may also occur in specific organs such as the stomach, breast or brain. These lymphomas are referred to as extra-nodal lymphomas. The lymphomas are sub-classified into two categories: Hodgkin lymphoma and Non-Hodgkin lymphoma. The presence of Reed-Sternberg cells in Hodgkin lymphoma diagnostically distinguishes Hodgkin lymphoma from Non-Hodgkin lymphoma.

2.2.6 Mixed types

The type components may be within one category or from different categories. Some examples are:

- adenosquamous carcinoma
- mixed mesodermal tumor
- carcinosarcoma
- teratocarcinoma

2.3 Breast Cancer

Breast cancer is a very common type of cancer in women; breast cancer is characterized by the uncontrolled growth of abnormal cells in the milk production glands of the breast or in the passages (ducts) that deliver milk to the nipples. It is a malignant tumor that starts in the breast tissue of male or female. Breast cancer affects predominantly women but only in rare cases can occur in men or children, (Ghoncheh *et al.,* 2019). The breast tissue comprises of fat, fibrous tissues, fine ducts and glandular elements or lobules, majority of breast cancers begin in the ducts (ductal cancer), while small number start in the sacs or lobules (lobular cancers) (Biplob and Arun, 2018). Within these two groups, there are different subtypes of breast cancer. Breast cancer can spread to lymph glands and to other parts of the body, such as the bones and liver (Biplob and Arun, 2018). Breast cancer is typically detected either during a screening examination, before symptoms development, or after the notice of a lump. It's important to understand that most breast lumps are benign (not cancerous) (Biplob and Arun,

2018). Non-cancer breast tumors are abnormal growths, but they do not spread outside of the breast. They are not life threatening, but some types of benign breast lumps can increase a woman's risk of getting breast cancer (Biplob and Arun, 2018). Any breast lump or change needs to be checked by a health care professional to find out if it is benign or malignant (Biplob and Arun, 2018).

2.3.1 Epidemiology of breast cancer

According to the WHO, malignant neoplasms are the greatest worldwide burden for women, estimated at 107.8 million Disability-Adjusted Life Years (DALYs), of which 19.6 million DALYs are due to breast cancer (WHO, 2018). Breast cancer is the most frequently diagnosed cancer in women worldwide with 2.26 million [95% UI, 2.24–2.79 million] new cases in 2020 and responsible for about 684,996 deaths [95% UI, 675,493–694,633] at an age-adjusted rate of 13.6/100,000 worldwide, but only 1% of this malignant tumor affect men (Religioni, 2020; Ferlay *et al*., 2020). In the United States, breast cancer alone is expected to account for 29% of all new cancers in women. In 2018, GLOBOCAN data shows that age-standardized incidence rates (ASIR) of breast cancer are strongly and positively associated with the Human Development Index (HDI) (Sharma, 2018). According to 2020 data, the ASIR was the highest in very high HDI countries (75.6 per

100,000) while it was more than 200% lower in medium and low HDI countries (27.8 per 100,000 and 36.1 per 100,000 respectively) (Ferlay *et al*., 2020). Besides being the most common, breast cancer is also the leading cause of cancer death in women worldwide. Although incidence rate was the highest in developed regions, countries in Asia and Africa shared 63% of total deaths in 2020 (Ferlay *et al*., 2020). Most women who develop breast cancer in a high-income country have a high tendency to survive; the opposite is true for women in most low-income and many middle-income countries (Ginsburg *et al*., 2019). In

2020 breast cancer mortality-to-incidence ratio (MIR) as a representative indicator of 5year survival rates was 0.30 globally (Ferlay *et al*., 2020). Current projections indicate that by 2030 the worldwide number of new cases diagnosed might reach 2.7 million annually, while the number of deaths 0.87 million (Ferlay *et al*., 2020). In low and medium income countries, the breast cancer incidence is expected to increase further due to the westernization of lifestyles (e.g., delayed pregnancies, reduced breastfeeding, low age at menarche, lack of physical activity, and poor diet), better cancer registration, and cancer detection (Porter, 2018; Sharma, 2019).

2.3.2 Symptoms of breast cancer

The first sign of breast cancer is a new lump or mass in the breast that can be felt; if the lump is painless, hard, or has uneven edges is more likely to be cancer. But sometimes

cancers can be tender, soft, and rounded. So, as soon as any unusual changes are seen, the person should go to the physician. Below are some of the main symptoms of breast cancer as seen in Figure 2.1

- Swelling of all or part of the breast
- Skin irritation or dimpling
- Breast pain
- Nipple pain or the nipple turning inward
- Redness, thickening of the nipple or breast skin
- A nipple discharges other than breast milk
- A lump in the underarm area (Chun, 2019).

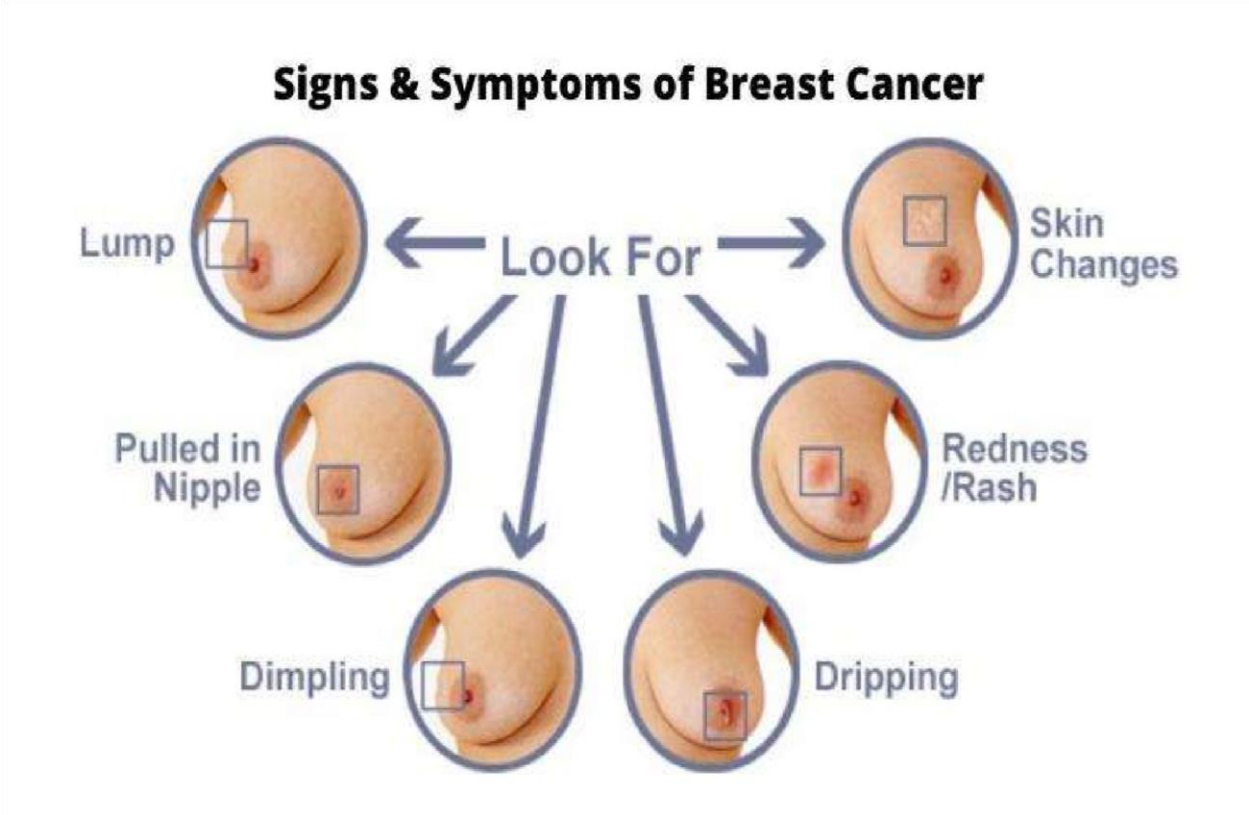


Figure 2.1: Sign and Symptoms of Breast Cancer

Source: Retrieved from www.howtorelief.com on 26th November, 2020.

2.3.3 Pathogenesis of breast cancer

The breast is a complex tubulo-alveolar organ fixed within an asymmetrical connective tissue (Stingl *et al*., 2019), that go through a chain of alteration from child bearing age to senility. The changes seen with every menstrual cycle and pregnancy guided us to assume the occurrence of precursor cells in the mature tissue that is able of synthesizing novel ductlobular units (Villadsen *et al*., 2017). The typical breast architecture contains a stratified epithelium bordered by a basement membrane and fixed in a template of blood vessels, lymphatic and stromal cells (Stingl *et al*., 2021) (Figure 2.2). In the usual breast, the stratified epithelium comprised of two dissimilar cell populations, myoepithelial and epithelial, which can be distinguished by way of immunohistochemical staining with antibodies against myosin and

cytokine (CK), correspondingly. It has been postulated that the creation of cellular heterogeneity in breast disorders depends on the primary developmental series of the normal breast.

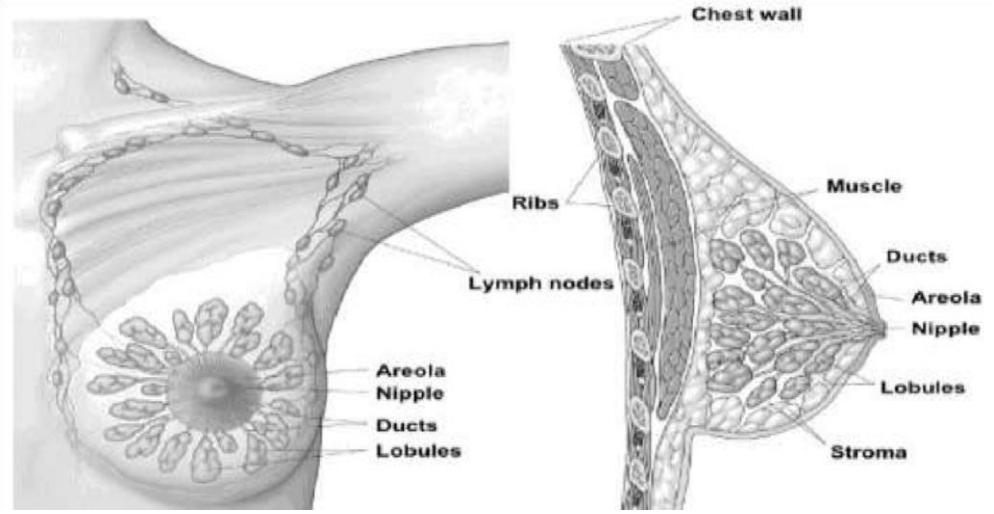


Figure 2.2: Features of Normal Breast Tissues

Source: Stingl *et al*., 2021

Recently, a new subclass of malignant cells within tumors called the cancer stem cells (CSCs) are observed and associated with tumor initiation, escape and recurrence. This small population of cells, which may develop from stem cells or progenitor cells in normal tissues, have self-renewal abilities and are resistant to conventional therapies such as chemotherapy and radiotherapy (Baumann *et al.,* 2008; Smalley *et al.,* 2013; Zhang *et al.,*

2017). Breast cancer stem cells (bCSCs) were first identified by Al-Hajj and even as few as 100 bCSCs could form new tumors in the immunocompromised mice (Al-Hajj *et al.,* 2003). bCSCs are more likely to originate from luminal epithelial progenitors rather than from basal

stem cells (Molyneux *et al.,* 2010). However, more studies are needed to understand bCSCs and to develop novel strategies to directly eliminate the bCSCs.

2.4 Risk Factors of Breast Cancer

The unambiguous cause of carcinogenesis has not yet been established, but several risk factors conducive to the development of breast cancer are known. These factors are significant; they include modifiable and non-modifiable factors.

2.4.1. Non-modifiable factors

2.4.1.1 *Sex*

The vast majority of cases of breast cancer, reaching 99%, occur in women. Only 1% of cases of this malignant tumor affect men (Religioni, 2020). The important factors that may increase a man's risk of breast cancer are: older age, BRCA2/BRCA1 mutations, increased estrogen levels, Klinefelter syndrome, family history of breast cancer, and radiation exposure (Giordano, 2018). Female gender constitutes one of the major factors associated with an increased risk of breast cancer, primarily because of the enhanced hormonal stimulation. Unlike men who present insignificant estrogen levels, women have breast cells which are very vulnerable to hormones (estrogen and progesterone in particular) as well as any disruptions in their balance. Circulating estrogens and androgens are positively associated with an increased risk of breast cancer (Key *et al*., 2019). The alternations within the physiological levels of the endogenous levels of sex hormones result in a higher risk of breast cancer in the case of premenopausal and postmenopausal women; these observations were also supported by the Endogenous Hormones and Breast Cancer Collaborative Group (Key *et al*., 2022).

2.4.1.2 *Age*

Age is one of the most important risk factors for breast cancer. The global increase in the incidence of breast cancer is observed in all age groups and is highest in women under 50 years of age (Benz, 2018). Currently, about 80% of patients with breast cancer are individuals aged >50 while at the same time more than 40% are those more than 65 years old (Benz, 2018). The risk of developing breast cancer increases with age; about 1.5% risk at age 40, 3% at age 50, and more than 4% at age 70 (Benz, 2018). Interestingly, a relationship between a particular molecular subtype of cancer and a patient's age was observed; aggressive resistant triple-negative breast cancer subtype is most commonly diagnosed in groups under 40 age, while in patients >70, it is luminal A subtype (McGuire *et al*., 2015). Generally, the occurrence of cancer in older age is not only limited to breast cancer; the accumulation of a vast number of cellular alternations and exposition to potential carcinogens results in an increase of carcinogenesis with time.

2.4.1.3 *Family history*

A family history of breast cancer constitutes a major factor significantly associated with increased risk of breast cancer. According to Collaborative Group on Hormonal Factors in Breast Cancer (CGHFBC, 2021), Approximately 13–19% of patients diagnosed with breast cancer report a first-degree relative affected by the same condition. Besides, the risk of breast cancer significantly increases with increasing number of first-degree relatives affected; the risk might be even higher when the affected relatives are under 50 years old (Baglia *et al*., 2018). The incidence rate of breast cancer is significantly higher in all of the patients with a family history despite the age. This association is driven by epigenetic changes as well as

environmental factors acting as potential triggers (Brewer *et al.*, 2017). A family history of ovarian cancer, especially those characterized by BRCA1 and BRCA2 mutations, might also induce greater risk of breast cancer (Elik *et al.*, 2018).

2.4.1.4 *Genetic mutations*

Several genetic mutations were reported to be highly associated with increased risk of breast cancer. Two major genes characterized by high penetrance are BRCA1 (located on chromosome 17) and BRCA2 (located on chromosome 13). They are primarily linked to the increased risk of breast carcinogenesis (Shahbandi *et al*., 2020). The mutations within the above mentioned genes are mainly inherited in an autosomal dominant manner; however, sporadic mutations are also commonly reported (Shahbandi *et al*., 2020). Apart from breast cancer, carriers of such mutation genes are also susceptible to ovarian cancer.

2.4.1.5 *Degree of economic development*

The incidence of breast cancer and mortality from this malignant tumor is related to the economic development of a country. This relationship has been documented in many studies (Duggan *et al*., 2020); breast cancer is increasing worldwide due to the continuous growth of the population and the ageing of the population (Sharma, 2019). The highest incidence rates are recorded in developed countries (Duggan *et al*., 2020). This phenomenon results from the so-called "Western lifestyle". In these countries, along with economic development, access to public health care becomes easier, prevention and screening programs are introduced (which increases detection), maternal, infant and child mortality decreases (Ferlay *et al*., 2020). On the other hand, the factors conducive to the development of breast cancer is growing, such as

late first birth, low number of babies born, use of hormone replacement therapy, obesity, lack of physical activity, or improper diet (Sharma, 2019). However, in lower, middle and low-income countries dominated by higher breast cancer mortality rates than in developed countries, despite lower incidence (Sharma, 2019; Duggan *et al*., 2020; Ferlay *et al*., 2020). This trend is associated with frequent diagnosis of cancer at an advanced stage, which results from the lack of resources for the effective implementation of primary prevention programs, diagnostic tests (primarily mammography), and finally modern methods of treatment (Sharma, 2019). **2.4.1.6** ***Reproductive history***

Numerous studies confirmed a strict relationship between exposure to endogenous hormones, estrogen and progesterone in particular and excessive risk of breast cancer in females. Therefore, the occurrence of specific events such as pregnancy, breastfeeding, menstruation, and menopause along with their duration and the concomitant hormonal imbalance, are crucial in terms of a potential induction of the carcinogenic events in the breast microenvironment. The first full-term pregnancy at an early age (especially in the early twenties) along with a subsequently increasing number of births is associated with a reduced risk of breast cancer (Bernstein, 2022). Besides, the pregnancy itself provides protective effects against potential cancer. However, protection was observed at approximately the 34th pregnancy week and was not confirmed for the pregnancies lasting for 33 weeks or less (Bernstein, 2022). The longer duration of the breastfeeding period also reduces the risk of both the ER/PR positive and negative cancers (Orgéas *et al*., 2018). Early age at menarche is another risk factor of breast cancer; it is also possibly associated with a tumor grade and lymph node involvement. Besides, the earlier age of the first menstruation could result in an overall poorer prognosis.

Contrarily, early menopause whether natural or surgical, lowers the breast cancer risk (Orgéas *et al*., 2018).

2.4.1.7 *Density of breast tissue*

The density of breast tissue remains inconsistent throughout the lifetime; however, several categories including low density, high density, and fatty breasts have been established in clinical practice. Greater density of breast is observed in females of younger age and lower BMI (body mass index), who are pregnant, or during the breastfeeding period, as well as during the intake of hormonal replacement therapy (Duffy *et al*., 2017). Generally, the greater breast tissue density correlates with the greater breast cancer risk; this trend is observed both in premenopausal and postmenopausal females. It was proposed that screening of breast tissue density could be a promising, non-invasive, and quick method enabling rational surveillance of females at increased risk of cancer (Duffy *et al*., 2017).

2.4.1.8 *History of breast cancer and benign breast diseases*

Personal history of breast cancer is associated with a greater risk of a renewed cancerous lesion within the breast (Schacht *et al*., 2019). Besides, a history of any other noncancerous alternations in breast such as typical hyperplasia, carcinoma in situ, or many other proliferative or non-proliferative lesions, also increases the risk significantly. The histologic classification of benign lesions and a family history of breast cancer are two factors that are strongly associated with breast cancer risk (Schacht *et al*., 2019).

2.5 Modifiable Factors

2.5.1 Chosen drugs

Data from some research indicates that the intake of diethylstilbestrol during pregnancy might be associated with a greater risk of breast cancer in children; this, however, remains inconsistent between studies and requires further evaluation (Hoover *et al*., 2021). The intake of diethylstilbestrol during pregnancy is associated with an increased risk of breast cancer not only in mothers but also in the offspring (Hoover *et al*., 2021). This relationship is observed despite the expression of neither estrogen nor progesterone receptors and might be associated with every breast cancer histological type (Hoover *et al*., 2021). The risk increases with age; women at age of ≥ 40 years are nearly 1.9 times more susceptible compared to women under 40 (Vinogradova *et al*., 2020). Moreover, breast cancer risk increases with greater diethylstilbestrol doses. Numerous researches indicate that females who use hormonal replacement therapy (HRT) especially longer than 5 or 7 years are also at increased risk of breast cancer (Vinogradova *et al*., 2020). Several studies indicated that the intake of chosen antidepressants, mainly paroxetine, and others selective serotonin reuptake inhibitors (SSRI) might be associated with a greater risk of breast cancer (Wernli *et al*., 2019). Studies showed that similar risk might be achieved due to the prolonged intake of antibiotics such as Tetracycline (Wernli *et al*., 2019). Attempts were made to investigate a potential relationship between hypertensive medications, non-steroidal antiinflammatory drugs, as an elevated risk of breast cancer; however, this data remains highly inconsistent (Denoyelle *et al*., 2021).

2.5.2 Physical activity

Even though the mechanism remains yet deciphered, regular physical activity is considered to be a protective factor of breast cancer incidence (Chen *et al*., 2018). Amongst females with a family history of breast cancer, physical activity was associated with a reduced risk of cancer but limited only to the postmenopausal period (Chen *et al*., 2018). However, physical activity is beneficial not only in females with a family history of breast cancer but also in those without such a history. However, there are several hypotheses aiming to explain the protective role of physical activity in terms of breast cancer incidence; physical activity might prevent cancer by reducing the exposure to the endogenous sex hormones, altering immune system responses or insulin-like growth factor-1 levels (Kolb and Zhang, 2020).

2.5.3 Body mass index (BMI)

According to epidemiological evidence, obesity is associated with a greater probability of breast cancer. This association is mostly intensified in obese post-menopausal females who tend to develop estrogen-receptor-positive breast cancer (Kolb and Zhang, 2020). Yet, independently to menopausal status, obese women achieve poorer clinical outcomes (Kolb and Zhang, 2020). Wang *et al*., (2019) reported that females above 50 years old with greater Body Mass Index (BMI) are at a greater risk of cancer compared to those with low BMI. Besides, studies show that greater BMI is associated with more aggressive biological features of tumor including a higher percentage of lymph node metastasis and greater size (Sun *et al*., 2018). Obesity might be the reason for greater mortality rates and a higher probability of cancer relapse, especially in premenopausal women (Sun *et al*., 2018).

Increased body fat might enhance the inflammatory state and affects the levels of circulating hormones facilitating pro-carcinogenic events. Thus, poorer clinical outcomes are primarily observed in females with BMI ≥ 25 kg/m^2 (Hopper *et al*., 2018). Interestingly, postmenopausal women tend to present poorer clinical outcomes despite proper BMI values but namely due to excessive fat volume. Greater breast cancer risk with regards to BMI also correlates with the concomitant family history of breast cancer (Hopper *et al*., 2018). Furthermore, according to studies, overweight and obesity are associated with a worse prognosis of breast cancer patients before and after menopause (Sellami and Bragazzi, 2020).

2.5.4 Alcohol intake

Evidences confirm that excessive alcohol consumption is a factor that might enhance the risk of malignancies within the gastrointestinal tract; however, it was proved that it is also linked to the risk of breast cancer. However, it is not alcohol type but rather the content of alcoholic beverages that mostly affect the risk of cancer. The explanation for this association is the increased levels of estrogens induced by the alcohol intake and thus hormonal imbalance affecting the risk of carcinogenesis within the female organs (Erol *et al*., 2017). Moreover, alcohol intake often results in excessive fat gain with higher BMI levels, which additionally increases the risk. Other hypotheses include direct and indirect carcinogenic effects of alcohol metabolites and alcohol-related impaired nutrient intake (Zeinomar *et al*., 2019). Alcohol consumption was observed to increase the risk of estrogen-positive breasst cancers in particular (Zeinomar *et al*., 2019).

2.5.5 Smoking

Carcinogens found in tobacco are transported to the breast tissue increasing the plausibility of mutations within oncogenes and suppressor genes (p53 in particular) (Terry and Rohan, 2022). Thus, not only active but also passive smoking significantly contributes to the induction of procarcinogenic events (Terry and Rohan, 2022). Besides, longer smoking history, smoking before the first full-term pregnancy, is additional risk factors that are additionally pronounced in females with a family history of breast cancer (Jones *et al*., 2017).

2.5.6 Insufficient vitamin supplementation

Vitamins exert anticancer properties, which might potentially benefit in the prevention of several malignancies including breast cancer; however, the mechanism is not yet fully understood. Attempts are continually made to analyze the effects of vitamin intake (vitamin C, vitamin E, B-group vitamins, folic acid, multivitamin) on the risk of breast cancer, nevertheless, the data remains inconsistent and not sufficient to compare the results and draw credible data (Jones *et al*., 2017). In terms of breast cancer, most studies are currently focused on vitamin D supplementation confirming its potentially protective effects. High serum 25-hydroxyvitamin D levels are associated with a lower incidence rate of breast cancer in premenopausal and postmenopausal women (Estébanez *et al*., 2018). Intensified expression of vitamin D receptors was shown to be associated with lower mortality rates due to breast cancer. Even so, further evaluation is required since data remains inconsistent in this matter (Zhou *et al*., 2020).

2.5.7 Intake of processed food/diet

According to the World Health Organization (WHO), highly processed meat was classified as a Group 1 carcinogen that might increase the risk of not only gastrointestinal malignancies but also breast cancer. Similar observations were made in terms of an excessive intake of saturated fats (Dandamudi *et al*., 2018). Ultra-processed food is rich in sodium, fat, and sugar which subsequently predisposes to obesity recognized as another factor of breast cancer risk. It was observed that a 10% increase of ultra-processed food in the diet is associated with an 11% greater risk of breast cancer. Contrarily, a diet high in vegetables, fruits, legumes, whole grains, and lean protein is associated with a lower risk of breast cancer (El-Sharkawy and Malki, 2020). Generally, a diet that includes food containing high amounts of n-3 PUFA, vitamin D, fiber, folate, and phytoestrogen might be beneficial as a prevention of breast cancer. Besides, lower intake of n-6 PUFA and saturated fat is recommended. Several in vitro and in vivo studies also suggest that specific compounds found in green tea might present anti-cancer effect which has also been studied regarding breast cancer. Similar properties were observed in case of turmeric-derived curcuminoids as well as sulforaphane (SFN), (Wright *et al*., 2018).

2.5.8 Exposure to chemicals

Prolonged exposure to chemicals can promote breast carcinogenesis by affecting the tumor microenvironment subsequently inducing epigenetic alterations along with the induction of pro-carcinogenic events (Casey *et al*., 2015). Prolonged exposure to chemicals presents significantly greater chances of cancer. The number of chemicals proposed to induce breast carcinogenesis is significant; so far, dichlorodiphenyltrichloroethane (DDT) and

polychlorinated biphenyl (PCB) are mostly investigated in terms of breast cancer since early exposure to those chemicals disrupts the development of mammary glands (Rodgers *et al*., 2018; Eve *et al*., 2020). A potential relationship was also observed in the case of increased exposure to polycyclic aromatic hydrocarbons (PAH), synthetic fibers, organic solvents, oil mist, and insecticides (Leso *et al*., 2019).

2.5.9 Other drugs

Other drugs that might constitute potential risk factors of breast cancer include antibiotics, antidepressants, statins, antihypertensive medications (e.g., calcium channel blockers, angiotensin II-converting enzyme inhibitors), as well as NSAIDs (including aspirin, ibuprofen), (Zhang *et al*., 2018).

2.6 Types of Breast Cancer

According to literatures Breast cancer is divided into invasive and noninvasive breast cancers with various subdivisions;

2.6.1 Non- invasive breast cancer

It is a cancer that has not extended away from the lobule or ducts where it situated (West *et al*., 2017). An example of non-invasive breast cancer is ductal carcinoma in situ. Ductal carcinoma in situ appears when atypical cells develop within the milk ducts, however have not extended to close proximity of tissue or outside. The word “in situ” describes “in place.” Even though the cells have not extended to tissues outer the lobules or ducts, they can progress and grow into invasive breast cancer (West *et al*., 2017).

2.6.2 Lobular carcinoma in situ (LCIS)

This type of breast cancer develops into breast lobules. The breast cancer has not extended exterior to the lobules into the breast tissue. Lobular carcinoma in situ is usually identified as non-invasive breast cancer (Chuba *et al*., 2019).

2.6.3 Ductal carcinoma in situ

It is the most general kind of non-invasive breast cancer, is limited to the breast duct. Example of ductal carcinoma in situ is ductal comedocarcinoma (Chuba *et al*., 2019).

2.6.4 Invasive breast cancer

Invasive breast cancer occurs when abnormal cells from within the lobules or milk ducts split out into close proximity of breast tissue (Harris *et al*., 2016). Cancer cells can pass through the breast to different parts of the body through immune system or the systemic circulation. They may move early in the development when the tumor is a minute or afterward when the tumor is huge Invasive breast cancer is most occurring general carcinoma in females. The regions of elevated threat are the prosperous populations of Australia and Europe wherever 6% of females suffer from invasive breast cancer prior to 75 years of age. The prevalence of breast cancer enhances quickly with increasing age (Prabhakaran *et al*., 2017). Invasive breast cancer that extends to different organs of the body is also recognized as metastatic breast cancer. Most common organs to which these cells spread include, brain, bones, lungs and liver. These cells once more segregate and expand irregularly and produce new cancers. The new forming cells are developing in different part of the body (Page *et al*., 2017).

2.6.5 Infiltrating lobular carcinoma (ILC)

Infiltrating lobular carcinoma is also recognized as invasive lobular carcinoma. ILC originates in the milk glands (lobules) of the breast, but frequently extends to other areas of the body (Page *et al*., 2017).

2.6.6 Infiltrating ductal carcinoma

Infiltrating ductal carcinoma is also recognized as invasive ductal carcinoma. IDC originates in the milk ducts of the breast and extends to the duct wall, invading the breast fatty tissues and probably other parts of the body (Somiari *et al*., 2018).

2.6.7 Medullary carcinoma

Medullary carcinoma is an invasive breast cancer that designs a discrete margin in normal tissue and medullary tissue (Mateo *et al*., 2017).

2.6.8 Inflammatory breast cancer

Inflammatory breast cancer is the form of swollen breasts (red and warm) with dimples and/or broad ridges due to cancer cells blocking lymph vessels or channels in the skin over the breast. Though inflammatory breast cancer is uncommon and is tremendously fastgrowing (Joglekar-Javadekar *et al*., 2017).

2.6.9 Paget's disease of the breast

It is the uncommon type of breast cancer that usually shows visible changes to the nipple of the breast (Errichetti *et al*., 2017). Its symptoms include red itchy rashes involving the nipple and then it can sometime spread to the normal skin as well. However, it resembles with the other skin conditions such as eczema and psoriasis but it can be differentiated as the other skin conditions usually affects both the breasts and can start from the areola rather than the nipple of the breast however Paget's disease of the breast most often affects only one breast and starts with the nipple of the breast instead of areola (Errichetti *et al*., 2017).

Nearly 1–3% of all the breast cancers is Paget's disease and can affect both men as well as women. The actual theory behind the pathogenesis or development of Paget's disease of the breast isn't clear yet however there are few theories supporting its pathogenesis. Their warning signs include bleeding and oozing of discharge from the nipple, flattening or inversion of nipple, lump found in the breast etc., (Merrill *et al*., 2017).

2.6.10 Triple- negative breast cancer

Triple-negative breast cancer is described by the deficiency of progesterone receptor, human epidermal growth factor receptor 2 and estrogen receptor expression. This type is mainly destructive, commonly observed in premenopausal females, and is responsible for 10–15% of cases in females (Liedtke *et al*., 2018).

2.7 Stages of Breast Cancer

According to the reports of breast cancer, stages of the breast cancer depend upon the size and type of tumor and how much the tumor cells have been penetrated in the breast tissues (Shaukat *et al*., 2018). Whereas stage 0 describes the non-invasive while stage 4 describes the invasive breast cancer. The following are stages of breast cancer:

2.7.1 Stage 0

This is the non-invasive stage of tumor which indicates that both cancerous and noncancerous cells are within the boundaries of that part of the breast in which the tumor begins to grow and no evidence found of their invasion in the surrounding tissues, the example of this tumor stage is ductal cell carcinoma in situ (DCIS) (Shaukat *et al*., 2018).

2.7.2 Stage 1

This stage describes as the invasive breast carcinoma; it has two categories that are 1A and 1B stage. The category 1A describes the tumor which measures up to 2 cm and none of the lymph nodes are involved in it while stage 1B describes that small group of cancer cells larger than 0.2 mm founds in lymph node (Moran *et al*., 2019).

2.7.3 Stage 2

Stage 2 also has two categories 2A and 2B. Stage 2A describes that the tumor is found in axillary lymph nodes but no tumor found in breast. The tumor can be smaller or larger than

2cm but not more than 5cm. However, stage 2B describes that the tumor could be larger than 5cm but can't reach to the axillary lymph nodes (Moran *et al*., 2019).

2.7.4 Stage 3

Stage 3 has been divided into three sub categories that are 3A, 3B and 3C. Amongst which stage 3A describes that no tumor is found in breast but it can be found in 4–9 axillary lymph nodes or in sentinel lymph nodes while stage 3B describes that the tumor can be of any size but have caused swelling or ulcer on the skin of the breast and can have spread up to 9 axillary lymph nodes or to sentinel lymph nodes, stage 3B can be considered as inflammatory breast cancer which includes red, warm and swollen skin of the breast. However, stage 3C describes the spread of tumor up to 10 or more than 10 axillary lymph nodes and it also have involved the lymph nodes above and below the clavicle (Neuman *et al*., 2022).

2.7.5 Stage 4

This is the advanced and metastatic stage of cancer and this stage describes the spread to other organs of the body that is lungs, bones, liver brain etc., (Neuman *et al*., 2022).

2.8 MCF-7 Cell Line

In recent years it has become clear that breast cancer does not represent a single disease but rather a number of molecularly-distinct tumors arising from the epithelial cells of the breast. Cell lines seem to be a key element for the molecular diagnosis in breast cancer as they can be widely used in many aspects of laboratory research, particularly, as *in vitro* models in cancer research. In breast cancer research, MCF-7 cell line represent a very important

candidate as they are used ubiquitously for estrogen receptor (ER)-positive breast cancer cell experiments and many sub-clones, which have been established, also as ERpositive tumors with varying nuclear receptor expression levels (Sweeney *et al*., 2013).

2.8.1 History of MCF-7 cell line

Sister Catherine Frances (Helen Marion) Mallon was born in 1901 and attended the Immaculate Heart of Mary Convent in Monroe, Michigan (Gunduz and Gunduz, 2011). In 1963 she had a mastectomy for a benign tumor in her right breast, and in 1967 she underwent a radical mastectomy for an adenocarcinoma in her left breast (Done, 2021).

That she developed breast cancer is perhaps not surprising given the early report by

Bernardino Ramazzini, the father of industrial medicine, that "tumors of the breast are found more often in nuns than any other women," and the subsequent epidemiologic literature indicating that nulliparous women are at higher risk for breast cancer. Just after she completed postoperative chest wall radiotherapy, a local recurrence in her left chest area was noted, but the recurrence was reportedly adequately controlled by radiation and hormone therapy of unknown type, perhaps diethylstilbesterol for period of three years. In 1970 Helen Marion developed metastatic disease to the pleura and chest wall, and researcher Herbert D. Soule at the Michigan Cancer Foundation attempted to develop a cell line from an excision of a chest wall nodule and from a pleural effusion (Done, 2021). At this time, many laboratories had documented technical difficulties in generating continuous stable cultures of cancer cell lines, including the overgrowth of fibroblasts, and several had tried to isolate cell lines using different substrates and nutrients. The process for cell line development that Soule used was relatively standard, and the cell cultures derived from the chest wall nodules were soon overgrown by fibroblasts and discarded (Done, 2021). However, the cells from the pleural

effusion grew initially in suspension and then ultimately formed a monolayer on plastic that grew as a continuous culture. The resulting cell line was called MCF-7, named after the Michigan Cancer Foundation, and represented Soule's seventh attempt at generating a cancer cell line. The popularity of the MCF-7 cell line for breast cancer research reflects its fidelity to many aspects of breast cancer in the clinical setting, particularly in the management of postmenopausal women with hormone receptor–positive breast cancer (Done, 2021).

2.8.2 Characterization of MCF-7 cell line

MCF-7 cell line is a commonly used breast cancer cell line that has been propagated for many years by multiple groups. It proves to be a suitable model cell line for breast cancer investigations worldwide, including those regarding anticancer drugs (Shirazi, 2011). With time, MCF-7 has produced more data of practical knowledge for patient care than any other breast cancer cell line. It is ER-positive and progesterone receptor (PR)-positive and belongs to the luminal-A molecular subtype (Done, 2021). MCF-7 is a poorly-aggressive and non-invasive cell line, normally being considered to have low metastatic potential (Shirazi, 2011). The human breast MCF-7 cell line, although often treated as a single entity, comprises of large number of individual phenotypes most of which constitute only small proportions of the total population. These phenotypes differ in gene expression profile, receptor expression and signaling pathway. Despite differences in the proliferation rate of individual phenotypes, a balance of multiple phenotypes is somehow maintained during progressive culturing of the cell line, perhaps by some type of signaling cooperation (Nugoli *et al*., 2018). The small sub-lines existing in the parental line can be expanded under appropriate selective conditions. The time scale of the *in vitro* selection process (6 months or more) is consistent with the long

period of time that occurs clinically in the development of resistance to anti-estrogen therapy or aromatase inhibitors in breast cancer patients. However, a critical question with regard to therapy is whether the emerging sublines express altered receptors and associated signaling pathways (Baguley and Leung, 2011). *In vitro*, MCF-7 models eventually evolved one step further toward clinical practice when they were adapted to *in vivo* models, which mirror more closely clinical care. *In vivo* models create a new dimension to assess the importance of the interaction between cancer cells, angiogenesis, cellular metabolism and respiration; processes that cannot be properly evaluated in cell culture (Sweeney *et al*., 2013).

2.9 Plant Remedies for Breast Cancer

2.9.1 Ethnomedicine and herbal compounds used for cancer treatment

Plants have played a key role in the survival and evolution of human beings as they have provided the basic need of mankind like food, clothing, shelter, and medicine since the beginning of human existance. Plants have formed the basis of traditional medicine systems that have served mankind over many years (Yahyea *et al*., 2022). A larger part of the population in developing and underdeveloped countries relies on herbal medicine for treating their primary health issues. Traditional herbal medicines become popular because of their cost-effectiveness, abundances, and less or no side effects (Yahyea *et al*., 2022). In recent years, global emphasis on plant research has increased to find out drug-like substances from traditionally used medicinal plants (Yahyea *et al*., 2022). Moreover, several naturally occurring plant-based compounds like curcumin, resveratrol, quercetin, and many more showed promising anticancer effects and are gaining interest as an adjuvant chemotherapeutic agent. Besides, naturally occurring compounds causes less toxicity to healthy cells and in

certain cases show selective toxicity against abnormal or diseased cells (Yuan *et al*., 2019). This might be the reason that today a large number of drugs being marketed are structurally similar to the structure of naturally occurring compounds.

Plants phytocompounds show a variety of anticancer activity mainly antioxidant, antiinflammatory, antimutagenic, and apoptosis-inducing activity that may help prevent cancer development in the early stage (Figure 2.3). Dietary consumption of adequate quantity of herbal products may help in prevention and treatment of breast cancer by cell cycle arrest, induction of apoptosis, regulating carcinogen metabolism and oncogenic expression, inhibiting cell adhesion, proliferation and migration, and blocking signaling pathways that are essential for cancer progression (Huang *et al*., 2020). Between the year 1981 and 2014, 136 anticancer drugs were brought to use around the globe, almost 83% were either herbal compounds or their derivatives (Amaral, 2019). A number of anticancer drugs have already in used for the treatment of breast cancer, including vincristine, vinblastine, paclitaxel, and docetaxel (Zyad *et al*., 2019). Despite the success of herbal products in curing breast cancer and its associated complexities, not many herbal products are making through preclinical or clinical trials. Hence, greater effort is necessary to successfully develop these agents to an ideal clinical setting to assess their potential for herbal therapies (Yahyea *et al*., 2022).

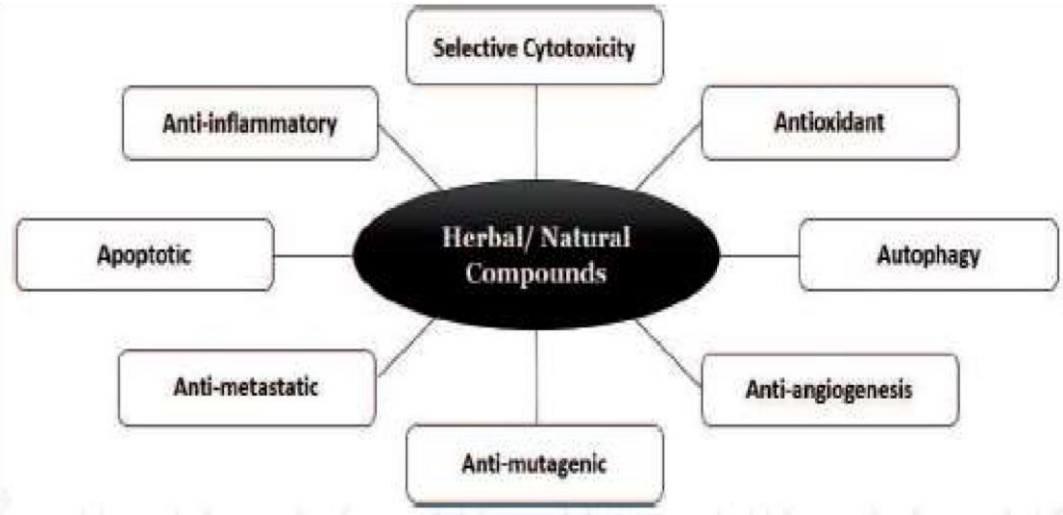


Figure 2.3: Features of Plants Phytocompounds that Attribute to their Anticancer Activity

Source: Yahyea *et al*., 2022.

2.9.2 Plant products used for prevention of breast cancer

Breast cancer is a preventable disease. Estrogens play a major role in promoting the proliferation of normal breast cells as well as neoplastic breast epithelium (Shamavat and Kurzer, 2015). Almost 40–70% of breast cancers are estrogen receptor positive. Hence, blocking the estrogen receptor for the treatment and chemoprevention of breast cancer is one of the significant approaches. Plant-based estrogen-like compounds or phytoestrogens were originally proposed as cancer-protective agents. These claims were strongly supported by epidemiological studies that revealed a low breast cancer incidence in the soyconsuming population (Russo, 2019). Phytoestrogens are structural analogues of the mammalian hormone, estrogen, and can bind weakly to the hormone receptor. Structurally, phytoestrogen can be grouped into flavones, flavanones, lignans, coumestans, and stilbenes (Alexander, 2019). The structure of important members of different classes of

phytoestrogens is given in Figure 2.4.

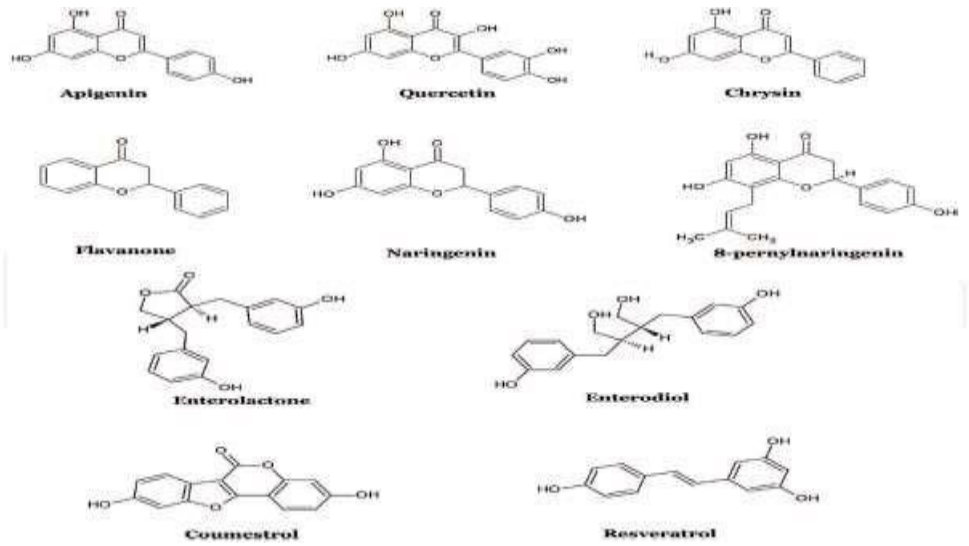


Figure 2.4: Some Important Members of Different Classes of Phytoestrogens Source: Kim, 2019.

Soybean and soy product is a rich source of isoflavones, other phytoestrogen classes are legumes and lignans found in seeds, nuts, whole grains, fruit, and vegetables (Rietjens *et al*., 2017). Historically, the rate of breast cancer occurrence in the United States is 4–7 times higher than that of Asian population where the consumption of dietary isoflavones is comparatively higher as 20-80mg/d (De-Kleijn, 2018). In addition, epidemiological observations also revealed a modest 30% reduction in breast cancer risk for women with a higher percentage of dietary lignan intakes. Therefore, consumption of phytoestrogen-rich diet is one of the many potential protective lifestyles against breast cancer. Recently, there are increasing pieces of evidence that phytoestrogen activity inhibits key steroidogenic enzymes activity involved in the synthesis of estradiol from circulating androgens and estrogen sulfate (Molina *et al*., 2018). Consequently, this activity could play a major role in protection against breast cancer. Besides inhibiting the estrogenic activity, phytoestrogens were also reported to activate the G-protein coupled receptor, GPR30, described as a novel estrogen receptor and play a significant role in estrogen-dependent diseases like breast cancer (Molina *et al*., 2018). However, the activity of phytoestrogens is unclear and depends on more than one factor that

includes; its structure, metabolism, its relative availability compared to that of endogenous estrogen (Rietjens *et al*., 2017). Naturally occurring phenolic compounds namely phenolic acids, flavonoids, tannins, quinones, anthocyanins, and others play an important role in cancer prevention and/or treatment. These phenolic compounds are ubiquitous and rich in medicinal herbs and dietary plants. Several phenolic compounds contribute toward inhibiting carcinogenesis mechanism and show chemopreventive activities by their diverse range of biological activities (Ambriz-

Perez *et al*., 2016).

2.9.3 Choosing a selectively cytotoxic herbal cure

One of the interesting features for herbal remedies is their selective toxicity toward cancer cells. There are a number of phytocompounds reported to be selective toxic toward breast cancer cells. Artemisinin is one among them, isolated from *Artemisia annua* L. proved to be selectively cytotoxic toward breast cancer cells when an adequate amount of iron (i.e., ferrous iron) is present in the cells. Because cancer cells have a higher iron influx, therefore, Artemisinin and its analogues can selectively destroy cancer cells under high iron concentration (Ambriz-Perez *et al*., 2016). Besides, polyphenols from *Artemisia annua* L. were reported to inhibit the adhesion and epithelial-mesenchymal transition (EMT) of highly metastatic breast cancer cells, MDA-MB-231 (Kim, 2019). Other than this, polyphenol-rich extracts of *Hibiscus sabdariffa* and aqueous extract of *Brucea javanica* were also reported to show selective cytotoxicity toward MCF7 and HTB-126 breast cancer cell lines, respectively (Wishart, 2018). However, further exploration is necessary to isolate the selective cytotoxic ingredients of these plants.

2.9.4 Combination therapy by herbal remedies and synthetic drugs

Combination therapy of herbal therapy and synthetic drugs possibly is the last resource for patients in the final stage of breast cancer, where surgery is not possible (Efferth, 2017). The combinatory effect of herbal drug with conventional cancer drugs might improve the bioavailability of one of them making the treatment more effective. Additionally, the combinatory use of herbal remedies with chemotherapy will reduce the dose of standard medicine resulting in lower toxicity and side effects (Patwardhan and Vaidya, 2020). Several researchers have suggested that herbal compounds can be used in a therapeutic modality as it enhances the anticancer activity of current drugs. Curcumin, a renowned anticancer herbal compound down-regulated the expression of breast cancer markers *in vivo* and *in vitro* when administered along with chemotherapeutic drugs cyclophosphamide and paclitaxel that made the cancer cells more viable to the drugs (Zhan *et al*., 2019). Similarly, 20(S)-protopanaxadiol, a metabolite of ginsenosides is one of the active ingredients in ginseng, inhibit cell proliferation in MCF-7 cells by interfering with estrogenic gene expression when used in combination with tamoxifen. Besides, this combination asynergistically improved the cytotoxicity of tamoxifen in ER-independent manner (Zhan *et al*., 2019). Hence, the benefits of these herbal compounds in synergistic therapy are considerable, and this might help to overcome chemotherapeutic drug resistance and toxicity in breast cancer treatment.

2.9.5 Herbal plants as nutraceuticals for breast cancer therapy

Cancer has been shown to be a preventable disease with changes in nutrition and dietary changes. A previous investigation showed that almost 35% of cancers are related to diet (Poprac *et al*., 2017). There are several confirmations from epidemiological and laboratory

studies that insufficient intake of fruit, vegetables, and herbal supplements are linked with breast cancer occurrence. A diet composed of adequate quantity of phytoestrogens, polyphenols, and rich sources of other chemo-preventive agents helps in reducing breast cancer risk. Dietary supplements of the herbal source are less toxic and easily metabolized. Besides, dietary consumption of these herbal remedies helps in fighting side effects in post chemotherapy patients (Poprac *et al*., 2017).

2.9.6 Mechanism of herbal compounds as anticancer agents

Evidences from previous researches shows medicinal plants have potency against cancerous cells, including antioxidant, cytotoxicity, antiproliferative effects, apoptotic activity, etc. Plant based cancer agents broadly classified into five groups which include, methyltranferase inhibitors, DNA protecting agents, antioxidants, histone deacetylases inhibitors and mitosis disruptors, others help in chemoprevention by preventing DNA damage, modulating carcinogenesis signaling, and inducing apoptotic cell death. Several *in-vitro* and *in-vivo* investigations support the activity of herbal compounds that linked with their anticancer activity (Yahyea *et al*., 2022).

2.9.7 Antioxidant activity of herbal compounds

Oxidative stress is developed when the balance between the production of reactive oxygen species (free radicals) and antioxidant defense is disturbed (Poprac *et al*., 2017). Oxidative stress development and consequently reactive oxygen species (ROS) generation are linked with several disease pathogenesis including cancer. Oxidative stress is dealt with by the body's antioxidant mechanism and several herbal compounds which help in boosting this

machinery. For instance, curcumin enhances the activity of antioxidant enzymes resulting in enhanced cellular resistance to oxidative damage (Poprac *et al*., 2017). Other plant-based compounds like Epigallocatechin gallate, a component of in green tea, found to reduce the levels of lipid peroxidation and protein carbonyl content in rats, possibly by enhancing the GSH redox status significantly when administered orally (Kumaran *et al*., 2018). Likewise, several herbal compounds help to reduce oxidative stress, hence play a preventive role against cancer onset.

2.9.8 Anti-angiogenesis activity of herbal compounds

Quite a few herbal compounds help to inhibit angiogenesis in breast cancer. Genistein, a flavonoid phytoestrogen, is a potent angiogenesis inhibitor (Pratheeshkumar, 2017). Curcumin was even found to be an effective inhibitor of angiogenesis that reduces the expression of various proangiogenic proteins such as vascular endothelial growth factor (VEGF) and basic fibroblast growth factor. Resveratrol and quercetin inhibited the migration and tube formation in bovine aorta endothelial cells consequently inhibiting angiogenesis in those cells (Singh *et al*., 2018). In addition, catechin derivatives, such as epicatechin (EC), epigallocatechin (EGC), epicatechin-3-gallate (ECG), and

epigallocatechin-3-gallate (EGCG), present in green tea are potent angiogenesis inhibitors. The anti-angiogenic activity of EGCG was demonstrated by inhibition of vascular endothelial growth factor (VEGF) production and reduction of matrix metalloproteinase-2 (MMP-2) activity in MDA-MB231 breast cancer cells (Wang *et al.*, 2019).

2.9.9 Apoptosis-inducing activity of herbal compounds

The apoptosis-inducing activity of herbal compounds is another favorable feature that contributes toward their anticancer effect. Curcumin was found to inhibit the proliferation and inducing apoptosis in several cancer cell lines including breast cancer cells such as T47D, MCF7, MDA-MB-231, and MDA-MB-468 (Hu *et al*., 2018). Protocatechuic acid was also found to be a potent apoptosis inducer in five types of human cancer cell lines including breast, lung, liver, cervix, and prostate cancer cells, which was confirmed by DNA fragmentation, changes in mitochondrial membrane potential, and measurement of caspase activity. The flavonoid 8-prenylnaringenin (8PN), a constituent of *Humulus lupulus*, is an effective phytocompound known for its growth-inhibiting and apoptotic activity in various human cancer types including breast cancer (Brunelli *et al*., 2018). This activity of 8-prenylnaringenin (8PN) in MCF7 cancer cells was possibly mediated by interference with an ER-associated PI3K pathway (Nahum, 2021). Interestingly, artemisinin, which is an ancient Chinese herbal compound for malarial fevers, has been recently found to have potent and selective toxicity against cancer cells. It reacts with iron to form free radicals with alkylating capacity that can kill cells. As cancer cells require a large quantity of iron uptake to proliferate, making them more susceptible to the cytotoxic effect of artemisinin (Konstat-Korzenny *et al*., 2018).

2.10 *Prosopis africana* (African Mesquite/Iron Tree)

Prosopis africana also known as African mesquite or iron tree is the only species of Prosopis that is indigenous to tropical Africa. *Prosopis africana* (Guill. & Perr) is a common flowering plant from the family Leguminosae-Mimosoideae. Its common names include; African

mesquite, also known as an iron tree. Because this species is not cultivated, it is often referred to as wild, endangered but edible, as a lost crop or as a lesser crop (Okafor, 2019). *Prosopis africana* has vast social, economic, cultural, medicinal and agricultural values. It has different names by the various ethnic groups in Nigerian, it's commonly called "kirya" (Hausa), "ayan" (Yoruba), "ubwo" (Igbo), 'Okpehe; (Idoma), Kpaaye, (Tiv) and "sanchi" (Nupe). *Prosopis africana* is very popular for its seeds, highly priced food condiment or seasoning, rich in protein, fatty acids and other vital nutrients and minerals which is widely used and consumed in the entire country and beyond. (Amusa *et al*., 2020). When dried, the roots are used by the Yoruba's to make chewing sticks called

"Ayan", which prevent dental root and gum decay in adults. The hardwood from *P. africana* offers good material for furniture, hoes, bows, pestles, mortars, and charcoal. The tree contributes to nutrient recycling and prevention of soil erosion (Alabi, 2018).

2.10.1 Scientific classification

Prosopis africana (Guill. & Perr) is a perennial leguminous tree found mostly to grow in savanna region of Senegal and Nigeria. This plant is classified into kingdom, phylum, class, order, family, genus and specie, below is the classification of *Prosopis africana*;

Kingdom: Plantae

Phylum: Tracheophytes

Class: Magnoliopsida

Order: Fabales

Family: Fabaceae

Subfamily: Caesalpinioideae

Genus: Prosopis

Species: *Prosopis africana*

2.10.2 Description of *Prosopis africana* plants

The genus Prosopis consists of about 44 species, out of which only 1(*Prosopis aficana*) is indigenous to Africa and it is popularly known all over the world as mesquite (Yarkwan *et al*., 2020). The tree can grow up to between 4-20m long. It is a very hard wood and characterized with a deep, fast–growing tap root, probable phreatophyte with very dark and scaly bark which is orange to red brown with white streaks when slashed. The branches and twigs are thornless, leaves alternate with bipinnate leaflets in 9–16 pairs, oblong lanceolate (12–30 mm) and shortly pubescent (Agboola, 2019).

Plate 1: (a) *Prosopis aficana* Tree Plant (b) Stem Bark of *Prosopis aficana*

2.10.3 Geographical distribution of *Prosopis aficana*

P. africana is a fast-growing hardy and drought resistant plant of about 4-20 m in height, found mainly in the semi-arid and arid regions of tropical Africa (Alagbe 2021). *Prosopis africana* is mostly found growing in the savanna regions of western Africa. The plant grows best in areas where the mean annual temperature falls within the range 22-35°c but can tolerate 18-40°c (Weber *et al*., 2018). In Nigeria, they can be found between latitute

70N and 100N in populations of two or more with such trees as *Parkia biglobosa* (Jacq.) (Fasidi *et al*., 2020). *Prosopis africana* is the only species found in the savanna, especially in Senegal and Nigeria. *Prosopis africana* tree is mostly found growing wild in Northern and the Middle Belt of Nigeria, in the area like Zamfara, Kaduna, Yobe and Enugu states. The other States where it can be found include Taraba, Kogi, Nassarawa, Benue etc. (Idoko *et al.*, 2018). Fruit setting, maturation, and dropping of *P. africana* takes place in Nigeria between November and March. The trees produce many legume pods during the months of January and March (Idoko *et al.*, 2018).

2.10.4 Ethnomedicinal and medicinal importance of *Prosopis africana*

The species belonging to the *Prosopis* genus have been traditionally used for the treatment of asthma, birth/postpartum pains, conjunctivitis, diabetes, diarrhea, expectorant, fever, flu, lactation, liver infection, malaria, pains, rheumatism, scabies, skin inflammations, stomach ache (Baturh, 2020). Studies also shows that the phytochemical constituents of *P. africana* includes flavonoids, tannins, alkaloids, phenols which demonstrate potentials in various bio-

functions, such as analgesic, anti-inflammatory, anthelmintic, antibiotic, antiemetic, microbial, antioxidant, antimalarial, pregnancy-related conditions (childbirth, breastfeeding, newborn diseases), in other biopharmaceutical applications, such as binding abilities for tablet production etc. (Olorunmaiye *et al*., 2019). Alagbe, (2021) shows that the stem bark of *Prosopis aficana* contains several bioactive compounds such as: alkaloids, flavonoids, tannins, oxalates, terpenoids, saponins and phenols. These bioactive compounds have significant therapeutic effects such as: anti-bacterial, antiviral, antioxidant, antifungal, immunomodulatory, hepatoprotective, cytotoxic, antispasmodic and neuroprotective and hypolipidemic activities (Akintayo *et al.*, 2020; Oluwafemi *et al*., 2020). The leaves, stem and roots of *Prosopis aficana* are used in traditional and folk medicine in the treatment of malaria, stomach ache, rheumatism, tooth ache, bronchitis, arthritis and several inflammatory conditions (Ayanwuyi *et al*., 2020).

2.11 *Tamarindus indica*

The *Tamarindus indica* is a fruit tree belonging to the Magnoliophyta, Order Fabales, Family Fabaceae (subfamily Caesalpinioideae). It is native to tropical Africa and its cultivation was widespread, developing well in all tropical continents (Amir *et al*., 2016).

There are different varieties of *T. indica* and they can be divided into acidic and sweet fruit. The sweet and sour at the same time in the fruit is unique and it is used popularly in cooking. In addition to the fruit, its various parts, as roots, wood, bark, and leaves, possess nutritional and pharmaceutical properties (Reis *et al*., 2016).

2.11.1 Biological description of *Tamarindus indica*

Tamarindus indica L. is an evergreen tree of the family Leguminoseae that grows up to 25m high and 7.5m girth with a canopy covering up to 12m radius. The tree stands strong, gracefully sloping down at the ends and highly wind-resistant; the stem bark is dark-gray and liberally fissured. The leaves are bright-green, fine feathery and pinnate, 7.5-15cm long each having 10-20 pairs of oblong leaflets. Although the leaves are essentially evergreen, they may be shed briefly in very dry region during the dry season. The flowers are perceptibly inconspicuous, borne in small racemes, are 5-petalled (2- petals reduced to bristles) and yellow with orange or red streaks. The flower buds are distinctly pink due to the outer colour of the four sepals, which are shed when the flowers open (PamplomaRoger, 2019). The fruits are hanging pods, 15-20cm long and 2-3.3cm in diameter with an interior that has a yellow-brown or reddish-brown flesh or pulp covering the seeds. The pulps in mature dry fruits dehydrate to a sticky paste enclosed by a few coarse strands of fiber extending lengthwise from the stalk. The pod usually contains 1-12 fully formed hard, glossy-brown, squarish seed of about 1.1-1.25cm in diameter, each enclosed in a parchment-like membrane (Pamploma-Roger, 2019). Tamarind as it is commonly called (French: Tamarin; Spanish: Tamarinde) serves as both food and medicine.

Plate 2: (a) *Tamarindus indica* Tree Plant, (b) Stem Bark of *Tamarindus indica*

2.11.2 Taxonomical classification of *Tamarindus indica*

Tamarin tree popularly known as Indian date is classified into the following (Santosh *et al*., 2021)

Kingdom: Plantae

Phylum: Spermatophyte

Class: Angiosperm

Sub class: Dicotyledon

Family: Leguminosae

Subfamily: Caesalpiniaceae

Genus: Tamarindus

Species: indica

2.11.3 Origin and distribution of *Tamarindus indica*

Native to tropical Africa, the tree grows wild throughout the Sudan and was so long ago introduced into and adopted in India; hence it has often been reported as indigenous to that

region. It was apparently from this Asiatic country that it reached the Persians and the Arabs who called it *"tamar hindi"* (Indian date, from the date-like appearance of the dried pulp), giving rise to both its common and generic names. Unfortunately, the specific name, *"indica"*, also perpetuates the illusion of its Indian origin. The fruit was well known to the ancient Egyptians and to the Greeks in the 4th Century B.C. In all tropical and near-tropical areas, including South Florida, it is grown as a shade and fruit tree, along roadsides and indoor yards and parks (Morton *et al*., 2019).

2.11.4 Medicinal benefits of *Tamarindus indica*

Medicinal benefits of the tamarind are uncountable. Tamarind preparations are universally recognized as antipyretics in fevers, laxatives and carminatives. Alone, or in combination with lime juice, honey, milk, dates, spices or camphor, the pulp is considered effective as a digestive for both human and animals, and as a remedy for biliousness and bile disorders, and as an anti-scorbutic (Raimondi *et al.*, 2018). In native practice, the pulp is applied on inflammations, used in a gargle for sore throat and, mixed with salt, as a liniment for rheumatism. The pulp is said to aid the restoration of sensation in cases of paralysis. In Colombia, an ointment made of tamarind pulp, butter, and other ingredients is used to rid domestic animals of vermin (Morton *et al*., 2019). Tamarind leaves and flowers, dried or boiled, are used as poultices for swollen joints, sprains and boils. Lotions and extracts made from them are used in treating conjunctivitis, as antiseptics, as vermifuges, treatments for dysentery, jaundice, erysipelas, hemorrhoids and various other ailments (Pamploma-Roger, 2019). The fruit shells are burned and reduced to an alkaline ash which enters into medicinal formulas. The bark of the tree is regarded as an effective astringent, tonic and febrifuge. Fried with salt and pulverized to an ash, it is given as a remedy for indigestion and colic. A decoction

made from the fruit pulp is used in cases of gingivitis and asthma and eye inflammations; and lotions and poultices made from the bark are applied on open sores and caterpillar rashes. The powdered seeds are made into a paste for drawing boils and, with or without cumin seeds and palm sugar, are prescribed for chronic diarrhea and dysentery. The seed coat, too, is astringent, and it, also, is specified for the latter disorders. An infusion of the roots is believed to have curative value in chest complaints and is an ingredient in prescriptions for leprosy. In addition to these, the leaves and roots contain the glycosides: vitexin, isovitexin, orientin and isoorientin. The bark yields the alkaloid, hordenine (Morton *et al*., 2019).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

3.1.1 Sample collection and identification

The stem barks of *Prosopis africana* and *Tamarindus indica* used in this study were obtained from Kontagora Local Government Area, Niger State, Nigeria on 28th October, 2019. The samples were identified and authenticated at Taxonomy unit of National Institute of Pharmaceutical Research and Development (NIPRD), with voucher numbers of NIPRD/H/7099 and NIPRD/H/7098 respectively.

3.1.2 Equipment/apparatus

The equipment and apparatus used in this study includes; centrifuge machine, luminometer (MODULUS, Promega), water bath, UV-1800 spectrophotometer (Shimadzu Corporation, Japan), light microscope, separating funnel, Serological pipette, measuring cylinder, beaker, conical flask, incubator, weighing balance, orbital shaker, soxhlet extractor, refrigerator, incubator, test tubes, thermometer and plastic cages.

3.1.3 Chemicals and reagents

The chemicals and reagents used in this study includes; CellTiter-Glo® 2.0 assay kit (Promega), Dulbecco's Modified Eagle's medium (DMEM) (Gibco® Invitrogen), 10% foetal bovine serum (FBS), 2mM glutamine, 1mM sodium pyruvate, 100µg/mL Streptomycin, 100 U/mL penicillin, trypsin, phosphate buffer saline (PBS) were all purchased from Invitrogen

UK. Methanol, n-hexane, ethyl acetate and other chemicals used in this study are of analytical grade and were purchased from Simbest chemicals Minna, Niger State.

3.1.4 Experimental animals

A total of eighteen (18) healthy Wistar rats with average weight of 194 g were used in acute toxicity screening. The rats were obtained from Federal University of Technology, Minna animal house. The animals were kept in dry and clean cages on temperature 25 ± 3°C with 12h:12h dark/light cycle; relative humidity of 45–55%. They were fed with standard laboratory diet and water was given ad libitum. They were allowed to acclimatize for 14 days to the laboratory conditions before the experiment. The use and care of the animals, and the experimental protocol was in strict compliance with the Institute of Laboratory Animals Research (ILAR) guidelines on the use and care of animals, in experimental studies. Sanitation and changing of the soiled wood shaving was observed on a daily basis

(Babayi *et al*., 2018)

3.1.5 Cancer cell line (MCF-7)

MCF-7 Cell line was purchased from Invitrogen UK. The cell line was maintained in a standard medium at 37^0C in a tissue culture incubator with an atmosphere containing 5% CO_2. Sub-culturing of cells was performed regularly to ensure their survival, regular supply for the study and maintain proper growth conditions.

3.2 Methods

3.2.1 Sample preparation

The stem bark of *Prosopis africana* and *Tamarindus indica* were rinsed with water to eliminate dust and other adhering particles and dried under laboratory conditions for 14 days. The dried samples were crushed into smaller sizes using wooden mortar and pestle and were preserved in a polythene bag before commencement of analysis.

3.2.2 Sample extraction

Each of the dried samples (300grams for each sample) were extracted with 99.5% methanol using soxhlet extractor. The extracts were filtered using Whatman No 1 filter paper, collected in separate clean beakers and heated in water bath at 70^0C to remove the solvent. The extracts were kept in an open space to dry completely for 72hours (3days). The percentage yields of each of the extracts were determined and extracts were preserved in plastic sample bottles before commencement of further analysis.

3.2.3 Phytochemicals screening

3.2.3.1 *Total phenol determination*

Singleton *et al.,* (1999) method was used to determine total phenol content of extracts. Briefly, 0.01 g of each extract was dissolved in 10 mL of distilled water, and 0.5 mL was oxidized by adding 2.5 mL of 10% Folin-Ciocalteu's reagent which was then neutralized by 2 mL of 7.5% sodium carbonate. The reaction mixture was incubated at 45^0C for 40 minutes. Absorbance

was read at 765 nm using double beam Shimadzu UV spectrophotometer, UV-1800. Standard garlic acid was used to prepare the calibration curve.

3.2.3.2 *Total flavonoids determination*

Flavonoids content of the extracts was determined using the method of Chang *et al.,* (2001). In this method, 0.01 g of each extract was weighed and dissolved in 10 mL of distilled water. Then 0.5 mL of each extract was added to a test tube containing 1.5 mL of absolute methanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1 M sodium acetate and 2.8 mL of distilled water and incubated at ambient temperature for 30 minutes. The absorbance was read at 415 nm with double beam shimadzu UV-spectrophotometer, UV1800. Quercetin was used to prepare the calibration curve.

3.2.3.3 *Total alkaloids determination*

Alkaloids content of the extracts was determined using method of Oloyed (2005). Briefly, 0.5 g of each of the extract was weighed and dissolved in 5 mL of mixture of 96% ethanol:20% H_2SO_4 (1:1) and then filtered using Whatman No 1 filter paper. 1 mL of each filtrate was then added to a test tube containing 5 mL of 60% H_2SO_4 and allowed to stand for 5 minutes. Thereafter, 5 mL of 0.5% formalin was added and allowed to stand at room temperature for 3 hours. The absorbance was read at wavelength of 565 nm. Vincristine extinction coefficient (E_{296}, ethanol {ETOH} = 15136 $M^{-1}cm^{-1}$) was used as reference alkaloid.

3.2.3.4 *Total tannins determination*

Tannin content of the extracts was determined using the method of Sofowora (1984). Briefly, 0.2 g of each of the extract was weighed into a 50 mL beaker and 20 mL of 50% methanol was added to it and covered with para film and heated in water bath at 80^0C for a period of 1 hour. The reaction mixture was shaken thoroughly to ensure uniformity. Each extract was then filtered into separate 100 mL volumetric flasks, and 20 mL of distilled water, 2.5 mL of Folin-Denis' reagent, and 10 mL of sodium carbonate were added and mixed properly. The reaction mixtures were then allowed to stand for 20 minutes at room temperature for the development of bluish-green coloration. The absorbance was recorded at 760 nm using double beam shimadzu UV-spectrophotometer, UV-1800. Standard tannic acid was used to prepare the calibration curve.

3.2.3.5 *Total saponins determination*

Saponins content of the extracts was determined using the method of Oloyed (2005). In this method, 0.5 g of each of the extract was weighed and dissolved in 20 mL of 1 NHCl and boiled in water bath at 80^0C for 4 hours. The reaction mixtures were cooled and filtered. 50 mL of petroleum ether was added and the ether layer was collected and evaporated to dryness. Thereafter, 5 mL of acetone-ethanol (1:1), 6 mL of ferrous sulphate and 2 mL of concentrated sulphuric acid were added and allowed to stand for 10 minutes. The absorbance was taken at 490 nm. Standard saponin was used to prepare the calibration curve.

3.2.4 Acute toxicity screening (LD_{50})

The acclimatized healthy albino rats were used in the acute toxicity studies of the methanol extracts of *P. africana* and *T. indica*. The acute toxicity of the extract was estimated in accordance with Lorke's method (Lorke, 1983). The method estimates dose of a certain compound or extract that kills 50% of the animal population (LD_{50}, 50% lethal dose) by oral administration route. On the day of treatment, food but not water was withheld overnight. The study was performed in two phases: in the first phase, 9 rats were randomly divided into three groups of 3 rats each. Groups 1, 2 and 3 animals were orally treated with a single dose of 10, 100 and 1000 mg/kg body weight (bw) of the extract, respectively, to possibly establish the range of doses producing any toxic effect, in the absence of toxicological signs and more importantly mortality in phase I, phase II was conducted. In the second phase, 9 rats were randomly divided into three equal groups and admistered a higher dose (1900, 2900 and 5000 mg/kg bw) of the methanolic extracts of *P. africana* and *T. indica* were administered to each animal to further determine the correct LD_{50} value. Food was withheld for another 3–4 h after giving the treatment and the animals were kept under close observation over a period of 14 days and monitored daily for signs of acute toxicity. Restlessness, respiratory distress, convulsions, diarrhea, motor activity, posture and reflexes were qualitatively determined. All observations were systematically recorded with individual records being maintained for each animal and the LD_{50} values were estimated using the formula below

$LD_{50} = \sqrt{}$ minimum dose without animal death x maximum dose with animal death

3.2.5 Brine shrimp lethality assay (BSLA)

Brine Shrimp Lethality Assay (BSLA) was carried out using the method of Gupta *et al.,* 1996 as reported by Ibikunle *et al.,* 2017. Two grams (2 g) of the Brine shrimp egg was placed in 800 mL of sea water in 1Litre conical flask under 60-watt bulb to provide illumination and aerator to provide oxygen. Aeration and illumination was carried out for complete 48hours for the hatching of the Brine shrimps into nauplii. The hatched eggs were left under illumination for another 48hours prior to the assay. After two days, when the shrimp larvae are ready, 3 mL of the sea water was dispensed into calibrated 5 mL vial and 10 brine shrimps was introduced into each making a total of 30 shrimps per dilution and the volume was adjusted with sea water to 5 mL per vials. The vials were left uncovered under the lamp. The number of surviving shrimps was counted and recorded after 24 hours. Using probit analysis, the Lethality Concentration at 50 and 90 (LC_{50} and LC_{90}) was assessed at 95% confidence intervals.

3.2.6 Solvent partitioning of *P. africana* crude extract

Solvent partitioning of crude extracts of *P. africana* was carried out according to the methods described by Gandhi *et al.* (2003) and Leila *et al.* (2007) with some modification in the choice of primary solvent (water) and partitioning (separating) solvents (n-hexane and ethyl acetate). The methanol extracts were dissolved in water (200 mL) to form a solution and exhaustively extracted by consecutive liquid/liquid partition with n-hexane (500 mL) and ethyl acetate (500 mL) using a separating funnel (1000 mL). The n-hexane fraction, ethyl acetate fraction and aqueous fractions was evaporated to obtain dry fractions.

The fractions were used for lethality test against brine shrimps as described in 3.2.5.

3.2.7 DPPH (2, 2-Diphenyl-1-Picrylhydrazyl) radical scavenging assay

The antioxidant activities of the solvent partitioned fractions were estimated using the DPPH radical scavenging assay as described by Oyaizu (1986). Briefly, different concentrations of extracts and ascorbic acid (50, 100, 200 and 400 µg/mL) were prepared from stock solutions (1000 µg/mL), prepared by weighing and dissolving 0.01g of the extracts and ascorbic acid, respectively in 10 mL of methanol. Thereafter, 2 mL of 0.004% DPPH in methanol added to 1 mL of various concentrations of plant extracts and ascorbic acid, respectively. The reaction mixtures were incubated at 25°C for 30 minutes. The absorbance of each test mixture was read against blank at 517 nm using double beam

Shimadzu UV-1800 series spectrophotometer. The experiment was performed in triplicates. The percentage antioxidant activity was calculated using the formula below:

$$\% \text{ Inhibition scavenged} = \frac{(\text{Absorbance of control} - \text{Absorbance of sample})}{\text{Absorbance of control}} \times 100$$

3.2.8 Mammalian tissue culture

3.2.8.1 *Measures taken for the use of cell culture*

Measures such as authentication of cell line, cryopreservation, and avoidance of crosscontamination of cell lines were all taken. Furthermore, the cells were checked for microbial contamination (regular mycoplasma test).

3.2.8.2 *Maintenance of cell lines*

The Dulbecco's Modified Eagle's medium (DMEM) were supplemented with filter sterilised 10% foetal bovine serum (FBS), 2 mM glutamine, 1 mM sodium pyruvate, 100 µg/mL

streptomycin and 100 U/mL penicillin all purchased from Invitrogen UK. MCF7 Cell line was maintained at 37^{o}C in a tissue culture incubator with an atmosphere containing 5% CO_2.

3.2.8.3 *Sub-culturing the cells*

Sub-culturing of cells used in this study was performed regularly to ensure their survival, regular supply for the study and maintain proper growth conditions. It was routinely done with cells that reached 80-90% confluence either in flask or tissue culture plates. All the procedures were done using sterile techniques performed in a sterile tissue culture hood. Briefly, in the process of sub-culturing, the media, buffers and trypsin were placed in water bath at 37°C at least 30 min prior to subculturing the cells. Tissue culture lab coat and gloves were worn. The gloves were sprayed with 70% ethanol. The laminar flow in the hood was turned and the working surface of tissue culture hood was swabbed with 70% ethanol. Any apparatus, media, trypsin and buffers taken inside the hood was first swabbed by 70% ethanol except for tissue culture flasks and plates. The tissue culture flask needed to be sub-cultured (>80% cell confluence) was taken out of the incubator, observed under the microscope and placed into the hood. With the help of aspirator attached to the suction pump, the old medium was removed from the flask. 4 mL of PBS (Phosphate buffered saline) (Invitrogen) was added to the cells and the flask gently swirled to wash away the old media and dead cells. PBS was aspirated out. 1 mL of pre-warmed 0.25% trypsin (Invitrogen) was gently added on top of cells with the help of serological pipette, and the flask swirled to ensure spreading of trypsin on the entire surface area of the flask. The flask was taken into tissue culture incubator maintained at 37^{o}C with 5% CO_2 atmosphere and incubated for 5 min (or until the cells were detached as observed under the light microscope). After the cells detached, 4 mL of pre-warmed cell culture media was added to the flask to stop the further action of trypsin and

dilute the cells. With the help of serological pipette, the cell suspension was mixed thoroughly by pipetting up and down to ensure total detachment and to break any cell clumps formed. After this, the number of cells in the suspension was determined and either seeded into other flasks for propagation and cell culture maintenance, or cryo-frozen for future use.

3.2.8.4 *Cell counting*

Throughout the course of this study, human cells were routinely counted for different experiments via counting chamber method. In this method, first of all, the counting chamber and the cover-slip used were cleaned with the help of lens paper and put under the light microscope. The coverslip was placed on top of the gridded area. The grid in the counting chamber was composed of squares of different areas. The cells to be counted were first trypsinized and then diluted in cell culture media. Every time micropipette is used to take 10 μL of cell suspension from the flask and put underneath the coverslip over the counting chamber where the cell suspension spreads quickly. The cells were counted in ten

0.04 mm^2 squares in the grid. Then, the number of cells per μL was calculated with the following formula:

Supposing, the number of cells counted in ten 0.04 mm^2 squares = 20 cells (supposed)

Total area in which 20 cells counted = (10 x 0.04) = 0.4 mm^2 Total

volume = 0.4 mm^2 x 0.1 (height of chamber) = 0.04 mm^3 So, 20

cells in 0.04 mm^3.

1 mm3 = 1 μL, hence, 0.04 mm^3 = 0.04 μL
If 0.04 μL has 20 cells, 1 μL has = 20 x 1 μL ÷ 0.04 μL = 500 cells.

Hence, 1 μL has 500 cells.

3.2.8.5 *Cryofreezing cells and reviving frozen cells*

In this case, the surplus cells were routinely cryo-frozen for future use. After the cells were trypsinized from a T75 flask, 5 mL of cell media was added to stop the action of trypsin, and the cell suspension was mixed thoroughly with the help of serological pipette attached to the pipette buoy. The cell suspension was transferred to a sterile 15 mL centrifuge tube, capped tightly and taken to the centrifuge machine set at 37°C. The cell suspension was centrifuged at 1000 rpm for 15 min to palette the cells. In the meantime, sterile cryotubes (Thermo Fischer) were labelled with cell details and passage number. After centrifugation, the tube was taken back to the hood, the supernatant was discarded and the cell palette was gently resuspended in 1 ml of freezing media. Following this, the cell suspension in freezing media was carefully transferred to cryotubes, capped properly and immediately taken for storage in -80°C freezer. When previously frozen cells were needed, the media was first warmed in the water bath at 37°C and 10 ml of media was transferred to the T75 flask. The cryotube with frozen cells was taken out and immediately put in the water bath at 37°C. As soon as the frozen mixture was thawed (usually after 1-2 min), the cryotube was taken to the tissue culture hood, and with the help of pipette, very gently transferred into the T75 flask with the media. After 16-24 h, old media was replaced by new media to replenish the nutrients and boost cell growth and after every 3-4 days, cell media was usually changed by first aspirating the old media, washing the cells with warm PBS, and transferring fresh media into the flask to boost the healthy condition of the growing cells.

3.2.8.6 *Cell treatments with solvent partitioned fraction*

Stock solutions of the partitioned aqueous fraction was prepared and stored as required. Working concentrations were achieved by diluting the stock in the media used and were based on prior literature. Prior to treatment with these Stock solutions, cells to be treated were taken out of the incubator; old media removed and pre-warmed PBS (Phosphate buffered saline) added. Pre-warmed media was then transferred to falcon tubes (corning) in the tissue culture hood and the required amount of drug was added to achieve the working concentrations. The PBS over the cells was taken out and the media with the drug carefully added. The amount of media added was dependent upon the type of tissue culture vial in which the cells were grown. For example, the cell lines were maintained in DMEM

(Gibco® Invitrogen) supplemented with 10% foetal bovine serum (FBS), 2 mM glutamine, 1 mM sodium pyruvate, 100µg/ml streptomycin and 100U/ml penicillin in an atmosphere of 5% CO_2 and incubated at 37°C. The Stock solutions were used by directly diluting it into media to desired concentrations.

3.2.8.7 *Cell viability assay*

The CellTiter-Glo® 2.0 assay kit (Promega) was used to determine cell viability, as described by Crouch *et al.* (1993) and Hall *et al.* (1998). Briefly, cells were seeded in a luminometer compatible 96-well plate and allowed to adhere for 18-24 h. After that, the cells were treated with different concentrations of the partitioned aqueous fraction (stock solution) for 24h and 48h. Following treatments, the plate and its contents were equilibrated to room temperature for approximately 30 min, a volume of CellTiter-Glo 2.0 reagent equal to the volume of cell culture medium present in each well the contents were then mixed for 2 min on an orbital

shaker to induce cell lysis and the plate was then incubated at room temperature for 10 min to stabilize the luminescent signal and finally the luminescence was recorded using luminometer (MODULUS, Promega). The luminescent signal is proportional to the amount of ATP in the sample, which indicates the presence of metabolically active cells.

Principle: The CellTiter-GloR 2.0 Assay relies on the properties of a proprietary thermostable luciferase (Ultra-Glo™ Recombinant Luciferase), which generates a stable

"glow-type" luminescent signal and improves performance across a wide range of assay conditions. The half-life of the resulting luminescent signal is greater than 3 hours. This extended half-life eliminates the need for reagent injectors and provides flexibility for continuous or batch-mode processing of multiple plates. Mono-oxygenation of luciferin is catalyzed by luciferase in the presence of Mg^{2+}, ATP, which is contributed by viable cells, and molecular oxygen (Patrick *et al.,* 2018).

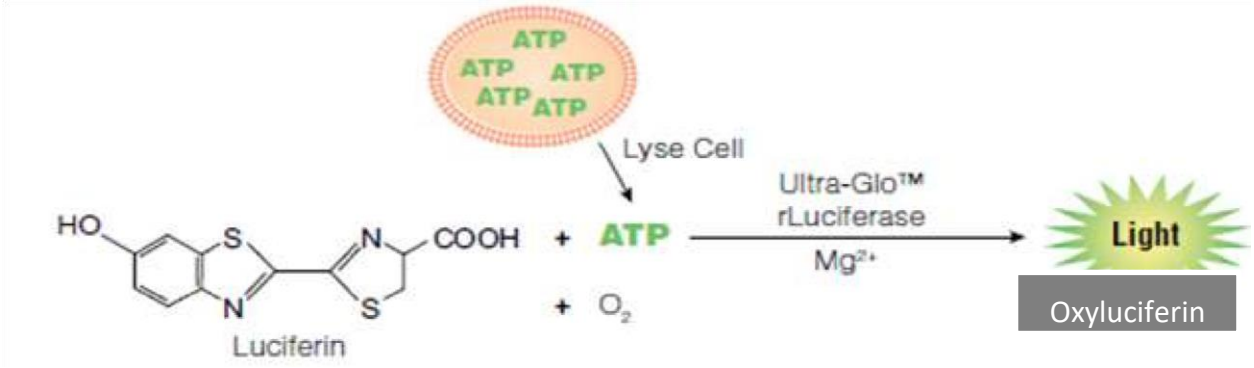


Figure 3.1: Overview of CellTiter-Glo® 2.0 Assay Principle

Source: Patrick *et al.,* (2018).

3.3. Data Analysis

All numeric data generated were expressed as the mean ± standard Error of mean (SEM). Comparison between different groups will be performed using analysis of variance (ANOVA Test). The significant difference between control and experimental groups will be assessed using Duncan's posthoc test using SPSS version 26.

CHAPTER FOUR

4.0. RESULTS AND DISCUSSION

4.1 Results

4.1.1 The Phytochemical compositions of *Prosopis africana* and *Tamarindus indica*

The phytochemical compositions of *P. africana* and *T. indica* are presented in Table 4.1. The result shows that the phytochemicals (phenols, tannins and alkaloids) present in *P. africana* were significantly higher (848.77±0.29, 105.72±0.04 and 560.23±0.77 mg/100g) than those in *T. indica.* However, flavonoids and saponins contents in *P. africana* were significantly ($p<0.05$) lower when compared to those in *T. indica.*

Table 4.1: Phytochemical Compositions of Methanol Extract of *P. africana* and *T. indica* Stem Bark

Phytochemical	*Prosopis africana* (mg/100g)	*Tamarindus indica* (mg/100g)
Phenol	848.77 ± 0.29^{b}	744.38 ± 0.17^{a}
Flavonoid	65.65 ± 0.06^{a}	174.68 ± 0.15^{b}
Tannin	105.72 ± 0.04^{b}	47.94 ± 0.01^{a}
Alkaloid	560.23 ± 0.77^{b}	359.62 ± 0.48^{a}
Saponin	236.27 ± 0.68^{a}	357.03 ± 0.03^{b}

Values are expressed as Mean ± standard error of mean. Values with different superscripts across a row are significantly different at $p<0.05$.

4.1.2 Acute toxicity study of *Prosopis africana* and *Tamarindus indica* crude extract

The acute toxicity study was performed according to Lorke's method, which specifies a limit test dose of 5000 mg/kg. No treatment-related mortality was observed at 5000 mg/kg, and throughout the 14-day observation period, there were no significant changes in behavior, such as apathy, hyperactivity, or morbidity, in any of the animals and no abnormal changes in body weight.

In the present study, *P. africana* and *T. indica* was found to be safe at a dose of 5000mg/kg, and therefore, the LD_{50} value for oral toxicity maybe assumed to be greater than

5000mg/kg (Table 4.2).

Table 4.2: Acute Toxicity Effects of *P. africana* and *T. indica* Crude Extracts

		P. africana		***T. indica***		
Groups	**Dose (g/kg)**	**No of death**	**% Mortality**	**No of death**	**% Mortality**	**Remarks**
I	10mg/kg	0	0	0	0	Normal
II	100mg/kg	0	0	0	0	Normal
III	100mg/kg	0	0	0	0	Normal
V	1900mg/kg	0	0	0	0	Normal
VI	2900mg/kg	0	0	0	0	Normal
VII	5000mg/kg	0	0	0	0	Normal

4.1.3 Cytotoxic effects of crude extracts of *Prosopis africana, Tamarindus indica* and fractions of *P. africana* to brine shrimps.

Table 4.3 below shows that crude extract of *Prosopis africana* is more cytotoxic (with an LC_{50} of 31.62 ppm) than the extract of *Tamarindus indica* (LC_{50} 109.784 ppm) against brine shrimps. However, the aqueous fraction of *P. african* (AqPA) was more lethal to the brine

shrimp with an LC_{50} and LC_{90} of 9.20 and 1.28 x 10^3 ppm respectively than the other fractions (n-hexane and ethyl acetate). Moreover, the LC_{90} values for both the aqueous fraction and crude extract of *P. africana* was greater than 1000ppm.

Table 4.3: Cytotoxicity of *Prosopis africana, Tamarindus indica* Crude Extracts and Fractions of *P. africana* against Brine Shrimps

Extracts	LC_{50} (ppm)	LC_{90} (ppm)
Prosopis Africana	31.62^b	5.71 $x10^3$
Tamarindus indica	109.78^d	$1.42x10^4$
n-hexPA	1156.04^e	2.76 $x10^6$
EtPA	49.24^c	$2.10x10^3$
AqPA	9.20^a	$1.28x10^3$

Values with different superscript on the same column are significantly different at $p<0.05$

Key:

n-hexPA = n-hexane fraction of *Prosopis africana* EtPA

= Ethylacetate fraction of *Prosopis africana*

AqPA = Aqueous fraction of *Prosopis africana*

LC_{50} = Lethal concentration that kills 50 % of the sample population

4.1.4 DPPH antioxidant scavenging activity of the aqueous fraction of *P. africana* The DPPH antioxidant scavenging ability of the aqueous fraction of *P. africana* is presented in Figure 4.1. The result revealed a concentration dependent increase in scavenging ability in both the standard ascorbic acid and aqueous fraction of *P. africana.* The maximum %

scavenging ability (88.51 and 67.45 %) for both ascorbic acid and aqueous fraction of *P. africana* were observed at 100 µg/ml with IC_{50} of 12.32 µg/ml and 50.55 µg/ml respectively.

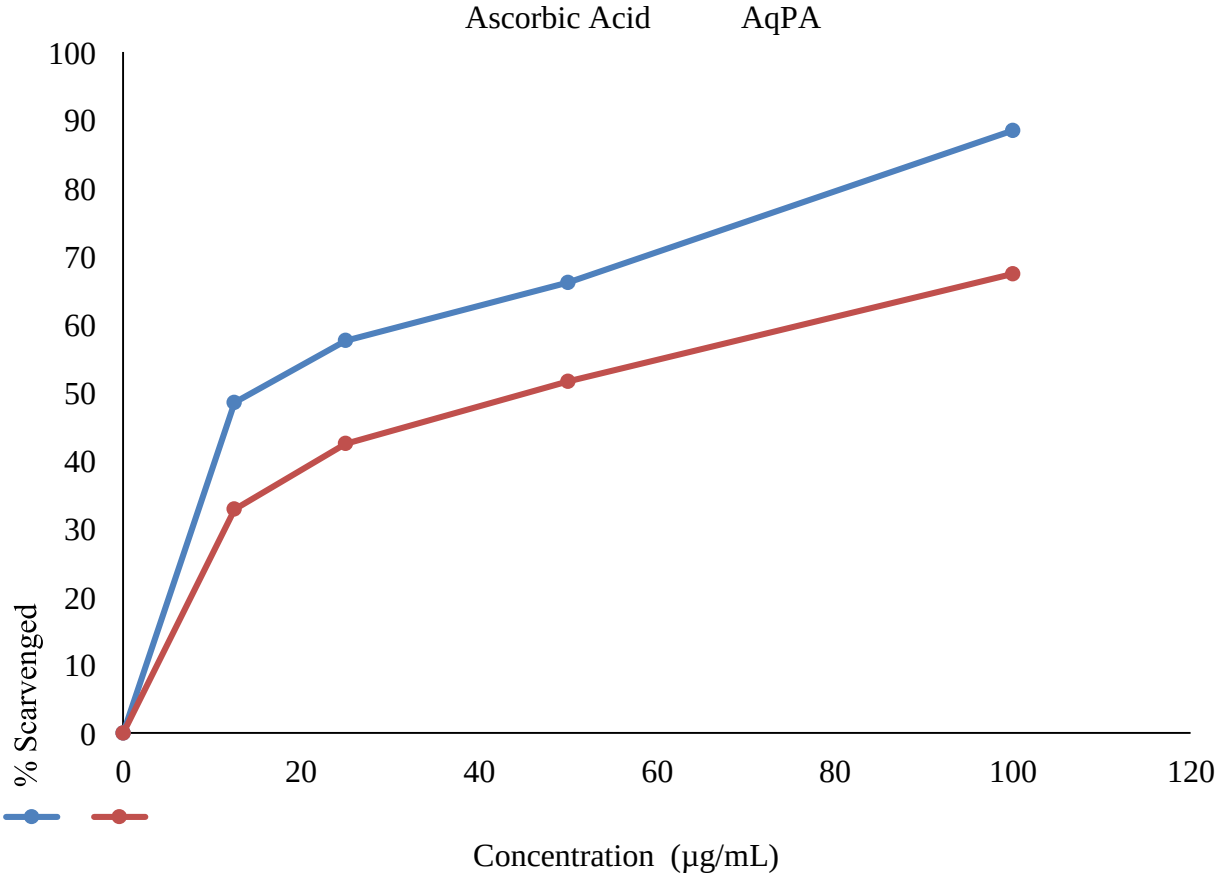


Figure 4.1: DPPH Antioxidant Assay for Aqueous Fraction of *Prosopis africana* Key:

AqPA = Aqueous fraction of *Prosopis africana*

4.1.5 Effects of different concentrations of doxorubicin (standard drug) on the cell count of MCF-7 cell lines after 24 and 48-hours exposure

The viable cell of the MCF-7 cell lines after exposure to varying concentrations of doxorubicin drug at 24 and 48 hours is presented in Table 4.4. At both 24 and 48 hours of exposure, a dose

dependent decrease in the viable cell of MCF-7 cell lines was observed. A significant increase in number of viable cells of the negative control group at 24 hours (79.93±0.61 $x10^6$) and 48 hours (82.69±0.88 $x10^6$) was observed when compared with other treatments (0.5-10 µg/mL). However, the cancer cells treated with 10 µg/mL of doxorubicin had the least number of viable cells (7.31±0.11 $x10^6$ and 4.75±0.04 $x10^6$) for both 24 and 48 hours' exposure time respectively.

Table 4.4: Effects of Different Concentrations of Doxorubicin Drug on the Cell Count of MCF-7 Cell Lines after 24 and 48-hours of Exposure

	Viable cells ($x10^6$)	
Treatment (µg/mL)	**24hr**	**48hr**
Control	$79.93±0.61^f$	$82.69±0.88^f$
0.5	$21.57±0.30^e$	$18.47±2.16^e$
1.00	$16.80±1.07^d$	$15.71±0.66^d$
2.50	$14.00±0.50^c$	$9.15±0.14^c$
5.00	$8.84±0.43^b$	$6.33±0.04^b$
10.00	$7.31±0.11^a$	$4.75±0.04^a$

Values are expressed as mean ± standard error of mean. Values with different superscript on the same column are significantly difference at $p<0.05$ while p<value less than 0.05 shows a significant difference between 24 and 48-hr MCF7 viable cell

4.1.6 Effects of different concentration of partitioned aqueous fraction of *Prosopis africana* (AqPA) on the cell count of MCF-7 cell lines after 24 and 48-hours of exposure

Table 4.5 presents the effect of varying concentration of AqPA fraction on the cell count of MCF-7 cell lines after exposure/treatment for 24hours and 48hours respectively. The result also revealed a dose dependent decrease in cell count with increase in concentration of AqPA

fraction at 24 and 48 hours' exposure. At concentration of 1000 µg/mL AqPA fraction, MCF-7 cells had the least cell count after 24 hours ($9.72\pm0.08 \times 10^6$) and 48 hours ($8.61\pm0.12 \times 10^6$) of exposure at significant level of ($p<0.05$). The influence of exposure time (24-48 hours) on MCF-7 cell count relative with the dose was observed, resulting in a significantly decrease in cell count as the exposure time increases.

Table 4.5: **Effects of Different Concentration of Partitioned Aqueous Fraction of *Prosopis africana* (AqPA) on the Cell Count of MCF-7 Cell Lines after 24 and 48hours of Exposure**

	Viable cells ($x10^6$)	
Treatment (µg/mL)	**24hr**	**48hr**
Control	82.93 ± 1.75^f	92.63 ± 6.84^f
50	58.77 ± 1.21^e	25.37 ± 0.35^e
100	50.27 ± 1.46^d	19.73 ± 1.27^d
250	28.60 ± 0.81^c	16.46 ± 0.57^c
500	18.67 ± 0.06^b	10.39 ± 0.50^b
1000	9.72 ± 0.08^a	8.61 ± 0.12^a

Values are expressed as mean ± standard error of mean. Values with different superscript on the same column are significantly difference at $p<0.05$ while p<value less than 0.05 shows a significant difference between 24 and 48-hr MCF7 viable cell

4.1.7 Percentage inhibition (PI) and cell viability of MCF-7 cell lines treated with Doxorubicin (standard drug) and aqueous fraction of *Prosopis africana* (AqPA)

The percentage cell viability and percentage inhibition of MCF-7 cell lines by Doxorubicin and AqPA fraction after 24 and 48 hours of exposure is presented in Table 4.6 and 4.7.

There was a dose dependent decrease in percentage cell viability and time of exposure of MCF-7 cell lines to the standard drug and AqPA fraction, at concentrations of 10 and 1000

µg/mL of doxorubicin and AqPA fraction, the results revealed the lowest % viable cell count (i.e. greater effect), (5.74 and 9.29 %) after 48 hours than 24 hours (9.05 and 11.72%) respectively. The PI (%) increases with increasing treatment dose and time of exposure for both the standard drug and working sample. At concentrations of 10 and 1000 µg/mL of doxorubicin and AqPA fraction, the result revealed the highest PI (%) (94.26 % and 90.71) after 48 hours of exposure against MCF-7 cell lines than 24 hours (90.85 and 88.21 %). The standard drug had lower IC_{50} (0.50 and 0.27 µg/mL) at 24 and 48 hours of treatment, while the AqPA fraction had IC_{50} of 235.87 and 52.04 µg/mL at 24 and 48 hours of treatment respectively.

Table 4.6: Percentage Inhibition (PI) and Cell Viability of MCF-7 Cell Lines Treated with Doxorubicin Drug after 24 and 48hrs

Treatments	**Cell Viability (%)**		**PI (%) (µg/mL)**	
	24hr	**48hr**	**24hr**	**48hr**
0.5	26.99	22.34	73.01	77.66
1.00	21.02	19.00	78.98	81.00
2.50	17.51	11.07	82.49	88.93
5.00	11.06	7.66	88.94	92.34
10.00	9.05	5.74	90.85	94.26
IC_{50}	----	----	0.50 µg/mL	0.27 µg/Ml

PI (%) == Percentage Inhibition

IC_{50} == Inhibition concentration of 50 % of the target subject

Table 4.7: Percentage Inhibition (PI) and Cell Viability of MCF-7 cell lines treated with Aqueous Fraction of *P. africana* after 24 and 48hrs

Treatments (µg/mL)	**Cell Viability (%)**		**PI (%)**	
	24hr	**48hr**	**24hr**	**48hr**

50	70.87	27.38	29.14	72.62
100	60.61	21.30	39.39	78.70
250	24.49	17.78	65.51	82.22
500	22.51	11.22	77.49	88.78
1000	11.72	9.29	88.28	90.71
IC_{50}	----	----	235.87 µg/mL	52.04 µg/mL

PI (%) == Percentage Inhibition

IC_{50} == Inhibition concentration of 50 % of the target subject

4.2 Discussion

4.2.1 Phytochemicals

The quantitative phytochemical composition of the stem bark crude extract of *Prosopis africana* and *Tamarindus indica* revealed the presence of phytoconstituents (Alkaloid, Saponin, Tannins, Phenols and Flavonoids). The presence of these phytoconstituents in both *P. africana* and *T. indica* may be responsible for their antioxidant and the antiinflammatory activities, as reported by (Singh, 2018; Prakash *et al*., 2019). The result in this study is in agreement with the results from previous studies (Agarwal and Paridhavi,

2017; Olorunmaiye *et al.,* 2019; Atawodi and Ogunbusola, 2019; Ayanwuyi *et al*., 2020). They reported that *P. africana* and *T. indica* contained the above highlighted phytoconstituents. These Phytochemicals are secondary plants metabolites that are responsible for many observed bioactivity of plant extract. They are known to possess antioxidant, anti-inflammatory, antibacterial, immunomodulatory, anti-sickling activities etc. The presence of these metabolites no doubt is an indication of the potential medicinal usefulness of the plant extracts which could serve as potential sources of drugs. Flavonoids and phenolic amongst other phytochemical compounds represent the two major and widely distributed polyphenols found in medicinal plants (Neveu *et al*, 2010). Outcomes of several

epidemiological studies are supporting the positive association between dietary polyphenol consumption and reduced cancer risk (Hui *et al*, 2018; Patrick *et al*, 2019). These classes of compounds are known to show curative activity against several pathogens and therefore could explain their use traditionally for the treatment and management of various diseases and ailments. However, there is a major drawback to their use, i.e. potential toxicity. It has been reported that there are more than 5000 plants being used as medicines in Africa, with very few of them being studied (Adebayo *et al.*, 2019). This could be as a result of the general belief that these plants are safe for consumption having been used for ages. However, this may not be the case as some studies have recorded some level of toxicity in organs of experimental animals treated with medicinal plant extracts perceived to be safe. Toxicity studies help to ascertain if there are possible lethal effects that could arise from consumption of such plants and also gives a guide on the dose to be consumed (Usman, *et al*; 2018).

4.2.2 Toxicity activity

In the acute toxicity assay, oral treatment with *P. africana* and *T. indica* crude extracts was well tolerated up to a dose of 5000 mg/kg administered to the rats and did not cause signs of toxicity, changes in behavior, or mortality. The LD_{50} value of both extracts was shown to be greater than 5000 mg/kg. According to the chemical labeling and classification of acute systemic toxicity recommended by OECD, *P. africana* and *T. indica* crude extracts was assigned class 5 status (LD_{50} > 5000 mg/kg), which is the lowest toxicity class. Any plant extracts with an LD_{50} 5000mg/kg is considered nontoxic (Zbinden and Flury-Roversi, 2019).

The brine shrimp test represents a rapid, inexpensive and simple bioassay for testing the plant extract lethality which in most cases correlates reasonably well with cytotoxic and anti-tumor

properties. The brine shrimp lethality assay has been proved to be a convenient system for monitoring biological activities of natural products (Chanda *et al*., 2021). In this study, the degree of lethality of *P. africana* and *T. indica* crude extracts to brine shrimps on exposure for 24 hours was directly proportional to the concentration of the extract with LC_{50} 31.62±0.41 and 109.78±2.62 ppm respectively, while the aqueous fraction of *P. africana* (AqPA) recorded the highest lethality with LC_{50} 9.20±0.27. The cytotoxic property of plants extract may be due to the presence of antitumor compounds present in the plant extracts. Standard brine shrimp lethality bioassay stipulates that an LC_{50} value

<1000 µg/mL is considered bioactive in toxicity evaluation of plant extracts (Meyer *et al*., 2018).

4.2.3 Antioxidant activity

Reactive oxygen species (ROS) and other free radicals are produced during inflammationassociated cellular injuries. ROS facilitates lipid peroxidation and breakdown of many macromolecules and cause injury to tissues. As a result, inflammatory mediators are released (Geronikaki and Gavalas, 2019). This strong association between ROS, and hence oxidative stress, and inflammation led to an investigation of the antioxidant activity of the extracts. The ability of the extracts to scavenge free radicals and ROS was evaluated using the DPPH assay. The % scavenging activity of the aqueous fraction of *P. africana* (AqPA)

(67.45%) was lower when compared with Ascorbic acid (Standard) (88.51%) (Figure 4.1). In the DPPH assay, the IC_{50} for the AqPA fraction and Ascorbic acid were 12.32 and 50.55 µg/mL, respectively. Many extracts and compounds exert antioxidant action by interfering with the formation of the initiator radical or terminate radicals during chain propagation. Some antioxidants also act indirectly by enhancing the activities of enzymes that neutralize ROS or

induce the expression of such proteins (Amorati *et al*., 2018). The significance of antioxidants in human health has been described extensively and many studies have shown they may play various roles as protection against cardiovascular disease (reducing chronic inflammation and improving endothelial function), certain types of cancer and cytotoxic effects (Ghumare and Dattatraya, 2021).

Since *P. africana* extract contains a number of different phytochemicals, the antioxidant effect exhibited is most likely a result of the synergistic action of the various constituents. The antioxidant activity of the extracts proves that it contains several antioxidant compounds with several possible chemical mechanisms of action. This assumption is in agreement with a study carried out by Nganso *et al*. (2018) and Elmezughi *et al*. (2013), who reported that 2 phenolic derivatives have been previously isolated from *P. africana* (Nganso *et al*., 2018; Elmezughi *et al*., 2013). Phenolic compounds are known to scavenge reactive oxygen species and free radicals such as singlet oxygen, superoxide anion, and hydroxyl radicals by donating hydrogens to stabilize the ROS (Azab *et al*., 2016: Miller and Ruiz-Larrea, 2022). It has therefore been suggested that there is a positive correlation between antioxidant activity and Phenolic compounds present in plants (Velioglu *et al*., 2018).

4.2.4 MCF-7 cell viability test

After 24 and 48 hours' incubation of MCF-7 cell lines with the fraction and standard drug, the results showed a time and dose dependent, with decrease in number of viable cells (i.e. increase in the dose and time of exposure, resulted in viable cells decrease) which was significant at ($P < 0.05$), compared to the negative control groups which showed a significant increase in number of viable cells from 24 to 48 hours' exposure as presented in

Table 4.4 and 4.5. The cytotoxic effect of AqPA fraction on the MCF-7 cell lines had an

IC_{50} of 235.87 and 52.04 µg/mL at 24 and 48 hours respectively. Result from the current study illustrates the cytotoxic effect of AqPA fraction on MCF-7 cell lines. Reduction in the (%) viable cell and the increase in the Percentage inhibition (%) after 24 and 48 hours of treatment with the aqueous fraction is a manifestation of its cytotoxicity as shown in Table 4.7. The results obtained show that AqPA fraction demonstrated anti-proliferation activity on MCF-7 cell lines which is an indication of the potential AqPA fraction to inhibit cell proliferation in rapidly dividing cells.

According to the American National Cancer Institute USA (NCI), the criteria of cytotoxicity activity for botanicals/crude extracts is IC50 < 20µg/mL or 10µM upon 48 h or 72 h incubation (Boik, 2021). The NCI considers an IC_{50} upper limit criteria of 30µg/mL as a promising crude extract for purification (Suffness and Pezzuto, 2019). Justifying the cytotoxicity activity of AqPA having IC_{50} 52.04 µg/mL recorded after 48 hours in this study, Ayoub *et al.,* (2014) considered that at higher IC_{50} values, a plant extract could be effective as being cytotoxic at concentrations up to 100 µg/mL. Isabel *et al.,* (2022) also reported that extracts were considered cytotoxically active at ≤100 µg/mL; therefore, AqPA fraction can be considered promising lead for the development of new plant-based anticancer drugs.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATION

5.1 Conclusion

The results of this study revealed that the aqueous fraction of *P. africana* significantly reduced the proliferation of MCF-7 cell lines with an IC_{50} of 52.04µg/mL after 48hours of exposure. The value falls under the range of ≤100µg/mL that means it is cytotoxic as described by Ayoub *et al*. (2014) and Isabel *et al*. (2022). Therefore, aqueous fraction of *P. africana* could potentially be a source of new plant-based anticancer compound.

5.2 Recommendations

i. Further studies on the isolation and preclinical trials of the bioactive compounds from the plant extracts are recommended.

ii. Efficacious of other non-polar solvent and their possible mechanism of action are recommended.

5.3 Contribution of the Research to Knowledge

The extract of *P. africana* is considered to be safe because it was well tolerated up to 5000mg/kg body weight in rats. This research revealed that the aqueous fraction of *P. africana* have the potency to inhibit proliferation of MCF-7 cell lines.

REFERENCES

Abarca, N.A., Campos, M.G., Reyes, J.A.A., Jimenez, N.N., Corral, J.H. & Valdez, L.G.S., (2007). Antioxidant activity of polyphenolic extract of monofloral honeybee collected pollen from mesquite (*Prosopis juliflora*, Leguminosae). *J. Food Compos. Anal*., 20, 119–124.

Adebayo, A.H., Zeng, G.Z., Fan, J.T., Ji, C.J., He, W.J., Xu, J.J., Zhang, Y.M, Akindahunsi, A.A, Kela, R, & Tan, N.H. (2019). Biochemical, haematological and histopathological studies of extract of *Ageratum conyzoides* L. in Sprague Dawley rats. *Journal of Medicine & Plants Research*, 4:2264–2272

Agarwal, S.S. & Paridhavi, M., (2017). Herbal drug Technology. University press pvt. Ltd. First edition, 104.

Agboola, A., (2019). Prosopis africana: stem, roots and seeds in the economy of the savannah areas of Nigeria. *Economy Botany*, 58(4): 34-42.

Ahmed, N., Mahmood, A., Ashraf, A., Bano, A., Tahir, S.S. & Mahmood, A., (2015). Ethnopharmacological relevance of indigenous medicinal plants from district Bahawalnagar, Punjab, Pakistan. *Journal of Ethnopharmacology*, 175, 109–123.

Akintayo, B., Omolere, M. & Alagbe, J.O., (2020). Probiotics and medicinal plants in poultry nutrition: A review. *United International Journal for Research and Technology*, 2(1): 7-13.

Alabi, D. A., (2018). Parkia biglobosa: An endangered species. Pages 265-285 in Proceedings of the Seminar on Lost Crops of Nigeria. University of Agriculture, Abeokuta, Nigeria.

Alagbe, J. O., (2021). Daniellia oliveri leaf extracts as an alternative to antibiotic feed additives in broiler chicken diets: meat quality and fatty acid composition. *International Journal of Clinical and Medical Informatics* 4(1): 15-24.

Alexander, S., (2019). Phytoestrogens and their effects. *European Journal of Pharmacology*, 741:230-236.

Al-Hajj, M., Wicha, M.S. & Benito-Hernandez, A., (2003). Prospective Identification of Tumorigenic Breast Cancer Cells. *Proc Natl Acad Sci.*, U S A., 100: 3983-3988

Amaral, R. G., (2019). Natural products as treatment against cancer: A historical and current vision. *Clinical Oncology*, 1-5.

Ambriz-Perez, D. L., Leyva-Lopez, N., Gutierrez-Grijalva, E.P., & Heredia, J.B., (2016). Phenolic compounds: Natural alternative in inflammation treatment. A review. *Cogent Food & Agriculture*, 2(1):1-14.

Amir, M., Khan, M. A., Ahmad, S., Akhtar, M., Mujeeb, M., Ahmad, A., Khan, S. A. & Al-Abbasi, F. A., (2016). Ameliorating effects of *Tamarindus indica* fruit extract on anti-tubercular drugs induced liver toxicity in rats. *Natural Product Research*, 30(6), 715–719.

Amorati, R., Foti, M. C., & Valgimigli, L., (2018). Antioxidant activity of essential oils. *Journal of Agricultural and Food Chemistry*, 61(46), 10835–10847.

Amusa, T. O., Jimoh, S.O., Aridanzi, P. & Haruna, M.A., (2020). "Ethno otany and conservation of plant resources of kainji lake national park, Nigeria". *Ethno-botany Research and Application*, 8: 18-194.

Atawodi, S. E, & Ogunbusola, F., (2019). Evaluation of anti-trypanosomal properties of four extracts of leaves, stem and root barks of *Prosopis africana* in laboratory animals. *Biokemistri,* 21:101–108.

Ayanwuyi, L. O., Yaro, A. H. & Abodunde, O.M., (2020). "Analgesic and antiinflammatory effects of methanol stem bark extract of Prosopis Africana". *Pharmaceutical Biological*, 48(3): 296-299.

Ayoub, I. M.; El-Shazly, M.; Lu, M. C.; & Singaba, A. N. B., (2014). Antimicrobial and cytotoxic activities of the crude extracts of Dietes bicolor leaves, flowers and rhizomes. *South African Journal of Botany*, 95, 97–101.

Azab, A., Nassar, A., & Azab, A. (2016). Anti-inflammatory activity of natural products. *Molecules*, 21(10), 1321.

Babayi, H., Praise, O. O., Fadipe, L., Ajoke, I. K. O., Baba, E., & Peter, O. O., (2018). Evaluation of the effects of Leech Salivary Extract (LSE) on haematological parameters in rats. *Journal of Hematology and Clinical Research,* 2, 006-014.

Baglia, M.L.; Tang, M.-T.C.; Malone, K.E.; Porter, P. & Li, C.I., (2018). Family History and Risk of Second Primary Breast Cancer after insitu Breast Carcinoma. *Cancer Epidemiol*. *Biomarker Previews,* 27, 315–320.

Baguley, B. C. & Leung, E., (2011). Heterogeneity of Phenotype in Breast Cancer Cell Lines. *In*: Breast Cancer - Carcinogenesis, Cell Growth and Signalling Pathways (Gunduz M, Gunduz E (eds.). Rijeka, *InTech*, 245-256.

Barretina, J., Caponigro, G., Stransky, N., Venkatesan, K. & Margolin, A., (2022). The Cancer Cell Line Encyclopedia enables predictive modelling of anticancer drug sensitivity. *Nature*, 483(7391): 603–607.

Baturh, Y., (2020). Phytochemical screening and antibacterial activity of dried Prosopis Africana. *Journal of Medicinal Plant Research,* 14(8): 359-365.

Baumann, M., Krause, M. and Hill, R., (2008). Exploring the Role of Cancer Stem Cells in Radioresistance. *Nat Rev Cancer*, 8: 545-554.

Benz, C. C., (2018). Impact of aging on the biology of breast cancer. *Critical Review of Oncology*, 66, 65–74.

Bernstein, L., (2022). Epidemiology of Endocrine-Related Risk Factors for Breast Cancer. J. Mammary Gland. Biol. *Neoplasia*, 7, 3–15.

Biplob, D., & Arun, K., (2018). A Review Article on Breast Cancer. *International journal of pharmacy & pharmaceutical research,* 11 (2): 284-298.

Boik, J., (2021). Natural Compounds in Cancer Therapy; Oregon Medical Press: *Princeton*, MN, USA.

Braakhuis, A.J., Campion, P., & Bishop, K.S., (2019). Reducing Breast Cancer Recurrence: The Role of Dietary Polyphenolics. *Nutrients,* 8:547.

Bray, F., Ferlay, J., Soerjomataram, I., Siegel, R.L., Torre, L.A. & Jemal, A., (2018). Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: *A Cancer Journal for Clinicians*, 3:31.

Brewer, H. R., Jones, M. E., Schoemaker, M. J., Ashworth, A. & Swerdlow, A. J., (2017). Family history and risk of breast cancer: An analysis accounting for family structure. *Breast Cancer Research Treatment*, 165, 193–200.

Brunelli, E., Minassi, A., Appendino, G. & Moro, L., (2018). 8-Prenylnaringenin, inhibits estrogen receptor-α mediated cell growth and induces apoptosis in MCF-7 breast cancer cells. *The Journal of Steroid Biochemistry and Molecular Biology*, 107(35):140-148.

Casey, S.C., Vaccari, M., Al-Mulla, F., Altemaimi, R., Amedei, A., Barcellos-Hoff, M.H., Brown, D., Chapellier, M., Christopher, J. & Curran, C. S., (2015). The effect of environmental chemicals on the tumor microenvironment. *Carcinogenesis*, 36, S160–S183.

Cattaneo, F., Sayago, J. E., Alberto, M.R., Zampini, I.C., Ordoñez, R.M., Chamorro, V., Pazos, A. & Isla, M. I., (2014). Anti-inflammatory and antioxidant activities, functional properties and mutagenicity studies of protein and protein hydrolysate obtained from *Prosopis alba* seed flour. *Food Chem.*, 161, 391–399.

CGHFBC., (2021). Familial breast cancer: Collaborative reanalysis of individual data from 52 epidemiological studies including 58,209 women with breast cancer and 101,986 women without the disease. *Lancet*, 358, 1389–1399.

Chanda, S. & Baravalia, Y., (2021). Brine shrimp cytotoxicity of *Caesalpinia pulcherrima* aerial parts, antimicrobial activity and characterization of isolated active fractions. *National Production Research*, (In Press).

Chang, J. H., Vines, E., Bertsch, H., Fraker, D. L., Czerniecki, B. J., Rosato, E. F., Lawton, T., Conant, E.F., Orel, S.G., Schuchter, L., Fox, K.R., Zieber, N., Glick, J.H. & Solin, L.J., (2001). The Impact of a Multidisciplinary Breast Cancer Center on Recommendations for Patient Management: The University of Pennsylvania experience. Cancer, 91: 1231-1237.

Chen, X., Wang, Q., Zhang, Y., Xie, Q. & Tan, X., (2018). Physical Activity and Risk of Breast Cancer: A Meta-Analysis of 38 Cohort Studies in 45 Study Reports. *Value Health*, 22, 104–128.

Chuba, P., Hamre, M., Yap, J., Severson, R., Lucas, D., & Shamsa, F., (2019). Bilateral risk for subsequent breast cancer after lobular carcinoma-in-situ: analysis of surveillance, epidemiology, and end results data. *Journal of Clinical Oncology*, 23:5534–41.

Chukwunomso, J. P., Bede, O.E. & Nonye, A.L., (2020). Effect of Diets with Iron Tree (*Prosopis africana*) Pulp on Performance and Blood Characteristics of Growing Rabbits. *Asian Journal of Animal Science*, 14: 61-68.

Chun, C., (2019). what are the sign and symptoms of Breast Cancer, Healthline. https://www.healthline.com/health/breast-cancer/warning-signs#causes

Crouch, S.P. *et al*. (1993). The use of ATP bioluminescence as a measure of cell proliferation and cytotoxicity. *Journal of Immunology Methods* 160, 81–8.

Dandamudi, A., Tommie, J., Nommsen-Rivers, L. & Couch, S., (2018). Dietary Patterns and Breast Cancer Risk: A Systematic Review. *Anticancer Research*, 38, 3209– 3222.

Darvishi, M., Esmaeili, S., Dehghan-Nayeri, N., Mashati, P. & Gharehbaghian, A., (2017). Anticancer effect and enhancement of therapeutic potential of Vincristine by extract from aerial parts of Juniperus excelsa on pre-B acute lymphoblastic leukemia cell lines. *Journal of Applied Biomedicine*, 15(3): 219- 226.

De-Kleijn, M.J.J., (2018). Intake of dietary phytoestrogens is low in postmenopausal women in the United States: The Framingham study. *The Journal of Nutrition*, 131:1826-1832.

Denoyelle, C.; Vasse, M.; Körner, M.; Mishal, Z.; Ganné, F.; Vannier, J.-P.; Soria, J. & Soria, C. (2021). Cerivastatin, an inhibitor of HMG-CoA reductase, inhibits the signaling pathways involved in the invasiveness and metastatic properties of highly invasive breast cancer cell lines: An in vitro study. *Carcinogenesis*, 22, 1139–1148.

Division induced dynamics in non-Invasive and invasive breast cancer. *Biophysics Journal*, 112:123–125.

Done, S.J., (2021). Preface. In Breast Cancer - Recent Advances in Biology, Imaging and Therapeutics. Rijeka, *InTech*, p IX.

Duffy, S.W., Morrish, O.W., Allgood, P.C., Black, R., Gillan, M.G., Willsher, P., Cooke, J., Duncan, K.A., Michell, M.J. & Dobson, H.M., (2017). Mammographic density and breast cancer risk in breast screening assessment cases and women with a family history of breast cancer. *European Journal of Cancer*, 88, 48–56.

Duggan, C., Dvaladze, A., Rositch, A.F., Ginsburg, O., Yip, C., Horton, S., Rodriguez, R.C., Eniu, A., Mutebi, M. & Bourque, J., (2020). The Breast Health Global

Initiative 2018 Global Summit on Improving Breast Healthcare Through Resource Stratified Phased Implementation: Methods and overview. *Cancer*, 126, 2339–2352

Efferth, T., (2017). Integration of Elmezughi, J. Shittu, H. Clements, C. Edrada-Ebel, A.R. Seidel, V. & Gray, A. (2020). "Bioactive natural compounds from *Prosopis africana* and *Abies nobili*," *Journal of Applied Pharmaceutical Science*, 3, 040–043.

Elik, A., Acar, M., Erkul, C.M., Gunduz, E. & Gunduz, M., (2018). Relationship of Breast Cancer with Ovarian Cancer. Concise Rev. Mol. Pathol. *Breast Cancer*, 87–202

Elmezughi J., Shittu H., Clements C., Edrada-Ebel R.A., Seidel V., & Gray A. (2013). Bioactive Natural Compounds from *Prosopis africana* and *Abies nobili*. *Journal of Applied Pharmaceutical Science*, 3(3):40-3.

Elnour, M.A., Penech, F. & Mesaik, M.A., (2017). Four Selected Sudanese Medicinal Plants Induce Anticancer and Cytotoxic Effects in Prostate Cancer Cell Line. *Clinical and Medical Biochemistry*, 3(2):134.

El-Sharkawy, A. & Malki, A. (2020). phytochemicals and phytotherapy into cancer precision medicine. *Oncotarget*, 8(30):50284-50304.

Erol, A., Ho, A.M.C., Winham, S.J. & Karpyak, V.M., (2017). Sex hormones in alcohol consumption: A systematic review of evidence. *Additives & Biology*, 24, 157–169.

Errichetti, E., Avellini, C., Pegolo, E. & De-Francesco, V., (2017). Dermoscopy as a supportive instrument in the early recognition of erosive adenomatosis of the nipple and mammary paget's disease. *Annual Dermatology*, 29:365–7.

Estébanez, N., Gómez-Acebo, I., Palazuelos, C., Llorca, J. & Dierssen-Sotos, T., (2018). Vitamin D exposure and Risk of Breast Cancer: A meta-analysis. *Science Report*, 8, 9039.

Eve, L., Fervers, B., Le Romancer, M. & Etienne-Selloum, N., (2020). Exposure to Endocrine Disrupting Chemicals and Risk of Breast Cancer. *International Journal of Molecular Science*, 21, 9139.

Fasidi, I., Samani, O.T., Kadiri, M. & Agboola. D. A., (2020). Dormancy types and water uptake in seeds of Parkia biglobosa. *Journal of Natural and Applied Sciences*, 1:1420.

Ferlay, J., Laversanne, M., Ervik, M., Lam, F., Colombet, M., Mery, L., Piñeros, M., Znaor, A., Soerjomataram, I. & Bray, F., (2020). Global Cancer Observatory: Cancer Tomorrow. *International Agency for Research on Cancer*: Lyon, France.

Ferlay, J., Shin, H.R. Bray, F. Forman, D. Mathers C. & Parkin D.M., (2018). Cancer Incidence and Mortality Worldwide. *International Agency for Research on Cancer GLOBOCAN*, 1.2.

Gandhi, A.P., Joshi, K.C., Jha, K., Parihar, V.S., Srivastav, D.C., Raghunadh, P., Kawalkar, J., Jain, S.K. & Tripathi, R.N., (2003). Studies on alternative solvents for the extraction of oil soybean. *International Journal of Food Science Technology,* 38(3):369-375.

Geronikaki, A. A. & Gavalas, A. M., (2019). Antioxidants and inflammatory disease: Synthetic and natural antioxidants with anti-inflammatory activity. *Combinatorial Chemistry & High Throughput Screening,* 9(6), 425–442.

Ghoncheh, M., Pournamdar, Z. & Salehiniya, H., (2019). Cancer. *Asian Pacific Journal of Cancer Preview,* 17(S3), 43–46.

Ghumare, P. & Dattatraya J. (2021). *Tamarindus Indica*: An Important Medicinal Plants.

Ginsburg, O., Bray, F., Coleman, M., Vanderpuye, V., Eniu, A., Kotha, S.R., Sarker, M., Huong, T.T., Allemani, C. & Dvaladze, A., (2019). The global burden of women's cancers: A grand challenge in global health. *Lancet*, 389, 847–860

Giordano, S.H. (2018). Breast cancer in men. *North England Journal of Medicine*, 378, 2311–2320.

GLOBOCAN. (2018). Global, regional, national burden of breast cancer in 185countries: evidence from globocan. *Breast cancer research and treatment,* 187, 557-567.

Gunduz, M. & Gunduz, E., (2011). Preface. *In*: Breast Cancer – Carcinogenesis, Cell Growth and Signalling Pathways. Rijeka, *InTech*, p XI.

Gupta M., Pal R.A.G.K., Tikoo D. (2013). Multiple myeloma: The disease and its treatment. A Review*; International Journal of Basic & Clinical Pharmacology*, 2(2):103-121

Gupta, M.P., Monge, A., Karikas, G.A., Lopez de Cerain, A., Solis, P.N., de Leon, E., Trujillo, M., Suarez, O., Wilson, F., Montenegro, G., Noriega, Y., Santana, A.I., Correa, M. & Sanchez, C., (1996). Screening of Panamanian Medicinal Plants for Brine Shrimp Toxicity, Crown Gall Tumor Inhibition, Cytotoxicity and DNA Intercalation. *International Journal of Pharmacognosy,* Vol. 34, No. 1, pp. 19-27.

Guruvayoorappan, C., Sakthivel, K.M., Padmavathi, G., Bakliwal, V., Monisha J.Y. & Kunnumakkara, A.B., (2020). Cancer preventive and therapeutic properties of fruits and vegetables: An overview, in Kunnumakkara (ed.), Anticancer Properties of Fruits and Vegetables: *A Scientific Review, World Scientific publishers, Singapore*, 1–52.

Hall, M. P., Gruber, M. G., Hannah, R. R., Jennens-Clough, M. L. and Wood, K.V. (1998). Stabilization of firefly luciferase using directed evolution. In: Bioluminescence and Chemiluminescence—Perspectives for the 21st Century. Roda A, Pazzagli M, Kricka LJ, Stanley PE (eds.), pp. 392–395. *John Wiley & Sons, Chichester, UK*.

Harris, L.N., Ismaila, N., McShane, L.M., Andre, F., Collyar, D.E. & Gonzalez-Angulo, A.M. (2016). Use of biomarkers to guide decisions on adjuvant systemic therapy for women with early-stage invasive breast cancer. *Journal of Clinical Oncology,*

34:1134–50.

Henciya, S., P. Seturaman, A.R. James, Y.H. Tsai & Nikam R., (2017). Biopharmaceutical Potentials of *Prosopis spp*. (Mimosaceae, Leguminosa). *Journal of Food Drug Anal.*, 25: 187-196.

Heravi, Karimovi M., Pourdehqan, M., Jadid, Milani M., Foroutan, S.K. & Aieen F., (2016). Study of the effects of group counseling on quality of sexual life of patients with breast cancer under chemotherapy at Imam Khomeini Hospital. *Journal of Mazandaran University of Medical Science*, 16(54):43-51.

Hoover, R.N., Hyer, M., Pfeiffer, R.M., Adam, E., Bond, B., Cheville, A.L., Colton, T., Hartge, P., Hatch, E. & Herbst, A.L., (2021). Adverse Health Outcomes in Women Exposed in Utero to Diethylstilbestrol. *North England Journal of Medicine*, 365, 1304–1314.

Hopper, J.L., kConFab Investigators, Dite, G.S., MacInnis, R.J., Liao, Y., Zeinomar, N., Knight, J.A., Southey, M.C., Milne, R.L. & Chung, W.K., (2018). Age-specific breast cancer risk by body mass index and familial risk: Prospective family study cohort (ProF-SC). *Breast Cancer Research,* 20, 132.

Howtorelief.com., (2019). Breast Cancer: Symptom, Causes, Treatment & Self-exam. Retrieved from https://www.howtorelief.com/breast-cancer-causes-diagnosis-treatment. On 26th November, 2020.

Hu, S., Xu, Y., Meng, L., Huang, L. & Sun H., (2018). Curcumin inhibits proliferation and promotes apoptosis of breast cancer cells. *Experimental and Therapeutic Medicine,* 16:1266-1272.

Huang, W.Y., Cai, Y.Z. & Zhang, Y., (2020). Natural phenolic compounds from medicinal herbs and dietary plants: Potential use for cancer prevention. *Nutrition and Cancer*, 62:1-20.

Hui, C., Qi, X., Qianyong, Z., Xiaoli, P., Jundong, Z., & Mantian, M., (2018): Flavonoids, flavonoid subclasses and breast cancer risk: a meta-analysis of epidemiologic studies. *PLoS One*, 8(1): e54318.

Hussein, S. I., Yaseen, N.Y., Jawad, S.Q. & Suha, T. A., (2017). Seeds of *Tamarindus indica* as Anti-cancer in some cell line. *International Journal of Advanced Biological Research*.7(2): 360-362. ISSN 2250 – 3579.

Ibikunle, G. F., Okwute, S. K. & Ogbadoyi, E. O., (2017). Cytotoxic agents from Nigerian Plants: A Case Study of Spondias mombin Linn (Anacardiaceae) Leaves. *FUW Trend in Science and Technology Journal*, 2(1B): 510- 513.

Idikio, H.A., (2011). Human cancer classification: A systems biology-based model integrating morphology, cancer stem cells, proteomics, and genomics. *Journal of Cancer*, 2:107-115

Idoko, S.S., Assa, E.A. & Gbande S (2018): Marketing and utilization of *Prosopis africana* condiment in makurdi, Benue state, Nigeria *Journal of Research in Forestry, Wildlife and Environmental*, 1:63-74.

Isabel, C., Pedro, V., Ana, I.O., María, A.C., & Cláudia, P., (2022). In Vitro Cytotoxic Activity of African Plants: A Review. *Molecules*, 27, 4989.

Jahromi, M.A.F., Etemadfard, H. & Zebarjad, Z., (2018). Antimicrobial and antioxidant characteristics of volatile components and ethanolic fruit extract of *Prosopis farcta* (Bank & Soland.). *Trends Pharmaceutical Science*, 4, 177–186.

Jiang, D., Rasul, A., Batool, R., Sarfraz, I., Hussain, G., Tahir, M.M., Qin, T., Selamoglu, Z., Ali, M., Li, J. & Li, X., (2019). Potential Anticancer Properties and Mechanisms of Action of Formononetin. A reviewed article. *BioMed Research International*, Article ID 5854315, 11 pages

Joglekar-Javadekar, M., Van-Laere, S., Bourne, M., Moalwi, M., Finetti, P. & Vermeulen, P.B. (2017). Characterization and targeting of platelet-derived growth factor receptor alpha (PDGFRA) in inflammatory breast cancer (IBC). *Neoplasia*, 19:564– 73.

Jones, M.; Schoemaker, M.J.;Wright, L.B.; Ashworth, A. & Swerdlow, A.J. (2017). Smoking and risk of breast cancer in the Generations Study cohort. *Breast Cancer Research*, 19, 118.

Key, T., Appleby, P., Barnes, I. & Reeves, G., (2022). The Endogenous Hormones and Breast Cancer Collaborative Group Endogenous Sex Hormones and Breast Cancer in PostmenopausalWomen: Reanalysis of Nine Prospective Studies. *Journal of National Cancer Institute*, 94, 606–616.

Key, T.J., Appleby, P.N., Reeves, G.K., Travis, R.C., Alberg, A.J., Barricarte, A., Berrino, F., Krogh, V., Sieri, S. & Brinton, L.A., (2019). Sex hormones and risk of breast cancer in premenopausal women: A collaborative reanalysis of individual participant data from seven prospective studies. *Lancet Oncology*, 14, 1009–1019.

Kim, S., (2019). Nucleic Acids Research. update: Improved access to chemical data. *PubChem*, 47(D1): D1102-D1109

Kolb, R. & Zhang, W. (2020). Obesity and Breast Cancer: A Case of Inflamed Adipose Tissue. *Cancers*, 12, 1686.

Konstat-Korzenny, E., Ascencio-Aragón, J., Niezen-Lugo, S. & Vázquez-López, R. (2018). Artemisinin and its synthetic derivatives as a possible therapy for cancer. Medical Science, 6(1):19.

Kumaran, V.S., Arulmathi, K. & Srividhya, R. (2018). Repletion of antioxidant status by EGCG and retardation of oxidative damage induced macromolecular anomalies in aged rats. *Experimental Gerontology*, 43:176-183.

Leila, Z., Eliandra de Sousa, Luisa, H. C., Anildo, C.J., Moacir, G. P., Bruno, S., Fatima, R. & Mena, B. S., (2007). Effect of crude extract andfractions from Vitex megapotamica leaves on hyperglycemia inalloxan-diabetic rats. Journal of Ethnopharmacology. 109:151-155.

Leso, V.; Ercolano, M.L.; Cioffi, D.L. |& Iavicoli, I. (2019). Occupational Chemical Exposure and Breast Cancer Risk According to Hormone Receptor Status: A Systematic Review *Cancers*, 11, 1882

Lewandowska, A.M., Rudzki, M., Rudzki, S., Lewandowski, T. & Laskowska, B., (2019). Environmental Risk Factors for Cancer. Review paper. *Ann Agric Environ Med*., 26(1): 1–7.

Liedtke, C., Mazouni, C., Hess, K.R., André, F., Tordai, A. & Mejia, J.A. (2018). Response to neoadjuvant therapy and long-term survival in patients with triple-negative breast cancer. *Journal of Clinical Oncology*, 26:1275–81.

Lorke, D., (1983). A new approach to practical acute toxicity testing. *Archives of toxicology*, 1:54(4):275-87.

Mateo, A., Pezzi, T., Sundermeyer, M., Kelley, C., Klimberg, V. & Pezzi, C. (2017). Chemotherapy significantly improves survival for patients with T1c-T2N0M0 medullary Breast cancer. *Annual Surgery Oncology*, 24:1050–6.

McGuire, A., Brown, J.A.L., Malone, C., McLaughlin, R. & Kerin, M.J., (2015). Effects of Age on the Detection and Management of Breast Cancer. *Cancers*, 7, 908–929.

Merrill, A., White, A. & Howard-McNatt, M. (2017). Paget's disease of the breast: an institutional review and surgical management. *Armstrong Surgery*, 83:96–8.

Meyer, B.N., Ferrigni, N.R., EPutnam J., Jacobson, L.B., ENichols, D. & Maclaughlin. J.L. (2018). "Brine shrimp, a convenient general bioassay for active plant constituents". *Planta Medica*, 45: 31-34.

Miller, N.J., & Ruiz-Larrea, M.B. (2022). Flavonoids and other plant phenols in the diet: Their significance as antioxidants. *Journal of Nutritional & Environmental Medicine*, 12(1), 39–51.

Mishra, B.B. & Tiwari, V. K., (2011). "Natural products: an evolving role in future drug discovery," *European Journal of Medicinal Chemistry*, 46(10): pp. 4769–4807

Molina, L., Bustamante, F.A., Bhoola, K.D., Figueroa, C.D. & Ehrenfeld, P. (2018). Possible role of phytoestrogens in breast cancer via GPER-1/GPR30 signaling. *Clinical Science*, 132(24): 2583-2598.

Molyneux, G., Geyer, F.C. and Magnay, F.A., (2010). BRCA1Basal-like Breast Cancers Originate from Luminal Epithelial Progenitors and not from Basal Stem Cells. Cell, 7: 403-417

Moran, M., Schnitt, S., Giuliano, A., Harris, J., Khan, S., & Horton, J. (2019). Society of surgical oncology–American society for radiation oncology consensus guideline on margins for breast-conserving surgery with whole-breast irradiation in stages I and II invasive breast cancer. *International Journal of Radiology, Oncology, Biology & Physics*, 88:553–64.

Morton, V.K.; Kearney, A.; Coleman, S.; Viswanathan, M.; Chau, K.; Orr, A. & Hexemer, A. (2019). Outbreaks of Salmonella illness associated with frozen raw breaded chicken products in Canada. *Epidemiology of Infections*, 147, 254.

Nahum, A. (2021). Lycopene inhibition of cell cycle progression in breast and endometrial cancer cells is associated with reduction in cyclin D levels and retention of p27 Kip1 in the cyclin E-cdk2 complexes. *Oncogene*, 20(26):3428-3436

Neuman, H., Morrogh, M. & Gonen, M. (2022). Stage IV breast cancer in the Era of targeted therapy, Does surgery of the primary tumor matter. *Cancer*, 116:1226–33.

Neveu, V., Perez-Jiménez, J., Vos, F., Crespy, V., Du-Chaffaut, L., Mennen, L., Knox, C., Eisner, R., Cruz, J., Wishart, D. & Scalbert, A. (2010): Phenol-Explorer: an online comprehensive database on polyphenol contents in foods. *Database (Oxford)*, bap024. doi: 10.1093/database/bap024.

Nganso, D. Y. O. Soh, D. Ndogo, E. O. Mala, O. M. T. G. & Nyasse, B. (2018). "Fatty alcohols Isolated from *Prosopis africana* and evaluation of antibacterial and antituberculosis activities," *Journal of Diseases and Medicinal Plants*, 4, 128–132.

Nguyen, C., Mehaidli, A. & Baskaran, K., (2019). "Dandelion Root and Lemongrass Extracts Induce Apoptosis, Enhance Chemotherapeutic Efficacy, and Reduce Tumour Xenograft Growth In-vivo in Prostate Cancer," Evidence-Based Complementary and Alternative Medicine, Article ID 2951428, 12 pages

Nugoli, M., Chuchana, P., Vendrell, J., Orsetti, B., Ursule, L., Nguyen, C., Birnbaum, D., Douzery, E.J.P., Cohen, P. & Theillet, C. (2018). Genetic variability in MCF-7 sublines: evidence of rapid genomic and RNA expression profile modifications. *BMC Cancer*, 12: 1-12.

Okafor, J.C. (2019). "Lost Crops of Nigeria: In Proceedings of the Seminar on Lost Crops of Nigeria". *University of Agriculture, Abeokuta, Nigeria*, 221.

Olorunmaiye, K.S., Apeh, L.E., Madandola, H.A & Oguntoye, M.O. (2019). Proximate and phytochemical composition of African mahoghany (Afzelia africana) seed and African mesquite (Prosopis africana) pod. *Journal of Applied Science and Environmental Management*, 23(2): 249-252.

Olorunmaiye, K.S., Apeh, L.E., Madandola, H.A. & Oguntoye M.O. (2019). Proximate and Phytochemical composition of African mahogany (*Afzelia africana*) seed and African

mesquite (*Prosopis africana*) pod, *Journal of Applied Science and Environmental Management*, 23(2): 249–252.

Oloyed, O.I., (2005). Chemical profile of unripe pulp of Carica pagaya. *Pak. Journal of Nutrition*, 4: 379-381.

Oluwafemi, R.A., Akinbisola, S.A. & Alagbe, J.O. (2020). Nutritional and growth performance of feeding *Polylathia longifolia* Leaf Meal as partial replacement of Wheat Offal in the diet of broiler chicks. *European Journal of Biotechnology and Bioscience.* 8(4): 17-21.

Orgéas, C.C.; Hall, P.; Rosenberg, L.U. & Czene, K. (2018). The influence of menstrual risk factors on tumor characteristics and survival in postmenopausal breast cancer. *Breast Cancer Research,* 10, R107.

Oyaizu, M., (1986). Studies on product of browning reaction prepared from glucose amine. *Japanese Journal of Nutrition*. 44; pp. 307-315.

Page, K., Guttery, D., Fernandez-Garcia, D., Hills, A., Hastings, R. & Luo, J. (2017). Next generation sequencing of circulating cell-free DNA for evaluating mutations and gene amplification in metastatic breast cancer. *Clinical Chemistry*, 63:532–41.

Pamploma-Roger, G. D. (2019). Madrid, Education and Health Library, *Encyclopaedia of Medicinal plants.*536.

Patrick A.W., David R.T. & Jeremy D. (2018). Chapter 8; Adenosine Triphosphate (ATP) measurement technology, Page 139.

Patrick, J.L., Steck, S.E., Bradshaw, P.T., Trivers, K.F., Abrahamson, P.E., Engel, L.S., He, K., Chow, W.H., Mayne, S.T., Risch, H.A., Vaughan, T.L. & Gammon, M.D., (2019). Dietary intake of flavonoids and oesophageal and gastric cancer: incidence and survival in the United States of America (USA). *Britain Journal of Cancer*, 112: 1291-300.

Patwardhan, B. & Vaidya, A.D.B. (2020). Natural products drug discovery: Accelerating the clinical candidate development using reverse pharmacology approaches. *Indian Journal of Experimental Biology*, 48(3):220-227.

Poorkiani, M., Hazrati, M., Abbaszadeh, A., Jafari, P., Sadeghi, M., Dejbakhsh, T. & Mohammadian, Panah M. (2019). Does a rehabilitation program improve quality of life in breast cancer patients? *Payesh*, 9(1):61-8.

Poprac, P., Jomova, K., Simunkova, M., Kollar, V., Rhodes, C.J.& Valko, M. (2017). Targeting free radicals in oxidative stressrelated human diseases. *Trends in Pharmacological Sciences*, 38(7):592-607.

Porter, P. (2018). Westernizing Women's Risks? Breast Cancer in Lower-Income Countries. *North England Journal of Medicine*, 358, 213–216.

Prabhakaran, S., Rizk, V., Ma, Z., Cheng, C., Berglund, A. & Coppola, D. (2017). Evaluation of invasive breast cancer samples using a 12-chemokine gene expression score: correlation with clinical outcomes. *Breast Cancer Research*, 19:71–4.

Prakash, O., Kumar, A., Kumar, P. & Ajeet, G. (2019). Anticancer Potential of Plants and Natural Products: A Review *American Journal of Pharmacological Sciences*, 1(6), 104-115.

Pratheeshkumar, P. (2017). Cancer prevention with promising natural products: Mechanisms of action and molecular targets. *Anti-Cancer Agents in Medicinal Chemistry*, 12(10):1159-1184.

Raimondi, L., Lodovici, M., Guglielmi, F., Banchelli, G., Ciuffi, M., Boldrini, E. & Pirisino, R. (2018). The polysaccharide from Tamarindus indica (TS-polysaccharide) protects cultured corneal-derived cells (SIRC cells) from ultraviolet rays. *Journal of Pharmacy and Pharmacology*. 55(3):333-8.

Reis, P.M.C.L., Dariva, C., Vieira, G.A.B. & Hense, H. (2016). Extraction and evaluation of antioxidant potential of the extracts obtained from tamarind seeds (*Tamarindus indica*), sweet variety. *Journal of Food Engineering*, 173(6):116- 123.

Religioni, U. (2020). Cancer Incidence and Mortality in Poland. *Clinical Epidemiology & Global Hearth*, 8, 329–334.

Rietjens, I.M.C.M., Louisse, J. & Beekmann, K. (2017). The potential health effects of dietary phytoestrogens. *British Journal of Pharmacology*, 174(11):1263-1280.

Rodgers, K.M.; Udesky, J.O.; Rudel, R.A. & Brody, J.G. (2018). Environmental chemicals and breast cancer: An updated review of epidemiological literature informed by biological mechanisms. *Environmental Research*, 160, 152–182.

Russo, M., (2019). Understanding Genistein in cancer: The 'good' and the 'bad' effects: A review. *Food Chemistry,* 196:589-600.

Santosh, S., Bhadoriya, A.G., Jitendra N.G.& Alok, P.J., (2021). *Tamarindus indica*: Extent of explored potential, *Pharmacogenomic Review*, 5(9):73-81.

Schacht, D.V., Yamaguchi, K., Lai, J., Kulkarni, K., Sennett, C.A. & Abe, H., (2019). Importance of a Personal History of Breast Cancer as a Risk Factor for the Development of Subsequent Breast Cancer: Results from Screening Breast MRI. Am. J. Roentgenology, 202, 289–292.

Sellami, M. & Bragazzi, N.L. (2020). Nutrigenomics and Breast Cancer: State-of-Art, Future Perspectives and Insights for Prevention. *Nutrients*, 12, 512

Shahbandi, A., Nguyen, H.D. & Jackson, J.G. (2020). TP53 Mutations and Outcomes in Breast Cancer: Reading beyond the Headlines. *Trends Cancer*, 6, 98–110.

Shamavat, H. & Kurzer, M.S. (2015). Estrogen metabolism and breast cancer. *Cancer Letters*, 356(2):231-243.

Sharma, R. (2018). Global, regional, national burden of breast cancer in 185 countries: Evidence from GLOBOCAN 2018. *Breast Cancer Research & Treatments*, 187, 557–567.

Sharma, R. (2019). Breast cancer incidence, mortality and mortality-to-incidence ratio (MIR) are associated with human development, 1999–2019: Evidence from Global Burden of Disease Study. *Breast Cancer*, 26, 428–445.

Shaukat, U., Ismail, M. & Mehmood, N., (2018). Epidemiology, major risk factors and genetic predisposition for breast cancer in the Pakistani population. *Asian Pacific Journal of Cancer Preview*, 14:5625–9.

Shirazi, F.H., (2011). Remarks in Successful Cellular Investigations for Fighting Breast Cancer Using Novel Synthetic Compounds. *In*: Breast Cancer – Focusing Tumor Microenvironment, Stem Cells and Metastasis (Gunduz M, Gunduz E (eds.). Rijeka, *InTech*, 85-102.

Singh, C.K., Chhabra, G. & Ahmad, N. (2018). Resveratrol and cancer cell biology. In: Resveratrol: State-of-the-Art Science and Health Applications. *World Scientific*, 183-207.

Singleton, V.L., Orthofer, R. & Lamuela-Raventos, R.M., (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods Enzymol*. 299: 152-179.

Smalley, M., Piggott, L. & Clarkson, R., (2013). Breast Cancer Stem Cells: Obstacles to Therapy. *Cancer Lett.*, 338: 57-62

Sofowora, E.A (1984). Phytochemical screening Medicinal Plants and Traditional Medicine in Africa. John Wiley and Sons Inc. New York, pp 35-50.

Somiari, R., Sullivan, A., Russell, S., Somiari, S., Hu, H., Jordan, R., George, A., Katenhusen, R., Buchowiecka, A., Arciero, C. & Brzeski, H. (2018). Highthroughput proteomic analysis of human infiltrating ductal carcinoma of the breast. *Proteomics*, 3(10):1863–73.

Stingl, J., Raouf, A., Eirew, P. & Eaves, C.J. (2021). Deciphering the mammary epithelial cell hierarchy. *Cell Cycle*, 5:1519–22.

Stingl, J., Raouf, A., Emerman, J.T. & Eaves, C.J. (2019). Epithelial progenitors in the normal human mammary gland. *Journal of Mammary Gland Biology Neoplasia*, 10:49–59.

Sudha, B. & Shankarrao, M. (2019). Cancer Biology-Causes & Biomarkers of Cancer. *Current research in Oncology,* 20(19), 01.

Suffness, M. & Pezzuto, J.M. (2019). Assays related to cancer drug discovery. In Methods in Plant Biochemistry: *Assays for Bioactivity*; Hostettmann, K., Ed.; Academic Press: London, UK, 71–133.

Sun, L., Zhu, Y., Qian, Q. & Tang, L., (2018). Body mass index and prognosis of breast cancer. *Medicine*, 97, e11220.

Sweeney, E.E., Mcdaniel, R.E., Maximov, P.Y., Fan, P. & Craig, V., (2013). Models and Mechanisms of Acquired Antihormone Resistance in Breast Cancer: Significant Clinical Progress Despite Limitations. *Hormonal Molecular Biology & Clinical Investigation*, 9: 143-163.

Terry, P.D. & Rohan, T.E., (2022). Cigarette smoking and the risk of breast cancer in women: A review of the literature. Cancer Epidemiol. *Biomarker Preview*, 11, 953–971.

Umair, M., Altaf, M. and Abbasi, A.M., (2017). An ethnobotanical survey of indigenous medicinal plants in Hafizabad district, Punjab-Pakistan. PLoS ONE, 12, e0177912.

Usman, H., Haruna, A.K., Akpulu, I.N., Ilyas, M., Ahmadu, A.A. & Musa, Y.M. (2018). Phytochemical and Antimicrobial Screenings of the Leaf Extracts of Celtisintegrifolia Lam. *Journal of Tropical Bioscience*, 5 (2): 72 – 76.

Velioglu, Y. S., Mazza, G., Gao, L., & Oomah, B. D. (2018). Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. *Journal of Agricultural and Food Chemistry*, 46(10), 4113–4117.

Villadsen, R., Fridriksdottir, A.J., Rønnov-Jessen, L. (2017). Evidence for a stem cell hierarchy in the adult human breast. *Journal of Cell Biology*, 177(1); 87–101.

Vinogradova, Y., Coupland, C. & Hippisley-Cox, J., (2020). Use of hormone replacement therapy and risk of breast cancer: Nested case-control studies using the QResearch and CPRD databases. *Biology & Medicine Journal*, 371, 3873.

Wang, X., Hui, T.L., Wang, M.Q., Liu, H., Li, R.Y. & Song, Z.C., (2019). Body Mass Index at Diagnosis as a Prognostic Factor for Early-Stage Invasive Breast Cancer after Surgical Resection. *Oncology Research & Treatments*, 42, 195–201.

Weber, J.C., Larwanou, M., Abasse, T.A. & Kallinganre, A., (2018). Growth and survival of *Prosopis africana* provenances tested in Niger.

Wernli, K.J., Ms, J.M.H., Trentham-Dietz, A. & Newcomb, P.A., (2019). Antidepressant medication use and breast cancer risk. *Pharmacoepidemiology & Drug Safety*, 18, 284–290.

West, A., Wullkopf, L., Christensen, A., Leijnse, N., Tarp, J.M. & Mathiesen, J., (2017).

WHO, (2018). Latest global cancer data: cancer burden rises to 18.1 million new cases and 9.6 million cancer death in 2018. *International Agency for Research on Cancer.* Press release No 263.

Wishart, D.S., (2018). Drug Bank 5.0: A major update to the Drug Bank database for 2018. *Nucleic Acids Research,* 46(D1): D1074-D1082.

Wright, L.; Frye, J.; Gorti, B.; Timmermann, B. & Funk, J. (2018). Bioactivity of Turmeric-derived Curcuminoids and Related Metabolites in Breast Cancer. *Current Pharmacological Design*, 19, 6218–6225.

Yadav, M., Chatterjee, P., Tolani, S., Kulkarni, J., Mulye, M. & Chauhan, N., (2018). A Nexus model of cellular transition in cancer. *Biological Research,* 51:23. DOI: 10.1186/ s40659-018-0173-8.

Yahyea, B., Laskar, R.., Lourembam M. & Pranab, B.M. (2022). Herbal Remedies for Breast Cancer Prevention and Treatment. *IntechOpen, DOI: http://dx.doi.org/10.5772/intechopen.89669*

Yarkwan, R.A., Akinbisola, S.A, & Alagbe, J.O. (2020). Nutritional and growth performance of feeding *Polylathia longifolia* Leaf Meal as partial replacement of Wheat Offal in the diet of broiler chicks. *European Journal of Biotechnology and Bioscience.* 8(4): 17-21.

Younis, W., Asif, H., Sharif, A., Riaz, H., Bukhari, I.A. and Assiri, A.M., (2018). Traditional medicinal plants used for respiratory disorders in Pakistan: A review of the ethno-medicinal and pharmacological evidence. *Chin. Med*. 13:48.

Yuan H, Ma Q, Ye L. & Piao G. (2019). The traditional medicine and modern medicine from natural products. *Molecules*, 21(5):559.

Zbinden, G. & Flury-Roversi, M. (2019). Significance of the LD50-test for the toxicological evaluation of chemical substances. *Arch Toxicology*, 47(2):77–99.

Zeinomar, N., kConFab Investigators, Knight, J.A., Genkinger, J.M., Phillips, K.A., Daly, M.B., Milne, R.L., Dite, G.S., Kehm, R.D. & Liao, Y. (2019). Alcohol consumption, cigarette smoking, and familial breast cancer risk: Findings from the Prospective Family Study Cohort (ProF-SC). *Breast Cancer Research*, 21, 128.

Zhan, Y., Chen, Y., Liu, R., Zhang, H. & Zhang, Y. (2019). Potentiation of paclitaxel activity by curcumin in human breast cancer cell by modulating apoptosis and inhibiting EGFR signaling. *Archives of Pharmacology Research,* 37(8):1086-1095.

Zhang, M., Lee, A.V. & Rosen, J.M., (2017). The Cellular Origin and Evolution of Breast Cancer. Cold Spring Harbor perspectives in Medicine, 7: a027128.

Zhang, S. M., Cook, N.R., Manson, J.E., Lee, I.M. & Buring, J.E., (2018). Low-dose aspirin and breast cancer risk: Results by tumour characteristics from a randomised trial. *Britain Journal of Cancer*, 98, 989–991.

Zhou, L.; Chen, B.; Sheng, L. & Turner, A. (2020). The effect of vitamin D supplementation on the risk of breast cancer: A trial sequential meta-analysis. *Breast Cancer Research & Treatments*, 182, 1–8

Zillich, A.J, Blumenschein, K., Johannesson, M. & Freeman, P. (2022). Assessment of the relationship between measures of disease severity, quality of life, and willingness to pay in asthma. *Pharmacoeconomics*, 20(4):257- 65.

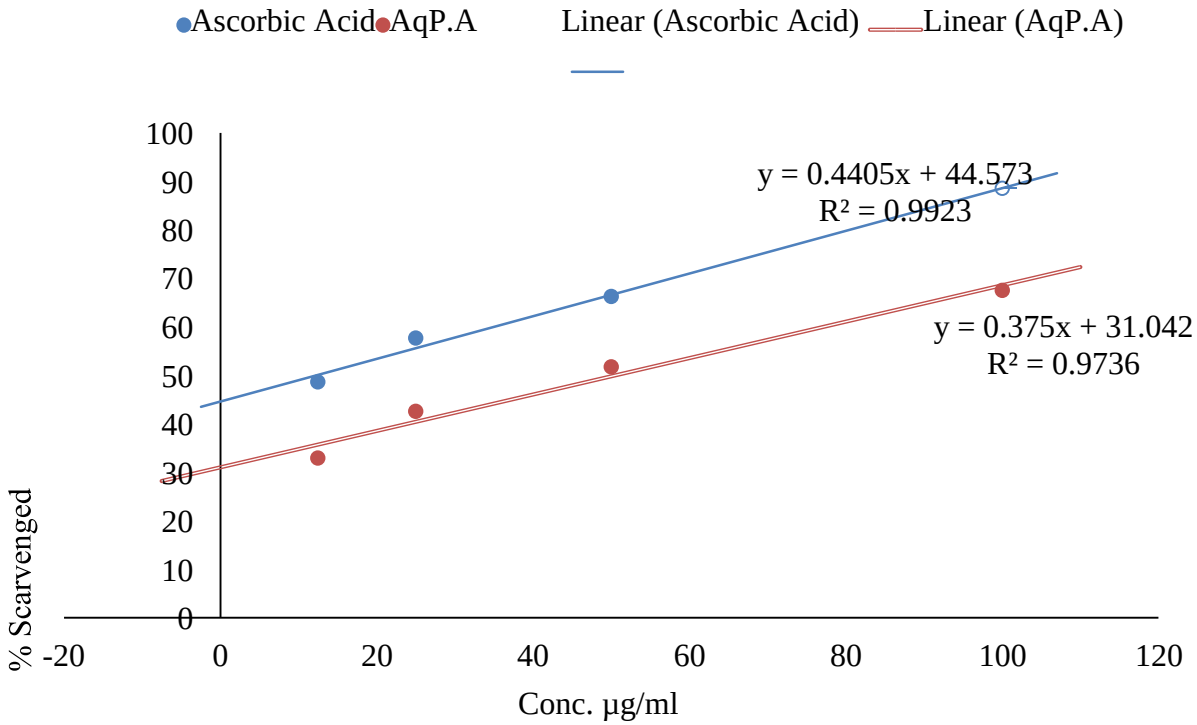
Zyad, A., Leouifoudi, I., Tilaoui, M., Mouse, H.A., Khouchani, M. & Jaafari, A. (2019). Natural products as cytotoxic agents in chemotherapy against cancer. In: Cytotoxicity. Vol. 1. Rijeka: *InTechOpen*, 13.

APPENDIX I

Phytochemical compositions of *P. africana* and *T. indica* crude extract

Sample	*Phytoc hemical (mg/1 00 g)*				
	Phenols	Flavonoids	Tannins	Alkaloids	Saponins
P. africana	849.26	65.54	105.79	561.55	235.09
	848.27	65.76	105.65	558.90	237.45
	848.77	65.65	105.72	560.23	236.27
T. indica	744.68	174.94	47.95	358.80	356.98
	744.08	174.41	47.92	360.43	357.08
	744.38	174.68	47.94	359.62	357.03

APPENDIX II



(b). DPPH scavenging effects of aqueous fraction of *P. africana* and standard (Ascorbic acid).
AqP.A: y = 0.375x + 31.042 (R^2 = 0.9736)

Ascorbic: y = 0.4405x + 44.573 (R^2 = 0.9923)

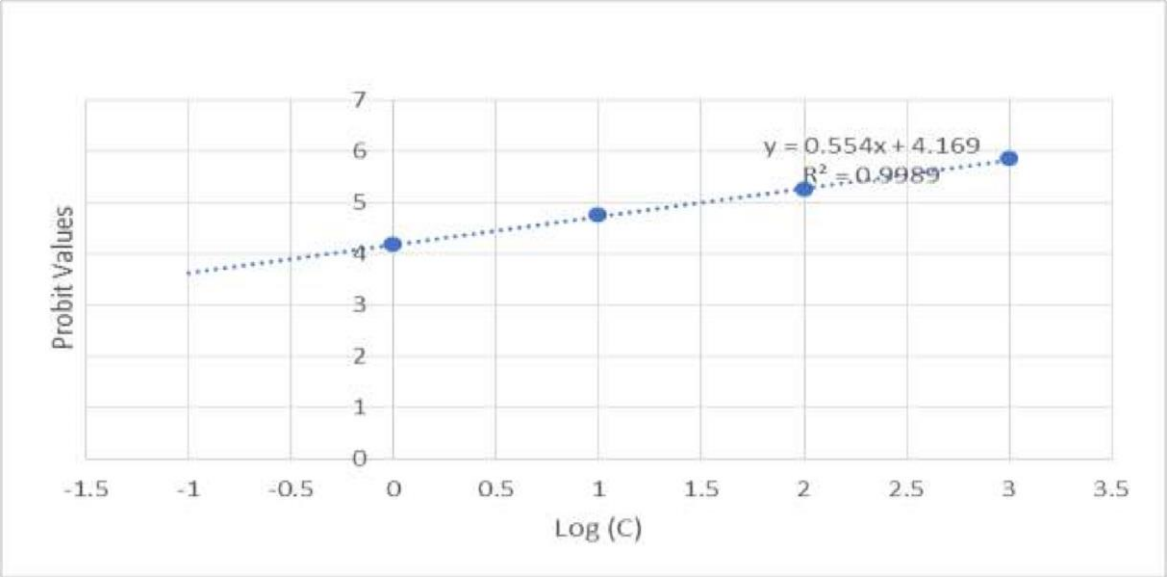
Key: AqPA = Aqueous fraction of *Prosopis Africana*

APPENDIX III

Cytotoxic effects of crude extract of *Prosopis africana* on brine shrimps

C	Log (C)	No of dead nauplii	% Mortality	Probit Value	LC_{50}	LC_{90}
0						
0.1	-1	0	0			
1	0	2	20	4.16		
10	1	4	40	4.75	31.623	5705.815

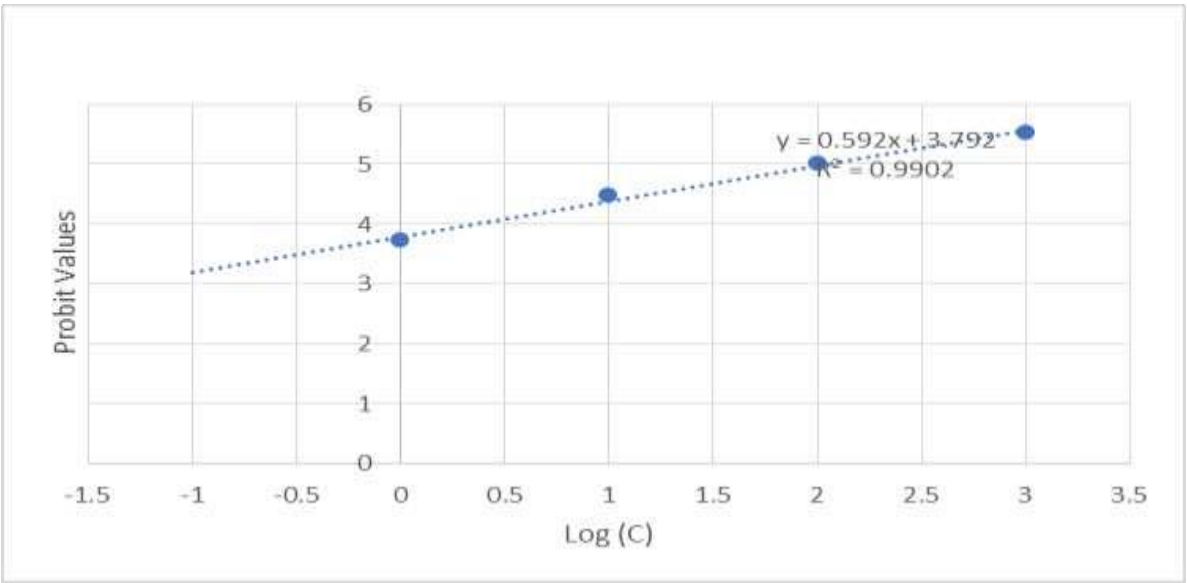
100	2	6	60	5.25		
1000	3	8	80	5.84		



APPENDIX IV

Cytotoxic effects of crude extract of *Tamarindus indica* on brine shrimps

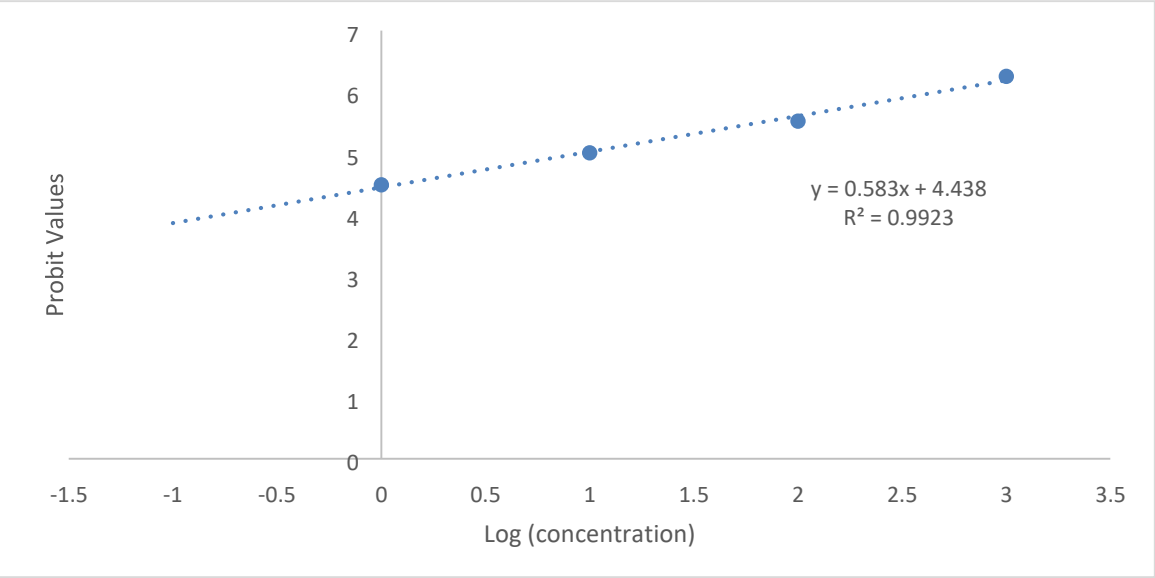
C	Log (C)	No of dead nauplii	% Mortality	Probit Value	LC_{50}	LC_{90}
0		0				
0.1	-1	0				
1	0	1	10	3.72	109.784	14191.46
10	1	3	30	4.48		
100	2	5	50	5		
1000	3	7	70	5.52		



APPENDIX V

Cytotoxic effects of aqueous fraction of *Prosopis africana* on brine shrimps

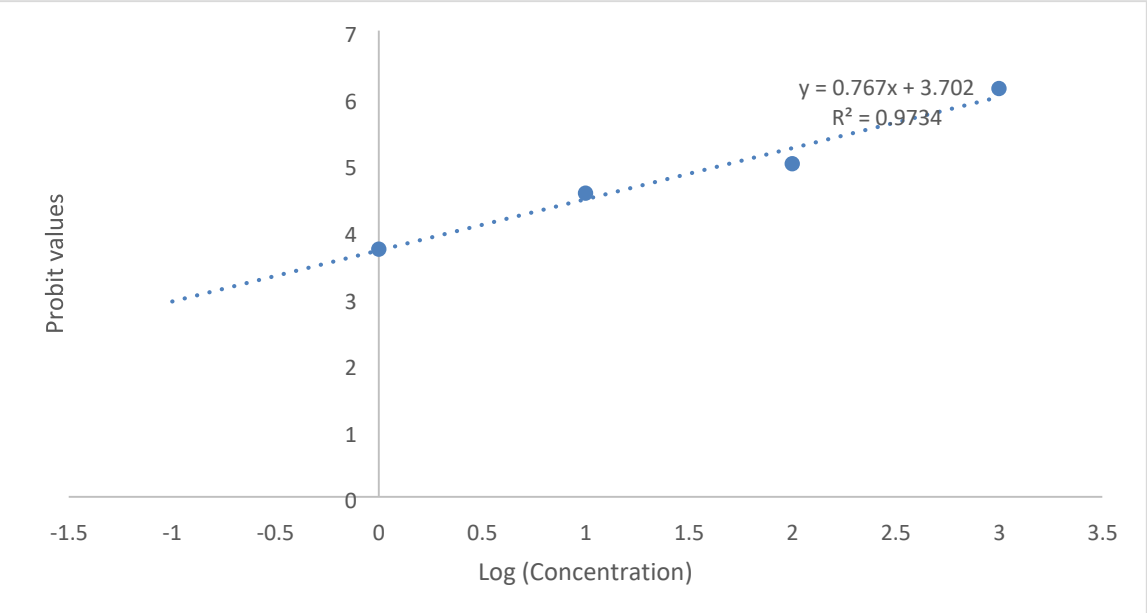
C	Log (C)	No of dead nauplii	% Mortality	Probit Value	LC_{50}	LC_{90}
0						
0.1	-1	0	0			
1	0	3	30	4.48	9.204	1,282.513
10	1	5	50	5		
100	2	7	70	5.52		
1000	3	9	90	6.25		



APPENDIX VI

Cytotoxic effects of ethylacetate fraction of *Prosopis africana* on brine shrimps

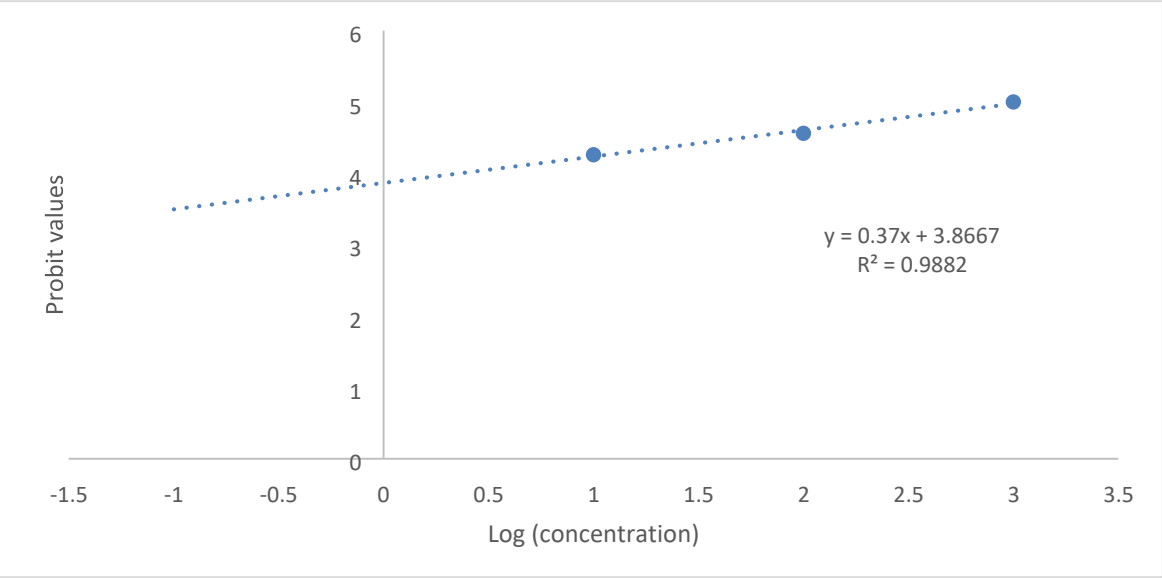
C	Log (C)	No of dead nauplii	% Mortality	Probit Value	LC_{50}	LC_{90}
0		0	0			
0.1	-1	0	0			
1	0	1	10	3.72	49.239	2,099.104
10	1	3.3	33	4.56		
100	2	5	50	5		
1000	3	8.7	87	6.13		



APPENDIX VII

Cytotoxic effects of n-hexane fraction of *Prosopis africana* on brine shrimps

C	Log (C)	No of dead nauplii	% Mortality	Probit Value	LC_{50}	LC_{90}
0		0	0			
0.1	-1	0	0			
1	0	0	0		1156.04	2.76 X 10^6
10	1	2.3	23	4.26		
100	2	3.3	33	4.56		
1000	3	5	50	5		



APPENDIX VIII

MCF-7 viable cell counts after 48-hours of exposure with different concentrations *P. afrcana* aqueous fraction

CONCENTRATION	A	B	C
CONTROL	9190 $x10^4$	9200 $x10^4$	9400 $x10^4$
1000	879 x 10^4	866 $x10^4$	837 $x10^4$
500	990 $x10^4$	988 $x10^4$	1140 $x10^4$
250	1580 $x10^4$	1760 $x10^4$	1600 $x10^4$
100	1760 $x10^4$	2200 $x10^4$	1960 $x10^4$

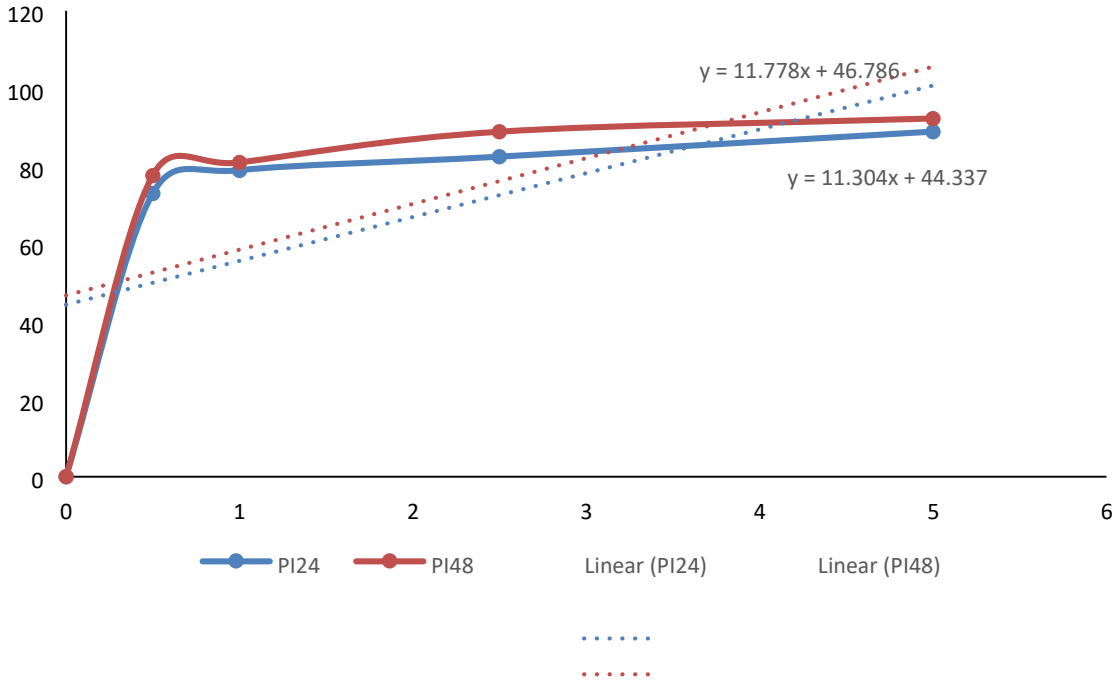
50		2590 $x10^4$	2470 $x10^4$
	2550 $x10^4$		

APPENDIX IX

MCF-7 viable cell counts after 24-hours of exposure with different concentrations of *P. africana* aqueous fraction

CONCENTRATION	**A**	**B**	**C**
CONTROL	7960 $x10^4$	8370 $x10^4$	8550 $x10^4$
1000	978 $x10^4$	981 $x10^4$	957 $x10^4$
500	1870 $x10^4$	1970 $x10^4$	1760 $x10^4$
250	2800 $x10^4$	2760 $x10^4$	3020 $x10^4$
100	4740 $x10^4$	5120 $x10^4$	5220 $x10^4$
50	6040 $x10^4$	5950 $x10_4$	5640 x 10^4

APPENDIX X

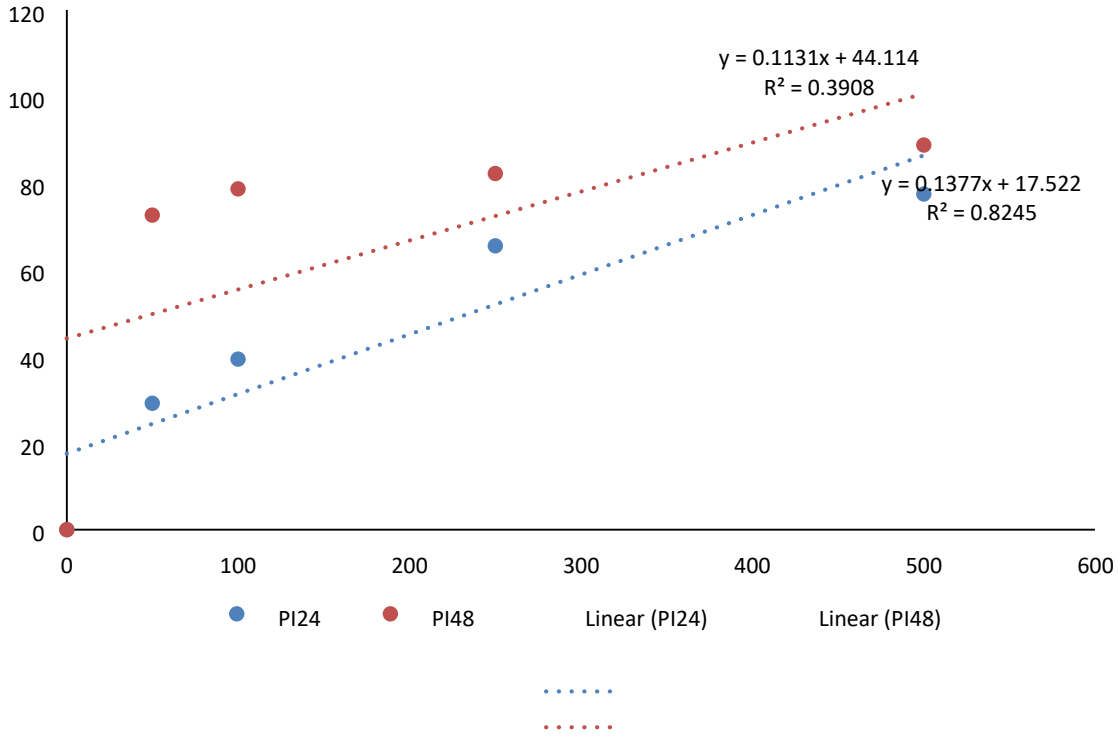


Cytotoxic effects of Standard drug (Doxorubicin) against MCF-7 cell lines after 24hrs and 48hrs of exposure

48hr $y = 11.778x + 46.786$ R^2 = 0.3822

24hr $y = 11.304x + 44.337$ R^2 = 0.3884

APPENDIX XI



Cytotoxic effects of aqueous fraction of P. Africana against MCF-7 cell lines after 24hrs and 48hrs of exposure

48hr $y = 0.1131x + 44.114$ R^2 = 0.3908

24hr $y = 0.1377x + 17.522$ R^2 = 0.8245

APPENDIX XII

Cell viability = $\frac{\text{Treatment}}{\text{Control}} \times 100$

Percentage inhibition (PI) = 100-Cell viability