BIOASSAY-LED ISOLATION OF CYTOTOXIC COMPOUNDS FROM EXTRACTS OF *AFRAMOMUM MELEGUETA* (ROSCOE) K. SCHUM. SEEDS AND *STROPHANTHUS HISPIDUS* DC. WHOLE PLANT

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ABSTRACT

Cancer is the second leading cause of death globally. Several chemotherapeutic agents used for the treatment of cancer have many side effects. This necessitated a search for new potent chemotherapeutic agents with high selectivity in order to minimise the occurrence of debilitating side effects. This led to the ethnobotanical survey of plants used for treatment of cancer in Akinyele Local Government area of Oyo State. This study was aimed at evaluating the cytotoxicity of the selected plants, isolate and characterise their active constituents.

Different morphological parts of six plants selected from the survey were macerated separately in redistilled methanol at room temperature (26-33 ^{o}C) and evaluated for preliminary *in vitro* cytotoxicity using Brine Shrimp Lethality (BSL) assay and MTT (3(4,5-dimethylthiazol-2-yl)-2diphenyltetrazoliumbromide) colorimetric assay against human carcinoma Rhabdomyosarcoma (RD), breast (AU565), cervix (Hep-2C) and African green monkey kidney epithelial (normal) cell lines. Cyclophosphamide and doxorubicin were used as standards. The two most active plants, *Aframomum melegueta* seeds (Ams) FHI 112374 and *Strophanthus hispidus* whole plant (Shw) FHI 112443 were selected based on their significant cytotoxic activities. The Methanolic extracts of Ams and Shw were successively partitioned into *n*-hexane, dichloromethane and ethyl acetate fractions and screened against cancer cell lines. The active fractions of Ams and Shw were then subjected to various chromatographic techniques (Vacuum Liquid Chromatography, column chromatography, HPLC) for isolation of bioactive compounds. Isolated compounds were characterised using spectroscopic techniques (UV, IR, and 1D and 2D NMR). Data were analysed using one-way ANOVA and Dunnet Multiple C mparis n test at $\alpha_{0.05}$.

The Methanolic extracts of Ams and Shw showed significant BSL activity at LC_{50} of 7.22±0.01 and 9.23±0.12 µg/mL, respectively. The Ams hexane fraction had the best cytotoxic activity with LC_{50} of 2.27±0.18 µg/mL against brine shrimp. *Strophanthus hispidus* whole plant (CC_{50} of 2.94±0.01; 1.66±0.03 µg/mL) and Ams (CC_{50} = 5.18±2.10 and 9.74±0.16 µg/mL) had good cytotoxic activity on RD and Hep -2C, respectively. The Ams hexane (CC_{50} of 0.98±0.1 µg/mL) showed significant cytotoxicity on RD cell, with a good selectivity of 98.97 compared to

cyclophosphamide (32.32). The *n*-hexane and dichloromethane fractions of Shw showed cytotoxicity of 10.72±0.18 and 1.26±0.06 µg/mL on AU565 with selectivity of 1.31 and 1.92, respectively. The Ams hexane afforded compounds 6-shogaol **(1)**, 6-paradol **(2)**, 1-dehydrogingerdione **(3)**, while Ams dichloromethane afforded zingerone **(4)**, 6-gingerol **(5)**, [6]-isodehydrogingerdione **(6)**, (Z)-3-hydroxy-1-(4-hydroxy-3-methoxyphenyl) dec-3-en-5-one **(7)**. The Shw hexane afforded Daucosterol **(8)**, stigmasterol **(9)** and a novel compound; 2-(4-ethoxy-3-methoxyphenyl)-5-hydroxy-3,7,8-trimethoxy-4H-chromen-4-one **(10),** while Shw dichloromethane afforded pityriacitrin **(11)**, ursolic acid **(12)**, strophanthidin digitoxoside **(13)** and urs-12-en-28-oic acid, 2,3,23,-trihydroxy-(2β,3β,4α) **(14)**. Compound 1 showed significant cytotoxicity (CC_{50} = 0.11±0.01 µg/mL) on RD with selectivity of 215.45, while ursolic acid (CC_{50} = 8.07 ± 0.02 µM) and strophanthidin-digitoxoside (CC_{50} = 11.42 ± 0.60 µM) had highest cytotoxicity on AU565 both with selectivity ˃100.

Aframomum melegueta seeds and *Strophanthus hispidus* whole plant exhibited significant cytotoxic properties. Ursolic acid, strophanthidin-digitoxoside isolated from *Strophanthus hispidus* and 6-shogaol isolated from *Aframomum melegueta* could serve as leads in anticancer drug discovery.

Keywords: *Aframomum melegueta*, *Strophanthus hispidus*, Ursolic acid, 6-shogaol,

Cytotoxicity

DEDICATION

This work is dedicated to Almighty Allah.

Also to my dear mother, Alhaja Qudrat Titilope Ajoke LABO-POPOOLA. May Allah forgive your sins and grant you Al-Jannah Firdaous, Ameen

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To my exclusive parents, Prof. and Prof. (Mrs.) Labo-Popoola; your struggles, the paths you cross and the lengths you go in certifying that I get the best in life cannot be overemphasized. You are to me a pillar. Thank you for your love and care, there is no way I would have made it in life but for you kind support and encouragement.

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To my husband, Mr. Kolawole Ambali, thanks so much for the love and support. Without your support, this work would not have been achieved. To my adorable children, Khaleedah Ewatomi and Iqbal Adebobola, thanks for the support, may you grow up well and be better than I am in goodness and greatness.

CERTIFICATION

I certify that this study was carried out by AMBALI, Owoola Azeezat in the Department of Pharmacognosy, Faculty of Pharmacy, University of Ibadan, Ibadan, Oyo State, Nigeria, under my supervision

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LIST OF ABBREVIATION

AKLGA……Akinyele L cal G vernment Area

AST…….. Aspartate transferase

CC…….C lumn Chr mat graphy

CDC……. Centre f r Disease C ntr l

CTX…… Cycl ph sphamide

CYP…… Cyt chr me P

DMSO …….Dimethyl Sulf xide

DPPH……2,2-diphenyl-1-hydrazine (DPPH)

HPLC……High Perf rmance Liquid Chr mat graphy

IARC……Internati nal Agency f r Research n Cancer

LDH……..Lact se Dehydrogenase

MTT……(3(4,5-dimethylthiazol-2-yl)-2diphenyltetrazolium bromide)

PKC…….Pr tein Kinase C

RD……...…Rhabd my sarc ma

ROS……..…..Reactive Oxygen Species

VLC……..Vacuum Liquid Chr mat graphy

WHO……W rld Health Organizati n

CHAPTER ONE

1.0 INTRODUCTION

Historically, plants with medicinal values have provided a means of stimulation for discovery of new and novel drugs. These drugs and supplements obtained from plant source have given a large contribution to human health (Elsenberg *et al.*, 1990.).

For several years, traditional medicine has been in existence for the world population (WHO, 2013.). Remote areas of some communities in the countries which are under developed and also some that are just developing, rely mainly on medicinal plants for treatment. Instances have shown in these communities whereby Primary health centres, (PHC) are all the people resort to and self-medications using plant medicines. Plants are accessible and affordable (Bussmann and Sharon, 2006.). The search for natural products for management of diseases represents an area of great interest. Plants, animals, marine organisms have been sources of treatments for many diseases and infections. Evaluation of these plants provides clues to the usage of the drugs for treatment (Okigbo and Ajalie, 2005.).

Natural products have been shown in several reports to contain a vast array of diverse classes of bioactive- compounds which include alkaloids, polyphenols, flavonoids and so on (Cimbora Zovko *et al.*, 2004.). Flavonoids can serve as antioxidants which help to neutralize free radicals that damage body tissues and which can be a potential cause of cardiovascular disease, strokes and cancers (Wu *et al.*, 2006; Rauter *et al.*, 2010.) .

Herbs have been used by humans as seasonings to yield useful nutrients (Tapsell *et al.*, 2006.). Plants possess varieties of compounds which can be useful medicinally or have undesirable property. Drugs derived from plants are being used as traditional means of treatment or remedy for these diseases both in developing and developed non-industrialized societies. Reports from a study by World Health Organization (WHO) indicate that a greater percentage of the people of Asia and Africa countries, place emphasis on plant medicinal usage for their basic primary health care. The main reason for this is as a results of the high cost of pharmaceutical medications (Hoareau and DaSilva, 1999.).

An increase in the demand for drugs which are derived from plants has been observed in developing countries due to their affordability and accessibility. Extracts from plants are often used for preparation of medicinal drugs and ointments. A major population of the world utilizes herbal drugs for treatment of different diseases (MacLennan *et al.*, 2006.). These herbs even though extracted from plants, exhibit some intrinsic side effects. This could be due to inability to identify the compounds in the plants and sometimes a deficiency in standardization methods as well as disastrous ways in which the drugs are being manufactured (Calixto, 2000.).

1.1 Cancer

Cancer is a collection of several disease whereby cells divide and produce new cells in an uncontrollable fashion. It undergoes metastasis and cause damage to essential organs. This disease has been projected to have increased to about 18.1 million cases which will results to death of approximately 9.6 million in 2018. One out of five men and six women respectively is prone to develop cancer in their lifetime while one out of eight men and one in eleven women die as a result of it (WHO, 2018.).

According to ATSDR public health announcement, several steps can lead to the formation of cancer cells in the body. An injury to the cell which could be caused by chemical intake or sometimes exposure to some toxic environmental conditions thereby leading to toxicity. Subsequently, the cell could undergo a program cell death, such that the body is able to get rid of abnormal cells without it leading to cancer disease. The DNA which is not completely destroyed by carcinogens might be repaired. This repair could be done immediately after DNA synthesis by a process called Mismatch DNA repair. Several other mechanisms can also be used for the repair such as excision repair, chemical reversal. The DNA repair can also be by double-stranded break repair which is carried out by the BRAC1 gene which prevents ovarian and breast cancer. Sometimes, the cell could repair its DNA maybe due to the intake of supplements or food with high nutritional contents and value. In most cases, once the DNA is not repaired and changed into a healthy cell, the denaturation of the genetic material could result in the formation of cancer cells. Figure 1.1 showed the steps leading to formation of cancer and dead cells by apopotosis (Williams-Fleetwood, 2009.).

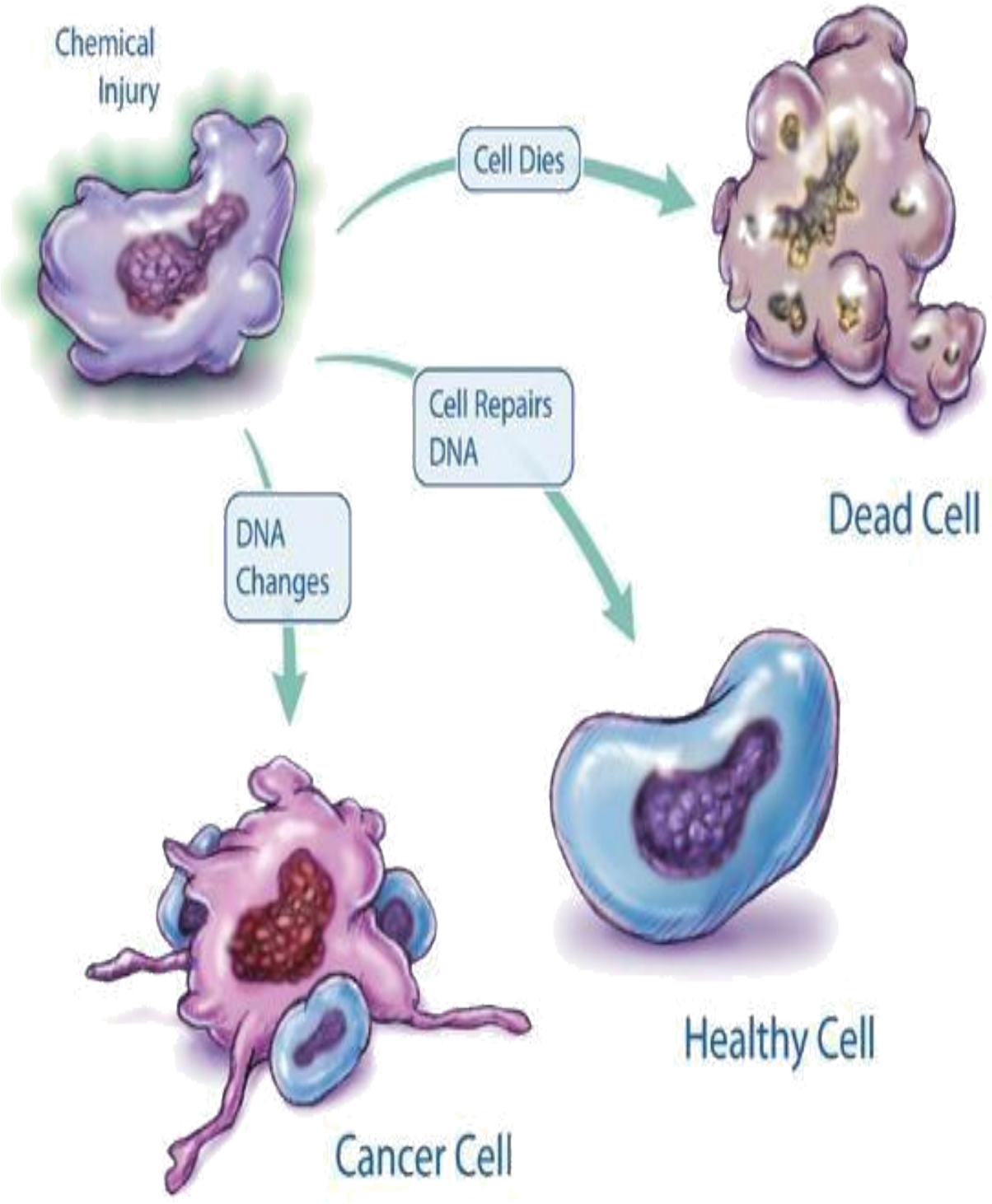


Figure 1.1: Steps leading to formation of Cancer (Williams-Fleetwood, 2009.).

1.2 Global Cancer Epidemic

The occurrence of this disease is highest in developed part of the world, predominantly in Northern America, Western Europe and Australia. Recently, there

is an alarming rate of growth in developing world. A large percentage of all cancer deaths is estimated to occur in countries that are not well developed and also lack fund for basic amenities. These continents are known to have about for two-thirds of all cases of cancer worldwide in about three decades to come (Bray and Møller, 2006.).

Economic impact of cancer is so enormous and often times not affordable. The cost of medical treatment the patients need which will include diagnosis, hospitalization with treatment such as surgery, radiotherapy and chemotherapy is huge. In 2010, the cost of initial phase of caring for a patient with female breast cancer was about $2,000 and almost double for lung cancer (Yabroff *et al.*, 2011.)

1.3 Causes of Cancers

A notable number of persons will one way or the other be affected with cancer during their life. Sometime exposure to some ecological risk factors can lead to the disease. Environmental risk which can be as a result of exposure to pollutants, tobacco smoking, exposure to chemicals which are toxic and the exposure to radiation in homes and work places could also be a factor leading to cancer.

Environmental

Pollutants such as Arsenic, cadmium, nickel and hexavalent chromium found in the environment are the most common and potent carcinogens which are classified as group 1 carcinogens to humans by the International Agency for Research on Cancer (IARC, 2012.). Chronic exposure to these metals are origins of various types of cancer in humans, thus, representing a significant environmental health issue (Wang and Yang, 2019.). Agricultural pesticides such as Chlorophenols (CPs), dioxin compounds and related phenoxyacetic acids (PAs) have been found to possess carcinogenetic effect on lung (Shankar *et al.*, 2019.).

Burning coal inside home for the purpose of heating or cooking produces particulate and gas emissions that may contain a number of cancer trigger factors, such as benzene, carbon monoxide, formaldehyde, and polycyclic aromatic hydrocarbons (PAHs). Indoor air pollution from household combustion of coal is carcinogenic to humans (Ferlay., 2019.).

Other types of environmental carcinogens include asbestos which can cause mesothelioma and lung cancers. Carcinogens from motor car exhausts also poses a risk of lung cancer (Shankar *et al.*, 2019.).

Lifestyle

Lifestyle factors such as obesity/overweight, age, gender, unhealthy diet, smoking and alcohol intake have contributed to cancer risk. Studies have shown a strong relationship between alcohol consumption and increased risk of cancer especially head and neck, oesophageal, breast, liver, and pancreatic cancers (Todoric *et al.*, 2016; Grewal and Viswanathen, 2012.).

A major percentage of cancers could be as a result of smoking tobacco leading to diseases of the lung, mouth, throat, larynx, oesophagus, as well as acute myeloid leukaemia. Survey among individuals have shown that exposure to constant smoke from a smoker, can lead to cancer of the lung in non-smoking persons and can augment the risk of other cancers if not properly managed. The whole record of people exposed to tobacco smoke from tobacco users in 2005 was proposed to be several millions (Mathers and Loncar, 2006.).

According to WHO, tobacco use is the single greatest avoidable risk factor for cancer mortality which kills approximately 6 million people each year, from cancer and other diseases. Tobacco smoke contain over 7000 chemicals, at least 250 are known to be harmful and more than 50 substances are carcinogenic (Georgia., 2019.).

Similarly in the United States, it was estimated that every year, 110,000 deaths due to cancer are caused by obesity, making it the second leading cause of cancer deaths after smoking. According to the WHO 2014 report, 39% adults worldwide are overweight and 13% are obese. Obese individuals are at a substantial elevated risk to develop cancer and at least 3.6% of all new cancer cases in adults older than 30 years are obesity-related (Todoric *et al.*, 2016.).

Hereditary factor

Cancers are commonly associated with genetic factors (Hunt and Ray, 2008.) and up to 10% of cancers occur through the inherited mutation of a group of genes called cancer predisposition genes in which individuals who carry a mutant allele of these genes have an increased susceptibility to cancer (Wang, 2016.). Genes that cause inherited cancer predisposition are passed on in an autosomal dominant manner (Pachter *et al.*, 2017). The hereditary cancer genes are located on the autosomal chromosomes, both males and females may inherit these cancer susceptibility genes. Expression of disease after inheriting the gene will differ between men and women depending on the types of cancer exhibited in the hereditary cancer syndrome as well as the type of mutation inherited (Mester and Eng, 2015.).

Mutations in BRCA1 and BRCA2 genes accounts for approximately 80-90% of hereditary breast and ovarian cancers incidence globally. Other forms of hereditary cancers include colorectal cancer (CRC), caused by mutation in one of the DNA MMR genes; while mutations within the APC gene are linked to colorectal adenomatous polyposis. Syndromes of breast cancer susceptibility are linked to mutations of BRCA1 and BRCA2, in addition to a smaller number of cases with germline mutations of ATM, PALB2, PTEN, p53, CHEK2, STK11, CDH1, and rarer syndrome (Walsh *et al.*, 2020.).

Among Nigerian women, one in eight cases of invasive breast cancer is a result of inherited mutations in BRCA1, BRCA2, PALB2,or TP53, and breast cancer risks associated with these genes are extremely high. Given limited resources, genetic testing can be carried out to identify high risk women for possible prevention, early detection and prompt treatment (Zheng *et al.*, 2018.).

1.4 Prevention

Several studies and reports have shown that some constituents of food help in the prevention of cancer and sometimes help in fighting the disease if it is in the body. The composition can be a form of antioxidants, which helps in the mopping up of the free radicals in the body. These radicals when in excess in the human body, could lead to oxidative stress in organs of the body. The human body itself can produce this antioxidant endogenously but there is still a need for exogenous sources to give a balance in the body (Bouayed and Bohn, 2010.).

The exogenous antioxidants can be obtained from vegetables, fruits and some grains. They can also be obtained through the intake of dietary supplements (Davis *et al.*, 2012; Giovannucci, 1999.). There is reduced danger of lung, colon and stomach malignancy in individuals with regular intake of vegetables as the form or part of diet (Biesalski *et al.*, 1998.). Fruits are known to contain several beneficial vitamins, minerals which can prevent or reduce the risk of cancer.

1.5 Treatment

Recently, there has been a remarkable development in understanding projected hallmarks of cancer treatment. Even though there is growing occurrence, the medical management of cancer continues to be a challenge. Treatment of cancer may involve any of each or a combination of treatments stated below:

1.51 Surgery

This is treatment method usually used for the removal of cancerous growths or a measure of obtaining small samples of tissue for examination. Surgical removal of a tumour may be adequate to cure the patient, but the cure is dependent mainly on the size, place where the cell is found, and stage of the disease. If the tissue contains cancerous cells, it might show what type of cancer it is and the rate of growth in the body. Surgery is not an appropriate means of treatment for an advanced stage of cancer. Surgery is often done in combination with or without radiation therapy and chemotherapy (Abeloff *et al.*, 2004; Temple *et al.*, 2004.).

1.5.2 Radiotherapy

Radiation is a physical agent which is used to kill cancerous cells in the body. High-energy radiation helps in destroying the genetic material, DNA of cells thereby hindering the cell division and proliferation (Jackson and Bartek, 2009.). Unfortunately, these rays cannot differentiate between the cancer cells and some other fast-growing normal cells in the body, thereby causing damages both to healthy and cancerous cells, but more of cancer cells.

A larger percentage of patients undergoing chemotherapy also utilizes the radiation therapy as a form of treatment to reduce the cancer cells (Delaney *et al.*, 2005; Begg *et al.*, 2011.). If radiotherapy is carried out before surgery, radiation will lead to

shrinking of the tumour, but if done after surgery, the process will terminate minute tumour cells that are not dead due to the sensitivity of different tumours to radiation.

1.5.3 Nanotechnology

Choice of nanotechnology in the treatment of cancer is as a result of late detection of cancer, ineffectiveness of most treatments, numerous side effects and also unavailability of immunization. Research has shown that 100% of mice treated with nanoparticles survived with low toxicity (Sunderland *et al.*, 2006.), though there has been no human trials yet. Several types of cancer cells can be exploited by targeting the cells with nanoparticles.

1.5.4 Chemotherapy

This is a method used for cancer treatment by the utilization of drugs in the destruction of cancerous cells. There are some peculiar types of cancers that can just be managed or treated with drugs, though sometimes other forms of treatments could be inclusive which could be surgery and radiotherapy. This form of treatment helps in the inhibition or reduction of the cancer cell growth. During cell replication, continuous proliferation occurs, therefore healthy cells might also be affected because of rate of division of the cells such as nail, hair and even the skin tissue.

Chemotherapy can be given to patient as a form of palliative care which will enable the shrinkage of tumorigenic cells which are causing severe pains in the body. It could also be used for the control of cancerous cell growth and also the inhibition of spread of the disease to other parts of the body. Better still, it could be administered for total destruction of the cancer cells. Due to its efficacy, chemotherapy can be given before surgery or radiotherapy so that the tumour can be reduced with less pain. It also helps in not allowing reoccurrence of the cancer or spread to surrounding tissues in the body.

The chemotherapeutic agent is administered as an injection in the upper arm or the thigh which is most often the fatty part of the patient's leg or arm. It could also be administered intra-arterial by directly injecting the cancerous cell through the artery. It can also be in form of cream which is administered topically. During the treatment, patients have several mixed feelings such as being frustrated, depressed and sometimes helplessness. So many side effects are associated with chemotherapy. They include vomiting, irritation, alopecia and sometimes mouth sores. The causes of these side effects could be as a result of drugs not being selective, that is, its interference with

normal fast-growing cells which could include hair growth, skin and even lining of the bone marrow.

An adequate intake of high-fibre foods, fruits and vegetables, can help in the management of the side effects and further healing of the patients(CHANGE, 2016; NIH, 2018.).

1.6 Cancer Incidence in Nigeria

After cardiovascular diseases such as atherosclerosis, cancer is another disease that constitutes the second source of death in developing countries (Mbaveng *et al.*, 2011.). The disease has been the major problem to the public health globally with an increase in cancer patients diagnosed yearly and deaths as an eventual result of the disease (Engel *et al.*, 2011.). About thirteen million new cancer cases was documented in 2008, while mortality rates varied with difference in regions and levels of income in the world (Ferlay *et al.*, 2010.).

In Nigeria the International Agency for Research on Cancer (IARC) gave the estimates of the worldwide burden of cancer through its 4 yearly publication GLOBOCAN (Thun *et al.*, 2009.). They reported that approximate number of 115,950 new cancer cases was recorded in 2019 in Nigeria, with about 70,327 cancer deaths. Nigeria also contributed to 8.3% of new cancer cases that occurred in Africa in 2012 which was estimated to be 847,000 (Jedy-Agba *et al.*, 2014.).

Table 1.0 below shows the cancer incidence and the mortality rate in Nigeria. The age standardised rate was estimated as a summary measure of the rate that a population would have if it had a standard age structure of the WHO standard population which is estimated per 100,000 persons.

Table 1.0: Evaluation of common Cancer cases, Incidence, and Mortality in Nigeria

Nigeria	Male	Female	Both Sexes
Population	99,227,846	96,597,394	195,875,239
Number of new cancer cases	44,928	71,022	115,950
Age standardised rate (World)	89.1	119.4	103.8
Risk of developing cancer before the age of 75 years (%)	9.8	12.5	11.1
Number of cancer death	28,414	41,913	70,327
Risk of dying from cancer before the age of 75 years (%)	6.5	8.4	7.4
5 year Prevalence cases	74,284	136,768	211,052
Common types of cancer	Prostrate, Non-Hodgkin, lymphoma	Breast, Cervix, Liver, Colorectum	Breast, Cervix, Prostrate, Liver

Adapted from (GLOBOCAN, 2019)

1.7 Plant Derived Anticancer Agents in Clinical use

Plants have been found to be the best alternative for treatment of diseases. Plants provide an unlimited range of efficacious agents for treatment of the disease. Plant constituents have always been utilized because of their potential to manage cancer (Farnsworth, 1988; Grabley and Thiericke, 1999; Kim and Park, 2002.). A great number of plants have been studied to be excellent means of obtaining therapeutically imperative agents which are important in the treatment of cancer. Active cancer drugs have been obtained from natural origin (Lee, 2010.), including plant derived agents, for example, vinca alkaloids vinblastine and vincristine, isolated from the *Catharanthus roseus* (Guéritte and Fahy, 2005.).

Alkaloids are class of compound that are nitrogen-containing. They are secondary metabolites of plant origin with physiological actions in man. Recent report showed that approximately half of therapeutic agents, anti-cancer drugs contain the alkaloids. Examples of such drugs include Homoharringtonine, Elliptnium, paclitaxel and vinblastine (Nwodo *et al.*, 2016.).

Triterpenes are a class of chemical compounds which re composed of three terpene units with the molecular formula $C_{30}H_{48}$. They are members of isoprenoids that are derived from a C30 precursor named squalene (Townsend and Ebizuka, 2010.). Triterpenes are predominantly present in roots, stem barks, leaves and sometimes the fruit of plants (Ja¨ger *et al.*, 2009.). These triterpenes can also be found in fungi and are attributed to possession of several medicinal importance to man in the present age (Yin, 2012.). Reports have shown the ability of triterpenes in the management of carcinogenic process by its influence in correcting pathway of its metastasis (Yin, 2012; Yang, 2010.). Triterpenes such as ursolic acid (Simin, 2013.); (Ogasawara *et al.*, 2013.), betulinic acid (Takada and Aggarwal 2003.) , asiatic acid, (Hsu *et al.*, 2005.) have shown activity against cancer cell lines. Some examples of the therapeutics are shown in table 1.1

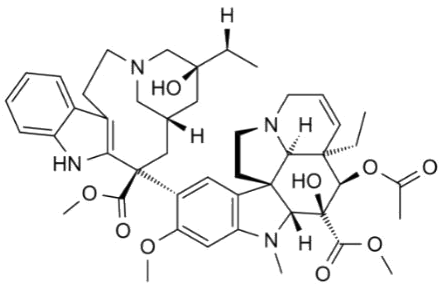
Below is table 1.1 showing chemotherapeutic agents derived from natural source, plants.

Table 1.1 Plants Derived Agents used in Cancer Chemotherapy

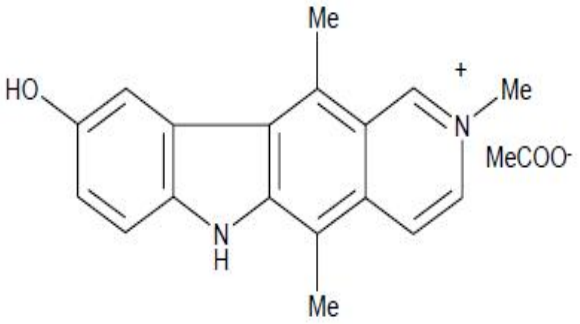
S/N	Semisynthetic analog of plant derivatives	Species and Genus name	Experiments on various cancer cells	Mechanism of action
*Catharantus roseus*1	Vindesine and Vinorebline			Mitotic block
			testicular cancer, breast cancer, lung cancer	
*Podophyllum*2	*peltatum*Etoposide and Teniposide,	*podophyllum emodi*	Lymphosas, bronchial and testicular cancers	----------
g, 3	Taxol®	*Taxus brevifolia Taxus baccata*		Anti-mitotic
			prostrate cancer and lymphoidmalignancies	
4	Taxotere®	*Taxus brevifolianutt. Taxus baccata*		
ctal cancer 5	DNAIrinotecantopoisimer	*mptothecaCse acuminate*		
				I inhibition
activity both 6	Exatecan	*Camptotheca acuminate*		DNA topoisomerase
			. *in vitro*and*vivo*	I inhibition
nes 7	LE-SN-38	*Camptotheca acuminate*		DNA topoisomerase I inhibition

8	*Berberi*Berbeline*samarnsis*			Caspase-3dependent apoptosis
9*Colchicum*eColchici*autumnale*			Leukemia and solid tumors	Anti-mitotic
*Cucurbitaceae*10tacinCucurb*speces*		*superb*		Inhibits signal transducer/ JAK2 activity and activates STAT-3 pathway
11	Curcumin	Curcuma longa	Colorectal cancer, multiple myeloma and pancreatic cancer	Exact mechanism of action is still unknown
12	Emodin	Rhizome of *rhubarb rhaonticum*	Lung, liver, ovarian and blood cancer	Apoptosis of cancer cells by several pathways
*Iridaceae*13Irisquinone*lataepallsii*		*kumaonsis*	Good activity in transplantable rodent tumor	Acts as a chemo-sensitizer
14	Perilly 1 alcohol	Many plant species like mints, cherries, lavender and many others	Non-small cell lung cancer, prostrate cancer, colon cancer and breast cancer	Exact mechanism is yet to be identified
nes in 15	SchisschkinninCentaureaschischkin		vitro	Not known

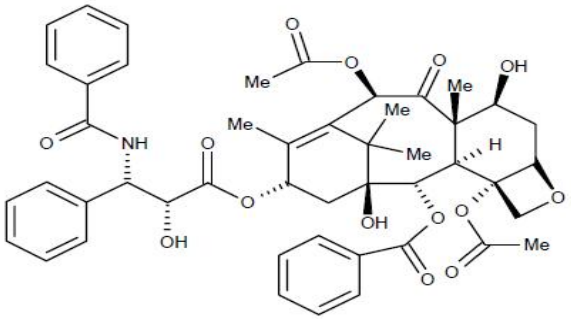
cell lines in vitro	16	Montamine	*Centaurea montana*	Not known



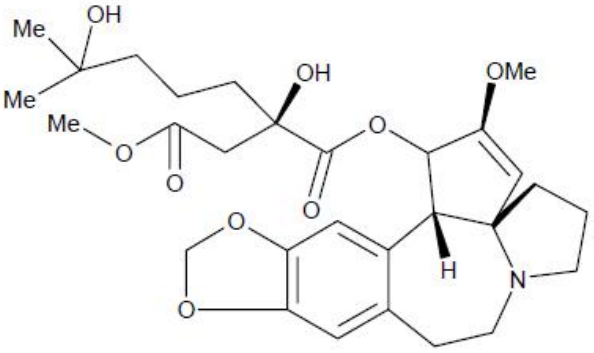
Vinblastine



Elliptnium



Homoharringtonine



Paclitaxel

Figure 1.2: Some plant-derived anticancer molecules in clinical trials

1.8 Justification for the study

Plants are excellent sources of medicinal natural products. The probabilities of obtaining pharmacologically active constituents from medicinal plants are high, thus contributing to the repository of plants used for cancer treatment is of great importance (Hamburger and Hostettmann, 1991.).

Several cytotoxic drugs used for cancer treatment such as taxane and viscristine have been obtained from natural plants and then synthesized for large scale production (Noble *et al.*, 1959; Wani *et al.*, 1971.). Recently, for example, omacetaxine mepesuccinate was initially discovered from *Cephalotaxus harringtonii* (Shigematsu *et al.*, 1994.). This was then manufactured by hemi-synthesis for production in large quantities for the treatment or management of chronic myeloid leukaemia.

Aframomum melegueta seeds scored highly in a recent ethno medical survey on therapeutic plants utilized for the management of cancer in south western part of Nigeria (Mephors *et al.*, 2017.). *Strophanthus hispidus* was selected based on another ethno medical survey conducted by Owoola A. AMBALI, for this research study on natural product, most especially plants being utilized for treatment of cancer in Akinyele local government area, Oyo state. These two plants were chosen for the study based on a high fidelity index generated after the analysis of information given by the TMPs in study areas. Thus selection of plants for Bioassay-guided isolation of cytotoxic plants which could serve as led to drug discovery and development of anticancer drugs from Nigeria Ethnobotany was aimed at for the study.

Research question

Can African medicinal plant contribute to the development of new anti-cancer Drugs?

Hypothesis

There have been several historical reports by Traditional Medical Practitioners (TMPs) using *Aframomum melegueta* and *Strophanthus hispidus* according to ethnopharmacological survey in Akinyele Local Government Area of Oyo state. Therefore, a hypothesis was proposed that a systematic investigation of the biochemical constituents of these two plants might be a lead in the discovery of valid

and more potent constituents which may be useful in the management, treatment and even prevention of cancer.

1.9 Aim and Objectives

1.9.1 Aim

To contribute to the repository of anti-cancer, cytotoxic compounds that could be developed for the chemotherapeutic management of cancer.

The objectives include;

1 Ethnobotanical survey of anticancer plants used in Akinyele Local government.

2 To determine the Brine Shrimp Lethality of *Aframomum melegueta* seeds and *Strophanthus hispidus* (whole plant) extracts and fractions.

3 Evaluation of the cytotoxic properties of *Aframomum melegueta* seeds and *Strophanthus hispidus* and fractions using human cancer cell lines in MTT colorimetric assay.

4 To subject the active fraction for isolation of active cytotoxic compounds using a combination of chromatographic techniques.

5 To elucidate the structure of cytotoxic compounds obtained using spectroscopic techniques.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 *Aframomum melegueta* (Roscoe) K. Schum.

Phylogeny
Kingdom: Plantae
Order: Zingiberales
Family: Zingiberaceae
Genus: *Aframomum*
Species: *Aframomum melegueta*
Local name: *Atare* (Yoruba), *Ose-orji/okwa* (Igbo); *Chilla/ Citta* (Hausa)
Common name: Alligator pepper, Grains of paradise
Synonyms: grain of paradise, guinea grains, guinea pepper, melegueta pepper

2.1.1 Botanical Description

Aframomum melegueta is a herb-like perennial plant which is found in swampy habitats along the West African coast of Africa. It has trumpet-shaped, purple flowers which is developed into 5-7 cm long pods that contain several small, brownish seeds. The plant produces tufted stem of about 1-4 meters tall from Rhizomatous (Standley and Steyermark, 1946.).

The plant has been grown as a spice which is used in cooking. Its medicinal value over several years has been recorded in ancient Rome, West Africa and South America (Bown, 1995.). This spice is aromatic and can be distinguished by its hot peppery taste. The seeds have pungent odour, peppery and sometimes with a bitter taste (Iwu *et al.*, 1999.). High humidity is needed for plant growth. It is therefore planted at daytime temperature of about 21^{o}C (Bown, 1995.).

2.1.2 Ethnobotanical uses

In Africa and throughout the tropics, *A. melegueta* fruits is cultivated and used extensively in ethno-medicine as a remedy for several ailments. It is one of the plants extensively used by African ethno-medicine. The seed is used as flavour in foods and the fruit is chewed raw as a stimulant, for digestion and also to relieve spasms (Bown, 1995.).

The prepared decoction is given to female patients with painful menstrual flow as a treatment for infertility and also to improve lactation (Bown, 1995.). One of the rare spices from Africa, melegueta pepper is more flavoured and stronger than pepper. It has a strong reputation of being traditionally a tonic. In Africa, is usually used as an aphrodisiac as well as to treat some pains arising from measles, leprosy and worms (Ajaiyeoba and Ekundayo, 1999; Fernandez *et al.*, 2006.).

2.1.3 Pharmacological and Biological Activity

Aframomum melegueta has the ability to inhibit cytochrome, CYP3A enzymes. *A. melegueta* at concentration of 10 mg/mL inhibited CYP3A enzymes *in vitro* (Agbonon *et al.*, 2010.). The Methanolic extract has been shown to be moderately inhibited by acetylch linesterase activity. The α-amylase and α-glucosidase inhibition has been found greatly influenced by *A. melegueta*, thus showing the antidiabetic property of the seeds (Adefegha and Oboh, 2012.). An ethanolic extract of *A. melegueta* was shown to cause dose-dependent increases in liver weight with a mild increase in LDH (liver enzyme) and non-significant elevation of liver function enzyme, AST level in the blood (Ilic *et al.*, 2010.). Therefore *A. melegueta* serves as a hepatoprotective plant.

Anti-ulcer, antimicrobial and cytoprotective effects of *A. melegueta* seeds has also been previously reported (Galal, 1996; Rafatullah *et al.*, 1995.). Two major compounds, 6-paradol and 6-shogaol previously isolated from the seeds of *A. melegueta* have been shown to have antibacterial and anti-fungal effects based on the previous study (Galal, 1996). *Aframomum melegueta* extract obtained by water extractions has also been reported to have aphrodisiac property (Kamtchouing *et al.*, 2002.).

2.1.4 Phytochemical constituents

The essential oil from *A. melegueta* has mainly several varieties and types of sesquiterpene hydr carb ns which include α- and β-caryophyllene, humulene, and their oxides (Ajaiyeoba and Ekundayo, 1999.). In the acetone extract of seeds of *A. melegueta*, hydroxyl arylalkanones include: 1-(4-hydroxy-3-methoxyphenyl)decan-3-one, 1-(4-hydroxy-3-methoxyphenyl)-3-hendecan-3-one and 1-(4-hydroxy-3-methoxyphenyl)-3-deca-4-ene-3-one in approximately equal part (El-Halawany *et al.*, 2014.) .

2.2 Compounds Previously Isolated from *A. melegueta*

Phenols

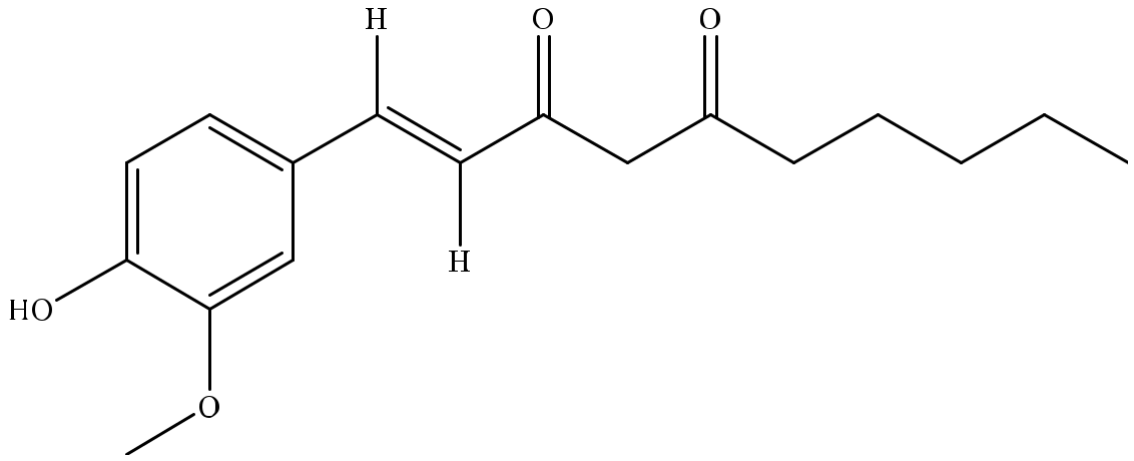


Figure 2.1: Chemical structure of 1-Dehydrogingerdione

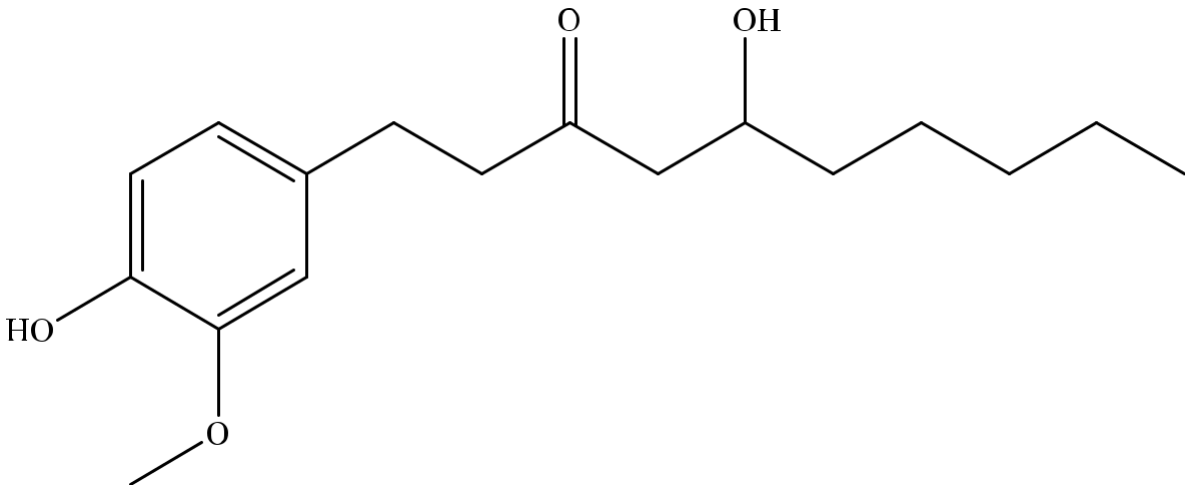


Figure 2.2: Chemical Structure of 5-hydroxy-1-(4-hydroxyphenyl) decan-3-one

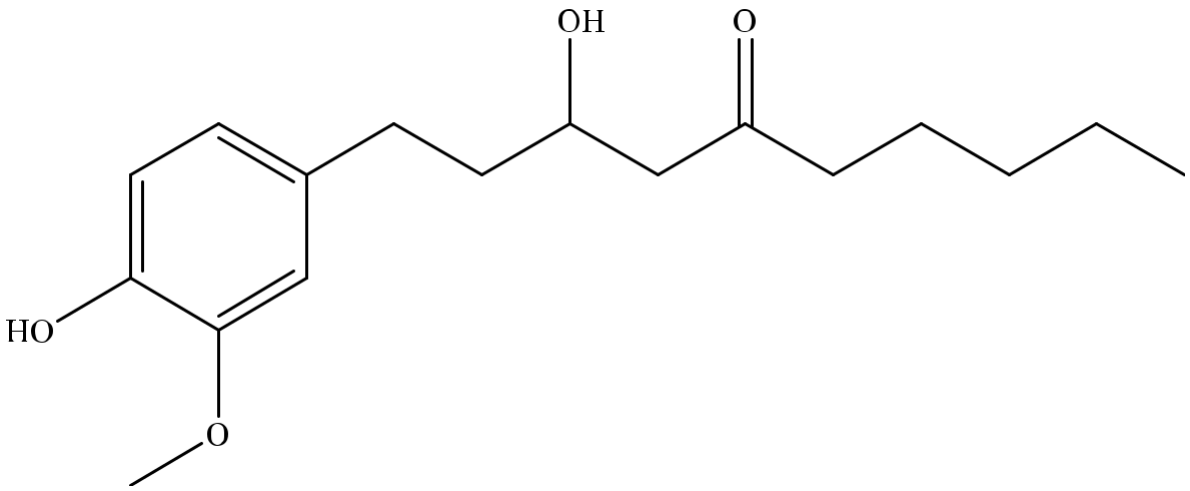


Figure 2.3: Chemical Structure of 3-hydroxy-1-(4-hydroxy-3-methoxphenyl) decan-5-one

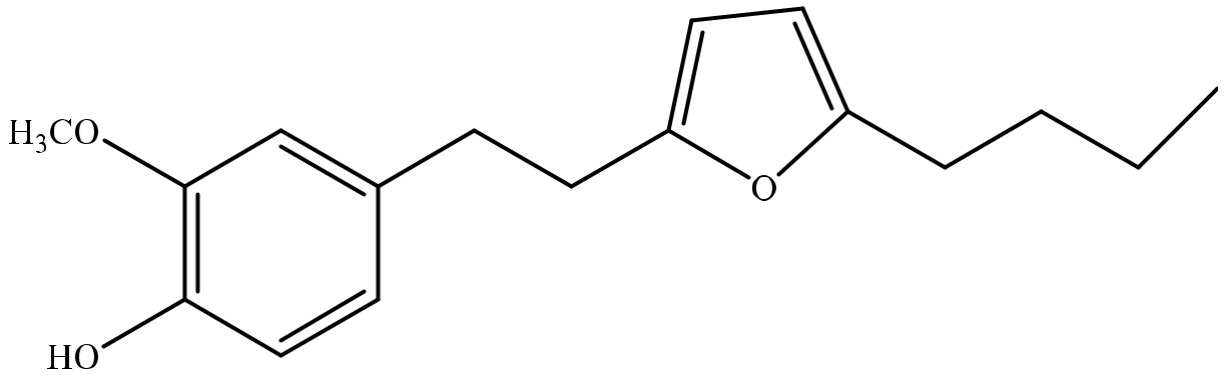


Figure 2.4: Chemical Structure of 4-(2-(5-butylfuran-2-yl)ethyl-2-methoxyphenol

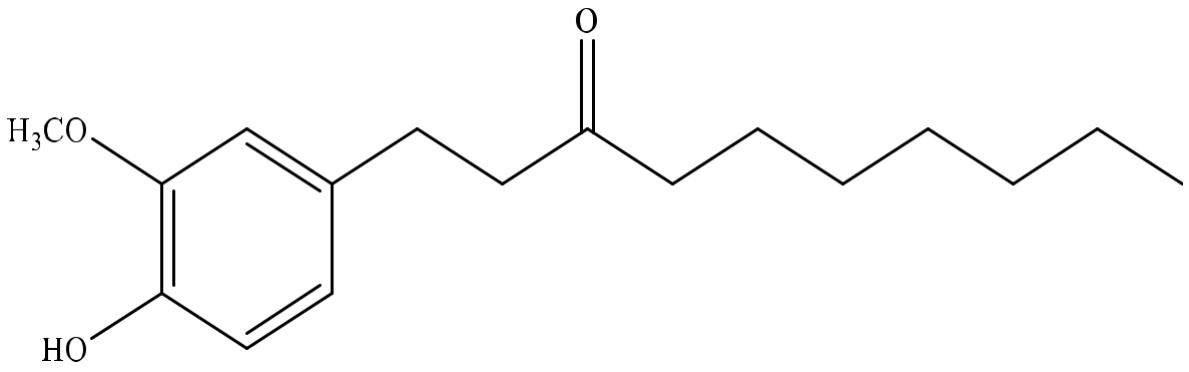


Figure 2.5: Chemical Structure of 1-(4-hydroxyl-3-methoxylphenyl)decan-3-one

Figure 2.6: *Aframomum melegueta* (Roscoe) K Schum showing fruits from Olokomeji Forest Reserve, Oyo State. (Image taken by myself, Owoola A. AMBALI).

2.3 *Strophanthus hispidus* DC.

Phylogeny

Kingdom: Plantae

Order: Gentianales

Family: Apocynaceae

Genus: *Strophanthus*

Species: *Strophanthus hispidus*

Local name: *Sagere*, *Isagere* (Yoruba), *Ota*, *Kwankwani*, (Igbo), *Kaguru* (Hausa)

Common name: Brown Strophanthus, Hairy Strophanthus

Synonyms: *Strophanthus hirtus* Poir., *Strophanthus bariba* Boye & Bereni, *Strophanthus tchabe* Boye & Bereni, *Strophanthus thierryanus* K. Schum. & Gilg

2.3.1 Botanical Description

Strophanthus hispidus is a climbing shrub of about 16 m long of the open savanna woodland, in more humid forest. It is found extensively in western part of Africa including Nigeria, Ghana and extending to other parts of Africa, from Senegal to W Cameroons, Congo, Cabinda and Uganda (Burkill, 1985; Ayoola *et al.*, 2008; Ojiako and Igwe, 2009.). The leaves with twigs are densely covered in long (1-2 mm) brown or yellow hairs. The leaves are up to 15 x 8 cm, rounded to sub cordate, papery, 6-11per laterals, they are channelled above; and often bullate. Petiole is about 1-5mm, 4-6 colleters in axils, follicles 24-50 x 1.3-1.8 cm, flowers with long hairs. The corolla is white to yellowish with 8-18 cm thread-like lobes, deciduous liana. The leaves are very shiny below with red midrib (Burkill, 1985.).

2. 3.2 Ethnobotanical uses

The seeds are known to be very toxic. It contains glycosides which are useful in production of drugs which help in stimulating the heart and circulation in the pharmaceutical industry (IITA, 2012.). The juice obtained from the seeds is mixed with other ingredients and used to make arrow poison. This is then rubbed on the arrow to be shot at an animal. Thereafter the heart stops as soon as the bullet penetrates. Several other parts of *S. hispidus* are utilized traditionally to treat snake bite, skin infections and sexually transmitted diseases (STDs) (Landis *et al.*, 2007.).

In Cote d'Voire, the decoction of the leaf or the aerial part is useful in the treatment of conjunctivitis and sometimes trachoma which in turn gives rapid relief and cure. The decoction is also used as an emetic and anti-diarrhoetic (Ezuruike and Prieto, 2014.). The sap from the leaves or latex is known to be used for wound, skin and cell healing in Ivory Coast (Kerharo and Bouquet, 1950.). In the traditional setting of the Senegalese, the extracts of the leaves, it is placed on sores and wounds to give prompt healing. An extract from pounded roots is taken on an empty stomach for treatment of pains in the joints, and also for hernia (Occhiuto *et al.*, 1989.).

2.3.3 Pharmacological and Biological Activities

Aqueous root extract of *S. hispidus* showed a mild inhibition but eventually increased concentration afforded a significant inhibitory activity. This signified a dose dependent inhibition of inflammation (Agbaje and Fageyinbo, 2012.). Studies on the potency of water and ethanolic extracts of *S. hispidus* (SH) stem bark in the treatment of diabetes mellitus showed great activity. This was illustrated by action of *S. hispidus* in the gradual reduction in the blood glucose level and the anti-lipidemic activity. (Osibemhe *et al.*, 2016.). Therefore, they concluded that *S. hispidus* could be said to be a potent anti-diabetic plant.

Examination of fractions *of S. hispidus* root extract on anti-nociceptive property was explored (Oluwatoyin and Samuel, 2014.). Central and peripheral mechanism of the analgesic activity was established with the post treatment effect of the rats on a hot plate model. The ethyl acetate (25.28%), n-butanol (50.67%) and the aqueous fractions (51.91%) possessed significant anti-nociceptive property comparable to aspirin and morphine which served as positive controls. It was then proposed that the presence of phytochemicals (alkaloids, tannins, anthraquinonoes and cardiac glycoside) which have been found to be analgesic have the ability of entering through the blood-brain barrier for effectiveness (Larkins and Wynn, 2004.). Ethanol extracts of *S. hispidus* root administered orally had significant ($p<0.05$) inhibition of writhing reflex with a maximum outcome of great inhibition at the highest dose (Ishola *et al.*, 2013.). This study was a confirmation of folklore medicine which records the utilization of *S. hispidus* in the management of inflammatory diseases.

Lethal dose of *S. hispidus* has been estimated in a study (Iheanacho *et al.*, 2016.) using the toxic rating (Lorke, 1983.) which states that LD_{50} between 50- 5,000mg/kg

is moderately toxic. The LD_{50} obtained for aqueous and ethanol extracts of this plant were 4020 and 4290 mg/kg respectively which indicated that the extracts were moderately toxic.

Study on the evaluation of trace elements and mineral in *S. hispidus* showed the existence of elements such as Calcium, Copper, potassium, Nickel, Magnesium, Chromium, sodium, iron, Zinc and Cadmium. The study reports that the metal content in *S. hispidus* were observed to have a reduction as compared with the parameters set by WHO (Akinlami *et al.*, 2015.). Though the LD_{50} showed it could be moderately toxic, the elements are needed for consumption because of the values in the human body. For instance, iron is an element which is very vital for the formation of haemoglobin which is needed for blood supply. At high concentration, iron is hazardous to human health. However, the iron content of *S. hispidus* root studied by Akinlami ,estimated it to be less than 10 ppm while the permitted level as set by WHO for iron content or concentration in a plant material used for consumption is 20 ppm (Akinlami *et al.*, 2015.). Wound healing and antimicrobial activities of the leaf, stem, bark and roots of *S. hispidus* extracted in methanol, have been confirmed in a different study (Agyare *et al.*, 2013.).

2.4 Classes of Compound previously isolated from *S. hispidus*

1. Ursolic acid (Pentacyclic triterpenes)

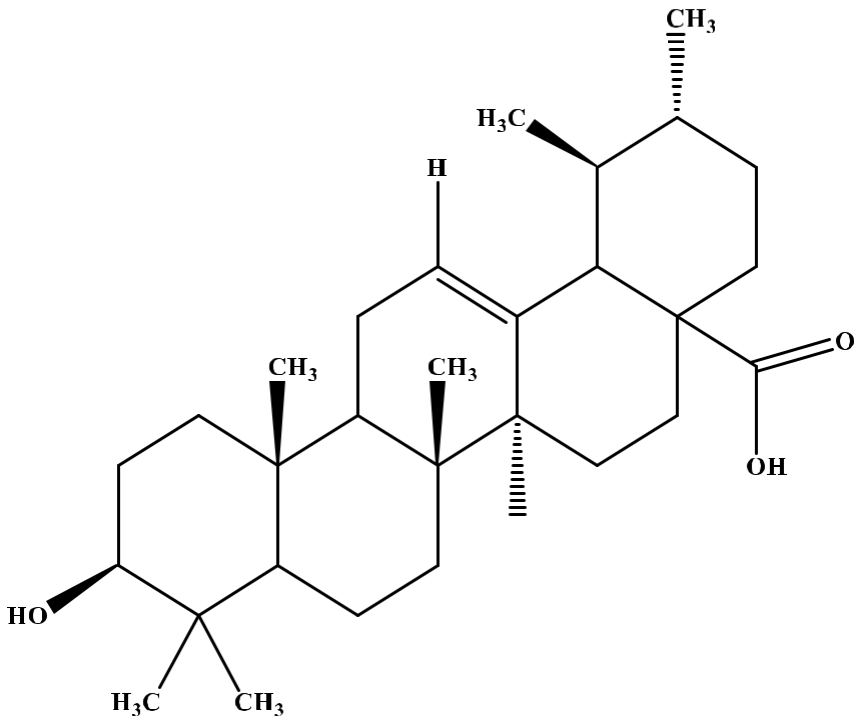


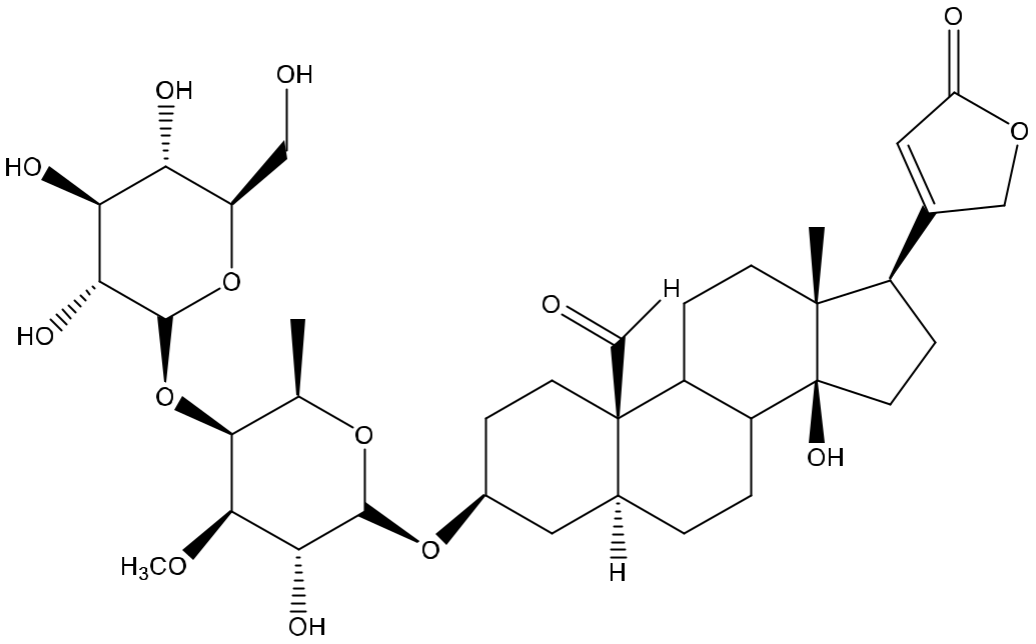
Figure 2.7: Chemical structure of Ursolic acid

2.4.1 Classes of compounds isolated from other species of *Strophanthus*

1. **Cardenolides**

Boivinides were isolated from *Strophanthus boivinii* in a study on isolation of cytotoxic cardenolides from the plant (Sampada, 2007.).

Boivinide A, is an amorphous solid which is white in colour. It has a molecular mass of 710 g/mol and molecular formula $C_{36}H_{54}O_{14}$.

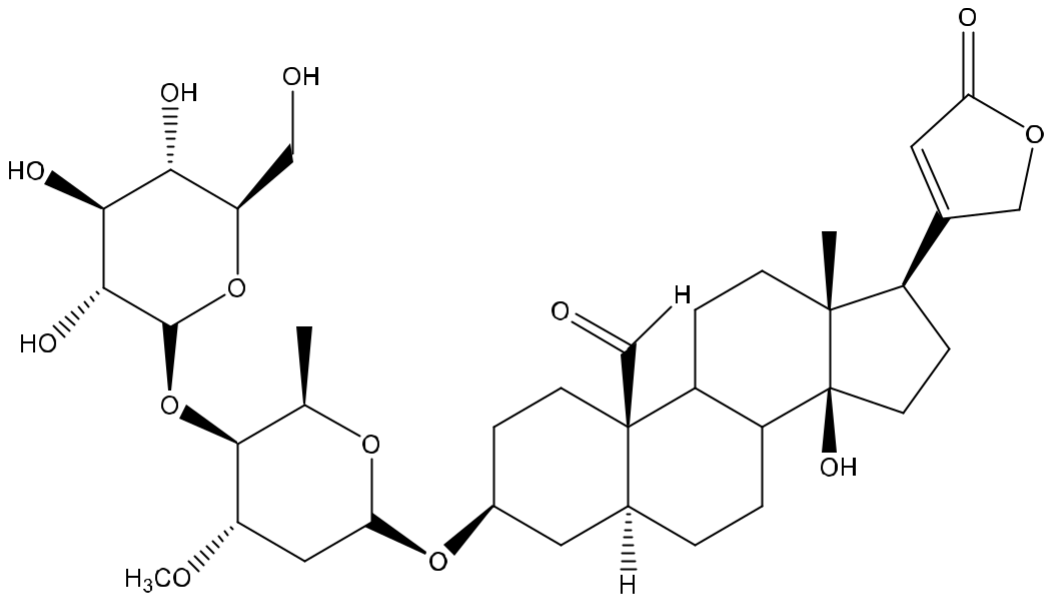


(3*S*,5*S*,10*R*,13*R*,14*S*,17*R*)-14-hydroxy-3-(((2*S*,3*R*,4*R*,5*S*,6*R*)-3-hydroxy-4-methoxy-6-methyl-5-(((2*R*,3*R*,4*S*,5*S*,6*R*)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2*H*-pyran-2-yl)oxy)tetrahydro-2*H*-pyran-2-yl)oxy)-1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,17a-octadecahydro-1*H*-cyclopenta[*a*]phenanthrene-10-carbaldehyde

Figure 2.8: Chemical structure of Boivinide A

2. Biovinide B

This is also an amorphous solid with a white coloration. This compound has a molecular mass of 694 with molecular formula $C_{36}H_{54}O_{13}$

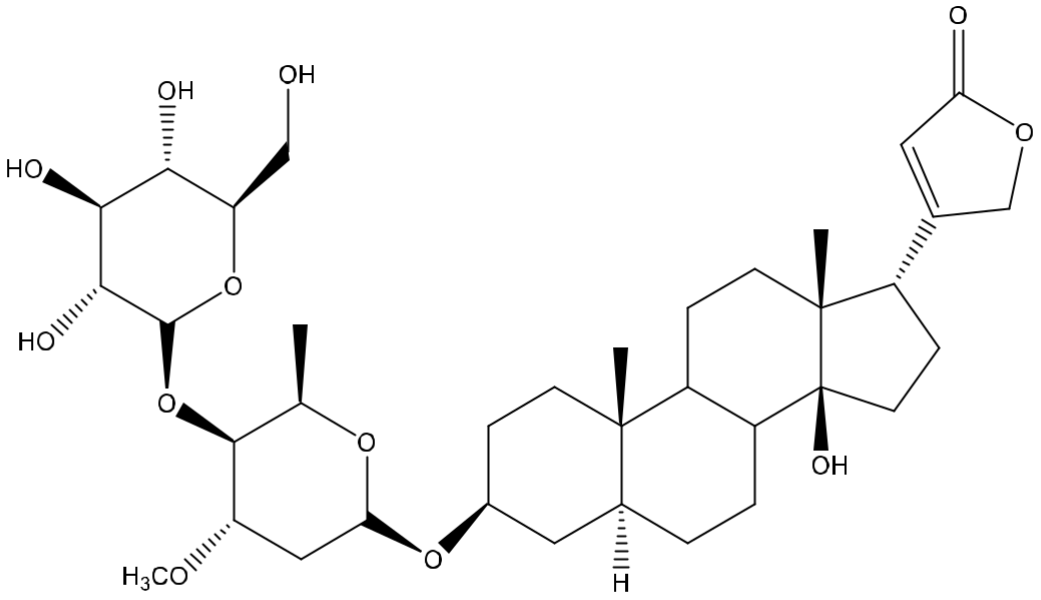


(3*S*,5*S*,10*R*,13*R*,14*S*,17*R*)-14-hydroxy-3-(((2*S*,4*S*,5*S*,6*R*)-4-methoxy-6-methyl-5-(((2*R*,3*R*,4*S*,5*S*,6*R*)-*H*-pyran-2-yl)oxy)tetrahydro-2*H*-pyran-2-yl)oxy)-*H*-cyclopenta[*a*]phenanthrene-10-carbaldehyde

Figure 2.9: Chemical structure of Boivinide B

3. **5α,17α-uzarigenin 3-*O*-[β-D-glucopyranosyl-(1→4)-β-D-sarmentopyranoside]**

This is an amorphous substance with molecular weight 680 and molecular formula of $C_{36}H_{56}O_{12}$.

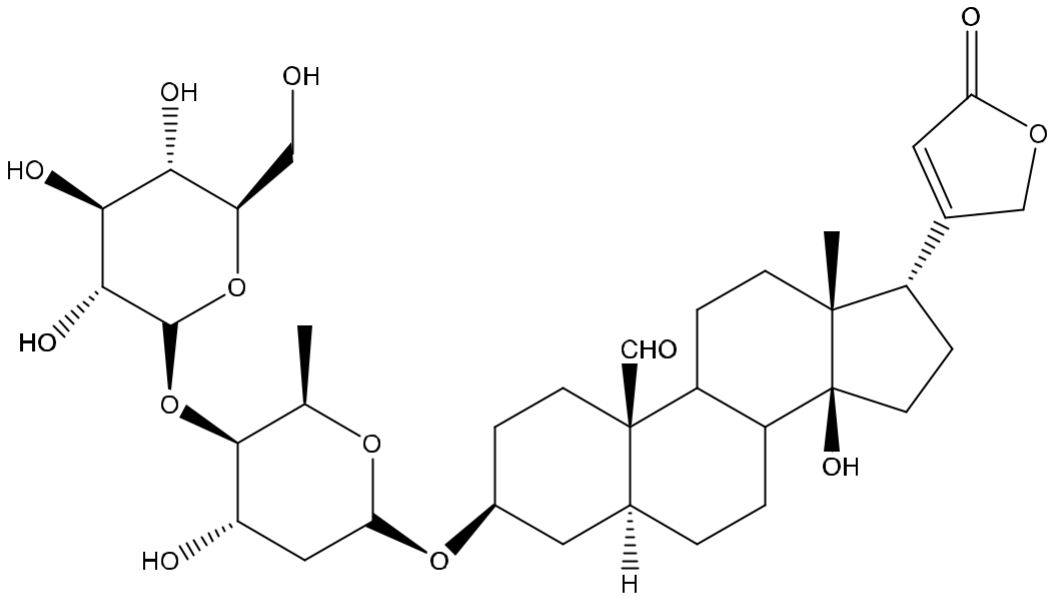


4-((3*S*,5*S*,10*S*,13*R*,14*S*,17*S*)-14-hydroxy-3-(((2*S*,4*S*,5*S*,6*R*)-4-methoxy-6-methyl-5-(((2*R*,3*R*,4*S*,5*S*,6*R*)-4-methoxy-6-methyl-5-((2*R*,3*R*,4*S*,5*S*,6*R*)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2*H*-pyran-2-yl)oxy)tetrahydro-2*H*-pyran-2-yl)oxy)tetrahydro-2*H*-pyran-2-yl)oxy)-2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17-hexadecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl)furan-2(5*H*)-one

Figure 2.10: Chemical structure of sarmentopyranoside

4. Cardiac-glycoside

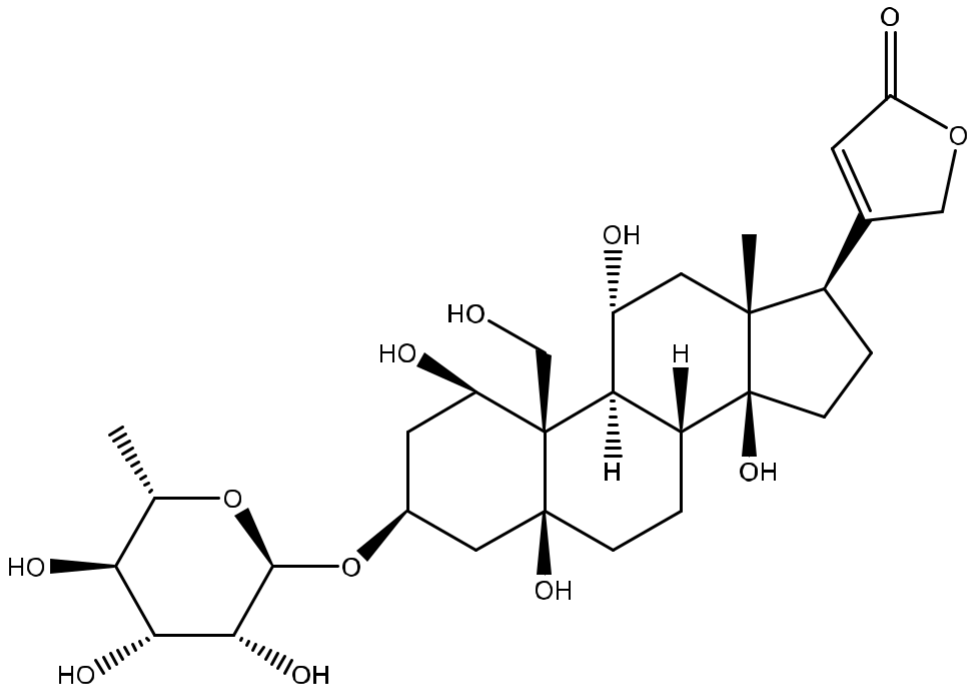
Corotoxigenin 3-*O*-[β--D-glucopyrano-(1→4)-β-D-boivinopyranoside], a white powder with molecular mass of 680g/mol and molecular formula, $C_{35}H_{52}O_{13}$



(3*S*,5*S*,10*R*,13*R*,14*S*,17*S*)-14-hydroxy-3-(((2*S*,4*S*,5*R*,6*R*)-4-hydroxy-6-methyl-5-(((2*R*,3*R*,4*S*,5*S*,6*R*)-*H*-pyran-2-yl)oxy)tetrahydro-2*H*-pyran-2-yl)oxy)-*H*-cyclopenta[*a*]phenanthrene-10-carbaldehyde

Figure 2.11: Chemical structure of boivinopyranoside

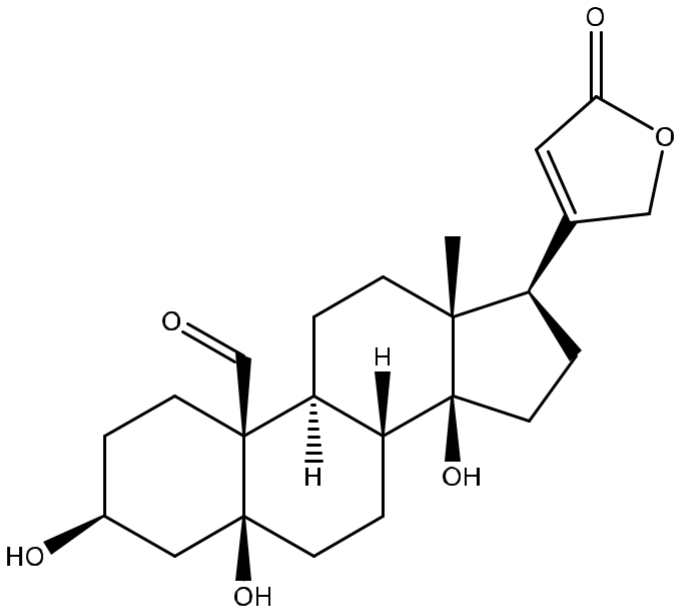
5. G-Strophanthin



4-((1*R*,3*S*,5*S*,8*R*,9*S*,10*R*,11*R*,13*R*,14*S*,17*R*
3-(((2*R*,3*R*,4*R*,5*R*,6*S* *H*-pyran-2-yl)oxy)hexadecahydro-1*H*-
cyclopenta[*a*]phenanthren-17-yl)furan-2(5*H*)-one

Figure 2.12: Chemical structure of G- strophanthidin

6. Strophanthidin



(3*S*,5*S*,8*R*,9*S*,10*S*,13*R*,14*S*,17*R*)-3,5,14-trihydroxy-13-methyl-17-(5-oxo-2,5-*H*-cyclopenta[*a*]phenanthrene-10-carbaldehyde

Figure 2.13: Chemical structure of Strophanthidin

Figure 2.14: Image Showing the Aerial part of *Strophanthus hispidus* at Ipara-Remo village, Ogun state. **(Image taken by myself, Owoola A. AMBALI)**

2.5 Isolation of Bioactive Compounds

2.5.1 Bioassay-led fractionation

Bioassay-led fractionation is ideal for isolation of bioactive constituents and prevention of isolation of isolation of in active compounds. When exploiting bioassay-led fractionation, the Methanolic extract and every fraction is tested for bioactivity. Ideally, after each fractionation step, bioactivity may have increased or decreased in some fractions, in comparison, to the parent extract and fractions.

If isolation is not properly guided or aided by bioassays, extracts will be fractionated and often times, only the major component are isolated and identified. After isolation and characterization, the compounds may then undergo bioactive analysis. This will then lead to the isolation of many compounds which do not possess any significant bioactivity.

2.5.2 Isolation methods

For successful isolation of natural products, a number of isolation methods should be used due to the uniqueness of plant extracts. Methods such as extraction of plant material, Liquid- liquid partitioning, Vacuum Liquid Chromatography, Column Chromatography and to further purify the compound isolated, High Perfomance Liquid Chromatography is used.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

Materials used include; 1000 mL conical flasks, Methanol, Water bath, Funnel, Cotton wool, weighing balance, Petri dishes, Rotary evaporator Separating funnel, conical flasks, Steam bath, retort clamp, stand and filter paper.

3.2 Chemicals and Reagents

Industrial and HPLC grade Hexane, Chloroform, Ethyl acetate, Methanol, Distilled water, De-ionized water, NMR grade: Pyridine, Chloroform, Methanol, Acetone and DMSO were used.

3.3 Ethnobotanical survey

3.3.1 Study Area

The research was carried out in some villages in Akinyele Local Government Area of Oyo state, Nigeria. The study was conducted in four traditional markets of Akinyele LGA. The herbal markets were Onidundun, Ayomaya, Asipa and Oloya.

3.3.2 Informed Consent

Before the beginning of the study, a consent form was administered to the group of traditional medical practitioners, TMPs, herb sellers and herbalist. The signed consent was obtained from individual before the commencement of the interview.

3.3.3 Sample Size and Sampling Technique

A well semi-structure questionnaires were administered to obtain information used for the study. A focus group discussion was carried out among the TMPs during one of their monthly meetings due to low level of education and understanding of English

language. The discussion was carried out in native language, Yoruba which was fully understood by all.

The information obtained from the TMPs were transcribed and the interpreted using the *use value index* (UVI), proposed (Phillips and Gentry, 1993.) was used to determine the level of importance attached to the plant used for treatment by the respondents.

$$UVI=\sum U/n_s$$

Where 'U' is the number f uses given by a resp ndent, 'n_s' is the number of respondents who use medicinal plant preparations.

3.3.4 Plant Selection and Authentication

Fresh whole plant of *S. hispidus* and seeds of *A. melegueta* were obtained from the herb sellers at Bode market, Ibadan, Oyo State. The seeds and whole plants were taken to the Forest Herbarium Ibadan were the voucher specimens were deposited for numbers and then kept in the Department of Pharmacognosy Herbarium, University of Ibadan (DPHUI) for references. *Aframomum melegueta* and S*trophanthus hispidus* were assigned FHI numbers, 112374 and 112443 respectively

3.4 Plant Preparation and Extraction, and Liquid-Liquid Partitioning of Methanolic Extracts

The air-dried plant materials, *A. melegueta* (4.5 kg) and *S. hispidus* (10 kg) were ground with a Hammer mill using a 5 KVA motor. *A. melegueta* was extracted by maceration at room temperature (30°C) in methanol for 72 h. *Strophanthus hispidus* was extracted by using Soxhlet apparatus in methanol at 50^{o}C. After removal of solvents by a rotary evaporator (Buchi), yields of extracts were obtained and the extracts stored in the refrigerator in amber coloured bottles. The Methanolic extract of active plant was fractionated, using liquid-liquid partitioning into hexane, Dichloromethane (chloroform), ethyl acetate and butanol successively.

.

The Methanolic methanol extract of *A. melegueta and S. hispidus* were each dissolved in 500 mL of methanol and water (1:3). This was then partitioned 5 times each using 1000 mL portions of n-hexane, dichloromethane, ethyl acetate successively as illustrated in Fig. 3.0. The fractions were evaporated to dryness *in vacuo* and stored at 10 ^{O}C until needed for analysis.

The percentage yield was calculated as:

$$\% \text{ yield} = \frac{\text{Weight of extract x 100}}{\text{Weight of powdered plant material}}$$

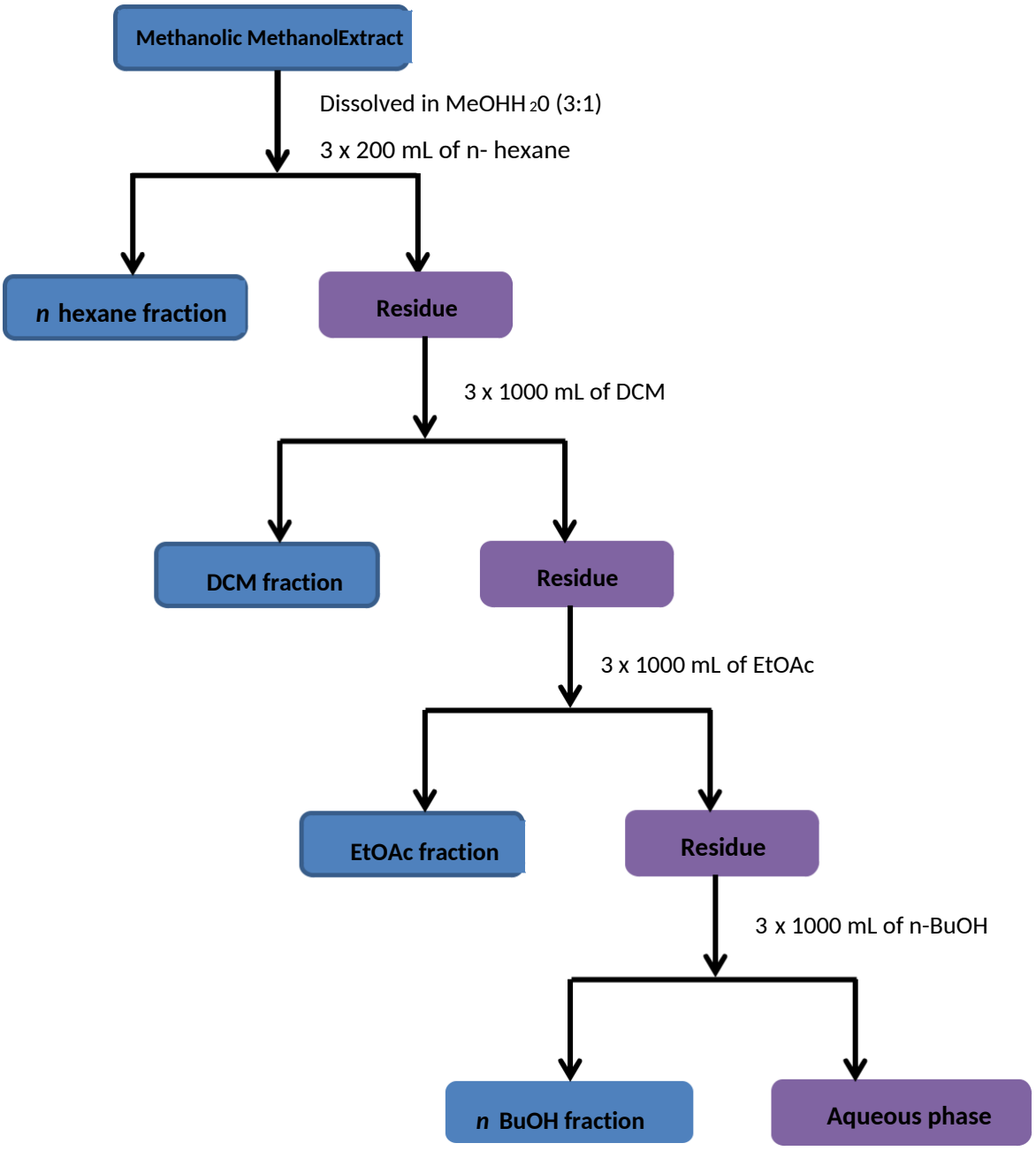


Figure 3.0: Extraction and Fractionation of Selected Plants

3.5 Brine Shrimp Lethality Assay (BSLA)

Artemia salina eggs were gotten from Prof Edith O. Ajaiyeoba. The protocol used for BSL described by McLaughlin (1991). This method checked the motility of shrimps before and after treatment with plant extracts.

The assay is a simple experiment which requires little or no sophisticated apparatus. The shrimps underwent the hatching process by placing them in sea water from a natural source collected from the Bar Beach, in Lagos state, Nigeria. To promote the hatching, a plastic container with two unequal compartments that was separated by a divider with several perforations was used. The eggs were put inside the bigger compartments which was covered with an aluminium foil to prevent penetration of light, while the small compartment was illuminated. After incubation at 25-29^{o}C for 48 h, a small Pasteur pipette was used to collect the larvae (nauplii) from the smaller compartment. Thereafter, the plant extracts solubilized before a stock solution (SS) (10mg/mL) was made. The SS was serially diluted to obtain five working concentrations (1000-1µg/mL). Ten larvae were picked and added into each test concentrations of the plant extract, sea water (negative control) and cyclophosphamide (positive control). After 24 h incubation period, the quantity of dead napuli was determined using the magnifying lens and the percentage mortality was estimated. The regression curve derived from the plots of the obtained data in the GraphPad Prism® statistical software, the LC50 value (50% lethal concentration) and the standard error of the mean (SEM) were estimated.

3.6 Statistical analysis

Graph-pad prism software was used to analyse the data. Lethal concentration at 50% (LC_{50}) of the test sample was determined using Non-linear regression (GraphPad Prism version 5.00 for Windows, GraphPad Software, San Diego California USA, www.graphpad.com).

3.7 Cytotoxicity Assay

Human Cancer cell line (RD, Hep-2C, HeLa)

Cancer cell lines were obtained from the Centre for Disease Control, Atlanta, Georgia and maintained in WHO Polio laboratory, situated in the Department of Virology, University of Ibadan, UI, Nigeria.

Human Rhabdomyosarcoma (RD), human epithelial type-2 (Hep 2C), cervical carcinoma, (HeLa), African green monkey kidney normal cell line (Vero) were obtained from the Virology laboratory stock in the Department. These cells were grown in Dulbecco's modified eagle medium (DMEM); all supplemented with 10% heat inactivated fetal bovine serum (FBS), 2 mM L-glutamine, and 1% penicillin-streptomycin. DMEM was also supplemented with 0.01 mg/mL insulin and 1mM sodium pyruvate. Cells were incubated in a 5% CO_2 humidified incubator at 37°C and passaged bi-weekly.

Splitting of Cancer Cell Lines

The flask with the cell lines containing liquid media were emptied into the waste container. The T-flask which was slightly washed with 2 mL phosphate buffered saline (PBS) and emptied into a waste container. The flask was slightly swished with 1 mL of trypsin and incubated for 3 minutes until cell detaches. The flask was removed from the incubator; the side of the flask was tapped repeatedly to ensure lumps of cells were dispersed. This was viewed under the microscope to ascertain if the cells had detached from the surface of the flask. The cells were then resuspended in freshly prepared DMEM media, growth medium ensuring even distribution by swishing media. Finally, 100 µL f mixture was seeded int a 96 micr well-plate and incubated for 2 days.

Cytotoxicity Assay of Samples using MTT

The cytotoxicity assay of the samples was determined using MTT (3-(4, 5-dimethyl thiazole2yl)- 2, 5-diphenyl tetrazolium bromide) assay following the protocol and procedure of Mosmann. (Mosmann, 1983; Buch, 2012.).

This was carried out to determine the cytotoxicity of extracts/ fractions at 50% (CC50). Each of the Methanolic extracts/fractions was dissolved in DMSO to give a

concentration of 10 mg/mL, respectively. The stock (0.1 mL) was added to 0.9 mL of maintenance medium c ntaining antibi tics, t btain a diluti n f 1000 µg/mL, and this was designated as “neat”. Serial ten-fold dilutions of the extracts were made from the “neat” using maintenance medium as diluent to obtain different concentrations. With the aid f a micr titre dispenser, 50 µL f each extract diluti n was dispensed into 96-well microtitre plates already seeded with monolayer of RD, Hep-2c, Hela or Vero cells in triplicates. The plates were incubated at 37^{o}C in a carbon-dioxide environment and the cells observed under microscope after 72 h (Buch *et al*., 2012). The CC_{50} was determined using the MTT colorimetric assay described below:

The percentage cytotoxicity was calculated as:

$$\% \text{ cytotoxicity} = \frac{(A - B)}{A} X100$$

Where A = the mean optical density of untreated cells.

B = the optical density of cells treated with plant extracts/ fractions.

The MTT colorimetric assay was used to evaluate the reduction of viability of cell cultures in the presence and absence of the extracts. The ability of the plant extract and fractions to be cytotoxic was measured using the tetrazolium dye (MTT), which is metabolized by mitochondrial enzymes of viable (surviving) cells to an insoluble, coloured formazan product (Fig. 3.1). The level of metabolism that occurs in the individual well of the 96-well microtitre plate is dependent on the number of healthy viable cells present. The procedure is described as follows: ^{o} Supernatants were removed from all wells after 72 h incubation at 37 C. 25 µL f 2 mg/mL MTT (sigma) solution in phosphate buffer^{o} saline (PBS) was added to each well and the plates were incubated for 2 h at 37 C. After that, 125 µL f DMSO was added to the wells to solubilize the MTT crystals formed. The plates were placed on a shaker for 15 min after which absorbance was read at wavelength of 492 nm on a multiwall spectrophotometer (TitertekUniskan).

MTT Assay Protocol (HeLa, AU565, BJ)

Cancer cell lines used for this experiment were obtained from Dr. Panjwani Centre for Molecular Biology, ICCBS, University of Karachi, Pakistan. Protocol used for the cytotoxicity of the plants and compounds obtained on the highlighted cell lines was according to Mosmann (Mosmann, 1983.).

The procedure for the MTT assay is same as described in 3.7.

$$\% \text{ inhibition} = \frac{\text{OD of sample} - \text{OD of blank}}{\text{OD of vehicle control} - \text{OD of blank}} \text{ x } 100$$

IC_{50} was calculated on EZFit5 software.

3.8 Data Analysis

The cytotoxic concentration (CC_{50}) and the inhibitory concentration (IC_{50}) for each extract/fraction were calculated from dose response inhibition curves using non-linear regression analysis.

ii). Selective Index (SI)

The selectivity index (SI), which indicates the cytotoxic selectivity (i.e. safety) of the Methanolic extract against cancer cells versus normal cells (Prayong *et al.*, 2008.), was calculated from the CC_{50} of the Methanolic sample in normal cells versus cancer cells. The therapeutic index or selective index is defined as the ratio CC_{50} of normal cell line compared to CC_{50} of the extracts or compounds on cancer cell line.

A high selective index is preferable to a low one. This corresponds to a situation in which a much higher dose of an agent would have to be taken to reach the lethal threshold than the dose that would be taken to elicit the therapeutic effect. Selective activities of the tested plant samples were calculated as follows:

$$\text{Selective Index (SI)} = \frac{\text{CC50 of extract on normal cell line (µg/mL)}}{\text{CC50 of extract on cancer cell line (µg/mL)}}$$

3.9 Vacuum Liquid Chromatography (VLC) for Hexane fraction of *A. melegueta*

The cylindrical sintered glass vacuum liquid chromatography column (25mm diameter and 100mm length) was packed with 500 g of dry silica gel. The silica gel was properly packed for proper separation to occur by tapping the glass column severally. After proper parking of the column, the solvent for elution, n-hexane was introduced and eluted with aid of a pump to ensure even packing of the column.

The solvent used for the process of VLC included Hexane, DCM, methanol at different concentrtions. The solvent, hexane was initially run at 100% followed by increasing concentration of DCM and later methanol asymmetrically. Eluents were collected at each concentration at a minimum of 250 mL in at least times.

The TLC profile of the constituents eluted were then carried out to pool similar constituents for further evaluation and isolation.

Application of Plant material

The *n*-hexane fraction from the Methanolic methanol extract weighing 75 g was dissolved in *n*-hexane and adsorbed with 50 g of silica gel then air-dried. It was then loaded on the VLC column and thereafter eluted with varying ratio of *n*-hexane: Ethyl acetate (H: E) and Dichloromethane : Methanol (D : M). A total of 22 fractions were obtained (11 H : E, 11 D : M) and was pooled to 7 subfractions (1-7) based on their TLC profile.

3.10 Isolation of Bioactive Compounds from n-Hexane fraction of A*framomum* melegueta

3.10.1 VLC fraction 3 of n-hexane fraction of *A. melegueta* seeds

The glass chromatography column was plugged with cotton wool. Thereafter a slurry of 40 g of Silica gel was made with n-hexane and then poured into the column. The column was tapped continuously to ensure uniformity of packing. Sub-fractions 3 (5 g) was loaded on the CC for further purification and a total of 20 fractions were collected and spotted on TLC plates to determine their TLC profiles.

Purification of Compounds by HPLC

Samples with high polarity were separated using RP-HPLC. Specification of column: 20mm internal diameter. 250mm length, 4µm particle size, 80Å pore size. Column name: ODS C-18 M80, Japan Analytical Industry Co. Ltd, HPLC model LC-908W.

Procedure

Preparation of sample

All samples were pre-treated by Column Chromatography using reagents such as silica gel, sephadex LH-20. Samples were soluble in solvent system used. Sample of 25mg was dissolved in 3 mL.

Injection of sample

The milli-pore filter fit for syringe (All pure PTFE 0.22µM) was used to remove impurities of insoluble constituents. A maximum of 3 mL of the solution was filled in an air-tight syringe for sample loading. The syringe-needle was placed to the port at the injection position for loading. The position of rhodyne was changed from inject to load position by turning the knob and the plunger of the syringe was used to introduced the sample solution to the loop. The solvent dropped from the siphon the knob is brought back to the inject position swiftly, then sample is injected therafter. The rhodyne was then changed back from load to inject. The marker on the recorder was then set to start. The Ultraviolent (UV) and refractive index (RI) serves as the detection methods for compound separation on the HPLC.

Purification of AM-2

Fraction AM-2 was purified by semi-preparative HPLC (MeOH–H_2O, 5 % MeOH at 5 min to 100% at 26 min, 5.0 mL/min) to afford compound **A** (2 mg, t_R = 29 min), **B** (2 mg, t_R = 29,6 min), and **C** (2 mg, t_R = 30.4 min).

3.10.2 Procedure for Thin Layer Chromatography (TLC)

Fractions from *A. melegueta* seed (n-hexane) VLC and column fractions were subjected to TLC pre-coated aluminium sheets (20x20) Silica gel 60 F_{254}Merck with the aid of capillary tubes at a distance of 1cm from the base. The spots were equivalent

from each other and were allowed to dry on the plates and were subsequently placed in a tank saturated with varying solvent systems such as hexane: ethyl acetate, hexane: chloroform at different ratios to ensure adequate separation. The plates were developed, brought out of the tank and dried. The ultravi let lamp (λmax f 254 and 365 nm) was used to view the compounds and then marked. Their retardation factors (R_f) were obtained using the formula:

$$R_f = \frac{\text{Distance moved by the sample}}{\text{Distance of the solvent front}}$$

3.11 Gas Chromatography-Mass Spectrometry (GC-MS) analysis

Sub fractions 2 and 3 from the VLC pooled fraction were subjected to GC-MS analysis. Agilent technologies 7890 GC system and Agilent technologies 5975 MSD (Mass Spect. Detector) were used for the GC-MS analysis. The mobile phase is the carrier gas (Helium, 99.99% purity), while the stationary phase is the column. The model of the column is HP5 MS with length 30 m, internal diameter 0.320 mm, while the thickness is 0.25 µm. The oven temperature program is initial temperature of 80°c to hold for 1 minute. It increases by 100 per minute to the final temperature of 240°c to hold for 6 minutes. The injection volume is 1 microlitre and the heater or detector temperature is 250°C.

The MS interpretation of the spectrum MZ (mass to charge ratio) with molar mass and structures. Interpretation of mass spectrum obtained from GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62000 patterns.

Nuclear Magnetic Resonance and Mass Spectrometry

NMR analyses were recorded on Bruker Avance III spectrometer operating at 600 MHz (^{1}H) and 150 MHz (^{13}C) using standard pulse sequences referenced to residual solvent signals. The high-resolution mass spectrum (HRMS) experiments were carried out on LTQ Orbitrap spectrometer (Thermo Scientific, USA) equipped with a HESI-II source. The spectrometer was equipped with an Agilent 1200 HPLC system (Santa Clara, USA) including pump, PDA detector, column oven (30°C) and auto-sampler (injection volume: 6 µL for Fullscan, 7µL for MS^n).

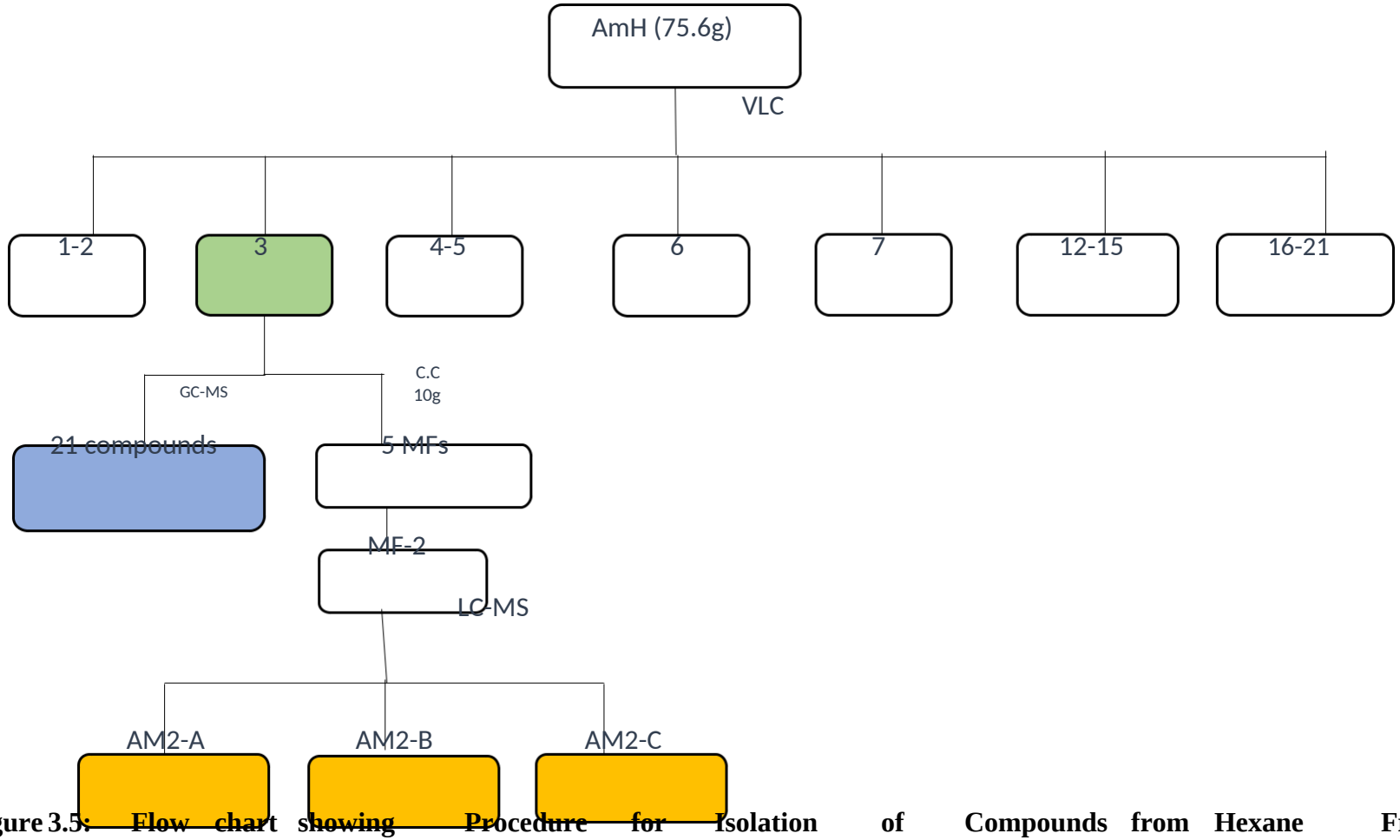


Figure 3.5: Flow chart showing Procedure for Isolation of Compounds from Hexane Fraction Aof. *melegueta*

3.14 Isolation of Bioactive compounds from DCM fraction of *Aframomum melegueta*

The *A. melegueta* DCM fraction (20g) was fractionated on column chromatography into 23 fractions using Hex: DCM: MeOH at varying concentration. It was then pooled into 8 major fractions. Major fractions include: AmD-4, AmD5-7, AmD-16, AMD 13-15. These major fractions were selected for further fractionation into different compounds.

AmD-4

Major fraction AmD4 with 61.1 mg weight was separated using a normal phase HPLC. Mobile phase Hex: EtOAc 8:2 was used for the separation to obtain compounds: 4-1,4-2,4-3 based on retention times 28.6, 42.4 and 53.3 minutes respectively. TLC profile was carried out to ascertain purity using mobile phase H: E: M 6:3:1 and sprayed with 20% sulphuric acid to give brown, bluish-green and yellow colour respectively.

AmD 13-15

This pooled fractions with a weight of 2.8g was further separated on a column by using solvent H: E: M. It was further fractionated into 13 sub-fractions, TLC profile was carried out and sub-fraction 6, SF 6 had two major spots. Afterwards, 2 g of SF 6 was then selected for purification on column chromatography using Hex: DCM: MeOH. The TLC profile of the fractions eluted afforded 4 major fractions that proceeded to normal phase HPLC. The fractions included AmD6-12/13, AMD6-14, AmD6-15, and AmD6-20 with weight of 51.8 mg, 46.8 mg and 9 mg respectively. AmD 6-15 afforded a pure compound AmD 6-15-2 of 23 mg which was visible only at 254 nm.

AmD 8-10, 400 mg was further purified using silica gel of 10 g into 5 sub-fractions (SF), 1-4, 5, 6-8, 9-12 and 14-17. SF 14-17 (80 mg) was then purified using normal phase HPLC, oily compound was eluted with retention time, 76 minutes. Then TLC was developed to ascertain purity.

3.15 Isolation of bioactive compounds from DCM fraction of

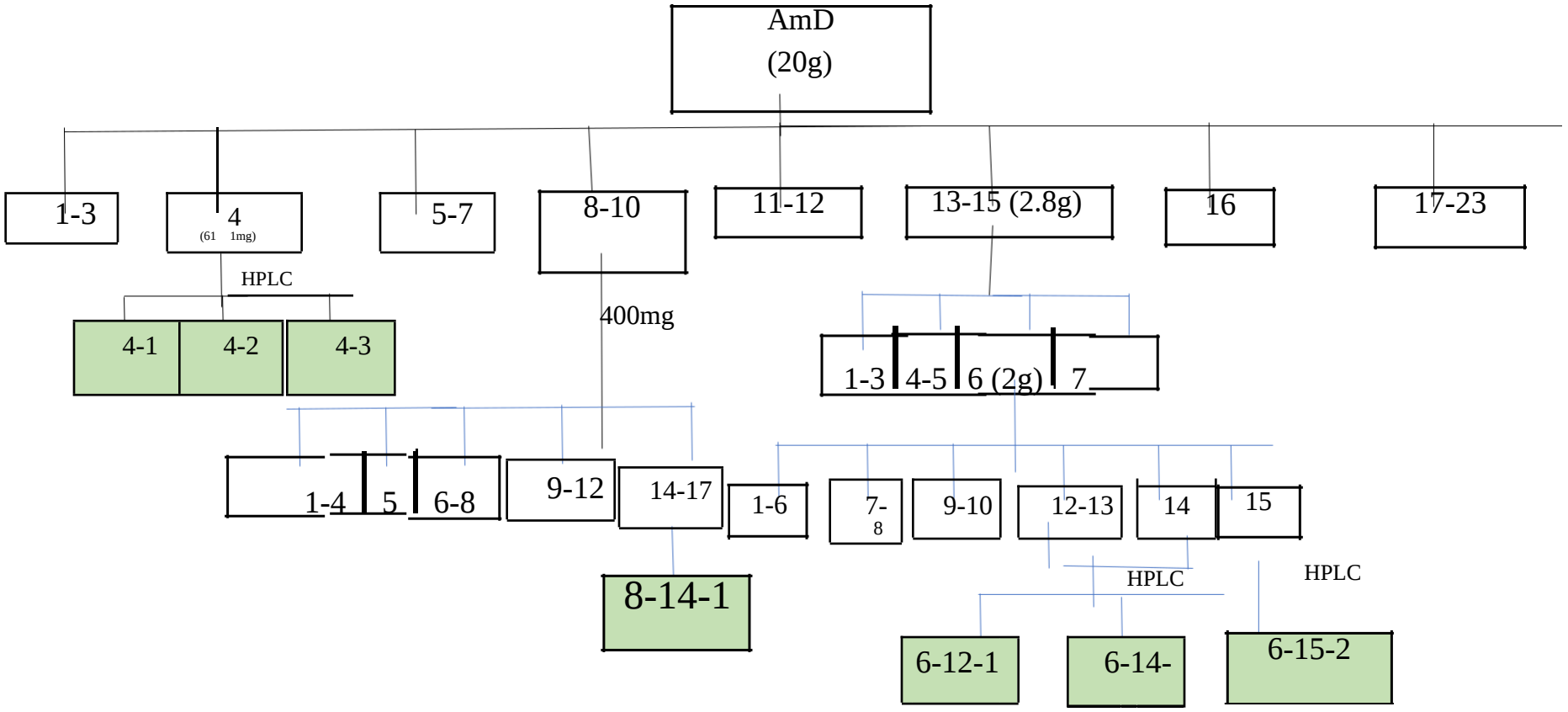


Figure 3.6: Flow chart showing Procedure for Isolation of Compounds from Dichloromethane Fraction of

3.16 Isolation of Bioactive Compounds from *Strophanthus hispidus*

Dried powdered whole plant of *S. hispidus* were extracted using Soxhlet apparatus into methanol, the temperature was set at 50^{o}C to avoid degradation of bioactive compounds. The methanol extract was thereafter concntrated *in vacuo* using a rotary evaporator (Buchi). Solvent-solvent patitioning was carried out on the Methanolic extracts using n-Hex, DCM, EtOAc, butanol. The obtained fractions were concentrated and kept in the refrigerator till needed for further analysis.

Isolation of Compounds from Dichloromethane fraction of *Strophanthus hispidus* (SHD)

A concentrated DCM fraction (30g) was semi-purified into fractions on a CC, using silica gel (6mm x 70mm). Mobile phase used include mixtures of Hexane: Dichloromethane and then Hexane: Ethyl Acetate, 0 to 100% and ethyl acetate: methanol, both of increasing polarity.

An aggregate of 126 fractions were obtained and pooled by TLC analysis to 25 major fractions (25 MF) based on similar TLC profile. The TLC of pooled fractions were developed with Hex: EtoAc; EtoAc: MeOH, and increasing polarity. The plates were observed under UV light at both 254 and 365 nm after development. It was then sprayed with 20% H_2SO_4, sulphuric acid or Drangerdoff reagent and then heated to observe the compounds present which could not be observed by absorbance of the UV light.

Investigation of Pure Compounds Obtained

SHD 58

Pure compound SHD 58 was eluted at Hex: DCM (70:30) and concentrated to obtain a yellow hair- like compound which was insoluble in hexane and soluble in DCM. It was thereafter dissolved in hexane and filtered. A colourless filtrate was obtained with a yellow residue which was left to dry. A compound of 43 mg was obtained. Purity was initially determined by spotting the compound on a TLC and developing with several varying mobile phase to obtain one clear band with a retardation factor R_f of 0.59.

SHD9

Major fraction 8-11 was obtained from mother column through elution with Hex: EtoAc 70:30. It was concentrated and a white powder was obtained in the flask. The concentrate was observed to be insoluble in Hex and then removed from the flask with EtoAc. Solution was left to evaporate in the hood (fume cupboard) and then a white powder emerged. Hexane was added to re-dissolve the compound and left to dryness in vial until the white powder was formed to obtain a pure compound (SHD9). Purity was confirmed by spotting the compound on a TLC and developing with several mobile phase to obtain one clear band with a retardation factor R_f of 0.510. SHD9 is a compound which is UV in active.

SHD21

This compound was obtained from major fraction 21. It was eluted with EtOAc: Methanol 90:10. After evaporation, EtoAc was added to obtain white substance which was filtered off and left to dry to obtain white crystalline compound 3 (SHD21). Purity was confirmed by spotting the compound on a TLC and developing with several varying mobile phase to obtain one clear band with a retardation factor RF of 0.5.

MF 13-15

This major fraction was obtained from H: E 60:40 and 50:50 with similar TLC profile. About 2g of MF 13-15 was loaded onto a column (1 mm X 75 mm) with 1 g extract: 100 g of silica gel factor due to the closeness of the compound separation on the TLC plate. Therefore, Silica gel of 200 g was utilized for a good separation to be obtained. A total number of 31 major fractions were obtained, MF 29-31 had 3 major spots with the weight of 73.6 mg. This fraction, MF 29-31 was then subjected to HPLC for purification of compounds.

MF 16-17

This major fraction with weight of 2.01 g obtained by H: E 40:60, 30:70 from the big column of DCM fraction. The fractions had similar TLC profile. Silica gel of about 200 g (1:100) was packed in a mini column (2 mm X 75 mm). The major fraction of 2.01 g was loaded to obtain 50 subfractions pooled to 26 sub-fractions (SF) based on

TLC profile. Solvents used in elution was Hex: DCM and DCM: MeOH with increasing polarity.

Sub-Fractions 25, 26-27

The two different fractions were obtained from small column (MF 16-17). The composition of compounds was observed by subjecting the subfractions to a reverse phase TLC plate using methanol: water at 95:5 for development of plate. Five major spots were observed at 366 nm and three major spots at 254 nm with SF 26 and 27 having similar spots with same R_f. SF 25, 26 were therefore subjected to RP-HPLC using HPLC model LC-908w. column size of 20 mm (internal diameter), 250 mm length and 4 µm particle size, pore size of 80 Å, column name ODS C-18 M80. The flow rate IN 4 mL/min, Fin- 3 mL/min with pressure of 27 and then 20.

Three injections were made on each fraction. A total of 79 and 110 vials were collected from SF 25 and SF 26 respectively for the entire injections. These were initially spotted of normal phase TLC using DCM: MeOH 95:5. Similar profile obtained in the 3 different injections of SF 26 were then pooled together to give three major fractions. The fractions were then subjected to analytical HPLC for profiling of the constituents of the fractions.

Further separation was carried out to obtain the major compounds from each MF. **SHD 26-MF-3** was eluted and evaporated to give a white crystalline which was soluble in methanol. Purity was confirmed by spotting the compound on a TLC and developing with several mobile phase to obtain one clear band with a retardation factor R_f of 0.60. SHD 26-MF3 is a compound which is UV- active. Visible spot was confirmed by spraying with 20% sulphuric acid and then heated to show brown colouration.

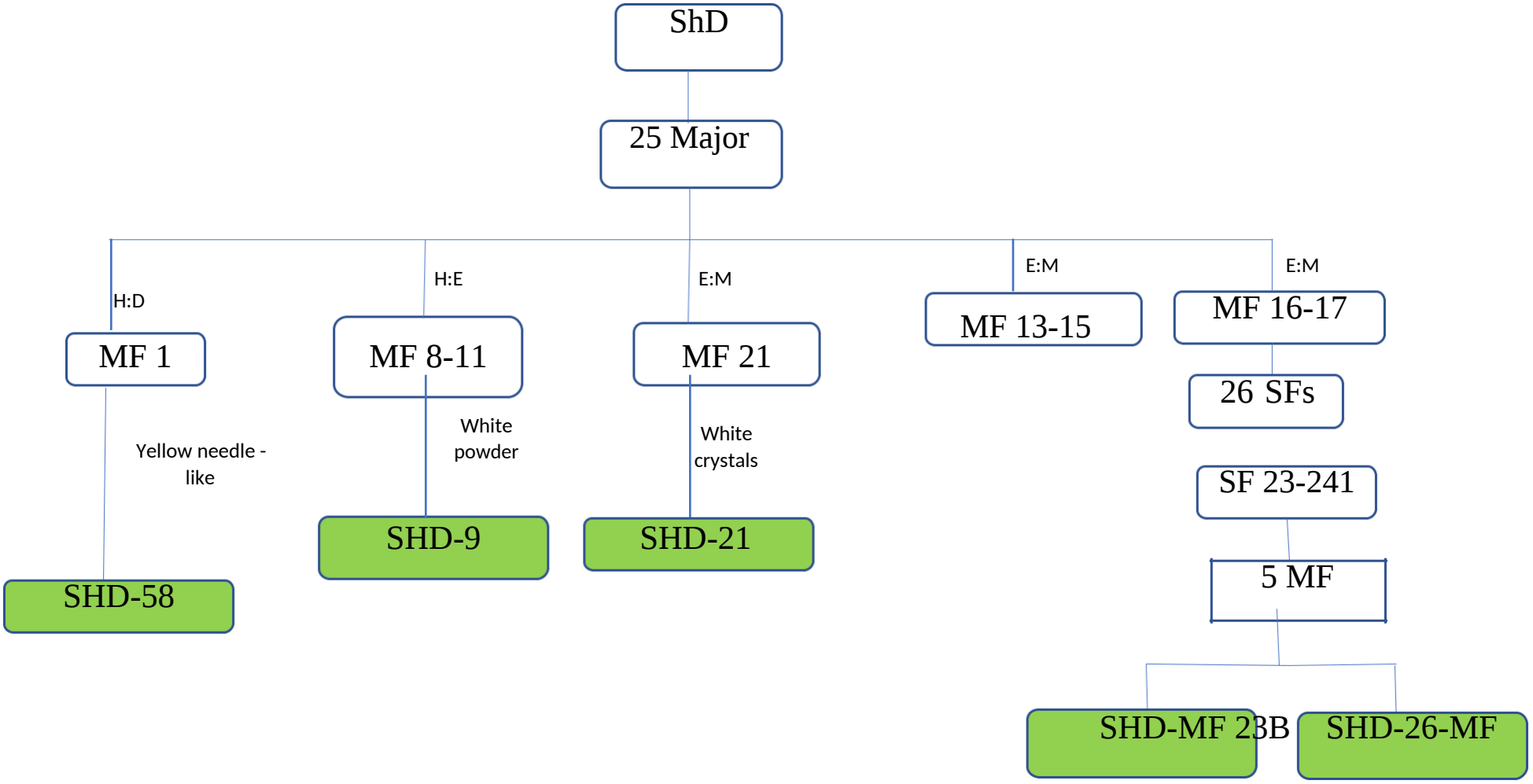


Figure 3.7: Flow chart showing Procedure for Isolation of Compounds from Dichloromethane Fraction of

3.17 Isolation of compounds from Hexane fraction of *Strophanthus hispidus*

The hexane fraction, (20 g) of S*trophanthus hispidus* (SHH) was loaded on the CC, packed with 300g of SiO_2 for fractionation using Hex: EtOAc: Methanol. A total number of 38 fractions were obtained.

SHH 14

At concentration of solvent (Hex: EtOAc 80:20), subfraction 14 which was attained as a white amorphous powder. It was insoluble in hexane but soluble in dichloromethane and chloroform. After concentration, it was thereafter placed in the fume cupboard. After purity was ascertained by TLC profile.

Major fraction 15-19

This major fraction (1g) was loaded for further purification on a smaller column. It was packed with 20 g of SiO_2 gel to obtain 12 sub fractions, SFs after TLC profile to pool similar compound or chemical constituents together. From the sub-fractions, three SFs (29-32 (50 mg), 33-41 (93.7 mg), 42-42 (194.4 mg)) were selected based on the TLC profile of compounds present. These SFs were further purified on the preparative normal phase HPLC using Hex: EtOAc 70: 30 with 1% acetic acid to obtain SHH 29-6, SHH 33-1, 33-4, and SHH 42-4.

SHH 32

Column chromatography of SF 29-30 mentioned above afforded a high viscous substance eluted directly at EtOAc: Methanol 90:10. The substance was removed from the elution and it was observed to be insoluble in both solvents of elution. It was further purified by washing with acetone, placed in a vial and then dried in the fume cupboard.

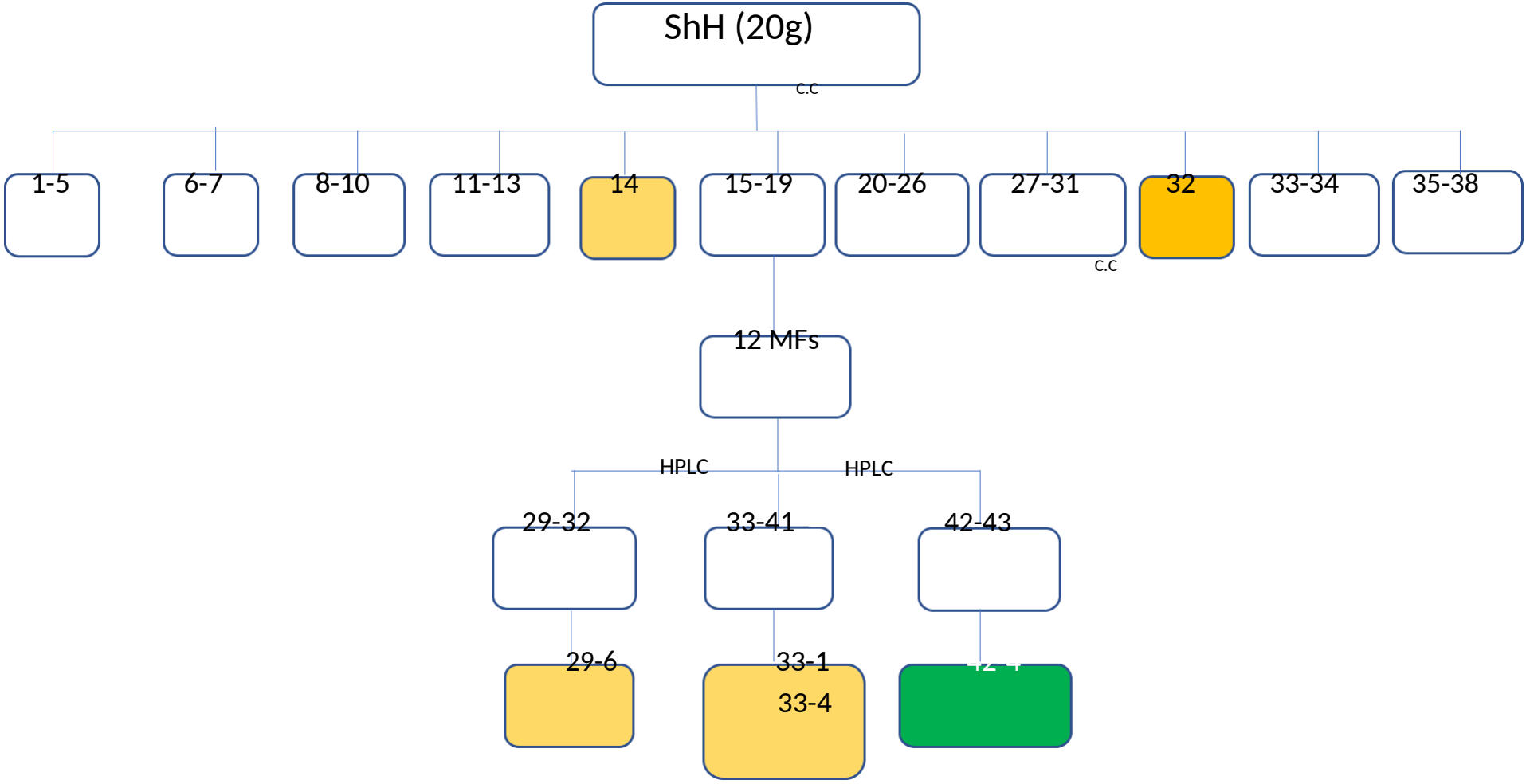


Figure 3.8: Flow chart showing Procedure for Isolation of Compounds from n-Hexane Fraction of

Ultraviolent

The UV data was recorded and analysed on THERMO ELECTRON-VISIONpro SOFTWARE V4.10

Fourier Transform-InfraRed

The InfraRed spectrum of the compounds were recorded on BRUCKER VECTOR 22 by using KBr pellets for the solid samples.

Melting point

The melting points of the isolates were recorded on an apparatus BUCHI M-560 and were uncorrected.

3.18 Structural Elucidation of Compounds

NMR Spectra were analysed based on Data obtained and then elucidated based on comparison with literature data using SciFinder and Google scholar. Structures were drawn with the aid of CS Chemdraw Drawing (cdx) professional software application.

CHAPTER FOUR

4.0 RESULTS

4.1 Ethnobotanical Survey

The results obtained from the study revealed age range of the respondents to be between 50 to 70 years. However, most of the respondents were female, this could be due to the fact that most of the herb sellers are women and the TMPs were mostly male. The data collected showed that the respondents used the medicinal plants for treatment of diseases in different ways due to the belief in the effectiveness of plants in treatment of diseases. Furthermore, most patients resort to the use of traditional herbs because of the high cost of allopathic drugs.

The disease, cancer was described t the TMPs in the y ruba language as '*Jejere'*. When inquired how the TMPs understood the disease state in the patients, they replied 'm st patients have already been diagn sed f the disease in the h spitals', thus n difficulties in identifying the disease.

The results obtained from different plants types used in management or treatment of Cancer in Akinyele Local Government area, Oyo state include 26 plants from 19 families. A total number of 7 plants from 5 families were selected based on literature survey and selection of plants for evaluation of cytotoxicity that have not previously been evaluated.

Table 4.1: Medicinal Plants used in the Management of Cancer in Akinyele LGA

S/N	Botanical name / Authority	Yoruba name	Family	Parts used	FI (%)
1	*Euphorbia lateriflora* & Thonn.	*Enu- kopire*	Euphorbiaceae	Aerial part, whole plant	66.6
2	*Kigelia africana*				62.2
3	*Khaya*Meliacea*nsisivor***e**			Stem bark	33.3
4	*Strophanthus hispidus*	Sagere	Apocynaceae	Aerial part, Root	44.4
5	Euphorbiaceae*Euporbiapoissoni*			Whole plant	22.2
6	*Acanthus monatus* Anders	Ahon ekun	Acanthaceae	Leaves	40.0
7	*Securidaca longependiculata* Fresen	Ipeta	Polygalaceae	Root, stem bark	22.2
8	*Calotrophis procera* Br.	Bomu bomu	Apocynaceae	Aerial part	33.3

k

Oganho

o adete

nn Ekuya funfun9 *Cleome*Capparidaceae*ciliate* Leaves 26.6

10	*Euphorbia hirta*	Akun esan	Euphorbiaceae	Whole plant	60.0
11	*Morinda lucida*	Oruwo	Rubiaceae	Leaves, stem bark	53.3
12	*Rauwolfia vomitora*	Asofeyege	Apocynaceae	Leaves	33.3
13	*Canavalia ensiformis* (L.)(DC.)	Sese nla	Fabaceae	Leaves, peas	24.4
14	*Fagara zanhoxyloides*	Ata	Rutaceae	Root	11.1
15	*Funtumia africana* Stapf	Ako-ire	Apocynaceae	Root	17.7
16	*Mitragyna inermis* Ktze.	Okobo	Rubiaceae	Bark	4.4
17	*Aframomum melegueta* Schum.	Atare	Zingiberarceae	Seed	71.1
18	*Calliandra haematocephala* Hassk.	Tude	Fabaceae	Root, leaves	33.3

	19	Plumbainaceae	*Plumbagozeylanica*			Root	44.4
Orogbo	20	*Garcinia* Guttiferae	*kola*			Seed, stem bark	48.8
Mistletoe	21	*Viscum album*	Santalaceae			Root	22.2
onola	22	*Boerhavia*	Punarnava *diffusa*			Root	53.3
gi aka	23	*Cynometra*	Leguminos*mnii***a**e			Stem bark, leaves	57.7
		Compositae	24*Ageratum conyzoides*			Whole plant	28.9
	Crassulaceae	25	*Bryophyllum pinnatum*			Leaves	22.2
	26	Cajanus cajan (L) Millsp		Otili	Fabaceae	Leaves	55.5

*FI- Fidelity index

4.1.1 Herbal recipes for cancer treatment

The twenty two herbal preparations obtained for treatment of cancer amongst the herb sellers and the traditional medical practitioners included decoctions (22.72%), concoctions (36.36%), paste (36.36%), and powder (4.54%).

Table 4.1.1: Enumeration of recipes, method of preparation, and mode of administration used in the treatment of cancer in Akinyele LGA, Oyo State

Disease	Recipe	Method of preparation	Mode of administration
Cervical and Prostrate cancer	Boil the stem back and root of *Kigelia africana* for about 2 hours and then allow to cool	Decoction	1 teacup twice daily for 1-2 months depending on the severity and stage of the disease
	Boil the bark of *Morinda lucida* with *omi dun (supernatant from previously sieved pap)*	Decoction	1 teacup daily
	The dried stem bark of *Securidaca longependiculata* is grinded into smooth powder and then mixed with boiled *E. hirta* and *E. poissni*	Concoction	1 shot 3 times daily
	The aerial part of *Calotrophis procera* is boiled for 1 hour	Decoction	1 teacup 3 times daily
	The whole plant of *S. hispidus*is boiled either with locally sourced water from stream or *omi dun (supernatant from previously sieved pap)*	Decoction	1 teacup each morning and night
	Thoroughly washed stem bark of *Fagara zanhoxyloides* and the root of *Funtumia africana* are boiled in water for about 2	Concoction	Drink when warm once daily

hours		
Fresh leaves of *Cleome ciliata* is ground and the juice is extracted and the mixed with *adi agbon* (coconut oil) in equal volume.	Paste	Lick morning and night and then administered on affected area
Fresh roots of *Beohivia diffusa* is parboiled and cooked with l cust beans, "alub sa-elewe"(onion leaves) and intestine of goat and then ate as soup	Concoction	Eat once a week
The aerial part of the *Ageratum conyzoides* is rinsed with water to remove attached insects and the boiled with water for about an hour	Decoction	Drink a glassful 3 times daily
The fresh leaves of *Cajanus cajana* and *Bryophyllum pinnata* are both cut into tiny pieces and the pounded. It is then soaked into *omi dun (supernatant from previously sieved pap)* for about 3 days	Concoction	One tablespoon must be taken at least 2 times daily for a period of 1 month
Dried leaves of *Acanthus monathus* with seeds of *Aframomum melegueta* is grounded and mixed with hot pap	Powder	1 teaspoon daily at dawn

Breast cancer	Pound the *Celosia laxa* leaves with black soap	Paste	Wash open wound daily
	Cook the fresh leaves of *Celosia laxa* with soup condiment	Concoction	2 times a week
	Properly air-dried stem and leaves of *E. lateriflora* boiled with water and *epo-orombo (lemon fruit peel)*	Concoction	1 shot Once daily
	Powdered stem of *E. lateriflora* mixed with shea butter	Paste	Rubbed of the open wound daily
	The root of *Morinda lucida,* completely air-dried, pound into fine powder with potash and then mixed with *adi-agbon* (coconut oil)	Paste	Rubbed of the open wound daily
	Rauwolfia vomitora leaves is boiled with water, a teaspoon of *adi-agbon (supernatant from previously sieved pap)*	Concoction	Administered 3 times daily
	Tude and inaberi is grinded into fine powder and then mixed with shea butter	Paste	Applied topically to the open wound
	Seeds of *Garcinia kola* and *A. melegueta* are macerated in alcohol for 2 days.	Concoction	Administered orally as tincture twice daily
	Ageratum conizoides leaves is dried and powdered smoothly	Paste	Rubbed of the open wound on

	and mixed with either sheabutter or *adi-agbon* (coconut oil)		the breast daily
	Viscum album (whole plant) is blended together with honey and taken as juice	Paste	Administered once a day
	The seeds and leaves of *Canavalia ensiformis* is dried at room temperature and grinded into fine powder, it is then mixed with honey	Paste	Administered morning and night till the open wound gets dried

4.2 Brine Shrimp Lethality Test for Plant Selected Based on Ethnobotanical Survey

The plants selected from ethnobotanical survey were screened using brine shrimp lethality assay. The brine shrimp eggs were hatched and artenia spp larva were obtained. Varying concentrations were used for screening with a range of 0.01-1000 µg/mL. Cyclophosphamide served as the positive control.

The evaluation showed that the aerial part of *S. hispidus* had the lowest lethal concentration, LC_{50} of 1.54 µg/mL and *Khaya ivorensis* stem back had the highest LC_{50}, 435.6 µg/mL. Activity was recorded based on the motility rate of the shrimps by extract used for treatment. *Strophanthus hispidus* was observed to have better activity than the positive control, cyclophosphamide

Table 4.2: Brine Shrimp Lethality of some Selected Plants

Methanolic extract	LC_{50} (µg/mL)
Aframomum melegueta	7.22
Acantus monantus	272.76
Euphorbia lateriflora	259.20
Khaya ivorensis	435.62
Euphobia poissni	336.79
Strophanthus hispidus aerial part	9.23
Strophanthus hispidus root	1.54
**Cyclophosphamide*	22.74

Key: *standard drug/ positive control

4.3 *In Vitro* Cytotoxic Activity of Plant Extracts on Human and Normal Cancer Cell Lines

Cytotoxicity of the plant extracts selected from ethnobotanical survey screened on cancer cell lines, and normal cell line. *Strophanthus hispidus* showed a significant level of cytotoxicity while *A. melegueta* also showed good level of cytotoxicity as observed in table 4.3. Both plant extract also showed good level of selectivity on the cancer cells which was comparable to the standard drug used for evaluation in the study.

Table 4.3: *In vitro* Cytotoxic Activity of Plant Extracts on Human Cancer and Cell lines and Normal Cell line

Methanolic extract	RD (µg/mL)	Hep-2C (µg/mL)	Vero (µg/mL)
Aframomum melegueta	5.18 ± 1.15	9.74 ± 0.16	60.87± 0.23
Acantus monantus	40.57 ± 0.03	39.21 ± 0.02	103.94±1.57
Euphorbia lateriflora	17.29 ± 0.08	26.79 ± 0.05	57.09±0.98
Khaya ivorensis	90.15 ± 0.19	115.9 ± 0.04	215.01±0.52
Euphobia poissni	28.17 ± 0.12	11.11 ± 0.09	78.32±3.21
Strophanthus hispidus aerial part	2.94 ± 0.01	1.66 ± 0.03	47.54±1.14
Strophanthus hispidus root	1.71 ± 0.04	1.96 ± 0.02	35.78±2.36
**Cyclophosphamide*	1.80 ± 0.01	2.2 ± 0.02	23.92±0.05

Key: *standard drug/ positive control

4.4 Percentage Yield of *A. melegueta* Extract and its Fractions

The n-hexane fraction of *A. melegueta* extract was observed to possess the highest yield of 52.4% while the ethyl acetate fraction had the least yield of 1.6% as stated in Table 4.4 below.

Table 4.4: The Percentage Yield of *Aframomum melegueta*

Aframomum melegueta	% yield
Methanolic extract	3.68
n-Hexane	52.4
Dichloromethane	46.5
Ethyl acetate	1.6

4.5 The Brine Shrimp Lethality Assay of *Aframomum melegueta* seed and its fractions

The BSLA of Methanolic extract of *A. melegueta* showed the Methanolic extract was active while the hexane fraction was observed to be the most cytotoxic with LC_{50} of 2.27µg/mL which was found to be more cytotoxic than the standard drug used in the study. The complete result is shown on the table below.

Table 4.5: Brine Shrimp Lethality Assay of *A. melegueta* seeds Extract and Fractions

***Aframomum melegueta* / Fractions**	**LC50 (µg/mL)**	**Cytotoxic Activity**
Methanolic extract	6.18	Active
n-Hexane	2.27	Active
Dichloromethane	2531.83	Inactive
Ethyl acetate	4.12	Active
Cyclophosphamide *	14.80	Active

Key: *standard drug/ positive control

4.6 *In vitro* Cytotoxic Activity, CC_{50} of *Aframomum melegueta* Seed Extract and Fractions on Cancer and normal Cell Line

Table below shows the cytotoxicity screening of *A. melegueta* on cancer cell lines and normal cell line. The Methanolic extract of *A. melegueta* was found to have high activity on the Methanolic extract with CC_{50} of 6.18µg/ mL while the Hexane fraction was the most active in RD cell line with CC_{50} of 0.98µg/ mL.

Table 4.6: *In vitro* Cytotoxic Activity, CC_{50} of *Aframomum melegueta* Seed Extract and Fractions on Cancer and normal Cell Line

	CC_{50} (µg/mL)			
Fractions	RD	MCF-7	Vero	SI
Methanolic extract	6.18 ± 1.0	9.25 ± 0.8	80.79 ± 5.4	15.59
n-Hexane	0.98 ± 0.1	3.48 ± 0.5	96.80 ± 1.6	98.77
DCM	486.00 ± 2.3	-	24.82 ± 0.5	0.05
EtOAc	7.70 ± 1.0	-	71.07 ± 1.4	9.22
Cyclophosphamide*	0.74 ± 0.8	0.71 ± 0.7	23.92 ± 2.6	32.32

Key: *standard drug/ positive control

4.7 Cytotoxicity of Extract and Fractions of *Aframomum melegueta*

In vitro cytotoxicity of *A. melegueta* fractions were carried out on cancer cell lines, then normal cancer cell line BJ. The dichloromethane and hexane fraction showed activity on HeLa cell line, though not comparable to the standard drug, doxorubicin used for the study. Table 4.7 showing the result is found below.

Table 4.7 Cytotoxicity of Extract and Fractions of *A. melegueta*

Extract/ Fractions	HeLa	AU565	BJ
AmH	11.1 ± 0.89	32.0 ± 1.80	19 ± 0.10
AmD	14.1 ± 1.95	36.9 ± 0.05	18.1 ± 3.10
AmE	Inactive	42.0 ± 1.80	20.12 ± 1.90
Doxorubicin*	1.2 ± 0.01	0.54 ± 0.45	2.84 ± 0.02

Key: *standard drug/ positive control

4.8 Vacuum Liquid Chromatography (VLC) of the n-Hexane fraction of *Aframomum melegueta*

Table 4.8 below shows the number of fractions obtained and pooled from the VLC. A total number of twenty-two fractions were pooled to 7 subfractions (1- 7) based on their TLC profiles.

Table 4.8: Weight and nature of resulting pooled fractions from VLC

S/N	Weight (g)	Nature
1	0.79	Oil
2	0.87	Oil
3	30.89	Oil (viscous)
4	2.43	Oil
5	1.22	Oil
6	0.48	Oil
7	0.37	Amorphous

4.9 Cytotoxicity of VLC sub-fractions of *Aframomum melegueta*

The VLC sub-fractions of the n-Hexane fraction of *A. melegueta* were screened on RD and MCF-7 cell lines to select the most cytotoxic. The VLC sub-fraction 2 and 3 were observed to be the most cytotoxic across the two cancer cell lines with CC_{50} of 0.52 µg/mL and 1.11µg/mL against both cell lines the sub-fraction 3. Result is shown on table 4.8 below.

Table 4.9 Cytotoxicity of VLC sub-fractions of *Aframomum melegueta*

	CC_{50} (µg/mL)	
Sub-fractions	RD	MCF-7
1	NA	NA
2	5.50±0.34	4.32±1.98
3	0.52±0.06	1.11±0.25
4	5.94±0.23	NA
5	49.30±4.34	23.9±5.34
6	11.13±3.73	87.22±0.56
7	22.50±1.34	40.97±0.47
Cyclophosphamide*	0.74 ± 0.8	0.71 ± 0.7

Key: *standard drug/ positive control

NA- not available

4.10 The GC-MS analysis of VLC sub-fraction 2 of n-Hexane fraction of *Aframomum melegueta* (AMNHV2)

Figure 4.1 and table 4.10 shows the analysis of the results the GC-MS. Seventeen compounds were observed in the *n*- hexane fraction of (AMNHV2**).** The most abundant compound is Methyl 9- Octadecanoate with peak area of 34.32% and retention time of 22.93 while Docosanoic acid, a methyl ester with a common name Methyl behenoate is the least abundant compound with a peak of 1.097% and a retention time of 28.53.

Table 4.10: Compounds identified from VLC sub-fraction 2 of n-Hexane fraction of *Aframomum melegueta* (AMNHV2)

Peak No	Retention time	Compound name	Molecular formula
1	12.677	Caryophyllene	$C_{15}H_{24}$
2	15.39	Caryophyllene oxide	$C_{15}H_{24}O$
3	15.50	(Z,E)-α- Farnesene	$C_{15}H_{24}$
4	15.74	Isocamphene	$C_{10}H_{18}O$
5	15.90	Caryophyllene oxide	$C_{15}H_{24}O$
6	15.94	2,3-Dimethyl-1,4-pentadiene	C_7H_{12}
7	19.49	4-Isopropyl-trans-bicyclo[4.3.0]-2-nonen-8-one, (4R,S)-	$C_{12}H_{18}O$
8	19.90	Palmitic acid	$C_{17}H_{34}O_2$
9	22.93	Methyl 9- Otadecanoate	$C_{19}H_{36}O_2$
10	23.38	Methyl stearate	$C_{19}H_{38}O_2$
11	25.935	11- Eicosenoic acid	$C_{21}H_{40}O_2$
12	26.24	Methyl-18-methyl nonadecanoate	$C_{21}H_{42}O_2$
13	28.52	Methyl behenoate	$C_{23}H_{46}O_2$
14	28.85	Phthalic acid	$C_{24}H_{38}O_4$
15	28.97	Octyl palmitate	$C_{24}H_{48}O_2$
16	30.60	(Z)-Decyl icos-9-enoate	$C_{30}H_{58}O_2$
17	31.27	Squalene	$C_{30}H_{50}$

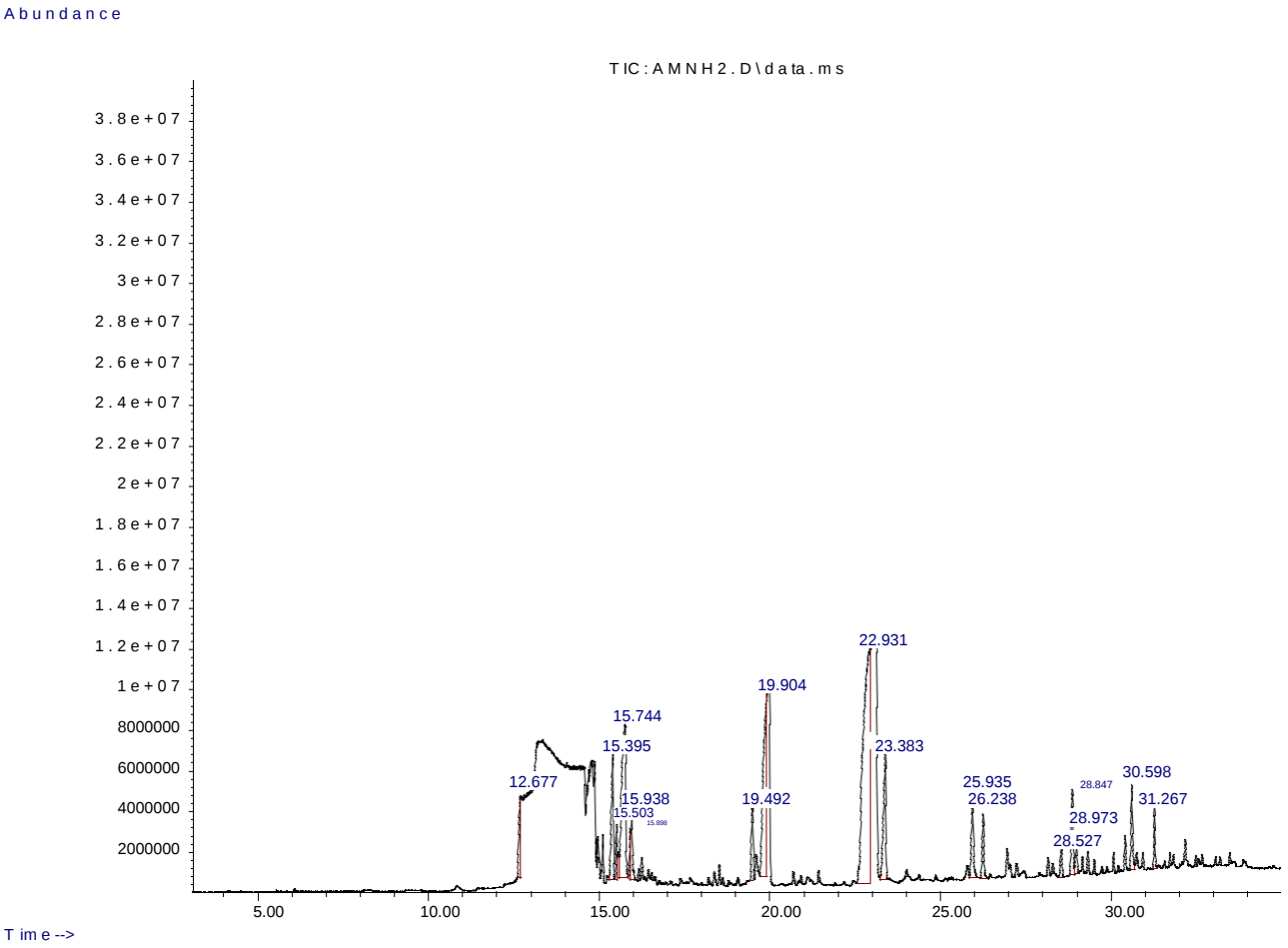


Figure: 4.1 Chromatogram of Compounds Present in VLC Sub-fraction 2

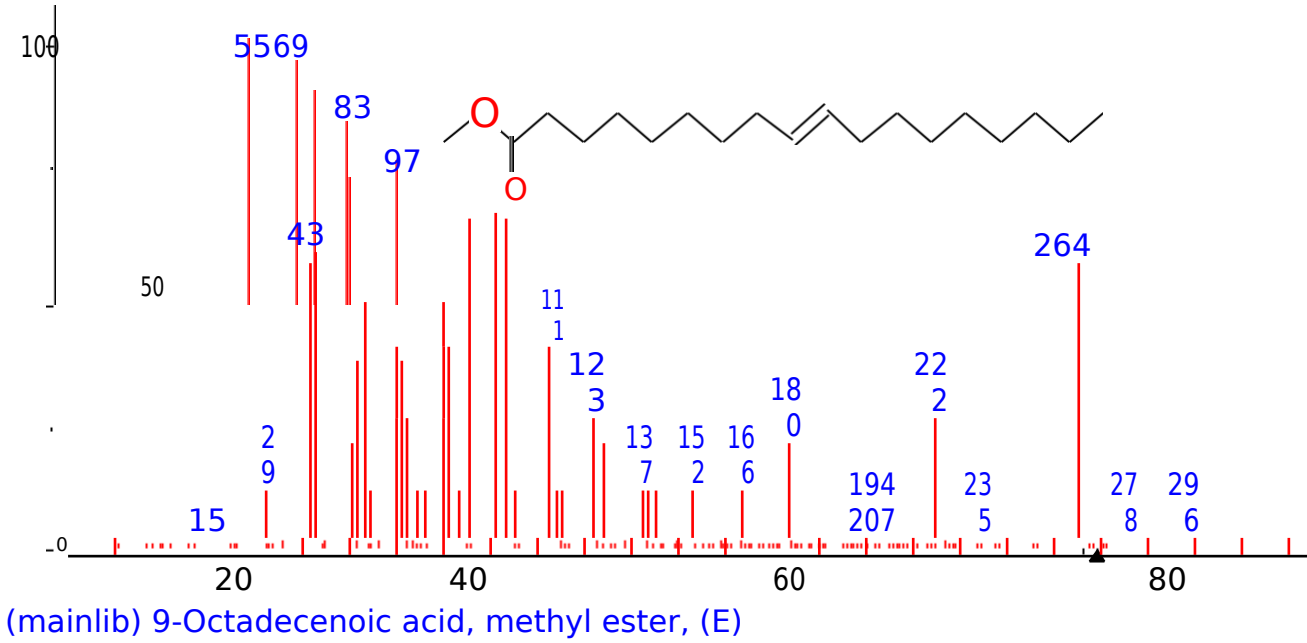


Figure 4.2: The Electron Ionization fragmentation of 9-Octadecenoic acid, methyl ester

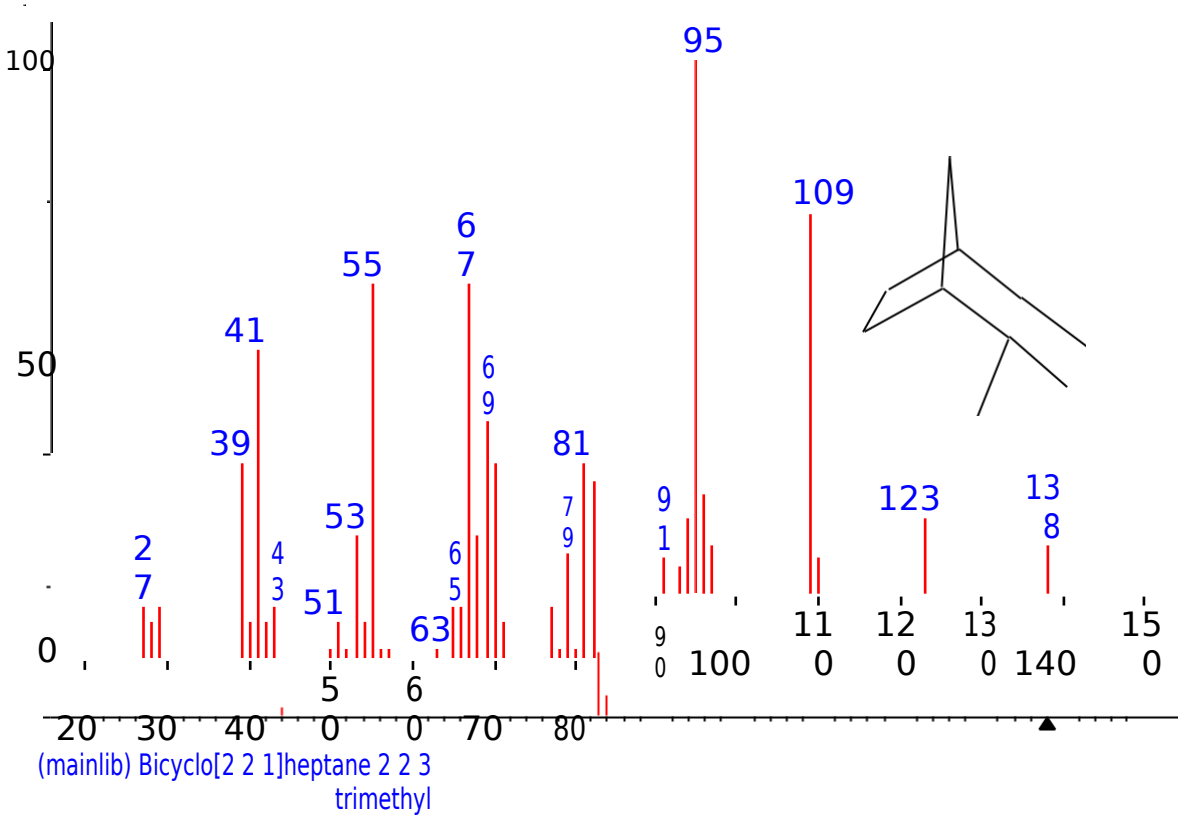


Figure 4.3: The Electron Ionization fragmentation of Bicyclo[2.2.1]heptane, 2,2,3-trimethyl-

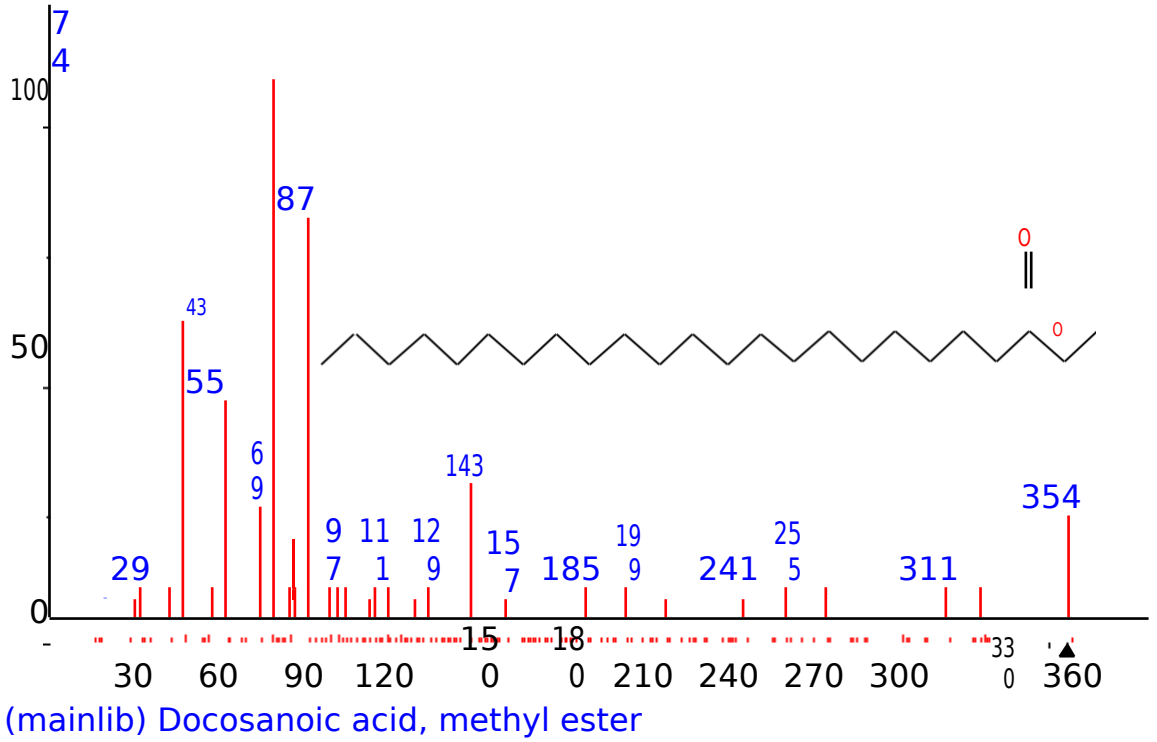


Figure 4.4: The Electron Ionization fragmentation of Docosanoic acid, methyl ester

4.11 The GC-MS analysis of VLC sub-fraction 3 of n-Hexane fraction of *Aframomum melegueta* (AMNHV3)

The results show the analysis of the GC-MS experiment on the sub-fraction 3 of *A. melegueta*, given in Figure 4.5 and Table 4.11. Twenty one compounds were detected in the *n*- hexane fraction of *A. melegueta* (AMNHV3**).**

Table 4.11: Compounds identified from VLC sub-fraction 3 of n-Hexane fraction of

Peak No	Retention time	Compound name	Molecular formula	Molecular weight	Area %
1	7.89	3-Carene	$C_{10}H_{16}$	136.23	0.46
.66 Humulene2-3	13.32		$C_{15}H_{24}$	204.35	4.04
4	15.09	Caryophyllene oxide	$C_{15}H_{24}O$	220.35	1.51
5	15.52	Isocamphane	$C_{10}H_{18}$	138.25	3.17
6	15.72	Caryophyllene oxide	$C_{15}H_{24}O$	220.35	0.09
7	15.78	7-(1,3-Dimethylbuta-1,3-dienyl)-1,6,6-trimethyl-3,8-dioxatricyclo[5.1.0.0(2,4)]octane	$C_{15}H_{22}O_2$	234.34	0.11
8- 10	16.21- 16.26	Zingeberone , Gingerone	$C_{11}H_{14}O_3$	194.23	5.59
11	18.19	7-Methyl-Z-8,10-dodecadienal	$C_{13}H_{22}O$	194.31	0.70
12-14	19.39- 19.57	Caffeine	$C_8H_{10}N_4O_2$	194.19	3.47
15	19.91	Palmitic acid	$C_{16}H_{32}O_2$	270.45	4.93

Peak No	Retention time	Compound name	Molecular formula	Molecular weight	Area %
16-17	21.31-21.39	n-Hexandecanoic acid	$C_{16}H_{32}O_2$	256.42	11.37
18	22.91	Oleic acid	$C_{19}H_{36}O_2$	296.49	12.95
19	23.23	3- Fluorophenyl isocyanate	C_7H_4FNO	137.11	1.42
20	23.32	2- Methoxyl-3-3 Isopropyl pyrazine	$C_8H_{12}N_2O$	152.19	3.52
21-28	24.2-25.12	Oleic acid	$C_{19}H_{36}O_2$	296.44	24.04
.66 [6]29--34Paradol	25.42		$C_{17}H_{26}O_3$	278.39	3.07
35-39	27.39-27.59	[6]- Shogaol	$C_{17}H_{24}O_3$	276.37	4.68
40-46	29.05-29.99	[8]- Paradol	$C_{19}H_{30}O_3$	306.44	25.39

47	30.39	[6]-Dehydro Gingerdione	$C_{17}H_{22}O_4$	290.35	2.44

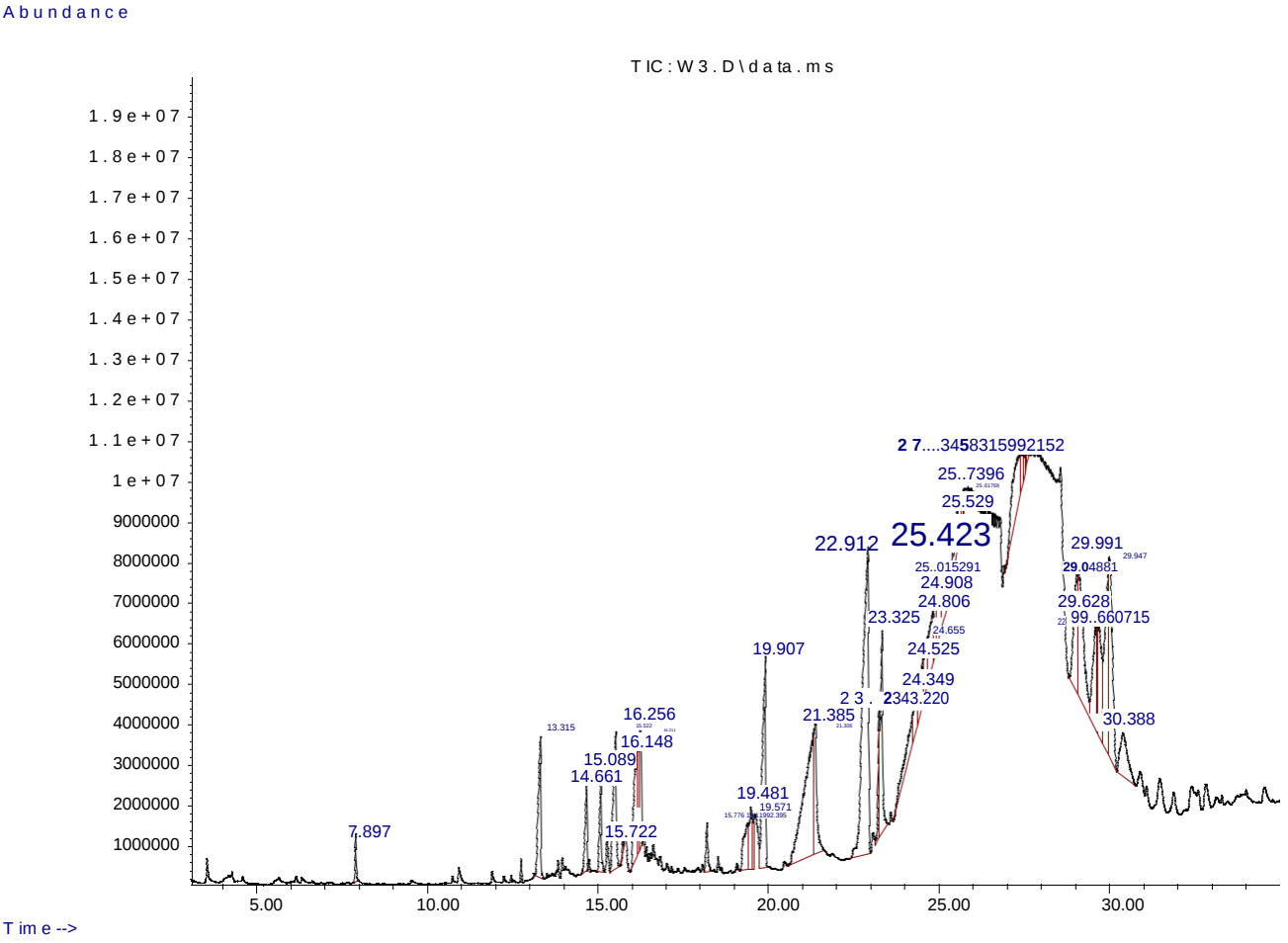


Figure 4.5: Chromatogram of compounds present in VLC sub-fraction 3

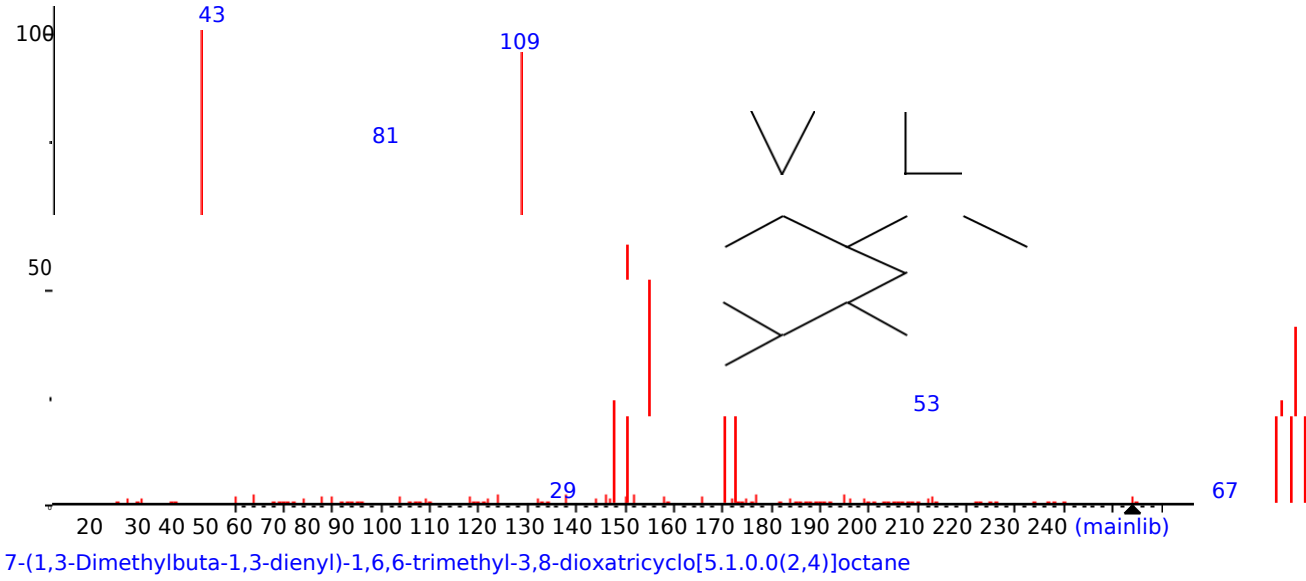


Figure 4.6: The Electron Ionization fragmentation of 7-(1,3-Dimethylbuta-1,3-dienyl)-1,6,6-trimethyl-3,8-dioxatricycleo[5.1.0.0(2.4)octane

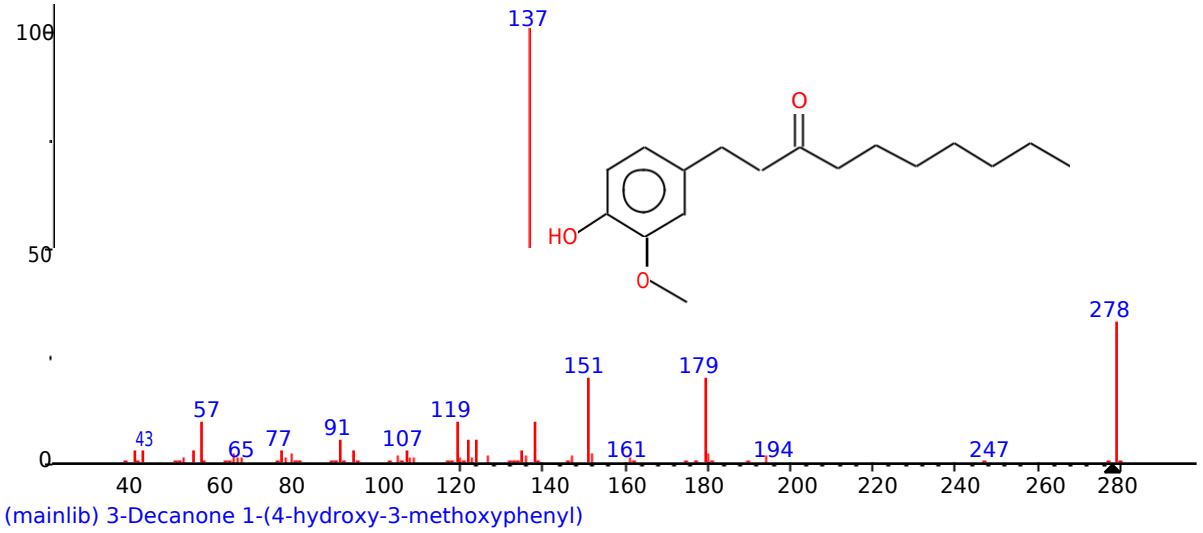


Figure 4.7: The Electron Ionization fragmentation of 3-Decanone, 1-(4-hydroxy-3-methoxyphenyl)-

4.12 Isolated compounds from *Aframomum melegueta*

4.12.1 Hexane fractions

4.12.1.1 AM-2A

This was isolated as a yellow syrup obtained from the HPLC. It has molecular ion peak at 276, thus giving the molecular weight. The structure was assigned as 6-Shogaol by comparison of the ^{1}H NMR (400 MHz) data with literature data (Shih *et al.*, 2014.).

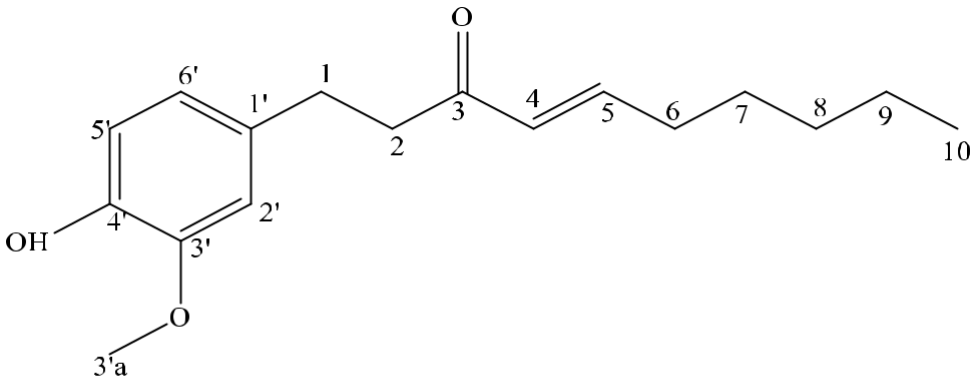
H^{+}-NMR (400 MHz) (Acet ne) δ ppm: 2.88 (H-1,-2); 6.11(H-4), 6.91-6.86 (H-5), 2.24-2.21(H-6), 1.50-1.45(H-7), 1.33-1.32(H-8,9), 0.90(H-10), 6.67-6.68 (H-2'), 6.91-6.86 (H- 5'), 6.73- 6.72(H- 6')

^{13}C-NMR data (100 MHz) (Acet ne) δ ppm: 31.2, 41.5, 198.6, 130.3, 146.9, 32.1, 27.7, 29.6, 22.2, 13.3, 132.9, 112.0, 147.3, 144.8, 114.7, 120.6, 55.3 ppm

EI-MS: 276 (M+, 43) 205(52), 151(16), 137 (100), 119 (10), 55(18)

Table 4.12: NMR Data of AM 2-A

Observed			Reported (Shih *et al.*, 2014.)	
H-No	^{1}H-NMR (400MHz,Acetone)	^{13}C-NMR (100MHz, Acetone)	^{1}H-NMR (400MHz,$CDCl_3$)	^{13}C-NMR (100MHz,$CDCl_3$)
1	2.88–2.79 (4H, m)	31.2	2.89–2.79 (4H, m)	31.3
2	2.88–2.79 (4H, m)	41.5	2.89–2.79 (4H, m)	41.9
3	-	198.6	-	199.8
4	6.11 (1H, d, J=17.1 Hz)	130.3	6.08 (1H, d, J = 16.0, 1.4 Hz)	130.2
5	6.91–6.86 (2H, m)	146.9	6.89–6.65 (4H, m)	147.8
6	2.24–2.21(q, 2H, *J*=21.78 Hz)	32.1	2.24–2.13 (2H, m)	32.4
7	1.50–1.45 (2H, m)	27.7	1.51–1.26 (2H, m)	27.1
8	1.33-1.32 (4H, m)	29.6	1.51–1.26 (4H, m)	29.8
9	1.33-1.32 (4H, m)	22.2	1.51–1.26 (6H, m)	22.4
10	0.90 (3H, t, *J*=12.96Hz)	13.3	0.88 (3H, t, J = 6.5 Hz)	13.9
1'	-	132.9	-	133.2
2 '	6.67 - 6.68 (1H, d, *J*=7.98Hz)	112.0	6.89–6.65 (4H, m)	111.0
3 '	-	147.3	-	146.3
4 '	-	144.8	-	143.8
5 '	6.91–6.86 (2H, m)	114.7	6.89–6.65 (4H, m)	114.2
6 '	6.73 – 6.72 (1H, d, *J*=7.68Hz)	120.6	6.89–6.65 (4H, m)	120.7
3 ' a	3.83 (3H, s)	55.3	3.86 (3H, s,)	55.8



(*E*

Figure 4.8: Elucidated Structure of AM 2-A

4.12.1.2 AM-2B

This compound was isolated as a colourless oil, with a molecular ion peak of the compound is 278 g/mol on the EI-MS fragmentation, thus the molecular weight.

The structure was assigned as 6-Paradol by comparing its ^{1}H NMR and ^{13}C-NMR (400MHz) data with literature data (Shih *et al.*, 2014.).

H^{+}-NMR (600 MHz) δ: 2.89 (H-1); 2.87 (H- 2); 2.42 (H- 4); 1.54 (H- 5), 1.28 (H6-9), 0.89(tH10); 6.73 (H-2'); 6.83 (H- 5'); 6.65 (H- 6'), 3.83 (s, -OCH_3, H-3a).

^{13}C-NMR (150 MHz) δ: 31.6, 42.2, 209.0, 44.1, 23.5, 29.0, 28.7, 29.2, 22.4, 13.4, 132.8, 111.9, 147.3, 144.7, 114.7, 120.5, and 55.3

EI-MS: 278(67), 179 (21), 151 (23), 137(100), 117(19), 99(23), 55(21).

Table 4.13: NMR Data of AM 2-B

Observed			Reported (Shih *et al.*, 2014.)	
H- No	^{1}H-NMR(600 MHz)	^{13}C-NMR(150 MHz)	^{1}H-NMR($CDCl_3$)	^{13}C-NMR ($CDCl_3$)
1	2.89-2.78 (4H, m)	31.6	2.86–2.63 (4H, m)	31.6
2	2.87-2.78 (4H, m)	42.2	2.86–2.63 (4H, m)	43.1
3	-	209.0	-	210.6
4	2.42(2H, t, *J*=14.52 Hz)	44.1	2.36 (2H, t, J = 7.4 Hz)	44.6
5	1.54 -1.51(2H, m)	23.5	1.58–1.51 (2H, m, H-5)	23.8
6	1.28 (8H, m)	29.0	1.24 (8H, m)	29.1
7	1.28 (8H, m)	28.7	1.24 (8H, m)	29.0
8	1. 28 (8H, m)	29.2	1.24 (8H, m)	29.5
9	1.28 (8H, m)	22.4	1.24 (8H, m)	22.5
10	0.89, (3H, t, *J*= 13.44 Hz)	13.4	0.88 (3H, t, J = 6.2 Hz)	14.0
1'	-	132.8	-	133.1
2'	6.73 (1H, d, *J*=7.98 Hz)	111.9	6.67 (1H, d, J = 1.8 Hz)	111.0
3'	-	147.3	-	146.3
4'	-	144.8	-	143.8
5'	6.83 (1H, s)	114.7	6.80 (1H, d, J = 8.0 Hz)	114.3
6'	6.65 (1H, d, *J*=7.98 Hz)	120.5	6.64 (1H, dd, J = 8.0, 1.8 Hz)	120.7
3'a	3.83 (3H, s)	55.3	3.85 (3H, s)	55.8

*NMR Solvent- Acetone

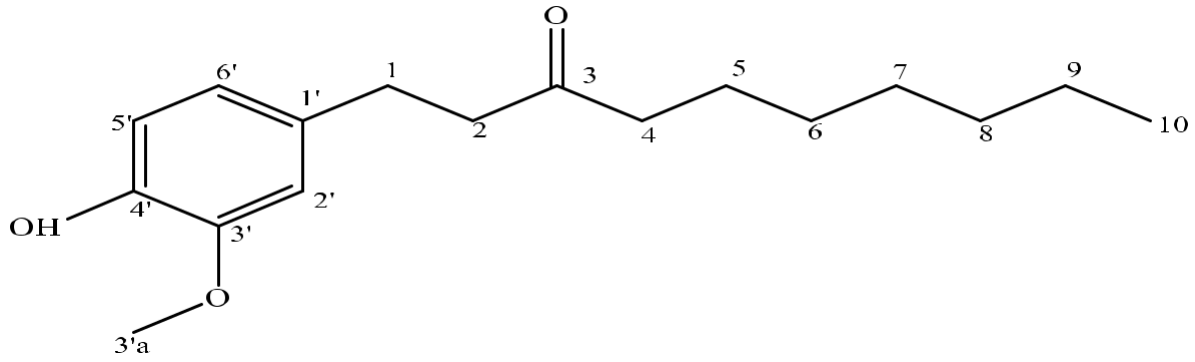


Figure 4.9: Elucidated Structure of AM 2-B

4.12.1.3 AM-2C

This is a yellow crystalline compound with molecular weight of 290g/mol. Electron Ionization -Mass Spectroscopy showed molecular peaks at 290.36 $[M]^{+}$, 219, 191, 177, 113, 86, 71. The structure was assigned as 1-Dehydro-6-gingerdione based on comparison of the data obtained from both NMR data with literature data (Charles *et al.*, 2000.).

^{1}H-NMR (600 MHz) (Acetone) δ: 7.54 (H-1), 6.32 (H-2), 5.63 (H-4), 2.39 (H-6), 1.65 (H-7), 1.34(H-8,9), 0.91(H-10), 7.02 (2'), 6.94(5'), 7.08(6'), 3.93(3a').

^{13}C-NMR (150 MHz) δ: 139.9, 1220.2, 177.5, 99.9, 199.6, 40.1, 31.6, 25.7, 22.6, 13.8, 127.1, 110.5, 148.8, 147.5, 115.5, 122.6, 55.7

Table 4.14: NMR Data of AM 2-C

Observed (Acetone d6)			Reported (Charles *et al.*, 2000.)	
H- No	^{1}H-NMR(600 MHz)	^{13}C-NMR (150MHz)	^{1}H-NMR(300MHz, $CDCl_3$)	^{13}C-NMR (75MHz, $CDCl_3$)
1	7.54, (1H, d, *J*=15.7Hz)	139.9	7.54, (1H, d, *J*=16.0Hz)	140.2
2	6.32, (1H, d, *J*= 15.8 Hz	120.2	6.32, (1H, d, *J*= 16.0 Hz	120.2
3	-	177.5	-	177.5
4	5.63 (2H, s)	99.9	5.62 (2H, s)	100.1
5	-	199.6	-	197.5
6	2.39 (2H, m)	40.1	2.37 (2H, m)	41.3
7	1.65 (2H, m)	31.6	1.65 (2H, m)	33.3
8	1.34(2H, m)	25.7	1.34(2H, m)	28.8
9	1.34(2H, m)	22.6	1.34(2H, m)	24.5
10	0.91(3H, t, *J*= 6.8 Hz)	13.8	0.91(3H, t, *J*= 6.8 Hz)	15.7
1'	-	127.1	-	126.1
2'	7.02 (1H, d, *J* =1.6 Hz)	110.5	7.01 (1H, d, *J* =1.5 Hz)	112.1
3'	-	148.8	-	149.5
4'	-	147.5	-	147.1
5'	6.94 (1H, d, J= 8.1 Hz)	115.5	6.93 (1H, d, J= 8.5 Hz)	115.3
6'	7.08 (1H, d, 8.3)	122.6	7.07 (1H, d, 8.5Hz)	122.3
3'a	3.93 (3H, s)	55.7	3.94 (3H, s)	56.8

*NMR Solvent- Acetone

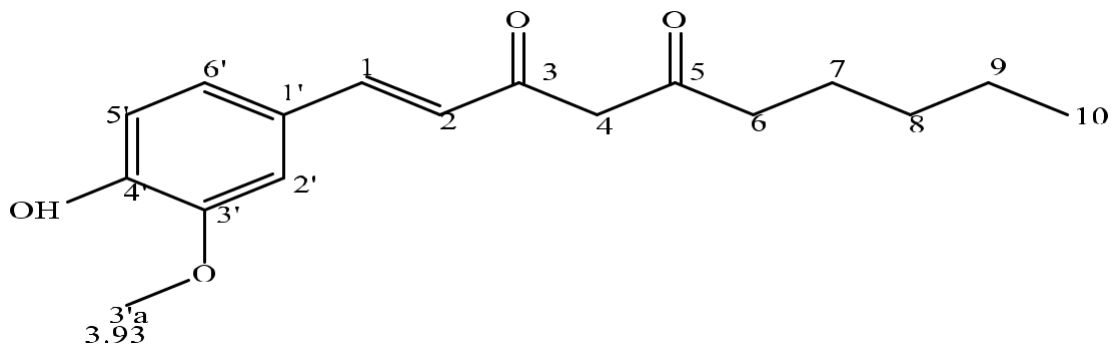


Figure 4.10: Elucidated Structure of AM 2-C

4.12.1.4 Cytotoxicity of Compounds Isolated from n-Hexane Fraction of *Aframomum Melegueta*

The isolated compounds, AM2-A, AM2-B and AM2-C were screened against cancer cell lines and normal cell line, Vero, AM-2A (6- Shogaol) showed lowest CC_{50}, of 0.11 on RD and 0.25µg/ mL, thus exhibiting the highest cytotoxicity amongst the three compounds. AM-2A showed better cytotoxicity on comparison to standard drug, cyclophosphamide used in for study. The Results showing cytotoxicity is as shown below.

Table 4.15 Cytotoxicity of Compounds Isolated from n-Hexane (*A. melegueta*)

Compound	RD	MCF-7	VERO	Selective Index
		IC_{50} (µg/mL)		
AM-2A	0.11±0.02	0.25±0.05	23.70±2.91	215.45, 95.00
AM-2B	9.40±0.45	0.68±.018	45.63±9.54	4.85, 67.10
AM-2C	26.72±0.21	46.39±0.13	4.74±1.05	0.18, 0.102
Cyclophosphamide	1.98±0.15	0.71± 0.7	23.92 ± 2.6	32.32, 33.69

4.12.2 Dichloromethane fraction

4.12.2.1 AmD 4-2

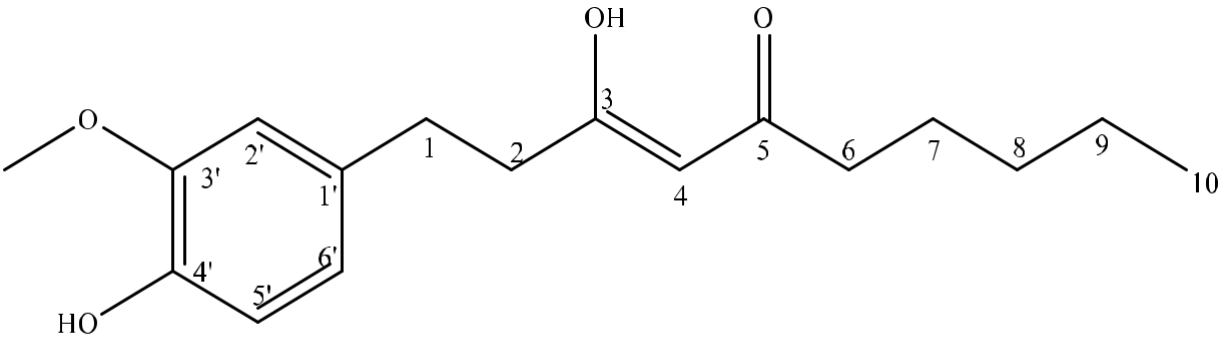
This is a colourless oil with molecular weight of 292.2 g/mol. Electron Ionization-Mass Spectroscopy peaks fragmentation showed a base peak of 278. Based on NMR data obtained for the compound and further comparison with literature (Groblacher *et al.*, 2012.), the name (Z)-3-hydroxy-1-(4-hydroxy-3-methoxyphenyl)dec-3-en-5-one and with a molecular formula: $C_{17}H_{24}O_4$ was given. The retardation factor was calculated for the compound 4-2 and the value obtained was 0.67.

^{1}H-NMR (400MHz) ($CDCl_3$):2.82 (H-1), 2.68 (H-2), 5.46 (H-4), 2.34 (H-6), 1.57(H-7), 1.26(H-8), 1.28(H-9), 0.85(H-10), 6.65(H-2'), 6.81(H-5'), 6.67(H-6').

EI-MS 292.2 (2.6), 278 (100), 179 (19.6), 151 (20), 137 (83.7), 119 (5.4) ,56 (46.7).

Table 4:16: NMR Data of AMD 4-2

Hydrogen number	$^{+}$H-NMR Observed(400MHz, $CDCl_3$)	$^{+}$H-NMR (600 MHz, $CDCl_3$) Reported (Groblacher *et al.*, 2012.)
1	2.82, t, J= 7.6Hz, 2H	2.86, t, J= 8.4Hz
2	2.68, t, J= 7.6Hz, 2H	2.56, t, J= 8.4Hz
3	-	-
4	5.46, s, 1H	5.54, s / 5.55, s
5	-	-
6	2.34, t, J= 7.6Hz, 2H	2.25, t, J=7.6Hz
7	1.57, m, 2H	1.59, m
8	1.26, m, 2H	1.30, m
9	1.28, m, 2H	1.31, m
10	0.85, t, J= 6.8Hz, 3H	0.89, t, J= 6.6Hz
1 '	-	-
2 '	6.65, (d, d J= 1.6Hz, 9.6Hz) 1H	6.69
5 '	6.81 (d, J= 8.0Hz) 1H	6.83
6 '	6.67 (d, J= 2Hz)	6.68
3 ' - OCH3	3.84 (s) 3H	3.86, s



(Z

Figure 4.11: Elucidated Structure of AMD 4-2

4.12.2.2 AmD 6-15-2

A colourless oil with molecular mass detected by electron ionization-mass spectra (EI/MS) as 294.3 g/mol. The retardation factor was calculated for the compound and the value obtained was 0.68. The structure was assigned as 6-Gingerol based on its ^{1}H NMR (400MHz) and literature data (Gan *et al.*, 2016; Agarwal *et al.*, 2001.).

Molecular formula $C_{17}H_{26}O_4$ was proposed for AmD 6-15-2 based on spectroscopic data and comparison with literature data. A melting point of 31^{o}C was obtained for 6-Gingerol.

^{1}H-NMR (400MHz,$CDCl_3$): 2.81(H-1), 2.69(H-2), 2.51(H-4a), 2.42(4b), 4.02(H-5), 1.36(H-6), 1.25(H-7-9), 0.86(H-1'),6.66(H-2'), 3.85(H-3'), 5.47(H-4'), 6.81(H-5'), 6.64(H-6')

EI/MS m/z 294 (33.3), 276 (7.4), 205 (7.5), 194 (41.0), 150 (45.9), and 137(100), 119 (14.4), 54 (10.5).

Table 4.17 NMR Data of AmD-6-15-2

H- No	^{1}H -NMR Observed (400MHz) ($CDCl_3$)	^{1}H -NMR Reported (Agarwal *et al.*, 2001.)
1	2.81 (2H, s, d, J=6.8Hz)	2.82-2.85 (2H, dd, J=6.8Hz)
2	2.69-2.71 (2H, d, t, J=1.2Hz, 7.2Hz)	2.72-2.75 (2H, dd, J=6.8, 6.9Hz)
3	-	-
4a	2.51-2.57 (m, 1H)	2.54-2.56 (1H, d, J=6.39Hz)
4b	2.42-2.49 (m, 1H)	2.50-2.53 (1H, d, J=9.93Hz)
5	4.02 (m, 1H)	4.02 (1H, m)
6	1.36-1.38 (m, 2H)	1.38 (2H, m)
7-9	1.25-1.35 (m, 6H)	1.27 (6H, m)
10	0.86 (t, 6.8Hz, 3H)	0.88 (3H, t, J= 6.64Hz)
2'	6.66 (s, 1H)	6.70 (1H, s)
3'	3.85 (3H, s, -OCH_3)	3.85 (3H, s, -OCH_3)
4'	5.47(1H, s)	5.69 (1H, s)
5'	6.81 (1H, d, J=8Hz)	6.81 (1H, d, J=8Hz)
6'	6.64 (dd, J=2Hz, 8Hz)	6.67 (dd, J=2Hz, 8Hz)

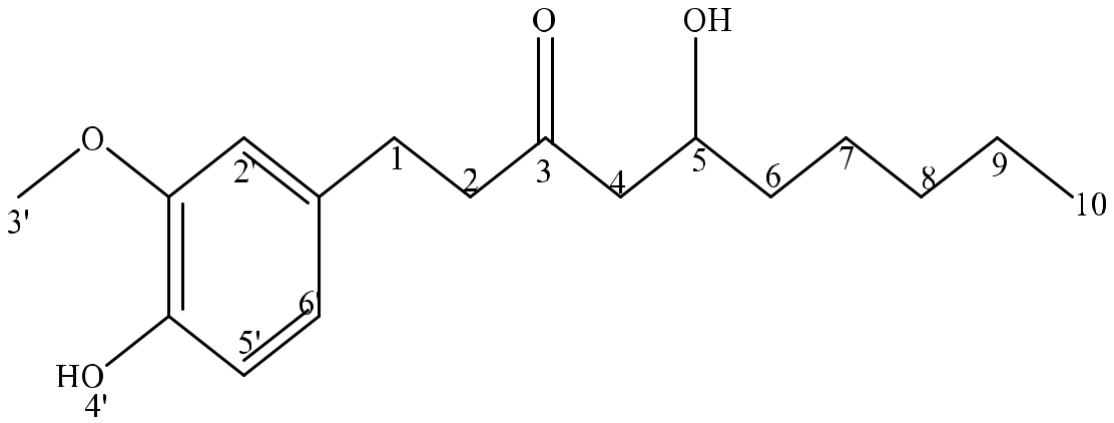


Figure 4.12: Elucidated Structure of AMD6-15-2

4.12.2.3 AmD-4-3

A yellow viscous oil with molecular mass detected by electron ionization-mass spectra (EI/MS) as 290.3g/mol. The retardation factor was calculated for the compound 4-3. The values obtained was 0.76. The structure was assigned as 1, 4- Decadien-3-one, 5-Hydroxyl-1-(4-hydroxyl-3-methoxyphenyl)-, (1E,4Z) based on the EI-MS, and ^{1}H and ^{13}C-NMR spectra obtained for the compound. The fragmentation pattern of the EI-MS showed a molecular ion peak at *m/z* 290.2, with major peaks at 216.1, 201, 191, 177 (100%: base peak), 137. According to Shih *et al*., 2014, a common name was given as [6]-Isodehydrogingerdione and it was obtained as a synthesized compound. The proton signal (1H, s) at 15.51ppm, observed in this compound is observed when it is run in deuterated chloroform and disappear by addition of Deuterated MeOH and or D_2O (oxide).

Molecular formula: $C_{17}H_{22}O_4$

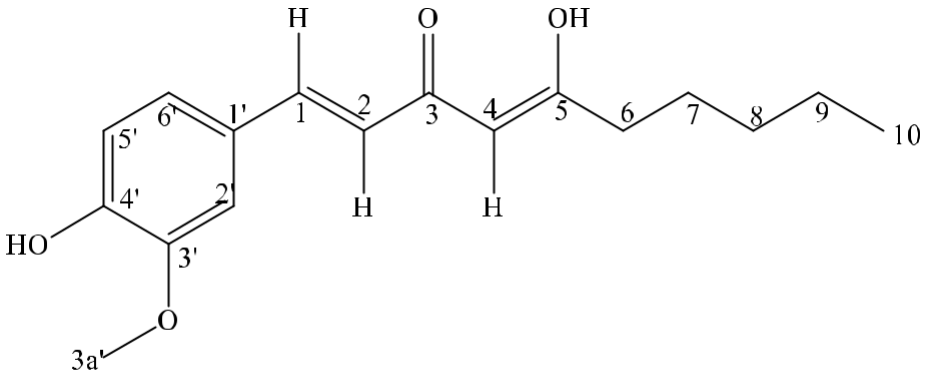
^{1}H-NMR (400MHz, $CDCl_3$): 7.52(H-1), 6.33(H-2), 5.60(H-4), 15.51(H-5), 2.37(H-6), 1.67(H-7), 1.32(H-8,9), 0.91(H-10), 7.01(H-2'), 3.92(3a'), 5.80(H-4'), 6.91(H-5'),7.08(H-6')

^{13}C-NMR (200MHz, $CDCl_3$): 139.8, 120.5, 178.0, 100.1,200.1, 40.1, 25.3, 31.4, 25.3, 331.4, 22.4, 13.9, 127.7, 109.4, 147.6, 56.0, 146.7, 114.7, 122.6.

EI-MS *m/z:* 290.2(72), 272(7), 216(82), 201(43), 191(97), 177(100), 145(51), 137(62),131(17), 89(2), 42(18).

Table 4.18 AmD 4-3

	Observed		Reported (Shih *et al.*, 2014.)	
H-No	$^{+}$H- NMR (400MHz, $CDCl_3$)	C-NMR (400MHz, $CDCl_3$)	^{1}H- NMR ($CDCl_3$)	^{13}C-NMR ($CDCl_3$)
1	7.52(d, J=16Hz, 1H)	139.8	7.51(d, J=15.8Hz, 1H)	139.8
2	6.33 (d, J=15.6Hz, 1H)	120.6	6.34 (d, J=15.8Hz, 1H)	120.5
3	-----	178.0	-----	178.0
4	5.60 (s, 1H)	100.1	5.61 (s, 1H)	100.1
5	15.51 (brs, 1H Enol proton)	200.1	-----	200.1
6	2.37 (t, 7.2Hz, 2H)	40.1	2.37 (t, 7.4Hz, 2H)	40.1
7	1.67 (m,2H)	25.3	1.69-1.57 (m,2H)	25.3
8	1.32 (m,2H)	31.5	1.35-1.28 (m,2H)	31.4
9	1.32 (m,2H)	22.4	1.35-1.28 (m,2H)	31.4
10	0.91 (m, 3H)	13.9	0.90 (t, J=6.2Hz 3H)	13.9
1'	-----	127.7	------	127.7
2'	7.01 (d, J=1.6Hz, 1H)	109.4	7.01 (d, J=1.8Hz, 1H)	109.4
3'	-----	147.6	-----	147.6
3a'	3.92 (s, 3H) OCH_3	56.0	3.94 (s, 3H) OCH_3	55.9
4'	5.8 (s, 1H)	146.7		146.7
5'	6.91 (d, J=8.4Hz, 1H)	114.7	6.90 (d, J=8.0Hz, 1H)	114.8
6'	7.08 (dd, J= 1.6Hz, 8.4Hz, 1H)	122.6	7.06 (dd, J= 1.8Hz, 8.0Hz, 1H)	122.6



(1*E*,4*Z*

Figure 4.13: Elucidated Structure of AMD4-3

4.12.2.4 Characterization of AmD8-14-1

Compound AmD8-14-1 was isolated as a pale-yellow oil with Rf of 0.46 and melting point of 41^{o}C. the structure of AmD8-14-1 was assigned as 4-(4-hydroxy-3-methoxyphenyl) butan-2-one with common name Zingerone (Agarwal *et al.*, 2001.).

H-NMR (400MHz, $CDCl_3$):2.82(H-1), 2.72(H-2), 2.11(H-4), 6.67(H-2'), 3.85(3''), 5.44(4'), 6.81(H-5'), 6.65(H-6')

^{13}C-NMR (100MHz, $CDCl_3$): 29.4, 45.5, 208.0, 30.1, 132.9, 111.0, 146.3, 55.8, 143.9, 114.3, 120.7.

EI-MS *m/z:* 194.1(52), 160(68), 151(14), 137(100), 119(12), 77(10), 43(9).

Molecular formula: $C_{11}H_{14}O_3$

Table 4.19 AmD8-14-1

	Observed		Reported (Agarwal *et al.*, 2001)	
H- No	^{1}H-NMR (400MHz, $CDCl_3$)	^{13}C-NMR (100MHz, $CDCl_3$)	^{1}H-NMR (400MHz, deu. $CDCl_3$)	^{13}C-NMR 100MHz, deu. $CDCl_3$)
1	2.82-2.79, t J=6.8Hz 2H	29.4	2.80-2.83 (t J=6.6Hz)	29.4
2	2.72-2.69, t J=6.8Hz 2H	45.5	2.70-2.72 (t J=6.6Hz)	45.5
3	--	208.0	--	208.4
4	2.11, s 3H	30.1	2.12, (s)	30.1
1'	--	132.9	--	132.8
2'	6.67 d, J=1.6Hz 1H	111.0	6.69 d (J=8Hz)	111.1
3'	--	146.3	--	146.4
3''	3.85, s 3H	55.8	3.84 s -OCH_3	55.8
4'	5.44, s 1H	143.9	5.76 (s)	143.9
5'	6.81 d, J=8Hz 1H	114.3	6.80 d (J=8Hz)	114.4
6'	6.65-6.63 dd, J=1.6, 8Hz 1H	120.7	6.64-6.66 (dd, J= 2, 8Hz)	120.7

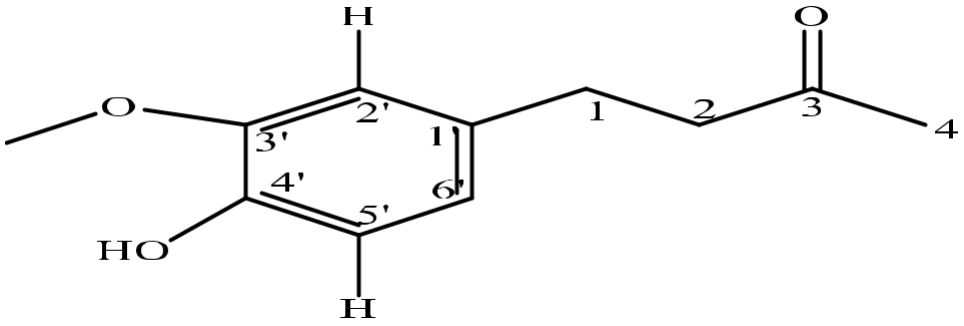


Figure 4.14: Elucidated Structure of AmD8-14-1

Table 4.20: Cytotoxicity of Isolated Compounds from DCM Fraction of *A. Melegueta*

Compound	AU565		3T3	
	% inhibition	CC50±SEM(µM)	% inhibition	CC50±SEM(µM)
AmD 4-2	47.86	Inactive	1.67	Inactive
AmD 4-3	100.00	14.90±0.7	43.8	Inactive
AmD 6-15-2	18.66	Inactive	6.39	Inactive
AmD 8-14-1	NA	NA	NA	NA

4.7 *Strophanthus hispidus*

4.7.1 Percentage yield of *S. hispidus*

The percentage yield obtained showed that the DCM fraction of *S. hispidus* had the highest yield while the ethyl acetate fraction was the lowest as shown in table 4.7.1.

Table 4.7.1 Percentage yield of *S. hispidus*

Extract/ Fraction	% yield
Methanolic (ShC)	6.77
Hexane (ShH)	0.07
Dichloromethane (ShD)	0.2
Ethyl Acetate (ShE)	0.03
Butanol (ShB)	0.06
Aqueous (ShAq)	25

4.7.2 Brine Shrimp Lethality Assay (BSLA)

The Methanolic extract of *S. hispidus* was found to be moderately cytotoxic while the DCM fraction was significantly toxic. The complete data is as shown in table 4.7.2.

Table 4.7.2 The BSLA of *S. hispidus*

Extract/ Fraction	% Mortality (1000µg/mL)	Cytotoxicity level
Methanolic (ShC)	40.00	Moderately cytotoxic
Hexane (ShH)	26.66	Not cytotoxic
Dichloromethane (ShD)	76.66	Significantly cytotoxic
Ethyl Acetate (ShE)	Insufficient	NA
Butanol (ShB)	NA	NA
Aqueous (ShAq)	46.66	Moderately cytotoxic
Etoposide*	46.66	Cytotoxic

*Standard drug for study

NA: No available data

4.7.3: **In-vitro cytotoxicity of *S. hispidus* on Cancer cell lines and Normal cell line**

The Methanolic extract was found to be cytotoxic across both cancer cell lines screened. The DCM fraction had the highest cytotoxicity and also selective to normal cell. The data is illustrated on table below.

Table 4.7.3: In-vitro cytotoxicity of *S. hispidus* on Cancer cell lines and Normal cell line

Sample	AU565	HeLa	BJ
	CC50 ± STD		
ShMethanolic	1.86 ± 0.01	0.32 ± 0.01	4.19 ± 0.10
ShH	10.72 ± 0.18	11.91 ± 1.25	14.26 ± 0.20
ShD	1.26 ± 0.06	0.21 ± 0.06	2.36 ± 1.10
ShE	8.13 ± 0.22	1.6 ± 0.02	4.23 ± 1.10
ShAq	18.55 ± 2.50	1.7 ± 0.5	3.1 ± 1.10
Doxorubicin*	0.54 ± 0.04	1.2 ± 0.01	2.84 ± 0.02

*Standard drug for the study

4.7.4 Selectivity of *S. hispidus* on Cancer cell against Normal cell line

The DCM fraction had the highest cytotoxicity and also selective to normal cell. The data is illustrated in table below.

Table 4.7.4: Selective index of *S. hispidus* on Normal Fibroblast (BJ) cell line against both cancer cell lines

Sample	HeLa	AU565
ShMethanolic	13.10	2.31
ShH	1.20	1.31
ShD	11.20	1.92
ShE	2.61	0.51
ShAq	1.80	0.20
Doxorubicin*	2.36	5.25

4.13 Compounds Isolated from *Strophanthus hispidus*

4.13.1 Dichloromethane fraction

4.13.1.1 ShD-58

The compound was isolated as a yellowish featherlike solid by direct elution from the column chromatography with the R_f.of 0.58 A mobile phase Hex: DCM 70: 30 was used for elution. This compound also had a melting point of 241.4^{0}C. ShD-58 had a molecular weight of 311.2g/mol based on data from EI-MS. The compound was identified according to the NMR data obtained and comparison with literature data as Pityriacitrin (Mayser *et al.*, 2002.).

^{1}H-NMR (400 MHz, DMSO):8.56 (H-3), 8.40(H-4), 8.31 (H- 5), 7.30 (H- 6), 7.60 (H-7), 7.83 (H-8), 9.24(H-2'), 8.55(4'), 7.30(H-5'-6'), 7.56(H-7'), 12.09(9-NH), 11.98(1'-NH).

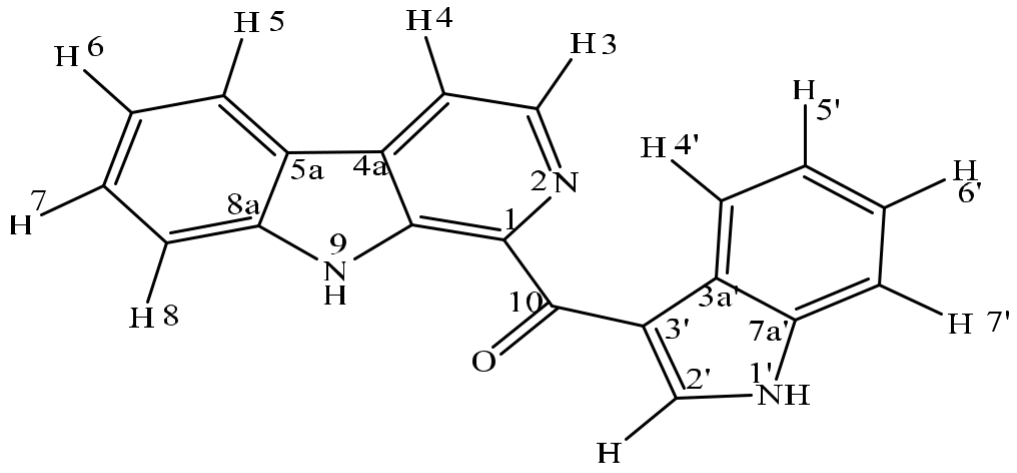
^{13}C-NMR (300 MHz, DMSO): 138.4, 135.9, 136.9, 117.9, 130.7, 122.8, 120.0, 119.8, 128.6, 113.0, 141.6, 187.3, 137.8, 114.2, 127.2, 122.0, 121.6, 121.7, 112.2, 135.0. EI-MS: 311.2 (71), 294 (100), 282 (43), 255 (3), 166 (9), 144 (27), 128 (4), 116 (12), 89 (8)

FTIR: 3411, 3223, 3151, 1594, 1555, 1441, 1233, 1137 and 737.2 cm^{-1}.

UV λmax : *220, 289, 310, and 391nm*

Table 4.8.1 NMR Data of ShD-58

	Observed		Reported (Mayser *et al.*, 2002.)	
Carbon no	^{1}H-NMR (400 MHz, DMSO)	^{13}C-NMR (300 MHz, DMSO)	^{1}H-NMR (600 MHz, CD_3CN)	^{13}C-NMR (150 MHz, CD_3CN)
1	-	138.4	-	139.4
1a	-	135.9	-	137.0
3	8.56 d, J= 4.80Hz	136.9	8.58 d, J= 4.80Hz	138.4
4	8.40 d, J= 4.80Hz	117.9	8.27 d, J= 4.80Hz	118.8
4a	-	130.7	-	132.1
5	8.31 d, J= 7.60Hz	122.8	8.26 d, J= 7.80Hz	122.6
5a	-	120.0	-	121.6
6	7.30, m	119.8	7.32, m	121.2
7	7.60, d, J= 7.90Hz	128.6	7.62, dd, J= 7.80, 7.80 Hz	129.9
8	7.83 d, J= 7.90Hz	113.0	7.77 d, J= 7.80Hz	113.4
8a	-	141.6	-	142.4
10	-	187.3	-	189.3
2'	9.24 s	137.8	9.40 d, J= 2.94Hz	138.6
3'	-	114.2	-	115.8
3a'	-	127.2	-	128.4
4'	8.55 d, J= 6.4Hz	122.0	8.60 d, J= 6.66Hz	123.1
5'	7.30, m	121.6	7.32, m	123.3
6'	7.30, m	121.7	7.32, m	124.2
7'	7.56, d J= 7.2Hz	112.2	7.57, dd J= 6.66, 1.86Hz	112.9
7a'	-	135.0	-	136.9
9 NH	12.09 br. S	-	10.96	-
1' NH	11.98 br. S	-	10.06	-



(1*H*-indol-3-yl)(9*H*-pyrido[3,4-*b*]indol-1-yl)methanone

Figure 4.15: Elucidated Structure of ShD-58

4.8.2 ShD-9

Compound ShD-9 was isolated by direct elution from the column (Hex: EtoAc 70:30) as a white amorphous powder, R_f 0.5 and with a mp of 267.4^{o}C. The compound was identified to be Ursolic acid with molecular formula $C_{30}H_{48}O_3$. Compound SHD9 formed a colourless needle when dissolved in methanol. Visible spot was confirmed with 20% sulphuric acid, H_2SO_4 and then heated to show bright pink colouration. A molecular ion peak at 456.2 in EI-MS which conforms to the molecular formula $C_{30}H_{48}O_3$ was observed. Compound ShD-9 has been previously isolated from *S. specious* (Simin, 2013.).

^{1}H-NMR (400 MHz, Pyridine): 3.47(H-3), 0.89(H-5), 1.65(H-9), 5.49(H-12), 0.89, 1.01, 1.01, 1.24, 1.02, 1.94(H-24-30)

^{13}C-NMR (75 MHz, Pyridine): 39.1, 28.1, 78.1, 39.2, 55.8, 18.8, 33.6, 39.9,48.1, 37.3, 23.6, 1225.6, 139.2, 42.5, 28.7, 24.9, 48.0, 53.6, 39.5, 39.4, 31.0, 37.4, 28.8, 16.5, 15.6, 17.5, 23.9, 179.8, 17.4, 20.8

EI-MS *m/z*: 456 (4.5), 438 (4.1), 410 (3.0), 300 (4.5), 248 (100), 235 (4.4), 219 (14.5), 203 (76.3), 189 (19.4), 133(40)

IR (KBr) *V* max cm^{-1}: 3446, 2930, 2869, 1660, 1586, 1462, 1363, 1101, 728.

UV λmax: 215, 229, 258, 260, 266 nm

Table 4.8.2 NMR Data of ShD-9

	Observed		Reported (Seebacher *et al.*, 2003.)	
C-No	^{1}H-NMR (400 MHz Pyr)	^{13}C-NMR (75 MHz Pyr)	^{1}H-NMR (600 MHz Pyr)	^{13}C-NMR (150 MHz Pyr)
1	α 0.94 (s) β 1.56 (s)	39.1	α 1.00 β 1.58	39.2
2	α 1.82 (s) β 1.82 (s)	28.1	α 1.00 β 1.58	28.2
3	α 3.47 (d, J=6.8Hz)	78.1	α 3.44 (dd)	78.2
4	---	39.2	---	39.6
5	α 0.89 (s)	55.8	α 0.88 (d)	55.9
6	α 1.56 (s) β 1.37 (s)	18.8	α 1.58 β 1.39	18.8
7	α 1.56 (s) β 1.37 (s)	33.6	α 1.58 β 1.39	33.7
8	---	39.9	---	40.1
9	α 1.65 (d, J=9.2Hz)	48.1	α 1.65	48.1
10	---	37.3	---	37.5
11	α 1.97 (d, J=4.0H z) β 1.97 (d, J=4.0H z)	23.6	α 1.96 β 1.96	23.7
12	α 5.49 (s)	125.6	α 5.49 (s)	125.7
13	---	139.2	---	139.3
14	---	42.5	---	42.6
15	α 1.28 (s) β 2.33 (dd, J=4.6Hz, 13.2Hz)	28.7	α 1.22 β 2.33	28.8
16	α 2.12 (dd, J=4.0, 13.2Hz) β 2.00 (s,)	24.9	α 2.14 β 2.01	25.0
17	---	48.0	---	48.1
18	β 2.63 (d, J=11.6Hz)	53.6	β 2.63 (d)	53.6
19	α 1.48 (s)	39.5	α 1.49	39.5
20	β 1.06 (s)	39.4	β 1.05	39.4

	Observed		Reported (Seebacher *et al.*, 2003.)	
C-No	^{1}H-NMR (400 MHz Pyr)	^{13}C-NMR (75 MHz Pyr)	^{1}H-NMR (600 MHz Pyr)	^{13}C-NMR (150 MHz Pyr)
21	α 1.53 (s) β 1.45 (s)	31.0	α 1.40 β 1.49	31.1
22	α 1.97 (d, J=4.0Hz) β 1.97 (d, J=4.0Hz)	37.4	α 1.97 β 1.97	37.4
23	α 1.24 (s)	28.8	α 1.24 (s)	28.8
24	0.89 (s)	16.5	β 1.02 (s)	16.5
25	β 1.01 (s)	15.6	β 0.92 (s)	15.7
26	β 1.01 (s)	17.5	β 1.06 (s)	17.5
27	α 1.24 (s)	23.9	α 1.24 (s)	24.0
28	---	179.8	---	179.7
29	β 1.02 (s)	17.4	β 1.02 (d)	17.5
30	α 1.94	20.8	α 0.97	21.4

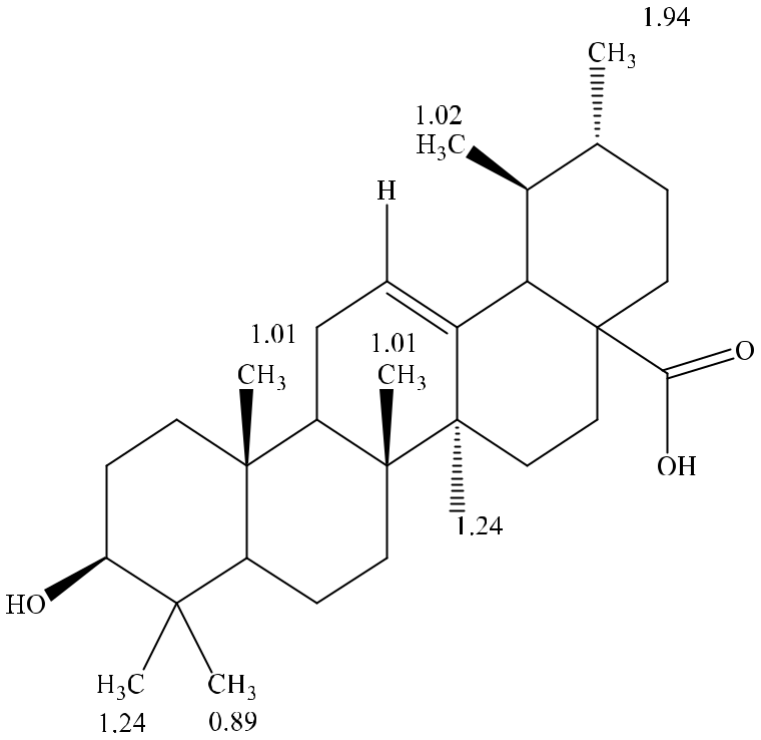


Figure 4.16: Elucidated Structure of ShD-9

4.8.3 ShD26-MF3

Compound ShD 26-MF3 was isolated by elution from a normal phase HPLC with a retention time of 15 minutes with mobile phase (DCM: MeOH 95:5) as a white crystalline solid, completely soluble in methanol and pyridine. It had a melting point of 248.9 ^{o}C. Molecular ion peak was observed at 488.2 in EI-MS corresponding to the molecular formula $C_{30}H_{48}O_5$.

^{1}H-NMR (600MHz, Pyridine): 4.29(H-2), 4.14(H-3), 2.01(H-5), 1.96(H-9), 5.46(H-12), 0.88, 1.01, 1.08, 1.16, 0.98, 0.93 (H-24-30) ppm

^{13}C-NMR (150MHz, Pyridine): 42.5, 67.2, 78.7, 48.5, 44.7, 18.9, 33.7, 42.3, 49.4, 39.1, 24.4, 126.7, 139.3, 40.2, 29.2, 25.3, 49.4, 54.4, 40.4, 40.4, 31.8, 38.1, 71.8, 17.9, 17.4, 17.7, 24.2, 181.7, 17.6, 21.6 ppm

EI-MS *m/z*: 488(2.5), 452 (3), 424 (4), 394 (3), 248 (100), 235 (16), 219 (29), 203 (93), 191 (40), 173 (56), 133 (79), 105 (23), 95 (19), 44 (29)

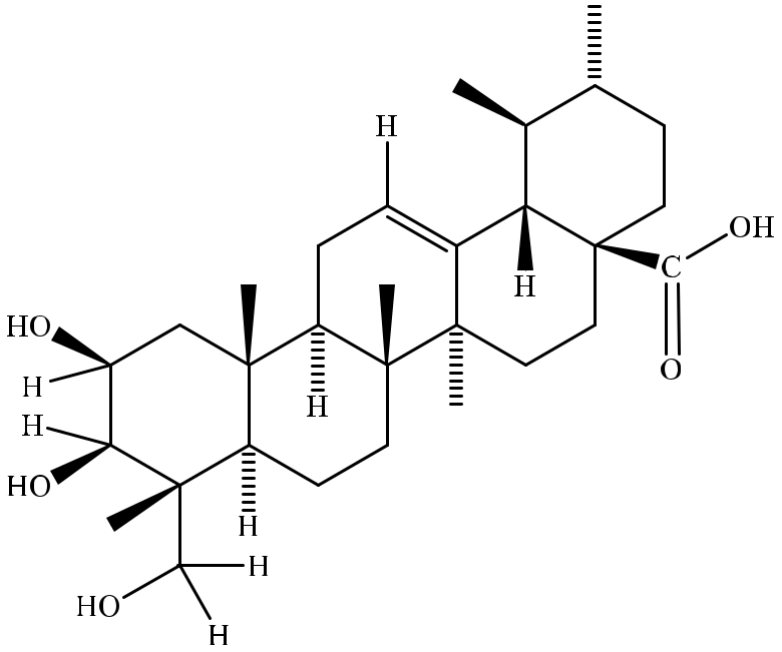
IR (KBr) *V* max cm^{-1} :3387, 2928, 1694, 1039,745,

667 UV λmax: 213, 229 nm

Table 4.8.3 NMR Data of ShD-26-MF3

Observed			Reported
H-No	^{1}H-NMR (600 MHz C_5D_5N)	^{13}C-NMR (600 MHz C_5D_5N)	^{1}H-NMR (500 MHz, C_5D_5N) (Khine, 2006; Kojima and Ogura, 1986.)
1	1.84, t, J=12Hz 1.96, m	42.5	1.82 1.94, m
2	4.29, m	67.2	4.89, m
3	4.11 (d, J=3.6Hz)	78.7	4.16 (d, J=2.3Hz)
4	-	48.5	-
5	2.01-2.07 (m)	44.7	2.02-2.08 (m)
6	1.46, 1.94 (m)	18.9	1.34, 1.60 (m)
7	2.09 (m), 1.75 (m)	33.7	1.34 1.72 (m)
8	-	42.3	-
9	1.96 (m)	49.4	1.94 (m)
10	-	39.1	-
11	1.96 2.10 (m)	24.4	1.96 2.08 (m)
12	5.46 (br, s)	126.7	5.48 (br, s)
13	-	139.3	-
14	-	40.2	-
15	1.62 (m), 2.34 (m)	29.2	1.14-2.36 (m)
16	1.99-2.06 (m)	25.3	1.98-2.06 (m)

H-No	^{1}H-NMR (600 MHz, C_5D_5N)	^{13}C-NMR(150 MHz, C_5D_5N)	^{1}H-NMR (500 MHz, C_5D_5N) (Khine, 2006; Kojima and Ogura, 1986.)
17	-	49.4	-
18	2.63 (br. d)	54.4	2.63 (br.d)
19	1.43 (m)	40.4	1.42 (m)
20	1.38 (m)	40.4	1.00 (m)
21	1.36 (m) 1.46 (m)	31.8	1.34 1.44 (m)
22	1.96 (m)	38.1	1.96 (m)
23	3.77, 3.93 (d, J=10.8Hz)	71.3	3.77, 3.94 (d, J=10.8 Hz)
24	0.88 (s)	17.9	0.87 (s)
25	1.01 (s)	17.4	1.00 (s)
26	1.08 (s)	17.7	1.07 (s)
27	1.16 (s)	24.2	1.14 (s)
28	-	181.7	-
29	0.98 (d, 6.6Hz)	17.6	0.97 (d, 6.4Hz)
30	0.93 (d, 6.6 Hz)	21.6	0.93 (d, 6.2Hz)



Urs-12-en-28-oic acid, 2, 3, 23-trihydroxy-, 2β,3β,4α

Figure 4.17: Elucidated Structure of ShD-26-MF3

4.8.4 ShD-21

Compound ShD-21 was obtained by direct elution from column as a white amorphous powder by mobile phase (EtoAc: MeOH 90:10) with Rf 0.5 and mp of 195^{o}C. NMR data obtained on comparison with literature data identified ShD-21 as Helveticoside (Nakamura *et al.*, 1998.).

^{1}H-NMR (400 MHz, Pyridine):4.36(H-3), 3.37 (H-17), 1.13 (H-18), 10.4(H-19), 6.06(H-24), 5.40(H-1'), 1.90, 2.32(H-2'), 4.38(H-3'), 3.60(H-4'), 4.25(H-5'), 1.55(H-6') ppm.

^{13}C-NMR (100 MHz, Pyridine): 24.9, 25.6, 74.9, 36.3, 74.1, 36.3, 74.1, 36.9, 21.7, 41.7, 39.5, 55.4, 18.6, 39.7, 49.1, 85.3, 30.8, 30.6, 48.6,18.3, 208.6, 174.1, 74.1, 116.7, 172.6, 97.8, 39.5, 68.5, 73.8, 70.5, 18.9 ppm

IR (KBr) *Vmax cm-*^{1}: 3499, 3427, 2939, 1810, 1735, 1422, 1164, 996, 672
UV λmax: 222, 230 nm

EI-MS: *m/z:* 386 $[M]^{+}$, 368 (4), 358 (32), 322 (15), 215 (14), 187 (24), 160 (56), 146 (20), 131 (28), 73 (28).

FABP-MS *m/z:* 535 $[MH]^{+}$, 405 $[MH-130]^{+}$, 387 $[MH-130-18]^{+}$

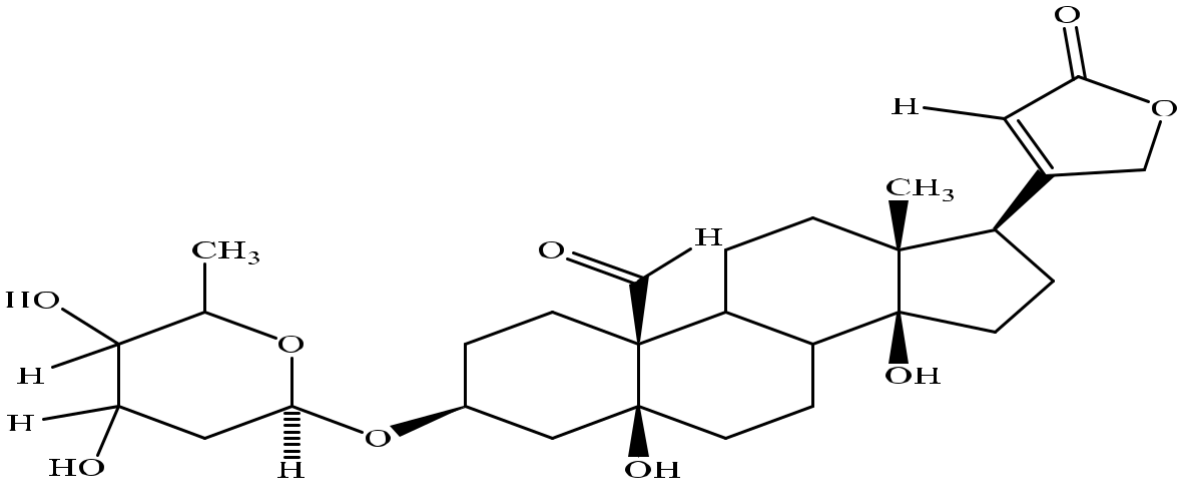
FABN-MS: *m/z:* 533 $[M-H]^{-}$, 403 [aglycone -H] $^{+}$
Molecular Formula: $C_{29}H_{42}O9$

Table 4.8.4 NMR Data of ShD-21

	Observed		Reported		
C-No	^{1}H-NMR (400 MHz, Pyr)	^{13}C-NMR (100 MHz, Pyr)	^{1}H-NMR (400 MHz, Pyr) (Sachdev-Gupta *et al.*, 1990.)	^{1}H-NMR (600 MHz, MeOH) (Nakamura *et al.*, 1998.)	^{13}C-NMR (150 MHz, MeOH) (Nakamura *et al.*, 1998.)
1	1.72, d, J=14.1Hz 2.05, m	24.9	--	--	25.1
2	2.20, m 1.72, d, J=14.1Hz	25.6	--	--	25.9
3	4.36	74.9	4.36 br.s	4.15 s	76.2
4	2.21, m 1.72, d, J=14.1Hz	36.3	--	--	36.7
5	3.58, m	74.1	--	--	75.3
6	1.79, s 2.32, m	36.9	--	--	37.1
7	1.32, m -1.60, dd, J=3.6, 13.6Hz	21.7	--	--	23.2
8	2.36, m	41.7	--	--	42.6
9	1.71, d, J=14.1Hz	39.5	--	--	40.4
10	--	55.4	--	--	56.1

C-No	^{1}H-NMR (400 MHz, Pyr)	^{13}C-NMR (100 MHz, Pyr)	^{1}H-NMR (400 MHz, Pyr) (Sachdev-Gupta *et al.*, 1990.)	^{1}H-NMR (600 MHz, MeOH) (Nakamura *et al.*, 1998.)	^{13}C-NMR (150 MHz, MeOH) (Nakamura *et al.*, 1998.)
11	1.94, q 2.60, m	18.6	--	--	18.9
12	1.70, d, J=14.1Hz 2.30, m	39.7	--	--	40.5
13	--	49.1	--	--	50.7
14	4.74, br s	85.3	--	--	85.9
15	1.05, q 2.07, m	30.8	--	--	32.4
16	1.81, m 2.07, m	30.6	--	--	27.9
17	3.37,t, J=9.6,18.4Hz	48.6	2.78, dd *J*=4Hz, 8Hz	2.82, m	51.7
18	1.13, s	18.3	0.98 s	0.84 s, 3H	16.1
19	10.4, s	208.6	10.41	10.04	210.0
20	--	174.1	--	--	178.1
21	4.90, d, J=2Hz 4.94, s	74.09	5.03 d J=18Hz 5.23 d J=18Hz	4.90 5.01	75.26
22	6.07 d, J=1.2Hz	116.7	6.12 s	5.89 s 1H	117.9

C-No	^{1}H-NMR (400 MHz, Pyr)	^{13}C-NMR (100 MHz, Pyr)	^{1}H-NMR (400 MHz, Pyr) (Sachdev-Gupta *et al.*, 1990.)	^{1}H-NMR (600 MHz, MeOH) (Nakamura *et al.*, 1998.)	^{13}C-NMR (150 MHz, MeOH) (Nakamura *et al.*, 1998.)
23	--	172.6	--	--	177.2
1'	5.4 J=16Hz, 9.6Hz	97.8	5.41 bd J=9.5Hz	4.93 dd, J=2.0Hz, 18Hz	98.3
2'	1.90, m 2.32, m	39.5	1.90 m 2.29 m	--	39.6
3'	4.38, m	68.5	4.39 m	4.01 m	69.0
4'	3.60, m	73.8	3.60 m	3.15 dd	74.2
5'	4.25 m	70.5	4.27 m	3.75 dq	70.9
6'	1.55 d, J= 6Hz	18.9	1.56 d J=7Hz	1.23 d J=6.5Hz	18.5



Strophanthidin- Digitoxoside (Helveticoside)

Figure 4.18: Elucidated Structure of ShD-21

4.8.2 Hexane fraction

SHH-32

Compound SHH-32 was obtained by direct elution from the column with mobile phase (EtOAc) as a viscous substance. With the NMR data analysis obtained from spectra, compared, it was identified as Dauc ster l (β-sitosterol-β-D-glycoside) with a melting point of 292 ^{o}C.

^{1}H-NMR (400MHz, Pyridine) ppm: 5.34(H-6), 0.66 (H- 18), 0.94 (H-19), 0.98 (H-21), 0.88(H-26, 27), 0.87((H-29), 5.05(H-1$^{'}$)

^{13}C-NMR (100MHz, Pyridine) ppm: 37.5, 29.3, 78.1, 39.8, 140.9, 121.9, 32.2, 32.1, 50.4, 36.9, 21.3, 39.9, 42.5, 56.8, 24.5, 28.5, 56.1,12.0, 19.2, 36.4, 19.2, 34.2, 29.3, 50.4, 29.5, 19.0, 19.9, 23.4, 12.0, 102.6, 75.3, 78.6, 71.7, 78.5, 62.9

EI-MS *m/z* = 412

FAB MS 577 $(M+ H)^{+}$

IR (KBr) V_{max} cm^{-1}: 3272, 2936, 2362, 1660, 1586, 1462, 1363, 1101, 728

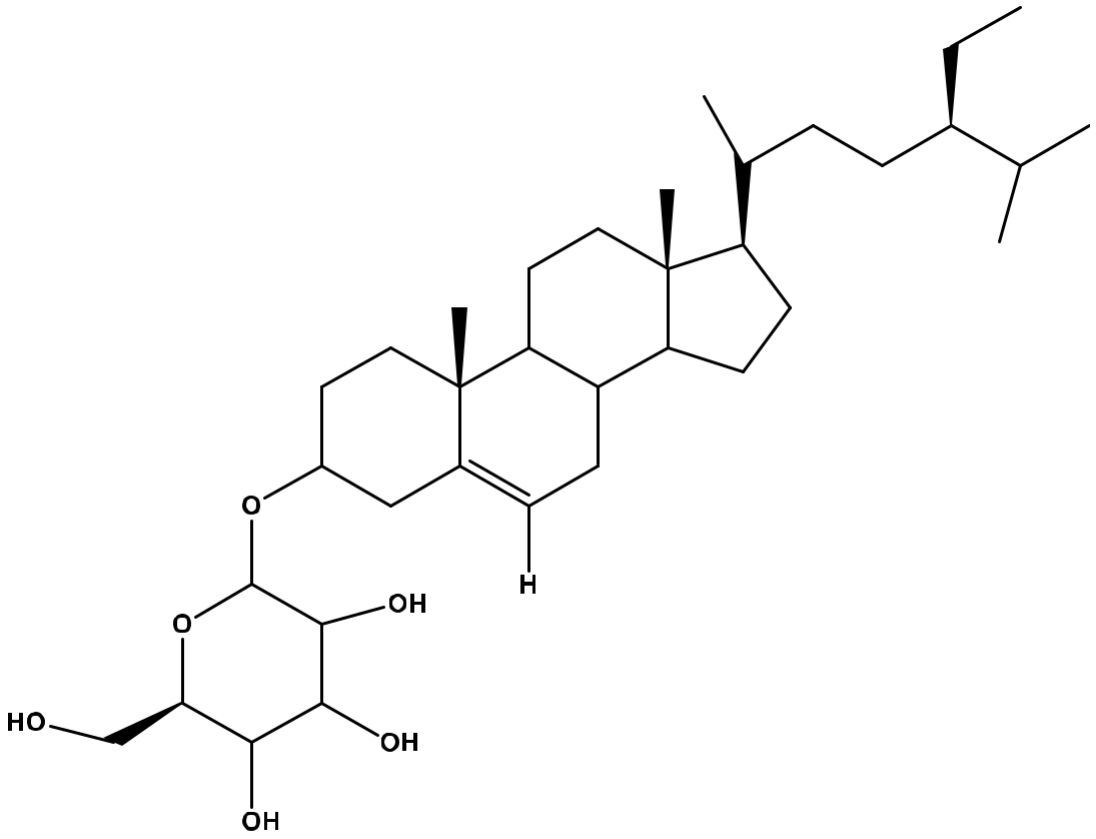
UV: Data not available due to insolubility of compound in Methanol.

Table 4.8.2.1: NMR Data of ShH-32

Observed			Reported (Lee *et al.*, 2013.)	
C- No	^{1}H-NMR (400 MHz, Pyridine)	^{13}C-NMR (100 MHz, Pyridine)	^{1}H-NMR (300 MHz, Pyridine)	^{13}C-NMR (75 MHz, Pyridine)
1	1.12, m 1.57, m	37.5	-	37.3
2	1.07, d *J*=6.8Hz	29.3	-	29.4
3	3.95, m	78.1	-	78.5
4	1.91 d, *J*=13.2Hz 1.98, d, *J*=13.2Hz	39.8	-	38.4
5	-	140.9	-	141.3
6	5.34, d, *J*=3.6Hz	121.9	5.35 (m)	122.3
7	1.27, d, *J*=6.0Hz 1.89, m	32.2	-	31.8
8	1.57, m	32.1	-	30.6
9	1.30, m	50.4	-	50.7
10	-	36.9	-	36.8
11	1.42, m	21.3	-	20.4
12	2.74, dd, *J*=2.4, 13.2Hz 2.49, m	39.9	-	40.3
13	-	42.5	-	42.7
14	1.12, d, *J*=10Hz	56.8	-	56.9
15	1.57, m	24.5	-	23.8
16	1.74, m	28.5	-	26.7
17	1.07, d, *J*=6.8Hz	56.1	-	56.6

C- No	^{1}H-NMR(400 MHz,	^{13}C-NMR(100	^{1}H-NMR(300	^{13}C-NMR

	Pyridine)	MHz, Pyridine)	MHz, Pyridine)	(75 MHz, Pyridine)
18	0.66, s	12.0	0.67 (s)	12.4
19	0.92, s	19.2	0.94 (s)	19.6
20	1.42, m	36.4	-	34.5
21	0.98, d *J*=6.4Hz	19.2	1.00 (d *J=6.3*)	19.4
22	1.42, m	34.2	-	32.6
23	1.25, m	29.3	-	24.9
24	0.91, t, *J*=3.6Hz	50.4	-	46.4
25	1.74, m	29.5	-	28.9
26	0.88, d, *J*=1.6Hz	19.0	0.94 (d, *J*=5.4)	19.8
27	0.88, d, *J*=1.6Hz	19.9	0.89 (d, *J*=6.3)	20.1
28	1.25, m	23.4	-	21.7
29	0.87, t, *J*=1.6Hz	12.0	0.87 (m)	12.5
1'	5.05, d, *J*=7.6Hz	102.6	5.09 (*J*=6.9)	103.0
2'	4.05, t, *J*=8.0Hz	75.3	-	75.8
3'	3.95, m	78.6	-	79.0
4'	4.31, m	71.7	-	72.1
5'	4.31, m	78.5	-	78.9
6'	4.42, dd, *J*=5.2Hz, 11.6Hz 4.57, dd, *J*=2.4Hz, 12.0Hz	62.9	-	63.2



Beta-Sitosterol glucoside (Daucosterol)

Figure 4.19: Elucidated Structure of ShH-32

4.8.3 ShH 42-4

SHH-42-2 was isolated from normal phase HPLC with mobile phase Hex: EtoAc 70:30+ 1% Acetic acid. It had a retention time of 62 minutes. Compound SHH-42-2 is a yellow solid with Rf of 0.5 and melting point of 128 ^{o}C

Compound showed a molecular weight of 402.2g/mol based on the molecular ion peak obtained by EI-MS. Based on NMR data obtained, the compound was elucidated and confirmed to be novel. Elucidation of compound with the spectra data conformed to the molecular formula $C_{21}H_{22}O_8$.

^{1}HNMR (400MHz, $CDCl_3$): 3.85 (H-3), 12.59 (H-5), 6.48 (H-6), 3.94 (H-7), 3.90 (H-8), 7.67 (H-2'), 3.94 (H-3"), 6.97 (H- 5'), 7.70 (H- 6'), 4.21(H-1"), 1.50 (H-2")

^{13}C-NMR (150MHz, $CDCl_3$): 155.9, 60.2, 138.8, 178.8, 152.7, 90.3, 56.1, 158.7, 90.3, 56.1, 158.7, 60.8, 132.2, 132.3, 106.6, 122.7, 111.5, 148.9, 56.3, 150.8, 111.8, 122.1, 64.4, 14.6

IR (KBr) V_{max} cm^{-1}: 3727, 3624, 2932, 1654, 1464, 1559, 1326, 1217, 805, 670

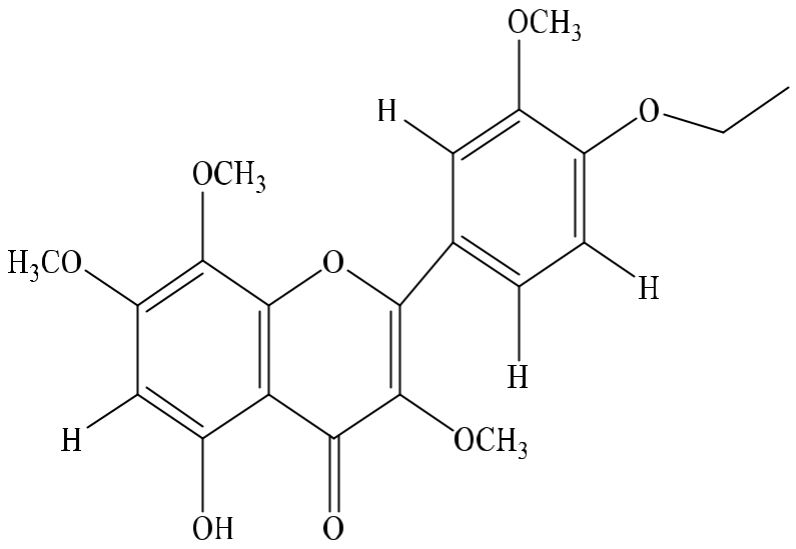
UV (MeOH) λmax: 232, 257, 270, 347nm

EI-MS *m/z:* 402.2 (M+) (100), 387 (91), 383 (31), 369 (22), 313 (8), 187 (7), 173 (18), 97 (8) 83 (12)

Table 4.8.3: NMR Data of ShH42-4

H-no	^{1}H-NMR(400MHz, $CDCl_3$)	^{13}C-NMR/ HSQC(150MHz, $CDCl_3$)	HMBC	COSY	Observed (Brito, 2018.) ^{1}H-NMR (300MHz, $CDCl_3$)	^{13}C-NMR (75MHz, $CDCl_3$)
1	--	--	--	--	--	--
2	--	155.9	--	--	--	158.4
3	3.85, s OCH_3	60.2	138.8		3.88	138.6
	--	138.8				
4	--	178.8	--		--	179.01
5	12.59, s	152.7	178.8, 155.9, 132.2, 106.6		12.5	157.3
6	6.48, s	90.3	178.8, 152.3, 132.2, 106.6		6.42	95.4
7	3.94, s OCH_3	56.1	158.7		3.94	56.0
	--	158.7				155.8
8	3.90, s	60.8	132.2		3.92	61.6
	--	132.2				128.7
9	--	132.3	--		--	148.5
10	--	106.6	--		--	105.3
1'	--	122.7	--		--	122.9

2'	7.67, dd, J=2.0Hz, 3.6Hz	111.5	150.8, 148.9, 122.1		7.80	110.8
3'	--	148.9	148.9		--	148.4
3''	3.94, s OCH_3	56.3				56.4
4'	--	150.8	--		6.09	146.4
5'	6.97, d, J=8.4Hz	111.8	150.8, 148.9, 122.7, 111.5	7.70	7.07	114.8
6'	7.70, d, J=2.0Hz	122.1	150.8, 111.5	6.97	7.79	122.6
1''	4.21, q, OCH_2	64.4	150.8, 14.6	1.50	--	--
2''	1.50, t, J=7.2Hz	14.6	64.4	4.21	--	--



H-chromen-4-one

Figure 4.20: Elucidated Structure of ShH-42-4

4.8.4 ShH -14

Compound ShH-14 was isolated by direct elution from column with a mobile phase of Hex: EtoAc 80:20. It was obtained as a white powder with R_f of 0.5

ShH-14 had a molecular weight of 412.2 g/mol based on the molecular ion peak with a base peak of 255 shown on the EI-MS fragmentation. NMR data obtained was elucidated and compared with existing data and identified as Stigmasterol with a molecular formula of $C_{29}H_{48}O$.

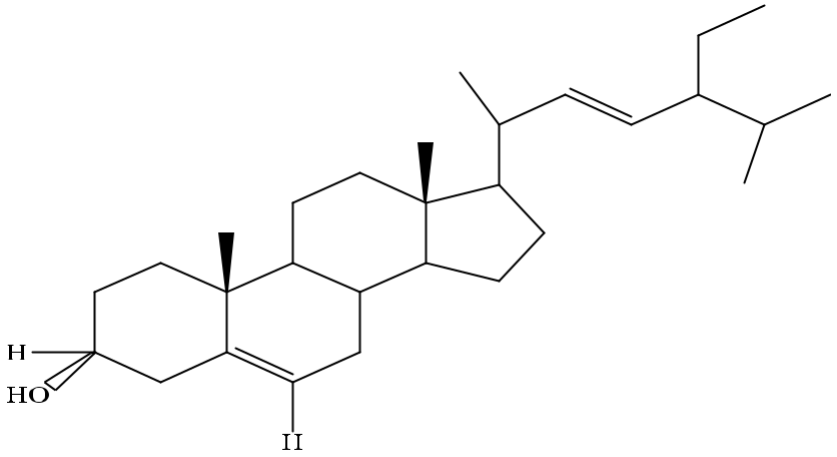
^{1}HNMR (500MHz, $CDCl_3$): 3.50 (H-3), 5.32 (H-6), 0.68 (H-18), 0.79 (H-19), 0.91 (H-21), 4.99 (H-22), 5.14 (H-23), 0.84 (H-26), 0.86 (H-27), 1.14 (H-28), 0.83 (H-29)

EI-MS: *m/z:* 412.2 (M^+) , 396 (36), 351(37), 300 (45), 271 (60), 255 (100), 213 (59), 159 (69), 133 54(), 107 (61), 81 (60), 55 (70)

Table 4.8.4: NMR Data of ShH-14

H-No	Observed (500MHz,$CDCl_3$)	Reported (Chaturvedula and Prakash, 2012.) (600MHz, $CDCl_3$)
1	1.46, m	
2	1.53, m	
3	3.50 (t,dd,J= 5.0, 4.5, 3.6 3.51 t,dd, 1H, J=4.5, 4.2, 3.8 Hz Hz)	
4	2.29, m	
6	5.32 (d,1H, 4.0 Hz)	5.31 t, 1H, J=6.1 Hz
7	2.03, m	
8	1.68, m	
9	1.53, m	
10	1.52, m	
11	1.51, m	
12	1.49, m	
13	1.58, m	
14	1.84, s, d,J= 3.5Hz	
15	1.45, m	
16	0.68 Overlapping Doublet	0.71 s, 3H
17	0.99, 3H, s	0.71 s, 3H
18	1.62, m	
19	0.91, d, 3H, J=6.5 Hz	0.91 d, 3H, J= 6.2Hz
20	4.99, dd, J=8.5 Hz	4.98 m, 1H

21	5.14, dd, J= 8.5Hz, 8.5 Hz, 1H	5.14 m, 1H
22	---	
23	---	
24	1.23, m	0.83 (t,3H, J=7.1Hz)
25	1.57, m	
26	0.84 (Overlapping, d)	0.82 d, 3H, J=6.6 Hz
27	0.86 d, 3H, J= 6.5Hz	0.80 d, 3H, J=6.6 Hz
28	1.14, m	
29	0.83 t, 3H, J=4.0 Hz	0.83 t, 3H, J= 7.1Hz



Stigmasterol

Figure 4.21 Elucidated structure of ShH-14

Table 4.8.5 Cytotoxicity of Isolated compounds on AU565, Breast Cancer cell line

Compound code	% Inhibition	IC50±SEM (μM)
SHD-9	88.82	8.07±0.02
ShD-21	81.88	11.42±0.60
SHD26MF3	99.50	18.27±1.73
SHH 32	N/A	N/A
Doxorubicin*	98.77	0.80±0.004

N/A: Not available

Table 4.8.6 Cytotoxicity of Isolated Compounds on 3T3, Normal cell line

Compound code	% Inhibition	IC50±SEM
SHD-9	7.75	INACTIVE
ShD-21	5.90	INACTIVE
SHD26MF3	0.50	INACTIVE
SHH 32	2.1	INACTIVE
Cyclohexamide*	89.19	0.80±0.1

CHAPTER FIVE

5.0 DISCUSSION AND CONCLUSION

The use of medicinal plants which are known to have bioactive compounds like alkaloids, tannins, terpenes, saponins and flavonoids has achieved a notable progress in the society today (Gordana, 2004.). The plants with medicinal importance may also help delay the development of cancer and the disease complications due to their rich source of antioxidants which help in the prevention of different diseases in humans. This study revealed the plants used for treatment of cancer by the people of Akinyele Local Government area of Oyo state. The most common plants mentioned for treatment was then selected for further study in relation to the cytotoxicity activity. The use of *Aframomum melegueta* and S*trophanthus hispidus* were investigated to validate the claims by the Traditional medical practitioners in the possible treatment and management of cancer, also to isolate and characterize the bioactive components responsible for the activity of the plants.

Ethnobotanical survey

The results obtained from the study revealed the age range of the respondents to be between 50 to 70 years. The respondents included healers (44.4%), herb sellers (33.3%), hunters and elders (22.2%). Most of the respondents were male (67%), consisting of the herb sellers and the TMPs. The study also showed that most of them were not educated. The data collected showed that the respondents used the medicinal plants for treatment of diseases due to the belief in the effectiveness of plants in treatment and management of the disease. Furthermore, most patients resort to the use of traditional herbs due to the high cost of conventional drugs used for the treatment of cancer.

The results obtained from types of plants used in management of cancer in Akinyele LGA, Oyo state included 26 plants from 18 families shown in Table 1.

Brine shrimp lethality assay (BSLA)

The level of toxicity of plant extract towards the *Artemia salina* napulii, was carried out using the Brine Shrimp Lethality (BSL) assay (Meyer *et al.*, 1982.). Table 4.3 shows the results of the BSLA after exposure of the Artemia *salina* nauplii to the different samples. Cyclophosphamide (CTX) and 6% DMSO served as the positive and negative controls respectively. Toxicity is classified based on values obtained: extracts with LD_{50} >1000 µg/mL are classified as non-cytotoxic, LD_{50} between 500 to 1000 µg/mL is classified as weakly cytotoxic, LD_{50} of 100 to 500 µg/mL as moderately cytotoxic while 0 to 100 µg/mL is considered strongly cytotoxic. The result showed that all the fractions were toxic except DCM fraction were cytotoxic. The n-Hexane fraction showed the highest LC_{50} value of 2.27 µg/mL while ethyl acetate and DCM fractions showed 4.12 and 2531.83 µg/mL, respectively. The n-hexane and the ethyl acetate fractions were significantly cytotoxic when compared to the positive control, cyclophosphamide with LC_{50} of 13.6 µg/mL.

Brine shrimp lethality assay does not give the mechanism of action by which bioactive molecules present in plants extract inhibit the growth of cancerous cells but it is a bench top assay which is accessible for basic screening of the plants before isolation process begins. Studies have shown that plants which are toxic to this species of shrimps, are most likely going to be best choice for drug development (Ramachandran *et al.*, 2011.). One of the desirable criteria for a chemotherapeutic drug is to have little or no side-effects on the normal cells of the individual being treated with the drug. To achieve this, lower doses of drugs are often utilized. Its selectivity to normal cells is indicative of appropriateness of a drug for further investigation.

The present study demonstrates the ability of the fraction of the methanolic extract of *A. melegueta* and *S. hispidus* for high selective toxicity at lower concentrations.

Cytotoxicity of Anticancer Plants

The disease cancer has been a major challenge globally. Several drugs are being used but they are not so good for the treatment. Thus, it is imperative to carry out advance

search for natural products that could lead to potent and effective drugs for the treatment of cancer.

A good number of cancer and normal cell lines were used for the evaluation of cytotoxicity in this study. Table 4.6 result showed when the methanolic extract of *A. melegueta* and its fractions were subjected to MTT (3-(4, 5Dimethylthiazol-2-yl)-2, 5 Diphenyltetrazolium Bromide) assay respectively. The assay is a colorimetric assay which assesses cell viability by the action of mitochondrial succinate dehydrogenase on the metabolic reduction of the yellow tetrazolium salt in living cells into insoluble purple formazan crystals (Mshana *et al.*, 1998.). These crystals produced are further solubilized with acidified alcohol or DMSO, by aiding the destruction of the cells (Kairo *et al.*, 1999.).

Regression analysis was done to evaluate the cytotoxic concentration required to produce a 50% reduction in cell viability (CC_{50}) of the plant extracts. According to the National Cancer Institute, extracts with CC_{50} less than or equal to 30 µg/mL is regarded as cytotoxic. Previous study by Al-Qubais et al highlighted the selective index helps in the determination of toxicity of a plant extract (Al-Qubaisi *et al.*, 2011.). Selective index value higher than two of a pure compound gives a good selectivity towards cancer cells (Demirgan *et al.*, 2016.). However, if an extract or a compound has a selective index less than 2, it is regarded as being a potential toxicant (Koch *et al.*, 2005.). The presence of this toxins can thereafter cause mutation, as a form of feedback mechanism, leading to cancer.

Cytotoxic Activity of *Aframomum melegueta*

Aframomum melegueta Methanolic extract showed over 50% inhibition at 6.18 µg/mL, with the hexane fraction having the major cytotoxic inhibition at 0.98 µg/mL while the ethyl acetate fractions had 7.7 µg/mL compared to the Methanolic fraction. Selectivity is classified as non-cyt t xic (SI ≥30), weak cyt t xic (SI ≥20) and cyt t xic (SI ≤10) (Ramesh, 2009.). The n-Hexane fraction was also more selective to the normal cells, Vero, used in the study with SI of 98 while cyclophosphamide had SI of 32 Selective index (SI) was calculated for the fractions.

The most active fraction of *A. melegueta*, n-hexane was thereafter subjected to VLC using mobile phase, Hex., DCM and MeOH for fractionation into twenty-one sub fractions. Thin layer chromatographic analysis was done to pool similar constituents of the subtractions together. The analysis on TLC profiling resulted in pooling the twenty-one sub fractions into seven sub-fractions.

Pooled sub-fractions were thereafter screened for cytotoxicity on the cancer cell lines including, lung cancer; AU549, cervical; Hep-2C, breast; MCF-7and skeletal muscle; RD cells. Sub-fraction 3 was found to be the most active across all cell lines used with CC_{50} of 0.50 µg/mL on RD cell line. Sub-fraction 2 was also relatively active on Hep-2C with CC_{50} of 2.03 µg/mL. Statistically, sub-fraction 3 was more active when compared to cyclophosphamide, the control drug used as standard in the study. Cytotoxic property of sub-fraction 3 (AmHV3) led to isolation of active principles in the plant, *A. melegueta.* Gas chromatography- mass spectroscopy (GC-MS) analysis carried out, showed the presence of at least twenty-one compounds. The most abundant compound was [8]- Paradol with peak area of 25.39% and retention time of 29.08- 29.99 minutes, while 7-(1,3-Dimethylbuta-1,3-dienyl)-1,6,6-trimethyl-3,8-dioxatricyclo[5.1.0.0(2,4)] octane is the least abundant compound with a peak of 0.11% and a retention time of 15.77 minutes.

Column chromatography and major purification of AmHV3 by normal phase HPLC led to isolation of three compounds. Compounds **1 (AM2A),** 2 mg, **2; (AM2B),** 2 mg and **3; (AM2C)** 2 mg were eluted at 23.17, 23.95 and 24.61 min, respectively. Spectroscopic analyses (^{1}HNMR, ^{13}CNMR and LC – MS) of the isolated compounds identified the compounds as 6-shogaol, 6-paradol, and 1-dehydro-6-gingerdione, respectively.

Characterization of AM2-A

This is a pale-yellow oil obtained by Normal phase HPLC using mobile phase, Hex: EtoAc. It was identified to be 1-(4)-Hydroxy-3-methoxyphenyl-4-decen-3-one with common name 6-shogaol. Compound 6-Shogaol are derivatives of ginger which has a main shogaol moiety: a benzene ring with dec-4-en 3-one, a methoxy and hydroxyl groups attached to positions 1', 3' and 4 ' respectively. These were observed as the quaternary carb ns at δ 132.9, 147.3 and 144.8 as well as a carb nyl ket ne signal at δ 198.6 as seen in figure 4.8. The meth xy signal reas nated at δ 3.86 (fig. 4.8). The methane carb n signals at δ 130.3 and 146.9 (fig. 4.8) and their corresponding methane pr t n at δ 6.11 (1H, d, J=17.1Hz) and δ 6.91 (1H, m) table 4.12 c nfirms the presence of the double bond at position 4 and thus the unsaturation of the compound. The carb n δ at p siti n 6-10 on the long aliphatic chain are evident to be similar in compounds Am-2A, AM-2B AM-2C and AMD 4-3

Characterization of AM 2-B

This phenolic compound was isolated as a colourless syrup and identified as (*E*)-1-(4-hydroxy-3-methoxyphenyl) dec-4-en-3-one. It has the basic moiety of a paradol which is similar to shogaol but for the absence of double bond at carbon position 4 on the aliphatic chain.

Characterization of AM 2-C

This compound was obtained as a crystalline yellow solid, belonging to gingerol family. The gingerol derivatives are similar to the shogaol but with the unsaturated double bond at position 1 and the presence of an additional carbonyl ketone. Elucidation of compound informed the presence of two carbonyl ketone groups at δ 177.5 and 199.6 were assigned to positions 3 and 5 on the aliphatic chain of the compound. The three quaternary carbons at δ 127.1 148.8, 147.5 (table 4.14) on the phenyl ring moiety were assigned to carbon positions 1', 3' and 4' of the ring system. They therefore have a long aliphatic side chain of dec-1-en-3, 5-dione moiety.

Cytotoxicity of Isolated Compound of the Hexane *A. melegueta*

The cytotoxic properties of the compounds (AM2-A, AM2-B, AM2-C) were assessed on two cancer cell and one normal cell lines. Cyclophosphamide and DMSO served as positive and negative control. Table 4. 15 showed the results of the cytotoxicity of the isolated compounds. The results revealed that AM2A, 6-shogaol had a strong inhibition concentration, CC_{50} of 0.25 µg/mL on the MCF-7, breast cancer cell line and CC_{50} of 0.11 µg/mL on RD cell. The results obtained from this study was consistent with the previous study by Ling et al., 2010 which reported a high inhibitory effect of 6-shogaol on PMA- induced invasion of breast cancer cell, MDA-MB-231 cells (Ling *et al.*, 2010.) and induce programmed cell death in human colorectal carcinoma cells.

Characterization of Compounds from the DCM fraction of *A. melegueta*

Characterization of AmD 4-2

Compound AmD 4-2 isolated from the DCM fraction of *A. melegueta* was recorded on NMR (400 MHz) using deuterated chloroform. This compound had a retention time of 42.5 minutes and visibility in the UV was observed at 254 nm. A bluish green coloration was observed after spraying with sulphuric acid. It had a retardation factor of 0.76. The NMR data obtained was compared with literature found to be an enolic

tautomer which shares some similarities with 5-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-dec-4-en-3-one (Groblacher *et al.*, 2012.). It was found that the ar matic m iety was similar but s me differences in the δ values n the aliphatic side chain. This revealed that AMD 4-2 is an enolic tautomer of 5- hydroxy-1-(4-hydroxy-3-methoxyphenyl)-dec-4-en-3-one Thus the name (Z)-3-Hydroxy-1-(4-hydroxy-3-methoxylphenyl) dec-3-en-5one was assigned to compound AMD 4-2.

Characterization of AmD 6-15-2

The compound AmD 6-15-2 was recorded on NMR (400MHz) using deuterated chloroform. This compound had a retention time of 41 minutes and RF of 0.68. The compound was visible at 254 nm. A bluish grey colouration was observed after spraying with sulphuric acid. The ^{1}H-NMR data obtained for this isolated compound confirmed it as 6-gingerol with a similarity to the enolic tautomer, 5-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-dec-4-en-3-one by Groblacher, (2012), the only difference being the absence of the double bond at position 4. AMD6-15-2 was therefore assigned as 5-hydroxy-1-(4-hydroxy-3-methoxy phenyl)-dec-3-one.

Characterization of AmD 4-3

The compound AmD 4-3 was recorded on NMR (400 MHz) using deuterated chloroform. This compound had a retention time of 53.3 minutes, visible under UV at both 254 and 365 nm. It gave a yellowish brown colour after spraying with sulphuric acid.. The proton signal 15.51 ppm, (1H,s) observed in this compound is observed when it is run in deuterium chloroform and disappears in Deuterated MeOH or D_2O. The presence of the four methylene groups present in the NMR data illustrates the assignment of the proton at positions H6-H9 on the structure of the compound. The methyl group present appeared at position 0.90 ppm which applied as a triplet with a coupling constant, *J-value* of 6.2Hz.

The carbon 13-NMR spectra revealed 17-carbon atoms which includes one methyl group assigned to C-10 at 13.9 ppm and a methoxyl group attached to C-3a' (55.9 ppm), four methylene (40.1, 25.3, 31.4, 31.4 ppm positions H6-H9). Six methine were also observed at δ 139.8, 120.5, 100.1, 109.4, 114.7 and 122.6. The presence of four quaterenary carbons where assigned to δ 178.0, 200.1, 127.7 and 147.6 respectively. The aromatic nucleus of AMD 4-3 is similar to AMD6-15-2 with two additional double bond and was assigned to structures (1*E*,4*Z*)-5-hydroxy-1-(4-hydroxy-3-

methoxyphenyl)deca-1,4-dien-3-one.

Characterization of AmD 8-14-1

The compound AmD 8-14-1 was recorded on NMR (400 MHz) using deteurated chloroform. Compound AmD 8-14-1 was isolated as a pale yellow sticky oil by elution from normal phase HPLC using solvent system Hex 70: EtOAc 30 . This compound had a retention time of 76 minutes and retardation factor of 0.48. The melting point of 41^{o}C was obtained in an uncorrected melting point apparatus.

The compound showed three methine proton observed in this compounds included δ 6.67 (1H, d, *J*= 1.6Hz), 6.81 (1H, d, *J*= 8Hz), 6.65-6.63 (1H, dd, *J*=1.6, 8.0Hz), characteristics of 2,3,5-trisubstituted aromatic system. The methoxyl group at δ 3.84 was assigned t p siti n 3' while the OH (1H,s) at δ 5.44 was attributed t p siti n 4'. The presence of two methylene groups were observed at δ 2.82- 2.79 (*J*-value = 6.8Hz) and 2.72 - 2.69 (*J*-value = 6.8Hz), both appearing as a doublet. The methyl proton attached to a carboxyl group had a δ 2.11 which appeared as a 3H singlet. These tw signals were assigned t Hs 1 and 2 which are attached t C1'.

The ^{13}C-NMR data sh wed the presence f three quaterenary carb ns at δ 143.9, 146.3 and 132.9 while the carb nyl ket ne res nated at δ208.0 The compound was identified as Zingerone based on comparism with literature in a study on derivatization of compounds from *Zingiber officinale* (Agarwal *et al.*, 2001.).

Cytotoxicity of Compounds Isolated from the Dichloromethane fraction of *A. melegueta*

The dichloromethane (DCM) fraction of *A. melegueta* also showed a good inhibitory activity on cervical cancer cell line, HeLa with CC_{50} of 14.1 µg/mL. The four pure compounds were obtained through further purification on normal HPLC with hexane and ethyl acetate as the mobile phase used for elution. Compounds isolated includes AMD4-2, AMD 4-3, AMD6-15-2, AMD8-14-1 with names, (Z)-3-hydroxy-3-methoxyphenyl) dec-3-en-5-one, 6-isogingerdione, 6-gingerol and zingerone, respectively.

Amongst the compound isolated from the DCM fraction of *A. melegueta*, compound AMD4-3 was the most cytotoxic with a 100% inhibition and a CC_{50} of 14.9 µM on

AU565, breast cancer cell line and non-cytotoxic on 3T3, normal cell line. The compound AmD4-2, showed only 47.8% cell inhibition at 50 µM concentration of compound screened on AU565 cell line while AmD8-14-1, zingerone, due to its small quantity, was not screened for cytotoxicity on the cell lines. No report was seen for the cytotoxicity of the compounds isolated from the DCM fraction, thus no comparison.

Cytotoxic Activity of *Strophanthus hispidus*

This study showed that *S. hispidus* had a good 50% inhibitory activity on the various cancer cell lines used and also good selectivity towards non cancer cells. The CC_{50} of 0.32 ± 0.01µg/mL at methanolic extract level on HeLa cell, cervical carcinoma and 1.86 ± 0.01 µg/mL on AU565, a breast cancer cell line, showed a good cytotoxic effect and had better activity on HeLa cell line when compared to doxorubicin, the standard drug used for the study. The methanolic extract had better selectivity 4.19±0.10 µg/mL on normal human fibroblast cell line, BJ as compared to doxorubicin with CC50 of 2.84 ± 0.02 µg/mL. Better selectivity was also seen in methanolic extract as compared to Doxorubicin.

The dichloromethane fraction showed most cytotoxic components of the plant with CC_{50} of 0.21±0.06 and 1.26±0.06 µg/mL on HeLa and AU565 cell lines respectively. The ethyl acetate fraction also showed good cytotoxicity followed by the hexane fractions. A good level of selectivity was also observed in the DCM fraction screened against HeLa cell as compared to standard.

Characterization of Isolates from the Dichloromethane fraction of *S. hispidus*

Characterization of ShD-58

This compound was isolated as yellow hair-like solid, eluted directly from column chromatography with solvent system, Hex: DCM 30:70. The EI-MS showed fragmentation with a molecular ion peak of 311.2, suggesting that the molecular weight of the compound as 311.2 g/mol. The NMR analysis was run in DMSO due to the excellent solubility of the compound in the solvent. Both EI-MS and FAB obtained supports the molecular weight of 311.2. Compound ShD 58 had a melting point of 241.4^{o}C which was recorded on a melting point apparatus BUCHI M-560, and were uncorrected. SHD-58 was found to be UV active and after spray with different spraying agent such as sulphuric acid and Drangerdoff, an orange spot was observed, showing the compound to be an alkaloid.

The infrared bands showed 3411.5, 3223.7, 1594.8, 1555.2, 1441.5, 1233.6, 1137.7 and 737.2 cm^{-1}. UV spectrum showed peaks at 220, 289, 310, and 391nm.

Structural elucidation with ^{1}H and ^{13}C-NMR data obtained suggest the compound to be an alkaloid. This class of alkaloid is found in nature both in plants, animals even human. It has also been found to be present in marine organisms. A previous study by Nagao et al., showed the isolation of Pityriacitrin from marine bacterium named *Paracoccus sp*, also isolated from Malassezia furfur (Mayser *et al.*, 2002.).

The ^{1}H-NMR showed the presence a broad singlet δ 12.09, which also suggest the presence of nitrogen at position 9-NH, 11.98 als a br ad singlet f r 1'NH. The ^{1}H-NMR spectra sh wed the presence f tw br ad singlets at δ 12.09 (1H, brs) and 11.98 (1H, brs) indicating the presence of nitrogen atoms which suggests a β-carboline alkaloid. This was supported by the presence of deshielded aromatic proton observed at δ 8.55 (1H, d, *J*= 6.4 Hz), 8.56 (1H, d, J=4.80 Hz), 8.40 (1H, d, J=4.80 Hz), 8.31,(1H d, J= 7.60 Hz), 7.83(1H, d, J =7.90 Hz and 7.56 (1H d, J =7.20 Hz at positions 4', 3, 4, 5, 8, and 7', respectively. The ^{13} C-NMR also showed the presence of twenty carbon atoms which consist of nine quaternary carbons with signals at δ 138.4, 135.9, 130.7, 120.0, 141.6, 187.3, 114.2, 127.2, and 135.0; also, eleven methine groups with signals at δ 136.9, 117.9, 122.8, 119.8, 128.6, 113.0, 137.8, 122.0, 121.6, 121.7, and 112.2.

The comparison of spectra NMR data obtained with literature data revealed that ShD-58 is similar to Pityriacitrin ($C_{20}H_{13}N_3O$), a β-carboline alkaloid and it was named (1*H*-indol-3-yl)(9*H*-pyrido[3,4-*b*]indol-1-yl)methanone.

Characterization of ShD-9

Compound ShD-9 was isolated as a white amorphous powder. It had a yield of 500mg in 20g of plant fraction. The melting point of the compound was 267.4^{o}C. This compound was eluted directly from the column chromatography with mobile phase of Hex: EtOAc 70:30.

The IR spectrum exhibited absorption for hydroxy group (OH) for alcohol at 3446.2 cm^{-1}, a broad OH for an acid at 2930 cm^{-1} , 2869 for C=C, and a carbonyl (1695cm^{-1})

and the double bond (763cm^{-1}). UV spectrum showed peaks at 215, 229, 248, 260 and 266 nm.

The Electron Ionization -Mass Spectroscopy (EI-MS) showed the M+ at m/z 456.2 g/mol, which corresponds to the molecular formula, $C_{30}H_{48}O_3$. Apart from the M+, the EI-MS exhibited several other diagnostic peaks at m/z 411, which represents the loss of COOH group from the M+. Another prominent peak at 248 represents the Retro-Diels Alders, (RDA) fragmentation at ring C and this showed a typical character of an ursane type triterpene with COOH group at C-17 also a molecular ion peak at m/z 203 characteristic of Δ^{12}- triterpenoids (Razbršek *et al.*, 2008.)

The ^{1}H-NMR spectrum of ShD-9 displayed five tertiary methyl at δ 0.89 (H-24), 1.24. (H-23), 1.24 (H-27), 1.01(H-25), 1.01 (H-26), and secondary methyl at 1.94, 1.02. The pr t n δ 3.47 at p siti n 3 attached t carb n signal δ 78.1 sh wed the hydr xyl gr up attached to the compound. The ^{13}C-NMR spectrum of SHD-9 showed the presence of 30 signals which consist of a total of seven quaternary carbons, seven methine, nine methylene and also seven methyl groups. This illustration was obtained by analysis of data from DEPT 90 and 135 obtained for the compound. The signal at δ 179.7 which is the found to be the most downfield was assigned for the carboxylic acid (C-28) attached to carbon 17. The presence of double bond was seen at signals δ : 125.7 and 139.3 thereby showing a property of an urs-12-ene triterpenoid. Based on the NMR data using ^{1}H and ^{13}C-NMR, DEPT, HSQC, HMBC, COSY spectra and comparison with literature, the compound observed to be ursolic acid, a pentacyclic triterpene.

Characterization of ShD 26-MF3

This compound was isolated as a white crystalline solid after repeated column chromatography and further purification on reverse phase HPLC using methanol and de-ionized water (95:5%) with a melting point of 248.9^{o}C. The compound was found to be UV active with a white fluorescent light at 365 nm. It has a retardation factor R_f of 0.8 with mobile phase of Hex: DCM: MeOH 1.5:0.5:2.5. The NMR analysis of the compound was run in pyridine due to the polarity and also for proper identification of the hydroxyl groups present in the compound.

The IR spectrum exhibited absorption for hydroxy (3387.4 cm^{-1}), carbonyl (1694cm^{-1}) and the double bond (825.3cm^{-1}), 2928.8 showing the OH of the carboxylic acid. Other

major absorptions also occurred at 1456.4, 1039.6 and 667.4 cm^{-1}. The UV spectrum showed peaks at 213 and 229 nm.

ShD26-MF3 was identified based on the ^{1}H and ^{13}C–NMR and MS data obtained by analysis and thereafter compared with previous studies on isolation and characterization of similar compounds. This compound showed a molecular mass of 488.3 g/mol by the means of EI-MS. The mass spectra presented a molecular ion peak ion at m/z 248 which is as a result of the retro-Diels-Alder fragmentation. This fragmentation is a basic characteristic of ursane and oleane triterpenes. Besides the molecular ion peak, the EI-MS exhibited some other diagnostic peaks at m/z 203 characteristic Δ^{12}- triterpenoids and m/z 410.3 which represents the loss of COOH group from the molecular ion. Additionally the compound had a molecular ion peak at m/z 203 characteristic of Δ^{12}- triterpenoids (Razb ršek *et al.*, 2008.).

In this compound, the presence of methyl groups with ^{1}H-NMR signals at δ 0.88, 0.93, 0.98, 1.01, 1.08, and 1.16 were observed. Three hydroxyl groups were observed at position of H- 2, 3, and 23 observed at positions Also, the ^{13}C-NMR showed the presence of 30 carbon atoms with seven quaternary carbons, nine methine, eight methylene and seven methyl groups. This illustration was obtained by analysis of data from DEPT 90 and 135 obtained for the compound. The signal at δ181.7 which is the found to be the most downfield was assigned for the carboxylic acid attached to carbon 28. The presence of double bond was seen at signals δ 126.7 and 139.3 thereby showing a property of an urs-12-ene triterpenoid. The spectra was similar to that of SHD-9 except that there were two additional OH signals which were assigned to position 2 and 23. Based on the NMR data using ^{1}H and ^{13}C-NMR, DEPT, HSQC, HMBC, COSY spectra and comparison with literature, the compound was confirmed to be Urs-12-en-28-oic acid, 2,3,23-trihydroxy-, 2β,3β,4α
], a pentacyclic triterpene.

Characterization of ShD-21

The IR spectrum band showed the decrease of hydroxyl at 3500 cm^{-1}, an increase of the CH2 band at 2939.3 cm^{-1} and 1422.5 $cm^{-1;}$ 1735 cm $^{-1}$(α, β-unsaturated five-membered ring lactone). The absorption band, 1619.5 $cm^{-1,}$ revealed the presence of the double bond carbon (C=C) in the compound. Other major bands reflected in the spectrum includes 2939, and 1072.2 cm^{-1}. UV spectrum showed peaks at 222 and

230nm. The spectroscopic analysis carried out using FAB negative and positive showed a mass of 535 $[M+1]^+$. The molecular ion of 386 also suggests the aglycone moiety, strophanthidin and a sugar moiety of 148. The molecular ion is similar to that of strophanthidin, previously isolated from *S. kombe* (William and Dahhne, 2009.).

The ^{1}H-NMR of this compound suggests the presence of an aldehyde group which appears as a singlet δ 110.4 attributed to H-19 in strophanthidin. Two methyl groups at signals δ 1.13 and 1.55 accounts for the methyl group at H-18 and H-6'. This signal at 1.55 informed the presence of the sugar moiety (deoxy), attached to the aglycone. The presence also of an anomeric proton at δ 5.4 (d, *J*= 9.6Hz) confirmed the presence of the sugar moiety attached to the strophanthidin.

The ^{13}C-NMR spectrum showed the presence of a total number of twenty-nine carbon atoms which consist of four quaternary carbons, twelve methine, eleven methylene and two methyl groups. The carbon signals obtained by NMR is stated in the table showing the NMR data of SHD-21 as seen in table 4.8.4.

Characterization of SHH-32

This compound was isolated directly from the column chromatography as a highly viscous substance. It was in soluble in the mobile phase of elution, EtoAc: MeOH (90:10). This compound was isolated for the first time from the *Strophanthus* genus. The compound after drying gave an off-white or slightly cream amorphous powder. The compound was found to be soluble in pyridine, thus the choice for solvent for NMR analysis.

The IR spectrum of this compound SHH-32 showed a broad band at 3272 cm^{-1} which specifies the presence of a hydroxyl group, illustrating the glycosidic bond linkage. An absorption was also seen at 2936 cm^{-1}, this was attributed to the -CH stretching of the methylene and methyl groups in the compound. An assignment of a very weak band which was observed at 1660 cm^{-1} to be for the C=C. The C-O-C linkage was illustrated based on the absorption seen 1101 cm^{-1}. Other major peaks observed in the spectrum of this compound include 1462, 1363 and 728 cm^{-1}.

The EI-MS gave molecular ion of *m/z* 412 suggesting a steroid, possible sitosterol. The FAB MS gave α-(m+H) peak of 577 suggesting that the molecule is a tautomeric glycoside. This is evident in the H-NMR data.

The ^{1}H-NMR data of the compound showed the presence of 6 methyl groups at δ 0.66 (s), 0.92 (s), 0.98 (d, *J*= 6.4Hz), 0.88 (d, *J*=1.6Hz), 0.88 (d, *J*=1.6Hz), and 0.87 (d, *J*=1.6Hz). The ccurrence als in the spectrum at δ 5.34 indicates an lefinic linkage of the molecule. The proton signal of the sugar moiety was observed as multiplet at positions H3'-H5' with signals at δ 3.95, 4.31 and 4.31, respectively. Signals for positions H-1' and H-2' were observed at 5.05 and 4.05 ppm, both as doublets with coupling constant, (*J*= 7.60 Hz and 8.00 Hz) respectively. Two hydrogen atoms were observed, both as double doublets: 4.42 (dd, J= 5.2, 11.6Hz) ppm and 4.57 (dd, J, 2.4, 12.0Hz) ppm. The proton at H-3 linking the β-sitosterol to the sugar moiety appeared at at δ 3.95, appearing as a multiplet.

The ^{13}C-NMR spectrum showed a total of thirty-five carbon signals: in which twenty-nine was for the aglycone/ steroidal moiety, while other six of the carbons were for the sugar moiety. The sugar carbons were observed and recorded at chemical shift signal at δ 102.6, 75.3, 78.6, 71.7, 78.5, and 62.9 for positions C1'-C6'. The aglycone or steroidal ring of the compound showed the presence of three quaternary, ten methine, ten methylene and 6 methyl carbons. All data obtained for SHH-32 and comparing with literature thereby results in the further confirmation of the identity of the compound as **Daucosterol** (Tania and Kar, 2017.).

Characterization of SHH42-4

About 2 mg of the compound was obtained from the normal phase HPLC with solvent system, Hex: EtoAc70:30 with 1 % Acetic acid. It was isolated as a yellow needle compound with a melting point of 128^{o}C.

The UV (MeOH) data sh wed wavelengths, λ max: nm 232, 257 suggesting a flavone nucleus. The IR (KBr) V_{max} cm^{-1} also showed bands at 3727 and 3624 showing the presence of hydroxy group located at C-5, 2932 (C-H$_{stretch}$) which is typical showing the non-aromatic part of the compound. The Vmax Cm^{-1} at 1654 indicated the presence of an–α, β unsaturated system which is typical of flavones. This was further supported by the stretch at Vmax Cm^{-1} 1217 (C-O$_{stretch}$).

The EI-MS data obtained gave the molecular weight as 402.2. Complete NMR data using the ^{1}H, ^{13}C , HMBC, HSQC, and DEPTs was evaluated and compared extensively with literature, it was observed that a flavone compound with similar

features had been isolated and elucidated as 2-(4-hydroxy-3-methoxyphenyl)5-hydroxy-3,7,8-trimethoxy-4*H*-chromene-4-one, with molecular weight of 374 g/mol. The previously isolated compound was obtained from a medicinal plant, *Parastrephia quadrangularis*, known for the production of flavonoids (Brito, 2018.).

The hydroxyl group at position 4 in the known compound was replaced with the ethoxyl group at the same position.

Methoxyl groups were observed with chemical shifts δ_H 3.85 (3H, s); 3.90 (3H, s), 3.94 (6H, s) ppm) and δ_C 60.2., 60.8, 56.1, 56.3 indicating presence at OCH_3-3, -8, -7and -3' respectively. Also, an ethoxy group was observed at 4.21 ppm which appeared as a quartet. This signal was absent in the known compound. A terminal methyl group at position2" with chemical shift of 1.50 which was also absent in the known compound was observed in compound SHH 42-4. A total number of 4 aromatic protons were bserved at δ_H 6.48, 6.92, 7.67, 7.70 with c rresp ndent δc at 90.3, 111.8, 111.5 and 122.1 indicating proton at 6, 5', 2' and 6' respectively.

Only ne hydr xyl pr t n singlet was bserved at δ_H 12.5 as opposed to two OH proton in the known compound. Compound SHH42-4 was found to possess some degree of unsaturation based on the UV and carbon-13 data (Dept 90 and 135).

The COSY correlation of SHH 42-4 showed a very strong correlation between the two vicinal protons H-5' and H-6', also at H-1" and H-2", thus confirming the presence of the ethoxy group observed at carbon position 4. The compound SHH 42-4 was therefore assigned the structure and name 2-(4-ethoxy-3-methoxyphenyl)-5-hydroxy-3, 7, 8- trimethoxy-4*H*-chromen-4-one which to the best of our knowledge from literature is novel.

Characterization of SHH-14

Compound SHH-14 was isolated as a white powder. The EI-MS data gave a molecular mass of 414 and a corresponding molecular formula $C_{29}H_{48}O$ which supports the ^{1}H-NMR.

The ^{1}H-NMR spectra showed the presence f tw methyl singlets at δ 0.99 and 1.14, position H-17, 28; three methyl d ublets at δ 0.84, 0.86 and 0.91 at p siti ns H-26,27 and 19; and a methyl triplet at δ 0.83 p siti n H-29 respectively.

The compound SHH-14 sh wed pr t n at δ 4.99, 5.14 and 5.31 corresponding to a trisubstituted and disubstituted olefinic bond at position H-20, H-21 and H-6

respectively. The pr t n at δ 3.50 at p siti n H-3 which appeared as a triplet of doublet of doublets is a characteristic feature of a sterol moiety. The ^{1}H-NMR spectrum data supports the presence of the sterol skeleton with the presence of the hydroxyl group at the C-3 position and two double bonds at C-5 and 6 also at C-20 and 21 with the presence of a total number of 6 methyl groups. The spectrum and physical data of the compound corresponds to the literature data reported earlier (Chaturvedula, 2012).

Cytotoxic Effects of Isolated Compounds from *Strophanthus hispidus* Cytotoxicity of Compounds from DCM Fraction

Column chromatography of SHD afforded compounds eluted based on different polarities. Classes of compound isolated from the DCM fractions included, triterpenes (SHD-9, SHD26-MF3), cardiac glycosides, (SHD-21) and alkaloid (SHD58).

Cytotoxicity screening of isolated compounds from DCM fraction of *S. hispidus* were carried out on AU565 breast cancer cell line and 3T3 normal cell line for selectivity. The results of the assays showed Ursolic acid, SHD9, to be the most cytotoxic compound with CC_{50} of 8.07±0.02 µM. This showed better activity than UA isolated from Green Walnut (*Juglans regia* L.) CC_{50} of 19 µg/mL (Tsasi, 2015.). Cytotoxic activity of UA isolated from *Dipterocarpus obtusifolius* was observed to have a CC_{50} 0f 18.3µM (Khiev *et al.*, 2012.).

Literature reviews have shown that ursolic acid (UA), a pentacyclic acid, sometimes exists as an aglycone of triterpenoid saponins (Liu, 1995.). Ursolic acid activity in management or progression of cancer has also been established from previous studies. Ursolic acid has been found to potentiate the inhibition of tumour growth, and spread (Lee *et al.*, 2001; Shishodia *et al.*, 2003.) which is similar to the activity observed when UA isolated from *S. hispidus* in this study. Ursolic acid was reported to account for the stoppage of induction of differentiation of tumour cells in the organism (Lee *et al.*, 1994.). Other pharmacological activities of ursolic acid have been reported in several other studies including its anti-inflammatory activity of UA isolated from *Perilla frutescens* (Banno *et al.*, 2004.), the reduction in level of liver toxicity in the rat (Binduja *et al.*, 1996.). Ursolic acid have also been reported on reduction or suppression of atherosclerosis in the heart, it has so possess immunoregulatory effects. Triterpenes have been resolved to undergo apoptosis as the mechanism of action in cancer treatment. Ursolic acid has been found to have inhibitory effect on the

proliferation and also induction of apoptosis in of cancerous cells with adequate level of selectivity. Cancerous cells found in blood (leukaemia) underwent apoptosis after treatment with Ursolic acid (Urech *et al.*, 2005.).

From existing literature search, Ursolic acid has never been isolated from *Strophanthus hispidus* but another plant from the *strophanthus* genus, *Strophanthus speciosus*, gave ursolic acid (Simin, 2013.).

Strophanthidin- digitoxoside (helveticoside), showed potent cytotoxic effect against AU565, CC_{50} of 11.42±0.60 µM. Reports have shown cytotoxicity of this compound, helveticoside on other cancer cell lines (Moon *et al.*, 2010.). Its cytotoxic effect was also observed in human liver, prostrate, ovary and also stomach cancer cell lines, which also shown to be more potent than standard drug, Doxorubicin used for the study (Lee; *et al.*, 2013.). This study is the first to report the isolation of Heleveticosede from *S. hispidus*.

Studies have shown the utilization of cardiac glycoside in treatment of cardiac congestion (López-Lázaro, 2005.). There could be a possibility of helveticoside being used for treatment of cardiac diseases, though mechanism of actions might differ.

ShD26-MF3, Urs-12-en-28-oic acid, 2,3,23-trihydroxy-(2β,3β,4α); SHD26-MF3, though showed cytotoxic cell inhibition of 99.5% but had a CC_{50} of 18.27±1.73 µM. Extensive literature search showed that this compound SHD 26-MF3 has not being isolated from *S. hispidus.* Also, cytotoxicity effect of the compound has never been established.

SHD-58, identified as Pityriacitrin, is a β-carboline alkaloid. Alkaloids containing the β-carboline skeleton are naturally found in plant, marine organisms and sometimes in animals. Previous work has shown the isolation of compound pityriacitrin in yeast *Malassezia furfur* (Mayser *et al.*, 2002.). SHD-58 was also found to show good cytotoxic activity at 66% inhibition of cell growth with a CC_{50} of 29.29±2.90 µM on AU565, breast carcinoma cell line used for this study. In previous study by Zhang et al, pityriacitrin was found to have a very low cell inhibition on breast cancer cell lines; MDA-231, MCF-7 and prostate cancer cell line; PC3. Though, inhibition concentration of pityriacitrin was better against PC3 with a CC_{50} of 55.11 µM (Zhang *et al.*, 2004.).

Cytotoxicity of Compounds from Hexane fraction

The column chromatography of the hexane fraction of *strophanthus hispidus* afforded several compounds directly and also by further purification of normal phase HPLC using Hex: EtOAc on both chromatographic techniques.

SHH14 identified based on proton NMR and Electron Ionization-Mass Spectroscopy as Stigmasterol, a white powder with molecular weight of 412 g/mol. In this study, cytotoxicity screen was not carried out on stigmasterol due to the little quantity of compound isolated purely. Though previous studies have shown activity of stigmasterol on cytotoxicity.

SHH 32, daucosterol showed non-cytotoxicity against 3T3, non- cancer cell with about 21.00% inhibition of the cells. Cytotoxicity was milder as compared to the standard dug used in the study, cycloheximide which showed an inhibition at 89.19%.

SHH42-4, with a molecular formula $C_{21}H_{22}O_8$ was obtained in small quantity of 1.5 mg, therefore could not be screened for cytotoxicity on cancer cell lines available. Though studies have shown that the previously isolated compound, 5-hydroxy-2-(4-hydroxy-3-methoxyphenyl)-3,7,8-trimethoxy-*4H*-chromene-4-one has a strong anti-inflammatory and antiproliferative activities, which led to the inhibition of leukocyte chemotaxis and also formation of oxygen free radical (Brito, 2018.).

All isolated compounds screened against the normal cell line showed non cytotoxic effects on the normal cell. This implies the selectivity of the compounds on non-cancerous cells.

5.1 CONCLUSION

This study has demonstrated that *Aframomum melegueta* seed extract and fractions have significant cytotoxic properties across a good number of cancer cell lines ranging from breast, cervical, lung and skeletal muscle carcinoma. *Aframomum melegueta* was also found not to be cytotoxic on normal cell lines, Vero, 3T3 and BJ cell lines. Furthermore, the extract exhibited high selectivity to normal cells.

The n-hexane fraction found to be cytotoxic on cancer cell but selective to normal cells afforded a total number of three isolated compounds, 6-Shogaol, 6-Paradol and dehydro-6-gingerdione. The cytotoxicity of 6-Shoagaol was confirmed by activity of RD and MCF-7 cancer cells and selective to Vero, normal cell line. Thus 6-shogaol can be translated into drug for cancer treatment. The DCM fraction which also showed activity of HeLa, cervical carcinoma and also selective on 3T3, normal cell line. This

fraction afforded four compounds with which 6-Isodehydrogingerdione, isolated for the first time, though synthesised previously was most cytotoxic on HeLa and all other compounds from AmD were selective to normal cell line.

Though studies have been shown on the isolation or synthesis of some these compounds but not from directly from *A. melegueta*.

This study on cytotoxicity of *Strophanthus hispidus* showed high potentials and likely hope in the management of cancer. Cytotoxicity was observed across all cancer cell lines used for the study and also moderately selective at Methanolic and fraction stages. The n-hexane fraction was slightly cytotoxic, though not as effective as the DCM fraction of *S. hispidus*. The hexane fraction afforded three compounds with which a novel compound, ShH42-4 was isolated. The DCM fraction evaluated to be cytotoxic afforded four isolated which were screened against cancer and normal cell lines. The compounds were found to be cytotoxic and also very selective to normal cells. Therefore, showing no toxicity and hence being safe for treatment or management of cancer patients. All compound isolated from *Strophanthus hispidus*, reported in this study are either new or novel thus the significance of this study.

The Anticancer properties of *Strophanthus hispidus* is being established for the first time based on extensive literature search using Google, GoogleScholar, SciFinder and other scientific search engines.

Beyond reasonable doubts, an *Aframomum melegueta* seed and *Strophanthus hispidus* justifies the traditional claims of effectiveness against cancer.

5.2 RECOMMENDATION

1. There should be proper documentation of knowledge and information obtained from the Traditional medical practitioners (TMPs) so that the knowledge is not lost as a result of the dissemination and transferring of the information from one generation to another.
2. Adequate and effective conservation of these information can be by organization of seminars thereby there will be proper of the dissemination of information to participants which will include TMPs, herb sellers, herbalist in conjunction with universities and also government parastatals.
3. A different but detailed cytotoxicity assay should be carried out on the plants which were utilized for this study to know other mechanism of action by which the plants and compounds isolated from them work in the management of the disease since this study was just by apoptosis, cancer line death.
4. There should be screening of other medicinal plants mentioned in the ethnobotanical study to see if there is potential activity on other cancer cell lines which were not utilized in this study.

References

Abeloff, M., Armitage, J., Niederhuber, J., Kastan, M. and Mckenna, W. 2004. Review of Clinical Oncology.

Adefegha, S. A. and Oboh, G. 2012. Inhibition of key enzymes linked to type 2 diabetes and sodium nitroprusside-induced lipid peroxidation in rat pancreas by water extractable phytochemicals from some tropical spices. *Pharmaceutical biology* 50.7**:** 857-865.

Agarwal, M., Walia, S., Dhingra, S. and Khambay, B. P. S. 2001. Insect growth inhibition, antifeedant and antifungal activity of compounds isolated/derived from Zingiber officinale Roscoe (ginger) rhizomes. *Pest Management Science: formerly Pesticide Science* 57.3**:** 289-300.

Agbaje, E. O. and Fageyinbo, M. S. 2012. Evaluating Anti-Inflammatory activity of aqueous root extract of Strophanthus hispidus DC.(Apocynaceae). *International Journal of Applied Research in Natural Products* 4.4**:** 7-14.

Agbonon, A., Eklu-Gadegbeku, K., Aklikokou, K., Gbeassor, M., Akpagana, K., Tam, T. W., Arnason, J. T. and Foster, B. C. 2010. In vitro inhibitory effect of West African medicinal and food plants on human cytochrome P450 3A subfamily. *Journal of ethnopharmacology* 128.2**:** 390-394.

Agyare, C., Dwobeng, A. S., Agyepong, N., Boakye, Y. D., Mensah, K. B., Ayande, P. G. and Adarkwa-Yiadom, M. 2013. Antimicrobial, antioxidant, and wound healing properties of Kigelia africana (Lam.) Beneth. and Strophanthus hispidus DC. *Advances in pharmacological sciences* 2013. 10.

Ajaiyeoba, E. and Ekundayo, O. 1999. Essential oil constituents of Aframomum melegueta (Roscoe) K. Schum. seeds (alligator pepper) from Nigeria. *Flavour and fragrance journal* 14.2**:** 109-111.

Akinlami, O. O., Osho, I. B., Owolabi, B. J. and Lajide, L. 2015. Trace Elements and Major Minerals Evaluation in Strophanthus hispidus,D.C. *Canadian Open Applied Chemistry Journal* 1.1**:** 1-10.

Al-Qubaisi, M., Rozita, R., Yeap, S.-K., Omar, A.-R., Ali, A. -M. and Alitheen, N. B. 2011. Selective cytotoxicity of goniothalamin against hepatoblastoma HepG2 cells. *Molecules* 16.4**:** 2944-2959.

Ayoola, G., Folawewo, A., Adesegun, S., Abioro, O., Adepoju-Bello, A. and Coker, H. 2008. Phytochemical and antioxidant screening of some plants of Apocynaceae from South West Nigeria. *African Journal of Plant Science* 2.10**:** 124-128.

Banno, N., Akihisa, T., Tokuda, H., Yasukawa, K., Higashihara, H., Ukiya, M., Watanabe, K., Kimura, Y., Hasegawa, J.-I. and Nishino, H. 2004. Triterpene acids from the leaves of Perilla frutescens and their anti-inflammatory and antitumor-promoting effects. *Bioscience, biotechnology, and biochemistry* 68.1**:** 85-90.

Begg, A. C., Stewart, F. A. and Vens, C. 2011. Strategies to improve radiotherapy with targeted drugs. *Nature Reviews Cancer* 11.4**:** 239.

Biesalski, H. K., De Mesquita, B. B., Chesson, A., Chytil, F., Grimble, R., Hermus, R., Köhrle, J., Lotan, R., Norpoth, K. and Pastorino, U. 1998. European consensus statement on lung cancer: risk factors and prevention. lung cancer panel. *CA: a cancer journal for clinicians* 48.3**:** 167-176.

Binduja, S., Visen, P., Dayal, R., Agarwal, D. and Patnaik, G. 1996. Protective action of ursolic acid against chemical induced hepato-toxicity in rats. *Indian Journal of Pharmacology* 28.4**:** 232.

Bouayed, J. and Bohn, T. 2010. Exogenous antioxidants—double-edged swords in cellular redox state: health beneficial effects at physiologic doses versus deleterious effects at high doses. *Oxidative medicine and cellular longevity* 3.4**:** 228-237.

Bown, D. 1995. *The Royal Horticultural Society encyclopedia of herbs & their uses*, Dorling Kindersley Limited.

Bray, F. and Møller, B. 2006. Predicting the future burden of cancer. *Nature Reviews Cancer* 6.1**:** 63.

Brito, I. J. B., Mario Simirgiotis and Alejandro Cárdenas. 2018. Crystal structure of 5-hydroxy-2-(4-hydroxy3-methoxyphenyl)-3,7,8-trimethoxy-4H-chromen4-one *Z. Kristallogr. NCS* 233.1**:** 61-64.

Buch, K. P., T., Nawroth, T. Sanger, M. Schmidberger, H., Langguth, P. 2012. Determination of cell survival after irradiation via clonogenic assay versus multiple MTT Assay-- A comparative study. *Radiation Oncology* 3.7**:** 1.

Burkill, H. 1985. The useful plants of West Africa Vol. 1. *Royal botanical gardens***:** 386-387.

Bussmann, R. W. and Sharon, D. 2006. Traditional medicinal plant use in Northern Peru: tracking two thousand years of healing culture. *Journal of Ethnobiology and Ethnomedicine* 2.1**:** 47.

Calixto, J. 2000. Efficacy, safety, quality control, marketing and regulatory guidelines for herbal medicines (phytotherapeutic agents). *Brazilian Journal of medical and Biological research* 33.2**:** 179-189.

Change 2016. *Chemotherapy Diagnosis and Treatment*, Macmillian Cancer Support.

Charles, R., Garg, S. and Kumar, S. 2000. New gingerdione from the rhizomes of Zingiber officinale. *Fitoterapia* 71.6**:** 716-718.

Chaturvedula, V. S. P. and Prakash, I. 2012. Isolation of Stigmasterol and ?-Sitosterol from the dichloromethane extract of Rubus suavissimus. *International Current*

Pharmaceutical Journal 1.9: 239-242.

Cimbora Zovko, T., Bombek, S., Kosmrlj, J., Kovacic, L., -Polanc, S., Katalinic, A. and Osmak, M. 2004. Development of potential anti cancer agents: Diazenes and derivatives. *Drug development research* 61.2**:** 95-100.

Davis, C. D., Tsuji, P. A. and Milner, J. A. 2012. Selenoproteins and cancer prevention. *Annual review of nutrition* 32. 73-95.

Delaney, G., Jacob, S., Featherstone, C. and Barton, M. 2005. The role of radiotherapy in cancer treatment. *Cancer* 104.6**:** 1129-1137.

Demirgan, R., Karagöz, A., Pekmez, M., Önay-Uçar, E., Artun, F. T., Gürer, Ç. and Mat, A. 2016. In vitro anticancer activity and cytotoxicity of some papaver alkaloids on cancer and normal cell lines. *African Journal of Traditional, Complementary and Alternative Medicines* 13.3**:** 22-26.

El-Halawany, A. M., El Dine, R. S., El Sayed, N. S. and Hattori, M. 2014. Protective effect of Aframomum melegueta phenolics against CCl 4-induced rat hepatocytes damage; Role of apoptosis and pro-inflammatory cytokines inhibition. *Scientific reports* 4. 5880.

Elsenberg, D., Davis, R. and Ethmer, S. 1990. Trend in alternative medicines use in the United States. *The Journal of the American Medical Association* 280. 1569-1575.

Engel, N., Oppermann, C., Falodun, A. and Kragl, U. 2011. Proliferative effects of five traditional Nigerian medicinal plant extracts on human breast and bone cancer cell lines. *Journal of ethnopharmacology* 137.2**:** 1003-1010.

Ezuruike, U. F. and Prieto, J. M. 2014. The use of plants in the traditional management of diabetes in Nigeria: Pharmacological and toxicological considerations. *Journal of Ethnopharmacology* 155.2**:** 857-924.

Farnsworth, N. R. 1988. Screening plants for new medicines. *Biodiversity* 15.3**:** 81-99.

Ferlay, J., Shin, H. R., Bray, F., Forman, D., Mathers, C. and Parkin, D. M. 2010. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *International journal of cancer* 127.12**:** 2893-2917.

Ferlay. 2019. Estimating the global cancer incidence and mortality in 2018_ GLOBOCAN sources and methods-. *International Journal of Cancer*.

Fernandez, X., Pintaric, C., Lizzani Cuvelier, L., Loiseau, A. M., Morello, A. and Pellerin, P. 2006. Chemical composition of absolute and supercritical carbon dioxide extract of Aframomum melegueta. *Flavour and fragrance journal* 21.1**:** 162-165.

Galal, A. M. 1996. Antimicrobial activity of 6-paradol and related compounds. *International journal of Pharmacognosy* 34.1**:** 64-69.

Gan, Z., Liang, Z., Chen, X., Wen, X., Wang, Y., Li, M. and Ni, Y. 2016. Separation and preparation of 6-gingerol from molecular distillation residue of Yunnan ginger rhizomes by high-speed counter-current chromatography and the antioxidant activity of ginger oils in vitro. *Journal of Chromatography B* 1011. 99-107.

Ge rgia. 2019. Review article Cancer : Cancer in the 21 st Century , The R le f Lifestyle and Nutrition, Food Controversies and Recommendations for Cancer preventi n *Article* 3.7**:** 173-178.

Giovannucci, E. 1999. Tomatoes, tomato-based products, lycopene, and cancer: review of the epidemiologic literature. *Journal of the national cancer institute* 91.4**:** 317-331.

Gordana, S., Sonja, M., Jasna, M., and Vesna, T. 2004. .Antioxidant properties of marigold extracts. *Food. Res. Int.* 37. 643-650.

Grabley, S. and Thiericke, R. 1999. Bioactive agents from natural sources: trends in discovery and application. *Thermal Biosensors, Bioactivity, Bioaffinitty*. Springer.

Grewal, P. A. and Viswanathen, V. A. 2012. Liver cancer and alcohol. *Clinical Liver Disease* 16. 839-850.

GroBlacher, B., Maier, V., Kunert, O. and Bucar, F. 2012. Putative myc bacterial efflux inhibitors from the seeds of Aframomum melegueta. *Journal of natural products* 75.7**:** 1393-1399.

Guéritte, F. and Fahy, J. 2005. The vinca alkaloids. *Anticancer agents from natural products* 10. 123-135.

Hamburger, M. and Hostettmann, K. 1991. 7. Bioactivity in plants: the link between phytochemistry and medicine. *Phytochemistry* 30.12**:** 3864-3874.

Hoareau, L. and Dasilva, E. J. 1999. Medicinal plants: a re-emerging health aid. *Electronic Journal of biotechnology* 2.2**:** 3-4.

Hsu, Y.-L., P-L, K., L-T, L. and C-C, L. 2005. Asiatic acid, a triterpene, induces apoptosis and cell cycle arrest through activation of extracellular signal-regulated kinase and p38 mitogenactivated protein kinase pathways in human breast cancer cells. *J Pharm Exp Ther* 313. 333–344.

Hunt, K. S. and Ray, J. A. 2008. Hereditary risk for cancer. *Fundamentals of cancer prevention.* Springer.

Iarc 2012. Agents Classified by the IARC Monographs.

Iheanacho, K., Ibegbulem, C., Emejulu, A., Okwu, G. and Igbo, N. 2016. Phytochemistry and LD50 of Aqueous and Ethanol Extracts of Strophanthus Hispidus.

Iita. 2012. Strophanthus hispidus. [Accessed 09-02-2019].

Ilic, N., Schmidt, B. M., Poulev, A. and Raskin, I. 2010. Toxicological evaluation of grains of paradise (Aframomum melegueta)[Roscoe] K. Schum. *Journal of ethnopharmacology* 127.2**:** 352-356.

Ishola, I. O., Awodele, O., Oreagba, I. A., Murtala, A. A. and Chijioke, M. C. 2013. Antinociceptive, anti-inflammatory and antiulcerogenic activities of ethanol root extract of Strophanthus hispidus DC (Apocynaceae). *Journal of basic and clinical physiology and pharmacology* 24.4**:** 277-286.

Iwu, M. W., Duncan, A. R. and Okunji, C. O. 1999. New antimicrobials of plant origin. *Perspectives on new crops and new uses. ASHS Press, Alexandria, VA***:** 457-462.

Ja¨Ger, S., H;, T., T;, K., Laszczyk M, N. and A., S. 2009. Pentacyclic triterpene distribution in various plants–rich sources for a new group of multi-potent plant extracts. *Molecules* 14. 2016-2031.

Jackson, S. P. and Bartek, J. 2009. The DNA-damage response in human biology and disease. *Nature* 461.7267**:** 1071.

Jedy-Agba, E., Oga, E., Odutola, M., Ekanem, I., Ezeome, E., Igbinoba, F., Ogunbiyi, O., Hassan, R., Osinubi, P. and Dakum, P. 2014. the Nigerian National System Of Cancer Registries-developing National Cancer Registration In Developing Countries.: 852. *Asia-pacific Journal of Clinical Oncology* 10. 150.

Kairo, S. K., Bedwell, J., Tyler, P. C., Carter, A. and Corbel, M. J. 1999. Development of a tetrazolium salt assay for rapid determination of viability of BCG vaccines. *Vaccine* 17.19**:** 2423-2428.

Kamtchouing, P., Mbongue, G., Dimo, T., Watcho, P., Jatsa, H. and Sokeng, S. 2002. Effects of Aframomum melegueta and Piper guineense on sexual behaviour of male rats. *Behavioural Pharmacology* 13.3**:** 243-247.

Kerharo, J. and Bouquet, A. 1950. Plantes médicinales et toxiques de la Côte d'Ivoire-Haute-Volta: mission d'étude de la pharmacopée indigène en AOF.

Khiev, P., Kwon, O. K., Song, H. H., Oh, S. R., Ahn, K. S., Lee, H. K. and Chin, Y. W. 2012. Cytotoxic terpenes from the stems of Dipterocarpus obtusifolius collected in Cambodia. *Chem. Pharm. Bull.* 60.8**:** 955-961.

Khine, M. M. 2006. Isolation and characterization of phytoconstituents from Myanmar medicinal plants. *Doktorarbeit), Martin-Luther-Universität, Mathematisch-Naturwissenschaftlich-Technischen Fakultät*.

Kim, J. and Park, E. 2002. Cytotoxic anticancer candidates from natural resources. *Current Medicinal Chemistry-Anti-Cancer Agents* 2.4**:** 485-537.

Koch, A., Tamez, P., Pezzuto, J. and Soejarto, D. 2005. Evaluation of plants used for antimalarial treatment by the Maasai of Kenya. *Journal of Ethnopharmacology* 101.1-3**:** 95-99.

Kojima, H. and Ogura, H. 1986. Triterpenoids from Prunella vulgaris. *Phytochemistry* 25.3**:** 729-733.

Landis, S., Ryan, S., Woo, K. and Sibbald, R. 2007. Infections in chronic wounds. *Chronic Wound Care: A Clinical Source Book for Healthcare Professionals. 4th ed. Malvern, PA: HMP Communications***:** 299-321.

Larkins, N. and Wynn, S. 2004. Pharmacognosy: phytomedicines and their mechanisms. *Veterinary Clinics: Small Animal Practice* 34.1**:** 291-327.

Lee, Lee, D. G., Lee, K. H., Cho, S. H., Nam, K.-W. and Lee, S. 2013. Isolation and identification of phytochemical constituents from the fruits of Acanthopanax senticosus. *African Journal of Pharmacy and Pharmacology* 7.6**:** 294-301.

Lee, Lee, J., Lee, Y. H. and Leonard, J. 2001. Ursolic acid-induced changes in tumor growth, O2 consumption, and tumor interstitial fluid pressure. *Anticancer research* 21.4A**:** 2827-2833.

Lee, H.-Y., Chung, H.-Y., Kim, K.-H., Lee, J.-J. and Kim, K.-W. 1994. Induction of differentiation in the cultured F9 teratocarcinoma stem cells by triterpene acids. *Journal of cancer research and clinical oncology* 120.9**:** 513-518.

Lee, K.-H. 2010. Discovery and development of natural product-derived chemotherapeutic agents based on a medicinal chemistry approach. *Journal of natural products* 73.3**:** 500-516.

Lee;, Y. J., Kim;, N. S., Kim;, H., Yi;, J.-M., Oh;, S.-M., Bang;, O.-S. and Lee, J. 2013. Cytotoxic and anti-inflammatory constituents from the seeds of Descurainia sophia. *Arch. Pharm. Res.* 36. 536-541.

Ling, H., Yang, H., Tan, S. H., Chui, W. K. and Chew, E. H. 2010. 6 Shogaol, an active constituent of ginger, inhibits breast cancer cell invasion by reducing matrix metalloproteinase 9 expression via blockade of nuclear factor κB activation. *British journal of pharmacology*.

Liu, J. 1995. Pharmacology of oleanolic acid and ursolic acid. *Journal of ethnopharmacology* 49.2**:** 57-68.

López-Lázaro, M. P. N., Azrak Ss, Ayuso Mj, Austin Ca, Cortés F. 2005. Digitoxin inhibits the growth of cancer cell lines at concentrations commonly found in cardiac patients. *Journal of Natural Product* 68. 1642-1645.

Lorke, D. 1983. A new approach to practical acute toxicity testing. *Archives of toxicology* 54.4**:** 275-287.

Maclennan, A. H., Myers, S. P. and Taylor, A. W. 2006. The continuing use of complementary and alternative medicine in South Australia: costs and beliefs in 2004. *Medical journal of Australia* 184.1**:** 27.

Mathers, C. D. and Loncar, D. 2006. Projections of global mortality and burden of disease from 2002 to 2030. *PLoS medicine* 3.11**:** e442.

Mayser, P., Schäfer, U., Krämer, H.-J., Irlinger, B. and Steglich, W. 2002. Pityriacitrin–an ultraviolet-absorbing indole alkaloid from the yeast Malassezia furfur. *Archives of dermatological research* 294.3**:** 131-134.

Mbaveng, A. T., Kuete, V., Mapunya, B. M., Beng, V. P., Nkengfack, A. E., Meyer, J. J. M. and Lall, N. 2011. Evaluation of four Cameroonian medicinal plants for anticancer, antigonorrheal and antireverse transcriptase activities. *Environmental toxicology and pharmacology* 32.2**:** 162-167.

Mephors, V., Ogbole, O. and Ajaiyeoba, E. 2017. Plants used in treatment of five cancers in two Local Government Areas in southwest Nigerian ethnomedicine. *Nigerian Journal of Natural Products and Medicine* 21.1**:** 54-60.

Mester, J. - and- Eng, C. 2015. Cowden syndrome: Recognizing and managing a not so rare hereditary cancer syndrome. *Journal of surgical oncology* 111.1**:** 125-130.

Meyer, B., Ferrigni, N., Putnam, J., Jacobsen, L., Nichols, D. J. and Mclaughlin, J. L. 1982. Brine shrimp: a convenient general bioassay for active plant constituents. *Planta medica* 45.05**:** 31-34.

Moon, S. S., Rahman, M. A., Manir, M. M. and Jamal Ahamed, V. S. 2010. Kaempferol glycosides and cardenolide glycosides, cytotoxic constituents from the seeds of Draba nemorosa (Brassicaceae). *Archives of Pharmacal Research* 33. 1169-1173.

Mosmann, T. 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *Journal of immunological methods* 65.1-2**:** 55-63.

Mshana, R. N., Tadesse, G., Abate, G. and Miörner, H. 1998. Use of 3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide for rapid detection of Rifampin-Resistant Mycobacterium tuberculosis. *Journal of clinical microbiology* 36.5**:** 1214-1219.

Nakamura, T., Goda, Y., Sakai, S., Kondo, K., Akiyama, H. and Toyoda, M. 1998. Cardenolide glycosides from seeds of Corchorus olitorius. *Phytochemistry* 49.7**:** 2097-2101.

Nih 2018. *Chemotherapy and You*, U.S. Department of Health & Human Services National Institutes of Health, NCI Office of Communications and Public Liaison.

Noble, R., Beer, C. and Cutts, J. 1959. Further biological activities of vincaleukoblastine—an alkaloid isolated from Vinca rosea (L.). *Biochemical Pharmacology* 1.4**:** 347-348.

Nwodo, N. J., Ibezim, A., Simoben, C. V. and Ntie-Kang, F. 2016. Exploring cancer therapeutics with natural products from African medicinal plants, part II: alkaloids, terpenoids and flavonoids. *Anti-Cancer Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Anti-Cancer Agents)* 16.1**:** 108-127.

Occhiuto, F., Circosta, C. and Costa, D. P. 1989. Studies on some medicinal plants of Senegal: effects on isolated guinea pig ileum. *Journal of ethnopharmacology* 26.2**:** 205-210.

Ogasawara, R., K;, S., K;, H., K;, N. and Fujita 2013. Ursolic acid stimulates mTORC1 signaling after resistance exercise in rat skeletal muscle.

. *Am J Physiol Endocrinol Metab* 305. E760–E765.

Ojiako, O. A. and Igwe, C. U. 2009. A time-trend hypoglycemic study of ethanol and chloroform extracts of Strophanthus hispidus. *Journal of herbs, spices & medicinal plants* 15.1**:** 1-8.

Okigbo, R. and Ajalie, A. 2005. Inhibition of some human pathogens with tropical plant extracts–Chromolaena odorata and Citrus aurantifolia, and some antibiotics. *International Journal of Molecular Medicine and Advance Sciences* 1.1**:** 34-40.

Oluwatoyin, A. E. and Samuel, F. M. 2014. Anti -nociceptive activity of fractionated root extract of Strophanthus hispidus DC (Apocynaceae). *Journal of Natural Remedies* 14.2**:** 164-173.

Osibemhe, M., Abdulrahman, B. and Onoagbe, I. 2016. Acute Toxicity of Aqueous and Ethanolic Extracts of Strophanthus hispidus Stem Bark. *International Journal of Biochemistry Research & Review* 9.1**:** 1.

Phillips, O. and Gentry, A. H. 1993. The useful plants of Tambopata, Peru: I. Statistical hypotheses tests with a new quantitative technique. *Economic Botany* 47.1**:** 15-32.

Prayong, P., Barusrux, S. and Weerapreeyakul, N. 2008. Cytotoxic activity screening of some indigenous Thai plants. *Fitoterapia* 79.7-8**:** 598-601.

Rafatullah, S., Galal, A., Al-Yahya, M. and Al-Said, M. 1995. Gastric and duodenal antiulcer and cytoprotective effects of Aframomum melegueta in rats. *International journal of pharmacognosy* 33.4**:** 311-316.

Ramachandran, S., Vamsikrishna, M., Gowthami, K., Heera, B. and Dhanaraju, M. 2011. Assessment of cytotoxic activity of Agave cantula using brine shrimp (Artemia salina) lethality bioassay. *Asian J. Sci. Res* 4.1**:** 90-94.

Ramesh, B. B., Selina, F., Patrick, J., John, S.C., Lekan, M.L. And Carl, B.G. 2009. Selective Cytotoxic Activities of Two Novel Synthetic Drugs on Human Breast Carcinoma MCF-7 Cells. *Anticancer Research*- 29.8: 2993-2996.

Rauter, A. P., Martins, A., Borges, C., Mota Filipe, H., Pinto, R., Sepodes, B. and Justino, J. 2010. Antihyperglycaemic and protective effects of flavonoids on streptozotocin–induced diabetic rats. *Phytotherapy Research* 24.S2**:** S133-S138.

Razb ršek, M. I., V nčina, D. B., D leček, V. and V nčina, E. 2008. Determinati n f oleanolic, betulinic and ursolic acid in Lamiaceae and mass spectral fragmentation of their trimethylsilylated derivatives. *Chromatographia* 67.5-6**:** 433-440.

Sachdev-Gupta, K., Renwick, J. and Radke, C. 1990. Isolation and identification of oviposition deterrents to cabbage butterfly, Pieris rapae, fromErysimum cheiranthoides. *Journal of chemical ecology* 16.4**:** 1059-1067.

Sampada, K. E. A., Shugeng Cao, Peggy Brodie, James S. Miller, N. M. Andrianjafy, J. Razafitsalama, Rabodo Andriantsiferana,(Vincent E. Rasamison,(and David G. I. Kingston*, 2007. Cytotoxic Cardenolide Glycosides of Roupellina (Strophanthus) boiWinii from the Madagascar Rainforest. *J. Nat. Prod.* 70. 1766–1770.

Seebacher, W., Simic, N., Weis, R., Saf, R. and Kunert, O. 2003. -Complete assignments f 1H and 13C NMR-res nances f lean lic acid, 18α oleanolic acid, ursolic acid and their 11 oxo derivatives. *Magnetic Resonance in Chemistry* 41.8**:** 636-638.

Shankar, A., Dubey, A., Saini, D., Singh, M., Prasad, C. P., Roy, S., Bharati, S. J., Rinki, M., Singh, N. and Seth, T. 2019. Environmental and occupational determinants of lung cancer. *Translational lung cancer research* 8.Suppl 1**:** S31.

Shigematsu, N., Ueda, H., Takase, S., Tanaka, H., Yamamoto, K. and Tada, T. 1994. FR901228, a novel antitumor bicyclic depsipeptide produced by Chromobacterium violaceum No. 968. *The Journal of antibiotics* 47.3**:** 311-314.

Shih, H.-C., Chern, C.-Y., Kuo, P.-C., Wu, Y. -C., Chan, Y. -Y., Liao, Y.-R., Teng, C.-M. and Wu, T. -S. 2014. Synthesis of analogues of gingerol and shogaol, the active pungent principles from the rhizomes of Zingiber officinale and evaluation of their anti-platelet aggregation effects. *International journal of molecular sciences* 15.3**:** 3926-3951.

Shishodia, S., Majumdar, S., Banerjee, S. and Aggarwal, B. B. 2003. Ursolic acid inhibits nuclear factor-κB activati n induced by carcin genic agents thr ugh suppressi n f IκBα kinase and p65 ph sph rylati n: c rrelati n with d wn-regulation of cyclooxygenase 2, matrix metalloproteinase 9, and cyclin D1. *Cancer research* 63.15**:** 4375-4383.

Simin, E.-Y. 2013. *Antiviral, antibacterial and cytotoxic activities of South African plants containing cardiac glycosides.* University of Pretoria.

Standley, P. C. and Steyermark, J. A. 1946. Flora of Guatemala. *Flora of Guatemala.*

Sunderland, C. J., Steiert, M., Talmadge, J. E., Derfus, A. M. and Barry, S. E. 2006. Targeted nanoparticles for detecting and treating cancer. *Drug Development Research* 67.1**:** 70-93.

Takada, Y. and Aggarwal , B. B. 2003. Betulinic acid suppresses carcinogen- induced NF-jB activation through inhibition of IjBa kinase and p65 phosphorylation: abrogation of cyclooxygenase- 2 and matrix metalloprotease-9. *J Immunol* 171. 3278–3286.

Tania, P. and Kar, H. K. 2017. Is lati n and Characterizati n f β-Sitosterol -3-O-β- D-glucoside from the Extract of the Flowers of Viola odorata. *British Journal of Pharmaceutical Research* 16.4**:** 1-8.

Tapsell, L. C., Hemphill, I., Cobiac, L., Sullivan, D. R., Fenech, M., Patch, C. S., Roodenrys, S., Keogh, J. B., Clifton, P. M. and Williams, P. G. 2006. Health benefits of herbs and spices: the past, the present, the future.

Temple, L. K., Hsieh, L., Wong, W. D., Saltz, L. and Schrag, D. 2004. Use of surgery among elderly patients with stage IV colorectal cancer. *Journal of Clinical Oncology* 22.17**:** 3475-3484.

Thun, M. J., Delancey, J. O., Center, M. M., Jemal, A. and Ward, E. M. 2009. The global burden of cancer: priorities for prevention. *Carcinogenesis* 31.1**:** 100-110.

Todoric, J., Antonucci, L. A. and Karin, M. 2016. Targeting inflammation in cancer prevention and therapy. *Cancer Prevention Research* 9.12**:** 895–905.

Townsend, C. and Ebizuka, Y. 2010. Natural products structural diversity-I secondary metabolites: organization and biosynthesis. Elsevier, UK.

Tsasi, G. S., P.; Tsitsilonis, O.; Jürgenliemk, G.; Skaltsa, H. 2015. Is lati n, identification and cytotoxic activity of triterpenes and flavonoids from Green Walnut (Juglans regia L.) Pericarps. *Rec. Nat. Prod.* 10.1**:** 83-92.

Urech, K., Scher, J. M., Hostanska, K. and Becker, H. 2005. Apoptosis inducing activity of viscin, a lipophilic extract from Viscum album L J. *Pharm. Pharmacol.* 57.1**:** 101-109.

Walsh, M. F., Cadoo, K., Salo-Mullen, E. E., Dubard-Gault, M., Stadler, Z. K. and Offit, K. 2020. Genetic Factors: Hereditary Cancer Predisposition Syndromes. *Abeloff's Clinical Oncology***:** 180-208. e11.

Wang, Q. 2016. Cancer predisposition genes: molecular mechanisms and clinical impact on personalized cancer care: examples of Lynch and HBOC syndromes. *Acta Pharmacologica Sinica* 37.2**:** 143-149.

Wang, Z. and Yang, C. Metal carcinogen exposure induces cancer stem cell-like property through epigenetic reprograming: A novel mechanism of metal carcinogenesis. Seminars in cancer biology, 2019. Elsevier, 95-104.

Wani, M. C., Taylor, H. L., Wall, M. E., Coggon, P. and Mcphail, A. T. 1971. Plant antitumor agents. VI. Isolation and structure of taxol, a novel antileukemic and antitumor agent from Taxus brevifolia. *Journal of the American Chemical Society* 93.9**:** 2325-2327.

Who 2013. *WHO traditional medicine strategy: 2014-2023*, World Health Organization.

Who 2018. Latest global cancer data: Cancer burden rises to 18.1 million new cases and 9.6 million cancer deaths in 2018. *In:* CANCER, I. A. F. R. O. (ed.). IARC, 150 Cours Albert Thomas, 69372 Lyon Cedex 08, France.

William, C. E. and Dahhne, E. 2009. Saponins, cardioactive drugs and other steroids. *Trease and Evans' Pharmacognosy*. Sixteenth ed.

Williams-Fleetwood, S. 2009. THE ATSDR PUBLIC HEALTH ASSESSMENT: A NOTE OF EXPLANATION.

Wu, L.-C., Hsu, H.-W., Chen, Y.-C., Chiu, C.-C., Lin, Y.-I. and Ho, J.-a. A. 2006. Antioxidant and antiproliferative activities of red pitaya. *Food Chemistry* 95.2**:** 319-327.

Yabroff, K. R., Lund, J., Kepka, D. and Mariotto, A. 2011. Economic burden of cancer in the United States: estimates, projections, and future research. *Cancer Epidemiology and Prevention Biomarkers* 20.10**:** 2006-2014.

Yang, H. D., Q.P 2010. Targeting apoptosis pathway with natural terpenoids: implications for treatment of breast and prostate cancer. *Curr Drug Targets* 11. 733.

Yin, M.-C. 2012. Anti-glycative potential of triterpenes: a minireview. *BioMedicine* 2. 2-9.

Zhang, J. A., Xuan, T., Parmar, M., Ma, L., Ugwu, S., Ali, S. and Ahmad, I. 2004. Development and characterization of a novel liposome-based formulation of SN-38. *International journal of pharmaceutics* 270.1-2**:** 93-107.

Zheng, Y., Walsh, T., Gulsuner, S., Casadei, S., Lee, M. K., Ogundiran, T. O., Ademola, A., Falusi, A. G., Adebamowo, C. A. and Oluwasola, A. O. 2018. Inherited breast cancer in Nigerian women. *Journal of clinical oncology* 36.28**:** 2820.

Appendix

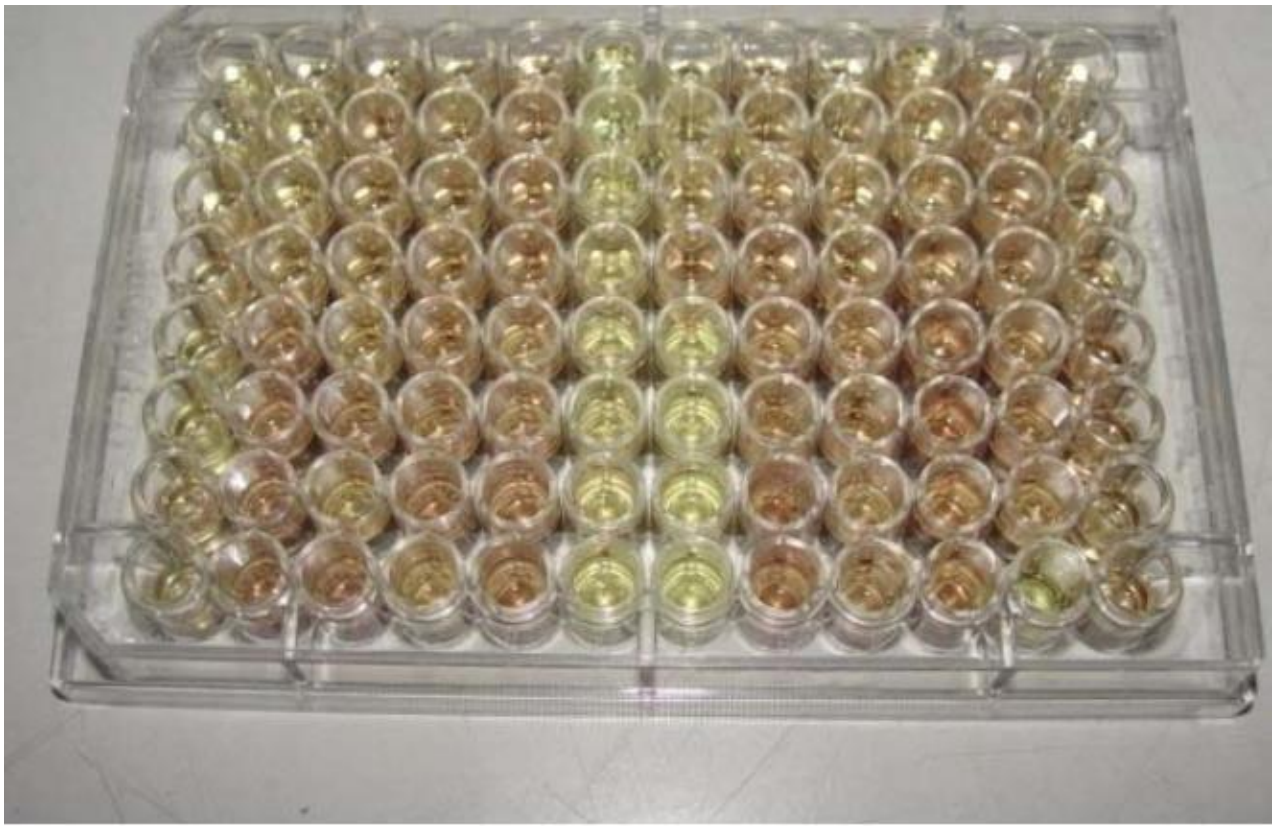


Figure 3.1: The 96 well plate containing cancer cell line and plant extracts showing yellow colour of MTT not reduced to formazan after dissolving in DMSO

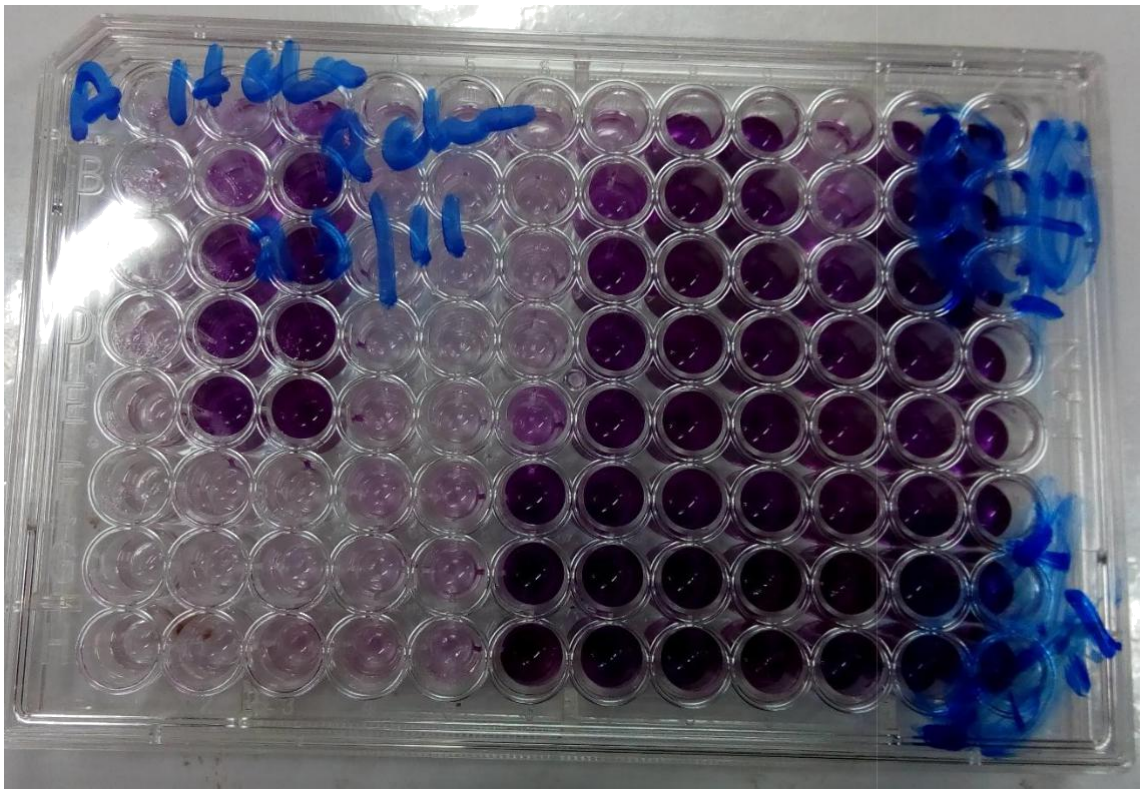


Figure 3.2: A 96 well plate showing purple colour of precipitated formazan after dissolving in DMSO

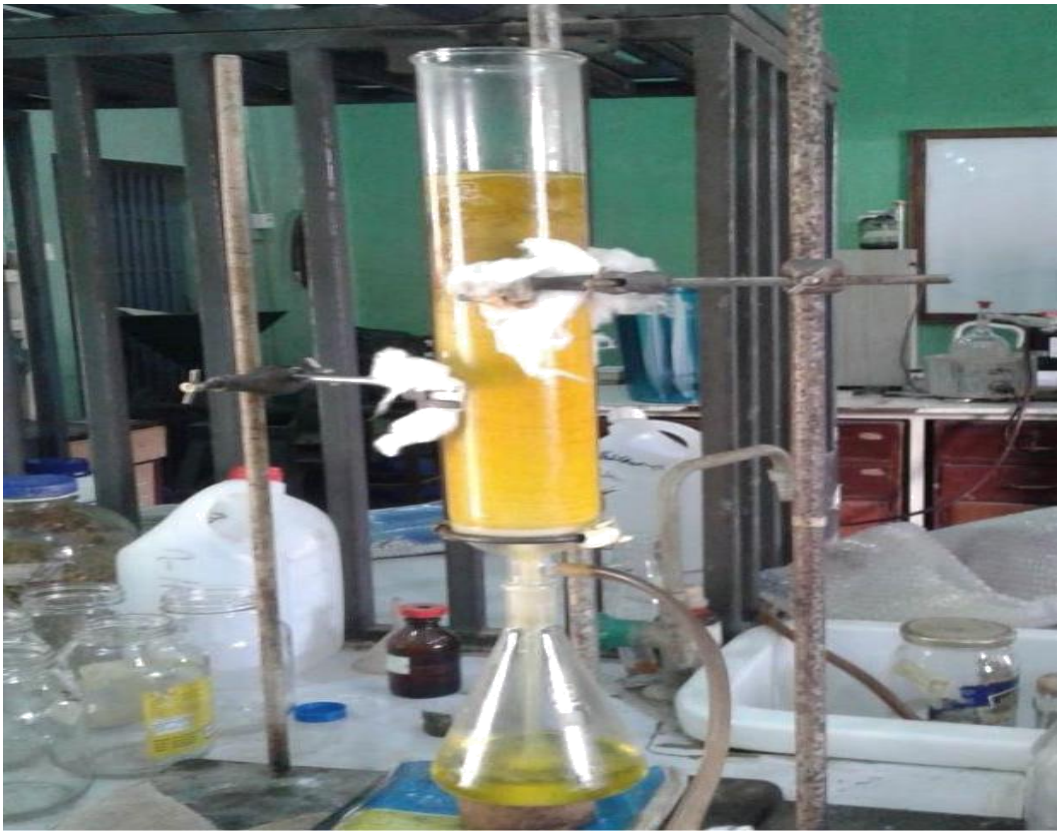


Figure 3.3: Procedure for Vacuum Liquid Chromatography of the n-Hexane fraction of *A. melegueta*

Figure 3.4: Vials showing the Vacuum Liquid Chromatography eluents of the n-Hexane fraction of *A. melegueta*

QUESTIONAIRE FOR FIELD-WORK SURVEILLANCE

To be administered to traditional healers, herb sellers, Elder, Mothers and other members of the community on cultural categorization of cancer and perceived efficacy of herbs.

INTRODUCTION

Greetings

My name is…….. I'm a student f the Faculty f Pharmacy, University f Ibadan where we are carrying out a study on the use of drugs (orthodox and traditional) in the treatment of cancer. This will assist us the researcher to know what you do and how we can work together and bring about progress into the treatment of cancer in our community. This research is important because cancer is one of the diseases causing ill health and death especially among men and woman. We will appreciate your honest responses to these questions. It is not compulsory for you to participate if you are not willing to do so and there will be no consequences for refusing. All your responses will be kept confidential, as your name will not be on this questionnaire. The outcome of this research will be used in drug development for cancer treatment. We will be happy if you answer the questions in detail and truthfully. Thank you.

Yours sincerely,

AMBALI, Owoola, A.

Willingness to participate: 1. YES ☐ 2. NO ☐

Place of interview: 1. RURAL ☐ 2. URBAN ☐

Study site: …… Date of Interview: ……

DEMOGRAPHIC CHARACTERISTIC

SECTION A

1. Type of respondents:
   i. Traditional healer ii. Herb seller iii. Other (Specify).
2. Age (years): i. 20-30 ii. 30-40 iii. 40-50 iv. 50-60
   v. 60-70: vi. Above 70
3. Sex: i. Male ii. Female
3. Religion: i. Christian ii. Muslim iii. Traditional worshiper iv. Others (Specify)
4. Education: i. None ii. Primary iii. Secondary iv. Tertiary
5. Occuati n: ……
6. Marital Status: i. Single ii. Married iii. Divorced iv. Widow/widower
7. State f Origin: ……..
8. Tribe: i. Yoruba ii. Hausa iii. Igbo iv. Others (Specify)

SECTION B

CULTURAL CATEGORIZATION OF CANCER

9. Have you ever heard of cancer?
10. If yes, mention the different types you know?

a)…….

b) ……

c) ……

d) ……..

e) ……..

11. Describe the different type you mentioned (A-E) with regards to the following features

	How person gets it	Complaints of ill person	Whom it commonly affects	When it commonly occurs	Where it usually occur	How it can be prevented
A						
B						
C						
D						
E						

12. Rank in ascending order the commonest or most popular of these types of cancer

ORDER	TYPE
1ST	
2ND	
3RD	
4TH	

13. Can the different type of cancer (A-E) be treated with traditional herbs?

TYPES OF CANCER	YES	NO
A		
B		
C		
D		
E		

14. List all the herbs that you know, that can be used to treat each type (A-E)

NAME OF HERBS USED IN TREATING EACH CANCER.

	A	B	C	D	E
1					
2					
3					
4					
5					

15. For cancer A, how are the herbs you mentioned collected and stored, prepared and processed?

Herb	Herb parts	Time of Collection	Stored	Processed
Herb 1				
Herb 2				

16. For cancer B, how are herbs you mentioned collected and stored, prepared and processed?

Herb	Herb parts	Time of Collection	Stored	Processed
Herb 1				
Herb 2				

17. For cancer C, how are herbs you mentioned collected and stored, prepared and processed?

Herb	Herb parts	Time of Collection	Stored	Processed
Herb 1				
Herb 2				

PERCIEVED EFFECTIVENESS

18. Rank an ascending order, the most efficacious herb for treatment in different type of cancer (A-E).

ORDER	CANCER A	CANCER B	CANCER C	CANCER D	CANCER E
1ST					
2ND					
3RD					
4TH					

19. What are the side effects of the herb you mention?

Herb	Side Effect 1	Side Effect 2	Side Effect 3	Side Effect 4
Herb 1				
Herb 2				
Herb 3				

20. Do you have any suggestions on how the local drugs for treating cancer could be improved?

……
……

Thank you very much for your time to respond to this questionnaire.