UNIVERSITY OF GHANA

COLLEGE OF HEALTH SCIENCES

SCHOOL OF PHARMACY

DEPARTMENT OF PHARMACOLOGY & TOXICOGNOSY

SCREENING OF SELECTED GHANAIAN MEDICINAL PLANTS

FOR ANTI-PROSTATE CANCER ACTIVITY

BY

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THIS THESIS / DISSERTATION IS SUBMITTED TO THE UNIVERSITY OF
GHANA, LEGON IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR
THE AWARD OF MPHIL. PHARMACOLOGY DEGREE.

JULY, 2018
DECLARATION

I, Joseph Abankwa Kofi of Pharmacology and Toxicology Department, University of Ghana, Legon, hereby declare that this thesis is the result of my own research work carried out at the Noguchi Memorial Institute of Medical Research under the watch of a research assistant Mrs. Eunice Dotse and supervised by Prof. Regina Appiah-Opong and Prof. Alexander Nyarko.

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Joseph Kofi Abankwa                   Date
(Student)

........................................  ........................................
Prof. Alexander Nyarko                   Date

........................................  ........................................
Prof. Regina Appiah-Opong                Date
DEDICATION

I dedicate this work to God whose unfailing love, mercies, unimaginable divine favor and blessings saw me through this M.Phil. studies. I also dedicate this project to my father Jacob Kofi Abankwa whose encouragement, financial support and advice has brought me to this stage of life. I also dedicate this thesis to my mother Leticia Annor and my siblings Grace Ama Abankwa and Moses Kwesi Abankwa for all their words of comfort.
ACKNOWLEDGMENT

My sincerest appreciation goes to Prof. Alexander Nyarko and Prof. Regina Appiah-Opong for all their invaluable contribution to this work. My special thanks go to Mrs. Eunice Dotse, Ms. Abigail Aning and Mr. Isaac Tuffuor for all the meticulous support I received in the determination of the total phenols, antioxidant activities and cytotoxic activities of my plant extracts. I wish to express my profound gratitude to all the lecturers and staff members of the School of Pharmacy, University of Ghana. My utmost thanks go to Mabel Owusu-Bediako and Georgina Ataa-Nyarko for their valuable support in this study.
### ABBREVIATION

**A**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>ABTS</td>
<td>2,2'-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid)</td>
</tr>
<tr>
<td>AQ</td>
<td>Aqueous</td>
</tr>
<tr>
<td>ASR</td>
<td>Age-standardized incidence rates</td>
</tr>
</tbody>
</table>

**B**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>BHT</td>
<td>Butylated hydroxyl toluene</td>
</tr>
<tr>
<td>BRCA1</td>
<td>Breast cancer 1 gene</td>
</tr>
<tr>
<td>BRCA2</td>
<td>Breast cancer 2 gene</td>
</tr>
</tbody>
</table>

**C**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>CE</td>
<td><em>Capparis erythrocarpus</em></td>
</tr>
<tr>
<td>COX2</td>
<td>Cyclooxygenase 2 enzyme</td>
</tr>
</tbody>
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**D**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>DCM</td>
<td>Dichloromethane</td>
</tr>
<tr>
<td>DMSO</td>
<td>Dimethyl sulfoxide</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>DPPH</td>
<td>2, 2-Diphenyl, 1-picrylhydrazil</td>
</tr>
<tr>
<td>DRE</td>
<td>Digital rectal examination</td>
</tr>
</tbody>
</table>

**E**

<table>
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<tr>
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<th>Description</th>
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<tbody>
<tr>
<td>EA</td>
<td>Ethylacetate</td>
</tr>
<tr>
<td>ERα</td>
<td>Estrogen receptor α</td>
</tr>
<tr>
<td>ETOH</td>
<td>Ethanol</td>
</tr>
</tbody>
</table>
F

FA : Ficus asperifolia

FAK : Focal adhesion kinase

FBS : Foetal bovine serum

FRAP : Ferric reducing ability of plasma

G

GAE : Gallic acid equivalent

GSH : Glutathione

GST : Glutathione S-Transferase

H

HPC1 : Hereditary prostate cancer 1 gene

HPC2 : Hereditary prostate cancer 2 gene

I

IC₅₀ : Inhibitory concentration at 50%

IFN γ : Inteferon gamma

IL-10 : Interleukin-10

IL1-α : Interleukin 1 alpha

IL-6 : Interleukin-6

IL-8 : Interleukin-8

J

JC : Jatropha curcas
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>PSA</td>
<td>Prostate specific antigen</td>
</tr>
<tr>
<td>PSG</td>
<td>Penicillin Streptomycin</td>
</tr>
<tr>
<td>PTEN</td>
<td>Phosphatase and tensin homolog</td>
</tr>
<tr>
<td>RNA</td>
<td>Ribonucleic acid</td>
</tr>
<tr>
<td>RNS</td>
<td>Reactive nitrogen species</td>
</tr>
<tr>
<td>ROS</td>
<td>Reactive oxygen species</td>
</tr>
<tr>
<td>RPMI</td>
<td>Rosewell Park Memorial Institute</td>
</tr>
<tr>
<td>RUNX</td>
<td>Runt related transcription factor 2</td>
</tr>
<tr>
<td>SHBG</td>
<td>Sex hormone binding globulin</td>
</tr>
<tr>
<td>SOD</td>
<td>Superoxide dismutase</td>
</tr>
<tr>
<td>TAEC</td>
<td>Trolox equivalent antioxidant capacity</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Tumour necrotic factor alpha</td>
</tr>
<tr>
<td>TNF-β</td>
<td>Tumour necrotic factor beta</td>
</tr>
<tr>
<td>TPC</td>
<td>Total phenolic content</td>
</tr>
<tr>
<td>UV</td>
<td>Ultra violet</td>
</tr>
<tr>
<td>Zip1</td>
<td>Zinc Transporter protein</td>
</tr>
</tbody>
</table>
Screening of selected Ghanaian medicinal plants for anti-prostate cancer activity.

ABSTRACT

Introduction: Prostate cancer is a major disease that affects adult males. The high cost of current drugs in use, treatment failure/drug resistance as well as toxic side effects warrants the development of more effective, less toxic and affordable anti-prostate cancer drugs. The aim of the study was to investigate the anti-prostate cancer properties of selected Ghanaian medicinal plants used for the management of prostate cancer.

Method: The leaves, fruit and seeds of selected plants claimed to have anti-prostate cancer properties were collected, dried and crushed. Each sample was sequentially extracted with solvents of increasing polarity (petroleum ether, dichloromethane, ethyl acetate, ethanol and aqueous) using cold maceration. The phenolic substances in the plants were determined by the Folin-Ciocalteau method. Antioxidant and anti-proliferative activities were determined using DPPH and MTT assays, respectively.

Results: Nine medicinal plants namely, *Latuca sativa*, *Tetrapleura tetraptera*, *Jatropha curcas*, *Capparis erythrocarpus*, *Ficus asperifolia*, *Mentha piperita*, *Carica papaya*, *Moringa oleifera* and *Phyllanthus amarus* were identified. Among them, the ethanolic extract of *P. amarus* recorded the highest (92.71 g GAE/100g) level of phenolic substances. The aqueous and ethanolic extracts of *P. amarus* showed the strongest antioxidant activities (EC$_{50}$ values of 19.32 ± 1.13 µg/ml and 60.03 ± 1.07 µg/ml, respectively). Ethyl acetate extract of *Latuca sativa* and *Mentha piperita* also showed significant (p<0.05) antioxidant activities (EC$_{50}$=121.4± 1.07 µg/ml, 91.13% and EC$_{50}$=129.7± 1.07 µg/ml, 88.37%, respectively) but did not meet the 100 µg/ml limit cut off for consideration as strong antioxidants. Most of the plant extracts
significantly inhibited proliferation of LNCaP and PC3 prostate cancer cell lines compared to normal prostate (PNT2) cells. The ethyl acetate extract of whole *Tetrapleura tetraptera* fruit showed the highest cytotoxic activity and was selective to PC3 (IC$_{50}$ =5.39 ± 1.24 µg/ml, SI=7.72) and LNCaP (IC$_{50}$=8.38±1.27µg/ml, SI=4.97)

**Conclusion:** All the plants contained phenolic substances and had antioxidant activities. All the samples demonstrated anti-proliferative activities with ethyl acetate extract of whole *Tetrapleura tetraptera* exhibiting the strongest anti-proliferative activity against LNCaP and PC3 prostate cancer cell lines.

**Recommendation:** Future studies should focus on investigating the phytochemical profile of *Tetrapleura tetraptera*, isolating the bioactive compounds responsible for the anti-prostate cancer activity and understanding the mechanisms involved in their anti-proliferative activity against LNCaP and PC3 cancer cells.

Keywords: Medicinal plants, Secondary metabolites, DPPH, Antioxidants, Total phenolic content.
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Chapter 1

1.0 Introduction

Cancer refers to the uncontrollable proliferation of a cell with the ability to metastasize to other areas of the body (Soladoye et al., 2010). Earlier reports have suggested that cancerous growth is parasitic and thrives well at the cost of the human body. Elledge (2009) has indicated that cancer cells have hall marks which include resistance to cell death, unlimited proliferative activity and are self-sufficing in cell growth signaling. Globally, various forms of cancers of the breast, liver, cervical, prostate and many more have been acknowledged and reported to have a great negative impact on our economy today. Jemal et al. (2011) has reported that cancer is the leading cause of deaths in many economically developed countries, killing about 7.6 million people in 2008. In a period of 9 years (1991-2000), the Korle bu Teaching Hospital, one of the biggest hospitals in Ghana, reported about 3659 deaths related to cancer (Wiredu and Armah, 2006). Wiredu and Armah (2006) reported the major types of cancer with high mortality rate among females to be breast cancer (17.25%) and hematopoietic malignancies (14.69%) and liver (21.15%) and prostate cancer (17.35%) being the highest in men.

Prostate cancer has been described to be the growth of malignant tumor in the prostate gland of the reproductive system in males (Alotaibi et al., 2017). It is reported to be the succeeding principal cause of cancer associated death after hepatocellular carcinoma in Ghanaian men (Wiredu and Armah, 2006). A few of the attributed risk factors to cancer as listed by the National Cancer Institute include old age, genetics, race and genealogy of prostate cancer (Pienta and Esper, 1993). Also, Bostwick et al. (2004) have reported lifestyle practices such as
smoking, excessive alcohol intake, lack of exercise and eating of meat to be some risk factors to prostate cancer. Pham-Huy et al. (2008) have revealed that free radicals and related reactive species generated from some of such lifestyle practices are implicated in prostate cancer hence attracting a great deal of alertness for a therapeutic strategy. It is noteworthy that, the reactive oxygen species (ROS) and reactive nitrogen species (RNS) are induced in the body through various exogenous sources and endogenous processes such as oxygen metabolism, mitochondria oxidative phosphorylation, protein glycation and lipid peroxidation (Valko et al., 2007; Pacher et al., 2007). These reactive radicals react with protein, genes, DNA and cause specific tumor suppressors and proto-oncogenes to become deregulated at organ sites (Taylor et al., 2010; Genestra, 2007; Baca et al., 2013; Barbieri et al., 2012). In Ghana, prostate cancer is documented to have the second highest mortality rate of 17.35% among 286 males (Wiredu and Armah, 2006). Laryae et al. (2014) also indicated that prostate cancer is the second most regular cancer case recording 13.2% among 76 males at the Komfo Anokye Teaching Hospital (KATH). Different reports have shown that, prostate cancer can be more aggressive and may advance to a castration-resistant disease which is mostly common in blacks (Gueye, 2003; Yarney, 2013 and Clegg-Lamptey, 2009). Besides, inheriting a faulty tumor suppressor gene (BRCA 2, BRCA 1, HPC 1, HPC 2 and p53) can make the disease severe (Benafif and Eeles, 2016; Yeboah et al., 2016).

Available treatment options for prostate cancer include surgery, chemotherapy, radiation therapy and hormone therapy. The choice of treatments largely depends on the stage and the gleason score of the prostate cancer. Oral chemotherapy which is usually administered by a medical oncologist often consists of giving the anti-cancer agent a specific number of cycles
over a set period of time. In general, standard chemotherapy begins with docetaxel (Docefrez, Taxotere) combined with a glucocorticoid prednisone. However, some of the cancers tend to resistant to the anti-cancer agents as some prostate cancer cells continue to grow (Petrylak et al., 2004; Tannock et al., 2004). Other substitutes such as mitoxantrone and carbazitaxel (Jevtana) have been reported to be toxic and do not improve lifespan (Tannock et al., 2004). In general, the side effects may depend on the individual, the type of chemotherapy, the dose used and the length of treatment. Prostate cancer treatment is expensive and some such as radiation and chemotherapies have toxic effects on non-targeted tissues (Fadeyi et al., 2013). Desai et al.(2008) have report ed inherent problems such as myelotoxicity, renal toxicity, cardiotoxicity and many more associated with oral chemotherapy. Due to these unwanted side effects, the search of other natural sources of drugs for treatment is of interest to many researchers.

Nature has blessed mankind with medicinal plants which are believed to have reduced side effects (Greenwell, 2015). Okwu (2004) has reported that these medicinal plants provide a rich supply of antioxidants which are able to scavenge these free radicals. Many therapeutic benefits of the medicinal plants are due to the high contents of superoxide dismutase, vitamin A, vitamin C, catalase, glutathione reductase, glutathione peroxidases and Vitamin E (Hercberg et al., 2004; Halliwell, 2005). Also, Giustarini et al.(2009) have indicated that antioxidants could be used in the treatment and management of oxidative stress associated diseases like prostate cancer. In addition, Raman (2006) has indicated that medicinal plants also contained anti-cancer therapeutic phytochemical compounds which include alkaloids, saponins, tannins, flavonoids, anthraquinones, cardiac glycosides and terpenoids.
Africa hosts about 57,704 plant species (Bramwell, 2002). Although there are volumes of oral histories of the therapeutic benefits of these plants, the scientific evidence of the claims are lacking compared to Chinese and Indian herbal medicines (Cordell et al., 1991). Hence, it is useful to review the claims that have been made and assess the quality of evidence available for better documentation of claims for research and development.

In Ghana, herbalist use medicinal plants and other herbal products for the management and treatment of prostate cancer (Mshana et al., 2000). For example, Ghanaian medicinal plants such as *Croton membranaceous* and *Zanthoxylum xanthoxyloides* have been shown to possess high cytotoxic activities against cancer cell lines and their use as anti-cancer agents are supported (Ayim et al., 2007). In this study, an ethnobotanical survey was conducted to identify other medicinal plants claimed to be useful for management of prostate cancer in Ghana. The plants identified are *Latsuca sativa*, *Tetrapleura tetraptera*, *Jatropha curcas*, *Capparis erythrocarpus*, *Ficus asperifolia*, *Mentha piperita*, *Carica papaya*, *Moringa oleifera* and *Phyllanthus amarus*. Information gathered on the plants are summarized in Table 1.
Table 1: Ethnobotanical information of medicinal plants

<table>
<thead>
<tr>
<th>CN</th>
<th>Scientific name of plant</th>
<th>Family</th>
<th>Local Name (Akan)</th>
<th>Parts used</th>
<th>Medicinal properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>TT</td>
<td><em>T. teretraperta</em></td>
<td>Mimosaceae</td>
<td>Prekese</td>
<td>Fruits, seeds</td>
<td>Antioxidant and anti-inflammatory and hypoglycemic activities (Gberikon et al., 2015; Ojewole and Adewummi, 2004).</td>
</tr>
<tr>
<td>FA</td>
<td><em>F. asperifolia</em></td>
<td>Moraceae</td>
<td>Nyankyerene</td>
<td>Leaves</td>
<td>Antioxidant and anticancer activity against DLD-1 and MCF-7 cell lines (Ojo and Akintayo, 2014; Bayor et al., 2007).</td>
</tr>
<tr>
<td>CP</td>
<td><em>C. papaya</em></td>
<td>Caricaceae</td>
<td>Brofere</td>
<td>Leaves</td>
<td>Antioxidant activity, antitumour activity against Jurkat, Molt-4, CCRF-CEM and HPB-AL (Pierson et al., 2012; Otsuki et al., 2010).</td>
</tr>
<tr>
<td>MO</td>
<td><em>M. oleifera</em></td>
<td>Moringaceae</td>
<td></td>
<td>Leaves</td>
<td>Antioxidants (Sultana et al., 2009); anti-inflammatory, anti-immunomodulatory activity (Das et al., 2013; Koolthot et al., 2014).</td>
</tr>
<tr>
<td>CE</td>
<td><em>C. erythrocarpus</em></td>
<td>Capparaceae</td>
<td>Woresenakyiame</td>
<td>Roots, leaves</td>
<td>Anti-arthritis, anti-inflammatory (Danquah et al., 2011).</td>
</tr>
<tr>
<td>PA</td>
<td><em>P. amarus</em></td>
<td>Euphorbiaceae</td>
<td>Bommagwufuki</td>
<td>Aerial parts</td>
<td>Antioxidant activity, anticancer activity Caco-2, HepG2, MCF-7, A549, DU 145 (Nguyen et al., 2017; Lawson-Exvi, 2008; Lee et al., 2011; Poompachee and Chudapongse, 2011).</td>
</tr>
<tr>
<td>JC</td>
<td><em>J. curcas</em></td>
<td>Euphorbiaceae</td>
<td>Abrototo</td>
<td>Leaves</td>
<td>Antioxidant, anti-inflammatory and anticancer (Staubmann et al., 1999; Balaji et al., 1999).</td>
</tr>
</tbody>
</table>
1.1 Problem statement and Justification

In many developed countries, cancer cases are rising and cancers are principal causes of death due to lifestyle changes, late reporting of the disease and treatment problems (Jemal et al., 2011; Segbefia et al., 2013). Globocan reports in 2012 indicated that globally, diagnosis stood at about 14.9 million cases with about 8.2 million deaths (Globocan, 2012). Also, the cost of orthodox medicine is often prohibitive to many individuals. The World Cancer Report valued cancer-related expenses at $1.16 trillion per annum as of 2010 (World Health Organization, 2014).

In Ghana, prostate cancer is prevalent among adult men and has led to the death of many (Wiredu and Armah, 2006; Yeboah et al., 2014). Currently, cancer drugs in use have been reported to be expensive, toxic and have not fulfilled their therapeutic expectations (Zhang et al., 2009; Hirose et al., 2011). Additionally, existing plant derived anti-cancer drugs such as vinblastine, taxol, paclitaxel have been shown to have side effects, ineffective due to drug resistance and are very expensive. Eid et al. (2015) have reported that 30-80% of cancers are resistant to anti-cancer drugs. This major setback necessitates the search for other sources for the treatment and management of the disease. Hence, the need to search the Ghanaian plant diversity for more effective, less toxic, affordable and readily available anti-prostate cancer drugs. Information obtained from the ethnomedical survey in Table 1 indicate that some of the selected plants are reported to have anti-cancer activity against cancer cell lines such as breast cancer, leukemia, lung cancer but not prostate cancer. Although, P. amarus is reported to have anti-proliferative activity on metastatic prostate cancer cells (DU 145), its effects on androgen dependent prostate cancer cell line has not yet been assessed. The same is true for Carica
papaya. Furthermore, though some of the selected plants are reported to have antioxidant properties, their cytotoxic potential against prostate cancer cell lines have not been assessed. Therefore, there is need to evaluate the effectiveness of the nine plants as antioxidants and anti-prostate cancer agents.

1.2 Hypothesis
The selected Ghanaian medicinal plants may have strong antioxidant and anti-prostate cancer properties.

1.3 Aim
The aim of the study was to investigate medicinal plants used in Ghana for the management of prostate cancer and determine their anti-cancer activity.

1.4 Objectives of the study
1. To identify and select plants used in Ghana for the management of prostate cancer.
2. To determine the total phenolic content of crude extracts of the identified plants using the Folin-Ciocalteau reagent.
3. To evaluate the total antioxidant activities of the extracts using the free radical scavenging assay.
4. To screen the selected plant extracts for cytotoxic activities against human prostate cancer cell lines PC3 and LNCaP using the tetrazolium-based colorimetric (MTT) assay.
CHAPTER 2

2.0 Literature review

2.1 Cancer

Cancer is the rapid growth of malignant cells and can move to various parts of the body (Gbadamosi and Erinoso, 2016). Bolarin (2009) has indicated that malignant tumors grow by invasion, infiltration and have the tendency to reappear after surgical treatment. Additionally, Hanahan (2011) reported that cancer cells can evade apoptosis by altering the apoptotic signaling pathway. Globocan reports indicated that about 14.1 million new cases and 8.2 million deaths occurred around the world (Globocan, 2012). It is estimated that the number of cancer related deaths may rise to 12 million by 2030 (Gbadamosi and Erinoso, 2016). The Globocan (2012), reports indicated that there were about 850,000 new cancers and almost 600,000 deaths recorded in Africa.

Studies in Ghana, have indicated that the age-standardized incidence rates (ASR) of cancers for males was 10.9/100,000 and 22.4/100,000 for females (Laryae et al., 2014). The reports indicate that cancer is a global burden.

2.1.1 Types and classification of Cancer

Cancer can be categorized based on the symptoms presented, cellular make up and the locations of the cells from which they originated. Classification may be based on categories reported by Sorensen et al. (1998), which include lymphomas, carcinomas, sarcomas and myelomas.
Lymphoma is a group of blood cancer that develop from lymphocytes and presents signs which include enlarged lymph nodes, fever, unintended weight loss and drenching sweats. Carcinoma is another type of cancer that occurs in cells that line the tissues of an organ such as the kidney, liver and prostate.

Sarcomas are known to arise from transformed cells of the mesenchymal connective tissue origin such as bone and cartilage whiles melanomas were reported to develop from the pigment-containing cells known as melanocytes particularly in the skin and can spread to other organs in the body.

Myeloma is a type of cancer of the plasma cells that prevent the normal production of antibodies, therefore compromising the body’s immune system and making it susceptible to infection. Glioma is a form of cancer that occurs in glial cells in the spine and the brain.

Histological classification of cancers is established on determining the kind of cells present and their origin (Goldstein, 2010). Reports by Jemal et al. (2005) have indicated that, the most prevalent sites of cancer in men are prostate, lung, colon and rectal while cancer of the breast, cervix and ovary has been reported in women.

2.1.2 Cancer risk factors

The proliferation of cancer cells is undesirable, parasitic and occurs rapidly in the human body (Olapade-Olaopa et al., 2014). Numerous studies show that malignant neoplasm can arise from a broad group of diseases such as hepatitis C and B and human papilloma virus (Perz, 2006; Trichopoulos, 1996). Viruses, bacteria and parasites are the common infectious agents
that cause DNA damage and invoke mutations causing irregularities in the gene function (Trichopoulos, 1996). Reports by Gbadamosi and Erinoso (2016) indicated that, risk factors to cancer in Africa may be attributed to numerous factors such as occupation, weather, lifestyle, rural area habitation, trauma, virus, food, pressure, drink and drugs. Other established factors include sex, age, obesity and race (Feigelson et al., 2004). Additionally, Danaei et al. (2005) have identified some general environmental factors that may give rise to cancer related death. These include radiation (both ionizing and non-ionizing), environmental pollutants, excessive tobacco smoking, fatty diets, infections and stress. These carcinogenic agents lead to the production of free radicals that induce oxidative stress. Additionally, the induced oxidative stress is very harmful and can damage the DNA, lipids and protein, leading to an increased risk of cancer development (Iweala, 2015).

2.1.3 Prostate Cancer

Prostate cancer is a form of cancer that occurs in the cells of the prostate gland and has the ability to metastasize to other areas of the body. The prostate is an exocrine gland that rest under the bladder of the male reproductive system. Early growth of prostate cancer has been documented to be androgen driven and normally occurs in the seminal prostate gland cells (Huggins and Hodges, 1941). The growth and development of the male reproductive system is primarily influenced by androgens which includes testosterone. Research shows that, early stages of prostate cancer growth require 5α-dihydrotestosterone derived from testosterone produced by the 5α-reductase enzyme system. On the other hand, other studies have shown that aromatase enzyme is able to convert testosterone to estradiol which binds to the estrogen receptor α (ERα) and act as transcription factors for prostate cancer cell proliferation (Hu et al., 2012). Amazingly, hormonal therapy or androgen ablation has been used for the treatment
and management of androgen dependent prostate cancer (Paul and Bruel, 2000). Yet, health complications have been associated with the use of androgen deprivation therapy. These complications include genetic damage from carcinogenic estrogen metabolites and increase incidence of osteoporosis (Kim and Freeland, 2010; Wibowo et al., 2010). Also, prostate cancer cells are able to adapt to the androgen deprived environment by generating hyper active receptors with broadened activation spectra (Hsieh, 2008).

A report by Laufer et al. (2000) has indicated that some prostate cancer cells can be androgen independent after prolonged clinical remission from the disease. Hence, hormonal intervention becomes futile and void as a result of mutations in the androgen receptor and amplification or loss of receptors (Wang and Uchida, 1997; Henshall et al., 2001; Kinoshita et al., 2000).

There are several types of unusual proliferation of cells in the prostate tissue. Generally, most prostate cancers are classified as adenocarcinoma (Bostwick, 1989). The few forms of cancers in the surrounding tissues of the prostate gland may include transitional cell (urothelial) cancer, ductal adenocarcinoma, squamous cell cancer, sarcomas and carcinoid. Also, several studies have shown that chronic inflammation is implicated in prostate cancer cell (Gurel et al., 2014; De Nunzio et al., 2011). Furthermore, some innate and adaptive immune cells such as inflammatory cytokines (IL-8 and IL-1α) and cyclooxygenase 2 enzyme (COX2) producing prostaglandins, have been reported to play a role in prostate cancer progression (Salazar et al., 2013; Shiao et al., 2016).
2.1.4 Description of Prostate Cancer

Studies have shown that, prostate cancer is heterogeneous in terms of its biological and molecular characteristics, ploidy, grade, oncogene expression and tumor suppression gene expression (Ahmed, 2012). In addition, many prostate cancer cells have been reported to break off and invade neighboring tissues (especially local lymph nodes and skeletal bone) and they grow faster than normal healthy cells (Gbadamosi and Erinoso, 2016).

2.1.5 Pathophysiology of Prostate Cancer

In adenocarcinoma, the normal semen-secreting prostate gland cells mutate into cancer cells, multiply and spread to the surrounding tissue of the prostate. In this case, the prostate that surrounds part of the urethra grows rapidly and eventually blocks or affect urination and ejaculation. Also, in prostate cancer some genes that suppress tumour growth are silenced. For example, the tumour suppressor gene product for the gene SLC39A1 (ZIP1) which transport and accumulates zinc in the prostate for production of citrate, a major component in semenare silenced (Costello and Franklin, 2006). Furthermore, there is upregulation of RUNX2 (a transcription factor that prevents apoptosis) and macrophage inhibitory cytokine-1 (MIC-1) which stimulates the focal adhesion kinase (FAK) signaling pathway which leads to survival and growth of prostate cancer (Senapati et al., 2010). Also, the downregulation of a tumour suppressor PTEN gene, E-cadherin and CD 44 have been observed. Prostate specific membrane antigen has been reported to increase available folates by hydrolyzing glutamated folates which stimulates the development of prostate cancer.

Oxidative stress caused by the presence of free radicals (ROS and RNS) have also been implicated in some adenocarcinoma (Valko et al., 2007; Parker et al., 2007). The free radicals
have been reported to increase as one ages due to the decreased levels of vitamin D, glutathione and tumor suppressor protein (p53) (Minelli et al., 2009). Also, inflammation has been shown to increase free radicals whiles modulation of androgen have been reported to produce hydrogen peroxide (Minelli et al., 2009). Increased oxidative stress has been related to prostate cancer and that supplementation using antioxidants have the potential to protect the prostate gland against prostate cancer (Khandrika et al., 2009; Chen et al., 2001; Wang et al., 2015).

2.1.6 Clinical Manifestation

The severity of prostate cancer disease increases with age and it is more prevalent in older individuals between 40 years and 80 years (Stangelberger et al., 2008; Yeboah et al., 2016). The various reported symptoms of prostate cancer include swelling of the prostate gland, blood in urine, blood in semen, nausea, pain, and difficulty urinating (Talcott, 2003). Also, pains in the bones, loss of bladder control and weak urinary stream have been implicated in prostate cancer. However, these symptoms may also arise due to other issues.

2.1.7 Prevalence of prostate cancer

It has been reported that 75% of all new prostate cancer cases are diagnosed in developed countries with global prostate cancer occurrence accounting for about 899,000 new cases, and about 258,000 deaths each year (Alotaibi et al., 2017). Also, cancer is projected to rise to 24 million by 2035, with consequent increase in expected deaths (Alotaibi et al., 2017). Figure 1 shows the mortality and incidence of prostate cancer across all ages in different areas of the world. The chart shows high incidence with low mortalities due to prostate in Australia / New Zealand and more developed countries. In contrast, though in Western Africa the incidence of
prostate cancer was lower, mortality rates associated with prostate cancer is high (Globocan, 2012; Hassanipour-Azgomi, 2016).

Figure 1A: The figure above shows the incidence and mortalities of 100,000 individuals suffering from prostate cancer across the ages in different areas of the world (Globocan, 2012).

In Ghana, 17.35% (n=286) of male cancer death is related to the prostate (Wiredu and Armah, 2006). The prevalence of the disease has been reported to be high among Ghanaian men (Yeboah et al., 2016). Reports by Laryae et al. (2014) also indicated that prostate cancer is the second most common cancer cases recording 13.2% (out of 76 males) at the Komfo Anokye Teaching Hospital (KATH).

2.1.8 Diagnosis of Prostate cancer

Diagnosis of prostate cancer helps in determining and managing the disease through various tests and therapies. Some of the approved test include digital rectal examination (DRE),
biomarker test, biopsy, prostate specific antigen test (PSA), prostate cancer antigen 3 (PCA3) test, trans-rectal ultrasound test, magnetic resonance imaging and many more. Physical examination of the prostate gland using the digital rectal examination (DRE) helps to identify abnormal parts of the prostate gland. The presence of prostate specific antigen (PSA) in either the urine or the blood is expressed by abnormal prostate gland cells and usually used for the screening of prostate cancer (Polascik et al., 1999). High levels of PSA suggest the existence of an inflammation in the prostate gland. Recent studies have, however, shown that some benign tumours have higher PSA levels (>4 ng/ml) hence PSA levels could have utility limitation in determining precisely the state of the tumour as being cancerous or benign (Loeb and Schaeffer, 2009). The precise state of the prostate gland as to whether it is cancerous or benign can be confirmed by biopsy. Biopsies involve the removal of a small amount of tissue for examination under the microscope. Gleason score and pathological staging are additional tests done to identify the aggressiveness and size of the cancer cells in order to inform urologists of appropriate treatment plan.

Specific biomarkers such as prostate specific membrane antigen (PSMA) which is produced in two different forms can help in differentiating a benign prostate gland tissue from cancer of the prostate gland (Su et al., 1995; Chang, 2004). Prostate specific membrane antigen (PSMA) is a transmembrane protein which forms immunoconjugates with anti-PSMA monoclonal antibodies 7E11 and still remains a therapeutic target (Chang, 2004). Immuno-histochemistry expression of PSMA in prostate specimens has been described, hence making it a useful diagnostic tool (Bostwick et al., 1998).
2.1.9 Treatment options and Limitation

The rationale behind the treatment is to exterminate cancer. Treatment options include radiation therapy, immunotherapy, surgery, hormone therapy, targeted therapy, chemotherapy and traditional herbal medicine (Gbadamosi and Erinoso, 2016). Combination chemotherapy has been the basis for treating cancer (Waterhouse et al., 2006). In cancer chemotherapy, anti-cancer agents are used in the treatment of prostate cancer. These chemotherapeutic agents include anti-metabolites, alkylating agents and natural products (Dardi et al., 2012). Chemotherapy is the use of drug (natural or synthetic) to annihilate or inhibit the growth of malignant tumours. Chemotherapy may be administered as a primary therapy or adjuvant chemotherapy. Palumbo et al. (2013) has classified cytotoxic agents as anti-metabolites (such as methotrexate and cytarabine), alkylating agents (such as cyclophosphamide and oxaliplatin), topoisomerase inhibitors (such as etoposide and irinotecan), anti-tumour antibiotics (such as doxorubicin and bleomycin) and microtubule stabilizers (such as paclitaxel and docetaxel). Drugs used for cancer treatment could be administered orally or intravenously. During drug distribution, the bioavailability of the drug act on rapidly growing cells, especially metastasized malignant cells. The toxic effects of these drugs are due to the inability to target not only cancer cells but affect all cells including normal cells.

Antimetabolites are chemical compounds that have a similar structure to the actual metabolite, such as altered nucleotides, and act to impair DNA replication machinery, thus preventing cell proliferation. Leumann (2005) has reported the pharmacodynamic action of anti-metabolites as directly integrated into DNA or RNA and halting cell growth by impairing DNA replication machinery.
Alkylating agents are highly reactive compounds that interfere with DNA replication by adding alkyl groups to nucleic acids such as guanine base of DNA molecule.

Topoisomerase inhibitors as anti-cancer agents have been shown to block the action of topoisomerase I and II which is responsible for unwinding DNA for replication (Vladimir et al., 2017). Also, other agents (anti-tumour antibiotics) such as anthracyclines have been reported to intercalate with the DNA and indirectly affecting topoisomerase II whiles mitomycin-C act more likely as an alkylating agent. The use of these drugs as anti-cancer agents has been successful due to different mechanisms of action on highly dividing cells during mitosis and the dependence on biomolecules by cancerous cells as compared to normal cells. However, these agents have been reported to target normal cells such as bone marrow cells, hair generating cells and cells lining the alimentary, which form the basis of their side effects such as alopecia, nausea, bone marrow depression resulting in anaemia and vomiting (Palumbo et al. 2013).

The growth of some cancers such as breast and prostate cancers in the body primarily depends on hormones. Thus, blocking the hormone concerned is important in treating such cancers. For example, the administration of a hormone-like drugs that block receptors (estrogen receptor α) or agents that stop the production of hormones in the prostate slows the primary growth of prostate cancer(Feldman and Feldman, 2001). In prostate cancer, drugs like bicalutamide, prednisone, have been used to block receptors on prostate gland cell from receiving testostorene. Although hormonal therapy provides a different therapeutic strategy it is not devoid of inherent side effects such as the lack of sexual drive or libido and erectile dysfunction. Other side effects include weight loss, osteoporosis and bone fracture.
Immunotherapy is a form of therapy which involves the use of biological agents that mimics growth signals and restrict tumour growth. These drugs include monoclonal antibodies, T-cell therapy and cancer vaccines are able to stimulate the body to produce immune cells to fight cancer cells via either by active immunity or passive immunity. Some examples of monoclonal antibodies are rituximab, adalimumab, bevacizumab, infliximab among others are administered to fight cancer. Sudhakar (2010) has reported the use of Sipuleucel-T as an immunotherapy drug which destroys prostate cancer. However, side effects such as cardiotoxicity, nausea, congestive heart failure head ache and optic neuritis has been implicated with this kind of treatment (Kaltsonoudis et al., 2014; Gasparyan et al., 2012).

Radiation therapy involves the use of high doses of ionizing radiation to effectively and safely treat cancer. It can be used to target the malignant tumour and functions by destroying the DNA of the cancerous cell. Fuccio et al. (2012) have reported side effects such as diarrhea and sore mouth.

These aforementioned cancer treatments have documented side effects, reduced efficacy, face challenges of drug resistance, high cost and lacks patient compliance. Also, experts who perform surgical operations and radiation therapies are inadequate in Ghana, making treatment of prostate cancer difficult. These limitations have necessitated the search for affordable, safer, less toxic and efficacious anti-cancer agents (Fadeyi et al., 2013).

Plant biodiversity has since time immemorial served as sources of medicines and many modern drugs have had plants as their source of origin. For example, in the case of anti-
cancer agents, vinca alkaloids, vinblastine, and vincristine have been isolated from the plant *Catharanthus roseus* (Gueritte and Fahy, 2005). Medicinal plants like *Croton membranaceous* and *Zanthoxylum xanthoxyloides* have also shown high cytotoxic activities against cancer cells and have potential use as anti-cancer agents (Ayim *et al*., 2007). However, some of the plants derived anti-cancer agents have also been shown to have side effects such as cardiotoxicity, kidney toxicities, and liver toxicities (Chen, 2013; Iqbal, 2017).

### 2.2.0 Plants in the management of Diseases

Medicinal plants are of huge significance to human health and are used to alleviate, treat disease or modify physiological processes (Rates, 2001). According to the reports by Kong *et al.* (2003) there are more than thirty-five thousand (35,000) plant species used as medicinal plants around the world. Medicinal plants have been used since time immemorial and are known to have curative properties as a result of their constituent chemical compounds (Tapsell *et al*., 2006). A plethora of traditional medicine references attest to the curative power, and scientific validation of these popular uses is in progress to support at least some of the claims. These pharmacologically active compounds include metabolites such as alkaloids, glycosides, polyphenols and terpenes (Raman *et al*., 2006). Previous reports have demonstrated tremendous role of secondary metabolites serving as antioxidants, anti-inflammatory agents and anti-cancer agents (Dias *et al*., 2012; Kim *et al*., 2011).

Most medicinal plants-derived substances such as vitamins, carotenoids phenolic compounds, flavonoids and functional metabolites have antioxidant that inhibit oxygen free radicals formation and degrade peroxides (Arabshahi *et al*., 2007). Medicinal plants are still used in the manufacture of many pharmaceutical drugs. Reports by Konczak *et al.* (2009) indicate that
some herbs and fruits have cytoprotective properties and plant-derived polyphenols in diets can lessen the threat of prostate cancer (Apostolou, 2013). The ethno-botanical survey for this study resulted in the identification of nine plants used for the management of prostate cancer. These are: *Latuca sativa*, *Tetrapleura tetraptera*, *Jatropha curcas*, *Capparis erythrocarpus*, *Ficus asperifolia*, *Mentha piperita*, *Carica papaya*, *Moringa oleifera* and *Phyllanthus amarus*.

2.2.0.1 *Carica papaya*

*Carica papaya* is a fruit mostly grown in the tropics. It is a species often used as medication for the treatment of diseases (Mello, 2008). It has a good effect as a remedy in dyspepsia and related ailments. The seeds are normally used as a dewormer when chewed. Also, the root and the juice are used for the management and treatment of cough, bronchitis, and other respiratory diseases. Although, the leaves are normally discarded, they have been used in folk medication for the treatment of conditions such as fever, gastric digestion problems, burns and amoebic dysentery (Canini *et al.*, 2007; Zunjar, 2011). Papain from crushed leaves is used traditionally to tenderize tough meat according to a report by Kadiri (2016). The leaf is used to expel parasitic worms. The dried leaves are smoked to alleviate asthma. Sealed packs of dried powdered leaves are sold in "health food" store. In Ghana, leaf decoction is administered as a purgative for horses. The dried leaf mixture is taken as a purgative and may cause abortion. A report by Owoyele *et al.* (2008) has indicated the anti-inflammatory activity of the leaves. Recently, black seeds of *Carica papaya* have been reported to have anti-prostate cancer activity (Alotaibi *et al.*, 2017).
The cytotoxic and total free radical scavenging activities of pawpaw leaves has been reported to be associated with the phytochemical compounds and their secondary metabolites such as proanthocyanidins and flavonoids (Pierson et al., 2012). Also, many antioxidant compounds such as flavonoid, tannin, anthraquinone, Vitamin A, and Vitamin C, have been found in pawpaw leaves (Imaga, 2010). Imaga et al. (2010) has reported the presence of tannins, saponins, alkaloids, vitamin B<sub>12</sub>, glycosides, folic acid and anthraquinones in different extracts of *C. papaya* leaves.

Also, Subramanian et al. (2014) has also reported the presence of carbohydrates, flavonoids, terpenoids, saponins and tannins in different extracts of *C. papaya* leaves. The phytochemical studies as reported by Subramanian indicated the absence of alkaloids, protein and amino acids in each solvent extract (methanol, ethanol, ethyl acetate and chloroform). However, tannins have been present in methanol and ethanol solvent extract but absent in ethyl acetate and chloroform extracts. Also, saponins were present only in methanol extract. In the leaves of *C. papaya*, some phytochemicals present has been reported to have anti-tumor activity and this include lycopene, tocopherol, flavonoid, and benzylisothiocyanate (Ching and Mohamed, 2001; Miean and Mohamed, 2001; Basu and Haldar, 2008; van Breemen and Pajkovic, 2008).

### 2.2.0 Phyllanthus amarus

*Phyllanthus amarus* is a small herb belonging to the family euphorbiaceae. This plant is mostly found in countries in the tropics. It is used traditionally in Nigeria and India to treat malaria associated symptoms and to ease genitourinary system, stomach pain, and liver, kidney, and spleen disorders (Iranloye et al., 2010). Nguyen et al. (2017) has reported that methanolic extract of *P. amarus* possessed more phenolic content than the aqueous extract. Previous studies have demonstrated the cytotoxic activity of *P. amarus* extracts on several
human cancer cell lines such as prostate cancer cell line DU145, colon cancer cell line Caco-2, lung cancer cell lines A549, breast cancer cell line MCF-7, and liver cancer HepG2 (Lawson-Evi, 2008; Lee et al., 2011; Poompachee and Chudapongse, 2011; Nguyen et al., 2017).

An earlier study showed that the aqueous extract of *P. amarus* inhibits DNA topoisomerase II of cell cultures. In addition, inhibition of cell cycle regulators responsible for cancerous growth has been reported in extracts of *P. amarus* (Rajeshkumar, 2002). Also, Kiemer (2003) has indicated that extracts of *P. amarus* inhibited induction of interleukin (IL)-1β, interferon-γ (IFN-γ), and interleukin (IL)-10 and reduced tumor necrosis factor (TNF-α) production.

Kassuya (2005) has also shown that the hexane and ethanol / water extracts of *P. amarus can lower the expression of COX-2 and endotoxin-induced nitric oxide synthase and prevent the onset of nuclear factor NF-κB.

2.2.0.3 *Moringa oleifera*

The leaves of *Moringa oleifera* are sometimes eaten raw or used for food preparations. In traditional medicine, aqueous extract of the leaves is used to treat several maladies including swellings, genito-urinary ailments, hypertension, malaria, diseases of the skin, arthritis and diabetes (Konmy et al., 2016; Aji et al., 2002). The leaves possess antioxidants, deficiency of which has adverse health implications (Konmy et al., 2016). *Moringa oleifera* leaves are rich in polyphenols, vitamins, saponins, flavonoids, carotenoids, alkaloids, tannins, isothiocyanates and glucosinolates (Sultana et al., 2009; Sreelatha and Padma, 2009).

Coppin et al. (2013) has indicated that *Moringa oleifera* leaves extract is able to inhibit nitric oxide (NO) produced by macrophage cells which was treated with bacterial
lipopolysaccharide (LPS). Moreover, anti-inflammatory and immunomodulatory effects of *Moringa oleifera* leaves have been reported in many *in vivo* and *in vitro* studies (Das *et al*., 2013). In addition, ethyl acetate extracts of *Moringa oleifera* leaves were reported to inhibit human macrophage cytokine production (TNF-α, IL-6 and IL-8) induced by extract of cigarette smoke (Kooltheat *et al*., 2014).

### 2.2.0.4 *Jatropha curcas*

*Jatropha curcas* is a woody plant belonging to the euphorbiaceae family. This medicinal plant is mostly used traditionally for the management of malaria, fever, diabetes, night blindness, headaches and arthritis (Iwu, 1993; Sandberg *et al*., 2005). The aqueous extract of the leaves is used locally for treating diabetes in Nigeria (Gbolade, 2009). In India, *J. curcas* leaves are used for treating liver troubles and jaundice (Sharma *et al*., 2010). Additionally, a report by Nath and Choudhury (2010) showed that the decoction of *J. curcas* potent for the treatment of wounds, chest inflammation and reducing pain. Uche and Aprioku (2008) have reported that methanolic extract of *J. curcas* leaves possessed anti-inflammatory activity on Wister albino rats. A previous study has reported that methanolic extracts of the leave had cytotoxic activity against HT 29 cell line (Oskoueian *et al*., 2011).

Odogwu *et al*. (2011) has reported that *Jatropha sp* contains secondary metabolites including alkaloids, sesquiterpenoids, triterpenes, flavonoids, steroid, saponins and phenolic. *J. curcas* leaves contain flavonoids, jatropholone, and tannins (El Diwani *et al*., 2009; Manpong *et al*., 2009). Previous studies have shown that aqueous and ethanolic extracts of the plant also possessed antioxidant activity (Igbinosa *et al*., 2011; El Diwani *et al*., 2009; Oskoueian *et al*., 2011). Additionally, the phosphomolybdate assay revealed high reducing power of methanolic extract of *J. curcas* (38.8mg/g Gallic acid equivalent).
2.2.0.5 *Mentha piperita*

*Mentha piperita* is commonly known as peppermint. The boiled leaf extract is used traditionally for treating bronchitis. As a folk medicine, the peppermint extract has been employed for centuries for the treatment of anorexia, vomiting and bronchitis (Iscan, 2002; Moreno, 2002). Previous studies have reported antifungal, antioxidant activities and anti-inflammatory properties (Nickavar, 2008; Sousa, 2010). Sun *et al.* (2014) reported that the essential oil of *M. piperita* has anti-inflammatory properties against SGC-7901, SPC-A-1 and K562 cancer cell lines.

2.2.0.6 *Ficus asperifolia*

The leaf extract of *Ficus asperifolia* is used as a purgative and for the treatment of helminths. Ojo *et al.* (2014) have reported the presence of saponins, glycosides, tannins, alkaloids and phenolic compounds in aqueous crude extract of *Ficus asperifolia*. Ojo and Akintayo (2014) has indicated that the DPPH scavenging ability of aqueous extract of *F. asperifolia* at the concentration of 5mg/ml was determined to be 78.65 ± 1.15%. Furthermore, a previous study has indicated that the methanolic extract of *F. asperifolia* possessed a good amount of total phenolic content (GAE: 31.96 mg/ml). Bayor *et al.* (2007) has reported that the methanolic leaves extracts possessed fair cytotoxic effects against breast cancer MCF-7 and malignant melanoma cell lines DLD-1. Wu *et al.* (2002) has indicated that the isolates from the leaves of *F. septica* have strong cytotoxic activity against NUGC and HONE-1 human cancer cell lines. Another report by Simon *et al.* (2001) has also indicated that the extract of *F. citrifolia* had therapeutic potential against multidrug resistance cancer cells.
2.2.0.7 *Tetrapleura tetraptera*

*Tetrapleura tetraptera* is a popular plant locally known as “Prekese” in the Eastern region of Ghana. It belongs to the mimosaceae family. It produces a four-winged brown fruit which hangs on sprout stalks. Traditionally, the fruit is used as an ingredient in most locally made soups for nursing mothers (Nwawu and Akah, 1986). The fruit is used in traditional medicine for the management of diseases such as arthritis, epilepsy, diabetes mellitus, and hypertension. Earlier investigations of *T. tertraptera* crude extracts have demonstrated diverse pharmacological properties including analgesic and anticonvulsant actions (Nwaiwu and Akah, 1986; Ojewole, 2005). The aqueous extract of the fruit of *T. tertraptera* has also been reported to have anti-inflammatory and hypoglycemic activities (Ojewole and Adewunmi, 2004). A report by Adesina (1982) indicates that caffeic acid from the fruit has anti-tumour and anti-inflammatory properties and also inhibits HIV replications. Further, it is claimed to be therapeutically useful in the treatment of leprosy and rheumatoid pains (Abii and Elegalam, 2007). The fruit contain phytochemical such as tannins, saponins, alkaloids, anthraquinones, glycosides, flavonoids and other phenolic compounds (Gberikon *et al.*, 2015) as well as minerals such as calcium, zinc, potassium and iron (Okwu, 2003). Ethanolic crude extract of *T. tertraptera* has previously been reported to be cytotoxic to Ehrlich ascites carcinoma cells (Ozaslan, 2016).

2.2.0.8 *Latuca sativa*

Lettuce is a vegetable that plays a major role for a healthy life (Rice- Evans *et al.*, 1997). This vegetable provides a good source of vitamins and bioactive compounds such as glycosides, flavonoids and many other polyphenols. Lettuce is often eaten raw or cooked. The chemical composition of lettuce is mostly affected by ecological, agronomical and genetic factors
(Llorach et al., 2008). Previous studies indicated that extract of L. sativa had higher total phenolic substances and antioxidant activity (Edziri et al., 2011; Lu and Foo 2002). Other investigators have reported that methanolic extract of the L. sativa leaves significantly decreases cyclooxygenase-2 expression and the liberation of nitric oxide (Pepe et al., 2015).

2.2.0 9 Capparis erythrocarpus

Capparis erythrocarpus is a local plant belonging to the family capparaceae. This plant has multiple traditional uses. It is used by Ghanaian traditional medical practitioners in the management of inflammatory conditions. Herbalists grind the leaves and use the juice for treatment of convulsive fever in children. The dried root powder has been shown to be beneficial when added to beverage or soup for the treatment of rheumatoid arthritis or any swellings of the joint (Danquah, 2011). Also, the liquid from the core of the root is used traditionally for the treatment of inflammation of the conjunctiva, hazy vision and inflammation of the ear (Danquah, 2010). The plant is used in the treatment of urinogenital pain and mastitis (Abbiw, 1990). Report indicates that extracts of the plant has antinociceptive effect as well as anti-arthritic properties (Woode et al., 2009; Danquah et al., 2011).

2.2.1 Phytochemical Compounds

Phytochemicals are naturally occurring compounds present in herbs. Some of the micronutrients have been documented to have pharmacodynamic properties such as acting as antioxidants, anti-cancer, anti-inflammatory and pain relievers (Pierson et al., 2012). The phytochemical compounds are the primary and secondary metabolites such as polyphenols and flavonoids serve various purposes such as normal development, reproduction and protection of the plants against harsh conditions (Shankar, 2015). Humans use these organic
Phytochemicals as medicines, recreational drugs and flavoring. The phenolic compounds possess strong therapeutic benefits (Cao et al., 1996).

There is a direct correlation between total phenolic content and antioxidant activity in plants (Yang et al., 2009; Lu and Foo, 2002). In the field of cancer research, secondary metabolites such as brassinosteroids, polyphenols and alkaloids have been documented to modulate cell cycle progression, proliferation and cell apoptosis (HemaIswarya and Doble, 2006).

### 2.2.2 Phytochemical compounds with anti-cancer properties

In the view of the toxicity of some chemotherapeutic agents to normal cells, scientific research is ongoing to obtain safer alternative treatments from medicinal plants. Some plant secondary metabolites have apoptosis inducing properties on cancer cells (Siriwantanmetanon, 2010). The phytochemicals used for anti-cancer treatment include polyphenols, brassinosteroids and taxol. Some polyphenols which include tannins, flavonoids, curcumin, gallacatechins and resveratrol have various mechanism of action. For example some of the plant polyphenols interfere with proteins which promote growth of the cancer cells, resveratrol regulates Cu (II) which binds to DNA and causes its degradation (Azmi et al., 2006) and curcumin has been shown to cause suppression of tumor necrosis factor (Gupta et al., 2014). Furthermore, saponins with foaming characteristicshave been reported to act as anti-cancer agents (Wang et al., 2012; Guclu-Ustundag and Mazza, 2007). Additionally, tannins which are phenolic compounds containing hydroxyls groups have anti-inflammatory properties, inhibits free-radicals and have antiviral and antimicrobial activities (Akiyama et al., 2001) which may be responsible for the therapeutic benefits of the medicinal plants.
Alkaloids are natural products that contain heterocyclic nitrogen atoms and are basic in nature. Research has indicated that alkaloids possess anti-cancer pharmacological activities. Alkaloids with anti-cancer properties include dimeric indoles, vincristine, and vinblastine (Jordan and Wilson, 2004; Solowey et al., 2014).

Flavonoids are phenolic substances found in vascular plants with over 8000 individually known compounds. Aside from their functional activities in the plants, flavonoids are important in food. Previous reports have recognized the pharmacological benefits of flavonoids which include antiviral, antiallergenic, vasodilatory actions and anti-inflammatory actions (Amin and Buratovich, 2007; Anand, 2016).

Currently, a report by Otsuki et al. (2010) has indicated that anti-cancer agents derived from medicinal plants have turned out to be recognized therapies. These have been grouped into four main classes of compounds which include vinca alkaloids (vindesine, vincristine, vinorelbine and vinblastine), podophyllotoxin (teniposide and etoposide), taxanes (paclitaxel and docetaxel), camptothecin (topotecan and irinotecan).

### 2.2.3 Synergic Medicine

Medicinal plants contain a mixture of compounds. Recent studies have acknowledged the synergistic and additive effects of phytochemical compounds via potentiation (Lila, 2005). Recent studies have indicated that potentiation can exist between two phytochemicals in a single plant extract or different plants (Schmidt, 2008; Wagner and Ulrich-Merzenich, 2009). Also, Becker et al. (2004) have indicated that synergism can occur between polyphenols exhibiting antioxidant activity through sacrificial oxidation, regeneration reactions and exertion of different pharmacodynamic action via different pathways.
2.2.4 Plant derived anti-cancer agents

Many parts of medicinal plants have been reported by Konczak et al. (2009), to have cytoprotective properties as they are a rich source of phytochemical compounds. Many of these active ingredients extracted are in clinical use all over the world for management of various cancers. Well known plant-derived anti-cancer compounds include vinblastine and vincristine from *Cataranthus roseus* (Gueritte and Fahy, 2005), irinotecan and topotecan from *Camptotheca acuminata* (Wall, 1966), paclitaxel and docetaxel have been isolated from pacific yew tree and *Taxus brevifolia*, respectively (Cragg, 1998). Anti-cancer agents which interfere with cell cycle include epipodophyllotoxin which was isolated from *podophyllum peltatum* (Unnati et al., 2013). Likewise, epigallacotechin 3 gallate has been isolated from green tea (Raza and John, 2005) and used for the management of prostate cancer. A previous research has demonstrated the anti-cancer activity of resins from the root of *Podophyllum hexandrum* and *Podophyllum peltatum*. Also, a report by Lopez-Lazaro (2009) has reported a broad pharmacological action of luteolin.

2.2.5 Approaches to Development of Anti-cancer Drugs

**Models**

*In vitro* and *in vivo* studies have been used to establish the antioxidants and anti-cancer activities of various substances (Fig. 1B). The *in vivo* model usually involves the induction of cancer in mice or rats and treatment with a test agent (Archana, 2013).

Advantages of *in vivo* models

- Cancer of interest could be induced in a living system and the metastatic process could be monitored and captured.
Disadvantages

- Use of *in vivo* models however take longer period of time to induce and have high failure rate of cancer induction (Evans, 2016). Also, visualization of the individual steps of the metastasis process is challenging and extracting quantitative mechanistic data is usually very difficult.

*In vitro* models however, involve use of established cancer cell lines for testing the cytotoxicity of new compounds (synthetic or natural products) (Carocho and Ferreira, 2013).

Advantages of *in vitro* models

*In vitro* studies allow for high-throughput testing of several compounds or agents in a short period of time. It involves low cost assays and is more reliable. Also, it allows control of most experimental variables and permits quantitative analysis.

Disadvantages of *in vitro* models

- *In vitro* studies captures only limited aspects of the tumour microenvironment, has reduced physiological relevance and may not directly reflect the *in vivo* situation (Katt, 2016).

![Anticancer plant materials](image)

**Figure 1B**: A scheme demonstrating approaches to cancer drug development
2.2.5.1 Application of Tumor Cell Lines

Many human prostate cancer cell lines have been reported. These include androgen-independent and androgen-dependent prostate cancer cell lines (Claas and van Steenbrugge, 1983; Horoszewicz, 1980). Androgen independent prostate cancer cell lines such as PC3, TSU-Prl and DU 145 have been established from metastatic deposits (Claas and van Steenbrugge, 1983). Metastatic sublines of PC-3 have been derived from mice (Wu et al., 2013). Androgen sensitive prostate cancer cell lines include LNCaP cell line established from a metastatic deposit in a lymph node (Horoszewicz, 1980). Likewise, new cell lines have been established by culturing LNCaP cells grown in castrated mice (Wu, 1994). Also, MDA PCa 2a and MDA PCa 2b prostate cancer cell lines were derived from a single bone metastasis in an African-American male who had androgen independent prostate cancer (Navone, 1997).

2.2.5.2.1 Cell lines used

The LNCaP cell line is commonly used in the field of oncology due to the inherent ability to closely mimic human disease progression. They are adherent elongated epithelial cells that can grow in either aggregates or as single cells (Horoszewicz, 1983). The LNCaP cell line has a mutated androgen receptor, lacks 5α-reductase enzyme and has a silent mutation on the tumor suppressor gene p53 (Carroll, 1993). The androgen and estrogen receptors have high affinity for 5α-dihydrotestosterone and estradiol respectively. The cells also express prostate specific antigen and are able to grow readily in vitro.

The PC3 cell line is also used in anti-prostate cancer drug development studies. Morphologically, the cell line appears round under the light microscope. It has a deletion mutation of p53 gene and lacks androgen receptor and 5α-reductase enzyme (Carroll, 1993).
This human prostate cancer cell line does not respond to androgens, glucocorticoids or fibroblast growth factors and has a highly metastatic ability than DU 145 and LNCaP cells which have low metastatic potentials (Pulukuri et al., 2005).

The PNT2 cell line on the other hand is a normal prostate epithelium which is employed in cancer drug development studies to assess the toxicities of the drug to normal cells. This cell line is also adherent and non-tumourigenic in nude mice. They possess a well differentiated morphology with the expression of cytokeratin 8, 18 and 19 with the latter being a feature of differentiated luminal cells of the glandular prostate. The cell cytotoxic value of the cell line is used in the calculation of the selectivity indices of the drug / extract.

2.2.5.2.1 Immortalized cell lines

Immortalized cell lines are either normal cells or tumor cells that have been manipulated artificially to grow and divide indefinitely in vitro under optimal culture condition. LNCaP and PC3cell lines are the most frequently used prostate cancer cell lines for toxicity studies (Horoszewicz et al., 1980; Horoszewicz et al., 1983; Kaighn, 1979). These cell lines can continuously divide and eventually fill the flask. A small fraction of these multiplying cells can be transferred into new flasks to continue proliferation by passaging. Immortalized cell lines are homogenous and have genetically identical population. These characteristics of the cell line aid in providing reliable and reproducible results. However, these cell lines are not normal and can divide indefinitely expressing unique gene patterns not found in any cell type in vivo. These prostate cancer cell lines grow in Roswell Park Memorial Institute media supplemented with 1% penicillin/ streptomycin and 10% foetal bovine serum (FBS) (Alonzeauet et al., 2013).
2.2.6 Assessment of antioxidant activity

Crude extracts of medicinal plants have been reported to contain huge variety of compounds which possess varying antioxidant activities. Several methods have been adopted in measuring the antioxidant capacity of each compound separately (Prasad et al., 2010). The accepted methods are based on the ability of the antioxidants in scavenging specific radicals by hydrogen atom transfer (DPPH (2, 2-Diphenyl-1-picrylhydrazyl)) free radical-scavenging assay), by inhibiting lipid peroxidation or chelating metal ions. The DPPH free radical-scavenging assay (Zlotek et al., 2015), ferric reducing / antioxidant power assay (FRAP assay) (Rajukar and Hande, 2011), Trolox antioxidant equivalent capacity (TAEC) (Prior et al., 2005) among others have been widely employed and used in antioxidant determination.

2.2.6.1 DPPH assay

The assessment of the antioxidant activity by DPPH assay has been widely employed due to its exclusive ability to assessing the total antioxidant activity. The DPPH is a stable radical, which forms a dark purple colour in methanolic solution. However, the dark purple colour disappears after the DPPH is reduced by antioxidants through hydrogen transfer to DPPH-H (non-radical form) (Fig1C). The reduction in absorbance at a wavelength 517 nm is as a result of the reaction between the phytochemicals and DPPH. Research has indicated that the extraction conditions and procedures can appreciably affect the efficacy of extracts, their stability and yield of polyphenols (Zlotek et al., 2015). The total antioxidant activity has been associated with the plant phenolic compounds as well as other secondary metabolites. Also, a report by Srikanth et al. (2010) has indicated that the total antioxidant activities of bioactive components are concentration-dependent. Furthermore, Blois (1958) has reported an inverse relationship between the antioxidant activity and the 50% effective concentration (EC$_{50}$).
Hence, the lower the value of EC_{50}, the higher the antioxidant activity. Plants extracts with EC_{50} values lower than 50 µg/ml are considered very strong antioxidants (Blois, 1958). Those with EC_{50} values of 50-100 µg/ml are considered strong antioxidant, whilst plant extracts that produce EC_{50} values of 101-150 µg/ml have medium antioxidant activity and those EC_{50} greater than 150 µg/ml are considered weak antioxidants (Blois, 1958).

Figure 1C: DPPH reaction

(Lewis, 2012)

### 2.2.6.2 FRAP assay

FRAP assay (Ferric Reducing Antioxidant Power Assay) is commonly used to study the antioxidant capacity of crude extract by measuring the reducing potential in a redox-linked reaction. The antioxidant capacity of plant extracts is determined by the ability of the antioxidants in the extracts to reduce ferric iron to ferrous in the FRAP reagent, which consists of 2, 4, 6-tris (1-pyridyl)-5-triazine (TPTZ) prepared in sodium acetate buffer, at low pH. The reduction of ferric iron in FRAP reagent (colourless complex) will result in the formation of a blue product (ferrous – TPTZ complex) whose absorbance can be read at 593 nm (Rajukar and Hande, 2011).
2.2.6.3 Trolox Equivalent Antioxidant Assay

Trolox Equivalent Antioxidant Assay is a chemical assay that measures the ability of antioxidants to scavenge the stable radical cation ABTS+ (2,2-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) to form a blue-green chromophore with maximum absorption at 734 nm that decreases in its intensity in the presence of antioxidants. The antioxidants neutralize the radical by donating an electron or by hydrogen atom donation. The extent of discoloration to the blue-green color depends on the duration of action, the intrinsic antioxidant activity and concentration in the sample (Prior et al., 2005).
CHAPTER 3

3.0 Materials and methods

3.1 Source of materials

All the reagents such as dimethyl sulfoxide (DMSO), methanol (MEOH), ethanol (ETOH), ethyl acetate (EA), dichloromethane (DCM), petroleum ether (PE), isopropanol were obtained from Daejung (South Korea) whereas Folin Ciocalteau reagent was obtained from Sigma Aldrich (USA). Also, butylated hydroxyl toluene (BHT), 2, 2-diphenyl-1-picrylhydrazyl (DPPH), gallic acid and Folin-Ciocalteu reagent were obtained from Sigma Aldrich (USA). Likewise, Rosewell Park Memorial Institute (RPMI) and Foetal Bovine Serum (FBS) were obtained from (USA). Also, sterile costar 96 well plate used for MTT assay was obtained from Sigma Aldrich (USA). Serological pipettes (5ml, 10ml and 25 ml), falcon tubes (15 ml and 50 ml), Finn pipette (Thermo scientific, OH 61352, Finland), eppendorf tubes and pipette filler (Thermo scientific, EN61010 Pudong, Shangai, China) were used in both DPPH assay and MTT assay. Centrifuge (Tomy LC-200, Japan), Biosafety Cabinet airstream (ESCO Biotech EQR/GL-64, Singapore) and incubator (Panasonic MCO-18 AC, China).All other reagents and supplies were obtained from standard suppliers.

3.2 Ethnobotanical/Literature Survey and Collection of Plants

Based on the literature review and ethnopharmacological details, nine (9) plants were selected and collected, extracts prepared to be evaluated for their total phenolic substances, antioxidants as well as cytotoxic activities. In the ethnobotanical survey, a list of eight plants used in the management of prostate cancer was selected after IRB approval to obtain information from herbalist. Also, one plant showing promising anti-cancer properties was
included after a thorough literature search (Google scholar, Pub Med and Elsevier). The list of plants was presented to taxonomists at the Center of Plant and Medicine Research Akuapem-Mampong and University of Ghana Herbarium, respectively. The collection was done early in the morning in the botanical gardens at Akuapem- Mampong and also at University of Ghana, respectively, between March and April 2017. The green fresh leaves, seeds and fruit were cleaned and air dried under room temperature (26°C). The samples were ground into fine powder using an electric blender and stored in appropriately labeled zip lock bag at 26°C. The seeds and the pulp of the *T. tetraptera* fruit were separated, grinded and kept in zip lock bags at room temperature.

### 3.2 Extraction Method

Sequential extraction method was used to obtain crude extracts from the powdered parts of the plant using solvent with increasing polarity (Prakash and Gupta, 2005). The powdered plant parts were sequentially extracted using petroleum ether (PE), dichloromethane (DCM), ethyl acetate (EA), ethanol (ETOH) and distilled water (AQ) in increasing polarity of solvents. The powdered plant parts were macerated at room temperature in portions of 50g, with 200 ml of each solvent in air tight conical flasks. The maceration process was conducted by placing the conical flask with the mixture on an electric shaker at a speed of 350 rpm for 3 days. The extracted material was collected by filtration using a double layered muslin cloth and Whatmann No 1 filter paper. The residues were then remacerated twice using 150 ml of fresh solvent. Hence, the extraction using petroleum ether was repeated three times using a total volume of 500 ml of solvent. The residue was dried completely to get rid of the first solvent and the dried residue was extracted three times
following the above procedure using the remaining solvent (using dichloromethane (DCM), ethyl acetate (EA) and ethanol (ETOH), respectively). The filtrates of the same solvents were pooled together and evaporated using rotary evaporator (BUCHI). The thick extracts obtained were dried in an oven at 50°C to constant weight. The aqueous extracts were prepared by boiling the dried residue in 500 ml of distilled water at 80°C. The procedure was repeated once again, filtrates were pooled and lyophilized in a freeze-dryer (Lyotrap Lf/ LYO/0411, United Kingdom). The percentage yield was calculated as follows:

\[
\text{percentage yield} = \frac{\text{final weight of dried extract}}{\text{Initial weight of plant powder}} \times 100
\]

The dried crude extracts were dispensed into eppendorf tubes and falcon tubes before storing at 4°C.

### 3.3 Determination of the total phenolic content (TPC)

Determination of total phenolics

A concentration of 5 mg/ml was prepared from each of the plant extracts. The total phenolic content of the extracts of each plant was assessed using the Folin-Ciocalteau method as described by Anim et al. (2016). Each sample was aliquoted (10µl) into a 24 well plate and was incubated with Folin-Ciocalteau reagent (50µl). Following addition of 150µl sodium carbonate 20% (w/v), it was incubated again for 2 h. The absorbance of the blue chromophore formed from the reduction of phosphotungstic phosphomolybdenum was assessed at a wavelength of 750 nm using the spectrophotometer (Tecan Infinite M200 Pro plate reader, Austria) (see Fig 2A). The total phenolic content was extrapolated from the calibration curve prepared from a standard, gallic acid. The total phenolic content was expressed as Gallic Acid Equivalents (GAE) in grams per 100 g dry weight of plant extract. All determinations were carried out in triplicates.
3.4 DPPH radical scavenging assay

The DPPH assay has been used in assessing the free radical scavenging activity of extracts because it is simple and fast (Appiah-Opong et al., 2016). It also has the ability to measure the total antioxidant oxidant activity without being specific to the kind of antioxidant present. The dried extracts and freeze-dried samples were weighed in 1 mg portions using a weighing balance (Mettler Toledo.XP 105 Delta Range, China) into different eppendorf tubes. After the solubility test, crude extracts of organic solvents (PE, DCM, EA and ETOH) dissolved completely in absolute ethanol whiles freeze dried extracts were dissolved in distilled water. A volume of 1 ml of absolute ethanol or distilled water was then added to each dried sample, vortexed (Taiyo Mixer S-5F) and sonicated (Ultrasonic Washer ASONE, China) for 5 min. A stock solution of BHT (1mg/ml in absolute methanol) and a two-fold serial dilution of the stock solutions (BHT and extracts) were prepared to obtain seven different concentrations. The 0.5 mM DPPH was prepared by dissolving a weight of 3.55g in 18ml of methanol to form a purple color. Aliquots of 100μl...
of each extract or standard (BHT) of concentrations (1-1000μg/ml) were pipetted into a properly labeled 96-well plate. Afterwards, a 100μl of 0.5 mM DPPH solution was added to each well to initiate the reaction. The 96-well plate was gently shaken to mix the content, incubated for 20 min and the absorbance was assessed at wavelength of 517 nm using a spectrophotometer (Tecan Infinite M200 Pro plate reader, Grodigi, Austria) (see Fig 2B). Ethanol, methanol and water were used as negative control and blank, respectively. The BHT was used as standard or positive control. Analysis was carried out in triplicate for standard and each extract. Antioxidant activity of each extract was determined by calculating the percentage of antioxidant activity using reduction of DPPH absorbance (Bedawey, 2010). Background interferences from absolute methanol, absolute ethanol and water were deducted from the activities of the corresponding extracts prior to the calculation. The results were expressed as percentage free radical scavenging activity which was calculated as shown below:

\[ \% \text{ scavenging activity} = \frac{\text{Blank solution} - \text{absorbance of extract}}{\text{Blank solution}} \times 100 \]

The 50% inhibition concentration (IC\(_{50}\)) of DPPH scavenging activity of each extract was determined from the log concentration response curve using a plot of % Antioxidant activity or scavenging activity versus concentration of sample tested ×100 using Graphpad prism software version 5(GraphPad Software Inc. San Diego, CA 92037).
3.5 Cell Culture

The PC3, LNCAP cells were cultured in 25mL culture flask whiles PNT2 cells were cultured in 75 mL culture flask containing RPMI media. The media was supplemented with 10% FBS and 1% streptomycin/ penicillin to make it complete. The cells were incubated in a humified incubator (Panasonic CO₂ Incubator MCO-18 AC, China) at 37 °C in 5% CO₂. The spent media was changed every three days with complete media and cells were passed at 80 - 90% confluence.

3.6 Preparation of test samples

3.6.1 Cell viability assay

Cell viability was assessed using the MTT assay (tetrazolium bromide solution in phosphate-buffered saline (2.5 mg/ml) as described (Appiah-Opong et al., 2016). The assay is based on the reduction of the tetrazolium bromide by the mitochondria dehydrogenase or reductases of the viable cells. The cells were seeded at 10,000 cells per well of the 96 -well
plate and incubated for 24 h to get cells attached to the well plate. Before treatment, a stock solution of each sample was prepared by dissolving in dimethylsulfoxide (100% DMSO) except the AQ extracts which were solubilised in distilled water and filtered using a 0.45 millipore. A working plant extract solution of 10% DMSO was prepared from the stock plant extract solution (100% DMSO) using RPMI media. Ten microlitre aliquots of different concentrations (concentration range 0-1000 µg/ml) of each medicinal plant extract and compound (0-200 µg/ml) were added to form 1% DMSO in appropriately labeled 96 well plate already containing $1 \times 10^4$ cells / well. Also, 100 µl aliquots of complete medium were added to the blank and untreated wells as a negative control. Each well for the negative control contained 10,000 cells /well and 1% DMSO. The final 1% DMSO was produced in each well by the addition of 10µl of the mixture of 10 % DMSO and 90% RPMI to the wells. A concentration of 10 µM curcumin was used as a positive control. The cells were then incubated with different concentrations of the test extracts and compound in triplicates at 37 °C in 5% CO$_2$. Following incubation of the plates for 72 h, 20 µl of 2.5 mg/ml solution of MTT was added to each well and re-incubated for 4 h. The purple formazan crystals formed in each well were dissolved in 150 µl of acidified isopropanol. Absorbance was read at a wavelength of 570 nm using the spectrophotometer (Tecan Infinite M200 Pro plate reader, Austria). The concentrations to exhibit 50% anti-proliferative activity (IC$_{50}$ value) were obtained through regression analysis of the results of five different concentrations of each extract and compound. The percentage cell viability of was evaluated using the formulae,

$$
\% \text{ Cell viability} = \left[ \frac{[\text{ODT0} - \text{ODT1}]}{[\text{ODU0} - \text{ODU1}]} \right] \times 100
$$
Where ODT0 is the absorbance of wells treated with test extracts or standard compound (Curcumin) for all the cell lines; ODT1 is the absorbance of wells with test extracts or Curcumin (Positive control); ODU0 is the absorbance of wells with negative control (untreated cells) for all cell lines; ODU1 is the absorbance of wells containing blank (culture media only). The inhibition concentration at 50% cell survival (IC$_{50}$) values was obtained from the plot of a dose response curve of the percentage cell survival versus concentration of the extracts/fractions. The IC$_{50}$ values obtained were used to determine the selectivity of the extracts/fractions for cancer cells. The IC$_{50}$ values of the cancerous cells (PC3 and LNCaP) and 50% cytotoxic concentration (CC$_{50}$) of normal cells (PNT2) obtained were used to determine the selectivity indices. The selective indices of the fractions were determined using the formula.

\[
SI = \frac{CC_{50} \text{ of normal cell}}{IC_{50} \text{ of cancer cell line}}
\]

SI values ≥ 2 were considered as significant or promising drug candidate (Badisa et al., 2009).

**MTT Assay**

Figure 2C: A schematic diagram showing the procedures used in MTT assay.
3.8 Statistical analysis

Data obtained were expressed as mean ± SD using statistical software Graph pad prism version 5 (GraphPad Software Inc. San Diego, CA 92037). Also one-way ANOVA was used to determine the level of significance between the extracts, followed by Tukey’s multiple comparison test. The results were considered statistically significant at P < 0.05.

3.6 Ethics Statement

Ethical clearance for this study was obtained from the Institutional Review Board Noguchi Memorial Institute for Medical Research (NMIMR). All procedures were in strict accordance and approved by the board members of Noguchi Memorial Institute for Medical Research (NMIMR) Institutional Review Board (IRB).
Chapter 4

4.0 Results

The ethno-botanical survey resulted in the identification of nine plants, namely: *Latuca sativa*, *Tetrapleura tetraptera*, *Jatropha curcas*, *Capparis erythrocarpus*, *Ficus asperifolia*, *Mentha piperita*, *Carica papaya*, *Moringa oleifera* and *Phyllanthus amarus*.

Fresh sample leaves of *Latuca sativa*, *Jatropha curcas*, *Carica papaya*, *Moringa oleifera*, *Phyllanthus amarus* were collected and authenticated by a plant taxonomist and kept at the University of Ghana Plant and Herbal medicine herbarium. Also, leaves of *Capparis erythrocarpus*, *Ficus asperifolia*, *Mentha piperita* and fruit of *Tetrapleura tetraptera* were obtained and authenticated at Centre of Plant and Medicinal Research, Akuapem Mampong.

Table 2: Identity of medicinal plants and their voucher specimen number

<table>
<thead>
<tr>
<th>S/N</th>
<th>Plant</th>
<th>Family</th>
<th>Part used</th>
<th>Voucher specimen number</th>
</tr>
</thead>
<tbody>
<tr>
<td>TT</td>
<td><em>Tetrapleura tetraptera</em></td>
<td>Mimosaceae</td>
<td>Fruit, seed</td>
<td>CPMR 4867,4868,4869</td>
</tr>
<tr>
<td>FA</td>
<td><em>Ficus asperifolia</em></td>
<td>Moraceae</td>
<td>Leaves</td>
<td>CPMR 4846,4865,4866</td>
</tr>
<tr>
<td>CP</td>
<td><em>Carica papaya</em></td>
<td>Caricaceae</td>
<td>Leaves</td>
<td>UGAJK-2017-001</td>
</tr>
<tr>
<td>PA</td>
<td><em>Phyllanthus amarus</em></td>
<td>Euphorbiacea</td>
<td>aerial part</td>
<td>UGAJK-2017-005</td>
</tr>
<tr>
<td>CE</td>
<td><em>Capparis erythrocarpus</em></td>
<td>Capparaceae</td>
<td>Leaves</td>
<td>CPMR 4873,4874,4875</td>
</tr>
<tr>
<td>MP</td>
<td><em>Mentha pepirita</em></td>
<td>Lamiaceae</td>
<td>Leaves</td>
<td>CPMR 4870,4871,4872</td>
</tr>
<tr>
<td>LS</td>
<td><em>Latuca sativa</em></td>
<td>Asteraceae.</td>
<td>Leaves</td>
<td>UGAJK-2017-001</td>
</tr>
<tr>
<td>JC</td>
<td><em>Jatropha curcas</em></td>
<td>Euphorbiacea</td>
<td>Leaves</td>
<td>UGAJK-2017-002</td>
</tr>
<tr>
<td>MO</td>
<td><em>Moringa oleifera</em></td>
<td>Moringaceae</td>
<td>Leaves</td>
<td>UGAJK-2017-004</td>
</tr>
</tbody>
</table>

After plant extraction using increasing polarity of solvents, the dried crude extracts were obtained and weighed. The results of the yield were tabulated as shown in Table 3. The percentage yield of the various solvent extracts of the different medicinal plants was
calculated, and presented in the table below. Among the medicinal plants, the highest yield was observed in the ETOH extract of the *T. tetraperta* pulp (30.38%). Also, the least yield was obtained from the PE extract of the *T. tetraperta* pulp (0.15%). The percentage yield is represented in Table 3 below.

Table 3: Percentage yield of medicinal plants

<table>
<thead>
<tr>
<th>Test plant</th>
<th>Petroleum ether (PE)</th>
<th>Dichloromethane (DCM)</th>
<th>Ethyl acetate (EA)</th>
<th>Ethanol (ETOH)</th>
<th>Aqueous (AQ)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>T. tetraperta</em> (pulp)</td>
<td>0.15</td>
<td>0.30</td>
<td>1.05</td>
<td>30.38</td>
<td>10.14</td>
</tr>
<tr>
<td><em>T. tetraperta</em> (seed)</td>
<td>5.40</td>
<td>1.34</td>
<td>0.25</td>
<td>1.52</td>
<td>7.52</td>
</tr>
<tr>
<td><em>L. sativa</em></td>
<td>2.20</td>
<td>2.36</td>
<td>1.36</td>
<td>8.86</td>
<td>9.87</td>
</tr>
<tr>
<td><em>C. erythocearpus</em></td>
<td>2.28</td>
<td>3.08</td>
<td>3.15</td>
<td>9.53</td>
<td>9.96</td>
</tr>
<tr>
<td><em>M. piperita</em></td>
<td>1.73</td>
<td>1.88</td>
<td>1.36</td>
<td>7.78</td>
<td>9.64</td>
</tr>
<tr>
<td><em>F. asperifolia</em></td>
<td>5.04</td>
<td>0.58</td>
<td>1.07</td>
<td>4.40</td>
<td>11.86</td>
</tr>
<tr>
<td><em>M. oleifera</em></td>
<td>3.89</td>
<td>0.68</td>
<td>0.70</td>
<td>3.12</td>
<td>13.94</td>
</tr>
<tr>
<td><em>C. papaya</em></td>
<td>3.93</td>
<td>1.52</td>
<td>0.62</td>
<td>2.36</td>
<td>13.84</td>
</tr>
<tr>
<td><em>J. curcas</em></td>
<td>0.94</td>
<td>0.16</td>
<td>0.17</td>
<td>1.37</td>
<td>6.26</td>
</tr>
<tr>
<td><em>P. amarus</em></td>
<td>4.07</td>
<td>1.14</td>
<td>1.15</td>
<td>4.67</td>
<td>11.09</td>
</tr>
<tr>
<td>Whole <em>T. tetraperta</em></td>
<td>0.38</td>
<td>0.43</td>
<td>0.33</td>
<td>20.32</td>
<td>17.29</td>
</tr>
</tbody>
</table>
4.1 Total Phenolic Content (TPC)

The calibration curve obtained from Gallic acid is shown in (Figure 3A). Table 4 shows the TPC levels of the various extracts derived from Figure 3A. The total phenolic contents of the aqueous and ethanol extract were significantly higher (p<0.05) in *P. amarus*, whole *T. tetraptera* fruit, *F. asperifolia*, pulp of *T. tetraptera* and *L. sativa* crude extracts when compared to their respective petroleum ether (PE) and dichloromethane (DCM) crude extracts. The AQ extract of *T. tetraptera* seeds showed a significantly higher (p<0.05) TPC than the rest of the solvent extracts.

The ETOH extract of *C. papaya* and *M. oleifera* showed a significantly higher total phenolic content when compared to the rest of their respective solvent extracts. However, there were no significant difference (p>0.05) in TPC of the aqueous, ethyl acetate and dichloromethane crude extracts of *C. papaya* and *M. oleifera*.

Furthermore, there was no significant difference (p>0.05) between ETOH and PE crude extracts of *C. erythrocarpus*. The TPC of ETOH and PE crude extracts of *C. erythrocarpus* were significantly higher (p<0.05) when compared with other solvent extracts. There was no significant difference between total phenolic content of aqueous and dichloromethane crude extracts.

The EA extract of *M. piperita* had a significantly higher total phenolic content when compared to the rest of the solvent extracts. The aqueous extract of *M. piperita* showed a significantly higher total phenolic content when compared to the rest of the solvents except ethyl acetate. Also, there were no significant difference between the PE extract and DCM crude extract of *M. piperita*. 
The ETOH extract of *J. curcas* possessed a significantly higher total phenolic content when compared with the rest of the extracts. There was no significant difference between aqueous and dichloromethane extracts of *J. curcas*. Also, the EA extract of *J. curcas* showed a significantly higher (p<0.05) total phenolic content than the petroleum ether extracts. The results indicate that most plants contain more polar phenolic compounds than least polar compounds.

Figure 3A: Calibration curve of Gallic acid

Where x is Gallic acid concentration and y is the absorbance
Table 4: Total phenolic content of extracts of selected medicinal plants

<table>
<thead>
<tr>
<th>Test plant</th>
<th>Petroleum ether (PE)</th>
<th>Dichloromethane (DCM)</th>
<th>Ethyl acetate (EA)</th>
<th>Ethanol (ETOH)</th>
<th>Aqueous (AQ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TTS</td>
<td>1.30±0.55</td>
<td>1.08±0.56</td>
<td>1.65±0.42</td>
<td>2.27±0.34</td>
<td>7.58±0.47a</td>
</tr>
<tr>
<td>LS</td>
<td>2.95±0.28</td>
<td>2.40±0.34</td>
<td>9.87±0.82</td>
<td>13.71±0.65a</td>
<td>11.51±0.47</td>
</tr>
<tr>
<td>MP</td>
<td>5.38±0.35</td>
<td>5.20±0.59</td>
<td>12.97±0.47a</td>
<td>3.97±0.34</td>
<td>9.01±0.46</td>
</tr>
<tr>
<td>CE</td>
<td>7.50±0.27a</td>
<td>3.47±0.35</td>
<td>1.80±0.40</td>
<td>8.10±0.23a</td>
<td>3.86±0.32</td>
</tr>
<tr>
<td>FA</td>
<td>4.38±0.73</td>
<td>8.22±0.16</td>
<td>5.51±0.87</td>
<td>30.77±1.68a</td>
<td>8.00±0.39</td>
</tr>
<tr>
<td>MO</td>
<td>2.43±0.19</td>
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<td>30.39±2.18a</td>
<td>6.51±0.17</td>
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<tr>
<td>TTP</td>
<td>3.06±0.13</td>
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<td>3.98±0.50</td>
<td>6.46±0.19</td>
<td>22.33±0.27a</td>
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<tr>
<td>JC</td>
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<td>9.58±0.85</td>
<td>35.72±3.08a</td>
<td>5.72±0.27</td>
</tr>
<tr>
<td>PA</td>
<td>4.89±0.25</td>
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<td>4.73±0.44</td>
<td>92.71±3.62a</td>
<td>23.69±0.97</td>
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<tr>
<td>WTT</td>
<td>2.75±0.53</td>
<td>6.49±0.13</td>
<td>4.79±0.20</td>
<td>18.13±0.99a</td>
<td>9.58±0.070</td>
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<tr>
<td>CP</td>
<td>5.46±1.45</td>
<td>10.91±0.89</td>
<td>9.08±0.94</td>
<td>41.50±5.78a</td>
<td>7.73±0.93</td>
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</tbody>
</table>

Values are given as means ± SD; total phenolic content (TPC g GAE/100g) is given in grams Gallic acid equivalent/ 100 g of plant extract. The TPC was assessed on 5mg/ml of dried crude plant extract. One-way ANOVA followed with Tukey’s was used to assess the level of significance between solvents of the same plant. “a” means the extract was significantly higher when compared to the rest of the extracts of the same plant (P<0.05).
A.

B.

Figure 3B: Total phenolic content of PE, DCM, EA, ETOH and AQ extracts of *P. amarus* leaf (A) and *T. tertraptera* seed (B) expressed as mean ± standard deviation.

The total phenolic content (TPC mg GAE/100g) was given in milligrams Gallic acid equivalent/ 100 g of plant extract. The TPC was assessed on 5mg/ml of dried crude plant extract. One-way Anova followed with Tukey’s was used to assess the level of significance between solvents of the same plant. “a” means the extract was significantly higher when compared to the rest of the extracts of the same plant (P<0.05).
Figure 3C: Total phenolic content of PE, DCM, EA, ETOH and AQ extracts of *T. tertraptera* pulp extracts (C) and *L. sativa* leaf extracts (D) expressed as mean ± standard deviation.

The total phenolic content (TPC mg GAE/100g) was given in milligrams Gallic acid equivalent/100g of plant extract. The TPC was assessed on 5mg/ml of dried crude plant extract. One way Anova followed with Tukey’s was used to assess the level of significance between solvents of the same plant. “a” means the extract was significantly higher when compared to the rest of the extracts of the same plant (P<0.05).
Figure 3D: Total phenolic content of PE, DCM, EA, ETOH and AQ extracts of *M. piperita* leaf (E) and *C. erythrocarpus* leaf (F) expressed as mean ± standard deviation.

The total phenolic content (TPC mg GAE/100g) was given in milligrams Gallic acid equivalent/ 100 g of plant extract. The TPC was assessed on 5mg/ml of dried crude plant extract. One-way Anova followed with Tukey’s was used to assess the level of significance between solvents of the same plant. “a” means the extract was significantly higher when compared to the rest of the extracts of the same plant (P<0.05).
G.

![Graph showing total phenolic content of PE, DCM, EA, ETOH and AQ extracts of J. curcas leaf (G) and F. asperifolia leaf (H).](image)

The total phenolic content (TPC mg GAE/100g) was given in milligrams Gallic acid equivalent/ 100 g of plant extract. The TPC was assessed on 5mg/ml of dried crude plant extract. One-way Anova followed with Tukey’s was used to assess the level of significance between solvents of the same plant. “a” means the extract was significantly higher when compared to the rest of the extracts of the same plant (P<0.05).

H.

![Graph showing total phenolic content of PE, DCM, EA, ETOH and AQ extracts of F. asperifolia leaf extract.](image)

Figure 3E: Total phenolic content of PE, DCM, EA, ETOH and AQ extracts of J. curcas leaf (G) and F. asperifolia leaf (H) expressed as mean ± standard deviation.
I. Figure 3F: Total phenolic content of PE, DCM, EA, ETOH and AQ extracts of *M. oleifera* leaf (I) and whole *T. Tetraptera* fruit (J) expressed as mean ± standard deviation.

The total phenolic content (TPC mg GAE/100g) was given in milligrams Gallic acid equivalent/ 100 g of plant extract. The TPC was assessed on 5mg/ml of dried crude plant extract. One-way Anova followed with Tukey’s was used to assess the level of significance between solvents of the same plant. “a” means the extract was significantly higher when compared to the rest of the extracts of the same plant (P<0.05).

J.
Figure 3G: Total phenolic content of PE, DCM, EA, ETOH and AQ extracts of *C. papaya* leaf (K) expressed as mean ± standard deviation.

The total phenolic content (TPC mg GAE/100g) was given in milligrams Gallic acid equivalent/100 g of plant extract. The TPC was assessed on 5mg/ml of dried crude plant extract. One-way Anova followed with Tukey’s was used to assess the level of significance between solvents of the same plant. “a” means the extract was significantly higher when compared to the rest of the extracts of the same plant (P<0.05).
4.2 Antioxidant Activities of the Plant extracts

The median effective DPPH scavenging values for extracts obtained from the various solvents and plants are recorded in Table 5. The table shows the EC$_{50}$ values obtained for antioxidant activities of the crude extracts of selected medicinal plant. All the samples exhibited a concentration dependent pattern of free radical scavenging. The AQ extract of $P. amarus$ leaves recorded strong antioxidant activity with the lowest EC$_{50}$ values of $(19.32\pm1.13 \, \mu g/ml)$ when compared to the ETOH and EA extracts, with EC$_{50}$ values of $60.03 \pm 1.07 \, \mu g/ml$ and $202.1 \pm 1.10 \, \mu g/ml$ respectively. The ETOH, EA and AQ extracts of $P. amarus$ however, recorded highest % antioxidant activities of 86%, 84% and 77%, respectively, at the highest concentration tested, whiles the PE extract showed the lowest activity of 15% as shown in figure A. (figure 4A.).

The AQ extract of the whole $T. tetraptera$ fruit had a strong free radical scavenging activity with EC$_{50}$ value of $83.46\pm1.07 \, \mu g/ml$ when compared with the rest of the other extracts. The AQ, EA and ETOH extract showed highest % antioxidant activities of 83.91%, 66.31% and 40.16% while the PE extract showed the least maximum efficacy values of 15%, as shown in Figure 4B. The free radical scavenging activity of the AQ extract of the whole $T. tetraptera$ fruit was not significantly different (p>0.05) when compared to the BHT (positive control). There was no significant difference between the crude extracts (p>0.05).

The AQ extract of the pulp of $T. tetraptera$ recorded the lowest EC$_{50}$ value of $87.50\pm1.10 \, \mu g/ml$ when compared to the rest of the organic solvents. The AQ, ETOH and EA extract
showed maximum efficacy values of 72.55%, 69.25% and 40.90% while petroleum ether extract showed the least activity of 8% as shown in figure I (Figure 4C.).

In addition, the AQ extract of *T. tetrapterea* seed had the lowest EC$_{50}$ values of (244.3±1.10 µg/ml) when compared with the other crude seed extracts. The AQ, ETOH and EA extracts showed a maximum efficacy values of (53.66%, 40.91% and 9.51%) while the DCM extract showed the least maximum efficacy values of 12.35% as shown in figure D (Figure 4B.).

The EA extract of *L. sativa* and *M. piperita* appeared to show stronger antioxidant activities with the lowest EC$_{50}$ values of 121.4±1.07 µg/ml and 129.7±1.07 µg/ml respectively, when compared with their respective solvent extracts. The EA, ETOH and AQ extracts of *L. sativa* showed a maximum efficacy values of 91.80%, 59.56% and 53.46% respectively, while PE extract showed the least maximum activity of 16.27% as shown in figure F (Figure 4B.). There was no significant difference (p>0.05) between the crude extracts. Additionally, the EA, AQ and DCM extracts of *M. piperita* showed maximum efficacy values of 88.36%, 52.99% and 67.01% respectively, while PE extract showed the least maximum activity of 38.72% as shown in figure E (Figure 4B.). Interestingly, the antioxidant activities of EA, AQ and DCM extracts of *M. piperita* had EC$_{50}$ values of 129.7±1.07 µg/ml, 307.5±1.10 µg/ml and 279.5±1.07 µg/ml, respectively. The antioxidant activity of BHT was significantly stronger (p<0.05) when compared with DCM, ethanol and aqueous extracts of *M. piperita.*
Additionally, the EA, AQ and ETOH extracts of *J. curcas* showed maximum efficacies values of 74.59%, 68.21% and 53.28% respectively, while PE extract showed the least maximum activity of 44.47 % as shown in figure J (Figure 4C.). This study reports that the EC$_{50}$ of EA, AQ and ETOH extracts of *J. curcas* have EC$_{50}$ values of 303.5±1.10 µg/ml, 435.1±1.07 µg/ml and 177.8±1.15 µg/ml, respectively. However, the difference between the antioxidant activities of standard BHT and ethyl acetate, aqueous and ethanolic extracts of *J. curcas* was significant (p<0.05).

Furthermore, the AQ, DCM and ETOH extracts of *M. oleifera* showed a maximum activity of 71.68%, 45.46% and 45.02% respectively, while PE extract showed the least maximum activity of 30.03 % as shown in figure K (Figure 4C.). Aqueous extract (AQ) of *M. oleifera* showed antioxidant activity with EC$_{50}$ value of 250.2±1.08 µg/ml. However, the antioxidant activity of BHT was significantly stronger than aqueous extracts and rest of the solvent extracts of *M. oleifera*.

Besides, the DCM, ETOH and AQ extracts of *C. erythrocarpus* showed maximum efficacy values of 77.04%, 39.72% and 36.03% respectively, while the PE extract showed the least maximum activities of 17.77 % as demonstrated in figure H (Figure 4C.). Dichloromethane extracts of *C. erythrocarpus* exhibited antioxidant activity with EC$_{50}$ value of (179.6±1.10 µg/ml). BHT (positive control) was significantly stronger than AQ extracts and rest of the solvent extracts.

Moreover, the AQ, ETOH and EA extracts of *F. asperifolia* showed maximum efficacy values of 68.21%, 59.09% and 55.97% respectively, while DCM extract showed the least
maximum activity of 41.04% as demonstrated in figure G (Figure 4C.). The AQ, ETOH and EA extract of *F. asperifolia* exhibited EC$_{50}$ values of 177.8±1.15 µg/ml, 303.5±1.10 µg/ml and 400.6±1.05 µg/ml, respectively.

Also, the AQ, ETOH and DCM extracts of *C. papaya* showed maximum antioxidant activities of 80.23%, 52.02% and 49.55% respectively, while EA extract showed the least maximum antioxidant activity of 31.09 % as shown in figure C (Figure 4B.). The AQ and ETOH extracts of *C. papaya* exhibited EC$_{50}$ values of (125.9 ±1.08 µg/ml and 439.7 ±1.07 µg/ml, respectively). BHT showed significantly stronger antioxidant activity (p<0.05) than rest of the solvent extracts except AQ extract of *C. papaya*.

The EA extract of *M. piperita* and *L. sativa* showed stronger antioxidant activities when compared to the rest of the remaining solvent extracts, respectively. However, AQ extracts of these plants did not show significantly higher antioxidant activity when compared to their respective solvent extracts (p>0.05). Also, the aqueous extracts of *J. curcas, M. oleifera, C. papaya, F. asperifolia, C. asperifolia, M. piperita and L. sativa* appeared to have weaker antioxidant activities when compared to the standard (BHT). The table below shows values of antioxidant activity (EC$_{50}$) of the crude extracts of the plants samples. The antioxidant activities of crude extracts and standard BHT were assessed at 1mg/ml of crude extracts.
<table>
<thead>
<tr>
<th>Test plant</th>
<th>Petroleum ether</th>
<th>Dichloromethane</th>
<th>Ethyl acetate</th>
<th>Ethanol</th>
<th>Aqueous</th>
</tr>
</thead>
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<tr>
<td>BHT</td>
<td>12.81± 1.07</td>
<td>13.31± 1.07</td>
<td>12.31± 1.07</td>
<td>13.31± 1.07</td>
<td>12.81± 1.07</td>
</tr>
<tr>
<td>TTS</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td>424.4± 1.20#'</td>
<td>244.3±1.10'a.#</td>
</tr>
<tr>
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<td>&gt;500</td>
<td>&gt;500</td>
<td>121.4± 1.07#</td>
<td>243.5± 1.12#</td>
<td>324.6±1.17a.#</td>
</tr>
<tr>
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<td>&gt;500</td>
<td>179.6± 1.10#</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td>&gt;500</td>
</tr>
<tr>
<td>MP</td>
<td>&gt;500</td>
<td>279.5± 1.07a.#</td>
<td>129.7± 1.07a</td>
<td>430.8± 1.17a.#</td>
<td>307.5±1.10a.#</td>
</tr>
<tr>
<td>FA</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td>400.6± 1.05a.#</td>
<td>303.5 ± 1.10a.#</td>
<td>177.8 ±1.15a.#</td>
</tr>
<tr>
<td>MO</td>
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<td>&gt;500</td>
<td>&gt;500</td>
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<td>&gt;500</td>
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<td>162.3 ±1.15a.#</td>
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<tr>
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<td>&gt;500</td>
<td>303.5 ±1.10#</td>
<td>435.1 ±1.07#</td>
<td>177.8 ±1.15#</td>
</tr>
<tr>
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<td>&gt;500</td>
<td>&gt;500</td>
<td>202.1 ± 1.10</td>
<td>60.03 ± 1.07</td>
<td>19.32 ±1.13a</td>
</tr>
<tr>
<td>TTW</td>
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<td>&gt;500</td>
<td>367.6± 1.07#</td>
<td>&gt;500</td>
<td>83.46 ±1.07a</td>
</tr>
<tr>
<td>CP</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td>439.7 ± 1.07#</td>
<td>125.9±1.08a</td>
</tr>
</tbody>
</table>

Values are given as means ± SD; Potential antioxidant activity of extracts (EC\textsubscript{50} µg/ml) is the concentration at 50% DPPH inhibition. The antioxidant activity was assessed on 1mg/ml of dried crude plant extract / compound. One-way Anova followed with Tukey’s was used to assess the level of significance between solvents of the same plant. The superscript “a” means the extract was significantly high when compared to the rest of the remaining extracts in the same row. Extract with # superscript indicated significant difference p< 0.05 compared to BHT.
Figures 4A: Antioxidant activities of aerial parts of *P. amarus* (A) and whole *T. tetraptera* fruit (B) sequentially extracted using various solvents. The figure shows a concentration dependent curve. Increasing the concentration of the extract increases its antioxidant potential. One milligram per milliliter of each extract was tested as described in the method section.
Figures 4B: Antioxidant activities of *C. papaya* leaves (C), *T. tetraperta* seed (D), *M. piperita* leaves (E) and *L. sativa* leaves (F) sequentially extracted using various solvents. The figure shows a concentration dependent curve. Increasing the concentration of the extract increases its antioxidant potential. One milligram per milliliter of each extract was tested as described in the method section.
Figures 4C: Antioxidant activities of *F. asperifolia* leaf extracts (G), *C. erythrocarpus* leaf extracts (H), *T. tetraperta* pulp extracts (I), *J. curcas* leaf extracts (J) and *M. oleifera* leaf extracts (K) sequentially extracted using various solvent. The figure shows a concentration dependent curve. Increasing the concentration of the extract increases its antioxidant potential. One milligram per milliliter of each extract was tested as described in the method section.
4.3 *In Vitro* Cell viability Assay

Fig 5A shows a representative plot of the cytotoxic profile of extracts of *P. amarus* on PC3 and LNCaP prostate cancer cell lines. The figure shows that all the fractions inhibited the growth of the cancer cells in a concentration dependent fashion. For the extracts of *P. amarus*, the aqueous extract exhibited the strongest growth inhibition of PC3 and LNCaP cells. Compared to all the plant extracts, positive control, curcumin demonstrated the strongest inhibition of cell growth as expected. The median inhibitory concentrations, IC$_{50}$ values, were derived from the log-concentration curve of the plots for the various crude extracts of plant and are recorded in Table 6.

Figure 5A: Representative plot of the cytotoxic profile of *P. amarus* extracts on PC3 (Figure A. and Figure B.) and LNCaP (Figure C. and Figure D.) prostate cancer cell lines.
The Table 6 below shows data on the cytotoxic effect of the plant extracts and curcumin on PC3, LNCaP and PNT2 cell lines. The EA extract of whole *T. tetraptera* fruit demonstrated a stronger inhibitory effect compared to all solvent extracts of the seed alone or the pulp alone and was more selective to PC3 and LNCaP prostate cancer cells with IC$_{50}$ values of 5.39 ±1.20 µg/ml and 8.38 ±1.11 µg/ml, respectively. The AQ extract of *P. amarus* leaves showed stronger inhibitory effect compared to their remaining solvent extracts and was more selective to PC3 and LNCaP prostate cancer cells with IC$_{50}$ values of 48.2±1.20 µg/ml and 55.54 ±1.11 µg/ml, respectively when compared with the other solvent extracts. The AQ extract of *P. amarus* thus showed good selective indices (SI) of 5.92 and 5.1 for PC3 and LNCaP, respectively. Also, the EA extract of *C. erythrocarpus* showed strong inhibitory effect on the PC3 and LNCaP prostate cancer cells with IC$_{50}$ values of 29.51±1.24 µg/ml and 15.75±1.23 µg/ml, respectively. The EA extract of *C. erythrocarpus* gave good selective indices (SI) of 16.42 and 28.63 for PC3 and LNCaP, respectively. The AQ extract of *J. curcas* also demonstrated a strong inhibitory activity on the PC3 prostate cancer cells with an IC$_{50}$ value of 48.56±1.29 µg/ml and a selective index value of 20.59. Similarly, the EA extract of *J. curcas* demonstrated a strong inhibitory activity against LNCaP with IC$_{50}$ value of 35.48±1.11 µg/ml and a selective index of 3.97. The DCM extract of *M. piperita* showed a strong inhibitory activity on PC3 with IC$_{50}$ value of 33.17±1.28 µg/ml. However, the selective index was less than two (SI<2) suggesting that it possessed strong inhibitory activity against normal cells PNT2. The EA extract of *M. piperita* showed a strong inhibitory effect on LNCaP prostate cancer cells with IC$_{50}$ value of 10.38±1.23 µg/ml and possessed a selective index value of 6.22. Furthermore, the AQ extract of *M. oleifera* possessed good selective indices and inhibited growth of PC3 and LNCaP prostate cancer cells with IC$_{50}$ values of
66.72±1.14µg/ml (SI=14.99) and 148.9±1.15µg/ml (SI=6.72), respectively. The DCM extract of *F. asperifolia* showed good selective indices and strong inhibitory effects yielding IC$_{50}$ values of 10.15±1.24 µg/ml (SI=14.79) and 25.57±1.33 µg/ml (SI=5.87) on PC3 and LNCaP prostate cancer cells, respectively. The AQ extract of *C. papaya* also possessed good selective indices and inhibited growth of PC3 and LNCaP prostate cancer cells. The IC$_{50}$ values were 45.68±1.16 µg/ml (SI=25.50) and 72.62±1.31 µg/ml (SI=13.77) for PC3 and LNCaP prostate cancer cells, respectively. The AQ extract of the pulp of *T. tetraperta* showed an anti-proliferative activity and was more selective for PC3 and LNCaP prostate cancer cells with IC$_{50}$ values of 47.74 ± 1.17µg/ml (SI=16.38) and 27.78 µg/ml (SI=28.15) when compared to the other remaining pulp extracts. Furthermore, the EA extract of *T. tetraperta* seed had stronger inhibitory activities on PC3 and LNCaP prostate cancer cells with IC$_{50}$ values of 55.28±1.23 µg/ml and 22.29±1.75 µg/ml, respectively when compared to the other remaining seed extracts. The IC$_{50}$ values of the prostate cancer cells and the normal cells are presented in Table 6.
Table 5: Cytotoxic activities and selective indices of plant extracts toward selected cancer and normal cell line.

<table>
<thead>
<tr>
<th>CODES/Compound</th>
<th>PC3 (IC\textsubscript{50} µg/ml)</th>
<th>Selective index</th>
<th>LNCaP (IC\textsubscript{50} µg/ml)</th>
<th>Selective index</th>
<th>PNT2 (CC\textsubscript{50} µg/ml)</th>
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<tr>
<td>Curcumin</td>
<td>7.35±1.27</td>
<td>1.37</td>
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<td>1.89</td>
<td>5.38±1.12</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>PE</td>
<td>465.1±1.58\textsuperscript{a}</td>
<td>0.48</td>
<td>228.7±1.43\textsuperscript{a}</td>
<td>0.99</td>
<td>226.3±1.36</td>
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<td>DCM</td>
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<td>43.97±1.14\textsuperscript{a}</td>
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<td>EA</td>
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<td>1.33</td>
<td>93.09±1.30</td>
</tr>
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<td>&gt;1000</td>
<td>NA</td>
<td>5.38±1.12</td>
<td></td>
</tr>
<tr>
<td>AQ</td>
<td>48.2±1.20\textsuperscript{a}</td>
<td>5.92</td>
<td>55.54±1.11\textsuperscript{a}</td>
<td>5.1</td>
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<td>LNCaP (IC&lt;sub&gt;50&lt;/sub&gt; µg/ml)</td>
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Values are given as means ± SD. IC<sub>50</sub>(µg/ml) is the concentration at 50% cytotoxic effect on PC3 and LNCaP cell lines. The cytotoxic activity was assessed on 1mg/ml of dried crude plant extract. CC<sub>50</sub> is the concentration of the 50% cytotoxic effect on normal cell line PNT2; SI (selective index) is the ratio CC<sub>50</sub>/IC<sub>50</sub>. One way ANOVA followed with Tukey’s was used to assess the level of significance between solvents of the same plant and on the same cell line. IC<sub>50</sub> values with superscript “a” means they have p values <0.05 when compared to the untreated wells. * denotes IC<sub>50</sub> less than 30 µg/ml which could be considered potential anti-cancer candidates.
MTT assay results for PC3

Figure 6A: Anti-proliferative activities of Curcumin (A), *P. amarus* leaf extracts (B) and *C. erythrocarpus* (C) leaf extracts on PC3 prostate cancer cell line. The cytotoxic activity was assessed on 1mg/ml of dried crude plant extract.
Figure 6B: Anti-proliferative activities of *J. curcas* leaf extracts (D), *L. sativa* leaf extracts (E), *M. piperita* leaf extracts (F) and *M. oleifera* leaf extracts (G) on PC3 prostate cancer cell line. The cytotoxic activity was assessed on 1mg/ml of dried crude plant extract.
Figure 6C: Anti-proliferative activities of *F. asperifolia* (H), *C. papaya* (I), *whole T. tetraperta* (J), *T. tetraperta* pulp (K) and *T. tetraperta* seed extracts on PC3 prostate cancer cell line. The cytotoxic activity was assessed on 1mg/ml of dried crude plant extract.
Mtt Assay results for LNCaP Cells

Figure 7A: Anti-proliferative activities of Curcumin (A), *P. amarus* (B), *C. erythrocarpus* (C) extracts on LNCaP prostate cancer cell line. The cytotoxic activity was assessed on 1mg/ml of dried crude plant extract.
Figure 7B: Anti-proliferative activities of extracts of *F. asperifolia* (D), *L. sativa* (E), *M. piperita* (F) and *J. curcas* (G) on LNCaP prostate cancer cell line. The cytotoxic activity was assessed at 1 milligram/milliliter of dried crude extract.
Figure 7C: Anti-proliferative activities of *M. oleifera* (H), *C. papaya* (I), whole *T. tetraperta* (J), *T. tetraperta* pulp (K) and *T. tetraperta* seed extracts (L) on LNCaP prostate cancer cell line. The cytotoxic activity was assessed on 1mg/ml of dried crude plant extract.
Mtt Assay results For PNT2 normal prostate cells

Figure 8A: Anti-proliferative activities of Curcumin (A), C. erythrocarpus extracts (B) and C. papaya (C) extracts on PNT2 normal prostate cell line. The cytotoxic activity was assessed on 1mg/ml of dried crude plant extract.
Figure 8B: Anti-proliferative activities of *F. asperifolia* extract (D), *J. curcas* extract (E), *L. sativa* extracts (F), *M. oleifera* extracts (G), *T. tetraptera* pulp extract (H) and whole *T. tetraptera* fruit extract (I) on PNT2 normal prostate cell line. The cytotoxic activity was assessed on 1 mg/ml dried crude extract.
Figure 8C: Anti-proliferative activities of *M. piperita* leaf extract (J), *P. amarus* leaf extract (K), and *T. tetraptera* seed extract (L) on PNT2 normal prostate cell line. The cytotoxic activity was assessed on 1 mg/ml dried crude extract.
Chapter 5

5.0 Discussion

Cancer is a serious health problem in the whole world. Not only does it present itself as a disease but also comes with burden such as high economic cost as well as the loss of life. Some of the available current treatments are faced with resistance and toxicity and these necessitate the search for new, safer, more affordable and effective medicines. Medicinal plants have been used since time immemorial for the treatment of cancer. Some herbalists in Ghana prepare herbal decoction or ethanolic extract from these medicinal plants and use them for the management of prostate cancer without any scientific credence on safety and toxicity. Recent studies have shown that some medicinal plants have constituent phytochemicals that have the ability to scavenge free radicals and are cytotoxic to cancer cells (Raman, 2006; Dias et al., 2012). In general, the sequential extraction employed was used to extract active principles of plants based on their solubility which in turn influence the anti-cancer activities of the extract.

The aim of the study was to identify medicinal plants used in Ghana for the management of prostate cancer and determine their anti-cancer activities. These medicinal plants used include \textit{Latuca sativa}, \textit{Tetrapleura tetraptera} (whole fruit, pulp and seed), \textit{Jatropha curcas}, \textit{Capparis erythrocarpus}, \textit{Ficus asperifolia}, \textit{Mentha piperita}, \textit{Carica papaya}, \textit{Moringa oleifera} and \textit{Phyllanthus amarus}. High yields of dried crude extracts were obtained for polar solvents than non-polar solvents. The highest yield was obtained in ETOH extract of \textit{Tetrapleura tetraptera} fruit and the lowest yield was obtained in PE extract of \textit{Tetrapleura tetraptera} fruit. According to classification of antioxidants by Blois (1958), the aqueous extract of \textit{P. amarus}
showed a very strong antioxidant activity ($EC_{50}=19.32\pm1.13 \ \mu g/ml$). Similarly the ethanolic extract of *P. amarus*, aqueous extracts of *T. tetraptera* pulp only and the whole fruit of *T. tetraptera* showed a strong antioxidant activity. The remaining extracts showed weak antioxidant activities.

Furthermore, all the extracts possessed phenolic compounds with the highest recorded in the ETOH extract of *P. amarus*. Phenolic compounds such as flavonoids, gallic acid and epigallocatechin have been reported to possess good antioxidant activities and anti-cancer activities making them good target compounds as therapies. Hence extraction using ethanol would be more ideal since most phenols are soluble in the ETOH solvent. Even though some phenols become more soluble when heated in AQ extracts, it appeared that the phenolic content was not as high as found in ethanolic extract of *P. amarus*. According to the US National Cancer Institute plant screening program, *in vitro* cytotoxic activity of a crude extract should have an $IC_{50}$ value less than $30 \ \mu g/ml$ before it can be considered as a potential anti-cancer agent for further development (Boik, 2001). Plant extracts with $IC_{50}$ values between $30\mu g/ml$ and $200 \ \mu g/ml$ have moderate potential to be developed into cancer therapeutic agents whilst those above $200 \ \mu g/ml$ are unlikely to be selected for further development (Itharat, 2004). Also, a good selectivity index and anti-cancer property is conferred on a plant crude extract when it possesses high cytotoxic activity in cancer cells whiles having low cytotoxicities to normal cells (Al-Rashidi *et al.*, 2011). Badisa *et al.* (2009) considered extracts with a selectivity index value greater than two (SI$>2$) to be a good drug candidate for drug development. Several compounds from medicinal plants have been reported to show strong cytotoxic activities ($<30 \ \mu g/ml$). However, these known drugs such as
docetaxel and paclitaxel suffer resistance against cancer cells and associated toxicities with these drugs warrants the investigation of other medicinal plants for source of anti-cancer agents.

5.1.1 Latuca sativa

In this study, higher yields of dried crude extracts were observed in the more polar solvents than the non-polar solvent therefore suggesting that most of the constituents of *L. sativa* were polar. Phenolic compounds in many herbs form a major group of secondary metabolites with bioactive pharmacological capacity attributed to anti-prostate cancer and antioxidant activities. Lu and Foo (2002) have indicated that antioxidant activity of vegetable extracts is associated with phenolic compounds.

A study by Edziri et al. (2011) has indicated that methanolic (MeOH) extract of *L. sativa* had a higher TPC than the AQ extract. The present study also shows that the ETOH extract appeared to possess the highest total phenolic content, followed by AQ extract and then EA extract (Figure 3C). However, the EA extract showed a potent antioxidant activity followed by ETOH and then the AQ extracts. This shows that phenolic substances which may possess good antioxidants activity are soluble in polar organic solvents and could be used in the management of prostate cancer. There was no correlation between the total phenolic compounds in the extract and their respective antioxidant activities. This could be due to the fact that not all phenolics have antioxidant activities. Also, antioxidants within a group can vary remarkably such that the same levels of phenolic substances do not necessarily correspond to the same antioxidant response.
Apart from EA extracts which demonstrated moderate antioxidant activities, all the other crude extracts showed weaker antioxidant activities. The present results are consistent with previous studies that have shown that *L. sativais* very rich in antioxidants (Sharma *et al.*, 2002; Patil *et al.*, 2003). This suggests that EA extract could be a promising agent in the management of oxidative stress related diseases like cancer. Indeed, the EA extract of *L. sativa* possessed the most potent antioxidant activity.

Pepe *et al.* (2015) have reported that the methanolic extract of the *L. sativa* leaves significantly decreased cyclooxygenase-2 expression, reactive oxygen species and the liberation of nitric oxide. However, there has not been any documentation of anti-prostate cancer activity of extracts of *L. sativa*. In this study the aqueous extract of *L. sativa* was found to be more selective for PC3 prostate cancer cells with IC$_{50}$ value of (59.38±1.19 µg/ml, SI=16.84). Although, the DCM extract was more cytotoxic to LNCaP prostate cancer cells, it appeared to possess low selective index (SI<2). Hence, suggesting that EA extract of *L. sativa* could be the next preferable choice for LNCaP prostate cancer cell treatment compared to the DCM extract, but it is unlikely to be selected for further anti-cancer drug development (Itharat, 2004).

**5.1.2 Tetrapleura tertraptera**

In this study, the AQ extract of the whole fruit had the most potent antioxidant activity when compared to their respective solvent extracts. This suggests that the aqueous extracts contained bioactive compound with strong antioxidant activities that could be used to manage oxidative stress related disease including prostate cancer.
The total phenolic compounds in the aqueous extract of the *T. tetraperta* pulp and seeds were very high suggesting the phenolic substances extracted are highly soluble in polar solvents. There was positive correlation between the total phenolic contents and antioxidant activity. However, the whole fruit showed the converse. The ETOH extract of the whole *T. tetraperta* fruit pulp had the highest content of phenolic substances. Although the AQ extract of the pulp and seed had high phenolic content which exhibited a positive correlation to the antioxidant activity, the AQ extract of the whole fruit had a lower phenolic content but stronger antioxidant activity than the pulp of the fruit only or seed. This suggests that the AQ extract of the whole fruit possesses a very polar compound with stronger antioxidant activity.

The present results are consistent with a previous study that showed that aqueous extract of whole *T. tetraperta* (with seed) possessed the strong antioxidant activity (Josiah *et al.*, 2017).

Ethanolic crude extract of *T. tetraperta* has previously been reported to be cytotoxic to Ehrlich ascites carcinoma cells (Ozaslan, 2016). In this study, it was also shown that all the extracts of the whole fruits and pulp only exhibited anti-proliferative activity of the cancer cells studied in a concentration dependent manner. Although, the EA and ETOH extract of the seed exhibited anti-proliferative activity of the cancer cells in a concentration dependent manner, the PE extract of the seed did not have any activity on the cancer cells. This suggests that the phytochemicals present in PE extract of seed do not possess anti-prostate cancer activity.

The aqueous extract of *T. tetraperta* pulp was more selective for PC3 and LNCaP with IC$_{50}$ values <50 µg/ml, SI values of about 16 and 28, respectively. The DCM, EA and ETOH extracts of the whole *T. tetraperta* met the cut off limit for good anti-cancer extracts (<30 µg/ml) and could be considered as potential anti-cancer agent for further development (Boik,
The EA extract of the whole *T. tetraperta* plant was more cytotoxic and selective to PC3 and LNCaP prostate cancer cells with IC$_{50}$ values of 5.39±1.24 µg/ml (SI=7.72) and 8.38±1.27 µg/ml (SI=4.97), respectively. The whole *T. tetraperta* fruit (with seed) extract showed a more potent anti-prostate cancer activity when compared to either the fruit alone or the seeds alone. This result suggests that the seed and the pulp produced a synergistic effect. The plant is reported to have phytochemical such as tannins, saponins, alkaloids, anthraquinones, glycosides, flavonoids and other phenolic compounds in AQ and ETOH extracts of the fruit (Gberikon *et al*., 2015) as well as minerals such as calcium, zinc, potassium and iron (Okwu, 2003).

**5.1.3 Capparis erythrocarpus**

The present study assessed the pharmacological benefits of the leaves of *C. erythrocarpus*. All the extracts of *C. erythrocarpus* leaves possessed antioxidant properties and phenolic substances. The ethanolic crude extract possessed the highest total phenolic content indicating that most of the phenols are soluble in ethanol. There was no correlation between the total phenolic content and antioxidant activity. The antioxidant activities of the extracts were concentration dependent. The DCM extract showed a stronger antioxidant activity when compared to the rest of the extracts.

The result showed that, the ethyl acetate extracts of *C. erythrocarpus* exhibited a stronger cytotoxic activity and was more selective for PC3 and LNCaP prostate cancer cells (IC$_{50}$=29.51±1.24 µg/ml, SI=16.42 and IC$_{50}$=15.75±1.23 µg/ml, SI=28.63 respectively) than normal cells. Strong pharmacological activities of root extracts of the plants have been attributed to active phytochemicals such as flavonoids and alkaloids (Danquah *et al*., 2011). Unlike previous studies focused on the roots, this study has shown that the ethyl acetate
extracts of *C. erythrocarpus* leaves have strong anti-proliferative activity against prostate cancer cells.

### 5.1.4 *Ficus asperifolia*

All the extracts showed antioxidant activities but were not that strong when compared to the AQ extract. The aqueous extract possessed a stronger antioxidant activity when compared to the remaining extracts of the plant. This suggests that AQ extract of *F. asperifolia* possessed compounds with strong antioxidant activity and could be a promising agent for the treatment and management of oxidative stress related diseases including prostate cancer. Similar findings were observed in a study done by Ojo and Akintayo, (2014). They indicated that the DPPH scavenging ability of AQ extract of *F. asperifolia* at the concentration of 5mg/ml was 78.65 ± 1.15%. Ojo *et al.* (2014) reported that the therapeutic benefit could be owed to the presence of saponins, glycosides, tannins, alkaloids and phenolic compounds in aqueous crude extract of *Ficus asperifolia*. All the extracts possessed phenols at 5 mg/ml. In the study, the total phenolic content was highest in ethanolic extracts with values (30.78 ±1.68 g/100g GAE) indicating that most of the phenols were soluble in the ethanolic extract. Moreover, the TPC values of the AQ extract appeared to be higher than the reported values by Ojo and Akintayo (2014). This could be due to the different extraction methods employed and different environmental factors affecting the yield of the plant constituents. Furthermore, a previous study has indicated that the methanolic extract of *Ficus asperifolia* possesses a good amount of total phenolic content (GAE: 31.96 mg/ml).

The present results are consistent with results obtained by Ojo and Akintayo (2014) which indicated good amounts of total phenolic content in alcoholic solvent extracts. Previous
studies focused on other cell lines such as breast cancer MCF-7 and malignant melanoma cell lines DLD-1. This study has confirmed anti-prostate cancer properties against PC3 and LNCaP prostate cancer cells. The anti-cancer properties were observed for all the extracts. The DCM extract of *Ficus asperifolia* showed a stronger anti-prostate cancer activity and was more selective to PC3 and LNCaP prostate cancer cell lines than the remaining extracts of *F. asperifolia*. The EA extract showed a moderate anti-cancer activity on PC3 and LNCaP prostate cell lines but possessed weak selectivity index (SI<2). The dichloromethane extract exhibited a stronger cytotoxic activity and was more selective to PC3 and LNCaP prostate cancer cells with IC$_{50}$ values of 10.15±1.24 µg/ml, SI=14.79 and 25.57±1.33 µg/ml, SI=5.87, respectively when compared to the rest of the extracts.

5.1.5 *Mentha piperita*

All the extracts showed antioxidant properties with the EA extract exhibiting a stronger activity at a concentration of 1 mg/ml. The ETOH extract in this study showed a higher phenolic content of 3.97± 0.34g/GAE100g with stronger antioxidant activity (EC$_{50}$= 0.43±0.01 mg/ml) than the previous study conducted by Farnad *et al.* (2014). Farnad *et al.* (2014) reported that the ethanolic extract showed a total phenolic content of 1.99 ± 0.32g/GAE 100g with a DPPH free radical scavenging activity (80.74 ± 0.83%, IC$_{50}$= 11.32 ± 0.71 mg/ml) at a concentration of 20 mg/ml.

This difference could be due to the different extraction techniques employed in the study and the different environmental factors affecting the yield of phenols. Also, all the extracts contained phenolic compounds. The ethyl acetate extract gave the highest total phenolic content indicating that most of the phenols were soluble in the ethyl acetate solvent. All the extracts showed antioxidant activities indicating that they could be used in scavenging
reactive free radicals responsible for oxidative stress related ailments like cancer. The ethyl acetate extract showed a stronger antioxidant activity when compared to the remaining extracts.

Sun et al. (2014) reported that the essential oil of *M. piperita* has anti-inflammatory properties against SGC-7901 gastric cancer cells, SPC-A-1 lung cancer cells and K562 cancer cell lines. Unlike previous studies focused on gastric cancer, lung cancer and erythroleukemia, this study has also shown that all the extracts of *M. piperita* possessed anti-prostate cancer activity. Among the extracts, the DCM extract of *M. piperita* showed the strongest cytotoxic activity against PC3 with IC$_{50}$ value of 33.17±1.28 µg/ml while EA extract exhibited the strongest cytotoxic activity against LNCaP prostate cancer cells with IC$_{50}$ value of 10.38±1.23 µg/ml.

### 5.1.6 *Carica papaya*

In this study, all the extracts of *C. papaya* possessed phenols. The ethanolic extracts possessed more phenols than the rest of the extracts. This shows that most of the phenols are soluble in ethanolic solvents. This result confirms similar findings in a study done by Fidrianny et al. (2016). Fidrianny et al. (2016) have established that the ethanolic extract possessed a significantly higher phenolic content (5.70 g GAE/100 g) whiles the hexane leaf extract possessed a lower phenolic content (1.88 g GAE/100 g). However, it did not confirm studies done by Vuong (2013) and Mandal (2015) who found the converse. This could be due to the different extraction methods employed in the study. Mandal et al. (2015) indicated that total phenolic content of aqueous, methanol and petroleum ether of *C. papaya* leaves extract were 57.6 ± 3.7 µg GAE/g dry weights, 47.8 ± 2.4 µg GAE/g dry weight and 8.4 ± 0.4 µg GAE/g dry weight, respectively. Additionally, Vuong (2013) has indicated that the aqueous extract of
C. papaya contained significantly higher polyphenols than ethanolic extract with values 23.06 ± 1.06 mg GAE/ g dry weight and 9.43 ± 0.14 mg GAE/ g dry weight, respectively.

A report by Fidrianny et al. (2016) has indicated that the ethanolic extract of pawpaw leaves possess a strong antioxidant activity with EC₅₀ value of 0.84 µg/ml. However, Mandal et al. (2015) showed that extracts of pawpaw leaves (aqueous, methanol and petroleum ether) had weak antioxidant activities (EC₅₀=247 ± 3.5 µg/ml, 262.2 ± 4.2 µg/ml and 171.5 ± 3.3 µg/ml respectively) using DPPH assay.

In this study, all the extracts showed antioxidant activities indicating that they could be used in the treatment of oxidative stress related diseases. The aqueous extract of C. papaya showed the most potent antioxidant activity.

An earlier study by Otsuki et al. (2010) has reported a strong anti-cancer activity of the aqueous extract of C. papaya leaves. Likewise, the aqueous extract was reported to have exerted immunomodulatory activity and anti-tumor activity in Jurkat, Molt-4, CCRF-CEM and HPB-ALL cell lines (Otsuki et al., 2010).

Unlike previous reports focused on cancers related to blood, this study has shown anti-prostate cancer activity. All extracts of C. papaya except petroleum ether and ethanolic extracts showed anti-proliferative activities against the prostate cancer cells. The aqueous extract exhibited a stronger anti-proliferative activity and was more selective to PC3 with IC₅₀ value of 45.68±1.16 µg/ml, SI=25.50 while the dichloromethane extract demonstrated
stronger anti-proliferative activity against LNCaP prostate cancer cell lines with IC\textsubscript{50} value of 70.87±1.31 µg/ml, SI=14.11. Alorkpa (2016) has reported the presence of alkaloids and tannins in non-polar solvent extracts of \textit{C. papaya} whereas phytochemical compounds such as flavonoids, glycosides and saponins were reported to be present in polar solvent extract of \textit{C. papaya}. In this current study the anti-prostate activity may be due to the phytochemical compounds present.

5.1.7 \textit{Moringa oleifera}

From the results in this study, all the extracts possessed some amount of phenolic substances which suggest that it could be used in the management of diseases since several phenols have been shown to have pharmacological activities. The ethanolic extracts possessed the highest amount of phenols suggesting that most of the phenols were soluble in ethanol. Previous reports have indicated that different solvent extracts of moringa leaves contain phenolic compounds, hence a great source of polyphenols (Sultana \textit{et al}., 2009; Sreelatha and Padma, 2009). Also, all the extracts showed antioxidant activities, suggesting that they could be used in the management of oxidative stress related diseases including prostate cancer. Among the extracts, the aqueous extract showed the strongest antioxidant activity and did not correlate with the phenolic content. This suggests that AQ extract may possess phytochemical compounds with stronger antioxidant activities. The percentage scavenging activity of the extract against free radicals was concentration dependent.

A report by Coppin \textit{et al}. (2013) have indicated that \textit{Moringa oleifera} leaf extract is able to inhibit nitric oxide (NO) produced by macrophage cells which was treated with bacterial lipopolysaccharide (LPS). Moreover, anti-inflammatory and immunomodulatory effects of \textit{Moringa oleifera} leaves have been reported in many \textit{in vivo} and \textit{in vitro} studies (Das \textit{et al}.,
2013). In addition, ethyl acetate extracts of *Moringa oleifera* leaves was reported to inhibit human macrophage cytokine production (TNF-α, IL-6 and IL-8) induced by extract of cigarette smoke (Kooltheat *et al.*, 2014). Unlike previous studies focused on anti-inflammatory and immunomodulatory effects, this study has shown anti-prostate cancer activity of *Moringa oleifera*. In this study, all the extracts except petroleum ether and ethanolic extracts exhibited anti-proliferative activity against the prostate cancer cell lines in a concentration dependent manner. Among the extracts, the aqueous extract showed the strongest anti-proliferative activity and were more selective to PC3 and LNCaP prostate cancer cells with IC$_{50}$ values of 66.72±1.15 µg/ml, SI=14.99 and 148.9±1.14 µg/ml, SI=6.72, respectively.

5.1.8 Phyllanthus amarus

In this study, all the extracts possessed varying amounts of polyphenols. The ETOH extract possessed the highest amount of phenols indicating that they are more soluble in the ethanolic solvent. Djeridane *et al.* (2006) indicated that the solubility of phenolic substances in an extract largely depends on the choice of the solvent used, due to the degree of polymerization of the phenols and the kind of interactions that exist between them. Also, all the *P. amarus* extracts showed antioxidant activities in a concentration dependent manner. The aqueous extract exhibited the most potent antioxidant activity which did not correlate with the phenolic content. This suggests that antioxidant activity may not be largely contributed by the phenols present in the extract. Numerous phytochemical compoundssuch as alkaloids, flavonoids, hydrolysable tannins, lignins, polyphenolic compounds and tetracyclic triterpenoids have been reported to be present in *P. amarus* extracts (Verma, 2014).
Furthermore, this study showed that, apart from ethanolic extract, all the extracts possessed anti-proliferative activity against both PC3 and LNCaP prostate cancer cell lines in a concentration dependent manner. From Figure 5A, the percentage cell viability decreased as the concentration of extract increased. The present results are consistent with the previous report, where *P. amarus* extract significantly inhibited the growth of DU 145 cell line (Nguyen, 2017). The aqueous extract showed the highest anti-prostate cancer activity against PC3 prostate cancer cells with IC\textsubscript{50} value of (48.2±1.20 µg/ml, SI=5.92) and was more selective to LNCaP cells with IC\textsubscript{50} value of (55.54±1.11 µg/ml, SI=5.1). Previous studies have reported the presence of phyto-compounds such as hydrolazable tannins, geraniin, gallic acid and ellagic acid in aqueous extracts of *P. amarus* (Verma, 2014; Okuda *et al.*, 1979).

### 5.1.9 *Jatropha curcas*

The results from this study revealed that all the extracts possessed some amount of phenolic substances. The ethanolic extract possessed more phenols than the remaining solvents extracts. This suggests that the phenolic compounds present were soluble in ethanolic solvent. From the results, Figure 4C showed that the scavenging ability of all the extracts were concentration dependent. The percentage scavenging activity increased as the concentration of the extract increased. Although, the ETOH extract possessed the highest content of phenolic substances it did not correlate with the antioxidant activity, as the aqueous extract showed a more potent antioxidant activity than the remaining extracts. This suggests that the polar phytochemicals of AQ extract possessed stronger antioxidant activities. Conversely, previous studies have reported that the methanolic extract of *J. curcas* possessed strong antioxidant activity using DPPH assay (Oskoueian *et al.*, 2011; Fu *et al.*, 2014).
Uche and Aprioku (2008) have reported that methanolic extract of *J. curcas* leaves possess anti-inflammatory activity on Wister albino rats. A previous study has reported that methanolic extracts of the leave had cytotoxic activity against HT 29 colon cancer cell line (Oskoueian *et al.*, 2011). Unlike previous studies focused on human colon cancer, this study has shown anti-prostate cancer activity. The aqueous extract showed potent anti-cancer activities and a good selective index against PC3 prostate cancer cells, whilst the ethyl acetate extract showed a stronger anti-cancer activity and a good selective index against LNCaP prostate cells when compared to the remaining solvent extracts of *J. curcas*. Rejila *et al.* (2012) have reported the presence of phytochemical compounds such as kaempferol, coumarin, catechin and quercetin in polar solvents extract of *J. curcas* leaves, which possesed anti-cancer activities.
CHAPTER 6

6.0 Conclusion and Recommendation

6.1 Conclusion

The study has shown that all the extracts contained phenolic substances and had antioxidant activities which may be useful in the management or treatment of oxidative stress related diseases such as prostate cancer. Among all the plant extracts, the ethyl acetate extract of the whole fruit of *T. tetraptera* exhibited the strongest anti-prostate cancer activity against LNCaP and PC3 prostate cancer cells. Also, other solvent extracts that possessed strong anti-prostate cancer activity as well as good antioxidant activities include AQ extracts of *P. amarus*, *C. erythrocarpus*, and *C. papaya*. Furthermore, ethyl acetate extract of *C. erythrocarpus* showed a strong anti-proliferative activity. These findings suggest that, these extracts could be potential anticancer agents. Also, these findings lend pharmacological credence to the suggested folkloric uses of the fruit and leaves of the plants in the management of prostate cancer.

6.2 Recommendation

Future studies should include characterization of the phytochemical profile of the plant extracts. Also, the toxicological safety of the bioactive fractions in an *in vivo* animal model should be elucidated. The bioactive compounds responsible for the anti-prostate cancer activity should be isolated and characterized. Further work involving the determination of the targets of intracellular signaling pathways must be done.
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APPENDIX A

Total Phenolic Content

Preparation of Gallic Acid Standard

Stock concentration = 1 mg/ml

Firstly, 1 mg of gallic acid was weighed and first dissolved in 100μL of absolute ethanol and then topped up to 1000 μl with 900μl of dH₂O.

Also, a two- fold serial dilutions was done to obtain working concentrations of; 1 mg/ml, 0.5mg/ml, 0.25mg/ml, 0.125mg/ml, 0.0625mg/ml, 0.3125mg/ml and 0.015625mg/ml.

Mathematical presentation

2-fold dilution = 1:1

Stock concentration = 1mg in 1ml (1000μL) of solution

For first dilution, volume needed = ½ × 1000μL = 500μL

Therefore, 500μL of stock solution + 500μL of solvent = 0.5mg/ml of GA.

Preparation of 0.09 M Sodium bicarbonate (Na₂CO₃)

The volume of required Na₂CO₃ for a plant sample = 150Μl × 96 = 14400μL.

Ten grams of Na₂CO₃ was dissolved in 40ml of distilled water and boiled.

The solution was cooled and pure crystals of Na₂CO₃ were added to induce recrystallization.

The solution was then filtered and 10 ml of distilled water was added to obtain 5ml of Na₂CO₃ solution.

Sample Preparation

Stock concentration = 5 mg/ml

Serial dilutions (2-fold) to obtain working concentrations of; 5mg/ml, 2.5mg/ml, and 1.25mg/ml. 2-fold dilution = 1:1
APPENDIX B

Free Radical Scavenging Activity (DPPH)

Sample Preparation

Stock concentration of crude extracts and standard BHT = 1mg/ml each

A two-fold serial dilutions was done to obtain working concentrations of; 1mg/ml, 0.5mg/ml, 0.25mg/ml, and 0.125mg/ml, 0.0625mg/ml, 0.03125mg/ml and 0.0015625mg/ml.

2-fold dilution = 1:1

Stock concentration = 1mg in 1ml (1000μL) of solution

For first dilution, volume needed = ½×1000μL = 500μL

Therefore, 500μL of stock solution + 500μL of solvent = 0.5mg/ml of sample.

The procedure above was used to prepare the successive working concentrations

Preparation of 0.05 DPPH solution

Preparation of 0.5mM DPPH for five crude extracts of a plant sample

Volume of DPPH required for free radical scavenging assay for a plant sample = 100μL×164 wells = 16400 μL

However, 18000μL (18 ml) of DPPH was prepared for each plant sample to cater for loss.

1 Molar mass of DPPH = 394.32g/mol

Also, Concentration (C) = number of moles (n)/volume (dm³)

This implies, n = C ×V (dm³)

Concentration (C) = number of moles (n)/volume (dm³)

Hence, n = C ×V (dm³)

n = (0.5 × 10⁻³) ×0.018= 9.0 × 10⁻⁶ moles

Theoretically, n = mass (m) /Molar mass (M)
Therefore, mass \( m \) = \( n \times M \)

\[
m = (9 \times 10^{-6}) \times 394.32 \text{ g/mol} = 3.5489 \times 10^{-3} \text{ g}
\]

\[m = 3.55 \text{ mg DPPH}\]

Preparation of 0.5mM DPPH solution was done by weighing 3.55 mg of DPPH and dissolving in absolute methanol and making up to 18 ml in a 50 ml falcon tube.
APPENDIX C

Preparation of Complete RPMI Media

Materials

RPMI, 10% FBS (foetal bovine serum), 1% streptomycin-penicillin, serological pipettes (5ml, 10ml and 25 ml) and falcon tubes (15 ml and 50 ml)

Procedure

The complete media was prepared in a sterilized laminar flow hood. A volume of 45 ml RPMI culture media (already containing 1% streptomycin-penicillin) was aliquoted into a 50 ml falcon tube using a serological pipette and a pipette filler machine. It was then followed by the addition of a volume of 5ml FBS and then mixed together gently to form a complete RPMI media

Cell Count

Material

Trypan blue, hemocytometer, coverslip, 70% ethanol, microscope, pipette tips (200 µl and 1000 µl) and cells (PC3, LNCaP or PNT2)

Method

A cell suspension of 1 x10^5 cells/mL was prepared after trypsinization of attached cells. A five times dilution of the cells was prepared in a 1:4 ratio of the cell suspension to trypan blue in an eppendorf tube. It was gently mixed and allowed to stand for 2 minutes.

Prior to counting, the hemocytometer and coverslip was washed with 70% ethanol and allowed to dry. The cell suspension in trypan blue was then transferred unto the hemocytometer already covered with a cover slip. The hemocytometer was then mounted on the light microscope and the unstained cells (viable cells) were counted at a × 40 magnification. The unstained cells in each of the 4 quadrant of the hemocytometer were
counted as viable. An average of the four different counts was calculated and multiplied by the dilution factor of 5 and $10^4$ to obtain the total number of cells/ml as shown below.

Number of viable cells = \[
\frac{(A+B+C+D) \times 10^4 \times 5}{4}
\]

where A, B, C, D are the number of viable cells in each of the four quadrants respectively. The cells were seeded at $1 \times 10^5/100 \mu l$ into each well of the 96 well plate and waited a day after to get the cells attached to the well before the curcumin or crude extracts were added.