Assessment of Cytotoxic Effects of Selected Medicinal Plants on Human Cervical, Breast and Lung Cancer Cell lines

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Certification

This is to certify that Oluwaseun Akinyemi, ADEDEJI with Matriculation Number LCU/PG/001398 carried out this research work titled "Assessment of Cytotoxic Effects of Selected Medicinal Plants on Human Cervical, Breast and Lung Cancer Cell lines" in the Department of Chemical Sciences (Biochemistry Unit), Faculty of Natural and Applied Sciences, Lead City University, Ibadan, Oyo State, for the award of Master Degree (M.Sc.) in Biochemistry and that this has not been previously submitted.

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Dedication

This research work is dedicated to the Almighty God through Jesus, the Wisdom of God.

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Abstract

Cancer remains a leading cause of death globally. Reports of severe adverse effects of anticancer drugs call for newer therapies from natural products. This study aim to investigate the cytotoxic effects of six medicinal plant extracts on human cervical (HeLa), breast (MCF-7) and lung (A549) cancer cell lines. The plant extracts were assayed for cytotoxicity using MTT assay method. The selectivity index was determined with the use of non-tumorigenic cell line (KMST-6). The most active plant was evaluated for its apoptotic effects and its effects on oxidative stress markers of the selected cell lines. Phytochemical composition of the most active plant extract was determined by GC-MS analysis. The leaf extract of *Ficus benjamina* has the highest cytotoxic effects on the cancer cell lines with IC_{50} values of 17.56, 33.35 and 33.57 µg/ml on HeLa, MCF-7, and A549 cell lines respectively. Other plant extracts exhibited low cytotoxic effects with IC_{50} >100µg/ml. The leaf extract of *Ficus benjamina* possesses a selectivity index (SI) of 2.2 in the HeLa cell line. At $p \leq 0.05$, Bax protein level was significantly higher in MCF-7, while caspase-9 and 3 were significantly higher in HeLa cell line. Activities of SOD increases in all the cell lines but significantly in MCF-7 ($p \leq 0.05$). The activities of GST and the levels of GSH were significantly reduced in MCF-7 and A549. LPO and NO were lowered significantly in all cancer cell lines ($p \leq 0.05$). The GC-MS analysis revealed the presence of phenolic compounds (Phytol and Tocopherols) and terpenoids (Eicosyne and Eicosane). The extract of *F. benjamina* induces apoptosis in HeLa and MCF-7. The analysis of bioactive compounds showed that the extract posseses antioxidant and antiflammatory properties. This study suggest that the leaf of *F. benjamina* could be a source of potential and safe anticancer drug against cervical cancer

Keywords: Medicinal plants, Cancer cell lines, Cytotoxicity, Selectivity index.

Word Count: 284

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List of Abbreviations

Abbreviation	Meaning
ATP	Adenosine Triphosphate
CPE	Cytopathic Effect
DMSO	Dimethylsulfoxide
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-linked Immunosorbent Assay
GST	Glutathione-S-Transferase

GSH	Reduced Glutathione
HRP	Horseradish Peroxidase
IC_{50}	Half maximal Inhibitory Concentration
LPO	Lipid Peroxidation
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NADH	Nicotinamide Adenine Dinucleotide Hydrogenase
NO	Nitric Oxide
OD	Optical Density
ROS	Reactive Oxygen Species
SI	Selectivity Index
SOD	Superoxidismutase

Chapter One

Introduction

1.1 Background of the Study

Cancer is a group of diseases involving abnormal cell growth with the potential to invade or spread to other parts of the body, the condition known as metastasis [1]. This is in contrast with benign tumors, which do not spread[2]. Cancer is a multifactorial disorder involving complex modification in the genome affected by the interactions between host and environment[1]. The significant characteristics of cancer include independence from growth signals, irresponsiveness to signals which halt the cell division, uncontrolled replication, evasion of apoptosis, sustained angiogenesis and finally the capacity to penetrate into other tissues[3]. The microenvironment of benign tumors manifests dysregulation of various regulatory proteins and extracellular environment which plays a vital role in the origination and development of cancer[4].

Cancer ranks as a leading cause of death and an important barrier to increasing life expectancy globally[5]. According to estimates from the World Health Organization (WHO) in 2019, cancer is ranked the first or second leading cause of death in 112 of 183 countries before the age of 70 years and ranks third or fourth in a further 23 countries[6]. The decline in the mortality rate of stroke and coronary heart diseases, relative to cancer in many countries account for the rising prominence of cancer as a leading cause of death[5]. The burden of cancer incidence and mortality overall are rapidly growing worldwide; this reflects both aging and growth of the population as well as changes in the prevalence and distribution of the main risk factors for cancer, several of which are associated with socioeconomic development[7].

Cancer of the cervix is one of the most common cancers among women worldwide, especially in the developing world accounting for 85% of the global burden of cervical cancer[8]. In Nigeria, It is the second most common cancer following the leading breast cancer in women. In Kano State, it is the leading female malignancy[9]. Cancer of the cervix is closely linked to human papilloma virus (HPV) infection which appears to be the major factor in the development of about 95% of all cervical cancers[10]. The genomes of all HPV types contain approximately eight open reading frames (ORFs) that are transcribed from only one DNA strand. The ORF´s are classified into three functional parts: the early (E) region that encodes proteins (E1–E7) necessary for viral replication, the late (L) region that encodes the structural proteins (L1–L2) required for virion assembly, and a largely non-coding part that is referred to as the long control region (LCR) which contains cis elements necessary for viral replication and transcription[11]. The E6 and E7 viral proteins play key roles in tumour progression in cervical cancers by abrogating the cell cycle control proteins such as- p53, Bak, Bax, retinoblastoma (Rb) protein inversely enhancing telomerase activity, steroid receptor co-activator (Src) family kinases, activator protein (AP-1) transcription complex, and activates histone deacetylases[11].

Breast Cancer is one of the most common cancer that usually occur in women. In 2018, World Health Organization (WHO) pointed out that around 2.1 million new cases of breast cancer incidence have been reported. The number of cancer related-death in women around the world were estimated to be around 626,679 (6.6%)[12]. In 2015, review from 15 studies using the International Federation of Gynaecology and Obstetrics (FIGO) staging system, the means of cumulative treatment costs weighted by sample sizes were $29,724 at stage I, $39,322 at stage II, $57,827 at stage III, and $62,108 at stage IV[13].

Lung cancer or lung carcinoma is a malignant lung tumor characterized by uncontrolled cell growth in the lung tissues. The highest rates are in North America, Europe, and East Asia, with over a one third of new cases in China. The rates in Africa and South Asia are much lower[14]. Worldwide in 2012, lung cancer occurred in 1.8 million people and resulted in 1.6 million deaths[15]. This makes it the most common cause of cancer-related death in men and second most common in women after breast cancer[16]. Lung cancer cases are often caused by a combination of factors which include behavioral, genetic, and exposure to radon gas, asbestos, second-handed smoke, or other forms of air pollution[17]. There are two main types of lung cancer; they are small–cell lung carcinoma (SCLC) and non-small-cell lung carcinoma (NSCLC). The most common clinical manifestations are coughing (including coughing of blood), weight loss, shortness of breath, and chest pain[18]. Diagnosis of lung cancer is mainly by chest radiographs and computed tomography (CT) scans. The diagnosis is confirmed with biopsy by bronchoscopy or CT-guidance[19]. Common treatment includes surgery, chemotherapy, and radiography. Non-small cell lung carcinoma (NSCLC) is sometimes treated with surgery, whereas SCLC usually respond to chemotherapy and radiotherapy[20]. Prevention of lung cancer is generally by avoiding risk factors including smoking and air pollution[21].

1.2 Statement of the Problem

Today, despite considerable efforts, cancer still remain an aggressive killer worldwide. Chemotherapy is routinely used for cancer treatment. Cancer cells are susceptible to chemotherapeutic treatment due to loss of many regulatory functions responsible for their abnormal growth. Approximately five decades of systemic drug discovery and development have resulted in the establishment of a large collection of useful chemotherapeutic agents. However, chemotherapeutic treatments are not devoid of their own intrinsic problems. Various kinds of

toxicities may occur as a result of chemotherapeutic treatments. For example, 5-fluorouracil, a common chemotherapeutic agent, is known to cause myelotoxicity and, cardiotoxicity and has even been shown to act as a vasospastic agent in rare but documented cases[22]. Another widely used chemo drug, doxorubicin causes cardiac toxicity, renal toxicity, and myelotoxicity[23]. Similarly, bleomycin a wellknown chemotherapeutic agent, is known for its pulmonary and cutaneous toxicity[24,25]. Cyclophosphamide, a drug to treat many malignant conditions, has been shown to have bladder toxicity in the form of hemorrhagic cystitis, immunosuppression, alopecia, and at high doses cardiotoxicity[26]. The toxicity of chemotherapeutic drugs sometimes creates a significant problem in the treatment of cancer using allopathy or established medicine.

Various therapies have been propounded for the treatment of cancer, many of which use plant-derived products. There are four classes of plant-derived anticancer agents in the market today, vinca alkaloids (vinblastine, vincristine and vindesine), the epipodophyllotoxins (etoposide and teniposide), the taxanes (paclitaxel and docetaxel) and the camptothecin derivatives (topotecan and irinotecan). Plants still have enormous potential to provide newer drugs and as such are a reservoir of natural chemicals that may provide chemoprotective potential against cancer. Recently, several researches have suggested a number of compounds from medicinal plants with potential anti-cancer activities[27].

1.3 Justification of the Study.

The alternative use of readily available and inexpensive medicinal plants is the panacea to the toxic side effects associated with synthetic drugs[28]. Medicinal plants have been in continuous use over the years for the management of cancer, particularly, in most developing countries of the world including Nigeria[28]. Plant extracts are widely used in Nigeria as important sources of chemotherapeutic agents in spite of the use of synthetic drugs by the vast majority of the

populace[27]. These medicinal plants are made up of bioactive compounds that are responsible for their pharmacological actions in the human body. Clinical studies and phytochemical screening have established the antitumor activity of herbal remedies against different types of cancers[30].

Notwithstanding, the fact that many anticancer agents have been derived from medicinal plants, many plants with anticancer potentials are yet to be discovered. There are over 114,000 plant extracts that are being analyzed for their anticancer activity in various cancer institutes[31]. Accordingly, there is a pressing need to carry out conclusive investigations to establish whether these extracts and others exhibit anticancer activity and are applied as chemotherapeutic agents.

In this study, six medicinal plants (*Ficus benjamina*, *Euphorbia milli, Parkia biglobosa, Piliostigma thonnigii, Vitex doniana,* and *Euphorbia heterophyilla)* were investigated based on the reports of their traditional uses as therapeutic agents. These plants have been reported in research articles to be indigenous folklore medicine to have anti-cancer properties.[32] However, there is currently no report supporting their *In vitro* cytotoxic effects on human cervical, breast and long cancer cell lines.

1.4 Aim and Objectives of the Study

The overall aim of this study is to investigate the cytotoxic effects of the extracts of six (*Ficus benjamina, Euphorbia milli, Parkia biglobosa, Piliostigma thonnigii, Vitex doniana,* and *Euphorbia heterophyilla)* medicinal plants on human cervical, breast, and lung cancer cell lines.

The specific objectives of this study include the following: To

i. determine the cytotoxic effect of the medicinal plant extracts on cervical cancer (HeLa), breast cancer (MCF-7), and lung cancer (A549) cell lines.

ii. determine the Selectivity Index (SI) using a non-cancerous cell line (KMST-6)

iii. determine the effects of the active plant on the levels of apoptotic proteins in the cell lines

iv. determine the effects of the active plant extract on the oxidative stress makers in the cell lines

v. determine the bioactive compound in the most active plant using GC-MS finger print.

1.5 Research Questions

i. Which of the selected medicinal plant is cytotoxic to the human Cervical, Breast and Lung Cancer Cell lines?
ii. What is the selectivity index of the medicinal plant that is cytotoxic to the cancer cell lines?
iii. What are the effects of that medicinal plant extract on the apoptotic protein levels?
iv. What are the effects of that medicinal plant extract on the Oxidative Stress makers of the Cell lines?
v. What are the bioactive compound present in that medicinal plant extract?

1.6 Significance of the Study

Cancer remains the leading cause of death worldwide. Chemotherapy is one of the ways to treat cancer, however there are several reports of severe side effects of chemotherapy drugs. This study is therefore being carried out in search for new anticancer agent with better efficacy and lesser side effects from medicinal plants. The results of this study will provide a baseline information on which of the selected medicinal plants could be a potential source of safe anticancer drug against human Cervical, Breast, and Lung Cancer.

1.7 Scope of the Study

This study considered six medicial plants (*Ficus benjamina*, *Euphorbia milli, Parkia biglobosa, Piliostigma thonnigii, Vitex doniana,* and *Euphorbia heterophyilla)* and three cancer cell lines (Human Cervical, Breast and Lung Cancer Cell Lines). KMST-6 was used as non-cancerous cell lines for Selectivity Index study.

1.8 Limitation of the Study

This study assessed the cytotoxicity effects of six selected medicinal plants on human Cervical, Breast and Lung Cancer Cell Lines. Howerver the work is limited to *In Vitro* assessment.

1.9 Operational Definition of Terms

Assessment: the collection of data to descreibe or better understand an issue

Cytotoxicity: a substance is said to be cytotoxic if it affects the attachment of cells, significantly alters morphology, adversely affects the rate of growth or cause cell to die.

Medicinal Plant: is any plant which in one or more of its organ contains substances that can be used for the therapeuthic purposes or which are precursors for the synthesis of useful drugs.

Cancer Cell Lines: are useful tools because they provide various model of the biological mechanisms involved in cancer development and progression.

Selectivity Index: is the ratio of the IC_{50} value of the non-cancerous cell line and cancer cell line.

Inhibition Concentration (IC_{50}): is the concentration of the plant extract that inhibit 50% of cell growth.

Apoptosis: is a programmed cell death.

Endnotes

1. Z. Abbas, & S. Rehman. *An Overview of Cancer Treatment Modalities*. **Neoplasm**, *1*, 2018, 139-157.

2. T. Kalaiselvi, S.T. Padmapriya, P. Sriramakrishnan & K. Somasundaram. *Deriving Tumor Detection Models Using Convolutional Neural Networks from MRI of Human Brain Scans.* **International Journal of Information Technology**, 12, 2020, 403-408.

3. A.A. Attama, P.O. Nnamani, O.B. Onokala, A.A. Ugwu &A.L. Onugwu. *Nanogels as Target Drug Delivery Systems in Cancer Therapy: A Review of the Last Decade.* **Front Pharmacol**. 13, 2022, 874510. doi: 10.3389/fphar.2022.874510.

4. W. Sun, J.D Luo, H Jiang & D.D Duan. *Tumor Exosomes: A Double-edged Sword in Cancer Therapy.* **Acta Pharmacol Sin**.39, 2018, 534–41.

5. F. Bray, M. Laversanne, E. Weiderpass & I. Soerjomataram. *The Ever-increasing Importance of Cancer as a Leading Cause of Premature Death Worldwide*. **Cancer**. 2021, In press.

6. World Health Organization (WHO). *Global Health estimates 2020: Deaths by Cause, Age, Sex, by Country and by Region, 2000-2019*. WHO; 2020. Accessed December 11, 2020.

7. L.T. Dean, S. Gehlert, M. L. Neuhouse, A. Oh, K. Zanetti, M. Goodman, B. Thompson, K. Visvanathan & K.H. Schmitz. *Social Factors Matter in Cancer Risk and Survivorship.* **Cancer Causes Control** 29, 2018, 611–618. https://doi.org/10.1007/s10552-018-1043-y.

8. M. Arbyn, E. Weiderpass., L Bruni, S de Sanjosé, M Saraiya, J, Ferlay & F. Bray. *Estimates of Incidence and Mortality of Cervical Cancer in 2018: A Worldwide Analysis.* **The Lancet Global Health**. 19, 2019, 30482-6. doi:10.1016/s2214-109x

9. A.A. Sule, & O. Ochicha. *A Histopathologic Review of Cervical Cancer in Kano, Nigeria*. **Sahel Medical Journal**. 20(1), 2017, 16.

10. R. Wang, P. Wei, J. Lei, H. WeiHuan, L. Yuehan, W. Di, G. Chun, M. Ding & L. Shujie. *Human Papillomavirus Vaccine Against Cervical Cancer: Opportunity and Challenge*. **CancerLetters.** 471, 2020, 88–102.

11. L. Mirabello, A.C Megan, W.N Chase, D Michael, W Nicolas, Y Meredith, C Michael, F Joseph. Boland, NCI HPV Workshop, S. Mark, & D.B Robert. *The Intersection of HPV Epidemiology, Genomics and Mechanistic Studies of HPV-mediated Carcinogenesis.* **Viruses.** 10(2), 2018, 80. https://doi.org/10.3390/v10020080.

12. World Health Organization (WHO). *Cancer Fact Sheet,* **Breast Cancer**. 2018 Retreived 01 Jul, 2019, from http://gco.iarc.fr/today/data/factsheets/cancers/20-Breast-fact-sheets.pdf

13. L. Sun, R. Legood, I. Dos-Santos-Silva, S.M. Gaiha, & Z Sadique. *Global treatment cost of breast cancer by stage: A systematic review*. **PloS one**, 13(11), 2018, 0207993-0207993. doi: 10. 1371/ journal.pone.0207993.

14. J.A Barta, C.A Powell, & J.P Wisnivesky. *Global Epidemiology of Lung Cancer.* **Ann Glob Health**;85(1), 2019, 8. doi: 10.5334/aogh.2419.

15. P.M de Groot, C.C. Wu, B.W Carter, & R.F Munden. *The Epidemiology of Lung Cancer.* **Transl Lung Cancer Res**. 7(3), 2018, 220-233. doi: 10.21037/tlcr.2018.05.06.

16. World Health Organization (WHO) *"CANCER"*. 12 September 2018. Retrieved 19 December 2018.

17. G. Zhou, *Tobacco, Air Pollution, Environmental Carcinogenesis, and Thoughts on Conquering Strategies of Lung Cancer.* **Cancer Biol Med**. 16(4), 2019, 700-713. doi: 10.20892/j.issn.2095-3941.2019.0180.

18. M.B Alexander, S.Y Kim, & H Cheng. Update 2020: *Management of Non-Small Cell Lung Cancer.* **Lung** 198, 2020, 897–907. https://doi.org/10.1007/s00408-020-00407-5

19. N. Shinagawa. *A review of Existing and New Methods of Bronchoscopic Diagnosis of Lung Cancer.* **Respiratory Investigation**, 2018. doi: 10.1016/j.resinv.2018.08.004.

20. H. Brett, D. Siddhartha & G. Ramaswamy. *Overcoming Chemotherapy Resistance in SCLC*. **Journal of Thoracic Oncology.** 16, 2021, 2002-2015.

21. A. Shankar, A. Dubey, D. Saini, M. Singh, C.P. Prasad, S. Roy, S.J. Bharati, M. Rinki, N. Singh, T. Seth & M. Khanna. *Environmental and Occupational Determinants of Lung ancer.* **Translational lung cancer research**, 8(1), 2019, S31.

22. T. Shiga & M. Hiraide. *Cardiotoxicities of 5-Fluorouracil and other Fluoropyrimidines.* **Curr. Treat**. **Options in Oncol**. 21, 2020; 27.

23. K. Tecza, J. Pamula-Pilat, J. Lanuszewska, D. Butkiewicz, & E. Grzybowska. *Pharmacogenetics of Toxicity of 5-fluorouracil, Doxorubicin and Cyclophosphamide Chemotherapy in Breast Cancer Patients.* **Oncotarget.** 9(10), 2018, 9114-9136. doi: 10.18632/oncotarget.24148.

24. M. Laprise-Lachance, P. Lemieux & J. P Grégoire. *Risk of Pulmonary Toxicity of Bleomycin and Filgrastim.* **Journal of Oncology Pharmacy Practice**. 25(7), 2019,1638-1644. doi:10.1177/1078155218804293

25. M. Del Re, T. Latiano & L. Fidilio. *Unusual Gastrointestinal and Cutaneous Toxicities by Bleomycin, Etoposide, and Cisplatin: A Case Report with Pharmacogenetic Analysis to Personalize Treatment.* **EPMA Journal** 8, 2017, 69–73.

26. M.S. Rania, M.N. Merihane, I.A. Jannatullah, K.R Omar & A.E Mohamed. *Alogliptin Attenuates Cyclophosphamide-induced Nephrotoxicity: A Novel Therapeutic Approach through Modulating MAP3K/JNK/SMAD3 Signaling cascade.* **Drug and Chemical Toxicology** 45(3), 2022, 1254-1263.

27. A.K. Kudva, S. Rao, P. Rao, R. Periera, G. Bhandari, J.M. Mathew & M.S. Baliga. *Piper betle Linn. In Cancer: Past, Present, and Future.* In: M Akhtar, M Swamy. (eds) **Anticancer plants: Properties and Application. Springer**, 2018. Singapore.

28. A.M. Reid, C. B. Oosthuizen, B.D. Fibrich, D. Twilley, I. A. Lambrechts, M.N de Canha, & N. Lall. *Traditional Medicine.* ***Medicinal Plants for Holistic Health and Well-being,*** 2018, 1–11. doi:10.1016/b978-0-12-812475-8.00001-9

29. F.O. Ohiagu, P. C. Chikezie, & C.M Chikezie. *Anticancer Activity of Nigerian Medicinal Plants: A Review.* **Futur J Pharm Sci**. 7, 2021, 70. doi.org/10.1186/s43094-021-00222-6

30. G. Sevgi & S. Nazim. *Current Perspective in the Application of Medicinal Plants Against Cancer.* **Novel Therapeutic Agent**. 19, 2019, 101-111. DOI 10.2174/18-715206196661812241210 04.

31. D. Cicka & C. Quave. *Bioprospecting for Pharmaceuticals: An Overview and Vision for Future Access and Benefit Sharing.* In: N. Joshee, S. Dhekney & P. Parajuli (eds) **Medicinal Plants.** 2019. Springer, Cham. https://doi.org/10.1007/978-3-030-31269-5_2

32. I.B. Abubakar, A.N. Ukwuani-Kwaja & A.D. Garba. *Ethnobotanical Study of Medicinal Plants Used for Cancer Treatment in Kebbi state, North-west Nigeria.* **Acta Ecologica Sinica**. 40(4), 2020, 306-314. https://doi.org/10.1016/j.chnaes.2020.02.007

Chapter Two

Literature Review

2.1 Cervical Cancer

Globally, cervical cancer is ranked fourth most common cancer among female after breast, colorectal, and lung cancer. It accounts for 600 000 new cases and 340 000 deaths annually[1]. Significantly, approximately 83% of all new cervical cancer cases and 88% of all deaths occur in Low and Medium Income Countries (LMICs) [2]. Cervical cancer is indeed the leading cause of cancer-related deaths in 36 countries which includes regions such as sub-Saharan Africa, Latin America and India[3]. This burden needs to be contextualized in terms of socio-economic conditions, health care infrastructure and competing health needs, which are not only risk factors of this disease, but significantly impact its prevention and management. Despite important advances in the understanding of cervical cancer as a potentially preventable disease, there have yet to be major improvements in patient survival and therefore the disease burden remains high [2].

2.1.1 Etiology of Cervical Cancer

Infection by high-risk Human Papillomavirus (HPV) is single most important etiological agent of cervical cancer [4]. Indeed, persistent infection with high-risk HPV types is responsible for up to 99.7% of cervical cancer cases[5]. The relatioship between HPV and cervical cancer was made known in the last 30 years based on the detection of HPV type 16 in cervical cancer tissue by Harald zur Hausen.[6] The prevalence of HPV infection is higher in women younger than 25 years and is estimated to infect around 291 million women globally[7]. The estimated worldwide prevalence of HPV among women with normal cytology is 11.7%, but there is considerable geographic variation with sub-Saharan Africa having the highest HPV prevalence (24.0%) [8].

Sub-Saharan Africa also has a high burden of HIV with over 70% of all global HIV positive individuals residing in sub-Saharan Africa [9]. There is compelling evidence that women infected with HIV are at increased risk of persistent infection with multiple types of HPV at an early age (13–18 years). These factors contribute to an increased risk of developing cervical cancer at an earlier age [10]. Indeed, HIV infected individuals have a six times higher risk of developing cervical cancer when compared to the general population [11]. Furthermore, the increased number of HIV positive women receiving anti-retroviral therapy results in improved life expectancy and therefore they have to be adequately screened because they have a higher risk of developing cervical cancer[12].

2.1.2 Initiation and Progression of Cervical Cancer

Cervical cancer originates in the cervix which is the narrow opening into the uterus and is connected to the vagina through the endocervical canal (see Figure1)[13]. The cervix is divided into the ectocervix and endocervix and while the ectocervix is covered with stratified squamous epithelial cells, the endocervix consists of simple columnar epithelial cells. Stratified squamous and columnar epithelium form the squamo-columnar junction in the endocervical canal. The meeting point of these regions is called the "transformation zone", which consists of metaplastic epithelium that replaces the columnar lined epithelium of the endocervix. This zone is the most likely site for the development of cervical cancer because it is a major site of premalignant transformation via persistent HPV infection[13]. There are two major histological sub-types of cervical cancer, squamous cell carcinoma (SCC) and adenocarcinoma. Whereas SCC develops from squamous cells in the ectocervix and accounts for approximately 75% of cervical carcinoma cases, adenocarcinoma originates from glandular cells that produce mucus in the endocervix [14]. During SCC progression, squamous cells in the cervical epithelium undergo

dysplastic changes following HPV infection and these precursor lesions are referred to as cervical intraepithelial neoplasia (CIN)[15]. The majority of HPV infections clear within a few years after exposure and only 10–20% of persistent infection potentially leads to the development of cervical cancer[16]. Upon establishment of persistent infection, HPV can integrate into the host genome with 80% of HPV 16- and 100% of HPV 18-positive cervical carcinomas displaying viral integration[17]. It is important to note that in the absence of viral DNA integration, a small percentage of women who are HPV positive develop cervical cancer and in these cases the HPV DNA remains in its episomal form[18]. The viral E5, E6 and E7 proteins contribute to the induction and maintenance of the cervical cancer phenotype by exploiting host cell machinery[19]. Actually, E5 does this by regulating and interacting with, among other host growth factor receptors, the epidermal-growth-factor receptor (EGFR), the platelet-derived growth-factor-β receptor and the colony-stimulating factor-1 receptor[20]. E5 was also shown to prevent apoptosis following DNA damage by disrupting the host FAS receptor and degrading the proapoptotic factor BAX[21]. Futhermore, E5 aids in the immune evasion of infected host cells by reducing the surface expression of major histocompatibility complex (MHC) class I and II as well as the surface receptor CD1d.[21]. E6 and E7 promote cervical cancer by disrupting cellular checkpoints and co-operating with host factors, including tumor suppressors and tumor promoters[22]. For example, E6 and E7 mediate malignant transformation through degradation of p53 and inactivation of retinoblastoma (pRb) tumor suppressor proteins, respectively[23]. When the HPV DNA integrates into host cells, a substantial loss of the HPV genome occurs, including the E5 coding sequence[24]. Integration of viral DNA however, results in the constitutive expression of E6 and E7 because the E2 repressor protein either cannot bind to the viral upstream regulatory regions (URR) due to methylation, or its open reading frame (ORF) is disrupted[22]. In cervical

cancer arising from HPV-integration into the host cells, E5 is therefore not a critical player and E6 and E7 are responsible for driving and maintaining the malignant phenotype[22]. HPV-infected cervical epithelial cells that undergo transformation, change from being well organized to highly dysplastic and the degree of dysplasia is graded based on severity[15]. CIN1 is characterized by mild dysplasia with the presence of koilocytes (cells with a perinuclear halo and enlarged and irregular nuclei), binucleate cells, and dyskeratotic cells (individual cell keratinisation). CIN2 is made up of heterogeneous lesions affecting two thirds of the epithelium, followed by CIN3 which affects greater than two thirds of the epithelium and represents severe dysplasia[15]. The invasive stage of cervical cancer is associated with poor prognosis and involves the spread of cancer cells either by direct extension into the parametrium, vagina, uterus and adjacent organs. While CIN staging refers to the precancerous condition, International Federation of Gynaecology and Obstetrics (FIGO) is the most widely used staging method for invasive cervical cancer guideline, which is divided into stages I, II, III, and IV [25]. When the cancer spreads beyond the inner lining of the cervix but is still confined to the cervix it is termed stage I. Once the cancer has spread beyond the cervix but not the pelvic wall and lower third of the vagina it is categorized as stage II, and when it reaches these regions it is categorized as stage III. Stage IV is characterized by cervical cancer cells having metastasized to the bladder, rectum (stage IVA) and other parts of the body, including the lungs, liver, and skeleton (stage IVB), by the hematogenous route[25]. It is important to note that it can take 10–30 years for the progression from the preinvasive CIN stage to invasive cervical cancer.

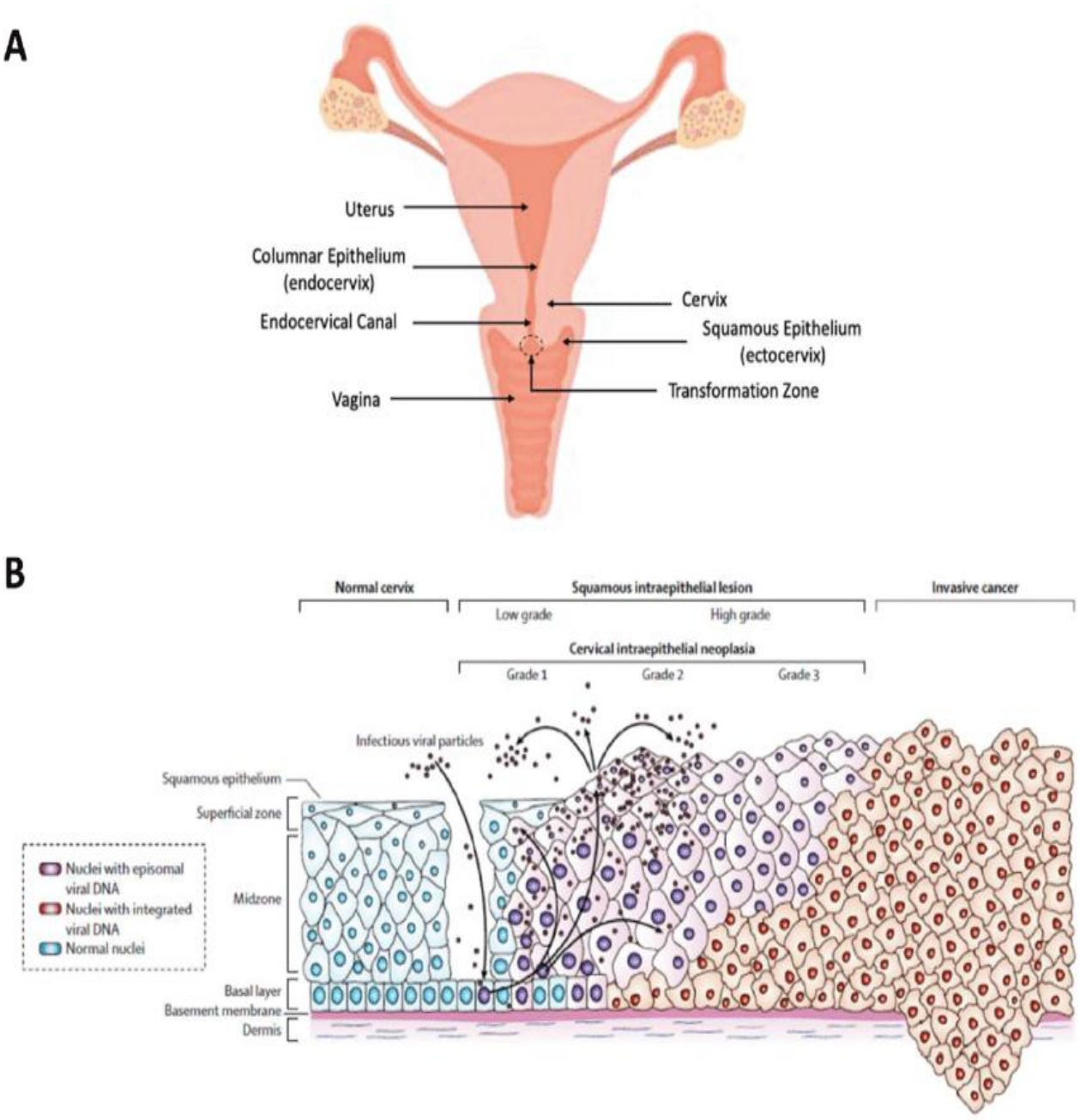


Figure 2.1. Anatomical location of cervical cancer origin and progression from a normal cervix to invasive squamous cell carcinoma mediated by HPV. **A)** Anatomical diagram representing the female reproductive organs. **B)** Schematic representation of HPV infection and cervical cancer development.

Source[204]

2.1.3 Treatment of Cervical Cancer

The stage and extent of cervical cancer progression determines the treatment strategy needed and may include one or a combination of surgery, radiation and chemotherapy.

2.1.3.1 Surgery

Surgery is a commonly used and successful technique in combatting various early-stage cancers as it involves the physical removal of cancerous tissue. It can, however, also be used to remove metastatic tissue [26]. Currently, the types of surgery performed to treat cervical cancer include total hysterectomy, radical hysterectomy, loop electro-surgical excision procedure (LEEP), conization, trachelectomy, and cryosurgery[27]. The choice of surgical procedure is highly dependent on the disease stage and extent of spread[28]. Total hysterectomy with or without salpingo-oophorectomy (the removal of one or both ovaries), remains the treatment of choice for women who have completed childbearing. Radical hysterectomy is most commonly used for larger cervical cancer lesions (up to 4 cm in size) and involves complete resection of the uterus, cervix, parametria, and cuff of the upper vagina[29]. The findings of the Laparoscopic Approach to Cervical Cancer (LACC) trial revealed that radical hysterectomy performed using laparoscopy was associated with an increased rate of recurrence, loss of fertility and potential urinary dysfunction in the long-term[30]. Radical hysterectomy using the open technique is therefore the preferred method, especially for tumors more than 2 cm in size. For women at childbearing age with early stage disease, a more conservative treatment approach is required and fertility-sparing surgeries include LEEP, conization and trachelectomy[29]. LEEP uses a thin wire to remove abnormal tissue from the cervix and can be done under local anaesthesia under low-cost clinical settings, such as in LMICs. Conization removes a cone-shaped wedge from the cervix including the transformation zone and either all or a portion of the endocervical canal which requires

hospital admission with significantly higher costs[29]. Radical trachelectomy involves the removal of the cervix, surrounding tissue (parametrium) and the upper vagina which is achieved via vaginal, laparoscopic, or robot-assisted methods[30].

2.1.3.2 Radiotherapy

Radiotherapy uses high energy x-rays and is a major treatment in the management of cervical cancer[31]. The three types of radiation therapy currently used to treat cervical cancer are external beam radiation therapy (EBRT), intensity-modulated radiotherapy (IMRT), and brachytherapy (internal RT). Superior diagnostic tools such as computerized tomography (CT) scans and magnetic resonance imaging (MRI) have also improved the evaluation of the primary tumour, extent of tumor invasion and metastasis which have further improved radiotherapy planning[31]. Briefly, EBRT aims high energy radiation beams from outside the body into the tumour and it is the most common form of radiotherapy used to treat cancer. IMRT, a more advanced form of radiotherapy, involves the manipulation of photon and proton radiation beams to correspond to the shape of the tumour and is used for both cancerous and non-cancerous tumours. Like IMRT, brachytherapy spares nearby tissues by either delivering a high dose of radiation to the tumour or a radioactive implant is inserted at the site of the tumour[31]. Despite important advances in radiotherapy, there are numerous adverse effects associated with this form of treatment which include diarrhoea, abdominal cramps and pelvic pain, skin toxicity, lymphedema and sexual dysfunction[32]. While there is a complete response in 68.3% of patients with stage IIA-IIIB cervical cancer, in 20–50% of women, radiotherapy alone fails to control the progression of locally advanced disease[33]. To enhance the efficacy of radiotherapy it is often used in conjunction with chemotherapy, especially for larger cervical cancer lesions (greater than 4 cm in width)[21]

2.1.3.3 Chemotherapy

Chemotherapy is an integral part of the standard cervical cancer treatment regimen and is typically administered as an adjuvant therapy following surgery when poor prognostic tumour features increase the risk of recurrent disease, in combination with radiotherapy as previously mentioned, and as a standalone treatment for locally advanced disease. The most effective single agent which has been used for the last three decades to treat cervical cancer is the platinum-based chemotherapeutic, cisplatin[34]. However, despite initial patient response to cisplatin, increased resistance during the course of the treatment is often reported and this reduces the efficacy of additional second-line platinum-based chemotherapeutics[35]. Subsequently, studies have found that combining cisplatin with other agents is potentially more effective than single drug treatment[36]. Indeed, a study showed that the response rate of cisplatin alone was lowered compared to when combined with topotecan [37]. Another study reported similar results when cisplatin was combined with paclitaxel[38]. Currently, topotecan, paclitaxel and other non-platinum-based chemotherapeutics such as 5-fluorouracil and bleomycin, are therefore commonly used in combination with cisplatin for treating cervical cancer. This results in significant and clinically meaningful improvement in median survival duration[39]. Chemotherapy is also often combined with radiotherapy (chemoradiotherapy) and is mostly used for locally advanced cervical cancer. This regimen aims to reduce disease recurrence but can result in adverse events and chronic morbidity. A systematic review and meta-analysis revealed that chemoradiotherapy improves overall and progression free survival and reduces the risks of local and distant cervical cancer recurrences[40]. Lastly, palliative chemotherapy is used to improve quality of life and relieve disease symptoms, though it may not effectively reduce tumour size[41].

The discovery and development of new and improved therapies is also important in terms of multidrug resistance in cancer cells which impacts the success of chemotherapy[42].

2.2 Breast Cancer

Breast cancer is the most common cancer among women and one of the most important causes of death among them[43]. Breast cancer is a multifactorial disease and various factors contribute to its occurrence[43]. Although the disease occurs all over the world, its incidence, mortality, and survival rates vary considerably among different parts of the world, which could be due to many factors such as population structure, lifestyle, genetic factors, and environment[44]. Changes in risk factors have led to an increase in the prevalence of breast cancer, which is increasing every day[43]. Although screening people can reduce the burden of breast cancer, side effects, over-diagnosis, and increased costs are the disadvantages of this method. Classification of women based on risk factors for breast cancer can be effective in improving risk-free methods and designing targeted breast cancer screening programs[45].

2.2.1 Genetic Predispositions as Important Risk Factors of Breast Cancer

A risk factor is defined at its most basic as anything that affects individual's chance of getting a disease, in this case breast cancer. Certain major risk factors for breast cancer are beyond individual's control[46]. For example, simply being a woman is the main risk factor for breast cancer as this disease is about 100 times more likely to occur in women than in men. Aging inevitably increases one's risk of breast cancer as evinced by the fact that most breast cancers are diagnosed in women age 55 and older. Beyond the inherent risks of gender and aging as they relate to breast cancer, it has been well documented that a woman's risk of developing breast cancer nearly doubles if she has a first-degree relative (mother, sister, or daughter) diagnosed

with breast cancer. Study showed close to 15% of US women who suffer from breast cancer also have a family member who has been diagnosed[46].

Overall, about 5-10% of breast cancers are linked to gene mutations inherited from a parent. The most common cause of hereditary breast cancer is an inherited mutation in the BRCA1 or BRCA2 gene[47]. Statistically, women with a BRCA1 mutation have a 55-65% lifetime risk of developing breast cancer. For women with a BRCA2 mutation, the lifetime risk is 45%. On average, a woman with a BRCA1 or BRCA2 gene mutation has about 70% chance of getting breast cancer by age 80[48]. The effect of the mutation is related to how many other family members have breast cancer, as breast cancer risk goes up if more family members are affected[49]. The impact of the BRCA1 and BRCA 2 mutation extend beyond just breast cancer, mutations in either of these genes is also linked to an increased ovarian cancer risk[46]. Conversely, BRCA1 mutations are found less common in breast cancers occurring in men while BRCA2 mutations are associated with a lifetime breast cancer risk of only about 6.8%[46].

Inherited mutations in many other genes can also lead to breast cancer development although less common and less drastic in their increase of breast cancer risk than the BRCA mutations[50]. Some of the mutated genes include ATM (inheriting 2 abnormal copies of this gene causes the disease ataxia-telangiectasia), TP53 (inherited mutations of this gene cause Li- Fraumeni syndrome with an increased risk of breast cancer, as well as some other cancers such as leukemia, brain tumors, and sarcomas), CHEK2 (a CHEK2 mutation can increase breast cancer risk about 2-fold), PTEN (inherited mutations in this gene can cause Cowden syndrome which is accompanied by a higher risk for both non-cancerous and cancerous tumors in the breasts, as well as growths in the digestive tract, thyroid, uterus, and ovaries), CDH1 (inherited mutations cause hereditary diffuse gastric cancer with an increased risk of invasive lobular breast cancer),

STK11 (mutations in this gene can lead to Peutz-Jeghers syndrome with a higher risk of many types of cancer, including breast cancer), and PALB2 (PALB2 gene makes a protein that interacts with the protein made by the BRCA2 gene, resulting in mutations in this gene causing a higher risk of breast cancer)[46].

Genetic testing of mutations in the BRCA1 and BRCA2 genes, as well as other less commonly mutated genes such as PTEN or TP53 in women in the high risk group if carefully and properly done can be beneficial for early detection and/or prevention of breast cancer development[50]. However, it is important to understand the limitations of genetic testing and what it can and can't tell an individual. In terms of practically making use of genetic testing for detection and prevention of breast cancer, it's also necessary to keep in mind that the testing is quite expensive and may not be covered by all health insurance plans. While genetic testing can be helpful in some cases, not every woman needs to be tested[46].

2.2.2 Non-genetic Risk Factors of Breast Cancer

2.2.2.1 Family History of Breast Cancer: While less than 15% of women with breast cancer have a family member with this disease, women who do have close blood relatives with breast cancer have a higher risk[51]. For instance, having a first-degree relative (mother, sister, or daughter) with breast cancer almost doubles a woman's risk while having two first-degree relatives with the disease increases the woman's risk about 3-fold. Interestingly, women with a father or brother who have breast cancer also have a higher risk of breast cancer. Within the context on an individual, a woman with cancer in one breast has a higher risk of developing a new cancer in the other breast or in another part of the same breast[46].

2.2.2.2 Race and Ethnicity: In general, Caucasian women are slightly more likely to develop breast cancer than African-American women although breast cancer is more common in African-American women under age 45. Furthermore, African-American women are more likely to die from breast cancer at any age. Other races such as Asian, Hispanic, and Native American women have a lower risk of developing and dying from breast cancer[51].

2.2.2.3 Certain Benign Breast Conditions: Women with dense breasts on mammogram have a risk of breast cancer that is about 1.5-2 times that of women with average breast density even though multi factors play a role in determining breast density, such as age, menopausal status, the use of certain drugs (such as menopausal hormone therapy) and pregnancy. Certain non-proliferative lesions may marginally affect breast cancer risk. These non-proliferative lesions include fibrosis and/or simple cysts, mild hyperplasia, adenosis, phyllodes tumor, single papilloma, duct ectasia, periductal fibrosis, squamous and apocrine metaplasia, epithelial-related calcifications, other tumors (lipoma, hamartoma, hemangioma, neurofibroma, adenomyoepithelioma), or mastitis.[46]

2.2.2.4 Certain Proliferative Breast Lesions: Some proliferative lesions without atypia seem to raise a woman's risk of breast cancer slightly[52]. Examples of such proliferative lesions are ductal hyperplasia, fibroadenoma, sclerosing adenosis, papillomatosis or radial scar. However, certain proliferative lesions with atypia in the ducts or lobules of the breast tissue will increase breast cancer risk 4-5-fold; and these include atypical ductal hyperplasia (ADH) and atypical lobular hyperplasia (ALH)[53].

2.2.2.5 Lobular Carcinoma *in situ* (LCIS) or Nobular Neoplasia: LCIS cells are cancer-like and grow in the lobules of the milk-producing glands of the breast, but are limited within the walls of the lobules[53]. LCIS is traditionally grouped with ductal carcinoma in situ (DCIS) as a

non-invasive breast cancer, while recent updates in the field consider LCIS to be benign. However, LCIS differs from DCIS in that it usually does progress to become invasive cancer if it is not treated. Women with LCIS also have a much higher risk of developing cancer in either breast.

2.2.2.6 Chest Radiation Therapy: Women, who were treated with radiation therapy to the chest for another cancer when they were younger, have higher risk for developing breast cancer[54]. The impact of this factor on increasing risk is highest if the individual had radiation as a teen or young adult, when the breasts were still developing. Conversely, radiation treatment after age 40 does not seem to increase breast cancer risk.

2.2.2.7 Exposure to Diethylstilbestrol (DES): From 1940s through early 1970s some pregnant women were given an estrogen-like drug DES because it was thought to lower the incidence of miscarriage[55]. These women have a slightly increased risk of breast cancer, and women whose mothers took DES during pregnancy may also have a slightly higher risk of breast cancer.

2.2.2.8 Lifestyle and Personal Behavior-Related Risk Factors of Breast Cancer Vast majority (about 85%) of breast cancers occur in women without apparent family history of breast cancer. These cancers may be caused by genetic mutations that occur as a result of the aging process and lifestyle-related risk factors, rather than inherited mutations.

2.2.2.9 Birth Control and Contraceptives: Many birth control methods use hormones, which may increase breast cancer risk[56]. Women using oral contraceptives have a slightly higher risk of breast cancer than women who have never used them, although the risk seems to go back to normal over time once the regimen is stopped. As an injectable form of progesterone, Depo-Provera has been shown to have an increase in breast cancer risk, but there is seemingly no

increased risk in women five years after they have stopped receiving the shots. Birth control implants, intrauterine devices (IUDs), skin patches, and vaginal rings usually also use hormones and thus in theory may increase breast cancer risk. Consequently, whenever considering the use of hormonal birth control, women should discuss the coupling of this impact with any other risk factors for breast cancer with their health care providers.

2.2.2.10 Hormone Replacement Therapy (HRT) after Menopause: The hormone estrogen (often combined with progesterone) has been used to relieve symptoms of menopause and to prevent osteoporosis[57]. Combined hormone therapy is needed in most cases as use of estrogen alone can increase the risk of cancer of the uterus. However, for women who have had a hysterectomy, estrogen by itself can be used. Postmenopausal combined hormone therapy increases the risk of breast cancer, the chances of dying from breast cancer, and the likelihood that the cancer may be found only at a more advanced stage. This increase in risk is usually seen with as little as two years of use. However, the increased risk from combined HRT is reversible and its risk applies only to current and recent users, as a woman's breast cancer risk seemingly returns to that of the general population within five years of stopping HRT. The use of bioidentical or "natural" estrogen and/or progesterone is not necessarily safer or more effective, and thus should be considered to have the same health risks as any other type of HRT. Short term use of estrogen alone after menopause does not seem to increase the risk of breast cancer much. However, long-term use of estrogen therapy (e.g., >15 years) was reported to increase the risk of ovarian and breast cancer. Thus, the decision to use any forms of HRT should be made by a woman and her physician after weighing the possible risks and benefits, and considering her other risk factors for heart disease, breast cancer, and osteoporosis.

2.2.2.11 Excessive Alcohol Consumption: Drinking alcohol is clearly linked to an increased risk of breast cancer, and the increase in risk caused by this factor correlates with the amount of alcohol consumed[46]. For example, women who have two to three drinks a day have approximately 20% higher risk of breast cancer compared to women who don't drink alcohol. Women who have only one alcoholic drink per day have a very small increase in risk.

2.2.2.12 Significant Overweight or Obese: Before menopause, women's ovaries make most of the body's estrogen, while fat tissue makes only a small amount[58]. However, when the ovaries stop making estrogen after menopause, most of a woman's estrogen comes from fat tissue. Thus, having more fat tissue after menopause will raise estrogen levels and increase breast cancer risk. Furthermore, being overweight tends to lead to higher blood insulin levels, and higher insulin levels are linked to certain cancers, including breast cancer. Nonetheless, the link between body weight and breast cancer risk is complex and remains to be fully understood.

2.2.2.13 Not Having Children or Not Breastfeeding: Women who have not had children or who have their first child after age 30 have a slightly higher overall risk for breast cancer. Conversely, having multiple pregnancies and/or becoming pregnant at an early age reduce breast cancer risk[59]. Nonetheless, pregnancy seems to have different effects on different types of breast cancer, and pregnancy seems to increase risk for triple-negative breast cancer. It has been suggested that breastfeeding may slightly lower breast cancer risk, especially if it is continued for 1.5-2 years. A possible explanation for this effect is that breastfeeding reduces woman's total number of lifetime menstrual cycles[60].

2.2.2.14 Starting Menstruation Early or Stopping Menopause After age 55: Women will have more menstrual cycles if they start menstruating early, especially before age 12, and thus they will have a longer lifetime exposure to the hormones estrogen and progesterone, leading to a

slightly higher risk of breast cancer[46]. Similarly, women will have more menstrual cycles if they go through menopause later, especially after age 55, and also have a longer lifetime exposure to estrogen and progesterone with a higher risk of breast cancer.

2.2.2.15 Lack of Physical Activity: Growing evidence indicates that regular physical activity, especially in women past menopause, may reduce breast cancer risk[61]. It is not completely clear how physical activity might reduce breast cancer risk, but it may be due to the fact that activity levels affect body weight, inflammation, hormones, and energy balance.

2.2.3 Cancer Gene Mutations in Breast Cancer

2.2.3.1 BRCA1/2 Mutations in Breast Cancer

Approximately 10%-20% breast cancer patients have at least one first-degree relatives affected with breast cancer.8,203 Among them, up to 20% of women with a family history of breast cancer have a mutation in the breast cancer susceptibility genes 1 or 2 (BRCA1 or BRCA2)[62]. The prevalence of germline BRCA mutations is relatively high in women of Ashkenazi Jewish ethnicity, where the risk is estimated to be 30%-35%[63] In male breast cancer cases, up to 14% have a BRCA2 mutation although 4.5% of Ashkenazi Jewish men presenting with breast cancer have a BRCA1 mutation[46].

Moreover, among women with ovarian cancer, regardless of family history, about 15% are attributable to BRCA mutations, while the proportion with a germline BRCA mutation may be as high as approximately 40% in Ashkenazi Jewish women with epithelial ovarian cancer[46]. The BRCA proteins share a similar, and cooperative, tumor suppressing mechanism by repairing DNA damage through homology-directed repair (HDR), which inhibits tumorigenesis[64]. Thus, deletion mutations and/or loss of function in the BRCA genes lead to decreased DNA repair

efficiency and possibly give rise to the expansion of cancerous cells, elevating the risk of developing breast cancer by five to six fold[65].

Even though BRCA mutations can be inherited and responsible for a certain proportion of familial cases, investigators have found no difference in the incidence of breast cancer in BRCA mutation carriers with or without intimate family history[66]. A recent study has shown that smoking also plays a minor role in increasing the likelihood of developing breast cancer, as well as other cancers for BRCA-mutation carriers[67]. These results will increase the accuracy of identifying the high-risk population and the efficiency of preventative measures. For more than two decades, the prevalence of genetic variations of the BRCA genes in breast cancer and other cancers has been well-investigated[68]. Interpretations that are more comprehensive were made available with the advancement of data collection and analysis. Consortium of Investigators of Modifiers of BRCA1/2 has made significant contributions to the characterization of the BRCA landscape. A latest update report of the CIMBA dataset summarized a total of 1650 and 1731 unique mutations in BRCA1 and BRCA2 respectively[69]. Of all the types of BRCA1 and BRCA2 mutations, frameshift is the most common, mostly leading to the generation of premature stop codons and therefore decreasing the levels of mature RNAs and functional proteins. Enduring efforts made by researchers or organizations such as CIMBA demonstrate the ubiquitous perception of higher ratio of BRCA mutations in younger patients with more aggressive subgroups of breast cancer[70]. Nonetheless, the majority of these studies imply a comparable outcome among patients regardless of BRCA status, limiting the application of BRCA mutation status in prognosis prediction.

Clinical data suggest that BRCA1 mutation-related breast cancers that do not overexpress ER or ERBB2 are related to the expression of the basal epithelial markers, which are already associated

with ER/ERBB2-negative tumors[71]. While the cells are phenotypically basal-like, there is evidence that BRCA-1 basal-like breast cancer cells as well as sporadic basal-like breast tumors are not originated from basal stem cells, but rather from luminal epithelial progenitors[74]. Cells with a potential for phenotypic plasticity do not necessarily reflect histology in tumor phenotypes[73].

2.2.3.2 Oncogenic Mutations of PIK3CA in Breast Cancer

Phosphatidylinositol 3-kinase (PI3K) is divided into three classes (I-III) based on their structure and substrate specificity. Class I PI3K is further categorized into class IA and IB, in which Class IA PI3K is the class most closely implicated in cancer[73]. Structurally, PI3K is constituted of a p110 catalytic subunit and p85 regulatory subunit. There are three isoforms of p110, namely p110a (encoded by PIK3CA), p110b, and p110d. While p110d is expressed exclusively in leukocytes, p110a and p110b are ubiquitously expressed. Conversely, human regulatory subunits p85a, p85b, and p55g are encoded by PI3K regulatory subunit 1 (PIK3R1), PIK3R2, and PIK3R3, respectively[73].

PI3K signaling is initiated by the growth factor activated receptor tyrosine kinase, or RAS protein, though direct interaction with p85 or via adaptor proteins, resulting PI3K being recruited to the membrane[74]. Activated PI3K subsequently activates critical downstream mediators AKT and mTOR, leading to enhanced growth, anti-apoptosis, cell-cycle progression, and translation. The PI3K/AKT/mTOR pathway is the most frequently enhanced oncogenic pathway in breast cancer. Among mechanisms of PI3K enhancement, PIK3CA mutations are most frequently (w30%) observed, along with protein loss of PTEN, although somatic mutations of PIK3CA coding p110a in various solid malignancies[75]. The majority of PIK3CA somatic mutations are located in the two “hot spots”, E542K or E545K in exon 9, and H1047R or H1047L in exon 20,

both of which are gain-offunction mutations and have transforming capacity[74]. Suprisingly, amplification of PIK3CA gene was reported even before PIK3CA mutations were identified, and was found in various malignancies, including approximately 10% of cases of breast cancer[76]. In addition to PIK3CA mutations, there are many other PI3K-enhancing mechanisms, such as HER2 amplification, dysfunction of PTEN, and AKT1 activating mutation. For example, PIK3R1 mutations were found in breast cancer with much lower occurrence (w3%) as the PIK3R1 gene product p85a plays a tumor-suppressor role by stabilizing p110a[74]. AKT1 mutations (E17K) have been found in 1.4%-8% of breast cancers, especially in tumors expressing both ER and PR[77]. PIK3CA mutations and gain of copy number of PIK3CA and PTEN loss and PTEN mutations have also been reported to coexist, although PIK3CA, PTEN, AKT1, and PIK3R1 mutations are reported to be mutually exclusive[77]. Oncogenic PIK3CA mutations are thought to cause dedifferentiation of luminal or basal mammary progenitor cells, allowing them to attain multi-lineage potential[46]. Mutations that upregulate the PI3K pathway in ER-positive breast cancers, such as downregulation of PTEN, overexpression of HER2 or IGF-1R, or the activation of mutant AKT1, can lead to the acquired resistance to hormone therapies in ER-positive tumors[78]. Overall, PIK3CA mutations are most likely found in luminal-type (HR-positive/ HER2-negative) tumors, in particular those with markers indicating less aggressive tumor characteristics[74]. Furthermore, it was reported that treatments with PI3K inhibitors in ER-positive, PIK3CA-mutant breast cancers had to be continuous to achieve optimal therapeutic effects, and to overcome proliferative rebound and antiestrogen resistance; and yet the occurrence of such resistance observed in one third of patients in this subtype and resulted in an exceptionally poor prognosis[79]. Nonetheless, it remains to be determined whether PIK3CA

mutations are valid prognostic or predictive biomarkers for the clinical management of breast cancer patients.

2.3 Lung Cancer

2.3.1 Incidence of Lung Cancer

Lung cancer has been the most common diagnosed cancer for the last several decades globally[80]. In 2018, there was an estimated 2.1 million new lung cancer diagnoses accounting for 12% of the global cancer burden[81]. Lung cancer remains the most common cancer diagnosis with approximately 1.37 million diagnoses in 2018 among men, with the highest incidence rates in Micronesia (54.1 per 100,000), Polynesia (52.0 per 100,000), Central and Eastern Europe (49.3 per 100,000), and Eastern Asia (47.2 per 100,000). Incidence rates among women are generally lower than men with approximately over 725,000 new lung cancer diagnoses in 2018[80]. Due to historical differences in cigarette smoking, there are geographic variations in incidence rates differ for women compared with men. Among women, the highest incidence rates occur in North America (30.7 per 100,000), Northern Europe (26.9 per 100,000), and Western Europe (25.7 per 100,000)[81].

In the United States, lung cancer is the second most common cancer in men after prostate cancer and the second most common cancer in women after breast cancer[82]. In 2019, an estimated 228,150 new cases of lung cancer are expected. The incidence rate among men is 71.3 per

100,000 and for women it is 52.3 per 100,000. Since the mid-1980s, the incidence rate has been declining in men, which later began to declining for women in the mid-2000s because of historical sex-specific differences of smoking uptake and cessation. The decline in incidence has gained momentum in the past decade with rates decreasing from 2011 to 2015 by nearly 3% per year in men and 1.5% per year in women. Geographically, lung cancer incidence is higher in the Midwest, East, and South with the highest rates observed in the South for both men and women[82].

2.3.2 Histologic Classification of Lung Cancer

Lung cancer tumors are divided into two broad histologic categories: Non Small Cell Lung Cancer (NSCLC) and Small Cell Lung Cancer (SCLC). NSCLC represents more than 80% to 85% of lung cancers of which approximately 40% are adenocarcinoma, 25% to 30% are squamous cell carcinoma, and 10% to 15% are large cell carcinomas. Bronchioloalveolar carcinoma (BAC) was a distinct histologic classification representing a subgroup of adenocarcinomas and has been replaced with adenocarcinoma *in situ*, minimally invasive adenocarcinoma, and invasive adenocarcinoma of the lung [82]. Other less common histologic subtypes include adenosquamous carcinoma, pleomorphic sarcomatoid carcinoma, large-cell neuroendocrine carcinoma, and carcinoid tumor.

Adenocarcinoma has been the most frequently diagnosed histologic subtype since at least the 1970s among women. The incidence rate of lung adenocarcinoma among men has been on the rise since the 1970s, and the incidence rate for lung adenocarcinoma surpassed squamous cell carcinoma around 1994. The incidence rate for squamous cell carcinomas has been on the decline since the early 1980s. This temporal shift in histologic diagnoses is largely attributed to the widespread use of filtered cigarettes and increasing amounts of tobacco-specific nitrosamines in tobacco[83]. Regarding the former, earlier in the 20th century, most mass-produced cigarettes

were non filtered, which discouraged deep inhalation and combusted tobacco smoke exposed primarily in the trachea and bronchus, resulting in observed higher rates of squamous cell carcinoma diagnoses especially among men [84]. When filtered cigarettes were introduced, combusted tobacco smoke dispersed deeper into the respiratory tree due to deeper inhalation resulting in adenocarcinomas with a more peripheral distribution[83]. The introduction of so-called "light" filtered cigarettes and changing tobacco blends, which decreased nicotine but increased nitrates and N-nitrosamines, had the paradoxical effect of increasing, rather than decreasing, lung cancer risk due to promotion of deeper and more frequent inhalation of combusted tobacco smoke [83].

Although the binary division of lung cancer into NSCLC and SCLC is still widely applied and relevant, advances in genomic profiling has resulted in a paradigm shift whereby lung cancers are also characterized and classified by tumor biomarkers and genetic alterations, such as gene expression, mutations, amplifications, and rearrangements, that are critical to tumor growth and survival and can be exploited with specific targeted agents or immune-checkpoint blockades[85].

2.3.3 Risk Factors of Lung Cancer

2.3.3.1 Tobacco Smoking

Without any doubt, tobacco smoking is the most important and prevalent lung cancer risk factor. A rare disease at the beginning of the 20th century, lung cancer was one of the first diseases to be causally linked to tobacco smoking[83]. Men pre-dominantly began smoking manufactured cigarettes earlier in the 20th century, during and after World War II. Although few women smoked regularly before World War II, average age at initiation continued to decrease and per capita in cigarette consumption increased through the 1960s. Tobacco consumption fell

drastically in the United States following publication of the landmark 1964 U.S. Surgeon General's Report that concluded cigarette smoking is causally related to lung cancer in men[83].

Tobacco smoke contains more than 4,000 chemicals, including at least 69 established carcinogens and other toxicants associated with major diseases [86]. Although only around 15% of smokers develop lung cancer, 80% to 90% of lung cancer diagnoses are attributed to tobacco smoking in the United States[87]. Numerous lung cancer risk models are available as web-based tools that provide risk assessment based on demographic information, including smoking history and intensity[88].

2.3.3.2 Exposure to Secondhand Smoke

Secondhand smoke, or side-stream smoke, is an indirect carcinogenic exposure resulting from the burning of tobacco pro-ducts. Carcinogens that have been measured in secondhand smoke include polycyclic aromatic hydrocarbons, nitrosamines, and aromatic amines. Studies have shown that nicotine and its metabolite cotinine as well as DNA adducts from tobacco carcinogens are present in the urine of never smokers who are exposed to sec-ondhand smoke [89.] A meta-analysis of 12 studies found that secondhand smoke exposure compared with never smokers without such exposure was associated with a 25% increased risk of lung cancer[90].

2.3.3.2 Electronic Cigarette

Electronic nicotine delivery systems, also referred to as electronic cigarettes and e-cigarettes, allow for the delivery of nicotine to the lung epithelium via an electronic device. Although a patent for this type of device was first issued in 1965, mass production of e-cigarettes did not occur until 2003 and became widely available in 2005 in the United States.

2.3.3.3 Radon

Because tobacco smoking is a potent and prevalent risk factor, secondary causes of lung cancer are often diminished in perceived importance. However, there are numerous other exposures that are causally linked to lung cancer risk. Radon is an invisible, odorless, tasteless radioactive gas that is found in soil and produced naturally during the radioactive decay of thorium and uranium. All humans are exposed to radon gas and there are substantial geographic variations and globally, 3% to 14% of lung cancers are attributed to radon exposure and the variance is attributed to geo graphic differences in radon concentration and on the method of calculation [91].

2.3.3.4 Occupational Exposures

Occupational exposure to carcinogens is one of the major cause of lung cancers of which asbestos exposure is historically the most common[92]. Asbestos is a com-mercial term for a group of naturally occurring mineral silicate fibers, including amphiboles (crocidolite, amosite, tremolite, anthophyllite, and actinolite) and chrysotile (the sole serpentine fiber). Asbestos is found on all continents, has been used commercially since the 19th century, and is still used in some countries today in numerous applications, including insulation, textile, cement, and roofing[93]. Although the mechanisms involved in asbestos-associated diseases are complex and the molecular pathways involved are not fully established, direct and indirect cellular and molecular effects likely contribute to lung cancer etiology, including oxidative stress, chronic inflammation, genetic and epigenetic alterations, and cellular toxicity and fibrosis [94].

2.3.3.5 History of Infectious-Related Respiratory Diseases

Pneumococcal disease is an umbrella term for a group of syndromes caused by a variety of organisms resulting in varied manifestations and sequelae[95]. Most commonly, pneumococcal disease is an infection caused by the Streptococcus pneumoniae bacterium that can infect the

lungs (pneumonia), bloodstream (bacteremia), and tissues and fluids surrounding the brain and spinal cord (meningitis). Pneumonia is a putative lung cancer risk factor through several possible mechanisms from mediators of chronic local inflammation, including elevated reactive oxygen species that can cause DNA damage and somatic mutations, antiapoptotic signaling, and increased angiogenesis [83].

2.3.4 Other Lifestyle Factors

There is also compelling evidence that other factors may be associated with an increased risk of lung cancer for both smokers and never smokers, including poor diet and low body mass index[96].

2.3.4.1 Inherited Genetics

The Genetic Epidemiology of Lung Cancer Consortium revealed the first evidence for a major susceptibility locus influencing lung cancer risk to a region on 6q23–25[97]. With the arrival of genome-wide association (GWA) studies about 17 years ago, it is now possible to interrogate the human genome more comprehensively for associations between inherited single-nucleotide polymorphisms (SNP) and human disease. GWA studies have successfully identified genetic factors significantly associated with lung cancer susceptibility with varying strengths of association evidence and some loci have been refined to specific subgroups, including sex, ethnicity, smoking status, and histo-logic subtypes. Data from these large GWA studies could be leveraged toward development of risk models based on polygenic risk scores defined by the combination of SNPs that yield the best predictive model[99].

2.3.5 Prevention

Most lung cancers are preventable and could be mitigated by reducing smoking initiation among adolescents and increasing smoking cessation among adults. Although primary prevention

(smoking prevention and cessation) mitigates risk and mortality, former smokers remain at significant risk of dying from lung cancer[99]. As such, early detection is currently the only option for those who have already quit smoking and among those individuals who are at high risk. Lung cancer will likely remain a major public health burden globally throughout the 21st century and advances in risk assessment, early detection, diagnosis, and treatment will be imperative in improving outcomes of this disease [100].

2.4 Cancer Cell Lines

Cancer cell lines are useful tools because they provide various models of the biological mechanisms involved in cancer development and progression. The utilization of cancer cell lines improved the knowledge of deregulated genes and signaling pathways involved in cancer progression; cell lines have also been used in several the studies involving potential molecular markers for cancer screening and prognosis[101]. Several cell lines with their unique properties and characteristics are currently available for in vitro study of different types of cancer[102]. Cell lines are easy to handle and manipulate genetically/epigenetically with the use demethylation agents, small interfering ribonucleic acid (siRNA), expression vectors, and they can be pharmacologically manipulated through cytostatics (cell growth inhibitors). Cell lines are homogenous, they provide identical tumor cells for easier analysis unlike in heterogeneous solid tumors. However, imitation of tumor characteristics *in vivo* as closely as possible can be achieved with the use of a cancer cell line panel representative of the heterogeneity as observed in the primary tumors. Cancer cell lines are pure populations of tumor cells and have a high degree of resemblance with the initial tumor. The results of experiments, using correct conditions are easily reproducible because of the homogeneity of cell lines[103]. In addition, there is a significant number and variety of cancer cell lines available. Despite these advantages, some

disadvantages of using cancer cell lines include cross-contamination with HeLa cells, genomic instability leading to differences between the original tumor and the specific cell line, such as changes in the morphology, gene expression, and cellular pathways of cell lines from culture conditions required to maintain them (i.e., culture adaption), and mycoplasma infection[104]. Furthermore, establishing long-term cancer cell lines of certain types of tumors is difficult, including prostate cancer tumors[105]. The limited number of cell line models for prostate cancer research arise from the difficulty in propagating prostate cancer cells in vitro for extended periods. Researchers have been able to generate only seven cell lines that were previously available through public cell line repositories, but with disadvantage that the spectrum of clinical disease is not represented. Therefore new cell lines, which demonstrate the commonly observed clinical phenotypes, are clearly needed[105]. Since the isolation of the first cell line in the 1950s (i.e., HeLa cells), different type of cancer cell lines have been developed for preliminary drug testing[106]. The viability of different cell lines over time is dependent on different media, growth factors, and supplements, as the constituents of culture media affect the cell lines. Reported in a study by Kim et al., human breast cancer cells (MDA-MB-231) cultured in minimum essential medium (MEM), Dulbecco's modified Eagle's medium (DMEM), or Roswell Park Memorial Institute (RPMI)-1640 medium and containing different concentrations of fetal bovine serum (FBS) or different sera (equine or bovine) showed significant changes in gene expression[107]. It was reported that about 25% of genes were expressed at significantly different levels by cells grown in DMEM, MEM or RPMI-1640 media based on genome-wide expression analysis[107]. Lung cancer cells (A549) and hepatocellular cancer cells (HepG2) cultured in Ham's F-12 nutrient mix (F12), RPMI, DMEM, and MEM showed a significantly increased proliferation rate for A549 cells in DMEM compared to the other media tested, and the lowest rate for both A549

and HepG2 cells in MEM, confirmed by assaying conditioned media for basal level ATP at 72 h[108]. This emphasize the significant effect of growth conditions and/or environment on cells in drug discovery experiments, and the need for specificity to ensure results reproducibility. There are few numbers of prostate cancer cell lines in use today, most of which were established from metastatic deposits[109].

2. 5 The Pathway to Cell Death in Cytotoxicity

There are various mechanism of cell death by which dysfunctional, damaged, and worn-out cells are cleared out in order to maintain the natural physiology and tissue function.The healthy, new cells that are better equipped to maintain normal body function are allowed to stay in the system[110]. Therefore, cell death, survival, proliferation, and diferentiation represent fundamental processes of life. Any deviations from the normal cell death processes may have calamitous signifcance and sometimes may lead to numerous diseases, including neurodegenerative, cardiovascular, autoimmune, immune, and infectious, either due to excessive or inadequate removal of cells from the system[111]. Cell death is a natural endpoint of normal cell physiology that results in the irreversible termination of cellular functions, including growth, division, and metabolic homeostasis; either due to the formation of new cells, disease factors/ morbidity, localized infection, or injury and mortality of an organism[112]. Cell death manifests with macroscopic morphological alterations. Most cell deaths utilize a caspase dependent pathway, commonly known as apoptosis. Genetic studies in model systems suggest that apoptosis accounts for the bulk of programmed cell death (PCD) [113]. PCD directly operates at the level of the organism or colony despite cellular homeostasis[114]. Such a homeostatic function refects not only the elimination of useless or potentially dangerous cells, but also the ability to die cells to expose or release molecules that alert the organism or colony about a potential threat. Such danger

signals are commonly referred to as damage associated molecular patterns (DAMPs) or alarmins[115]. In addition to apoptosis, several regulated cell death (RCD) modalities have come to light in recent years. Initially, cell death was divided into three distinct types: apoptosis, autophagy, and necrosis[116].

Apoptosis, as type I cell death, is a form of self-killing and happens due to the proteolytic activity of an enzymatic cascade system[117]. Apoptosis is characterized by cytoplasmic shrinkage, chromatin condensation (pyknosis), nuclear fragmentation (karyorrhexis), and plasma membrane blebbing, culminating with the formation of apparently intact small vesicles (commonly known as apoptotic bodies) that are efficiently taken up by neighboring cells with phagocytic activity and degraded within lysosomes. The apoptosis execution occurs by two pathways, the intrinsic and extrinsic, that center on the activation of cysteine-proteases called caspases[118].

Autophagy or type II cell death is a self-eating process to remove damaged cells, proteins, dysfunctional organelles and commences by forming autophagosomes[119]. Autophagy manifests with extensive cytoplasmic vacuolization and similarly culminating with phagocytic uptake and consequent lysosomal degradation. Autophagy is critical for maintaining cellular homeostasis, and its dysregulation has implications for health and disease.

Type III cell death or necrosis is defned as a type of uncontrolled cell death that can occur in response to infection, toxins, chemicals, injury, or lack of blood supply. It displays no distinctive features of type I or II cell death, terminating with the disposal of cell corpses in the absence of obvious phagocytic or lysosomal involvement[117]. Cells that die by necrosis generally have diffuse nuclei and a loss of organelles structures[120]. It contains a great diversity of cell death processes, such as necroptosis and pyroptosis.

Lately, multiple novel cell death modalities have been identifed and characterized concerning their corresponding stimuli, molecular mechanisms, and morphologies. Some of these modalities share overlapping, but not identical, signal pathways and fail to be incorporated into the type I–III categories. In 2018, the Nomenclature Committee on Cell Death listed multiple cell death based on mechanisms of disposal of fragments in a molecule-oriented manner[121].

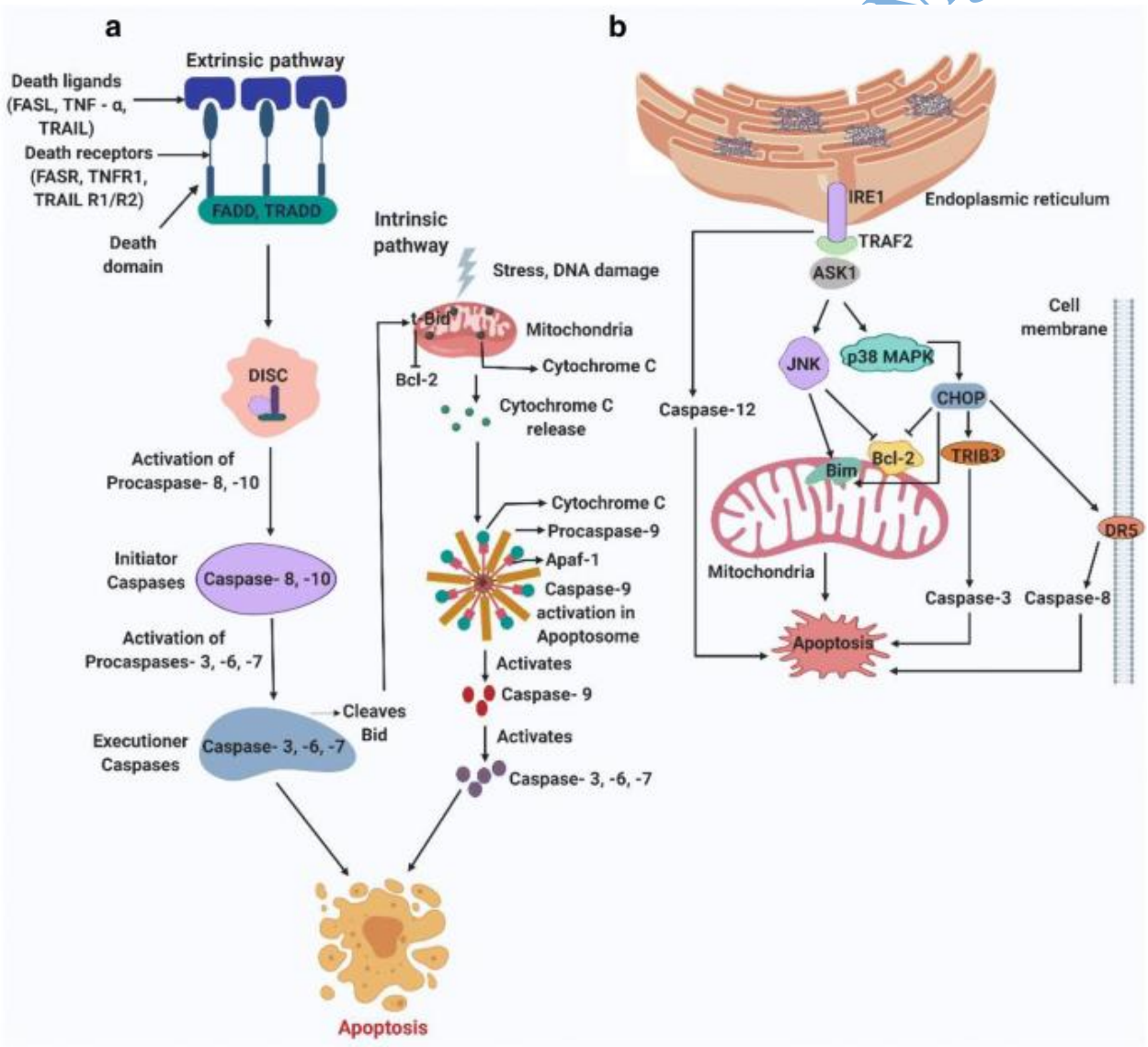


Figure: 2.1 Cell death Pathway

Source[203]

2.6 Medicinal Plants

A medicinal plant according to WHO is any plant which in one or more of its organs contains substances that can be used for the therapeutic purposes or which are precursors for the synthesis of useful drugs[122]. Medicinal plants have been in use for thousands of years as therapeutic sources worldwide, particularly in Africa for healthcare[123]. Owing to structural diversity of plant metabolites and their previously demonstrated pharmacological properties, medicinal plants also can serve as starting materials for drugs. In fact, the therapeutic use of plants continues today due to their biomedical benefits and cultural beliefs in many parts of the world. The economic reality of the inaccessibility of modern medication for several African peoples also has played a key role in the large use of herbal medicines[124]. The World Health Organization (WHO) has recognized the contribution and value of the herbal medicines used by a large segment of the world's population. Iwu et al., reported that infectious diseases account for one-half of all deaths among the tropical countries[125]. As a result, people of all continents have long applied poultices and imbibed infusions of indigenous plants dating back to prehistory for health purposes[126]. Many scientific studies have shown various pharmacological properties of medicinal plants. Isolation of active compounds, in most cases, provided scientific explanation for traditional

medicine that make use of plants. World Health Organization (WHO) has defined traditional medicine (including herbal drugs) as comprising therapeutic practices that have been in existence, often for decades, before the development and spread of modern medicine and are still in use today[127]. The traditional preparations comprise medicinal plants, minerals, organic matter, etc. Herbal drugs constitute only those traditional medicines which primarily use medicinal plant preparations for therapy. "Despite the benefits derived from medicinal plants, some of them have some unpleasant side effects which may be related to overdoses, toxic principles, or other factors. The dominant effect may depend on the condition, growth stage, or part of the plant, the amount consumed, and the species and susceptibility of the victim[128]. This may lead to acute toxicity and death of patients; scientific evidence of the safety of herbal preparation will help to harness the therapeutic potentials of medicinal and aromatic plants for further drug development in the future. Toxic plants can affect the entire spectrum of organ systems, with some plants having several toxic principles that affect different systems.

2.7 Plants of Study

2.7.1 *Ficus benjamina*

Weeping fig (*Ficus benjamina* L.) is an annual tree belonging to the family mulberry (Moraceae). It has been used for thousands of the year as an ornamental plant and hedge plant[129]. *Ficus i*s a large genus of about 40 genera and 1000 species of trees, shrubs, lianas, or rarely herbs and many varieties are native to Asia, Malaysia, Australia, and parts of the Pacific region. The uncertainty in the exact number of spices within the genus is largely attributed to the great variability among the constituent. *Ficus benjamina* crosspollinates very easily resulting diversity and variation. Wasps play an important role in pollination for reproduction of these species. Members of this genus are hard to differentiate by their flowers, but can be distinguished by their

pattern, whether they are weeping fig or not, by leaf shape, and by their fruits[130]. *Ficus benjamina* is the most diverse species within the range. Large numbers of cultivars are available but the exact numbers are unknown while they also vary in flavors and uses. *Ficus benjamina* is a tree reaching 30 meters under natural conditions. Some famous species that represent the sort of the genus include the common place fig, a small temperate deciduous tree whose recognized fig leaf is known in artwork and iconography, the weeping fig *(F. benjamina*), a hemi-epiphyte with thin hard leaves on pendulous stalks adapted to its rain wooded area habitat; the difficult-leaved sandpaper figs from Australia; and the creeping fig (*F. pumila*), a few species are more not unusual but some aren't a vine whose small, tough leaves form a compact carpet of foliage over rocks or garden walls. But 'Variegate' is most common in spices than that of several spices in Europe. Variegate are leaves with white and gray inexperienced along edges, new leaves regularly begin white and age with extra green. Often greater common cultivars are Amstel King-upright with long leathery banana fashioned leaves[130]. Leaves are ovate-elliptic, slender pointed, thin leathery, dark green and shiny, glossy and 2-5 cm long. Flower are insignificant and minute time unknown and sometime it borne in a hollowedout stem in axils but these flowers are not showy[129]. Wide range of the species is due to the glossy oval foliage. The plant is available as a natural-looking bush and grow as an ornamental plant in different places such as park and around the roads[131]. Different factors like plant part, maturity of the plant part and agro climatic conditions affects the percentage yield of the extracts. *Ficus benjamin*a fruits, leaves and bark contains various bioactive components like stigma sterol, quercetin, cinnamic acid, and lactose naringenin[132].

Weeping fig contains some measure of oil fat substance and brings less caloric worth. *Ficus benjamina* demonstrated the presence of phenolic mixes, carbohydrates, saponins, flavonoids,

alkaloids, proteins and tannins. Mineral synthesis (Ca, Fe, K, Cu, Si and Zn), all out phenols, flavonoids, tartaric esters and anthocyanins. It additionally contains latex which is 30% caoutchouc, alongside 59% gum. Furthermore, wax contains cerotic corrosive. Extraction from leaves, bark, and organic products contains six mixes, for example, cinnamic corrosive, lactose, naringenin, coumaric quercetin, caffeic corrosive and stigma sterol. Fundamental oil yielded four mixes in stem and eight mixes in root. Phenolic mixes, for example, chlorogenic p-, ferulic and syringic acids are available in root, three mixes, for example, chlorogenic, p-coumaric, and ferulic acids are available in stems, and leaves, just one compound is available, for example, caffeic acid. 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and Quercetin is additionally present in this plant[133].

Ficus benjamina has explicit aromatic odor because of the existence of essential or volatile oil, which is largely confined in green leaves. These oils are generally characterized by GC-MS analysis[134]. This scented volatile oil from leaves is chiefly comprised of alkaloids, saponins, flavonoids and tannins[135, 136]. While oil also extracted from seed are mainly composed of naringenin, quercetin and cinnamic acid lactose. Its organic product contains caffeic corrosive; its bark contains stigma sterol while its root bark contains Benjamin amide. The leaf oil of *Ficus benjamina* collected during the day, contained high contents of alpha-Pinene, abietadiene, cis-alpha-bisabolene, gas, reticuline, calycanthidine, anabasine, tomatidine, acridine derivative, sophocarpine, neblinine, harmine, obscurinervinediol, ergoline, ellipticine, indicine, matridine, scoulerine, hydroxyl morphine, aspidospermidin, nicodicodine, adenocarpine, lycocernuine, isoclaurine, dasycarpidan, retronecine, and clemastine[134].

Ficus benjamina and other spices contribute significantly for the ornamental purpose that is why it is found in various countries outside its natural range. Sometimes, it is also used for the

landscaping in housing estates and urban areas. It is also planted beside roadsides. Furthermore, latex of this plant possesses toxic property. Twigs can be used to control insects in field. Leaf juice can be used as bug and flea repellant. Latex can be applied on the boils as this plant has number of antioxidants. Free radicals are responsible for the several clinical disorders like cancer, diabetes mellitus, degenerative disease, renal failures because they disturbed the natural defense mechanisms. However, natural antioxidant present in the body provides defense against these diseases. It was found that plant extracts strengthen the antioxidant defense system of human and are antioxidant of choice as they lower the side effect as well as toxicity over the synthetic ones. *Ficus benjamina* has a number of medicinal importance as it is used in medicine for malaria, influenza, dysentery, airways inflammation (bronchitis), acute enteritis, whooping cough (pertussis) and hot seizures in children[137].

Ficus benjamina has various uses ranging from culinary to religious; its uses are often high in ritual. There are a number of curious beliefs associated with the historical uses of weeping fig. Presence of various types of alkaloids in *Ficus* species is responsible for the potent biological activities like, antioxidant, anticancer, antimicrobial and anti-muscarinic activity. *F. benjamina* has showed potent anti-oxidant activities. Methanolic extracts of *F. benjamin* has been observed for the anti-oxidant pastime via 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and discovered that extract exhibit precise antioxidant assets. The antioxidant activity of *F. benjamina* serves as the medical basis for the traditional use in the prevention and therapy of diseases[137].

Plate 2.1: *Ficus benjamina* leaf
Source; Field work, 2022.

2.7.2 Euphorbia Milli

The euphorbiaceae family currently having around 2000 species. Genus Euphorbia is the largest genus of medicinal plant. Euphorbia milii is commonly known as “crown of thown” [138]. The part of plants that grow above ground that was used for make medicine. The genus E. milii is the

largest genus of medicinal plants widely distributed in tropical countries. Different species of Euphorbia are used for the treatment of various ailments such as skin diseases, intestinal parasites and warts. It has been reported that Euphorbia possesses antiarthritis, anticancer, anticonvulsant, antidiabetic, anti-eczema, antiinflammatory, antimicrobial, antioxidant, antispasmodic, antitumor, antitussive properties, hormonal and myelopoiesis properties[139]. Some species of Euphorbia have been traditionally used for the treatment of skin diseases, gonorrhea, migraine, intestinal parasites and as wart cures[140]. The genus Euphorbia has been studied widely for its antiproliferative[141]. Fungi of the genus Aspergillus produce a toxic substance called aflatoxin, which contaminates crops (e.g., corn and peanuts) and causes human diseases. Aflatoxin has even been implicated as a contributing factor in liver cancer. *Euphorbia milii* flowers, when dried and processed as powder, inhibit the growth of Aspergillus[138]. Milin, an extract of *E. milii* latex, is a glycosylated serine protease (an enzyme that breakdown it as protein and has a sugar attached to it). Because it is more stable than most of the proteases, it is useful to food processors and makers of detergents who have been using proteases in their operations[138].

Phytochemical studies of *E. milii* revealed the presence of flavonoids, terpenoids, and tannins. Flavonoids are yellow pigments, which occur in the plant kingdom either in a free state or as glycosides or associated with tannins. These are known as anthoxanthins.

E. milii is a wide variety of secondary metabolites. Biological activity such as antioxidant properties of ethanol extracts of thorn and stem parts of *E. milii* depicted that *E. milii* could be a potential drug agent of folk medicines[139].

Plate 2.2: *Euphorbia milli* leaf
Source; Field work, 2022.

2.7.3 Vitex Doniana

Vitex doniana belongs to a family of Lamiaceae, and is a deciduous small to medium-sized tree. Vernacular names include Uchakiri (Igbo) Dinyar (Hausa) Galbihi (Fulani) Ori-nla (Yoruba) Black plum (English) and is extremely widespread in tropical Africa, occurring from West Africa (Senegal) to East Africa (Somalia) and down to South Africa. In Nigeria, various parts of the plant are used by traditional medicine practitioners in the management and treatment of several disorders which include cancer, hypertension, rheumatism, and other inflammatory diseases[142]. Traditional medicine is considered as a strong amalgamation of ancestral experience and dynamic medical know-how. The World Health Organization (WHO) described traditional medicine as the totality of the practice and knowledge which can or cannot be explained, that are used for diagnosing, preventing, and eliminating social, mental, or physical imbalance exclusively reliant on observations and practical experiences passed down from one generation to another. In Africa especially in West Africa, the use of traditional medicine and traditional healers is of extreme importance to the health of the populace. The use of traditional remedies for various ailments by the indigenous population cannot be over emphasized

Quercetin, the penultimate most abundant flavonoid in *V. doniana* reduces oxidation of LDL and decreases platelet aggregation. Quercetin is frequently used therapeutically in allergic conditions, including asthma and hay fever, eczema and hives[143]. Apigenins, another abundantly found flavonoid in *V. doniana*, have antioxidant, diuretic, hypotensive, smooth muscle relaxation and antitumor properties whereas casticins have confirmed role enhancing the antimalarial property of artemisinin[143]. Vitexin has recently received increased attention due to its wide range of pharmacological effects such as anti-cancer, antioxidant, anti-inflammatory, and neuroprotective effects while quercetin has shown effectiveness in treatment of colitis and regenerative effects on

human gingival fibroblasts and mesenchymal stem cells. Catechins have anticarcinogenic, anti microbial and hypocholestrolemic activities[144].

Plate 2.3: *Vitex doniana* leaf
Source: Field work, 2022.

2.7.4 Euphorbia heterophylla

Euphorbia species are used in traditional medicine for the treatment of various diseases[145]. Plants of the Euphorbia genus are common herbs that are applied in the treatment of respiratory diseases, healing of wounds, relieving skin irritations, indigestion, inflammation, microbial infestations and also as a food source[61]. Prehistorical records show that Euphorbia species were used in the treatment of scorpion and snake bites, liver diseases, respiratory disorders, asthma and rheumatism in the Chinese and Ayurveda medicine systems[147]. The medicinal applications of these species have been attributed to the presence of diverse secondary metabolites such as flavonoids and terpenes. The abundance of these chemical constituents in Euphorbia species qualifies them as a rich source of therapeutic natural products possessing various pharmacological activities. These constituents can provide potential lead molecules for drug discovery. The genus Euphorbia is also among the largest of genera in the spurge family, and consists of several other subsections and subgenera, having more than 2000 species with promising research potential. Flavonoids are among the dominant constituents of Euphorbia species after macrocyclic diterpenes and triterpenoids[63]. Flavonoids mainly occur as isoflavonoids (3- phenylbenzopyrans), neoflavonoids (4-phenylbenzopyrans), chalcones, flavonols, flavanone, flavanonol, flavanol and anthocyanidins[148]. These constituents are structurally and biogenetically related as they share a common precursor, the chalcone. They have also been reported to possess various pharmacological activities and have promising therapeutic potential[149]. Apart from their biological functions in protecting plant species against herbivores and other pathogens as well as acting as stress-protecting agents, they also perform important pharmacological activities in humans. Reports indicate that plant flavonoids exhibit antiulcer, antidepressant, antimicrobial, antiviral, antibacterial, anti-diabetic, anti-inflammatory,

anti-agiogenic, antiproliferative and anticancer activities *in vitro*[150]. Even though they have not been classified as nutrients, the intake of flavonoids is considered to be significant for human health[151]. They are also used as natural dyes as well as for cosmetics and skin-care products[152]. Related studies have shown that flavonoids from the Euphorbia species also have a wide range of pharmacological activities such as cytotoxic, anti-inflammatory properties and tumor-promoting abilities[148]. Furthermore, various reports have stated the significance of flavonoids in metabolism of the thyroid hormone, which is commonly reported to be vitamin P and is considered useful in counteracting hemorrhage. They are also functional foods for promotion of good health and prevention of diseases[153]. As a result, significant efforts have been made in isolation, identification and characterization of flavonoids from the latex, aerial parts, roots, stems, seeds, stem bark and whole plant extracts of some Euphorbia species since early times. Indeed, several reviews have been published about the role and significance of plant flavonoids as a source of bioactive compounds. For instance, in 2019, Avtar and Bhawna reviewed the chemistry and pharmacology of flavonoids[154]. Similarly, Muhammad et al. reviewed the significance of flavonoids as prospective neuroprotectants in ageing associated with neurological disorders[155]. In addition, the role of plant flavonoids in cancer and apoptosis mechanisms was also reported, as well as the commercial application of flavonoids as anti-infective agents[156].

Plate 2.4: *Euphorbia heterophylla* leaf
Source; Field work, 2022.

2.7.5 Parkia Biglobosa

Parkia biglobosa (Jacq.) Benth is an economic tree found in African savannahs and dry forests. It belongs to the family Fabaceace – pea family, of the order Fabaceae. It is popularly known as the Africa locust bean, Igba or Irugba (Yoruba), Dorowa (Hausa) and Orgili (Ibo). It is an important multipurpose tree and is well known in many African countries. Apart from providing building materials, wood, food, fodder, weapons and other commodities, Parkia biglobosa plant is especially important as traditional medicine. In Africa, 47% of the various identified uses of Parkia biglobosa were medicinal[157]. The fermented seeds of Parkia biglobosa are used in all parts of Nigeria and indeed the west coast of Africa for seasoning traditional soups. The yellow pulp is a high energy giving food with up 60% sugar[158]. The trees are often grown as shade trees. Parkia biglobosa have found use traditionally as food and medicinal agent. Depending on the part, the plant is used treating dental caries, pneumonia, bronchitis, violent colic, severe cough, diarrhea, wounds, otitis, dermatoses, amoebiasis, hemorrhoids, bilharziosis, leprosy, hookworms, tracheitis, conjunctivitis[159].

The bark is employed in wound healing, treatment of bronchitis, pneumonia, skin infection, sores, ulcer, bilharziasis, malaria, diarrhea and hypertension. In Gambia, the leaves and roots are used in preparing a lotion for sore eyes. A decoctions of the bark of Parkia biglobosa is also used as a bath for fever and as a hot mouth washes to steam and relieve toothache. The pulped bark used along with lemon for wound and ulcer. Fibres from pods and root are used as sponges and as string for musical instrument[160]. The powdered pods are used to paint traditional Hausa buildings in northern Nigeria. Parkia biglobosa has been identified as source of tannin, saponins, gum, fuel and wood. Seeds of various species of Parkia have also been investigated for their protein and mineral content[161]. Indigenous healers in Africa use different parts of the locust bean

tree for health benefits. In a survey conducted on healers in Togo, Parkia biglobosa was one of the highest cited plants used for treating hypertension[162]. The tree was also one of two plants "listed as having real wound-healing properties in South-Western Nigeria, influencing the proliferation of dermal fibroblasts significantly[163]. In a survey conducted in relating to their use of antimalarial plants, Parkia biglobosa was cited among those most often successfully used[164]. In an analysis on the antibacterial properties of the plant, another study found that "these properties compare favourably with those of streptomycin, making it a potential source of compounds used in the management of bacterial infections[165].

Dietary intake of raw seeds was also reported to significantly lower the occurrence of esophageal cancer in southern Thailand[166]. Lectin obtained from the *P. speciosa* seeds exerted mitogenic activity in both rat thymocytes and human lymphocytes by stimulating the incorporation of thymidine into DNA cell, which activity was comparable to the known T-cell mitogens like pokeweed mitogen, concanavalin A and phytohemagglutinin[167].

Plate 2.5: *Parkia biglobosa* tree
Source; Field work, 2022.

2.7.6 Piliostigma Thonningii

Piliostigma thoningii Schum is a deciduous, single stem; leguminous tree belonging to the family Caesalpiniaceae. It is a perennial plant with large, simple, two-lobed, leathery leaves which resemble a camel's foot which account for the common name. The name 'Pilio-stigma' means cap-shaped stigma, while specific name, thonningii was given after the Danish Botanist, Peter Thonning[168,169]. It was formerly called Bauhinia thonningii, but later differentiated from Bauhinia by its unisexual flowers and indehiscent pods[170]. The tree bears flowers with five white to pinkish pendulous petals and are unisexual with male and female on a separate tree produced during November and April[171]. The fruit is a hairy, hard, flattish pod which turns rusty brown at ripening and split; it is usually persistent on the tree and produced between June and September[138]. Piliostigma thonningii Schum is a common plant across most sub-Saharan African Countries. In Nigeria, it grows abundantly in the wild in some places such as Enugu, Nsukka, Zaria, Bauchi, Ilorin, Plateau, Lagos and Abeokuta[171]. The plant is commonly called Okpoatu, abefe and kalgo in Igbo, Yoruba and Hausa languages respectively[170]. The root and twig of *Piliostigma thonningii* Schum have been used to treat dysentery, fever, respiratory ailments, snake bites, hookworm and skin diseases[170]. It is also used in the treatment of malaria fever, wounds, ulcers, gastric and heart pain, arthritis, headache, hemorrhoids backache and gingivitis[170]. Traditional healers in "Doila" refer to this plant as "child remedy" as it is mainly used as a remedy for children.

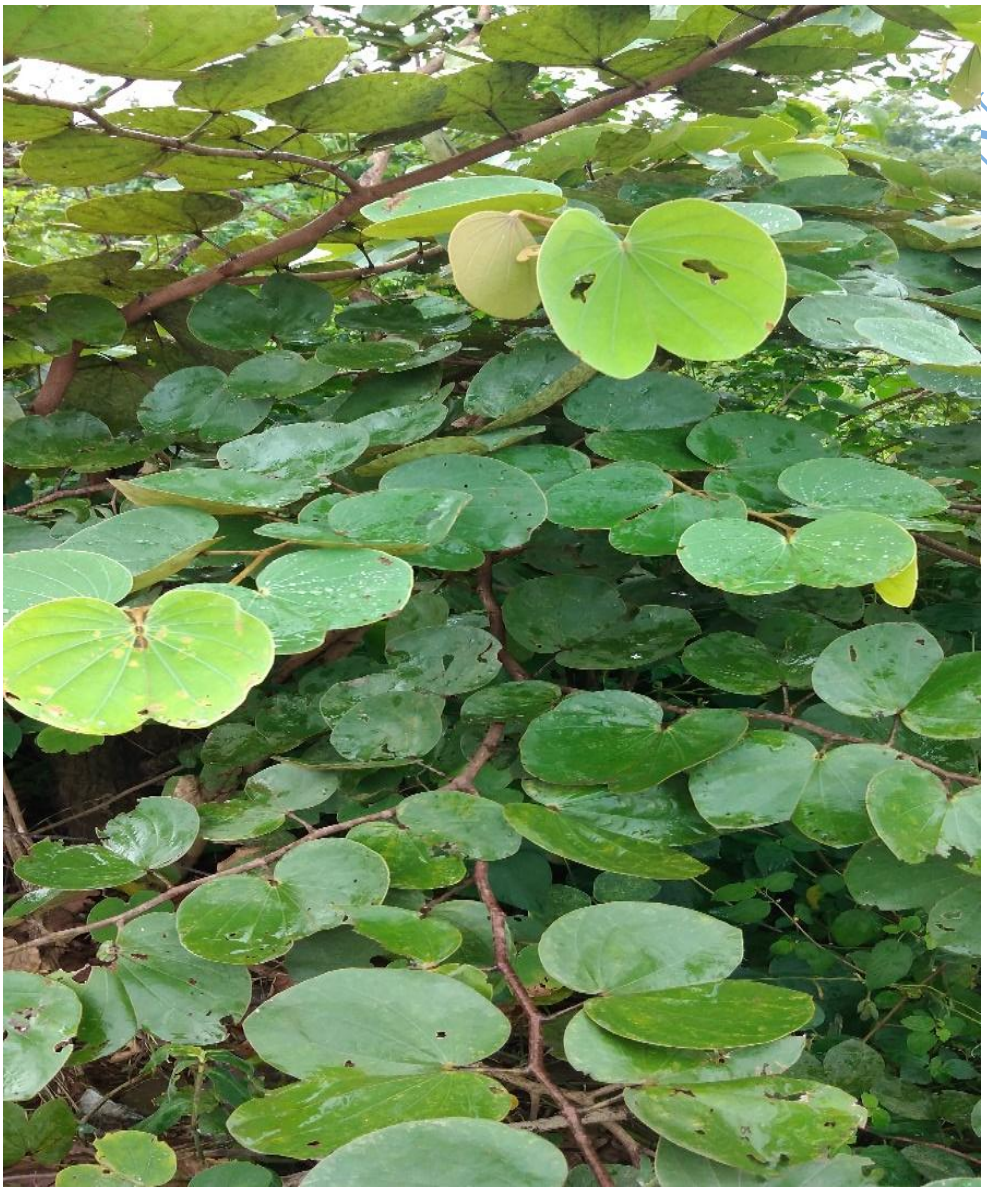


Plate 2.6: *Piliostigma thonningii* tree
Source: Field work, 2022.

2.8 Cytotoxicity of Medicinal Plants.

Cytotoxicity is a broad term and sometimes with vague meaning. A test substance is considered to be cytotoxic with regard to in vitro cell culture systems, if it affects the attachment of cells, significantly alters morphology, adversely affects the rate of cell growth, or causes cells to die. There has been much attention devoted to cytotoxicity studies as a first step in evaluating the toxicity of test substances. This is especially valid in connection with screening biological activity of plant extracts and active compounds isolated from plants. Cytotoxicity assays are widely performed because the test compounds, including plant-derived extracts and purified compounds, may be intended for use as pharmaceuticals or cosmetics, in which case minimal to no toxicity is important[172]. Alternatively, the chemicals may be designed to act as anticancer drugs, in which case selective cytotoxicity to cancerous cells is essential. A low cytotoxic profile exerted in non-cancerous cells indicates that these extracts do not act as indiscriminate cellular toxins, but have a specific cell type-based cytotoxicity. This process allows the identification and prioritization of test substances useful for further biological activity studies.

Cell viability or cytotoxicity assay results may be heavily influenced by the design of the experiment, so it is useful to take into consideration a number of factors when comparing results obtained using similar methods. When designing cell culture experiments, it is critically important to note the growth state of a culture and its kinetic parameters. As many of the properties of cell cultures vary significantly between the lag phase, the log phase (logarithmic or exponential growth phase), and the stationary phase, it is necessary to determine the status of the culture when the experiment begins and at the time of sampling[173]. It is generally found that cultures in the log phase are more consistent and uniform, and when sampling is performed at the end of the log phase, reproducibility is high. Cells in the stationary phase may be more

differentiated, have a different morphology, and may become polarized, often secreting more extracellular matrix[174]. The duration of an experiment is also an important consideration. Addition of a test substance at a certain time in the growth phase and then assaying when the cells are still undergoing exponential growth, or when their growth has plateaued, will influence cytotoxicity results[174]. Detailed knowledge of the growth cycle, including population doubling time of the cells during exponential growth, is necessary in planning cytotoxicity experiments.

Toxicity is a complicated process in human and animal systems, potentially involving direct cellular damage (e.g., with a cytotoxic anticancer agent), physiological effects (such as membrane transport in the kidney or neurotoxicity in the brain), inflammation, and other systemic effects. As it is difficult to measure systemic and physiological effects in vitro, most assays investigate effects at the cellular level. Cells may die by necrosis, apoptosis, or autophagy (self-digestion), or they may cease proliferating (cytostasis) or become terminally differentiated. A cytocidal, or cell killing, effect is required to demonstrate efficacy of an anticancer agent, but verifying a lack of toxicity of a pharmaceutical substance is far more complicated and may require analysis of specific targets, such as alteration of gene transcription, cell signaling, or cell-cell interactions[174]. More intricate assays focusing on metabolic pathway regulation and signaling are necessary to supplement standard cytotoxicity assays, such as the induction of an allergic or inflammatory response. Cytotoxicity tests are therefore still useful, and new drugs, cosmetics, and food additives must be subjected to extensive cytotoxicity testing, usually involving many animal experiments. Motivation for performing as many cytotoxicity tests as possible *in vitro* originates from an economic as well as an ethical perspective. *In vitro* tests are carried out widely in current ethnopharmacological research, partly because of the aforementioned ethical and financial constraints of using animals or animal tissue, and also

because they facilitate bioassay-guided isolation of "active" compounds, that is, those responsible for any activity shown as a result of the bioassay using the total extract. The significantly lower quantity of material required for testing is also an important aspect of using in vitro assays. The selectivity index (SI) is defined as the ratio of cytotoxicity to biological activity. A lack of selectivity in the cytotoxic effect between cancer cell lines and noncancerous cell lines minimizes the prospect that these plants contain compounds which could serve as leads for novel anticancer drugs.[175]

2.9 Types of Cytotoxicity Assay

The nature of the test substance, the expected response, and the target cell are the major factors to be considered in the choice of assay when investigating cytotoxicity[176]. Cell growth or survival, the traditional measure of cytotoxicity, can be measured in various ways, including evaluating the net change in population size, a change in cell mass (total protein or DNA) or metabolic activity (e.g., DNA, RNA, or protein synthesis, reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide). General viability criteria for cell cultures can also be grouped in terms of the indicator and method of evaluation used for examples, (i) Cell differentiation (ii) Cell division (iii) Cell membrane (iv) Lysosomes (v) Mitochondria. According to Niles and coworkers, cytotocicity assays which are the most practical, useful and popular for drug discovery were classified into (i) viability by metabolism reductase activities; (ii) viability by bioluminescent ATP (adenosine triphosphate) assays; and (iii) cytotoxicity by enzymes released into culture medium[177]. Each of these methods has its limitations as well as merits, but the inherent shortcomings of single assays may be mitigated by employing multiparametric methods using various viability and cytotoxicity markers[177]. For practical purposes, most researchers refer to cytotoxicity when conducting what are better classified as viability assays.

2.9.1 Metabolism Reductase Viability Assays

In metabolism and ATP-based viability assays, the scientific premise is that activity is proportional to viable cell number, inferring that reduction in activity after treatment when compared with the control results from cytotoxicity. On the other hand, cytotoxicity assays measure parameters proportional to the degree of cell death. Viability and cytotoxicity measures in most cases are inversely proportional, but differences between the approaches may be emphasized depending on the length of compound exposure[178].

2.9.2 Tetrazolium Assays

In recent years, many assay methods have been developed for assessing cellular viability based on cell metabolism using tetrazolium and resazurin reduction. The first tetrazolium salt to be employed in a multi-well viability assay for mammalian cells was MTT, or 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide[179]. In this assay, the MTT is reduced by metabolically active cells (via mitochondrial and cytosolic enzymes) to a blue-purple formazan product. This product accumulates within cells as it cannot pass through intact cell membranes, but upon removal of the aqueous medium and solubilizing in DMSO (dimethyl sulfoxide) or isopropanol, the colored product can be measured spectrophotometrically. Only viable cells can reduce MTT, so the amount of reduced MTT formazan is proportional to the intensity of color and the greatest cell viability[180]. As a substitute for MTT, XTT (2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-5-carboxanilide-2H-tetrazolium) can be used as it is soluble in most aqueous media. The XTT assay is also suitable for cells in suspension, which is a useful advantage. The assay is based on the extracellular reduction of XTT by NADH (a form of NAD, or nicotinamide adenine

dinucleotide) produced in the mitochondria via trans-plasma membrane electron transport and an electron mediator. Third-generation tetrazoliums with good solubility and better stability in the form of stock solutions are MTS (5-(3-carboxymethoxyphenyl)-2-(4,5-dimethylthiazoly)-3-(4-sulfophenyl) tetrazolium, inner salt) and WST-1 ((4-[3-4-iodophenyl]-2-(4-nitrophenyl)-2H-5-tetrazolio)-1,3-benzene disulfonate)[177]. There have been reports that some plant extracts and compounds, such as flavonoids, have the ability to reduce MTT nonspecifically in the absence of cells. It is therefore recommended to include steps to wash the cells with phosphate-buffered saline between removal of the plant extract or test substance and dissolving of the MTT crystals. Cell-free controls should also be included. The MTT assay is well characterized and widely used and is often a gold standard to which new viability/cytotoxicity assays are compared[177].

2.9.3 Resazurin Assays

Various formulations of resazurin are becoming more commonly used as viability reagents in drug screening. The original patented commercial product, Alamar Blue, contained resazurin and possibly also a mixture of stabilizer salts to minimize spontaneous background reduction in the absence of cells. Resazurin assays are based on the reduction by living cells of the oxidized blue dye to a pink fluorescent resorufin product. The assay also can be monitored by absorbance although there is a slight loss of sensitivity. The reduction of resazurin is believed to be accomplished by reductase or diaphorase-type enzymes from mitochondria and the cytosol. A more recently available resazurin-based colorimetric agent is PrestoBlue, which is flexible in that the results of an assay can be measured visually, using absorbance, or by fluorescence reading of the reduced resorufin product. PrestoBlue is a fast, live assay for measuring cell viability with incubation steps as short as 10 min. It is also sensitive, being able to detect as few as 12 cells per

well[181]. The time of color development following addition to treated cells is, however, dependent upon the metabolic rates of various bacteria and cell lines being tested[181].

2.9.4 Bioluminescent ATP Assays

Measuring the ATP concentration in cells in vitro is widely accepted as a valid marker of the number of cells present[181]. Eukaryotic cells growing in culture have a constant amount of ATP that is regulated to maintain homeostasis. When cells die, they lose the ability to synthesize ATP, and endogenous ATPases remove remaining ATP. The most popular method for measuring ATP is based on the ability of firefly luciferase to generate a luminescent signal. It is purported to be the most sensitive microplate assay for detecting viable cells growing in culture, as it can detect fewer than 10 cells per well, mainly because of the low background luminescence present in biological samples[177].

2.9.5 Enzyme Release-Based Cytotoxicity Assays

A reliable method for evaluating cell death is to detect and quantify the leakage of cellular constituents from affected cells into the culture medium. Lactate dehydrogenase (LDH) is a preferred marker of cell death for in vitro systems[182]. LDH activity can be indirectly measured by subjecting the sample to a coupled enzymatic chemistry reagent containing lactate, NAD 1, diaphorase, and an appropriate redox dye such as resazurin, which yields a change in absorbance or a shift in the fluorescence profile. Other highly sensitive bioluminescent assays for cytotoxicity measure ATP generation through the activity of released ATP cycling enzymes. For example, adenylate kinase (AK) activity can be measured in the culture medium by providing ADP (adenosine diphosphate) to serve as a substrate for the production of ATP. In a similar way,

GAPDH (glyceraldehyde-3-phosphate dehydrogenase) activity can be measured by adding coupled glycolytic pathway enzymes and other constituents necessary for the generation of ATP. As these assays are based on luminescence, they have excellent signal-to-noise ratios from relatively few dead cells, but they are limited in terms of net signal decay owing to limiting reagents and luciferase inactivation[177].

2.9.6 Brine Shrimp Assay

A simple test that has been widely used as an indication of cytotoxicity is based on the lethality of the larvae of the brine shrimp *Artemia salina*[183]. The brine shrimp lethality bioassay is a rapid and inexpensive test requiring a relatively small amount of sample (2-20 mg). This bioassay has been proposed to have a good correlation with cytotoxic activity in some human solid tumors and with pesticidal activity, leading to the discovery of annonaceous acetogenins as a new class of natural pesticides and antitumoral agents[185]. However, the value of a crustacean model in drawing conclusions regarding mammalian cytotoxicity is likely to be less than that of mammalian cell culture models.

2.10 Chemotherapeutic in Cancer Treatment

The most common and effective treatment against most types of malignancies is known as chemotherapy. Chemotherapeutic drugs are well known to target cancer cells that invade other tissues of the body apart from the primary tumor. They are classified based on their mechanism of action such as alkylating agents, platinum agents, antimetabolites, topoisomerase inhibitors, anti-microtubule agents, and antitumor antibiotics[186]. However, despite their beneficial effect on cancer cells, conventional chemotherapeutic agents are sometimes toxic to normal cells and healthy body tissues, leading to dose-dependent side effects and secondary problems for the

patients, suggesting that chemotherapy is a non-selective process[187]. Physical activity, quality of life and mental health are mostly affected by these chemotherapeutic side effects. Many of these drugs can cause toxicity, even at a normal therapeutic dose. For example, myelosuppression is one of the most common toxicities of chemotherapy and leads to the significant clinical problem of thrombocytopenia[188]. In addition, diarrhea, constipation, fatigue nausea and vomiting, peripheral neuropathy, infertility, and cardiotoxicity are some of the most common side effects of chemotherapy[189]. Poorly understood toxicities and signs of alimentary mucositis, a condition that affects the gastrointestinal tract (GI) are diarrhea and constipation[190]. Chemotherapy-induced peripheral neuropathy is associated with depression, ataxia, and insomnia[191]. Cardiotoxicity is induced by the production of free radicals that cause damage to cardiomyocytes, leading to cardiomyopathy and even heart failure[192]. All these side effects remain a major source of concern for both patients and clinicians despite the improved efficacy and advanced efforts of cancer treatment[193]. The limited efficacy of cancer treatment in several forms of cancer along with the adverse side effects and the disadvantages of cancer screening programs led to increasing interest and attention in chemoprevention.

2.11 Phytochemicals in Cancer Treatment

Phytochemicals and derivatives present in plants have been found to be promising options to improve treatment efficiency in cancer patients and decrease adverse reactions. A number of these phytochemicals are naturally occurring biologically active compounds with significant antitumor potential. The development of effective and side-effects free phytochemical based anticancer therapy begins with the testing of natural extracts (from dry/wet plant material) for potential anticancer biological activity followed by purification of active phytochemicals based on bioassay-guided fractionation and testing for *in vitro* and *in vivo* effects.

The use of plants in the treatment of various diseases aliment have been from time immemorial. In the ancient period, the knowledge of selection of right plants, a specific time for their collection, method of drug preparation with their specific use was transferred verbally from one generation to the next generation. The folklore system has documented all parameters about the drugs and their specific uses in the disease conditions. These drugs were prepared as tinctures, teas, powders, poultices, decoctions, and other types of formulations which were the most common methods of drug preparation until 18th century. Unfortunately, none of them could fit into the modern scientific definition of a drug[194].

With advances in organic chemistry and chemical analysis, an analytical investigation of active components of medicinal plants and herbal remedies was pursued in late 18th or early 19th centuries, which opened the doors toward the isolation/purification and characterization of numerous active principles of plants. This increased the pace of drug discovery and led to a miracle innovation in the medical field. The first breakthrough which launched the first generation of drugs came with the isolation of analgesic (pain killing) drugs morphine from the plant *Papaver somniferum*. Later, many well-defined 20th century drugs were derived from plants, including salicylic acid, the precursor of aspirin (*Salix* sp.), cocaine (*Erythroxylum coca*), quinine (*Cinchona officinalis*), digitoxin (*Digitalis purpurea* and *Digitalis lanata)*, and many others with pharmaceutical and clinical potential[195]. Over the period from around 1981 to the end of 2014, more than half of all approved small-molecule drugs originated from natural products, where they served as drug precursors, templates for synthetic modification, and pharmacological probes[196]. This in itself demonstrates the enormous medicinal potential of plants that has been known for thousands of years in traditional medicine.

There are various phytochemicals that have been tested for anti-cancer efficacy at both in vitro and in vivo levels. They possess complementary and overlapping mechanisms to slow down the carcinogenic process by scavenging free radicals, suppressing the survival and proliferation of malignant cells, as well as diminishing invasiveness and angiogenesis of tumors[197]. They exert wide and complex range of actions on different molecular targets and signal transduction pathways including membrane receptors, kinases, downstream tumor-activator or -suppressor proteins, transcriptional factors, microRNAs

2.11.1 Phytochemicals Used in Current Cancer Therapy

The four major classes of clinically used plant-derived anticancer compounds include vinca alkaloids, taxane diterpenoids, camptothecin derivatives, and epipodophyllotoxin. Apart from these phytochemical classes, other plant-derived anticancer agents from different classes such as combretastatins, homoharringtonine (omacetaxine mepesuccinate, cephalotaxine alkaloid), and ingenol mebutate are also used. Poor aqueous solubility and significant toxic side effects still remain the major concern, in this context, several analogues and prodrugs have been synthesized and methods have been devised to enhance aqueous solubility and tumor specificity. Brief description of a few phytochemicals which are used in cancer therapy is given below

2.11.1.1 Vinca Alkaloids

Vinca alkaloids are a subset of drugs obtained from the pink periwinkle plant *Catharanthus roseus* (Apocynaceae). The Vinca alkaloids achieve cytotoxic effects by binding to β-tubulin at a site distinct from that of the taxanes thereby inhibiting polymerization and assembly of microtubules, leading to metaphase arrest and cell death. As the microtubules are associated with several other cellular functions such as maintenance of cell shape, motility, and transport

between organelles, the vinca alkaloids affect both malignant and non-malignant cells in the non-mitotic cell cycle. Vinblastine and vincristine are the two naturally isolated alkaloids that have been used in clinical oncology for almost 50 years. A series of semisynthetic analogues of these two alkaloids have been developed. Vinorelbine and vindesine are the two effective semisynthetic analogues that are approved for clinical use. These agents have been generally included in combination chemotherapy for the treatment of a variety of cancers, including leukemia, Hodgkin and non-Hodgkin lymphomas, advanced testicular carcinoma, breast and lung cancers, and Kaposi's sarcoma. Recently, vinflunine, a second-generation gem-difluoromethylenated derivative of vinorelbine, has been approved for the treatment of second-line transitional cell carcinoma of the urothelium (TCCU)[198].

2.11.1.2 Taxanes

Taxanes represent promising anticancer drugs that were first isolated from the bark of the Yew tree. Taxanes exert an anticancer affect by stabilization of microtubules, resulting in cell cycle arrest and aberrant mitosis. Paclitaxel, a natural product isolated from the bark and leaf of *Taxus brevifolia* and docetaxel, a semi synthetic derivative, is primarily used in breast, ovarian, pancreas, prostate, and lung cancer therapies. A number of semisynthetic derivatives have been developed with improved cytotoxicity in resistant tumors, decreased toxicity, and improved solubility. For example, cabazitaxel a second-generation docetaxel derivative exhibits cytotoxic activity against various docetaxel-resistant tumors with less overall toxicity[197]. An additional characteristic of cabazitaxel is its ability to penetrate the blood–brain barrier *in vivo*, which is not achievable with other taxanes. Some of the paclitaxel analogues such as larotaxel, milataxel, ortataxel, and tesetaxel are currently undergoing clinical evaluation.

2.11.1.3 Camptothecins

Camptothecin is a quinolone alkaloid isolated from the Chinese tree *Camptotheca acuminata*. Camptothecin complexes with type I DNA topoisomerase preventing both cleavage and religation of DNA leading to a DNA double-strand break and cytotoxicity[199]. Irinotecan a derivative of Campothecin is being prescribed for treatment of advanced cancers of the large intestine and rectum. Whereas, Topotecan is approved for the treatment of recurring ovarian, small cell lung cancer, and cervical cancer.

2.11.1.4 Podophyllotoxins

Podophyllotoxin is a natural product isolated from *Podophyllum peltatum* and *Podophyllum emodi (Berberidaceae).* Podophyllotoxin reversibly binds to tubulin, whereas its key derivatives etoposide and teniposide inhibit topoisomerase II, inducing topoisomerase II-mediated DNA cleavage. Moreover, podophyllotoxin also exhibits potential anti-multidrug resistant (MDR) activity against diverse drug-resistant tumor cells. For example, CIP-36, a podophyllotoxin derivative, has been shown to overcome the MDR of adriamycin-resistant human leukemic cell line K562/ADR by regulating the activity of topoisomerase-IIa[197]. However, CIP-36 failed in clinical trials due to lack of efficacy and unacceptable toxicity.

2.11.2 Other Plant-Derived Anticancer Agents

2.11.2.1 Ingenol Mebutate

Ingenol mebutate (IM) is a hydrophobic ester of the diterpene ingenol isolated from common Australian plant *Euphorbia peplus* (Euphorbiaceae). IM is approved for the topical treatment of actinic keratosis, a common skin condition that results from exposure to chronic ultraviolet radiation which can lead to squamous cell carcinoma, if not treated. IM presents two mechanisms of action: at high concentrations (~200–300 μM), it induces rapid induction of cell death in the

treated area and at low concentrations (~0.1 μM) it activates inflammatory response, capable of eliminating the residual cells. Skroza et al. reviewed in more detail the pharmacology, mode of action, pharmacokinetics, dosing, and rout of administration of ingenol mebutate[200].

2.11.2.2 Homoharringtonine

Homoharringtonine (HHT) is a naturally-occurring ester of the alkaloid cephalotaxine isolated from various trees of the *Cephalotaxus* genus (Cephalotaxaceae) and is approved for the treatment of chronic myeloid leukemia. HHT binds to the A-site cleft in the large ribosomal subunit, which affects chain elongation and prevents protein synthesis. A semi-synthetic version of HHT, also known as omacetaxine mepesuccinate, has been reported to be an effective treatment for myelodysplastic syndromes (MDS) and chronic myelomonocytic leukemia (CMML) in patients with resistance and intolerance toward hypomethylating agents such as azacitidine and decitabine[201].

2.11.2.3 Combretastatins

The combretastatins are a family of several cis-stilbenes from Cape bushwillow (*Combretum caffrum,* Combretaceae), a shrub from South Africa. Compounds in the combretastatin class indirectly act on cancer cells by inhibiting tubulin polymerization causing disruption of the tumor endothelial cells lining the tumor vasculature, inducing rapid vascular collapse in solid tumors[202]. Combretastatin A1 and combretastatin A4 are the two naturally isolated compounds. Combretastatin A4 phosphate (CA4P) is a phosphate prodrug of combretastatin A4 which has been designated as an orphan drug by the US Food and Drug Administration (FDA) and is approved for the treatment of a range of thyroid and ovarian cancer.

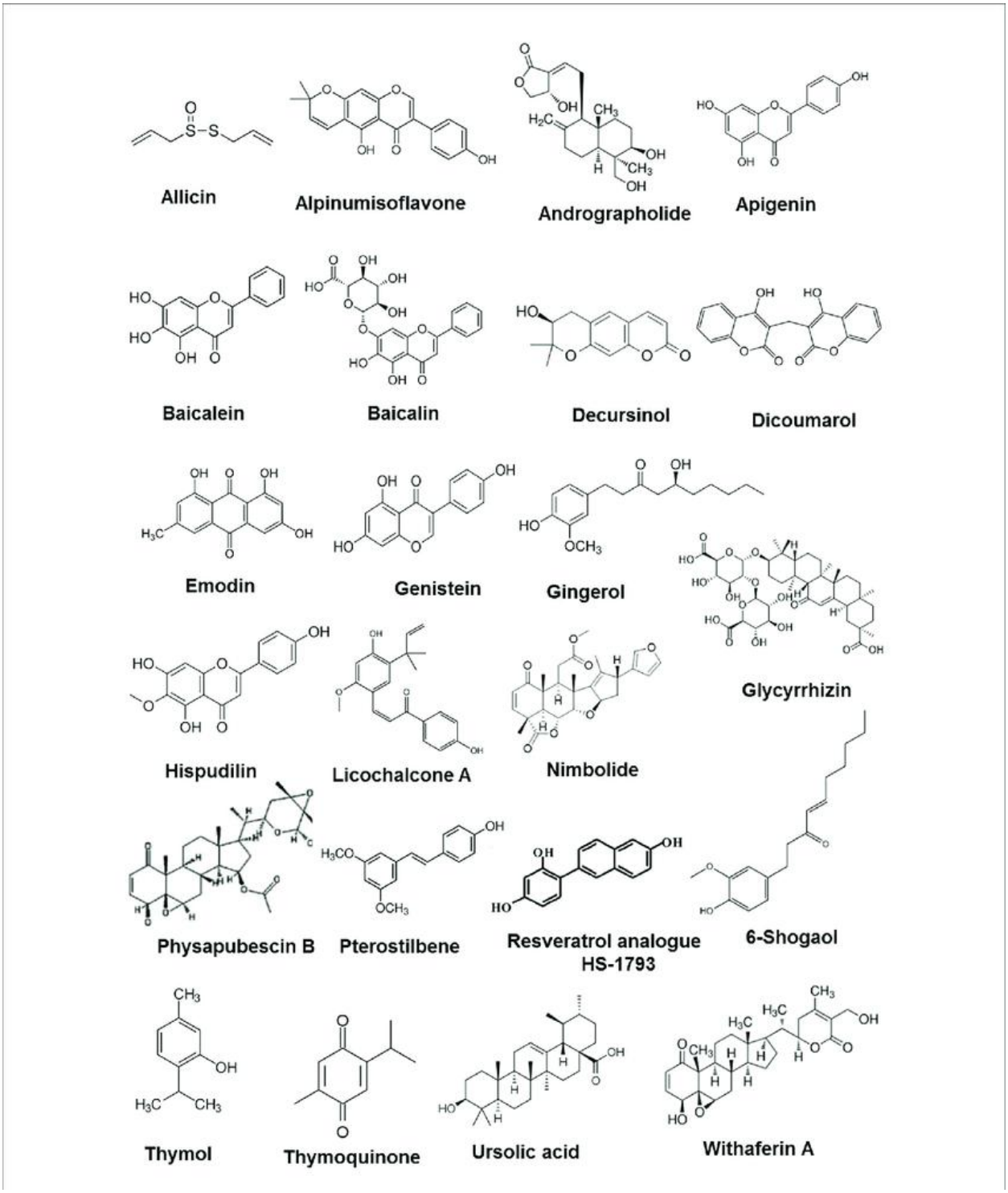


Figure 2: Chemical structures of some anticancer phytochemicals in preclinical trials.

Source: [197]

Endnotes

1. H. Sung, J. Ferlay, R.L. Siegel, M. Laversanne, I. Soerjomataram, A. Jemal, & F. Bray. *Global cancer statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries*, CA: **A Cancer Journal for Clinicians,** 2021. https://doi.org/10.3322/caac.21660.

2. P.A. Cohen, A. Jhingran, A. Oaknin & L. Denny. *Cervical Cancer.* **Lancet,** 2019. https://doi.org/10.1016/S0140-6736(18)32470-X

3. M. Arbyn, E. Weiderpass, L. Bruni, S. de Sanjos´e, M. Saraiya, J. Ferlay & F. Bray, *Estimates of Incidence and Mortality of Cervical Cancer in 2018*: *A Worldwide Analysis*. **Lancet Global Health**, 2020. https://doi.org/10.1016/S2214-109X(19)30482-6.

4. K. Baskran, P.K. Kumar, K. Santha & Sivakamasundari II. *Cofactors and their Association with Cancer of the Uterine Cervix in Women Infected with High-risk Human Papillomavirus in South India*. **Asian Pac J Cancer Prev.** 20(11), 2019, 3415-3419. Doi: 10.31557/APJCP.2019.20.11.3415.

5. C.A. Johnson, D. James, A. Marzan, & M. Armaos. *Cervical Cancer: An Overview of Pathophysiology and Management.* **Semin. Oncol. Nurs,** 2019. https://doi.org/ 10.1016/j.soncn.2019.02.003.

6. Tewari, K.S. *Therapeutic Vaccination Using HPV 16 E7 to Eradicate CIN3.* **Journal of gynecologic oncology.** 30, 2019, 6.

7. K. Kombe, J. Arnaud, L. Bofeng, Z. Ayesha, M.M. Hylemariam, B. Guy-Armel, Z. Ying, & J. Tengchuan. *Epidemiology and Burden of Human Papillomavirus and Related Diseases, Molecular Pathogenesis, and Vaccine Evaluation*. **Frontiers in public health.** 8, 2021, 552028.

8. D. Feng, W. Sitian, Z. Y. Jun Chen, L. Yeshe, W. Hongbo, & Z. Xiaowu. *Human Papillomavirus Prevalence and Genotype Distribution Landscapes in Shannan City, Tibet Tibetan Autonomous Region, China.* **Virology Journal.** 19(1), 2022, 1-7.

9. J. Velloza, C.G. Kemp, F.M Aunon, M.K. Ramaiya, E. Creegan & J.M. Simoni. *Alcohol Use and Antiretroviral Therapy Non-Adherence Among Adults Living with HIV/AIDS in Sub-Saharan Africa: A Systematic Review and Meta-analysis.* **AIDS and Behavior**. 24, 2020, 1727-42.

10. G. Olorunfemi, N. Ndlovu, G. Masukume, A. Chikandiwa, P.T. Pisa & E. Singh. *Temporal Trends in the Epidemiology of Cervical Cancer in South Africa (1994–2012)*, **Int. J. Cancer.** 143, 2018. https://doi.org/10.1002/ijc.31610.

11. L., Gui, S. Monisha, T. Nicholas & V.B. Ruanne. *HIV-positive Women Have Higher Risk of Human Papilloma Virus Infection, Precancerous Lesions, and Cervical Cancer*. **Aids** 32(6), 2018, 795-808.

12. M. Lieber, A. Omara, S. Kathryn, M. Adrienne, Christine Du Preez, & M. B. Ann. *Cervical Cancer Screening in HIV-positive Farmers in South Africa: Mixed-method Assessment.* **Annals of Global Health** 85(1), 2019.

13. I. Peate. *Cervical Cancer.* **British Journal of Healthcare Assistants** 13(3), 2019, 110-116.

14. F. Yarandi, A. Mahdi, R. Sara, & S. Elham. *Endocervical Adenocarcinoma Implantation in Episiotomy Scar: A Case Report and Review of the Literature***. Journal of Medical Case Reports** 17(1), 2023, 1-6.

15. S.D. Balasubramaniam, V. Balakrishnan, C.E. Oon & G. Kaur. *Key Molecular Events in Cervical Cancer Developmen*t. **Medicinal (Lithuania)** 2019, https://doi.org/10.3390/ medicina55070384.

16. R. Hull, M. Mbele, T. Makhafola, C. Hicks, S.M. Wang, R.M. Reis, R. Mehrotra, Z. Mkhize-Kwitshana, G. Kibiki, D.O. Bates & Z. Dlamini. *Cervical Cancer in Low and Middle-Income Countries*. **Oncology letters**. 20(3), 2020, 2058-2074.

17. G. Gao, W. Jintu, L. Jan, N.M. Kasperbauer, F. T. Tombers, H. Gou, Y. Zhao, Z. Bao, & D. I. Smith. *Whole Genome Sequencing Reveals Complexity in both HPV Sequences Present and HPV Integrations in HPV-positive Oropharyngeal Squamous Cell Carcinomas*. **BMC cancer.** 19, 2019, 1-15.

18. M. Ibragimova., M. Tsyganov, O. Shpileva, O. Churuksaeva, V. Bychkov, L. Kolomiets & N. Litviakov. *HPV Status and its Genomic Integration Affect Survival of Patients with Cervical Cancer.* **Neoplasma.** 65(3), 2018, 441-448.

19. H. zur Hausen. *Papillomaviruses and Cancer: From Basic Studies to Clinical Application*, **Nat. Rev. Cancer** 2:2002, 342–350. https://doi.org/10.1038/ nrc798.

20. J. A. Scarth, R. Molly, Patterson, L.M. Ethan & M. A. Macdonald. *The Human Papillomavirus Oncoproteins: A Review of the Host Pathways Targeted on the Road to Transformation***. The Journal of general virology.** 102(3), 2021.

21. C.A. Burmeister, S.F. Khan, G. Schäfer, N. Mbatani, T. Adams, J. Moodley & S. Prince. *Cervical Cancer Therapies: Current Challenges and Future Perspectives*. **Tumour Virus Research**. 2022, 200238.

22. A. Pal & R. Kundu. *Human Papillomavirus E6 and E7: The Cervical Cancer Hallmarks and Targets for Therapy*. **Front. Microbiol**. 10, 2020. https://doi.org/10.3389/ fmicb.2019.03116

23. N.S.L. Yeo-Teh, Y. Ito, & S. Jha. *High-Risk Human Papillomaviral Oncogenes E6 And E7 Target Key Cellular Pathways to Achieve Oncogenesis.* **Int. J. Mol. Sci.** 2018. https://doi.org/10.3390/ijms19061706.

24. M.A. Oyervides-Muñoz, A.P. Antonio, F.R. Hazyadee, S. G. Gabriela, R.F. Oscar, T. Víctor, A.B.S. Hugo, & L.G.R. María Lourdes. *Understanding The HPV Integration and Its Progression to Cervical Cancer.* **Infection, Genetics and Evolution**. 61, 2018, 134-144.

25. J.D. Wright, K. Matsuo, Y. Huang, A. I Tergas, J.Y. Hou, F. Khoury-Collado, C.M.S. Clair, C.V. Ananth, A. I Neugut, & D.L. Hershman. *Prognostic Performance of the 2018 International Federation of Gynecology and Obstetrics Cervical Cancer Staging Guidelines.* **Obstetrics and Gynecology**. 134(1), 2019, 49.

26. H. Li, Y. Pang & X. Cheng, *Surgery of Primary Sites for Stage IVB Cervical Cancer Patients Receiving Chemoradiotherapy: A Population-Based Study*, **J. Gynecol. Oncol.** 31, 2020. https://doi.org/10.3802/Jgo.2020.31.E8.

27. S. Gupta, P. Kumar & B.C. Das. *HPV: Molecular Pathways and Targets*. **Curr. Probl. Cancer.** 2018, https://doi.org/10.1016/j.currproblcancer.2018.03.003.

28. N. Bhatla, D. Aoki, D.N. Sharma & R. Sankaranarayanan. *Cancer of the Cervix Uteri*. **Int. J. Gynecol. Obstet.** 143, 2018, 22–36. https://doi.org/10.1002/ijgo.12611.

29. W.-J. Koh, N.R. Abu-Rustum, S. Bean, K. Bradley, S.M. Campos, K.R. Cho, H. S. Chon, C. Chu, R. Clark, D. Cohn, M.A. Crispens, S. Damast, O. Dorigo, P.J. Eifel, C.M. Fisher, P. Frederick, D.K. Gaffney, E. Han, W.K. Huh, J.R. Lurain, A. Mariani, D. Mutch, C. Nagel, L. Nekhlyudov, A.N. Fader, S.W. Remmenga, R.K. Reynolds, T. Tillmanns, S. Ueda, E. Wyse, C.M. Yashar, N.R. Mcmillian & J.L. Scavone. *Cervical Cancer, Version 3.2019, NCCN Clinical Practice Guidelines in Oncology*. **J. Natl. Compr. Cancer Netw**. 17, 2019, https://doi.org/10.6004/ Jnccn.2019.0001.

30. P.T. Ramirez, M. Frumovitz, R. Pareja, A. Lopez, M. Vieira, R. Ribeiro, A. Buda, X. Yan, Y. Shuzhong, N. Chetty, D. Isla, M. Tamura, T. Zhu, K.P. Robledo, V. Gebski, R. Asher, V. Behan, J.L. Nicklin, R.L. Coleman & A. Obermair. *Minimally Invasive Versus*

Abdominal Radical Hysterectomy for Cervical Cancer. **N. Engl. J. Med.** 379, 2018. doi.org/10.1056/Nejmoa1806395.

31. C.A. Johnson, D. James, A. Marzan & M. Armaos. *Cervical Cancer: An Overview of Pathophysiology and Management*. **Semin. Oncol. Nurs**. 2019. https://doi.org/ 10.1016/J.Soncn.2019.02.003.

32. K. Wang, & J.E. Tepper. *Radiation Therapy-Associated Toxicity: Etiology, Management, and Prevention*. **CA, Cancer J. Clin**. 71, 2021. https://doi.org/ 10.3322/caac.21689.

33. E. Rahakbauw & H. Winarto. *Radiotherapy Response and Related Clinicopathological Factors of Patients with Cervical Cancer,* In: **Journal of Physics**: **Conference Series**, 2018. https://doi.org/10.1088/1742-6596/1073/3/032040.

34. S. Ghosh, *Cisplatin: The First Metal Based Anticancer Drug*. **Bioorganic Chemistry**. 88, 2019, 102925.

35. H. Zhu, H. Luo, W. Zhang, Z. Shen, X. Hu & X. Zhu. *Molecular Mechanisms of Cisplatin Resistance in Cervical Cancer*. **Drug Des. Dev. Ther**. 2016. https://doi. org/10.2147/dddt. s106412.

36. H. Hirte, E.B. Kennedy, L. Elit & M.F.K. Fung. *Systemic Therapy for Recurrent, Persistent, or Metastatic Cervical Cancer: A Clinical Practice Guideline*. **Curr. Oncol**. 2015. https://doi.org/10.3747/Co.22.2447

37. J.Y. Moon, I.C. Song, Y.B. Ko, & H.J. Lee. *The Combination of Cisplatin and Topotecan as a Second-Line Treatment for Patients with Advanced/Recurrent Uterine Cervix Cancer*. **Medicine.** 97(14), 2018.

38. A.M. Heeren, I.F. Van Luijk, J. Lakeman, N. Pocorni, J. Kole, R.X. De Menezes, G.G. Kenter, Bosse, T., De Kroon, C.D. & E.S. Jordanova, *Neoadjuvant Cisplatin and Paclitaxel Modulate Tumor-Infiltrating T Cells in Patients with Cervical Cancer*. **Cancer Immunology, Immunotherapy.** 68, 2019, 1759-1767.

39. R. Rieu, & G. Eminowicz. *Chemotherapy for Cervical Cancer*. **Uterine Cervical Cancer**: **Clinical and Therapeutic Perspectives**, 2019, 215-231.

40. N. Horeweg, P. Mittal, P.L. Gradowska, I. Boere, R.A. Nout, & S. Chopra. *A Systematic Review and Meta-Analysis of Adjuvant Chemotherapy After Chemoradiation for Locally Advanced Cervical Cancer.* **Critical Reviews in Oncology/Hematology**, 2022, 103638.

41. S. Mailankody, M. Dhanushkodi, T.S. Ganesan, V. Radhakrishnan, V. Christopher, S. Ganesharajah & T.G. Sagar. *Recurrent Cervical Cancer Treated with Palliative Chemotherapy: Real-World Outcome*. **Ecancermedicalscience** 14, 2020. https:// doi.org/10.3332/ecancer.2020.1122.

42. Bukowski, K., Kciuk, M. & R. Kontek. *Mechanisms of Multidrug Resistance in Cancer Chemotherapy*. **International Journal of Molecular Sciences**. 21(9), 2020, 3233.

43. Z. Momenimovahed & H. Salehiniya. *Epidemiological Characteristics of and Risk Factors for Breast Cancer in the World*. Breast Cancer: **Targets and Therapy**. 2019, 151-164.

44. F. Bray, J. Ferlay, I. Soerjomataram, R.L. Siegel, L. A. Torre & A. Jemal. *Global Cancer Statistics 2018: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers In 185 Countries.* **CA: A Cancer Journal for Clinicians.** 68(6), 2018, 394-424.

45. C. Costantino, D. Alba, L. Cimino, A. Conforto, &W. Mazzucco *The Role of Vaccination and Screening in Limiting the Worldwide Disease Burden of Preventable Female Cancers*: A Review. **Women**, 1(1), 2020, 2.

46. Y. Feng, M. Spezia, S. Huang, C. Yuan, Z. Zeng, L. Zhang, X. Ji, W. Liu, B. Huang, W. Luo & B. Liu. *Breast Cancer Development and Progression: Risk Factors, Cancer Stem Cells, Signaling Pathways, Genomics, and Molecular Pathogenesis*. **Genes & Diseases**.5(2), 2018, 77-106.

47. C.Wendt & S. Margolin, *Identifying Breast Cancer Susceptibility Genes–A Review 0f The Genetic Background in Familial Breast Cancer.* **Acta Oncologica**, 58(2), 2019, 135-146.

48. V. Giannakeas & A.N. Steven, *The Expected Benefit of Preventive Mastectomy on Breast Cancer Incidence and Mortality in BRCA Mutation Carriers, By Age at Mastectomy*. **Breast Cancer Research and Treatment**: 167, 2018, 263-267

49. A.F. Patenaude, *Cancer Susceptibility Testing: Risks, Benefits and Personal Beliefs*. In **The Genetic Testing of Children.** 2020, 145-156. Garland Science.

50. M.E. Barnard, C. E Boeke & R.M. Tamimi. *Established Breast Cancer Risk Factors and Risk of Intrinsic Tumor Subtypes*. **Biochim Biophys Acta**. 1856(1), 2015, 73-85.

51. A.N. Giaquinto, K.D. Miller, K.Y. Tossas, R.A. Winn, A. Jemal & R.L. Siegel. *Cancer Statistics for African American/Black People 2022.* **CA: A Cancer Journal for Clinicians**, *72*(3),2022, 202-229.

52. V.G. Vogel*, Epidemiology of Breast Cancer*. **In The Breast**, 2018, 207-218. Elsevier.

53. K. Allison & K. Mooney. *The Spectrum of Risk Lesions in Breast Pathology: Risk Factors or Cancer Precursors?* Atypical Breast Proliferative Lesions and Benign Breast Disease. 2018:1-9.

54. D.L. Monticciolo, M. S. Newell, L. Moy, B. Niell, B, Monsees & E.A. Sickles. *Breast Cancer Screening in Women at Higher-Than-Average Risk: Recommendations from the ACR*. **Journal of the American College of Radiology**. 15(3), 2018, 408-14.

55. A. Tiwari M. Singh & B. Sahu. *Risk Factors for Breast Cancer.* **International Journal of Nursing Education and Research.** 10(3), 2022, 276-82.

56. Z. Momenimovahed & A.H. Salehiniya. *Epidemiological Characteristics of and Risk Factors for Breast Cancer in The World. Breast Cancer:* **Targets and Therapy**. 10. 2019, 151-64.

57. T. Fait. *Menopause Hormone Therapy: Latest Developments and Clinical Practice.* **Drugs In Context**. 8, 2019.

58. X.R Yang, M.E. Sherman, D.L. Rimm, J. Lissowska, L.A Brinton, B. Peplonska, S.M. Hewitt, W.F Anderson, N. Szeszenia-Dabrowska, A. Bardin-Mikolajczak & W. Zatonski. *Differences in Risk Factors for Breast Cancer Molecular Subtypes in A Population-Based Study*. **Cancer Epidemiol Biomark** Prev. 16(3), 2007.

59. K.L. Britt, J. Cuzick, & K.A. Phillips. *Key Steps for Effective Breast Cancer Prevention.* Nature **Reviews Cancer**. 20(8), 2020, 417-36.

60. C.Todd, A.M. Lagman-Bartolome & C. Lay. *Women and Migraine: The Role of Hormones.* **Current Neurology and Neuroscience Reports**. 18, 2018, 1-6.

61. P. De Cicco, M.V. Catani, V. Gasperi, M. Sibilano, M. Quaglietta & I. Savini. *Nutrition and Breast Cancer: A Literature Review on Prevention, Treatment and Recurrence*. **Nutrients**. 11(7), 2019, 1514.

62. F.J. Couch, K. L. Nathanson & K, Offit. *Two Decades After BRCA: Setting Paradigms in Personalized Cancer Care and Prevention.* **Science**. 343(6178), 2014, 1466-1470.

63. A. Rathore, S. Ranjan, S. Vishwanath, A. Pathak, A. Dubey & P. Nikhil. *BRCA 1 and 2 Mutations in Carcinoma Breast: An Indian Study.* **Clinical Cancer Investigation Journal.** 7(3), 2018, 97-.97.

64. C.C. Chen, W. Feng, P.X. Lim, E.M. Kass & M. Jasin. *Homology-Directed Repair and The Role of BRCA1, BRCA2 and Related Proteins in Genome Integrity and Cancer*". **Annual Review of Cancer Biology**. 2, 2018, 313-36.

65. K.B. Kuchenbaecker, J. L. Hopper, D.R. Barnes, K.A. Phillips, T.M. Mooij, M.J. Roos-Blom, S. Jervis, F.E. Van Leeuwen, R.L. Milne, N. Andrieu & D.E. Goldgar. *Risks of Breast, Ovarian and Contralateral Breast Cancer for BRCA1 and BRCA2 Mutation Carriers.* **JAMA**, 317(23), 2017, 2402-2416.

66. K.A. Metcalfe, J. Lubinski, J. Gronwald, T. Huzarski, J. Mccuaig, H.T. Lynch, B. Karlan, W.D. Foulkes, C.F. Singer, S. L. Neuhausen & L. Senter. *The Risk of Breast Cancer in BRCA1 and BRCA2 Mutation Carriers Without a First-Degree Relative with Breast Cancer.* **Clin Genet**. 93(5), 2018, 1063-1068.

67. K.P. Ko, S.J. Kim, T. Huzarski, J. Gronwald, J. Lubinski, H.T. Lynch, S. Armel, S.K. Park, B. Karlan, C.F. Singer & S.L. Neuhausen. *The Association Between Smoking and Cancer Incidence in BRCA1 and BRCA2 Mutation Carriers.* **Int J Cancer**. 142(11), 2018, 2263-2272.

68. E.R. Copson, T.C. Maishman, W.J. Tapper, R.I. Cutress, S. Greville-Heygate, D.G. Altman, B. Eccles, S. Gerty, L.T. Durcan, L. Jones & D.G. Evans. *Germline BRCA Mutation and Outcome in Young-Onset Breast Cancer (POSH): A Prospective Cohort Study*. **Lancet Oncol.** 19(2), 2018, 169-180.

69. T.R. Rebbeck, T.M. Friebel, E. Friedman, U. Hamann, D. Huo, A. Kwong, E. Olah, O.I. Olopade, A.R. Solano, S.H. Teo & M. Thomassen. *Mutational Spectrum in A Worldwide Study of 29,700 Families with BRCA1 or BRCA2 Mutations.* **Hum Mutat**. 39(5), 2018, 593.

70. R. Koron. *DNA Signatures as A Predictor of Breast Cancer Risk.* **The University of Liverpool (United Kingdom),** 2018.

71. R.C. Brianese, K.D.D.M. Nakamura, F.G.D.S.R.D. Almeida, R.F. Ramalho, B.D.D.F. Barros, E.N.E. Ferreira, M.N.D.C. Formiga, V. P. De Andrade, V.C.C. De Lima & D. M. Carraro. *BRCA1 Deficiency Is a Recurrent Event in Early-Onset Triple-Negative Breast Cancer: A Comprehensive Analysis of Germline Mutations and Somatic Promoter Methylation".* **Breast Cancer Res Treat**. 167(3), 2018, 803-814.

72. G. Molyneux, F.C. Geyer, F.A. Magnay, A. Mccarthy, H. Kendrick, R. Natrajan, A. Mackay, A. Grigoriadis, A. Tutt, A. Ashworth & J.S. Reis-Filho. *BRCA1 Basal-Like Breast Cancers Originate from Luminal Epithelial Progenitors and Not from Basal Stem Cells*. **Cell Stem Cell**. 7(3), 2010, 403-417

73. F.M. Elmenier D.S. Lasheen & K.A. Abouzid. *Phosphatidylinositol 3 Kinase (PI3K) Inhibitors as New Weapon to Combat Cancer***. European Journal of Medicinal Chemistry.** 183, 2019, 111718**.**

74. T. Mukohara. *PI3K Mutations in Breast Cancer: Prognostic and Therapeutic Implications.* **Breast Cancer (Dove Med Press).** 7, 2015, 111-123.

75. F. Mundt, S. Rajput, S. Li, K.V. Ruggles, A.D. Mooradian, P. Mertins, M.A. Gillette MA, K. Krug, Z. Guo, J. Hoog & P. Erdmann-Gilmore. *Mass Spectrometry–Based Proteomics Reveals Potential Roles of NEK9 and MAP2K4 in Resistance to PI3K Inhibition in Triple-Negative Breast Cancersproteomic Profiling of PI3K Inhibitor Resistance*. **Cancer Research.** 78(10), 2018, 2732-46.

76. D. Miricescu, A. Totan, Stanescu-Spinu II, S.C. Badoiu, C. Stefani & M. Greabu. *PI3K/AKT/Mtor Signaling Pathway in Breast Cancer: From Molecular Landscape to Clinical Aspects*. **International Journal of Molecular Sciences.** 22(1), 2020, 173.

77. K. Stemke-Hale, A.M. Gonzalez-Angulo, A. Lluch, R.M. Neve, W.L. Kuo, M. Davies, M. Carey, Z. Hu, Y. Guan, A. Sahin & W.F. Symmans, W.F. *An Integrative Genomic and Proteomic Analysis of PIK3CA, PTEN, And AKT Mutations in Breast Cancer.* **Cancer Res**, 68(15), 2008, 6084-6091.

78. J.N. Mills, A.C. Rutkovsky & A. Giordano. *Mechanisms of Resistance in Estrogen Receptor Positive Breast Cancer: Overcoming Resistance to Tamoxifen/Aromatase Inhibitors*. **Current Opinion in Pharmacology** 41, 2018, 59-65.

79. W. Yang, S.R. Hosford, L.M. Dillon, K. Shee, S.C. Liu, J.R. Bean, L. Salphati, J. Pang, X. Zhang, M.A. Nannini & E. Demidenko, E. *Strategically Timing Inhibition of Phosphatidylinositol 3-Kinase to Maximize Therapeutic Index in Estrogen Receptor Alpha-Positive, PIK3CA-Mutant Breast Cancer*. **Clin Cancer Res**. 22(9), 2016, 2250-2260.

80. F. Bray, J. Ferlay, I. Soerjomataram, R. L. Siegel, L.A. Torre & A. Jemal, *Global Cancer Statistics 2018: GLOBOCAN Estimates of Incidence and Mortality Worldwide For 36 Cancers In 185 Countries*. **CA Cancer J Clin**. 68, 2018, 394–424.

81. American Cancer Society. *Global Cancer Facts & Figures*. 4th Edition. Atlanta, GA: **American Cancer Society**, 2018.

82. I. Yambayev, T.B. Sullivan, K. Suzuki, Q. Zhao, S.E. Higgins, O.H. Yilmaz, V.R. Litle, P. Moreira, E.L. Servais, C.T. Stock & S.M. Quadri. *Pulmonary Adenocarcinomas of Low Malignant Potential: Proposed Criteria to Expand the Spectrum Beyond Adenocarcinoma In Situ And Minimally Invasive Adenocarcinoma*. **The American Journal of Surgical Pathology**.45 (4), 2021, 567-76.

83. M.B. Schabath & M.L. Cote, *Cancer Progress and Priorities: Lung Cancer*. **Cancer Epidemiology, Biomarkers & Prevention.** *28*(10), 2019, 1563-1579.

84. P.T. Nguyen, K. Katanoda, E. Saito, M. Hori, T. Nakayama &T. Matsuda, *Trends in Lung Cancer Incidence by Gender, Histological Type and Stage at Diagnosis in Japan, 1993 To 2015: A Multiple Imputation Approach.* **International Journal of Cancer**, 151(1), 2022, 20-32.

85. Q.G. Zhu, S.M. Zhang, X.X. Ding, He B & H.Q. Zhang, *Driver Genes in Non-Small Cell Lung Cancer: Characteristics, Detection Methods, And Targeted Thera-Pies*. **Oncotarget**. 8, 2017, 57680–92.

86. A. Ghaderi, P. Nasehghafoori, M. Rasouli-Azad, M. Sehat, F. Mehrzad, M. Nekuei, J. Aaseth H.R. Banafshe & O. Mehrpour. *Examining of Thallium in Cigarette Smokers.* **Biological Trace Element Research**.182, 2018, 224-30.

87. American Cancer Society. *Cancer Facts & Figures 2019*. **Atlanta, GA: American Cancer Society;** 2019.

88. M. Markaki, I. Tsamardinos, A. Langhammer, V. Lagani, K. Hveem & O.D. Roe, *A Validated Clinical Risk Prediction Model for Lung Cancer in Smokers of All Ages and Exposure Types: A HUNT Study*. **Ebiomedicine**, 31, 2018, 36–46.

89. S.S. Hecht, & D.K Hatsukami, *Smokeless Tobacco and Cigarette Smoking: Chemical Mechanisms and Cancer Prevention*. **Nature Reviews Cancer**, *22*(3), 2022, 143-155.

90. A.S. Kim, H.J. Ko, J. H. Kwon & J.M. Lee. *Exposure To Secondhand Smoke and Risk Of Cancer In Never Smokers: A Meta-Analysis Of Epidemiologic Studies.* **Int J Environ Res Public Health**. 15, 2018, 1981.

91. J. Elío, Q. Crowley, R. Scanlon, J. Hodgson & L. Zgaga, *Estimation of Residential Radon Exposure and Definition of Radon Priority Areas Based on Expected Lung Cancer Incidence*. **Environment International**, 114, 2018, 69-76.

92. K.C. Thandra, A. Barsouk, K. Saginala, J.S. Aluru & A. Barsouk, *Epidemiology of Lung Cancer*. **Contemporary Oncology/Współczesna Onkologia**, *25*(1), 2021, 45-52.

93. T. Sofilić, A.B. Hadžipašić, & U.S. Šimić, *Experiences in Asbestos-Containing Waste Management in The Republic of Croatia*. **Sigurnost**. 64(1), 2022, 47-62.

94. D. Ospina, V.E. Villegas, G. Rodríguez-Leguizamón & M. Rondón-Lagos, *Analyzing Biological and Molecular Characteristics and Genomic Damage Induced by Exposure to Asbestos*. **Cancer Management and Research,** 11, 2019, 4997.

95. V. Jain & A. Bhardwaj. *Pneumonia, Pathology*. In: Statpearls [Internet]. Treasure Island, FL: **Statpearls Publishing,** 2018.

96. H. Zhu & S. Zhang. *Body Mass Index and Lung Cancer Risk in Never Smokers: A Meta-Analysis*. **BMC Cancer**. 18, 2018, 635.

97. L.A. Cannon-Albright, C.C. Teerlink, J. Stevens, J.C. Facelli, S.R. Carr, K. Allen-Brady, S. Puri, J.E. Bailey-Wilson & A.M. Musolf. *Genetic Epidemiology of Lung Cancer Consortium* & A.M. Musolf. *A Rare FGF5 Candidate Variant (Rs112475347) For Predisposition to Non-Squamous, Non-Small-Cell Lung Cancer.* **International Journal of Cancer**.2023.

98. C.M. Lewis & E. Vassos. *Polygenic Risk Scores: From Research Tools to Clinical Instruments*. **Genome Medicine**, 12(1), 2020, 1-11.

99. J. Jassem, *Tobacco Smoking After Diagnosis of Cancer: Clinical Aspects*. **Translational Lung Cancer Research**: 8(Suppl 1), 2019, S50.

100. M.B. Schabath. *Risk Models to Select High Risk Candidates for Lung Cancer Screening*. **Ann Transl Med.** 6, 2018, 65.

101. P. Mirabelli, C. Luigi, & S. Marco. *Cancer Cell Lines Are Useful Model Systems for Medical Research.* **Cancers** 11(8), 2019, 1098.

102. V. Sailer, A. Gunhild, D. Stefan, K Jutta, L Verena, M Eric & O Anne. *Experimental In Vitro, Ex Vivo and In Vivo Models in Prostate Cancer Research.* **Nature Reviews Urology**. 2022,1-21.

103. L. Zhou, Z. Caiqin, Z. Yongbin, & S. Changhong. *Application of Organoid Models in Prostate Cancer Research.* **Frontiers in Oncology** 11, 2021, 736431.

104. W.G. Dirks. *Ethical Challenges Using Human Tumor Cell Lines in Cancer Research*. **In *Ethical Challenges in Cancer Diagnosis and Therapy***. 2021, 39-46.

105. G.P. Risbridger, R. Toivanen & R.A Taylor. *Preclinical Models of Prostate Cancer: Patient-Derived Xenografts, Organoids and Other Explant Models*. **Cold Spring Harbor Perspectives in Medicine.** 8(8), 2018, A030536.

106. L.J Gordon, A.B Mark, & M.R Melissa. *Cell-Based Methods for Determination of Efficacy for Candidate Therapeutics in The Clinical Management of Cancer.* **Diseases** 6(4), 2018, 85.

107. S.W. Kim, S.J Kim, R.R Langley & I. J Fidler. *Modulation of the Cancer Cell Transcriptome by Culture Media Formulations and Cell Density***. Int. J. Oncol**. 46, 2015, 2067–2075.

108. S.L Arodin, W.L Marita, J Rim, D. Olof, & B. Mikael. *The Cell Culture Medium Affects Growth, Phenotype Expression and the Response to Selenium Cytotoxicity in A549 and Hepg2 Cells.* **Antioxidants** 8(5), 2019, 130.

109. C Pascoe, M Todd, W David, & L Nathan. *Ureteral Intramural Metastatic Deposit of Prostate Cancer with Ureteric Obstruction***. Case Reports** 2018: Bcr-2017.

110. A.S. Gudipaty, M.C. Christopher, R. Jody, & J.M. Denise. *Unconventional Ways to Live and Die: Cell Death and Survival in Development, Homeostasis, And Disease.* **Annual Review of Cell and Developmental Biology.** 34, 2018, 311.

111. A. Rossin, M. Giorgia, & H. Anne-Odile. *TRAIL and Fasl Functions in Cancer and Autoimmune Diseases: Towards an Increasing Complexity.* **Cancers** 11(5), 2019, 639.

112. Y. Lee, & I. Michael. *Focus: Death: After-Death Functions of Cell Death.* **The Yale Journal of Biology and Medicine** 92, 4: 2019, 687.

113. R. Singh, L. Anthony, & S. Kristopher. *Regulation of Apoptosis in Health and Disease: The Balancing Act of BCL-2 Family Proteins.* **Nature Reviews Molecular Cell Biology** 20(3), 2019, 175-193.

114. M.P Durand, M.F Barreto, & E.M Richard. *Focus: Death: Cell Death in Evolutionary Transitions in Individuality.* **The Yale Journal of Biology and Medicine** 92 (4), 2019, 651.

115. M. Zhou, A. Monowar, & W. Ping. *Damage-Associated Molecular Patterns as Double-Edged Swords in Sepsis.* **Antioxidants & Redox Signaling** 35(15), 2021, 1308-1323.

116. D.R Green, & F. Llambi. *Cell Death Signaling.* **Cold Spring Harb Perspect Biol**. 7(12), 2015, A006080. https://doi.org/10.1101/ Cshperspect.A006080.

117. Q. Zia, A. Azhar, N. Hassan, P. Jain, M. Singh, M.A. Mirza, A. Ali, S. Parveen, S. Hasan, A.S. Alothaim & A. Jamal. *Cell Death: A Molecular Perspective*. **Current Molecular Biology Reports,** 2021, 1-26.

118. S. Nagata. *Apoptosis and Clearance of Apoptotic Cells.* **Annual Review of Immunology** 36 2018, 489-517.

119. M. Condello, P. Evelin, C. Michele, & M. Stefania. *Targeting Autophagy to Overcome Human Diseases.* **International Journal of Molecular Sciences** 20, 3, 2019: 725.

120. N Khalid, & A Mahzad. *Necrosis*. Study Guide from **Statpearls Publishing, Treasure Island** (FL). 4, 2020.

121. L Galluzzi, I Vitale, S.A Aaronson. *Molecular Mechanisms of Cell Death: Recommendations of The Nomenclature Committee on Cell Death 2018.* **Cell Death Difer**. 25(3), 2018, 486–541. https://doi.org/10.1038/S41418-017-0012-4.

122. World Health Organization. *WHO Global Report on Traditional and Complementary Medicine 2019*. World Health Organization, 2019.

123. A.E. Sofowora. *Medicinal Plants and Traditional Medicines in Africa. 2nd Ed. Ibadan, Nigeria*: Spectrum Books. 1993, 289.

124. World Health Organization (WHO). *Research Guidelines for Evaluating the Safety and Efficacy of Herbal Medicines.* Manila: WHO Regional Office for The Western Pacific; 1993.

125. M.M. Iwu, A.R. Duncan & C.O Okunji. *New Antimicrobials of Plant Origin.* In: Janick J, Editor. **Perspectives in New Crops and New Uses.** Alexandria, VA: ASHS Press; 1999, 457-62.

126. S. A Rankoana. *Indigenous Plant-Derived Medical Applications for Primary Health Care.* **International Journal of Social Science Research and Review,** 5(11), 2022, 163-168. 2022.https://doi.org/10.47814/Ijssrr.V5i11.719

127. World Health Organization (WHO). In Progress Report by The Director General, Document No. A44/20. Geneva: World Health Organization; 1991. *Toxic Plants Used in African Traditional Medicine* 171

128. J.M Lyndy, M.F Ibukun, T.K Edward, & O.A Adeyemi, *Ethnoveterinary Botanical Medicine in South Africa: A Review of Research from The Last Decade (2009 To 2019).* **Journal of Ethnopharmacology.** 257,2020, 112864. https://doi.org/10.1016/J.Jep.2020.112864.

129. U.S. Ezealigo, B. N Ezealigo & M.G Plaza, E.N. Dim, F. Kemausuor, L.E.K. Achenie & A.P. Onwualu, A.P., *Preliminary Characterisation and Valorisation of Ficus benjamina*

Fruits for Biofuel Application. **Biomass Conv. Bioref**. 2022, 1-12 https://doi.org/10.1007/S13399-021-02230-1

130. F.M. Mahomoodally, A Farwa, R Rafia & I.S Aniqa. *A Review of the Pharmacological Potential and Phytochemical Profile of Weeping Fig-Ficus benjamina L*. **Int J Chem Biochem Sci** 16, 2019, 70-75.

131. R.S Patel, R Kadia, B Vrushali, & P. Vipra. *Study of Ornamental Plants Found from Butterfly Park and One Tree Hill Garden of Kankariya, Ahmedabad, Gujarat, India*. 2018.

132. A. Pękala-Safińska, T. Halyna, B. Lyudmyla, K. Natalia, G. Ivan, H. Vitaliy, & P. Andriy. *Comparative Assessment of the Antibacterial Efficacy of Leaf Extract Obtained from Ficus benjamina L. (Moraceae) And Its Cultivars Against Aeromonas Sobria Strain.* **Agrobiodiversity for Improving Nutrition, Health and Life Quality** 5, 1, 2021.

133. A. Ashraf, S.A Zafar, M.Y Ashraf, M.U Ijaz, S Muzammal, F Asad, F Jabeen &.M Shahid. *Phytochemical Composition and Potent Biological Activities of Ficus benjamina Var. comosa Leaves Extract.* **Pakistan Journal of Botany.** 52(2), 2020, 531-5**.**

134. I. Batool, S. Nisar, L. Hamrouni, & M.I Jilani. *Extraction, Production and Analysis Techniques for Menthol: A Review*. **Int. J. Chem**. **Biochem. Sci.** 14, 2018,71-6.

135. M.A. Hanif, S. Nisar, G.S. Khan, Z. Mushtaq & M. Zubair, *Essential Oils*. *In Essential Oil Research,* **Springe***r:* 2019, 3-17.

136. M. Naz, S Nisar, A. El Zerey-Belaskri, A Umer & M. Idrees. *Synthesis and Uses of Various Essential Oil Based Derivatives in Biomedicine.* **International Journal of Chemical and Biochemical Sciences.** 13, 2018, 92-99.

137. P. Deepa, S. Kandhasamy, K Songmun & J. P Se. *A Role of Ficus Species in The Management of Diabetes Mellitus: A Review.* **Journal of Ethnopharmacology** 215, 2018, 210-232.

138. C. O. Okonkwo, & O. C. Ohaeri. *Essential Oils from The Leaves of Euphorbia milii Exert Insecticidal Activity Through Disruption in Ionic Composition.* **IOSR J. Phar**m. **Blogica Sci.** 13(4) 2018, 46-53.

139. S. Patil. *Phytochemical Study and Antioxidant Properties of Ethanolic Extracts of Euphorbia milii.* R Haleshappa, E Keshamma, C.R Girija, M Thanmayi, C.G Nagesh, G.H Lubna Fahmeen, M Lavanya and J.P Sharangouda.2020.

140. E.J Mavundza, R. Street, & H. Baijnath. *A Review of the Ethnomedicinal, Pharmacology, Cytotoxicity and Phytochemistry of the Genus Euphorbia in Southern Africa.* **South African Journal of Botany** 144, 2022, 403-418.

141. H.Y Ibrahim, A.O Shaimaa, R.H Hesham & Z.H Abedltawab. *In-Vitro Screening of Some Plant Extracts for Their Potential Anticancer Activity*. **African Journal of Traditional, Complementary and Alternative Medicines** 17(1), 2020, 1-8.

142. M. Ali, F. Aminu & I.S. Ibrahim. *In-Vitro Assessment of Antibacterial Activity and Phytochemical Screening of Vitex doniana on Clinical Isolate of Salmonella Typhi*. **IN-VITRO** 3(1), 2017.

143. I.M Ifeanacho, C.O Shedrack, & U.A Peter. *Phytochemical Composition of Vitex Doniana*. **Analytical Chemistry Letters** 9(6), 2019, 863-875.

144. M. Gupta, S. Naresh, G. Monica, G. Reena, S. Kalvatala, & K. Bhupinder. *Herbal Bioactives in Treatment of Inflammation: An Overview*. **South African Journal of Botany** 143, 2021, 205-225.

145. S Johari, & K Ashwani. *Euphorbia Spp. and Their Use in Traditional Medicines: A Review.* **World J. Pharm. Res** 9, 2020, 1477-1485.

146. D. Kemboi, P. Xolani, L. Moses, & T. Jacqueline. *A Review of the Ethnomedicinal Uses, Biological Activities, and Triterpenoids of Euphorbia Species.* **Molecules** 25(17), 2020, 4019.

147. T. De Freitas, B. João, J.R.S Antonio, & M.K. Ricardo. *Isolation and Characterization of Polyphenols from Euphorbia Heterophylla L.(Euphorbiaceae) Leaves.* **Revista Fitos** 13(1), 2019, 49-60.

148. D.K. Magozwi, D. Mmabatho, M. Nthabiseng, S.N Xavier, W.K Rui, S Molahlehi, J.M Lyndy, A.A Wilma, & J.T Vuyelwa. *Flavonoids from the Genus Euphorbia: Isolation, Structure, Pharmacological Activities and Structure–Activity Relationships*. **Pharmaceuticals** 14(5), 2021, 428.

149. S. Aboush & A.R Gadir. *Isolation and Characterization of Flavonoids and Oils from Some Saudi Plants and Their Biological Activity.* Phd Diss., Sudan University of Science and Technology, 2017.

150. M.M Jucá, F.M Cysne Filho, J.C De Almeida, D.D Mesquita, J.R Barriga, KC Dias, T.M Barbosa, L.C Vasconcelos, L.K Leal, J.E Ribeiro & S.M Vasconcelos. *Flavonoids: Biological Activities and Therapeutic Potential.* **Natural Product Research** 34(5), 2020, 692-705.

151. A. Ullah, M. Sidra, L.B Syed, K. Noreen, G. Lubna, G.P Benjamin, E. Abdul-Hamid & J. Mariusz. *Important Flavonoids and Their Role as A Therapeutic Agent.* **Molecules** 25(22), 2020, 5243.

152. O. Deveoğlu & K. Recep. *A Review on The Flavonoids–A Dye Source*. **International Journal of Advances in Engineering and Pure Sciences** 31(3), 2019, 188-200.

153. S.A Adefegha. *Functional Foods and Nutraceuticals as Dietary Intervention in Chronic Diseases; Novel Perspectives for Health Promotion and Disease Prevention*. **Journal of Dietary Supplements** 15(6), 2018, 977-1009.

154. A.C Rana & G. Bhawna. *Chemistry and Pharmacology of Flavonoids-A Review.* **Indian Journal of Pharmaceutical Education and Research** 53(1), 2019, 8-20.

155. M.A. Ayaz, J. Muhammad, U. Farhat, O. Muhammad, U Ikram, A. Jawad & S. Muhammad. *Flavonoids as Prospective Neuroprotectants and Their Therapeutic Propensity in Aging Associated Neurological Disorders.* **Frontiers in Aging Neuroscience** 11, 2019, 155.

156. M. Abotaleb, M.S Samson, E. Varghese, V. Sharon, K Peter, L Alena, & B. Dietrich. *Flavonoids in Cancer and Apoptosis.* **Cancers** 11(1), 2018, 28.

157. O. T. Osuntokun, T. A. Jemilaiye, & A. R. Akinrodoye. *Comparative Study Between the Effect of Parkia biglobosa (JACQ) Benth and Conventional Antibiotics Against Multiple Antibiotic Resistant Uropathogenic Bacteria (MARUB).* **MOJ Bioequiv Availab** 5(4), 2018, 200-212.

158. I.A. Airaodion, O. A. Edith, O. O Emmanuel, O Uloaku & U.O Etinosa. *Effect of Oral Intake of African Locust Bean on Fasting Blood Sugar and Lipid Profile of Albino Rats*. **Asian Journal of Research in Biochemistry** 4(4), 2019, 1-9.

159. W. G Balogun, A. A. Ismail, Y Usman & S. Azman. *A Review of the Phytochemistry and Medicinal Activities of the Popular African Food Additive: Parkia biglobosa Seed.* **Oriental Pharmacy and Experimental Medicine** 18(4), 2018, 271-279.

160. M. Muhammed, A.A. Yusuf, B.O. Odey, A.R. Alawode, G.A. Adegbola & R. A. Agboola. *A Systematic Review of Domestication, Ethnopharmacological Use, Phytochemistry, Nutritional Composition, And Biological Activities of Parkia biglobosa (Jacq.) R*. Br. Ex G. Don. **BIOMED Natural and Applied Science** 1(1), 2021, 01-12.

161. F.U. Okwunodulu, F. Chisom, N.O. Innocent, & I.C. Vintan. *Comparative Study on The Phytochemicals, Proximates, Vitamins and Mineral Elements Compositions of Unfermented and Fermented Seeds of Parkia Biglobosa.* **Bioscience and Biotechnology Journal** 2(21), 2021, 7-10.

162. S. Compaore, B. Lazare, H. Achille, I. Rodrigue, Z. Issouf, O. Sylvin, & T. Adjima. *Diversity of Plants Used in The Management of Hypertension by Three Associations of Traditional Healers Along a Climate Gradient in Burkina Faso.* **Advances in Traditional Medicine** 21, 2021, 151-162.

163. M.I. Builders, S.J. Oyepata & R.V. Akpobome. *Effect of Parkia biglobosa Extract on Open Skin Wound Healing in Dexamethasone-Induced Hyperglycaemia and Histological*

Assessment in Rats. **African Journal of Pharmacy and Pharmacology (AJPP)**13(8), 2019.

164. S.M Abbas, M.E Hattem, S.S Amira & A.S Mai. *Optimization of Germination, Callus Induction, And Cell Suspension Culture of African Locust Beans Parkia Biglobosa (Jacq.) Benth*. **Journal of Genetic Engineering and Biotechnology** 16(1), 2018, 191-201.

165. O.T Osuntokun, T.A. Jemilaiye, & A.R. Akinrodoye. *Comparative Study Between the Effect of Parkia biglobosa (JACQ) Benth and Conventional Antibiotics Against Multiple Antibiotic Resistant Uropathogenic Bacteria (MARUB).* **MOJ Bioequiv Availab.** 5(4), 2018, 200-212.

166. S.M. Saleh, J Juriyati, Z Satirah, Y.A. Ahmad, H.M. Nor, & K. Yusof. *Genus Parkia: Phytochemical, Medicinal Uses and Pharmacological Properties.* **International Journal of Molecular Sciences.** 22(2), 2021, 618.

167. J.V Santos, A.L Porto & M.I Cavalcanti. *Potential Application of Combined Therapy with Lectins as A Therapeutic Strategy for The Treatment of Bacterial Infections*. **Antibiotics**. 2, 10(5), 2021, 520.

168. U. Chukwunonye, E. Christopher, P.E Obi, T.M. Kenne & S. P. G. Anze. *Phytochemical Screening and Antimicrobial Activity of Methanol Extract and Fractions of the Leaf of Piliostigma thonningii Schum (Caesalpiniaceae)*. **World Applied Sciences Journal.** 35(4), 2017, 621-625.

169. U. Ginikachukwu, O Onugwu, C.E Chukwujindu & P.E Obi. *Pharmacognostic Study of the Leaves of Piliostigma thonningii Schum (Ceasalpiniaceae).* **Journal Of Advances in Medical and Pharmaceutical Sciences.** 202, 1-9.

170. M.O. Afolayan, R.O. Omosimua, A.E. Fadeyi, O.C. Aguzue, O. T. Asekun & O.B. Familoni. *Phytochemical, Antioxidant, And Flavonoid Investigation of Methanolic Leaf Extract of Piliostigma thonningii (Schum.).* **In Natural Product Experiments in Drug Discovery.** 2022, 1-26.

171. A.A. Ajao, O.M. Yusuf, & S. Saheed. *Wandering Through Southwestern Nigeria: An Inventory of Yoruba Useful Angiosperm Plants.* **Heliyon**. 2021, E08668.

172. S.O. Aslantürk. *In Vitro Cytotoxicity and Cell Viability Assays: Principles, Advantages, And Disadvantages.* **Genotoxicity-A Predictable Risk to Our Actual World.** 2, 2018, 64-80.

173. F.M Luna, T.S Freitas, F.F Campina, M.S Costa, J.E Rocha, R.P Cruz, D.S Júnior, Z.S Silveira, N.S Macedo, J.C Pinheiro & F.N Pereira-Júnior. *Evaluation Of Phytochemical Composition, Toxicity in Drosophila Melanogaster and Effects on Antibiotics*

Modulation of Plathymenia Reticulata Benth Extract. **Toxicology Reports**. 1(8), 2021, 732-9.

174. E.T. Verjans, D. Jordi, L. Walter, L. Bart & S. Liliane. *Three-Dimensional Cell Culture Models for Anticancer Drug Screening: Worth the Effort?* **Journal of Cellular Physiology.** 233(4), 2018, 2993-3003.

175. M. Rizwanullah, A. Saima, R.M Showkat, F.U Khalid, & M.A Mohd. *Phytochemical Based Nanomedicines Against Cancer: Current Status and Future Prospects.* **Journal of Drug Targeting.** 26, No. 9;2018: 731-752.

176. R.I Freshney. *Culture of Animal Cells: A Manual of Basic Technique and Specialised Applications.* 6th Ed. Hoboken, NJ: John Wiley & Sons, Inc.; 2010

177. T. Riss, N. Andrew, M. Rich, K. Natashia & V. Jolanta. *Cytotoxicity Assays: In Vitro Methods to Measure Dead Cells.* Assay Guidance Manual [Internet],2019.

178. T. Riss & R. Moravec. *Use of Multiple Assay Endpoints to Investigate the Effects of Incubation Time, Dose of Toxin, And Plating Density in Cell-Based Cytotoxicity Assays.* **Assay Drug Dev Technol.** 2, 2004, 51-62.

179. T. Mosmann. *Rapid Colorimetric Assay for Cellular Growth and Survival: Application to Proliferation and Cytotoxicity Assays.* **J Immunol Method**s. 65, 1983, 55-63.

180. F.A Barile. *Principles of Toxicology Testing.* Boca Raton, FL: **CRC Press** (Taylor and Francis Group); 2008.

181. N. Lall, C.J Henley-Smith, M.N De Canha, C.B Oosthuizen & D. Berrington. *Viability Reagent, Prestoblue, In Comparison with Other Available Reagents, Utilized in Cytotoxicity and Antimicrobial Assays.* **Int J Microbiol**. 5, 2013, 420601.

182. S.P. Crouch, R Kozlowski, K.J. Slater & J Fletcher. *The Use of ATP Bioluminescence as A Measure of Cell Proliferation and Cytotoxicity.* **J Immunol Methods.**160,1993, 81-8.

183. C. Korzeniewski, D. Callewaert. *An Enzyme-Release Assay for Natural Cytotoxicity.* **J Immunol Methods**. 64, 1983, 211-24.

184. P Soli´S, C Wright, M Anderson, M Gupta & J.D Phillipson. *A Microwell Cytotoxicity Assay Using Artemia Salina (Brine Shrimp).* **Planta Med**. 59, 1993, 250-2.

185. G.A Durán, M.G Teresa, J.R Francisco Mejías, M.G José & A.F Macías. *An Overview of The Chemical Characteristics, Bioactivity and Achievements Regarding the Therapeutic Usage of Acetogenins from Annona Cherimola Mill.* **Molecules.** 26(10), 2021, 2926.

186. C.Y Huang, D.T Ju, C.F. Chang, P.R Muralidhar, & B.K Velmurugan. *A Review on The Effects of Current Chemotherapy Drugs and Natural Agents in Treating Non-Small Cell Lung Cancer.* **Biomedicine (Taipei)** 7, 23, 2017

187. M. Serhani, H Essaadi, K Kassara, & A. Boutoulout. *Control by Viability in A Chemotherapy Cancer Model.* **Acta Biotheor**. 2019.

188. K. Kilpatrick, L. S. Jaime, J. Renee, T. Andrew, E. Melissa, S. Laura & A. S. Gerald. *Occurrence and Management of Thrombocytopenia in Metastatic Colorectal Cancer Patients Receiving Chemotherapy: Secondary Analysis of Data from Prospective Clinical Trials*. **Clinical Colorectal Cancer** 20(2), 2021, 170-176.

189. T. Amjad, A.C. Muhammad & K. Anup. *Cancer Chemotherapy*. 2020.

190. C. Scarpignato & B. Ingvar. *Drug-Induced Small Bowel Injury: A Challenging and Often Forgotten Clinical Condition.* **Current Gastroenterology Reports.** 21, 2019, 1-12.

191. R.K. Nurgali, J. Thomas, & A. Raquel. *Adverse Effects of Cancer Chemotherapy: Anything New to Improve Tolerance and Reduce Sequelae?* **Frontiers in Pharmacology.** 9, 2018, 245.

192. M. Sheibani, A. Yaser, S. Maryam, N. Sadaf, E. Faezeh, H. F. Mohammad, & R. D. Ahmad. *Doxorubicin-Induced Cardiotoxicity: An Overview on Pre-Clinical Therapeutic Approaches*. **Cardiovascular Toxicology.** 22(4), 2022, 292-310.

193. V. Jain, S. Bindal, P.K. Bhatia, & V.S. Nanduri, V.S. *Managing Pain and Side Effects of a Hodgkin Lymphoma Female Patient Undergoing Chemotherapy Using Yoga Prana Vidya System as Complementary Medicine: A Case Report*. **International Journal of Medical Sciences and Academic Research.** 2(05), 2021, 5-11.

194. P Limonta, M.M Roberta, M Monica, F Fabrizio, R Michela & M.M Marina. *Role of Endoplasmic Reticulum Stress in The Anticancer Activity of Natural Compounds.* **International Journal of Molecular Sciences**. 20(40), 2019, 961.

195. A.S. Chime & M. P. C. Phoebe. *Herbal Medicines as Potential Immune Boosters Against Coronavirus Diseases*. **Current Traditional Medicine.** 9(2), 2023, 64-74.

196. D.J. Newman & G.M. Cragg. *Natural Products as Sources of New Drugs from 1981 To 2014*. **J. Nat. Prod**. 79 (3), 2018, 629–661. doi: 10.1021/Acs.Jnatprod.5b01055

197. S.A Choudhari, C.M Pallavi, D Manasi, R Prabhakar & P. Om. *Phytochemicals in Cancer Treatment: From Preclinical Studies to Clinical Practice.* **Frontiers in Pharmacology**. 2020, 161410.

198. E. Martino, C. Giuseppe, C. Sonia, C. Edoardo, P. Serena, P. Francesca, R. Marta, M.S. Angela, & C. Simona. *Vinca Alkaloids and Analogues as Anti-Cancer Agents: Looking Back, Peering Ahead.* **Bioorganic & Medicinal Chemistry Letters** 28, 2018, 2816-282617.

199. C. Mei, L. Lin, T. Li-Ming, X. Xiao-Jing, H. Bai-Mei, L. Chao & Y. Ji-Ye. *The Role of Single Strand Break Repair Pathways in Cellular Responses to Camptothecin Induced DNA Damage*. **Biomedicine & Pharmacotherapy** 125, 2020, 109875.

200. N. Skroza, B. Nicoletta, P. Ilaria, & P. Concetta. *Clinical Utility of Ingenol Mebutate in The Management of Actinic Keratosis: Perspectives from Clinical Practice.* **Therapeutics and Clinical Risk Management.** 14, 2018, 1879.

201. J.N. Short, J. Elias, N. Kiran, P. Ami, N. Jing, S. Koji, M. G. Nogueras-Gonzalez, P. Bose, S.M. Kornblau, K. Takahashi & M. Andreeff. *A Phase II Study of Omacetaxine Mepesuccinate for Patients with Higher-Risk Myelodysplastic Syndrome and Chronic Myelomonocytic Leukemia After Failure of Hypomethylating Agents.* **American Journal of Hematology** 94(1), 2019, 74-79.

202. V. Čermák, D Vojtěch, J. Michael, L. Lenka, K. Jan, R. Daniel & B. Jan. *Microtubule-Targeting Agents and Their Impact on Cancer Treatment.* **European Journal of Cell Biology.** 99(4), 2020, 151075.

203. J.G. Nirmala & M. Lopus, *Cell Death Mechanisms in Eukaryotes*. **Cell Biol Toxicol** 36, 2020, 145–164. https://doi.org/10.1007/s10565-019-09496-2

204. B.W. Stewart & P. Kleihues. *World cancer report*. Lyon: IARC press. 57, 2003.

Chapter Three

Methodology

3.1 Materials

3.1.1 Collection of Plants

Mature plant specimens comprising leaves and stem barks as applicable for the study were collected in their natural habitat at two different locations. *Ficus benjamina* and *Euphorbia milli* were collected at the premises of the University of Ibadan, Oyo State, Nigeria while *Parkia biglobosa, Piliostigma thonningii, Vitex doniana,* and *Euphorbia heterophyilla* were collected at Iyana-Ofa, Lagelu Local Government Area, Ibadan. The plants were identified and authenticated at the Botany Depatment, University of Ibadan by Mr. Esimekhuei. Samples were deposited at the UI Herbarium and assigned with herbarium numbers (See Appendix I).

3.1.2 Cell lines

Two T75 flasks of HeLa (Cervical cancer) cell line were obtained as gifts from the Cell Culture Unit of the National Institute of Communicable Disease (NICD), Johannesburg, South Africa. Breast cancer (MCF-7) and Lung cancer (A549) cell lines were obtained from the Cell Culture

Unit of the WHO National Polio Laboratory, Department of Virology, University of Ibadan, Nigeria. A flask of non-tumorigenic epidermal cell line (KMST-6) was obtained as a gift from Prof. S. O. Omilabu of the Central Research Laboratory, College of Medicine, University of Lagos, Nigeria.

3.1.3 Chemicals and Reagents

All chemicals and reagents used in this study are of analytical grade.

3.2 Methods

3.2.1 Preparation of Plant extracts

Leaf plant materials collected were properly rinsed in distilled water and labeled appropriately in separate drying trays. The stem bark materials were cut into small pieces to increase their surface area and were allowed to air dry at room temperature. Dried plant specimens were milled into powder and 300g of each were weighed into 500mL of Methanol. The mixtures were allowed to soak for 72hrs at room temperature. The extracts were filtered with the use of muslin bags. The filtrates were further filtered using 125mm pore size of Whatman filter papers. Filtered methanolic extracts were concentrated under reduced pressure using a rotatory evaporator. Exhaustive extraction was carried out by adding fresh solvent to marcs and resultant filtrates were pooled with the initial extracts.

3.2.2 Cytotoxicity Assay

MTT (3-(4,5-dimethylthiazol-2yl)-2,5 diphenyltetrazolium bromide assay was carried out to detect the cytotoxic effect of the extracts on both cancer and Non-tumorigenic cell lines.

3.2.2.1 Principle of MTT Assay

Tetrazolium salts (MTT) are cleaved to formazan by cellular enzymes. An increase the mitochondrial dehydrogenases occur as a result of the proliferation of viable cells. The number of metabolically active cells in the culture is directly proportional to formazan dye due to the enzyme activities. Therefore, metabolic active cells are quantified by measuring the amount of soluble formazan formed spectrophotometrically[1].

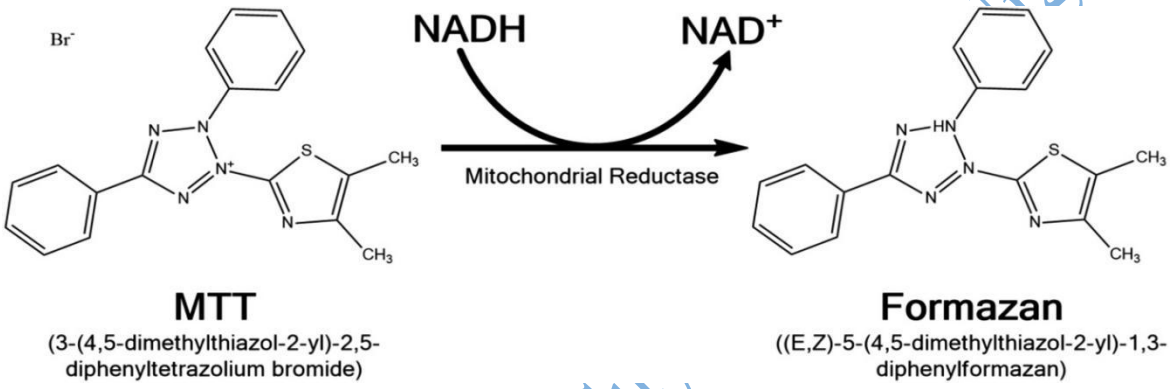


Fig. 3.1: MTT assay equation

Source: [9]

3.2.2.2 MTT Assay Procedure

Each plant extract was pre-solubilized in dimethyl sulphoxide (DMSO) at 37°C to give a stock solution of 1000µg/mL. Ten-fold serial dilutions were made from the stock solution to give working concentrations of 0.01-1000µg/mL ensuring that the final concentrations of DMSO in the tested dilution were less than 1%.

Confluent monolayers of HeLa, A549, MCF-7 and KMST-6 grown in Mimmum Essential Medium (MEM GIBCO) fortified with 10%FBS and Penicillin/Streptomycin were split separately with the use of 0.25% Trypsin, 1:5000 EDTA. Cells were counted with the use of an

automatic cell counter (EVE Nanotek) and the concentration of each cell line was adjusted to $1x10^5$ cells/mL. 96 well-microtitre plates were seeded for each cell line and incubated. After 24hrs of incubation, cells were treated with different concentrations of test extracts (0.01-1000µg/mL) in triplicates and incubated at 37°C for 72hrs. The negative control was performed using growth medium alone instead of plant extract, while Doxorubicin was used as the positive control. The cell viability was examined microscopically with the use of an Olympus Inverted Microscope for the presence of cytopathic effect (CPE). At the expiration of the 72hrs treatment. The supernatant was removed from the well and 25µL of the MTT solution (2mg/mL in PBS) was added to each well. The plates were incubated for 1.5hrs at 37°C and 125µL of Dimthylsulphoxide (DMSO) was added to each well to dissolve the formazan crystals. The plates were placed on a shaker for 15mins and optical density was determined at 492nm on a multi-well spectrophotometer (Thermo/Lab System 352 Multiscan Microplate reader).

Calculation:

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

3.2.3 Preparation of Cell lysate for Biochemical Assays.

Two T75 flasks of each of the cell lines were cultured at a cell density of $1x10^5$ /mL with MEM containing 10% FBS and Pen/strep. After 24hrs of incubation, the cells were treated with methanol extract of *Ficus benjamina* at the concentration of IC_{50} value, while the control flasks received 2% MEM. The treated flasks were further incubated at 36°C for 24 hours. At the expiration of 24hrs, the medium was discarded and washed twice with ice-cold Phosphate Buffer Saline (PBS). 1mL of prechilled lytic buffer (see Appendix II) was added to each flask and was placed on ice for 20mins, occasionally rocked. The lysate was removed with a pipette and transferred into the appropriately labeled centrifuge tube. This was repeated for all the flasks. Lysates collected were centrifuged at 20,000g for 10min at 4°C. The supernatant was carefully removed into appropriately labeled cryovials and stored at -80°C till when needed for Biochemical assays.

3.2.4 Quantitative Determination of Human Bcl-2, BAX, Caspase-9 and Caspase-3 in Cell lysates.

Test principle

The ELISA Kit (Elabscience®) uses the sandwich-ELISA principle. The micro ELISA plate provided in the kit has been pre-coated with an antibody specific to Human Bcl-2. Samples (or

standards) are added to the micro ELISA plate wells and combined with the specific antiboby. Then a biotinylated detection antibody specific for Human Bcl-2 and Avidin-Horseradish Peroxide (HRP) conjugates is added successively to each microplate and incubated. Free components are washed away. The substrate solution is added to each well. Only those wells that contain Human Bcl-2, biotinylated detection antibody, and Avidin-HRP conjugate will appear in blue color. The enzyme-substrate reaction is terminated by the addition of stop solution and the color turns yellow. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 ± 2nm. The OD value is proportional to the concentration of the Human Bcl-2. The concentration of the Human Bcl-2 of the unknown sample can then be calculated from the standard curve.

Reagent Preparation (see Appendix III)

Assay Procedure

100µl each dilution of standard, blank and sample were added into the appropriate wells. The plates were covered with the sealer and incubated for 90 mins at 37°C. The liquid from each well was decanted. 100ml of Biotinylated detection antibody working solution was added to each well. The plates were covered with the sealer and incubated for an hour at 37°C. The solution from each well was decanted and 350µL of wash buffer was added to each well. This was allowed to soak for 1min and aspirated from each well and patted till dry against clean absorbent paper. This step was repeated three times. 100µL of HRP conjugate working solution was added to each well. The plates were covered with the sealer and incubated for 30mins at 37°C. The solution was decanted from each well and washing was repeated five times. 90µL of substrate reagent was added to each well. The plate was covered and incubated for about 15mins at 37°C. The

plate was kept away from light. Microplate Reader was preheated for 15mins before OD measurement. The Optical Density value (OD) of each well was determined at once with a micro-plate reader set to 450nm. (Thermo/Lab System 352 Multiscan Microplate reader).

3.2.5 Assessment of Lipid Peroxidation (LPO) Activity

Lipid peroxidation was determined by measuring the formation of Thiobarbituric acid reactive substances (TBARS) present in the test sample according to the method of Varshney and Kale[2].

Test Principle

Under acidic conditions, malondialdehyde (MDA) produced from the peroxidation of fatty acidreactsct with the chromogenic reagent 2-thiobarbituric acid to yield a pink coloured complex with maximum absorbance at 532nm.

Reagent Preparation (see Appendix IV)

Procedure

An aliquot of 0.2ml of test sample (in duplicate) was mixed with 0.8ml of Tris-KCl buffer to which 0.25 ml of 30% TCA was added. Then 0.25ml of 0.75% TBA was added and placed in a water bath for 45 minutes at 80°C. This was then cooled in ice to room temperature and centrifuged at 3000rpm for 15 minutes. The clear supernatant was collected and absorbance measured against a reference blank of distilled water at 532nm.

Calculation

The MDA level was calculated using an extinction coefficient of $0.156uM^{-1}cm^{-1}$

3.2.6 Estimation of Reduced Glutathione (GSH) level.

The method of Beutler *et al* was followed in estimating the level of reduced glutathione (GSH)[4].

Principle

This method is based upon the development of a relatively stable yellow coloured product when 5'5-dithiobis-2-nitrobenzoic acid (DNTB; Ellman's reagent) is added to sulfhydryl compounds of which glutathione comprises the bulk in tissues. The resulting chromophoric product possesses maximum 412nm.

Reagents (see Appendix V)

GSH Standard curve preparation

Serial dilutions of the GSH stock solution were prepared as shown in the table below. The absorbance of the yellow colour formed upon the addition of Ellman's reagent was read within 30 minutes at an absorbance of 412nm against a blank of 4.5ml of Ellman's reagent and 0.5ml phosphate buffer. A plot of absorbance against concentration of reduced GSH was then plotted.

Procedure

250µl of the sample was added to 750µl of precipitating solution which was vortexed and centrifuged at 300rpm for 300seconds. Thereafter, 65µl of the supernatant was added to 560µlof Ellman's reagent. The absorbance of the reaction mixture was read at 412nm against a reagent blank using a spectrometer.

3.2.7 Determination of Superoxide Dismutase (SOD) Activity.

The activity of SOD was determined by the method of Misra and Fridovich[5].

Principle

The ability of SOD to inhibit the autoxidation of epinephrine at pH 10.2 makes this reaction a basis for a simple assay for this dismutase. Superoxide radical causes the oxidation of

epinephrine to adrenochrome and the yield of adrenochrome produced per superoxide radical introduced increases with increasing pH and concentration of epinephrine.

Reagent (see Appendix VI)

Procedure

100µl of the sample was added to 1.25ml of 0.05M carbonate buffer (pH 10.2) and 0.15ml of Adrenaline in a cuvette, inverted gently to mix properly, and change in absorbance monitored every 30 seconds for 2 and half minutes at 480nm. The reference cuvette was the same as for the samples with water replacing the samples.

Calculation

$$\% \text{ Inhibition} = 100 - \left(100 \times \frac{\text{Increase in absorbance per min for sample}}{\text{Increase in absorbance per min for blank}}\right)$$

1unit SOD activity was given as the amount of SOD necessary to cause 50% inhibition of the auto-oxidation of adrenaline.

3.2.8 Estimation of Glutathione S-Transferase (GST) Activity

Glutathione S-transferase activity was determined according to the method of Habig *et al.*[6]

Principle

The assay is based on the principle that all known glutathione S-transferase isoenzyme demonstrate a relatively high activity with 1-choloro-2,4-dinitrobenzene (CDNB) as the second substrate. When CDNB is conjugated to reduced glutathione, its absorption maximum shifts to a longer wavelength of 340nm providing a direct measurement of the enzymatic reaction.

Reagents (see Appendix VII)

Procedure

The medium for the estimation was prepared as shown in the table (see Appendix) and the reaction was allowed to run for 3 minutes with readings taken every 60 seconds against the blank at 340nm using a spectrometer.

Calculation

The extinction coefficient of CDNB at 340nm =9.6$Nm^{-1}cm^{-1}$

GSH S-transferase activity = *A_{340}/min × reaction volume × dilution factor

$$\frac{}{9.6 \times \text{sample volume} \times \text{mg protein/ml}}$$

=µmole/min/mg protein

Dipotassium hydrogen phosphate, K_2HPO_4 (0.6448g), and potassium dihydrogen phosphate, KH_2PO_4 (1.2649g) were dissolved in 90ml of distilled water, the pH adjusted to 7.4 and the volume made up to 100ml with distilled water.

3.2.9 Determination of Nitric Oxide (NO) Level

Principle

As NO rapidly combines into its stable oxidative metabolites ($NO_3$and NO_2) in an aqueous solution Palmer *et al,* serum concentrations of NO_3 and NO_2 were estimated as an index of NO production[7]. The NO radicals play an important role as a physiological messenger. NO is formed from L- arginine by NO synthase, which exists in several isoforms. Constitutive calcium-dependent isoforms (cNOS) modulate the control of vascular tone in endothelial cells or the neurotransmission in neurons, whereas inducible calcium-dependent isoforms (cNOS) are

located in macrophages, chondrocytes, hepatocytes and are induced by cytokines and endotoxin. Pathological Conditions associated with increased release of cytokines and endotoxin e.g, inflammation or sepsis can therefore increase NO production. Upon coming into the bloodstream, nitrite immediately reacts with oxyhemoglobin to form methemoglobin.

Reagent (see Appendix VIII)

Procedure

The amount of nitrite in supernatants or in serum was measured following the Griess reaction by incubating a 0.5ml of the sample,0.5ml of Griess reagent a room temperature for 20minutes, the absorbance was measured spectrophotometrically at 550nm. Nitrite concentration was calculated by comparison with standard solution of known sodium nitrite concentration.

3.2.10 GC-MS Analysis

GC-MS characterization of the bioactive compound of the extract of *Ficus benjamina* was carried out using a specific software: GCMS-QP2010SE. Operating conditions were as follows: splitless mode, initial oven temperature, 60°C for 1min, then 240°C for 2min at 20°C min^{-1} and finally to 302°C for 4min, injector and detector temperatures, 250˚C and 300˚C; carrier gas, Helium, 1.0 mL·min−1; injection volume, 8 µL. Mass spectrometer conditions are: the voltage ionization 1.44kV, ion source temperature 230˚C and MS transfer line it 250˚C.

Qualitative data were obtained with the comparison of spectra registered in a NIST library (NIST08) included in the software GC-MS solution. F. benjamina extracts composition is expressed as % area of each compound identified with NIST library in the sample chromatogram with respect to the total alkaloids peak areas in the same chromatogram[8].

3.2.11 Statistical Analysis

The results were expressed as mean ± standard error of the mean (SEM). Differences between the groups were analysed by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test with GraphPad Prism 6.0. P-values <0.05 were considered statistically significant for differences in means.

Endnotes

1. Ö. S. Aslantürk. *In Vitro Cytotoxicity and Cell Viability Assays: Principles, Advantages, And Disadvantages*. **Genotoxicity-A Predictable Risk to Our Actual World.** 2, 2018, 64-80.

2. R. Varshney & R.K Kale. *Effects of Calmodulin Antagonists on Radiation-Induced Lipid Peroxidation in Microsomes.* **International Journal of Radiation Biology.** 58, 1990, 733-43.

3. M.A Trush, P.A Egner & T.W Kensler. *Myeloperoxidase as a Biomarker of Skin Irritation and Inflammation.* **Food Chemtoxicol**. 32(2), 1994, 143-7.

4. E. Beutler, O. Duron & B.M Kelly. *Improved Method for The Determination of Blood Glutathione*. **J Lab Clin Med.** 61, 1963, 882-8.

5. H.P Misra & I. Fridovich. *The Role of Superoxide Anion in The Autoxidation of Epinephrine and A Simple Assay for Superoxide Dismutase.* **Journal of Biological Chemistry**. 247, 1972, 3170-3175.

6. W.H Habig, M.J Pabst & W.B Jakoby. *Glutathione-S-Transferases. The First Enzymatic Step in Mercapturic Acid Formation*. **J Biolchem.** 249, 1974, 7130–7139.

7. R.M Palmer, A.G Ferrige & S. Moncada. *Nitric Oxide Release Accounts for The Biological Activity of Endothelium-Derived Relaxing Factor.* **Nature**. 327(6122), 1987, 524-6.

8. G. Angelo, C. Lorena, G. Marta & C. Antonella. *Composition and Antioxidant Properties of Lavandula Angustifolia Miller Essential Oil Are Shielded by Propolis Against Uv Radiations.* **Photochemistry And Photobiology**, 90, 2014,702-708. http://dx.doi.org/10.1111/Php.12229.

9. S. Kamiloglu, G. Sari, T. Ozdal, & E. Capanoglu, *Guidelines for Cell Viability Assays.* **Food Frontiers.** 1(3), 2020, 332-349.

Chapter Four

Results and Discussion of Findings

4.1 Result

4.1.1 Cytotoxicity Test

Table 4.1 below is showing the results of the cytotoxicity study of the extracts of *Ficus benjamina* (Leaf), *Euphorbia milli* (Leaf), *Parkia biglobosa* (Bark), *Vitex doniana* (Leaf), *Euphorbia heterophyilla* (Leaf) and *Piliostigma thonningii* (Bark) on Cervical (HeLa), Breast (MCF-7), Lung (A549) Cell lines and nontumorigenic Cell line (KMST-6). Doxorubicin was used as the positive control. *F. benjamina* has the highest cytotoxicity with IC_{50} value of 17.56±4.68 µg/mL in HeLa cell line.

Table 4.1: Cytotoxicity of the plant extracts on Human cancer cell lines (IC_{50} µg/ml, Mean±SEM, n=3)

		Cell Lines			
S/N	**Plant Exract**	**HeLa**	**MCF-7**	**A549**	**KMST-6**
		IC_{50}(µg/mL)			
1.	*Ficus benjamina* (Leaf)	**17.56±4.68**	33.35±5.47	33.5±4.10	38.6±3.32
2.	*Euphorbia milli* (Leaf)	44.76±5.50	51.73±2.55	39.07±3.03	56.98±3.20
3.	*Parkia biglobosa* (Bark)	233	ND	315.3	326.8
4.	*Vitex doniana* (Leaf)	185.5	ND	296.5	283.2
5.	*Euphorbia heterophyilla* (Leaf)	210.1	ND	237.9	286.3
6.	*Piliostigma thonningii* (Bark)	241	ND	281.7	303.7
7.	*Doxorubucin (Positive Control)*	11.93±5.5	13.77±5.30	28.55±2.70	22.39±4.75

ND=Not determined IC_{50}=50% Inhibition of cell growth.

Source: Author's Analysis 2023

4.1.2 Selectivity Index Test

Table 4.2 below is showing the selectivity index of *Ficus benjamina, Euphorbia milli, Parkia biglobosa Piliostigma thonnigii, Vitex doniana,* and *Euphorbia heterophyilla* extracts to cancer cell line. Doxorubicin was used as the positive control, and KMST-6 Cell line was used as a nontumorigenic cell line. *F. benjamina* has the highest Selectivity Index in Cervical cancer cell lines (HeLa).

Table 4.2: Selectivity index of some medicinal plants on cancer cell lines

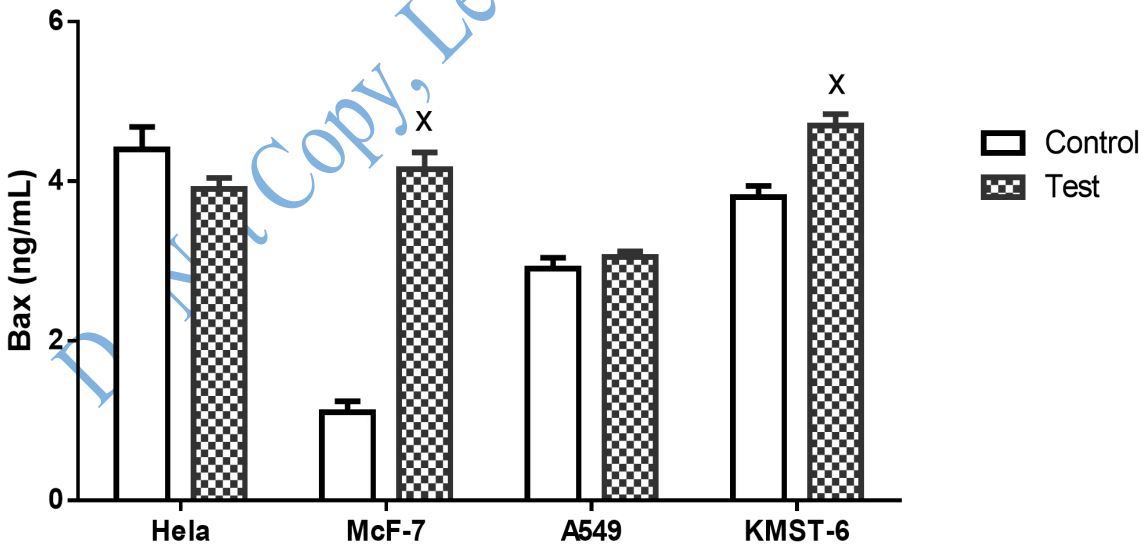
		Cell Lines		
S/N	**Plant Extracts**	**HeLa**	**MCF-7**	**A549**
1.	*Ficus benjamina*	**2.2**	1.2	1.1
2.	*Euphorbia milli*	1.3	1.1	1.5
3.	*Parkia biglobosa*	1.4	ND	1.0
4.	*Piliostigma thonnigii*	1.3	ND	1.1
5.	*Vitex doniana*	1.5	ND	1.0

6.	*Euphorbia heterophyilla*	1.4	ND	1.2
7.	Doxorubicin	1.9	1.6	0.8

Source: Author's Analysis 2023

4.1.3 BAX Protein Level

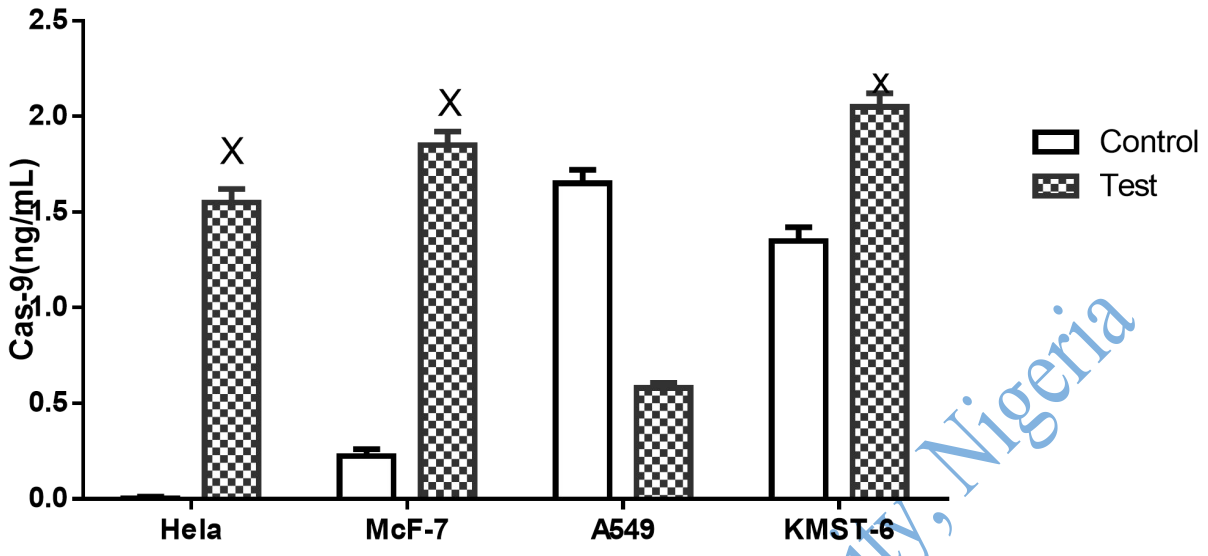
Figure 4.1 below is showing the effect of Methanol extract of *Ficus benjamina* leaf on the BAX protein level after 24hrs of treatment. Each value represents Mean ±SD, X represents significant different from control at $p \leq 0.05$



Source: Author's Analysis 2023

4.1.4 Caspase-9 Protein Level

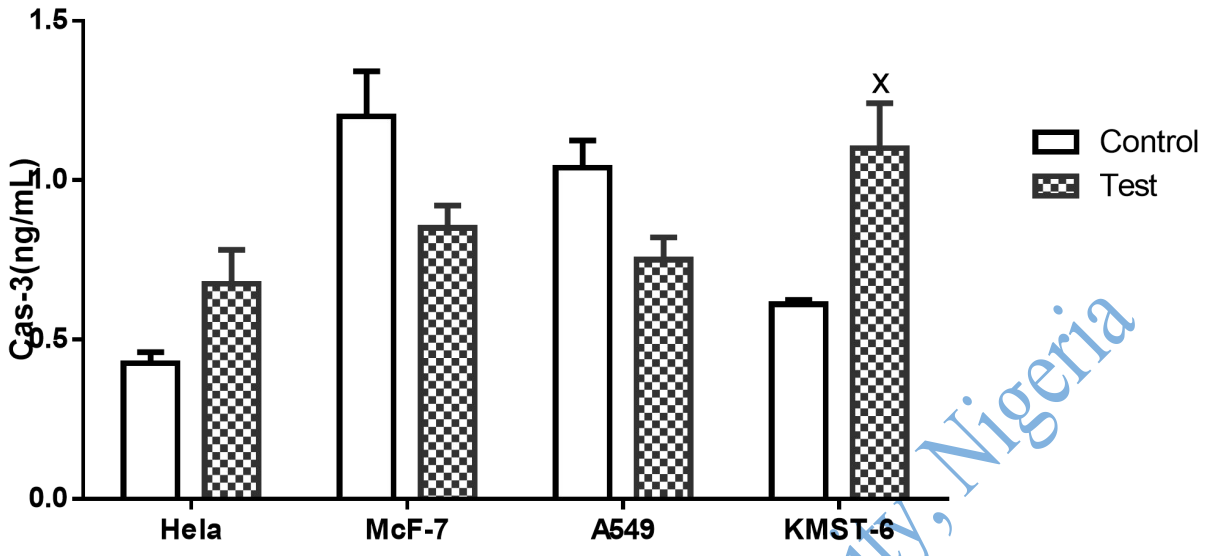
Figure 4.2 below is showing the effect of Methanol extract of *F. benjamina* leaf on Cas-9 protein level after 24hrs of treatment. Each value represents Mean ±SD, X represents significant difference from control at $p \leq 0.05$. The levels of Cas-9 protein were significantly higher in Cervical and Breast cancer cell lines.



Source: Author's Analysis 2023

4.1.5 Caspase-3 Protein levels

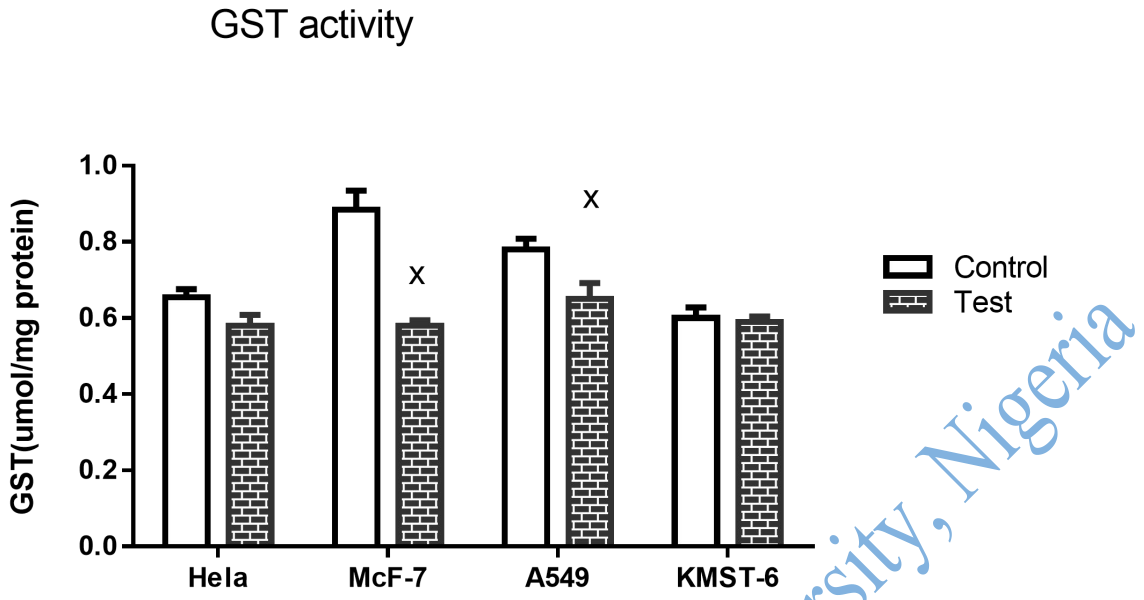
Figure 4.3 below is showing the effect of Methanol extract of *Ficus benjamina* leaf on th Cas-3 protein levels after 24hrs of treatment. Each value represents Mean ±SD, X-represent significance difference from control at $p \leq 0.05$. The level of Cas-9 was significantly higher in Cervical cancer cell line.



Source: Author's Analysis 2023

4.1.6 Activity of Glutathione-S- Transferase

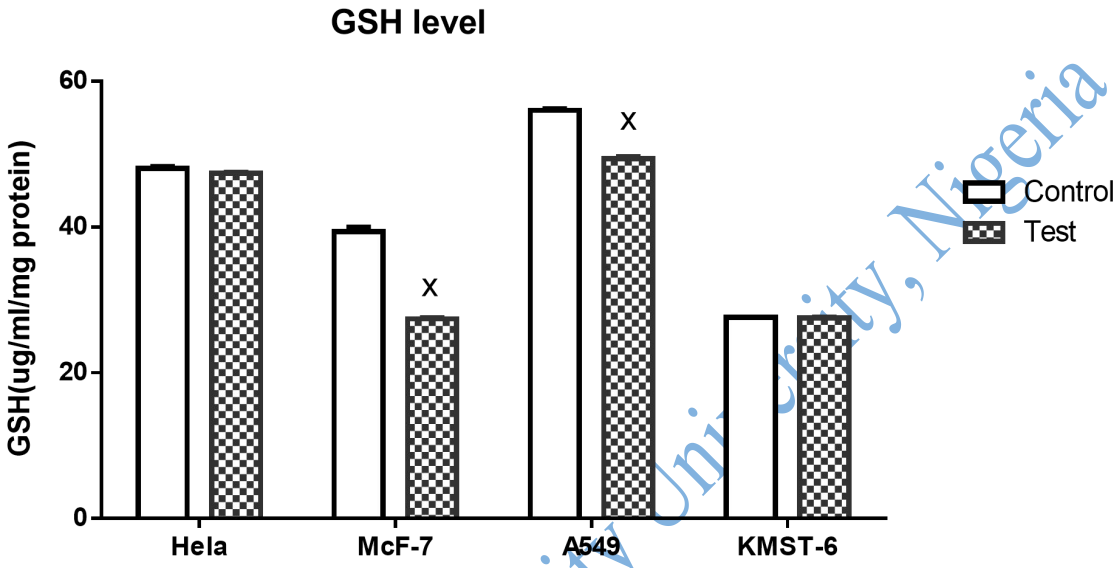
Figure 4.4 below is showing the effect of methanol extract of *F. benjamina* on Glutathione-S-Transferase (GST) activity after 24hrs of treatment of cancer cell lines. Each value represent mean± S.D, X- represent significant difference from control at $p \leq 0.05$. The activities of GST were significantly reduced in both MCF-7 and A549 cell lines.



Source: Author's Analysis 2023

4.1.7 Level of Glutathione (GSH)

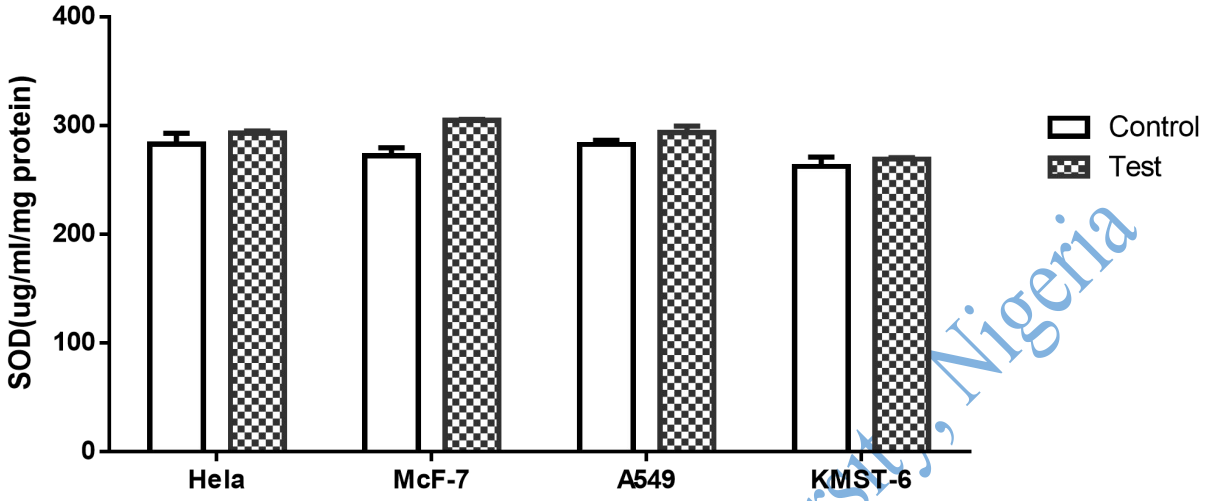
Figure 4.5 below is showing the effect of methanol extract of *F. benjamina* leaf on the level of Reduced Glutathione (GSH) after 24hrs of treatment of cancer cell lines. Each value represents mean±S.D, X-represent significant difference from control at $p \leq 0.05$. The levels of GST significantly reduced in MCF-7 and A549 cell lines.



Source: Author's Analysis 2023

4.1.8 Level of Superoxide Dismutase (SOD)

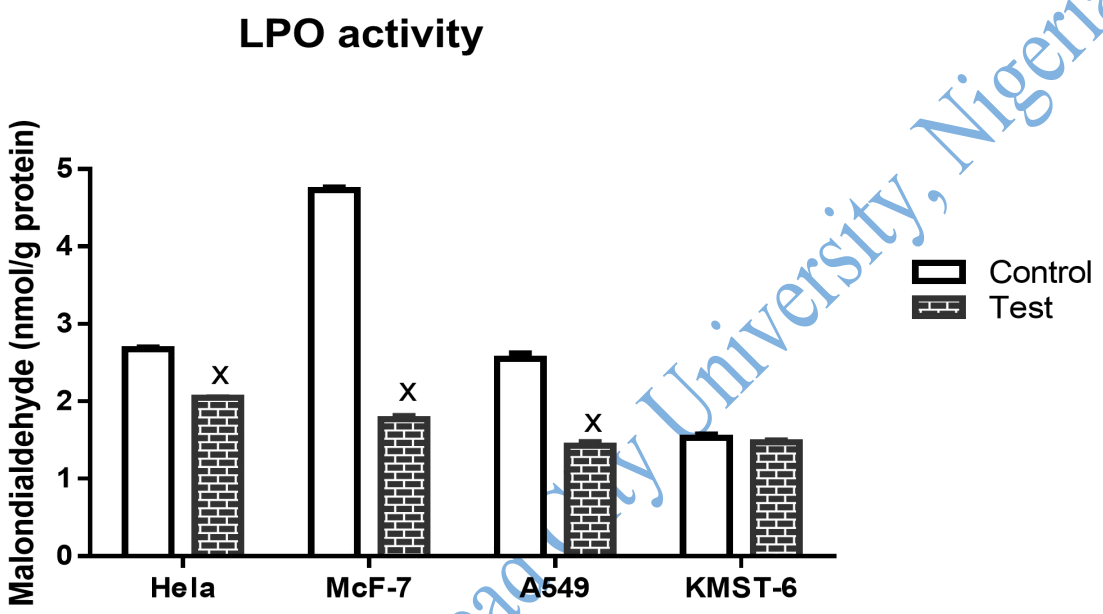
Figure 4.6 below showing the Effect of Methanol extract of *F. benjamina* leaf on the level of Superoxide Dismutase after 24hrs of treatment. Each value represents Mean ±SD, X-significantly different from control at $p \leq 0.05$. The levels of SOD were increased in all the cell lines but not statistically significant.



Source: Author's Analysis 2023

4.1.9 Lipid Peroxidation

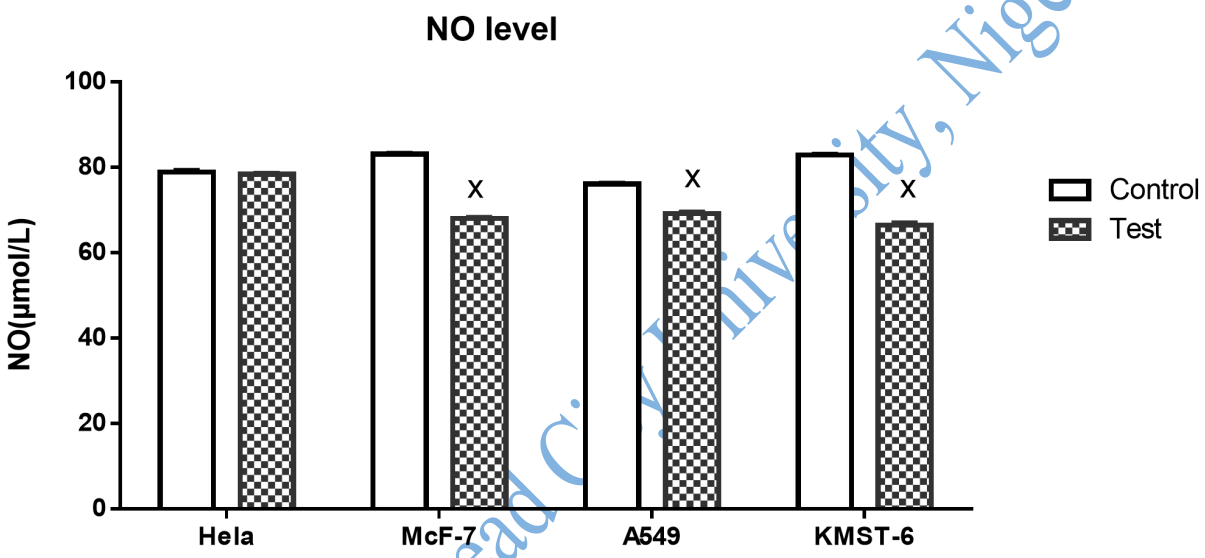
Figure 4.7 below is showing effect of methanol extract of *F. benjamina* leaf on LPO after 24hrs of treatment of cancer cell lines. Each value represents mean±S.D, X- significant difference from control at p≤0.05. The LPO activities were significantly reduced in all the cell lines.



Source: Author's Analysis 2023

4.1.10 Nitric Oxide Level

Figure 4.8 below is showing the effect of methanol extract of *F. benjamina* leaf on the level of Nitric Oxide (NO) on cancer cell lines after 24hrs of treatment. Each value represents mean±S.D, X-represent significant difference from control at p≤0.05. The levels of NO were significantly reduced in MCF-7 and A549.



Source: Author's Analysis 2023

4.1.11 GC-MS Analysis

Table 4.3: GC-MS Analysis of Bioactive compounds in Methanol extract of *F. bejamina* leaf.

Peak	R.Time	% Area	%Height	Name
1	10.456	0.16	0.49	alfa.-Copaene
2	11.012	0.12	0.20	5,9-Undecadien-2-one, 6,10-dimethyl-, (Z)-
3	11.667	0.12	0.13	Phenol, 2,5-bis(1,1-dimethylethyl)-
4	11.771	0.14	0.23	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,
5	11.920	0.14	0.22	Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dim
6	12.225	0.15	0.10	Fumaric acid, ethyl 2-methylallyl ester
7	13.983	0.21	0.22	Acetic acid, 2-(2,2,6-trimethyl-7-oxa-bicyclo[
8	14.133	0.20	0.15	Tetradecanoic acid
9	14.282	0.32	0.33	Bis(cyclohex-3-enylmethyl)amine
10	14.901	**2.19**	**4.00**	**3-Eicosyne**
11	15.000	0.17	0.28	(3E,5E,7E)-6-Methyl-8-(2,6,6-trimethyl-1-cycl
12	15.093	0.6	0.96	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
13	15.255	0.69	1.41	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
14	15.333	0.20	0.21	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione
15	15.411	0.28	0.60	5,9,13-Pentadecatrien-2-one, 6,10,14-trimethyl-, (
16	15.508	0.76	2.13	Hexadecanoic acid, methyl ester
17	15.771	0.29	0.78	Isophytol
18	15.940	0.78	0.68	n-Hexadecanoic acid

Peak	R.Time	% Area	%Height	Name
19	16.000	0.18	0.23	Fumaric acid, 3-fluorophenyl hexadecyl ester
20	16.390	0.14	0.28	Nerolidyl acetate
21	16.902	0.63	1.11	12,15-Octadecadienoic acid, methyl ester
22	16.943	0.57	0.84	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-
23	17.183	**2.47**	**4.17**	**Phytol**
24	17.233	0.21	0.44	Heptadecanoic acid, 16-methyl-, methyl ester
25	17.325	0.11	0.11	Cholest-5-ene-3,16,22,26-tetrol
26	17.416	0.28	0.16	(1R,2'S)-[1,1'-[Bicyclopentyl]-2,2'-diol
27	17.691	0.12	0.15	Octadecanamide
28	17.843	0.18	0.24	2,2,4-Trimethyl-3-(3,8,12,16-tetramethyl-hep
29	18.868	0.38	0.56	8,11,14-Eicosatrienoic acid, (Z,Z,Z)-
30	19.045	0.14	0.12	Z-8-Methyl-9-tetradecen-1-ol formate
31	19.442	1.20	1.66	Octadecenamide, (Z)-
32	19.720	0.51	0.54	6,10,14,18,22-Tetracosapentaen-2-ol, 3-bromo-
33	20.196	0.25	0.21	Lup-20(29)-en-3-one
34	20.768	0.88	0.97	Hexanoic acid, undecyl ester

Peak	R.Time	% Area	%Height	Name

35	20.957	0.36	0.28	1,1,6-trimethyl-3-methylene-2-(3,6,9,13-tetra
36	21.075	0.22	0.34	Bis(2-ethylhexyl) phthalate
37	21.214	0.54	0.80	2H-Pyran-2-one, tetrahydro-6-tridecyl-
38	21.923	0.24	0.44	2,2,4-Trimethyl-3-(3,8,12,16-tetramethyl-heptadeca-3,7,11,15-tetraenyl)-cyclohexanol
39	22.192	0.57	0.24	2,5-Octadecadiynoic acid, methyl ester
40	22.410	0.68	0.95	1-Heptacosanol
41	22.863	0.20	0.35	2H-Pyran-2-one, tetrahydro-6-tridecyl-
42	23.158	0.28	0.29	Tricosyl trifluoroacetate
43	23.229	1.31	0.82	Acetic acid, [(Z,Z)-3,7,11-trimethyl-2,6,10-dodecatrien-1-yl] ester
44	23.413	**6.56**	**8.21**	**2,2,4-Trimethyl-3-(3,8,12,16-tetramethyl-heptadeca-3,7,11,15-tetraenyl)-cyclohexanol**
45	23.602	**3.32**	**4.80**	**Heptadecafluorononanoic acid, undecyl ester**
46	23.731	**5.15**	**3.68**	**1-Pyrrolidinebutanoic acid, 2-[(1,1-dimethylethoxy)carbonyl]-.alpha.-nitro-, 2,6-bis(1,1-dimethylethyl)-4-methoxyphenyl ester,**
47	23.993	**2.67**	**2.39**	**1-Heptacosanol**
48	23.993	**2.11**	**1.67**	**Eicosane**
49	24.098	**3.68**	**3.79**	**6,10,14,18,22-Tetracosapentaen-2-ol, 3-bromo-2,6,10,15,19,23-hexamethyl-, (all-E)-**
50	24.159	1.56	2.41	trans-Geranylgeraniol

Peak	R.Time	% Area	%Height	Name
51	24.199	1.41	2.20	2H-1-Benzopyran-6-ol, 3,4-dihydro-2,8-dimethyl-2-(4,8,12-trimethyltridecyl)-, [2R-[2R*(4R*,8R*)]]-
52	24.259	1.21	2.14	2,2,4-Trimethyl-3-(3,8,12,16-tetramethyl-heptadeca-3,7,11,15-tetraenyl)-cyclohexanol
53	24.292	2.17	2.11	Hexadeca-2,6,10,14-tetraen-1-ol, 3,7,11,16-tetramethyl-
54	24.485	**3.13**	**1.83**	**4-Oxo-.beta.-isodamascol**
55	24.596	**3.11**	**2.63**	**Succinic acid, di(dodec-9-yn-1-yl) ester**
56	24.676	1.79	2.35	Hexadeca-2,6,10,14-tetraen-1-ol, 3,7,11,16-tetr
57	24.739	**3.21**	**2.79**	**2,2,4-Trimethyl-3-(3,8,12,16-tetramethyl-heptadeca-3,7,11,15-tetraenyl)-cyclohexanol**
58	24.948	**7.38**	**4.46**	**beta.-Tocopherol**
59	25.059	**5.05**	**2.92**	**gamma.-Tocopherol**
60	25.271	**4.57**	**2.40**	**Cholestane-3,6-diol, (3.beta.,5.alpha.,6.alpha.,17.alpha.,20S)-**
61	25.398	2.70	1.86	Heptadecyl 2,3,4,5,6-pentafluorobenzoate
62	25.491	1.98	1.94	Stigmasta-5,22-dien-3-ol, acetate, (3.beta.)-
63	25.550	1.92	1.84	4,4,6a,6b,8a,11,11,14b-Octamethyl-1,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-octadecahydro-2H-picen-3-one
64	25.633	2.66	1.83	Hexacontane
65	25.825	**7.05**	**6.21**	**Vitamin E**
66	26.011	**3.19**	**3.37**	**.alpha.-Tocopheryl acetate**
67	26.225	2.23	0.79	Cholest-7-en-3-ol, acetate, (3.beta.)-6

Source: Author's Analysis 2023

4.2 Discussion of Findings

Since ancient times plants have been used in medicine to treat different diseases. As a result, there is an increasing interest in the study of plant bioactive compounds as potential therapeutic agents[1]. Phytochemicals, such as essential oils, containing active chemical compounds have been one of the main focuses of the design of novel anti-cancer therapies[2]. The use of *in-vitro* cytotoxicity assay to determine the degree of cytotoxic activity of plant extract using tumor and non-tumor cells is considered as specialized and a rapid screening method.

In this study, cytotoxic effect of methanol extract of selected medicinal plants *F. benjamina, E. milli, P. biglobosa, P. thonnigii, V. doniana,* and *E. heterophyilla* were investigated on human cervical cancer (HeLa), breast cancer (MCF-7), lung cancer (A549) cell lines and a non-tumorigenic cell line (KMST-6). The IC_{50} values obtained (Table 4.1) revealed that leaf extracts of *F. benjamina* and *E. milli* have high cytotoxic effect on the all cancer cell lines, while the extracts of *E. heterophylla, P. biglobosa, P. thonningii* showed very weak cytotoxicity on all the cancer cell lines. Also, it was observed that *F. benjamina* has the highest cytotoxicity in the HeLa cell line with an IC_{50} of 17.56µg/ml. These indicate the possibility of the cytotoxic active compound present in the extract inducing a plasma membrane destabilization effect on the cancer cell line either through necrosis or apoptosis which will further lead to the loss of mitochondrial dehydrogenase activities of the cell as evaluated with the MTT assay used in this study.

As at this study, no cytotoxic effect of methanol extract of *F. benjamina* leaf on HeLa, MCF-7 and A549 has been reported. However, several studies reported the cytotoxicity of *Ficus* family. A study done with the leaf oil extract of *F. religiosa* reported a marginally signal in the brine shrimp lethality test (LC_{50} 50 µg/ml) and also observed *in vitro* cytotoxic activity against MCF-7 cell

line (80% kill at 100 µg/ml)[3]. Crude aqueous and ethanolic extracts in concentrations of 0–620 µg/ml prepared from *F. religiosa* bark were evaluated in cervical cancer cell lines, SiHa and HeLa. The *in vitro* results obtained showed that both extracts produced significant cytotoxicity in cervical cancer cell lines SiHa (human papillomavirus 16 serotype– HPV16 positive) and HeLa (human papillomavirus 18 serotype– HPV18 positive)[4]. Jasmine investigated the cytotoxic effect of *F. carica* on MCF-7 cells, and several concentrations were tested. The results showed that the growth of human breast cancer cells MCF-7 is inhibited in a dose-dependent manner by the ethanolic extract from fruits of F. carica, with an IC50 of 31.2 µg/ml.[5]

In this study, extracts of *Parkia biglobosa, Piliostigma thonnigii, Vitex doniana,* and *Euphorbia heterophyilla* exhibit very low cytotoxic effects on the cancer cell lines, though it was reported by Abubarkar et al., that these plants are being used for cancer treatment by traditional medicinal practitioners in Kebbi State, Nigeria[6]. These other plants may be used as adjuvants to balance other physiological functions in the body to improve patient's well-being.

To determine whether the extract will be cytotoxic to cancer cell lines with very minimal toxicity to normal cells, the selectivity index (SI) of the extracts were determined. In this study, KMST-6 a non-tumorigenic cell line was used as normal cell lines. The result showed that *F. benjamina* has highest SI of 2.2 for HeLa cell line, (Table 4.2). Other plant extracts were found to have SI value <2.0. Selectivity Index is the ratio between IC_{50} values of KMST-6 (normal cell) and cancer cell lines. The greater the SI value, the more selective it is. SI value more than 2 indicates that the herbal extract is toxic against cancer cells but less toxic to normal cells. SI value less than 2 indicates the general toxicity of the extract[7].

Apoptosis, also known as programmed cell death, is a fundamental process required for morphogenetic homeostasis during early development and in pathophysiological conditions at

any stage during individual life. Apoptosis is an active signaling process triggered by a variety of stimuli such as deprivation of growth factors, exposure to cytotoxic drugs or DNA damaging agents, activation of death receptors, and action of cytotoxic cells. Apoptosis can be initiated via two pathways, the intrinsic and the extrinsic pathway. The process of apoptosis plays an important role in controlling cell numbers and eliminating harmful or virus-infected cells to maintain cell homeostasis throughout development. Apoptosis is tightly regulated by the family of cysteine aspartic proteases termed caspases (cysteineaspases) which function by cleaning their substrates following on as particle residue. There are two major functions assigned to caspases. While caspases 1,4,5 and 11 are primarily involved in the processing and activation of the proinflammatory cytokine, several others including caspases 2,3,6,7,8 and 9 have been implicated in the execution phase of apoptosis. Induction of apoptosis is almost always associated with the activation of caspases; therefore, measurement of caspase activity is a convenient way to assess whether the cells are undergoing apoptosis[8].

The BCL-2 family proteins are found on outer mitochondrial membranes and suppress the apoptosis process by inhibiting the release of cytochrome C from membrane permeabilization.

In this study, the BCL-2 were found to be below detection level both in treated and non treated cell lines suggesting that the extract of *F. benjamina* at the IC_{50} concentration of 17.6µg/ml did not induce or activate BCL-2 protein. It thus implies that there were no suppression of apoptosis. Anti-apoptotic protein family members maintain cell survival by regulating or maintaining the pro-apoptotic protein level[9].

In the event of apoptosis, Bax and Bak undergo homodimerization and oligomerization in outer mitochondrial membrane and release cytochrome C in the cytoplasm. Our result showed a significant increase in the level of Bax protein in breast cancer. The level of Bax protein inreases

in A549 and decreases in HeLa but not statistically significant in both cell lines. These results suggesting the possibility of the extract of *F. benjamina* leaf of inducing intrinsic apoptosis in breast cancer. (Figure 4.1)

The caspase-9 enzyme is encoded by the CASP-9 gene located on the chromosome 1[10]. Procaspase-9 which acts as an initiator caspase for the intrinsic apoptosis pathway exists as a monomer and consists of three domains: N-terminal pro-domain, large subunit, and a small subunit pi. Our results showed significant increase of caspase-9 in both HeLa and MCF-7 after 24hrs of treatment with methanol extract of *F. benjamina* leaf further suggesting the induction of apoptosis through the intrinsic pathway (Fig.4.2)

Caspases are important cysteine proteases activated during apoptosis. The prominent execution of caspases (3, 6 and 7) are capable of targeting a large number of structural and functional proteins between cells. It was observed in this study that the level of caspase -3 in the HeLa cell line increases but not statistically significant, it could be inferred that the cytotoxic effect of extract of *F. benjamina* was based on intrinsic apoptosis. The level of caspase-3 in MCF-7 and A549 was reduced but not significantly, while it was observed to be significantly increased in the non-cancerous KMST-6 cell line (Fig.4.3).

Increased reactive oxygen species (ROS) levels have been found in almost all cancers and are thought to play an important role in the initiation and progression of cancers. These highly reactive ions and molecules are produced during normal metabolism of cells but are present in higher levels in cancer cells due to increased metabolic activity, mitochondrial dysfunction, peroxisome activity, increased cellular receptor signaling, oncogene activity, increased activity of oxidases, cyclooxygenases, lipoxygenases, and thymidine phosphorylase, or through crosstalk with infiltrating immune cells. ROS are managed under normal physiological conditions, through

detoxification by non-enzymatic molecules such as glutathione, or through antioxidant enzymes, which specifically scavenge different kinds of ROS.[11]

First-line antioxidants act to suppress or prevent the formation of free radicals or reactive species in cells. There are three key enzymes: superoxide dismutase, catalase, and glutathione peroxidase are top on the list. These enzymes respectively dismutase superoxide radical, and breakdown hydrogen peroxides and hydroperoxides into harmless molecules (H2O2/alcohol and O2)[12].

In this study, it was observed that the GST activities were significantly reduced ($p \leq 0.05$) in MCF-7 and A549 after 24hrs of treatment with *F. benjamina* (Fig. 4.4). The activity was also reduced in HeLa cells but not statistically significant. Similarly, GSH levels were also observed to be significantly reduced in MCF-7 and A549 while no difference was observed in the HeLa cell line and non-cnanerous KMST-6 (Fig. 4.5). These results further suggests the increase in metabolic activities that generates ROS that lowered the activities of GST and reduced the level of GSH compared to non-cancerous cell lines.

Superoxide dismutase (SOD) constitute a very important antioxidant defense against oxidative stress. Therefore the enzyme acts as a good therapeutic agent against reactive oxygen species-mediated diseases. In this study, the results showed that the cancer cell lines treated with *F.benjamina* increases the activities of SOD in all the cell lines but most significant in breast cancer cell line and non-tumorigenic cell line (Fig.4.6). This suggest that the leaf extract of *F. benjamina* has bioactive compounds that enhances the activities of SOD. This is in concordance with the report of Almira et. al., associating the increase in the activities of SOD to phytochemicals in plant[17].

Peroxidation of membrane lipids is known to substantially alter the physical properties of lipid bilayers. In particular, peroxidation alters lipid-lipid interactions, ion gradients, membrane fluidity, and membrane permeability. In this study, it was observed that the lipid peroxidation activities were significantly reduced ($p \leq 0.05$) in all cancer cell lines treated with *F. benjamina*

compared to non–treated cancer cell lines (Fig.4,7). This protective effect of *F. benjamina* is probably due to the antioxidant activity which reduced the oxidative damage by blocking the production of free radicals and thus inhibited lipid peroxidation. The antioxidant activity of *Ficus* plants were reported by Saleh [13].

Nitric oxide is a maker of inflammation and it serves as an essential antioxidant by reacting rapidily with peroxyl radicals. After 24 hrs of treatment of the cancer cell lines and non-cancer cell line with methanol extract of *F. benjamina*, we observed that the levels of NO were significantly reduced in MCF-7, A549 and KMST-6, while there was no difference in the level of NO in HeLa (Fig. 4.8) This can be inferred that *F. benjamina* possess an anti-inflammatory activity as reported by Saleh[13].

In this study, GC-MS analysis of *F.benjamina* showed the presence of phenolic compounds and essential oil (Table 4.3). Among the most abundant compounds are alpha-Tocopheryl acetate, Vitamin E, gama-Tocopherol, beta-Tocopherol, and Phytol. Several studies have shown the anticancer effect of these compounds. Phytol was reported to have anticarcinogenic and antitumor properties[14]. Vitamin E is a lipid-soluble vitamin comprising of eight natural isoforms, namely, α, β, δ, γ isoforms of tocopherol and α, β, δ, γ isoforms of tocotrienol. Study has been performed to elucidate its role in cancer prevention[15]. Succinic acid or its derivatives, a major compound in the leaf extract of *F. benjamina* have shown to exhibit anticancer activity by inducing apoptosis [16].

Endnotes

1. M. Asadi-Samani, M. Rafieian-Kopaei, Z. Lorigooini, & H. Shirzad. *A Screening to Determine Total Phenol and Flavonoid Content of Some Iran's Medicinal Plants Grown in Chaharmahal Va Bakhtyari Province*. **Indian Journal of Natural Products and Resources.** 9, 2018, 296-302.

2. K. Blowman, M. Magalhães, M. Lemos, C. Cabral & I. Pires. *Anticancer Properties of Essential Oils and Other Natural Products.* **Evidence-Based Complementary and Alternative Medicine**. 2018, 1-12. doi:10.1155/2018/3149362

3. K. Putra, E Winanda, P. Ari, D.N Gilang, R Mahendra, & D.S Ahmad. *Phytochemical Activities of Ficus (Moraceae) In Java Island, Indonesia*. **International Journal of Bonorowo Wetlands** 10(2), 2020.

4. B. Salehi, P.M Abhay, N Manisha, K Natallia, S Ila, K.D Anna, S Barbara. *Ficus Plants: State of The Art from A Phytochemical, Pharmacological, And Toxicological Perspective.* **Phytotherapy Research** 35(3), 2021, 1187-1217.

5. R. Jasmine, K Manikandan, & K Karthikeyan. *Evaluating The Antioxidant and Anticancer Property of Ficus Carica Fruits.* **African Journal of Biotechnology**, 14(7), 2015 634–641.

6. I.B Abubakar, A.N Ukwuani-Kwaja, A.D Garba, D Singh, I Malami, T. S Salihu, S.J Ahmed. *Ethnobotanical Study of Medicinal Plants Used for Cancer Treatment in Kebbi State, North-West Nigeria.* **Acta Ecologica Sinica**, 40(4), 2020, 306–314. doi: 10.1016/J.Chnaes.2020.02.007

7. M. Wardihan, G Rusdi, L Alam, & M.A Manggau, *Selective Cytotoxicity Evaluation in Anticancer Drug Screening of Boehmeria Virgata (Forst) Guill Leaves to Several Human Cell Lines: Hela, Widr, T47D and Vero*. **Dhaka University Journal of Pharmaceutical Sciences**. 12(2), 2013, 87–90.

8. J. Kale, E.J Osterlund & D.W Andrews. *BCL-2 Family Proteins: Changing Partners in The Dance Towards Death.* **Cell Death Differ**.25(1), 2018, 65-80.

9. T.J Opferman & K Anisha. *Anti-Apoptotic BCL-2 Family Members in Development. Cell Death & Differentiation* 25(1), 2018, 37-45.

10. P. Li, L Zhou & T. Zhao. *Caspase-9: Structure, Mechanism and Clinical Application.* **Oncotarget**. 8 (14), 2017, 23996 – 24008.

11. G. Y Liou & P Storz. *Reactive Oxygen Species in Cancer.* **Free Radic. Res**. 44, 2010, 479–496.

12. O.M Ighodaro & O.A. Akinloye. *First Line Defence Antioxidants-Superoxide Dismutase (SOD), Catalase (CAT) and Glutathione Peroxidase (GPX): Their Fundamental Role in The Entire Antioxidant Defence Grid.* **Alexandria Journal of Medicine**. 54(4), 2018, 287-293.

13. B. Salehi, A. Prakash Mishra, M. Nigam, N. Karazhan, I. Shukla, A. Kiełtyka-Dadasiewicz, & J. Sharifi-Rad. *Ficus Plants: State of The Art from A Phytochemical, Pharmacological and Toxicological Perspective.* **Phytotherapy Research**. 35(3), 2021, 1187-1217. doi:10.1002/Ptr.6884

14. M. V. O. B De Alencar, M. T Islam, R. M. T De Lima, M. F. C. J Paz, A. C Dos Reis, A. M. O. F Da Mata, J.W.G.D.O. Filho, G.S. Cerqueira, P.M.B. Ferreira, J.M.D.C E Sousa, & M.S. Mubarak. *Phytol as an Anticarcinogenic and Antitumoral Agent: An in Vivo Study in Swiss Mice with DMBA-Induced Breast Cancer.* **IUBMB Life.** 71(2), 2019, 200-212. Doi:10.1002/Iub. 1952.

15. A. Abraham. A.J Kattoor, T. Saldeen, & J.L Mehta. *Vitamin E and Its Anticancer Effects.* **Critical Reviews in Food Science and Nutrition**, 2018, 01–23. Doi:10.1080/10408398.2018.1474169

16. G. Kasarci, B. Ertugrul, E.S Iplik & B. Cakmakoglu. *The Apoptotic Efficacy of Succinic Acid on Renal Cancer Cell Lines.* **Med Oncol**. 38(12), 2021, 144. Doi: 10.1007/S12032-021-01577-9. PMID: 34687367.

17. V.G. Almira, N. Widyastuti, G. Anjani & N.N Widyastiti. *The Increasing of Superoxide Dismutase (SOD) In Metabolic Syndrome Rat by Robusta Coffee Leaves (Coffea Canephora).* **Food Research** 4 (3), 2020, 155-162.

Chapter Five

Conclusion

5.1 Summary of Findings

Medicinal plants have been in continuous use over the years for the management of cancer particularly in most developing countries. Phytochemicals and derivatives present in plants are promising options to improve treatment efficiency in cancer patients and decrease adverse reactions. Also, a number of phytochemicals isolated from medicinal plants have been shown to decrease cell proliferation, induce apoptosis, retard metastasis and inhibit angiogenesis.

In this study, six medicinal plants were selected (*Ficus benjamina*, *Euphorbia milli, Parkia biglobosa, Piliostigma thonnigii, Vitex doniana,* and *Euphorbia heterophyilla)* from Ibadan, South West Nigeria which have been reported in research articles to be indigenous folklore medicine to have anti-cancer properties. These plants were investigated for their *in vitro* cytotoxic effect on human cervical cancer, breast cancer and long cancer cell lines. The plant parts such as leaf or bark were extracted with methanol accordinly. The cancer cell lines were treated with the plant extracts for 72hrs. At the expiration of 72hrs, cytotoxicity effects were evaluated with the use of MTT assay. The effect of methanol extract of *F. benjamina* leaf that has highest cytotoxicity was evaluated for the possibility of apoptosis in the all the cell lines with the use of ELISA kits. Also, the effect of methanol extract of *F.benjamina* leaf on the antioxidant makers were biochemically assayed for the all the cell lines. Bioactive compounds of the methanol extract of *F. Benjamina* leaf was analysed with the use of GC-MS.

The results from this study showed that extracts of *F.benjamina* and *Euphorbia milli* have high cytotoxic effect on all the cancer cell lines while extract of the other plants showed low cytotoxic effect. Selectivity index which is the ratio of cytotoxicity in non-cancer cell lines to cancer cell lines showed that the leaf extract of *F. benjamina* is more selective to human cervical cancer cell than non-cancerous cells. Increase in the level of caspase-9 and caspase-3 on the treated cervical cancer cells showed the induction of apotosis by the leaf extract of *F.bejamina.* The acitivity of SOD was higher in treated MCF-7 and unaltered in HeLa and A549 showed that ROS were scavenged. LPO activities and NO level were lowered in all the treated cancer cell lines compared with non-treated cell lines indicates the antioxidant and antiflammatory protective effect of the extract of *F.benjamina* leaf. The GC-MS analysis of leaf extract of *F. benjamina* revealed the presence of Terpenoids (Eicosyne and Eicosane), Phenolics compounds (Phytol and Tocopherol) which are phytochemicals with anticancer properties.

5.2 Conclusion

This study showed that, of the six plant extracts used, *F. benjamina* has the highest cytotoxic effect on human cancer cervical cancer cell line. The extract is more selective to cervical cancer cells and less toxic to non-cancer (normal) cell line. The cytotoxicity was as a result of apoptosis induction as revealed by the increase in the levels of apoptosis makers. There were statistically significant reduction in the activities of lipid peroxidation and the level of nitric oxide in all the cancer cell line after the treatment with leaf extract of *F.benjamina*. The presence of flavonoid, terpenoid and phenolic compound indicates that leaf possesses antioxidants and antiflammatory activity. Therefore, the leaf of *F.benjamina* could be a potential and safe anticancer drug against human cervical cancer cell lines.

5.3 Recommendations

1. Medicinal plants required laboratory investigations to know the levels of their cytotoxicity before use as alternative therapy for the treatment of cancer.
2. There is a need to develop interaction between the researchers and traditional users of medicinal plants for proper orientation on the appropriate herbs for different cancer treatment.

5.4 Contributions to Knowledge

1. This study has established that the leaf of *Ficus benjamina* has cytotoxic effect on human cervical cancer cells, and it is less toxic to non-cancer (normal) human cells.
2. This study showed that *Parkia biglobosa, Piliostigma thonnigii, Vitex doniana, and Euphorbia heterophyilla* has low cytotoxic effects on human cervical cancer, breast cancer and lung cancer cells.

5.5 Suggested Areas for Further Research.

1. *In silico* and *in vivo* study involving molecular techniques on the cytotoxic effect of *F. benjamina* leaf on human cervical cancer cell line should be investigated.

Bibliography

Books

Aslantürk, Ö. S. *In Vitro Cytotoxicity and Cell Viability Assays: Principles, Advantages, And Disadvantages.* **Genotoxicity-A Predictable Risk to Our Actual World** 2 ,2018, 64-80.

Barile F.A, *Principles of Toxicology Testing*. **Boca Raton, FL: CRC Press (Taylor and Francis Group);** 2008.

Freshney, R.I*., Culture of Animal Cells: A Manual of Basic Technique And Specialized Applications*. 6th Ed. **Hoboken, NJ: John Wiley &Sons, Inc**.; 2010.

Koron, R., *DNA Signatures as A Predictor of Breast Cancer Risk*. **The University of Liverpool (United Kingdom).**

Pecorino, L. *Molecular Biology of Cancer: Mechanisms, Targets, and Therapeutics*. **Oxford University Press,** 2021.

Sofowora A.E, *Medicinal Plants and Traditional Medicines In Africa*. 2nd Ed. Ibadan, **Nigeria**: **Spectrum Books;** 1993, 289.

Conference

Rahakbauw, E., Winarto, H., *Radiotherapy Response and Related Clinicopathological Factors of Patients with Cervical Cancer*, In: **Journal of Physics: Conference Series**, 2018, https://doi.org/10.1088/1742-6596/1073/3/032040

Internet

Jain, V., & Bhardwaj, A. *Pneumonia, Pathology*. **In: StatPearls** [Internet]. Treasure Island, FL: StatPearls Publishing; 2018.

Riss, T, Andrew N, Rich M, Natashia K, and Jolanta V. *Cytotoxicity Assays: In Vitro Methods to Measure Dead Cells.* **Assay Guidance Manual** [Internet]; 2019, 95.

World Health Organization (WHO) *Cancer Fact Sheet, Breast Cancer.* Retreived 01 Jul, 2019, from http://gco.iarc.fr/today/data/factsheets/cancers/20-Breast-fact-sheets.pdf

World Health Organization (WHO) *CANCER*. 12, 2018.

World Health Organization (WHO). *Cancer Fact Sheet, Breast Cancer.*2018. Retreived 01 Jul, 2019, from http://gco.iarc.fr/today/data/factsheets/cancers/20-Breast-fact-sheets.pdf.

World Health Organization (WHO). *Research Guidelines for Evaluating the Safety and Efficacy of Herbal Medicines*. Manila: **WHO Regional Office for the Western Pacific**; 1993.

World Health Organization (WHO). *In Progress Report by The Director General, Document No. A44/20. Geneva*; **Toxic Plants Used in African Traditional Medicine.** 1991.

Journals

Abbas, M. S., Hattem, M. E, Amira, S. S, & Mai, A. S., *Optimization of Germination, Callus Induction, And Cell Suspension Culture of African Locust Beans Parkia biglobosa (Jacq.) Benth*." **Journal of Genetic Engineering and Biotechnology** 16(1) 2018, 191-201.

Abotaleb, M., Samson, M. S., Elizabeth, V., Sharon, V., Peter, K., Alena, L., & Dietrich, B. *Flavonoids in Cancer and Apoptosis.* **Cancers** 11(1), 2018, 28.

Abraham, A., Kattoor, A. J., Saldeen, T., & Mehta, J. L. *Vitamin E and Its Anticancer Effects.* ***Critical Reviews in Food Science and Nutrition***, 2018, 01–23. doi:10.1080/10408398.2018.1474169

Abubakar, I. B., Ukwuani-Kwaja, A. N., Garba, A. D., Singh, D., Malami, I., Salihu, T. S. & Ahmed, S. J. *Ethnobotanical Study of Medicinal Plants Used for Cancer Treatment in Kebbi State, North-West Nigeria.* **Acta Ecologica Sinica.** 40(4), 2020, 306–314. doi: 10.1016/J.Chnaes.2020.02.007

Adefegha, S. A. *Functional Foods and Nutraceuticals as Dietary Intervention in Chronic Diseases; Novel Perspectives for Health Promotion and Disease Prevention.* **Journal of Dietary Supplements** 15(6), 2018, 977-1009.

Afolayan, M.O., Omosimua, R.O., Fadeyi, A.E., Aguzue, O.C., Asekun, O.T. & Familoni, O.B., *Phytochemical, Antioxidant, And Flavonoid Investigation of Methanolic Leaf Extract of Piliostigma Thonningii (Schum.)*. **In Natural Product Experiments in Drug Discovery.** 2022,1-26

Airaodion, A. I., Edith, O., Airaodion, E. O., Ogbuagu, U. O., & Etinosa, U. O. *Effect of Oral Intake of African Locust Bean On Fasting Blood Sugar and Lipid Profile of Albino Rats*. **Asian Journal of Research in Biochemistry.** 4(4), 2019,1-9.

Ajao, A. A., Yusuf, O. M., & Saheed, S. *Wandering Through Southwestern Nigeria: An Inventory of Yoruba Useful Angiosperm Plants*. **Heliyon**: 2021, 08668.

Alexander, M., Kim, S.Y. & Cheng, H. *Management of Non-Small Cell Lung Cancer.* **Lung** 198, 2020, 897–907. https://doi.org/10.1007/S00408-020-00407-5

Ali, M., F. Aminu, & I. S. Ibrahim. *In-Vitro Assessment of Antibacterial Activity and Phytochemical Screening of Vitex Doniana on Clinical Isolate of Salmonella Typhi*. **IN-VITRO** 3(1), 2017.

Allison, K., & Mooney, K., *The Spectrum of Risk Lesions in Breast Pathology: Risk Factors or Cancer Precursors*? **Atypical Breast Proliferative Lesions and Benign Breast Disease.** 2018, 1-9.

Almira, V.G., Widyastuti, N., Anjani, G., Widyastiti, N.N. *The Increasing of Superoxide Dismutase (SOD) In Metabolic Syndrome Rat by Robusta Coffee Leaves* (Coffea *Canephora*)**. Food Reasearch** 4(3), 2020,155-162.

American Cancer Society. *Cancer Facts & Figures*. Atlanta, GA: **American Cancer Society**; 2019.

American Cancer Society. *Global Cancer Facts & Figures.* 4th Edition. Atlanta, GA: **American Cancer Society**; 2018.

Amjad, M. T., Anusha C., & Anup, K. *Cancer Chemotherapy*. Study Guide from **Statpearls Publishing**, **Treasure Island** (FL). 26, 2020.

Angelo, G., Lorena, C., Marta, G. & Antonella, C. *Biochemical Composition and Antioxidant Properties of Lavandula Angustifolia Miller Essential Oil Are Shielded by Propolis Against UV Radiations*. **Photochemistry and Photobiology,** 90, 2014, 702-708. http://dx.doi.org/10.1111/Php.12229

Arbyn, M. Weiderpass, E. Bruni, L. De Sanjos´E, S. Saraiya, M. Ferlay, J. & Bray, F. *Estimates of Incidence and Mortality of Cervical Cancer in 2018*: *A Worldwide Analysis*, **Lancet Global Health,** 2020. https://doi.org/10.1016/S2214-109X(19) 30482-6.

Arodin S., Lisa, M., Wallenberg, L., Rim, J., Olof, D., & Mikael, B., *The Cell Culture Medium Affects Growth, Phenotype Expression and The Response to Selenium Cytotoxicity in A549 And Hepg2 Cells*. **Antioxidants** 8(5), 2019, 1309.

Asadi-Samani, M., Rafieian-Kopaei, M., Lorigooini, Z., & Shirzad, H., *A Screening to Determine Total Phenol and Flavonoid Content of Some Iran's Medicinal Plants Grown in Chaharmahal Va Bakhtyari Province*. **Indian Journal of Natural Products and Resources**. 9, 2018, 296-302.

Ashraf, A. S., Zafar, M. Yasin A, Muhammad, U. I., Saima, M., Farkhanda, A., Farhat, J., & Muhammad, S. *Phytochemical Composition and Potent Biological Activities of Ficus Benjamina Var. Comosa Leaves Extract*. **Pakistan Journal of Botany.** 52(2), 2020, 531-535.

Attama, A.A, Nnamani, P.O, Onokala O.B, Ugwu, A.A, & Onugwu, A.L. *Nanogels as Target Drug Delivery Systems in Cancer Therapy: A Review of the Last Decade.* **Front Pharmacol**. 8(13), 2022, 874510. doi: 10.3389/Fphar.2022.874510.

Ayaz, M., Abdul, S., Muhammad, J., Farhat, U., Muhammad, O., Ikram, U., Jawad, A., & Muhammad, S., *Flavonoids as Prospective Neuroprotectants and Their Therapeutic Propensity in Aging Associated Neurological Disorders.* **Frontiers in Aging Neuroscience** 11, 2019, 155.

Balasubramaniam, S.D., Balakrishnan, V., Oon, C.E., & Kaur, G., *Key Molecular Events in Cervical Cancer Development.* **Medicina (Lithuania).** 2019, Https://Doi.Org/10.3390/ Medicina55070384.

Balogun, W. G., Ismail A. A., Usman, Y., & Azman, S. *A Review of the Phytochemistry and Medicinal Activities of the Popular African Food Additive: Parkia Biglobosa Seed.* **Oriental Pharmacy and Experimental Medicine** 18(4), 2018, 271-279.

Barnard, M.E, Boeke, C. E.& Tamimi, R.M. *Established Breast Cancer Risk Factors and Risk of Intrinsic Tumor Subtypes*. **Biochim Biophys Acta**.1856(1), 2015,73-85.

Barta, J.A, Powell C.A, Wisnivesky J.P. *Global Epidemiology of Lung Cancer*. **Ann Glob Health**. 85(1), 2019, 8. doi: 10.5334/Aogh.2419.

Baskran, K. Kumar, P.K. Santha, K. & Sivakamasundari II. *Cofactors and Their Association with Cancer of the Uterine Cervix in Women Infected with High-Risk Human Papillomavirus in South India.* **Asian Pac J Cancer Prev.** 20(11), 2019, 3415-3419. doi: 10.31557/apjcp.2019.20.11.3415.

Batool, I., Nisar S, Hamrouni L, & Jilan M.I. *Extraction, Production and Analysis Techniques for Menthol: A Review*. **International Journal of Chemical and Biochemical Sciences**. 14, 2018, 71-76.

Beutler, E, Duron, O & Kelly, B.M. *Improved Method for The Determination of Blood Glutathione*. **J Lab Clin Med.** 61, 1963, 882-8.

Bhatla, N., Aoki, D., Sharma, D.N., & Sankaranarayanan, R., *Cancer of The Cervix Uteri*, **Int. J. Gynecol. Obstet.** 143, 2018, 22–36. https://doi.org/10.1002/Ijgo.12611.

Blowman, K, Magalhães, M, Lemos, M, Cabral, C, & Pires, I. *Anticancer Properties of Essential Oils and Other Natural Products.* **Evidence-Based Complementary and Alternative Medicine.** 2018, 1-12. Doi:10.1155/2018/3149362.

Bray F, Ferlay J, Soerjomataram I, Siegel R.L, Torre L.A & Jemal A. *Global Cancer Statistics: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers In 185 Countries.* **CA Cancer J Clin.** 68, 2018, 394–424.

Bray, F., Laversann, M., Weiderpass & E., Soerjomataram, I., *The Ever-Increasing Importance of Cancer as A Leading Cause of Premature Death Worldwide*. **Cancer.** 127(16), 2021, 3029-3030.

Brett H. Herzog, Siddhartha D, & Ramaswamy G, *Overcoming Chemotherapy Resistance in SCLC,* **Journal of Thoracic Oncology**, 16(12), 2021, 2002-2015.

Brianese, R.C, Nakamura K.D.M., & Almeida, F. *BRCA1 Defi-Ciency Is a Recurrent Event in Early-Onset Triple-Negative Breast Cancer: A Comprehensive Analysis of Germline Muta-Tions and Somatic Promoter Methylation*. **Breast C Ancer Res Treat**.167(3), 2018, 803-814

Britt, K.L, Cuzick, J., & Phillips, K.A. *Key Steps for Effective Breast Cancer Prevention.* **Nature Reviews Cancer**.20(8), 2020, 417- 436

Builders, M. I., Oyepata, S.J., & Akpobome, R.V., *Effect of Parkia Biglobosa Extract on Open Skin Wound Healing in Dexamethasone-Induced Hyperglycaemia and Histological Assessment in Rats.* **African Journal of Pharmacy and Pharmacology (AJPP**)13(8), 2019.

Bukowski, K., Kciuk, M. & Kontek, R. *Mechanisms of Multidrug Resistance in Cancer Chemotherapy*. **International Journal of Molecular Sciences**, 21(9), 2020, 3233.

Burmeister, C.A., Khan, S.F., Schäfer, G., Mbatani, N., Adams, T., Moodley, J., & Prince, S., *Cervical Cancer Therapies: Current Challenges and Future Perspectives*. **Tumour Virus Research**, 2022, 200238.

Cannon-Albright, L.A., Teerlink, C.C., Stevens, J., Facelli, J.C., Carr, S.R., Allen-Brady, K., Puri, S., Bailey-Wilson, J.E., & Musolf, A.M., *Genetic Epidemiology of Lung Cancer Consortium* & Musolf, A.M. *A Rare FGF5 Candidate Variant (Rs112475347) For Predisposition to Non-Squamous, Non-Small-Cell Lung Cancer*. **International Journal of Cancer,** 2023

Čermák, V., Vojtěch, D., Michael, J., Lenka, L., Jan, K., Daniel Rösel, & Jan Brábek. *Microtubule-Targeting Agents and Their Impact on Cancer Treatment.* **European Journal of Cell Biology** 99(4), 2020, 15107

Chen, C.C., Feng, W., Lim, P.X, Kass, E.M., & Jasin, M., *Homology-Directed Repair and The Role of BRCA1, BRCA2, And Related Proteins in Genome Integrity and Cancer*. **Annual Review of Cancer Biology.** 2, 2018, 313-336.

Chime, S. A., & Chika, P. M. *Herbal Medicines as Potential Immune Boosters Against Coronavirus Diseases*. **Current Traditional Medicine** 9(2), 2023, 64-74.

Choudhari, A. S., Pallavi, C. M., Manasi, D., Prabhakar, R., & Om, P. *Phytochemicals in Cancer Treatment: From Preclinical Studies to Clinical Practice.* **Frontiers in Pharmacology.** 10, 2020, 1614.

Chukwunonye, U., Christopher, E., Obi, P. E., Kenne, T. M. & Anze, S. P. G. *Phytochemical Screening and Antimicrobial Activity of Methanol Extract and Fractions of the Leaf of Piliostigma Thonningii Schum (Caesalpiniaceae)*. **World Applied Sciences Journal** 35(40), 2017, 621-625.

Cicka, D., & Quave, C. *Bioprospecting for Pharmaceuticals: An Overview and Vision for Future Access and Benefit Sharing.* In: Joshee, N., Dhekney, S., Parajuli, P. (Eds) **Medicinal Plants. Springer, Cham**. 2019. https://doi.org/10.1007/978-3-030-31269-5_2.

Çoban, E.A., Tecimel, D., Şahin, F., & Deniz, A.A.H. *Targeting Cancer Metabolism and Cell Cycle by Plant-Derived Compounds.* In: Turksen, K. (Eds) **Cell Biology and Translational Medicine, Volume 8. Advances in Experimental Medicine and Biology**, 1247,2019. Springer, Cham. https://doi.org/10.1007/5584_2019_449.

Cohen, P.A. Jhingran, A. Oaknin, & A. Denny, L. *Cervical Cancer,* **Lancet.** 2019. https://doi.org/10.1016/S0140-6736(18)32470-X

Compaore, S., Lazare B., Achille, H., Rodrigue, Idohou, I. Z., Sylvin O., & Adjima T. *Diversity of Plants Used in The Management of Hypertension by Three Associations of Traditional Healers Along a Climate Gradient in Burkina Faso.* **Advances in Traditional Medicine** 21, 2021, 151-162.

Condello, M., Evelin P., Michele, C., & Stefania, M. *Targeting Autophagy to Overcome Human Diseases.* **International Journal of Molecular Sciences** 20(3), 2019, 725.

Copson, E.R, Maishman, T.C, & Tapper W.J. *Germline BRCA Mutation and Outcome in Young-Onset Breast Cancer (POSH): A Prospective Cohort Study*. **Lancet Oncol**.19(2), 2018, 169-180.

Costantino, C., Alba, D., Cimino, L., Conforto, A. & Mazzucco, W., *The Role of Vaccination and Screening in Limiting the Worldwide Disease Burden of Preventable Female Cancers: A Review***. Women**, 1(1), 2020, 2.

Crouch, S.P, Kozlowski, R., Slater K.J, & Fletcher, J. *The Use of ATP Bioluminescence as A Measure of Cell Proliferation and Cytotoxicity.* **J Immunol Methods**.160, 1993,81-8.

De Alencar, M.V.O.B., Islam, M.T., De Lima, R.M.T., Paz, M.F.C.J., Dos Reis, A.C., Da Mata, A.M.O.F., Filho, J.W.G.D.O., Cerqueira, G.S., Ferreira, P.M.P., E Sousa, J.M.D.C. & Mubarak, M.S., *Phytol as an Anticarcinogenic and Antitumoral Agent: An In Vivo Study in Swiss Mice With DMBA-Induced Breast Cancer***. IUBMB Life,** 71(2), 2019, 200-212.

De Cicco, P., Catani, M.V, Gasperi, V., Sibilano M., Quaglietta, M., & Savini, I. *Nutrition and Breast Cancer: A Literature Review on Prevention, Treatment and Recurrence.* **Nutrients.**11(7), 2019, 1514.

De Freitas, T., João, B., Antonio, J., Ribeiro Da Silva, & Ricardo, M. K. *Isolation and Characterization of Polyphenols from Euphorbia Heterophylla L. (Euphorbiaceae) Leaves*. **Revista Fitos** 13, 1, 2019, 49-60.

De Groot P.M., Wu C.C., Carter, B.W., & Munden, R.F., *The Epidemiology of Lung Cancer.* **Transl Lung Cancer Res.**;7(3), 2018, 220-233. doi: 10.21037/Tlcr.2018.05.06. PMID: 30050761; PMCID: PMC6037963

Dean, L.T., Gehlert, S, & Neuhouser, M.L. *Social Factors Matter in Cancer Risk and Survivorship.* **Cancer Causes Control.** 29, 2018, 611–618. https://doi.org/10.1007/S10552-018-1043-Y.

Deepa, P., Kandhasamy, S., Songmun, K., & Se, J. P., *A Role of Ficus Species in The Management of Diabetes Mellitus: A Review.* **Journal of Ethnopharmacology.** 215, 2018, 210-232.

Del Re, M., Latiano, T., & Fidilio, L. *Unusual Gastrointestinal and Cutaneous Toxicities by Bleomycin, Etoposide, And Cisplatin: A Case Report with Pharmacogenetic Analysis to Personalize Treatment.* **EPMA Journal.** 8, 2017, 69–73. https://doi.org/10.1007/S13167-017-0080-Z

Deveoğlu, O., & Recep, K., *A Review on The Flavonoids–A Dye Source*. **International Journal of Advances in Engineering and Pure Sciences.** 31(3), 2019, 188-200.

Dirks, W.G., *Ethical Challenges Using Human Tumor Cell Lines in Cancer Research.* **In Ethical Challenges in Cancer Diagnosis and Therapy**. 2021, 39-46

Durand, P. M., Marcelo, M., Barreto, F., & Richard, E. M., *Focus: Death: Cell Death in Evolutionary Transitions in Individuality.* **The Yale Journal of Biology and Medicine.** 92(4), 2019, 651.

Ebrahim, H. Y., Shaimaa, A. O., Hesham, R. H., & Zeinab, A. H., *In-Vitro Screening of Some Plant Extracts for Their Potential Anticancer Activity.* **African Journal of Traditional, Complementary and Alternative Medicines.** 17(1), 2020, 1-8.

Elío, J., Crowley, Q., Scanlon, R., Hodgson, J. & Zgaga, L. *Estimation of Residential Radon Exposure and Definition of Radon Priority Areas Based on Expected Lung Cancer Incidence.* **Environment International**. 114, 2018, 69-76.

Elmenier, F.M, Lasheen, D.S, & Abouzid, K.A., *Phosphatidylinositol 3 Kinase (PI3K) Inhibitors as New Weapon to Combat Cancer*. **European Journal of Medicinal Chemistry**.183, 2019, 111718.

Ezealigo, U.S., Ezealigo, B.N., Plaza, M.G., Dim, E.N., Kemausuor, F., Achenie, L.E.K. & Onwualu, A.P., *Preliminary Characterisation and Valorisation of Ficus benjamina Fruits for Biofuel Application*. **Biomass Conversion and Biorefinery**. 2022, 1-12.

Fait, T. *Menopause Hormone Therapy: Latest Developments and Clinical Practice*. **Drugs in Context.** 2019,8.

Feng, D., Sitian, W., Jun, Z.Y., Chen, L., Yeshe, W., Hongbo, & Xiaowu, Z., *Human Papillomavirus Prevalence and Genotype Distribution Landscapes in Shannan City, Tibet Tibetan Autonomous Region, China.* **Virology Journal.** 19(1), 2022, 1-7.

Feng, Y., Spezia, M., Huang, S., Yuan, C., Zeng, Z., Zhang, L., Ji, X., Liu, W., Huang, B., Luo, W. & Liu, B., *Breast Cancer Development and Progression: Risk Factors, Cancer Stem Cells, Signaling Pathways, Genomics, And Molecular Pathogenesis.* **Genes & Diseases.** 5(2), 2018, 77-106.

Galluzzi, L., Vitale, I., Aaronson S.A., Abrams, J.M., Adam, D., Agostinis, P., Alnemri, E.S., Altucci, L., Amelio, I., Andrews, D.W. & Annicchiarico-Petruzzelli, M. *Molecular Mechanisms of Cell Death: Recommendations of The Nomenclature Committee on Cell Death* 2018. **Cell Death Difer.** 25(3), 2018,486–541. https://doi.org/10.1038/S41418-017-0012-4.

Gao, G., Jintu, W., Jan, L., Kasperbauer, N. M., Tombers, F. T., Honglan, G., Yonggang, Z., Zhenhong, B., & David, I. S., *Whole Genome Sequencing Reveals Complexity in Both HPV Sequences Present and HPV Integrations in HPV-Positive Oropharyngeal Squamous Cell Carcinomas.*" **BMC Cancer** 19, 2019, 1-15.

Ghaderi, A., Nasehghafoori, P., Rasouli-Azad, M., Sehat, M., Mehrzad, F., Nekuei, M., Aaseth, J., Banafshe, H.R., & Mehrpour, O. *Examining of Thallium in Cigarette Smokers*. **Biological Trace Element Research**. 182, 2018, 224-230

Ghosh, S., *Cisplatin: The First Metal Based Anticancer Drug.* **Bioorganic Chemistry**. 88, 2019, 102925.

Giannakeas, V., & Steven, A.N. *The Expected Benefit of Preventive Mastectomy on Breast Cancer Incidence and Mortality in BRCA Mutation Carriers, by Age at Mastectomy.* **Breast Cancer Research and Treatment** 167, 2018, 263-267.

Giaquinto, A.N., Miller, K.D., Tossas, K.Y., Winn, R.A., Jemal, A. & Siegel, R.L., *Cancer Statistics for African American/Black People 2022.* **CA: A Cancer Journal for Clinicians**, 72(3), 2022, 202-229.

Ginikachukwu, U., Onugwu, O., Chukwujindu, C. E., & Obi, P. E., *Pharmacognostic Study of the Leaves of Piliostigma Thonningii Schum (Ceasalpiniaceae).* **Journal of Advances in Medical and Pharmaceutical Sciences**. 2021,1-9.

Gordon, J. L., Mark, A. B., & Melissa, M. R., *Cell-Based Methods for Determination of Efficacy for Candidate Therapeutics in The Clinical Management of Cancer*. **Diseases.** 6(4), 2018, 85.

Green, D.R, & Llambi, F., *Cell Death Signaling.* **Cold Spring Harb Perspect Biol.**7(12), 2015, A006080. https://doi.org/10.1101/

Gudipaty, S. A., Christopher, M. Conner, J. R., & Denise, J. M. *Unconventional Ways to Live and Die: Cell Death and Survival in Development, Homeostasis, And Disease.* **Annual Review of Cell and Developmental Biology** 34, 2018, 311.

Gupta, M., Naresh, S., Monica, G., Reena, G., Kalvatala, S., & Bhupinder, K. *Herbal Bioactives in Treatment of Inflammation: An Overview.* **South African Journal of Botany.** 143, 2021, 205-225.

Gupta, S., Kumar, P., & Das, P.C., *HPV: Molecular Pathways and Targets.* **Current Problems in Cancer,** 42(2), 2018, 161-174. https://doi.org/10.1016/J.Currproblcancer.2018.03.003.

Habig, W.H, Pabst M.J, & Jakoby, W.B. *Glutathione-S-Transferases. The First Enzymatic Step in Mercapturic Acid Formation.* **J Biolchem.** 249,1974, 7130–7139.

Hanif, M.A., Nisar, S., Khan, G.S., Mushtaq, Z., & Zubair, M., *Essential Oils*. In **Essential Oil Research, Springer**: 2019, 3-17.

Hausen, H.Z., *Papillomaviruses and Cancer: From Basic Studies to Clinical Application.* **Nat. Rev. Cancer.** 2, 2002,342–350. https://doi.org/10.1038/ Nrc798.

Hecht, S.S. & Hatsukami, D.K., *Smokeless Tobacco and Cigarette Smoking: Chemical Mechanisms and Cancer Prevention*. **Nature Reviews Cancer**, 22(3), 2022, 143-155.

Heeren, A.M., Van Luijk, I.F., Lakeman, J., Pocorni, N., Kole, J., De Menezes, R.X., Kenter, G.G., Bosse, T., De Kroon, C.D. & Jordanova, E.S., *Neoadjuvant Cisplatin and Paclitaxel Modulate Tumor-Infiltrating T Cells in Patients with Cervical Cancer.* **Cancer Immunology, Immunotherapy**, 68, 2019,1759-1767.

Hirte, H., Kennedy, E.B., Elit, L., & Fung, M.F.K., *Systemic Therapy for Recurrent, Persistent, Or Metastatic Cervical Cancer: A Clinical Practice Guideline,* **Curr. Oncol**. 2015. https://doi.org/10.3747/Co.22.2447.

Horeweg, N., Mittal, P., Gradowska, P.L., Boere, I., Nout, R.A. & Chopra, S., *A Systematic Review and Meta-Analysis of Adjuvant Chemotherapy After Chemoradiation for Locally Advanced Cervical Cancer*. **Critical Reviews in Oncology/Hematology.** 2022:103638.

Huang, C. Y., Ju, D. T., Chang, C. F., Muralidhar Reddy, P. & Velmurugan, B. K. *A Review on the Effects of Current Chemotherapy Drugs and Natural Agents in Treating Non-Small Cell Lung Cancer.* **Biomedicine (Taipei),** 7, 2017, 23.

Hull, R., Mbele, M., Makhafola, T., Hicks, C., Wang, S.M., Reis, R.M., Mehrotra, R., Mkhize-Kwitshana, Z., Kibiki, G., Bates, D.O. & Dlamini, Z., *Cervical Cancer in Low and Middle-Income Countries*. **Oncology Letters***,* 20(3), 2020, 2058-2074.

Ibragimova, M., Tsyganov, M., Shpileva, O., Churuksaeva, O., Bychkov, V., Kolomiets, L., & Litviakov, N. *HPV Status and Its Genomic Integration Affect Survival of Patients with Cervical Cancer.* **Neoplasma** 65(3), 2018, 441-448.

Ifeanacho, M. O., Odoguwa, S.C., & Amadi, P.U. *Phytochemical Composition of Vitex Doniana.* **Analytical Chemistry Letters** 9, 6(2), 2019, 1.

Ighodaro, O. M., & Akinloye, O. A. *First Line Defence Antioxidants-Superoxide Dismutase (SOD), Catalase (CAT) And Glutathione Peroxidase (GPX): Their Fundamental Role in The Entire Antioxidant Defence Grid.* **Alexandria Journal of Medicine**.54(4), 2018, 287-93doi: 10.1016/J.Ajme.2017.09.

Iwu, M.M., Duncan A.R., & Okunji C.O., *New Antimicrobials of Plant Origin. In: Janick J, Editor. Perspectives in New Crops and New Uses*. Alexandria, VA**: ASHS Press**; 1999, 457-462.

Jasmine, R., Manikandan, K., & Karthikeyan, K. *Evaluating the Antioxidant and Anticancer Property of Ficus Carica Fruits*. **African Journal of Biotechnology**, 14(7), 2015, 634–641.

Jassem, J., *Tobacco Smoking After Diagnosis of Cancer: Clinical Aspects*. **Translational Lung Cancer Research,** 8(1), 2019, S50.

Johari, S., & Ashwani, K. *Euphorbia Spp. and Their Use in Traditional Medicines: A Review*. **World J. Pharm**. **Res.** 9, 2020, 1477-1485.

Johnson, C.A. James, D. Marzan, A. & Armaos, M. *Cervical Cancer: An Overview of Pathophysiology and Management*. **Semin. Oncol. Nurs.** 35(2), 2019, 166-174.

Jucá, M. M., Francisco, M. S. C. F, Cunha De Almeida, J., Dara Da Silva, M. Rodrigues De Moraes B. J., Kátia, C. F. D., & Talita, M. B. *Flavonoids: Biological Activities and Therapeutic Potential*. **Natural Product Research.** 34(5), 2020, 692-705.

Kale, J, Osterlund E. J, & Andrews, D.W. *BCL-2 Family Proteins: Changing Partners in The Dance Towards Death*. **Cell Death Differ**.25 (1), 2018, 65-80. doi:10.1038/cdd.2017.186.

Kamiloglu, S., Sari, G., Ozdal, T. & Capanoglu, E., *Guidelines for Cell Viability Assays.* **Food Frontiers,** 1(3), 2020, 332-349.

Kasarci, G., Ertugrul, B, Iplik, E.S, & Cakmakoglu, B. *The Apoptotic Efficacy of Succinic Acid On Renal Cancer Cell Lines.* **Med Oncol**. 38(12), 2021,144. Doi: 10.1007/S12032-021-01577-9.

Khalid, N., & Mahzad, A. *Necrosis.* Study Guide from **Statpearls Publishing**, **Treasure Island** (FL). 4, 2020.

Kilpatrick, K., Jaime, L., Shaw, R. J., Andrew, T., Melissa, E., Laura, S., & Gerald, A. S. *Occurrence and Management of Thrombocytopenia in Metastatic Colorectal Cancer Patients Receiving Chemotherapy: Secondary Analysis of Data from Prospective Clinical Trials***. Clinical Colorectal Cancer.** 20(2), 2021, 170-176.

Kim, A.S., Ko, H.J., Kwon, J.H, & Lee, J.M. *Exposure to Secondhand Smoke and Risk of Cancer in Never Smokers: A Meta-Analysis of Epidemiologic Studies*. **Int J Environ Res Public Health.** 15(9), 2018,1981**.**

Kim, S.W.; Kim, S.-J.; Langley, R.R.; & Fidler, I.J. Modulation of The Cancer Cell Transcriptome by Culture Media Formulations and Cell Density. **Int. J. Oncol**. 46, 2015, 2067–2075.

Ko, K.P, Kim S.J, & Huzarski, T. *The Association Between Smoking and Cancer Incidence in BRCA1 and BRCA2 Mutation Carriers.* **Int J Cancer**.142(11), 2018, 2263-2272.

Koh, W.J., Abu-Rustum,N.R., Bean, S., Bradley, K., Campos, M.S., Cho, K.R., Chon, H.S., Chu, C., Clark,R., Cohn, D.,Crispens, M.A., Damast, S O. Dorigo, P.J. Eifel, C.M. Fisher, P. Frederick, D.K. Gaffney, E. Han, W.K. Huh, J.R. Lurain, A. Mariani, D. Mutch, C. Nagel, L. Nekhlyudov, A.N. Fader, S.W. Remmenga, R.K. Reynolds, T. Tillmanns, S. Ueda, E. Wyse, C.M. Yashar, N.R. Mcmillian, & Scavone J.L., *Cervical Cancer, Version 3.2019, NCCN Clinical Practice Guidelines In Oncology***, J. Natl. Compr. Cancer Netw**. 17, 2019. Https://Doi.Org/10.6004/ Jnccn.2019.0001.

Kombe, K. Arnaud. J. Bofeng, Li. Ayesha Z, Hylemariam, M. M. Guy-Armel, B., Ying, Z., &Tengchuan, J. *Epidemiology and Burden of Human Papillomavirus and Related Diseases, Molecular Pathogenesis, And Vaccine Evaluation***. Frontiers in Public Health** 8, 2021, 552028.

Korzeniewski, C., & Callewaert, D. *An Enzyme-Release Assay for Natural Cytotoxicity*. **J Immunol Methods** 64, 1983, 211-224.

Kuchenbaecker, K.B., Hopper, J. L, & Barnes, D.R. *Risks of Breast, Ovarian, And Contralateral Breast Cancer for BRCA1 and BRCA2 Mutation Carriers.* **JAMA.** 317(23),2017, 2402-2416.

Kudva, A.K., Rao, S., Rao, P., Periera, R., Bhandari, G., Mathew, J.M., Ashwini, K., Pais, M.L., Swamy, M.K. And Baliga, M.S., *Piper Betle Linn. In Cancer: Past, Present, And Future.* **Anticancer Plants: Properties and Application:** 1, 2018,.327-347.

Kumar, S., Allegra F., & Elgene, L. *Type 1 Nuclear Receptor Activity in Breast Cancer: Translating Preclinical Insights to The Clinic.* **Cancers** 13(19),2021,4972.

Lall, N., Henley-Smith, C.J, De Canha, M.N., Oosthuizen C.B, & Berrington D. *Viability Reagent, Prestoblue, In Comparison with Other Available Reagents, Utilized in Cytotoxicity and Antimicrobial Assays.* **Int J Microbiol** 2013; Article ID 420601, 5 Pages.

Laprise-Lachance, M., Lemieux, P., & Grégoire J-P. *Risk of Pulmonary Toxicity of Bleomycin and Filgrastim.* **Journal of Oncology Pharmacy Practice**.25(7),2019,1638-1644. doi:10.1177/1078155218804293

Lee, Y., & Michael, O. *Focus: Death: After-Death Functions of Cell Death*. **The Yale Journal of Biology and Medicine** 92(4), 2019, 687.

Lewandowska, A. M., Marcin, R., Sławomir, R., Tomasz, L., & Barbara, L., *Environmental Risk Factors for Cancer-Review Paper.* **Annals of Agricultural and Environmental Medicine** 26(1),2018, 1-7.

Lewis, C.M. & Vassos, E., *Polygenic Risk Scores: From Research Tools to Clinical Instruments.* **Genome Medicine**, 12(1), 2020, 1-11.

Li, H., Pang, Y., & Cheng, X. *Surgery of Primary Sites for Stage Ivb Cervical Cancer Patients Receiving Chemoradiotherapy: A Population-Based Study*, **J. Gynecol. Oncol.** 31, 2020. https://doi.org/10.3802/Jgo.2020.31.E8.

Li, P., Zhou, L., & Zhao, T., *Caspase-9: Structure, Mechanism and Clinical Application.* **Oncotarget**, 8, 2017, 23996 – 24008.

Lieber, M. Omara, A. Kathryn, S., Adrienne, M., Christine Du Preez, & Ann.M. B, "*Cervical Cancer Screening in HIV-Positive Farmers in South Africa: Mixed-Method Assessment.* **Annals of Global Health** 85(1), 2019.

Limonta, P., Roberta, M. M., Monica, M., Fabrizio, F., Michela, R., & Marina, M. M., *Role of Endoplasmic Reticulum Stress in The Anticancer Activity of Natural Compounds.* **International Journal of Molecular Sciences** 20(4), 2019, 961.

Liu, G., Monisha, S., Nicholas, T. & Ruanne, V.B., *HIV-Positive Women Have Higher Risk of Human Papilloma Virus Infection, Precancerous Lesions, And Cervical Cancer.* ***Aids*** 32, 6 2018, 795-808.

Luna, E.M., Freitas, T.S., Campina, F.F., Costa, M.S., Rocha, J.E., Cruz, R.P., Júnior, D.S., Silveira, Z.S., Macedo, N.S., Pinheiro, J.C.A. & Pereira-Júnior, F.N., *Evaluation of Phytochemical Composition, Toxicity in Drosophila Melanogaster and Effects on Antibiotics Modulation of Plathymenia Reticulata Benth Extract*. **Toxicology Reports,** 8, 2021, 732-739.

Lyndy J. Mcgaw, Ibukun, M. Famuyide, Edward, T. Khunoana, Adeyemi, O., *Ethnoveterinary Botanical Medicine in South Africa: A Review of Research from The Last Decade (2009 To 2019).* **Journal of Ethnopharmacology**, 257, 2020, 112864. https://doi.org/10.1016/J.Jep.2020.112864.

Magozwi, D. K., Mmabatho, D., Nthabiseng, M., Xavier, S-N., Rui, W.M. K., Molahlehi, S., Lyndy, J. Mcgaw, Wilma, A. A. & Vuyelwa, J. T., *Flavonoids from The Genus Euphorbia: Isolation, Structure, Pharmacological Activities and Structure–Activity Relationships.* **Pharmaceuticals.** 14(5), 2021, 428.

Mahomoodally, M. F., Farwa, A., & Rafia Rahman2 & Aniqa, I. S., *A Review of the Pharmacological Potential and Phytochemical Profile of Weeping Fig-Ficus benjamina* L. **Int J Chem Biochem Sci.** 16, 2019, 70-75

Mailankody, S. Dhanushkodi, M., Ganesan, T.S., Radhakrishnan, V., Christopher, V., Ganesharajah, S., Sagar, T.G., *Recurrent Cervical Cancer Treated with Palliative Chemotherapy: Real-World Outcome.* **Ecancermedicalscience.** 14, 2020. 110.3332/ECANCER.2020.1122.

Markaki, M., Tsamardinos, I., Langhammer, A., Lagani, V., Hveem, K., & Roe, O.D. *A Validated Clinical Risk Prediction Model for Lung Cancer in Smokers of All Ages and Exposure Types: A HUNT Study*. **Ebiomedicine** 31, 2018, 36–46.

Martino, E., Giuseppe, C., Sonia, C., Edoardo, C., Serena, P., Francesca, P., Marta, R., Angela, M. S., & Simona, C. *Vinca Alkaloids and Analogues as Anti-Cancer Agents: Looking Back, Peering Ahead.* **Bioorganic & Medicinal Chemistry Letters** 28(17), 2018, 2816-2826.

Mavundza, E. J., Street, R. & Baijnath, H. *A Review of the Ethnomedicinal, Pharmacology, Cytotoxicity and Phytochemistry of the Genus Euphorbia in Southern Africa.* **South African Journal of Botany** 144, 2022, 403-418.

Mei, C., Lei, L., Tan, L.M., Xu, X.J., He, B.M., Luo, C., Yin, J.Y., Li, X., Zhang, W., Zhou, H.H. & Liu, Z.Q., *The Role of Single Strand Break Repair Pathways in Cellular Responses to Camptothecin Induced DNA Damage*. **Biomedicine & Pharmacotherapy,** 125, 2020, 109875.

Metcalfe, K.A, Lubinski, J., Gronwald, J., Huzarski, T., Mccuaig, J., Lynch, H.T., Karlan, B., Foulkes, W.D., Singer, C.F., Neuhausen, S.L. & Senter, L., *The Risk of Breast Cancer in BRCA1 and BRCA2 Mutation Carriers Without a First-Degree Relative with Breast Cancer.* **Clin Genet**. 93(5), 2018, 1063-1068.

Mills, J.N., Rutkovsky, A.C., & Giordano, A., *Mechanisms of Resistance In Estrogen Receptor Positive Breast Cancer: Overcoming Resistance To Tamoxifen/Aromatase Inhibitors*". **Current Opinion in Pharmacology**. 41, 2018, 59-65.

Mirabelli, P., Luigi, C., & Marco, S. *Cancer Cell Lines Are Useful Model Systems for Medical Research.* **Cancers.** 11(8), 2019, 1098.

Mirabello, L., Megan, A., Clarke, C. W. N., Michael, D., Nicolas, W., Meredith, Y., Michael, C., Joseph, F. B., NCI HPV Workshop, Mark, S., & Robert, D. B. *The Intersection of HPV Epidemiology, Genomics and Mechanistic Studies Of HPV-Mediated Carcinogenesis* **Viruses** 10, 2, 80. https://doi.org/10.3390/V10020080.

Miricescu, D., Totan, A., Stanescu-Spinu II, Badoiu, S.C, Stefani, C. & Greabu, M. *PI3K/AKT/Mtor Signaling Pathway in Breast Cancer: From Molecular Landscape to Clinical Aspects.* **International Journal of Molecular Sciences**. 22(1), 2020,173.

Misra, H.P. & Fridovich, I. *The Role of Superoxide Anion in The Autoxidation of Epinephrine and A Simple Assay for Superoxide Dismutase*. **Journal of Biological Chemistry**. 247,1972, 3170-3175.

Molyneux, G., Geyer, F.C, & Magnay, F.A, *BRCA1 Basal-Like Breast Cancers Originate from Luminal Epithelial Progenitors and Not from Basal Stem Cells*. **Cell Stem Cell**. 7(3), 2010, 403-417.

Momenimovahed, Z., & Salehiniya, H., *Epidemiological Characteristics of and Risk Factors for Breast Cancer in The World*. **Breast Cancer: Targets and Therapy.** 2019, 151-164.

Monticciolo, D.L, Newell, M.S., Moy, L., Niell, B., Monsees, & Sickles, E.A. *Breast Cancer Screening in Women at Higher-Than-Average Risk: Recommendations from the ACR*. **Journal of the American College of Radiology.** 15(3), 408-142018

Moon, J.Y., Song, I.C., Ko, Y.B. & Lee, H.J., *The Combination of Cisplatin and Topotecan as A Second-Line Treatment for Patients with Advanced/Recurrent Uterine Cervix Cancer*. **Medicine**. 97(14), 2018.

Mosmann T. *Rapid Colorimetric Assay for Cellular Growth and Survival: Application to Proliferation and Cytotoxicity Assays*. **J Immunol Methods.** 65, 1983,55-63.

Muhammed, M., A. A. Yusuf, B. O. Odey, A. R. Alawode, G. A. Adegbola & R. A. Agboola. *A Systematic Review of Domestication, Ethnopharmacological Use, Phytochemistry, Nutritional Composition, And Biological Activities of Parkia Biglobosa (Jacq.*) R." *Br. Ex G. Don.* **BIOMED Natural and Applied Science** 1(1), 2021, 01-12.

Mukohara, T. *PI3K Mutations in Breast Cancer: Prognostic and Therapeutic Implications*. **Breast Cancer (Dove Med Press).** 7, 2015,111-123.

Mundt, F., Rajput, S., Li, S., Ruggles, K.V., Mooradian, A.D, Mertins, P., Gillette MA, Krug K, Guo Z, Hoog J, & Erdmann-Gilmore P., *Mass Spectrometry–Based Proteomics Reveals "Potential Roles of NEK9 and MAP2K4 in Resistance to PI3K Inhibition in Triple-Negative Breast Cancers Proteomic Profiling of PI3K Inhibitor Resistance*. **Cancer Research**, 78(10), 2018, 2732-2746.

Nagata, S., *Apoptosis and Clearance of Apoptotic Cells.* **Annual Review of Immunology.** 36, 2018, 489-517.

Newman, D. J., & Cragg, G. M. *Natural Products as Sources of New Drugs from 1981 To 2014*. **J. Nat. Prod.** 79 (3), 2016,629–661. doi: 10.1021/Acs.Jnatprod.5b01055

Nguyen, P.T., Katanoda, K., Saito, E., Hori, M., Nakayama, T. & Matsuda, T. *Trends in Lung Cancer Incidence by Gender, Histological Type and Stage at Diagnosis in Japan, 1993 To 2015: A Multiple Imputation Approach.* **International Journal of Cancer,** 151(1),20-32.

Nurgali, K., Jagoe, R. T. & Abalo, R. Editorial: *Adverse Effects of Cancer Chemotherapy: Anything New to Improve Tolerance and Reduce Sequelae*? **Front Pharmacol**, 9, 2018, 245.

Nurgali, K. R., Thomas, J., & Raquel, A. *Adverse Effects of Cancer Chemotherapy: Anything New to Improve Tolerance and Reduce Sequelae*? **Frontiers in Pharmacology** 9, 245, 2018.

Ohiagu, F.O., Chikezie, P.C., Chikezie, C.M. & Enyoh, C.E., *Anticancer Activity of Nigerian Medicinal Plants: A Review*. **Futur J Pharm Sci.** 7, 70, 2021. https://doi.org/10.1186/S43094-021-00222-6

Okonkwo, C. O., & Ohaeri O. C. *Essential Oils from The Leaves of Euphorbia Milii Exert Insecticidal Activity Through Disruption in Ionic Composition.* **IOSR J. Pharm. Blogica Sci.** 13(4) 2018, 46-53.

Okwunodulu, F. U., Chisom, F., Innocent, N. O., & Vintan, I. C., *Comparative Study on The Phytochemicals, Proximates, Vitamins and Mineral Elements Compositions of Unfermented and Fermented Seeds of Parkia biglobosa*. **Bioscience and Biotechnology Journal**. 2(1), 2021, 7-10.

Olorunfemi, G., Ndlovu, N., Masukume, G., Chikandiwa, A., Pisa, P.T., & Singh, F., *Temporal Trends in The Epidemiology of Cervical Cancer in South Africa* (1994–2012), **Int. J. Cancer.** 143, 2018. https://doi.org/10.1002/Ijc.31610.

Opferman, J. T., & Anisha, K., *Anti-Apoptotic BCL-2 Family Members in Development.* **Cell Death & Differentiation.** 25(1), 2018, 37-45.

Ospina, D., Villegas, V.E., Rodríguez-Leguizamón, G. & Rondón-Lagos, M., *Analyzing Biological and Molecular Characteristics and Genomic Damage Induced by Exposure to Asbestos*. **Cancer Management and Research**.11, 2019, 4997.

Osuntokun, O. T., Jemilaiye, T. A & Akinrodoye, A. R., *Comparative Study Between the Effect of Parkia biglobosa (JACQ) Benth and Conventional Antibiotics Against Multiple Antibiotic Resistant Uropathogenic Bacteria (MARUB).* **MOJ Bioequiv Availab** 5(4), 2018, 200-212.

Oun, R., Moussa, Y. E., & Wheate, N. J. *The Side Effects of Platinum-Based Chemotherapy Drugs: A Review for Chemists.* **Dalton Transactions**, 47(19), 2018, 6645–6653. doi:10.1039/C8dt00838h.

Oyervides-Muñoz, M. A., Antonio, A. P-M., Hazyadee, F. R-G., Gabriela, S. G-M., Oscar, R. F-R., Víctor, T., Hugo, A. B-S, & María, L. G-R. *Understanding the HPV Integration and Its Progression to Cervical Cancer.* **Infection, Genetics and Evolution** 61, 2018, 134-144.

Pal, A., Kundu, R., *Human Papillomavirus E6 and E7: The Cervical Cancer Hallmarks and Targets for Therapy*, **Front. Microbiol**. 10, 2020. https://Doi.Org/10.3389/ Fmicb.2019.03116

Palmer, R.M, Ferrige, A.G, Moncada, S. *Nitric Oxide Release Accounts for The Biological Activity of Endothelium-Derived Relaxing Factor.* **Nature.** 327(6122), 1987, 524-526.

Pascoe, C., Tod, G., Manning, D. W., & Nathan, L, *Ureteral Intramural Metastatic Deposit of Prostate Cancer with Ureteric Obstruction.* **Case Reports** 2018, 2018, Bcr-2017.

Patel, R. S., Kadia, R., Vrushali, B., & Vipra, P., *Study of Ornamental Plants Found from Butterfly Park and One Tree Hill Garden of Kankariya, Ahmedabad, Gujarat, India*. **International Journal of Scientific Research in Science and Technology**. 4(5), 2018, 560-8.

Patil, S. *Phytochemical Study and Antioxidant Properties of Ethanolic Extracts of Euphorbia Milii. Haleshappa R., Keshamma E., Girija CR, Thanmayi M, Nagesh CG, Lubna Fahmeen GH, Lavanya M. & Sharangouda J. Patil* (2020).

Peate, I., *Cervical Cancer*. **British Journal of Healthcare Assistants.** 13(3), 2019, 110-116.

Pękala-Safińska, A., Halyna, T., Lyudmyla, B., Natalia, K., Ivan, G., Vitaliy, H., & Andriy Prokopiv. *Comparative Assessment of the Antibacterial Efficacy of Leaf Extract Obtained from Ficus benjamina L. (Moraceae) And Its Cultivars Against Aeromonas Sobria Strain.* **Agrobiodiversity for Improving Nutrition, Health and Life Quality.** 5(1), 2021.

Putra, K., Winanda, E., Ari, P., Gilang, D. Nugroho, M. R., & Ahmad, D. S., *Phytochemical Activities of Ficus (Moraceae) In Java Island, Indonesia.* **International Journal of Bonorowo Wetlands** 10(2), 2020.

Ramirez, P.T., Frumovitz, M., Pareja, R., Lopez, A., Vieira, M., Ribeiro, R., Buda, A., Yan, X., Shuzhong, Y., Chetty, N., Isla, D., Tamura, M., Zhu, T., Robledo, K.P., Gebski, V., Asher, R., Behan, V., Nicklin, J.L., Coleman, R. L., Obermair, A., *Minimally Invasive Versus Abdominal Radical Hysterectomy for Cervical Cancer.* **N. Engl. J. Med.** 379, 2018. https://doi.org/10.1056/Nejmoa1806395.

Rana, A. C., & Bhawna, G. *Chemistry and Pharmacology of Flavonoids-A Review.* **Indian Journal of Pharmaceutical Education and Research** 53(1), 2019.

Rania, M. S., Merihane, M. N., Jannatullah I. A., Omar, K. R., & Mohamed, A. E. *Alogliptin Attenuates Cyclophosphamide-Induced Nephrotoxicity: A Novel Therapeutic Approach*

Through Modulating MAP3K/JNK/SMAD3 Signaling Cascade. **Drug and Chemical Toxicology** 45(3), 2022, 1254-1263.

Rankoana, S. A. *Indigenous Plant-Derived Medical Applications for Primary Health Care*. **International Journal of Social Science Research and Review**, 5(11), 163-168. https://doi.org/10.47814/Ijssrr.V5i11.719

Rathore, A., Ranjan, S., Vishwanath, S., Pathak, A., Dubey, A., & Nikhil, P. *BRCA 1 And 2 Mutations in Carcinoma Breast: An Indian Study*. **Clinical Cancer Investigation Journal**. 7(3), 2018, 97-97.

Rebbeck, T.R, Friebel, T.M, Friedman, E., Hamann, U., Huo, D., Kwong, A., Olah, E., Olopade, O.I., Solano, A.R., Teo, S.H. & Thomassen, M., *Mutational Spec-Trum in A Worldwide Study of 29,700 Families with BRCA1 or BRCA2 Mutations.* **Hum Mutat** 39 (5), 2018, 593.

Reid, A.-M., Oosthuizen, C. B., Fibrich, B. D., Twilley, D., Lambrechts, I. A., De Canha, M. N., & Lall, N. *Traditional Medicine***. Medicinal Plants for Holistic Health and Well-Being,** 2018, 1–11. doi:10.1016/B978-0-12-812475-8.00001-9.

Rieu, R. & Eminowicz, G., *Chemotherapy for Cervical Cancer. Uterine Cervical Cancer*: **Clinical and Therapeutic Perspectives.** 2019, 215-231.

Riss, T, Moravec, R. *Use of Multiple Assay Endpoints to Investigate the Effects of Incubation Time, Dose of Toxin, And Plating Density in Cell-Based Cytotoxicity Assays*. **Assay Drug Dev Technol**. 2, 2004, 51-62.

Rizwanullah, M.D, Saima, A., Showkat, R. M., Khalid, U.F., & Mohd, M. A. R. *Phytochemical Based Nanomedicines Against Cancer: Current Status and Future Prospects*. **Journal of Drug Targeting** 26(9), 2018, 731-752.

Rossin, A, Giorgia, M, & Anne-Odile, H., *TRAIL and Fasl Functions in Cancer and Autoimmune Diseases: Towards an Increasing Complexity***. Cancers.** 11(5), 2019, 639.

Sailer, V., Gunhild Von Amsberg, Stefan, D., Jutta, K., Verena, L, Eric, M., Anne, O. Pantel, K., Schuele, R., Taubert, H. & Wach, S., *Experimental In Vitro, Ex Vivo And In Vivo Models in Prostate Cancer Research.* **Nature Reviews Urology.** 20(3), 2023, 158-178.

Saleh, Mohammed, S.M, Juriyati, J., Satirah, Z., Ahmad, Y, A., Nor, H. M., & Yusof, K. *Genus Parkia: Phytochemical, Medicinal Uses, And Pharmacological Properties.* **International Journal of Molecular Sciences.** 22(2), 2022, 618.

Salehi, B., Prakash Mishra, A., Nigam, M., Karazhan, N., Shukla, I., Kiełtyka-Dadasiewicz, A., & Sharifi-Rad, J. *Ficus Plants: State of The Art from A Phytochemical, Pharmacological, and Toxicological Perspective.* **Phytotherapy Research**. 35(3), 2021, 1187-1217. doi:10.1002/Ptr.6884.

Salehi, B., Abhay, P. M., Manisha, N., Natallia, K., Ila, S., Anna, K-D., & Barbara, S. *Ficus Plants: State of The Art from A Phytochemical, Pharmacological, And Toxicological Perspective.* **Phytotherapy Research.** 35(3), 2021, 1187-1217.

Santos, J., Victor De Oliveira, A., Lúcia, F.P., & Isabella, M. F. C. *Potential Application of Combined Therapy with Lectins as A Therapeutic Strategy for The Treatment of Bacterial Infections*. **Antibiotics.** 10(5), 2021, 520.

Scarpignato, C., & Ingvar, B. *Drug-Induced Small Bowel Injury: A Challenging and Often Forgotten Clinical Condition.* **Current Gastroenterology Reports** 21, 2019, 1-12.

Scarth, J.A., Molly, R., Patterson, L.M. Ethan, M., & Andrew, M., *The Human Papillomavirus Oncoproteins: A Review of the Host Pathways Targeted on The Road to Transformation.* **The Journal of General Virology** 102(3), 2021.

Schabath, M.B. *Risk Models to Select High Risk Candidates for Lung Cancer Screening*. **Ann Transl Med.** 6, 2018, 65.

Schabath, M.B. & Cote, M.L., *Cancer Progress and Priorities: Lung Cancer*. **Cancer Epidemiology, Biomarkers & Prevention**. 28(10), 2019, 1563-1579.

Serhani, M., Essaadi, H., Kassara, K. & Boutoulout, A. *Control by Viability in A Chemotherapy Cancer Model*. **Acta Biotheor**. 67(3), 2019, 177-200.

Sevgi, G. & Nazim, S. *Current Perspective in The Application of Medicinal Plants Against Cancer*. **Novel Therapeutic Agent.** 19(1), 2019, 101-111.

Sheibani, M., Yaser, A., Maryam, S., Sadaf, N., Faezeh, E., Mohammad, H. F., & Ahmad, R. D. *Doxorubicin-Induced Cardiotoxicity: An Overview on Pre-Clinical Therapeutic Approaches.* **Cardiovascular Toxicology.** 22(4), 2022, 292-310.

Shiga, T., Hiraide, M. *Cardiotoxicities of 5-Fluorouracil and Other Fluoropyrimidines*. **Curr. Treat. Options in Oncol**. 21, 27, 2020.

Shinagawa, N. *A Review of Existing and New Methods of Bronchoscopic Diagnosis of Lung Cancer.* **Respiratory Investigation**. 57(1), 2019, 3-8. doi: 10.1016/J.Resinv.2018.08.004.

Short, N.J., Elias J, Kiran N, Ami P, Jing N, Koji S, & Graciela M. Nogueras-Gonzalez. *A Phase II Study of Omacetaxine Mepesuccinate for Patients with Higher-Risk Myelodysplastic Syndrome and Chronic Myelomonocytic Leukemia After Failure of Hypomethylating Agents.* **American Journal of Hematology** 94(1), 2019, 74-79.

Singh, R., Anthony, L., & Kristopher, S. *Regulation of Apoptosis in Health and Disease: The Balancing Act of BCL-2 Family Proteins*. **Nature Reviews Molecular Cell Biology.** 20(3), 2019, 175-193.

Skroza, N., Nicoletta, B., Ilaria, P., & Concetta, P. *Clinical Utility of Ingenol Mebutate in The Management of Actinic Keratosis: Perspectives from Clinical Practice.* **Therapeutics And Clinical Risk Management.** 14, 2018, 1879.

Sofilić, T., Hadžipašić, A.B. & Šimić, U.S., *Experiences in Asbestos-Containing Waste Management in The Republic of Croatia*. **Sigurnost,** 64(1), 2022, 47-62.

Solı´S, P., Wright, C., Anderson, M., Gupta, M., & Phillipson, J. D., *A Microwell Cytotoxicity Assay Using Artemia Salina*. **Plant Medicin**, 59,1993, 250-25.

Stemke-Hale, K., Gonzalez-Angulo, A.M, & Lluch, A. *An Inte-Grative Genomic and Proteomic Analysis of PIK3CA, PTEN, And AKT Mutations in Breast Cancer.* **Cancer Res.** 68(15), 2008, 6084-6091.

Sule, A.A, & Ochicha, O.A. *Histopathologic Review of Cervical Cancer in Kano, Nigeria*. **Sahel J.** 20, 2017, 16-20

Sun, W., Luo, J.D, Jiang, H. & Duan, D.D. *Tumor Exosomes: A Double-Edged Sword in Cancer Therapy*. **Acta Pharmacol Sin**.39, 2018, 534–41.

Sun, L., Legood, R., Dos-Santos-Silva, I., Gaiha, S.M., & Sadique, Z. *Global Treatment Cost of Breast Cancer by Stage: A Systematic Review*. **Plos One**, 13(11), E0207993-E0207993. Doi: 10. 1371/ **Journal.Pone**.0207993; 2018.

Sung, H. Ferlay, J. Siegel, R.L. Laversanne, M. Soerjomataram, L. Jemal, A. & Bray, F. *Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide For 36 Cancers In 185 Countries,* CA: **A Cancer Journal for Clinicians,** 2021, Https://Doi.Org/10.3322/Caac.21660.

Tecza, K, Pamula-Pilat, J., Lanuszewska, J., Butkiewicz, D., & Grzybowska, E. *Pharmacogenetics of Toxicity of 5-Fluorouracil, Doxorubicin and Cyclophosphamide Chemotherapy in Breast Cancer Patients.* **Oncotarget.** 9(10), 2018, 9114-9136. Doi: 10.18632/Oncotarget.24148.

Tewari, K. S. *Therapeutic Vaccination Using HPV 16 E7 to Eradicate CIN3.* **Journal of Gynecologic Oncology.** 30, 6, 2019.

Thandra, K.C., Barsouk, A., Saginala, K., Aluru, J.S. & Barsouk, A. *Epidemiology of Lung Cancer.* **Contemporary Oncology/Współczesna Onkologia**.25(1), 2021: 45-52.

Tiwari, A, Singh, M, & Sahu, B. *Risk Factors for Breast Cancer*. **International Journal of Nursing Education and Research.** 10(3), 2022, 276-82.

Todd, C., Lagman-Bartolome, A.M, & Lay, C., *Women and Migraine: The Role of Hormones*. **Current Neurology and Neuroscience Reports**. 18, 2018,1-6.

Trush, M.A, Egner, P.A, & Kensler, T.W. *Myeloperoxidase as a Biomarker of Skin Irritation and Inflammation.* **Food Chemtoxicol**. 32(2), 1994, 143-7.

Ullah, A., Sidra, M., Syed, L. B, Noreen, K., Lubna, G., Benjamin, G. P., Abdul-Hamid, E., & Mariusz, J. *Important Flavonoids and Their Role as A Therapeutic Agent.* **Molecules** 25(22), 2020, 5243.

Umer, M. I., *Synthesis and Uses of Various Essential Oil-Based Derivatives in Biomedicine*. **International Journal of Chemical and Biochemical Sciences**. 13, 2018, 92-99.

Varshney, R. & Kale, R.K. *Effects of Calmodulin Antagonists on Radiation-Induced Lipid Peroxidation in Microsomes*. **International Journal of Radiation Biology**, 58, 1990, 733-743;

Velloza, J., Kemp, C.G., Aunon, F.M., Ramaiya, M.K., Creegan, E., & Simoni. J.M., *Alcohol Use and Antiretroviral Therapy Non-Adherence Among Adults Living with HIV/AIDS In Sub-Saharan Africa: A Systematic Review and Meta-Analysis.* **AIDS and Behavior**.24, 2020, 1727-1742.

Verjans, E.T., Doijen, J., Luyten, W., Landuyt, B. & Schoofs, L., *Three-Dimensional Cell Culture Models for Anticancer Drug Screening: Worth the Effort?* **Journal of Cellular Physiology,** 233(4), 2018, 2993-3003.

.

Vogel, V. G. *Epidemiology of Breast Cancer*. In **The Breast**, 2018, 207-218.

Wang, K., & Tepper, J. E., *Radiation Therapy-Associated Toxicity: Etiology, Management, And Prevention*, **CA, Cancer J. Clin.** 71, 2021. https://doi.org/ 10.3322/Caac.21689.

Wang, R., Wei, P., Lei, J., Weiming, H., Yuehan, Li., Di, W., Chun, G., Ding, M., & Shujie, L. *Human Papillomavirus Vaccine Against Cervical Cancer: Opportunity and Challenge.* **Cancer Letters**. 471, 2020, 88–102. https://doi.org/10.1016/J.Canlet.2019.11.03.

Wardihan, M. Rusdi, G. Alam, L, & Manggau, M. A. *Selective Cytotoxicity Evaluation in Anticancer Drug Screening of Boehmeria Virgata (Forst) Guill Leaves to Several Human Cell Lines: Hela, Widr, T47D and Vero.* **Dhaka University Journal of Pharmaceutical Sciences**, 12(2), 2013, 87–90.

Wendt, C., & Margolin, S. *Identifying Breast Cancer Susceptibility Genes–A Review of the Genetic Background in Familial Breast Cancer.* **Acta Oncologica**. 58(2) 2019, 135-46.

Wright, J.D., Matsuo, K., Huang, Y., Tergas, A.I., Hou, J.Y., Khoury-Collado, F., Clair, C.M.S., Ananth, C.V., Neugut, A.I. & Hershman, D.L., *Prognostic Performance of the 2018 International Federation of Gynecology and Obstetrics Cervical Cancer Staging Guidelines*. **Obstetrics and Gynecology**. 134(1), 2019, 49.

Yambayev, I., Sullivan, T.B, Suzuki, K., Zhao, Q., Higgins, S.E., Yilmaz, O.H., Litle, V.R, Moreira, P., Servais, E.L, Stock, C.T., & Quadri, S.M., *Pulmonary Adenocarcinomas of Low Malignant Potential: Proposed Criteria to Expand the Spectrum Beyond Adenocarcinoma in Situ and Minimally Invasive Adenocarcinoma.* **The American Journal of Surgical Pathology**. 45(4), 2021, 567-576.

Yang, W., Hosford, S.R., Dillon, L.M., Shee, K., Liu, S.C., Bean, J.R., Salphati, L., Pang, J., Zhang, X., Nannini, M.A. & Demidenko, E., *Strategically Timing Inhibition of Phosphatidylinositol 3-Kinase to Maximize Therapeutic Index in Estrogen Receptor Alpha–Positive, PIK3CA-Mutant Breast Cancerstrategic PI3K Inhibition for ER+ Breast Cancer*. ***Clinical Cancer Research.*** 22(9), 2016, 2250-2260.

Yang, X.R., Sherman, M.E, & Rimm, D.L. *Differences In Risk Factors For Breast Cancer Molecular Subtypes In A Population-Based Study.* **Cancer Epidemiol Biomark Prev**. 16(3), 2017.

Yarandi, F., Mahdi, M., Sara, R.& Elham, S., *Endocervical Adenocarcinoma Implantation in Episiotomy Scar: A Case Report and Review of the Literature***. Journal of Medical Case Reports.** 17(1), 2023, 1-6.

Yeo-Teh, N.S.L, Ito, & Y. Jha. S. *High-Risk Human Papillomaviral Oncogenes E6 and E7 Target Key Cellular Pathways to Achieve Oncogenesis*, **Int. J. Mol. Sci.** 19(6), 2018, 1706. https://doi.org/10.3390/Ijms19061706

Zhou G. *Tobacco, Air Pollution, Environmental Carcinogenesis, And Thoughts on Conquering Strategies of Lung Cancer.* **Cancer Biol Med. 2019.** 16(4), 2019, 700-713. doi: 10.20892/J.Issn.2095-3941.2019.0180.

Zhou, L., Caiqin, Z., Yongbin, Z., & Changhong, S. *Application of Organoid Models in Prostate Cancer Research.* **Frontiers in Oncology** 11, 2021, 736431.

Zhou, M., Monowar, A., & Ping, W. *Damage-Associated Molecular Patterns as Double-Edged Swords in Sepsis*. **Antioxidants & Redox Signaling.** 35(15), 2021, 1.

Zhu, H., Luo, H., Zhang, W., Shen, Z., Hu, X., & Zhu, X., *Molecular Mechanisms of Cisplatin Resistance in Cervical Cancer*, **Drug Des. Dev. Ther**. 2016, 1885-1895 https://doi. org/10.2147/DDDT.S106412.

Zhu, H., & Zhang, S., *Body Mass Index and Lung Cancer Risk in Never Smokers: A Meta-Analysis.* **BMC Cancer.** 18, 2018, 635.

Zhu, Q.G., Zhang, S.M., Ding, X. X, He B, & Zhang, H.Q. *Driver Genes in Non-Small Cell Lung Cancer: Characteristics, Detection Methods, And Targeted Thera-Pies*. **Oncotarget** 8, 2017, 57680–57692;

Zia, Q., Asim, A., Nazia, H., Pooja, J., Manvi, S., Mohd, A. M., & Asgar, A. *Cell Death: A Molecular Perspective***. Current Molecular Biology Reports.** 2021, 1-26.

Thesis

Aboush, S. A, & Gadir, A. R. *Isolation and Characterization of Flavonoids and Oils from Some Saudi Plants and Their Biological Activity*." Phd Diss., Sudan University of Science and Technology, 2017.

Appendices

Appendix I

Selected Plants of Study

SN	Plant Used	Common Name	Part Used	Voucher No
1	*Ficus benjamina*	Weeping Fig	Leaf	UIH-23165
2	*Euphorbia milli*	Crown of Thorns	Leaf	UIH-23166
3	*Vitex doniana*	Black Plum	Leaf	ND
4	*Euphorbia heteropyilla*	Fire Plant	Leaf	ND
5	*Parkia biglobosa*	Locus bin	Bark	ND
6	*Piliostgma thonnigii*	Camel foot	Bark	ND

Appendix II

Preparation of Lytic Buffer.

Tris-Hydrochloride 50mM

Tween-20	0.5%
Sodium Chloride (NaCl)	250mM
Ethylenediaminetetraacetic acid (EDTA)	5mM
Sodium Fluoride (NaF)	50mM

The pH of the buffer was adjusted to 7.0with 1M Sodium Hydroxide (NaOH) and refrigerated at 4°C.

Appendix III

Reagent Preparation for Bax, Bcl, Cas-9 and Cas-3 Determination

All reagents were brought to room temperature (18-25°C) before use. 30ml of concentrated Wash buffer was diluted with 720ml of deionized water to prepare 750ml of wash buffer. Crystals formed in the concentrate were dissolved by gentle warming in the water bath at 40°C. The standard was centrifuged at 10,000 xg for 1 min. 1ml of Reference Standard & Sample Diluent was added and stand for 10 minutes. This was inverted gently several times until finally dissolved and was mixed thoroughly with a pipette. This reconstitution produces a working solution of 10ng/ml. Serial dilutions were made as follows: 10,5,2.5,1.25,0.63,0.31,0.16,0ng/ml.

Appendix IV

Reagent Preparation for LPO Detrmination

1.30% Trichloroacetic acid (TCA)

30g of TCA (CCl_3COOH) was dissolved in 100 ml of distilled water and stored at 4°C

2. 0.75% Thiobarbituric acid (TBA)

0.075g of TBA was dissolved in 10ml of 0.1M HCl and made up to 10ml with the same. Dissolution was aided by stirring in a hot water bath (50°C).

0.15M Tris-KCl buffer (pH 7.4)

1.12g of KCl and 2.36g of Tris base were dissolved separately in distilled water and the volume was made up to 100ml with distilled water, the pH was then adjusted to pH 7.4 with HCl.

Appendix V

Reagent Preparation for GSH Determination

1. Phosphate buffer (0.1M, pH7.4)

Dipotassium hydrogen phosphate trihydrate

GSH standard curve protocol

GSH Stock (ml)	Phosphate buffer (ml)	Ellman's reagent (ml)	GSH conc. (µg/ml)
0.02	0.48	4.5	1.6
0.05	0.45	4.5	4
0.10	0.40	4.5	8
0.20	0.30	4.5	16

0.30	0.20	4.5	24
0.40	0.10	4.5	32

Source: Author's Analysis 2023

Appendix VI

Reagent Preparation for SOD Determination

1. 0.05M Carbonate buffer (pH 10.2)

1.608g of $Na_2CO_3.10H_2O$ and 0.4725g of $NaHCO_3$ were dissolved in 225ml of distilled water. The pH was adjusted to 10.2 and then made up to 250ml with distilled water.

Appendix VII

Reagent Preparation for GSH Determination

1. 1-choloro-2,4-dinitrobenzene (CDNB) (20mM)

0.074g of 1-choloro-2,4-dinitrobenzene (CDNB) was dissolved in 22ml of absolute ethanol.

2. Reduced Glutathione (0.1M)

0.138g of glutathione (GSH) was dissolved in 4.5ml of 0.1M phosphate buffer (pH 6.5)

3. Phosphate buffer (0.1M, pH 6.5)

Dipotassium hydrogen phosphate, K_2HPO_4 (2.0336g), and potassium dihydrogen phosphate, KH_2PO_4 (3.9893g) were dissolved in 90ml of distilled water, the pH adjusted to 6.5 and the volume made up to 100ml with distilled water.

GST Assay medium

Reagent	Blank	Test
CDNB (20mM)	150µl	150µl
Reaction mixture (2.8ml buffer, 0.03ml GSH, and 10.5ml H_2O)	30µl	30µl
Sample	-	30µl

Source: Author's Analysis 2023

Appendix VIII

Reagent Preparation for NO Determination

Phosphate Buffer Saline (PBS) pH 7.4

10 mM sodium nitropruside prepare in PBS (298mg in 100ML of PBS)

1% sulfanilamide (Sulf) (1g in 100mL of distilled water)

2% Phosphoric acid (2.35mL in 100mL of distilled water)

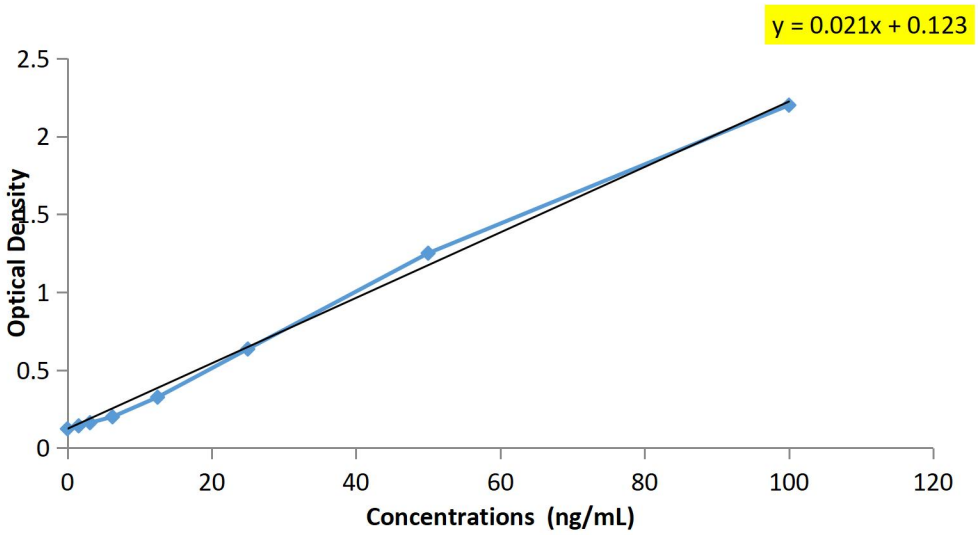
0.1% N-1-napthylethylenediamine dihydrochloride (100mg in 100mL of 2% phosphoric acid)

Griess reagent was prepared by mixing the equal volume of 1% sulfanilamide (Sulf) and 0.1% N-1-napthylethylenediamine dihydrochloride.

Appendix IX

Standard curve graph for Cas-9 determination

Sample ID	Concn (ng/mL)	O.D
Std 1	**0**	**0.123**
Std 2	**1.56**	**0.142**
Std 3	**3.13**	**0.162**
Std 4	**6.25**	**0.2**
Std 5	**12.5**	**0.326**
Std 6	**25**	**0.635**
Std 7	**50**	**1.25**
Std 8	**100**	**2.2**

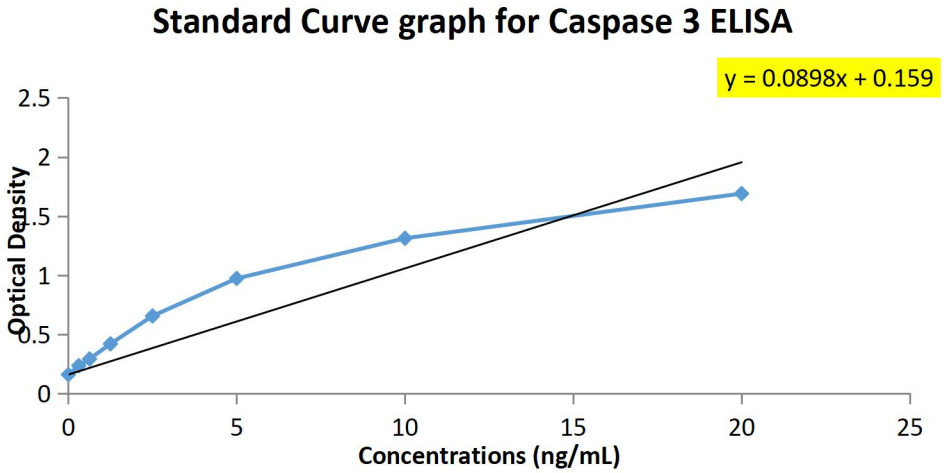


Appendix X

Standard curve graph for Caspase-3 determination.

Sample ID	Concn (ng/ml)	O.D
Std 1	0	0.159
Std 2	0.31	0.235
Std 3	0.63	0.291
Std 4	1.25	0.419
Std 5	2.5	0.655
Std 6	5	0.972

Std 7	10	1.312
Std 8	20	1.69

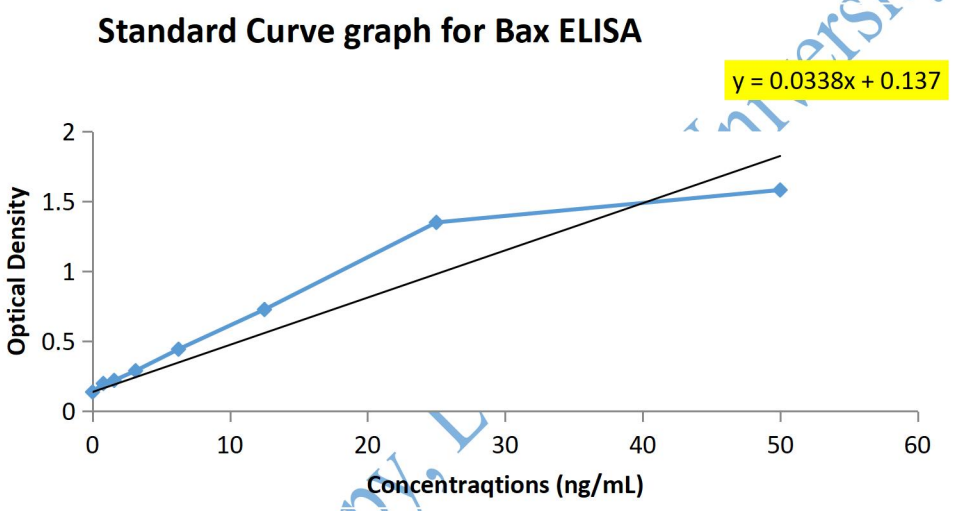


Appendix XI

Standard curve graph for Bax determination

Sample ID	Concn	O.D
Std 1	**0**	**0.137**
Std 2	**0.78**	**0.198**
Std 3	**1.57**	**0.22**

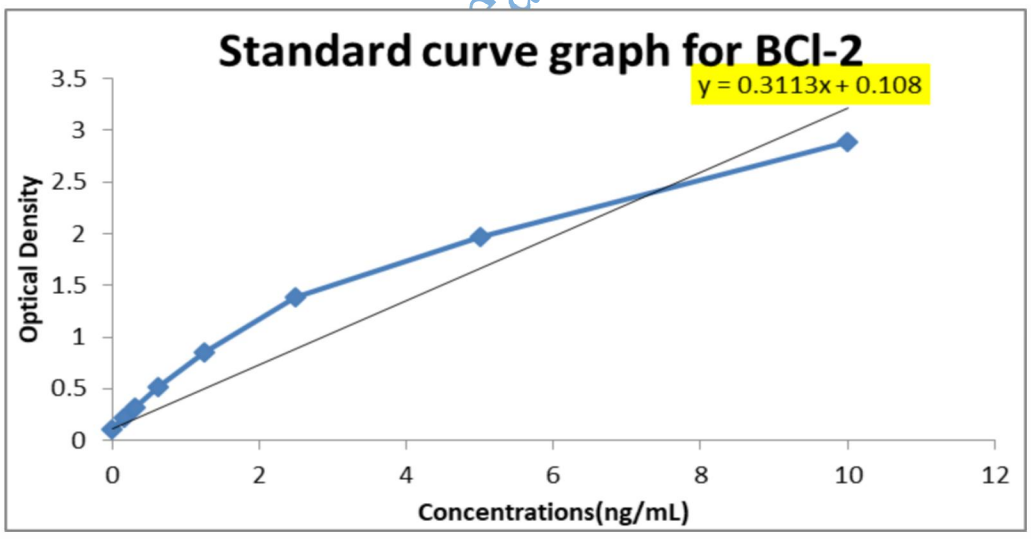
Std 4	**3.13**	**0.289**
Std 5	**6.25**	**0.443**
Std 6	**12.5**	**0.727**
Std 7	**25**	**1.35**
Std 8	**50**	**1.582**



Appendix XII

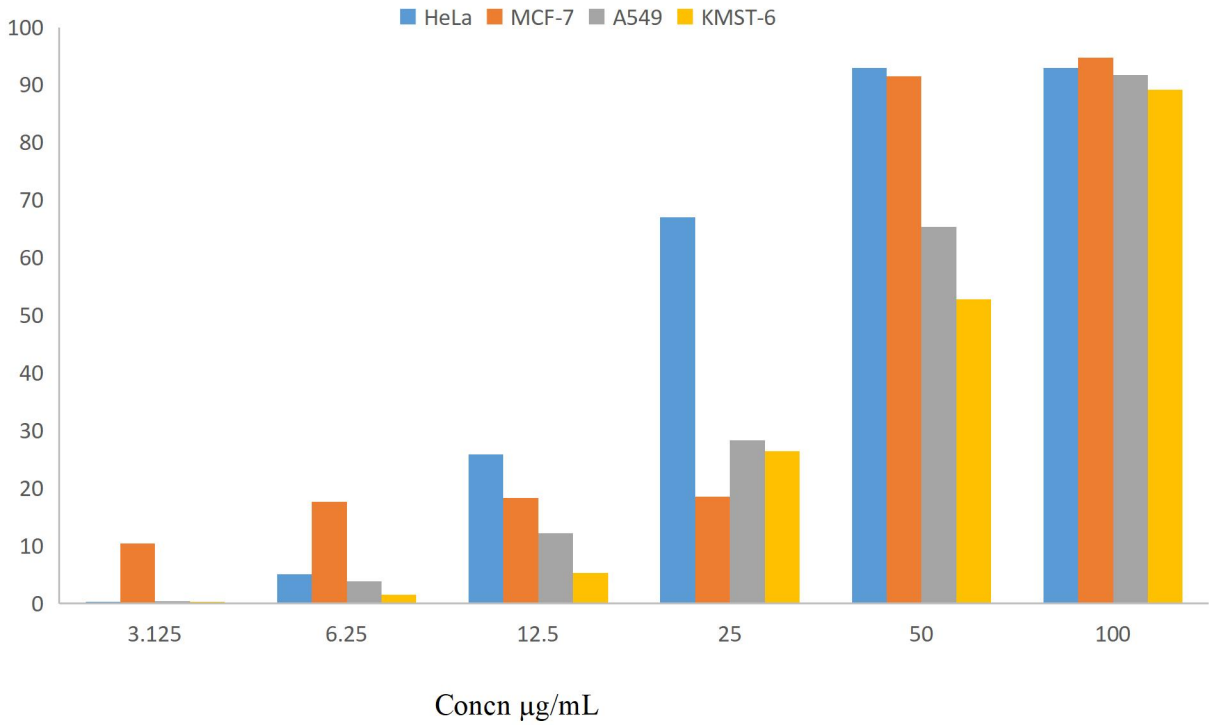
Standard curve graph for Bcl-2 determination

Sample ID	Concn (ng/mL)	O.D
Std 1	0	0.108
Std 2	0.16	0.213
Std 3	0.31	0.323
Std 4	0.63	0.518
Std 5	1.25	0.855
Std 6	2.5	1.382
Std 7	5	1.971
Std 8	10	2.881



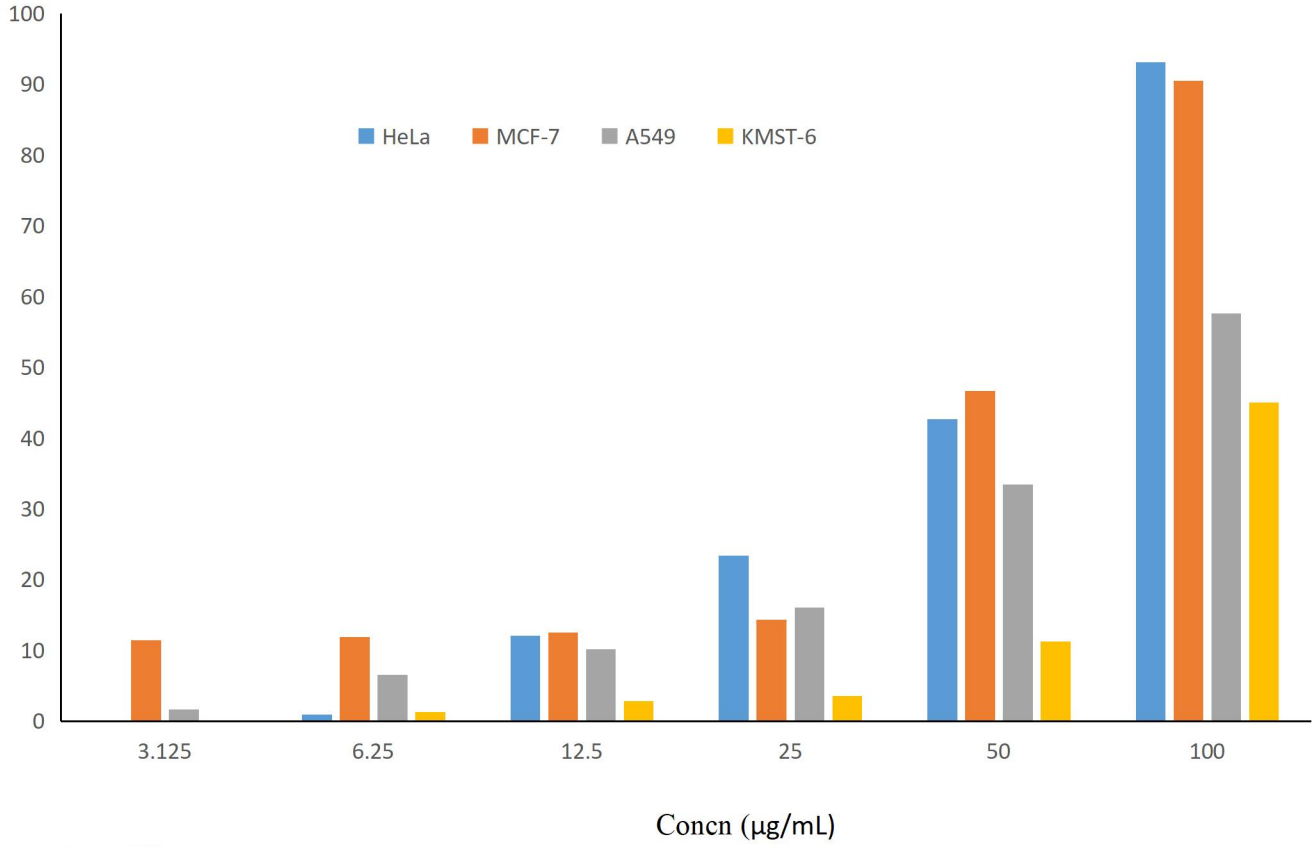
Appendix XIII

Graph showing %inhibition of the leaf extract of *F. benjamina* on HeLa, MCF-7, A549, and KMST-6 cell lines.



Appendix XIV

Graph showing %inhibition of the leaf extract of *E. milli* on HeLa, MCF-7, A549, and KMST-6 cell lines.



Biodata

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B. Education background

Educational Institutions attended with dates and qualifications

- Lead City University, (**M.Sc. Biochemistry)** 2019- till date
- National Open University of Nigeria (**B.Sc. Chemistry)** 2015-2020
- Federal University of Technology, Akure (**PGD Clinical Biochemistry**) 2006 -2008
- University of Lagos, Final Diploma in SLT **(Chem/Biochem Option)** 1997-2002

C. Work experience with dates

- WHO National Polio Laboratory, Department of Virology, UCH, Ibadan 2001 - till date
- College of Medicine, University of Lagos 1997 - 2001

D. Award and fellowship (if any) Non

E. Membership of academic professional bodies

- Associate Member of Nigeria Institute of Science Laboratory Technology {**AISLT}** 2007-till dat

F. Publications: **Thesis and Dissertation**

1. Isolation and characterization of beta amylase from cassava peel.
2. Concentration-dependent effects of amodiaquine on human cervical cancer cell.

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Signature **Date**

The University Compliance Certification

This is to certify that this Thesis written by Oluwaseun Akinyemi, ADEDEJI, with matriculation number LCU/PG/001398 in the Department of Chemistry, Faculty of Applied Sciences, Lead City University, Ibadan, Oyo State is in full compliance with the approved University format and style.

_____ _____

Signature Date