2019-10

Evaluation of anti-cancer potential of crude extracts of Annona senegalensis Pers. and Allophylus africanus P Beauv.

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NM-AIST

https://doi.org/10.58694/20.500.12479/1037

EVALUATION OF ANTI-CANCER POTENTIAL OF CRUDE EXTRACTS OF *Annona senegalensis* Pers. AND *Allophylus africanus* P Beauv.

Emiliana Zacharia Biseko

A Dissertation Submitted in Partial Fulfillment of the Requirements for the Degree of Master's in Life Sciences of the Nelson Mandela African Institution of Science and Technology

Arusha, Tanzania

October, 2019

ABSTRACT

The medicinal plants *Annona senegalensis* Pers. and *Allophylus africanus* P Beav. are traditionally used for the treatment of cancer in Tanzania. However, there is no scientific documentation on their therapeutic effectiveness. To evaluate the anticancer potential of *A. senegalensis* and *A. africanus* plant species from Tanzania, stem bark of the two plants were collected from Ugweno village at Kilimanjaro, Tanzania. Pulverized plant materials were soaked in dichloromethane/methanol (DCM:MeOH), petroleum ether (PE), DCM, ethyl acetate (EtOAC), MeOH and water to obtain DCM-MeOH, PE, DCM, EtOAC and MeOH extracts respectively. Anticancer activity against breast (HCC 1396), throat (HEp- 2) and colon (CT 26) cancer cell lines was assessed by the MTT cell viability assay. Results showed that anticancer activity varied between plant extracts and the cancer cell lines. The highest anticancer activity was achieved with *A. senegalensis* petroleum ether extract against HEp-2. The findings justify traditional use of *A. senegalensis* and *A. africanus* in treatment of cancer. This study found petroleum ether extract of *A. senegalensis* to have high potential for development of an anticancer agent against throat cancer. Further studies involving the isolation of pure anticancer compounds from the two plants are recommended to elucidate bioactive molecules with anticancer activity.

DECLARATION

I, Emiliana Zacharia Biseko, do hereby declare to the Senate of The Nelson Mandela African Institution of Science and Technology that this dissertation is my own original work and that it has neither been submitted nor being concurrently submitted for degree award in any other institution.

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CERTIFICATION

The undersigned certify that has read and found the dissertation acceptable by the Nelson Mandela African Institution of Science and Technology.

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ACKNOWLEDGEMENT

I praise the Almighty God for the success of this work. The completion of this work is due to His mercies and grace. I am extremely thankful and deeply indebted to the African Development Bank (AfDB) for financial support that facilitated my academic life. I sincerely submit my respectful regards to my supervisors, Prof. Hulda S. Swai and Dr. Jeremiah W. Gathirwa, for the useful comments, expertise, guidance, encouragement, and patience which added considerably to my experience. This accomplishment would not have been possible without you.

I would also like to acknowledge my friends, Ms. Regina Mbugua, Ms. Jecinta Ndung'u, Ms. Jean Chepng'etich, Mr. Mohammed Mpina and Mr. Rogers Mwanga for their support through the process of researching and writing this thesis. I thank my family for providing me with unfailing support and continuous encouragement throughout my years of study. I am grateful to everyone that has put an effort to the success of this academic work.

DEDICATION

I dedicate this dissertation work to my dear parents, Mr. and Mrs. Biseko who encouraged me and formed part of my vision.

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LIST OF ABBREVIATIONS

µL	Micro litres
^0C	Degrees celcius
Ab	Absorbance value of a blank
Ac	Absorbance value of a negative control
ANOVA	Analysis of Variance
At	Absorbance value of a test compound
ATCC	American Type Culture Collection
CC_{50}	Cytotoxic Concentration inhibited normal cells by fifty percent
CT 26	Colon carcinoma cell line
CTMDR	Centre for Traditional Medicine and Drug Research
DCM	Dichloromethane
DCM:MeOH	Dichloromethane/methanol
DMEM	Dulbeco Modified Essential Medium
DMSO	Dimethyl sulfoxide
EtOAC	Ethyl acetate
MeOH	Methanol
HCC 1396	Breast carcinoma cell line
HEp-2	Human larynx carcinoma cell line
IC_{50}	Drug concentration inhibiting proliferation of cultured cells by fifty percent
KEMRI	Kenya Medical Research Institute
MTT	Three-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide
OD	Optical Density
SI	Selectivity index
TPRI	Tropical Pesticides Research Institute
VERO	African green monkey kidney cell lines
WHO	World Health Organization

CHAPTER ONE

INTRODUCTION

1.1. Background Information

Cancer is one of the most fatal diseases worldwide (Max Parkin, Bray, Ferlay, & Pisani, 2005; Biemar & Foti, 2013). Around one third of deaths from cancer are due to the five leading behavioral and dietary risks, which are: high body mass index, low fruit and vegetable intake, lack of physical activity and the use of tobacco and alcohol (Prakash, Kumar, Kumar, & Ajeet, 2013). In 2012, the worldwide burden of cancer rose to an estimated 14 million new cases per year, a figure expected to rise to 22 million annually within the next two decades. Over the same period, cancer deaths were predicted to rise from an estimated 8.2 million annually to 13 million per year. During this period, lung cancers were responsible for the highest death, about 1.6 million (19.4%) followed by liver (0.8 million, 9.1%) and gastric (0.7 million, 8.8%) (World Health Organization (WHO), 2014).

Cancer treatments approach involve chemotherapy, surgery, radiation therapy, immunotherapy, targeted therapy, and hormonal therapy, of which are associated with side effects (Baskar, Lee, Yeo, & Yeoh, 2012; WHO, 2014). Of all treatments, chemotherapy is the most effective but due to high dose requirements it kills normal cells hence causing side effects such as fatigue, nausea, hair loss, vomiting, loss of appetite, constipation, anemia, diarrhea etc, (Aslam *et al*., 2014; Conklin, 2004). Therefore, patients' preferences to use herbal medicines as alternative source of treatment has gained attention in many parts of the world including Tanzania (Priya, Priya, Kotakadi, & Josthna, 2015). According to the Institute of Traditional Medicine at Muhimbili National Hospital (MUHAS), Tanzania is estimated to have over eighty thousands traditional healers with varying specialties and they play a crucial role of providing primary health care. It was reported that more than 60 % of the population in Tanzania depends on traditional medicines for management of various diseases (Kisangau, Lyaruu, Hosea, & Joseph, 2007).

Plants produces secondary metabolites which has been reported to possess therapeutic effect which can be tolerated by the body (Priya *et al*., 2015). Apart from that, they could be combined to obtain synergism, which would enhance efficacy while reducing drug resistance and toxicity to normal tissues (Pinmai, Chunlaratthanabhorn, Ngamkitidechakul, &

Soonthornchareon, 2008). This property has allowed traditional healers, including here in Tanzania to use combinations of medicinal plants to cure various diseases. According to the information obtained through carrying out interviews of traditional healers and communities, *Annona senegalensis* and *Allophylus africanus* are among of medicinal plants used in treatment of cancer in Tanzania. *Annona senegalensis* is reported to be used by local populations all over Africa in treatment of various diseases such as respiratory infections, guinea worms, pneumonia, diarrhea, gastroenteritis, snake bites, toothache and dizziness (Awa, Ibrahim, & Ameh, 2012). *Allophylus africanus* is used traditional by some part of Africa in treatment of cough, fever, dysentery, and malaria (Sofidiya *et al*., 2012). The stem bark of the two plants have been claimed to be used by Pare tribe from Ugweno village of Kilimanjaro region in Tanzania for the treatment of different types of cancer such as throat, breast, liver, cervical and colon cancer. However, unlike in several other African countries (Graham, Quinn, Fabricant, & Farnsworth, 2000; Awa *et al*., 2012; Oladosu, Balogun, & Ademowo, 2013), the scientific proof of the therapeutic effectiveness of the plants has not been documented in Tanzania. In an attempt to fill the gap, this study was implemented to evaluate the anticancer activity of the aforementioned plants.

1.2. Statement of Research Problem and Justification

Cancer is the leading cause of death worldwide (Deshmukh *et al*., 2017). Various approaches have been employed to treat and control cancer but all of them have been associated with side effects. Therefore herbal medicines have been used as alternative source of treatment since they are available and are not harmful as conventional medicine (Yasser, 2016).

Annona senegalensis and *Allophylus africanus* are medicinal plant that are claimed to be used in treatment of cancer in Tanzania, but the scientific evidence on performance of these plants against cancer has not been documented. However, the scientific evidence in treatment of various diseases including cancer, malaria and bacterial diseases have been reported from different part of Africa (Ajaiyeoba, Falade, Ogbole, Okpako, & Akinboye, 2006; Sofidiya *et al*., 2012; Mustapha, 2013). Regarding the studies which revealed the effect of ecological variation on the production of active substances in the medicinal plants (Devkota, Dall'Acqua, Jha, & Innocent, 2010; Liu, Liu, Yin, & Zhao, 2015), there is a need of evaluating bioactivity of these two plants in Tanzania habitat and validate its traditional use.

Since the scientific information for the plants' therapeutic value against cancer is limited in Tanzania, this study envisaged evaluating anticancer potentials of *A. senegalensis* and *A. africanus* against throat (HEp-2), breast (HCC 1396) and colon (CT 26) human cancer cell lines.

1.3. Objectives

1.3.1. General Objective

To determine the effects of *Annona senegalensis* and *Allophylus africanus* plant extracts on human breast (HCC 1396), throat (HEp-2) and colon (CT 26) cancer cell lines.

1.3.2. Specific Objectives

(i) To determine phytochemical composition of *A. senegalensis* and *A. africanus* plant extracts.

(ii) To determine *in vitro* anticancer activity of *A. senegalensis* and *A. africanus* against selected human cancer cell lines.

(iii) To determine cytotoxic activity of *A. senegalensis* and *A. africanus* against VERO cell lines.

1.4. Hypothesis

(i) The plants have no phytochemical compounds.

(ii) The plants have no anticancer effect against selected cancer cell lines.

(iii) The plants have no cytotoxic activity against VERO cell line.

1.5. Significance of the Study

This study will contribute evidence to the existing traditional knowledge for the treatment of cancer. Also, will provide opportunity for further studies which may lead to development of cancer therapy that has little or no potential side effects.

CHAPTER TWO

LITERATURE REVIEW

2.1. Overview

Cancer is a malignant condition in which the spread of abnormal cellular growth become uncontrollable (Priya *et al*., 2015). It is commonly due to mutation of two genes which are oncogenes and tumor -suppressor genes. Oncogenes normally promote cell growth, however when overexpressed they transform healthy cells into cancer cells. Tumor- suppressor genes normally restrain growth so when under expressed allow inappropriate cell division which can facilitate carcinogenesis (Boik, 2001). The mutation can arise due to several factors such as tobacco use, lack of physical exercise, unhealthy diet, alcohol consumption, automobile exhaust pollutant, UV radiation and bacterial or viral infection (Prakash *et al*., 2013). This mutation causes DNA damage which results to precancerous cells that divides to produce daughter cells having the ability to invade and metastasize other tissues (Idikio, 2011).

2.2. Cancer Barden

Cancer is the second leading cause of death in high-income countries following cardiovascular diseases but the third leading cause of death in low- and middle-income countries, following cardiovascular diseases and infectious diseases (WHO, 2014). According to the International Agency for Research on cancer, lung cancer is the most diagnosed and leading cause of cancer death. This is followed by breast, prostate and colorectal cancer (Delancey, Jemal, & Ward, 2010). Global, the type of cancer and distribution is economically dependent. Cancer burden is increasing in developing countries due to population growth, changing of lifestyles and aging. The number of cases is expected to increase most in middle and low countries to 24 million in 2050 which would be twice the number in 2002 which was 10.8 million (Siegel, Miller, & Jemal, 2018).

2.3. Cancer Chemoprevention

Cancer cells rely on processes that are fundamentally similar to the processes used by normal cells hence become hard to kill without damaging normal cells. The best way to inhibit a cancer cell is not to destroy its structural properties but to normalize the signals that drive it

(Boik, 2001). Chemotherapy is the most effective and widely used treatment in most types of cancer though there are other cancer treatments approach such as surgery, radiation therapy, immunotherapy, targeted therapy, and hormonal therapy (Baskar *et al*., 2012). However, it is none specific as it affects both cancer cells and normal cells resulting to side effects (Conklin, 2004). According to the study conducted in Pakistan, the most frequently reported of these side effects including weakness, fatigue, nausea, hair loss and vomiting (Prakash *et al*., 2013). Other prominent side effects include mouth sores, dry mouth, temperature reaction, constipation, mood swings, weight loss and numbness whereas diarrhea, abdominal cramps and memory impairment is less commonly occurring side effects. These side effects limit the efficiency of chemotherapy. So, there is a need of searching alternative cancer treatment with minimum or no side effects (Aslam *et al*., 2014).

2.4. Plant Phytochemicals and Cancer Prevention

For many years herbal medicines have been used and are still being used in developing countries as the primary source of medical treatment (Yasser, 2016). Since 1997 medicinal plants had proven scientifically to associate with fewer side effects by National Institute of Health of United States. Medicinal plants produces secondary metabolites which are used to perform important biological functions, and to defend against attack from predators such as insects, fungi and herbivorous (Ajuru *et al*., 2017). These metabolites have been reported to possess therapeutic effect which are non-toxic to normal cells hence not harmful to the body (Greenwell & Rahman, 2015). The metabolites including tannins, alkaloids, terpenoids, flavonoids and saponins which contain good immunomodulatory and antioxidant properties which lead them to be potential anticancer drugs.

Tannins possess antioxidant and haemostatic properties. Also has a tendency of reducing the digestibility of proteins in foods. Alkaloids have been reported to have a wide range of pharmacological properties such as antimalarial, antiasthma and anticancer properties. It was also reported to have antibacterial, anti-hyperglycemic and analgesic activity (Bako, Bakfur, John, & Bala, 2005). Terpenoids protect plants from their natural enemies and play role as growth regulator. In addition have medicinal properties such as antimalarial, anti-ulcer, antimicrobial and anticancer. Flavonoids have reported to possess antioxidant, anti-inflammatory, anti-allergic, anti-carcinogenic, anti-microbial, and anti-viral activities (Ajuru *et al*., 2017). Saponins have reported to be very useful in the treatment of upper respiratory

tract inflammations. They also have been reported to have anti-diabetic and anti-fungal properties (Yessuf, 2015). Phytochemicals can be classified by function as an individual compound and may have more than one biological function (Table 1) (Saxena, Saxena, Nema, Singh, & Gupta, 2013). Plant-derived drugs are preferred for cancer treatment as they are natural and available (Madhu, Sailaja, Satyadev, & Satyanarayana, 2016).

Table 1: Bioactive and disease preventing phytochemicals present in plant

Classification	Main groups of compounds	Biological function
Non-starch polysaccharides	Cellulose, hemicellulose, gums, mucilages, pectins, lignins	Water holding capacity, delay in nutrient absorption, binding toxins and bile acids
Antibacterial and Antifungal	Terpenoids, alkaloids, phenolics	Inhibitors of micro-organisms, reduce the risk of fungal infection
Antioxidants	Polyphenolic compounds, flavonoids, carotenoids, tocopherols, ascorbic acid	Oxygen free radical quenching, inhibition of lipid peroxidation
Anticancer	Carotenoids, polyphenols, curcumine, Flavonoids	Inhibitors of tumor, inhibited development of lung cancer, anti-metastatic activity
Detoxifying Agents	Reductive acids, tocopherols, phenols, indoles, aromatic isothiocyanates, coumarins, flavones, carotenoids, retinoids, cyanates, phytosterols	Inhibitors of procarcinogen activation, inducers of drug binding of carcinogens, inhibitors of tumourogenesis
Other	Alkaloids, terpenoids, volatile flavor compounds, biogenic amines	Neuropharmacological agents, antioxidants, cancer chemoprevention

(Sexane *et al*., 2013)

2.5. Commonly Medicinal Plants Used in Tanzania

More than sixty percent of the population in Tanzania depends on traditional medicines for management of various diseases including cancer (Kisangau *et al*., 2007). Medicinal plants play an important role in providing primary health care to the rural and urban communities of Tanzania. It also provides a source of income to traditional healers within the country (Kitula, 2007). Apart from that, it was reported that, the use of traditional medicine in Tanzania is associated with belief in the power of medicinal plants to bring good health during pregnancy and child growth, preventing damage from evil eyes and witchcraft (Stanifer *et al*., 2015).

Also are used as contraceptives for birth control. Many ethnic groups such as Pare, Haya, Mbulu etc. have been practicing medicinal plants for treatment of various diseases. These medicinal plants include *A. senegalensis* and *A. africanus* which are used in different regions of the country (Matata, Ngassapa, Machumi, & Moshi, 2018). According to the study conducted by Wenzel (2011), the most reason why patients opt for the use of traditional medicines is because treatment at the hospital did not heal them. However, the findings indicated that a large majority of the patients surveyed believed both traditional and western, although traditional medicine is often not the first choice.

2.6. Annona senegalensis

Annona senegalensis, popular known as African custard apple or wild custard apple is a shrub that belongs to the family Annonaceae and is usually found growing in semi-arid to sub-humid regions of Africa (Okoli *et al*., 2010). It is native and widely distributed in Africa (Okoye, Akah, Ezike, Omeje, & Odoh, 2012). In Tanzania, it is known as Mkisha by Pare tribe of Kilimanjaro region. Stem barks of *A. senegalensis* are claimed by this community to be used to cure several types of cancer such as liver, cervical, breast and colon cancer. The plant has reported scientifically from different part of Africa to possess antimicrobial, antioxidant, antiparasitic, anti-inflammatory, anticonvulsant, antimalarial, trypanocidal, anti-snake venom, anti-nociceptive, and anthelmintic activities (Ajaiyeoba *et al*., 2006; Awa *et al*., 2012; Mustapha, 2013). It has also has been reported to be effective against cervical, skin and pancreatic cancers (Graham *et al*., 2000; Okoye, Akah, Nworu, & Ezike, 2014).

Figure 1: *Annona senegalensis*

2.7. Allophylus africanus

Allophylus is the largest genus of a family Sapindaceae (Balogun, Oladosu, & Liu, 2016). This genus is widely distributed in tropical and subtropical regions of the America, Africa, Asia, Indian Archipelago and Pacific (Chavan & Gaikwad, 2016). *Allophylus africanus* is commonly called Mlunguu by the Pare tribe in Tanzania. Its stem barks are used in treatment of throat and breast cancer. Scientifically, it has reported to have strong antimalarial (Oladosu *et al*., 2013; Balogun, Oladosu, & Liu, 2014), antibacterial and antioxidant activities (Sofidiya *et al*., 2012). One of the species from the same genus, *Allophylus cobbe*, was confirmed to have anticancer activity against human prostate cancer cell lines (Ghagane, Puranik, Nerli, & Hiremath, 2017).

Figure 2: *Allophyus africanus*

CHAPTER THREE

MATERIALS AND METHODS

3.1. Plant Collection

Fresh stem barks of each plant were collected from Ugweno village in Kilimanjaro region of Tanzania. This is mountainous area comprising of evergreen rainforest assemblages. The plants were collected from traditional healers and identified by a taxonomist at the Tropical Pesticides Research Institute (TPRI) herbarium located in Tanzania where specimen were deposited after being assigned voucher specimen numbers EB.01 and EB.02 for *A. senegalensis* and *A. africanus* respectively.

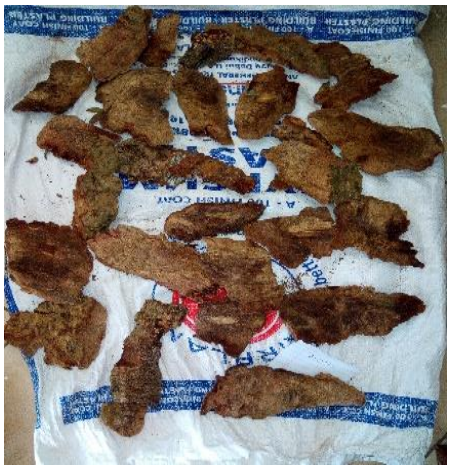

Figure 3: Stem barks of *A. senegalensis*

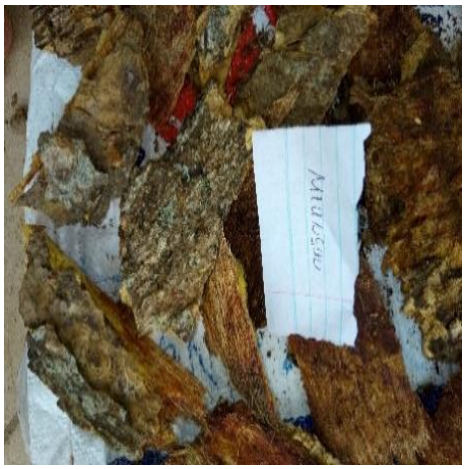

Figure 4: Stem barks of *A. africanus*

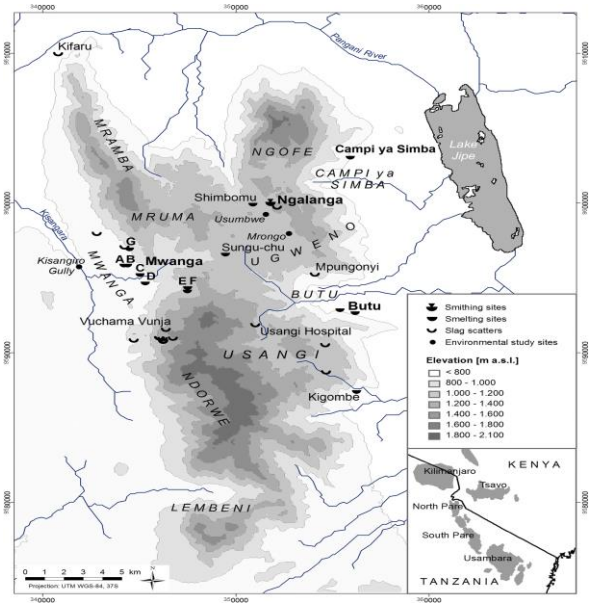

Figure 5: Sampling area at Ugweno village.

3.2. Extraction Methods

Plant material were air dried and ground to fine powder using an electric blender then stored at room temperature until used. Extraction was done using six solvents for each plant making a total of twelve extracts. Extraction was done using dichloromethane/ methanol (DCM:MeOH) at a ratio of 1:1 (Fouche *et al*., 2008). About 500 g of each plant powder were soaked completely into a mixture of 1 L of DCM and 1 L of MeOH for 72 h. The extract solutions were filtered and concentrated using a rotary evaporator. Extraction was done sequentially with petroleum ether (PE), DCM, ethyl acetate (EtOAC) and MeOH starting from least polar to most polar solvent respectively. For sequential extraction, 500 g of each plant powder were soaked in 1 L of petroleum ether, and then the filtrate re-soaked in the rest of solvents sequentially. All solvents were filtered after every 48 h and extracts concentrated through the vacuum using a rotary evaporator (Kigondu *et al*., 2011). The remaining powder material were further extracted in aqueous medium by soaking 500 g of fine powder of each plant material in 1 L of water at 60 ˚C for 60 min. The filtrate were then freeze dried to free powder (Rukunga *et al*., 2009).

Figure 7: Plants powder soaked into respective solvents.

Figure 6: Solvent filtration after incubated for 48 h

3.3. Study Design

In vitro laboratory based (pre-clinical) experimental study design method was used.

3.4. Qualitative Phytochemical Screening

Qualitative phytochemical screening was done to determine secondary metabolites which were present in the *Annona senegalensis and Allophylus africanus* extracts. The screening was done as described by Ajuru *et al.* (2017). Secondary metabolites tested were alkaloids, tannins, glycosides flavonoids, saponins and terpenes.

3.4.1. Test for Alkaloids

Alkaloids were tested by pouring 2 ml of the extracts into a watch glass and followed by addition of 1% of hydrochloric acid and three drops of Mayer's reagent. The formation of a white precipitate indicated the presence of alkaloids.

3.4.2. Test for Saponins

Saponins were tested by mixing 2 ml of the extracts with 2 ml of distilled water and the mixture was shaken vigorously. After shaking the test tube was allowed to stand. Formation of a persistent layer of foam indicated the presence of saponins.

3.4.3. Test for Flavonoids

Flavonoids were tested by mixing 2 ml of the extracts with 5 ml of dilute ammonia in a test tube. Then 2 ml of concentrated sulphuric acid was added and shaken. Formation of intense yellow color indicated the presence of flavonoids.

3.4.4. Test for Glycosides

Glycosides were tested by mixing 2 ml of the extracts with 2 ml of chloroform. Then was followed by addition of 2 ml of sulphuric acid and mixed well. Formation of a brown color indicated the presence of glycosides.

3.4.5. Test for Terpernoids

Terpernoids were tested by adding 2 ml of chloroform into 2 ml of the plant extracts and shaken vigorously. Then, 2 ml of concentrated sulphuric acid was added and heated for 2 min. Formation of grey color indicated presence of terpernoids.

3.4.6. Test for Tannins

Tannins were tested by adding 5 ml of distilled water into 2 ml of the plant extracts and heated to boil. Two percent of iron chloride was then added. A green precipitate indicated the presence of tannins.

3.5. Determination of Anticancer Activity

3.5.1. Cell Lines Culturing

The following cancer cell lines were used in this study: CT 26 (Colon cancer), HEp -2 (Throat cancer), and HCC 1396 (Human breast cancer). VERO P23 (African green monkey kidney) was used as the normal cells for reference purpose. The cell lines were originally obtained from the American Type Culture Collection (ATCC) and sub-cultured at the Center for Traditional Medicine and Drug Research (CTMDR), Kenya Medical Research Institute (KEMRI). The cell lines were cultured in Dulbecco Modified Essential Medium (DMEM) supplemented with 10% Fetal Bovine Serum (FBS), 1% L-glutamine and 100 µg/ml of streptomycin at 37 ˚C in a 5% CO_2 and 95% humidity.

3.5.2. Methyl Tetrazolium Bromide (MTT) Assay

(i) Principal

This is a colorimetric assay based on cleavage of tetrazolium salt to form a blue formazan product by enzymatic activity of mitochondria succinate dehydrogenase enzymes in living cells. The Formazan formed is direct proportional to the number of the living cells during MTT exposure. It is measured spectrophotometrically in an optical density reader. The activity of the enzyme to produce formazan is directly proportional to the level of cell viability and inversely proportional to the level of cell inhibition (Twentyman & Luscombe, 1987).

(ii) Procedure

Upon attainment of confluence, cells were washed with saline phosphate buffer and harvested by trypsinization. The number of viable cells was determined using Trypan blue exclusion method (cell density count) using a hemocytometer. They were then seeded in 96 well plates at a concentration of 2 x 10^5 cell/ml in 100 µl per well and incubated for 24 h at 37°C in a 5% CO_2 and 95% humidity for 24 h to let cells adhere onto to the surface of the wells. Zero point zero one gram of each extracts was then weighed and diluted to a concentration of 100 µg/ml. Fifteen micro litres of each extract was then added onto row H of the plate. This was followed by three folds serial dilution to get different concentrations from 100 µg/ml, 33.33 µg/ml, 11.11 µg/ml, 4.0 µg/ml, 1.33 µg/ml, 0.44 µg/ml and 0.146 µg/ml from row H to B respectively. Row A was left as a negative control. Doxorubicin, a standard drug for cancer treatment was used as the positive control (Wang *et al*., 2004). All concentrations were replicated two times for each plant extracts, then incubated for 48 h at 37 °C in a 5% CO_2 and 95% humidity. After 48 h incubation, 10 µl of MTT dye was added to each well and incubated for 2 h. The insoluble formazan product which is directly proportional to the number of living cells present during MTT exposure was then dissolved by 50 µl DMSO. Absorbance was then read at a wavelength of 540 nm and a reference wavelength of 720 nm using ELISA Reader (MULTSKAN GO Thermo scientific, USA). The effect of the plant extracts on the cells was expressed as IC_{50} values (drug concentration inhibiting cell growth by 50% compared to untreated cells).

Figure 8: Appearance of cells in 96 well plate after adding MTT dye

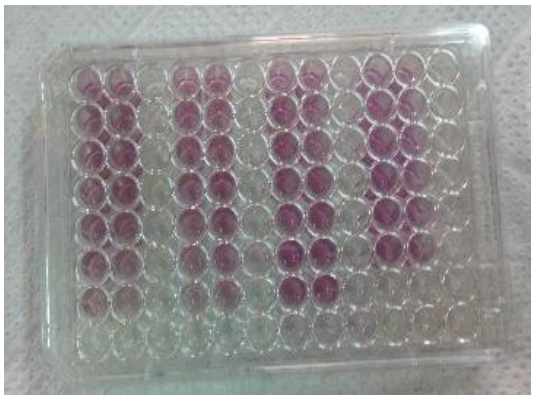

Figure 9: Appearance of cells in 96 well plate after incubated for 2 h with MTT dye

3.6. Determination of Percentage Inhibition

The percentage cells inhibition after treatment was calculated using the formula developed by Patel, Gheewala, Suthar and Shah (2009) as follows;

$$\text{Proliferation rate}=\frac{At-Ab}{Ac-Ab}\text{x } 100$$

$$\text{Percentage inhibition}=100-\frac{At-Ab}{Ac-Ab}\text{x } 100$$

Where,
At= Absorbance value of test compound (cells plus extracts)

Ab= Absorbance value of blank (media only)

Ac=Absorbance value of negative control (cells plus media)

3.7. Selectivity Index (SI)

Selectivity index is the value calculated to determine which plant extracts can select cancer cells and sparing normal cells. The selectivity index is corresponded to the CC_{50} value determine activity of plant extracts on VERO cells divided by the IC_{50} determine activity on cancer cells. The selectivity index was considered as interesting for values higher than three.

$$SI=\frac{CC50\ (VERO\ cells)}{IC50(\ Cancer\ cells)}$$

3.8. Data Management and Statistical Analysis

Raw data were entered into excel data sheets where by the concentrations inhibiting growth of the cells by 50% (IC_{50}) were calculated. A dose response curve was plotted and used to determine the (IC_{50}) values. The IC_{50} data were subjected to One Way Analysis of Variance (ANOVA, MiniTab Version 18) to determine differences ($p \leq 0.05$) among plant extracts IC_{50}. Multiple comparison of IC_{50} was done by Tukey test. Experimental results are expressed as mean ± SEM and all measurements were in duplicate.

The data generated from phytochemical screening of *A. senegalensis* and *A. africanus* extracts were qualitative and tabulated.

3.9. Ethical Consideration

Samples were collected following permission from traditional healers and owners of the farms at Pare Mountain. Prior to commencement of study, clearance was sought from the Scientific and Ethics Review Unit (SERU) in KEMRI. All safety and standards laboratory procedures were considered. There was no involvement of animal or human in this study.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1. Percentage Yields

Annona senegalensis and *Allopylus africanus* were collected from Ugweno village of Kilimanjaro region in Tanzania and evaluated for anticancer activity. Twelve extracts were made by extracting the stem bark of the two plant in six solvents. The percentage yield of each extract is shown in Table 2. There was variation of extraction yields from 1 to 6.8% depending on the type of solvent used for extraction. Highest yields were obtained with aqueous extractions which could be due to the high solubility of different plant compounds in this solvent (Senguttuvan, Paulsamy, & Karthika, 2014)

Table 2: Extraction yield (%) of *A. senegalensis and A. africanus*

Plant sample (stem bark)	Sample weight(g)	Extracted weight(g)	Yield (%*W/W*)
AS Pet ether	500g	5g	1.0%
AA Pet ether	500g	6g	1.2%
AS DCM:MeOH	500g	25g	5.0%
AA DCM:MeOH	500g	10g	2.0%
AS DCM	500g	6g	1.2%
AA DCM	500g	8g	1.6%
AS Ethyl acetate	500g	9g	1.8%
AA Ethyl acetate	500g	11g	2.2%
AS MeOH	500g	15g	3.0%
AA MeOH	500g	26g	5.2%
AS Aqueous	500g	34g	6.8%
AA Aqueous	500g	32g	6.4%

AS: *Annona senegalensis*, AA: *Allophylus africanus*

4.2. Phytochemical Screening of Plant extracts

The twelve stem bark extracts of *A. senegalensis and A. africanus*, contained phytochemical compounds shown in Table 3 and 4. Flavonoids were found present in all extracts of both plants. Flavonoids is commonly known to have antioxidant nature and it has been reported to have antiproliferative activity against many cancers (Widyawati, Dwi, Budianta, Kusuma, & Wijaya, 2014). Therefore, its presence in *A. senegalensis* and *A. africanus* extracts could be related with their anticancer activity. All metabolites were present in ethyl acetate extracts of both plants except terpenes which were absent in ethyl acetate extract of *A. senegalenses*. The

presence of these metabolites could be related with previous researches which informed that ethyl acetate is semi polar solvents hence can dissolve both polar and non-polar compounds (Ajuru *et al*., 2017). The results suggested ethyl acetate as good extraction solvent for active phytochemical compounds from these two plant species. The absence of metabolites in other plant extracts could be due to inability of these components to dissolve into respective solvents regarding their difference in polarity (Bandar *et al*., 2013). Regarding the study of Al-asady, Suker and Hassan (2014) which indicated that the glycoside fraction I from *Convolvulus arvensis* had more cytotoxic inhibition at 10 mg/ml against rhabdomyosarcoma (RD) tumour cell line *in vitro* after 72 h, compared with other extracts (aqueous and methanol), crude extracts of the leaves, stems and roots, the presence of these phytochemicals in the extracts could be implicated in the medicinal value of the two plants.

Table 3: Phytochemical analysis of different solvent extracts of *A. africanus*

Solvent	**Alkaloids**	**Saponins**	**Flavanoids**	**Glycosides**	**Terpenes**	**Tannins**
Pet ether	-	-	+	-	+	-
DCM:MeOH	+	-	+	+	+	+
DCM	-	-	+	-	+	-
Ethyl acetate	+	+	+	+	-	+
MeOH	+	+	+	+	-	+
Aqueous	+	+	+	+	-	-

(+) sign indicates the presence of compounds tested and (-) sign indicates the absence of compounds tested

Table 4: Phytochemical analysis of different solvent extracts of *A. senegalensis*

Solvent	**Alkaloids**	**Saponins**	**Flavanoids**	**Glycosides**	**Terpenes**	**Tannins**
Pet ether	-	-	+	-	+	-
DCM:MeOH	+	+	+	+	+	+
DCM	+	-	+	-	+	+
Ethyl acetate	+	+	+	+	+	+
MeOH	+	+	+	+	-	+
Aqueous	+	+	+	+	-	+

(+) sign indicates the presence of compounds tested and (-) sign indicates the absence of compounds tested.

4.3. Anticancer Activity of *A. senegalensis and A. africanus* Plant Extracts against HEp- 2, HCC 1396, CT 26 Cell lines.

Generally, stem bark of *A. senegalensis* and *A. africanus* inhibited proliferation rate of the HCC 1396, HEp- 2, CT 26 cell lines. All the twelve plant extracts showed different levels of cell growth inhibition at different concentrations against the tested cell lines. There was a concentration dependent cell inhibition, as the concentration of plant extracts decreased from 100 µg/ml to 0.146 µg/ml, the percentage cell inhibition decreased (Appendix I-VI). The proliferation rate was lowest at 100 µg/ml in row H and highest at 0.146 µg/ml in untreated cells (row A) (Figure 10). These results could be compared with that of Fadeyi, Fadeyi, Adejumo and Okoro (2013) where by twenty four plants were screened for anticancer activity, and results indicated that the activity is dose dependent. No anticancer activity was detected to exposure of low concentration (0.5 µg/ml).

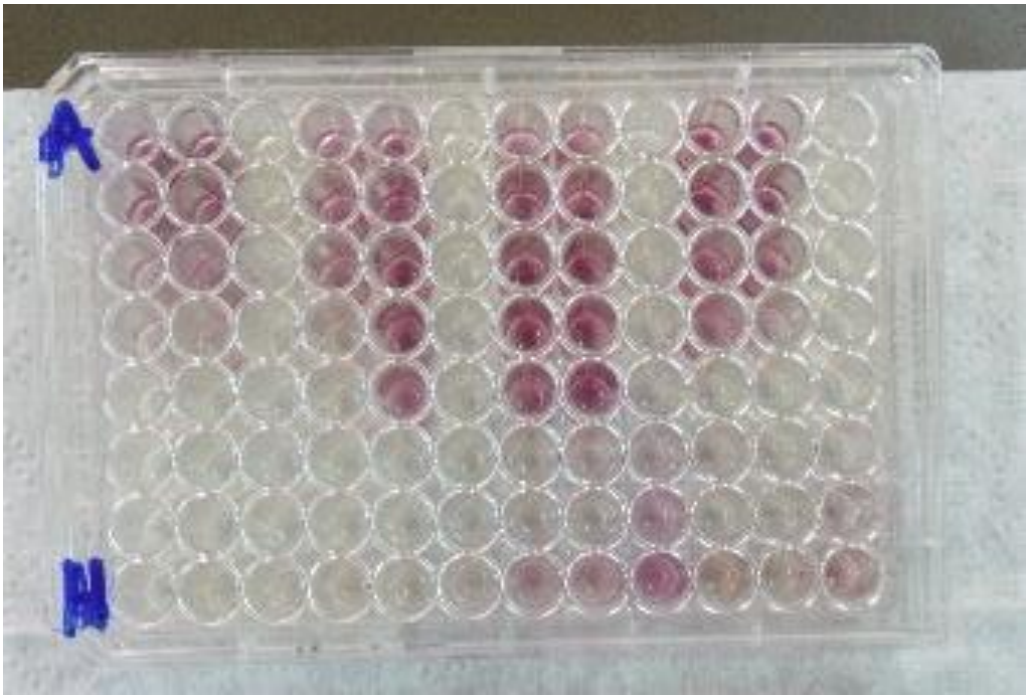

Figure 10: Ninety six well plate showing the decrease of proliferation rate with decreasing of concentration

4.4. Determination of IC_{50} of *A. senegalensis and A. africanus* Plant Extracts against HEp- 2, HCC 1396, CT 26 and VERO Cell Lines

Concentration of plant extracts that inhibited cell growth by 50% (IC_{50}) for the twelve plant extracts tested was calculated and the results displayed in Table 5. Anticancer activity was

classified according to the standards of the National Cancer Institute (NCI) as follows: high anticancer when an IC_{50} <20 μg/ml, anticancer for an IC_{50} between 20 μg/ml to 30 μg/ml, moderate anticancer for IC_{50} between 30 μg/ml to 100 μg/ml and inactive with IC_{50} >100 μg/ml (Boik, 2001). Tabulated results show that anticancer activity varied between plant extracts and cancer cell lines tested. The highest anticancer activity was achieved with petroleum ether extract of *A. senegalensis* against HEp-2 with IC_{50} value of 0.42 ± 0.09 μg/ml. This demonstrated the efficiency of petroleum ether over the other extraction solvents for extracting anticancer compounds against HEp-2 from *A. senegalensis* stem bark. Comparing with phytochemical results, the unknown anticancer compound extracted with this petroleum ether extract could be less polar flavanoids or terpenes (Table 4). Among all plant extracts, the following exhibited high activity. Dichloromethane extract of *A. senegalensis*: IC_{50} 10.41± 2.07 μg/ml and MeOH extract of *A. africanus*: IC_{50} 7.33 ± 0.43 μg/ml against HCC 1396. Petroleum ether extract of *A. senegalensis*: IC_{50} 0.42 ± 0.09 μg/ml and DCM:MeOH extract of *A. africanus*: IC_{50} 1.00 ± 0.4 μg/ml against HEp-2 cancer cells. Petroleum ether extract of *A. senegalensis*: IC_{50} 9.19 ± 0.81 μg/ml and MeOH extracts of *A. africanus*: IC_{50} 9.04 ± 1.05 μg/ml against CT 26 cancer cells. In this study, the aqueous extracts (water) which is the common solvent used by traditional healers for extraction of medicinal plants due to its availability (Mekonnen & Abebe, 2017), exhibited anticancer activity ranging from moderate to none.

The findings could be related with previous study by Okoye *et al*. (2014) which indicated that root bark of *A. senegalensis* has anticancer activity against pancreatic and cervical cancer cells. This study, therefore, revealed that the stem bark of the same plant species has anticancer activity against colon, breast and throat cancer cells. Likewise, the study support a previous study conducted by Sofidiya *et al*. (2012) which showed that *A. africanus* had the best antioxidant activity which could be related to anticancer activity of the plant.

Table 5: Mean IC_{50} of the plant extracts on HCC 1396, HEp-2, CT 26 and VERO cell lines

Plant extracts	**IC_{50}(µg/ml)** HCC 1396	**IC_{50} (µg/ml)** HEp- 2	**IC_{50} (µg/ml)** CT 26	**IC_{50}(µg/ml)** VERO
AS Pet Ether	21.88 ± 2.18^{cd}	0.42 ± 0.09^{a}	11.59 ± 2.58^{b}	39.56 ± 1.73^{b}
AA Pet Ether	41.10 ± 1.42^{e}	2.37 ± 1.45^{a}	9.19 ± 0.81^{b}	62.70 ± 2.04^{bc}
AS DCM:MeOH	27.41 ± 2.28^{d}	4.50 ± 0.72^{a}	54.02 ± 4.13^{f}	>100
AA DCM:MeOH	12.61 ± 1.67^{bc}	1.00 ± 0.41^{a}	21.52 ± 0.06^{c}	78.20 ± 1.47^{cd}
AS DCM	10.41 ± 2.07^{b}	12.36 ± 3.20^{b}	12.19 ± 2.70^{b}	52.21 ± 1.95^{b}
AA DCM	8.76 ± 0.43^{b}	5.02 ± 0.71^{a}	19.04 ± 0.78^{c}	57.73 ± 1.05^{b}
AS Ethyl acetate	17.19 ± 0.19^{c}	12.00 ± 1.11^{b}	26.08 ± 0.04^{d}	93.33 ± 0.67^{d}
AA Ethyl acetate	18.60 ± 0.28^{c}	9.48 ± 0.42^{b}	27.61 ± 4.57^{d}	68.33 ± 3.79^{c}
AS MeOH	47.98 ± 4.52^{f}	97.12 ± 2.88^{f}	36.52 ± 3.23^{e}	>100
AA MeOH	7.33 ± 0.43^{b}	25.38 ± 2.57^{c}	9.04 ± 1.05^{b}	55.72 ± 1.00^{b}
AS Aqueous	76.31 ± 1.22^{g}	76.20 ± 2.38^{e}	65.03 ± 0.04^{g}	>100
AA Aqueous	28.58 ± 0.71^{d}	65.10 ± 3.49^{d}	>100	>100
Doxorubicin	1.14 ± 0.01^{a}	0.21 ± 0.04^{a}	2.94 ± 0.05^{a}	10.94 ± 0.06^{a}

Values are expressed as Mean±SEM. Doxorubicin was used as a positive control. The IC_{50} values of the plant extracts were compared with the doxorubicin for each cell line. Values that do not share a letter are significantly different ($p\leq0.05$). AS=*A. senegalensis* and AA=*A. africanus.*

Doxorubicin a standard drug for cancer treatment was used as the positive control. The results showed that doxorubicin was more potent than all the plant extracts with IC_{50} value of 1.14 ± 0.01 µg/ml for HCC 1396, 0.21± 0.04 µg/ml for HEp- 2, and 2.94 ± 0.05 µg/ml for CT 26. This was expected as the drug is purified as opposed to the extracts which were in crude form. Of particular interest, petroleum ether extract of *A. senegalensis* depicted high activity against HEp- 2 at an IC_{50} value of 0.42 ± 0.09 µg/ml comparing well to the reference standard doxorubicin. Selectivity index for the same was also high (SI = 94.19). This implied its high potential for development of a safe anticancer agent. Potency of plant extracts varied with plant species and the screened cancer cell lines. High potency (IC_{50} <20 µg/ml) coupled with high selectivity (SI>3) was observed on extracts of *A. senegalensis* extracted using DCM against HCC 1396, petroleum ether on HEp- 2 and CT 26. For *A. africanus* this was observed on DCM:MeOH against HEp- 2, MeOH against HCC 1396 and CT 26 (Table 5). This indicated that, the aforementioned are suitable extraction solvents for anticancer compounds from these plants respectively. Extracts from both polar and non-polar solvents showed

varied levels of activity. This signified the possibility of *A. senegalensis* and *A. africanus* to possess both polar and non-polar compounds with anticancer activity. Regarding the variation on performance of plant extracts shown by the solvent used in extraction, the results supported previous studies which showed that, the solvent type used in extraction has effect on the potency of medicinal plants (Koffi *et al*., 2010; Dhawan & Gupta, 2016).

4.5. Cytotoxic Activity against VERO Cell Lines and Selectivity Index

The cytotoxicity activity was determined using VERO P23 cell lines (African green monkey kidney cells). The results indicated that all the plant extracts investigated were less toxic to VERO cells (IC_{50} >39 µg/ml) than the positive control, doxorubicin. Four extracts were observed to be inactive (>100 µg/ml) while the rest moderate with IC_{50} ranging between 39 µg/ml and 100 µg/ml. The IC_{50} of doxorubicin on VERO cells was low (10.94 ± 0.06 µg/ml), this support previous study which reported that doxorubicin provide side effect against normal tissue (Wang *et al*., 2004). There was a variation of selectivity among plant extracts and cancer cell lines tested (Table 6). Selectivity index value >3 were considered selective for cancer cell line while SI values <3 were considered non selective to specific cancer cell line (Bézivin, Tomasi, Lohézic-Le Dévéhat, & Boustie, 2003). Most of the extracts were observed selective active to one cancer cell line while were not selective to the other cell lines tested. However, at least one extract for each plant species showed selectivity to all cancer cell lines. These are DCM:MeOH for *A. africanus* and DCM for *A. senegalensis*. The variation of selectivity could be related with different phytochemical composition in the plant extracts (Mwitari *et al*., 2018). Generally, aqueous extract of *A. senegalensis* was found not to be selective (SI<3) to any cancer cell line. This could be due to the presence of toxic compounds that affect the performance of active compounds (Dzoyem, Mcgaw, & Eloff, 2014). Petroleum ether extract of *A. senegalensis* depicted the highest selectivity on HEp- 2 cancer cell lines with SI value of 94.19. This indicated that, the extract has high potential of producing safe herbal medicine against throat cancer.

Table 6: Selectivity index of *A. senegalensis and A. africanus* plant extracts

Plant extracts	**HCC 1396**	**HEp-2**	**CT 26**
AS Pet Ether	1.81	94.19	3.41
AA Pet Ether	1.53	26.46	6.82
AS MeOH: DCM	3.65	22.22	1.85
AA MeOH: DCM	6.20	78.20	3.63
AS DCM	5.02	4.22	6.42
AA DCM	6.60	9.92	2.74
AS Ethyl acetate	5.43	7.78	3.58
AA Ethyl acetate	3.67	7.21	2.47
AS MeOH	2.08	1.03	2.74
AA MeOH	7.60	2.20	6.16
AS Aqueous	1.31	1.31	1.54
AA Aqueous	3.49	1.54	N/A
Doxorubicin	9.6	52.1	3.8

N/A*; Not applicable because the test drug did not inhibit the growth of the cell AS=*A. senegalensis* and AA=*A. africanus*

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1. Conclusion

This study indicated that, *A. senegalensis* and *A. africanus* have potential anticancer activity on throat, breast and colon cancer cells. Different solvents used for extraction showed varied activity and selectivity against the cancer cells tested. Petroleum ether extract of *A. senegalensis* was in particular found to have high potential for development of an anticancer agent against throat cancer. These findings justify the use of *A. senegalensis* and *A. africanus* in traditional practice. The findings also support previous studies which indicated that, extraction solvents used on extraction of bioactive molecules affect the performance of medicinal plants.

5.2. Recommendations

From the conclusion the following recommendations are made;

(i) Further studies are recommended on evaluation of anticancer pure compounds from the active extracts.

(ii) Evaluation of *in vivo* anticancer activity of *A. senegalensis* and *A. africanus* in animal model is recommended.

(iii) Further studies on anti-cancer activity of petroleum ether extract of *A. senegalensis* against throat cancer can be made so as to recommend the development of anti-cancer agent from this plants species.

REFERENCES

Ajaiyeoba, E., Falade, M., Ogbole, O., Okpako, L., & Akinboye, D. (2006). Short communication. *African Journal of Traditional Complementary and Alternative Medicines*, 3(1), 137–141.

Ajuru, M. G., Williams, L. F., & Ajuru, G. (2017). Qualitative and quantitative phytochemical screening of some plants used in ethnomedicine in the Niger Delta Region of Nigeria. *Journal of Food and Nutrition Sciences*, 5(5), 198–205.

Al-asady, A. A. B., Suker, D. K., & Hassan, K. K. (2014). Cytotoxic and cytogenetic effects of *Convolvulus arvensis* extracts on rhabdomyosarcoma (RD) tumor cell line *in vitro*. *Journal of Medicinal Plant Research*, 8(15), 588–598.

Aslam, M. S., Naveed, S., Ahmed, A., Abbas, Z., Gull, I., & Athar, M. A. (2014). Side effects of chemotherapy in cancer patients and evaluation of patients opinion about starvation based differential chemotherapy. *Journal of Cancer Therapy*, 5, 817–822.

Awa, E. P., Ibrahim, S., & Ameh, D. A. (2012). GC/MS Analysis and antimicrobial activity of diethyl ether fraction of methanoolic extract from the stem bark of *Annona senegalensis* Pers. *International Journal of Pharmaceutical Sciences and Research*, 3(11), 4213–4218.

Bako, S., Bakfur, M., John, I., & Bala, E. (2005). Phytochemical of medicinal plants. *Cytotechnology*, 9, 167–177

Balogun, O. S., Oladosu, I. A., & Liu, Z. (2016). Fatty acid profile and in vitro biological activities of *Allophylus africanus* (P. Beauv). *Journal of Herbs, Spices & Medicinal Plants*, 22(3), 238–246.

Balogun, S. O., Oladosu, I. A., & Liu, Z. (2014). Chemical compositions and antioxidant potential of essential oils from leaves and flowers of *Allophylus africanus*. *Journal of Essential Oil Bearing Plants*, 17(5), 769–775.

Bandar, H., Hijazi, A., Rammal, H., Hachem, A., Saad, Z., & Badran, B. (2013). Techniques for the extraction of bioactive compounds from *Lebanese Urtica dioica*. *American Journal of Phytomedicine and Clinical Therapeutics*, 1(6), 507–513.

Baskar, R., Lee, K. A., Yeo, R., & Yeoh, K. (2012). Cancer and radiation therapy : current advances and future directions. *International Journal of Medical Science*, 9(3), 193–199.

Bézivin, C., Tomasi, S., Lohézic-Le Dévéhat, F., & Boustie, J. (2003). Cytotoxic activity of some lichen extracts on murine and human cancer cell lines. *Phytomedicine*, 10, 499–503.

Biemar, F., & Foti, M. (2013). Global progress against cancer — challenges and opportunities. *Cancer Biolology Medicine*, 10, 183–186.

Boik, J. (2001). *Natural Compounds in Cancer Therapy* (first edition). United State of America.

Chavan, R. B., & Gaikwad, D. K. (2016). The ethnobotany, phytochemistry and biological properties of *Allophylus* species used in traditional medicine. *World Journal of Pharmacy and Pharmaceutical Sciences*, 5(11), 664–682.

Conklin, K. A. (2004). Chemotherapy-associated oxidative stress: Impact on chemotherapeutic effectiveness. *Integrative Cancer Therapies*, 3(4), 294–300.

Delancey, J. O., Jemal, A., & Ward, E. M. (2010). The global burden of cancer : priorities for prevention. *Carcinogenesis*, 31(1), 100–110.

Deshmukh, S. K., Azim, S., Ahmad, A., Zubair, H., Tyagi, N., & Sanjeev, K. (2017). Biological basis of cancer health disparities : resources and challenges for research. *American Journal of Cancer*, 7(1), 1–12.

Devkota, A., Dall'Acqua, S., Jha, P. K., & Innocent, G. (2010). Variation in the active constituent contents in Centella asiatica grown in different habitats. *Journal of Plant Science*, 7, 43–47.

Dhawan, D., & Gupta, J. (2016). Comparison of different solvents for phytochemical extraction potential from *Datura metel* plant Leaves. *International Journal of Biological Chemistry*, 11, 17–22.

Dzoyem, J. P., Mcgaw, L. J., & Eloff, J. N. (2014). *In vitro* antibacterial , antioxidant and cytotoxic activity of acetone leaf extracts of nine under-investigated *Fabaceae* tree species leads to potentially useful extracts in animal health and productivity. *BMC Complementary and Alternative Medicine*, *14*(1), 1–7.

Fadeyi, S. A., Fadeyi, O. O., Adejumo, A. A., & Okoro, C. (2013). In vitro anticancer screening of 24 locally used Nigerian medicinal plants. *BMC Complementary and Alternative Medicine*, 13(79), 1–9.

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

Ghagane, S. C., Puranik, S. I., Nerli, R. B., & Hiremath, M. B. (2017). Evaluation of *in vitro* antioxidant and anticancer activity of *Allophylus cobbe* leaf extracts on DU-145 and PC-3 human prostate cancer cell lines. *Cytotechnology*, 69, 167–177.

Graham, J. G., Quinn, M. L., Fabricant, D. S., & Farnsworth, N. R. (2000). Plants used against cancer – an extension of the work of Jonathan Hartwell. *Journal of Ethnopharmacology*, 73, 347–377.

Greenwell, M., & Rahman, S. M. (2015). Medicinal plants : their use in anticancer treatment. *International Journal of Pharmacological Science Research*, 6(10), 4103–4112.

Idikio, H. A. (2011). Human cancer classification: A systems biology-based model integrating morphology, cancer stem cells, proteomics, and genomics. *Journal of Cancer*, 2(1), 107–115.

Kigondu, E. V. M., Rukunga, G. M., Gathirwa, J. W., Irungu, B. N., Mwikwabe, N. M., Amalemba, G. M., … Kirira, P. G. (2011). Antiplasmodial and cytotoxicity activities of some selected plants used by the Maasai community, Kenya. *South African Journal of Botany*, 77, 725–729.

Kisangau, D. P., Lyaruu, H. V. M., Hosea, K. M., & Joseph, C. C. (2007). Use of traditional medicines in the management of HIV/AIDS opportunistic infections in Tanzania : a case in the Bukoba rural district. *Journal of Ethnobiology and Ethnomedicine*, 29(3), 1–8.

Kitula, A. R. (2007). Use of medicinal plants for human health in Udzungwa Mountains Forests : a case study of New Dabaga Ulongambi Forest Reserve. *Journal of Ethnobiology and Ethnomedicine*, 7(3), 1–4.

Koffi, E., Sea, T., Dodehe, Y., & Soro, S. (2010). Effect of solvent type on extraction of polyphenols from twenty three Ivorian plants. *Journal of Animal and Plant Sciences*, 5(3), 550–558.

Liu, W., Liu, J., Yin, D., & Zhao, X. (2015). Influence of ecological factors on the production of active substances in the anti- cancer plant Sinopodophyllum hexandrum. *PLoS ONE*, 10(4), 1–22.

Madhu, M., Sailaja, V., Satyadev, T., & Satyanarayana, M. V. (2016). Quantitative phytochemical analysis of selected medicinal plant species by using various organic solvents. *Journal of Pharmacognosy and Phytochemistry*, 5(2), 25–29.

Matata, D. Z., Ngassapa, O. D., Machumi, F., & Moshi, M. J. (2018). Screening of plants used as traditional anticancer remedies in Mkuranga and Same Districts, Tanzania, Using Brine Shrimp Toxicity Bioassay. *Evidence-Based Complementary and Alternative Medicine*, 1–16.

Max Parkin, D., Bray, F., Ferlay, J., & Pisani, P. (2005). Global Cancer Statistics, 2002. *A Cancer Journal for Clinicians*, 55, 74–108.

Mekonnen, N., & Abebe, E. (2017). Ethnobotanical knowledge and practices of traditional healers in Harar, Haramaya, Bati and Garamuleta, Eastern Ethiopia. *Ethiopian Veterinary Journal*, *21*(2), 40.

Mustapha, A. A. (2013). Annona senegalensis Persoon : A Multipurpose shrub, its phytotherapic , phytopharmacological and phytomedicinal uses. *International Journal of Science and Technology*, 2(12), 862–865.

Mwitari, P. G., Mutuku, N. C., Keter, L., Gathirwa, J. W., Kimani, F., Kirira, P., & Wachira, S. (2018). Immune modulating and antiproliferative potential of *Withania somnifera* crude and pre-purified fractions on selected cancerous and normal cells. *Journal of Complementary and Alternative Medical Research*, 5(1), 1–12.

Okoli, C. O., Onyeto, C. A., Akpa, B. P., Ezike, A. C., Akah, P. A., & Okoye, T. C. (2010). Neuropharmacological evaluation of *Annona senegalensis* leaves. *African Journal of Biotechnology*, 9(49), 8435–8444.

Okoye, T. C., Akah, P. A., Nworu, C. S., & Ezike, A. C. (2014). Kaurenoic acid isolated from the root bark of *Annona senegalensis* induces cytotoxic and antiproliferative effects against PANC-1 and HeLa cells. *European Journal of Medicinal Plants*, 4(5), 579–589.

Okoye, T. C., Akah, P. A. O., Ezike, A. C., Omeje, E. O., & Odoh, U. E. (2012). Antimicrobial effects of a lipophilic fraction and kaurenoic acid isolated from the root bark extracts of *Annona senegalensis*. *Evidence-Based Complementary and Alternative Medicine*, 1–10.

Oladosu, I. A., Balogun, S. O., & Ademowo, G. O. (2013). Phytochemical screening, antimalarial and histopathological studies of *Allophylus africanus* and *Tragia benthamii*. *Chinese Journal of Natural Medicines*, 11(4), 371–376.

Patel, S., Gheewala, N., Suthar, A., & Shah, A. (2009). In-vitro cytotoxicity activity of *Solanum nigrum* extract against. *International Journal of Pharmacy and Pharmaceutical Sciences*, 1(1), 38–47.

Pinmai, K., Chunlaratthanabhorn, S., Ngamkitidechakul, C., & Soonthornchareon, N. (2008). Synergistic growth inhibitory effects of *Phyllanthus emblica* and *Terminalia bellerica* extracts with conventional cytotoxic agents : Doxorubicin and cisplatin against human hepatocellular carcinoma and lung cancer cells. *World Journal Gastroenterol*, 14(10), 1491–1497.

Prakash, O., Kumar, A., Kumar, P., & Ajeet. (2013). Anticancer potential of plants and natural products. *American Journal of Pharmacological Sciences*, 1(6), 104–115.

Priya, L. M., Priya, B. K., Kotakadi, V. S., & Josthna, P. (2015). Herbal and medicinal plants molecules towards treatment of cancer. *American Journal of Ethnomedicine*, *2*(2),136-142

Rukunga, G. M., Gathirwa, J. W., Omar, S. A., Muregi, F. W., Muthaura, C. N., Kirira, P. G., … Kofi-Tsekpo, W. M. (2009). Anti-plasmodial activity of the extracts of some Kenyan medicinal plants. *Journal of Ethnopharmacology*, 121(2), 282–285.

Saxena, M., Saxena, J., Nema, R., Singh, D., & Gupta, A. (2013). Phytochemistry of Medicinal Plants. *Phytojournal*, 1(6), 168–182.

Senguttuvan, J., Paulsamy, S., & Karthika, K. (2014). P hytochemical analysis and evaluation of leaf and root parts of the medicinal herb, Hypochaeris radicata L . for *in vitro* antioxidant activities. *Asian Pacific Journal of Tropical Biomedicine*, 4, S359–S367.

Siegel, R. L., Miller, K. D., & Jemal, A. (2018). Cancer Statistics, 2018. *Cancer Journal for Clinicians*, 68, 7–30.

Sofidiya, M. O., Jimoh, F. O., Aliero, A. A., Afolayan, A. J., Odukoya, O. A., & Familoni, O. B. (2012). Evaluation of antioxidant and antibacterial properties of six Sapindaceae members. *Journal of Medicinal Plants Research*, 6(1), 154–160.

Stanifer, J. W., Patel, U. D., Karia, F., Thielman, N., Maro, V., Shimbi, D., … Boyd, D. (2015). The determinants of traditional medicine use in northern tanzania : a mixed-methods Study. *PLoS ONE*, 10(4), 1–17.

Twentyman, P. R., & Luscombe, M. (1987). A study of some variables in a tetrazolium dye (MTT) based assay for cell growth and chemosensitivity. *British Journal of Cancer*, 56, 279–285.

Wang, S., Konorev, E. A., Kotamraju, S., Joseph, J., Kalivendi, S., & Kalyanaraman, B. (2004). Doxorubicin induces apoptosis in normal and tumor cells via. *The Journal of Biological Chemistry*, 279(11), 25535–25543.

Wenzel, T. L. (2011). Western and Traditional Medicine Use Practices in Shirati, Tanzania. *Spring*, 3, 1–17.

WHO. (2014). World Cancer Report. The International Agency for Research on Cancer. Lyon, London.

Widyawati, P. S., Dwi, T., Budianta, W., Kusuma, F. A., & Wijaya, E. L. (2014). Difference of solvent polarity to phytochemical content and antioxidant activity of *Pluchea indicia* less leaves extracts. *International Journal of Pharmacognosy and Phytochemical Research*, 6(4), 850–855.

Yasser, H. E. M. (2016). Biochemistry & analytical biochemistry in-vitro anti-cancer activity of extracts Dracaen Cinnabari Balf . F Resin from Socotra Island in Yemen Republic. *Biochemistry and Analytical Biochemistry*, *5*(3), 1–17.

Yessuf, A. M. (2015). phytochemical extraction and screening of bio active compounds from black phytochemical extraction and screening of bio active compounds from black Cumin (Nigella Sativa) seeds extract. *American Journal of Life Sciences*, 3(5), 358–364.

APPENDICES

Appendix 1: Percentage cell inhibition against four cell lines by petroleum ether extracts

Concentration	Vero AS	VeroAA	HepAS	HepAA	CTAS	CTAA	HCCAS	HCCAA
0.146	3.109215	1.990551	35.79141	30.35112	12.8377	5.975294	7.783968	8.745881
0.44	5.408872	6.650528	51.02494	37.75747	20.60153	10.73077	11.46489	10.32553
1.33	10.69637	11.36135	71.35304	49.04817	29.12688	22.22418	20.97959	16.96158
4	17.01382	16.88385	85.40934	74.24477	35.73491	40.88149	29.2209	25.64314
11.11	26.49965	24.60198	100.3033	100.0679	53.09883	55.49115	36.44813	30.62045
33.33	45.6563	30.7047	100.5067	100.6611	100.7437	100.3946	98.21393	92.50762
100	88.44631	78.85177	100.9903	100.8744	100.9752	100.9402	100.9998	100.3954

Appendix 2: Percentage cell inhibition against four cell lines by dichloromethane: methanol extracts

Concentration	Vero AS	VeroAA	HepAS	HepAA	CTAS	CTAA	HCCAS	HCCAA
0.146	0.8.754341	0.2.884512	21.10726	30.9613	4.37352	5.83884	7.07493	12.89962
0.44	2.8415793	1.9768973	27.69811	41.8097	8.66742	11.1487	13.6328	17.7897
1.33	6.1542581	4.9122449	38.83832	52.6301	11.1452	15.327	17.2612	26.35619
4	9.9201977	6.0827326	50.07779	60.3073	21.9717	23.8576	23.8725	33.23073
11.11	15.381717	14.544101	79.6383	85.2922	37.6422	39.3352	45.9368	48.52601
33.33	29.942929	22.775279	99.34776	100.074	44.1067	57.1214	61.584	95.28508
100	47.939222	63.697144	100.2516	100.546	100.922	100.139	100.396	100.9543

Appendix 3: Percentage cell inhibition against four cell lines by dichloromethane extracts

Concentration	Vero AS	VeroAA	HepAS	HepAA	CTAS	CTAA	HCCAS	HCCAA
0.146	1.568688	0.607232	13.19412	30.26855	10.20426	12.72226	15.19781	31.65944
0.44	6.678865	4.161618	22.73479	34.17425	22.80155	16.9199	26.64458	36.11432
1.33	11.99676	9.606829	30.73108	40.95545	30.85601	20.1247	35.23833	40.22862
4	18.8007	14.36968	35.14755	45.31353	36.96416	24.23811	47.16388	44.19285
11.11	27.98112	24.57353	48.75756	98.76875	48.06989	45.78486	51.4184	53.14578
33.33	37.21242	33.71729	100.041	100.0071	100.0056	100.1236	100.2887	100.4422
100	97.43571	95.12137	100.5156	100.0569	100.0686	100.3808	100.7152	100.78

Appendix 4: Percentage cell inhibition against four cell lines by ethyl acetate extracts

Concentration	Vero AS	VeroAA	HepAS	HepAA	CTAS	CTAA	HCCAS	HCCAA
0.146	0.231027	0.904217	5.749881	29.01362	14.57699	11.34678	17.7004	13.19061
0.44	4.75939	5.713936	8.551389	32.54415	17.28636	15.70814	21.39388	18.133
1.33	11.75161	11.94548	14.78123	40.91393	25.56982	27.18327	28.98725	25.03783
4	14.08674	14.73363	26.78115	43.68418	31.59973	33.48175	34.40078	32.13801
11.11	28.72646	20.7796	46.72101	59.01005	41.04459	39.99705	45.79794	43.0322
33.33	40.3436	31.5698	100.0976	100.1557	60.61488	59.68164	99.91097	99.58771
100	53.88398	69.29952	100.7316	100.6141	100.0858	100.416	100.0632	100.2509

Appendix 5: Percentage cell inhibition against four cell lines by Methanol extracts

Concentration	Vero AS	VeroAA	HepAS	HepAA	CTAS	CTAA	HCCAS	HCCAA
0.146	0.064629	5.117588	7.9763	16.46567	5.848756	18.68598	3.598511	20.78965
0.44	2.626243	10.66204	11.87596	25.01827	14.75913	27.92778	7.714212	26.72455
1.33	5.683982	14.97508	16.70033	29.2312	22.36342	36.04466	10.15068	32.97834
4	8.791458	19.58329	21.79765	37.03491	26.39888	42.30013	16.4059	47.13884
11.11	15.08628	26.90871	28.76747	48.63545	33.30377	52.36983	27.57141	55.99092
33.33	25.02201	42.42361	36.31202	57.42991	48.08798	84.4163	42.01472	89.96474
100	48.07844	81.90062	51.07767	100.8381	99.46752	100.5861	98.21734	100.9806

Appendix 6: Percentage cell inhibition against four cell lines by Water (aqueous) extracts

Concentration	Vero AS	VeroAA	HepAS	HepAA	CTAS	CTAA	HCCAS	HCCAA
0.146	0.183648	0.146814	6.893887	11.58941	15.11442	0.001493	0.712619	10.34706
0.44	0.970823	0.678579	9.186239	17.67058	19.13455	0.970047	3.107263	22.13535
1.33	4.0967	3.752795	18.96768	21.36069	23.92801	4.197933	9.349022	28.592
4	8.908407	7.365114	21.26221	26.27096	29.1807	8.777549	11.19515	33.56486
11.11	15.26641	11.70284	30.58765	32.87458	35.62713	11.50783	20.8576	41.0112
33.33	24.33912	19.25259	35.61668	38.53816	41.26326	15.77658	34.76984	53.43236
100	48.30747	42.0986	64.02359	74.1801	77.50445	37.09513	62.11372	99.52996