AN INVESTIGATION INTO THE ANTINEOPLASTIC PROPERTIES OF *SCHINZIOPHYTON RAUTANENII* AND *COLOPHOSPERMUM MOPANE*

A THESIS SUBMITTED IN FULFILMENT

OF THE REQUIREMENT FOR THE DEGREE OF

MASTER OF SCIENCE

OF THE

UNIVERSITY OF NAMIBIA

BY

DUSHIMEMARIA FLORENCE

200815431

MARCH 2014

MAIN SUPERVISOR: DR DAVIS R MUMBENGEGWI

CO-SUPERVISOR: DR RONNIE BOCK

ABSTRACT

Cancer incidences are on the rise in Namibia, because of late diagnosis, cancer cases often have poor prognosis and treatment options are limited especially in resource poor rural settings. Ethnomedicinal plants present a treatment option which is used in such settings, is accessible and inexpensive. However evidence on safety and efficacy of these traditional medicinal plants is lacking, which prevents mainstream use. This study evaluated two Namibian indigenous plants, *Schinziophyton rautanenii* and *Colophospermum mopane* used medicinally in Zambezi region, for their phytochemical content and anti-protease activity. Furthermore, phytochemicals where quantified as percentage yields of dry plant material, antioxidant activity measured and plants were evaluated *in vitro* for anti-cancer activity against breast, renal and melanoma cancer cell models. Additionally, plant extracts were screened against a human fetal lung fibroblast cell line to determine cytotoxicity. Extracts were also assayed *in vivo* in Planaria, for toxicity. Alkaloids, coumarins, flavonoids and triterpenes were found in all plant extracts, except anthraquinones which were only found in the root extract of *S. rautanenii*. Antioxidant activity shown by plant extracts was in correlation with the observed alkaloid content of plants, (correlation =0.58, n=12, p=0.048). The highest *in vitro* anticancer activity was exhibited by the organic root extract of *C. mopane,* with IC_{50} 48.2 µg/ml although it also showed mild cytotoxicity against fibroblast cells, IC_{50} 162.4 µg/ml. *In vivo* toxicity evaluation further revealed the strong toxicity level of the organic *C. mopane* root extract against the freshwater flatworm planaria at 20 µg/ml. The aqueous bark and root extracts of *C. mopane* showed some anticancer activity

towards the breast cancer model (IC_{50} 86.8 µg/ml and 87.9 µg/ml respectively) while the organic bark and root extracts of *C. mopane* showed anticancer activity against a melanoma (IC_{50} 92.5 µg/ml and 64.1 µg/ml), breast (IC_{50}70.2 µg/ml and 48.2 µg/ml) cancer models respectively. The melanoma cell line UACC-62 displayed sensitivity towards the aqueous bark and root extracts of *S. rautanenii* with low IC_{50} values of 116.7 µg/ml and 128.7 µg/ml respectively. The organic root extract of *S. rautanenii* also showed sensitivity towards the UACC-62 melanoma cell line at high extract concentration although IC_{50} 102.6 µg/ml, was similar to that obtained against MCF-7 breast cancer cells, 102.4 µg/ml. The organic and aqueous root extracts of *S. rautanenii* displayed the highest IC_{50} values of 315.5 µg/ml and 444.8 µg/ml against the human fetal lung fibroblast cell. In conclusion, the use of *C. mopane* and *S. rautanenii* within the traditional settings is rational and a readily accessible alternative. However, further studies are required to assess the extent of toxicity before the extracts can be recommended for mainstream usage.

Table of Contents

LIST OF ABBREVIATIONS

ABTS-2,2 Azino-bis (3-ethylbenzo-thiazoline-6-sulfonic acid) diammonium salt.

AIDS-Acquired immunedeficiency syndrome.

ATCC-America Type Culture Collection

Bcl2-B-cell lymphoma 2

CAN-Cancer Association of Namibia

CDK-Cyclin dependant kinase

COX-2-Cyclooxgyenase 2

CSIR-Council for Scientific and Industrial Research

DCC-Deleted in colorectal cancer

DMSO-Dimethyl sulphoxide

ECACC-European Collection of Cell Cultures

FC-Folin-Ciocalteu

FDA-Food and Drug Administration

HBV-Hepatitis B virus

HIV-Human Immune Virus

HPV-Human papillomavirus

LOH-Loss of heterozygosity

LPS-lipopolysaccharides

P53-Tumor suppressor protein 53

ROS-Reactive oxygen species

SMAD4-Mothers against decapentaplegic homology 4

TGFβ-Transforming growth factor β

LIST OF FIGURES

LIST OF TABLES

LIST OF PUBLICATIONS

This work has been partially published in the form of peer-reviewed articles and conference poster presentations.

1. Dushimemaria, F. and Mumbengegwi, D. (2013). Palliative treatment of cancer in resource poor settings: Traditional medicine perspective. Manuscript under review.
2. Dushimemaria, F and Mumbengegwi, D. (2013). Evaluation of a low cost field assay for the determination of anti-proliferative properties of indigenous plants. Manuscript under review.

3. Dushimemaria, F. and Mumbengegwi, D. (2013). Namibian Plants: *Colosphospermum mopane* and *Schinziophyton rautanenii* exhibit selective cytotoxicity against a three cell line panel. Unpublished manuscript.
4. Dushimemaria, F., Mumbengegwi, D. and Bock, R. (2013). Ethno-medicine: Indigenous Knowledge of medicinal plants used for treatment of cancer. Book chapter in Indigenous scientific knowledge of Namibia. Faculty of Science book project.

LIST OF CONFERENCES AND POSTERS

1. Dushimemaria, F., Mumbengegwi, D. and Bock, R. (2012). Screening of ethnomedicinal plants from the Caprivi region for potential anti-proliferative and apoptosis-inducing properties. The 2nd indigenous knowledge systems information symposium. 8-9 October, 2012. University of Namibia library auditorium.
2. Dushimemaria, F., Mumbengegwi, D. and Bock, R. (2013). Anticancer and cytotoxicity properties of phytochemical compounds present in Namibian indigenous plants. 1st annual science research conference. 25-26th October, 2013.
3. Dushimemaria, F., Mumbengegwi, D. and Bock, R. (2013). Investigation of the antineoplastic properties of Namibian indigenous plants: *Colophospermum mopane* and *Schinziophyton rautanenii*. 21-24th November, 2013. AORTIC 2013.

ACKNOWLEDGEMENTS

I wish to acknowledge the following individuals, institutions and programmes for their support and contribution to my studies: I wish to thank God, first and foremost. Dr Davis Ropafadzo Mumbengegwi, a mentor, supporter, counselor, for investing time, his patience and energy into my professional growth and always wanted the best professional exposure and experience for me. Dr Ronnie Bock, who helped shape me professionaly by his guidance, patience and encouragement. I will forever be grateful to both Dr Mumbengegwi and Dr Bock for their inspirations and interests in my studies.

Mr Emmanuel Vellemu and Mr Jesaya Nakanyale, who offered statistical guidance and selflessly volunteered to advise, correct through all the statistical tests even when they had other commitments.

Ms Sylvia Nafuka, Ms Lucia Heita, Mr Munyaradzi Tambo, Mr Werner Embashu, Ms Iwanette Du Preez, Mr Tatenda Malan Probert, Ms Kapanga Kalistu, Ms Hatago Stuumann for their assistance in the laboratory, support and encouragement thoughout the study period.

Natasha Kolesnikova from the Council for Scientific and Industrial Research (CSIR), who trained me in cell culture maintenance, cytotoxicity and anticancer screening, and the opportunity to shadow her in the laboratory, as well as her patience and assistance.

The Malaria research laboratory management, Department of Biological sciences and Multidisciplinary Research Center (MRC) for hosting this research in their facilities.

The German Academic Exchange programme for the scholarship, Research and Publication Office and the Directorate of Research, Science and Technology (Ministry of Education) for funding this research.

DECLARATION

I, Florence Dushimemaria, declare that this study is a true reflection of my own research, and that this work, or part thereof has not been submitted for a degree in any other institution of higher education.

..... Date.....

DEDICATION

I wish to dedicate my work to cancer sufferers, especially those in rural areas. To my family: Verediane, Bizimungu, Given, and my late father, Mathew.

To all those who encouraged me every step of the way.

CHAPTER ONE: INTRODUCTION

1.1. Orientation of study

Plants have been a valuable resource in the history of mankind, both as a source of food and medicine. As medicines, plants were often used to prepare poultices, powders, tinctures and other forms of medicinal portions for the treatment of different ailments (Balunas and Kinghorn, 2005). Presently, plants still remain as the basis for traditional medicinal systems being the source of primary health care for many individuals worldwide today (Ganesan, 2008) and have proven their value as contributing novel entities to modern medicine (Bellik et *al.,* 2013). Modern synthetic medicinal entities have only supplanted traditionally prepared crude extracts as major healthcare provisions within the past few centuries with many drugs in clinical use owing their origin to plants, such as salicyclic acid (Karuppusamy, 2009). In fact, about 1141 different Traditional Chinese Medicine derived drugs were registered for their different pharmaceutical activities in 2002, (Patwardhan et al., 2005). Certainly, medicinal plants, not only contribute positively to health management but are valuable as revenue sources in local economics and cultural heritage (Okigbo et al., 2008).

Plants owe their therapeutic properties to the occurrence of phytochemicals, which are non-nutritional secondary metabolites of plant origin (Doughari et al., 2009). These beneficial compounds have the potential to alleviate many different diseases including cancer and metabolic diseases. Phytochemicals consist of polyphenols, flavonoids,

terpenoids, alkaloids and saponins and other classes of compounds. The presence of particular phytochemical compounds in plants is usually viewed or used to infer the therapeutic properties of such plants, e.g, vinblastine and vincristine known as vinca alkaloids, for anticancer (Mutee et al., 2012). Corroboration of the usefulness of traditional plants can be provided through investigations which determine the efficacy and safety of these plants.

This study focuses on the evaluation of two Namibian indigenous plants, *Schinziophyton rautanenii* and *Colophospermum mopane* as anticancer extracts. Firstly, *S. rautanenii* and *C. mopane* were analysed for the presence of selected phytochemical compounds, antioxidant activity and antiprotease activity. Organic and aqueous extracts prepared from the two plants were evaluated for their anticancer activity *in vitro* against a panel of three human cancer cell lines, representing melanoma, renal and breast carcinomas. In addition, cytotoxicity analysis was conducted *in vitro* and and *in vivo* toxicity analysis against a human fibroblast cell line and *Dugesia dorotocephala* (planaria) respectively.

1.2. Statement of the problem

The Namibian Cancer Registry (2009) shows that different cancers are on the increase in Namibia. Prostate cancer and Kaposi's sarcoma are the leading cause of morbidity and mortality among males while breast and cervical cancer are the most widespread among the Namibian female population. Cancer patients in distant remote areas of

Namibia have to endure a long referral process. This translates into poor prognosis of cancer cases before treatment commences. In addition, cancer is often incurable and patient's access to treatment is often limited, leading to increased morbidity. In such instances, palliation of cancer is mostly the solution. Traditional herbal remedies are an option for treatment and palliation of cancer, however, there is stigma towards traditional medicine because it does not always have a scientific basis for its pharmacological activities. This further restricts the inclusion of traditional herbal medicines into mainstream clinical usage, worsening the associated stigma even deeply. To date, no scientific studies have been conducted to evaluate the efficacy and safety of plants used traditionally for the treatment of cancer similar symptoms in various Namibian communities. Such information can also be used to promote their use, discover an alternative anticancer treatment option and help reduce the existing stigma.

1.3. Research objectives

i. To screen for selected phytochemical compound classes in plant extracts: *Colophospermum mopane* and *Schinziophyton rautanenii* with anticancer properties and quantify them.

ii. To determine anti-proliferative and cytotoxicity effects of these plant extracts *in vitro*.

iii. To evaluate the toxicity effect of the metabolic-end products of plant extracts in a fresh water animal model: planaria.

1.4. Hypothesis

Plant extracts of *Colophospermum mopane* and *Schinziophyton rautanenii* contain known anticancer phytochemical class compounds: the extracts exhibit *in vitro* antineoplastic properties and low cytotoxicity, as well as low toxicity effects based on their usage in the traditional setting, Zambezi region, Namibia.

1.5. Significance of study

This study makes a contribution by filling the knowledge gap on the use of traditional plants for the treatment of cancer related symptoms in Namibia. Also, the evaluation of the selected plants: *S. rautanenii* and *C. mopane* provides science based evidence for the efficacy and safety for the use of plants medicinaly, which may help reduce the stigma associated with traditional medicine. Furthermore, the plants may be applicable as herbal supplements for the palliation and treatment of cancer, especially in distant resource-poor areas. Ultimately, this may reduce delays in patients receiving treatment and palliation for cancer and improve prognosis while bridging the gap between traditional medicine and orthodox medicine with common interest in the well being of the cancer patient. In addition, this study may lead to the discovery of novel pharmaceutical entities for the palliation and treatment of cancer.

1.6. Limitations of study

This study will be conducted within the following limitations. Firstly, cancer cell lines are laboratory homogenous immortal models (Kashyap et al., 2011) and their use in this study may not reflect the real clinical presentation of a tumor and neither does it represent the behavior of clinical cases (HogenEsch and Nikitin, 2012). Moreover, only a few selected phytochemical classes are researched in this study and likewise does not present the entire spectrum. This study uses a fresh water flatworm, Planaria, for the analysis of toxicity. Planaria is not routinely used for analysis of toxicity, in fact, this is the first use of the worm in this manner. Disadvantages of use of Planaria for toxicity analysis included its low relatability to frequently used models such as mice or man. Lastly, extract preparation in this study may differ from the method used in the traditional setting; hence, obtained results should be viewed in this light.

CHAPTER TWO: LITERATURE REVIEW

2.1. Cancer and its development.

Cancer is an umbrella term used to refer to a group of diseases, which are characterized by unregulated division of cells (Ganesan and Muthuchelian, 2011). Cancer can further be classified into two major groups: a localized mass of cells forming a tumor, i.e. benign cancer or can spread from point of origin, and is referred to as a malignant cancer. Cancer development begins when the initiating cell develops mutations in its cellular division machinery (Khan et al., 2011), see figure 1. It undergoes uncontrolled cellular division, resulting in the first initiated cell which multiplies, grows and survives at the primary site, leading to invasion, angiogenesis, intravasation, extravasation and eventually, growth in another distant site, termed as secondary site (Varsale et al., 2010). Cancer initiation can be as result of endogenous factors such as genetic disposition and others such as failure of apoptosis, inflammation reactions in immunological responses or oxygen metabolism. However, exogenous sources such as ionization radiation, chemicals or bacteria and viruses are capable of initiating carcinogenesis. The next step involves promotion. Normal intracellular defense systems including antioxidant enzymes such as superoxide dismutase, catalase, glutathione peroxidase and additional non-enzymes such as carotenoids, flavonoids, vitamin C and E work together to prevent oxidative stress and thereby protect cellular macromolecules such as DNA and proteins from damage (Min and Ebeler, 2008). However, oxidative stress is unavoidable because mechanisms set in place to circumvent it, which are

antiproteases are sometimes inbalanced or are absent. Sedelnikova et al, (2010), described the effects of reactive oxygen species (ROS), resulting oxidative stress and the role ROS play in cancer pathogenesis. In fact, cancer development and progression is influenced by different factors occurring at the molecular level such as genetic abberations (Abdel-Hamid, 2009).

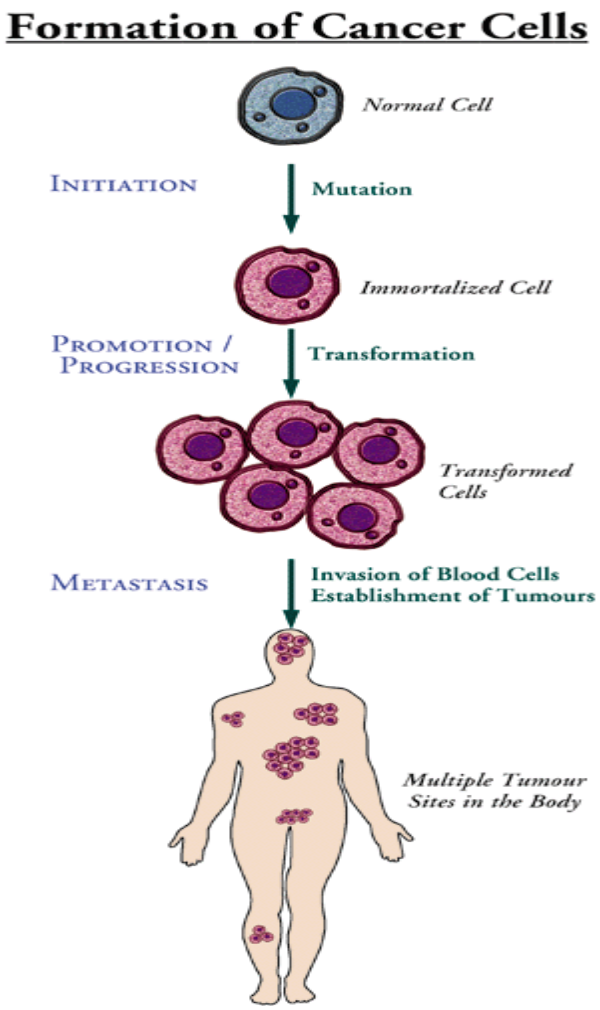

Figure 1. Initiation, promotion and progression of cancer. Adopted from Brudnak (2000).

Six characteristics are evident in abnormal cancer cells. Firstly, cancer cells exhibit self-sufficiency by mimicking growth factors. Normal cell cycle progression is mediated by extracellular stimulatory signals for a cell to proliferate, which are often produced by other cells. In cancer cells, oncogenes often mimic these growth factors such as in the case of glioblastomas and sarcoma and this allows cancer cells to replicate independent of mitogenic extracellular signals. In other examples such as breast cancer, the overexpression of a cell surface receptor HER2/neu on cell surface transduces growth signals into cells, promoting proliferation (Lam et al., 2008).

Secondly, cancerous cells exhibit unrestrained replicative potential. Normal cells have an intrinsic inbuilt mechanism of tracking cell generations, which has direct implications in cellular DNA stability because, that determines when a cell undergoes apoptosis (Rampazzo et al., 2010). This intrinsic mechanism uses the number of telomere repeats at the end of chromosomes, which become worn during successive replications (Kelland, 2007). However, cancerous cells mutate their genes in order to up-regulate the telomerase enzyme and thereby obtaining unrestrained replicative power (Lebrun, 2012). Thirdly, initiating cancerous cells also display callousness towards response to anti-growth factors, such as in the case of loss of the transforming growth factor β (Meulmeester and Dijike, 2011) which plays a role in controlling cellular proliferation, differentiation, apoptosis, adhesion, and invasion among others. Antigrowth factors can regulate progression of the cell cycle by two mechanisms: forcing cells into the G0 stage of quiescence or inducing cell differentiation. These

signals are received and processed *via* the retinoblastoma (RB) pathway mostly. However, in about 15 % of all breast cancer cases, mutations in the RB gene have been found, which results in disruption of the RB pathway when the gene is over-expressed (Aderonke *et al.,* 2013). The RB pathway is regulated by three additional factors: transforming growth factor β (TGF-β), mothers against decapentaplegic homology 4 (SMAD4) and cyclin-dependent kinase (CDK) inhibitors. Loss of these, besides direct loss of RB may lead to malignant transformation.

Abnormal/ cancerous cells display phenotypes which offer them an advantage in order to survive. Firstly, cancerous cells oppose the mechanism of apoptosis, as mentioned earlier, *via* different resistance contrivances. Resistance to apoptosis signals stems in loss of the tumor suppressor protein 53 (p53) or activation of anti-apoptotic B-cell lymphoma 2 (Bcl2) signal (Thomas et al., 2013). Additional characteristics which enable the survival and proliferation of cancer cells include angiogenesis as well as tissue invasion and metastasis. Angiogenesis is the establishment of fine vascular networks to supply the developing cancerous mass of cells with oxygen and nutrients (Shokrzadeh and Saravi, 2010) as well as provide an avenue for metastasis (Wei et al., 2011). Lastly, loss of contact inhibition, loss of adhesiveness, anchorage independent growth precedes spread of abnormal cells from the primary site to other distant secondary body sites through the vascular system, which is directed by changes in the glycans in the cell membrane (Fujii et al., 2010). Proteases such as threonine, serine, aspartate, cysteine and metalloproteases aid in the initiation as well as metastasis of cancer (Rakashanda et al., 2012).

2.2. Endogenous factors contributing to cancer

2.2.1. Free radicals

During normal cell metabolism, reactive oxygen species, such as hydroxyl radicals, carbon, nitrogen reactive compounds, hydrogen peroxide, superoxide radicals and other singlet oxygen atoms form as a normal by-product of oxygen metabolism (Min and Ebeler, 2008), having both advantageous and disadvantageous effects (Lone et al., 2013). Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) have the tendency to accumulate within living systems, originating from the mitochondria (Bernard and Olayinka 2010; Sedelnikova et al., 2010). These free radicals are charged molecules which are unstable, due to the electrical charge that they carry. In the presence of free radicals, molecules within cells such as DNA and proteins, polysaturated fatty acids sustain damage causing changes in genetic expression and consequently affect cellular metabolism (Bernard and Olayinka, 2010). Proteins and polysaturated fatty acids found in cell membranes can undergo oxidative damage resulting in loss of membrane integrity and enzyme function loss (Venditti et al., 2013). Despite the presence of endogenous antioxidants, reactive oxygen species can still accumulate within cells. Continued oxidative damage to genetic material such as DNA, and lipids and cellular proteins has been shown to lead to the development of cancer and other diseases such as gastrointestinal inflammation, liver disease, asthma, cardiovascular disease, cataract, diabetes and other general inflammation related illnesses (Thambiraj et al., 2012).

2.2.2. Exogenous factors causing cancer

There are external factors which play a role in the development of cancer and they can be classified as physical, biological or chemical carcinogens. Unhealthy lifestyle choices such as poor diet, smoking, lack of exercise and other bad life style habits have been shown to predispose individuals to cancer (Trovato, 2012).

Exposure to harmful ultraviolet rays from the sun is responsible for the increased incidences of diagnosed melanomas and an estimated new cases, 132 600 were reported worldwide in 2000 (Parkin et al., 2001). Depletion of the ozone layer has played a part in increased UV infiltration into the earth's atmosphere, (Prasad et al., 2010). Ultraviolet radiation, especially UVB rays are very harmful as they penetrate the skin, causing several effects such as thickening of skin, accumulation of melanin for longer periods, resulting in cancer (Akaydin, 2010).

Cancer can also be a result of viruses acting as carcinogens. This is because viruses insert their genetic material into the host's genome at random places during replication, which can contribute to defective genes. Kremsdorf et al, (2006) has shown a correlation between infection with the Hepatitis B virus and the development of hepatocellular carcinoma. Cervical cancer is among the leading causes of morbidity for women worldwide and its development has been closely linked to mucosatropic Human papilloma viruses (HPV), (Bosch et al., 2002). HPV expresses an E6 protein upon infection, which mediates ubiquitination of p53, subsequently targeting p53 for proteasome degradation. Bacteria also play a role in the development of particular

human cancers. *Salmonella enteritidis* has been implicated in the development of hepatocellular carcinoma due to their production of lipopolysaccharides (LPS) (Rastegar et al., 2011). *Clostriduim perfringes, Streptococcus bovis and Bacteriodes fragilis* have also been associated with malignant transformation in the development of colon cancer (Rahimkhani et al., 2010) whilst *Heliobacter pylori* (Marie and Lory, 2012) and Schistosoma species (Deribew and Petros, 2013) have been shown to be involved in causing stomach and bladder cancer respectively.

The multi-step induction of cancer, beginning with initiation, followed by promotion and progression of damage, leading to masses of abnormal cells has seen the involvement of chemical carcinogens (Klaunig et al., 2011). To date, the prevalence of cancers such as colon, esophageal, stomach, colon, liver and prostate cancers have been among leading causes of cancer morbidity worldwide (Jemal et al., 2011). Farombi (2004) and Zaki et al. (2012) discusses some toxins that are found in animal and plant products of which humans use as food. Mycotoxins such as aflatoxins, present considerable health risks such as hepatoxicity, liver cancer, Reye's syndrome, kwashiorkor (Atanda et al., 2011), and originate from fungi such as *Aspergillus flavus* and *Aspergillus parasiticus* (Eaton and Gallagher, 1994). Fumonisin toxins are commonly found in fields of maize, a staple crop in most African countries. Alone Fusarium species: *Fusarium verticillioides, fusarium prolifratume, Fusarium nygamai and Aternaria alternaria* (Mohanlall et al., 2013) produce about 100 toxins and these pose substantial dangers in initiating cancer. A particular fumonisin, FB1 has been found to have a positive correlation with the development human esophageal cancer as

well as liver damage (Bokhari and Aly, 2013). Ochratoxins were first discovered from *Aspergillus ochraceus* in 1965. Lately, Ochratoxin A has been isolated from other molds from the penicillium species such as *P. verrucosum* and *P. nordicum* (Mboya et al., 2011) and has also been found on medicinal and herbal plants in Egypt contaminated with *Aspergillus niger* (Allam et al., 2012). Another carcinogenic risk is presented by polycyclic aromatic hydrocarbons, which are found in soot, tar and untreated mineral oils (Mastrangelo et al., 1996) and these accumulate in the atmosphere, rivers, oceans and soil, which end up in the food chain and in processed foods (Ana et al., 2010). In addition, increased industrialisation worldwide with factories producing chemicals which are also carcinogenic contribute to this scourge. Individuals that come into contact with these chemicals during work (in what is termed as occupational exposure) and those who find it in their environment are at repeated risk (Anetor et al., 2008).

2.3. Prevalence of cancers in the developing world

Cancer remains and continues to be a fatal disease in most populations around the world and continues to cause significant morbidity (Kummalue et al., 2012; Wong et al., 2011). Jemal et al. (2011) reported an estimated 7.6 million cancer resulting deaths and 12.7 million new cancer incidences in 2008 alone. Incidences of cancers such as lung/ bronchus cancer and breast cancer continue to dominate as the highest cause of both morbidity and mortality in male and female populations worldwide (Jemal et al., 2011).

The cancer burden remains heavy in the developing world as compared to developed nations, with 56% new cases and 64% deaths estimated (Jemal et al., 2011) being reported in developing countries. Cancer causes a higher proportion of mortality worldwide in comparison to HIV/AIDS, tuberculosis, malaria, with ranking 2, 8,9 and 12 respectively (American Cancer Society, 2011).

In both the developed and developing countries, cardiovascular disease and cancer were cited as the most common cause of mortality in older women (Stevens et al., 2013). In developing countries, breast, lung/bronchus, stomach, cervix uteri and liver cancers where the top five carcinoma by site (Jemal et al., 2011). According to Kavanos (2006), breast cancer accounted for the highest cancer incidence in both the developed and developing world, figure 2. Breast cancer continues to be the foremost prevalent cancer in females, causing both morbidity and mortality (Parkin et al., 2005) and has continued to increase with over 1.4 million incidences and 458 400 deaths reported in 2008 (Jemal et al., 2011). In developing countries, factors such as increased life expentancy, dietary changes, lack of physical activity, late age of first child conception, absent breast awareness programmes and screening opportunities and others are indicated as contributing to the high breast cancer incidences (Al-Foheidi et al., 2013).

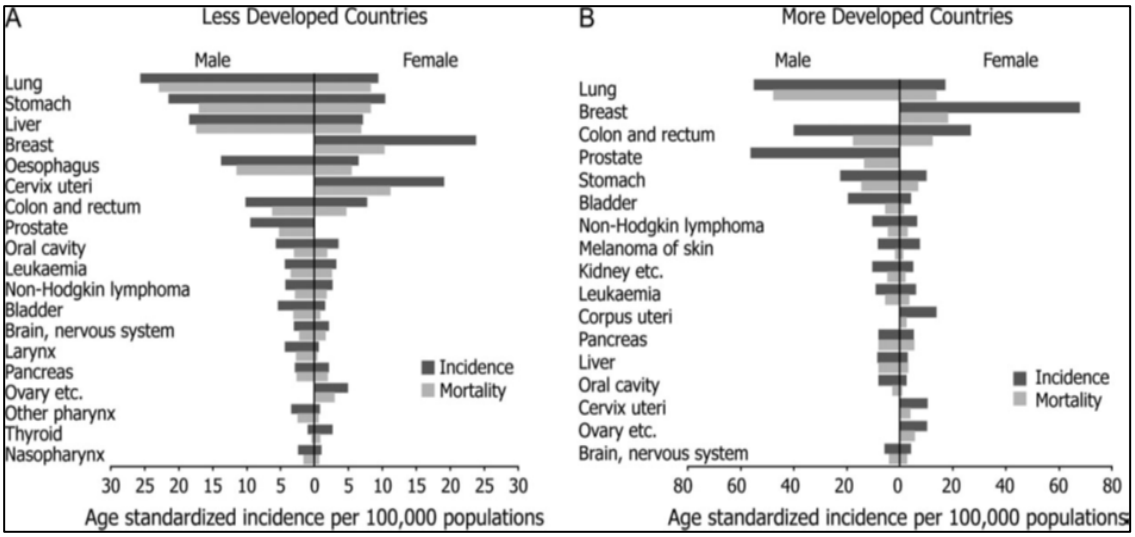

Figure 2. The proportion of different cancers in the developing countries,(A) and developed countries (B), adopted from Kavanos (2006).

The most common types of lung cancers are non-small cell lung cancer and small-cell lung cancer (Zang et al., 2011). Jemal et al. (2011), reported lung and bronchus associated cancers being the most frequently diagnosed cancers and indeed being the leading cause of cancer mortality in males while it was the fourth cause of cancer morbidity in females. Stomach cancer was among the top five most common causes of morbidity and mortality in both sexes in 2008 (Jemal et al., 2011). According to Jemal et al. (2011), stomach cancer incidences were more observed in the developing countries and the burden was more felt by the male population. Infection with *Heliobacter pylori*, has been implicated as a major risk factor for the development of gastric cancer (Crew and Neugut, 2006). Cervical cancer affects over 493 000 women worldwide, each year and 4/5 proportions of these are diagnosed to women from developing countries (Yumin et al., 2012). According to Saonere (2010) about 5% of all

cervical cancers were diagnosed to pregnant women, of which 1/3 were under the age of 35, globally. Cervical cancer has been linked irrevocably to infections with the Human papillomavirus (HPV), a common infection transmitted sexually (Schluterman et al., 2013). Hepatocellular carcinoma and intrahepatic cholangiocarcinoma are the most common cancers of the liver (Yeap et al., 2012), with the former accounting for over 70% of all liver cancers (Jemal et al., 2011). According to Machana et al. (2012), about 90% of all liver cancers are hepatocellular while, 5% are intrahepatic cholangiocarcinoma.

2.4. Treatment options and mode of action

2.4.1. Chemical therapy

Sites onto which cancerous cells develop influence the treatment option. Usually the placement of the tumor or cancer eliminates treatment options such as radiation and surgery, leaving chemotherapy as the only alternative (Ganesan and Muthuchelian, 2011). Due to poor recovery rates of patients treated with radiotherapy and surgery during the early periods of the 20th century, there was a need for better efficacious options (Tohme et al., 2011). This gave rise to the use of chemotherapeutic drugs such as paclitaxel, vinflunine, ixabepilone, erubin, larotaxel and trabectin for treatment of different cancers. Chemotherapeutic agents mostly exert anticancer action by targeting the cell cycle at mitosis (Hashemi et al., 2011), with three main modes of action. Firstly, cytostatic drugs which interfer with DNA synthesis. This is achieved through different

mechanisms such as alkylating the genetic template, DNA. Alkylation of DNA strands makes it difficult for the DNA polymerase to read the genetic information thus prevents multiplication of faulty cells. Examples of drugs that help fight cancer in this manner are procarbazine, busulfan, cyclophosphamide, melphan and ifosfamide to mention a few (Yamaguch and Fujisawa, 2011).

Secondly, chemotherapeutic agents which cause DNA abberations, such as cisplatin, bind DNA but do not alkylate DNA strands. This class of cytostatics may have side effects such as irreversible reproductive toxicity, hepatotoxicity and renal damage (Beytur et al., 2012). Cytostatic antibiotics insert themselves into DNA strands and inhibit transcription of DNA. Examples are mithramycin and chromomycin, which are isolated from *Streptomyces plicatus* (Devi et al., 2009).

And thirdly, cytostatics which interfer with the mitotic spindle apparatus. The proteins of the spindle apparatus form part of the chromosomes before cell division can occur. However, some cytostatics act at this point by preventing the formation/assembly of the microtubule apparatus, leading to mitotic cell arrest at the metaphase stage. Examples of such drugs are the vinca alkaloids, vincristine and vinblastine, derived from the Madagascar periwinkle plant, *Catharanthus roseus* (Nirmala et al., 2011). The discovery and development of antineoplastic drugs has advanced cancer treatment and helped reduce morbidity as result of cancer worldwide. For instance, the discovery of camptothecin and taxol from *Camptotheca acuminate* and *Taxus brevifolia* respectively (Wall and Wani, 1995).

Adverse effects of chemotherapy include the following but differ from one individual to another. Firstly, loss of hair ocurs due to damage of hair follicles, nausea and vomiting caused by the stimulation of chemoreceptors found in the postrema area. Diarrhea and other gastrointestinal disturbances resulting from inadequate replacement of enterocytes are additional side effects of chemotherapy. Furthermore, patients undergoing treatment with cytostatics are more prone to different infections due to the weakening of the immune system. Cytostatics also cause bone marrow depression, leading to a condition called anemia, through a step by step process, involving arrest of mitotic division of myeloid cell, leading to granulocyte proliferation arrest and lastly the supply of erythrocytes is affected. Another adverse effect of chemotherapy involves the reproductive systems as maturation of sperm and follicles is impaired. Brilhante et al. (2011) discussed the effect of doxorubicin, which causes germ cell depletion, tubular vacuolization, formation of multinucleated spermatids and germ cell showing apoptotic signs. Despite the success of cancer therapy using chemotherapy, a problem still persists in the form of developing resistance to chemotherapeutic agents, limiting treatment options (Tao et al., 2011).

2.4.2. Radiotherapy

Radiation therapy for the treatment of cancer uses high-energy radiation to kill off cancerous cells, however, treatment often does not discriminate between normal and cancer cells. The treatment employs the use of X-rays, gamma rays and charged

particles to cause damage in cellular DNA and eventually force cell apoptosis. Advances in radiotherapy have lead to various ways of delivering the radiation to the tumor site, which can be achieved by directing the radiation from an external machine onto the tumor site or by injecting the radioactive material into the blood circulation system. Due to the indiscriminating nature of radiation therapy, side effects such as loss of nerve function occur, impotency and incontinence (Fransson et al., 2001) side effects differ depending on the tissue receiving radiotherapy and other symstems associated with it. In fact, the nature of the cancer sometimes limits treatment options. For instance, non-small cell lung cancer patients with locally advanced disease have limited response to radiation therapy (Marnitz et al., 2002). Also, choice of therapy and subsequent dosage of radiation is restricted by the siting of cancerous tissue to closeness of radio-sensitive tissues such as brain, spinal cord and parotid gland (González-Arriagada et al., 2013). In other instances, a combination of radiation and chemotherapy is given to effectively achieve the goal of cancer therapy (Shapiro and Recht, 2001).

2.4.3. Surgery

Surgery is another option for cancer treatment, which involves removal of the cancerous tumor by means of surgery. Depending on the position of the cancerous tissue, surgery is the treatment of choice. In rectal cancer, surgery is the first treatment option (Peeters et al., 2005).

However, surgery as a mode of treatment of cancer has its advantages as well. For instance patients diagnosed with metastatic prostate cancer have a low survival rate, 18-20 months after surgical treatment/medical castration, at which point the tumor becomes androgen-independent, complicating the condition since no chemotherapeutic agent offers better response against androgen- independent prostate cancer (Petrylak et al., 2004). According to González-Arriagada (2013), some side effects arising from surgical treatment of head and neck cancers include damage to speech, taste, smell, feeding functionalities of patients.

2.4.4. Palliative care

Palliative care involves different disciplines and is solely focused on providing holistic care and support to individuals (patients) and their relatives who are facing life threatening illnesses. Palliative care, unlike hospice begins at diagnosis and continues all throughout duration of illness to the very end of life. It addresses the physical, sociocultural, psychological and spiritual aspects that come with receiving news of a life threatening disease, such as cancer (Maciasz et al., 2013). Palliative care is typically offered to a patient by a specialist who works directly with the patient and the specialist is usually assisted with a team of other medical professionals such as nurses, physicians, pharmacists, social workers and pastors, who provide comfort to aid patients, such as those diagnosed with cancer, live better with the disease while making the appropriate decisions towards treatment (Jung et al., 2013).

Forerunners of modern day palliative care engineered the holistic approach in the developed countries but presently, health care providers are seeking expansion of this service, even in developing countries (Harding, 2008). The need for appropriate palliative care is great and yet, to date, this essential service is still illusive to attain. In many developing countries, the burden of non communicable diseases such as cancer (Opare et al., 2013), infectious and the HIV/AIDS pandemic are on the rise, further necessitates the need for affordable, culturally acceptable palliative as the majority of individuals are stricken from a vast range of life threatening illnesses (Powell et al., 2011).

With regards to cancer management, Kris et al. (1987) stressed the importance and need of anticancer supportive treatments to be administered to patients undergoing chemotherapy, especially to control nausea and vomiting.

Various interventions have been adopted for the effective palliation of cancer, (Ernst, 2001). Petrylak et al. (2004) advised on the use of docetaxel in combination with estramustine for palliation of androgen independent prostate cancer, as compared to an earlier approach, a combination of prednisone and mitoxantrone.

According to Yan et al. (2009), cancer patients facing palliative care have turned to traditional medicines such as Traditional Chinese medicine, where specific formulations have been developed for cancer sufferers. Lee *et al.* (2000), found that women who had been using alternative therapies to supplement their treatment when suffering from breast cancer found the therapies helpful and would recommend therapies such as

acupuncture, spiritual healing, herbal portions, dietary, physical therapy to others. Patel et al. (2010) concurred that phytochemicals contained in herbal medicinal plants may be a viable option in symptom management during chemotherapy.

2.5. Use of plants as traditional medicines

The use of plant-derived entities for medicinal purposes has been in existence for many centuries and has different earliest records among different cultures: The Chinese Traditional medicine has its earliest record of use as far back as 2500 BC (Xun-Li, 2013), and continues to be a reliable resource for the discovery and development of effective medicines (Farombi, 2003; Wang and Lee, 1997). Instincts and experience guided early humans in the use of plant components, minerals and animal products for alleviation of various ailments (Folashade et al., 2012) such that this history has led to the acceptance of these naturally derived implements in the modern society as safe alternatives for maintenance of good health. Different communities retain a wide array of plants used traditionally for disease treatment. Up to 80 % of Africa's population have been reported to depend on traditional medicinal plants to meet their primary health care needs (Elujoba et al., 2005). Chinese Traditional Medicine is a well-documented traditional system, which has received considerable scientific-based support. Ayurveda, from India is another folklore practice, which is well established in various societies but lacks scientific research and supporting evidence, to permit equal competing footage in international markets (Patwardhan et al., 2005). Several other

traditional practices such as siddha, kampo, unani, acupuncture, African and South American traditional medicinal systems exist. Pandey et al. (2011) lists several drugs currently in mainstream usage and their initial folklore origin. These include well-known drugs such as codeine, ephedrine, emetine, digoxin and quinine, which were initially reported among Sumarians, Chinese, South Americans, Europeans and Indians respectively.

Efforts on the drug discovery road map have been met with several difficulties. For instance, in an attempt to synthesize drug alternatives using templates derived from nature has sometimes proven difficult, owing to the chemical complexity of the template compound structure. In some instances, acquiring enough plant materials from nature has posed difficulties, further impending research progress (Nikolic et al., 2011).

2.5.1. Use of traditional plants for treatment of cancer

Some medicinal traditional plants have found use in the prevention and treatment of cancer as antioxidants, which are molecules that terminate the cascading reactions initiated by free radicals by being oxidized themselves (Hamid et al., 2010). Many compounds from nature have been implicated in their effect of retarding, reversing or delaying the multistep process of cancer development (Farombi and Owoeye 2011). In recent years, interest has increased in the search of natural sources of antioxidants, especially in medicinal plants and plants used as food (Boubaker et al., 2011), which would be promising candidates for prevention of oxidative stress resulting illnesses.

Compounds which possess antioxidant or radical scavenging potential are termed chemopreventative, and numerous studies have demonstrated antioxidant properties of plant products (Latha et al., 2012; Hodzic et al., 2009) which can imply potential anticancer therapeutic properties (Jing et al., 2010).

Proteolytic activity is one important aspect of biological systems, which involves endogenous proteases. However, endogenous proteases have been implicated in the initiation and progression of cancer. This warrants the need for treatment options whose mode of actition is protease inhibition activity. Root extracts of a plant called *Zanthoxyllum zanthoxyloides* were found as an active antiprotease which can be used in management of sickle cell disease (Imaga, 2010). In the case of HIV, plant extracts from *Crotalaria pallida* were found much more effective as HIV antiproteases as compared to a known antiprotease, Pepstatin A (Govindappa et al., 2011). Tuber extracts of *Amorphaphallus paeonifolius,* possess antitumor, antioxidant and protease inhibition activity while possessing phytochemicals such as alkaloids, flavonoids steroids, carbohydrates, tannins and proteins (De et al., 2010).

2.5.2. Phytochemicals

Phytochemicals are non-nutritive, secondary metabolites of plant origin (Doughari et al., 2009). Phytochemicals have generated interest due to the pharmacological properties that they lend plant extracts; antibacterial, antioxidant, antidiabetic, anticancer, antiallergic, anti-inflammatory and chemopreventative properties

(Ramkumar et al., 2007). Examples of phytochemicals includes alkaloids, glycosides, phenols, tannins, coumarins, flavonoids, terpenes and many more (Okigbo et al., 2009). Phenolic plant compounds encompass a wide variety of plant secondary metabolites (Harborne, 1998) which are characterized by one or more aromatic rings, to which one or more hydroxyl structures are attached (Shukla and Dwivedi, 2013). Examples of phenolic acids includes flavonoids, coumarins, anthraquinones, xanthonoids, polyphenols, derived from medicinal plants have been shown to confer chemopreventative properties in the prevention of cancer (Amusan et al., 2007; Huang et al., 2009).

Biological properties of phenolics are diverse (Ojieh et al., 2013) and include radical scavenging/antioxidant activity (Hodzic et al., 2009), anti-inflammation (Oskoueian et al., 2011), anti-proliferation/ anticancer (Neeraj et al., 2011), and anti-angiogenesis (Badrhadad et al., 2012). Several plant derived anticancer compounds and their derivatives worth mentioning are discussed (Wang and Lee, 1997; Nirmala et al., 2011). Okigbo et al. (2009) discussed some advances in medicinal and aromatic plants native to the African continent.

2.5.2.1. Phenolics: Flavonoids, coumarins and anthraquinones.

Flavonoids have made considerable contributions in laboratory research as anticancer agents over different cancer models. Eupatilin, isolated from the antimalaria plant,

Artemisia argyi (Rasul and Ma, 2012) has been found potent in causing arrest of melanoma cells in the G2/M phase and subsequent apoptosis (Shawi et al., 2011).

Flavone-8-acetic acid, a synthetic compound derived from the basic flavonoid structure was extensively assayed in phase 1 clinical trials during the 1990's, in independent studies on different continents against malignant melanoma but was found to be potentially cytotoxic and not potent in humans (Krestzschmann and Furst, 2013). A more potent derivative was developed, 5,6-Dimethylxanthenone-4-acetic acid, whose mode of action was the disruption of already established vascular vessels supplying large tumors (Krestzschmann and Furst, 2013). In another report, Wang et al. (2012), the anticancer properties of various flavonoids are discussed, as well as their derivatives. Figure 3 shows structural examples of flavonoids, coumarins and anthraquinones.

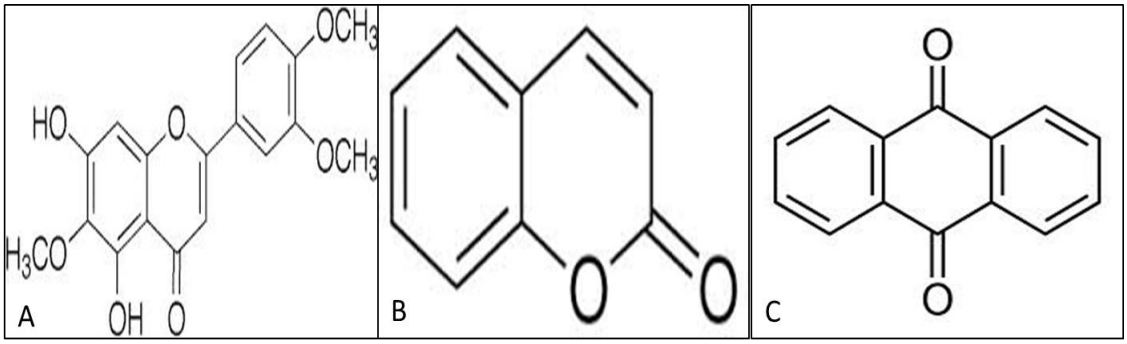

Figure 3. A) A Flavonoid: Eupatilin, B) a coumarin, C) an Anthraquinone structure.

2.5.2.2. Alkaloids

A compound, isolated originally from *Camptotheca acuminate* (Nyssaceae), camptothecin is an alkaloid (Pasqua et al., 2004). The parent molecule, which consists of a pentacyclic ring structure, is flanked by two hexacyclic moieties, one of which is a pyridine ring and can be isolated from other plant families; Apocynaceae, Olacaceae and Rubiaceae (Agarwal et al., 2012). Camptothecin displayed broad activity against a range of tumor models, but demonstrated poor solubility and severe toxicity (Nirmala et al., 2011). Subsequent studies resulted in the development of an analogue, camptothecin sodium and several others such as irinotecan, topotecan, 9-aminocamptothecin, 10-hydroxycamptothecin (Nirmala et al., 2011). The development of these analogues counteracted a disadvantage of the parent molecule, camptothecin. Camptothecin sodium was more water soluble, enabling its advancement in clinical trials (Wall and Wani, 1995). Camptothecin's anticancer activity is largely due to the structure of the molecule, the s-configured lactone and its carboxylic form which exerts inhibition of DNA topoisomerase I activity (Nirmala et al., 2011).

Vinca alkaloids were discovered and isolated in 1958 accidently from a plant known as the Madagascar periwinkle, *Catharanthus roseus* (Apocynaceae) (Sharma et al., 2011). Initially, vinblastine and vincristine were the two major isolated compounds and to date, several other isolates exist. In fact, vincristine and vinblastine have been used as templates for the semi synthesis of newer compounds such as vinorelbine and vindesine (Nirmala et al., 2011)

Homoharringtonine is an alkaloid drug from the cephalotaxanes drug class, originally isolated from a plant called *Cephalotaxus harringtonia* (Bhanot et al., 2011). Since then, harringtonine and homoharringtonine have been isolated from other species of Cephalotaxus, such as *C. hainanensis* and *C. qinensis* and are in clinical use against acute and chronic myeloid leukemia due to their protein synthesis and chain elongation inhibition activities during translation (Nirmala et al., 2011).

The alkaloid colchicine, figure 4, was extracted from *Colchicum autamnale*, an important herbal component of Unani practice. The plant has since been used for the alleviation of ailments such as gonorrhea, prostate enlargement, gout, rheumatism and cancer (Akram et al., 2012). Its mechanism of action involves inhibiting the assembly of microtubule (Maheshwari et al., 2008) but its toxicity has prevented its clinical use (Kupper et al., 2010) and is not approved by the Food and Drug Administration (FDA) despite it current use for treatment (Nasr et al., 2011). However, several derivatives such as colchicinamide, have been developed which showed improved activity in comparison to colchicine (Wang and Lee, 1997) with applications for treatment of solid tumors such as breast cancer (Wang and Lee, 1997) and leukemia (Nirmala et al., 2011).

Ellipticine and its analogues were isolated originally from *Ochrosia elliptica* (Farombi 2003) and there has been evidence that its antitumor activity stems from its ability to intercalate with DNA (Lamani et al., 2010).

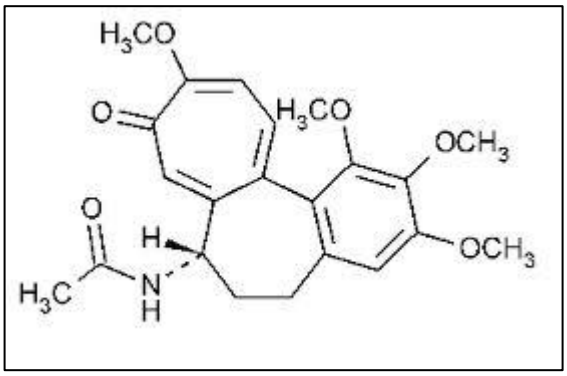

Figure 4.Colchicine, an example of an alkaloid.

2.5.2.3. Triterpenes

Triterpenes are a subclass of a group called terpenes, which is inclusive of hemiterpenes, monoterpenes, sesquiterpenes, diterpenes, tetraterpenes, sesterterpenes and polyterpenes. More commonly, terpenes are known as turpentine or resin gum. Within the plant kingdom, terpenes and its members act as deterrents to herbivores (Paduch et al., 2007) and this contributes to the application of terpenoids in medicine. Terpenes are also responsible for the bright colours, pleasant smells, and spicy taste of members of the plant kingdom. Triterpenes have been shown to confer a range of pharmacological properties which has lead to investigations of resinous plant material such as frankincense, figure 5, for anti-inflammatory, asthma, bowel disease, brain tumors and others pharmacological activity (Zhang et al., 2013). Triterpenes with anticancer activity are not only found in plant sources but have also been found in a traditional mushroom called *Ganoderma lucidum* (Chan et al., 2008).

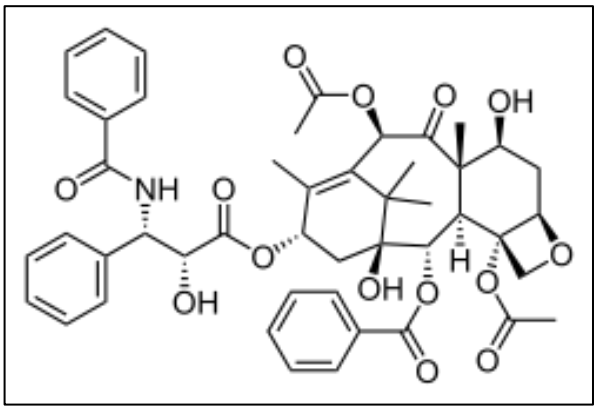

Figure 5. Structure of frankincense, a triterpenen.

2.5.3. Plant derived drugs under development

Butler (2005) outlines a number of plant derived anticancer compounds in various stages of the drug development pipeline. These drugs, (Butler, 2005) target cellular machinery such as topoisomerase I and II, tubulin binding and stabilization, caspase 3, 8, 9, protein kinases C activation, T-cell anti-proliferation, protein synthesis inhibition, expression and activation of NFκ-β activation. These drugs are derived from well-known anticancer drugs already in clinical use such as camphothecin, epipodophyllotoxin, combretastatin, homoharrington, ingenol, daidzein, paclitaxel, protopanaxadiol, vinblastine and triptolide (Butler 2005). In another report, anticancer compounds, alkaloids in nature, such as berberine, matrine, evodiamine, piperine, tetrandrine and sanguinarine are discussed and their antineoplastic properties (Lu et al., 2012). Several other mechanisms are in the pipeline for improvements of current and future anticancer drugs, which are often insoluble, lacking delivery specificity and causing side effects. Nanoparticles are already receiving considerable attention in the

drug delivery system arena, utilizing such materials as folate (Shakeri-Zadeh et al., 2010), lipids (Jahanshahi and Babaei, 2008) or methoxy poly (Ding et al., 2011). Some drugs in clinical trials and clinical use are available, fashioned using nanoparticles as a drug delivery system (Matsumura and Kataoka, 2009). Doxorubicin, has been fashioned in several different materials such as magnetic (Aljarrah et al., 2012), barium titanate (Ciofani et al., 2010), poly-2-hydroxyethyl methacrylate (Chouhan and Bajpai, 2009) to enhance the treatment of multidrug resistant breast cancer.

2.6. Cancer statistics in Namibia and risk factors

The incidences of cancer in Namibia have been shown to be on the rise. Cases such as skin, Kaposi sarcoma, breast, prostate and cervix cancer have consistently been among the top five cancer types, as confirmed by histologically proven cases, data courtesy of the Namibian cancer association. Figure 6 depicts the general increment in cancer cases since 2005. According to the Namibian Cancer Registry (2009), a total of 4949 neoplasmas were reported over a period of six years while the Namibian Cancer Registry (2011), reported an increased cancer incidence of about 6363 cases during a four-year period. This shows an alarming rate of cancer incidences. Furthermore, the Namibian Cancer Registry (2011) reported the cancer incidence rate as 142.3 per 100 000 and 109.3 per 100 000 in males and females respectively. The three most common cancers per gender were breast, cervix and Kaposi sarcoma for females , while Kaposi

sarcoma, prostate and mouth cancer were more commonly diagnosed in males (Namibian Cancer Registry, 2011).

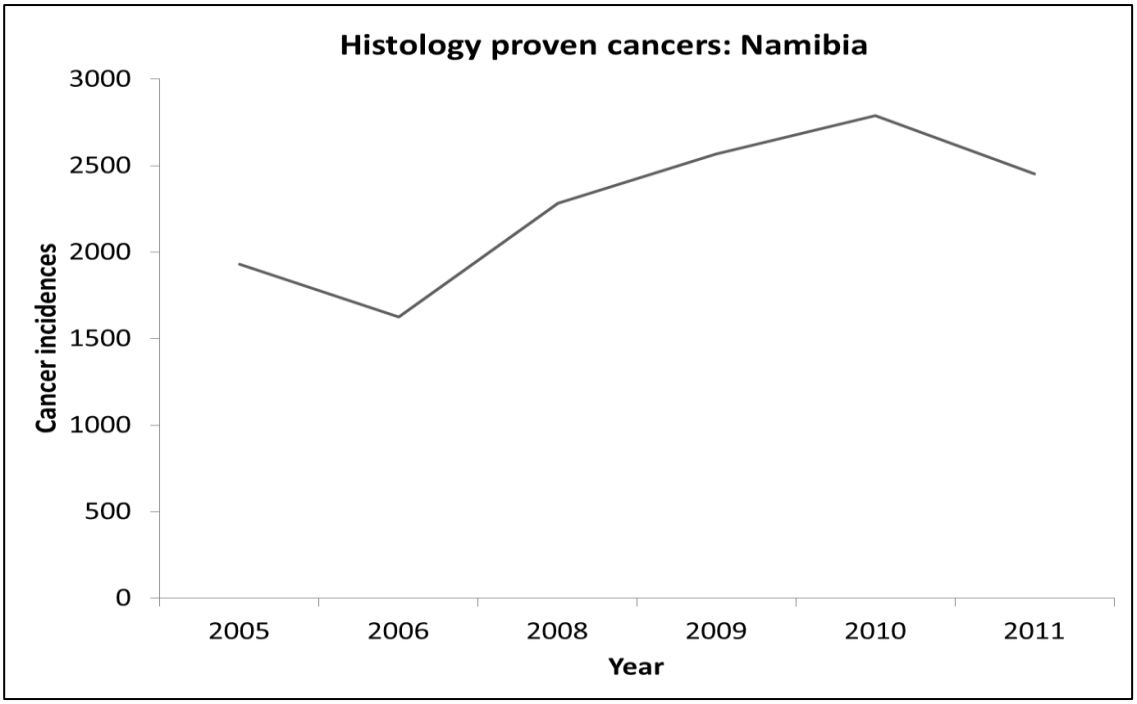

Figure 6.Trend of histologically proven cancer incidences in Namibia. Courtesy of Cancer Association of Namibia.

According to Calitz (2013), the Cancer Association of Namibia (CAN) concurred that breast cancer in Namibia is diagnosed to women over the age of forty, further confirming that the risk of developing breast cancer increases with age. Breast cancer risk factors within the Namibian population, according to Calitz (2013) include family history, excessive alcohol use, obesity, in addition to age. Additional studies have confirmed the increased risk of developing breast cancer with obesity and hormonal serum content (Endogenous hormones and breast cancer collaborative group, 2003).

An important cause of certain kinds of cancers such as lung carcinoma is use of tobacco (Iwalewa et al., 2007). Tobacco will become increasingly popular in developing nations in years to come (Owusu-Dabo *et al.*, 2011). Research findings have also implicated tobacco in the development of cancers such as cervical cancer (Bosch et al., 2002). According to Saonere (2010), females who smoke were twice more likely to suffer cervical cancer as compared to non-smokers, also longer exposure to tobacco smoke increased the risk even more. Yeap et al, (2012) also indicated tobacco smoking as a factor in the development of hepatocellular cancer. Oropharyngeal cancer develops as well when individuals partake of tobacco as snuff or even chewing (smokeless tobacco) (Lee and Hamling, 2009).

Alcohol abuse is rampant within the Namibian population. A recent media report, Kaira (2013), reported on the escalating alcohol consumption within the Namibian population, while another article, Nickols et al. (2012), drew a connection between density of drinking establishments/ alcoholic consumption and elevated HIV incidences in Namibia. Alcohol consumption not only has implications for risky behavior but also liver, breast, esophagus, ovarian, prostate, lung, coleractal and other forms of cancer (Everatt et al., 2013; Toriola et al., 2008).

Namibia has a high HIV prevalence rate, estimated at 15.3% and among the highest in the whole world (de Beer et al., 2012). Despite HIV incidences showing a decline over the past few years (Ministry of Health and Social Services, 2008), HIV/AIDS continues to be the highest cause of death 23%, followed by cancer 8%

(http://www.cdc.gov/globalhealth/countries/namibia/pdf/namibia.pdf). The HIV/AIDS prevalence predisposes much of Namibia's population to the development of HIV associated cancers such as Kaposi sarcoma, non-hodgkin's lymphoma and invasive cervical carcinoma (Crum-Cianflone et al., 2009). Namibian statistics support this, because the Namibian Cancer Registry (2011) cited an increase in HIV associated cancers, including eye cancer. Kaposi sarcoma and Non-Hodgkin lymphoma were among the top seven cancer incidences between 2000-2005 (Namibian Cancer Registry, 2009) and were among the top six diagnosed cancers between 2006-2009 (Namibian Cancer Registry, 2011). Kaposi sarcoma was the leading cancer incidence in males, rating at 22.1% and 10.3% in females. The high HIV/AIDS pandemic also predisposes the Namibia population to infections which are sexually transmitted such as those caused by the Hepatitis viruses (Wild and Montesano, 2009), and Human papillomaviruses (Ribassin-Majed *et al.,* 2012; Huang et al., 2009; Bosch et al., 2002), which have also been known to cause cancer. In addition, Namibia's HIV/AIDS prevalence rate has implications for the conception of children born to HIV/AIDS parents or also those who are carriers of certain cancer-risky microbial infections.

Other risk factors, according to the Nambian Cancer Registry (2009) and (2011), that predispose the Namibian population with the development of cancer are further given, such as human herpes virus, diets low in vegetables, and lack of physical activity, The overall ageing population of the world is a cause of different problems, including cancer. On average, by the year 2020, on average, an individual will live to be about 73 years old as compared to the life expectancy of 66 years in 1997 (Reeler et al., 2008).

Namibia's life expectancy increased from 57.9 years in the year 1980 to 62.6 years in 2012 (Human development report, 2013), meaning that individuals live longer. Namibia's increased life expectancy reflects that it's population is aging, which posses certain health risks such as development of cancer.

2.7. Use of medicinal plants in Namibia

Plants are used in different Namibian communities to alleviate symptoms similar to cancer (Cheikhyoussef et al., 2011; Chisembu et al., 2011). Patients, both originating from rural and urban areas seek the assistance of traditional healers and other knowledge holders for the treatment of cancer-like symptoms. In certain situations, indigenous medicinal preparations are taken together with orthodox medicines. Traditional medicines are popular although there is no scientific support of their safety and efficacy in literature.

2.7.1. Traditional medicinal plants in study

2.7.1.1. *Colophospermum mopane*

Description

Colophospermum mopane is popularly known as mopane. This plant can be found growing as a shrub or a small tree (Van Wyk and Gericke, 2000). It can reach 18 meters in height. Its bark is usually grey in color, with fissures. Its small greenish flower give

rise to a flattened featherly pod copiously dotted with resin glands (Palgrave, 1981). However, its most distinguishing feature is its butterfly-shaped leaf, consisting of two symmetrical leaves, joined by a small appendage in between. In addition, the leaves have a distinct turpentine odour.

Figure 7. Leaves of *Colophospermum mopane*. Source Florence Dushimemaria.

Distribution in Namibia

In Namibia, *C. mopane* inhabits a wide range of areas, figure 8. Its distribution extends from the Kunene river and towards north eastern Namutoni. Small patches of the tree are also found in the Zambezi region and Grootfontein. Other ears such as Kaokoland, Etosha, Owambo, Outjo and Omaruru boost a wide population of *C. mopane*. Grootfontein, , Kunene River towards the Ugab and northeastwards towards Namutoni. All in all, C. mopane covers about 9% of Namibia's surface area.

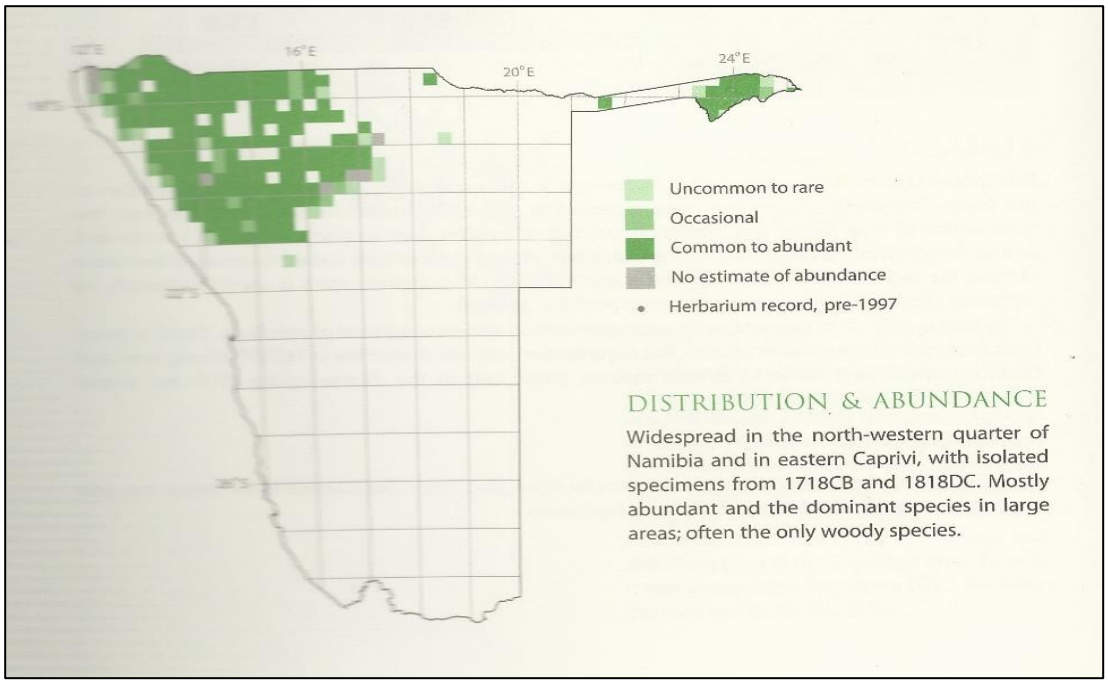

Figure 8. The distribution of *C. mopane* in Namibia. Adapted from Curtis and Mannheimer, 2005.

2.7.1.2. Uses of *C. mopane* within the Namibian traditional context

C. mopane has various applications within the traditional settings of Namibian indigenous people. The mopane tree is consumed by livestock as fodder. Within the Kwanyama people, *C. mopane* is used in ceremonies such as weddings or cleansing from spirits. Gum that exudes from the stem of *C. mopane* is also used for treatments of cuts and wounds. According to Van den Eynden et al, (1992), *C. mopane* is locally known as tsaurahais and is used mainly as a pain relieving decoction prepared from the leaves, on wounds, body parts inflammation. Roots are known to contain tannins and resin. Resin is used to treat wounds, stomach problems, inflamed eye, and syphilis. Leaves are also used as enemas (von Koenen, 2001).

2.7.1.3. *Schinziophyton rautanenii*

Figure 9. *Schinziophyton rautanenii* leaves. Source Florence Dushimemaria

Description

Schinziophyton rautanenii belongs to a family called *Euphorbiaceae*. The tree grows up to 20 meters in height. Its bark is grey to brown in colour, with a tendency to peel. Its tender shoots are covered with soft rusty furry hairs (Palgrave, 1981). Its leaves are palmately compounded with five obovate, margins entire and the leaves are attached to the tree by a slightly reddish stem. *S. rautanenii*'s fruit is egg shaped, hard, woody, light grey to green, which is covered by a velvetish covering.

Distribution of *S. rautanenii.*

S. rautanenii is commonly to uncommonly dispersed in the north-eastern parts of Namibia. Locations such as Owamboland, Grootfontein, Kavango and Grootfontein north

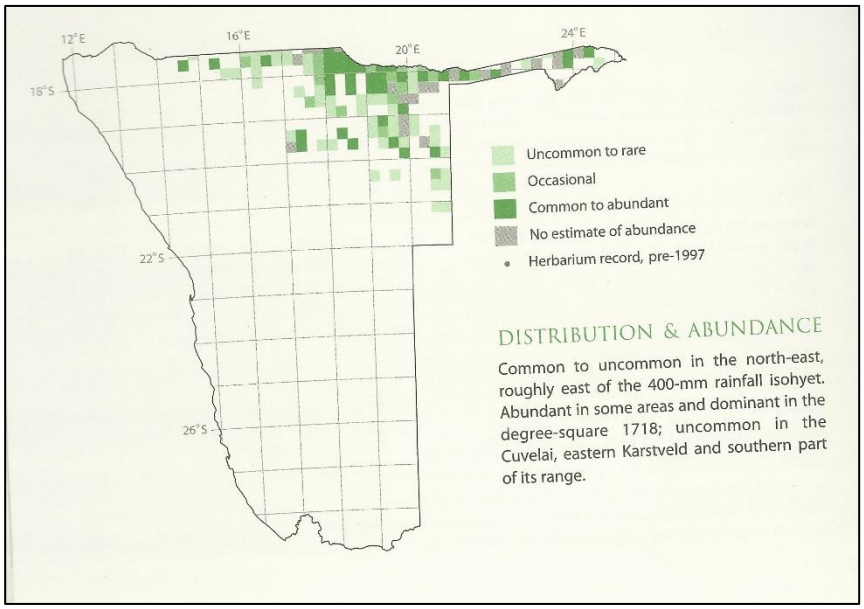

Figure 10. The distribution of *S. rautanenii* in Namibia. Adapted from Curtis and Mannheimer, 2005.

2.7.1.4. Uses of *S. rautanenii* within Namibian traditional context

S. rautanenii also known as *Ricinodendron ratanenii* in some literatures is used as a cosmetic among the Kwanyama people (Rodin, 1985). Enemas prepared from *S. rautanenii* have been known to kill babies (Robin, 1985). The white kernel found inside the nut is extracted for cooking oil, while the red flesh fruit aril is edible (Graz, 2002). Von Koenen (2001) reported only on the use of *S. rautanenii* for food.

Table 1. Traditional uses of C. mopane and S. rautanenii among the natives of the Zambezi region, Namibia.

Scientific name	**Family**	**Local names**	**Uses**
1. S. rautanenii	*Euphorbiaceae*	Mankettii	Sores on body surface
2. C. mopane	*Fabaceae*	Mopane, Omusati	Swollen testis

CHAPTER THREE: MATERIALS AND METHODS

3.1. Research design

This study employed both qualitative and quantitative approaches. Qualitative design yielded information such as preliminary antioxidant and anti-protease activity as well as the presence or absence of a phytochemical in thin layer chromatography. Quantitative approaches resulted in data such as quantification of selected phytochemical compound classes (phenols, alkaloids and saponins), cell vaiability and IC_{50} values, and toxicity investigation using planaria. The study is designed in a manner in which chemical preliminary assays are used as a basis for further biological assays.

3.2. Procedure

3.2.1. Plant collection and processing

Plant specimens of *S. rautanenii* and *C. mopane* were collected from the Zambezi region of Namibia during April 2012 and March 2013. These plants were selected based on a survey conducted in 2010, on the ethnobotanical knowledge of traditional knowledge holders in the Zambezi region, formerly known as the Caprivi region, Namibia, (Du Preez et al., 2011). Voucher specimens, were prepared using a plant press, and deposited with the National Botanical Research Institute (NBRI) in Windhoek, Namibia for scientific validation. Coordinates were taken at sites of plant

collection and these were included into the NBRI voucher collection forms. An axe, pruner and spade were used to obtain plant material for laboratory analysis. Plant parts collected for analysis were roots and bark of both plants, *S. rautanenii* and *C. mopane*. The plant material was then taken to the laboratory and bench top dried at room temperature for a period of two weeks.

Table 2. Subject plants collected and voucher specimen numbers deposited with NBRI.

Scientific name	**Local name**	**Family**	**NBRI Voucher specimen #**
S. rautanenii	Mankettii	*Euphorbiaceae*	FD03
C. mopane	Omusati	*Fabaceae*	FD02

3.2.2. Extraction of phytochemicals

Air dried plant material was ground to powder using an industrial blender, model 37BL85(240CB6) and this was followed by extract preparations. To prepare extracts for biological assays, absolute methanol was used for the organic extracts while distilled water was used for aqueous extracts. Powdered plant material, 10 g, was macerated in 100 ml of the respective solvent. After 7 days, the mixture was filtered using a funnel and 110 mm Whatmann filter paper, employing gravity force. Filtration was followed by rotary evaporation and freeze drying at reduced pressure to remove extraction solvent. Dry extracts were scraped off the round bottom flasks and were then stored at -20°C until further use, in Epperdorf tubes.

3.2.3. Chemical assays

3.2.3.1. Phytochemical profiling

Antioxidant and anti-protease activity

Plant extracts were prepared by maceration, using 2 g plant material and 4ml absolute ethanol (Promark chemicals). The mixture was filtered using a 110 mm Whatmann filter paper and funnel and the filtrate was collected into a glass vial and these extracts were used to determine the antioxidant and antiprotease activities of *Colophospermum mopane* and *Schinziophyton rautanenii*, using the GIBEX screens-to-nature screening kit (Screen-to-Nature manual for Namibia, 2012). In order to determine antioxidant activity, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) (Sigma Aldrich) was used. An aliquote, 200 µl of the ABTS solution was added to each well of a 96 well plate, with appropriate labeling. Further, 10 µl of each plant extract was added to its corresponding labeled well, each plant extract was assayed in triplicate, see figure 9. An aliqoute of 10 µl 60% methanol served as negative control while 10 µl ascorbic acid solution (17.6 mg/ml) was used as positive control. The colorimetric change was observed and graded using (-,+, ++, +++) with (-) being the absence of antioxidant activity while (+++) was very good antioxidant activity. The grading was as follows, (-) = solution remained dark green in color, meaning no antioxidant activity, while (+++)=solution become clear or colorless, meaning high antioxidant activity.

Prepration of the ABTS Solution

The ABTS colorimetric solution was prepared by dissolving 7mg ABTS in 1 ml distilled water, which was kept in the dark by wrapping the container of ABTS solution with foil. An amount of 50 mg of potassium persulfate was dissolved in 1 ml distilled water. An aliqoute, 20 µl of the potassium persulfate solution was pipetted into the ABTS solution and the mixture was shaken to mix manually. The mixture was then diluted by transferring the content of photosensitive mixture into 20 ml distilled water, to produce the ABTS solution used in the procedure above.

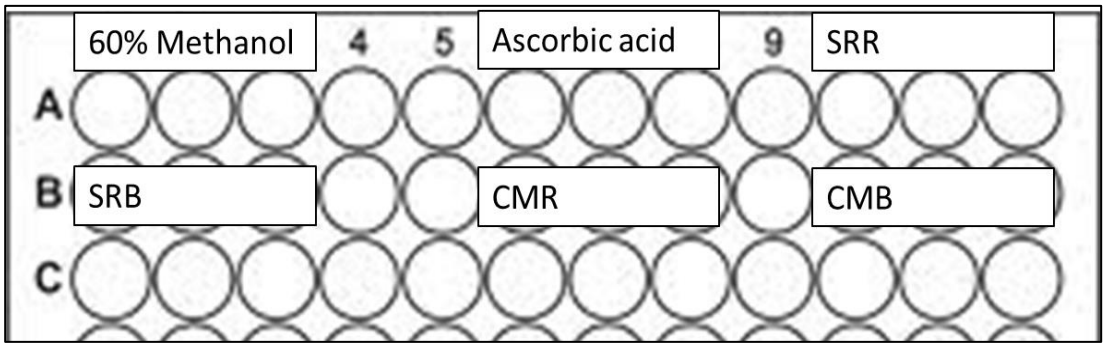

Figure 11. A 96 well plate set up for the antioxidant assay. SRR= *Schinziophyton rautanenii* root, SRB= *Schinziophyton rautanenii* bark, CMR= *Colophospermum mopane* root and CMB= *Colophospermum mopane* bark.

To determine the anti-protease activities of the plant extracts, a method derived from GIBEX, Screens-to-Nature Manual for Namibia (2012) was also employed. The assay used a radiofilm coated with gelatin and a digestive protease, trypsin. The trypsin being a protease digests the gelatin coat on radiofilm revealing a blue backgorund, in the

absence of a protease inhibitor in the plant extract. The methanolic plant extract, 10 µl of each was pipetted onto the radiograph film and 10 µl trypsin solution (2.5 mg/ml) was added to each drop of the extract. Distilled water (W) and a trypsin inhibitor (I) (MP Biomedicals) were used as controls. The radiofilm was left undisturbed for 10 minutes, which was followed by gentle rinse of the strip with water. The results were scored based on the effectiveness of the extract to prevent the trypsin from digesting the gelatin, (P+): the lack of a blue spot, while (N-) was a score for no anti-protease activity, as seen in figure 10.

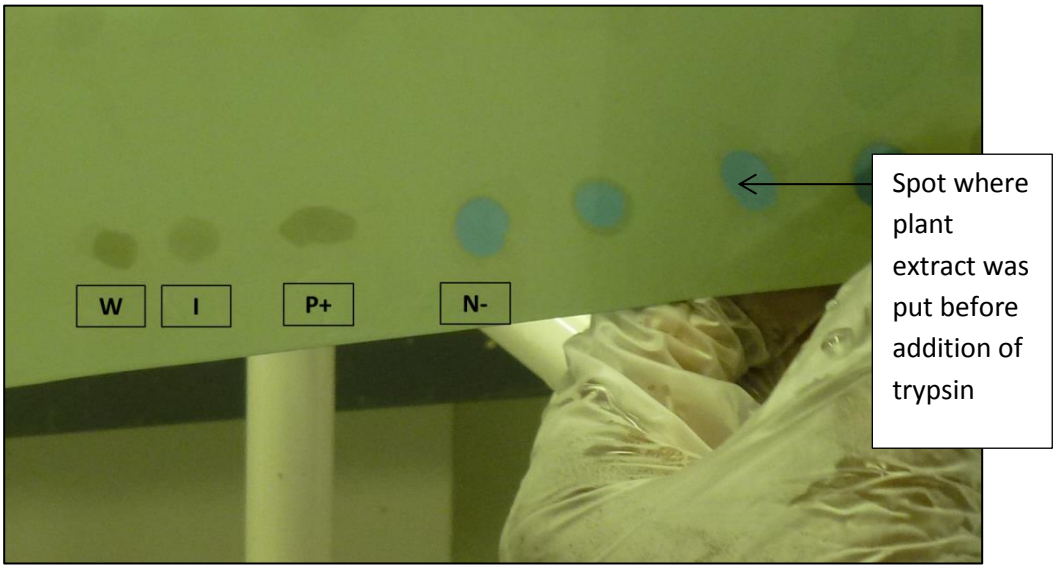

Figure 12. Radiofilm coated with gelatin displaying P+ and N- results showing positive antiprotease and negative antiprotease activity respectively. Source Florence Dushimemaria

Thin layer chromatography (TLC)

The presence of five phytochemicals: coumarins, anthraquinone, alkaloids, terpenoids and flavonoids in the plant extracts of *S. rautanenii* and *C. mopane* was determined using thin layer chromatography For this, a method adapted from Harborne (1998), was used and chromogenic reagents were used. An aluminium plate coated with silica was used. A line was drawn, 1 cm from the bottom edge of the plate. The extract was spotted onto the plate using capillary tubes dipped into the methanolic plant extracts. The extract was applied in successive applications while allowing drying of spots. The spots were spotted 2 cm from the left and right edges of the plate. An approriate control was used in respect to the particular phytochemical being investigated (Quercetin= Flavonoids, Quinine= Alkaloid, Alizarin= Anthraquinone, B-sitosterol, Coumarin=Coumarin). Quercetin dehydrate ans Alizarin were purchased from Alfa Aesar, Quinine hydrochloride dehydrate and B-sitosterol were obtained from Sigma Aldrich while coumarin was purchased from Merck KGaA. Solvent systems were prepared as shown in table 3 below. To the chromatographic tank, a prepared solvent system as seen in table 3, was added to not more than 1 cm depth. The tank was then covered in order to saturate the tank with the mobile phase prior to running the plates. Gently, the TLC plate was lowered into the tank using tweezers and the tank covered for the duration of the TLC run. The mobile phase was allowed to run up the stationary silica phase. The plate was removed and the end solvent point was marked with a soft pencil. Chromogenic reagents, table 3, were used to detect the phytochemicals on the

TLC plate. The presence or absence of the phytochemical group was scored based on the colors seen after spraying with chromogenic reagents.

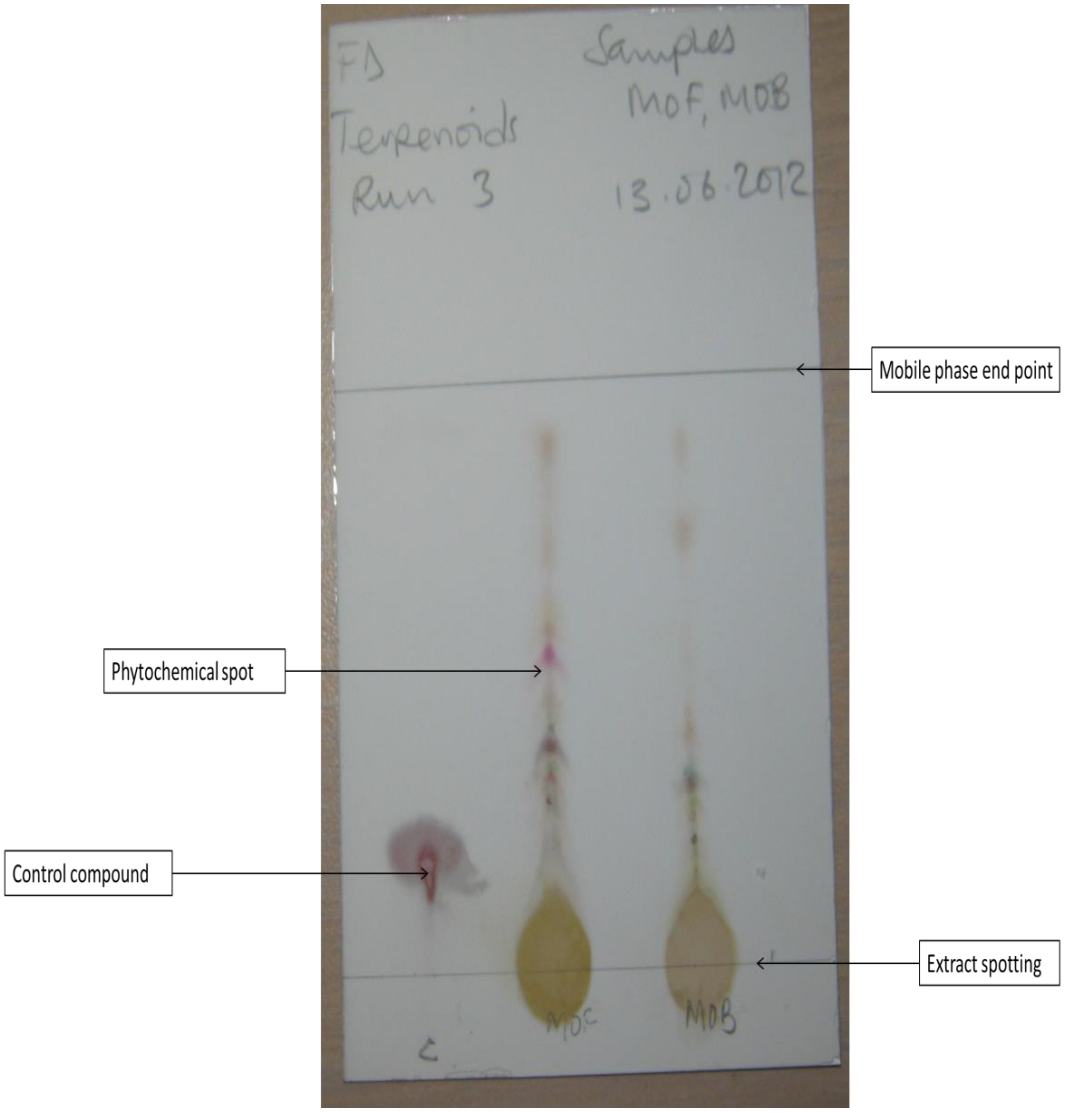

Figure 13. TLC plate set up for phytochemical detection. Source Florence Dushimemaria

Table 3. Phytochemical solvent systems and chromogenic reagents.

Phytochemical compound	Solvent system	Chromogenic solvent
Alkaloid	Ammonium hydroxide: Methanol. 3:200	Dragendorff reagent
	Ethyl acetate: Methanol:Water,	
Anthraquinone	100:17:13	10% KOH in methanol
Triterpenoid	Hexane:Ethyl acetate, 17:3	Liebermann burchard reagent
Coumarin	Chloroform	10% KOH in methanol
		1% Aluminium chloride in
Flavonoid	Butanol:Acetic acid:Water, 4:1:5	methanol

3.2.3.2. Quantification of phytochemical class compounds

Alkaloids

Total alkaloids in the powdered plant material were determined according to a method contained in Edeoga et al. (2005) with slight modifications. To quantify alkaloids, 1 g of powdered plant material was macerated in 150 ml of 10% acetic acid in ethanol. The mixture was filtered using a 110 mm Whatmann filter paper, after four hours of maceration at room temperature. The filtrate was concentrated to ¼ of its initial volume using a rotary evaporator (Heidolph). To the remaining extract, ammonium hydroxide

(PAL chemicals) was added dropwise in order to precipitate the alkaloid. Each plant sample was precipitated for alakoids in three independent trials. The precipitate was allowed to settle and it was collected on preweighed 110 mm Whatmann filter papers. The collected precipitate was washed with dilute ammonium hydroxide and air dried until a constant mass was achieved. Total alkaloid yield was calculated based on the initial powdered plant material's weight and the average of three replicates was noted.

Saponin

Total saponin content was measured according to a method also adapted from Edeoga *et al.* (2005) with slight modifications. To quantify saponins, 3 g ground plant material was macerated in 150mL 20% ethanolic aqueous solvent in a water bath at 55 °C, for 4 hours. The mixture was filtered using the force of gravity and a 110 mm Whatmann filter paper. The residue was washed with an additional 10 ml 20% ethanolic aqueous solution and the combined filtrates were concentrated on a rotary evaporator to a third of its initial volume. The concentrated plant extract volume was transferred to a 250 ml separatory funnel and 10 ml diethyl ether (Merck KGaA) was added, the mixture was shaken vigorously with intermittant venting. The organic layer was discarded while the aqueous layer was recovered. To the aqueous layer, 5 mL butanol was added, followed by vigorous shaking and venting. This was followed by the addition of two portions, each 5 ml of 5% aqueous sodium hydroxide for washing. The combination was evaporated to dryness in pre-weighed crucibles in an oven. The saponin content was

calculated as a percentage yield based on the initial dry plant material and each extract was quantified for saponin content in triplicate

Phenolic acids

Total phenolic content determination was conducted according to a slightly modified method obtained from Jing et al. (2010), using the Folin-Ciocalteu (FC) reagent. Dilutions of plant extracts were prepared in methanol in order to achieve a spectrophometric reading in obedience to Beer-Lambert's law of linearity. A 100 µl volume of the methanolic plant extract was mixed with 750 µl FC reagent and 750 µl of sodium bicarbonate solution (60 g/L) in a cuvette. The mixture was allowed to stand for 90 minutes before taking the absorbance at 725 nm. Gallic acid was used to develop a standard curve from which phenolic content was extrapolated. Total phenol content was expressed as gallic acid equivalence.

Antioxidant activity

Free radicals, produced during normal cell metabolism can accumulate in cells and cause damage to cell components such as DNA, leading to the development of cancer (Gunassekaran *et al.*, 2010), due to their unstable nature (Min and Ebeler, 2008). However, free radical scavenging molecules such as phytochemicals have come highly appraised as being able to quench radicals (Subhashini and Arunachalam, 2010) and are known as antioxidants, since they prevent oxidation of cell macromolecules by being

themselves targets for radicals. Quantification of antioxidant potential of plant extracts was investigated based on a method adopted from Jing et al. (2010) and Re et al. (1999). Equal volumes of 7mM ABTS and 2.45mM potassium persulfate solutions were incubated in the dark for 16 hours. Then, the solutions were diluted with distilled water to obtain an absorbance of 0.7±0.2 at 734nm. Meanwhile, plant extracts were dissolved in methanol to yield 1mg/ml concentrations of which about 200µl was mixed with 2ml ABTS/potassium persulfate solution in plastic cuvettes. Colorimetric change was observed for five minutes before taking absorbance at 734nm at four different concentrations of the ascorbic acid. No repeated measures were conducted for each ascorbic acid concentration. A standard graph, figure 12, was developed using ascorbic acid as the standard, which was used to estimate the antioxidant potential using the linear regression model. Four points were used for the plot in order to obtain a good fit as shown by the R^2 value, indicating goodness of fit. Ascorbic acid is a known compound with antioxidant properties.

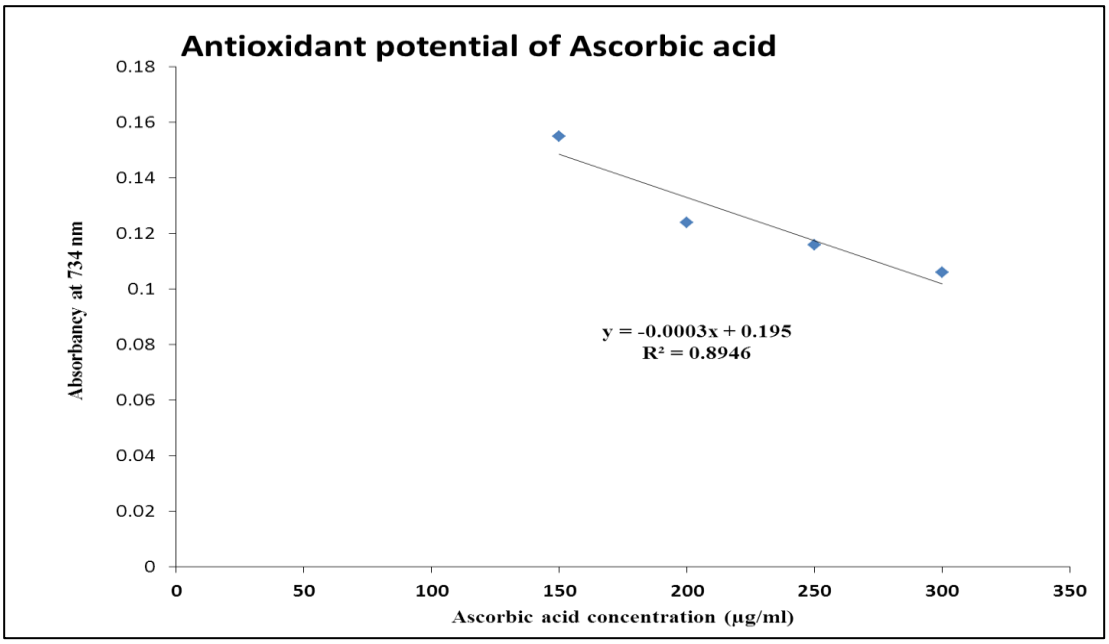

Figure 14. Standard graph developed from ascorbic acid for use to estimate the antioxidant activity of plant extracts in relation to ascorbic acid.

3.3. Biological assays

3.3.1. Anticancer Sulforhodamine B (SRB) assay

A protein stain, sulforhodamine B (SRB) (Sigma Aldrich) was used to measure total cell protein, which is proportional to cell number and stains the basic amino acids in mild acidic conditions (Vichai and Kirtikara, 2006) and this is achieved by staining with trichloroacetic acid (TCA) (Sigma aldrich). The assay measures viable cells as a direct corroboration of cell protein. The anticancer effect of plant extracts was determined as a percentage reduction in cell viability using a method adopted from Fouche et al. (2008). Screening for anticancer activity was conducted on a panel of three cancer cell lines

consisting of breast cancer MCF-7, renal cancer TK-10 and melanoma UACC-62 cell lines obtained from the American Type Culture Collection (ATCC). Cell lines were grown in RPMI media supplemented with 2mM L-glutamine, 5% foetal bovine serum and gentamycin (50 µg/ml). Flasks with 100% confluent cells were rinsed with PBS containing gentamycin (50 µg/ml), three times. Cells were trypsinized using 2 ml of 0.25% trypsin/EDTA for 2 minutes. After trypsinizing, 8 ml complete media was added, in order that the FBS neutralizes the trypsin. Cells suspended in media were transferred to a 50ml falcon tube, which was centrifuged at 2000 rpm for 2 minutes to obtain a pellet. The media containing trypsin/EDTA was decanted off the cell pellet and fresh complete media was added to resuspend cells. Cell plating densities were between 7-10 000 cells/ well. The assay was performed on a 96 well plate, see figure 13, for the plating plan. A 100 µl aliqoute of cell suspension in media was pipetted in each well of a 96 well plate with regular aspirating, while 100 µl media was added to wells which served as negative controls around the perimeter of the well plate. The plate was incubated for 24 hours in 5% CO_2 humidified environment at 37°C. Concentrations of the bark and root organic and aqueous extracts of *S. rautanenii* and *C. mopane* were diluted in DMSO and further diluted in complete cell media to achieve a thousand fold dilution of the DMSO. Final extract concentrations in the wells after addition of 100 µl media containing plant extracts were as follows: 6.25, 12.5, 25, 50 and 100 µg/ml. The extract concentrations deceased from outward of the plate to inward from both sides and with two different extracts on each plate, figure 13.

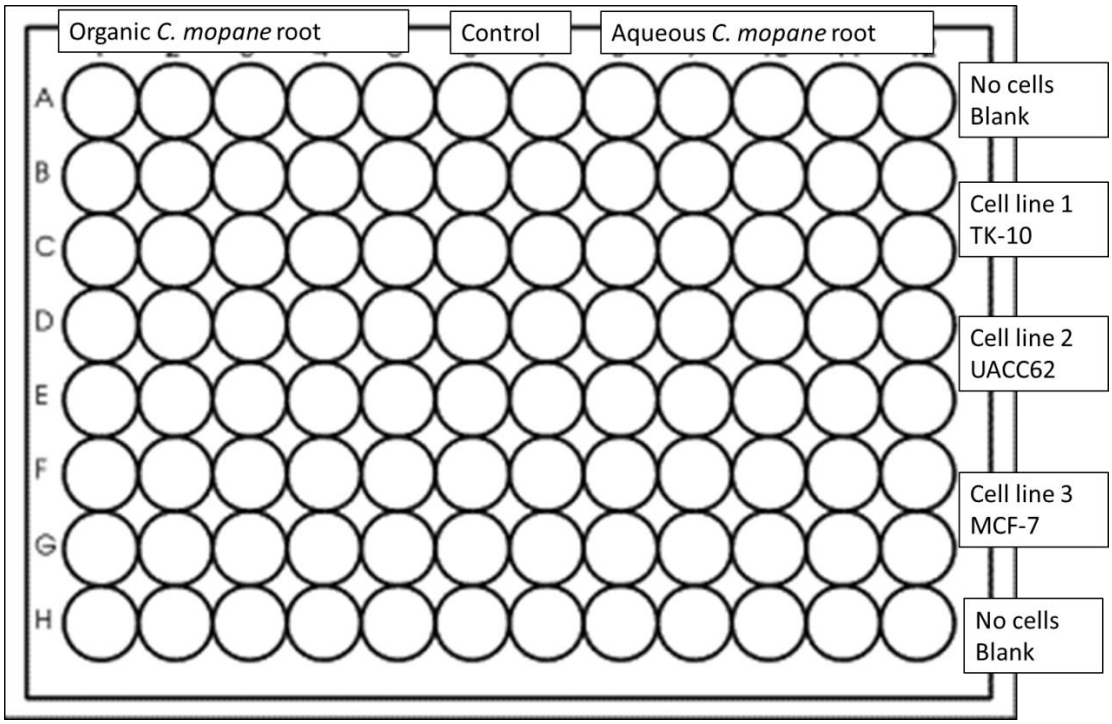

Figure 15. SRB assay 96 well plating pattern.

Cells were incubated for 48 hours after treatment with experimental extracts before fixing live cells by addition of 50 µl/well of 50% trichloro acetic acid (TCA) in dH_2O. Plates were fixed by keeping them at 4°C for 2 hours. Well plates where rinsed several times using tap running water, blotted on paper towel to dry and left to dry at room temperature. An aliquote, 100µl/well of 0.4% sulforhodamine B constituted in 1% acetic acid aqueous solution was incubated for half an hour with TCA fixed cells. The dye was rinsed off with copious amounts of 1% acetic acid and the plate was left to dry at room temperature. This was followed by addition of 100µl/well of 10mM Tris Base to dislodge the fixed cells. Absorbance was measured at 540 nm using a multiwall spectrophotometric plate reader, to determine the total protein content, as a direct

indication of cell viability. Percentage cell viability resulting from the plant extracts was calculated as follows, in relation to the viability of cells without treatment (control wells) for each cell line. The concentrations (µg/ml) were expressed in $\log_{10}$ form and used to plot the non-linear graphs against percentage cell viability. Further, the IC_{50} value, which is the extract concentration which reduces cell viability by 50% was determined using a non-linear sigmoidal curve of cell viability and $\log_{10}$ extract concentrations.

Cell viability %= (OD_{540} treatment-OD_{540}blank)/(OD_{540}control-OD_{540}blank)*100%

3.3.2. SRB *In vitro* cytotoxicity assay

This assay investigated the effects of the plant extracts of *S. rautanenii* and *C. mopane* on a non-cancerous cell line, W138. The assay was conducted according to a method adapted from Fouche *et al.*, 2008 using the SRB protein stain. For this assay, a human fetal lung fibroblast, W138 cell line (obtained from ECACC) was used for the assay and was maintained in EMEM media supplemented with 10% FBS, 2 mM L-glutamine and 50 µg/mL gentamycin. Seeding of W138 cells was at 10 000 cells/ well. Plant extracts, at various concentrations, 100, 50, 25, 12.5, 6.25 µg/ml, were incubated with fibroblast cells in a 96 well plate in 5% CO_2, 100% humidified incubator at 37°C. After 48 hours, cells were fixed with TCA, stained with 0.4% SRB and solubilizing with tris base. Etoposide was used as the positive control. Absorbance was measured at 540 nm in a plate reader spectrophotometer as an indicator of total cell protein. Percentage cell

viability resulting from the plant extracts was calculated as follows, in relation to the viability of cells without treatment (control wells) for each cell line. The concentrations (µg/ml) were expressed in $\log_{10}$ form and used to plot the non-linear graphs against percentage cell viability. Also, the IC_{50} value, which is the concentration which reduces cell viability by 50% was determined using a non-linear sigmoidal curve of cell viability and log extract oncentrations.

Cell viability %= (OD_{540} treatment-OD_{540}blank)/(OD_{540}control-OD_{540}blank)*100%

3.3.3. *In vivo* cytotoxicity assay with planaria

3.3.3.1. Maintenance of fresh water Planaria (*Dugesia dorotocephala*)

Flatworm planaria were obtained from Carolina biological laboratories in the United States of America. The worms were maintained in a glass tank containing shallow mineral water. The tank was covered with aluminium foil to protect them from direct sunlight because planarian are photophobic in nature. Planaria were fed once each week with raw chicken liver, which was followed by cleaning of the tank and addition of fresh water. Each planaria worm was sliced into two parts, head and tail, of which the head section was retained for propagation while the tail section was used for the *in vivo* toxicity screen. The planaria were fed prior to each experiment.

3.3.3.2. Toxicity assessment *in vivo*

The assessment for plant extract *in vivo* toxicity was conducted based on a method modified from the GIBEX-screens-to-nature manual for Namibia (2012). Each planaria was transferred to a petri dish containing mineral water using a soft bristle paintbrush. The flatworm was then cut using a sterile scalpel, below the sensory lobes, see figure 14, for the appearance of planaria before and after excision of head. A clear ruler placed below the petri dish was used to measure the tail section of each worm. The length was recorded as the initial length of each planaria on day 0 in millimeters.

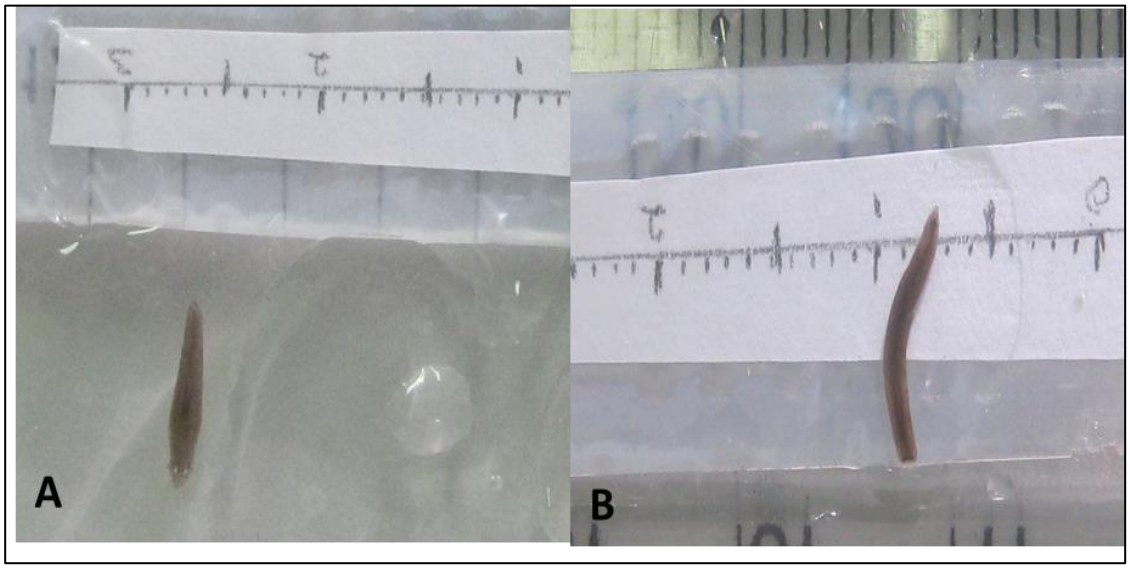

Figure 16. (A) whole planaria, (B) tail section used in the *in vivo* cytotoxicity screen after measuring initial planaria length. Source Florence Dushimemaria.

Plant extract dilutions in DMSO were performed and these were further diluted in mineral water to achieve final concentrations of 20 µg/ml and 5 µg/m and further reduce the concentration of the DMSO carrier solvent. Planaria were also maintained in

mineral water containing 20 µg/ml and 5 µg/ml DMSO in order to determine the effect of the carrier solvent on the growth of the planaria. A 24 well plate was labeled with appropriate labels: which were plant extracts and respective concentrations, negative control and non-treatment wells. Each treatment was done in triplicate. The tail section was then transferred to a well in a 24 well plate, containing the appropriate treatment. The entire 24 well plate was maintained in the dark by wrapping with aluminium foil. Observations on the length of each planaria was done every second day. On every second day of observation, contents of each well were replaced with a fresh preparation of the appropriate treatment to ensure a constant presence of plant extracts, before returming the planaria tail section. The change in planaria length was calculated by subtracting the length of the planaria on each observational day from the planaria's initial length on day 0. Furthermore, change in planaria length was normalized by deducting the effects of the control vehicle solvent on planaria length: DMSO, on each observational day. The mean change in planaria length under each treatment was used to determine the effect of plant extracts on the regenerative ability of planaria. See the following equestion.

Change in planaria length= (Observed length-Length on Day 0)-Length of planaria in DMSO

3.4. Data analysis

The analysis of quantitative data obtained from the phytochemical class compounds quantification, antioxidant activity, percentage cell viability of the anticancer assay, *in vito* cytotoxicity screen and mean change in planaria length from the *in vivo* cytotoxicity were analysed. A one-way Student's t-test, at 95% confidence level, was used to determine differences in the level of phytochemicals quantified and antioxidant activities of the four methanolic plant extracts. A two-way Student's t-test, at 95% confidence level, was used to compare phytochemical levels obtained between the two plant parts, roots and bark. The percentage yields of alkaloids, saponin and phenolic content was compared with the antioxidant activity using spearman correlation analysis to determine which phytochemical was responsible for observed antioxidant activity. Cell viability data resulting from the two extracts from the same plant part were also compared using a two-way Student's t-test. Multiple linear regression analysis of the effect of extract, concentration and their combined effect was also done for the *in vitro* cytotoxicity analysis. Multiple analysis of variance was used to determine whether there was a difference in the mean change in planaria length each day. For each Student's t-test performed, the decision criterior was $p<0.05$ for a significant difference while $p>0.05$ was considered as no significant difference.

3.5. Research ethics

This study involved the use of plant material and for the collection of the plant material for use in research experiments and for preparation of voucher specimen submitted to the National Botanical Research Institute of Namibia, a plant permit was obtained from the Ministry of Environment and Tourism of Namibia to permit collection of plant material for research use. Sustainable harvesting of plant material was conducted. No ethical approval was required for the use of planaria as an *in vivo* model for toxicity.

CHAPTER FOUR: RESULTS

4.1. Phytochemical profiling

In this study, methanolic plant extracts of *S. rautanenii* and *C. mopane* were screened for the presence of five phytochemical classes, antioxidant activity and antiprotease activity, Table 4 shows the results. According to the GIBEX-screens-to-nature assay method, all plant extracts displayed high antioxidant and antiprotease activity. *S. rautanenii* and *C. mopane* root and bark extracts both displayed a significant high presence of antioxidant activity.

This study reports high antiprotease activity of all methanolic extracts in this study, derived from *S. rautanenii* and *C. mopane*. In addition, this study revealed the presence of phytochemicals such as alkaloids, flavonoids, coumarins, anthraquinones and triterpens. Alkaloids where found in both *S. rautanenii* and *C. mopane* root and bark extracts. Triterpenes were also detected in all plant extracts, especially in the root extract of *S. rautanenii*. Only one band was detected indicating the presence of flavonoids in all extracts. Coumarins were detected on the same level in almost all plant extracts except for *S. rautanenii* root. Anthraquinones where only detected in one plant extract, the root extract of *S. rautaneniii*.

Table 4. Detection of phytochemical compounds, antioxidant and antiprotease activity of S. rautanenii and C. mopane.

Test compound	**Plant name and Part**			
	***S. rautanenii* bark**	***S. rautanenii* root**	***C. mopane* bark**	***C. mopane* root**
Antioxidant	+++	+++	+++	+++
Anti-protease	+++	+++	+++	+++
Alkaloid	+	+	+	+
Anthraquinone	-	+++	-	-
Coumarin	++	+	++	++
Flavonoid	+	+	+	+
Triterpenes	+++	++++	+++	+++

Key: ++++ Very high presense, +++ high presence, ++ moderate presence, + present, - absent

4.2. Phytochemical class compounds quantification

4.2.1.Total phenolic content

Quantification of the total phenolic content of the root and bark extracts of *S. rautanenii* and *C. mopane* yielded the following quantities, graphed in figure 15, derived from an ascorbic acid graph. The root and bark of *C. mopane* contained 205.4 ±9.3 and 12.6 ±1.7 GAE µg/ml respectively. *S. rautenenii* root and bark contained 41.4 ± 1.2 and 48.6 ± 1.2 GAE µg/ml respectively. The plant extract with highest phenolic content was *C.*

mopane root while the least phenolic content was displayed by the bark extract of *C. mopane*. Data analysis found a very significant difference in the mean phenolic content of all plant extracts, ($p=0.006$). Meanwhile, both comparisons of each root and bark extract of *C. mopane* and *S. rautanenii* also showed a significant difference ($p<0.001$ and $p=0.002$) respectively.

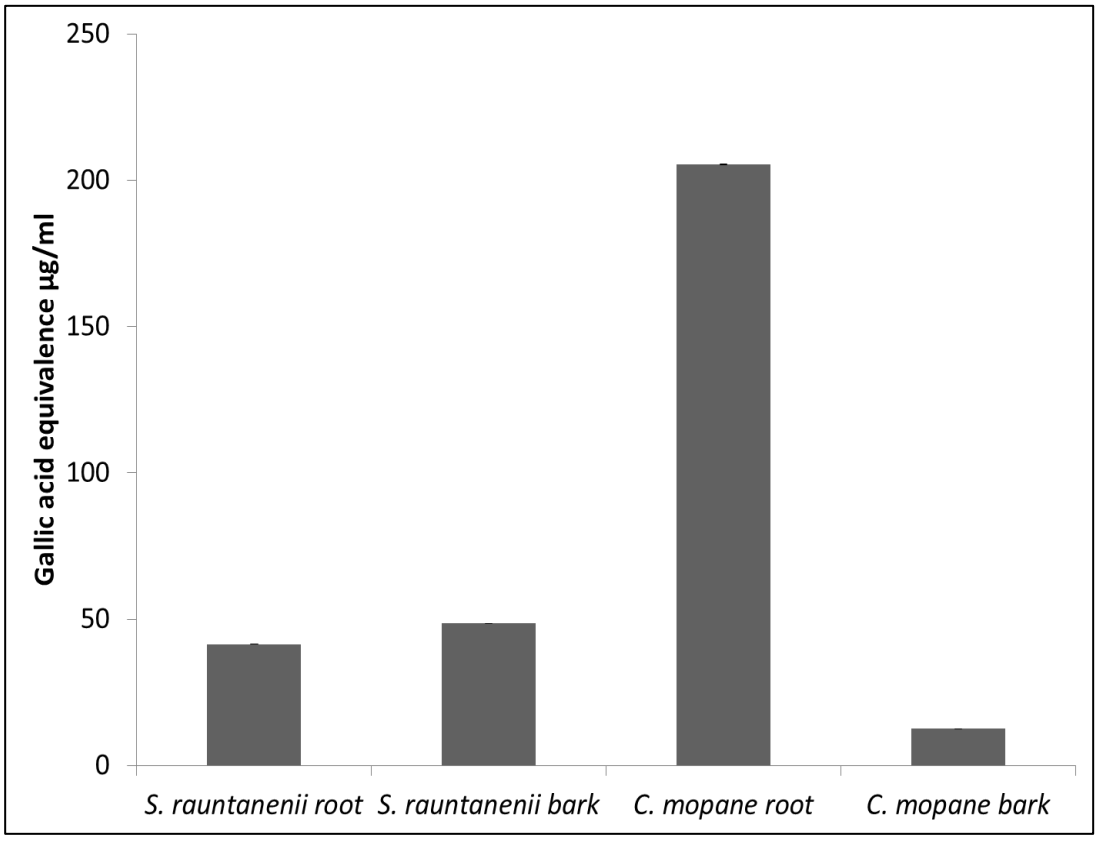

Figure 17. Quantification of total phenolics contained in the root and bark of *S. rautanenii* and *C. mopane.*

4.2.2.Total alkaloid content

Quantification of total alkaloids by the precipitation method revealed that data obtained was normally distributed as determined by the Shapiro-Wilk test for normality,

p=0.539. Alkaloid percentage yields ranged between 11.1 ±2.7 percent and 2.1±0.6 percent, with *C. mopane* root and *S. rautanenii* bark exhibiting the highest and lowest alkaloid percentage yields respectively. Data was further analysed using a two way Student's t-test, and analysis between *S. rautanenii* root and *S. rautanenii* bark showed no significant difference in alkaloid content (p=0.102), while there was a significant difference between the alkaloid content of *C. mopane* root and bark extracts, (p=0.049), figure 16.

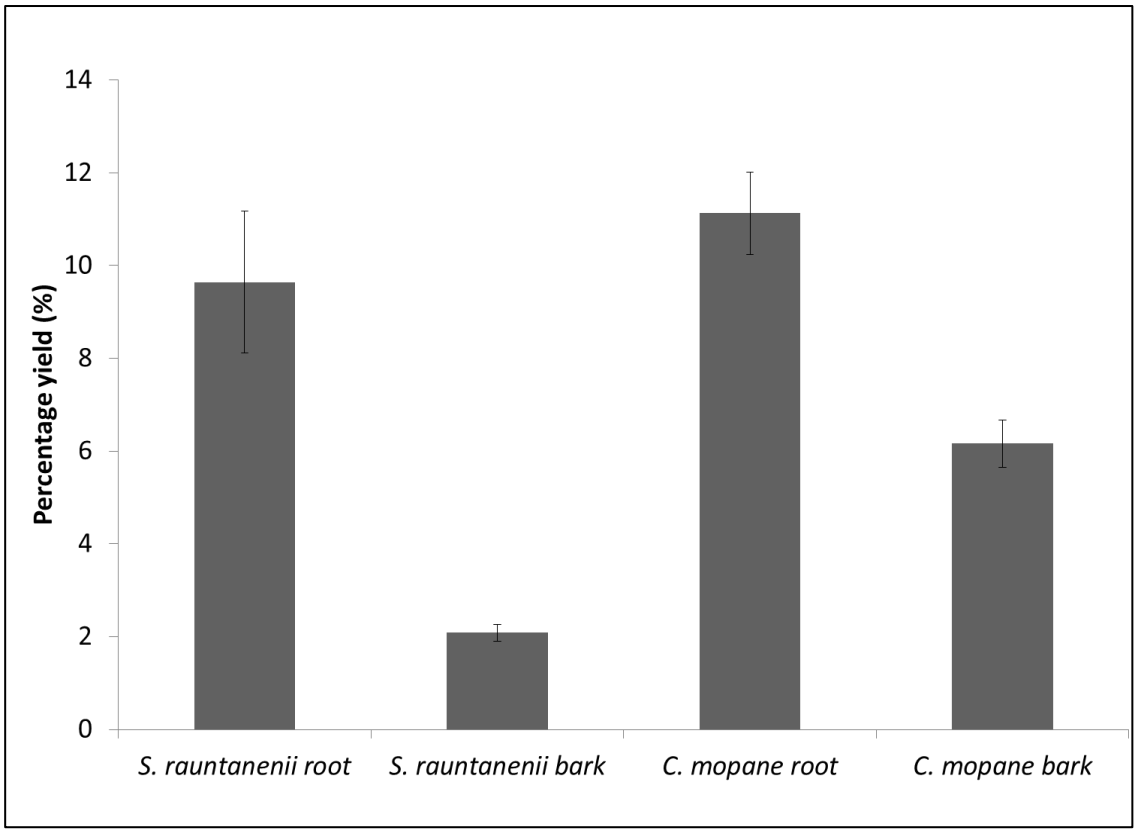

Figure 18. Total alkaloid content in *S. rautanenii* and *C. mopane* roots and bark.

4.2.3.Total saponin content

Quantification of total saponin in the methanolic extracts of *S. rautanenii* and *C. mopane* root and bark plant parts gave the following yields, figure 17. The sample with the highest saponin content was *S. rautanenii* bark extract 13.6 ± 3.5 %, which was significantly different in comparison to the second plant extract with high saponin content, *C. mopane* bark 7.5 ± 2.5 % ($p=0.032$). The bark and root extracts of *C. mopane* contained significantly different saponin contents ($p=0.035$) however, extracts of *S. rautanenii* contained similar levels of saponins since the yield was not significantly different at 95 % confidence level.

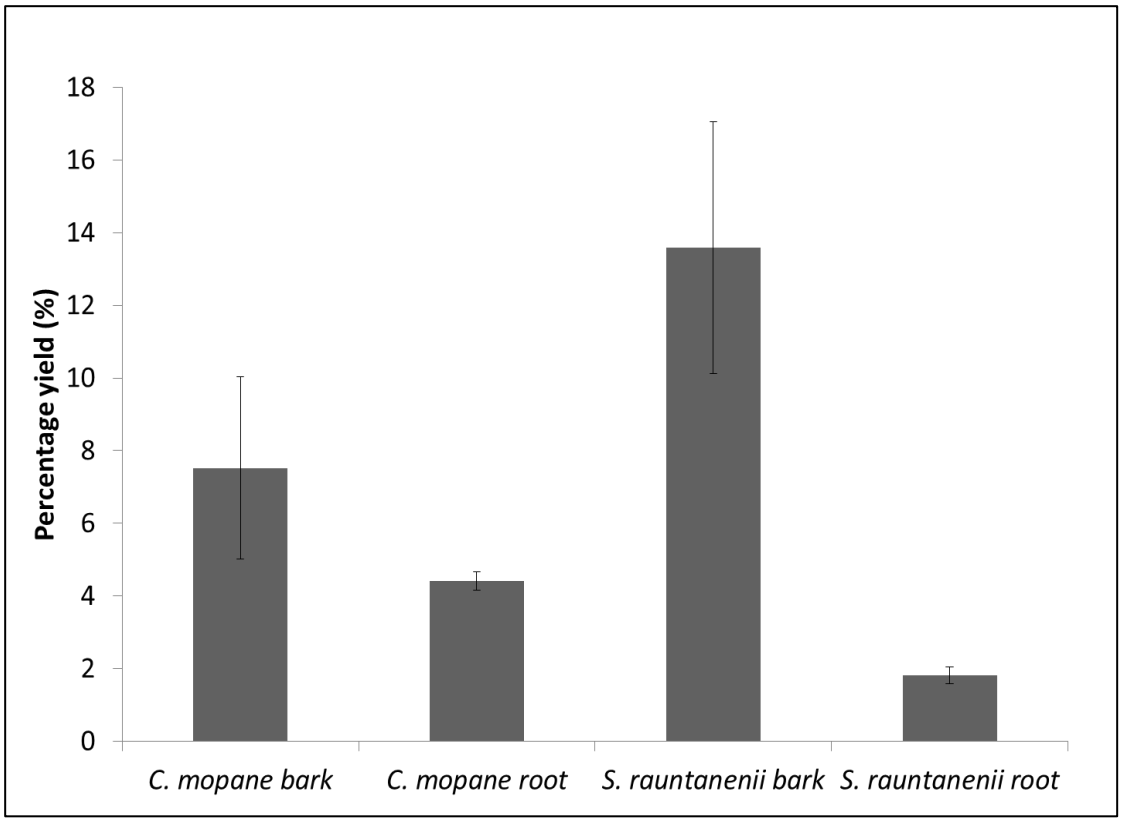

Figure 19. Quantification of total saponins from root and bark extracts of *S. rautanenii* and *C. mopane*.

4.2.4. Antioxidant potential

The plants, *S. rautanenii* and *C. mopane* bark and root organic extracts had varying antioxidant activity, figure 18, which ranged between 945.6 ± 231.1 AAE µg/ml and 226.7 ± 17.6 AAE µg/ml. Using a one-way Student's t-test, there was a significant difference in the antioxidant potential of all plants ($p<0.001$). A significant difference was observed between *S. rautanenii* root and bark extracts ($p=0.032$), but no difference was observed in the activities of *C. mopane* plant parts.

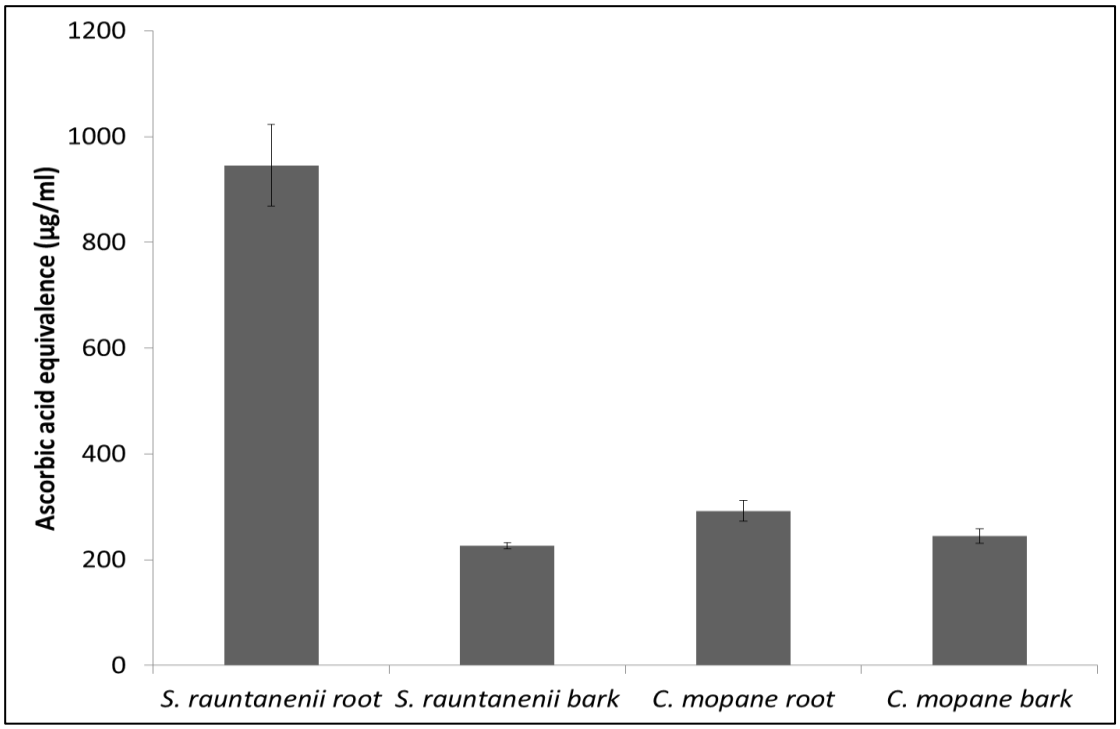

Figure 20. Antioxidant potential of two Namibian indigenous plants: *S. rautanenii* and *C. mopane*.

4.3. SRB anticancer screening

The effect of plant extracts on the cell viability of three cancer cell lines was investigated using the SRB protein dye assay. Screening of the eight plant extracts against a panel of three cancer cell lines, TK10 renal, MCF-7 breast and UACC-62 melanoma carcinoma cell lines revealed the activities as seen in table 5, figure 20-23. Two known cytostatic drugs were used as positive controls, etoposide and parthenolide, the controls exhibited differing activity across the three cancer cell line panel, figure 19. parthenolide exerted a greater effect on the cells as compared to etoposide, because it greatly reduced the cell viability of all three cell lines. Only etoposide gave an IC_{50}

value against renal cancer TK-10 cell line of 21.43 µg/ml while the rest yielded values below 6.25 µg/ml.

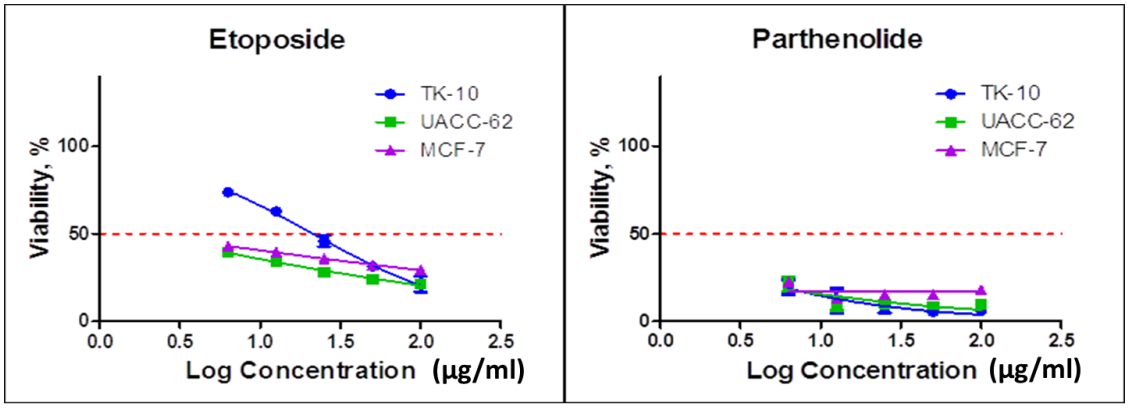

Figure 21. Anticancer activity of etoposide and parthenolide against a panel of three cancer cell lines, which were used as positive controls in the Sulforhodamine B assay.

Both organic and aqueous extracts of *S. rautanenii* bark produced the responses below against a panel of the three cancer cell lines, figure 20. The organic extract appears to display higher activity against the three cell lines, especially the breast MCF-7 cancer cell line with an IC_{50} value of 74 µg/ml in comparison to 120.1 µg/ml and 167.8 µg/ml obtained against melanoma UACC-62 and renal TK10 cell lines, table 5. However, no statistically significant difference was found using a Student's t-test at 95% confidence level by comparison of the mean cell viability plots of each cell line of both organic versus aqueous extracts.

Comparison of the effect of the plant extract of aqueous *S. rautanenii* bark using a two way Student's t-test, on the three cancer cell lines revealed no significant difference when the mean cell viability percentage of each cancer cell line was compared against

the other, except in the case of renal against melanoma cancer (p=0.015). On the other hand, no significant difference on the effect of organic *S. rautanenii* bark was observed on the three cell lines at 95% confidence level.

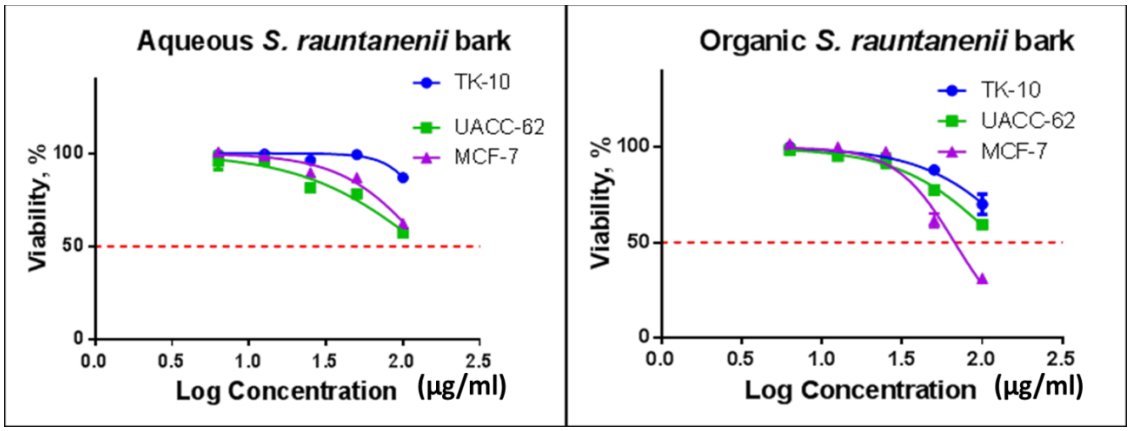

Figure 22. Antiproliferetive activity of S. rautanenii bark aqueous and organic extracts, with breast cancer MCF-7 cell line demonstrating sensitivity towards the organic extract.

The organic and aqueous extracts prepared from *S. rautanenii* root affected the cell viability of a panel of three cell lines as seen in figure 21. The cancer cell model with the greatest sensitivity to both the organic and aqueous extracts of *S. rautanenii* was the melanoma model, figure 21. Student's t-test analysis of the organic and aqueous *S. rautanenii* root extracts cell viability revealed that the extracts affected each cell line by the same magnitude since no significant differences were found at 95% confidence level. However, there was a significant difference when mean cell viability percentages

obtained from the organic and aqueous extracts on melanoma and renal cancer cells were compared (p=0.009 and p=0.026) respectively.

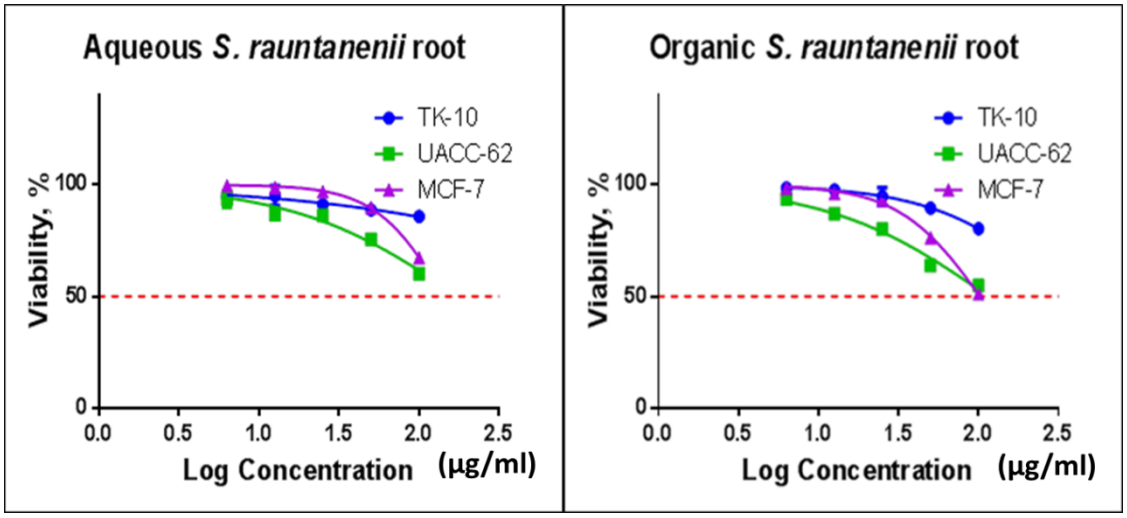

Figure 23. Activity of the aqueous and organic root extracts *S. rautanenii* against a panel of three cancerous cell lines.

An IC_{50} value of 83.09 µg/ml was obtained against the breast cancer cell line MCF-7 as resulting from anticancer effect of aqueous *C. mopane* bark, figure 22. Meanwhile,the organic extract of *C. mopane* root displayed IC_{50} values of 94.39 µg/ml and 61.26 µg/ml on human melanoma UACC-62 and MCF-7 cell lines respectively, figure 22. Conversely, statistical analysis using a Student's t-test revealed no significant difference when the percentage cell viability of the different cell lines were compared. Additionally, no significant difference was found when the effects of the organic and aqueous extracts on causing cancer cell line death was compared.

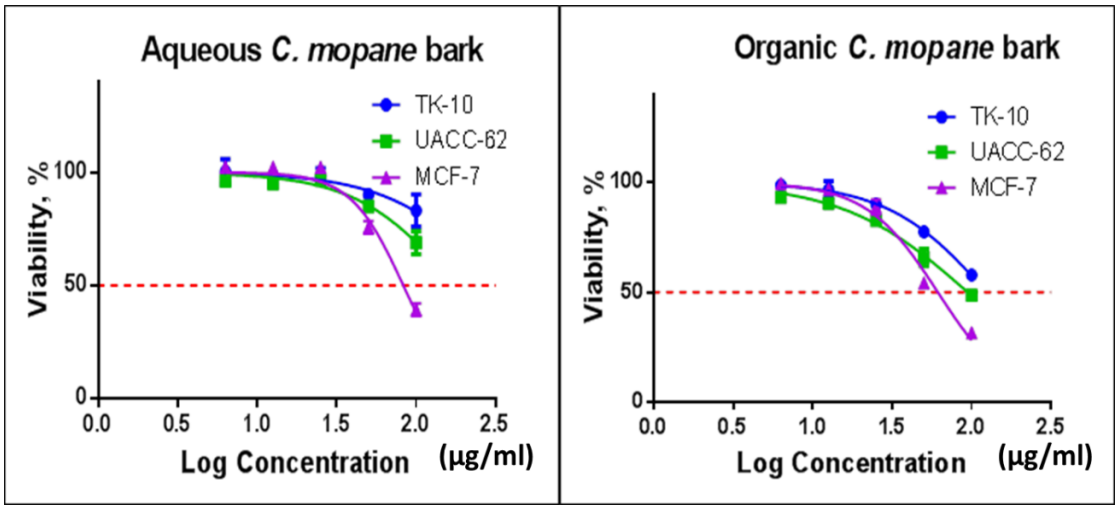

Figure 24. Anticancer activity exhibited by *C. mopane* bark aqueous and organic extracts against three cancer cell lines.

The *C. mopane* root extracts displayed the following effects on the three cancer cell types, figure 23. The aqueous extract displayed cell viability reduction within the tested range of concentrations yielding an IC_{50} value of 87.9 µgml against breast cancer. The organic root extract of *C. mopane* yielded the following IC_{50} values, 64.1 µg/ml and 48.2 µg/ml against melanoma and breast cancer cell lines respectively. The effect of the *C. mopane* root organic extract observed against breast cancer cells was the most potent anticancer activity in this study. However, no significant difference was obtained when establishing a differential effect of the organic and aqueous extracts on the cancer cell lines at a 95% confidence level against all three cell lines, renal ($p=0.201$), melanoma ($p=0.55$) and breast ($p=0.106$).

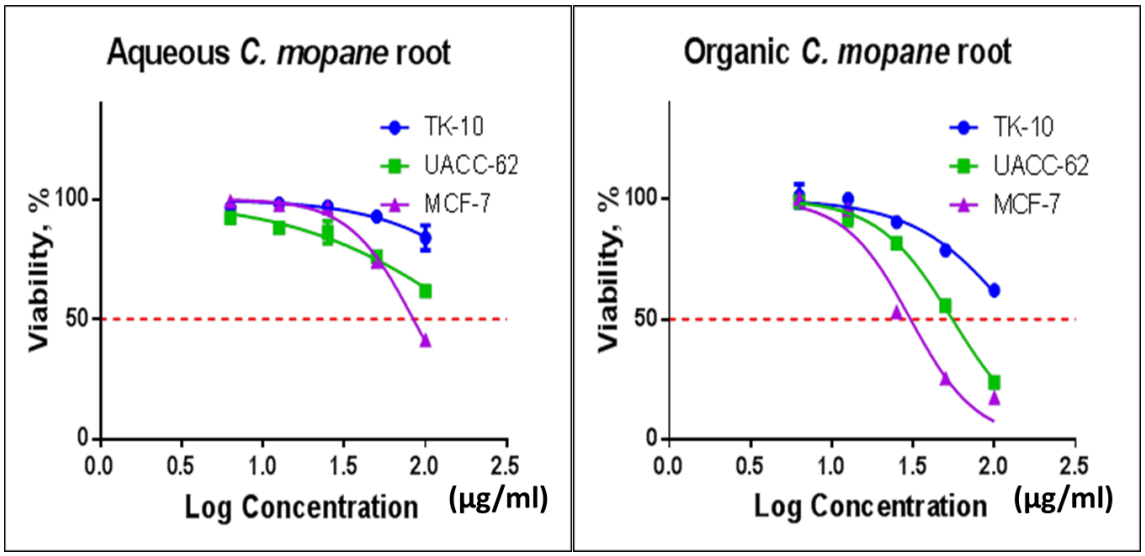

Figure 25. Antiproliferative effect of *C. mopane* root extracts against three cell lines with the organic extract displaying activity against both breast MCF-7 and UACC-62 cell lines.

The aqueous root and bark plant extracts derived from *S. rautanenii* displayed selectivity towards the UACC-62 melanoma cell lines while organic root and bark extracts of *C. mopane* displayed selectivity towards breast cancer MCF-7, table 5. However, the selectivity index could be determined empirically. In addition, both the organic and aqueous extracts of *C. mopane* displayed IC_{50} values below 100µg/ml for MCF-7 breast cancer while the organic *C. mopane* extract displayed similar activity below the same threshold against UACC-6, table 5.

Table 5. Anticancer evaluation and resulting IC_{50} values of different plant extracts on a panel of three cancer cell lines.

Solvent	Extract	IC_{50} µg/ml		
		TK10	UACC62	*MCF7*
Aqueous	***S. rautanenii* bark**	411.6	116.7	*134.0*
	***S. rautanenii* root**	452.4	128.7	*154.5*
	C. *mopane* bark	273.7	164.3	*86.8*
	***C. mopane* root**	332.3	135.2	*87.9*
Organic	***S. rautanenii* bark**	167.8	120.1	*74.0*
	***S. rautanenii* root**	252.5	102.6	*102.4*
	***C. mopane* bark**	115.8	92.5	*70.2*
	***C. moane* root**	124.1	64.1	*48.2*
	Etoposide	21.43	1.668	*1.759*
	Parthenolide	<6.26	<6.26	*<6.26*

4.4. Cytotoxicity analysis

The cytotoxicity analysis of the aqueous and organic extracts of both *S. rautanenii* and *C. mopane* bark and root extracts displayed the following activities as depicted in table 6, figures 25-28. Etoposide was used as the positive control, with an IC_{50} value below 6.25 µg/ml, figure 24.

Extracts displayed mild cytotoxicity against the human W138 fibroblast cell line. All IC_{50} values obtained were above the highest extract concentration of 100 µg/ml. However, when cell viability percentage was plotted against extract concentration in linear plot, IC_{50} values as seen in table 6, were obtained. The organic root extract of *C. mopane* yielded the lowest IC_{50} value of 162.4 µg/ml, while the organic root extract of *S. rautanenii* gave the highest IC_{50} value of 444.8 µg/ml. No significant difference

between aqueous and organic plant part extracts was observed at 95% coefficient level, indicating that the plant extracts were equally cytotoxic

Table 6. IC_{50} values obtained during cytotoxicity analysis of *S. rautanenii* and *C. mopane* plant extracts compared to etoposide as control against W138 cell line.

	IC_{50} µg/ml	
	Aqueous	**Organic**
***C. mopane* bark**	202.5	197.6
***C. mopane* root**	211.5	162.4
***S. rautanenii* bark**	174.4	248.8
***S. rautanenii* root**	444.8	315.5
Etoposide	5.1	

A known cytostatic drug, etoposide was used as a positive control in the cytotoxicity assay. Figure 24 shows the response of the human fetal lung fibroblast cell line, W138 to etoposide, which is measured as percentage cell viability. Etoposide showed increasing cytotoxicity with increasing concentrations of etoposide. The graph seen in figure 24 shows expontential decay dosage response.

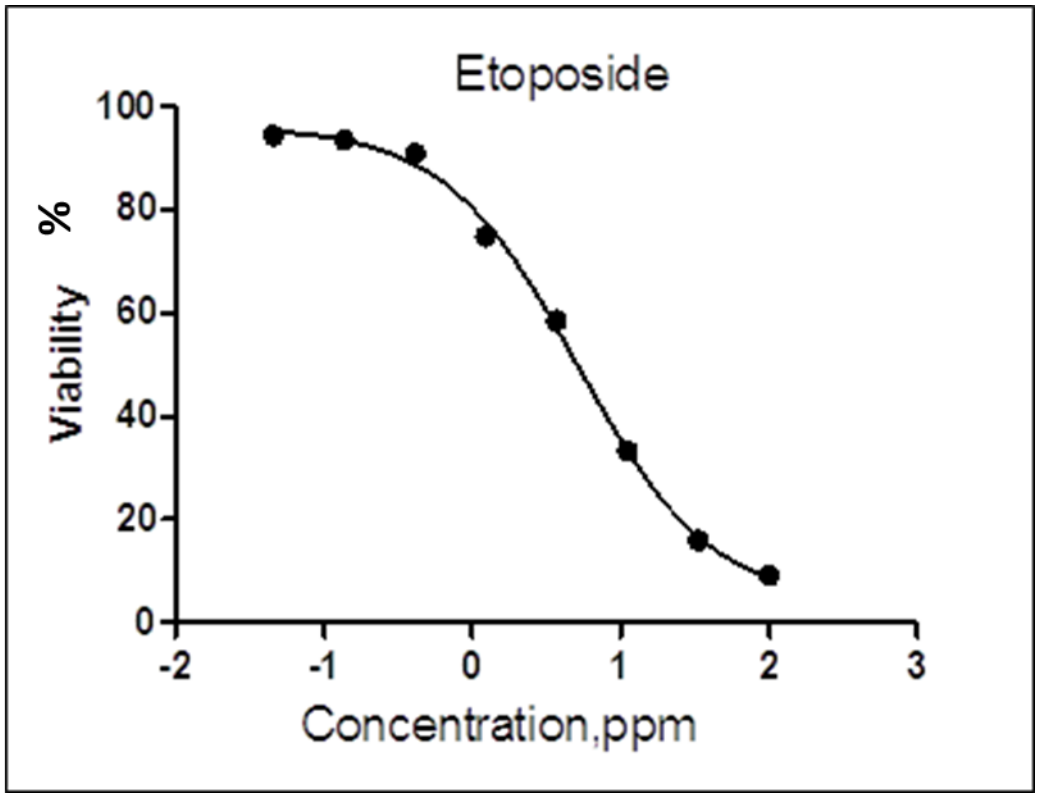

Figure 26. Antiproliferative activity of positive control, etoposide against the human fetal lung fibroblast cell line W138.

Analysis of cytotoxicity *in vitro* of *S. rautanenii* bark aqueous and organic extracts resulted in the dosage response sigmoidal curves in figure 25. The decrease in cell viability is observed more strongly in the case of the aqueous extract, in comparison with the organic bark. This is further supported by the IC_{50} values, table 6, of the *S. rautanenii* bark aqueous and organic extracts, 174.4 µg/ml in comparison to 248.8µg/ml respectively. Further data analysis reveaveled that despite the extract concentration effect on cell viability, no difference could be accounted to the different plant extracts. In other words, only an increase in extract concentration made a difference to cell

viability but the extraction solvent did not affect cell viability in the same manner, table 7.

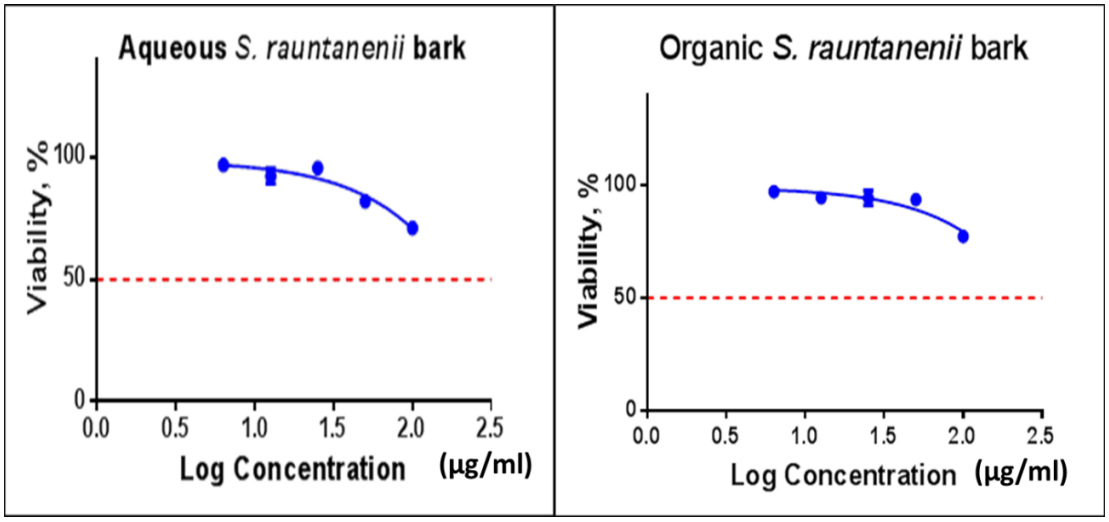

Figure 27. Cytotoxicity evaluation of *S. rautanenii* bark aqueous and organic extracts against the human fetal lund fibroblast cell line W138.

Table 7. Multiple linear regression analysis results of *S. rautanenii* bark extracts.

Change	Degree of freedom	Sum of squares	Mean of squares	F	P. value
Concentration	1	655.366	655.366	67.1	<.001
Extract	1	33.893	33.893	3.47	0.112
Concentration and Extract	1	19.011	19.011	1.95	0.212
Residual	6	58.601	9.767		
Total	9	766.872	85.208		

Evaluation of cytotoxicity of the aqueous and organic extracts of *S. rautanenii* root revealed that the organic extract was more cytotoxic as compared to the aqueous extract, figure 26. There was a significant difference in cell viability resulting from the effect of the organic extract in the one-way Student's t-test, ($p<0.001$). PostHoc analysis using the least significant difference indicated that the difference was at low extract concentrations. IC_{50} values were 444.8 µg/ml and 315.5 µg/ml for the aqueous and organic extracts of *S. rautanenii* root respectively. Multiple linear regression of the combined effect of extract concentration and extract revealed that extract concentration significantly affects cell viability ($p=0.001$) but the cell viability did not differ significantly in response to extract type ($p=0.448$).

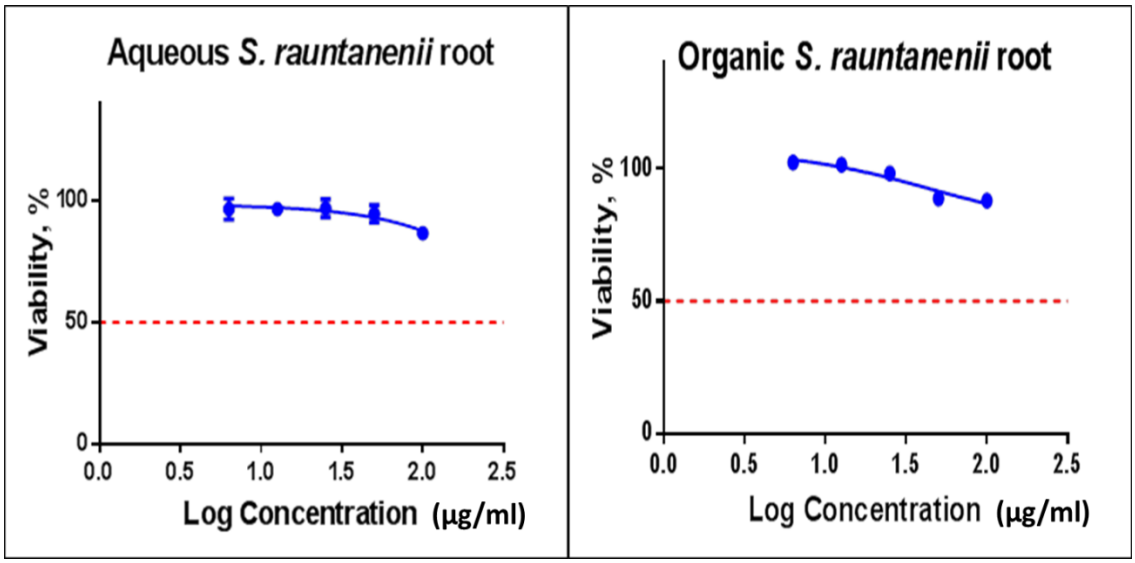

Figure 28. Evaluation of aqueous and organic extracts of *S. rautanenii* root extracts against the human fetal lung fibroblast cell line W138.

Analysis of cytotoxicity of both aqueous and organic extracts derived from the bark of *C. mopane* revealed the following, see figure 27. The IC_{50} value obtained against the extract's effect on the human fetal lung fibroblast cell line, W138 was 202.5 µg/ml for the aqueous extract, which was slightly higher in comparison to that obtained from the organic extract, 197.6 µg/ml. Furthermore, a significant concentration effect was observed in both extracts ($p<0.001$), however the two plant extracts did not differ significantly in causing a decrease in cell viability ($p=0.101$). In other words, figure 27, shows that the rate of decrease in cell viability changed in a similar manner for both the organic and aqueous extract. Subsequently, analysis of the combination of extract type and concentration on cell viability also not significantly different ($p=0.594$).

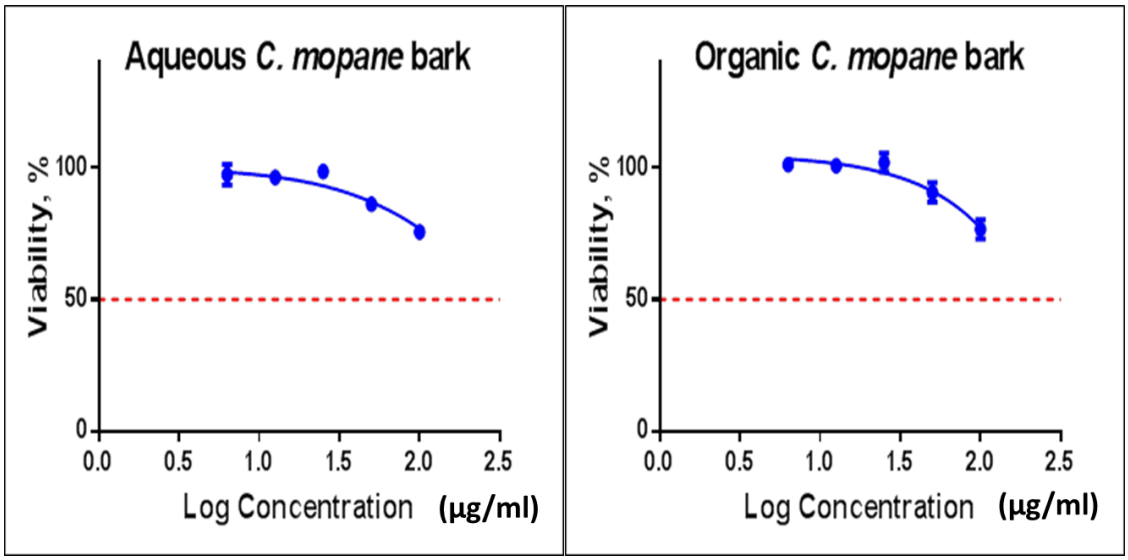

Figure 29. Cytotoxicity investigation of both extracts of *C. mopane* bark against a fetal lung fibroblast cell line, W138.

Investigation of the cytoxicity effects of the aqueous and organic extracts of *C. mopane* root yielded IC_{50} values of 211.5 µg/ml and 162.4 µg/ml respectively. The two curve responses appeared similar, figure 28 and this was confirmed in that there was no difference in cell viability depending on extract being prepared either from an aqueous or organic solvent, table 8. However, there was a concentration response observed in both extracts ($p<0.001$), the curve resulting from the effect of organic *C. mopane* root extract shows a much greater change in decreasing cell viability as extract concentration doubles, at the highest extract concentration. Generally, cell viability decreased with increasing extract concentration, that implies that cytotoxicity increased with extract concentration.

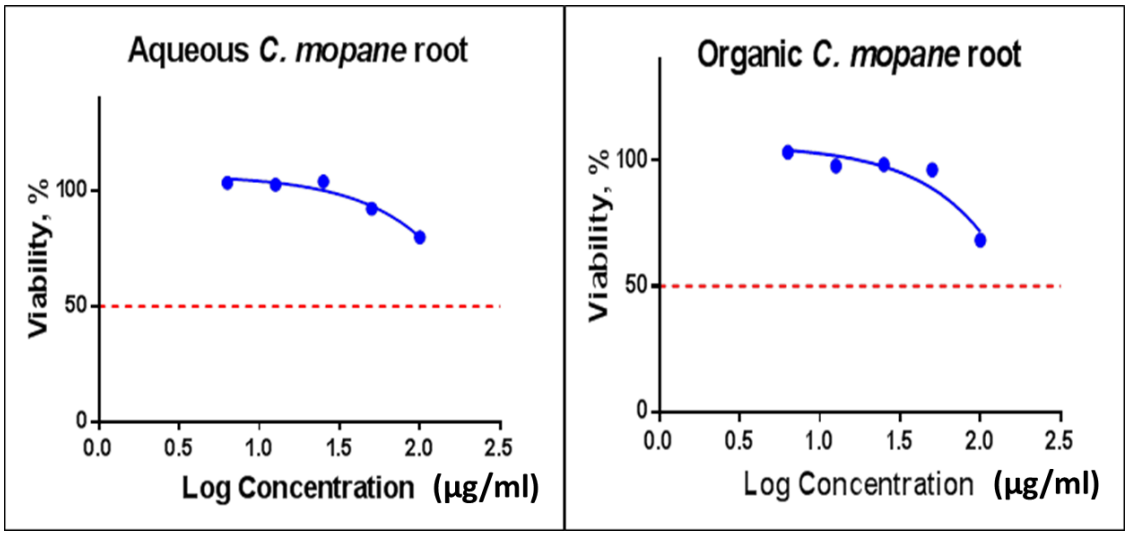

Figure 30. Evaluation of cytotoxicity effect of the root aqueous and organic extracts of *C. mopane* root on a fibroblast cell line, W138.

Table 8. Multiple linear regression analysis of *C. mopane* root extracts against cell viability of human fibroblast cells, W138.

Change	Degree of freedom	Sum of squares	Mean square	F	P. Value
Concentration	1	1092.8	1092.8	63.16	<.001
Extract	1	36.5	36.5	2.11	0.197
Concentration and extract	1	16.68	16.68	0.96	0.364
Residual	6	103.82	17.3		
Total	9	1249.8	138.87		

The extracts with the highest and lowerest IC_{50} values where aqueous *S. rautanenii* root and organic *C. mopane* root extracts respectively, table 6. A multiple linear regression analysis of the cell viabilities obtained from the two extracts revealed that a concentration effect was evident between the two plants ($p<0.01$). The effect of differences in extracts was not significant ($p=0.529$), however, a combined effect of the concentration and extract type was significant ($p=0.017$), table 9.

Table 9. Multiple linear regression analysis of the effects of aqueous *S. rautanenii* root and organic *C. mopane* root extracts on the cell viability of the human fibroblast cell line W138.

Change	Degree of freedom	Sum of squares	Mean square	F	P. Value
Concentration	1	599.13	599.13	39.77	<.001
Extract	1	6.71	6.71	0.45	0.529
Concentration and extract	1	160.38	160.38	10.65	0.017
Residual	6	90.38	15.06		
Total	9	856.6	95.18		

Table 10. Selectivity indeces of plant extracts and etoposide against a panel of here cancer cell lines.

Solvent	Extract	Selectivity index		
		TK10	UACC62	MCF7
Aqueous	*S. rautanenii* bark	0.4	1.5	1.3
	S. rautanenii root	1.0	3.5	2.9
	C. mopane bark	0.7	1.2	2.3
	C. mopane root	0.6	1.6	2.4
Organic	*S. rautanenii* bark	1.5	2.1	3.4
	S. rautanenii root	1.2	3.1	3.1
	C. mopane bark	1.7	2.1	2.8
	C. moane root	1.3	2.5	3.4
	Etoposide	4.2	0.3	0.3

The selectivity index (SI) is ratio of the measure/ indicator of how much effective a compound is as a therapeutic emtity as compared to its cytotoxicity. Since the SI is indicative of the compound's differential activity of a compound, then, the greater the SI value, the greater the selectivity of the compound (Wardihan et al., 2013) and the better its potential use as a new therapeutic entity. For this study, high selectivity was considered as SI≥3 (Machana et al., 2012; Mahavorasirikul et al., 2010). Results displayed in figure 10, shows high selectivity of certain plant extracts against against cancer cell lines. The aqueous root extract of *S. rautanenii* displayed high selectivity cytotoxicity against the UACC62 melanoma cell line with SI=3.5. Organic extracts of *S.*

rautanenii bark, *S. rautanenii* root and *C. mopane* root also displayed high selective cytotoxicity against the breast cancer cell line, MCF7 with SI values of 3.4, 3.1 and 3.4 respectively. The organic root extract of *S. rautanenii* also showed high cytotoxicity against the UACC62 melanoma cell line, SI=3.1. Worth notice is the anticancer and cytotoxic effect of the positive control, eoposide, which was highly selective against the renal cancer cell line, TK10, with SI=4.2.

4.5. *In vivo* toxicity investigation

Planaria are among the few organisms capable of regenerating complete body parts after they are lost. Planaria was cut and maintained in the presence of different plant extracts and concentrations. The length of the planaria was measured every second day for eight days. The following graphs were obtained by plotting the mean change in planaria length over the experimental period of eight days, figure 29-32. Data analysis for normality of data obtained from the mean change in planaria length every observational day using Shapiro-Wilks test proved data to be normally distributed, therefore, additional parametric data analysis tests were performed.

The effect of growing planaria in the presence of the organic *S. rautanenii* bark extract displayed both growth promoting and growth retarding effects at different concentrations. Overall, there was an increase in planaria length at the lower extract concentration of 5 µg/ml over the eight observational days, and yet this was not comparable to the growth seen at no treatment, 0 µg/ml, figure 29. However, a four fold increase in extract concentration resulted in a decrease in planaria length, figure 29.

Multivariate analysis of the mean lengths on different observation days and different extract conconcentrations reveal a significant difference over the eight days, p=0.010. This depicted that *S. rautanenii* organic bark extract negatively affected planaria regeneration, indicating that as extract concentration increased, the cytotoxicity effects of *S. rautanenii* bark extracts were more visibly seen as affecting planaria regeneration in comparison to low extract concentration.

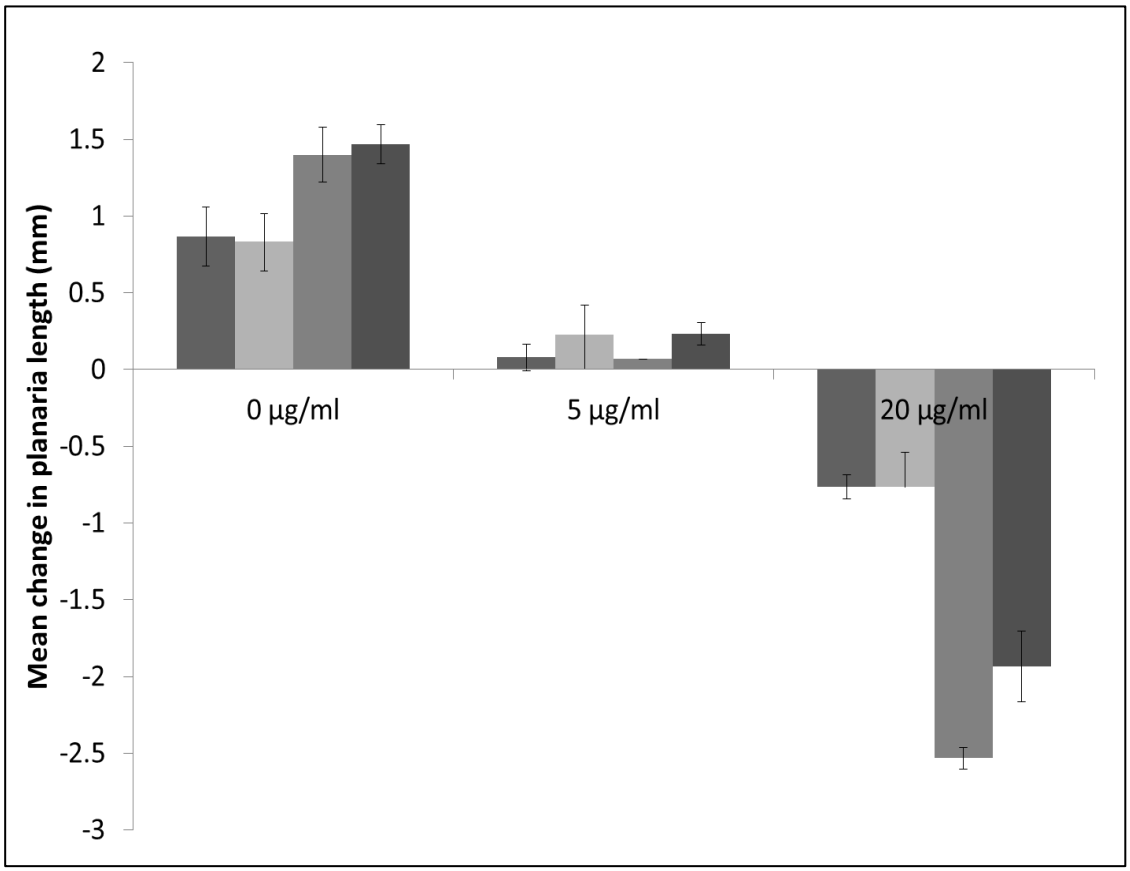

Figure 31. Effect of the organic bark extract of *S. rautanenii* on planaria length.

S. rautanenii root extract affected the planaria growth in a concentration dependent manner (figure 30). At low extract concentration of 5 µg/ml, planaria length was comparable to that of the planaria growing in mineral water that did not contain any treatment as no significant difference was found in the mean change in length of planaria gowing in the presence of 5 µg/ml or 0 µg/ml extract ($p=0.9$). Multivariate analysis of the mean growth of the planaria as affected by the different extract concentrations of *S. rautanenii* root showed they were comparable, ($p=0.870$). This means that the extract of *S. rautanenii* was not toxic to the planaria overall since growth was similar in the instance of treatment and no treatment.

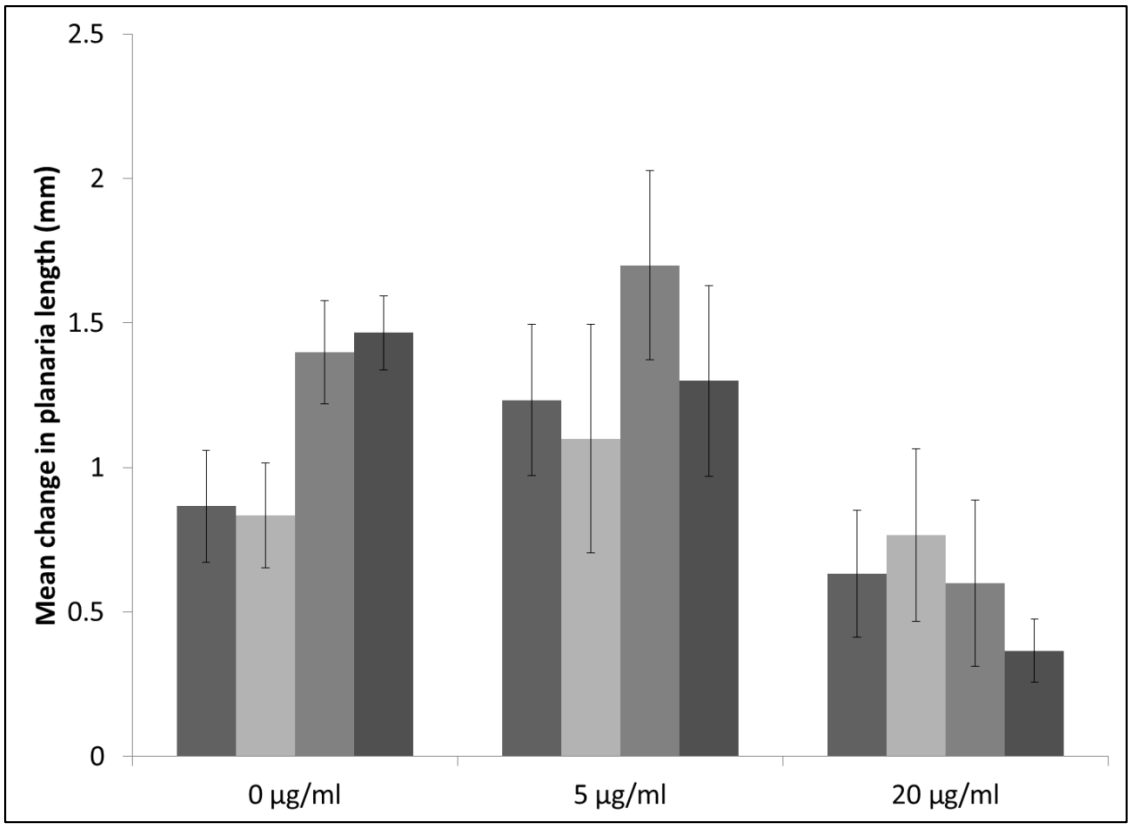

Figure 32. Effect of the organic root extract of *S. rautanenii* on planaria length.

C. mopane bark extract affected planaria growth as seen in figure 31. The extract at different concentrations showed an initial strong antigrowth effect as extract concentration increases, planaria length decreases. However, there is no difference in the effect of the different extract concentrations over the eight observational days, p=0.215. This shows that the bark extract, is not toxic to the planaria.

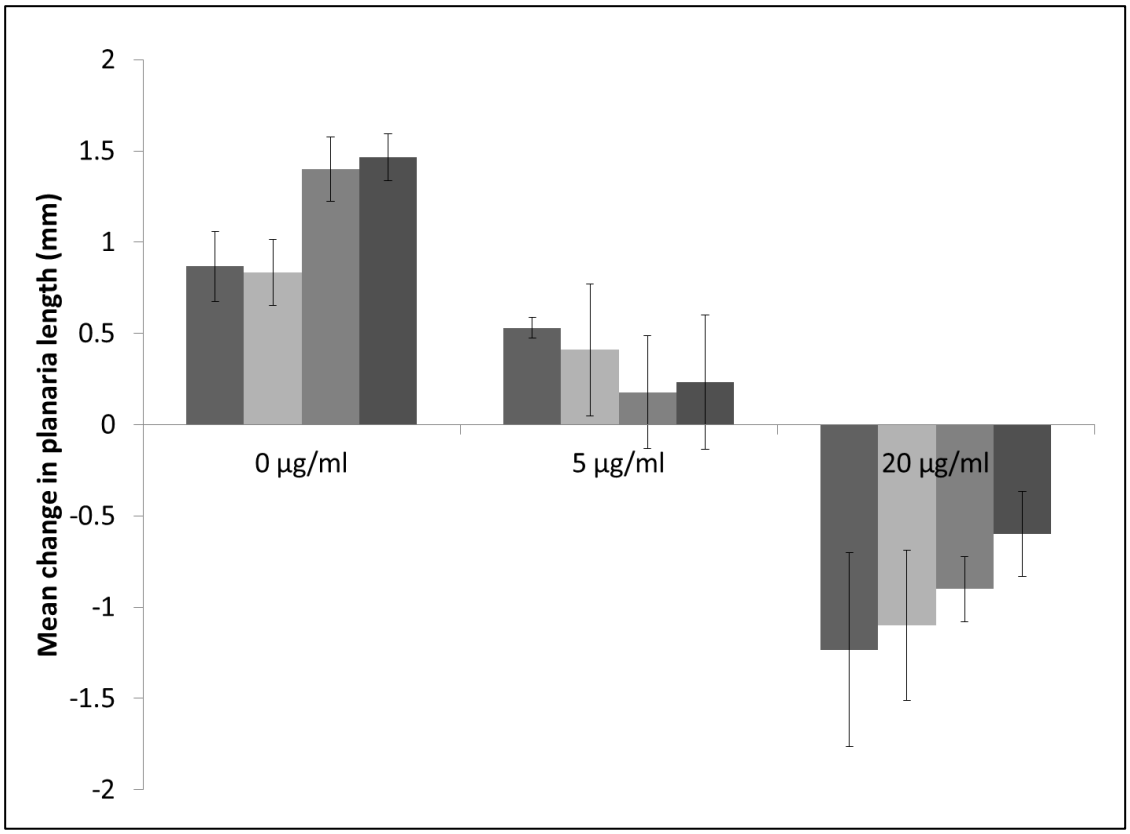

Figure 33. Influence of the organic bark extract of *C. mopane* on planaria length.

The organic extract prepared from the root part of *C. mopane* displayed growth inhibiting and toxicity effects on regenerating planaria, figure 32. At low extract concentration, 5 µg/ml, the extract appears to cause a growth inhibition effect as compared to no treatment. Statistical analysis shows that there is no significant difference in the change in planaria length when data at low extract concentration and no treatment was compared over the eight days, ($p=0.257$). However, when extract concentration was increased to 20 µg/ml, *C. mopane* root extract was very toxic to the

planaria, resulting in planaria death. Overall, there exists a significant change in planaria length as a result of differing concentrations, (p=0.033).

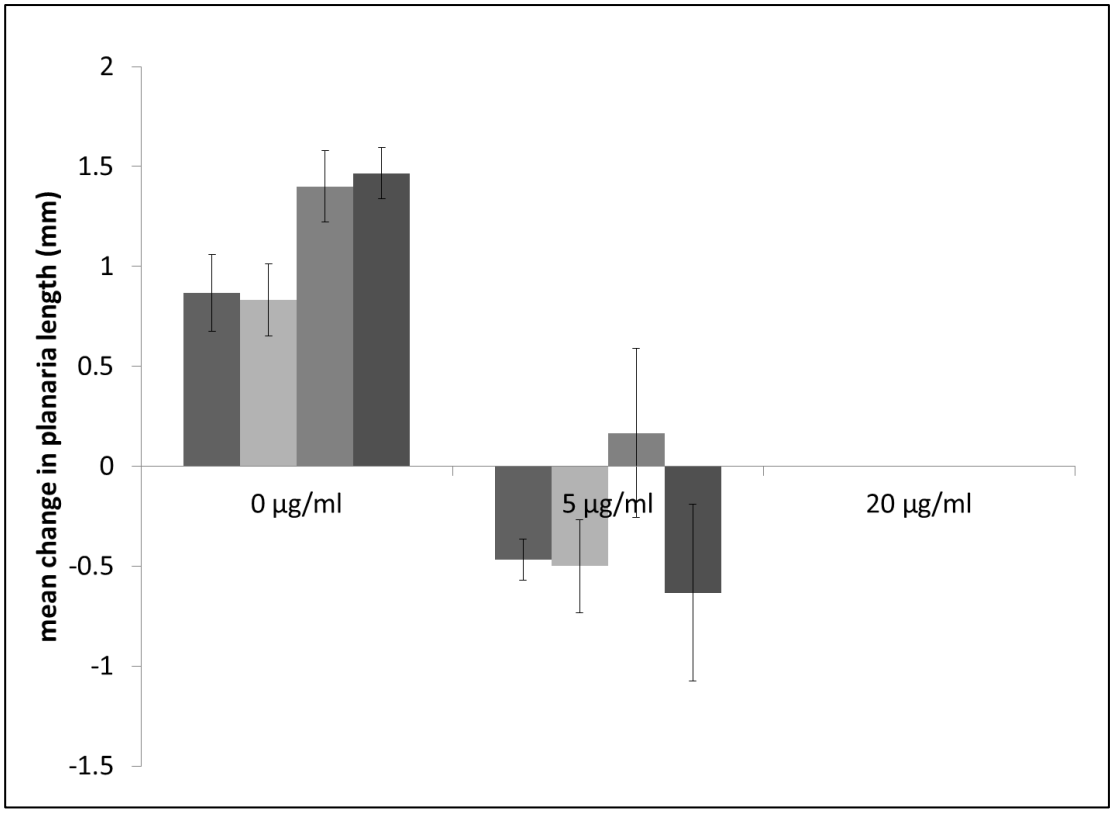

Figure 34. Effect of *C. mopane* root organic extract on planaria length.

CHAPTER FIVE: DISCUSSION

The need for anticancer remedies is undoubtably important, owing to the increasing cancer incidences in Namibia (Namibia Cancer registry, 2011) and world wide (Jemal et al., 2011). Plants have proven to be a valuable resource for novel medicinal entities over the years. For the treatment of diseases such as cancer, plants such as *Catharanthus roseus*, *Camptotheca acuminate*, *Taxus brevifolia*, to mention a few, have revolutionized cancer drug discovery with the inclusion of vinca alkaloids, camphothecin and paclitaxel (Nirmala et al., 2011) as clinically approved treatments for the treatment of different cancer forms such as leukemia, Kaposi sarcoma, solid tumors and others. Many of these plant derived anticancer drugs in clinical use such as paclitaxel were derived from plants with a long history of use within traditional settings for treatment of cancer (Thaware, 2012). Namibia already has a rich heritage of traditional medicinal plants (Cheikhyoussef et al., 2011a; Cheikhyoussef et al., 2011b; Chinsembu, 2009; Hedimbi and Chinsembu, 2012;), however, there is a lack of scientific evidence supporting the efficacy of Namibia's plants.

This study revealed the presence of various phytochemical compounds, in both the root and bark extracts of *S. rautanenii* and *C. mopane*, with the exception of anthraquinones, which were only detected in *S. rautanenii* roots. Alkaloids were detected in all four plant extracts, table 4. The pharmacological properties of alkaloids include antioxidant (Tiong et al., 2013), analgesic and anticancer properties (Lu et al., 2012). However,

alkaloids have been known to be toxic to man and livestock, acting as deterants to prevent the plant from being eaten as food (Harborne, 1998), which may explain their useful applications as medicines. Phenolic compounds such as flavonoids, coumarins, anthraquinones, lignans and curcuminoids (Huang et al., 2009), are well documented for their roles in chemoprevention of cancer (Hail, 2005), as a result of their hydrogen donating properties, radical scavenging ability, metal ion and singlet oxygen species chelators (Ida Christi and Meona, 2013). This diverse group includes 8000 biologically active members (Stalikas, 2007). In a study in Botswana, Madibela et al. (2006), reported that a phenolic compound accounted for 27g/kg tannin content in folder leaves, citing anti-nutritional factors. Both the root and bark of *C. mopane* tested positive for the presence of flavonoids. In a recent report (Kapoor and Pandita, 2013) quantified three flavonoid compounds from the leaves of *C. mopane*. This study also demonstrated the presence of several terpenoidal compounds, table 4. The presence of triterpenens in this study are in agreement with another report, on the phytochemical composition of the chloroform extracts of the bark and seed of *C. mopane,*which revealed the presence of diterpenes, dihydrogrindelic acid and an aldehyde: dihydrogrindelaldehyde (Mebe, 2001). Extracts prepared from the bark and seed of *C. mopane* proved cytotoxic towards breast cancer cells (Mebe, 2001). Triterpenes were also detected in all extracts of both plants. Pharmacological properties of terpenes includes chemoprevention of cancer (Salminen et al., 2008), anti-inflammatory, anticancer and many others (Paduch et al., 2007). A member of the *Euphorbiaceae*

family, *Euphorbia kansui* is a Chinese plant, from which potent anticancer diterpenes have been isolated in the past (Itokawa et al., 2008).

High antioxidant activity of a compound refers to its ability to protect other compounds or molecules from being oxidized. Antioxidant activity is an important property of plant extracts which is especially beneficial for chemoprevention (Spanou et al., 2007). Several members of the *Fabaceae* family have recently been shown to contain strong antioxidant activity (Godevac et al., 2008) using several methods such as DPPH, ABTS radical scavenging and inhibition of liposome peroxidation. Plant products are a good source of antioxidants and have been motivated for inclusion into diets and also the use of medicinal herbal supplements for the reduction of diseases such as cancer and cardiovascular diseases, whose development is influenced by free radicals (Ida Christi and Meona, 2013). Therefore, the findings of this research regarding the high level of antioxidant activity, especially in the root extract of *S. rautanenii* implies that application as a chemopreventative remedy to reduce cancer risk.

Saponins are widely distributed in different plant species and are characterised by their bitter taste and foaming abilities. Saponins are glycosidic in structure comprising an aglycone structure attacted to sugar moieties. Saponins can either be triterpenoid or steroidal, with the former being more common, and they have been shown to confer a variety of physiological activities such as anticancer (Man et al., 2010) and other therapeutic properties in man. Podolak et al. (2010), recently revealed the cytotoxic properties of various saponins. This study revealed the presence of saponins in the plant

extracts, especially in the bark and roots of *S. rautanenii* and *C. mopane* respectively, figure 6. Literature contains reference of triterpenoid saponins in a member of the *Euphorbiaceae* family, (Acharya and Khan, 2013).

The root extracts of both plants had greater antioxidant activity in comparison to the bark extracts. This may be due to the presence of phenols: anthraquinones, coumarins and flavonoids as found in the extracts during phytochemical profiling of plant extracts, table 3. The highest antioxidant activity was observed in *S. rautanenii* root extract, which can be attributed to the presence of phytochemicals, table 4. In another study, a plant called *Acalypha manniana* (Noumedem et al., 2013), belonging to the *Euphorbiaceae* family possessed antioxidant properties. Antioxidant activity is commonly attributed to phenolics (Farombi and Owoeye, 2011; Oueslati et al., 2012). However, analysis of correlation between the antioxidant potential, figure 18 and total phenolic content, figure 15 using the spearman correlation of the four plant extracts, *S. rautanenii*, *C. mopane* root and bark extracts (n=12) revealed a very weak correlation, (-0.112), p=0.729 indicating that the observed antioxidant activity does not depend on the phenolic content of plant extracts. In a study conducted by Motlhanka (2008), in Botswana, both water and methanol extracts of *C. mopane* displayed free radical scavenging activity of close to 70% using the DPPH method at extract concentrations between 50-100µg/ml. A correlation analysis of the total alkaloid content (figure 16) against the antioxidant potential revealed a significant positive association with correlation=0.58 (n-12, p=0.048). This shows that the total alkaloid content of the plant extracts may have been responsible for the observed antioxidant

activity, figure 18. In another study, four alkaloids isolated from the *Catharanthus roseus* plant were demonstrated to possess antioxidant properties with potential applications in the treatment of type 2 diabetes (Tiong et al., 2013).

Vertebrates possess a defense system which circumvents attack by microorganisms. Anti-proteases are ubiquitous and are found free floating within vertebrate's serum and act as a non-specific immune response (Rao and Chakrabarti, 2004). Indeed, phytochemicals such as triterpenes have been known to mediate protease inhibition activity (Hodges et al., 2003). Cancer progression has been linked to reduced pH values within and in the immediate periphery of cancer cells (Rothberg et al., 2013), resulting in increased proteolytic activity of enzymes such as cysteine cathepsins. Proteases such as cysteine cathepsin are pro- angiogenic, involved in degrading extracellular matrix (Dabrosin, 2005) to allow cancer metastasis. The methanolic extracts of *C. mopane* and *S. rautanenii* all displayed anti-protease activity in this study, table 4. Zha et al. (2013) showed the activity of various extracts of several plants, prepared using methanol and chloroform as potent inhibitors of urokinase type plasminogen activator, a protease believed to play an important role in cancer metastasis. The findings of this research imply that plant extracts have antiprotease activity which may be applicable in the control of cancer progression by inhibiting angiogenesis and hence reduce cancer metastasis.

To date, many plant candidates from various traditional systems have been examined for anticancer medicinal properties (Elkady 2012; Muhamad et al., 2011; Wang et al.,

2011). Here, two Namibian plants, *S. rautanenii* and *C. mopane* roots and bark extract displayed different levels of anticancer activity, table 5. *C. mopane* root and bark organic and aqueous and *S. rautanenii* organic bark extracts displayed anticancer activity against the breast MCF-7 cancer cell line with IC_{50} values below 100 µg/ml. The organic *C. mopane* bark and root extracts were of particular interest because they displayed activity below 100 µg/ml extract oncentration for at least two cell lines, and this was moderate activity, according to the Council for Scientific and Industrial Research (CSIR) criteria (Mashele and Kolesnikova, 2010). However, according to the US NCI cancer screening criteria, an extract with observed IC_{50} value above 30 µg/ml is considered as not possessing *in vitro* anticancer activity (Karimi et al., 2012).

All the extracts derived from *C. mopane* and *S. rautanenii* did not exhibit anticancer activity against the renal cell line, TK-10, with IC_{50} values above 100 µg/ml, table 5, despite their phytochemical profiles, table 4 and their use within the traditional settings (table 1). According to Fouche *et al.* (2008), the cell lines, UACC-62, MCF-7 and TK-10 are equally sensitive for detection of anticancer activity, but this research displayed differences in the sensitivity of the three cancer cell lines, as evidenced by the IC_{50} values, table 5. Garner and Eastman (2011), noted that the TK-10 cells have lower metabolism as compared to MCF-7, which might help explain its insensitivity to plant extracts. Furthermore, Shen et al. (2007), describes the renal TK10 cell line as being resistant towards chemotherapeutic agents, even towards cisplatin.

In the traditional setting, water is used as the main solvent for preparation of medicinal portions. However, results obtained in this study, table 5, show that the organic extracts are more potent, in comparison to their aqueous extract equivalents as shown by lower IC_{50} values. This can be attributed to the type of solvent system used for the isolation/extraction of bioactive compounds in plants. Organic solvent usually yield greater activity as compared to the aqueous extracts (Clarkson et al., 2004; Fouche et al., 2008), despite the fact that water is the main solvent of choice in the traditional setting. Laboratory preparation of extracts differs from traditional preparation of medicinal remedies. In the traditional setting, medicinal portion reparation often involve long hours of boiling (Clarkson et al., 2004). *S. rautanenii* is prepared by simple soaking of fresh plant material in water and not boiling while *C. mopane* root is applied in the form of a dry powder to swollen testis, in Namibia's Zambezi region. In the laboratory, extraction methods such as soxlet or ultrasonication extraction methods have repeatedly yielded elevated activities as compared with extracts prepared by simple maceration (Oh et al., 2011). Drying and preparation of extracts in the laboratory setting may lead to the degrading of bioactive compounds, essential for pharmacological activity. Compounds such as terpenoids, and phenols, which were detected during phytochemical screening are volatile and are subject to loss during the extraction process (Omar et al., 2013). Further, the bioactive constituents may exist in an inactive state, which requires activation through metabolic breakdown to release the active compounds (Huttunen et al., 2011), and hence may not show activity *in vitro.*

Phyllanthus niruri, is a member of the *Euphorbiaceae* family, from which a potent anticancer compound, corilagin has been isolated with activity against ovarian cancer (Jia *et al.*, 2013). Meanwhile other members of the *Fabaceae* family have also recently displayed anticancer activity against a range of cell lines (Dos Santos et al., 2012), in Brazil.

An important aspect of cytostatic treatment options for the treatment of cancer is the ability of the chemotherapeutic drug to differentiate between cancer and non cancerous cells. The indiscriminate nature of many cytostatic clinical drugs results in side effects such as dyspnea, hair loss, nausea and vomiting, tiredness and many others (Ihbe-Heffinger et al., 2013). Side effects such as hot flashes, vaginal discharge, irregular menstruations were discussed in a study by Shapiro and Recht (2001) as associated negative effects arising from the use of tamoxifen as a chemotherapeutic drug. Such side effects support the search for alternative less toxic treatment options. Some plants extracts in this study, table 10, appeared to display selective cytotoxicity and did not adversely affect non-cancerous cells in comparison to cancer cells, table 5 and 6. These findings show potential use of plant extract for the development of an alternative treatment option with possibility for inclusion into mainstream usage, provided the mode of action of the plant extracts can further be established *in vitro*, *in vivo* small animal studies and eventually clinical trials.

In vivo models for evaluating anticancer or cytotoxicity properties of extracts or compounds include transplantable cancer models such as sarcoma 180, leukemia L1210

and carcinoma 755, which can be studied in a host such as a rodent (Suggitt and Bibby, 2005). An ideal *in vivo* cancer model should be characterised by a simple, fast, low research cost and modeling close to *in situ* pharmacodynamic activity. Planaria is a fresh water flatworm with the perculiar regenerative abilities not so common in all animals, because only a few other animals are capable of complete tissue regeneration. Planaria regeneration is influenced by its immediate environment, either supporting or retarding the rate of regeneration. Planaria is a multicellular organism, whose growth is rapidly affected by its immediate environment, it offers a cost effective, simple, and sensitive method for *in vivo* toxicity screening (Dushimemaria and Mumbengegwi, 2013). Data in figures 20-23 revealed the effects of bark and root extracts of *S. rautanenii* and *C. mopane* on planaria. Planaria treatment was measured every two days with a fresh change of the extract preparation. Organic root extract of *C. mopane* proved cytotoxic to planaria, causing death of all Planaria. This implies that the products of the metabolic breakdown of *C. mopane* root extracts are toxic at high concentration. The use of planaria in this study for the assessment of cytotoxicity is a novel and cheap method but is disadvantaged by the fact that Planaria does not compare to more frequently used models such as mice. Both planaria and mice are multicellular organisms, able to show the cytotoxic effect of plant extracts and their metabolic end by products, however, mice models are more versatile, allowing assessment of toxicity experienced by different body organs. Toxicity results obtained in this study can serve as a baseline, for further studies using, frequently used experimental models. Other cheap models for investigating cytotoxicity include brine shrimp assays (Ali et al.,

2013), and drosophila flies for anticancer screening and cytotoxicity (Willoughby et al., 2013).

There are three different ideas on the origin of phytochemical compounds (Karuppusamy, 2009), and one school of thought suggests that pharmacological properties displayed by medicinal plants owe them to symbiotic interactions with endophytic fungi that reside in them (Chandra, 2012). In fact, paclitaxel, was isolated from a fungus, *Taxomyces andreanae*, which exists in a symbiotic relationship with *Taxus brevifolia*, (Nikolic et al., 2011) and also the isolation of a gene involved in the biosynthesis of taxol, 10-deacetylbaccatin-III-10-O-acetyl transferase from *Clasdosporium cladosporiodes*, from *Taxus* media (Karuppusamy, 2009). This and many other findings have affirmed the principle that endophytic fungi are able to produce bioactive substances similar to the host plant's secondary metabolites, with biological properties including antioxidants, antineoplastic, cytotoxicity and others, (Wang and Dai, 2011). *C. mopane* has been shown to host a range of endophytes such as *Phoma, Phomopsis* and *Alternaria*, (Wang and Dai, 2011). No research has been conducted to show that the fungal species isolated from *C. mopane* produce secondary metabolites similar to those observed in literature, contained in the plant host. To date, it is not possible to differentiate secondary metabolites such as taxol, produced by the plant or the endophytic fungal taxol produced by organisms such as *Taxomyces andreanae* since both the fungi and it host may share a similar pathway to produce the metabolite (Soliman et al., 2013).

CHAPTER SIX: CONCLUSION AND RECOMMENDATIONS

Namibian indigenous plants, *S. rautanenii* and *C. mopane* were observed to possess phytochemical compounds such as alkaloids, flavonoids, anthraquinones, coumarins and triterpenenes, which are known to confer either chemopreventative activity against cancer initiation and progression or antiproliferative properties. Antiprotease activity and antioxidant activity were also detected in all plant extracts. *In vitro* anticancer activity against renal, breast and melanoma cell lines revealed that the organic root and bark extracts of *C. mopane* had moderate anticancer activity, with the the root organic extract showing the lowest IC_{50} values, especially against the breast MCF7 cell line. Cytotoxicity screening of all extracts against the human fetal lung fibroblast cells displayed low cytotoxicity, while the lowest IC_{50} value was obtained from the organic root extract of *C. mopane*. However, *in vivo* toxicity evaluation of the plant extracts in a

fresh water flatworm revealed that *C. mopane* organic root extract was toxic to planaria at 20 µg/ml, and resulted in the death of the planaria.

In conclusion, the findings of this report provide only moderate support for the use of *C. mopane* for palliation and treatment of cancer in resource poor settings to meet the healthcare needs of patients from distant rural areas who cannot easily access Western treatment or have to endure the long referral process. However, due to the toxicity results displayed by the extracts against Planaria, it is essential to investigate dosaging regimen of the plant extracts, *in vitro*, *in vivo* mechanistic studies and randomized clinical trials are required before recommendation of plant extracts for cancer treatment and palliation.

This research forms the basis for further research on the anticancer properties of the medicinal plants used in this study. Future studies should focus on optimization of the extraction process using different solvent systems to achieve a greater yield of bioactive compounds. The extracts can also be fractionated to identify the bioactive constituents in the extracts as well as any sources of toxicity. In addition, the panel of cancer cell lines can be extended to yield a wide range of activity profiles of the two plant extracts. Furthermore, plant extracts should be evaluated for anticancer activity and toxicity in a mammalian *in vivo* cancer model such as xenographts in mice. Finaly, future research should distinguish between endophytic originating phytochemicals and those originating from the two plants investigated in this study.

REFERENCES

Abdel-Hamid, N. (2009). Update to risk factors for hepatocellular carcinoma. *Int. J. Med. Med. Sci*, 1(3), 033-043.

Acharya, C., & Khan, N. A. (2013). A triterpenoid saponin from the seeds of *Ricinus communis* and its antibacterial activity. *Chem. Nat. compd*, 49(1), 54-57.

Aderonke, S. T., Babatunde, J. A., Adesola, O. T., Okereke, O. U., Innocent, C., Elisha, M. O., Abolaji, O. L., & Abiola, M. O. (2013). Evaluation of retinoblastoma (Rb) and protein-53 (p53) gene expression levels in breast cancer cell lines (MCF-7) induced with some selected cytotoxic plants. *J. Pharmacognosy*

Phytother, 5(7), 120-126.

Agarwal, N., Majee, C., & Chakraborthy, G. S. (2012). Natural herbs as anticancer drugs. *Int. J. Pharmatech. Res*, 4(3), 1142-1153.

Akaydin, M. (2010). Research of UV permeability properties of basic weft knitted structures. *Sci. Res. Essays,* 5(16), 2169-2178.

Akram, M., Alam, O., Usmanghani, K., Akhter, N., & Asif, H. M. (2012). *Colchicum autumnale*: A review. *J. Med. Plants Res,* 6(8), 1489-1491.

Al-Foheidi, M., Al-Mansour, M. M., & Ibrahim, E. M. (2013). Breast cancer screening: review of benefits and harms, and recommendations for developing and low-income countries. *Med Oncol*, 30, 471.

Ali, N., Aleem, U., Shah, S. W. A., Sha, I., Junaid, M., Ahmed, G., Ali, W., & Ghias, M. (2013). Acute toxicity, brine shrimp cytotoxicity, anthelmintic and relevant potentials of fruits of *Rubus fruticosus* Agg. *BMC Complem Altern M,* 13, 138

Aljarrah, K., Mhaidat, N. M., Al-Akhras, M-A, H., Aldaher, A. N., Albiss, B. A., Aledealat, K., & Alsheyab, F. M. (2012). Magnetic nanoparticles sensitize MCF-7 breast cancer cells to doxorubicin-induced apoptosis.*World J Surg*

Oncol, 10,62.

Allam, N. G., El-Shanshoury, A. E. R., Emara, H. A., & Zaky, A. Z. (2012). Biological activity of *Streptomyces noursei* against ochratoxin A producing *Aspergillus niger*. *Afr. J. Biotechnol*, 11(3), 666-677.

American Cancer Society. (2011). *Global Cancer Facts & Figures 2nd Edition.* Atlanta:American Cancer Society.

Amusan, O. O. G., Sukati, N. A., Dlamini, P. S., & Sibandze, F. (2007). Some Sawi phytomedicines and their consituents. *Afr. J Biotechnol*, 6(3), 267-272.

Ana, G. R. E. E., Sridhar, M. K. C., & Asuzu, M. C. (2010). Environmental risk factors and hospital based cancers in two Nigerian cities. *J Public Health Epidemiol*, 2(8), 216-223.

Anetor, J. I., Anetor, G. O., Udah, D. C., & Adeniyi, F. A. A. (2008). Chemical carcinogenesis and chemoprevention: Scientific priority are in rapidly industrializing developing countries. *Afr. J. Environ. Sci. Technol*, 2(7),150-156.

Atanda, S. A., Pessu, P. O., Agoda, S., Isong, I. U., Adekalu, O. A., Echendu, M. A., & Falade,T. C. (2011). Fungi and mycotoxins in stored foods. *Afr. J. Microbial. Res,* 5(25), 4373-4382.

Badrhadad, A., Piri, Kh., & Mansouri, K. (2012). *In vitro* anti-angiogenesic activity of fractions from hydroalcoholic extract of *Elaeagnus angustifolia* L. flower and *Nepeta crispa* L. arial part. *J. Med. Plants. Res*, 6(31), 4633-4639.

Balunas, M. J., & Kinghorn, D. A. (2005). Drug discovery from medicinal plants. *Life Sci*, 78, 431-441.

Bellik, Y., Boukraa, L.,Alzahrani, H. A., Bakhotmah, B. A., Abdellah, F., Hammoudi, S. M., & Iguer-Ouada, M. (2013). Molecular mechanism underlying anti-inflammatory and anti-allergic activities of phytochemicals: an update. *Molecules*, 18, 322-353.

Bernard, S. A., & Olayinka, O. A. (2010). Search for a novel antioxidant, Anti-inflammatory /analgesic or anti-proliferative drug: Cucurbitacins hold the ace. *J. Med. Plant. Res*, 4(25), 2821-2826.

Beytur, A., Ciftci, O., Oguz, F., Oguzturk, H., & Yilmaz, F. (2012). Montelukast

attenuates side effects of cisplatin including testicular, spermatological, and hormonal damage in male rats. *Cancer chemoth Pharm,* 69, 207-213.

Bhanot, A., Sharma, R., & Noolvi, M. N. (2011). Natural sources as potential anti-cancer agents: A review. *International journal of phytomedicine*, 3, 09-20.

Bokhari, F. M., & Aly, M. M. (2013). Unexpected hazard due to fumosins contaminating herbal teas used traditionally by Saudi people. *Afr. J. Microbial. Res*, 7(1), 35-40.

Bosch, F. X., Lorincz. A., Munoz, N., Meiier, C. J. L., & Shah, K. V. (2002). The causal relation between human papillomavirus and cervical cancer. *J. Clin. Pathol*, 55, 244-265.

Boubaker, J., Mansour, H. B., Ghedira, K., & Chekir-Ghedira, L. (2011). Antimutagenic and free radical scavenger effects of leaf extracts from *Accacia salicina*. *Ann. Clin. Microbiol. Antimirob,* 10, 37-47.

Brilhante, O., Stumpp, T., & Miraglia, S. M. (2011). Long-term testicular toxicity caused by doxorubicin treatment during pre-pubertal phase. *Int. J. Med. Med. Sci,* 3(2), 52-60.

Brudnak, M. (2000). Cancer-preventing properties of essential oil monoterpenes D-limonene and perillyl alcohol. Retrieved from http://www.freeorangeoil.com/html/dr_brudnak.php on 28.11.2013.

Butler, M. S. (2005). Natural products to drugs: natural product derived compounds in clinical trials. *Nat. Prod. Rep.*, 22, 162-195.

Calitz, T. (2013). *Breast cancer most common cancer among Namibian women.* Retrieved 10.30.2013 from *http://www.namibindependent.com.na/cms_data/dfault/photos/stories/629/3179151.pdf#page=15*.

CDC in Namibia. Retrieved from *http://www.cdc.gov/globalhealth/countries/namibia/pdf/namibia.pdf.* on 18.11.2013

Chan, W. K., Cheung, C. C. H., Law, H. K. W., Lau, Y. L., & Chan, G. C. F. (2008). *Ganoderma lucidum* polysaccharides can induce human monocytic leukemia cells into dentritic cells with immune-stimulatory function. *J. Hematol. Oncol*, 1, 9.

Chandra, S. (2012). Endophytic fungi: novel sources of anticancer lead molecules.

Appl. Microbiol. Biotechnol, 95, 47-59.

Cheikhyoussef, A., Mapaure, I., & Shapi, M. (2011). The use of some indigenous plants for medicinal and other purposes by local communities in Namibia with emphasis on Oshikoto region: A review. *Res. J. Med. Plant,* 5(4), 406-419.

Cheikhyoussef, A., Shapi, M., Matengu, K., & Mu Ashekele, H. (2011). Ethnobotanical study of indigenous knowledge on medicinal plant use by traditional healers in Oshikoto region, Namibia. *J. Ethnobiol. Ethnomed,* 7,10.

Chinsembu, K. C. (2009). Model and experiences of initiating collaboration with traditional healers in healers in validation of ethnomedicines for HIV/AIDS in Namibia. *J. Ethnobiol. Ethnomed,* 5, 1-13.

Chinsembu, K. C., & Hedimbi, M. (2010). An ethnobotanical survey of plants used to manage HIV/AIDS opportunistic infections in Katima Mulilo, Caprivi region, Namibia. *J. Ethnobiol. Ethnomed,* 6,25.

Chinsembu, K. C., Hedimbi, M., & Mukaru, W. C. (2011). Putative medicinal properties

of plants from the Kavango region, Namibia. *J. Med. Plants. Res,* 5(31), 6787-

6797.

Chouhan, R., & Bajpai, A. K. (2009). Real time in vitro studies of doxorubicin release from PHEMA nanoparticles. *J. Nanobiotechnology,* 7,5.

Ciofani, G., Danti, S., D'Alessandro, D., Moscato, S., Petrimi, M., & Menciassi, A. (2010). Barium titanate nanoparticles: Highly cytocompatible dispersions in glycol-chitosan and doxorubicin complexes for cancer therapy. *Nanoscale Res Lett,* 5,1093-1101.

Clarkson, C., Maharaj, V. J., Crouch, N. R., Grace, O. M., Pillay, P., Matsabisa, M. G., Bhagwandin, N., Smith, P. J., & Folb, P. I. (2004). *In vitro* antiplasmodial activity of medicinal plants native o or naturalized in South Africa. *J. ethnopharmacol,* 92, 177-191.

Crew, K. D., & Neugut, A. I. (2006). Epidemiology of gastric cancer. *World. J. Gastroenterol,* 12(3), 354-362.

Crum-Cianflone, N., Hullsiek, K. H., Marconi, V., Weintrob, A., Ganesan, A., Barthel, R. V., Fraser, S., Agan, B. K., & Wegner, S. (2009). Trends in he incidence of

cancers among HIV-infected person and the impact of antiretroviral therapy: A 20-year cohort study. *AIDS*, 23(1), 41-50.

Curtis, B., & Mannheimer, C. (2005). *Tree atlas of Namibia.* Windhoek: National Botanical Research Institute.

Dabrosin, C. (2005). Sex steroid regulation of angiogenesis in breast tissue. *Angiogenesis*, 8, 127-136.

De Beer, I. H., Gelderblom, H. C., Schellekens, O., Gaeb, E., van Rooy, G., McNally, A., Wit, F. W., de Wit, R., & Tobias, F. (2012). University students and HIV in Namibia: an HIV prevalence survey and a knowledge and attitude survey. *J. Int. AIDS. Soc*, 1, 9.

De, S., Dey, Y. N., & Ghosh, A. K. (2010). Phytochemical investigation and chromatographic evaluation of the tuber of *Amorphaphallus paeonifolius* (Araceae). *Int. J. Pharm. Biomed. Res,* 1(5), 150-157.

Deribew, K., & Petros, B. (2012). Efficacy of praziquantel for the treatment of schistosomiasis in Ethiopia. *Int. J. Med. Med. Sci*, 5(3), 131-139.

Devi, P. G., Chakraborty, P. K., & Dasgupta, D. (2009). Inhibition of a Zn(II)-containing enzyme, alcohol dehydrogenase, by anticancer antibiotics,

mithramycin and chromomycin A_3. *J. Biol. Inorg. Chem,* 14, 347-359.

Ding, B-Y., Zhang, W., Wu, X., Wanh, X., Fan, W., Gao, S., Gao, J., Ma, L-I., Ding, X-Y., & Hao, Q. (2011). Biodegradable methoxy poly (ethylene glycol)-poly (lactide) nanoparticles for controlled delivery of dacarbazine: preparation, characterization and anticancer activity evaluation. *Afr. J. Pharm. Pharacol,* 5(11), 1369-1377.

dos Santos Junior, H. M., Oliveira, D. F., de Carvalho, D. A., Pinto, J. M. A., Campos, V. A. C., Mourao, A. R. B., Pessoa, C., de Moraes, M. O., & Costa-Lotufo, L. V. (2010). Evaluation of Native and exotic Brazilian plants for anticancer activity. *J. Nat. Med,* 64, 231-238.

Doughari, J. H., Human, I. S., Bennade, S., & Ndakidemi, P. A. (2009). Phytochemicals as chemotherapeutic agents and antioxidants: Possible solution to the control of antibiotic resistant verocytotoxin producing bacteria. *J. Med. Plant. Res,* 3(11), 839-848.

Dushimemaria, D., & Mumbengegwi, D. (2013). Palliative treatment of cancer in

resource poor settings: Traditional medicine perspective. *Unpublished manuscript.*

Du Preez, I., Nepolo, E., Siyengwa, R., Shapi, M., Cheikhyoussef, A., & Mumbengegwi, D. (2011). Study on indigenous medicinal knowledge in Caprivi Region. *Multidisciplinary research center. Windhoek.*

Eaton, D. L., & Gallagher, E. P. (1994). Mechanism of aflatoxin carcinogenesis. *Annu. Rev. Pharmacol. Toxicol*, 34, 135-172.

Edeoga, H. O., Okwu, D. E., & Mbaebie, B. O. (2005). Phytochemical constituents of some Nigerian medicinal plants. *Afr. J. Biotechnol*, 4(7), 685-688.

Elkady, A. I. (2012). Crude extract of *Nigella sativa* inhibits proliferation and induces apoptosis in human cervical carcinoma HeLa. *Afr. J. Biotechnol*, 11(64), 12710-12720.

Elujoba, A. A., Odeleye, O. M., & Ogunyemi, C. M. (2005). Traditional medicine development for medical and dental primary health care delivery system in Africa. *Afr. J. Trad. CAM*, 2(1), 46-61.

Endogenous hormones and breast cancer collaborative group. (2003). Body mass index,

serum sex hormones , and breast cancer risk in postmenopausal women. *J. Natl. Cancer. Inst*, 95(16), 1218-1226.

Ernst, E. (2001). Complementary therapies in palliative cancer care. *Cancer,* 91(1), 2181-2185.

Everatt, R., Tamosiunas, A., Virviciute, D., Kuzmickiene, I., & Reklaitiene, R. (2013). Consumption of alcohol and risk of cancer among men: a 30 year cohort study in Lithuania. *Eur. J. Epidemiol*, 28, 383-392.

Farombi, E. O., & Owoeye, O. (2011). Antioxidant and chemopreventative properties of *Vernonia amygdalina* and *Garcinia biflavonoid*. *Int. J. Environ. Res. Publ. Health*, 8 2533-2555.

Farombi, E. O. (2004). Diet-related cancer and prevention using anticarcinogens. *Afr. J. Biotechnol*, 3(12), 651-661.

Farombi, E. O. (2003). African indigenous plants with chemotherapeutic potential and biotechnological approach to the production of bioactive prophylactic agents. *Afr. J. Biotechnol*, 2(12), 662-671.

Folashade, K. O., Omoregie, E. H., & Ochogu, A. P. (2012). Standardization of herbal

medicines-A review. *Int. J. Biodivers. Conserv*, 4(3), 101-112.

Fouche, G., Cragg, G. M., Pillay, P., Kolesnikova, N., Maharaj, V. J., & Senabe, J. (2008). *In vitro* anticancer screening of South African plants. *J. Ethnopharmacol*, 119, 455-461.

Fransson, P., Tavelin, B., & Widmark, A. (2001). Reliability and responsiveness of a prostate cancer questionnaire fot radiotherapy-induced side effects. *Support. Care. Cancer,* 9, 187-198.

Fujii, M., Yusa, A., Yokoyama, Y., Kokuryo, T., Tsunoda, N., Oda, K., Nagino, M., Ishimaru, T., Shimoyama, Y., Utsunomiya, H., Iwara, H., Itoh, Y., Itoh, J., Kannagi, R., & Kyogashima, M. (2010). Cytoplasmic expression of the JM403 antigen GIcA-GIcNH$_3^+$ on he heparin sulfate glycosaminoglycan in mammary carcinomas-a novel proliferative biomarker for breast cancers with high malignancy. *Glycoconj. J*, 27, 661-672.

Ganesan, A. (2008). The impact of natural products upon modern drug discovery. *Curr. Opin. Chem. Biol*, 12, 306-317.

Ganesan, R. M., & Muthuchelian, K. (2011). Antitumor potential of an acetogenin

isolated from the seed extract of *Annona sequamosa linn*. *J. Cancer. Res. Exp. Oncol,* 3(8), 95-104.

Garner, K. N., & Eastman, A. (2011). Variations in Mre11/Rad50/Nbs1 status and DNA damage-induced S-phase arrest in the cell lines of the NCI60 panel. *BMC Cancer,* 11, 206.

Godevac, D., Zdunic, G., Savikin, K., Vajs, V., & Menkovic, N. (2008). Antioxidant activity of nine fabaceae species growing in Serbia and Montenegro. *Fitoterapia*, 79, 185-187.

González-Arriagada, W. A., de Andrade, M. A. C., Ramos, L. M. A., Bezerra, J. R. S., Santos-Silva, A. R., & Lopes, M. A. (2013). Evaluation of an educational video to improve the understanding of radiotherapy side effects in head and neck cancer patients. *Support. Care. Cancer,* 21, 2007-2015.

Govindappa, M., Kumar, A., & Santoyo, G. (2011). *Crotalaria pallida* extracts as a putative HIV-protease inhibitors. *J. Res. Biol*, 4, 285-291.

Graz, F. P. (2002). Description and ecology of *Schinziophyton rautanenii* (Schinz) aaRadcl.-Sm. In Namibia. *Dinteria*, 27, 19-35.

Gunassekaran, G. R., Gayathri, R., Priya, D. K. D., Murugan, S., & Sakithsekaran, D. (2010). Protective role of gossypol against N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) induced gastric carcinogenesis in experimental rats. *Int. J. Med. Med. Sci*, 2(4), 121-127.

Hail, N. (2005). Mitochondria: A novel target for the chemoprevention of cancer. *Apoptosis*, 10, 687-705.

Hamid, A. A., Aiyelaagbe, O.O., Usman, L. A., Ameen, O. M., & Lawal, A. (2010). Antioxidants: Its medicinal and pharmacological applications. *Afr. J. Pure. Appl. Chem*, 4(8), 142-151.

Harding, R. (2008). Palliative care in resource-poor settings: Fallacies and misapprehesnsions. *Commentary*, 36(5), 515-517.

Hashemi, M., Behrangi, N., Borna, H., & Entezari, M. (2011). Evaluating new targets for natural anticancer molecules through bioinformatics tools. *World academy of science, engineering and technology*, 59, 477-480.

Harborne, J. B. (1998). *Phytochemical methods: A guide to modern techniques of plant analysis*. 3ed. London:Chapman & hall.

Hedimbi, M., & Chinsembu, K. C. (2012). Ethnomedicinal study of plants used to manage HIV/AIDS-related disease conditions in the Ohangwena region, Namibia. *Int. J. Med. Plant. Res,* 1(1), 004-011.

Hodges, L. D., Kweifio-Okai, G., & Macrides, T. A. (2003). Antiprotease effect of anti-inflammatory lupeol esters. *Mol. Cell. Biol,* 252, 97-101.

Hodzic, Z., Pasalic, H., Memisevic, A., Srabovic, M., Saletovicm M., & Poljakovic, M. (2009).The Influence of total phenols content on antioxidant capacity in the whole grain extracts. *Eur. J. Sci. Res*, 28(3), 477-477.

HogenEsch, H., & Nikitin, A. Y. (2012). Challenges in pre-clinical testing of anti-cancer drugs in cell culture and in animal models. *J. Control. Release*, 164, 183-186.

Huang, W. Y., Cai, Y. Z., & Zhang, Y. (2009). Natural phenolic compounds from medicinal herbs and dietary plants: Potential use for cancer prevention. *Nutr. Cancer,* 62(1), 1-20.

Human development report. (2013). *The rise of the south: Human progress in a diverse world.* New York: United Nations Development Programme.

Huttunen, K. M., Raunio, H., & Rautio, J. (2011). Prodrugs-from serendipity to retional design. *Pharmacol. Rev*, 63(3), 750-771.

Ida Christi, V. E., & Meona, S. (2013). Comparative in-vitro antioxidant study of three species from *Euphorbiaceae* family. *Int. J. Res. Pharma. Chem*, 3(2), 228-234.

Ihbe-Heffinger, A., Paessens, B., Berger, K., Shlaen, M., Bernard, R., von Schilling, C., & Peschel, C. (2013). Impact of chemotherapy-induced side effects on medical care usage and cost in German hospital care-an observational analysis on non-small-cell lung cancer patients. *Support. Care. Cancer*, 21, 1665-1675.

Imaga, N. O. A. (2010). Use of phytomedicines as effective therapeutic agents in sickle cell anemia. *Sci. Res. Essays*, 5(24), 3803-3807.

Itokawa, H., Morris-Natschke, S. L., Akiyama, T., & Lee, K-H. (2008). Plant-derived natural product research aimed at new drug discovery. *J. Nat. Med*, 62, 263-280.

Iwalelwa, E. O., McGaw, L. J., Naidoo, V., & Eloff, J. N. (2007). Inflammation: the foundation of diseases and disorders. A review of phytomedicines of South African origin used to treat pain and inflammatory conditions. *Afr. J.*

Biotechnol, 6(25), 2868-2885.

Jahanshahi, M., & Babaei, Z. (2008). Protein nanoparticle: A unique system as drug delivery vehicles. *Afr. J. Biotechnol,* 7(25),4926-4934.

Jemal, A., Bray, F., Center, M. M., Ferlay, J., Ward, E., & Forman, D. (2011). Global cancer statistics. *CA Cancer J Clin,* 61, 69-90.

Jia, L., Jin, H., Zhou, J., Chen, L., Lu, Y., Ming, Y., & Yu, Y. (2013). A potential anti-tumor herbal medicine, corilagin, inhibits ovarian cancer cell growth through blocking the TGF-B signaling pathways. *BMC. Complem. Alter. Med,* 13, 33.

Jing, J. L., Mohamed, M., Rahmat, A., & Bakar, M. F. A. (2010). Phytochemicals, antioxidant properties and anticancer investigations of the different parts of several gingers species (*Boesenbergia rotunda, Boesenbergia pulchella var attenuata* and *Boesenbergia armeniaca*). *J. Med. Plant. Res,* 4(1), 27-32.

Jung, H., Sinnarajah, A., Enns, B., Vononey, J-P., Murray, A., Pelletier, G., & Wu, J. S-Y. (2013). Managing brain metastases patients with and without radiotherapy: initial lessons from a team-bases consult service through a multidisciplinary integrated palliative oncology clinic. *Support. Care. Cancer*, 21, 3379-3386.

Kaira, C. (2013, November 13). Namibians drink more, spend less on education now. *The Namibian,* p.1.

Kapoor, B. B. S., & Pandita, S. (2013). Flavonoid contents from some exotic tree species growing in Rajasthan desert. *Indian J. Pharm. Biol. Res,* 1(3), 20-22.

Karimi, E., Oskoueian, E., Hendra, R., Oskoueian, A., & Jaafar, H. Z. E. (2012). Phenolic compounds characterization and biological activities of *Citrus aurantium* Bloom. *Molecules,* 17, 1203-1218.

Karuppusamy, S. (2009). A review on trends in production of secondary metabolites from higher plants in vitro tissue, organ and cell cultures. *J. Med. Plant. Res,* 3(13), 1222-1239.

Kashyap, C. P., Tikka,B., Sharma, S., Kumari, S., Verma, P., Sharma, S., & Arya, V. (2011). Human cancer cell line-A brief communication. *J. Chem. Pharm. Res,* 3(6), 514-520.

Kavanos, P. (2006). The rising burden of cancer in the developing world. *Ann. Oncol,* 17(8), viii15–viii23

Kelland, L. (2007). Targeting the limitless replicative potential of cancer: the

telomerase/telomere pathway. *Clin. Cancer. Res*, 13, 4960-4963.

Khan, N. P., Pandith, A. A., Hussain, M. U. I., Yousuf, A., Khan, M. S., Siddiqi, M. A., Wani, K. A., & Mudassar, S. (2011). Loss of heterozygosity (LOH) of deleted in colorectal cancer (DCC) gene and predisposition to colorectal cancer: Significant association in colorectal cancer patients of Kashmir. *J. Cancer. Res. Exp. Oncol*, 3(8), 88-94.

Klaunig, J. E., Wang, Z., Pu, X., & Zhou, S. (2011). Oxidative stress and oxidative damage in chemical carcinogenesis. *Toxicol. Appl. Pharm*, 245, 86-99.

Kremsdorf, D., Soussan, P., Paterlini-Brechot, P., & Brechot, C. (2006). Hepatitis B virus-related hepatocellular carcinoma: paradigms for viral-related huma carcinogenesis. *Oncogene*, 25, 3823-3833.

Kretzschmann, V. K., & Furst, R. (2013). Plant-derived vascular disrupting agents: compounds, actions, and clinical trials. *Phytochem. Rev.* DOI 10.1007/s11101-013-9304-6.

Kris, M. G., Gralla, R. J., Clark, R. A., Tyson, L. B., & Groshen, S. (1987). Antiemetic

control and prevention of side effects of anti-cancer therapy with lorazepam or diphenhydramine when used in combination with metoclopramide plus dexamethasone. *Cancer,* 60, 2816-2822.

Kummalue, T., Suntiparpluacha, M., & Jiratchariyakul, W. (2012). Antiproliferative activity of combination of thai herbal remedy and chemotherapeutic agents on human cancer cell lines. *J. Med. Plant. Res,* 6(2), 200-205.

Kupper, J., Pentsch, K., Mittelholzer, A., Artho, R., Meyer, S., Kupferschmidt, H., & Naegeli, H. (2010). A fatal case of autumn crocus (*Colchicum autumnale*) poisoning in a heifer: confirmation by mass-spectrophotometric colchicine detection. *J. Vet. Diagn. Invest,* 22, 119-122.

Lam, P. B., Burga, L. N., Wu, B. P., Hofstatter, E. W., Lu, K. P., & Wulf, G. M. (2008). Prolyl isomerase Pin I is highly expressed in Her2-positive breast cancer and regulates erbB2 protein stability. *Mol. Cancer,* 7, 91-103

Lamani, D. S., Reddy, K. R. V., & Naik, H. S. B. (2010). An efficient synthesis and DNA binding interaction study of some novel heterocyclic fused-pyarzolequinolines: A potent antimicrobial agent. *Afr. J. Pure. Appl. Chem*,

4(11), 247-255.

Latha, S., Selvamani, P., Thirunavukkarasu, C., Kadambavadani, R., & Rathi, K. (2012). Comparative evaluation of antioxidant activity of crude extracts of commiphora spp and formulated polyherbal tablets. *J. Med. Plant. Res*, 6(43), 5532-5545.

Lebrun, J-J. (2012). The dual role of TGFβ in human ancer: From tumor suppression to cancer metastasis. *ISRN molecular biology,* 1-28.

Lee, M. M., Lin, S. S., Wrensch, M. R., Adler, S. R., & Eisenberg, D. (2000). Alternative therapies used by women with breast cancer in four ethnic populations. *J. Natl. Cancer. Inst*, 92, 42-7.

Lee, P. N., & Hamling, J. (2009). Systematic review of the relation between smokeless tobacco and cancer in Europe and North America. *BMC. Med*, 7, 36.

Lone, A. A., Ganai, S. A., Ahanger, R. A., Bhat, H. A., Bhat, T. A., & Wani, I. A. (2013). Free radicals and antioxidants: Myths, facts and mysteries. *Afr. J. Pure. Appl. Chem*, 7(3), 91-113.

Lu, J-J., Bao, J-L., Chen, X-P., Huang, M., & Wang, Y-T. (2012). Alkaloids isolated

from natural herbs as the anticancer agents. *Evid. Based Complement. Alternat. Med.* doi:10.1155/2012/485042.

Machana, S., Weerapreeyakul, N., & Barusrux, S. (2012). Anticancer effects of the extracts from *Polyalythia evecta* against human hepatoma cell line (HepG2). *Asian. Pac. Trop. Biomed*, 368-374.

Maciasz, R. M., Arnold, R. M., Chu, E., Park, S. Y., White, D. B., Vater, L. B., & Schenker, Y. (2013). Does it matter what you call it? A randomized trial of language used to describe palliative care services. *Support. Care. Cancer,* 21, 3411-3419.

Madibela, O. R., Seitshiro, O., & Mochankana, M. E. (2006). Deactivation effects of polyethylene glycol (PEG) on in vitro dry matter digestibility of *Colophospermum mopane* (Mophane), Acacia Browse trees in Botswana. *Pakistan journal of nutrition,* 5(4), 343-347.

Maheshwari, P., Garg, S., & Kumar, A. (2008). Taxoids: Biosynthesis and *in vitro* production. *Biotechnol. Mol. Biol. Rev*, 3(4), 071-087.

Mahavorasirikul, W., Viyanant, V., Chaijaroenkul, W., Itharat, A., & Na-Bangchang, K.

(2010). Cytotoxic activity of thai medicinal plants against human cholangiocarcinoma, laryngeal and hepatocarcinoma cells *in vitro. BMC. Complem. Altern. Med,* 10, 55.

Man, S., Gao, W., Zhang, Y., Huang, L., & Liu, C. (2010). Chemical study and medical application of saponins as anti-cancer agents. *Fitoterapia,* 81, 703-714.

Marie, M. A. M., & Lory, S. (2012). *Heliobacter pylori* and asthma pathogenesis, role of HP-NAP? *Afr. J. Microbiol. Res,* 6(3), 481-485.

Marnitz, S., Stuschke, M., Bohsung, J., Moys, A., Wurm, R., & Budach, V. (2002). Intraindividual comparison of conventional three dimensional radiotherapy and intensity modulated radiotherapy in the thereapy of locally advanced non-small cell lung cancer. *Strahlenther onkol,* 178, 651-8

Mashele, S., & Kolesnikova, N. (2010). *In vitro* anticancer screening of *Asparagus laricinus* extracts. *Pharmacologyonline,* 2, 246-252.

Mastrangelo, G., Fadda, E., & Marzia, V. (1996). Polycyclic aromatic hydrocarbons and cancer in man. *Environ. Health. Perspect*, 104(11), 1166-1170.

Matsumura, Y., & Kataoka, K. (2009). Preclinical and clinical studies of anticancer agents-incorporating polymer micelles. *Cancer Sci*, 100(4), 572-579.

Mboya, R., Tongoona, R., Yobo, K. S., Derera, J., Mudhara, M., & Langyuntuo, A. (2011). The quality of maize stored using roof and sack storage methods in katumba ward, rungwe district, Tanzania: implications on household food security. *J. Stored. Prod. Postharvest. Res*, 2(9), 189-199.

Mebe, P. P. (2001). Diterpenes from the bark and seeds of *Colophospermum mopane*. Abstract. *Phytochemistry*, 57(4), 537-571.

Meulmeester, E., & Dijike, P. T. (2011). The dynamic role of TGF-β in cancer. *J. Pathol*, 223, 205-218.

Ministry of Health and Social Services. (2008). *Estimates and projections of the impact of HIV/AIDS in Namibia.* Windhoek.

Min, K., & Ebeler, S. E. (2008). Flavonoid effects on DNA oxidation at low concentrations relevant to physiological levels. *Food. Chem. Toxicol*, 46, 96-104.

Mohanlall, R., Odhav, B., & Mohanlall, V. (2013). The effects of thermal processing on

fumonisin B1 (FB1) levels in maize-based foods. *Afr. J. Food. Sci,* 7(3), 45-50.

Motlhanka, D, M. (2008). Free radical scavenging activity of selected medicinal plants of Eastern Bostwana. Abstract. *Pak. J. Biol. Sci*, 11(5), 805-808.

Muhamad, S., Pihie, A. H. L., Latif, J., Rha, C., & Sambandan, T. G. (2011). Induction of apoptosis in MCF-7 via the Caspase pathway by longilactone from *Eurycoma longifolia* Jack. *Res. Pharm. Biotech*, 3(1), 1-10.

Mutee, A. F., Salhimi, S. M., Ghazali, F. C., Al-Hassan, F. M., Lim, C. P., Ibrahim, K., & Asmawi, M. Z. (2012). Apoptosis induced in human breast cancer cell line by *Acanthaster planci* starfish extract compared to tamoxifen. *Afr. J. Pharm. Pharmacol*, 6(3), 129-134.

Namibia 2011 population and housing census preliminary results. Retrieved from http://www.gov.na/documents/10180/34849/2011_Preliminary_Result.pdf/0ea026d4-9687-4851-a693-1b97a1317c60 on 5/6/2012.

Namibian cancer registry. (2009). *Cancer in Namibia 2000-2005*. Cancer association of Namibia:Windhoek.

Namibian cancer registry. (2011). *Cancer in Namibia 2006-2009*. Cancer association of Namibia: Windhoek.

Nasr, A., Lauterio, T. J., & Davis, M. W. (2011). Unapproved drugs in the United States and the food drug administration. *Adv. Ther*, 28(10), 842-856.

Neeraj, G. K., Sharad, M., Tejram, S., Abhinav,M., Suresh, V., & Rajeev, T. K. (2011). Evaluation of anti-apoptotic avtivity of different dietary ntioxidants in renal cell carcinoma against hydrogen peroxide. *Asian. Pac. J. Trop. Biomed*, 1(1), 57-63.

Nickols, B. E., Nkalamo, D., & Whitcomb, B. W. (2012). Density of drinking establishments and HIV prevalence in a migrant town in Namibia. *AIDS behav,* 16, 829-834.

Nikolic, V. D., Savic, I. M., Savic, I. M., Nikolic, L. B., Stankovic, M. Z., & Marinkovic, V. D. (2011). Paclitaxel as an anticancer agent: isolation, activity, synthesis and stability. *Cent. Eur. J. Med*, 6(5), 527-536.

Nirmala, J,M ., Samundeerswari, A., & Sankar, D. P. (2011). Natural plant resources in anti-cancer therapy-A review. *Res. Plant. biol*, 1 (3), 01-14.

Noumedem, J. A. K., Tamokou, J. D. D., Teke, G. N., Momo, R. C. D., Kuete, V., &

Kuiate, J. R. (2013). Phytochemical analysis, antimicrobial and radical-scavenging properties of *Acalypha manniana* leaves. *Springerplus,* 2, 503.

Oh, S-H., Ahn, J., Kang, D-H., & Lee, H-Y. (2011). The effect of ultrasonificated extracts of *Spirulina maxima* on the anticancer activity. *Mar Biotechnol*, 13, 205-214.

Ojieh, A. E., Adegor, E. C., & Lawrence, E. O. (2013). Preliminary phytochemical screening, analgesic and anti-inflammatory properties of *Celosia isertii. Eur. J. Med*, 3(3), 369-380.

Okigbo, R. N., Anuagasi, C. L., & Amadi, J. E. (2009). Advances in selected medicinal and aromatic plants indigenous to Africa. *J. Med. Plant. Res*, 3(2), 86-95.

Okigbo, R. N., Eme, U. E., & Ogbogu, S. (2008). Biodiversity and conservation of medicinal and aromatic plants in Africa. *Biotechnol. Mol. Rev*, 3(6), 127-134.

Omar, J., Alonso, I., Garaikoetxea, A., & Etxebarria, N. (2013). Optimization of focused ultrasound extraction (FUSE) and supercritical fluid extraction (SFE) of citrus peel volatile oils and antioxidants. *Food. Anal. Method*, 6, 1244-1252.

Opare, J., Ohuabunwo, C., Agongo, E., Afari, E., Sackey, S., & Wurapa, F. (2013).

Improving surveillance for non-communicable diseases in the eastern region of Ghana-2011. *J. Public. Health. Epidemiol*, 5(2), 87-94.

Oskoueian, E., Abdullah, N., Saad, W. Z., Omar, A. R., Ahmad, S., Kuan, W. B., Zolkifli, N. A., Hendra, R., & Ho, Y. W. (2011). Antioxidant, anti-inflammatory and anticancer activities of methanolic extracts from *Jatropha curcas* Linn. *J. Med. Plant. Res*, 5(1), 49-57.

Oueslati, S., Ksouri, R., Falleh, H., Pichette, A., Abdelly, C., & Legault, J. (2012). Phenolic content, antioxidant, anti-inflammatory and anticancer activities of the edible halophyte *Suaeda fruticosa* Forssk. *Food. Chem*, 132, 943-947.

Owusu-Dabo, E., Lewis, S., McNeill, A., Gilmore, A., & Britton, J. (2011). Support for smoke-free policy, and awareness of tobacco health effects and use of smoking cessation therapy in a developing country. *BMC. Public. Health*, 11, 572.

Palgrave, K. C. (1981). *Trees of southern Africa*. Cape town: Struik publishers.

Paduch, R., Kandefer-Szerszen, M., Trytek, M., & Fiedurek, J. (2007). Terpenens: substances useful in human healthcare. *Archivum immunologiae et therapia experimentalis*, 55, 315-327.

Pandey, M., Debnath, M., Gupta, S., & Chikara, S. (2011). Phytomecine: An ancient approach turning into future potential source of therapeutics. *J. Pharmacognosy. Phytother,* 3(3), 27-37.

Parkin, D. M., Bray, F., Ferlay, J., & Pisani, P. (2005). Global cancer statistics, 2002. *CA cancer J Clin,* 55, 74-108.

Parkin, D. M., Bray, F., Ferlay, J., & Pisani, P. (2001). Estimating the world cancer burden: GLOBOCAN 2000. *Int. J. Cancer,* 94, 153-156.

Pasqua, G., Monacelli, B., & Valleta, A. (2004). Cellular localization of the anti-cancer drug camptothecin in *Camptotheca acuminate* Decne (Nyssaceae). *Eur. J. Histochem,* 48(3),321-328.

Patel, B., Das, S., Prakash, R., & Yasir, M. (2010). Natural bioactive compounds with anticancer potential. *Int. J. Adv. Pharm. Sci,* 1, 32-41.

Patwardhan, B., Warude, D., Pushpangadan, P., & Bhatt, N. (2005). Ayurveda and traditional Chinese medicine: A comparative overview. *eCAM,* 2(4), 465-473.

Peeters, K. C. M. J., van de Velde, C. J. H., Leer, J. W. H., Martijn, H., Junggburt, J. M.C., Klein Kranenbarg, E., Steup, W. H., Wiggers, T., Rutten, H. J., &

Marijnen, C. A. M. (2005). Late side effects of short-course preoperative radiotherapy combined with total mesorectal excision for rectal cancer: increased bowel dyfunction in irradiataed patients-A Dutch coleractal cancer group study. *J. Clin. Oncol,* 23(25), 6199-6206.

Petrylak, D. P., Tangen, C. M., Hussain, M. H. A., Lara, P. N., Jones, J. A., Taplin, M. E., Burch, P. A., Berry, D., Moinpour, C., Kohli, M., Benson, M. C., Small, E. J., Raghavan, D., & Crawford, E. D. (2004). Docetaxel and estramustine compared with mitoxantrone and prednisone for advanced refractory prostate cancer. *N. Engl. J. Med,* 351, 1513-1520.

Podolak, I., Galanty, A., & Sobolewska, D. (2010). Saponins as cytotoxic agents: a review. *Phytochem Rev*, 9, 425-474.

Powell. R. A., Mwangi-Powell, F. N., Kiyange, F., Radbruch, L., & Harding, R. (2011). Palliative care development in Africa: How can we provide enough quality care. *BMJ Support Palliat Care*, 1(2), 113-114.

Prasad, N. V. K., Niranjan, K., & Madhavi, N. (2010). Estimation of different biological effective irradiances at Visakhapatnam (17.7°N, 83.3°E) from standard action spectra. *Int. J. Phys. Sci,* 5(1), 39-46.

Rahimkhani, M., Mohagheghi, M. A., & Yaraei, K. (2010). Fecal microbial flora in colorectal cancer. *Afr. J. Microbiol. Res,* 4(23), 2622-2625.

Rakashanda, S., Rana, F., Rafiq, S., Masood, A., & Amin, S. (2012). Role of proteases in cancer:A review. *Biotechnol. Mol. Biol. Rev,* 7(4), 90-101.

Ramkumar, K. M., Rajaguru, P., & Ananthan, R. (2007). Antimicrobial properties and phytochemical constituents of an antidiabetic plant *Gymnema montanum. Adv. Biol. Res,* 1(1-2), 67-71.

Rampazzo, E., Bertorelle, R., Serra, L., Terrin, L., Candiotto, C., Pucciarelli, S., Del Bianco,P., Nitti, D., & De Rossi, A. (2010). Relationship between telomere shortening, genetic instability, and site of tumor origin in colorectal cancers. *Br. J. Cancer,* 102, 1300-1305.

Rao, Y. Y., & Chakrabarti, R. (2004). Enhanced anti-protease activity in *Labeo rohita* fed with a diet containing herbal ingredients. *Indian. J. Clin. Biochem,* 19(2), 132-134.

Rastegar, H., Ashtiani, H. R. A., Hedayati, M., & Mirzaei, A. (2011). The effect of extracted bacterial LPS from *Salmonella enteritidis* on COX-2 in hepg2 cell

line in induction and inhibition conditions. *Sci. Res. Essays,* 6(27), 5771-5775.

Rasul, A., & Ma, T. (2012). *In vitro* cytotoxic screening of 300 selected Chinese medicinal herbs against human gastric adenocarcinoma SGC-7901 cells. *Afr. J. Pharm. Pharmacol*, 6(9), 592-600.

Reeler, A. V., Sikora, K., & Solomon, B. (2008). Overcoming challenges of cancer treatment programmes in developing countries: A sustainable breast cancer initiative in Ethiopia. *Clin. Oncol,* 20, 191-198.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free. Radic. Biol. Med*, 26(9/10), 1231-1237.

Ribassin-Majed, L., Lounes, R., & Clemencon, S. (2012). Impact of human papillomavirus vaccination on anal cancer incidence in French women. *J. Public. Health. Epidemiol*, 4(5), 141-149.

Rodin, R. J. (1985). *The ethnobotany of the kwanyama ovambos.* Kansas:Allen press.

Rothberg, J. M., Bailey, K. M., Wojtkowiak, J. W., Ben-Nun, Y., Bogyo, M., Weber, E., Moin, K., Blum. G., Mattingly, R. R., Gillies, R. J., & Sloane, B. F. (2013).

Acid-mediated tumor proteolysis: Contribution of cysteine cathepsins [1,2]. *NEOPLASIA,* 15(10), 1125-1137.

Salminen, A., Lehtonen, M., Suuronen, T., Kaarniranta, K., & Huuskonen, J. (2008). Terpenoids: natural inhibitors of NF-kB signaling with anti-inflammatory and anticancer potential. *Cell. Mol. Life. Sci*, 65, 2979-2999.

Saonere, J. A. (2010). Awareness screening programme reduces the risk of cervical cancer in women. *Afr. J. Pharm. Pharmacol*, 4(6), 314-323.

Schluterman, N. H., Sow, S. O., Traore, C. B., Bakarou, K., Dembele, R., Sacko, F., Gravitt, P. E., & Tracy, J. K. (2013). Differences in patterns of high-risk human papillomavirus infection between urban and rural low-resource settings: cross-sectional findings. *BMC Womens Health*, 13, 4.

Screens-to-nature manual for Namibia. (2012). *The 2nd GIBEX workshop in Namibia. January 24-26,2012.* Rutgers University.

Sedelnikova, O. A., Redon, C. E., Dickey, J. S., Nakamura, A. J., Georgakilas, A. G., & Bonner, W. M. (2010). Role of oxidatively induced DNA lesions in human

pathogenesis. *Mutat. Res,* 704, 152-159.

Shakeri-Zadeh, A., Mansoori, G. A., Hashemian, A. R., Eshghi, H., Sazgarnia, A., & Montazerabadi, A. R. (2010). Cancerous cells targeting and destruction using folate conjugated gold nanoparticles. *Dyn. Biochem. Process. Biotech. Mol. Biol*, 4(1), 6-12.

Shapiro, C. L., & Recht, A. (2001). Side effects of adjuvant treatment of breast cancer. *N. Engl. J. Med,* 344(26), 1997-2008.

Shawi, A. A., Rasul, A., Khan, M., Iqbal, F., & Tonghui, M. (2011). Eupatilin: A flavonoid compound isolated from the artemesia plant, induces apoptosis and G2/M phase cell cycle arrest in human melanoma A375 cells. *Afr. J. Pharm. Pharmacol,* 5(5), 582-588.

Shen, L., Kondo, Y., Ahmed, S., Boumber, Y., Konishi, K., Guo, Y., Chen, X., Vilaythong, J. N., & Issa, J-P, J. (2007). Drug sensitivity predicition by CpG island methylation profile in the NCI-60 cancer cell line panel. *Cancer Res*, 67(23), 11335-11343.

Shokrzadeh, M., & Saravi, S. S. S. (2010). The chemistry, pharmacology and clinical

properties of *Sambucus ebulus*: A review. *J. Med. Plant. Res,* 4(2), 095-103.

Shukla, A., & Dwivedi, S. K. (2013). Antifungal approach of phenolic compounds against *Fusarium udum* and *Fusarium oxysporum f.sp.ciceri*. *Afr. J. Agri. Res*, 8(7), 596-600.

Soliman, S. S. M., Trobacher, C. P., Tsao, R., Greenwood, J. S. & Raizada, M. N. (2013). A fungal endophyte induces transcription of genes encoding a redundant fungicide pathway in its host plant. *BMC Plant Biol*, 13,93.

Spanou, C., Stagos, D., Tousias, L., Angelis, A., Aligiannis, N., Skaltsounis, A. L., & Kouretas, D. (2007). Assessment of antioxidant activity of extracts from unique greek varieties of the leguminosae plants using *in vitro* assays. *Anticancer Res*, 27, 3403-3410.

Stevens, G. A., Mathers, C. D., & Beard, J. R. (2013). Global mortality trends and patterns in older women. *Bull. World. Health. Organ,* 91, 630-639.

Subhashini, S., & Arunachalam, K. D. (2010). Investigations on the phytochemical

activities and wound healing properties of *Adhatoda vasica* leave in swiss albino mice. *Afr. J. Plant. Sci*, 4(12), 467-479.

Suggitt, M., & Bibby, m. C. (2005). 50 years of preclinical anticancer drug screening: Emperical to target-driven approaches, *Clin. Cancer. Res*, 11, 971-981.

Stalikas, C. D. (2007). Extraction, separation, and detection methods for phenolic acids and flavonoids. *J. Sep. Sci*, 30, 3268-3295.

Tao, L., Li, J., & Zhang, J. (2011). Brazilein overcame ABCB1-mediated multidrug resistance in human leukemia K562/AO2 cells. *Afr. J. Pharm. Pharmacol*, 5(16), 1937-1944.

Thambiraj, J., Paulsamy, S., & Sevukaperumal, R. (2012). Evaluation of *in vitro* antioxidant activity in the traditional medicinal shrub of western districts of Tamilnadu, India, *Acalypha fruticosa* forssk. (*Euphorbiaceae*). *Asian Pac. J. Trop. Biomed*, 2(1), 127-130.

Thaware, J. (2012). Screening pollen of *Catharanthus roseus* L.-An anticancer plant. *Int. J. Drug. Discov. Herbal. Res*, 2(2), 403-407.

Thomas, S. A., Vasudevan, S., Thamkachy, R., Lekshmi, S. U., Santhoshkumar, T. Y,

Rajasekharan, K. N., & Sengupta, S. (2013). Upregulation of DR5 receptor by the diaminothiazole DAT1 [4-amino-5-benzoyl-2-(4-methoxy phenyl amino) thiazole] triggers an independent extrinsic pathway of apoptosis in colon cancer cells with compromised pro and antiapoptotic proteins. *Apoptosis*, 18, 713-726.

Tiong, S. H., Looi, C. Y., Hazni, H., Arya, A., Paydar, M., Wong, W. F., Cheah, S-C., Mustafa, M. R., & Awang, K. (2013). Antidiabetic and antioxidant properties of alkaloids from *Catharanthus roseus* (L.) G. Don. *Molecules,* 18, 9770-9784.

Tohme, R., Darwiche, N., & Gali-Muhtasib, H. (2011). A journey under the sea: the quest for marine anticancer alklaoids. *Molecules,* 16, 9665-9696.

Toriola, A. T., Kurl, S., Laukanen, J. A., Mazengo, C., & Kauhanen, J. (2008). Alcohol consumption and risk for colorectal cancer: the findrink study. *Eur. J. Epidemiol*, 23, 395-401.

Trovato, G. M. (2012). Behavior, nutrition and lifestyle in a comprehensive health and disease paradigm: Skills and knowledge for a predictive, preventive and personalized medicine. *The EPMA Journal*, 3,8.

Van den Eynden, V., Vernemmen, P., & Van Damme, P. (1992). *The ethnobotany of*

the topnaar. Universiteit Gent.

Van Wyk, B-E., & Gericke, N. (2000). *People's plants. A guide to useful plants of Southern Africa.* Pretoria: Briza publication.

Varsale, A. R., Wadnerkar, A. S., & Mandage, R. H. (2010). Cancer investigation: A genome perspective. *Biotechnol. Mol. Biol. Rev*, 5(5), 79-86.

Venditti, P., Di Stafano, L., & Di Meo, S. (2013). Vitamin E management of oxidative damage-linked dysfunctions of hyperthyroid tissues. *Cell. Mol. Life. Sci*, 70, 3125-3144.

Vichai, V., & Kirtikara, K. (2006). Sulforhodamine B colorimetric assay for cytotoxicity screening. *Nat. Protoc*, 1(3), 1112-1116.

Von Koenen, E. (2001). *Medicinal poisonous and edible plants in Namibia.* Windhoek: Klaus hess publishers

Wall, M. E., & Wani, M. C. (1995). Camptothecin and taxol: Discovery to clinic-Thirteenth Bruce F. Cain memorial award lecture. *Cancer Res,* 55, 753-760.

Wang, H. K., & Lee, K. H. (1997). Plant-derived anticancer agents and their analogs currently in clinical use or in clinical trials. *Bot Bull Acad Sin*., 38, 225-235.

Wang, X-B., Liu, W., Yang, L., Guo, Q-L., & Kong, L-Y. (2012). Investigation on the substitution effects of the flavonoids as potent anticancer agents: a structure-activity relationships study. *Med. Chem. Res,* 21, 1833-1849.

Wang, X., Xu, J., Yang., M., & Zhou, H. (2011). Chloroform extract of Tibetan herbal medicine Dracocephalum tanguticum Maxim. inhibits proliferation of T98G glioblastomas cells by modulating Caspase-3 cleavage and expression of Bax and p21. *J. Med. Plant. Res,* 5(25), 6024-6031.

Wang, Y., & Dai, C-C. (2011). Endophytes: a potential resource for biosynthesis, biotransformation, biodegradation. *Ann. Microbiol,* 61, 207-215.

Wardihan., Rusdi, M., Alam, G., Lukman., & Manggau, M. A. (2013). Seelctive cytotoxicity evaluation in anticancer drug screening of Boehmeria virgate (Forst) guill leaves to several human cell lines: Hela, WiDr, T47D and Vero. *Dhaka Univ. J. Pharm. Sci,* 12(2), 123-126.

Wei, L., Lin, J., Xu, W., Hong, Z., Liu, X., & Peng, J. (2011). Inhibition of tumor angiogenesis by *Scutellaria barbata* D. Don via suppressing proliferation, migration and tube formation of endothelial cells and downregulation of the

expression of VEGF-A in cancer cells. *J. Med. Plant. Res,* 5(14), 3260-3268.

Wild, C. P., & Montesano, R. (2009). A model of interaction: Aflatoxins and hepatitis viruses in liver cancer aetiology and prevention. *Cancer lett,* 286, 22-28.

Willoughby, L. F., Schlosser, T., Manning, S. A., Parisot, J. P., Street, I. P., Richardson, H. E., Humbert, P. O., & Brumby, A. M. (2013). An *in vivo* large-scale chemical Screening platform using Drosophila for anticancer drug discovery. *Dis. Model. Mech,* 6, 521-529.

Wong, H. C., Sagineedu, S. R., Lajis, N. H., Loke, S. C., & Stanslas, J. (2011). Andrographolide induces cell cycle arrest and apoptosis in PC-3 prostate cancer cells. *Afr. J. Pharm. Pharmacol,* 5(2), 225-233.

Xun-Li, X. (2013). Traditional medicine. History of Chinese medicinal wine. *Chin. J. Integr. Med,* 19(7), 549-555.

Yamaguchi, K., & Fujisawa, M. (2011). Anticancer chemotherapeutic agents and testicular dysfunction. *Reprod. Med. Biol*, 10, 81-87.

Yan, L.L., Zhang, Y. J., Gao, W. Y., Man, S. L., & Wang, Y. (2009). *In vitro* and *in vivo* anticancer activity of steroid saponins of *Paris polyphylla* var.

yunnanensis. *Exp. Oncol,* 31(1), 27-32.

Yeap, S. K., Tamilselvan, S., Al-Qubaisi, M., Omar, A. R., Ho, W. Y., Beh, B. K., & Alitheen, N. B. (2012). A review of risk factors, incidence and solutions for hepatocellular carcinoma. *Sci. Res. Essays,* 7(2), 94-99.

Yumin, W., Jie, C., Wenhui, Z., Wangdong, H., & Fangyou, Y. (2012). Study of the prevalence of human papillomavirus infection in Chinese women with cervical cancer. *Int. J. Microbiol. Res,* 6(5), 1048-1053.

Zaki, M. M., El-Midany, S. A., Shaheen, H. M., & Rizzi, L. (2012). Mycotoxins in animals: occurrence, effects, prevention and management. *J. Toxicol. Environ. Health. Sci,* 4(1), 13-28.

Zang, W-D., Liu, J., Wang, L-S., & Pan, T-W. (2012). Identifying genes related with non-small cell lung cancer via transcription factors-target genes relationships. *Int. J. Phys. Sci,* 6(28), 6450-6457.

Zha, X., Diaz, R., Franco, J. J. R., Sanchez, V. F., Fasoli, E., Barletta, G., Carvajal, A., & Bansal, V. (2013). Inhibitors of urokinase type plasminogen activator and

cytostaic activity from crude plants extracts. *Molecules*, 18, 8945-8958.

Zhang, Y., Ning, Z., Lu, C. Zhao, S., Wang, J., Liu, B., Xu, X., & Liu, Y. (2013). Triterpenoid resinous metabolites from the genus Boswellia: phamacolofical activities and potential species-identifying properties. *Chem. Cent. J*, 7,135.