# EVALUATION OF ANTIBREAST AND ANTIPROSTATE CANCER ACTIVITIES OF SELECTED MEDICINAL PLANTS FROM SOME PARTS OF KENYA

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**A THESIS SUBMITTED IN FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF DOCTOR OF PHILOSOPHY (PHARMACOGNOSY AND COMPLEMENTARY MEDICINE) IN THE SCHOOL OF PHARMACY OF KENYATTA UNIVERSITY**

**NOVEMBER, 2019**

## DECLARATION

This thesis is my original work and has not been presented for a degree in any other University or any other award.

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## DEDICATION

This work is dedicated to my family, Eucabeth Bosibori (wife), sons: Enosh Osano, David Onyancha and daughters: Tabitha Moke, Ruth Moraa and Esther Kemunto.

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## ABBREVIATIONS AND ACRONYMS

| | |
|---|---|
| $^{13}$C NMR | Carbon 13 Nuclear Magnetic Resonance |
| $^{1}$H NMR | Proton Nuclear Magnetic Resonance |
| 22Rv1 | Human prostate cancer cell line |
| 4T1 | Mouce breast cancer cell line |
| ATCC | American Type Culture Collection |
| CC | Column chromatography |
| $CC_{50}$ | Drug concentration that is toxic to 50% against the target cells |
| $C_3D_6CO$ | Deuterated acetone |
| CTMDR | Centre for Traditional Medicine Development and Research |
| CVR | Centre for Viral Research |
| d | Doublet |
| DMSO | Dimethyl sulfoxide |
| DMEM | Dulbecco's modified Eagle medium |
| DU-145 | Human prostate cancer cell line |
| EMEM | Eagle's Minimum Essential Medium |
| EI | Electron impact |
| ELISA | Enzyme linked immunosorbent assay |
| FAB | Fast atomic bombardment |
| FBS | Foetal bovine serum |
| FTIR | Fourier transform Infrared |
| GLOBOCAN | Global burden of cancer website |
| HCC 1395 | Human breast carcinoma |

| | |
|---|---|
| Hz | Hertz |
| $IC_{50}$ | Drug concentration that inhibit cell prolifetration by 50% |
| IARC | International Agency for research on Cancer |
| IR | Infrared |
| *J* | Coupling constant |
| m | Multiplet |
| MDGs | Millennium development goals |
| MeOD | Deuterated methanol |
| MeOH | Methanol |
| MHz | Megahertz |
| MKU | Mount Kenya University |
| ml | Millilitre |
| MS | Mass spectrometry |
| MTT | 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyltetrazolium bromide |
| M/z | Mass to charge ratio |
| NCCs | National centre for cell sciences |
| OECD | Organization for economic cooperation and development |
| PBS | Phosphate Buffered Saline |
| ppm | Parts per million |
| $R_f$ | Retention factor |
| RPMI | Roswell Park Memorial Institute medium |
| s | Singlet |
| SEM | Standard error of the mean |
| SI | Selectivity index |

| | |
|---|---|
| SR | Solvent recrystallization |
| Syn. | Synonym |
| t | Triplet |
| TLC | Thin layer chromatography |
| TMS | Tetramethylsilane |
| µg | Microgram |
| UV | Ultraviolet |
| vero | African green monkey kidney epithelial cells |
| WHO | World Health Organization |
| δ | Chemical shift |
| $\lambda_{max}$ | Maximum wavelength |

## ABSTRACT

Cancer is a group of diseases characterised by uncontrolled proliferation of cells. Of all the types of cancers worldwide, breast cancer is the most commonly diagnosed in women while prostate cancer is the second in men. The current cancer management methods have challenges including unpleasant side effects, high cost and even not effective. As the number of patients is on the rise, physicians look forward with hope to the discovery and development of safe, effective and less toxic anticancer drugs. More than 67 % of prescribed anticancer drugs have been developed based on natural products. The objective of this study was to evaluate anticancer activities of extracts obtained from *Fagaropsis angolensis*, *Hydnora abyssinica*, *Launaea cornuta*, *Spermacoce princeae*, *Combretum tanaense*, *Uvariodendron anisatum*, *Marsidenia schimperi* and *Prunus africana* against breast and prostate cancer cells. Methanol and water extracts from the seven plants were evaluated for anticancer activities using methyl thiazole tetrazolium cell viability (MTT) assay and microtiter 96 well plates. Breast cancer (HCC 1395 and 4T1) and prostate cancer (DU-145 and 22RV1) cell lines were used in this study. The contols that were used in this study were cyclophosphamide and fluorouracil for positive chemotherapeutic agent and African green monkey kidney epithelia normal cell (vero) for for cancer cells. Enzyme linked immunosorbent assay (ELISA) scanning multiwell spectrophotometer was used to measure optical densities to calculate cell viability. Analysis of concentrations that inhibited 50% of cell growth ($IC_{50}$) was done using Prism Graphpad version 8.0. Remarkable activities of extracts ($IC_{50}$ < 50 µg/ml) were demonstrated by the methanol extracts of *C. tanaense* root, *U. anisatum* root, *H. abyssinica* rhizome, *M. schimperi* husks, *M. schimperi* leaves and *F. angolensis* stem bark. High selectivity indices were revealed *F. angolensis* extrcats. Bioassay-guided isolation of these extracts resulted to isolation of seven compounds. The active fractions were those *F. angolensis* and *C. tanaense* extracts, dichloromethane and ethyl acetate fractions, respectively, the two fractions exhibited anticancer activities with moderate ($1 \leq SI \leq 3$) to high ($SI > 3$) selectivity indices. The isolated compounds were coded as $FC_1$, $FC_2$, $FC_3$, $CC_1$, $CC_2$, $UC_1$ and $UC_2$. The $FC_{1-3}$ compunds were active against cancer cell lines, $CC_{1-2}$ revealed moderate activities and $UC_{1-2}$ were not active. $FC_1$ revealed high selectivity indices against the cancer cell lines. All extracts that demonstrated remarkable anticancer activities revealed no toxic effects upon acute oral toxicity studies on swiss mice. It was therefore established that plants that were selected on the basis of ethnopharmacological approach had potential anticancer activities and were also relatively safe. Moreover, the compounds that were isolated were remarkably active and less toxic. This study therefore provided scientific basis for validating the use of extracts from *Fagaropsis angolensis* stem bark and *Hydnora abyssinica* rhizome in the management and treatment of breast and prostate cancers.

# CHAPTER ONE: INTRODUCTION

## 1.1 Background information

Cancer is a term used for a large group of diseases characterized by a shift in the control mechanisms that govern cell survival, proliferation and differentiation (American Cancer Society, 2019). The cancerous cells multiply excessively and form solid or liquid tumors which are benign, malignant, invasive or metastatic in nature (Katzung *et al*.,, 2012). Cancer cells are characterized by chromosome aberration resulting to a series of metabolic abnormalities which cause illness and death of the patients and this situation calls for medical intervention (Katzung *et al*., 2012). The oldest evidence of cancer in the world was around 3000 BC, and it is revealed among fossilized bone tumors, human mummies in ancient Egypt (Morrison, 2010; Kamil and Kamil, 2015).

Based on the International Agency for Research on Cancer (2018), the annual cancer incidences are estimated to have increased to 18.1 million worldwide. At the same time, cancer related deaths have also increased to 9.6 million (International Agency for Research on Cancer, 2018). Assuming that the underlying rates of cancer will remain unaltered, the number of new cancer cases and deaths will double by the year 2030 (Atun and Cavalli, 2018). Cancer is expected to be the leading cause of deaths worldwide in the 21$^{st}$ century (Bray *et al*., 2018). Most of all worldwide cancer deaths occur in low and middle-income countries (Ferlay *et al*., 2015). In Africa, over 847,000 new cancer cases and 635,400 cancer deaths are recorded annually (Dent *et al*., 2017) with the highest incidences (287,300) and deaths (208,500)

recorded in East Africa (Boyle *et al*., 2016). Like in the rest of the world, incidences and deaths in East African countries are expected to increase at an alarming rate due to aging, growth of population, adoption of behavior associated with western lifestyles (Westernization) such as smoking tobacco, unhealthy diet, physical inactivity and poverty (Kuete *et al*., 2016).

In Kenya cancer is the third killer disease after cardiovascular and infectious disease with annual incidences and mortalities of over 47,887and 32,987 respectively (Ministry of Health, 2018). This indicates that over 70 Kenyan people die daily from cancer related illness. The republic of Kenya like many other developing countries is characterized by ever increasing cancer burdened. Though it has made tremendous advances in the fight against infectious disease including acquired immunodeficiency syndrome (HIV/AIDS), malaria, schistosomiasis, polio, tuberculosis among others, the country is not fully prepared to deal with the cancer burden satisfactorily (Topazian *et al*., 2016). However, Kenya is making great efforts towards reduction of cancer mortalities like establishment of act of parliament and the Cancer National Institute (Government of Kenya, 2012), National Policy Guidelines and National Cancer Control and Prevention Strategies (Ministry of Health, 2012; 2013a; 2013b; 2017; Ministry of Public Health and Sanitation and Ministry of Medical Services, 2012).

### 1.2 Problem statement

The management of cancer by surgery, radiotherapy, chemotherapy and immunotherapy are attributed to conventional medicine system (global standard medical care). These treatment options have serious drawbacks resulting from a

delicate balance between the destruction of cancerous tissues and sparing normal health cells. The side effects range from mild forms like loss of appetite, nausea, vomiting, fatigue, alopecia, skin and nail changes, diarrhea to severe forms such as anemia, leukemia, pain, fertility and sexual problems, loss of memory and even infections or therapeutic related deaths (National Cancer Institute, 2018). In addition to the challenges experienced with conventional methods of treatment, the situation becomes more complicated with chemoresistance of the cancer cells against the available treatment regimens (Teoh and Pavelka, 2016). Many of the cancer patients in Africa including Kenya face limited access to cancer treatments due to inadequate facilities and high costs, a great proportion of patients never get cured due to delayed diagnosis (Kenyan Network of Cancer Organizations, 2016). It is known that over 49% cancer patients use complementary and alternative medicines (CAM) for management of cancer (Yalcin *et al*., 2018). Scientific evidence on efficacy of some of plants used in Kenya and Africa in general is scarce or lacking or together. Secondly, data on side effects which may result from use of such treatment is lacking. Therefore, potential for development of Kenyan plant extract into viable anticancer medicines should be explored.

### 1.3 Justification

In some parts of the world and history, the plant used in complementary alternative remedies have scientific backing to provide rationale for their use. Plants have been reported to play a significant role in the discovery of anticancer drugs and still have potential in future discoveries (Mishra and Tiwari, 2011). Ethnomedicine reports indicate that over 78% of Kenyan population use traditional medicines to cure and prevent diseases in primary health care (Ochwang'i *et al*., 2014). However, less than

10% of products obtained from nature have been pharmacologically and chemically assessed (Dias *et al*., 2012). Therefore, the current study on the documentation and assay of anticancer activities of *Combretum tanaense*, *Fagaropsis angolensis*, *Hydnora abyssinica*, *Launaea cornuta*, *Spermacoce princeae*, *Marsidenia schimperi* and *Uvariodendron anisatum* was necessary to increase the percentage of anticancer plants that have scientific data as a requirement of Kenya stated in the Health Act 2017 as an effort to strengthen traditional medicine system in the country. This study also provides information which other researchers can build on during drug development.

**1.4 Research questions**

(i) Are there anticancer plants used by traditional medicine practitioners in selected regions of Kenya?

(ii) What are the *in vitro* anticancer activities of the crude extracts from selected medicinal plants against the human breast carcinoma (HCC 1395 and 4T1) and prostate cancer (DU-145 and 22RV1) cell lines?

(iii) Which fraction(s) of the active crude extract(s) has/have *in vitro* anticancer activities against the Human breast carcinoma (HCC 1395) and prostate cancer (DU-145) cell lines?

(iv) What compound (s) are present in fractions that has/have *in vitro* anticancer activities against the human breast carcinoma (HCC 1395 and 4T1) and prostate cancer (DU-145 and 22RV1) cell lines?

(v) What are the cellular toxicity levels and selectivity indices of extracts, fractions and isolated compounds against normal cell line?

(vi) What are the toxic effects of active crude extract(s) on swiss albino mice?

## 1.5 Hypothesis

Extracts and compounds from *Combretum tanaense* root, *Fagaropsis angolensis* bark, *Prunus africana*, *Hydnora abyssinica* rhizome and flower, *Launaea cornuta* aerial parts, *Spermacoce princeae* aerial parts, *Marsidenia schimperi* leaves and husks and *Uvariodendron anisatum* root do not have activities against breast and prostate cancer cells.

## 1.6 Objectives

### 1.6.1 General objective

To establish and evaluate anticancer activities of some medicinal plants used for management of breast and prostate cancers from selected parts of Kenya

### 1.6.2 Specific objectives

(i) To identify anticancer plants used by traditional medicine practitioners in selected regions of Kenya

(ii) To determine *in vitro* anticancer activities of the crude extracts from the selected medicinal plants against the human breast carcinoma (HCC 1395 and 4T1) and prostate cancer (DU-145 and 22RV1) cell lines.

(iii) To determine *in vitro* anticancer activities of fractions obtained from active crude extracts against human breast carcinoma (HCC 1395) and prostate cancer (DU-145 and) cell lines

(iv) To identity compounds, present in fractions having anticancer activities against human breast carcinoma (HCC 1395 and 4T1) and prostate cancer (DU-145 and 22RV1) cell lines

(v) To determine cellular toxicity levels and selectivity indices of extracts, fractions and isolated compounds against normal (vero) cell line.

(vi) To evaluate acute oral toxicity of active extract(s) using swiss albino mice

### 1.7 Study significance and outputs

The study was intended to contribute to knowledge of plants used in Kenya for management of cancer. Three out of the seven plants in this study were reported for the first time. Further *in vitro* anti-breast and anti-prostate cancer studies of the seven plants and their fractions were reported for the first time in this study. Isolation of compounds with anticancer activities from the plants laid a basis for validating the plants in the use of managing cancer in the Kenya traditional medicine system. Some of the findings were disseminated through relevant peer reviewed journals so that this information may be accessible by other researchers (Appendix 1 and 2). The findings provide a baseline for evidence based complementary and alternative medicines and the pharmaceutical industry could use these findings to develop new anticancer drugs. The extracts or fractions would also be standardised and improved by the pharmaceutical industry for commercialization.

# CHAPTER TWO: LITERATURE REVIEW

## 2.1 The biology and pathophysiology of cancer

Cancer is a broad group of various diseases characterized by unregulated cell growth and proliferation. It is as a result of transformation of normal cells into cancer cells (neoplasms) in a process known as oncogenesis (Croce, 2008). Typically, multiple genes are required to transform a normal cell into a cancer cell. In addition, a single cancer cell undergoes successful rounds of mutations and selective expansion which result to the formation of a tumor mass, subsequent mutations and expansion lead to tumor growth and progression (Knudson, 2001; Momna, 2010). Accumulated mutations that involve oncogenes, tumour suppressor genes, DNA repair genes (genes that govern cell growth and division processes) and chromosomal alterations are the primary cause of cancer (Baylin and Ohm, 2006). Different mammalian cells have similar molecular networks that control cell proliferation, differentiation and cell death. The mutations that alter the network at molecular, biochemical and cellular levels results to cancer cells which invade or spread to the entire body (WHO, 2018).

All cancer cells are characterized by low sensitivity to anti-growth signals, sustained angiogenesis, unlimited replicative potential, tissue invasion, apoptosis avoidance, change in cell metabolism and behavioural changes. The cancer cells can form benign (*in situ*) tumors which grow uncontrollably but do not invade the neighbouring tissues nor spread throughout the body, while other cancer cells form malignant tumors which invade neighbouring tissues and spreading to distant parts of the body through lymphatic and blood circulation systems (Bora and Parihar, 2018). Over 90% of cancer related deaths are due to metastastic tumor (Momna, 2010). The

cancer cells do not contribute to body functions, they deplete body nutrients leading to malnutrition, weakness and wasting of the body (Younes and Noguchi, 2000). Increase in size of tumors exert pressure on neighbouring organs and cause distortion of the tissues and interfere with blood, nervous and lymphatic access. Cancer cells can invade bone and bone marrow, this cause haematological neoplasms which affect blood, bone marrow and lymphatic system. Some of their metabolites induce anorexia, inflammation, coagulation, blood pressure, ulceration, cancer related fatigue and pain. Furthermore, cancerous tissues are susceptible to infections too (Doll, 2018).

## 2.2 Aetiology of cancer

Transformation of normal body cells to cancerous cell is a multistage process. It results to precancerous lessons which usually grow into malignant tumours (American Cancer Society, 2019). More than 90% of cancer cells arise due to the interaction between genetic and environmental (modifiable) factors, these cause cellular alterations that lead to carcinogenesis. On the other hand, up to 10% of the cancer cells are caused by non-modifiable factors which include inherited gene mutations, hormones and immune conditions (Islami *et al*., 2018; American Cancer Society, 2019). The environmental carcinogenic factors further categorised into as physical, chemical and biological factors (Islami *et al*., 2018).

### 2.2.1 Physical carcinogens

The physical carcinogens cause cancer through their physical effects (Maltoni and Holland, 2000). These include ionizing radiations which are characterized by high energy, enough to damage deoxyribonucleic acid (DNA) and cause cancer (IARC;

Working Group on the Evaluation of Carcinogenic Risks to Humans, 2009). The X-rays, radon, gamma rays, prolonged exposure to ultraviolet radiations, frequent application of heat objects onto the body, physical trauma and chronic inflammation are known physical carcinogens (Santosh *et al*., 2017).

### 2.2.2 Chemical carcinogens

Exposure to specific chemicals has been associated with specific types of cancer (Santosh *et al*., 2017). While tobacco is responsible for causing 25-30% of all cancers, over 50 carcinogenic compounds most of them being nitrosamines and polycyclic aromatic amines are known in tobacco (Biesalski *et al*., 1998). Other chemical substances known to cause cancer also occur in alcohol, polluted air, water and food. More than 90% of lung cancer cases are caused by tobacco smoke. Some other cancers associated with tobacco smoking are cancers of the larynx (voice box), mouth, oesophagus, throat, bladder, kidney, liver, colon, stomach, pancreas, cervix, rectum, liver and acute myeloid leukemia (Kuper *et al*., 2002a; Kuper *et al*., 2002b).

Acetaldehyde is an alcohol metabolite produced in the human body with carcinogenic effects. Other carcinogenic contaminants like phenols, hydrocarbons, asbestos fibres and nitrosamines which may be introduced into alcohol during production exacerbate carcinogenicity of alcoholic beverages (IARC;Working Group on the Evaluation of Carcinogenic Risks to Humans, 2010;2012). Alcohol cause liver, neck, head, colorectal and breast cancers (Santosh *et al*., 2017).

### 2.2.3 Biological carcinogens

The known biological factors that infect humans are associated with carcinogenesis are viruses, bacteria and parasites. Globally, about 18% of cancer deaths are related

to these infectious agents (Anand *et al*., 2008) whereas in Africa the proportion is higher (about 25%) compared to developed countries (less than 10%) (Anand *et al*., 2008). Some viruses that cause cancer (oncoviruses) can disrupt signals that normally keep cell growth and proliferation in check. Examples include Human papillomavirus (HPV) cause cervical, oropharyngeal, vaginal, vulvar and penile cancers. Herpesvirus (KSHV) also known as human herpesvirus-8 (HHV-8) cause Kaposi's sarcoma and primary effusion lymphomas. Chronic infections with hepatitis B virus and hepatitis C virus (HBV and HCV) cause liver or hepatocellular carcinoma. Epstein-Barr Virus cause lymphoma and cancers of the nose and throat, Merkel Cell Polyomavirus (MCPyV) (skin cancer) and Human T-Cell Leukemia/Lymphoma Virus Type 1 (non-Hodgkin lymphoma called adult T-cell leukemia/lymphoma) (Pagano *et al*., 2004).

Human Immunodeficiency Virus (HIV) does not cause cancer but supresses the immune system of the body and makes it less able to fight other infections that cause cancer (Ljubojevic and Skerlev, 2014). Bacterial infections like *Helicobacter pylori* are known to cause stomach cancer (gastric carcinoma) while parasitic infections like *Opisthorchis viverrini* and *Clonorchis sinensis* induce cholangiocarcinoma cancer (cancer of the bile ducts in the liver) and *Schistosoma haematobium* increase the likelihood of developing squamous cell carcinoma of the bladder (Samaras *et al*., 2010; Ljubojevic and Skerlev, 2014).

### 2.2.4 Diet, inactivity and obesity

Intemperate diet schedule and inactivity may cause obese (body overweight). This is an indispensable lifestyle subject because overeating (overnutrition) contributes to

more than half of food related cancer deaths (National Cancer institute, 2017). In addition, physical inactivity has negative effects on body weight, immune and endocrine systems and contribute to unhealthy amount of body fats. Obesity and inactivity are known to cause about 30-35% cancer deaths (Islami *et al*., 2018). Consistent evidence indicates that there are a number of obesity related cancers including liver, breast, ovarian, thyroid, gallbladder, colorectal, meningioma, pancreatic, kidney, gastric, endometrial oesophageal and multiple myeloma (National Cancer institute, 2017). Specific foods and food additives also induce cancer, example are Betel nut that induce oral cancer and high salted diet that promote carcinogenesis of gastric and colon cancer (Park *et al*., 2008; Brenner *et al*., 2009). Processed meat like bacon, ham, hot dogs, sausages and, to a lesser degree, red meat, pickled foods and refined carbohydrates are linked to some cancers (Wicki and Hagmann, 2011; Bouvard, 2015; Hauser, 2015). Food contaminants like aflatoxins are also associated with liver cancer (Park *et al*., 2008).

### 2.4.5 Hereditary

The American Society of Clinical Oncology (2018) indicate that about 5-20% of all cancers are due to inherited genetic defects (germline mutations) while the rest of the cancers are caused by acquired mutations. Gene alterations that occur are as a result of multiple uncorrected mutations that occur over a long period, these may be due to failure of tumour suppressor genes, DNA repair genes and presence of oncogenes. Protective genes (tumour suppressor genes) limit cell growth by checking on cell multiplication rate, correction of altered DNA and controlling apoptosis. They are similary called DNA repair genes. Examples include, breast cancer gene one (BRCA1), breast cancer gene two (BRCA2) which are associated with inheritary

breast, ovarian, prostate, pancreatic and melanoma cancers (Roukos, 2009; American Society of Clinical Oncology, 2018a).

Tumor Protein p53 (TP53) is associated to more than 50% of acquired cancers (American Society of Clinical Oncology, 2018a). Other mutations that are known to have increased risk of cancer are Phosphatase and TENsin homolog (PTEN) gene, Partner And Localizer of BRCA2 (PALB2), Checkpoint Kinase 2 (CHEK2), CaDHerin 1 (CDH1) and Serine/Threonine Kinase 11 (STK11). The Phosphatase and TENsin homolog (PTEN) gene which is responsible for limiting tumor growth by controlling the rate of cell division, induce autolysis of damaged cells before they can become cancerous. Mutation of PTEN is associated with Cowden syndrome, an inherited disorder that increases the risk of breast, thyroid, endometrial, and other types of cancer (Gammon *et al*., 2016). PALB2 enables BRCA2 gene to correct DNA damage and prevent breast cancer. CDH1 like PTEN causes cancer cells to stick together therefore preventing their spread, CHEK2 synthesize a protein that suppresses tumor growth and STK11 suppresses Peutz-Jeghers syndrome which is associated with increased risk of many types of cancers (https://www.national, 2018).

### 2.4.6 Hormones

Gender related cancers like cancers of the breast, prostate, cervix, endometrium, ovary, testis are induced by hormones which promote cell proliferation. Oestrogen and progesterone are associated with breast and cervical cancers, on the other hand, high levels of testosterone in men are associated with prostate cancer (Chan and Yeung, 2006). Also insulin-like growth factors and growth hormones promote cancer

development (Pollak *et al*., 1999; Rieunier *et al*., 2019). In addition, cancers of the thyroid and bones are also induced by hormones (Henderson *et al*., 2000).

### 2.4.7 Autoimmune diseases

Some autoimmune diseases are associated with cancer development. Celiac disease which affect the small intestines is associated with all cancers. Chronic inflammatory conditions in the gastrointestinal tract may result to autoimmune diseases such as Crohn's disease and ulcerative colitis. These conditions are responsible for gastrointestinal cancers. In addition, immunomodulators and biologic agents may cause extraintestinal cancers (Han *et al*., 2015; Axelrad *et al*., 2016).

## 2.5 Staging systems for cancers

Cancers develop through various stages in the respective body sites, the description of the extent or spread of a particular cancer at the time of diagnosis is called staging. Most cancers are staged on the basis of two factors; first, size of the primary tumour and secondly, the extent to which cancer has spread to the nearby lymph nodes or other parts of the body (American Cancer Society, 2019). Staging systems for cancer are classified into three; the summarised system, the tumour, node and metastasis (TNM) and the surgical resection (R). Summarised classification categorise cancer into two stages as *in situ* and invasive cancers. In the *in-situ* stage cancer cells are confined to where the layer of cells develops and do not spread. On the other hand the invasive stage is characterised by the spread of cancer cells to other tissues other than the original layer of tissue (American Cancer Society, 2019). The invasive stage is further categorized into three sub-stages based on the extent of spread as local, regional and distant. Local if the cancer is confined to the organ of origin, regional

when cancer has spread to the nearby organs or lymph nodes or both and finally, distant when the cancer cells have spread to far tissues or organs. The spread is through direct extension or discontinuous metastasis that involves tissues, organs and lymph nodes (American Cancer Society, 2019).

Tumour, node, metastasis (TNM) staging system was developed by International Union Against Cancer (IUAC) (American Cancer Society, 2019). It is the mostly used system in clinical set ups. Alphanumeric notations are used to define the extent of the growth and spread of cancer into three, first assessment of the size of the main or primary tumour (T), the involvement of the regional lymph nodes (N) and the distance of metastases (M). Numbers of 0, I, II, III or IV are assigned after the T, N and M to provide more details about the advancement of each of cancer spread. Assignment of numbers as *in situ* stage (0), early stage (I) and late stage (IV), the higher the number denotes more advancement of the cancer (Ministry of Health, 2013b; American Cancer Society, 2019).

The third classification system is based on the evaluation of surgical resection margin. It is also called the R classification system. Completeness of surgical excision of primary tumour and regional nodes are assessed in reference to tumour (T) and node (N) without the assessment of metastasis (M). The system classifies cancer stages into four, residual tumour undetectable (RX), absence of residual tumour (R0), microscopic residual tumour (R1) and macroscopic residual tumour (R2). Complete removal of all local tumour corresponds to resection margins that are free of tumour. The staging systems play a significant role in the determination of

choices of treatment and guidance of prognosis of the disease (Ministry of Health, 2013b).

### 2.6 Classification of cancers

There are more than 100 types of cancers named after the organ or type of cell in which they arise (Pinto *et al*., 2018). The cancers are classified based on the type of normal body cell the tumour cells resemble, therefore they are presumed to be the origin of the tumour. Four major commonly known types of cancers are carcinomas, sarcomas, Leukemia and lymphomas, multiple myeloma and melanoma (https://www.cancerresearchuk.org. 2019).

#### 2.6.1 Carcinomas

The cancers formed by epithelial cells that cover the inner or outer surface of the body are called carcinomas. Carcinomas are further classified as adenocarcinoma, squamous cell carcinoma (epidermoid carcinomas), transitional cell carcinoma and basal cell carcinoma. Adenocarcinoma form in tissues or glands that produce fluids or mucus such as breast, colon, lung, pancreas and prostate. Squamous cell carcinoma is also called epidermoid carcinomas. It forms in the epithelial cells that lie just beneath the outer surface of the skin, it also involves the cells that line many other other organs such as the stomach, intestines, lungs, bladder and kidneys. Transitional cell carcinoma arises from the transitional epithelium, or urothelium, this tissue is made up of many layers of epithelial cells that can get bigger and smaller, is found in the linings of the bladder, ureters, and part of the kidneys (renal pelvis), and a few other organs. Some of the transitional cell carcinoma include cancers of the bladder, ureters and kidneys. Lastly, basal cell carcinoma is a cancer

that begins in the lower or basal (base) layer of the epidermis, which is a person's outer layer of skin (Ministry of Health, 2013b; National Comprehensive Cancer Network, 2014).

### 2.6.2 Sarcomas

Sarcomas forms in connective (bone, soft and fibrous) tissues of the body, including muscle, tendons, fat, blood vessels, lymph vessels, nerves and tissue around joints. They develop from cells originating in mesenchymal cells outside the bone marrow. Osteosarcoma is the most common cancer of bone. The most common types of soft tissue sarcoma are leiomyosarcoma, Kaposi sarcoma, malignant fibrous histiocytoma, liposarcoma, and dermatofibrosarcoma protuberans (National Comprehensive Cancer Network, 2014).

### 2.6.3 Leukemia, lymphoma and multiple myeloma

Leukemia, lymphoma and multiple myeloma are cancers that develop from hematopoietic cells (blood forming cells) and do not form solid tumors. Leukemias arise from abnormal cells of blood forming tissue of the bone marrow, uncontrolled division of cells results in large numbers of abnormal white blood cells (leukemia cells and leukemic blast cells) in the blood and bone marrow, excessive population of abnormal cells interfere with oxygen circulation in tissues, control of haemorrhage, or protection against infections (Ray, 2012).

There are different forms of leukemia which occur mostly in adults of over 55 years of age. However, leukemia is also reported to be common in children of below 15 years. The disease can be divided into myelogenous (myeloid) or lymphocytic

(lymphoblastic) on the basis of the types of white blood cells they are formed from or into acute or chronic depending on the rate of growth of the cancer (Ministry of Health, 2013b).

Lymphoma cancer involve the abnormal growth of lymphocytes (B cells and T cells), they spread and accumulate in the lymph nodes and lymph vessels, as well as in other organs of the body. There are two main types of lymphomas, the Hodgkin lymphoma characterized with abnormal lymphocytes that are called Reed-Sternberg cells and the Non-Hodgkin lymphoma which constitute a large group of cancers that start in lymphocytes (Varricchio, 2004).

Myeloma is cancer of the bone marrow, it involves plasma cells, which are white blood cells that produce distinctive proteins known as antibodies. The cancerous cells grow and multiply in the bone marrow. The disease is referred to as multiple myeloma because it involves different sites of the body at the time of diagnosis. There are two forms commonly known myeloma, the localized and extramedullary myeloma. The former involves a few neighbouring sites while the latter involves other tissues other than the bone marrow including the dermal, pulmonary and muscular tissues (Grace, 2013; Kiraka *et al*., 2014).

### 2.6.4 Melanoma

Melanoma is a type of skin cancer that is formed by uncontrolled multiplication of melanocytes (melanin containing cells on the skin). Melanoma may also occur in the mouth, eye and intestines (WHO, 2014b). Ultra violet light is the main predisposing factor to the development of melanoma. However, family history, poor immune function, devices that produce ultra violet light for darkening skin colour and genetic

defects like xeroderma pigmentosum also increase the risk of developing melanoma (Kanavy and Gerstenblith, 2011; Azoury and Lange, 2014).

### 2.7 The burden of cancer (incidences and mortality)

The burden of cancer is greater in women that it is in men globally. Among men the five most common sites of cancer diagnosed are lung (16.7%), prostate (15.0%), colorectal (10.0%), stomach (8.5%), liver (7.5%) of the total cancers in men. On the other hand, the cancers that cause most deaths in men are lung cancer (23.6% of the total), liver cancer (11.2%) and stomach cancer (10.1%) of the total cancer deaths (WHO, 2014a). Among women, five types of cancers that are commonly reported are cancer of the breast (25.2%), colorectal (9.2%), lung (8.7%), cervix (7.9%) and stomach (4.8%). The most cancer deaths in women are due to breast cancer (14.7%) and lung cancer (13.8%) (WHO, 2014a). In both sexes, five most common incident sites of cancers are lung (13.0%), breast (11.9%), colorectal (9.7%), prostate (7.9%), and stomach (6.8%) (WHO, 2014a).

The situation of cancer in Africa is critical and is exacerbated by the fact that most countries in African continent are low and middle-income countries (LMICs), therefore many patients do not seek or cannot access professional medical services on time. Many cancer patients in Africa are more often diagnosed at an advanced stage when cure is no longer possible. Among African men, prostate cancer has the highest incidence and mortality rates while among women, breast cancer was the most commonly diagnosed in Africa and was the leading cause of cancer deaths in 2012 (Boyle *et al*., 2016).

Breast cancer has the second highest prevalence (19.6 %) after cervical cancer (23.3%) in Africa. However, in Kenya breast cancer is the most commonly diagnosed cancer in women and accounts for 23% of all the cancers followed by cervical cancer (21.1%) (Ministry of Public Health and Sanitation and Ministry of Medical Services, 2012; Ferlay *et al*., 2015; Korir, *et al*., 2015). In comparison to countries with low Human Development Index (HDI), like most African countries including Kenya, cervical cancer is the second most cause of incidences and mortalities where it was cause one out of five cancer deaths estimated in women (Boyle *et al*., 2016; WHO, 2014b)

On the other hand, prostate cancer incidents and deaths are higher in African Americans who have recorded more than 72% mortality rate (Schroder and Roobol, 2012). The incidence rate of prostate cancer in Kenya and other African countries is about 9.4%. It is the most common type of cancer comprising of 15.6% all cancers diagnosed in men with age standardised rate of 40.6 per a population of 100,000 men in Kenya (Korir *et al*., 2015).

### 2.7.1 Breast cancer

Cancer of the breast is as a result of uncontrollable growth of the breast cells which usually form a tumour, it begins from any part of the breast but the two types of breast cancers are known, first, ductal cancers (begin in the ducts that carry milk to the nipple) and second, globular cancers (begin at the gland that makes breast milk). Though the causes of breast cancers are not explicitly known, certain inherited or acquired DNA changes have been found to increase a woman's chances of developing cancer. Germline mutations of breast cancer (BRCA1 and BRCA2) genes

lead to increased risk of breast and ovarian cancer. BRCA genes are known as tumour suppressor genes, they repair DNA breaks which may result to carcinogenesis and therefore when they are altered normal breast cells divide and grow to cancer cells (Pecorino, 2012). Breast cancer is recorded to have about 2.1 million new cancer cases and 0.6 million cancer deaths in the year 2018 (Bray *et al*., 2018).

### 2.7.2 Prostate cancer

Prostate cancer is a prostatic disease of men together with non-malignant form benign prostatic hyperplasia (BPH) and inflammation of the prostate gland (Emmanuel, 2010). These conditions develop when abnormal cells of the prostate start rapid and uncontrollable growth, growth progression of the prostatic conditions is usually slow than other types of cancer and therefore become more common in advanced age (Rohrmann *et al*., 2017).

Benign prostatic hyperplasia which is not cancer in itself but the normal process of aging, is characterized by lower urinary tract symptoms, painful prostate, abscesses and dysuria. Androgen receptors and chronic prostatic inflammation precipitate the development and progression of prostate cancer and BPH, therefore, androgen deprivation therapy (ADT), anti-inflammatory and immunotherapy approaches in the management of these prostatic disease are necessary (Nunzio *et al*., 2011).

Prostate cancer is the second most prevalent and commonly diagnosed cancer in men worldwide (American Cancer Society, 2019). It is the fifth leading cause of cancer deaths among men worldwide and approximately 1.3 million incidences and over 0.3 million deaths recorded in the year 2018 (Bray *et al*., 2018). A decrease in mortality

of men suffering from prostate cancer has been observed in developed countries. However, the incidences and deaths are still high in developed and developing countries (Bray *et al*., 2018).

### 2.7.3 Cervical cancer

Cancer of the cervix affects the cervix in women. It was the third most commonly diagnosed cancer in women. It ranked the third cause of cancer related deaths in women in 2018 worldwide (Bray *et al*., 2018). Cervical cancer is mainly caused by persistent human papilloma virus (HPV) infection. There are about 15 HPV types that are associated with increases risk of cervical cancer. However, HPV 16 and 18 are most common among cervical cancer patients. HPV 16 and 18 are responsible for causing more than 70% of cervical cancers worldwide. In addition, long term use of oral contraceptives, high parity (high number of childbirths), immunosuppression and cigarette smoking also increase the risks of cervical cancer (WHO, 2014b; American Cancer Society, 2019).

## 2.8 Management of cancer

The management of cancer is an old practice that dates to over 50 centuries ago by Egyptian physicians. The Egyptian papyri have earliest records of patients and treatments on uterine and breast cancer. The treatments of cancer of the old time included surgery, cautery, caustic pastes, bloodletting and mineral and herbal medicines (Morrison, 2010). Over time, the treatment options for cancer therapy have improved. Currently there are a number of cancer management modalities with the objectives of curing, prolonging and improving the quality of life (Katzung *et al*., 2012). The most common cancer management options are grouped as local

therapy (surgery and radiation) and chemotherapy (Katzung *et al*., 2012). There are other modern developments in cancer therapy which are based on hormones, immunity of the body, light amplification by stimulated emission radiation, precision medicine (drugs specifically targeted towards cancerous cells), palliative care and complementary and alternative medicine (Katzung *et al*., 2012; Kamil and Kamil, 2015).

### 2.8.1 Radiotherapy

Ionizing radiations are used at high doses to destroy deoxyribonucleic acid (DNA) of cancerous tissues or tumours. This method may be used alone to treat cancer. It has been found to be highly successful when used together with other methods like surgery and chemotherapy for cancer of the head, neck and painful bone metastasis. Though, it has limitations like lifetime dose limit, high cost and side effects related to the destruction of nearby normal cells (Kufe *et al.*, 2003).

### 2.8.2 Surgery

The use of small thin knives (scalpels) to remove solid tumours is a common treatment for cancers. The whole tumour that is contained in one area can be removed or debulked to facilitate other treatment and relieve cancer symptoms that cause pain or pressure. Surgery is used to remove the lymph node and also to obtain biopsies that are usually required in the diagnosis of cancer. The common problems associated with surgery are pain and infection (Santosh *et al*., 2017).

### 2.8.3 Chemotherapy

Chemotherapy is a systemic use of anticancer drugs to destroy cancer cells in the body. It is practiced in three clinical settings, first, primary induction chemotherapy

which is used for the treatment of advanced metastatic disease or cancers with no other effective treatment approaches. Secondly, neoadjuvant treatment which is for patients who present with localized cancers, for whom local forms of therapy such as surgery or radiation, or both, are inadequate by themselves and thirdly, adjuvant treatment to local methods of treatment, adjuvant therapies are used to prevent recurrence of cancer (Katzung *et al*., 2012).

Chemotherapeutic agents are categorized into two classes, cell cycle specific drugs (CCSD) and cell cycle non-specific drugs (CCNSD). Anticancer drugs exert their effects on dividing cells either at S, G1, M, and G2 phases (Fig 2.1) or destroy tumour cells whether dividing, resting (G0) or not dividing. The dividing cancer cells are more sensitive compared to resting and non-dividing cancer cells. Table 2:1 provides a summary of cell cycle specific and cell cycle non-specific anticancer drugs. Drug combination chemotherapy is important because anticancer drugs are selected on the basis of different levels of cell cycle they target (Fig. 2.1) (Katzung *et al*., 2012).

The use of combined chemotherapy, where different classes of anticancer agents are used together confer advantage over use of single chemotherapeutic agents. First, it provides maximal cell kill within the range of toxicity tolerated by the host. Second, it provides a broader range of interaction between drugs and tumour cells with different genetic abnormalities in a heterogeneous tumour population. Finally, it may prevent or slow the subsequent development of cellular drug resistance (Santosh *et al*., 2017)

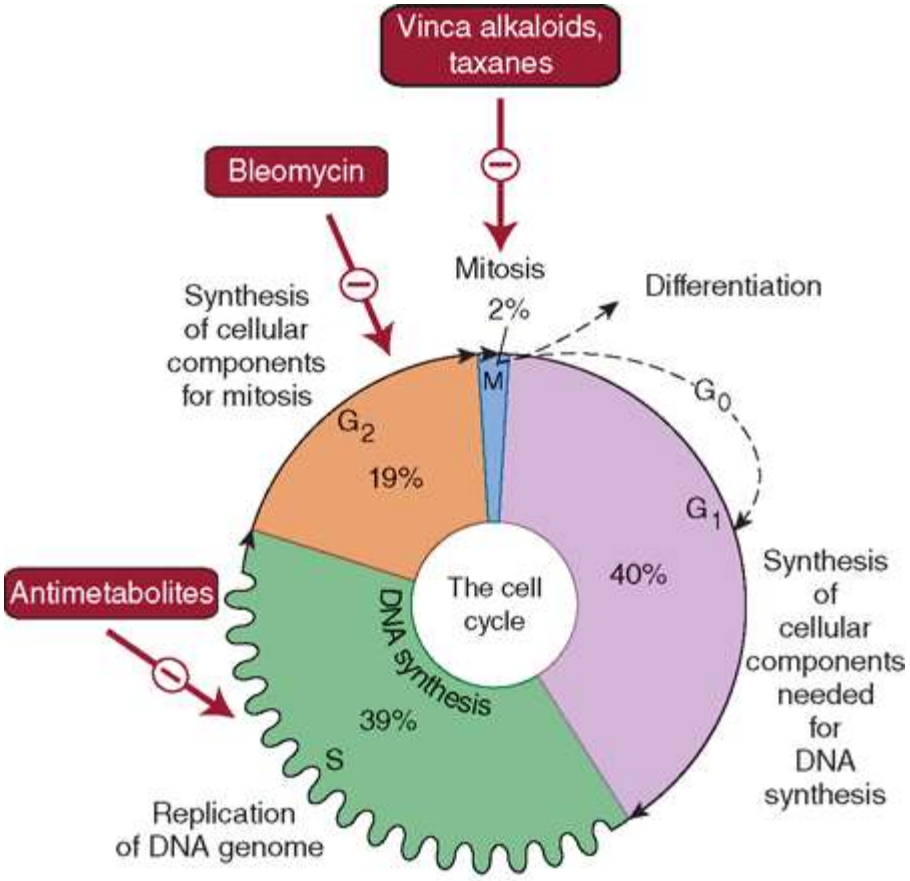



**Figure 2.1: Cell cycle and effects of some anticancer drugs**

**Table 2.1: Effects of major classes of anticancer drugs on cell cycle (Adapted from Katzung *et al.*, 2012)**

| Cell cycle phase | Class of anticancer drugs | Cell cycle Specific agents | Cell cycle phase | Class of anticancer drugs | Cell cycle non-specific agents |
|---|---|---|---|---|---|
| S | Antimetabolites | Capecitabine | S | Alkylating agents | Altretamine |
| | | Cladribine | | | Bendamustine |
| | | Clofarabine | | | Busulfan |
| | | Cytarabine (ara-C) | | | Carmustine |
| | | Fludarabine | | | Chlorambucil |
| | | 5-Fluorouracil | | | Cyclophosphamide |
| | | Gemcitabine | | | Dacarbazine |
| | | 6-Mercaptopurine | | | Lomustine |
| | | Methotrexate | | | Mechlorethamine |
| | | Nelarabine | | | Melphalan |
| | | Pralatrexate | | | Temozolomid |
| | | 6-Thioguanine | | | Thiotepa |
| G1-S | Topoisomerase II inhibitors | Etoposide | G1-S | Antitumor antibiotics | Dactinomycin Mitomycin |
| M | Taxanes | Paclitaxel | M | Topoisomerase I inhibitors | Irinotecan |
| | | Cabazitaxel | | | Topotecan |
| | Vinca alkaloids | Vinblastine | | Platinum analogs | Carboplatin |
| | | Vincristine | | | Cisplatin |
| | | Vinorelbine | | | Oxaliplatin |
| | Antimicrotubule inhibitor | Ixabepilone | | Anthracyclines | Daunorubicin |
| G2-M | Antitumor antibiotics | Bleomycin | | G2-M | Doxorubicin |
| | | | | | Epirubicin |
| | | | | | Idarubicin |
| | | | | | Mitoxantrone |

### 2.8.4 Hormonal therapy

The treatment of cancers whose development and growth depends on hormones is referred to as hormonal, endocrine or hormone therapy. The cancers treated by this

approach are prostate and breast cancers (Chan and Yeung, 2006; Abraham and Staffurth, 2016). Hormone therapy can block the body's ability to produce hormones or can interfere with how hormones behave in the body (Hickey, 2006). Hormonal therapy can also be used in the treatment of endometrial cancers apart from breast and prostate cancers in three ways. First, shrinking tumours before surgery or radiation therapy (neo-adjuvant therapy), second, reducing the risk that cancer will come back after the main treatment (adjuvant therapy) and finally, destroying cancer cells that have returned or spread to other parts of the body (Katzung *et al*., 2012). Nevertheless, hormonal therapy has side effects which include weak bones, enlarged and tender breasts and diarrhoea in men while vagina dryness, changes in moods and menstrual periods is experienced in women. Hot flushes, loss of interest in sex, fatigue and nausea are experienced in both sexes (Taylor, 2018).

### 2.8.5 Immunotherapy

Immunotherapy comprise the use of biologicals that stimulate the body's immune system to fight diseases and cancer or destroy the cancer cells (Waldmann, 2003). Most of the immunotherapy approaches are classified into two, first, those that kill cancer cells such as monoclonal antibodies, checkpoint inhibitors therapy, treatment vaccines and adoptive cell transfer and secondly, those that enhance the body's immune response to fight cancer like Bacillus Calmette-Guérin (BGC) and cytokinin (Waldmann, 2003). Though, immunotherapy has been used to treat cancers, it is commonly associated with side effects such as pain, swelling, soreness, redness, itchiness, rash, weight gain from retaining fluid, heart palpitations, sinus congestion, diarrhoea, risk of infection and flu like symptoms (American Society of Clinical Oncology, 2018b).

### 2.8.6 Laser therapy

The term “laser” stands for light amplification by stimulated emission of radiation. Laser light has specific wavelength which is focused in a narrow beam and creates a very high-intensity light. The beam of light can be used to destroy or shrink tumours and limit precancerous growth. Lasers can focus very accurately on tiny areas, and therefore can be used for very precise surgical work or for cutting through tissue (in place of a scalpel) (National Cancer Institute, 2011). Lasers are most commonly used to manage cancers on the surface of the body or the lining of internal organs (superficial cancers) such as basal cell skin cancer. Early stages of some cancers, such as cervical, penile, vaginal, vulvar, and non-small cell lung cancer are also treated with laser therapy. Laser therapy can be used to seal nerve endings and therefore reduce pain after surgery, seal lymph vessels to reduce swelling and limit the spread of tumour cells. in addition to relieve of certain symptoms of cancer like bleeding or obstruction (Santosh *et al*., 2017).

Laser therapy can be used alone, but most often it is combined with other treatments, such as surgery, chemotherapy, or radiation therapy. The advantages of laser therapy include high precision compared to standard surgical tools. Therefore, less damage to normal tissues. Patients usually experience less pain, bleeding, swelling, and scarring. Durations of operation are usually shorter, less healing time and less likelihood of infections. On the other hand, can be limited due to need for specialized training of surgeons, strict safety precautions to be adhered to, high cost of the equipment and repeat treatments (National Cancer Institute, 2011).

### 2.8.7 Palliative care

Palliative care comprise of interdisciplinary treatments and is aimed at improvement of feelings of cancer patients, therefore promoting quality of life for patients with advanced illness and their families (Ferrell *et al*., 2017). It is a holistic approach that integrates an individual's specific needs and constitutes actions to reduce physical, emotional, spiritual and psycho-social distress in serious debilitating illnesses like cancer, heart disease, lung disease, kidney failure, multiple sclerosis, AIDS, and cystic fibrosis (National Institute of Nursing Research, 2018). It is usual for people at all stages of cancer treatment to receive some kind of palliative care. In some cases, medical specialty and professional organizations recommend that patients and physicians respond to cancer only with palliative care (Temel *et al.*, 2010; Ferrell *et al.*, 2017). This applies to patients who have low performance status, implying limited ability to care for themselves, patients who received no benefit from prior evidence-based treatments. Patients who are not eligible to participate in any appropriate clinical trial or have no strong evidence to show that treatment would be effective (Ferrell *et al*., 2017).

Palliative care may be confused with hospice and therefore only indicated when people approach end of life. Like hospice care, palliative care attempts to help the patient cope with their immediate needs and to increase comfort. Unlike hospice care, palliative care does not require people to stop treatment aimed at the cancer (Ferrell *et al*., 2014). Multiple national medical guidelines recommend early palliative care for patients whose cancer has produced distressing symptoms or who need help coping with their illness. In patients first diagnosed with metastatic cancer, palliative care may be immediately indicated. Palliative care is indicated for patients

with a prognosis of less than 12 months of life even given aggressive treatment plan (Temel *et al*., 2010; Ferrell *et al*., 2014).

### 2.8.8 Photodynamic therapy (PDT)

Photodynamic therapy employs photosensitisers which are exposed to specific type of radiations to generate a form of oxygen killing cells (Dolmans *et al*., 2003). The activation of each photosensitizers is dependent of particular wavelength of light, for instance, blue light is used to activate aminolevulinic acid, while red laser light activates the porfimer sodium, through a thin fiber-optic glass filament (Brown *et al*., 2004).

A photosensitizing agent is usually administered intravenously or subcutaneously and is absorbed by both normal and cancerous cells, non-cancerous cells excrete these agents between 24 to 72 hours of injection while it may take a couple of days to weeks to be excreted from cancerous cells after injection (Dolmans *et al*., 2003). In this way, therefore latter subjection of photosensitizers in cancer cells to specific light wavelengths get to destroy the cells by three ways, first, production of an active form of oxygen that destroys nearby cancer cells, secondly by destruction of blood vessels in the tumour and thirdly by activating the immune system to fight the tumour (Santosh *et al*., 2017).

Photodynamic therapy is a painless procedure with higher adherence compared to radiotherapy, chemotherapy and surgery. It is mostly used for treatment of local cancers such as melanomas and mouth cancers, this is because the wavelength for photosensitization can only penetrate about 1 cm of the tissue which in this case is

the skin. Under specialized cases it can be used with the aid of endoscope to treat lung and oesophagus cancers (Capella and Capella, 2003).

Photodynamic therapy is preferred because it is less intrusive with a minute scar after treatment and temporary side effects like coughing, trouble swallowing, stomach pain, painful breathing, or shortness of breath (Capella and Capella, 2003; Vrouenraets *et al*., 2003).

### 2.8.9 Targeted therapy

Targeted therapy is tailored forms of treatments that address patients' individual needs. They are the foundation of precision medicine whereby appropriate therapies are selected for patient based on the knowledge of genetic composition of their disease. Target therapies can work for cancer patients when they interfere with particular molecules that promote cell growth, division and spread of the cancer cells. It is also referred to using other forms like precision therapies, molecular target therapies or sometimes product of "rational" drug design. Though target therapies are not part of routine care for a majority of cancer patients, a number of therapies have been approved is United States of America by Food and Drug Administration (FDA) to treat cancers or to be used in combination with surgery, radiotherapy or chemotherapy (Miller *et al*., 2016; Yadav *et al*., 2017).

### 2.8.10 Complementary and alternative medicine

Complementary and alternative medicines (CAM) is a term used for medical products and practices that are not part of standard medical care (mainstream) (Mohd *et al*., 2017). The CAM methods promote health, relieve symptoms of disease and side effects of conventional treatments, or cure disease. The employment of

acupuncture or special diet to treat cancer are examples of CAM (Vickers, 2004). Cancer patients have benefited from a total approach of care called integrative medicine, it employs the combination of standard medical care and CAM practices that are safe and effective (integrative medicine). This approach has been found to offer cancer patients improved treatment outcomes (American Cancer Society, 2016).

### 2.9 Anticancer drugs of natural origin

Out of total 250,000- 500,000 known plant species on earth, approximately 1-10% have been screened for pharmacological activity and about one thousand have been found to have anticancer potential. The fact that about 60% of current chemotherapeutic agents are derived from natural sources and 69 clinically relevant compounds are of plants origin, it explains the resurgence of efforts towards the discovery of anticancer agents from natural sources (Cragg and Newman, 2004).

### 2.10 Classes of phytochemicals used for management of cancer

Phytochemicals are compounds formed during the plants metabolic processes, they are referred to as secondary metabolites which are classified as alkaloids, phenols, flavonoids, coumarins, glycosides, polysaccharides, tannins, terpenes and terpenoids. The extraction, screening, detection and identification of active plant constituents (phytochemical screening) is not only vital for authentication of folkloric medicines but also the first fundamental step towards studying medicinal properties of plants (Neha, 2013). Most of the phytochemical constituents are potent bioactive compounds and have been used as drugs or lead compounds for drug development. Some of phytochemicals have demonstrated potent anticancer activity and have

applications either in clinical cases or are under development (Table 2.2) while others are approved for use as phytomedicines (Table 2.3).

**Table 2.2: Anticancer drugs in clinical trials derived from plants (Kebebe *et al*., 2018)**

| **Anticancer drug** | **Plant source** | **Chemical class** |
|---|---|---|
| Combrestatin A4 | *Combretum caffrum* | Stilbenes |
| Flavopiridol | *Dysoxylum binectariferum* | Flavonoid alkaloid |
| Roscovitine | *Raphanus sativus* | Flavonoid alkaloid |
| Resveratrol | *Veratum grandiflorum* | Flavonoid alkaloid |
| Pervilleine A | *Erythroxylum pervillei* | Tropane alkaloid |
| 4-Ipomeanol | *Ipomea batata* | Monoterpene |
| Bruceantin | *Brucea antidysenterica* | Quassinoid |
| Lapachol | *Tabebuia avellanedae* | Naphthoquinone |
| Silvesterol | *Aglaila silvestre* | Cyclopenta[b]benzofurans |
| Pancratistatin | *Pancratium littorale* | Alkaloid |
| Betulinic acid | *Betula* species | Triterpene |

**Table 2.3: Anticancer drugs in clinical use derived from plants (Kebebe *et al*., 2018)**

| Anticancer drug | Plant source (family) | Chemical class |
|---|---|---|
| Vincristine, vinblastine, vinorelbine and vindesine | *Catharanthus roseus* (Apocynaceae) | Vinca alkaloids |
| Topotecan and irinotecan | *Campotheca acuminate* (Nyssaceae) | Alkaloids |
| Homoharingtonine | *Cephalotaxus harringtonia* (Cephalotaxaceae) | Alkaloid |
| Elliptinium | *Bleekeria vitensis* (Apocynaceae) | Alkaloid |
| Docetaxel, Paclitaxel | Plants of the genus Taxus (Taxaceae) including *T. brevifolia, T. buccata, T. canadensis*). | Taxane |
| Etoposide and Teniposide | Podophyllum species (*P. peltatum*, *P. emodii*) | Lignans |

## 2.11 Screening of plants for discovery of anticancer drugs

The goal of screening plants is to isolate therapeutically active compounds. The bioactive compounds provide novel structures which can be semisynthetisised to produce patentable molecules of higher positive bioactivity. On the other hand, screening may result to standardization of whole plant or plant part as herbal remedy (Lahlou, 2007;2013).

The search of drugs from natural products for biologically active compounds takes several years. The process involves four steps, first, plant material collection, second, development of method for measuring biological activity, third, bioassay-guided fractionation and determination of structures of biologically active

compounds and finally evaluation of clinical trials of the extracts, fractions or isolated pure compounds (Lahlou, 2007).

### 2.12 Selection of plants for screening anticancer activities

Scientific efforts for the discovery and development of anticancer drugs more especially from plants embrace modern strategies for selecting plants for biological screening (Lahlou, 2007). The appropriate approaches that are commonly used for selection of plants are divided into random and non-random screening (Atanasov *et al*., 2015).

#### 2.12.1 Random screening approach

Random screening is the selection of extracts, fractions and isolated compounds on the basis of their availability. The method is especially applicable when samples are collected from habitats of high biodiversity and endemism (Atanasov *et al*., 2015). Materials selected in this manner have potential of yielding unexpected novel chemical entities, that otherwise, could have not been predicted using the existing knowledge.

The random screening approach is associated with limited success rate (about 1 %). It takes long time and vigorous work and therefore, knowledge based approaches (non-random approaches) including ethnopharmacological, chemosystematic, ecological and computational approaches are encouraged to increase the probability of identifying relevant bioactive (Atanasov *et al*., 2015; Ramesha *et al*., 2011).

### 2.12.2 Ethnopharmacological approach

The plants to be screened are selected on the basis of traditional or folkloric use of the plant. This approach is multidisciplinary and combines knowledge from a number of studies including pure sciences (botany, chemistry, biochemistry and pharmacology) and natural sciences (language, anthropology, archaeology and history). It is the most preferred approach of selecting material in the discovery of new drug (Atanasov *et al*., 2015). The likelihood of isolating compounds that have similar activity or related to that reported traditionally is higher. Success rates of about 80% have been reported in early studies which used ethnopharmacological approach for selection of study plants.

Ethnopharmacological approach, first demonstrates the validity of folkloric use(s) in the light of scientific evidence, second, provide leads in search for modern drugs from plants based on their indigenous use(s) and thirdly, to provide data for cultural medical heritage, in addition, ethnopharmacological studies can be applied in making cross-cultural comparisons of plant families or genera used for various diseases (Lahlou, 2007; Saslis-Lagoudakis *et al*., 2011).

The development of antimalarials mefloquine and chloroquine was based on the traditional use of quinine from *Cinchona* species. Similarly, artemether and sodium artesunate (antimalarials) were developed based on artemisinin from *Artemisia annua*. Chromoglicic acid and the sodium salt used for management of allergy and asthma was developed based on khellin that was isolated from *Ammi visnaga* while biguanidine-type antidiabetics were synthesized on the basis of galegine that was isolated from *Galega officinalis*. Papaverine from *Papaver somniferum* led to the

development of antihypertensive drug verapamil and opium from the same plant is the basis for the synthesis of morphine. The search of developing some anticancer agents has also benefited from ethnopharmacological approach of study, examples include the development of teniposide and etoposide based on podophyllotoxin isolated from *Podophyllum* species.

Though it is usually reputable to employ pharmacological approach when reliable information is available, bioassays may frequently fail to yield promising results. The reason being that cancer is poorly defined in folklore or traditional medicine, and is mostly referred to conditions such as hard swellings, abscesses, calluses, corns, warts, polyps or tumours (Albuquerque *et al*., 2014; Newman and Cragg, 2016). The use of other approaches like bioinformatics and broad taxonomic diversity are known to increase the success rates of ethnopharmacological approach (Gordon and Boyd, 1996; Bernard *et al*., 2001).

### 2.12.3 Chemosystematic approach

The chemosystematic approach is also known as phylogenetic. It utilizes chemotaxonomic knowledge and molecular phylogenetic data to guide the selection of plants species for study. Plants from genus or families which are known to have compounds or class of compounds associated with certain pharmacological activities are identified in a more targeted manner. The plant species bearing a common ancestry have enzymes that form a unique biosynthetic pathway and therefore responsible for the production of related secondary metabolites. The enzymes are strictly related to the genetic makeup of the organism and the organisms that seem to have evolved together are assumed to have developed similar group of compounds

for survival purposes. A correlation between phylogeny and biosynthetic pathways (phytochemistry) is sometimes assumed to offer plant exploratory power of prediction in bioprospecting (Atanasov *et al*., 2015).

Phylogenetic ethnobotany is a new field whereby phylogenetic information and traditional ethnobotanical knowledge are used together in search for active compounds from organisms, it is proven that this method has high success rate in drug discovery. A good example is the isolation of galanthamine from *Galanthus woronowii* Losinsk (Amaryllidaceae) and other alkaloids with acetylcholinesterase inhibition activities from plants of the same family (Rønsted *et al*., 2008; Mellergaard *et al*., 2010). The combination of chemotaxonomy and ethnobotany is considered to be a powerful tool for identifying highly promising plant groups, in the case where phylogenetically related plant species collected from various regions of the world are used for medical conditions in the same therapeutic areas (Atanasov *et al*., 2015).

### 2.12.4 Ecological approach

The ecological approach is also known as field observation technique (Luiz and Barbosa, 2012). It is based on production of various classes of compounds by living organisms as a result of their environmental interaction. This approach has been explained by use theories that include biodiversity and chemodiversity theory (Ramesha *et al*., 2011), apparency theory (De Almeida *et al.*, 2011; De Almeida *et al*., 2005), life strategy theory (Coley *et al*., 2003), chemical defences (Albuquerque, *et al*., 2012) and animal behaviour theory (Obbo *et al*., 2013). The increase in biodiversity leads to an increase in chemical diversity. This means that

bioprospecting should lead to new chemical entities. In addition, specific phylogenetic groups should be studied to enhance the probability of isolating a particular compound-type of interest (Ramesha *et al*., 2011). In many species, the produced compounds have ecological functions which may be used as medicines by human beings and his livestock. It is believed that there is high proportion of biochemical architecture to all living things, this makes it justifiable that secondary metabolites from bacteria, sponges, algae, fungi, plants and animals interact reasonably with the macromolecules of the human body (Atanasov *et al*., 2015).

Life strategies include apparency, chemical defences and animal behaviour theories, they are related to adaptations of plants against predation, microbial pathogens or harsh environment. On the basis of apparency, plants can be divided long life cycle plants like trees and shrubs (apparent) and short life cycle like herbs (non-apparent). Apparent plants accumulate high quantities of less toxic chemicals and this makes them less palatable to herbivores. On the other hand non-apparent plants accumulate small amounts of toxic chemicals for their defence (De Almeida *et al*., 2005; De Almeida *et al*., 2011). In environments characterized by inadequate resources, plants accumulate more defence chemicals compared to plants growing in resource rich areas. Young leaves defend themselves by accumulating high quantities of defence chemicals compared to older leaves. In addition, leaves of slow growing shade tolerant species should display better chemical defences than those fast growing species found in better-lit conditions (Coley *et al*., 2003).

Finally, observation of animal behaviour also referred to as ethological approach is based on habits of animals (Luiz and Barbosa, 2012). It is a variant of ecological

approach whereby the use of non-nutritional plants that are bitter in taste by animals provide insight into potential plant activity. This approach is important and leads to self-medication in animals (zoopharmacognosy) and animals ingest the plants to increase alertness, reduce pain, microbial and parasitic infestations especially in primates (Atanasov *et al*., 2015). Though the ecological approach is little explored, it has achieved excellent results, traditional practitioners, as well as bioprospectors for new bioactive compounds still find ecological approach significant. For example, compounds with antimalarial and antiprotozoal activity could be isolated from plant species that were ingested by chimpanzees and baboons in the wild in unusual feeding behaviour, supposedly in order to control intestinal parasite infection (Obbo *et al*., 2013).

### 2.12.5 Computational approach

The materials for research are selected based on *in silico* bioactivity predications for constituents of certain plant species. Computer assisted drug design has made great advances in all steps in drug discovery process. It involves rational drug design and inventions based on the knowledge of a biological target, the process postulate that bioactive compounds act by interacting with macromolecule targets, mainly proteins or nucleic acids (Geromichalos, 2013). Computational also called in silico methods have undergone revolutions, starting with bioinformatics, cheminformatics, visual screening and reverse pharmacognosy. Virtual screening discovers new ligands on the basis of biological structure, large libraries of chemical compounds that complement targets of known structure are screened to select a small number of likely candidates for experimental testing (Geromichalos, 2013).

Virtual screening is divided into two major groups, structure-based and ligand-based, depending on the experimental information available. Ligand-based approaches utilize structure activity data of a set of known activities, whereas, structure-based methods use the three-dimensional structure of the biological target (Medina-Franco, 2013). The aim of virtual screening is identification of new ligands for known targets. However, the identification of putative targets based on the chemical similarity of the known ligands is an inverse approach. It exemplifies a new era of pharmacognosy called reverse pharmacognosy, which deals with the finding new biological targets from structurally similar chemicals, and finally finding the natural sources of the biologically active natural compound which contain them (Soni *et al*., 2015).

**2.13 Bioassay techniques for anticancer activities**

Measurement and quantification of biological activity is one of the important steps in isolation of active compounds from natural sources during drug discovery. There are many protocols developed for testing bioactivity using suitable models. When the drug is evaluated using intact animals, the test is referred to as *in vivo*. Whereas when it is done using cell cultures on growth media or isolated organs, the test is referred to as *in vitro*. The protocols used to study natural products for anticancer potency have been developed by National Cancer Institute (NCI) and implemented in extensive research programs. Until 1985, NCI used murine P-388 AND L-1210 leukemia by *in vivo* models to study anticancer activities of drugs, thereafter use of cytotoxic (*in vitro*) screening protocols were developed for large scale tests due to

favourable economic advantages in terms of cost, time and number of animals used (Pagé, 2004).

The evaluation methods basically test the antiproliferative or cytotoxic effects on the cultured cells or tumour bearing animals. The assays are broadly categorized into two, mechanism-based assays and cell-based assays. The measurement of specific activity of the drug towards a specific enzyme is an example of mechanism-based assay, the target systems are assayed in an artificial environment that is isolated and physiologically dissimilar from their natural environment.

Proper configuration of mechanism-based assays provides results that can be dependable in terms of accuracy and effectiveness. However, these methods only appropriate the *in vivo* environment which is many at times incomplete due to the likelihood of missing some pathways or mechanisms, furthermore compounds or extracts and fractions may be found active in the assay, may be inactive *in vivo* models due to inability to penetrate the cell membrane.

Cell based assays have often been adopted by National Institute of Cancer for screening of natural products for antiproliferative or cytotoxic effects. The principle of cell-based assay is the quantification viable or death cells at the end of the test, measurement of some aspects of general metabolism or an enzymatic activity as a marker of viable cells is achieved by use of tetrazolium salts, resazurin dye, Sulforhodamine B dye, protease markers, and ATP detection. The major methods use multi-well formats where data are recorded using a plate reader.

### 2.13.1 Tetrazolium reduction assays

The tetrazolium salts are used in mult-well plate cell cultures to perform various antiproliferative assays. The assays are named in respect to the salt used. In the current study 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was used. The method was based on the cleavage of a tetrazolium salt by a mitochondrial enzyme, succinate dehydrogenase, leading to the formation of a coloured product, formazan, which can be quantified spectrophotometrically. In practice, most of these tetrazolium salts have been found to be of variable efficacy for the quantification of viable cells (Marine *et al*., 2014; Terry *et al*., 2016).

### 2.13.2 Resazurin reduction assay

Resazurin dye (7-hydroxy-3H-phenoxazin-3-one 10-oxide) branded as AlamarBlue™, is been broadly used as an indicator of cell viability in several types of proliferation and cytotoxicity assays (Ricardo *et al*., 2009). Resazurin is a redox indicator, it is deep blue and non-fluorescent when dissolved in physiological buffers. However, it can be reduced to resorufin by the dehydrogenase activity of mitochondria to give rise to a pink molecule. Resorufin is pink and fluorescent in viable cells which are metabolically active ant the quantity of resorufin produced is proportional to the number of viable cells (Terry *et al*., 2016). Quantification of life cells is done using a microplate fluorometer equipped with a 560 nm excitation / 590 nm emission filter set. Resorufin also can be quantified by measuring a change in absorbance. However, absorbance detection is not often used because it is far less sensitive than measuring fluorescence. The resazurin reduction assay is slightly more sensitive than tetrazolium reduction assays and there are numerous reports using the

resazurin reduction assay in a miniaturized format for high throughput screening (HTS) applications (Terry *et al*., 2016).

### 2.13.3 Sulforhodamine B (SRB) assay

Vital stains are useful in assessment of vital functions of cells. Sulforhodamine B dye is a protein stain that binds to basic amino acids of cellular macromolecules. The Sulforhodamine B (SRB) assay is based on determination of cell density by measurement of cellular protein bound content spectrophotometrically at 510 nm (López-Lázar, 2015). It was the first method used by the National Cancer Institute (NCI) for large screening of new drugs (Pagé, 2004). The SRB assay is sensitive compared to other fluorometric methods. It is neither affected by cell metabolic activities nor susceptible to chemical interferences caused by reducing compounds (Tonder *et al*., 2015). Though this method does not distinguish between viable and non-viable cells, its ability to detect cytotoxic effects of a drug is not compromised. Several studies have shown that results from the SRB assay correlate well with those of the MTT assay. However, the $IC_{50}$ values of compounds tested using the SRB method generally are slightly higher (López-Lázar, 2015; Tonder *et al*., 2015).

### 2.13.4 Protease viability marker assay

The selective detection of protease activity that is restricted to viable cells is used as an indicator for viable cells. Glycylphenylalanyl-aminofluorocoumarin (GF-AFC) is fluorogenic protease substrate, it penetrates the cells into the cytoplasm where it is broken down by aminopeptidase into respective amino acids and fluorescent aminofluorocoumarin (AFC). Since dead cells are devoid of aminopeptidase, this selective activity of the enzyme is marker of the viable cell population. The number

of life cells is proportional to the intensity of the fluorescent signal of a fluorometer measured at at 380–400 nm. The protease viability marker assay has been shown to correlate well with other established methods of determining cell viability such as an ATP assay. The compound GF-AFC is also known to be relatively non-toxic to cultured cells (Terry *et al*., 2016).

### 2.13.5 Adenosine triphosphate (ATP) assay

ATP assay is based on the evaluation of energy molecule (ATP) to determine cell viability. ATP is a widely acceptable biomarker for viable cells and therefore its detection and measurement is used to estimate viable cells. The ATP assay is a fast and highly sensitive luminescence method widely used for estimating cell viability in high-throughput screening. ATP assay is also called luciferase assay, it constitutes a kit that allows the conversion of luciferin to luminescent oxyluciferin. The commercial kit contains a detection reagent detergent to lyse the cells, ATPase inhibitors to stabilize the ATP that is released from the lysed cells, luciferin as a substrate, and the stable form of luciferase to catalyse the reaction that generates photons of light. Intensity of emitted light is measured by a luminometer, the amount of light is linearly related to ATP concentration from the number viable cells (López-Lázar, 2015; Terry *et al*., 2016). The ATP assay is a rapid cell viability assay, it is also most sensitive and is less prone to artefacts than the other viability assay methods.

### 2.13.6 Real-time assay

The instant measurement of viable cell number is an advanced approach. The method employs reagent components that constitute of engineered stable form of a marine

shrimp derived luciferase and a cell permeable pro-substrate. The test reagent is added directly to culture medium where viable cells convert the pro-substrate into a substrate. The substrate is broken down by luciferase to produce a luminescent signal which is directly proportional to the viable cells and indirectly proportional to the death cells (Terry *et al*., 2016). Measurements of light intensity can be made from the same sample for days without renewal of the pro-substrate given that the reagent is compatible with cell medium and stable at 37°C for about 72 hours (Terry *et al*., 2016).

### 2.13.7 Lactate dehydrogenase (LDH) assay

Lactate dehydrogenase assay is based on measurement of lactate dehydrogenase (LDH. Damaged cells loose membrane integrity and release lactate dehydrogenase (LDH) into the surrounding cell culture medium. Initially LDH catalyses lactase to pyruvate with the association of reduction of nicotinamide dinucleotide (NAD+) to NADH. Secondly, diaphorase uses NADH to reduce appropriate redox reagents like tetrazolium salt or resazurin reagent into a coloured formazan or fluorescent product. The levels of subsequent products are measured spectrophotometrically and quantities are directly proportional to the amount of released LDH in the medium. Therefore LDH assay is used to quantify the population of viable or dead cells in culture media (Smith *et al*., 2011; López-Lázar, 2015).

## 2.14 Bioassay- guided isolation and structural elucidation of active compounds

Bioassay-guided isolation entails the evaluation of crude extracts for biological activities, followed by fractionation process and assessment of the fractions for biological activities up to the time pure active compounds are obtained. Fractions can

be prepared through liquid-liquid extraction, using immiscible organic solvents which is also called partitioning. Stepwise fractionation with subsequent bioassay of active fractions lead to separation mainly by column chromatography, flash chromatography, vacuum liquid chromatography and thin-layer chromatography (Gnanaraj *et al*., 2017). Though, bioassay-guided isolation is a gold standard in the discovery of anticancer agents from natural products, the process is expensive, labourious, tiresome and takes long time to complete. Sometimes fractionation results into isolation of inactive compounds which may be explained that activity in the crude extract was from additively or synergistically weak active compound in the extract (Gnanaraj *et al*., 2017).

Structure elucidation of isolated compounds is the end point of natural product research. However, it sometimes takes long time and can be an obstacle in the search for drugs from nature in spite of the availability of modern analytical instrumental techniques, the most ones being the use of spectroscopic, chromatographic and tandem methods. Various spectroscopic techniques including Ultraviolet-visible spectroscopy (UV-vis), Infrared spectroscopy (IR) and Nuclear magnetic resonance (NMR) have proven exceptionally important for elucidating structures of isolated compounds. First, spectroscopic data for known compounds is simply compared with published data in literature or with that of the standard. Secondly, when the isolated compound is unknown and multiplex, additional data like retention factor, melting point, mass spectrometry, polarimetry and crystal crystallography will be necessary for structural elucidation (Satyajit *et al*., 2006).

Furthermore, natural product research has advanced with the evolution of modern analytical techniques. The combination of different instrumentations as hyphenated devices, such as capillary electrophoresis- mass spectrometry (CE-MS), high performance liquid chromatography-mass spectrometry (HPLC-MS) and high performance liquid chromatography-nuclear magnetic resonance (HPLC-NMR) is most preferred for separation, detection and resolution of structures of compounds from natural products (Ke *et al*., 2015).

### 2.15 African Traditional medicine and plants with anticancer activities

Africa is considered the cradle of mankind with rich biological and cultural diversity. African traditional medicine is therefore the oldest and perhaps the most diverse of all the medicine systems (Gurib-Fakim, 2006). It is a holistic approach, techniques used by traditional medical practitioners are derived from basic understanding of the etiology of the disease not only as physical ailments and psychological causes (as in Western medicine), but also from astronomical influences, spiritual and also magical causes (Sofowora, 1996).

The World Health Organization (WHO) estimates that up to 80% of the population in some developing countries use traditional medicine and about 80-90% of the populations of African countries are dependent on traditional medicine for their primary health care (Hostettmann *et al*., 2000; WHO, 2002). The practitioners of traditional medicine in Africa include herbalists, herb sellers, traditional birth attendants, bone setters, diviners, faith healers, traditional surgeons, spiritualists and others (Sofowora, 1996). Vegetables, animals and mineral sources and certain other methods are used by African traditional medicine practitioners. The practice is based on social, cultural and religious backgrounds as well as on the knowledge, attitudes

and beliefs that are prevalent in the community regarding physical, mental and social well-being and the causes of disease and disability (Sofowora, 1996).

The African continent is endowed with about 65,000 plants out of which approximately 5,000 are known for their use in traditional medicine (Vasisht and Kumar, 2004; Mahomoodally, 2013). Some of the African plants whose extracts have evidence of use in treating cancer are *Cryptolepis sanguinolenta* root, *Catharanthus roseus* (L.) (Vasisht and Kumar, 2004). In addition, other African plants have demonstrated potential antiproliferative activities against cancer cells (Table 2.4). Despite the large plant diversity in Africa in addition to diverse cultural practices, Africa medicinal plants are characterized by under commercialization (Cunningham, 1993; Mcmullin, 2012). The plants require research to provide scientific information regarding to their efficacy and safety to increase the confidence of use.

**Table 2.4: Some African plants that have antiproliferative potency (Kuete *et al*., 2016)**

| Family | Plant species | Country of collection | Extract or isolated compounds | Cell line and $IC_{50}$ values |
|---|---|---|---|---|
| Anarcadiaceae | *Anarcadium occidentale* | Not specified | Leaf EtOH extract Flavonoid | Jurkat ($IC_{50}$ of 62.6 µg/mL) Jurkat and HL-60 ($IC_{50}$ of 2.04 and 11.03 µg/ml) respectively |
| Annonaceaea | *Xylopia aethiopica* | Cameroon | Fruit EtOH extract | HCT116, U937 and KG1a ($IC_{50}$ of 12, 7.5 and 25 µg/ml) respectively |
| Astaraceae | *Acanthospermum hispidum* | Nigeria | Roots MeOH extract | COR-L23 ($IC_{50}$ of 8.87 µg/ml) |
| | *Echinops giganteus* | Cameroon | Seed MeoH extract | CCRF-CEM ($IC_{50}$ of 6.68 µg/ml), CEM/ADR5000 ($IC_{50}$ of 7.96 µg/ml) and MiaPaCa-2 ($IC_{50}$ of 9.84 µg/ml) |
| Bignoniaceae | *Newbouldia laevis* | Cameroon | | PF-382 ($IC_{50}$ of 0.57 µg/mL), Colo-38 ($IC_{50}$ of 0.67 µg/mL), HeLa ($IC_{50}$ of 0.40 µg/mL) and Caski ($IC_{50}$ of 0.17 µg/ml) |
| Celestraceae | *Elaeodendron alluaudianum* | Madagacar | Stem EtOH extract | A2780 ($IC_{50}$ of 3.3 µg/ml) |
| | *Maytenus senegalensis* | Kenya | Bark MeoH extract | CT26 ($IC_{50}$ of 2.32 µg/ml) |

| | | | | |
|---|---|---|---|---|
| Cupressaceae | *Cupressus lusitanica* | Cameroon | Bark MeOH extract | MCF-7 ($IC_{50}$ of 13.1 µg/ml), |
| Euphorbiaceae | *Croton barorum* | Madagascar | | P388 ($IC_{50}$ of 10 µg/ml) |
| | *Croton goudotii* | Madagascar | | P388 ($IC_{50}$ of 10 µg/ml) |
| Fabaceae | *Acacia nilotica* | Egypt | | |
| | *Albizia gummifera* | Madagacar | Root EtOH extract | A2780 ($IC_{50}$ of 7.2 µg/ml) |
| | *Cajanus cajan* | Nigeria | | |
| | *Guibourtia tessmannii* | Cameroon | Bark MeOH extract | MCF-7 ($IC_{50}$ of 13.1µg/ml) and HeLa ($IC_{50}$ of 8.8 µg/ml), |
| Guttiferae | *Pentadesma butyradecea* | Cameroon | Xanthones | MCF-7 ($IC_{50}$ of less than 4 µg/ml) |
| | *Symphonia globulifera* | Cameroon | Xanthones | KB ($IC_{50}$ of around 2 µg/ml) |
| | *Visimia laurentii* | Cameroon | Xanthones | CCRF-CEM, HL-60, 786-0, U87 MG, A 549, Colo-38 and Caski ($IC_{50}$ of below or around 4 µg/ml) |
| Melianthaceae | *Bersama engleriana* | Cameroon | Leaf MeOH extracts | MCF-7 and DU-145 ($IC_{50}$ of 8.6 and 15.7 µg/mL respectively). |
| | | | Bark MeOH extract | MCF-7 ($IC_{50}$ of 18.7 µg/ml) |
| | | | Root MeOH extract | MCF-7, HeLa and HepG2 ($IC_{50}$ of 8.6, 10.9 and 19.5 µg/ml) respectively |

| | | | | |
|---|---|---|---|---|
| Lamiaceae | *Salvia africana* | South Africa | Aerial parts Methanol Chloroform (1:1) extract | SF-268 ($IC_{50}$ of 8.72 µg/ml) |
| | *Salvia radula* | South Africa | Aerial parts Methanol Chloroform (1:1) extract | MCF-7 ($IC_{50}$ of 9.69 µg/ml) |
| | *Salvia stenophylla* | South Africa | Aerial parts Methanol Chloroform (1:1) extract | HT-29 ($IC_{50}$ of 17.41 µg/ml) |
| Moraceae | *Dorstenia psilirus* | Cameroon | Root MeOH extract | MiaPaCa-2, CCRF-CEM, and CEM/ADR5000 ($IC_{50}$ of 9.17, 7.18 and 7.79 µg/ml) respectively |
| Poaceae | *Imperata cylindrica* | Cameroon | Root MeOH extracts | MiaPaCa-2 ($IC_{50}$ of 12.11 µg/mL), CCRF-CEM ($IC_{50}$ of 8.4 µg/ml) and CEM/ADR5000 ($IC_{50}$ of 7.18 µg/ml) |
| Polygonaceae | *Polygonum limbatum* | Cameroon | Aerial parts MeOH extract | THP-1 and MCF-7 ($IC_{50}$ of 10 and 20 µg/ml) respectively |
| Piperaceae | *Piper capense* | Cameroon | Seed MeOH extract | MiaPaCa-2, CCRF-CEM and CEM/ADR5000 ($IC_{50}$ of 8.92, 7.03 and 6.56 µg/mL) respectively |

| | | | | |
|---|---|---|---|---|
| Rosaceae | *Prunus africana* | Not specified | Bark EtOH extract | PC-3 and LNCaP ($IC_{50}$ of about 2.5 µg/ml in both cell lines) |
| Umbelliferae | *Ferula hermonis* | Egypt | | |
| Zingiberaceae | *Zingiber officinale* | Cameroon | Rhizome MeOH extract | MiaPaCa-2, CCRF-CEM and CEM/ADR5000 ($IC_{50}$ of 16.33, 8.82 and 6.83 µg/ml) respectively |

**Cancer cells: human ovarian cancer (A2780), lung adenocarcinoma (A549), leukemia (CCRF-CEM, CEM/ADR5000, HT-29, TPH-1, HL-60, U937 and KG1a), human prostate (DU-145, PC-3 and LNCaP), human pancreatic (MiaPaCa-2), human large- cell lung (COR-L23), human cervical (Caski, HeLa), human hepatocellular (HepG2), human breast (MCF-7), murine lymphocytic leukemia (P388), skin melanoma (Colo-38), human oropharyngeal epidermoid (KB), glioblastoma cell line (SF-268 and U87 MG), Colon cancer cell line (HCT116), Acute lymphoblastic leukemia cells (Jurkat), renal carcinoma (786-0).**

### 2.16 Plants used in Kenya for management of cancer

Kenya has approximately 1,200 medicinal plant species of which over 41 are reported to have anticancer activities (Kokwaro, 2009; Kigen *et al*., 2013; Misonge *et al*., 2016; Tariq *et al*., 2017). This number of plants is only second to that of India where 45 medicinal plant species have anticancer activities (Ochwang'i *et al*., 2014; Tariq *et al*., 2017).

Traditional medicine plays an important role in the treatment of cancer and other chronic disease in Kenya. The country has not relented the efforts of incorporating traditional medicine in the health policy since the late 1970s (Vasisht and Kumar,

2004). There are efforts by the republic of Kenya to recognize the role of traditional medicine practitioners formerly into the mainstream health care system, this may improve capacity for cancer treatment in the country (Government of Kenya (GoK), 2017; Ministry of Health, 2017). The country has also invested in research to strengthen research on alternative medicines for management of cancer (Ministry of Health, 2017). The current study evaluated plants used by herbalists to manage cancers of the prostate and the breast in Kenya. The plants belong to hydnoraceae, annonaceae, combretaceae, asteraceae, rutaceaea and rubiaceae families.

**2.17 Hydnoraceae family**

Hydnoraceae is a small family with seven species that belong to only two genera (*Prosopanche* and *Hydnora*). This family comprise of some unusual plants in the world. The plants are terrestrial parasites and lack leaves and chlorophyll. The genus *Prosopanche* is found in Central and South America with two species *P. americana* and *P. bonacinae*. *Hydnora* on the other hand, is essentially distributed in Africa with about twelve species. However, fewer species have been recognized due to its underground nature and concealed appearance (Tennakoon *et al*., 2007).

The genus *Hydnora* is part of a remarkable basal angiosperm composed entirely of root holoparasites with extremely reduced vegetative morphology. Its distribution ranges from South Africa across sub- Saharan Africa to the Arabian Peninsula and Madagascar. There are currently five species of the genus *Hydnora* that have been recognized, *H. africana* Thumb., *H. abyssinica* A. Braun., *H. Esculanta* Jum. & H. Perrier, *H. Triceps* Drege & Meyer and *H. Sinandevu* Beetje & Q. Luke (Maass and

Musselman, 2004; Tennakoon *et al*., 2007). *Hydnora abyssinica* was evaluated in this study.

### 2.17.1 General description of *Hydnora abyssinica* A. Braun

*Hydnora abyssinica* A. Braun Schweinf is synonymous to *H. johannis* Becc.Nouv. and *H. solmsiana* Dinter (Tennakoon *et al*., 2007). It is commonly known as Nyambo or Mnyambo (Swahili), Muthigira (Kikuyu), Mũtũmũra Nthí (Embu/Mbeere) and Kimela (Kamba). Toga (Borana), Oyusu or Osugo (Luo), Erkunyi or Erukunyi (Maasai), Auriong'o (Turkana) Auriong'o or Kaworiongo (Pokot), Guli (Burji) and Liki, Like, Laka or Dingah (Somali) (Maundu, 1999; Kokwaro, 2009; Ndwigah *et al*., 2014). The plant is a parasitic herb with bad smell, 10-15 cm high, usually growing on *Acacia nilotica* roots or on several other *Acacia* species. The floral parts are the only visible structures above the ground. The rhizome looks like a root; it is thick, hard, dark brown almost black, warty and attached to the host's root by haustoria (Gachathi, 2007).

The floral buds burst out of the ground and open out, they are angled, and the perianth lobes are joined and fleshy with a red surface. Flesh of the buds thick, white, turning rusty red on exposure to air. Flower large, up to 15 cm long, brown, scaly, edges of lobes pink or red, covered with coarse bristles. Stamens inside 4, joined to form a cream, convoluted fold. Anthers numerous without visible stalk. Fruits produced underground with numerous seeds embedded in a gelatinous pulp (Maundu, 1999; Gachathi, 2007).

### 2.17.2 Biological activity of *Hydnora abyssinica* A. Braun

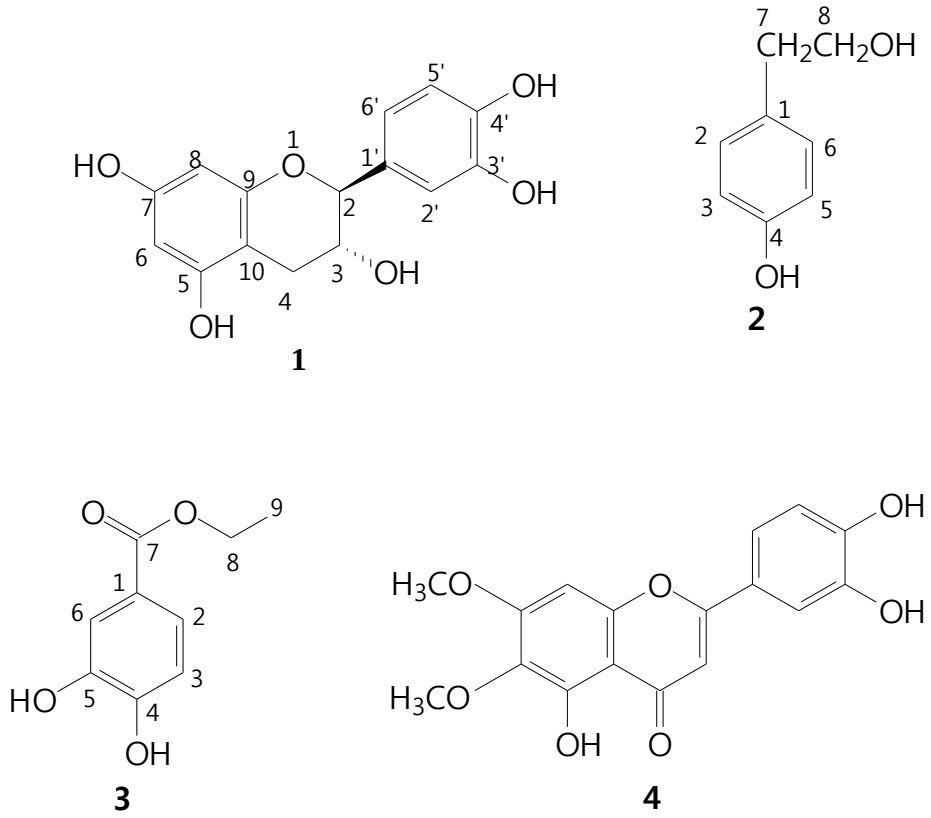
The 80% ethanolic extract of *Hydnora abyssinica* has been reported to have moderate antioxidant activity (59% inhibition of superoxide anion generation). In addition, 2-2-diphenyl picryl hydrazyl (DPPH) free radical scavenging activity activity with $IC_{50}$ = 26.7 µg/ml was reported by Onyancha *et al*. (2015). Cytoxicity studies done by Waleed *et al*. (2009) and Yagi *et al*. (2012) revealed that the extracts of *H. abyssinica* inhibited proliferation of mouse fibroblast (3T3), human mouth epidermoid carcinoma (KB) and normal human foetal lung (MRC5) cell lines. *In vitro* antimicrobial activities *H. abyssinica* extracts have also been observed against bacterial and fungal strains (Saadabi and Ayoub, 2009; Yagi *et al*., 2012; Ndwigah *et al*., 2014).

### 2.17.3 Ethnobotanical information of *Hydnora abyssinica* A. Braun

In Kenya, *Hydnora abyssinica* is used as food, whereby fleshy parts of the flower bud (calyx), whole flower and the mealy underground fruits are eaten raw by the Somali, Pokot, Turkana, Massai, Boran and Samburu. Squirrels, cattle and other animals also feed on the plant (Maundu, 1999). Infusions and decoctions of the rhizome is used to treat evil eyes, cholera, sore throat, oral thrush, amoebic dysentery, diarrhea, stomachache, pneumonia, typhoid, East coast fever, anthrax, cancer, and wounds (Ibrahim *et a*l., 1998; Ruffo, 2002; Musa *et al*., 2011; Ndwigah *et al*., 2014; Wanzala *et al*., 2016). It is also useful in management of female conditions like retained afterbirth, postpartum haemorrhage, uterine problems and breast cancer (Gachathi, 2007; Kokwaro, 2009; Kaingu *et al*., 2011; Kamau *et al*., 2016).

### 2.17.4 Phytochemicals isolated from *Hydnora abyssinica* A. Braun

Phytochemical investigations of *Hydnora abyssinica* rhizome synonymous to *H. johannis* reports the isolation of tetradecanoic acid (2-hydroxhexadecyl ester), catechin **(1)**, tyrosol **(2)**, ethyl 3,4-dihydroxybenzoate **(3)**, cirsiliol **(4)**, sigmasterol **(5),** oleic acid **(6)**, myristic acid **(7)**, palmitic acid **(8),** trans 3′5-dihydroxy-4′7-dimethoxydihydroflavonol **(9)**, vanillin **(10)** and protocathechuic acid **(11)** as shown in Figure 2.2 below (Waleed *et al*., 2009; Yagi *et al*., 2012; Waleed *et al*., 2015). Several phytochemical groups have also been detected in the plant and they include phenols, tannins, proanthocyanins, flavonoids, mucilage, alkaloids, glycosides, triterpenes and sterols (Waleed *et al*., 2009; Onyancha *et al*., 2015).




**1** **2** **3** **4**

**Figure 2.2: Some compounds isolated from *H. abyssinica* rhizome**

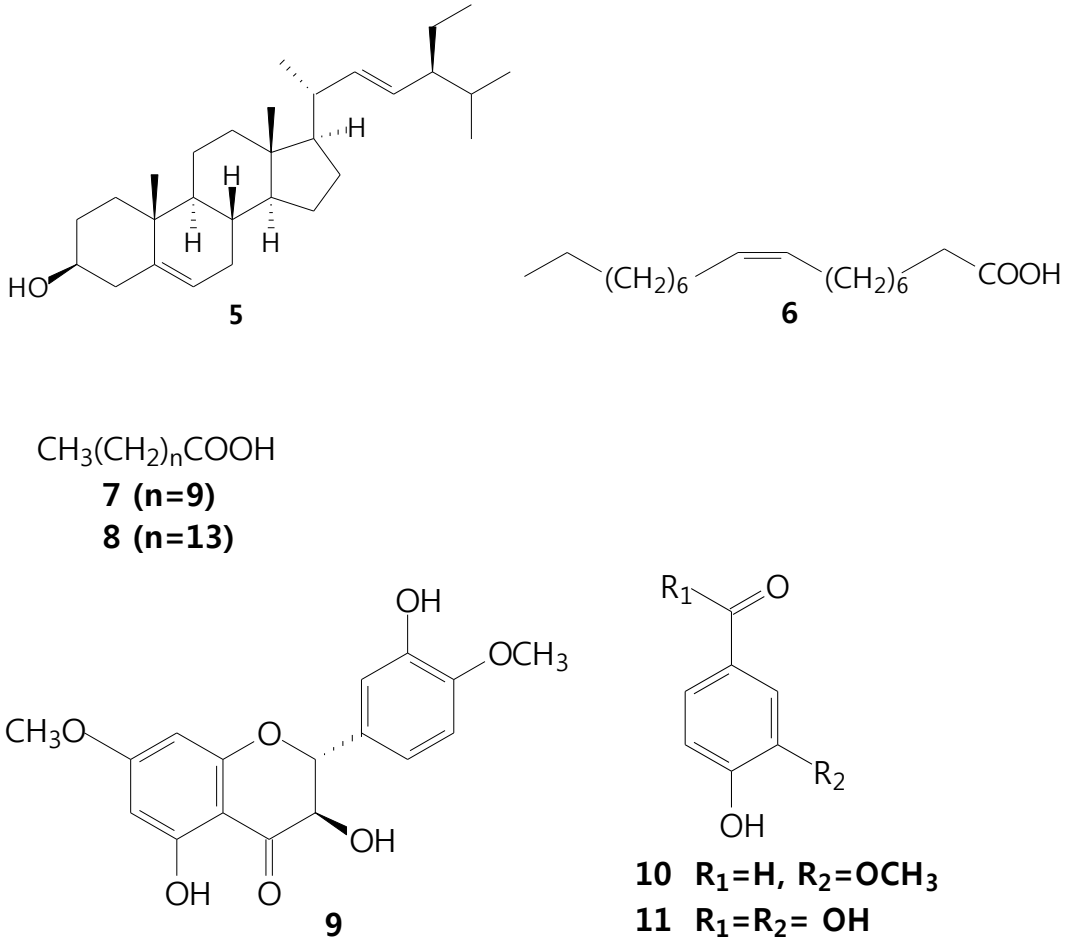



**Figure 2.2: Some compounds isolated from *H. abyssinica* rhizome continued**

### 2.18 Annonaceae family

Annonaceae is called the custard apple or "Sour sop" family and is the most diverse family of the order Magnoliales comprising more than 130 pan tropical genera. The family is constituted of about 2300-2500 flowering plant species with only five genera named as *Annona, Rollinia, Uvaria, Melodorum* and *Asimina*. Besides their edible fruits, the bark, leaves and roots of some plants of this family are also very useful in folk medicine (Mukeu *et al*., 2011).

Many plants in this family are known to possess cytotoxic compounds (De Mesquita *et al*., 2009; Kuete *et al*., 2011; Puvanendran *et al*., 2011; Choumessi *et al*., 2012). The Annonaceous acetogenins have been found in the seeds, leaves, twigs, barks and roots of the plants in this family, which are found to have potent and diverse biological effects such as cytotoxic, antitumor, antioxidant, antimalarial, pesticidal, antibacterial, antifungal and insecticidal activities (Castillo-Sanchéz *et al*., 2010; Manjula *et al*., 2011; Chandraju *et al*., 2012). *Uvariodendron anisatum* was evaluated for its anticancer activities in this study.

### 2.18.1 General description of *Uvariodendron anisatum* Verdec

*Uvariodendron anisatum* Verdec is a shrub or small tree, up to 9 m tall, it is known locally known as Mutonga (Kikuyu) and Mutongu (Meru). Most of the aerial parts are aniseed scented; bark grey-brown; branchlets longitudinally rugose, lenticillate, obscurely pale silky when very young soon glabrous. Leaf buds golden or ferruginous silky; juvenile foliage red, hanging. Leaf –blades oblong –elliptic or rarely oblong-lanceolate, 8-29.5 cm. long, 3.4-11.2 cm wide, broadest near the middle. Acute or obtuse at the apex, rounded or cuneate at the base, somewhat coriaceous, glabrous and very shining above, obscurely adpressed pilose beneath; midrib impressed above, very prominent beneath; lateral veins 16-21, prominent on both sides; venation reticulate; petiole 4-7 mm. long, thick (Bernard and Verdcout, 1971; Beentje, 1994).

*Uvariodendron anisatum* is a rare, indigenous and endemic to Central and Eastern parts of Kenya (Beentje, 1994), it was first reported in Emali forest in 1941, Nairobi district: 8 km, North Nairobi, Karura forest, by Karura stream in 1950 and in almost

same locality, near Kenya Limited breweries (Nairobi) in 1961 (Verdcout, 1971). It occurs in altitude ranging from 1170-1770 m. The plant is also found in Meru and Mbeere districts of Kenya at Kianjiru and Kiangombe hills (Kareru *et al*., 2007).

### 2.18.2 Biological activity of *Uvariodendron anisatum* Verdec

There are limited bioassay reports of *Uvariodendron anisatum* extracts. The water extract of the root of *U. anisatum* was reported by Misonge *et al*. (2014) to posses oxytocic like effects on isolated rat uterus. Antimicrobial activity against positive bacteria like *Staphylococcus aureus* and gram negative bacteria (*Escherichia coli*) have also been reported (Mutembei *et al*., 2018).

### 2.18.3 Ethnobotanical information of *Uvariodendron anisatum* Verdec

In Kenya, the root decoction of *Uvariodendron anisatum* is used to easy labour or remove after birth if it is late or retained while the root infusion is used to manage impotence in men (Gachathi, 2007). The wood is used as a walking sticks and axe handles (Beentje, 1994).

### 2.18.4 Phytochemicals isolated from *Uvariodendron anisatum* Verdec

The phytochemistry of genus *Uvariodendron* has limited studies. Only a few chemicals groups have been recorded for example, the phenylpropanoids like eugenol and acetyl eugenol from the *Uvariodendron usambarense* and *Uvariodendron pycnophyllum* of Tanzania (Kihampa *et al*., 2009). There is no report of previously isolated constituents from *Uvariodendron anisatum*. However, phytochemical groups including alkaloids, saponins, glycosides, terpenoids, volatile

oils, steroids and phenols have been detected in the leaf and root powder (Misonge *et al*., 2014; Mutembei *et al*., 2018).

### 2.19 Combretaceae family

The Combretaceae family comprises about 600 species and 20 genera of which 11 occur in the tropical Africa. *Combretum*, *Terminalia* and *Pteolepsis* are the most knoen genera in Africa due to their traditional medicinal uses (Fyhrquist, 2007). The genus *Combretum* is the largest of the Combretaceae family comprising of about 250 species, at least 24 species of which are well known in traditional medicine in Kenya for varied uses including abortifacients, aphrodisiacs scorpion and snake bites, mental problems, heart and worm remedies, fever and microbial infections. *Combretum caffrum* (a South African plant) has been reported to be important in the search for anticancer substances from plants, it is the source of combrestatin A-4, which is one of the most potent antimitotic agents (Evans, 2009; Kokwaro, 2009).

#### 2.19.1 General description of *Combretum tanaense* J. Clark

*Combretum tanaense* J. Clark is a liana, it is vulnerable and endemic to Kenyan riverine forests. It is collections in Kenya are limited to Embu /kitui district, Tana River, Fort Hall district and Thika River (Wickens, 1973). It has long whippy branches at least 10 m. Leaves (sub-) opposite, elliptic or obovate, base cuneate or rounded, apex rounded or shortly cuminate, 4-15 by 2-7 cm, glabrous. Flowers creamy white, in sub terminal spikes (or panicles) to 8 cm long, petals about 1 by 1.2-1.5 cm. Fruit yellow-green, 4 winged, 20-23 by 18-20 mm, glabrous and scaly (Beentje, 1994). It is selected in this study owing to the fact that it belongs to

combretaceae, a family known in the treatment of many diseases including cancer. In this case, anticancer activities of *C. tanaense* were studied.

### 2.19.2 Phytochemistry and biological activity of *Combretum tanaense* J. Clark

There are no reports of previously isolated constituents from *Combretum tanaense*. However, the root powder has been reported to contain glycosides, phenols, tannins and saponins (Onyancha *et al*., 2017). In addition, Onyancha *et al*. (2017) also reported antioxidant and antibacterial activities of *Combretum tanaense* extracts.

### 2.19.3 Ethnobotanical information of *Combretum tanaense* J. Clark

There is no report ethnopharmacological information of *Combretum tanaense* that is so far in record.

### 2.20 Asteraceae family

The family Asteraceae (Compositae) is one of the eight families of the order campanulales. It is the largest family of the flowering plants and contains about 960 genera and about 13,000 species (Evans, 2009). Compared with some large families such as fabaceae (Leguminosae), the number of important economic products derived from this family is relatively small. Currently, there is increased interests in asteraceae family a number of plants are uninvestigated for antitumor or antibacterial activities (Evans, 2009). This study focused on anticancer activities of *Launaea cornuta* (Hochst.) C. Jeffrey.

### 2.20.1 General description of *Launaea cornuta* (Hochst.) C. Jeffrey

It is an erect perennial herb with milky juice and hollow stems up to 1.5 m height and creeping rhizomes. Leaves are deeply divided form a rosette at the base, alternate on the stem, sessile, up to 2.5 cm long by 3 cm wide, entire or with two to three pairs of lobes acute-pointed near the base. Inflorescence large, diffuse with numerous yellow flower heads on peduncle about 2.5 cm long involucres up to 10 cm long by 4 mm cross, glabrous or shortly pubescent, phyllares in two to three rows, 2-4 mm long outside, up to 10 mm long inside. Florets 10-25, yellow up to 15 mm long, ligules often reddish outside seeds pale brown, elliptical, ribbed 2-4 mm long with white pappus 5 mm long,and (Agnew and Agnew, 1994; Maundu, 1999).

The herb is native to Africa and commonly known as wild or bitter lettuce, moleita and merlot (Sudan), muthunga (Kikuyu) muthunga (Meru and Embu) mchunga (Swahili), Mnyinya (Taita), and Achak (Luo). It occurs on alluvial soils in cultivated areas, including irrigated crops, on roadsides, near rivers and bush vegetation. A single plant can cover a large area because of spread by rhizomes. It is the commonest species of *Launaea* around Nairobi, Kenya (Agnew and Agnew, 1994; Maundu, 1999).

### 2.20.2 Biological activities of *Launaea cornuta* (Hochst.) C. Jeffrey

Recent studies have revealed potent hypoglycaemic, cytotoxic, antimicrobial, thromolytic and haemolytic activities (Musila *et al*., 2013; Kaigongi *et al*., 2014; Karau *et al*., 2014; Misonge *et a.l*, 2015; Khan *et al*., 2016). Karau *et al*. (2014) also demonstrated the safety of the ethyl acetate and water extracts by sub-acute toxicity studies.

### 2.20.3 Ethnobotanical information of *Launaea cornuta* (Hochst.) C. Jeffrey

*Launaea cornuta* is used as a wild vegetable in African communities for instance Kilifi, the coastal region of Kenya and Nigeria as a source of vitamin C (Schippers, 2004). In East Africa the decoction is used to treat typhoid, leaf juice is dripped into the ear to stop pain. The herb is boiled with water and the extract used to wash the body for the treatment of measles. In Tanzania the leave decoction is used in the treatment of gonorrhoea, ascariasis, stomach pains and fresh roots are chewed to cure swollen testicles (Kokwaro, 2009).

In Kenya, it is good for browser like goats and rabbits. The roots are used for the treatment of warts and also administered orally for the management of chronic joint pains (Wambugu *et al*., 2011). The concoction the whole plant is used to manage breast cancer and benign prostate hyperplasia and diabetes (Kareru *et al*., 2007).

### 2.20.4 Phytochemicals isolated from *Launaea cornuta* (Hochst.) C. Jeffrey

There is no report on previously isolated chemical compounds of *Launaea cornuta*, however preliminary phytochemical screening indicates the presence of alkaloids, saponins, flavonoids and sesquiterpene lactones (Kareru *et al*., 2007; Musila *et al*.,

2013; Misonge *et al*., 2015). Karau *et al*. (2014) used gas chromatography coupled with mass spectrometery to reveal the presence of sigmasterol **(5)**, lanosterol **(12)**, heinecosane **(13)**, octadecanoic acid **(14)**, octadic-9-enoic acid **(15)**, n-hexadecanoic acid **(16)**, 6-methylheptyl acetate **(17)**, cholest-5-en-3-ol (3-beta),- carbonochlorinate **(18)**, β-amyrin **(19)**, lup-20(29)-en-3-one **(20)**, fern-7-en-3.beta.-ol **(21),** benzimidazol (2,1-a) isoquinolone, 2-propenoic acid, and 1-decanol, 2-hexyl, 2H-1-Benzopyran-2-one, 6-acetyl-7-(acetyloxy)-4-methyl and 9,12-octadecanoic acid (Figure 2.3).

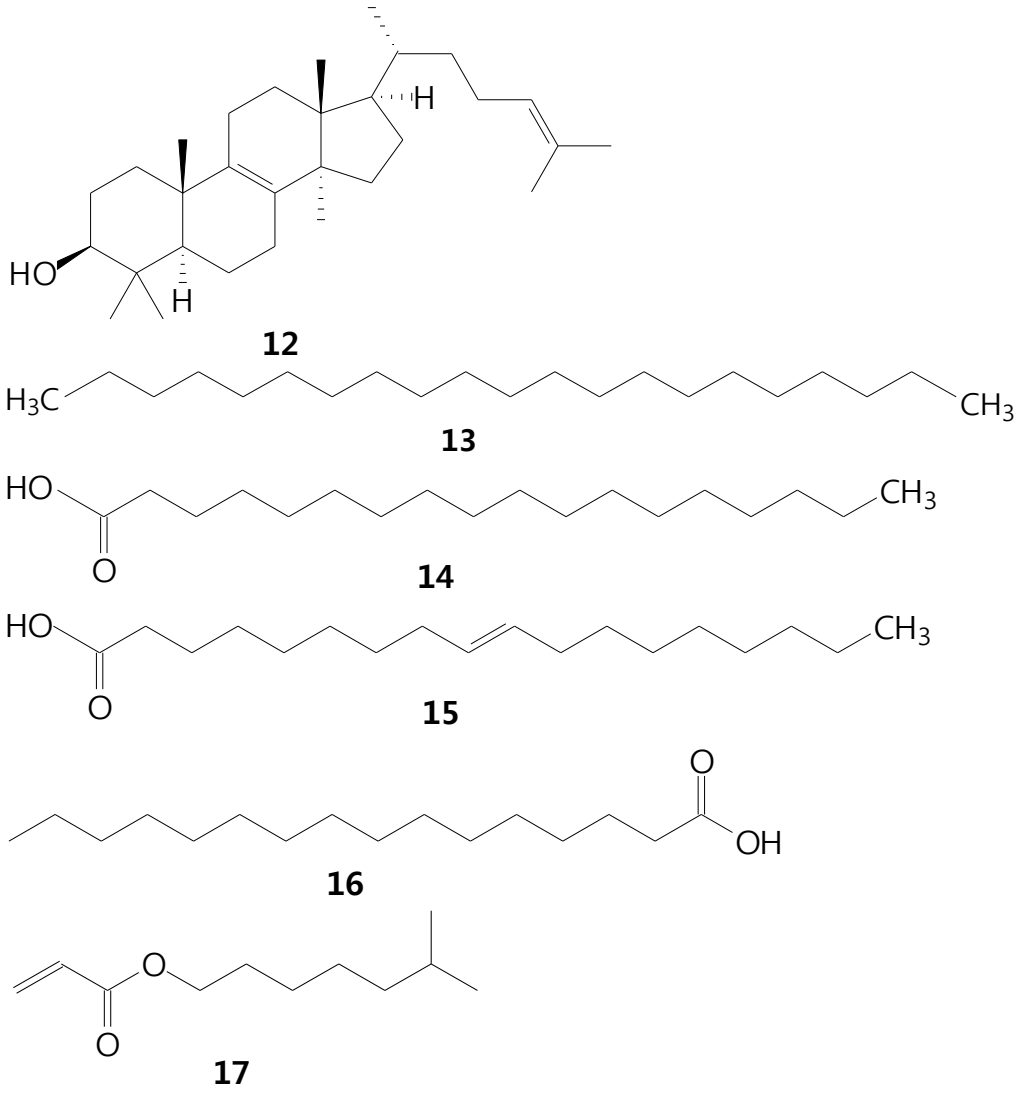
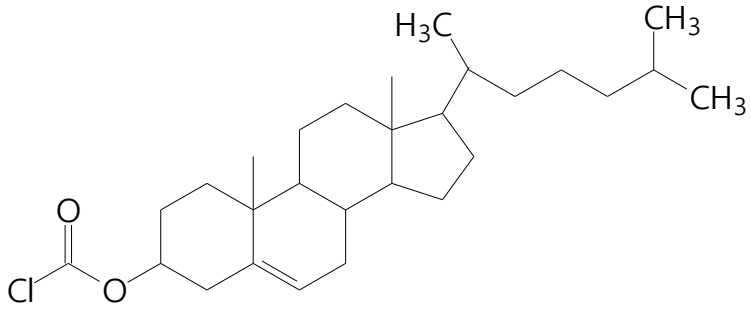



**Figure 2.3: Some compounds isolated from *L. cornuta***

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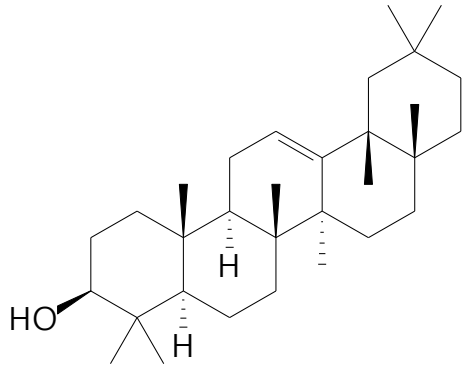


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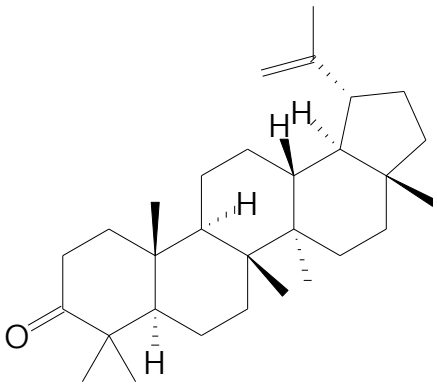


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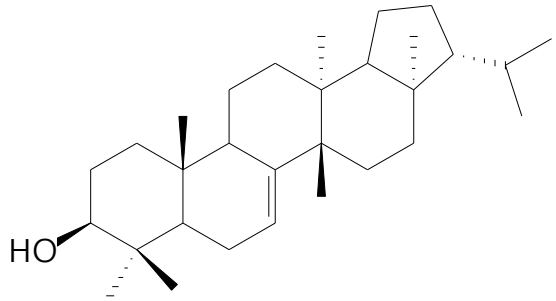



**21**

**Figure 2.3: Some compounds isolated from *L. cornuta* continued**

## 2.21 Rutaceae family

Rutaceae family comprises about 150 genera and 1600 species of trees, shrubs and climbers distributed throughout the tropical and temperate regions of the world. The main genera of this family are Citrus, Ruta, Ptelea, Murrya and Fortunella. In Kenya there are 10 general and 28 species. The plants rutaceae are characterized by varied secondary metabolites including alkaloids, flavonoids, coumarins, and volatile oil,

plants of this family have for a long time been used in the perfumery industry, gastronomy and traditional medicine (Supabphol and Tangjitjareonkun, 2014).

### 2.21.1 General description of *Fagarospis angolensis* (Engl.) Dale

*Fagaropsis angolensis* (Engl.) Dale is commonly known as Mukuriampungu or Murumu (Meru), Mũkaragatĩ (Kikuyu), Shingulosto (Luhya), Kwiril (Marakwet), Noiywet (Nandi), Myinja (Trade name and Mafu (Standard name). It is a deciduous tree which grows up to 20 m tall, bark is pinkish-grey, slightly rough and sometimes covered with purple corky outgrowths that have white dots. Leaves are compound, glabrous except the midrib, each leaf has 5-11 leaflets measuring 4-9 by 2-5 cm and are smooth with gland dots near the margin. Flowers are cream or yellowish in terminal panicles that are 3-12 cm long, petals are 3.5-6 mm long. The fruit is round, 6-8 mm diameter and is purple when ripe (Beentje, 1994; Gachathi, 2007).

### 2.21.2 Biological Activity of *Fagaropsis angolensis*

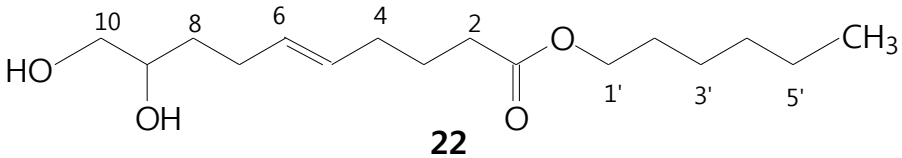
Methanol and aqueous extracts of the stem bark has been reported to show considerable *in-vitro* activity against both chloroquine resistant and chloroquine-sensitive *Plasmodium falciparum* strains, also the chloroform extract and volatile oil from the leaves have compounds with larvicidal activity against *Anopheles gambiae* making the plant important in both curative and preventive medicine in the fight against malaria (Kirira *et al*., 2006; Mudalungu, 2013). The ethanol extract of the stem bark has also demonstrated *in vitro* antimicrobial and antioxidant effects. Methanol extracts have significant toxicity in the brine shrimp lethality test (Kirira *et al*., 2006).

### 2.21.3 Ethnobotanical information of *Fagaropsis angolensis*

In Kenya, *Fagaropsis angolensis* wood is used for construction and making furniture (Gachathi, 2007). Ethnomedically the root decoction of *Fagaropsis angolensis* is used for the management of cancer and malaria (Jeruto *et al*., 2010). The leaf decoction is also useful in treatment of malaria and back and joint aches (Jeruto *et al*., 2010; Kareru *et al*., 2007). In some Kenyan neighbouring countries like Ethiopia, the bark concoction is used for the management of babesiosis in livestock, while in Uganda the stem bark decoction is used in the management of pneumonia, respiratory infections and bovine or caprine pleuropneumonia (Kuglerova *et al*., 2011; Fenetahun and Eshetu, 2017).

### 2.21.4 Phytochemicals isolated from *Fagaropsis angolensis*

Active chemicals have been isolated from *F. angolensis* bark and leaf extracts and include hexyl-9, 10-dihydroxydec-5-enoate **(22)**, rutaevin **(23)**, 5-methoxycanthin-6-one **(24),** canthin-6-one **(25),** phenanthrene carboxylic acid derivative **(26)**, diosphenol **(27)**, dihydrosanguinarine **(28)**, dihydronitidine **(29)**, 5-chloroindole **(30)**, methyl-10(-3-phenylpropanoyxyl)-7-hydroxyl-19-methylhenico-4,13,16-trienoate **(31)**, n-methyl-p-Chlorobenzenesulfonamide **(32)**, 11-cyclopentylheneicosane **(33)**, hexadecane **(34)**, 1-butyl-2-ethyloctahydro-4,7-epoxy-1H-inden-5-ol **(35)**, 3-methylhelheneicosane **(36)**, nitidine, 6-Hy-droxymethyldihydronitidine, 1,1-dicyano-2-methyl-4-(p-cyanophenyl) propene and Hahnfett (Figure 2.4) (Waterman and Khalid, 199; Khalid and Waterman, 1985; Kuglerova *et al*., 2011; Mudalungu *et al*., 2013).

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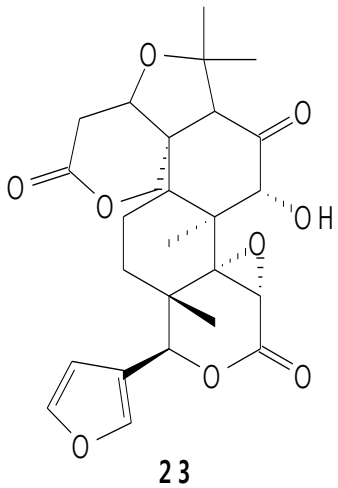


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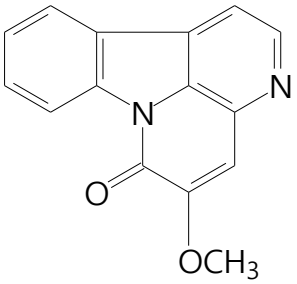


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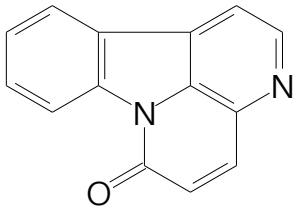


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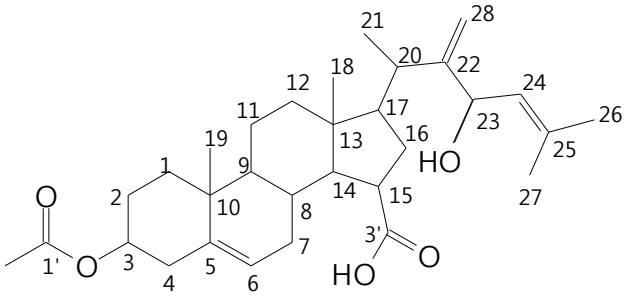


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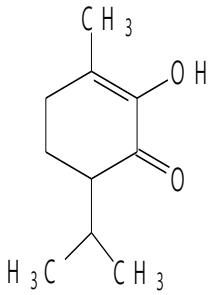


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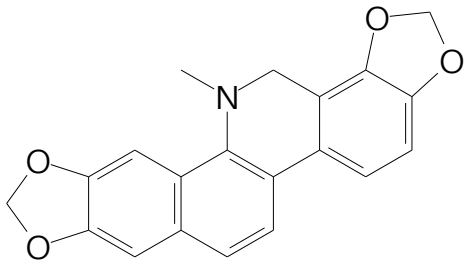


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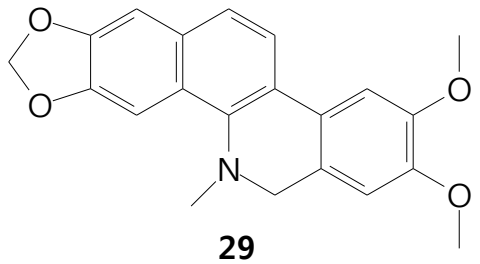



**29**

**Figure 2.4: Some compounds isolated from *F. angolensis***

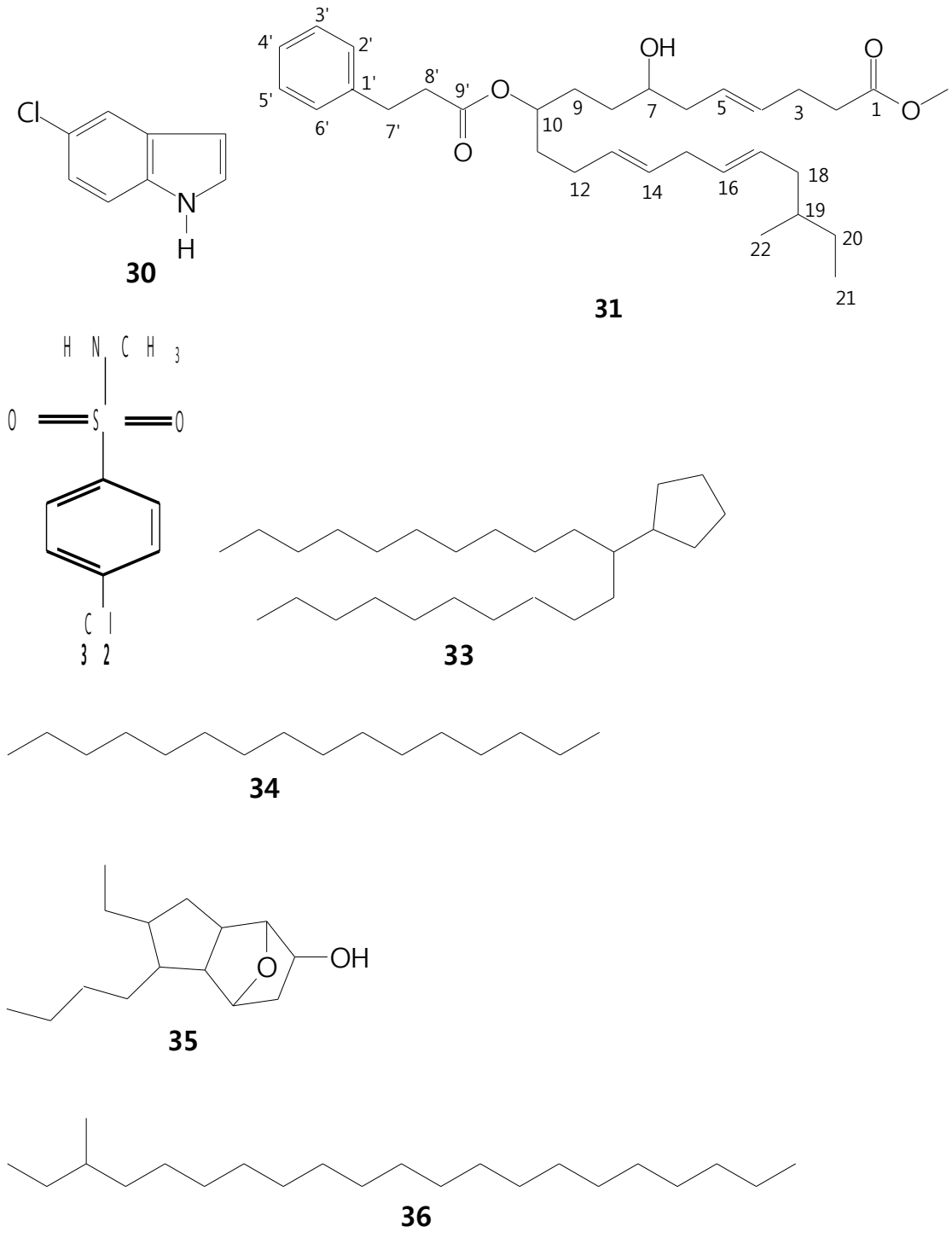



**Figure 2.4: Some compounds isolated from *F. angolensis* continued**

**Figure 2.4: Some compounds isolated from *F. angolensis* continued**

## 2.22 Rubiaceae family

Rubiaceae also called the madder, bedstraw or coffee family comprises one of the largest angiosperm families with about 650 genera and about 13,000 species, distributed in the tropical, subtropical and temperate regions. The plant in this family are most commonly shrubs but other mambers are trees, lianas and less than 20% herbs (Karou *et al*., 2011). In Africa the family has approximately 73 species of medicinal importance distributed into 34 genera.

### 2.22.1 General description of *Spermacoce princeae* (K. Schum.) Verdec

*Spermacoce princeae* (K. Schum.) Verdec. Synonymous to *Borreria princeae* (K. Schum.) is a trailing hairy perennial herb native to Africa, it is locally known by some Kenyan tribes as Omoutakiebo (Gusii), Gakungathe (Kikuyu), Murkugwet (Kipsigis), Nyamoch (Luo) and Chemurguiyweti (Nandi) (Jeruto *et al*., 2011; Kokwaro, 2009). It is 0.3-0.6 m long with ascending branches, stem often dark in color with hairs; roots develop from the nodes; leaves elliptic leaves with deeply impressed more or less parallel veins on the upper side; flowers are to 15 mm long, white in clusters of about 12; fruits capsule about 15 mm long (Agnew and Agnew, 1994). Mainly found in streamside, forest edges, road sides, hedgerows and swampy places.

### 2.22.2 Phytochemistry and Biological activity of *Spermacoce princeae*

Phytochemical screening studies indicate that the plant contains alkaloids, terpernoids, saponins and flavonoids (Jeruto *et al*., 2011).

### 2.22.3 Ethnomedical information of *Spermacoce princeae*

In Kenya, the whole plant of *Spermacoce princeae* is used for the management of cancer, diarrhea, wounds, chronic asthma, eye problems, skin diseases, venereal diseases, pneumona, typhoid, caterpillar bites and hepatic diseases (Jeruto *et al*., 2011; Kokwaro, 2009).

## 2.23 Asclepiadaceae family

Asclepiadaceae family consist of herbs, shrubs and rarely treelike plants with milky or less often clear latex. It has about 250 genera and over 2000 species which are spread in tropical and subtropical regions, especially in Africa and Southern South America. Some authorities include this family in the Apocynaceae. All plant parts especially the seeds and latex are often poisonous. They contain various alkaloids and glycosides which are used in medicine and as insecticides and several species are currently the objects of active research for antidiabetic and antitumor activity (Onyancha *et al*., 2017).

The genus *Marsidenia* belongs to the family Asclepiadaceae and is botanically synonymous to *Dregea* (Schmelzer and Gurib- Fakim, 2013). It is a large genus containing 200-300 species. In continental tropical Africa and Madagascar about 7 and 15 species occur respectively and several of them are used medicinally. The root decoctions and infusions are used to treat problems of urine retention, constipation, infectious diseases, and abdominal pain during pregnancy, relieving breast pain of women, aphrodisiacs and the aerial parts infusions are used to treat snakebites (Mitsuo, 1987; Schmelzer and Gurib- Fakim, 2013). It is reported that petroleum

ether extract of the fruits of *Dregea volubilis* Benth. Showed antitumor activity against Enlrich Ascites carcinoma bearing mice (Biwas *et al*., 2010).

### 2.23.1 General description of *Marsidenia schimperi* Decne

*Marsidenia schimperi* Decne. Synonym *Dregea schimperi* (Decne.) (Bullock) which is a plant selected in this study occur in Cameroon and widely in East Africa. The plant is a robust climber, 1-5 m. Leaves broad- ovate to circular and merely-tomentose beneath. Inflorescences cymose and loose, stalked. Flowers white or yellow, corolla 8-12 mm. Fruits follicles (narrowly) ovoid, 6-8 by 2-4cm, pods with numerous wrinkles but not winged. It occurs in dry forest margins, riverine woodland and bush land near forest edges (Agnew and Agnew, 1994).

### 2.23.2 Phytochemistry and Biological Activity of *Marsidenia schimperi*

There is no report of previously isolated constituents or biological activity of *Marsidenia schimperi* so far documented.

### 2.23.3 Ethnomedical information of *Marsidenia schimperi*

In Kenya the stem of *Marsidenia schimperi* is crushed, burned, mixed with fat and given to children improve digestion; the infusion is also used for treatment of diarrhoea, snakebite wounds and indigestion. Its roots are also used for aphrodisiac activity. The wood is used to make tool handles as ropes for binding cows and tying beehives in trees (Mitsuo, 1987; Kokwaro, 2009; Lulekal, *et al*., 2014; Schmelzer and Gurib- Fakim, 2013). In Somalia the plant is considered poisonous to livestock and cause animals to stand stiffly, shake and have diarrhoea (Schmelzer and Gurib-Fakim, 2013).

# CHAPTER THREE: MATERIALS AND METHODS

## 3.1 Study design and area

Analytical design which involved both observational and experimental studies were used in the current work. Cross-sectional ethnobotanical surveys were conducted in Embu county. The other counties that were involved in this study were Nyeri, Kiambu and Nyamira. Laboratory experiments for *in vitro* anticancer activities and oral acute toxicity were done at Kenya Medical Research Institute (KEMRI).

## 3.2 Ethnobotanical documentation of anticancer plants in Embu County

A guided reconnaissance was conducted during August 2016 to map out the key informant herbalists that were used to provide data for this study. The actual study was carried out between December 2016 to September 2017. A total of 16 key informants were recruited (four from each constituency) for the study with the assistance of the chairperson of the association of herbalists and the administrative officers in the Ministry of Gender, Sports, Culture and Social Service. The key informants were sampled purposively and data was collected using community participatory appraisal method. A workshop of sixteen key informants was held at Embu social hall where interviews, questionnaires, face to face consultations techniques were used to document names of plants used to manage cancer, the parts of plant used and methods of preparation of the medicines. Later on, the key informants were visited at their clinics for informal discussions, participant observations and confirmation of the information gathered during the interviews.

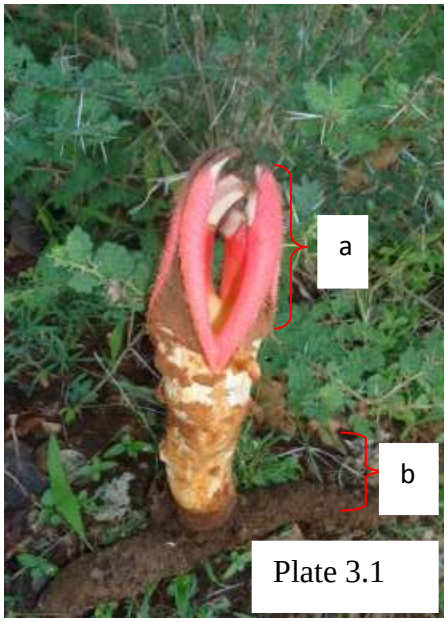
### 3.3 Selection and collection of plant materials

The selection of plants for study in this work was based on three approaches, first, ethnopharmacological approach where the plant samples were chosen by the fact that they were known as anticancer plants from folkloric medicine. Plant parts that were selected using this criterion were stem barks of *Fagaropsis angolensis* (Engl.) Dale, rhizomes and flowers of *Hydnora abyssinica* A. and aerial parts of *Launaea cornuta* C. The second approach was chemotaxonomical, the approach was useful for selecting plant species from genera or families that are known to produce compounds or class of compounds that are known to have anticancer activities, whole roots of *Combretum tanaense* J. (Combretaceae) and *Uvariodendron anisatum* V. (Annonaceae) were chosen following chemotaxonomical approach. Finally, *Marsidenia schimperi* D. selected on the basis of morphological features of the dry open fruit that resemble woman reproductive organ (Plate 3.10).

The five plants collected from Embu County included roots of *U. anisatum* from Kiangombe forest, rhizomes and flowers of *H. abyssinica* from Ishiara Karuri village, stem bark of *F. angolensis* from the ground of Ministry of Social and Cultural Services (Embu township), stem bark of *P. africana* and aerial parts of *L. cornuta* were obtained from Gatunduri Village (Embu county). Three other plants were collected from different counties. Roots of *C. tanaense* were collected from Mount Kenya University botanical garden in Kiambu County, aerial parts of *S. princeae* from Mabariri village, Bomwagamo location in Nyamira County and *Marsidenia schimperi* from the entrance of The Ark gate of Aberdares slopes, Mweiga-Nyeri in Nyeri County.

The collected specimens were identified and authenticated by a taxonomist at the National Museums of Kenya (East Africa Herbarium) where the voucher specimen numbers were prepared and deposited. Plant voucher specimens were as provided in parentheses as; *U. anisatum* (JMO-1-2015), *Hydnora abyssinica* (JMO-2-2014), *Fagaropsis angolensis* (JMO-3-2015), *Prunus africana* (JMO-3-2014), *Launaea cornuta* (JMO-1-2014), *Combretum tanaense* (JMO-2-2015), *Marsidenia schimperi* (JMO-5-2015) and *Spermacoce princeae* (JMO-4-2015). The photographs of the collected plant samples are provided in plates 3.1 to 3.11.

The collected plant samples were transported to Mount Kenya University on the day of harvesting. The samples were sorted and cleaned, thereafter they were cut into small pieces and air-dried under shade on the drying bench for ten days. After drying the plant samples were ground into course powder using an electric mill at KEMRI laboratories.



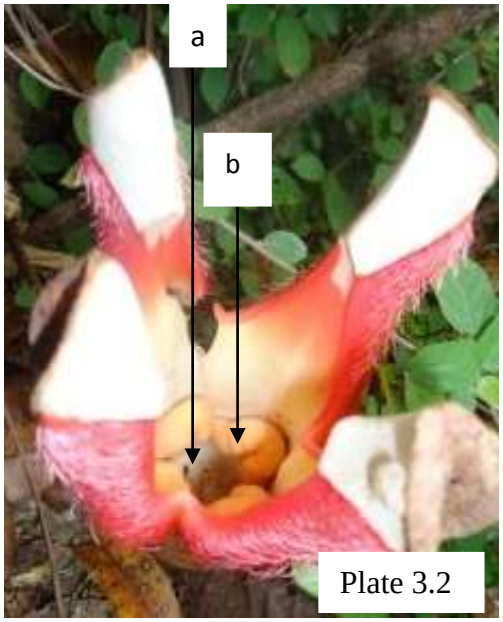




**Plate 3.1: Photograph of *Hydnora abyssinica* flower attached to rhizome**, a; Perianth and b; Rhizome

**Plate 3.2: Photograph of *Hydnora abyssinica* open flower**, a; pistilate and b; androecium

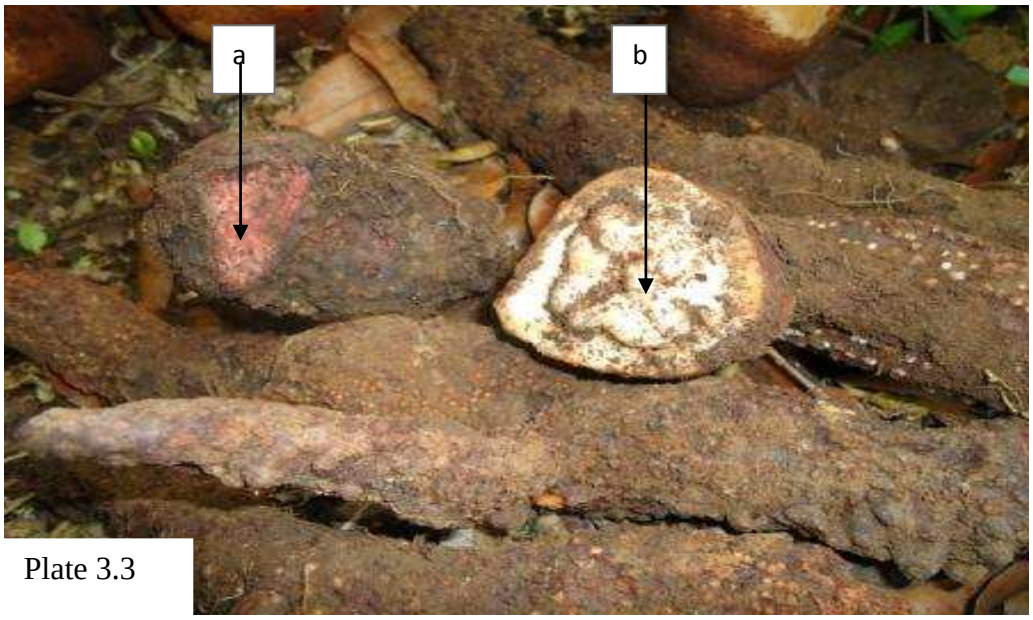



**Plate 3.3: Photograph of *Hydnora abyssinica* fresh rhizome and cross section** a; cross section fresh rhizome, b; cross section of ovary attached to rhizome

Plate 3.4

Plate 3.5

**Plate 3.4: Photograph showing branches of *Uvariodendron anisatum* with juvenile red foliage and mature green foliage**

**Plate 3.5: Photograph showing branches of *Uvariodendron anisatum* with mature fruits**

Plate 3.6

**Plate 3.6: Photograph of *Launaea cornuta* mature plant showing the leaves and flowers**


**Plate 3.7: Photograph of *Combretum tanaense* plant**


**Plate 3.8: Photograph of a mature *Spermacoce princeae* herb**

Plate 3.9

**Plate 3.9: Photograph of *Marsidenia schimperi* plant showing the leaves, fruits and feathery seeds**

Plate 3.10

**Plate 3.10: Photograph of *Marsidenia schimperi* pair of dry fruits and seeds**

Plate 3.11

**Plate 3.11: Photograph of *Fagaropsis angolensis* mature plant**

### 3.4 Preparation of crude plant extracts

Methanol extracts were prepared by maceration. Two hundred and fifty (250 grams) of ground powders were soaked in 1 L of methanol (Sigma-Adrich GmbH) for 48 hours using 2.5 L conical flasks. Methanol extracts were filtered and concentrated *in vacuo* at 50°C and final drying was done in an oven at 35°C. Water extracts were obtained by boiling 50 g of the powdered plant materials in distilled water (0.5 L) for 5 minutes, the water extracts were then allowed to cool at room temperature, after cooling the water extracts were filtered and lyophilized (Edward, Britain). The dried

extracts were weighed and labelled as indicated in Table 3.1. All labelled crude extracts were stored in a freezer at 4°C waiting for subsequent experiments.

**Table 3.1: Crude drugs extracts and respective percentage yield**

| Plant | Part used | Solvent of extraction | Yields (% w/v) |
|---|---|---|---|
| *Combretum tanaense* | Whole root | methanol | 22.7 |
| | | water | 17.2 |
| *Fagaropsis angolensis* | Stem bark | methanol | 21.7 |
| | | water | 9.8 |
| *Hydnora abyssinica* | Flower | methanol | 8.9 |
| | | water | 5.2 |
| *Hydnora abyssinica* | Rhizome | methanol | 77.6 |
| | | water | 18.8 |
| *Launaea cornuta* | Aerial | methanol | 28.8 |
| | | water | 19.7 |
| *Marshidenia schimperi* | Husks | methanol | 6.2 |
| *Marshidenia schimperi* | Leaves | methanol | 8.6 |
| *Spermacoce princeae* | Aerial | methanol | 25.7 |
| | | water | 13.9 |
| *Uvariodendron anisatum* | Whole root | methanol | 26.2 |
| | | water | 15.6 |
| *Prunus africana* | Stem bark | methanol | 38.8 |
| | | water | 10.2 |

### 3.5 Experimental animals

Adult female Swiss albino mice (eight weeks old) and weighing 20 ± 2 g were used to investigate acute toxicity of active crude extracts. A total of 84 experimental mice were obtained from Kenya Medical Research Institute (KEMRI) animal house following random selection. Mice were obtained after approval of use of animals for experimental procedures was granted by the Institutional Scientific and Ethics Review Unit (KEMRI/SERU/CTMDR/001/3024) (Appendix 3 and 4). The mice

were housed at KEMRI in a well-ventilated animal house with 12-h/12-h normal light/dark cycle under standard laboratory conditions (temperature 25 ± 3$^{o}$C with natural light and relative humidity between 50-60%). The experimental mice had access to water and standard pellet diet. The mice were labeled using picric acid and were kept separately in polycarbonate cages for five days prior to dosing (OECD, 2001).

### 3.6 Instrumental analyses

Infrared (FTIR 8400S, Japan) spectrophotometer and Ultraviolet Spectrophotometer (ENF-240 C/F UV lamp, Spectronics Co., Westbury, UK) were used at Jomo Kenyatta University (Kenya). The Nuclear magnetic resonance (NMR) spectrophotometer and spectrometer were used at the Analytical testing Centre of Tianjin International Joint Academy of Biotechnology and Medicine (Republic of China).

### 3.7 Cell culture procedures

#### 3.7.1 Cell line, Cell culture media and laboratory animals

Mouse breast carcinoma (4T1), human prostate carcinoma (22Rv1 ATCC®CRL-2505™) and (DU-145 ATCC® HTB-81™), and human breast carcinoma (HCC 1395 ATCC® CRL-2324™) (Rockville, USA) and African Green Monkey, Kidney epithelial cells (vero) cell lines (KEMRI, Kenya), Eagle's Minimum Essential Medium (EMEM) powder (Sigma® Aldrich), Roswell Park Memorial Institute (RPMI-1640) powder (Gibco® life technologies) and Swiss mice (KEMRI animal house, Kenya) were used.

### 3.7.2 Cell growth and passage

Human breast carcinoma (HCC 1395) and human prostate carcinoma (22Rv1) were cultured and maintained using Roswell Park Memorial Institute (RPMI-1640) medium while the DU-145, 4T1 and Vero cells lines were cultured and maintained using Eagle's Minimum Essential Medium (EMEM), all cultutres were supplemented with 100 units/ml penicillin, 100 µg/ml streptomycin and 10% fetal bovine serum (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) and maintained at 37 °C in a humidified atmosphere of 5% $CO_2$ in air.

All types of cells were cultured in T75 flasks and cell growth was controlled three times a week, usually on Monday, Wednesday and Friday. When the cells attained 90-100% confluence, they were subcultured using a new flask whereby old media was replaced by fresh maintenance media. Subculturing (cell passage) technique was performed and it involved removing old cell media, washing the cells using PBS (two times) as this was to achieve sufficient removal of media, thereafter trypsin (200 µl) was added and the cells were incubated for 5-10 minutes to detach them from the base of the flask. Cell detachment was confirmed by observing the flask under inverted microscope (Nicon eclipse TS100, Japan) at (x40) after which fresh media (5 ml) was added to the flask to provide cells with media for suspension and also to stop further enzymatic activity.

The cells were resuspended by gently repeated purging to separate the cells that were detached in clamps to form a homogenous suspension. Thereafter the cell population was determined using haemacytometer and then were seeded onto 96 well plate for MTT assay. The remaining cell suspensions were split and seeded onto a new flask with a predetermined splitting rate. Usually, a rate of 1:4 was enough for the cells to

acquire complete confluency within 48 hours.

### 3.7.3 Cell counting procedure

Cell density and viability assay was done by sing the trypan blue dye (Sigma® Adrich GmbH) exclusion technique. A homogenous cell suspension (40 µl) were diluted by a factor of 4 using trypan blue dye (40 µl cell suspension +120 µl trypan blue dye). Using a micropipette, 10 µl of the diluted cell suspension were aspirated and transferred to the counting chamber of a clean dry hematocytometer (Neubauer, Esco, Japan). The loaded hematocytometer was coverd with a cover slip and was placed under inverted microscope (Nicon eclipse TS100, Japan) (x40). Focus was made on the quadrants labelled A, B, C and D as shown in Figure 3.1 and viable cells were observed, counted and recorded as they appeared in 16 small squares of each squared quadrants (Viable cells fluoresced while the dead cells stained blue). In all cases the cells that touched the upper and right lines (marked x in Figure 3.1) were not counted to avoid double counting (as show in the side counting of quadrant C).

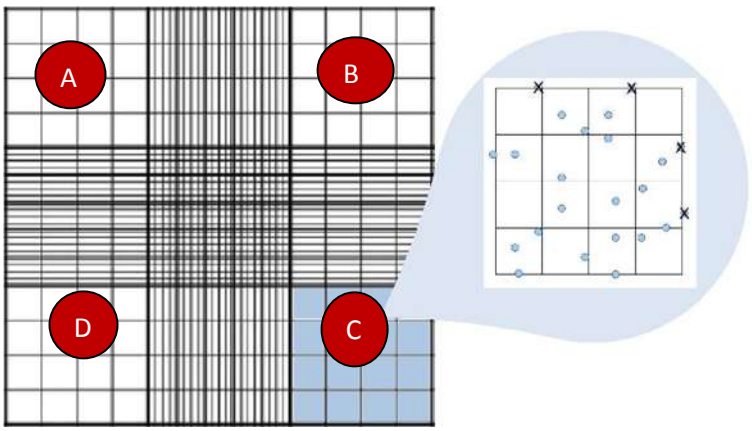



**Figure 3.1: Four quadrants each with 16 small squares**

The counting of the viable cells that were within the 16 small squares in each quadrant were recorded and the average was computed as per WHO (2004).

Total viable cells for the four corners (t) = (A) + (B) + (C) + (D)

Number of viable cells $ml^{-1}$= t x tb x $\frac{1}{4}$ x $10^4$

Where **t** = total viable cell count of four corner squares, **tb** = correction factor for trypan blue dilution (counting dilution was $\frac{1}{b}$), $\frac{1}{4}$ = correction to give mean cells per corner square and $10^4$ = conversion factor for the counting chamber. The working concentrations were then prepared by diluting the initial concentration using suitable media (WHO, 2004).

### 3.7.4 *In vitro* anticancer activity assay (MTT ASSAY) of the crude extracts

Crude extracts were dissolved in dimethylsulfoxide (DMSO) at a concentration of 10 mg/ml. Required serial dilutions were prepared under sterile conditions by adding calculated amounts of phosphate buffer solution (PBS) to obtain working concentration ranging from 1000 - 0 µg/ml. All prepared drugs were stored at 4 $^oC$ and retrieved only during use.

Standard 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was used to evaluate cancer cell line (HCC 1395, 4T1, DU-145 and 22Rv1) viability in the presence or absence of extract(s) was used. Microtitre plates (Castor®, USA) with 96 well were used, 100 µl of respective growth medium was placed in each well and seeded with 2 x $10^4$ cell lines per well. Cells were allowed to attach overnight and then various dilutions of the crude extracts were added in duplicate to respective wells.

The plates were then incubated for 48 h at 37$^{o}$C, 5% $CO_2$ and relative humidity 95%. 10 µl of MTT reagent was added to each well and be incubated further for 4 hours after which the supernatant was aspirated. 100 µl of dimethyl sulfoxide (DMSO) (Sigma, USA) solution was added to each well to solubilize MTT crystals and the plates were read for colour absorbance on an Enzyme linked immunosorbent assay (ELISA) scanning multiwell spectrophotometer (Multiskan Ex labsystems) at 562 nm. Cyclophosphamide and 5-fluorouracil were used as positive controls. Figure 3.2 indicates the plate design for the cytotoxicity studies. Percentage cell cytotoxicity was calculated using the formula:

$$\text{Percentage cytotoxicity} = \frac{\text{Ac} - \text{At}}{\text{Ac}} \times 100$$

Where Ac is absorbance of cells without treatment (control cells), At is absorbance of treated cells (Siti Syarifah *et al*., 2011).

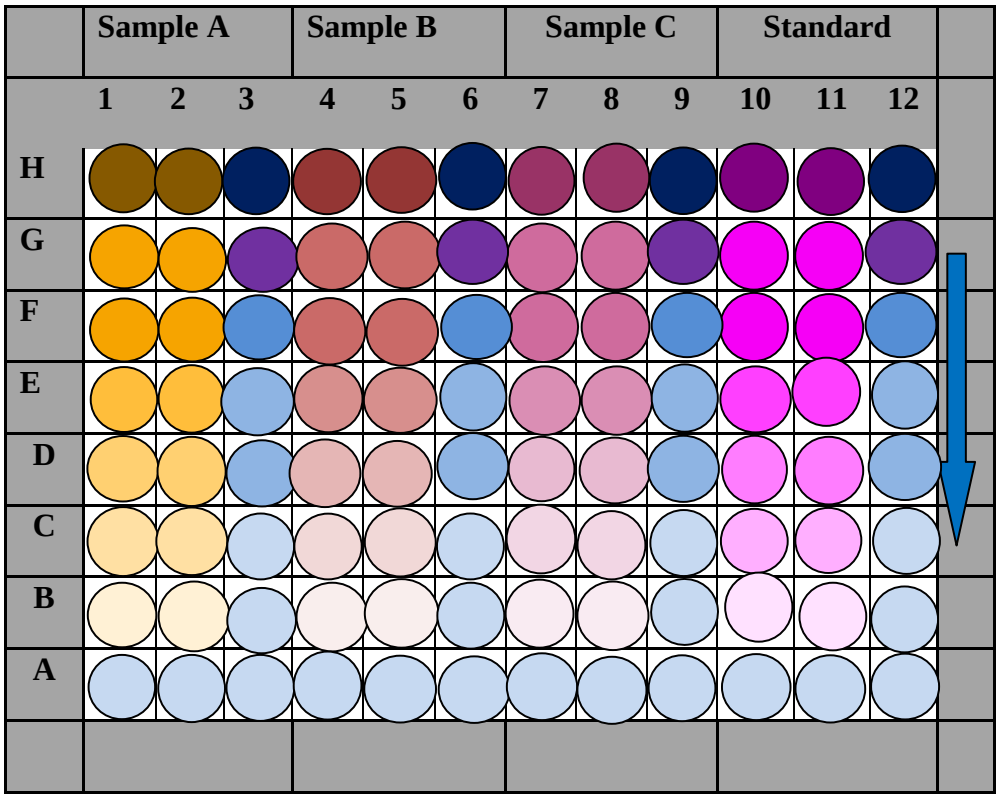



**Figure 3.2:** Diagrammatic representation of 96-well microtitre plate design and drug dilutions

Entire row A represents negative control (wells with media and untreated cells) with no test drug.

Column pairs (1, 2), (4, 5), (7, 8) and (10, 11) contained duplicates of different test drugs samples.

The arrow direction indicates decreasing concentration of extracts from H (1000 µg/ml to B (0 µg/ml)

Column 3,6,9, and 12 contained media and extracts in decreasing concentrations from H-B (wells without cells).

### 3.8 Bioactivity guided fractionation of active extracts

Crude extracts that demonstrated high activity with $IC_{50}$ of less than 50 µg/ml (methanolic extracts of *C. tanaense* root, *F. angolensis* stem bark, *H. abyssinica* and *U. anisatum*) in this study were selected for bioactivity-guided fractionation using column chromatography. Isocratic elution was done starting with petroleum ether,

dichloromethane, ethyl acetate, acetone and methanol (Sigma-Adrich GmbH) as mobile phases. Thin layer chromatography (TLC) of the individual fractions was done, after which the fractions that had similar TLC profile were combined. The combined fractions were allowed to air dry in the hood, thereafter they were weighed and packed into labelled sample bottles as indicated in Table 3.2. The dry fractions obtained were stored at 4 $^{o}$C until the time they were used for evaluation of anticancer activities using the method described in Section 3.7.4.

**Table 3.2: Fractions and respective percentage yields**

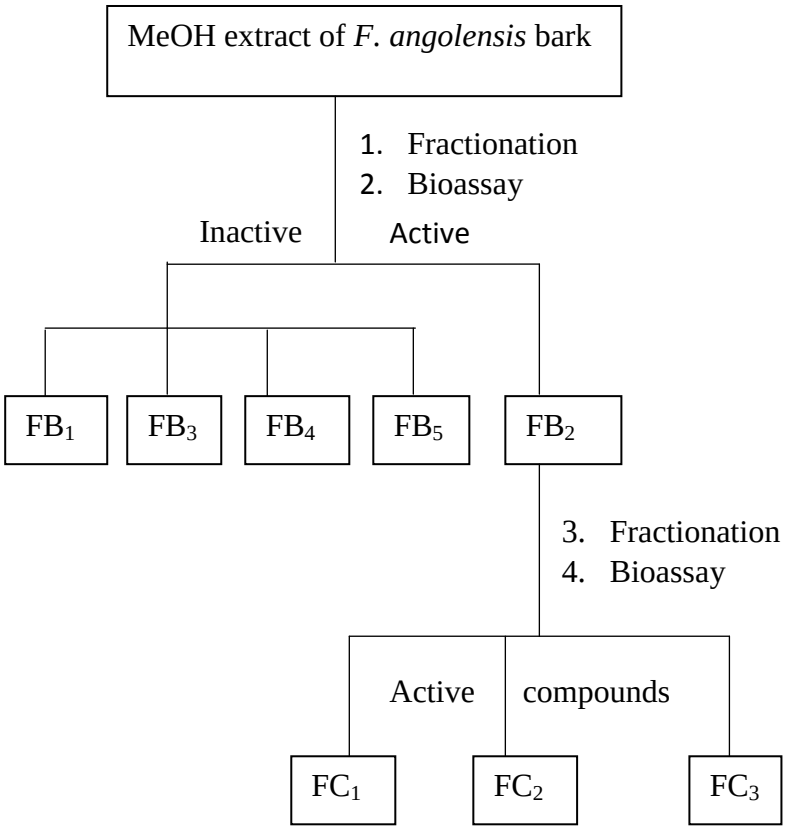
| Plant extract | Fractions | Code | Yields (% w/v) |
|---|---|---|---|
| *F. angolensis* stem bark methanol extract | Petroleum ether | $FB_1$ | 11 |
| | Dichloromethane | $FB_2$ | 31 |
| | Ethyl acetate | $FB_3$ | 6 |
| | Acetone | $FB_4$ | 8 |
| | Methanol | $FB_5$ | 34 |
| *C. tanaense* whole root methanol extract | Dichloromethane | $CT_1$ | 16 |
| | Ethyl acetate | $CT_2$ | 22 |
| | Acetone | $CT_3$ | 20 |
| | Methanol | $CT_4$ | 31 |
| *U. anisatum* whole root methanol extract | Petroleum ether | $UA_1$ | 6 |
| | Dichloromethane | $UA_2$ | 6.3 |
| | Ethyl acetate | $UA_3$ | 2.2 |
| | Acetone | $UA_4$ | 0.4 |
| | Methanol | $UA_5$ | 75 |
| *H. abyssinica* rhizome methanol extract | Petroleum ether | $HA_1$ | 3 |
| | Dichloromethane | $HA_2$ | 11 |
| | Ethyl acetate | $HA_3$ | 3.2 |
| | Acetone | $HA_4$ | 12 |
| | Methanol | $HA_5$ | 61 |

### 3.9 Bioactivity guided isolation of active compounds from active fractions

The dichloromethane fraction from *Fagaropsis angolensis* stem bark ($FB_2$) and ethyl acetate fraction from *Combretum tanaense* root ($CR_3$) demonstrated remarkable anticancer activities. These fractions were purified by column chromatography (Scheme 3.1 and 3.2).

### 3.9.1 Purification of compounds from dichloromethane fraction of *F. angolensis* stem bark

The dried greenish white powder (3.1 g) of active fraction $FB_2$ was purified by column chromatography. Gradient elution was done first using a mixture of petroleum ether and dichloromethane in the ratio of 1:0, 3:1, 1:1:, 1:3, 0:1. Secondly, a mixture of dichloromethane and ethylacetate were used in the ration of 1:0, 3:1, 1:1:, 1:3, 0:1. The eluates were collected into test tube (3 ml each), thin layer chromatography (TLC) was used to establish the eluates that had similar profiles, similarily eluates that were characterised with single spot and similar Retention factors ($R_f$) values were combined and allowed to evaporate and dry on the bench. Upon drying the dichloromethane fraction ($FB_2$) yielded three physically different powders. The purity of the samples was ascertained by thin layer chromatography (TLC) profiles in two solvent systems, the difference in their $R_f$ values was recorded. The samples were removed from the test tubes using a spatula and they were packed in clean amber coloured sample bottles. The compounds were labelled as *Fagaropsis angolensis* compound 1 to 3 ($FC_1$, $FC_2$, and $FC_3$) (Scheme 3.1). The pure samples were later subjected to *in vitro* anticancer asssessment the protocols as in Section 3.7.4. Structural elucidation was carried out as described in Section 3.11.

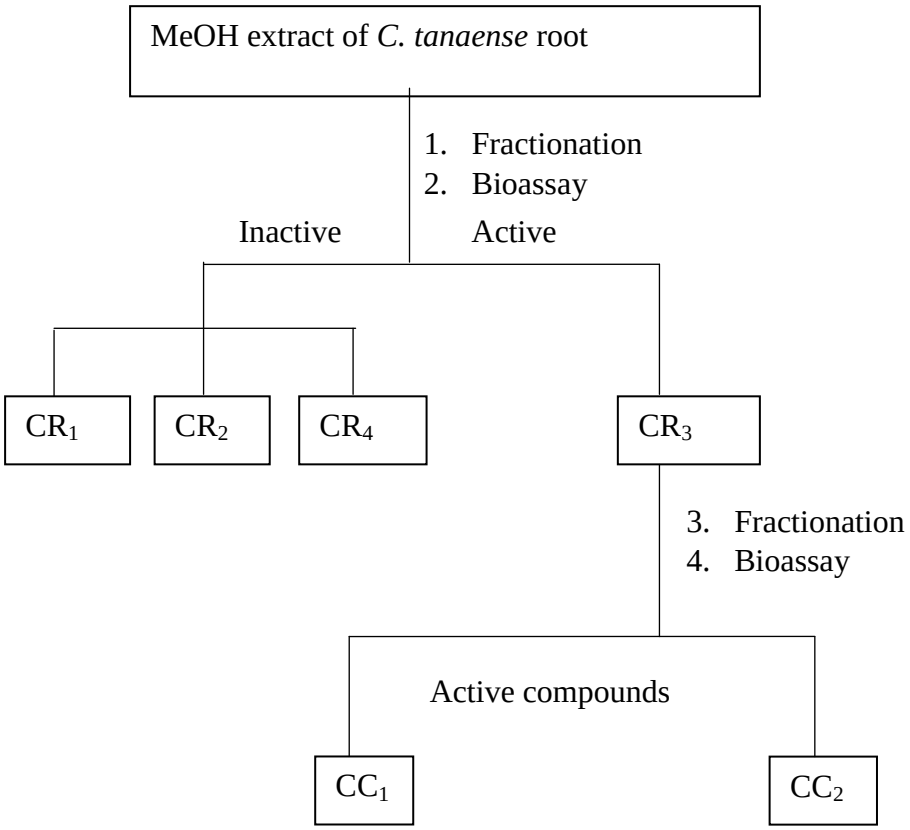



**Scheme 3.1: Bioassay guided fractionation of *F. angolensis* stem bark methanol extract**

### 3.9.2 Purification of compounds from acetone fraction of *C. tanaense* whole root

Two grams of dry greenish white powder ($CR_3$) were loaded onto a column packed silica gel and subjected to gradient elution using mixtures of petroleum ether and dichloromethane in the ratio of 1:1, 0:1. Therafter mixture of dichloromethane and ethylacetate in the ratio 1:0, 1:1, 0:1 were also used. Thin layer chromatography was performed and the eluates that had common Rf values were combinesd, they were allowed to evaporate and dry on the bench and thereafter they were removed by a spatula and kept in clean and labelled sample bottle. Two samples were obtined and were kept in dry cabinet awaiting *in vitro* antiancer activity asssessment the

protocols as in section 3.7.4. Structural elucidation was carried out as described in Section 3.1.




**Scheme 3.2: Bioassay guided fractionation of *C. tanaense* whole root**

### 3.9.3 Purification of compounds from methanol fraction of *Uvariodendron anisatum* root

About seven and half grams (7.5 g) of the acetone and methanol fractions, that were obtained from *Uvariodendron anisatum* root methanol extract ($UA_4$ and $UA_5$) were loaded onto column and subjected to gradient elusion. Mixture of solvents were used as mobile phases starting with ethyl acetate 100% (500 ml), ethyl acetate and acetone 25:75% (500 ml), ethyl acetate and acetone 50:50% (500 ml), ethyl acetate and acetone 75:25% (500 ml), acetone 100% (500 ml), acetone and methanol 25:75% (500 ml), acetone and methanol 50:50% (500 ml), acetone and methanol 75:25%

(500 ml) and finally methanol 100% (500 ml). Thin layer chromatography (TLC) was used to monitor the profiles of the fractions and similar fractions were combined. Fractions that had single spot on a TLC in two different solvent systems were considered to have pure compounds and were harvested using a spatula. The samples were weighed and stored in clean amber coloured sample bottles. The labels of the samples were *Uvariodendron anisatum* compound **1** and **2** ($UC_1$ and $UC_2$). The two samples were obtained and were kept in dry cabinet awaiting *in vitro* antiancer activity asssessment (protocols as in Section 3.7.4). Structural elucidation was carried out as described in Section 3.11.

### 3.10 Acute toxicity studies

#### 3.10.1 *In vitro* acute toxicity studies of against vero cell line

Normal kidney epithelial cells from African green monkey (vero E6) were used to evaluate toxicity effects of the plant extracts, fractions and isolated compounds. Microtitre plates with 96 well were designed as described in Section 3.7.4 above and MTT- formazan viability assay was performed. Optical densities readings were used to compute percentage cell viability using the formula described in Section 3.7.4. Thereafter selectivity indices (SI) values were determined which indicated the ability of the drug to discriminate against cancerous cells in favour of normal cells using the formula:

$SI = CC_{50}$ values for vero cell line/$IC_{50}$ values for cancer cell lines (Siti Syarifah *et al*., 2011).

Where $CC_{50}$ is the concentration of the extracts that exerted toxic effects to half of the population of normal cells and $IC_{50}$ is the concentration of the extracts that

inhibited growth or proliferation of half of the population of cancerous cells. Selectivity index values were assigned following criteria by Mahavorasirikul, *et al*. (2010) whereby $SI \leq 1$ (not selective), $1 < SI < 3$ (moderately selective) and $SI \geq 3$ (selective).

### 3.10.2 *In vivo* acute toxicity of the active crude extracts

Acute toxicity of the active crude extracts (methanol extracts of *F. angolensis* stem bark, *C. tanaense* root, *U. anisatum* and *H. abyssinica* rhizome) were evaluated according to Organization for Economic Co-operation and Development (OECD) Guideline 423 on Swiss albino mice. A single dose was administered stepwise to groups of normal 8 weeks old mice orally. Each group constituted three randomly selected mice. Drug administration was repeated for every step since using different mice based on the fact that there were no deatths observed. A total of 84 mice were used with dosing done at 50, 300 and finally 2000 mg/kg (OECD, 2001). The first three animals were given a single dose of 50 mg/kg orally, similar dosing was repeated for another set of three mice. Absence of extract-related mortality of the three animals dosed at this step led to administration of the next subsequent higher doses of 300 and 2000 mg/kg to three other additional animals in duplicate, respectively.

The drugs doses were reconstituted using phosphate buffered solution, mice were fasted for four hours and weighed before oral administration of 0.2 ml of the drug containing respective concentrations. Phosphate buffered solution was administered to the control group. Observations of wellness parameters (skin, fur, eye colour, mucus membrane, salivation, lethargy, sleep, coma, convulsions, tremors and

diarrhoea) were recorded at intervals of 30 minutes, 4 hours, 24 hours, 48 hours, 1 week and 2 weeks for each individual mouse. Weights for each mouse were recorded on the $7^{th}$ and $14^{th}$ day of the experiment. The experiment was terminated on the $14^{th}$ day whereby all mice in various groups were euthanized in carbon dioxide ($CO_2$) chamber and incinerated.

### 3.11 Structural elucidation methods

#### 3.11.1 Thin layer chromatography

Commercial aluminium plates that were pre-coated with 250 μm thick layer of normal silica gel were used for thin layer chromatography of the active crude extracts, fractions and isolated compounds. The developed plates were air dried and then visualized using UV lamp at wavelengths of 254 and 365 nm, respectively, secondly the plates were stained with iodine vapour. Lastly the plates were sprayed with 1% vanillin and heated in an oven at $110^0$C for 5 minutes. In every case, various spots were marked and their respective $R_f$ values calculated.

#### 3.11.2 Melting point

Melting point was determined using Micro Melting Point Apparatus (Yanaco, Japan).

#### 3.11.3 Infrared (IR) spectroscopy

Isolated compounds (1 mg) were mixed with 10 mg of Potassium bromide (KBr) discs (RFCL Limited, New Delhi India) and ground into fine mixture using a mortar and pestle. The ground material (compound and KBr) were sandwiched in a metal disc and pressed using a min-hand press (MHP-Shimadzu, Japan) till a transparent

thin film was formed. The film was placed in a sample holder and then scanned at wavenumber range 4000 to 400 $cm^{-1}$ ten times using Infrared (IR) spectrophotometer (FTIR 8400S, Japan).

### 3.11.4 Ultraviolet (UV) spectroscopy

The isolated compounds were analysed using Ultraviolet (UV) spectroscopy. Two milligrams of each compound was dissolved in 3 ml of methanol in a quartz cuvette. The prepared samples were introduced into Ultraviolet (UV) spectrophotometer (ENF-240 C/F UV lamp, Spectronics Co., Westbury, UK). The scanning range was set at wavelength of 190 nm to 900 nm and the spectra was produced as a ratio between the reference beam and the sample beam intensities ($I_0/I$).

### 3.11.5 Nuclear magnetic resonance spectroscopy

Proton Nuclear Magnetic Resonance ($^1$HNMR) spectra were recorded using Bruker spectrometer that was operated at 400 MHz and 500 MHz using deuterated acetone and methanol as solvents for the sample compounds. Tetramethylsilane (TMS) was used as an internal reference against which all the proton chemical shifts (δ) in parts per million (ppm) were made. The chemical shift for proton NMR were recorded as singlets (s), doublet (d), doublet of doublet (dd), triplet (t), quartet (q), multiplet (m) and coupling constants (*J* values) in Hertz (Hz). Carbon 13 Nuclear Magnetic Resonance ($^{13}$CNMR) was undertaken using the same spectrometer operated at 101 MHz and 126 MHz using deuterated acetone and methanol as solvents for the samples. TMS was used as a reference as chemical shift values (δ) were assigned and recorded in parts per million (ppm).

### 3.11.6 Mass spectroscopy

Electrospray was used as a mode of ionization (ESI) and mass to charge ratio (m/z) scan range of between 50 and 3000. The molecular ions for individual compounds were recorded as $M^+$ or $M^-$

## 3.12 Physical and spectroscopic data of the isolated compounds

### 3.12.1 Compound 37; 3-oxoolean-12-en-28-oic acid

White needle like crystals (Petroleum ether), melting point; 154.4 °C, chemical formula $C_{30}H_{46}O_3$; Rf values 0.74 and 0.63 (mobile phase; 9:1 and 9.5:0.5) dichloromethane: methanol, respectively), UV: $\lambda_{max}$ (MeOH); Appendix 5: 230, 235 nm. IR: $\nu^{KBr}{}_{max}$ ($cm^{-1}$); Appendix 6: 3165, 2942, 2865, 1695, 1595, 1400, 1331, 1210, 1113, 702. ESI-MS m/z; Appendix 7: 453.39 ($M^+$). $^1$H NMR (400 MHz, $C_3D_6CO$); Appendix 8 & 9; δ (ppm): 5.32 – 5.23 (m, 1H), 2.92 (dd, *J* = 13.8, 4.7 Hz, 1H), 2.55 (ddd, *J* = 15.8, 11.0, 7.3 Hz, 1H), 2.33 (ddd, *J* = 15.9, 7.0, 3.8 Hz, 1H), 2.13 – 1.85 (m, 5H), 1.90 – 1.68 (m, 4H), 1.73 – 1.30 (m, 7H), 1.33 – 1.22 (m, 1H), 1.27 – 1.16 (m, 4H), 1.21 – 1.11 (m, 1H), 1.16 – 1.01 (m, 8H), 0.95 (d, *J* = 10.7 Hz, 8H), 0.90 – 0.80 (m, 4H). $^{13}$C NMR (101 MHz, $C_3D_6CO$); Appendix 10 & 11; δ (ppm): 215.1, 178.1, 144.0, 122.0, 55.0, 46.9, 46.8, 46.0, 45.9, 41.8, 41.4, 39.3, 38.9, 36.6, 33.6, 33.6, 33.6, 32.5, 32.2, 30.4, 27.6, 26.0 25.3, 23.3, 23.0, 22.9, 20.9, 19.4, 16.6, 14.5.

### 3.12.2 Compound 38; 3-deoxyolean-12-en-28-oic acid

Colourless plate like crystals (dichrolomethane), melting point; 283.8 °C, chemical formula $C_{30}H_{48}O_2$; Rf values; 0.57 (mobile phase; 9:1) and 0.4 (9.5:0.5 dichloromethane: methanol), UV: $\lambda_{max}$ (MeoH); Appendix 12: 195 nm. IR: $\nu^{KBr}{}_{max}$

($cm^{-1}$); Appendix 13: 3131, 2943, 2864, 1698, 1595, 1460, 1398, 1326, 1209, 1113, 996, 919, 703, 648 and 579. ES-MS m/z; Appendix 14: 441.45 (M+H). $^{1}$H NMR (500 MHz, $CD_3OD$); Appendix 15 - 17; δ (ppm): 5.30 (t, *J* = 3.7 Hz, 1H), 2.89 (dd, *J* = 14.1, 4.6 Hz, 1H), 2.59 (ddd, *J* = 16.0, 10.8, 7.3 Hz, 1H), 2.40 (ddd, *J* = 16.1, 7.1, 3.8 Hz, 1H), 2.10 – 1.89 (m, 4H), 1.84 – 1.69 (m, 3H), 1.61 – 1.52 (m, 4H), 1.48 (s, 1H), 1.41 (dt, *J* = 9.6, 3.6 Hz, 3H), 1.22 – 1.12 (m, 6H), 1.12 – 1.05 (m, 10H), 0.99 – 0.88 (m, 10H). $^{13}$C NMR (126 MHz, $CD_3OD$); Appendix 18-20; δ (ppm): 181.8, 145.2, 123.5, 56.5, 48.2, 47.7, 47.2, 43.0, 42.8, 40.5, 40.2, 37.9, 35.1, 34.9, 33.8, 33.6, 33.5, 31.6, 28.8, 27.0, 26.3, 24.6, 24.1, 24.0, 21.9, 20.7, 17.6, 15.5.

**3.12.3 Compound 39;** 3β-Hydroxyolean-12-en-28-oic acid

White pellet crystals (acetone), Melting point; 272-274 °C, chemical formula $C_{30}H_{48}O_3$; Rf values; 0.63 and 0.69 for mobile phase of 9:1 and 9.5:0.5 (dichloromethane: methanol, respectively), UV: $\lambda_{max}$ (MeoH); Appendix 21: 240 nm. FTIR: $\nu^{KBr}_{max}$ ($cm^{-1}$); Appendix 22: 3165, 2942, 1695, 1595, 1400, 1331, 1210, 1113 and 702. $^{1}$H NMR (500 MHz, $CD_3OD$); Appendix 23 - 25; δ (ppm): 5.14 (t, *J* = 3.7 Hz, H), 3.21 (p, *J* = 1.6 Hz, 2H), 1.19 (d, *J* = 3.6 Hz, 1H), 1.06 (s, 1H), 0.88 – 0.80 (m, 4H), 0.72 (s, 1H), 0.68 (s, 1H). $^{13}$C NMR (126 MHz, $CD_3OD$); Appendix 26; δ (ppm): 181.9, 145.2, 123.6, 79.7, 56.7, 49.2, 47.6, 47.2, 42.9, 42.7, 40.5, 40.2, 39.8, 38.2, 34.5, 34.0, 33.8, 33.6, 31.6, 28.8, 28.8, 27.9, 26.4, 24.5, 24.0, 19.5, 17.7, 16.3, 15.9.

**3.12.4 Compond 40;** Anhydrous Bergenin

White needle like crystals (MeOH), melting point; 235-238$^{o}$C; chemical formula $C_{14}H_{16}O_9$; Rf values; 0.29 and 0.53 for mobile phase; 9:1 and 8:2 (dichloromethane:

methanol respectively), UV: $\lambda_{max}$ (MeOH); Appendix 27: 273 nm. IR: $\nu^{KBr}{}_{max}$ ($cm^{-1}$); Appendix 28: 3388, 3198, 1702, 1602, 1461, 1401, 1343, 1234, 1098, 1070, 991, 697 and 617. ES-MS m/z; Appendix 29: 327.06 $(M-H)^-$. $^1H$ NMR (400 MHz, $C_3D_6CO$); Appendix 30 – 33; δ (ppm): 8.44 (s, 1H), 8.34 (s, 1H), 7.08 (s, 1H), 5.04 (d, *J* = 10.4 Hz, 1H), 4.91 (dd, *J* = 4.6, 0.8 Hz, 1H), 4.64 (dd, *J* = 4.9, 0.8 Hz, 1H), 4.20 – 4.01 (m, 3H), 3.89 (s, 3H), 3.93 – 3.66 (m, 3H), 3.51 (ddd, *J* = 9.7, 8.5, 4.9 Hz, 1H), 2.88 – 2.80 (m, 3H), 2.05 (p, *J* = 2.2 Hz, 2H). $^{13}C$ NMR 101 MHz, ($C_3D_6CO$); Appendix 34; δ(ppm): 162.8, 150.8, 148.2, 140.4, 118.7, 116.1, 109.3, 82.0, 80.0, 74.6, 73.0, 71.1, 61.8, 59.8.

### 3.12.5 Compound 41

White prismatic cystals (MeOH), melting point; 152-155°C. UV: $\lambda_{max}$ (MeOH); Appendix 35: 273 nm. The IR ($\nu^{KBr}{}_{max}$ ($cm^{-1}$); Appendix 36: 3388, 3198, 1702, 1602, 1461, 1401, 1343, 1234, 1098, 1070, 991, 697 and 617.

### 3.12.6 Compound 42

White star like crystals from ethyl acetate (11.6 mg), melting point; 313.3-314.9; FTIR: $\nu^{KBr}{}_{max}$ ($cm^{-1}$); Appendix 37: 3156, 1594, 1401, 1338, 1212, 1112, 1069, 699, 615 and 488. ES-MS m/z; Appendix 38: 487.42 $(M-H)^-$. $^1H$ NMR (400 MHz, $C_3D_6CO$); Appendix 39 – 43; δ (ppm): 5.27 (t, *J* = 3.7 Hz, 1H), 4.07 (q, *J* = 7.1 Hz, 1H), 3.57 (d, *J* = 10.6 Hz, 1H), 3.40 (d, *J* = 9.5 Hz, 1H), 3.30 (d, *J* = 10.6 Hz, 1H), 2.91 (dd, *J* = 14.0, 4.6 Hz, 1H), 2.08 – 1.87 (m, 3H), 1.87 – 1.65 (m, 3H), 1.67 – 1.55 (m, 1H), 1.46 – 1.24 (m, 2H), 1.26 – 1.16 (m, 3H), 1.05 (d, *J* = 0.8 Hz, 2H), 0.95 (d, *J* = 11.1 Hz, 4H), 0.82 (s, 2H), 0.74 (s, 2H). $^1H$ NMR (400 MHz, $C_3D_6CO$) δ 5.27 (t, *J* = 3.7 Hz, 1H), 4.07 (q, *J* = 7.1 Hz, 1H), 3.57 (d, *J* = 10.6 Hz, 1H), 3.40 (d, *J* = 9.5 Hz, 1H), 3.30 (d, *J* = 10.6 Hz, 1H), 2.91 (dd, *J* = 14.0, 4.6 Hz, 1H), 2.08 – 1.87 (m,

3H), 1.87 – 1.65 (m, 3H), 1.67 – 1.55 (m, 1H), 1.46 – 1.24 (m, 2H), 1.26 – 1.16 (m, 3H), 1.05 (d, *J* = 0.8 Hz, 2H), 0.95 (d, *J* = 11.1 Hz, 4H), 0.82 (s, 2H), 0.74 (s, 2H). $^{13}$C NMR (101 MHz, $C_3D_6CO$); Appendix 44 – 45; δ (ppm): 178.0, 144.1, 122.1, 77.6, 68.0, 66.3, 59.6, 47.6, 47.3, 46.5, 46.0, 45.9, 42.5, 41.7, 41.3, 39.3, 37.8, 33.6, 32.5, 32.3,27.5, 25.5, 23.4, 23.0, 22.9, 17.8, 16.8, 16.6, 12.9.

### 3.12.7 Compound 43

White plate like crystals from ethyl acetate (7.7 mg), melting point; 346.7-349.5. FTIR: $\nu^{KBr}_{max}$ ($cm^{-1}$); Appendix 46: 3148, 1594, 1401, 1113 and 699. ES-MS m/z; Appendix 47: 487.42 $(M-H)^-$. $^1$H NMR (500 MHz, $CD_3OD$); Appendix 48 to 51; δ (ppm): 5.16 (t, *J* = 3.7 Hz, H), 4.52 (s, 0H), 3.40 (d, *J* = 11.1 Hz, H), 3.27 – 3.15 (m, 6H), 2.75 (dd, *J* = 14.1, 4.6 Hz, H), 1.97 – 1.78 (m, 1H), 1.72 – 1.56 (m, 1H), 1.20 (d, *J* = 5.2 Hz, 1H), 1.08 (s, 1H), 0.93 (s, 1H), 0.85 (d, *J* = 2.5 Hz, 1H), 0.81 (s, 1H), 0.72 (s, 1H), 0.59 (s, 1H). $^{13}$C NMR (126 MHz, $CD_3OD$); Appendix 52; δ (ppm): 145.4, 123.4, 78.1, 69.7, 66.2, 44.1, 43.0, 42.7, 40.6, 39.0, 34.9, 33.8, 33.6, 33.3, 31.6, 28.8, 26.5, 24.6, 24.0, 19.1, 17.8, 17.5, 13.9.

## 3.13. Disposal of cancer cells and experimental animals

All the used cell lines and mice were disposed in accordance with the protocols that are set by Kenya Medical Research Institute (KEMRI)'s Animal Care and Use Committee (ACUC).

## 3.14. Ethical considerations

Permission to carry out the study and ethical clearance was obtained from Kenyatta University School of Postgraduate Studies (Appendix 53), the Kenya Medical

Research Institute's Scientific and Ethics Review Unit (Appendix 3) and Animal Care and Use Committee (Appendix 4).

### 3.15 Data analysis

Ethnobotanical data was analysed by computation of the ratio a given plant species is mentioned as medicine to the total number of the traditional medicine practitioners who are interviewed in the study. The ratio is known as familiarity index ($F_i$) and calculated using the formula:

$F_i = Na/Nb \times 100$ (Tabuti *et al*., 2004; Tabuti *et al*., 2010).

Where **Na** was the number of traditional medicine practitioners who mentioned the plant as being used for treatment of cancer while **Nb** was the total number of herbalists who were interviewed (16).

*In vitro* anticancer and cellular toxicity results were processed using Microsoft Excel 2010 to compute $IC_{50}$ and $CC_{50}$ values. Observations for $LD_{50}$ were recorded systematically and individual records were maintained for each animal in table form. The $IC_{50}$ of the three independent experiments for anticancer, cellular toxicity and the weight changes of experimental animals were expressed as mean ± standard error of the mean (SEM). Statistical significance in the changes of body weights of experimental animals (mice) before and after treatments were calculated using student t-test. Graphpad Prism Version 7 was used to perform all the statistical analyses in the current study. The limit for potent anticancer activities in this study was set at $IC_{50} < 50$ µg/ml for crude extracts and at $IC_{50} < 5$ µg/ml for isolated pure compounds. However, the American National Cancer Institute criteria puts the cut

off of activity of crude extracts at $IC_{50}$ < 20 µg/ml and that of isolated compounds at $IC_{50}$ < 4 µg/ml (Boik, 2001; Mahavorasirikul, *et al*., 2010; Siti Syarifah *et al*., 2011).

The Nuclear magnetic resonance raw data of the isolated compounds were analysed using MestReNova software (Mestrelab Research Chemistry Software Solutions). The spectrometric data that was obtained was printed out and compared with authentic data for structural prediction. The resolved structures were drawn using ChemDraw Ultra 8.0 (Cambridgesoft Corp.).

# CHAPTER FOUR: RESULTS ANALYSIS

## 4.1 Anticancer plants

A total of thirteen plants from different families were reported for ttreatment and management of cancer from selected parts of Kenya. Nine of the plants were revealed by herbalist in Embu county (Table 4.1). Five of the plants, *Fagaropsis angolensis*, *Hydnora abyssinica*, *Launaea cornuta*, *Vitex keniense*, *Maytenus obscura*, *Flueggea virosa*, *Grewia villosa* and *Prunus africana* were reported to manage breast cancer. Three of the plants used by herbalists to treat breast cancer, namely, *Fagaropsis angolensis*, *Hydnora abyssinica* and *Prunus africana* were as well reported to treat prostate cancer. *Indigofera swaziensis* roots, was the only plant that was recorded to manage throat cancer.

The popilarity of use of the plants among the herbalists in Embu revealed that *Fagaropsis angolensis*, *Hydnora abyssinica* and *Prunus africana* were known to treat both breast and prostate cancers by all herbalists. This was indicated by Familiarity index of 100% for the three plants. The familiarity indices of the other six plants is indicated in Table 4.1.

Additionally, two other plants were recorded on the basis of chemotaxonomy, namely, *Uvariodendron anisatum* and *Combretum tanaense*, from Embu and Kiambu counties, respectively. *Marsidenia schimperi* from Nyeri County was recorded following the shape of the dry open fruit that resembled female reproductive organ (doctrine of signatures), a criterion with an assumption that plant parts resemble the human organs they treat. Finally, *Spermacoce princeae* recorede from Nyamira on the basis of literature review.

**Table 4.1: Plants used for management of cancer by Traditional Medicine Practitioners in Embu County, Kenya**

| Scientific name of plant (family), local names | Life form | Type of cancer treated | Part used and method of preparation and administration | Familiarity index |
|---|---|---|---|---|
| *Fagaropsis angolensis* (Engl.) Dale (Mukuriambungu) (Rutaceae) | Tree | Breast and prostate cancers | The stem bark and whole roots are boiled and solution taken | 100 |
| *Hydnora abbyssinica* Schweinf. (synonym: *H. johannis* Becc.Nouv. and *H. solmsiana* Dinter.) (Ndonga or Mutumurathi) (Hydnoraceae) | Herb | Breast and prostate cancers | The whole rhizome is boiled and the decoction taken with soup | 100 |
| *Prunus africana* Hook. F. (Mwiria) (Rosaceae) | Tree | Breast and prostate cancers | The stem bark is boiled and the decoction taken with soup | 100 |
| *Flueggea virosa* (Willd.) Voigt (Euphorbiceae) (Mukururu) | Shrub | Breast cancer | The roots are boiled and solution taken | 67 |
| *Launaea cornuta* (Hochst. ex Oliv. & Hiern) C. Jeffrey (Muthunga) (Asteraceae) | Herb | Breast cancer | Aerial parts (leaves and stems) are boiled and the vapor inhaled | 63 |
| *Grewia villosa* Willd. (Mubuu) (Tiliaceae) | Shrub | Breast cancer | The roots are boiled and solution taken | 56 |
| *Vitex doniana* Sweet (Muburu) (Verbenaceae) | Tree | Breast cancer | Leaves are boiled and drunk | 50 |
| *Maytenus obscura* (A. Rich.) Cuf. (Muraga) (Celestraceae) | Shrub | Breast cancer | The roots are boiled and solution taken | 44 |
| *Indigofera swaziensis* Bolus (Unknown) (Papilionaceae) | Shrub | Throat cancer | Root are boiled and drunk | 6 |

## 4.2 *In vitro* anticancer activities of selected crude plant extracts

Out of eighteen plant extracts that were investigated for anti-breast and anti-prostate cancer activities, fifteen extracts demonstrated varied activities ranging from remarkably high to low anticancer activities. The lowest with $IC_{50}$ value was 1.8 ± 0.1 µg/ml (most active extract), while the highest $IC_{50}$ value was recorded as 911.5 ± 76.5 µg/ml (the least active extract) (Table 4.2). Generally, methanol extracts revealed high anticancer activities compared to water extracts (Table 4.2 and 4.3).

### 4.2.1 Anticancer activities of crude plant extracts against breast cancer cell lines

Mice breast cancer (4T1) and human breast cancer (HCC1395) cell lines were used for evaluating the plant extracts for anti-breast cancer activity. All the eighteen extracts inhibited growth of 4T1 cell line. On the other hand, cell growth of the HCC 1395 cell line was inhibited by seventeen extracts out of the eighteen tested extracts, only *Combretum tanaense* root water extract was reported to be inactive ($IC_{50}$ > 1000 µg/ml) against HCC 1395. Four methanol plant extracts obtained from *Uvariodendron anisatum* root, *Hydnora abyssinica* rhizome, *C. tanaense* root and *Fagaropsis angolensis* stem bark exhibited remarkably high activities against either one or both of tested breast cancer cell lines (HCC 1395 and 4T1). These crude extracts revealed low $IC_{50}$ values of less than 30 µg/ml (a concentration that inhibited viability of cancer cell lines by half) (Table 4.2). Most of $IC_{50}$ values of the aforementioned extracts were similar to that of the reference drugs. This observation may suggest that the crude extracts were more potent as compared to the conventional reference drugs (Cyclophosphamide and 5 fluorouracil) which were pure compounds. *Prunus africana* stem bark extracts were also used as crude plant extract

references. The crude extracts in this study demonstrated activities that had comparable $IC_{50}$ values to that of *P. africana* as indicated in Table 4.2.

Methanol *U. anisatum root* extract, methanol and water *H. abyssinica* flower extracts and methanol *F. angolensis* stem bark extract revealed high anticancer activities against HCC 1395 cancer cell lines. High activities against 4T1 cell line were also observed with *H. abyssinica rhizome* water extract, *H. abyssinica* flower methanol and water extracts and *F. angolensis* stem bark water extract. Plant extracts that showed $IC_{50}$ values that ranged between 30 to 100 µg/ml were classified as having high anticancer activities. Other extracts from aerial parts of *Launaea cornuta* and *Spermacoce princeae* exhibited low activities ($IC_{50}$ values ranging between 100-1000 µg/ml) as shown (Table 4.2).

**Table 4.2: *In vitro* anticancer activities of plant extracts against breast cancer cells**

| Plant (part) | Solvent of extraction | $IC_{50}$ values (µg/ml) of tested cancer cell lines | |
|---|---|---|---|
| | | HCC 1395 | 4T1 |
| *Uvariodendron anisatum* whole root | methanol | 50.6 ± 2.9 | 1.8 ± 0.1 |
| *Uvariodendron anisatum* whole root | water | 248.0 ± 5.8 | 150.7 ± 4.9 |
| *Hydnora abyssinica* rhizome | methanol | 27.2 ± 1.1 | 22.9 ± 0.1 |
| *Hydnora abyssinica* rhizome | water | 499.3 ± 1.3 | 79.8 ± 1.0 |
| *Hydnora abyssinica* flower | methanol | 79.7 ± 1.0 | 56.1 ± 11.1 |
| *Hydnora abyssinica* flower | water | 37.2 ± 3.9 | 81.6 ± 16.6 |
| *Launaea cornuta* aerial | methanol | 231.7 ± 2.0 | 300.5 ± 5.5 |
| *Launaea cornuta* aerial | water | 381.0 ± 15.3 | 700.5 ± 14.5 |
| *Combretum tanaense* whole root | methanol | 193.0 ± 13.2 | 19.5 ± 0.00 |
| *Combretum tanaense* whole root | water | >1000 | 289.7 ± 2.9 |
| *Fagaropsis angolensis* stem bark | methanol | 53.9 ± 5.6 | 12.9 ± 1.2 |
| *Fagaropsis angolensis* stem bark | water | 553.6 ± 15.4 | 80.0 ± 1.7 |
| *Spermacoce princeae* aerial | methanol | 533.0 ± 56.6 | 204.0 ± 6.6 |
| *Spermacoce princeae* aerial | water | 911.5 ± 76.5 | 562.0 ±1 0.0 |
| *Prunus africana* stem bark | methanol | 10.6 ± 0.7 | 4.8 ± 1.0 |
| *Prunus africana* stem bark | water | 81.9 ± 8.04 | 36.8 ± 8.6 |
| *Marsidenia schimperi* leaf | methanol | 45.0 ± 5.1 | 23.1 ± 7.3 |
| **Cyclophosphamide (positive control)** | | **32.8 ± 1.1** | **22.8±1.1** |
| **5 Fluorouracil (positive control)** | | **38.8 ± 7.6** | **NT** |

**Values are represented as Mean ± SEM of $IC_{50}$ (µg/ml) from three independent experiments (Appendices 54 - 61), NT: Not tested, HCC 1395 and 4T1: Breast cancer cell lines, %: percentage. Anticancer activities of extracts; $IC_{50}$ < 50 µg/ml: highly active, $IC_{50}$ between 50 and 100 µg/ml: moderately active, $IC_{50}$ between 100 and 1000 µg/ml: weakly active, and $IC_{50}$ > 1000 µg/ml: non-active**

### 4.2.2 Anticancer activities of crude plant extracts against prostate cancer cell lines

Majority of selected plant extracts (> 95%) in this study demonstrated anti-prostate cancer activities against prostate cancer cell lines (DU-145 and 22RV1). The activities ranged from remarkably high to low ($12.8 \pm 1.1 < IC_{50} < 763.7 \pm 61.5$) µg/ml. *F. angolensis* stem bark methanol extract exhibited remarkable activity against DU-145 while *U. anisatum* root methanol, *H. abyssinica* rhizome methanol and *C. tanaense* root methanol extracts were reported to have high activity against DU-145 cell line ($30 > IC_{50} < 100$ µg/ml). On the other hand, three methanol extracts (*U. anisatum* root, *H. abyssinica* flower, *F. angolensis* stem bark) and one water extract from *H. abyssinica* flower exhibited high activities against 22RV1 prostate cancer cell line ($52.8 \pm 19 > IC_{50} < 165.0 \pm 10.1$ µg/ml) (Table 4.3). Extracts from aerial part of *L. cornuta* and *S. princeae* were reported to have low anti-prostate cancer activities together with other extracts (Table 4.3) that had $IC_{50}$ values between 100 to 1000 µg/ml. However, water extract from *C. tanaense* root was reported to be inactive against the tested prostate cell line ($IC_{50} > 1000$ µg/ml) (DU-145).

**Table 4.3: *In vitro* anticancer activities of plant extracts against of prostate cancer cells**

| Plant extract | Solvent of extraction | $IC_{50}$ values (µg/ml) of cancer cell lines | |
|---|---|---|---|
| | | DU-145 | 22RV1 |
| *Uvariodendron anisatum* whole root | methanol | 81.7 ± 3.9 | 35.8 ± 15.5 |
| *Uvariodendron anisatum* whole root | water | 671.5 ± 65.5 | 824.0 ± 88.2 |
| *Hydnora abyssinica* rhizome | methanol | 66.5 ± 56.0 | 141.7 ± 14.8 |
| *Hydnora abyssinica* rhizome | water | 521.7 ± 26.0 | 125.0 ± 5.8 |
| *Hydnora abyssinica* flowers | methanol | 372.2 ± 222.5 | 65.0 ± 10.1 |
| *Hydnora abyssinica* flowers | water | 763.7 ± 61.5 | 52.8 ± 19 |
| *Launaea cornuta* aerial | methanol | 289.7 ± 2.3 | 596 ± 8.2 |
| *Launaea cornuta* aerial | water | 142.7 ± 41.7 | 635 ± 69.7 |
| *Combretum tanaense* whole root | methanol | 73.9 ± 3.1 | 203.0 ± 22.9 |
| *Combretum tanaense* whole root | water | > 1000 | NT |
| *Fagaropsis angolensis* stem bark | methanol | 12.8 ± 1.1 | 61.7 ± 6.9 |
| *Fagaropsis angolensis* stem bark | water | 314.0 ± 96.3 | 35.0 ± 0 |
| *Spermacoce princeae aerial* | methanol | 151.7 ± 16.7 | 340.0 ± 67.6 |
| *Spermacoce princeae aerial* | water | 328.7 ± 37.9 | 530.0 ± 47.7 |
| *Prunus africana* stem bark | methanol | 24.4 ± 3.6 | 19.6 ± 5.8 |
| *Prunus africana* stem bark | water | 19.9 ± 0.9 | 20.7 ± 0.8 |
| *Marsidenia schimperi* leaf | methanol | 36.7 ± 3.1 | 44.8 ± 2.3 |
| *Marsidenia schimperi* husk | methanol | 47.6 ± 2.7 | 27.3 ± 2.3 |
| **Cyclophosphamide (positive control)** | | **28.9 ± 2.9** | **NT** |
| **5 Fluorouracil (positive control)** | | **18.3 ± 6.1** | **25.0 ± 12.1** |

**Values are represented as Mean ± SEM of $IC_{50}$ (µg/ml) from three independent experiments (Appendices 62 - 69), NT: Not tested, DU-145 and 22Rv1: prostate cancer cell lines.**

**Anticancer activities of extracts; $IC_{50}$ < 50 µg/ml: highly active, $IC_{50}$ between 50 and 100 µg/ml: moderately active, $IC_{50}$ between 100 and 1000 µg/ml: weakly active, and $IC_{50}$ > 1000 µg/ml: non-active**

## 4.3 Bioassay- guided fractionation of crude extracts with remarkable anticancer activities

The methanolic extracts of *F. angolensis* stem bark, *C. tanaense* root, *U. anisatum* root and *H. abyssinica* rhizome demonstrated remarkable activity (Table 4.2 and Table 4.3) for they exhibited antiproliferative potency of around concentrations of 30 ± 5 µg/ml (Kuete *et al*., 2016). These extracts gave different fractions with varied anticancer activities against the tested cancer and normal cell lines.

### 4.3.1. Bioassay-guided fractionation of *F. angolensis* stem bark methanol extract

Bioassay guided fractionation yielded five fractions (petroleum ether, dichloromethane, ethyl acetate, acetone and methanol fractions) as shown in Table 4.4. This study indicated that the fraction obtained from dichloromethane had remarkable activity against both prostate (DU-145) and breast cancer (HCC 1395) cell lines. The findings revealed that there were improved anticancer activity levels that was shown by reduction of $IC_{50}$ values from 53.9 ± 5.6 to 8.33 ± 1.7 µg/ml against HCC 1395. However, there was an increase in $IC_{50}$ values from 12.8±1.1 to 31.07 ± 3.3 µg/ml against DU-145 (prostate cancer cell lines). Though there was a decrease in antiprostate cancer activities, the $IC_{50}$ values (31.07 ± 3.3 µg/ml) that were recorded was still within the category of remarkable activity ($IC_{50}$ < 30 µg/ml). These results suggests probably that purification makes the extract more potent as an anticancer agent.

**Table 4.4: Concentration of *F. angolensis* fractions that inhibited proliferation of cancer cells by 50% ($IC_{50}$)**

| ***F. angolensis* stem bark fractions/Control** | **$IC_{50}$ values (µg/ml) of cancer cell lines** | |
|---|---|---|
| | **DU-145** | **HCC 1395** |
| *F. angolensis* bark petroleum ether fraction | 181.7 ± 4.4 | 63.3 ± 14.5 |
| *F. angolensis* bark dichloromethane fraction | 31.1 ± 3.3 | 8.3 ± 1.7 |
| *F. angolensis* bark ethyl acetate fraction | 106.7 ± 14.5 | 203.3 ± 14.2 |
| *F. angolensis* bark acetone fraction | 215.0 ± 55.1 | 88.8 ± 9.3 |
| *F. angolensis* bark methanol fraction | 31.9 ± 1.6 | 236.7 ± 27.3 |
| **Cyclophosphamide (positive control)** | **28.9 ± 2.9** | **32.8 ± 1.1** |
| **5 Fluorouracil (positive control)** | **18.3 ± 6.1** | **38.8 ± 7.6** |

**Values are represented as Mean ± SEM of $IC_{50}$ (µg/ml) from three independent experiments (Appendices 70 & 71), DU-145-prostate cancer cell line and HCC 1395-Breast cancer cell line, %: percentage. Anticancer activities of fractions; NT: Not tested, DU-145 and 22Rv1: prostate cancer cell lines. Anticancer activities of extracts; $IC_{50}$ < 10 µg/ml: remarkably active, $IC_{50}$ between 10 and 50 µg/ml: moderately active and $IC_{50}$ > 50 µg/ml: weak activity.**

### 4.3.2 Bioassay-guided fractionation of *Combretum tanaense* root methanol extract

Activity guided fractionation of *C. tanaense* root methanol extracts provided four different fractions (dichloromethane, ethylacetate, acetone and methanol fractions). The four fractions and their percentage yield values were recorded as shown in Table 4.5. Acetone fraction revealed remarkable activities, the estimated $IC_{50}$ values of acetone fraction were much lower (22.5 ± 3.8 µg/ml) against DU-145 compared to higher $IC_{50}$ values of the crude extract **(**73.9 ± 3.1 µg/ml) against the same cell line. A similar trend was demonstrated for activities against breast cancer cell lines (HCC 1395) where $IC_{50}$ values of the fraction had

reduced to 33.3 ± 3.0 µg/ml compared to that of the crude extract (193.0 ± 13.2 µg/ml). The methanol fraction was aslo active against HCC 1395 ($IC_{50}$ = 20.8 ± 0.8 µg/ml) (Table 4.4).

**Table 4.5: Concentration of *Combretum tanaense* root fractions that inhibited proliferation of cancer cells by 50% ($IC_{50}$)**

| ***C. tanaense* whole root fractions/Control** | **$IC_{50}$ values (µg/ml) of cancer cell lines** | |
|---|---|---|
| | **DU-145** | **HCC 1395** |
| *C. tanaense* root dichloromethane fraction | 60.0 ± 1.5 | 147.0 ± 12.4 |
| *C. tanaense* root ethyl acetate fraction | 69.2 ± 6.0 | 51.7 ± 3.01 |
| *C. tanaense* root acetone fraction | 22.5 ± 3.8 | 33.3 ± 3.0 |
| *C. tanaense* root methanol fraction | 74.2 ± 11.7 | 20.8 ± 0.8 |
| **Cyclophosphamide (positive control)** | **28.9 ± 2.9** | **32.8 ± 1.1** |
| **5 Fluorouracil (positive control)** | **18.3 ± 6.1** | **38.8 ± 7.6** |

**Values are represented as Mean ± SEM of $IC_{50}$ (µg/ml) from three independent experiments (Appendices 72 - 73), DU-145-prostate cancer cell line and HCC 1395: Breast cancer cell line, %: percentage. Anticancer activities of fractions; NT: Not tested, DU-145 and 22Rv1: prostate cancer cell lines. Anticancer activities of extracts; $IC_{50}$ < 10 µg/ml: remarkably active, $IC_{50}$ between 10 and 50 µg/ml: moderately active and $IC_{50}$ > 50 µg/ml: weak activity.**

### 4.3.3 Bioassay-guided fractionation of *Uvariodendron anisatum* root methanol extract

Anticancer activities of five fractions that were obtained during fractionation of *U. anisatum* root methanol extract are as shown in Table 4.6. Petroleum ether fraction did not exhibit anticancer activity against breast cancer (HCC 1395) and and prostate cancer (DU-145) cell lines. However, low activities were observed with dichloromethane and ethly acetate fractions. The activities of acetone and methanol fractions against DU-145 and HCC 1395

revealed $IC_{50}$ values of 53.3 ± 11.6 and 74.2 ± 0.8 µg/ml respectivly. It was noted that tere was decrease of anticancer activities of fractions compared to the activities of the crude extracts. The anticancer activities were at the same range with that of the crude extract ($IC_{50}$ = 50.6 ± 2.9 and 81.7 ± 3.9 µg/ml against HCC 1395 and DU-145 cell lines, respectively). The crude extract and fractions (acetone and methanol) of *Uvariodendron anisatum* root had $IC_{50}$ categorized as high anticancer activity with $IC_{50}$ between 30 and 100 µg/ml).

**Table 4.6: Concentration of *Uvariodendron anisatum* root fractions that inhibited proliferation of cancer cells by 50% ($IC_{50}$)**

| ***U. anisatum* whole root fractions/Control** | **$IC_{50}$ values (µg/ml) of cancer cell lines** | |
|---|---|---|
| | **DU-145** | **HCC 139** |
| *U. anisatum* root petroleum ether fraction | > 1000 | > 1000 |
| *U. anisatum* root dichloromethane fraction | 533.0 ± 15.3 | 473.7 ± 3.0 |
| *U. anisatum* root ethyl acetate fraction | 468.3 ± 24.2 | 296.3 ± 44.8 |
| *U. anisatum* root acetone fraction | 66.7 ± 18.3 | 87.7 ± 7.8 |
| *U. anisatum* root methanol fraction | 53.3 ± 11.6 | 74.2 ± 0.8 |
| **Cyclophosphamide (positive control)** | **28.9 ± 2.9** | **32.8 ± 1.1** |
| **5 Fluorouracil (positive control)** | **18.3 ± 6.1** | **38.8 ± 7.6** |

**Values are represented as Mean ± SEM of $IC_{50}$ (µg/ml) from three independent experiments (Appendices 74 - 75), DU-145-prostate cancer cell line and HCC 1395: Breast cancer cell line, %: percentage. Anticancer activities of fractions; NT: Not tested, DU-145 and 22RV1: prostate cancer cell lines. Anticancer activities of extracts; $IC_{50}$ < 10 µg/ml: remarkably active, $IC_{50}$ between 10 and 50 µg/ml: moderately active and $IC_{50}$ > 50 µg/ml: weak activity.**

### 4.3.4 Bioassay-guided fractionation of *Hydnora abyssinica* rhizome methanol extract

Semi-purification of *H. abyssinica* rhizome methanol extract gave five fractions. Evaluation of anticancer activities of fractions showed lower anticancer activities, this was shown through high $IC_{50}$ values of more than 100 µg/ml for most of fractions compared with whole crude methanol extract ($IC_{50}$ < 30 µg/ml). Nevertheless, methanol fraction mantained activities of silmilar range with that of the crude extract ($IC_{50}$ < 100 µg/ml) for prostate cancer cell line (Table 4.7). However, low activities of methanol fraction were recorded compared to that of the crude extract against breast cancer cell lines ($IC_{50}$ < 30 µg/ml).

**Table 4.7: Concentration of *Hydnora abyssinica* rhizome fractions that inhibited proliferation of cancer cells by 50% ($IC_{50}$)**

| ***H. abyssinica*** **rhizome fractions/Control** | **$IC_{50}$ values (µg/ml) of cancer cell line** | |
|---|---|---|
| | **DU-145** | **HCC 1395** |
| *H. abyssinica* rhizome petroleum ether fraction | 618.3 ± 18.8 | > 1000 |
| *H. abyssinica* rhizome dichloromethane fraction | 332.0 ± 23.9 | 610.3 ± 6.4 |
| *H. abyssinica* rhizome ethyl acetate fraction | 238.0 ± 31.2 | 632.7 ± 3.7 |
| *H. abyssinica* rhizome acetone fraction | 506.3 ± 62.7 | 233.7 ± 23.0 |
| *H. abyssinica* rhizome methanol fraction | 90.0 ± 3.8 | 40.8 ± 5.8 |
| **Cyclophosphamide (positive control)** | **28.9 ± 2.9** | **32.8 ± 1.1** |
| **5 Fluorouracil (positive control)** | **18.3 ± 6.1** | **38.8 ± 7.6** |

**Values are represented as Mean ± SEM of $IC_{50}$ (µg/ml) from three independent experiments (Appendices 76 & 77). DU-145: prostate cancer cell line and HCC 1395: Breast cancer cell line, %: percentage. Anticancer activities of fractions; NT: Not tested, DU-145 and 22rv1: prostate cancer cell lines. Anticancer activities of extracts; $IC_{50}$ < 10 µg/ml: remarkably active, $IC_{50}$ between 10 and 50 µg/ml: moderately active and $IC_{50}$ > 50 µg/ml: weak activity.**

### 4.3.5 Anticancer activities of the pure isolated compounds

Seven pure compounds were isolated in this study, the compounds were coded as **$FC_1$**, **$FC_2$**, **$FC_3$**, **$CC_1$**, **$CC_2$**, **$UC_1$** and **$UC_2$**. Compounds **$FC_1$**, **$FC_2$** and **$FC_3$** were obtained from dichloromethane fraction of *F. angolensis* stem bark extract. **$UC_1$** and **$UC_2$** were obtained from methanol fraction of *U. anisatum* root extract while compounds **$CC_1$** and **$CC_2$** from acetone fraction of *C. tananese* root extract. Evaluation of anti-breast and anti-prostate cancer activitities against selected cancer cell lines revealed that compounds **$FC_1$**, **$FC_2$**, **$FC_3$**, **$CC_1$** and **$CC_2$** were remarkably active with $IC_{50}$ values ranging from 3.6 ± 0.1 to 25.9 ± 0.5 µg/ml (Table 4.8) against all cell lines. The exception was $IC_{50}$ values of 63.7 ± 1.7 µg/ml which where recorded with compound **$CC_2$** against 22RV1 prostate cancer cell line. Nevertheless, compounds **$UC_1$** was inactive even though it was isolated from an active fraction (Table 4.8). Anticancer activities of Compound **$UC_2$** are yet to be determined.

**Table 4.8: Concentration of isolated compounds that inhibited proliferation of cancer cells by 50% ($IC_{50}$)**

| Cmpounds | $IC_{50}$ values (µg/ml) of cancer cell lines | | | |
|---|---|---|---|---|
| | HCC 1395 | 4TI | DU-145 | 22RV1 |
| **$FC_1$** | 3.6 ± 0.1 | 3.7 ± 0.1 | 3.7 ± 0.7 | 5.7 ± 1.1 |
| **$FC_2$** | 3.9 ± 2.2 | 7.2 ± 1.1 | 7.4 ± 0.3 | 6.1 ± 0.9 |
| **$FC_3$** | 6.8 ± 0.7 | 5.6 ± 0.5 | 22.9 ± 0.1 | 12.5 ± 2.3 |
| **$CC_1$** | 22.3 ± 0.2 | 19.2 ± 0.0 | 20.6 ± 5.6 | 25.9 ± 0.5 |
| **$CC_2$** | 23.2 ± 0.3 | 25.6 ± 0.2 | 20.7 ± 5.6 | 63.7 ± 1.7 |
| **$UC_1$** | >1000 | 769 ± 4.4 | 748 ± 80 | >1000 |
| **Cyclophosphamide** | **32.8 ± 1.1** | **22.8 ± 1.1** | **28.9 ± 2.9** | **NT** |
| **5 Flurouracil** | **38.8 ± 7.6** | **NT** | **18.3 ± 6.1** | **25.0 ± 12.1** |

**Values are represented as Mean ± SEM of $IC_{50}$ (µg/ml) from three independent experiments (Appendices 78 & 79), NT: Not tested. HCC 1395 and 4T1: Breast cancer cell line, DU-145 and 22Rv1: prostate cancer cell line. Anticancer activities of isolated pure compounds; $IC_{50}$ < 5 µg/ml: remarkably active, $IC_{50}$ between 5 and 10 µg/ml: moderately active, $IC_{50}$ between 10 and 50 µg/ml: weakly active, and $IC_{50}$ > 50 µg/ml: non-active.**

## 4.4 Cellular toxicity and selectivity indices

### 4.4.1 Toxicity studies of plant extracts against vero cells

Normal vero cell line (a mammalian cell line) established from the kidney of the African green monkey (*Cercopithecus aethiops*) was used to evaluate safety levels of crude extracts, fractions and isolated compounds in the current study. Vero cells are recommended for testing chemical toxicity (National Standards Authority of Ireland (NSAI), 2009). Screening for toxic levels of extracts against vero cells revealed the extracts under this study had

varied levels of toxicity with concentrations that were cytotoxic to 50% of the vero cells ($CC_{50}$) having range values from 3.3 ± 0.2 to >1000 µg/ml. Following American National Cancer Institute criteria for assigning activity in relation to $CC_{50}$ values, the extracts that exhibited the low values ($CC_{50}$<30 µg/ml) were regarded to be remarkably toxic. The extracts that demonstrated remarkable toxicity in this study were the methanol extracts from *U. anisatum* root and *F. angolensis* stem bark with $CC_{50}$ values that were 3.3 ± 0.2 and 21.7 ± 3.8 µg/ml, respectively. Other extracts that expressed $CC_{50}$ values of between 30 µg/ml to 100 µg/ml were considered to be highly toxic in this study, they included methanol extracts of *C. tanaense* root, *H. abyssinica* rhizome and flower ($CC_{50}$=84.2 ± 6.3, 77.2 ± 1.1 and 36.2 ± 4 µg/ml respectively (Table 4.9).

As indicated in Table 4.8, all the other extracts from the plants in the current study exhibited low toxicity with $CC_{50}$ values of between 100 and 1000 µg/ml, an exception being water extract from *L. cornuta* and *C. tanaense* that demonstrated $CC_{50}$ values of greater than 1000 µg/ml and were considered non-toxic.

**Table 4.9: Toxicity of crude extracts against vero cells ($CC_{50}$ µg/ml)**

| Crude extracts | $CC_{50}$ values (µg/ml) of extracts against vero cells |
|---|---|
| **Cyclophosphamide** | **2.8 ±1.1** |
| *Uvariodendron anisatum* root methanol | 3.3 ± 0.2 |
| *Prunus africana* stem bark methanol | 20.5 ± 0.6 |
| *Fagaropsis angolensis* stem bark methanol | 21.7 ± 3.8 |
| *Combretum tanaense* root methanol | 36.2 ± 4 |
| *Hydnora abyssinica* flower methanol | 77.2 ± 1.1 |
| *Hydnora abyssinica* rhizome methanol | 84.2 ± 6.3 |
| *Uvariodendron anisatum* root water | 153.5 ± 1.5 |
| *Hydnora abyssinica* rhizome water | 184.0 ± 12 |
| **5 Flurouracil (positive control)** | **185 ± 8** |
| *Prunus africana* stem bark water | 196 ± 6 |
| *Spermacoce princeae* aerial methanol | 203 ± 4.9 |
| *Fagaropsis angolensis* stem bark water | 296.0 ± 11 |
| *Launaea cornuta* leaf methanol | 365 ± 15.3 |
| *Hydnora abyssinica* flower water | 443 ± 169.5 |
| *Spermacoce princeae* aerial water | 576 ± 36.7 |
| *Launaea cornuta* leaf water | > 1000 |
| *Combretum tanaense* root water | > 1000 |

**Values are represented as Mean ± SEM of $CC_{50}$ (µg/ml) from three independent experiments (Appendix 80), vero: Normal kidney epithelial cells from African green monkey. Cytotoxic activities of extracts; $IC_{50}$ < 50 µg/ml: remarkably toxic, $IC_{50}$ between 50 and 100 µg/ml: highly toxic, $IC_{50}$ between 50 and 100 µg/ml: moderately toxic, $IC_{50}$ between 100 and 1000 µg/ml: weakly toxic, and $IC_{50}$ > 1000 µg/ml: non-toxic**

### 4.4.2 Toxicity of fractions from active extracts against vero cells

Evaluation of fractions from active methanol extracts (*F. angolensis* stem bark, *C. tanaense* root, *U. anisatum* root and *H. abyssinica* rhizome) disclosed varied levels of toxicity against vero cells as indicated in Table 4.10. Three out of four fractions from *C. tanaense* root

methanol extracts were found to be remarkably toxic while one was equally highly toxic. Acetone fraction from *C. tanaense* was the most toxic fraction ($CC_{50}$=15.8 ± 0.8) (Table 4.9), it was followed by methanol, ethyl acetate and dichloromethane fractions ($CC_{50}$=16.7 ± 6.7, 20.0 ± 1.4 and 35.8 ± 0.8, respectively). Three fractions from *F. angolensis* (petroleum ether, dichloromethane and ethyl acetate fractions) exhibited high toxicity (30 µg/ml > $CC_{50}$ < 100 µg/ml). The rest of the fractions (all fractions from *U. anisatum*, *H. abyssinica*, acetone and methanol fractions from *F. angolensis*) showed low toxicity for they recorded high $CC_{50}$ values of between 102.5 ± 27.5 to 789 ± 24.7 µg/ml (Table 4.9) and therefore they were lowly toxic.

It was noted that fractionation led to a decrease in $CC_{50}$ values from 36.2 ± 4 µg/ml to lower values of up to half of that recorded for the crude extract for *C. tanaense* ($CC_{50}$ = 15.8 ± 0.8 µg/ml) therefore increasing toxicity (Table 4.8 and 4.9). However, this trend of decrease of $CC_{50}$ values upon fractionation was not consistent with fractionation of the methanol extracts of *F. angolensis* stem bark, *U. anisatum* root and *H. abyssinica*. The later fractions exhibited an increase of $CC_{50}$ values ranging from 56.7 ± 4.4 to 789 ± 24.7 µg/ml compared to the $CC_{50}$ values of extracts that ranged from 3.3 ± 0.2 to 84.2 ± 6.3 µg/ml of the crude extracts. The low toxicity levels were revealed by increasing $CC_{50}$ values that were observed with fraction. The highest increases of $CC_{50}$ values were recorded with the fractionation of *U. anisatum* root and *H. abyssinica* rhizome and finally *F. angolensis* stem bark.

**Table 4.10: Concentration of fractions that was cytotoxic to 50% of normal cells ($CC_{50}$)**

| Fractions | $CC_{50}$ values (µg/ml) of extracts against vero cells |
|---|---|
| **Cyclophosphamide** | **2.8 ±1.1** |
| *C. tanaense* root acetone fraction | 15.8 ± 0.8 |
| *C. tanaense* root methanol fraction | 16.7 ± 6.7 |
| *C. tanaense* root ethyl acetate fraction | 20.0 ± 1.4 |
| *C. tanaense* root dichloromethane fraction | 35.8 ± 0.8 |
| *F. angolensis* bark petroleum ether fraction | 56.7 ± 4.4 |
| *F. angolensis* bark dichloromethane fraction | 74.2 ± 0.8 |
| *F. angolensis* bark ethyl acetate fraction | 84.2 ± 11.6 |
| *F. angolensis* bark acetone fraction | 102.5 ± 27.5 |
| *F. angolensis* bark methanol fraction | 167.0 ± 4.9 |
| *U. anisatum* root methanol fraction | 167.0 ± 8.6 |
| **Flurouracil** | **185 ± 8** |
| *H. abyssinica* rhizome Dichloromethane fraction | 231.7 ± 5 |
| *H. abyssinica* rhizome methanol fraction | 269 ± 135 |
| *H. abyssinica* rhizome mthyl acetate fraction | 305 ± 4.9 |
| *U. anisatum* root ethyl acetate fraction | 369.3 ± 23 |
| *H. abyssinica* rhizome petroleum ether fraction | 381.7 ± 15.3 |
| *U. anisatum* root acetone fraction | 399.3 ± 72.6 |
| *U. anisatum* root petroleum ether fraction | 653.3 ± 53.3 |
| *U. anisatum* root dichloromethane fraction | 784.3 ± 23.5 |
| *H. abyssinica* rhizome acetone fraction | 789 ± 24.7 |

**Values are represented as Mean ± SEM of $CC_{50}$ (µg/ml) from three independent experiments (Appendix 81), vero: Normal kidney epithelial cells from African green monkey**
**Cytotoxic activities of fractions; $IC_{50}$ < 10 µg/ml: remarkably toxic, $IC_{50}$ between 10 and 50 µg/ml: moderately toxic, and $IC_{50}$ > 50 µg/ml: weakly toxic**

### 4.4.3 Toxicity studies of isolated compounds against vero cell line

A study of toxicity levels of isolated compounds revealed that compounds **$FC_1$**, **$FC_2$**, **$FC_3$**, **$UC_1$** and **$CC_1$** were remarkably toxic, compounds **$UC_2$** was found to be nontoxic gainst the vero cells while compound **$CC_2$** was revealed relatively low toxicity (Table 4.11). Standard reference drugs for pure compounds also demonstrated varied toxicity. Cyclophosphamide recorded remarkable toxicity that was shown by its low $CC_{50}$ values (2.8 ± 1.1 µg/ml) while 5 fluorouracil revealed low toxicity ($CC_{50}$ = 185 ± 75 µg/ml).

**Table 4.11: Concentration of pure compounds that was cytotoxic to 50% of normal cells ($CC_{50}$)**

| Isolated compounds | $CC_{50}$ values (µg/ml) of extracts against vero |
|---|---|
| **Cyclophosphamide** | **2.8 ± 1.1** |
| **$CC_1$** | 15.5 ± 0.5 |
| **$FC_3$** | 18.50 ± 0.3 |
| **$FC_2$** | 18.7 ± 15.7 |
| **$UC_1$** | 20.6 ± 1.1 |
| **$FC_1$** | 21.7 ± 0.0 |
| **$CC_2$** | 58.67 ± 2.8 |
| **5 Fluorouracil** | **185 ± 75** |
| **$UC_2$** | 326.7 ± 68.7 |

**Values are represented as Mean ± SEM of $CC_{50}$ (µg/ml) from three independent experiments (Appendix 82 & 83), vero: Normal kidney epithelial cells from African green monkey. Cytotoxic activities of isolated pure compounds $IC_{50}$ < 5 µg/ml: remarkably toxic, $IC_{50}$ between 5 and 10 µg/ml: moderately toxic, $IC_{50}$ between 10 and 50 µg/ml: weakly toxic, and $IC_{50}$ > 50 µg/ml: non-toxic**

### 4.4.6 Selectivity indices (SI) of crude extracts

Selectivity index (SI) which indicated the ability of the drug to discriminate against cancerous cell in favour of normal cells was recorded as indicated in Table 4.12. Extracts that demonstrated SI ≤ 1 were regarded as not selective, 1 < SI < 3 moderately selective and SI ≥ 3 were described as highly selective in this study. It was found that extracts demonstrated different selectivity (1 < SI ≥ 3) against different cancer cell lines. Water extract from *H. abyssinica* flower was selective for most cell lines in this study. The extract was highly selective against HCC 1395 (SI = 12), 4T1 (SI = 5.5) and 22RV1 (SI = 8.5). Methanol extract of *H. abyssinica* rhizome which was also highly selective against HCC 1395 (SI = 3.1) and 4T1 (SI = 3.7). Higher selectivity values were also observed with *Fagaropsis angolensis* stem bark water extract against 4T1 (SI = 3.8 and 22Rv1 (SI = 8.6) and *C. tanaense* root methanol extracts against 4T1 (SI = 3.5). The remaining extracts showed moderate selectivity for 4T1 cell line (1 < SI <3) while majority of extracts were not selective (SI ≤ 1) for the cancer cell lines.

*Prunus africana* stem bark extracts are used in this study as standard references for crude extracts, these extracts demonstrated selectivity against cancerous cell lines in this study as shown in Table 4.12. Especially water extract exhibited high selectivity indices against prostate cancer cell lines (DU-145 = 9.9 and 22Rv1 = 9.5). The extracts (*P. africana* stem bark water extract) equally showed high selectivity against 4T1 (SI = 5.3) though it was moderately selective against HCC 1395 (SI = 2). Methanol extract of *P. africana* stem bark was also highly selective against 4T1 (SI = 4.3) nevertheless, the extract was reported to have lower selectivity against HCC 1395 and 22RV1 (SI = 2 and 1 respectively) and it was

not selective against DU-145 (SI = 0.8). The classification of methanol extract of *P. africana* as highly selective against 4T1 cell line in the current study is consistent with previous studies done by Nabende *et al*. (2015), it was established that methanol extract of *P. africana* stem bark distinguished normal vero cell line from 4T1 breast cancer and CT26 human colon cancer cell lines by SI values of 7.3 and 1.1, respectively, this means that the methanol extract of *P. africana* stem bark had high anticancer effects against cancer cell lines, whereas it was lowly toxic against normal cell line.

In reference to selectivity indices therefore water extracts from *H. abyssinica* flowers, *F. angolensis* stem bark and methanol extract from *H. abyssinica* rhizome demonstrated considerably high selectivity. The three extracts were found to have ability to destroy cancerous cells at a dose concentration that was non-toxic to normal body cells. The methanol extracts obtained from *U. anisatum* root and *C. tanaense* root demonstrated moderate selectivity for one breast cancer cell line (4T1) but the extracts were non-selective for the other breast cancer cell line (HCC 1395), the two prostate cancer cell lines (DU-145 and 22RV1) in this study (Table 4.12).

**Table 4.12: Selectivity index (SI) of extracts for selected cancer cell lines**

| Extracts | SI values of extracts against cell lines | | | |
|---|---|---|---|---|
| | HCC 1395 | 4T1 | DU-145 | 22RV1 |
| *Uvariodendron anisatum* root methanol | 0.1 | 1.8 | 0.04 | 0.1 |
| *Uvariodendron anisatum* root water | 0.6 | 1 | 0.2 | 0.2 |
| *Hydnora abyssinica* rhizome methanol | 3.1 | 3.7 | 1.3 | 0.6 |
| *Hydnora abyssinica* rhizome water | 0.4 | 2.3 | 0.4 | 1.5 |
| *Hydnora abyssinica* flower methanol | 1 | 1.4 | 0.1 | 1.2 |
| *Hydnora abyssinica* flower water | 12 | 5.5 | 0.6 | 8.5 |
| *Launaea cornuta* leaf methanol | 1.7 | 1.3 | 0.9 | 0.6 |
| *Launaea cornuta* leaf water | 2.6 | 1.4 | 7 | 1.6 |
| *Combretum tanaense* root methanol | 0.2 | 1.9 | 0.5 | 0.2 |
| *Combretum tanaense* root water | < 1 | 3.5 | <1 | NT |
| *Fagaropsis angolensis* stem bark methanol | 0.4 | 1.7 | 1.7 | 0.4 |
| *Fagaropsis angolensis* stem bark water | 0.6 | 3.8 | 1 | 8.6 |
| *Spermacoce princeae* aerial methanol | 0.4 | 1 | 1.3 | 0.6 |
| *Spermacoce princeae* aerial water | 0.6 | 1 | 1.1 | 1.1 |
| *Prunus africana* stem bark methanol | 2 | 4.3 | 0.8 | 1 |
| *Prunus africana* stem bark water | 2.4 | 5.3 | 9.9 | 9.5 |
| **Cyclophosphamide** | **0.1** | **4.8** | **0.1** | **NT** |
| **5-Fluorouracil** | **0.1** | **NT** | **10.1** | **7.4** |

**SI > 3: Highly selective, SI between 1 - 3: Moderately selective and SI < 1: none selective, HCC 1395 and 4T1: Breast cancer cell line, DU-145 and 22RV1: prostate cancer cell line.**

### 4.4.7 Selectivity indices (SI) of fractions

It was observed that less than 10% of the fractions of the 19 fractions in this study demonstrated high selectivity, about 26% moderate selectivity while over 64 % were not selective. The fractions with high selectivity index were dichloromethane fraction from *F. angolensis* stem bark and *U. anisatum* root with high SI values (8.9 and 3.1) against HCC 1395 and DU-145 cell lines, respectively (Table 4.12). The fractions that had moderate selectivity with ($1.2 < SI < 2.7$) were acetone and methanol fractions from *U. anisatum* root against breast cancer (HCC 1395) and prostate cancer (DU-145) cell lines, acetone and dichloromethane fractions from *F. angolensis* stem bark against breast cancer (HCC 1395) and prostate cancer (DU-145) cell lines, respectively. Acetone and methanol fractions from *H. abyssinica* rhizome also demonstrated moderate selectivity against prostate cancer (DU-145) and HCC 1395 cell lines (Table 4.13).

Most fractions under this study had selectivity index of less than one, meaning that the fractions were non-selective (Table 4.13). Three out of the five fractions from *F. angolensis* were none selective (petroleum ether, ethyl acetate and methanol fractions) against breast cancer cell line (HCC 1395) while petroleum ether, ethyl acetate and acetone fractions where none selective against prostate cancer cell line (DU-145). All the fractions from *C. tanaense* root extract were none selective against the prostate and breast cancer cell lines (DU-145 and HCC 1395) in the current study.

**Table 4.13: Selectivity indices (SI) of fractions for selected cancer cell lines**

| Fraction | SI values of fractions against cell lines | |
|---|---|---|
| | **HCC 1395** | **DU-145** |
| *F. angolensis* bark petroleum ether fraction | 0.9 | 0.3 |
| *F. angolensis* bark dichloromethane fraction | 8.9 | 2.3 |
| *F. angolensis* bark ethyl acetate fraction | 0.4 | 0.8 |
| *F. angolensis* bark acetone fraction | 1.2 | 0.4 |
| *F. angolensis* bark methanol fraction | 0.8 | 5.2 |
| *C. tanaense* root dichloromethane fraction | 0.2 | 0.6 |
| *C. tanaense* root ethyl acetate fraction | 0.4 | 0.3 |
| *C. tanaense* root acetone fraction | 0.5 | 0.7 |
| *C. tanaense* root methanol fraction | 0.8 | 0.2 |
| *U. anisatum* root petroleum ether fraction | 0.7 | 0.7 |
| *U. anisatum* root dichloromethane fraction | 0.7 | 0.7 |
| *U. anisatum* root ethyl acetate fraction | 0.1 | 0.0 |
| *U. anisatum* root acetone fraction | 2.0 | 2.7 |
| *U. anisatum* root methanol fraction | 2.3 | 3.1 |
| *H. abyssinica* rhizome petroleum ether fraction | 0.4 | 0.6 |
| *H. abyssinica* rhizome dichloromethane fraction | 0.4 | 0.7 |
| *H. abyssinica* rhizome ethyl acetate fraction | 0.5 | 1.3 |
| *H. abyssinica* rhizome acetone fraction | 3.5 | 1.6 |
| *H. abyssinica* rhizome methanol fraction | 1.9 | 1.5 |
| **Cyclophosphamide** | **0.1** | **0.1** |
| **5-Fluorouracil** | **0.1** | **10.1** |

**SI > 3: Highly selective, SI between 1 - 3: Moderately selective and SI < 1: none selective, HCC 1395: Breast cancer cell line, DU-145: prostate cancer cell line**

It was observed that though all fractions that were obtained from methanol extract of *C. tanaense* root exhibited anti-breast and anti-prostate cancer activities, the fractions lacked selectivity (SI ≤ 1) for cancer cell lines and these very low selectivity indices indicated that all fractions (dichloromethane, ethyl acetate, acetone and methanol fractions) that were obtained from *C. tanaense* root extract were non selective against breast and prostate cancer cell lines (HCC 1395 and DU-145) in this study. The fractions were also toxic to the normal cell line (vero cell).

### 4.4.8 Selectivity indices of isolated compounds

A study on discriminatory ability of the purified compounds to destroy cancer and not normal cells revealed that compounds **$FC_1$**, **$FC_2$**, **$FC_3$** and **$CC_2$** were selective against the cancer cell lines in this study. Compound **$FC_1$** (SI > 3) was highly selective against all selected cancer cell lines as indicated in Table 4.13. Compound **$FC_2$**, was also highly selective against two cancer cell lines (HCC 1395 (SI = 4.8) and 22Rv1 (SI = 3.1) and moderately selective against 4T1 (SI = 2.6) and DU-145 (SI = 2.5). Compound **$FC_3$** showed high selectivity against 4T1 (SI = 3.3) and moderate selectivity against HCC 1395 (SI = 2.7) and 22Rv1 (SI = 1.5). Equally, compound **$CC_2$** demonstrated moderate selectivity against HCC 1395 (SI = 2.5), 4T1 (SI = 2.3) and DU-145 (SI = 2.8) but it was none selective against 22Rv1 (SI =0. 9). Compounds **$UC_1$** and **$CC_1$** were found to be non-selective (SI ≤ 1) (Table 4.14).

The reference standard drugs 5-fluorouracil exhibited high selectivity index against breast cancer (HCC 1395, SI =4.8) and prostate cancer (DU-145, SI =10.1 and 22Rv1, SI = 7.4)

cell lines. However, cyclophosphamide showed low selectivity indices (SI = 0.1) against breast cancer (HCC 1395, 4T1) and prostate cancer (DU-145) cell lines in the current study (Table 4.13).

**Table 4.14: Selectivity indices (SI) of isolated pure compounds for selected cancer cell lines**

| Compounds | SI values of extracts against cell lines | | | |
|---|---|---|---|---|
| | HCC 1395 | 4T1 | DU-145 | 22RV1 |
| **$FC_1$** | 5.9 | 5.7 | 5.7 | 3.8 |
| **$FC_2$** | 4.8 | 2.6 | 2.5 | 3.1 |
| **$FC_3$** | 2.7 | 3.3 | 0.8 | 1.5 |
| **$CC_1$** | 0.7 | 0.8 | 0.8 | 0.6 |
| **$CC_2$** | 2.5 | 2.3 | 2.8 | 0.9 |
| **$UC_1$** | 0.02 | 0.03 | 0.03 | 0.02 |
| **Cyclophosphamide** | **0.1** | **0.1** | **0.1** | **ND** |
| **5 Fluorouracil** | **4.8** | **ND** | **10.1** | **7.4** |

**SI > 3: Highly selective, SI between 1 - 3: Moderately selective and SI < 1: none selective, HCC 1395 and 4T1: Breast cancer cell line, DU-145 and 22Rv1: prostate cancer cell line**

### 4.5 Toxic effects of active crude extracts on swiss albino mice

The methanol extracts of *U. anisatum* root, *F. angolesnsis* stem bark, *C. tanaense* root and *H. abyssinica* rhizome were found non-toxic following the analyses made on wellness parameters, weight and occurrence of death on the experimental mice. The observations were similar at all doses (0, 50, 300 and 2000 mg/kg) and therefore the results of the highest dose were analyzed.

### 4.5.1 Analyses of wellness parameters

The wellness parameters the were taken into consideration in this study were skin and fur, discharge from eyes, mucous membrane, salivation, lethargy, sleep, coma, convulsions, tremors, diarrhoea and behavioral patterns. It was observed that there were no changes after acute oral exposure of swiss albino mice to methanol extracts of *U. anisatum* root, *F angolensis* stem bark, *C. tanaense* root and *H. abyssinica* rhizome (Table 4.15). The observations were made for dose range from 50 mg/kg to 2000 mg/kg. However, there were no signs of toxicity that we observed even up to the highest dose (2000 mg/kg) in this study.

**Table 4.15: Effects of methanol extracts of *F. angolensis* stem bark, *C. tanaense* root, *H. abyssinica* rhizome and *U. anisatum* root on appearance and behaviour for control and treated groups mice at 2000 mg/kg dose**

| Observation | Control group (**0 mg/kg)** | | | Test group (**2000 mg/kg)** | | |
|---|---|---|---|---|---|---|
| | 4 hrs | 24 hr | $14^{th}$ day | 4 hrs | 24 hr | $14^{th}$ day |
| Skin and fur | Normal | Normal | Normal | Normal | Normal | Normal |
| Eye discharge | Normal | Normal | Normal | Normal | Normal | Normal |
| Mucous membrane | Normal | Normal | Normal | Normal | Normal | Normal |
| Salivation | NO* | NO | NO | NO | NO | NO |
| Lethargy | NO | NO | NO | NO | NO | NO |
| Sleep | NO | NO | NO | NO | NO | NO |
| Coma | NO | NO | NO | NO | NO | NO |
| Convulsions | NO | NO | NO | NO | NO | NO |
| Tremors | NO | NO | NO | NO | NO | NO |
| Diarrhoea | NO | NO | NO | NO | NO | NO |

NO -Not Observed

### 4.5.2 Body weight statistical analyses

The methanol extracts of *F. angolensis* stem bark, *C. tanaense* root, *H. abyssinica* rhizome and *U. anisatum* root did not show significant effect on body weights on mice. The increase of body weight was not different when compared between the control and the treatment groups. It was found that there was increase in weight of all the mice after the oral administration of the extracts. The increase of weights of mice were considered insignificant following the calculated test of significance using student unpaired t-test where *p* was found to be greater than 0.05 in all extracts (Table 4.16).

**Table 4.16: Effects of methanol extracts (2000 mg/kg) of *F. angolensis* stem bark, *C. tanaense* root, *H. abyssinica* rhizome and *U. anisatum* root on body weight of mice**

| Concentration (mg/kg) | Body weights (g) | | P-values |
|---|---|---|---|
| | Before treatment ($M_1 \pm SEM_1$) | After treatment ($M_2 \pm SEM_2$) | |
| Control (0 mg /kg) | $19.5 \pm 0.5^a$ | $24.0 \pm 1.5^a$ | 0.1 |
| C. *tanaense* root extract | $18.5 \pm 0.6^a$ | $19.5 \pm 0.5^a$ | 0.2 |
| *F. angolensis* root extract | $19.5 \pm 0.5^a$ | $22.5 \pm 1.5^a$ | 0.4 |
| *H. abyssinica* rhizome extract | $20.5 \pm 0.5^a$ | $22.5 \pm 0.5^a$ | 0.1 |
| *U. anisatum* root extract | $20.5 \pm 0.0^a$ | $24.5 \pm 1.5^a$ | 0.2 |

**N = 3; $M_1$, $SEM_1$ and $M_2$, $SEM_2$ are mean weights and standard error of the mean before and after treatment respectively; means sharing same letter superscript are not significantly different at $p < 0.05$ while means with different letter subscripts were significantly different $p < 0.05$**

### 4.5.3 Mortality

The current study revealed that the methanol extracts of *F. angolensis* stem bark, *C. tanaense* root, *H. abyssinica* rhizome and *U. anisatum* root were non-toxic. These extracts did not cause any fatal effects on swiss mice following oral administration at a maximum dose of (2000 mg/kg).

## 4.6 Structural elucidation

### 4.6.1 Identified compounds

The structures of compounds **37**, **38,** **39**, **40** were determined by comparing their spectroscopic, spectrometric and other physical chemical data of the pure compounds with existing literature. Compounds **41**, **42** and **43** were not completely elucidated and require more spectroscopic data for structural determination. Compound **37**, **38** and **39** were isolated from the methanol extract of *Fagaropsis angolensis* stem bark, compound **40** and **41** from the methanol extract of *Uvariodendron anisatum* whole root while **42** and **43** were obtained from the methanol extract of *Combretum tanaense* whole root.

#### 4.6.1.1 Compound 37

Compound **37** was obtained as white needle like crystals (18.4 mg) from petroleum ether fraction of *F. angolensis* bark methanol extract. Proton NMR spectrum (Appendices 8 and 9) indicated presence of seven tertiary methyl protons at δ 0.90 – 0.80 (m, 4H) and 0.95 (d, $J$ = 10.7 Hz, 8H), olefinic (δ 5.2-5.3) and methine (2.92 (dd, $J$ = 13.8, 4.7 Hz, 1H) protons (Liu *et al*., 2008). This data gave a prediction of oleanane type of pentacyclic triterpenoid skeleton.

Carbon 13 Nuclear magnetic resonance spectra (Appendices 10 and 11) of this compound indicated thirty (30) resonance signals, the signal peaks corresponded to thirty (30) magnetically non-equivalent carbons, and this provided a further confirmation of pentacyclic triterpenoid. The $^{13}C$ NMR spectra revealed four downfield signals δ 215.1, 178.1, 144.0 and 122.0 which indicated that compound **37** had a ketone, a carbonyl and tri-substituted carbon respectively, they were assigned to C-3, C-28, C-13 and C-12 of the oleanane skeleton (Kwon *et al*., 2011; Kwon *et al*., 1997). The other chemical shifts for $^{13}C$ NMR spectrum were compared with literature information as shown in Table 4.19 and compound **37** was identified as 3-oxooleanoic acid (3-oxoolean-12-en-28oic acid) (Irungu *et al*., 2014). The structure of compound **37** was as shown in Figure 4.1.

Other physical chemical characteristics that provided additional information included melting point (154.4°C), IR: $\nu^{KBr}{}_{max}$ ($cm^{-1}$) functional groups at 3165 $cm^{-1}$ (OH), 2942 and 2865 $cm^{-1}$ (CH), 1695 $cm^{-1}$ (C=O) and 1595 $cm^{-1}$ (C=C) which are constituent groups of oxo-oleanolic acid (Appendix 6). The UV spectrum revealed UV: $\lambda_{max}$ (MeOH) at 205 nm due to the presence of nonconjugate carbonyl (C=O) or carbon-carbon double bond (C=C) groups (Appendix 5). The molecular formula of 3-oxooleanolic acid is given as $C_{30}H_{46}O_3$ and it's molecular mass is 454 g, therefore the ESI-MS m/z: 453.39 $(M-H)^-$ (Appendix 7) for for compound **37** were consistent with that found in literature for 3-oxo-oleanolic acid (Majid *et al*., 2016; Van Doelen, 2016).

**Table 4.17: Assignment of $^{13}C$ NMR chemical shifts for compound 37 (Irungu *et al.*, 2014)**

| Position | Compound 37 | | | Position | Compound 37 | | |
|---|---|---|---|---|---|---|---|
| | $\delta_H$, ppp, (J Hz) | $\delta_C$ | $\delta_C$ Literature | | $\delta_H$, ppp, (J Hz) | $\delta_C$ | $\delta_C$ Literature |
| 1 | - | 39.3 | 38.9 | 16 | - | 23.3 | 23.5 |
| 2 | - | 33.6 | 34.1 | 17 | - | 46.8 | 46.4 |
| 3 | - | 215.1 | 216.2 | 18 | 2.92 (dd,13.8, 4.7) | 41.4 | 41.3 |
| 4 | - | 46.9 | 48.4 | 19 | - | 45.9 | 45.9 |
| 5 | - | 55.0 | 54.1 | 20 | - | 30.4 | 30.2 |
| 6 | - | 19.4 | 20.9 | 21 | - | 33.6 | 33.5 |
| 7 | - | 32.2 | 31.7 | 22 | - | 32.5 | 32.6 |
| 8 | - | 38.9 | 38.6 | 23 | 1.12 | 26.0 | 26.1 |
| 9 | - | 46.0 | 46.4 | 24 | 0.92 | 20.9 | 21.5 |
| 10 | - | 36.6 | 36.1 | 25 | 1.05 | 14.4 | 14.5 |
| 11 | - | 22.9 | 22.8 | 26 | 0.86 | 16.6 | 16.5 |
| 12 | 5.27, m | 122.0 | 121.2 | 27 | 1.17 | 25.3 | 25.3 |
| 13 | - | 144.0 | 143.6 | 28 | - | 178.1 | 179.4 |
| 14 | - | 41.8 | 41.9 | 29 | 0.95 | 33.6 | 33.1 |
| 15 | - | 27.6 | 27.0 | 30 | 1.07 | 23.0 | 23.1 |

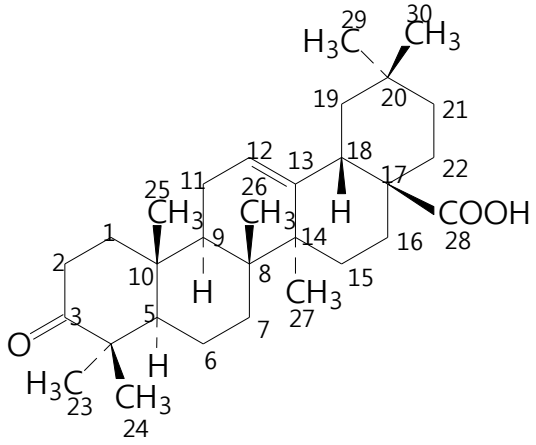



**Figure 4.1: Structure of compound 37**

### 4.6.1.2 Compound 38

Compound **38** (22.4 mg) was isolated as colourless plate like crystals with melting point 283.8°C. The compound was isolated from dichloromethane fraction of the methanol extract *F. angolensis* stem bark. The nuclear resonance spectra revealed compound **38** as an oleanane triterpenoid, Proton NMR exhibited seven methyl carbons at the shielded region between δ 0.8 to 1.2 ppm (Appendix 15) which are characteristic of triterpenoids (Kwon *et al*., 1997; Martins *et al*., 2013). Further, proton NMR spectrum (Appendices 17 and 18) show δ 5.30 (t, J = 3.7 Hz, 1H) and 2.89 (dd, J = 14.1, 4.6 Hz, 1H) tri-substituted and methine carbons which were assigned to C-12 and C-18, respectively. Tri-substituted carbon was equally represented by $^{13}$C NMR spectrum (Appendix 19) at δ 123.5 and 145.2 which were assigned to C-12 and C-13, respectively (Kwon *et al*., 1997; Mahato and Kundu, 1994). The more deshielded with a downfield signal at δ 181.8 presented the presence of a carbonyl group in compound **38**.

The comparison of $^{13}$C NMR spectrum with that of available literature (Table 4.20) and further superimposing the spectrum using MestReNova and ChemDraw Ultra 8.0 softwares

(Appendix 84), the compound was predicted as 3-deoxyoleanolic acid (3-deoxyolean-12-en-28-oic acid) with the structure shown in Figure 4.2. The molecular formula of deoxyoleanolic acid is given as $C_{30}H_{48}O_2$ with calculated molecular mass 440 g. ESI-MS m/z: 441.45 (M+H) (Appendix 14). Further spectral data included UV: $\lambda_{max}$ (MeoH): 195 nm (Appendix 12) which was due to the nonconjugate double bond (C=C) in the compound, IR: $\nu^{KBr}{}_{max}$ ($cm^{-1}$): indicated the presence of OH of an acid centred at 3131, $CH_2$ (2943 and 2864), C=O (1698), (C=C) 1595, and (gem-dimethyl (1398) (Appendix 13).

**Table 4.18: Assignment of $^{13}C$ NMR chemical shifts for compound 38 (Uddin *et al*., 2011)**

| Position | Compound 38 | | | Position | Compound 38 | | |
|---|---|---|---|---|---|---|---|
| | $\delta_H$, ppp, (J Hz) | $\delta_C$ | $\delta_C$ Literature | | $\delta_H$, ppp, (J Hz) | $\delta_C$ | $\delta_C$ Literature |
| 1 | - | 40.2 | 39.0 | 16 | - | 24.6 | 23.8 |
| 2 | - | 21.9 | 18.9 | 17 | - | 47.7 | 46.7 |
| 3 | - | 48.5 | 42.0 | 18 | 2.9 (ddd, 14.1, 4.6) | 42.8 | 42.1 |
| 4 | - | 35.1 | 39.4 | 19 | - | 47.2 | 46.6 |
| 5 | - | 56.5 | 55.9 | 20 | - | 31.6 | 31.0 |
| 6 | - | 20.7 | 18.8 | 21 | - | 34.9 | 34.3 |
| 7 | - | 33.8 | 33.4 | 22 | - | 33.5 | 32.2 |
| 8 | - | 40.5 | 39.8 | 23 | 1.10 | 27.0 | 28.8 |
| 9 | - | 48.2 | 48.2 | 24 | 0.93 | 15.5 | 16.5 |
| 10 | - | 37.9 | 37.4 | 25 | 1.07 | 15.5 | 15.6 |
| 11 | - | 24.0 | 23.8 | 26 | 0.89 | 17.6 | 17.5 |
| 12 | 5.2 (t, 3.7) | 123.5 | 122.6 | 27 | 1.2 | 26.3 | 26.2 |
| 13 | - | 145.2 | 144.8 | 28 | - | 181.8 | 180.0 |
| 14 | - | 43.0 | 42.2 | 29 | 0.93 | 33.6 | 33.4 |
| 15 | - | 28.8 | 28.4 | 30 | 1.09 | 24.1 | 23.8 |

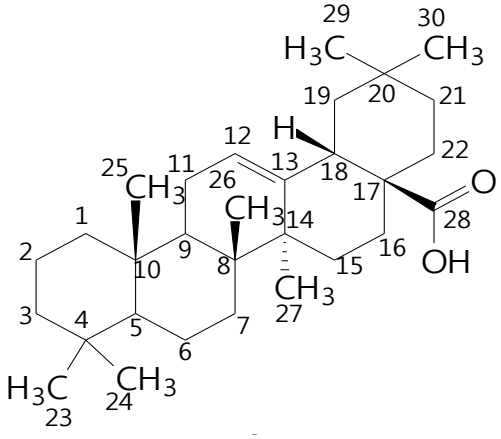



**Figure 4.2: Structure of compound 38**

### 4.6.1.3 Compound 39

Compound **39** was obtained from the dichloromethane fraction of the methanol extract of *F. angolensis* stem bark as white pellets (138.9 mg). Thin layer chromatography indicated that it was pure compound with different $R_f$ values; 0.63 and 0.69 using dichloromethane: methanol in the ration of 9:1 and 9.5:0.5, respectively as mobile phases. The $^{13}C$ NMR of compound **39** showed 30 carbon peaks (Appendix 26), the peak signal at δ 181.9 indicated the presence of a carbonyl group which is characteristic of a high magnetically deshielded atom. The peaks at δ 123.6 and 145.2 indicate that compound **39** has two olefinic carbons (Mahato and Kundu, 1994; Kwon *et al*., 1997; 2011). The three signals at δ 181.9, δ 123.6 and δ 145.2 are characteristic of an oleanane type of skeleton assigned at C-28, C-12 and C-13, respectively. The oxygen deshielding chemical shift at δ 79.9 was assigned to C-3. Proton NMR spectra (Appendix 24), revealed seven tertiary methylene groups at chemical shifts between δ (0.68-1.19), which were assigned as 1.19 (3H, s, $CH_3$-27), 1.06 (3H, s, $CH_3$-23), 0.87 – 0.81 (9H, m, $CH_3$-25, 29.30), 0.72 (3H, s, CH-24), and 0.68 (3H, s, CH-26). A doublet of one proton at δ 2.7 and triplet of one vinyl proton at δ 5.1 were assigned

to C-18 and C-12, respectively (Appendix 25). The NMR spectral data of compound **39** was similar to that of oleanolic acid from literature as indicated in Table 4.21 (Uddin *et al*., 2011). Analysis of $^{13}C$ NMR spectra of compound **39** was graphically compared with that of oleanolic acid using MestReNova and ChemDraw Ultra 8.0 softwares was indicated in Appendix 85.

Additional spectral data from IR: $\nu^{KBr}_{max}$ ($cm^{-1}$): 3165, 2942, 1695, 1595, 1400, 1331, 1210, 1113 and 702 can be interpreted as the presence of OH, $CH_2$, C=O, C=C, $CH_3$ and C-O (Appendix 22). Moreover, other physical properties including melting point (272-274°C), colour (white), shape (needle like), UV spectra: ($\lambda_{max}$ (MeoH): 240 nm) (Appendix 21) are comparable with those reported in literature ( Irungu, 2014). Therefore compound **39** was identified as oleanolic acid (3β-Hydroxyolean-12-en-28-oic acid) with a chemical structure as shown in Figure 4.3.

The spectra of compounds **37**, **38** and **39** revealed close similarity, except for the $^{13}C$ NMR that compound **37** revealed a keto group represented by δc 215.1. The chemical shifts δc 79.69 represented oxygen deshileded chemical shift and was assigned to carbon three (C-3) for compound 39**.** This chemical shift was abscent in compound **37**.

**Table 4.19: Assignment of $^{13}C$ NMR chemical shifts for compound 39 (Uddin *et al*., 2011)**

| Position | Compound 39 | | | Position | Compound 39 | | |
|---|---|---|---|---|---|---|---|
| | $\delta_H$, ppp, (J Hz) | $\delta_C$ | $\delta_C$ Literature | | $\delta_H$, ppp, (J Hz) | $\delta_C$ | $\delta_C$ Literature |
| 1 | 1.59, m | 40.2 | 39.0 | 16 | - | 24.0 | 23.8 |
| 2 | 1.54, m | 27.9 | 28.1 | 17 | - | 47.6 | 46.7 |
| 3 | 3.21, p (1.6) | 79.7 | 78.2 | 18 | 2.76, m | 42.7 | 42.1 |
| 4 | - | 39.8 | 39.4 | 19 | 3.05, m | 47.2 | 46.6 |
| 5 | - | 56.7 | 55.9 | 20 | - | 31.6 | 31.0 |
| 6 | - | 19.5 | 18.8 | 21 | - | 34.5 | 34.3 |
| 7 | - | 34.0 | 33.4 | 22 | - | 33.6 | 33.2 |
| 8 | - | 40.5 | 39.8 | 23 | 1.06, m | 28.8 | 28.8 |
| 9 | - | 49.2 | 48.2 | 24 | 0.71, m | 16.3 | 16.5 |
| 10 | - | 38.2 | 37.4 | 25 | 0.84, m | 15.9 | 15.6 |
| 11 | - | 24.5 | 23.8 | 26 | 0.68, m | 17.7 | 17.5 |
| 12 | 5.14, t (3.7) | 123.6 | 122.6 | 27 | 1.19, m | 26.4 | 26.2 |
| 13 | - | 145.2 | 144.8 | 28 | - | 181.9 | 180.0 |
| 14 | - | 42.9 | 42.2 | 29 | 0.81 m | 33.8 | 33.4 |
| 15 | - | 28.8 | 28.4 | 30 | 0.87, m | 24.0 | 23.8 |

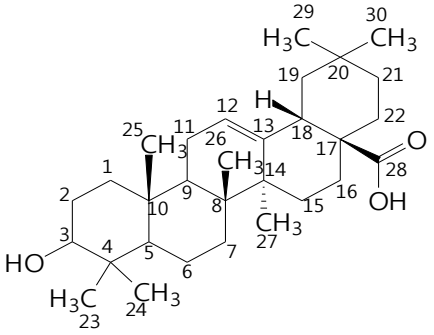


**Figure 4.3: Structure of compound 39**

### 4.6.1.4 Compound 40

Compound **40** was isolated as transparent prism like crystals (115 mg) from methanol fraction of *U. anisatum* root methanol extract. It had a melting point of 235-238°C, was observed as a dark and yellow spot at λ 254 nm under UV and resublimed iodine respectively, however it was inactive at λ 365 nm and 1% vanillin spray. The electron spray mass spectrometry (ES) revealed a molecular ion peak $[M-H]^-$ with mass-to-charge ratio (*m/z*) of 327.06 (Appendix 29). Proton NMR spectrum revealed a signal for one aromatic proton (s, 7.08, 1H) and a signal for methoxy proton (s, 3.89, 3H) (Appendix 30 to 34) as indicated by Subramanian *et al*. (2015). Carbon 13 spectrum exhibited signal at δ 162.8 and δ 59.8 which confirmed the presence carbonyl (C=O) and methoxy (O-$CH_3$) groups, respectively in compound **40** (Appendix 35). Further IR spectrum at wavenumber 1702 $cm^{-1}$ indicated the presence of a strong carbonyl bond (C=O), 1599 $cm^{-1}$ C=C group and absorption bands at 3201-3643 $cm^{-1}$ showed OH groups with intermolecular hydrogen bonds (Appendix 28). Comparison of both $^{1}H$ and $^{13}C$ NMR spectral data with existing literature provided a strong prediction of compound **40** to be bergenin hydrous (Table 4.4).

Further data that was used to identify compound **40** as bergenin included UV absorption maximum which was at 273 nm (Appendix 27), melting point at 235-238°C and mass ion peak at 327 $[M-H]^-$ or 328 $[M-H]^+$ as published literature (Nasser *et al*., 2009; Lin *et al*., 2012; Sariga *et al*., 2015). Therefore compound **40** was strongly suggested to be bergenin anhydrous with molecular formula $C_{14}H_{16}O_9$ (Dung *et al*., 2004; Nasser *et al*., 2009). The calculated molecular weight of anhydrous bergenin is 328.273 g and its molecular structure is as shown in Figure 4.6. A graphical presentation of $^{13}C$ NMR spectra of bergenin with that of compound **40** is also indicated in Appendix 86.

**Table 4.20: Assignment of $^{13}C$ NMR (101 MHz, ($C_3D_6CO$) and 1H NMR (400 MHz, MeOD) chemical shifts for compound 40**

| Postition | $^{1}$ H NMR δ of compound **40** | | $^{13}C$ NMR δ of compound **40** | $^{13}C$ NMR δ of compound **40** in literature |
|---|---|---|---|---|
| | $\delta_H$, ppm (J, Hz) | H | | |
| 2 | 3.51, ddd (9.7, 8.5, 4.9) | 1H, H-2 | 81.9 | 81.8 |
| 3 | 3.66, m, | 1H, H-3 | 71.1 | 70.8 |
| 4 | 4.64, dd (5.9, 0.8) | 1H, 4-H | 74.6 | 73.8 |
| 4a | 4.91, dd (4.6, 0.9) | 1H, 4a-H | 80.0 | 79.9 |
| 6 | - | - | 162.8 | 163.5 |
| 6a | - | - | 118.7 | 118.2 |
| 7 | 7.08, s | 1H, 7-H | 109.3 | 109.6 |
| 8 | 8.44, s | 1H, 8-H | 150.8 | 151.1 |
| 9 | - | - | 140.4 | 140.7 |
| 10 | 8.34, s | 1H, 10-H | 148.2 | 148.2 |
| 10a | | | 116.1 | 116.1 |
| 10b | 5.04, d (10.4) | 1H, 10b-H | 73.0 | 72.2 |
| 11 | - | - | 61.8 | 61.2 |
| 12 | 3.89, s | 3H, 12-H | 59.8 | 60.0 |

Literature data derived from (Nasser *et al*., 2009; Silva *et al*., 2009; Lin *et al*., 2012; Subramanian *et al*., 2015).

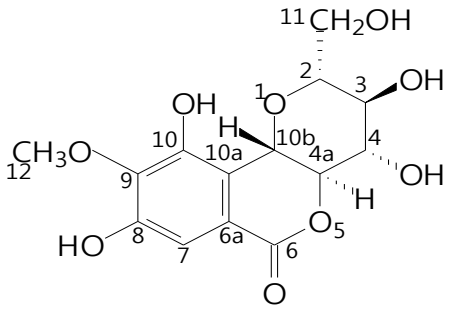



**Figure 4.4: Structure of compound 40**

### 4.6.2 Partially identified compounds

#### 4.6.2.1 Compound 41

Compound **41** was isolated as white prism like crystals (963 mg) from methanol fraction of *U. anisatum* root methanol extract. It had a melting point of 152-155°C and was observed as a dark and yellow spot at λ 254 nm under UV and resublimed iodine respectively. The UV absorption maximum of Compound **41** was revealed at 273 nm (Appendix 54), the FTIR spectra exhibited $\nu^{KBr}_{max}$ at 1703 $cm^{-1}$, this was indicative of a carbonyl (C=O) bond and at 3198-3385 $cm^{-1}$ which suggest OH groups in this compound (Appendix 55). The compound was not completely elucidated thereby in this study mass spectrometry and nuclear magnetic resonance had not been carried out for compound **41.** The quantities of the compound were insufficient at the time of study.

#### 4.6.2.2 Compound 42

Compound **42** was isolated as white star like crystals (11.6 mg) from ethyl acetate fraction of methanol extract of *Combretum tanaense* root. Proton NMR spectrum (Appendix 56) shows seven peaks between δ 0. 73-1.04 which are typical methyl signals in a triterpenoid skeleton (appendix 57). δ 5.27 (t, J = 3.7 Hz, 1H) which was due to olefinic hydrogen and therefore compound **42** was predicted as an oleanane triterpenoid. $^{13}C$ NMR spectrum (Appendix 58) confirmed the identification by having δ at 122.1 and 144.1 due to a double bond and was characteristic of C-12 and C-13 unsaturation. The δ at 178.0 was associated to a carbonyl functional group in compound **42** spectra. Though other spectra data including molecular ion peak of compound **42** at 487.42 $(M-H)^-$ (Appendix 39) and fourier transform infra-red spectrum which revealed band wave numbers ($cm^{-1}$) at 3156 and 1594 which were

characteristic of the OH and C=C absorbing groups (Appendix 59), it was not possible to determine the structure of sample **42** precisely due to poor NMR spectra resolution.

### 4.6.2.3 Compound 43

Compound **43** was isolated as white plate like crystals (7.7 mg) from ethyl acetate fraction of *Combretum tanaense* root methanol extract. Proton NMR spectrum (Appendix 50) showed seven peaks at the range of δ 0.59 to 1.2 parts per million. Furthermore, the presence of chemical shifts at δ 5.16 (t, *J* = 3.7 Hz, 0H) was ascribed to olefinic proton and 2.75 (dd, *J* = 14.1, 4.6 Hz, 0H) to methine protons. $^{13}$C NMR of compound **43** indicated chemical shifts at δ 145.4 and 123.4 which are characteristics of a tri-substituted carbon (Appendix 53) in oleanane skeleton. δ 78.1 revealed hydroxylated carbon atom in compound **43**.

The compound was not completly elucidated due to poor NMR spectra resolutions. Compound **42** and **43** were closely related, they revealed similar molecular ion peak of compound at 487.42 $(M-H)^-$ (Appendix 39 and 48). The difference occurred in $^{13}$C NMR spectra where compound **42** revealed a carbonyl carbon at chemical shift δ 178.0 and this was absent in the spectra of compound **43**. The FTIR spectra of compound **42** and **43** indicated that the compounds had similar absorption bands at 3156 and 3148 $cm^{-1}$ indicating the presence of OH groups and at 1594 $cm^{-1}$ which is attributed to the presence of C=C group.

# CHAPTER FIVE: DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

## 5.1 Discussion

### 5.1.1 Documentation of anticancer plants used in selected parts of Kenya

According to reports in literature, the nine plants that were documented in the current study to have anticancer activities in selected regions of Kenya, had also been reported to be used in the management of cancers by other ethnic communities. Cross-references of the plants parts that were documented for management of cancer were consistent with published data. Besides the current study, the root decoctions of *Flueggea virosa* and *Grewia villosa* have been indicated for prostate and breast cancers (Kareru *et al*., 2007; Musa *et al*., 2011). The root and leaf decoctions of *Maytenus obscura, Fagaropsis angolensis* and stem bark of *Prunus africana* have also been reported to treat cancer (Kareru *et al*., 2007; Jeruto *et al*., 2011; Ochwang'i *et al*., 2014). However, the bark of *Fagaopsis angolensis* is reported for management of breast and prostate cancer in the current study for the first time.

The rhizome of *Hydnora abyssinica* was reported to manage breast and prostate cancer in this study, this is in agreement with early documentation that the plant is useful in the management of cancer (Mwangi *et al*., 2001). In addition, the infusions of *Vitex doniana* leaves and *Launaea cornuta* whole plant were reported for the treatment and management of breast and prostate cancers. However, they had also been documented in early studies (Kareru *et al*., 2007; Kayombo, 2016). The decoction *Spermacoce princeae* whole plant has also been reported by other researchers for management of cancers (Jeruto *et al*., 2011). The use of *Indigofera swaziensis* root decoction for treatment and management of throat cancer for the first time in this study.

The other plants that were included in this study namely, *Uvariodendron anisatum*, *Marsidenia schimperi* and *combretum tanaense* were documented following doctrine of signatures and phylogenetic approach. Though *Uvariodendron anisatum* had been indicated as an anticancer plant in early studies by Kareru *et al*. (2007), it wasn't revealed by herbalists in this study as a plant with anticancer activities. *U. anisatum* and *C. tanaense* were documented due to phylogenetic studies, the family annonaceae and combretaceae have provided compounds including acetogenins and combretastatins respectively that exhibit promising anticancer activities (Evans, 2009; Biba *et al.*, 2018). The study of these specific phylogenetic groups were included to increase the possibility of isolating particular type of compounds with anticancer activities (Schwikkard and Mulholland, 2014). Finally, *M. schimperi* was reported in this study for the first time based on the fact that the shape of open dry fruit resembles female reproductive organ (Bennett, 2007; Salim *et al*., 2008; Pandita *et al*., 2016).

### 5.1.2 *In vitro* anticancer activities of plant extracts

All the plant extracts that were selected for investigation in this study were active against selected cancer cell lines. The fact that all studied extracts demonstrated anticancer activities, the findings can be attributed to the reason that the plants were selected by ethnopharmacological and chemosystematic or phylogenetic approaches that led to plant material and natural products with higher likelihood of biological activities. Furthermore, it has been documented in many studies that over 74% of the pharmacologically active plant derived medicines have been discovered following the ethnomedical uses of the plants (Demain and Vaishnav, 2011).

*In vitro* anticancer activities of plant extracts were dependent on concentration, the anticancer activities increased with increasing concentration. The observed variations of anticancer activities of the extracts against four different cancer cell lines is commonly reported in various studies (Mahavorasirikul *et al*., 2010). Anti-breast cancer activities of extracts of *F. angolensis* stem bark, *C. tanaense* root, *H. abyssinica* rhizome, *U. anisatum* root, *L. cornuta* aerial parts and *S. princeae* aerial parts have not been previously reported. Similarly, anti-prostate cancer activities of the aforementioned extracts were also reported for the first time in the current study. However, studies conducted using water and 70% ethanol extracts *Hydnora johannis* (synonym *H. abyssinica*) (Yagi *et al*., 2012), showed that the extracts had anticancer activity against human mouth epidermoid carcinoma (KB) cell line.

Anticancer activities of aerial parts of *L. cornuta* and *S. princeae* were reported with the range of $100 < IC_{50} < 1000$ µg/ml against breast and prostate cancer cell lines. Therefore the extracts were classified to have low in vitro antibreast and antiprostate

cancer activities. Though *L. cornuta* and *S. princeae* appear in previous ethnobotanical documentations as anticancer plants, the current study doesn't validate their use due to the high $IC_{50}$ values that were found from this study.

### 5.1.3 Bioactivity-guided fractionation of active crude extracts

There are many strategies for identifying active compounds from plant extracts. The strategies can be classified as older and modern whereby bioassay-guided isolation is widely acceptable and modern strategy. It involves consecutive fractionation cycles coupled with bioactivity testing in order to gradually enrich the active compounds and finally isolate the pure active principles. The increase in bioactivity of fractions containing the active constituents was expected following each fractionation cycle compared to the crude extract, because the relative abundance of the active constituents in these fractions was increasing (Harbone, 1989; Houghton and Raman, 1998). This was true for fractions that were obtained from methanol extracts of *F. angolensis* stem bark and *C. tanaense* root. In contrast, the decreased anticancer activities of fractions that were obtained from methanol extracts of *H. abyssinica* rhizome and *U. anisatum* root was also consistent with common knowledge of plant extracts, where activity is known to diminish due to either decomposition of active compounds during fractionation or loss of synergistic interactions of the active components in the crude extract (Atanasov *et al*., 2015; Houghton and Raman, 1998).

### 5.1.4 Anticancer activity of isolated compounds

Lead compounds have been isolated from plant extracts for long time. In the current study, five oleanane triterpenoids with anticancer activities against selected cell

breast and prostate cancer cell lines were isolated. Three of the oleanane triterpenoids were oleanolic acid naturally occurring derivatives named as **37**, **38** and **39** which were isolated from *F. angolensis* stem bark extract. In this study, bergenin was also isolated but it did not exhibit anticancer activities against the selected cell lines.

#### 5.1.4.1 Oleanane triterpenoids

Compounds **$FC_1$**, **$FC_2$** and **$FC_3$** were oleanane triterpenoids which occur naturally in many plant extracts. However, their isolation from *F. angolensis* bark methanol extract was reported for the first time in this study. A part from their occurrence in stem barks, they are also found in fruits and leaves of many plants that are used as food or medicine.

$FC_1$ is found to occur in free or glycosidic form. Its commercial source is *Olea europea* (Oleaceae) and it can also be obtained from other species of the oleaceae family (Sultana and Ata, 2008; Shanmugama *et al*., 2014). There are few cases where the **$FC_1$**, **$FC_2$** and **$FC_3$** have been isolated from one species. **$FC_1$** acid has been realized by other researchers from more than one thousand six hundred plant species including but not limited to *Prunus africana*, *Lantana camara*, *Ekebergia capensis*, *Ocimum sanctum*, *Aspilia africana*, *Grewia optiva*, *Syzygium aromaticum* and *Solanum incanum* (Hossain and Ismail, 2013; Srivastava *et al*., 2013; Irungu *et al*., 2014; Rali *et al*., 2016).

The partially identified compounds that were isolated from *C. tanaense* were proposed to be oleanane triterpenoids (Compound **$CC_1$** and **$CC_2$**). They revealed anticancer potency against cancer cell lines. The findings about anticancer potency of both the identified and unidentified oleanane group of compounds are consistent with

known anticancer activities of triterpenoids (Thoppil and Bishayee, 2011; Lu *et al*., 2012; Yan *et al*., 2014).

### 5.1.4.2 Isolated bergenin

Anhydrous bergenin (compound **$UC_1$**) was isolated for the first time from methanol extract of *Uvariodendron anisatum* root. However, it is a common compound that has been isolated from a number of higher plants. Some of the plants that have bergenin include *Bergenia ligulate* (Kashima *et al*., 2013), *Dryobalanops aromatica* (Wibowo *et al*., 2011), *Astilbe rivularis* (Rajbhandari *et al.*, 2011), *Vatica odorata* (Latip *et al.*, 2011), *Ficus glomerate* (Hoang *et al*., 2004), *Endopleura uchi* (Nunomura *et al.*, 2009; Silva *et al*., 2009), *Caesalpinia decapetala* (Wei *et al.*, 2013), *Syzygium cumin* (Sariga *et al*., 2015) and *Peltophorum pterocarpum* (Subramanian *et al*., 2015).

The anhydrous bergenin (compound **$UC_1$**) was inactive against the cancer cell lines in this study. $IC_{50}$ > 1000 µg/ml for HCC 1395 and 769±4.4 for 4T1 were recorded for breast cancer cell lines while for prostate cancer cell lines, $IC_{50}$ > 1000 µg/ml (22Rv1) and 748±80 (DU-145) were revealed. These findings are consistent with studies done by early researchers, Kim *et al*. 2013, found that bergenin was inactive against prostate cancer cell lines (DU-145 and LNCaP) with $IC_{50}$ values being > 100 µM/mL. Latip *et al*. (2011) also reported inactivity of bergenin against murine leukemia p-388 cells. in addition, Wibowo *et al.* (2011) also reported inactivity of bergenin against HL-60, MCF-7, HepG2, A-549 and WRL-68. Nevertheless, bergenin has been reported to be responsible of many other activities in plant extracts such as antioxidant (Sumino *et al*., 2002; Kim *et al*., 2013), anti-inflammatory and

antitussive (Swarnalakshmi *et al*., 1984), immunomodulatory (Nazirn *et al*., 2007), antidiabetic, antifungal (Saulo *et al.*, 2011), hepatoprotective (Lim *et al*., 2000), neuroprotective (Takahachi *et al*., 2003), antiviral (Rajbhandari *et al*., 2011) and anti-HIV (Piacente, 1996).

### 5.1.5 Acute toxicity studies

Plant extracts and the compounds thereof have great potential as anticancer agents. The toxicity of the plant extracts and the isolated compounds require to be assessed before their impact in drug discovery and development is considered (Tshikalange and Hussein, 2010).

#### 5.1.5.1 Cellular toxicity of extracts

Cellular toxicity of methanol extracts of *U. anisatum* root, *F. angolensis* stem bark, *C. tanaense* root and *H. abyssinica* rhizome against normal vero cells were reported for the first time in this study. The toxicity of *U. anisatum* root methanol extract was ($CC_{50}$ = 3.3 ± 0.2) and this justified the extract to be classified toxic, other researchers who have worked on plants of annonaceae family where *U. anisatum* is a member, have equally found that the extracts of those plants contain compounds which are toxic against normal vero cell lines. The studies include those done by Sumithra *et al.* (2014) and Suresh, *et al*. (2012), they reported cytotoxic activities of *Annona reticulata* and *Annona squamosa* against vero cell lines with $CC_{50}$ values of 22.0 to 26.0 and 75 µg/ml respectively.

Kirira *et al*. (2006) demonstrated toxicity of *F. angolensis* stem bark against brine shrimp larvae with $LD_{50}$ of 173.48 ± 0.6 and 57.09 ± 1.4 for water and methanol extracts respectively. These finding depicted *F. angolensis* stem bark extracts as

toxic and were comparatively similar to the finding of this study where the extracts were toxic against vero cells ($CC_{50}$ = 21.7 ± 3.8 µg/ml). On the other hand, *H. abyssinica* rhizome was relatively toxic ($CC_{50}$ = 84 µg/ml) and this observation was consistent with the studies done by Waleed *et al*. (2009) and Yagi, *et al.* (2012). These workers found that *H. abyssinica* extracts had toxic effects against 3T3 mouse fibroblast cell line and MRC5 (derived from non-cancer human fetal lung) respectively.

The vero African green Monkey epithelial kidney normal cells have also been used to investigate cellular toxicities of other plants from combretaceae, many plants of this family including *C. woodii*, *C. vendae*, *C. padoides* and *C. bracteosum* have exhibited toxicity against vero cells with $CC_{50}$ values of 3.51 ± 2.03, 5.70 ± 1.25, 9.03 ± 0.20 and 48.81 ± 6.15 µg/ml, respectively (Shahid, 2012). These findings are consistent with those of *C. tanaense* root ($CC_{50}$ = 36.2 ± 4 µg/ml) in this study where the extracts are shown to have toxic effects against vero cells.

### 5.1.5.2 Cellular toxicity of fractions

Cellular toxicities of fractions of *C. tanaense* root, *F. angolensis* bark, *H. abyssinica* rhizome and *U. anisatum* root against normal vero cells are reported for the first time in this study. *C. tanaense* fractions were found to be highly cytotoxic ($CC_{50}$ between 15.8 ± 0.8 and 35.8 ± 0.8) compared to crude methanol extract of *C. tanaense* root ($CC_{50}$ = 36.2 ± 4). These findings indicate that crude extract contain toxic compounds of varied polarity that range from polar to non-polar. Second in toxicity was *F. angolensis* bark fractions with $CC_{50}$ values of 56.7 ± 4.4 µg/ml (petroleum ether fraction), 74.2 ± 0.8 µg/ml (dichloromethane fraction), 84.2 ± 11.6

µg/ml (ethyl acetate fraction), 102.5 ± 27.5 µg/ml (acetone fraction) and 167.0 ± 4.9 µg/ml (methanol fraction). Cytotoxic compounds were found to be in non-polar and fractions of middle polarity fractions (petroleum ether, dichloromethane and ethyl acetate) while non-toxic compounds were found to occur in polar fractions (acetone and methanol).

*H. abyssinica* rhizome which was weekly toxic ($CC_{50}$ = 84.2 ± 6.3 µg/ml) yielded fractions that were non-toxic ($CC_{50}$ between 231.7 ± 5 and 789 ± 24.7 µg/ml). *U. anisatum* fractions were also non-toxic ($CC_{50}$ between 167.0 ± 8.6 and 784.3 ± 23.5 µg/ml) compared to the methanol extract of *U. anisatum* root that was recorded highly toxic in this study ($CC_{50}$ = 3.3 ± 0.2 µg/ml). These observations of reduction of toxicity upon fractionation if *U. anisatum* can be explained that the toxicity of the crude extract was due to sum total of toxicity of constituents thereof, whose toxicity was reduced by separation.

### 5.1.5.3 Cellular toxicities of isolated compounds

*In vitro* toxicity assay of some of the isolated compounds in this study has been reported by early researchers, Irungu *et al*. (2014) found that **$FC_1$** was toxic against normal vero cells ($CC_{50}$ = 35.8 ± 1.3 µM) while in this study, the compound exhibited strong toxicity against vero cell lines ($CC_{50}$ = 47.8 µM conversion equivalent of 21.7 ± 0.0 µg/ml). Toxicity of **$FC_3$** against vero cell lines has also been reported by (Ge *et al.*, 2010; Mukherjee *et al*., 2013; Irungu *et al.*, 2014) with $CC_{50}$ values of 43 µg/ml, 112.0 ± 5.1 µM and 98 µg/ml, respectively. The findings implied that the compound was found to be nontoxic against the vero cell lines.

Anhydrous bergenin (compound **$UC_1$**) was found to be weekly cytotoxic against vero cells with $CC_{50}$ = 20.6 ± 1.1. Similarly, Mukherjee (2009), found that anhydrous bergenin was also weekly cytotoxic against vero cell ($CC_{50}$ = 88 µg/ml. In 2007, Bizimenyera *et al*. (2007) found that hydrous bergenin $CC_{50}$ > 1000 µg/ml and was considered inactive against vero cells. In the current study, anhydrous bergenin was also found to be inactive with $CC_{50}$ = 326.7 ± 68.7 µg/ml. The $CC_{50}$ values of more than 100 µg/ml are assigned non-toxic status of the compound and therefore it implied that the findings in this study are consistent with the early studies.

### 5.1.5.4 Acute oral toxicity of crude extracts

Evaluation of a cute toxicity of medicinal plants is a crucial primary step in drug research and development. In this study, the methanol extracts of *U. anisatum* root, *F angolensis* stem bark, *C. tanaense* root and *H. abyssinica* rhizome plant extracts were found to be not toxic to swiss mice. At doses of 2000 mg/kg there were no observable changes in behaviour, appearance or deaths that resulted from oral administration of the crude extracts. The extracts were therefore assigned the lowest toxicity class as recommended by the Organization for Economic Cooperation and Development where the dose that would kill half of the tested animals ($LD_{50}$) was more than 2,000 mg/kg (OECD, 2001; Kumar and Lalitha, 2013; Naidu *et al*., 2014).

Except for *H. abyssinica* water extract that was reported to be non-toxic ($LD_{50}$ = 1600 mg/kg) when given orally to rats by Osman (2010), investigation of toxicity levels of the other extracts (*F. angolensis* bark, *C. tanaense* root and *U. anistaum* root) were reported for the first time in this study. It is however evident that all these extracts revealed cellular toxicity in contrast to non-toxicity in mice. This

phenomenon has been attributed to possible interactions in the gastrointestinal tracts and drug bioavailability issue (Shahid, 2012).

## 5.2 Conclusions

The findings from this study provide additional knowledge of the plants used to manage cancer in Kenya. A scientific justification for the traditional use of some of these plants for management of cancer is also provided. The isolation of five compounds that demonstrated *in vitro* anti-breast and anti-prostate cancer activities gave an impression that potent lead drugs can be obtained from these plants. This study aimed at establishing of anticancer activities of some Kenya plants that were selected using ethnopharmacological and chemosystematic strategies. It was concluded that:

(i) *Fagaropsis angolensis*, *Hydnora abyssinica*, *Launaea cornuta*, *Vitex doniana*, *Maytenus obscura*, *Prunus africana*, *Grewia villosa*, *Spermacoce princeae* and *Flueggea virosa* were documented following ethnopharmacological approach and had reports of use in the management and treatment of cancers. The other plants that were selected following chemosystematic approach included *Combretum tanaense*, *Uvariodendron anisatum* and *Marsidenia schimperi* had and no previous reports for managing or treating cancers.

(ii) The extracts from *Fagaropsis angolensis*, *Hydnora abyssinica*, *Launaea cornuta*, *Prunus africana*, *Spermacoce princeae*, *Combretum tanaense*, *Uvariodendron anisatum* and *Marsidenia schimperi* demonstrated anticancer activities against the human breast carcinoma (HCC 1395 and 4T1) and prostate cancer (DU-145 and 22RV1) cell lines. However, methanol extracts

of *F. angolensis* bark, *C. tanaense* root, *U. anisatum* root and *H. abyssinica* rhizome were potentially active against the breast and prostate cancer cell lines.

(iii) The increased anticancer activities of methanol extracts of *Fagaropsis angolensis* bark, *Combretum tanaense* root and *H. abyssinica* rhizome was due to seperation of inert compounds from active compounds during fractionation. On the other hand, decrease of anticancer activities in fractionating methanol extract of *Uvariodendron anisatum* root was associated with separation of compounds with synergestic effects and therefore there was loss of anticancer activities.

(iv) The anticancer activities against human breast carcinoma (HCC 1395 and 4T1) and prostate cancer (DU-145 and 22Rv1) cell lines of the crude extracts (*F. angolensis and C. tanaense*) is ascribed to the isolated compounds identified with codes as $\mathbf{FC_1}$, $\mathbf{FC_2}$, $\mathbf{FC_3}$, $\mathbf{CC_1}$ and $\mathbf{CC_2}$.

(v) Methanol extracts of *Fagaropsis angolensis* bark, *Combretum tanaense* root, *Uvariodendron anisatum* root were non-toxic against normal cell lines. In addition, dichloromethane fraction of *F. angolensis* methanol extract, acetone fraction of *C. tanaense*, $\mathbf{FC_1}$, $\mathbf{FC_2}$ and $\mathbf{FC_3}$ were also non-toxic against normal cell lines.

(vi) Methanol extracts of *Fagaropsis angolensis* bark, *Combretum tanaense*, *Uvariodendron anisatum* and *Hydnora abyssinica* are non-toxic because these extracts did not exhibit lethality or changes in general appearance or behavioural patterns at a dose of 2000 mg/kg.

Therefore, research questions formulated in this study, were answered affirmatively.

## 5.3 Recommendations

Based on the results from the current study the following recommendations were made:

(i) The documented plants that have long evidence of use in management of cancer are recommended for use in other parts of Kenya, these plants were *Hydnora abyssinica*, *Maytenus obscura*, *Grewia villosa*, *Flueggea virosa*, *Fagaropsis angolensis*, *Vitex doniana*, *Launaea cornuta* and *Prunus africana*.

(ii) The plant extracts that have folkoric claim, *Fagaopsis angolensis* stem bark, *Hydnora abyssinica* rhizome and flower, *Launaea cornuta* aerial parts and *Spermacoce princeae* aerial parts can be used as such for management of breast and prostate cancer, provided good production processes are observed.

(iii) Dichloromethane fraction from *Fagaropsis angolensis* and acetone fraction from *Combretum tanaense* can be used for improved anticancer activities against breast and prostate cancer cell lines.

(iv) *Fagaropsis angolensis* stem bark and *Combretum tanaense* root extracts can be used as anticancer agents owing to $\mathbf{FC_1}$, $\mathbf{FC_2}$, $\mathbf{FC_3}$, $\mathbf{CC_1}$ and $\mathbf{CC_2}$ compounds that were present in their extracts.

(v) Extracts, fractions and isolated compounds from *F. angolensis* can be used to develop herbal product for management of breast and prostate cancer.

## 5.4 Suggestions for further research

(i) Fractions of middle to high polarity, *F. angolensis* stem bark, *C. tanaense* root, *H. abyssinica* rhizome and *U. anisatum* root extracts should be completely purified for chemical analysis since they exhibited anticancer activities against cancer cell lines.

(ii) *In vivo* anticancer activities should be carried out for *F. angolensis* stem bark, *C. tanaense* root, *H. abyssinica* rhizome and *U. anisatum* root extracts using balb/c mice.

(iii) Chromatographic finger prints and other qualitative parameters should be developed for *F. angolensis* bark *C. tanaense* root, *H. abyssinica* rhizome and *U. anisatum* root.

(iv) Further studies on structural modification and combinatorial effects of the active compounds should be investigated to enhance anticancer activity and reduce toxic effects.

(v) Evaluation of sub chronic and chronic toxic effects of active crude extracts should be done.

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# APPENDICES

## Appendix 1: Abstract of part of the work published in the Journal of Pharmacognosy and Phytotherapy

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Journal of Pharmacognosy and Phytotherapy

*Full Length Research Paper*

# Anticancer activities and safety evaluation of selected Kenyan plant extracts against breast cancer cell lines

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**Breast cancer is a leading cause of deaths among women suffering from cancer in Kenya. The current study was done to determine anticancer activities of medicinal plant extracts against breast cancer cell lines (HCC 1395 and 4T1). Vero cells were used for evaluation of safety of extracts. Thiazoly blue tetrazolium bromide (MTT) assay was used in this study. Reference drugs were 5 fluorouracil and cyclophosphamide. Extract concentrations that inhibited growth of cell growth by half ($IC_{50}$) were estimated using GraphPad prism version 7 and 90 % of extracts showed anticancer activities. Methanol extracts of *Uvariodendron anisatum*, *Fagaropsis angolensis*, *Combretum tanaense*, *Hydnora abyssinica* and water extract of *F. angolensis* exhibited remarkable anticancer activities ($IC_{50}$< 30 µg/ml). Methanol extracts of *F. angolensis* and *H. abyssinica* demonstrated high selectivity index (SI ≥3). Evaluation for safety, indicated that about 64% of the extracts under this study were non-toxic ($CC_{50}$ >100 µg/ml). Findings from plants in this study support folklore claims. Phytochemical analysis, bioassay guided fractionation and toxicity studies are underway on extracts of *C. tanaense*, *F. angolensis*, *H. abyssinica* and *U. anisatum*.**

**Key words:** 4TI, ethnomedicine, HCC 1395, $IC_{50}$ values, medicinal plants, MTT assay, selectivity index, vero E6.

### INTRODUCTION

Breast cancer is the most frequently diagnosed and leading cause of cancer deaths among women. It caused about 522,000 deaths in 2012 worldwide and these estimations are expected to double by the year 2030(WHO, 2014). Of all reported cases of cancer in Kenya, breast cancer has a prevalence of 23.3% and is

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# Appendix 2: Abstract of part of the work presented at a NAPRECA workshop

NAPRECA – K International Conference 8 -10 Feb. 2018 SAJOREC, JKUAT

**OL XI** **Anticancer Efficacy and Safety of *Launaea cornuta* H. (Asteraceae) against Prostate and Breast Cancer Cell Lines**

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Prostate and breast cancer are the leading cause of cancer related deaths in Kenya. Various methods of mitigation are being exploration. However, ethnopharmacological approach is the most preferred option in drug discovery. Traditional medical practitioners claims on the cures for cancer require scientific backing and some of the medicinal plants used in Kenya warrant validation. It is against this background that study was carried out to determine *in vitro* anticancer activity and safety levels of *Launaea cornuta* water and methanol extracts against human breast adenocarcinoma (HCC 1395 and 4TI) and prostate cancer (DU-145 and 22Rvl). Vero cell lines were used for cytotoxicity studies to establish cellular toxicity levels of the extracts. Thiazoly blue tetrazolium bromide (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) (MTT) assay studies were conducted using 96 microtiter plates. Experimental design was done in triplicates for each of the tested extracts and reference drug (cyclophosphamide). Data that was obtained was expressed as a mean ± SEM of the three independent experiments. Estimation of extract or drug concentration that inhibited growth of cancer cell lines by half ($IC_{50}$) and that which inhibited growth of normal cell line (vero) by half ($CC_{50}$) were done using Graph Pad Prism Version 7. The results revealed that both the water and methanol extracts had $IC_{50}$ values ranging between 100 to 1000 µg/ml, this indicated that the extracts were active against the tested prostate and breast cancer cell lines. On the other hand, it was observed that $CC_{50}$ values of both water and methanol extracts against normal cell lines (vero) were greater than 100 µg/ml, this indicated that the extracts were non-toxic safe. It was therefore concluded that ethnopharmacological claims for use of *L.cornuta* in managing prostate and breast cancer is valid. Efforts to isolate anticancer compounds was recommended.

**Key Words:** Antiproliferative, Cell lines, Cytotoxicity, Ethnopharmacology, $IC_{50}$, $CC_{50}$ and Selectivity index (SI)

**Appendix 3: Approval letter obtained from KEMRI /Scientific and Ethic Review Unit**


# KENYA MEDICAL RESEARCH INSTITUTE

P.O. Box 54840 - 00200 NAIROBI - Kenya
Tel: (254) (020) 2722541, 254 (020) 2713349, 0722-205901, 0733-400003 Fax (254) (020) 2720030
Email: director@kemri.org info@kemri.org Website:www.kemri.org


**KEMRI/RES/7/3/1** **August 13, 2015**

**TO:** **JARED ONYANCHA,**
**PRINCIPAL INVESTIGATOR**

**THROUGH:** **DR. PETER MWITARI,**
**THE DIRECTOR, CTMDR,**
**NAIROBI**

Forwarded
19.5.2015

Dear Sir,

**RE:** **PROTOCOL NO. KEMRI/SERU/CTMDR/001/3024 (*RESUBMISSION OF INITIAL SUBMISSION*): EVALUATION OF ANTICANCER ACTIVITY OF SOME MEDICINAL PLANTS USED FOR MANAGEMENT OF CANCER BY TRADITIONAL MEDICAL PRACTITIONERS IN KENYA.**

Reference is made to your undated letter. KEMRI/Scientific and Ethics Review Unit (SERU) acknowledges receipt of the revised study documents on August 10, 2015.

This is to inform you that the Committee noted that the issues raised during the 237th meeting of the KEMRI ERC on 17th March, 2015 have been adequately addressed.

Consequently, the study is granted approval for implementation effective this day, **13th August 2015** for a period of one year. Please note that authorization to conduct this study will automatically expire on **August 12, 2016.** If you plan to continue data collection or analysis beyond this date, please submit an application for continuation approval to SERU by **July 1, 2016.**

You are required to submit any proposed changes to this study to SERU for review and the changes should not be initiated until written approval from SERU is received. Please note that any unanticipated problems resulting from the implementation of this study should be brought to the attention of SERU and you should advise SERU when the study is completed or discontinued.

You may embark on the study.

Yours faithfully,

**PROF. ELIZABETH BUKUSI,**
**ACTING HEAD,**
**KEMRI/SCIENTIFIC AND ETHICS REVIEW UNIT**

## Appendix 4: Approval letter for use of laboratory mice obtained from KEMRI Animal Use and Care Committee


**KENYA MEDICAL RESEARCH INSTITUTE**

Centre for Virus Research, P.O.Box 54628 - 00200 NAIROBI - Kenya
Tel: (254) (020) 2722541, 254 02 2713349, 0722-205901, 0733-400003 Fax (254) (020) 2726115
Email: cvr@kemri.org

**KEMRI/ACUC/ 01.07.15** **1st July 2015,**

Misonge Jared Onyancha,
Centre for Traditional Medicine and Drug Research,
Nairobi.

Mr. Onyancha,

**RE: Animal use approval for KEMRI-SERU- CTMDR -001 -3024 – "Evaluation of anticancer activity of some medicinal plants used for management of cancer by traditional medical practitioners in Kenya" protocol**

The KEMRI ACUC committee acknowledges the resubmission of the above mentioned protocol. It has been confirmed that all the issues raised earlier have been addressed appropriately and acknowledges that the use of laboratory animals is justified in achieving the study objectives.

The committee grants you the approval to use laboratory mice in your study but recommends that you proceed after obtaining all the other necessary approvals that may be required.

Approval is granted for a period of two years starting from when the final ethical approval will be obtained. If you still intend to use laboratory animals 2 years after the initial approval, you are required to submit an application for continuing approval to the ACUC 1 month before the expiry of this initial approval.

The committee expects you to adhere to all the animal handling procedures as described in the protocol. The committee wishes you all the best in your work.

Yours sincerely,

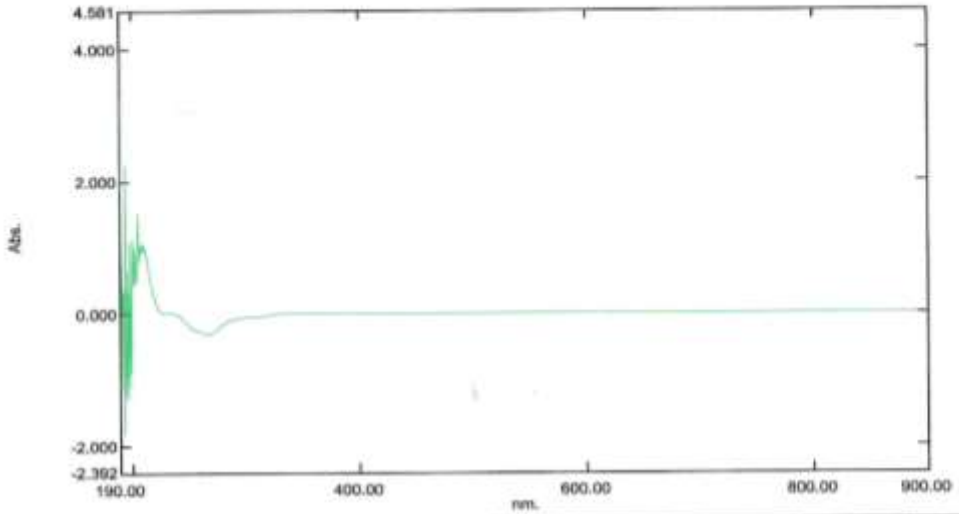
Dr. Konongoi Limbaso
Chairperson KEMRI ACUC

**Appendix 5: UV spectrum of compound 37**

## Spectrum Peak Pick Report

06/22/2017 12:20:36 PM

Data Set: 7 - RawData




| No. | P/V | Wavelength | Abs. | Description |
|---|---|---|---|---|
| 1 | | 350.50 | 0.012 | |
| 2 | | 302.50 | -0.056 | |
| 3 | | 234.50 | 0.016 | |
| 4 | | 205.50 | 1.515 | |
| 5 | | 306.00 | -0.059 | |
| 6 | | 267.00 | -0.303 | |
| 7 | | 230.00 | 0.010 | |

[Measurement Properties]
Wavelength Range (nm.): 190.00 to 900.00
Scan Speed: Fast
Sampling Interval: 0.5
Auto Sampling Interval: Enabled
Scan Mode: Single

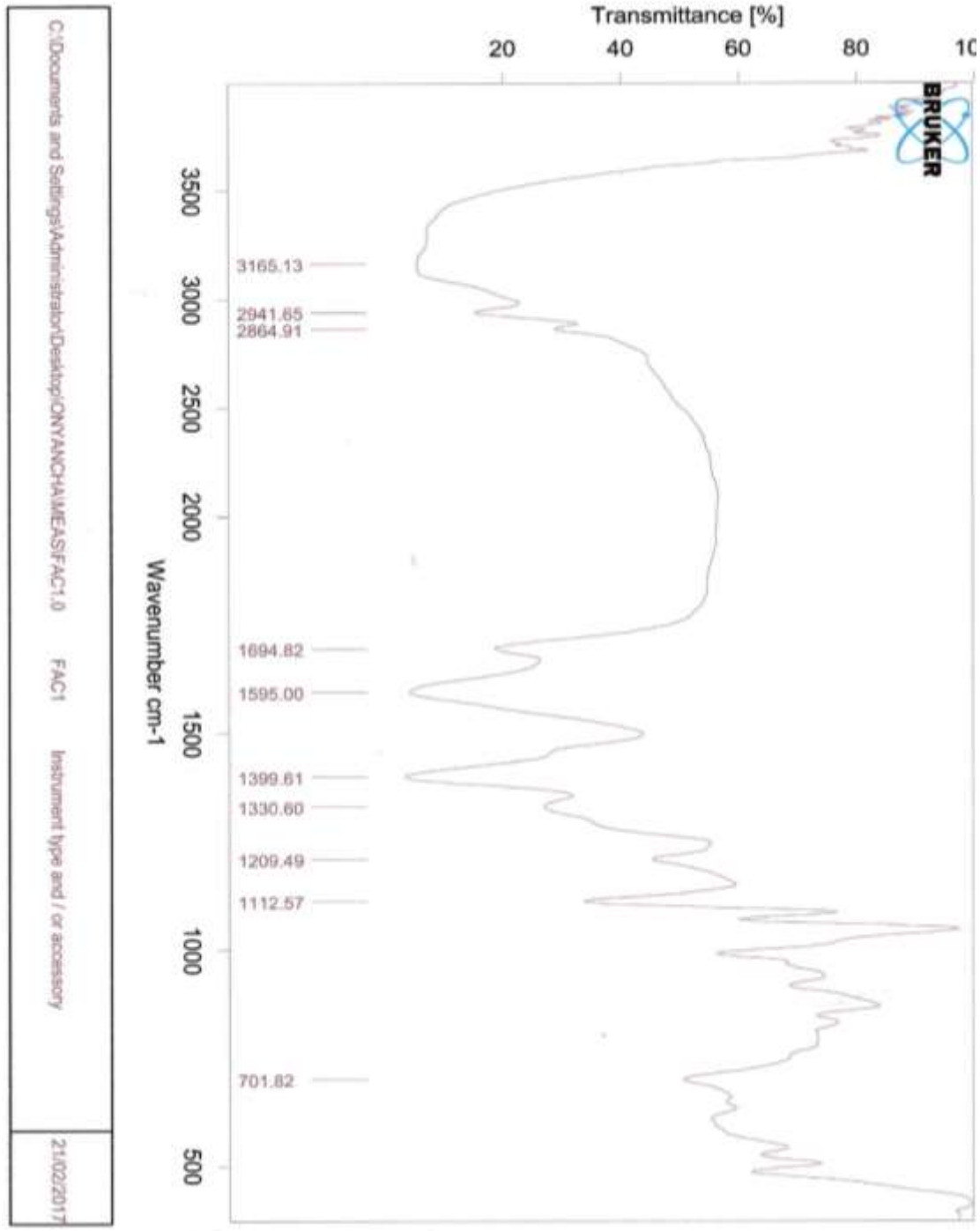
[Instrument Properties]
Instrument Type: UV-1800 Series
Measuring Mode: Absorbance
Slit Width: 1.0 nm
Light Source Change Wavelength: 340.0 nm
S/R Exchange: Normal

[Attachment Properties]
Attachment: None

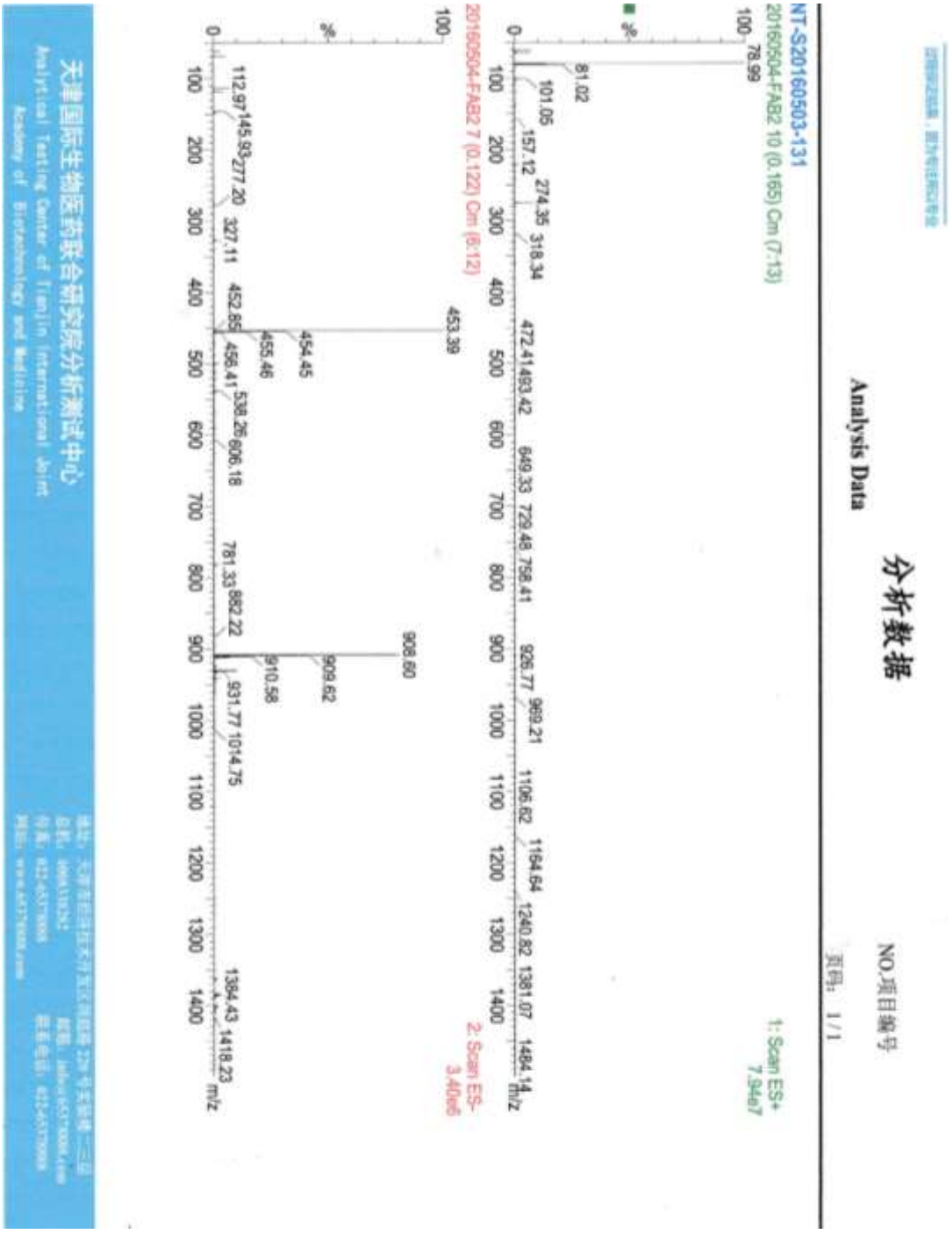
[Operation]
Threshold: 0.0010000
Points: 4
InterPolate: Disabled
Average: Disabled

[Sample Preparation Properties]
Weight:
Volume:
Dilution:
Path Length:
Additional Information:

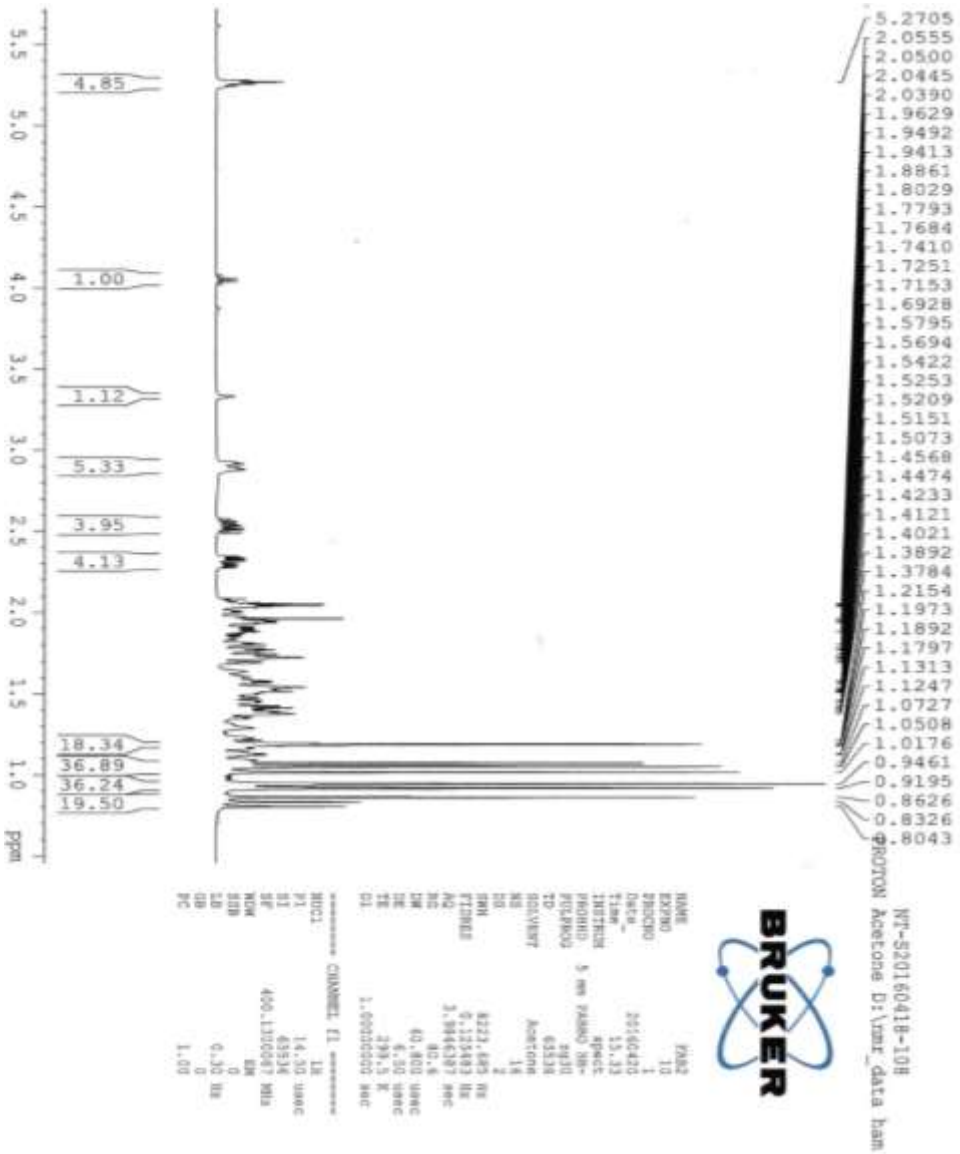
**Appendix 6: FTIR spectrum of compound 37**



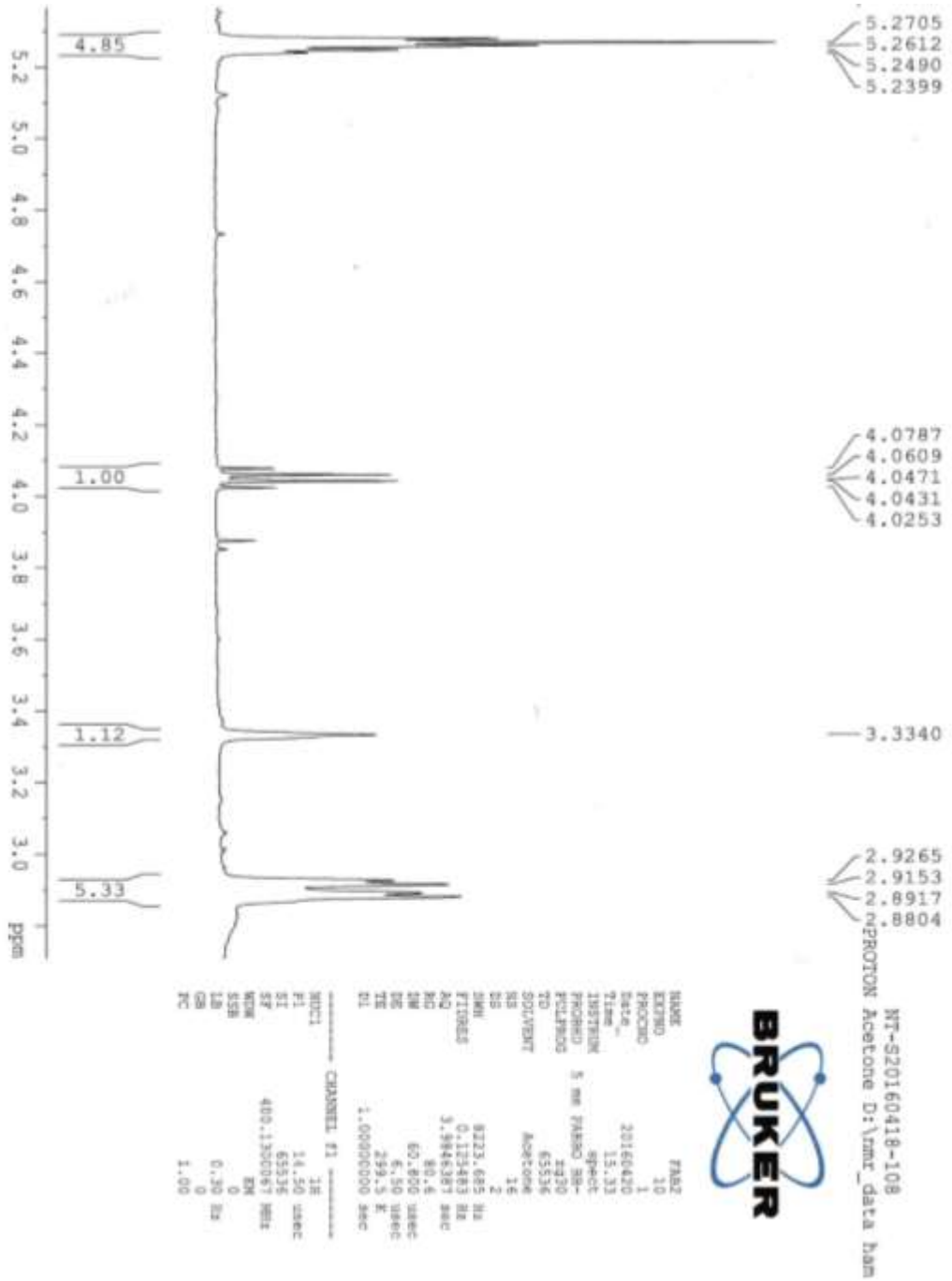
**Appendix 7: MS spectrum of compound 37**



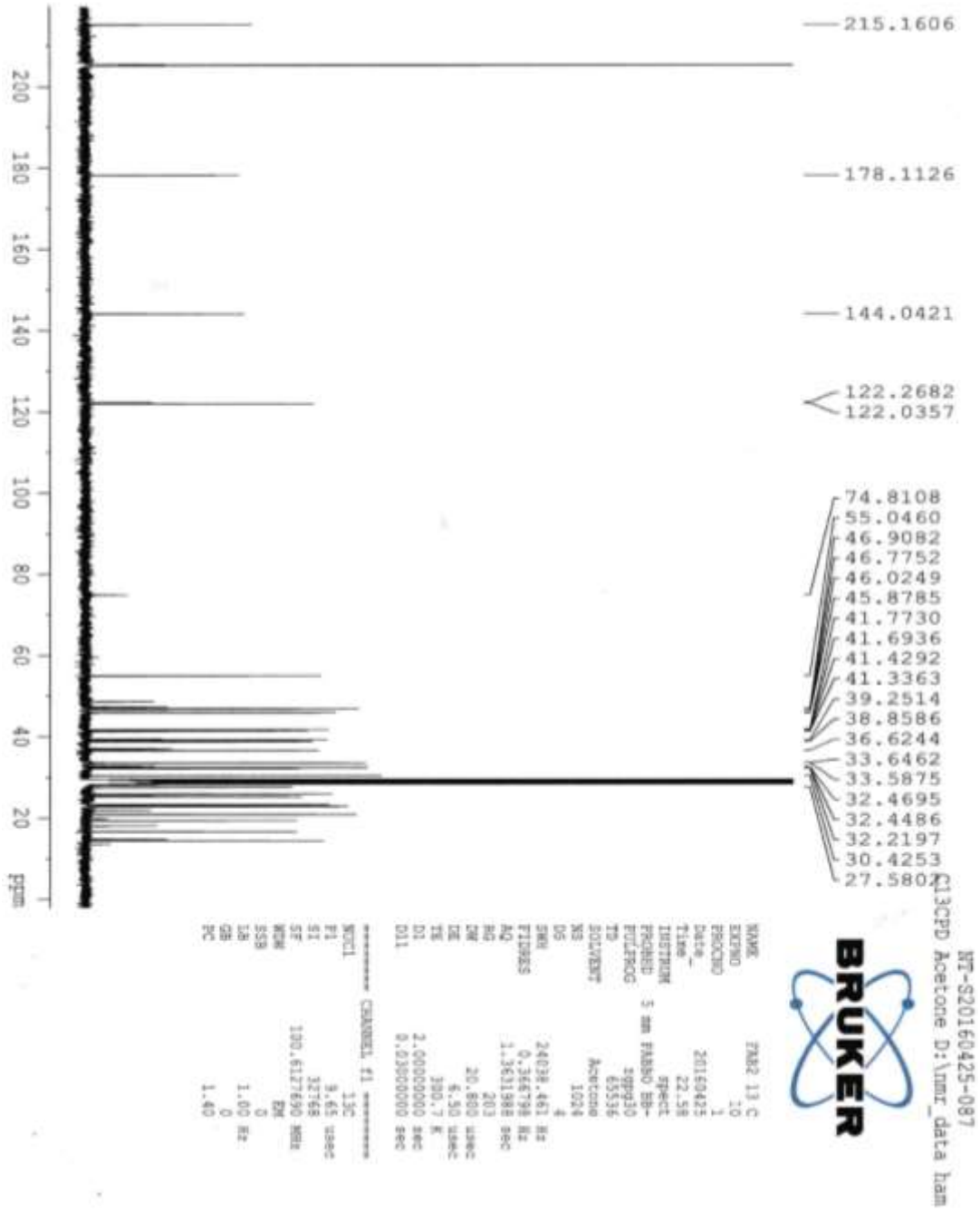
**Appendix 8: $^{1}$H NMR spectrum of compound 37 (δH 0.80 to 5.27)**



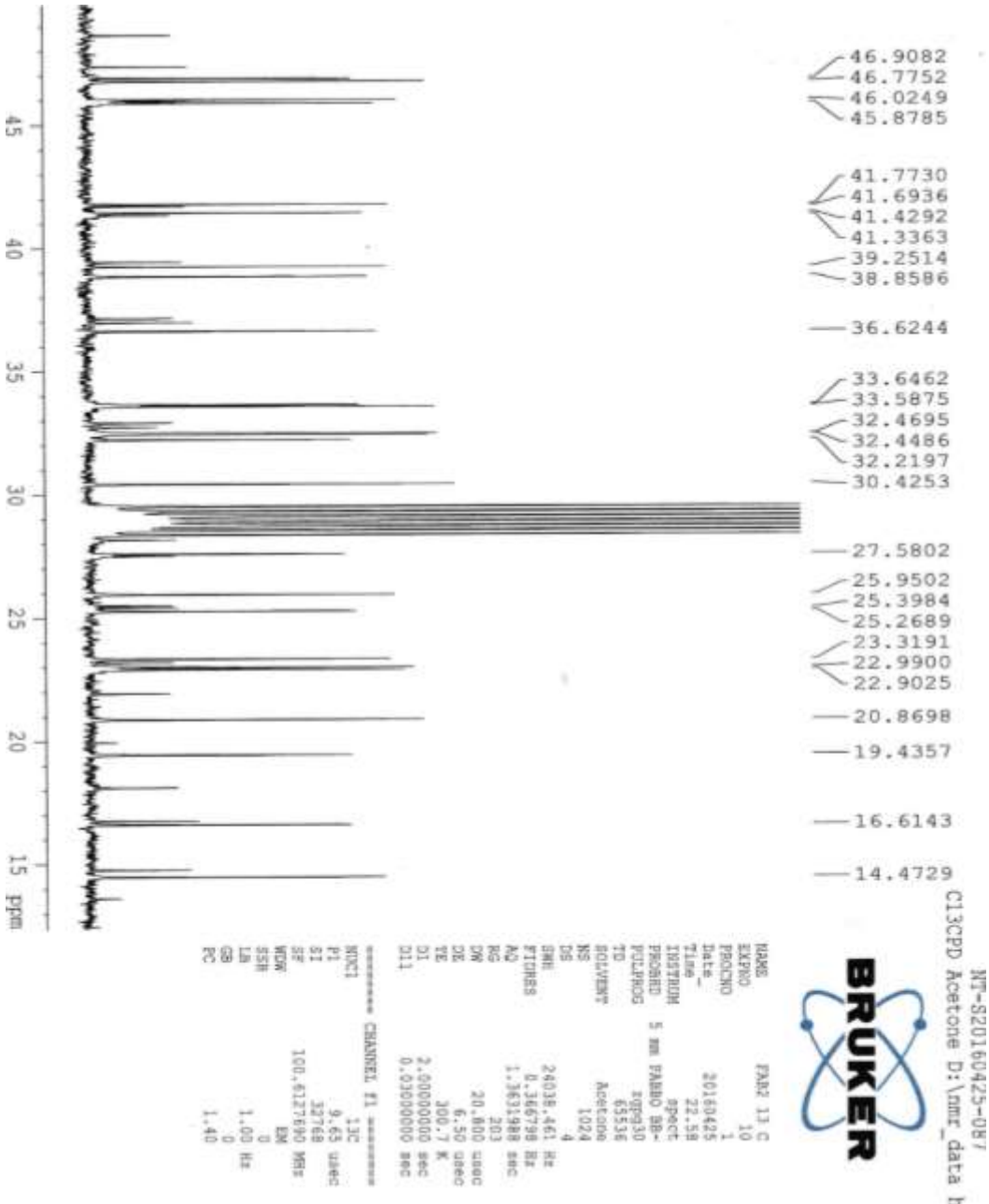
**Appendix 9: $^{1}$H NMR spectrum of compound 37 (Expanded δH 2.88 to 5.27)**



**Appendix 10: $^{13}$C NMR spectrum of compound 37 (Expanded δC 27.58 to 215.16)**



**Appendix 11: $^{13}$C NMR spectrum of compound 37 (Expanded δC 14.47 to 46.91)**

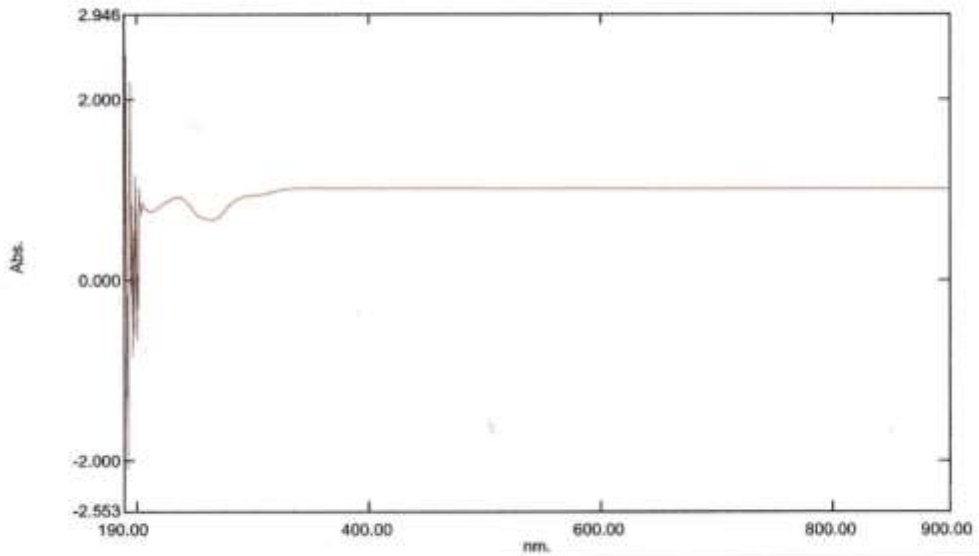


**Appendix 12: UV spectrum of compound 38**

## Spectrum Peak Pick Report

06/22/2017 12:16:34 PM

Data Set: 5 - Dataset 5




| No. | P/V | Wavelength | Abs. | Description |
|---|---|---|---|---|
| 1 | ▼ | 355.00 | 1.017 | |
| 2 | ▼ | 236.50 | 0.922 | |
| 3 | ▼ | 195.00 | 2.198 | |
| 4 | ○ | 264.50 | 0.668 | |
| 5 | ○ | 213.50 | 0.758 | |

[Measurement Properties]
Wavelength Range (nm.): 190.00 to 900.00
Scan Speed: Fast
Sampling Interval: 0.50
Auto Sampling Interval: Enabled
Scan Mode: Single

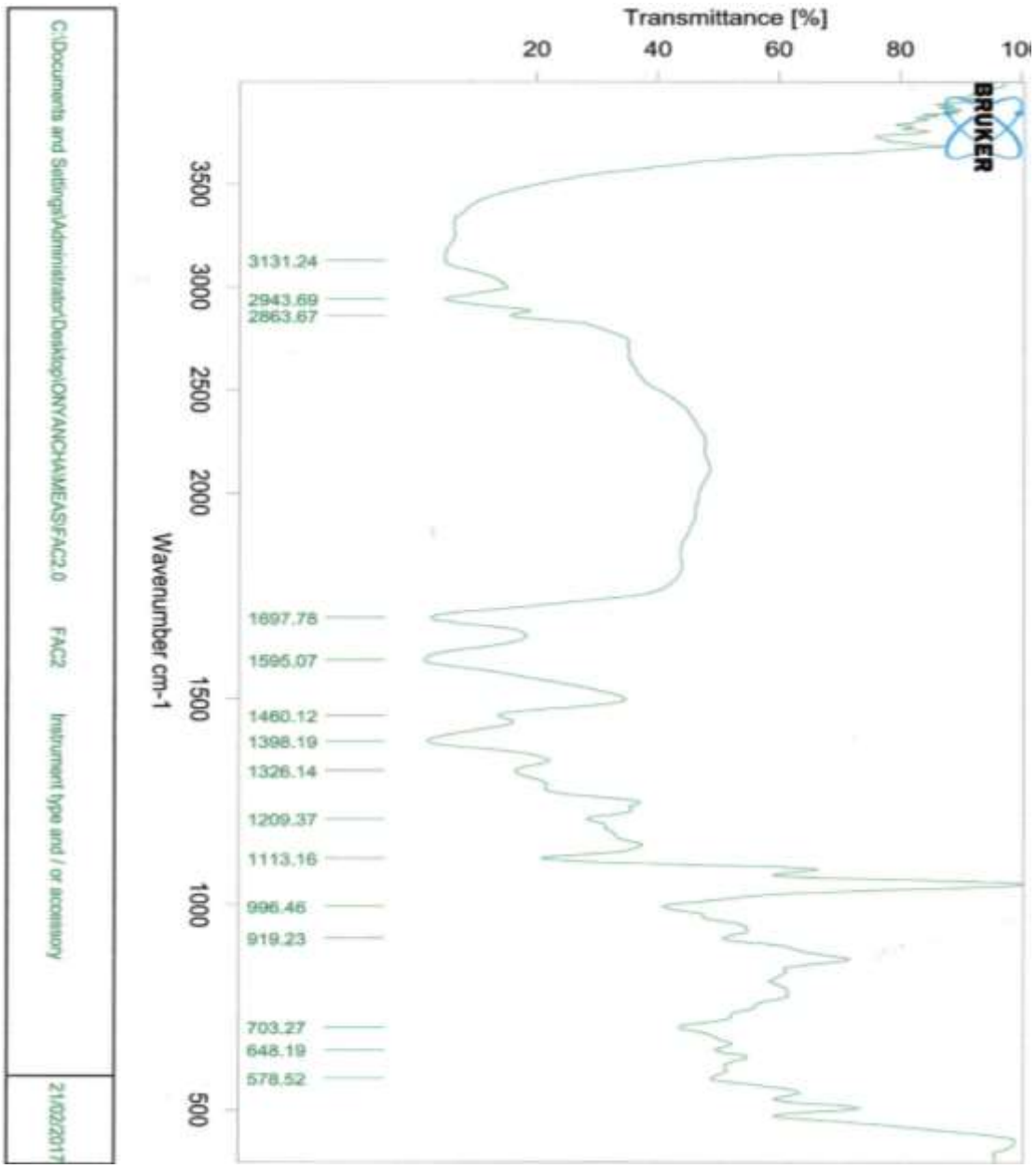
[Instrument Properties]
Instrument Type: UV-1800 Series
Measuring Mode: Absorbance
Slit Width: 1.0 nm
Light Source Change Wavelength: 340.0 nm
S/R Exchange: Normal

[Attachment Properties]
Attachment: None

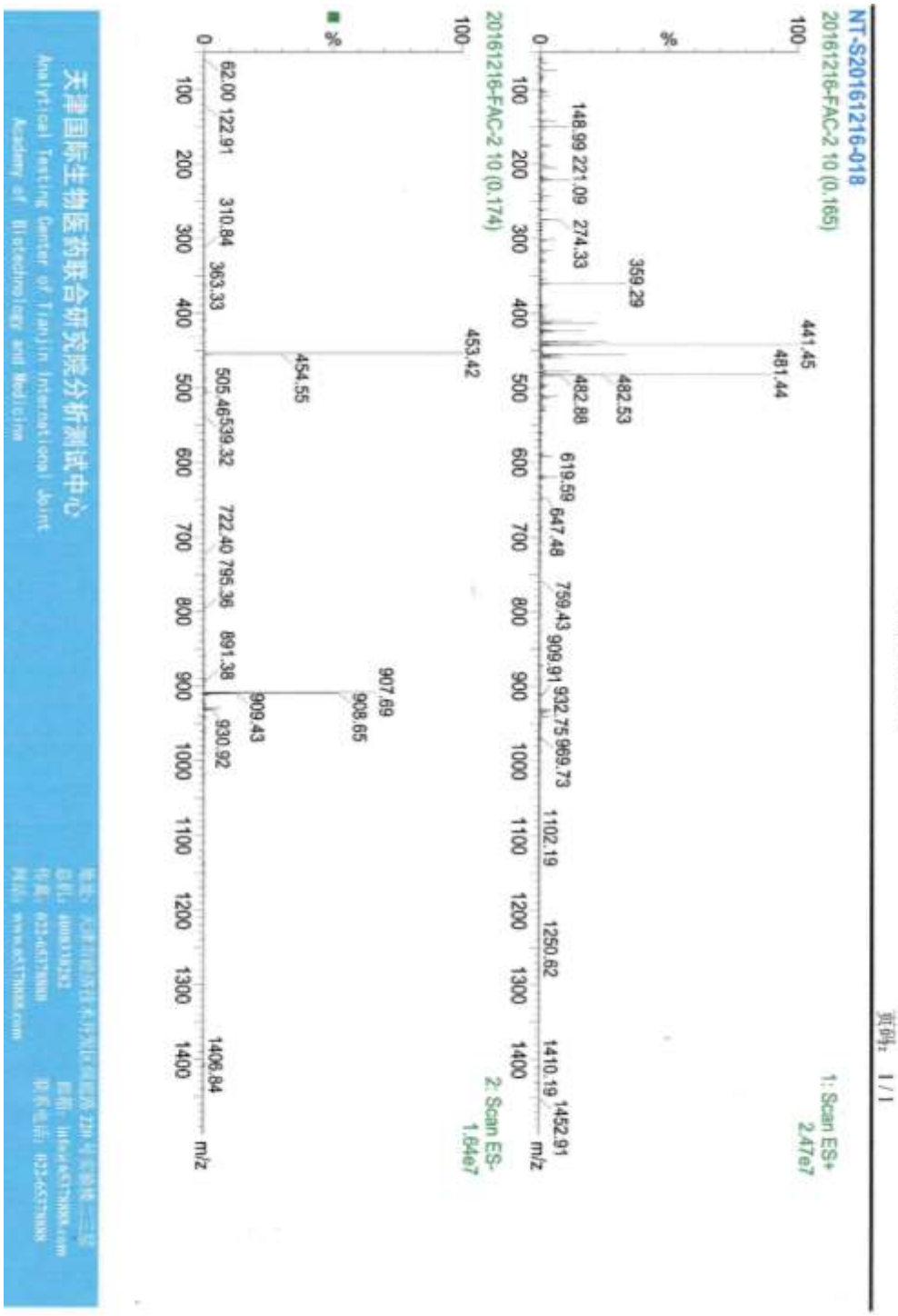
[Operation]
Threshold: 0.0010000
Points: 4
InterPolate: Disabled
Average: Disabled

[Sample Preparation Properties]
Weight:
Volume:
Dilution:
Path Length:
Additional Information:

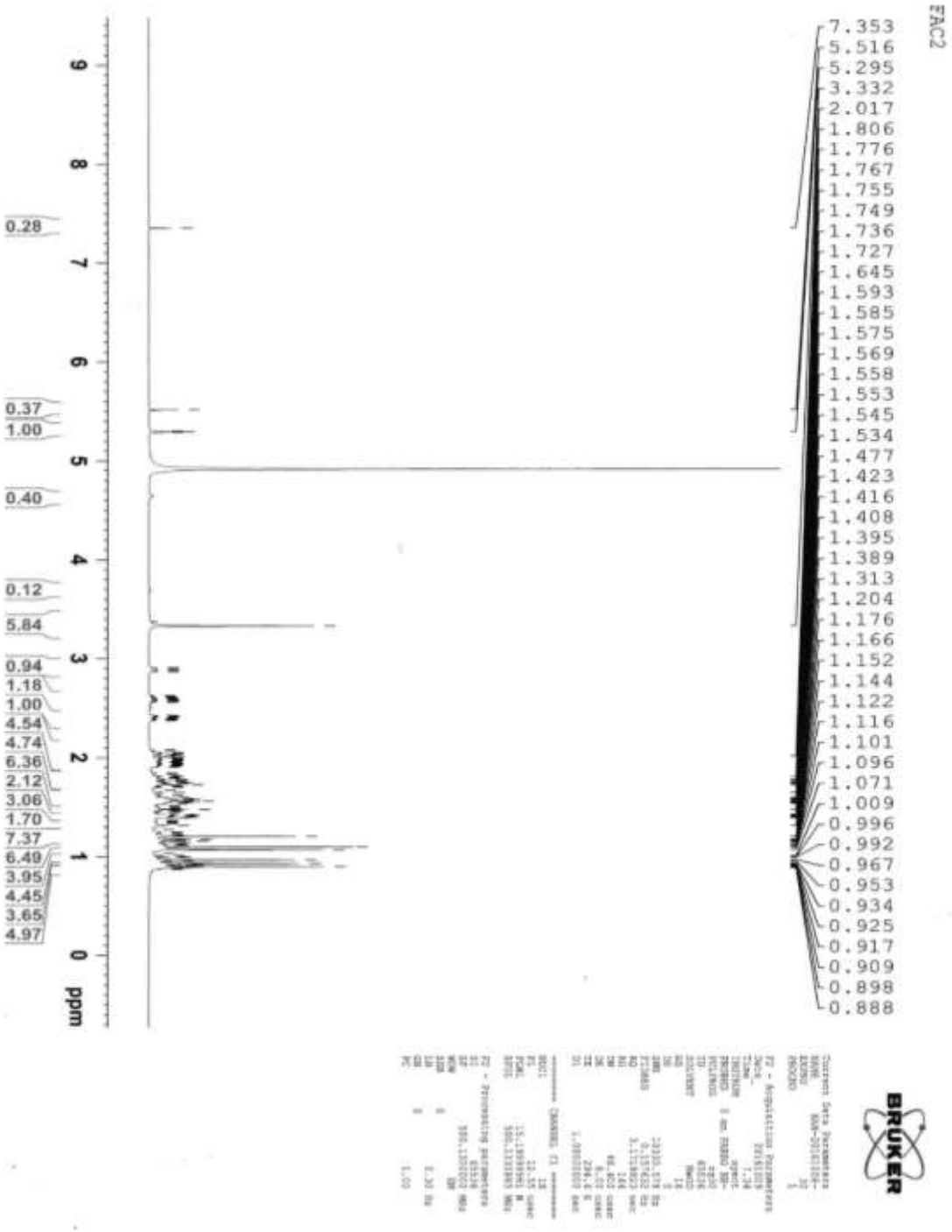
**Appendix 13: FTIR spectrum of compound 38**



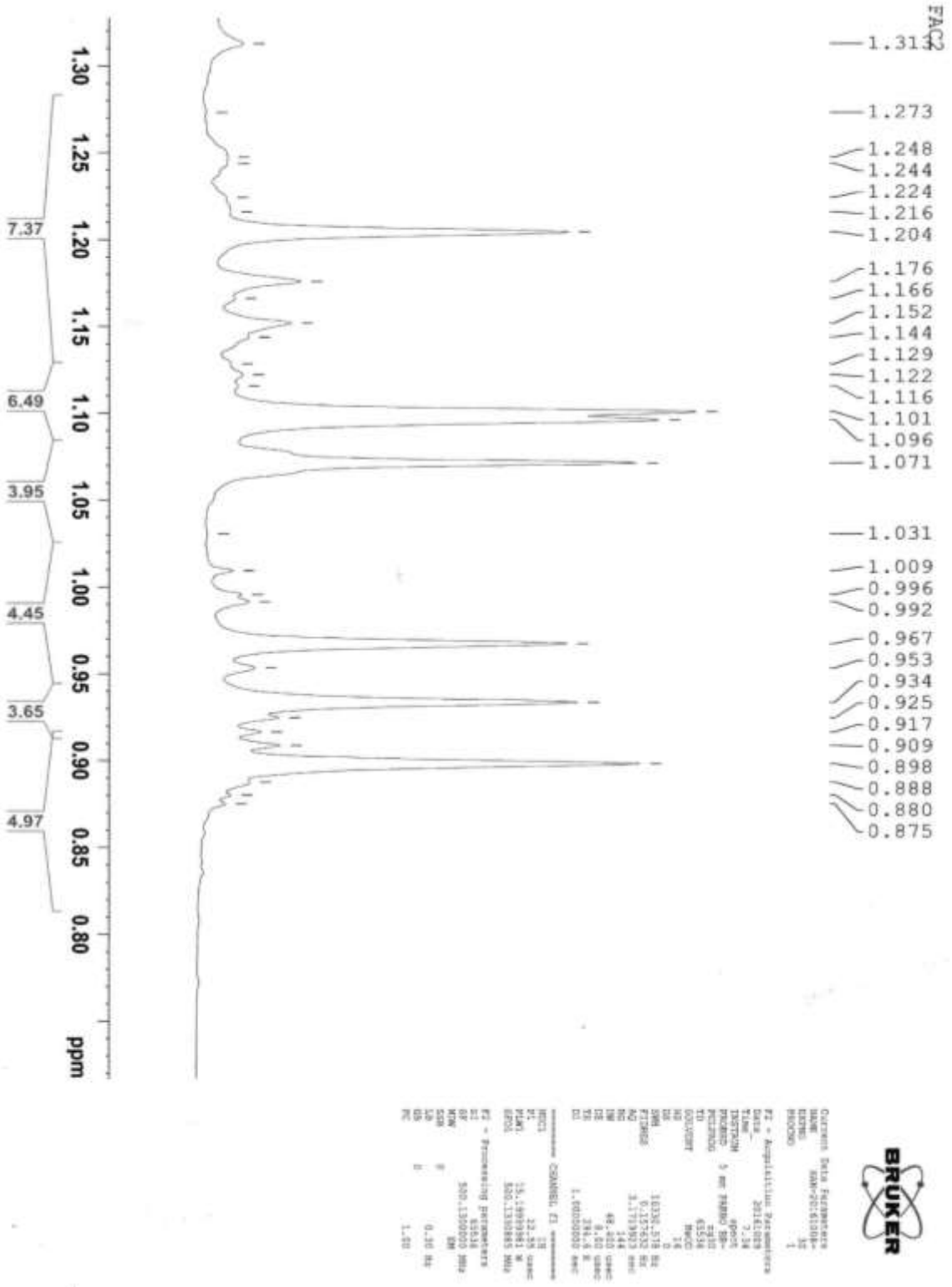
**Appendix 14: MS spectrum of compound 38**



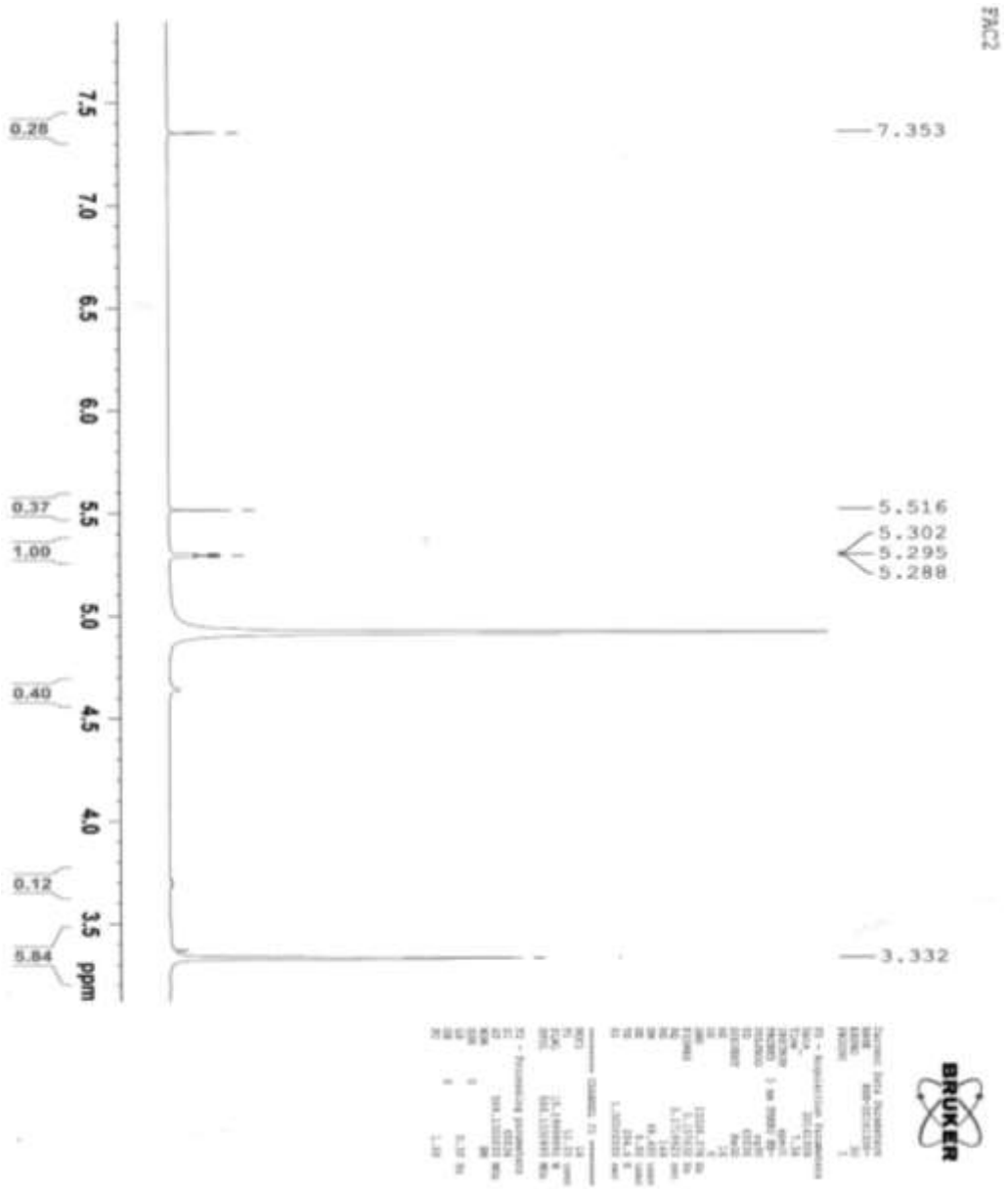
**Appendix 15: $^{1}$H NMR spectrum of compound 38 (δH 0.89 to 7.35)**



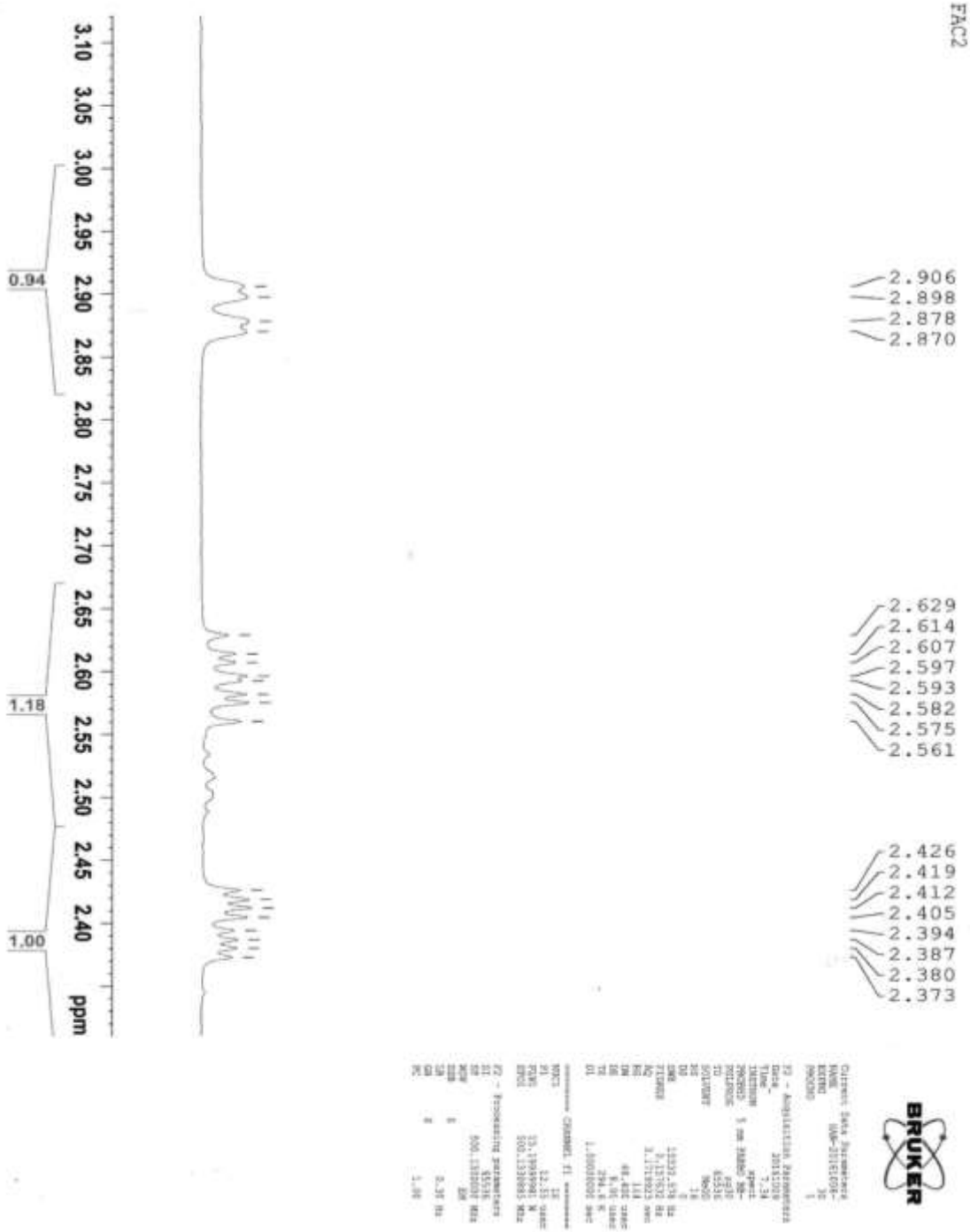
**Appendix 16: $^{1}$H NMR spectrum of compound 38 (Expanded δ H 0.88 to 1.31)**



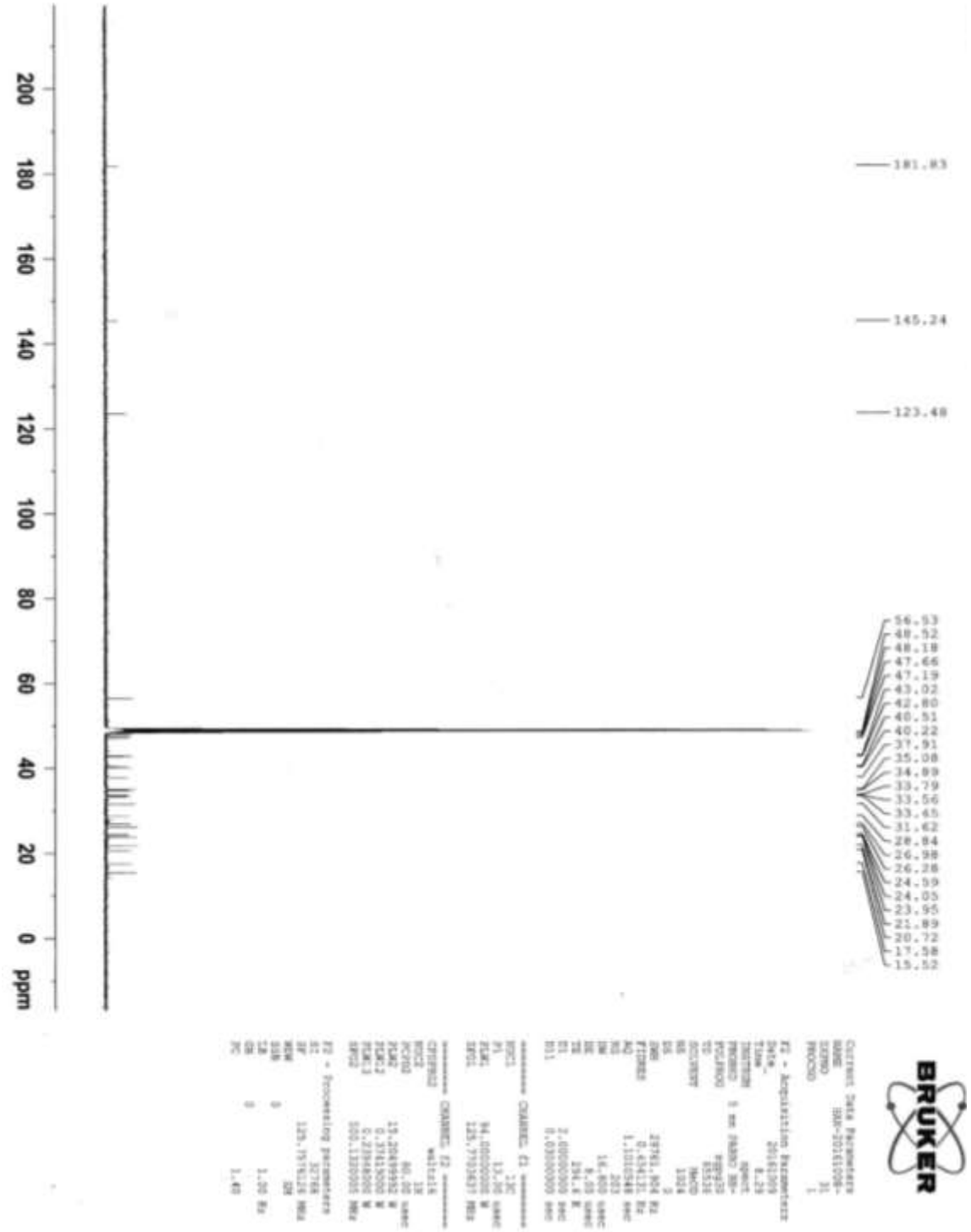
**Appendix 17: $^{1}$H NMR spectrum of compound 38 (Expanded δ H 3.33 to 7.35)**



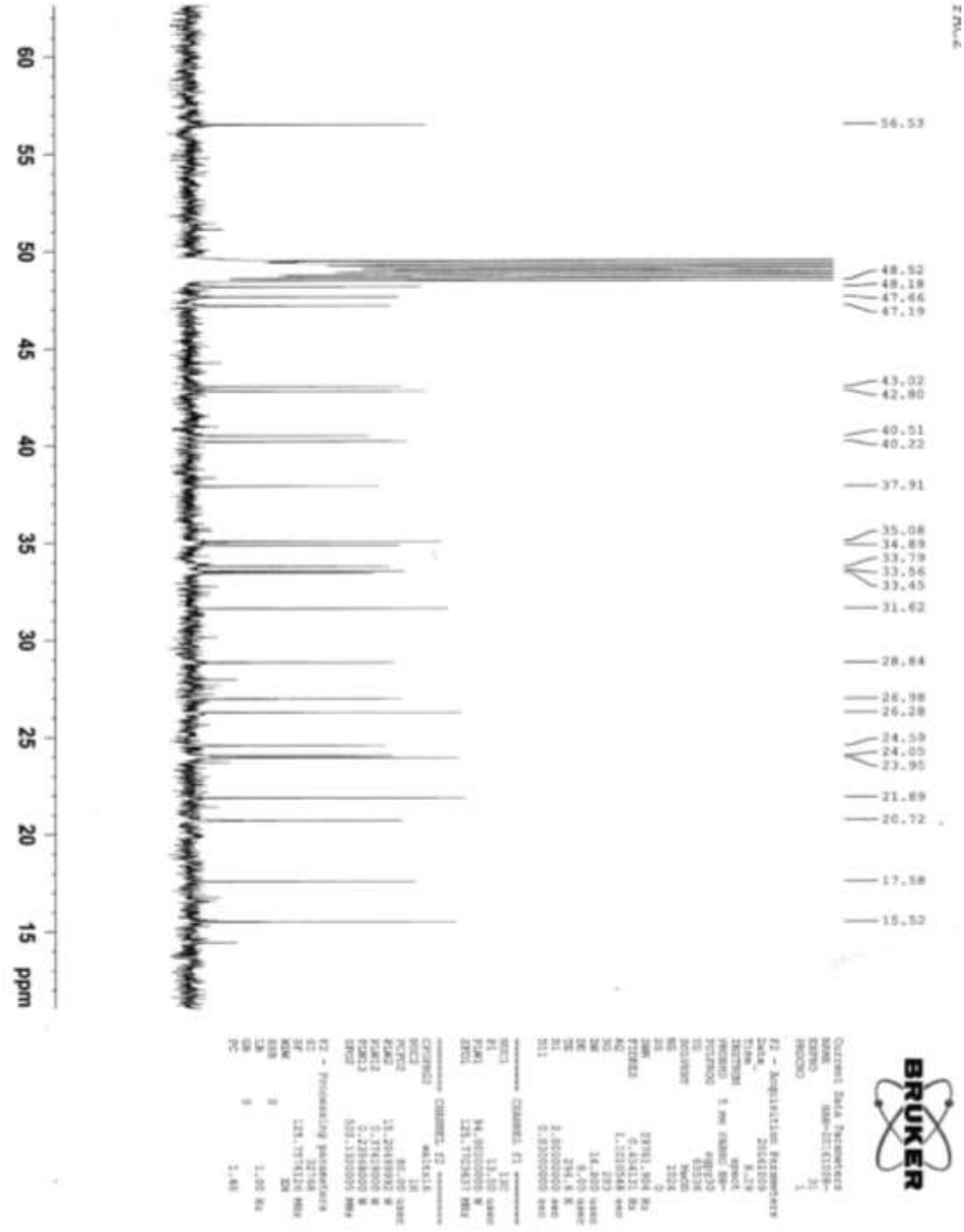
**Appendix 18: $^{1}$H NMR spectrum of compound 38 (Expanded δH 2.37 to 2.91)**



**Appendix 19: $^{13}$C NMR spectrum of compound 38 (δC 15.52 to 181.83)**



**Appendix 20: $^{13}$C NMR spectrum of compound 38 (Expanded δC 15.52 to 56.53)**

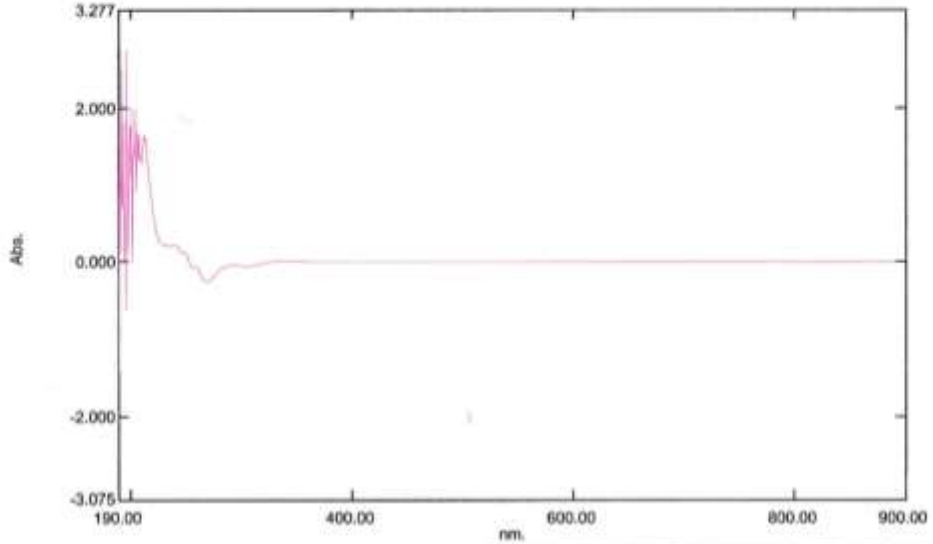


**Appendix 21: UV spectrum of compound 39**

## Spectrum Peak Pick Report

06/22/2017 12:20:14 PM

Data Set: 8 - RawData




| No. | P/V | Wavelength | Abs. | Description |
|---|---|---|---|---|
| 1 | P | 340.00 | 0.014 | |
| 2 | P | 293.50 | -0.035 | |
| 3 | P | 240.50 | 0.222 | |
| 4 | V | 418.50 | 0.002 | |
| 5 | V | 307.00 | -0.056 | |
| 6 | V | 268.50 | -0.257 | |
| 7 | V | 237.00 | 0.208 | |

[Measurement Properties]
Wavelength Range (nm.): 190.00 to 900.00
Scan Speed: Fast
Sampling Interval: 0.5
Auto Sampling Interval: Enabled
Scan Mode: Single

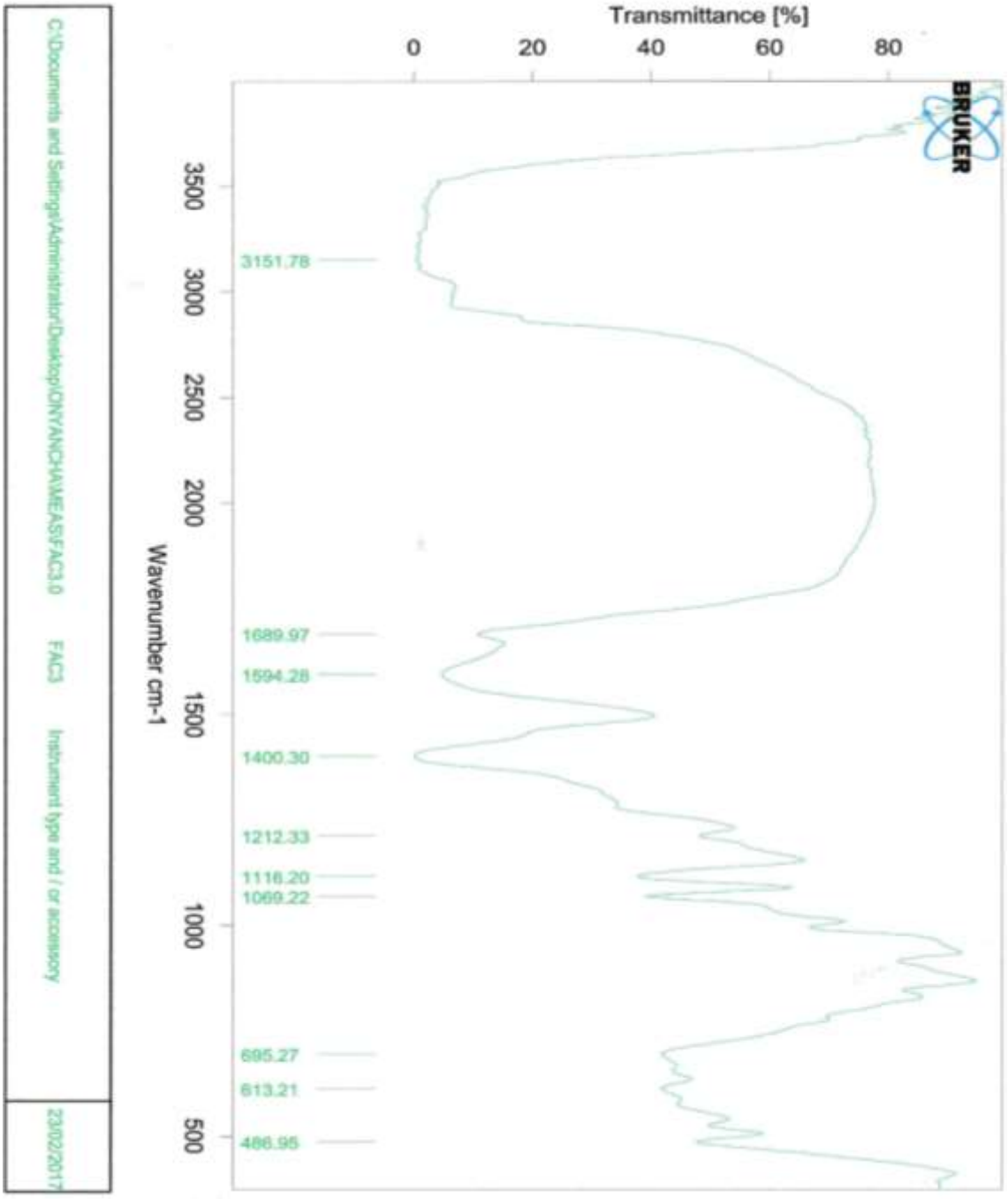
[Instrument Properties]
Instrument Type: UV-1800 Series
Measuring Mode: Absorbance
Slit Width: 1.0 nm
Light Source Change Wavelength: 340.0 nm
S/R Exchange: Normal

[Attachment Properties]
Attachment: None

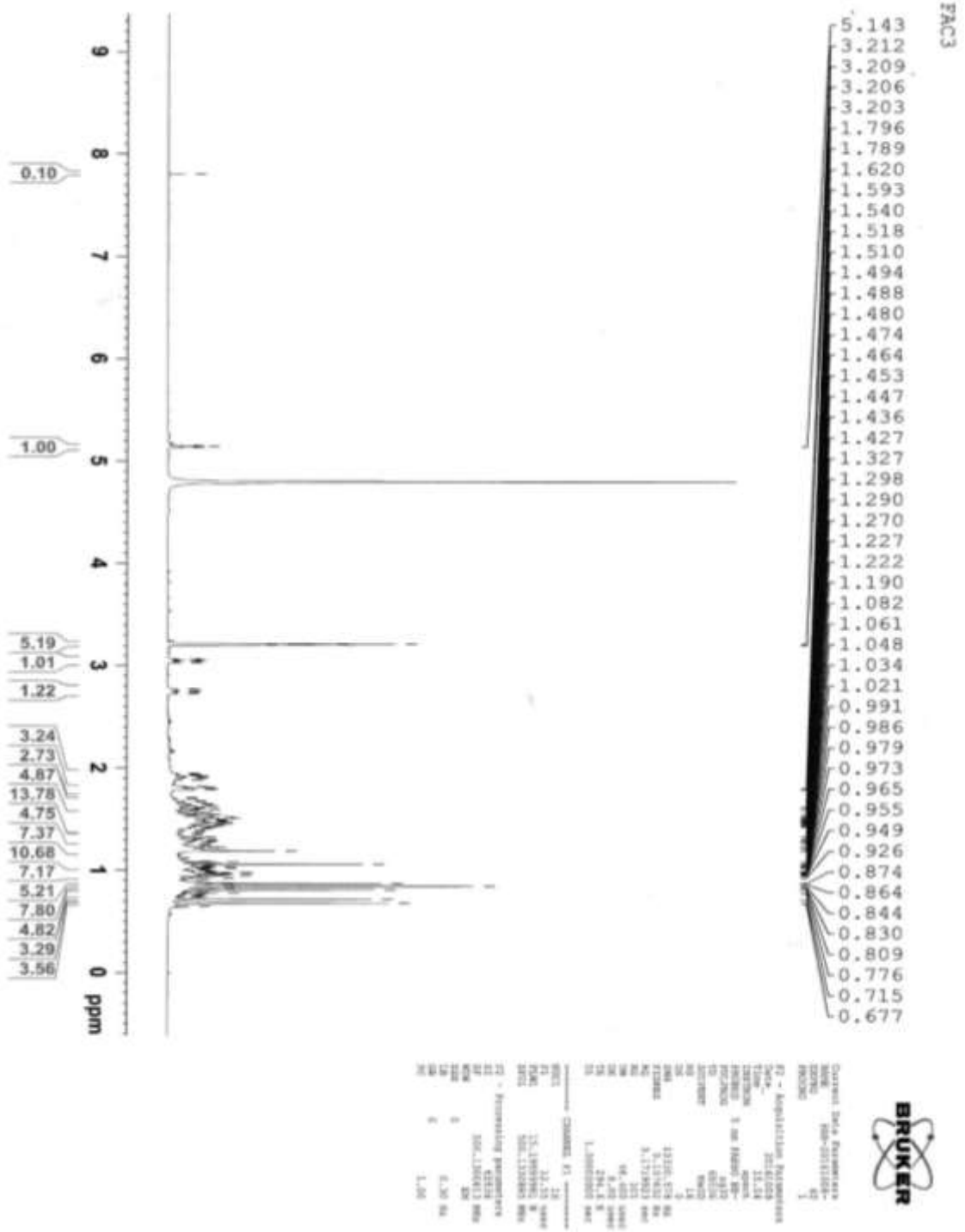
[Operation]
Threshold: 0.0010000
Points: 4
InterPolate: Disabled
Average: Disabled

[Sample Preparation Properties]
Weight:
Volume:
Dilution:
Path Length:
Additional Information:

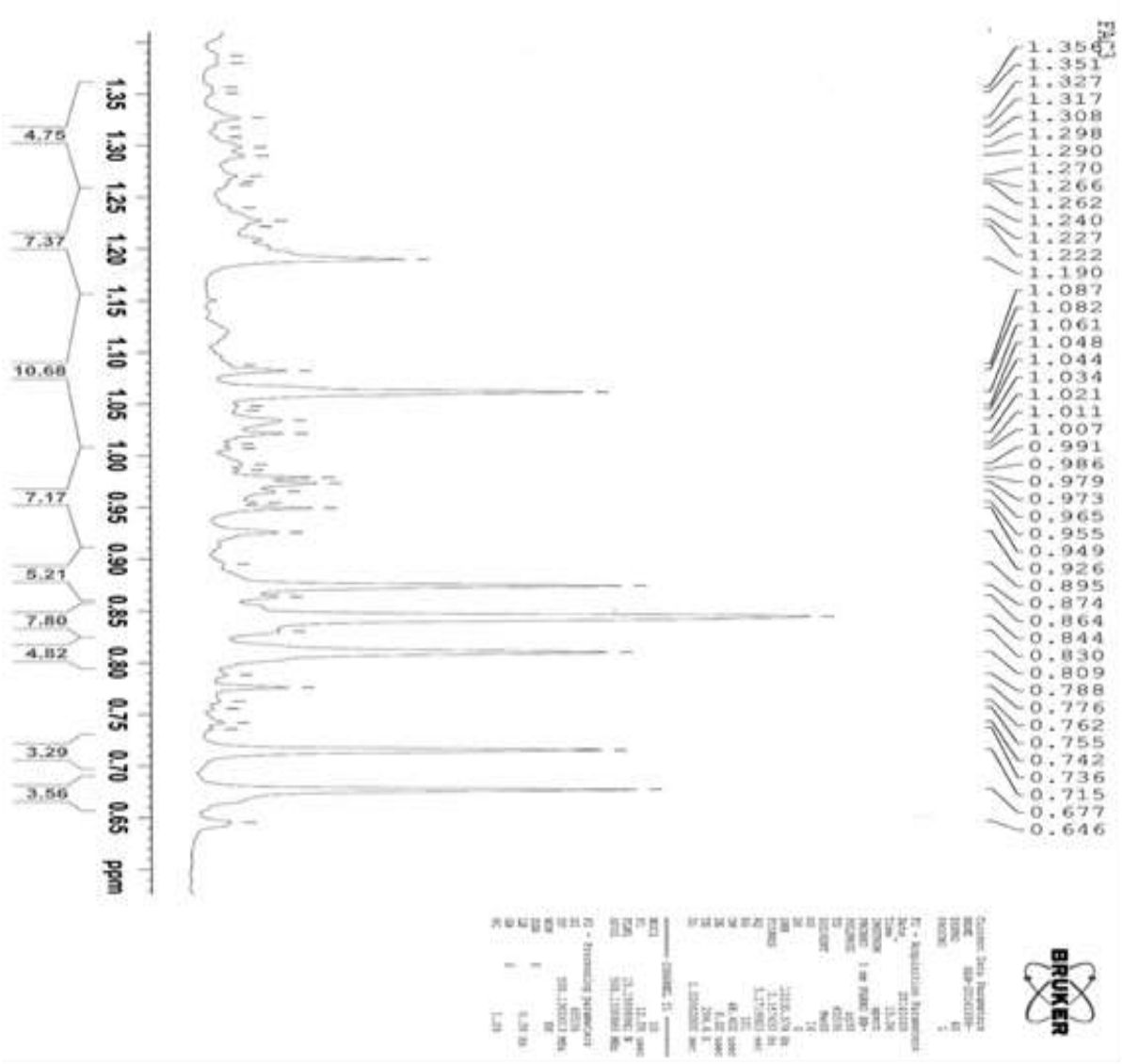
**Appendix 22: FTIR spectrum of compound 39**



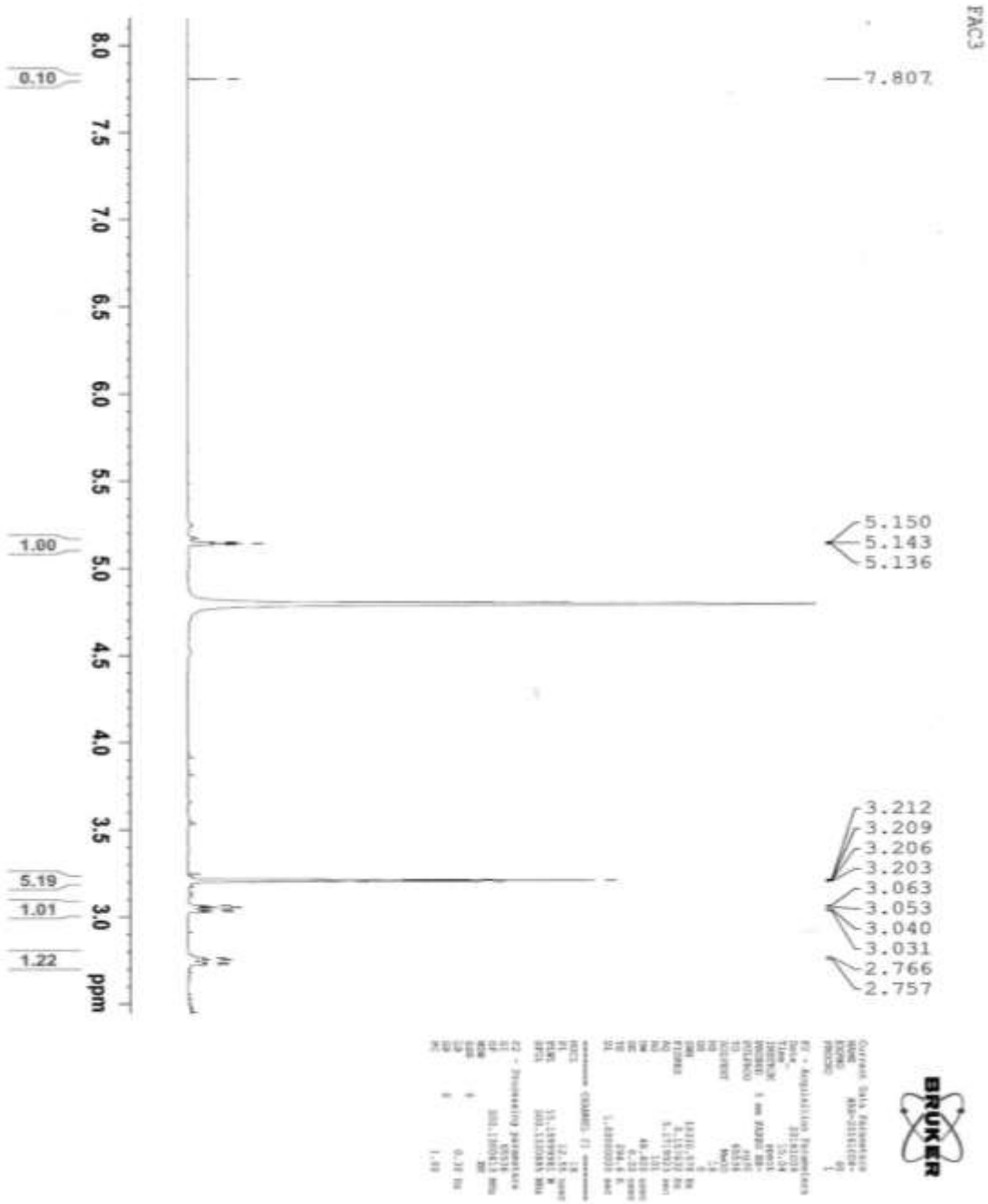
**Appendix 23: $^{1}$H NMR spectrum of compound 39 (δH 0.68 to 5.14)**



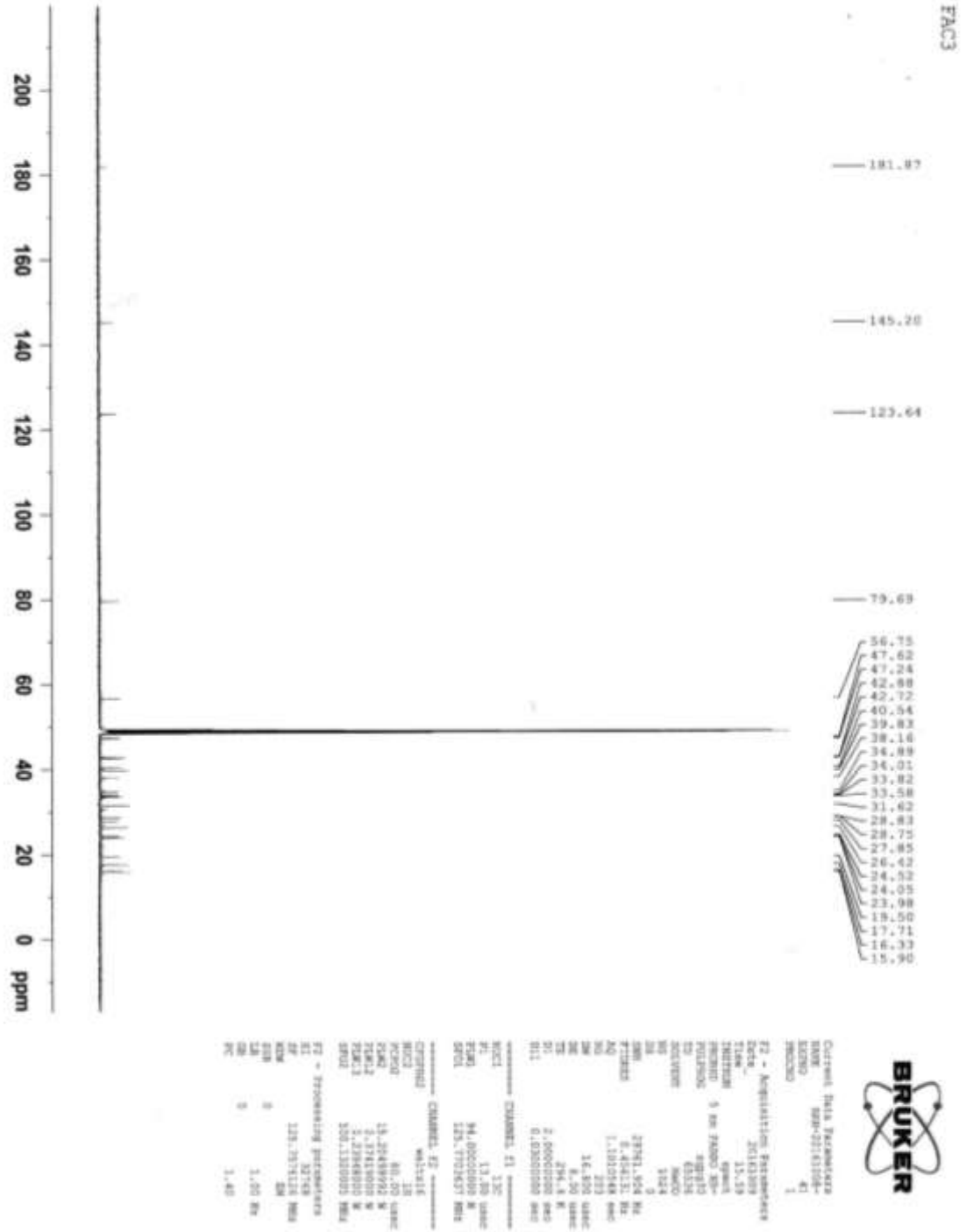
**Appendix 24: $^{1}$H NMR spectrum of compound 39 (Expanded δH 0.65 to 1.36)**



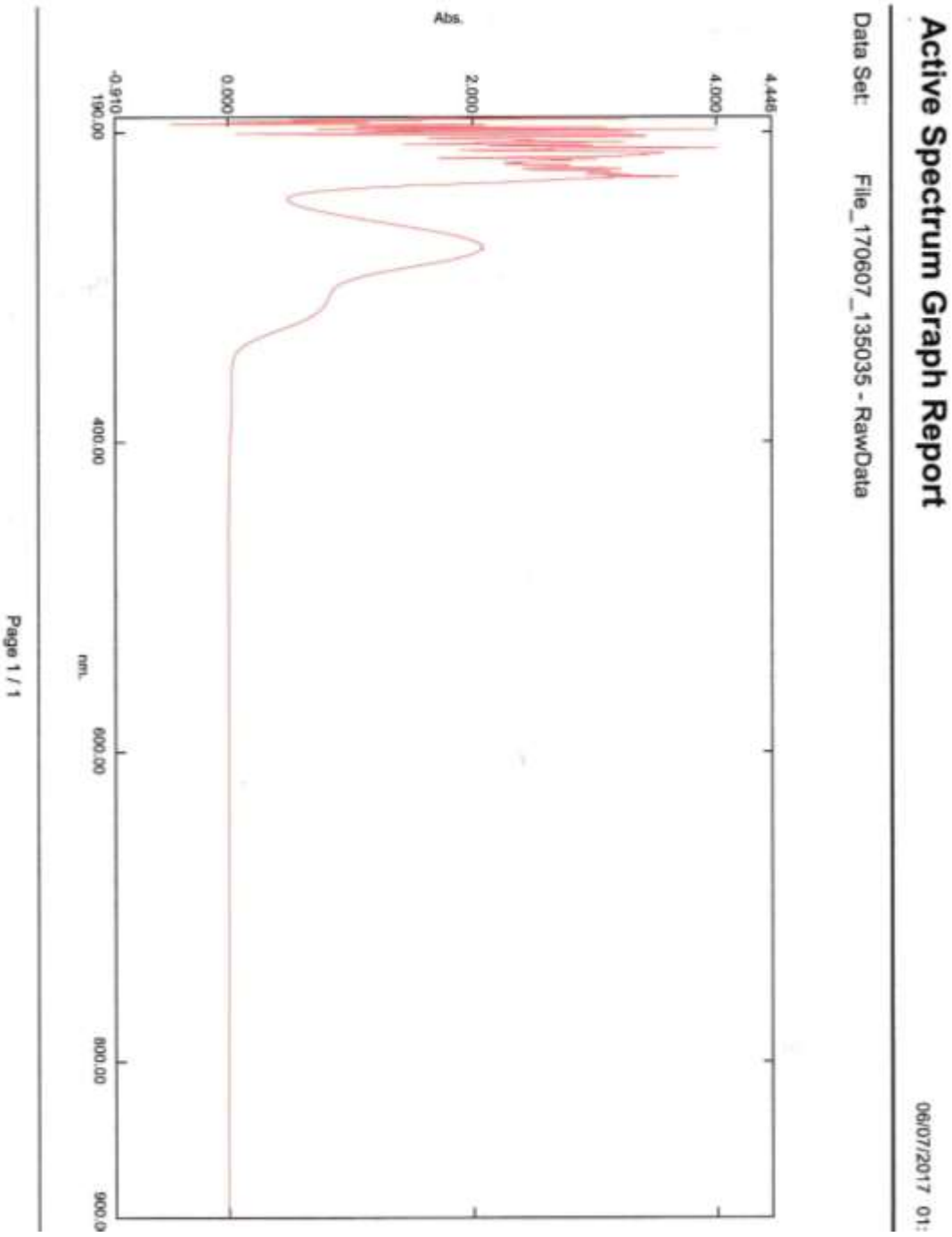
**Appendix 25: $^{1}$H NMR spectrum of compound 39 (Expanded δH 2.76 to 7.81)**



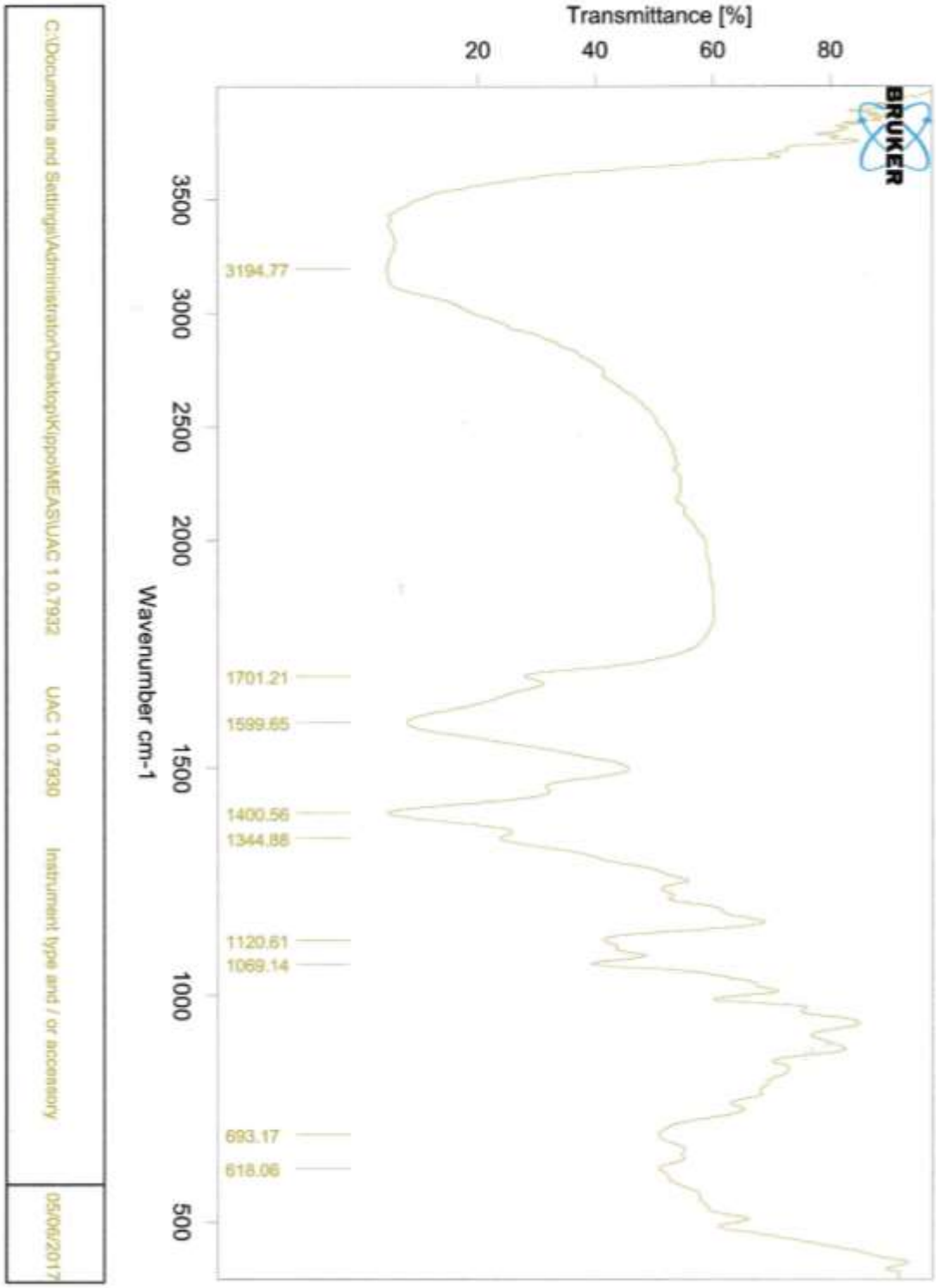
**Appendix 26: $^{13}$C NMR spectrum of compound 39 (δC 15.90 to 181.87)**



**Appendix 27: UV spectrum of compound 40**

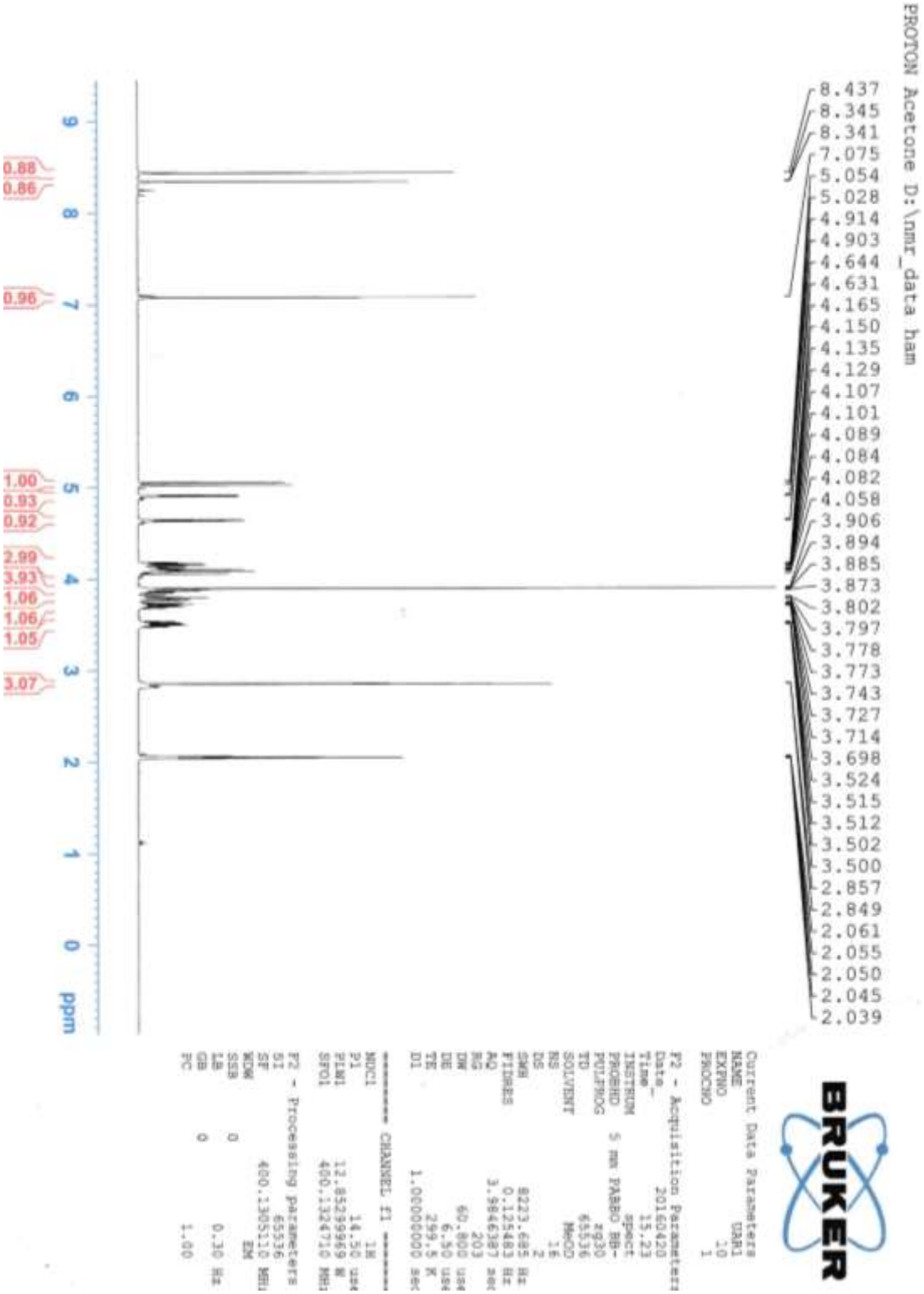


**Appendix 28: FTIR spectrum of compound 40**

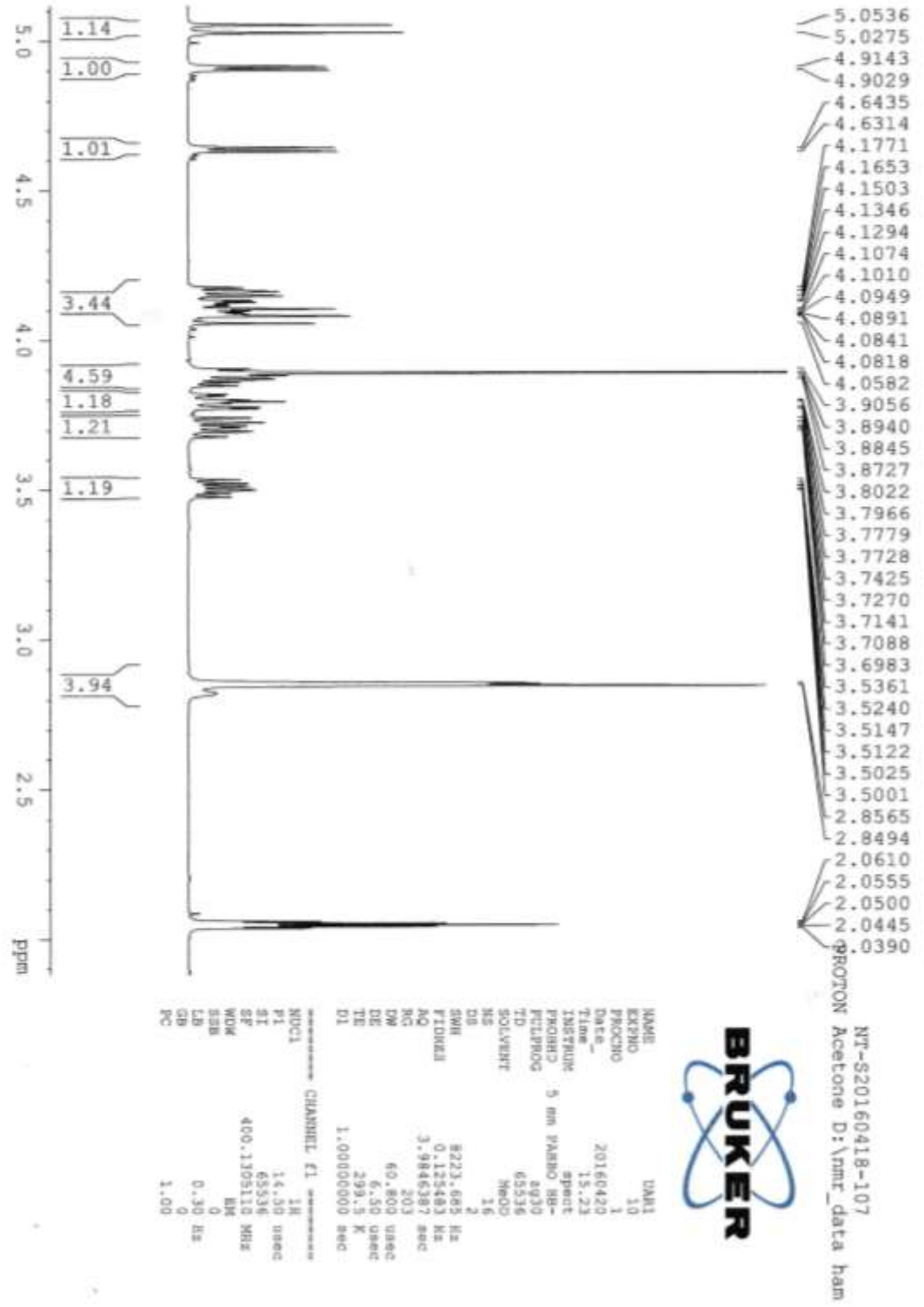


**Appendix 29: MS spectrum of compound 40**

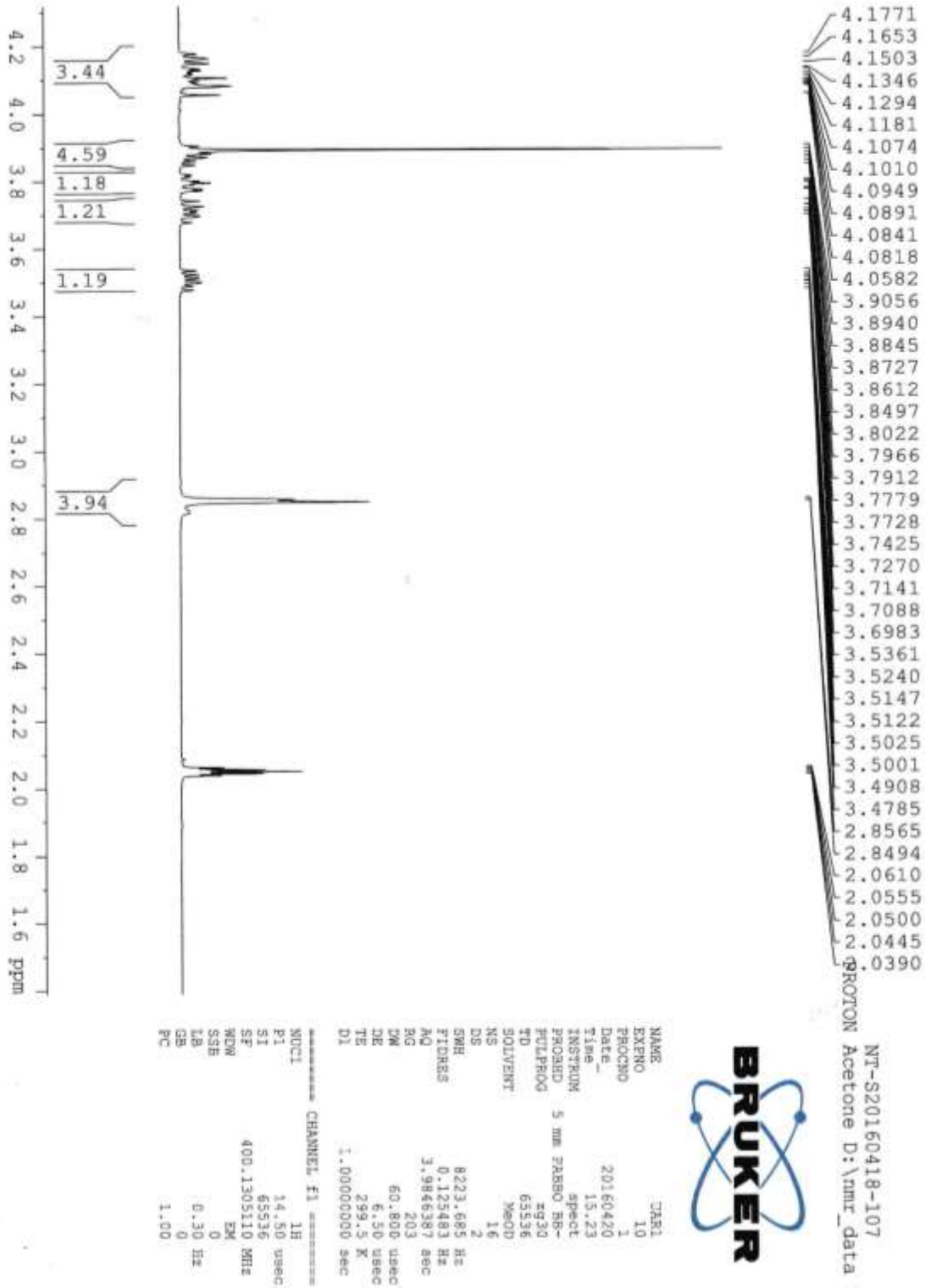
**Appendix 30: $^{1}$H NMR spectrum of compound 40 (δ H 2.04 to 8.44)**



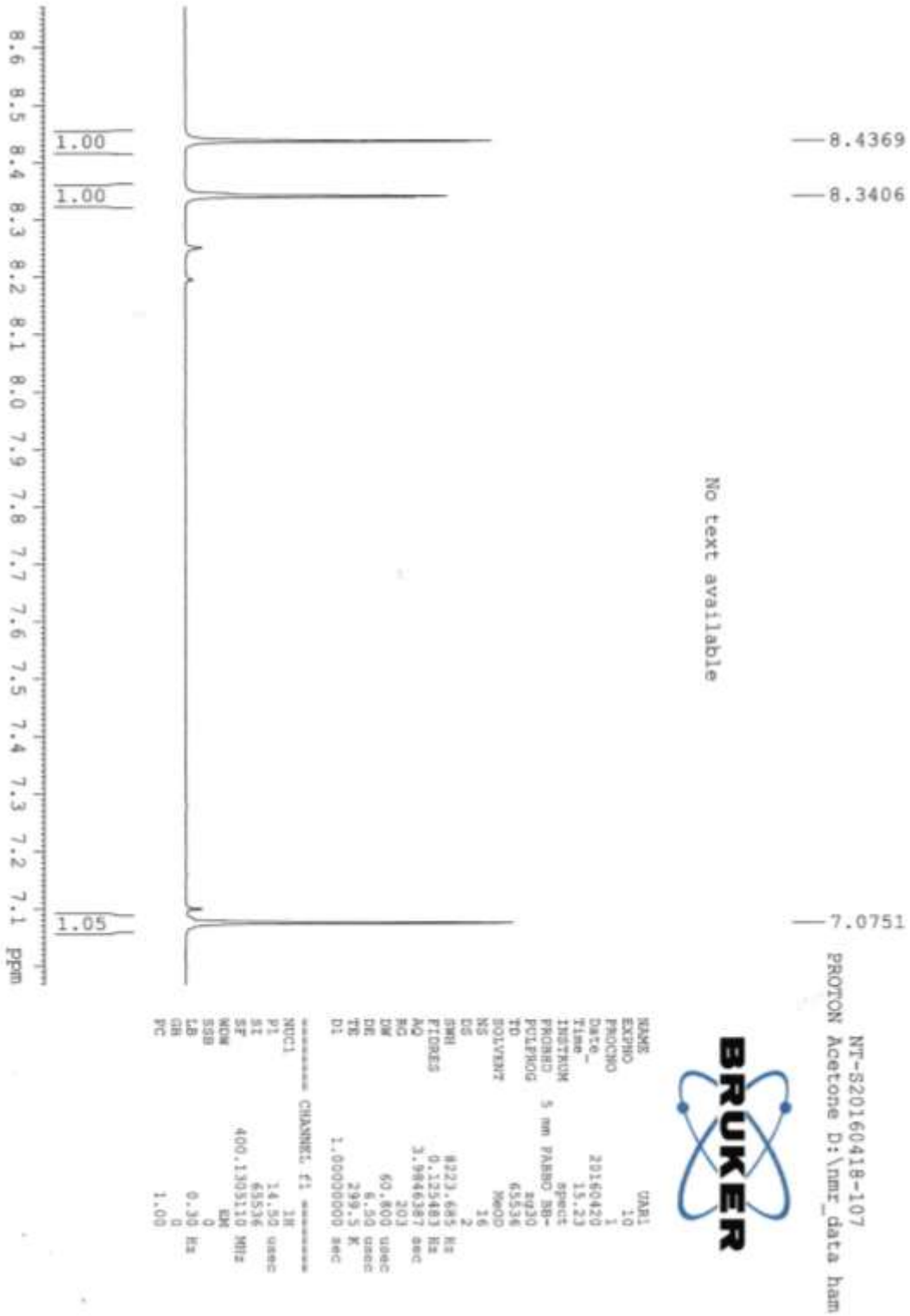
**Appendix 31: $^{1}$H NMR spectrum of compound 40 (Expanded δ H 2.04 to 5.05)**



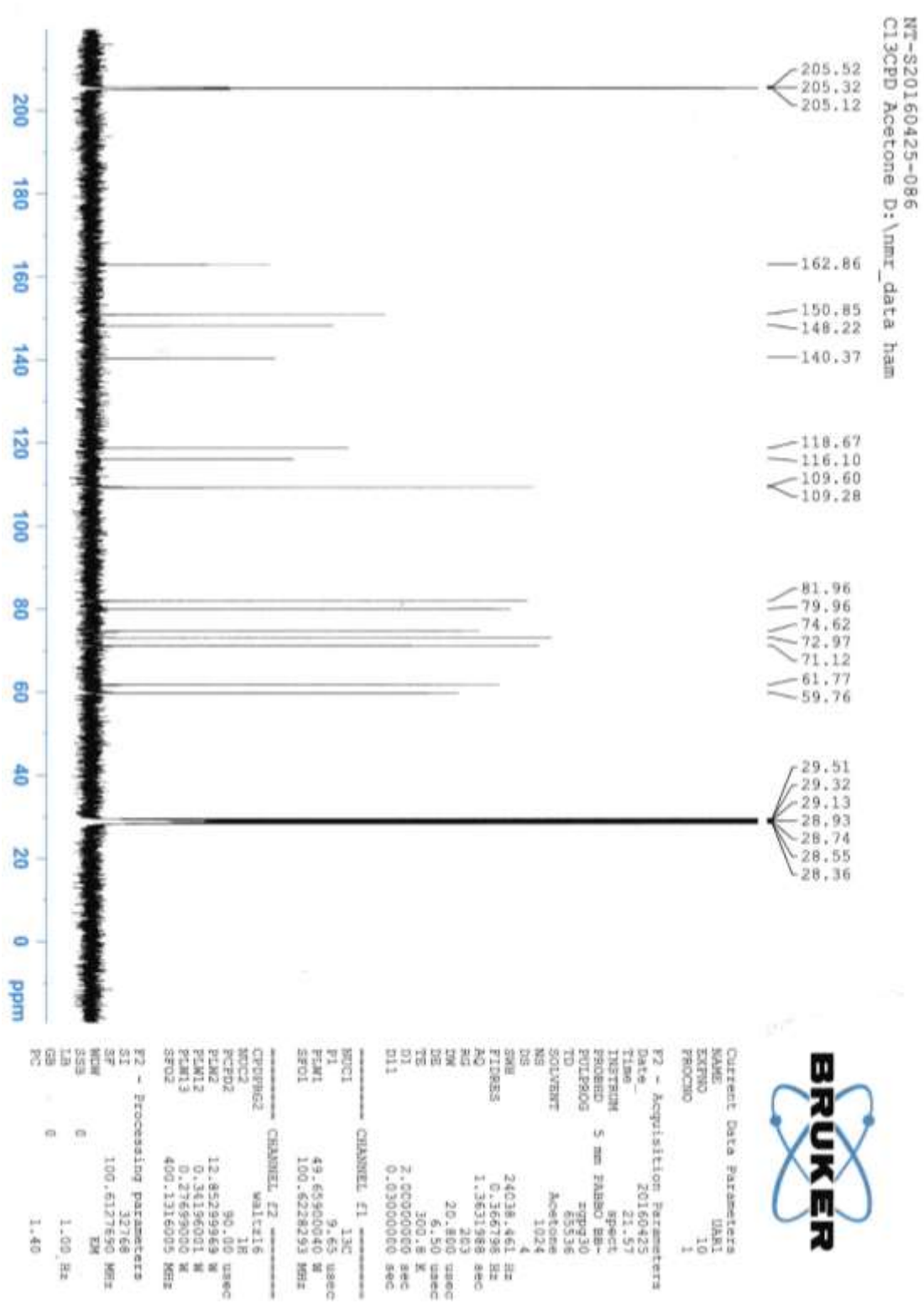
**Appendix 32: $^{1}$H NMR spectrum of compound 40 (Expanded δ H 2.04 to 4.18)**



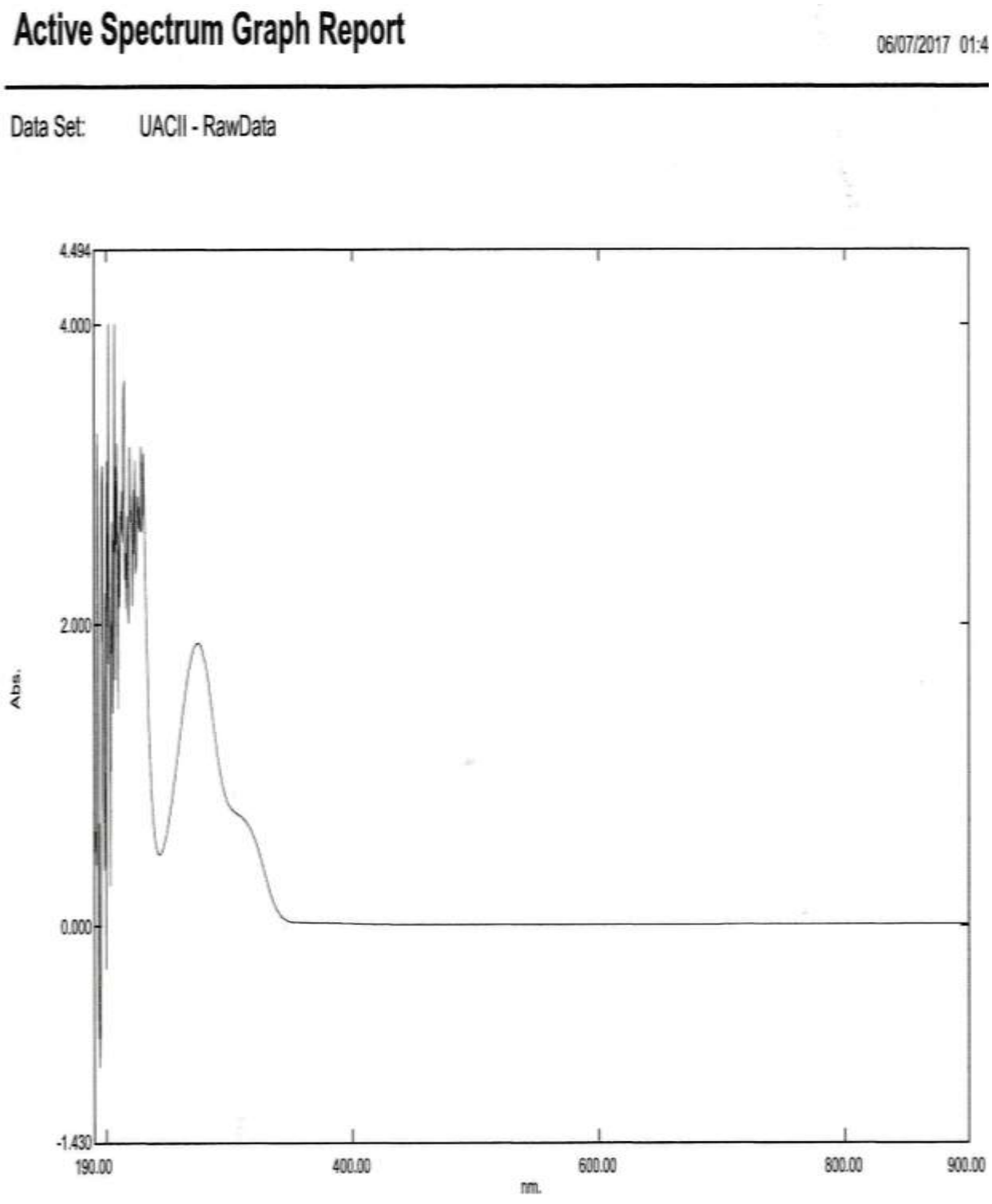
**Appendix 33: $^{1}$H NMR spectrum of compound 40 (Expanded δ H 7.08 to 8.44)**



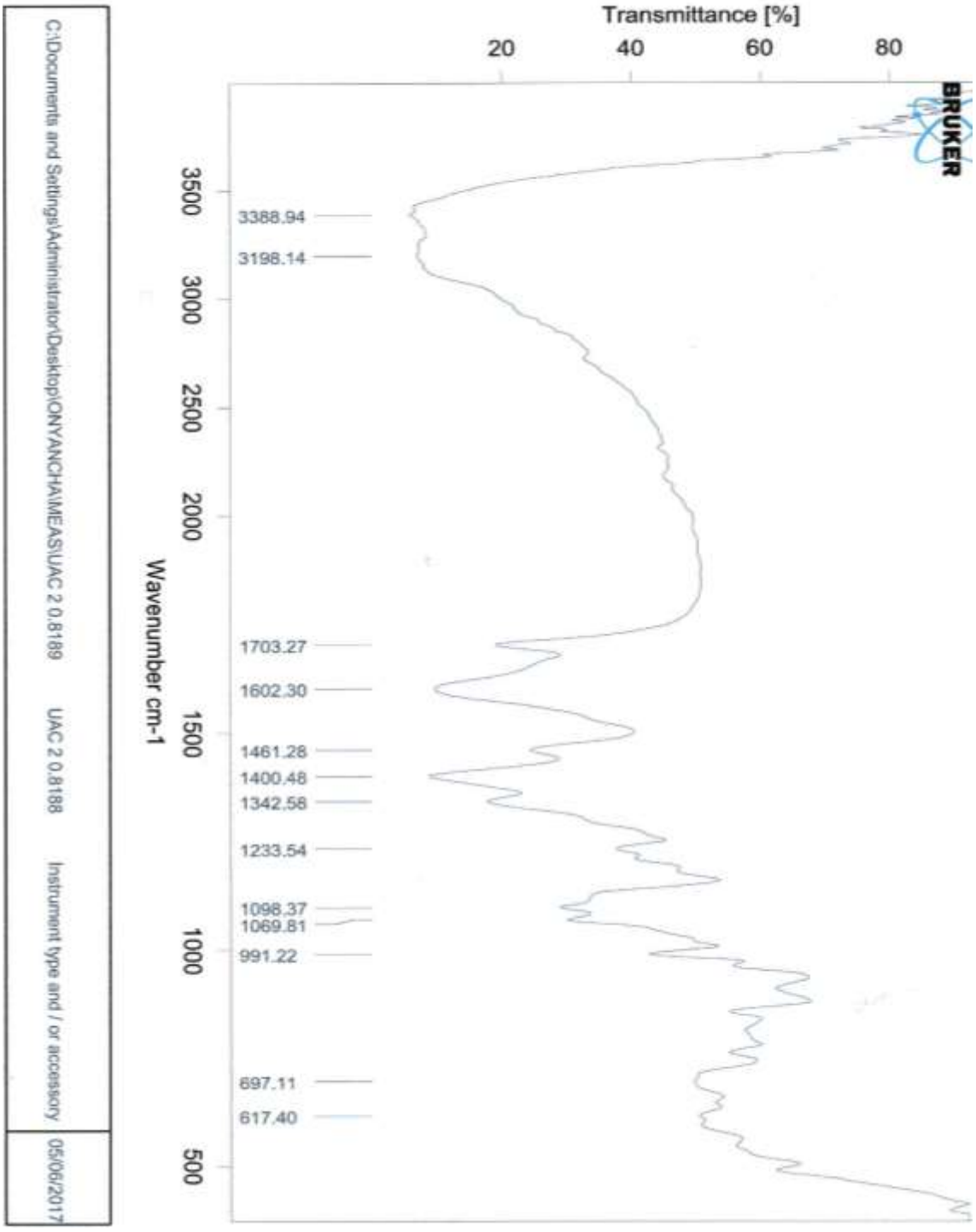
**Appendix 34: $^{13}$C NMR spectrum of compound 40 (δC 28.36 to 162.52)**



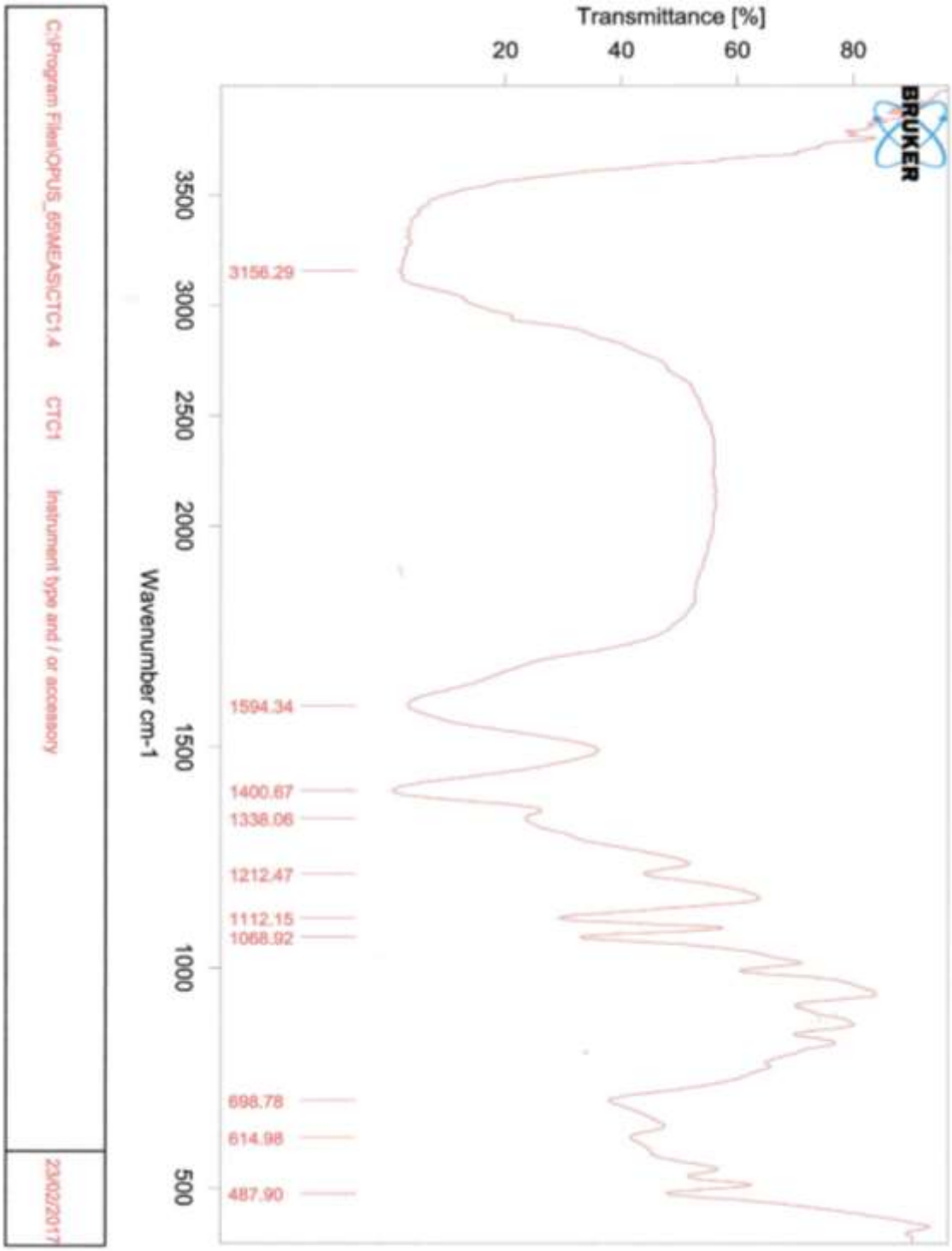
**Appendix 35: UV spectrum of compound 41**



**Appendix 36: FTIR spectrum of compound 41**

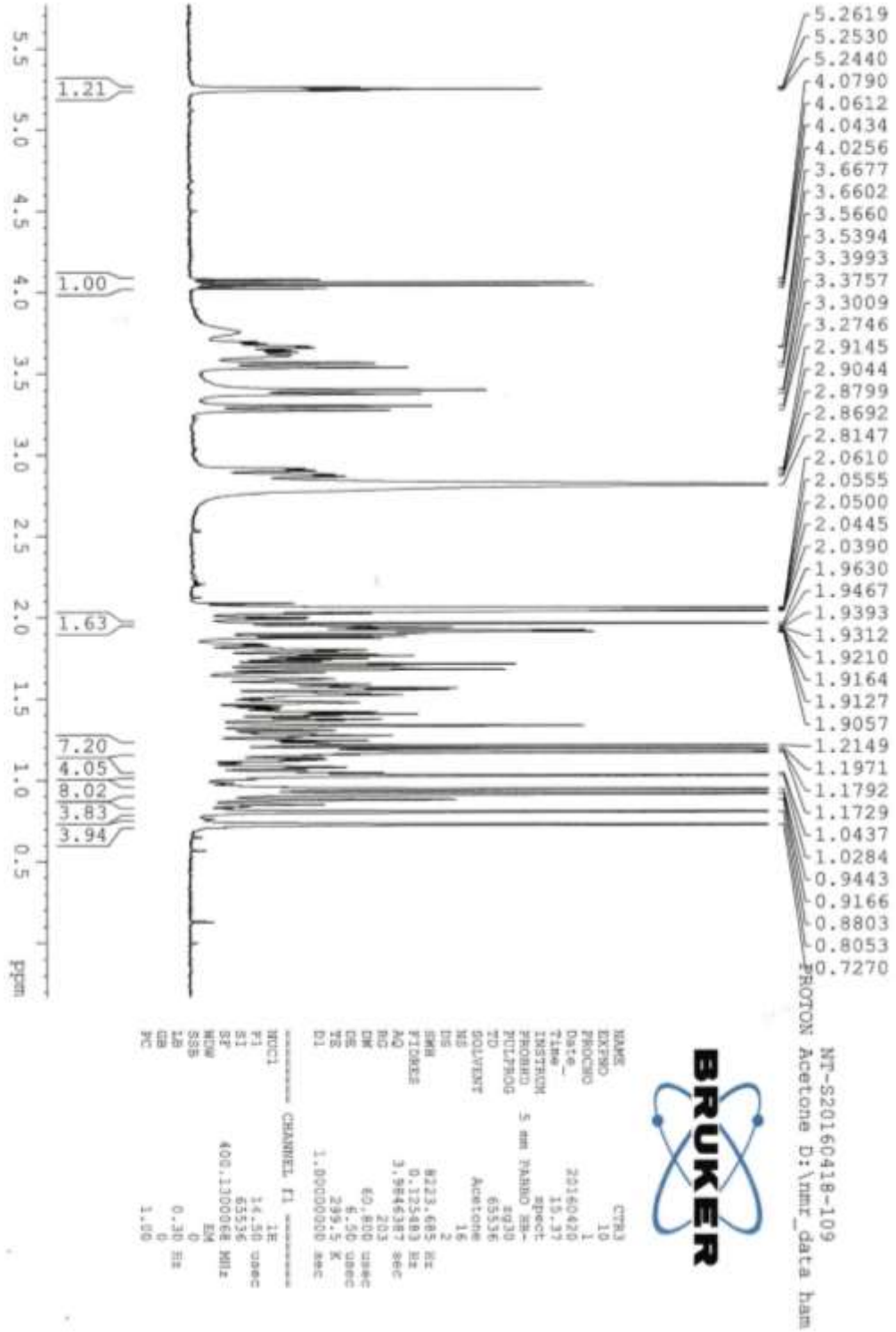


**Appendix 37: FTIR spectrum of compound 42**

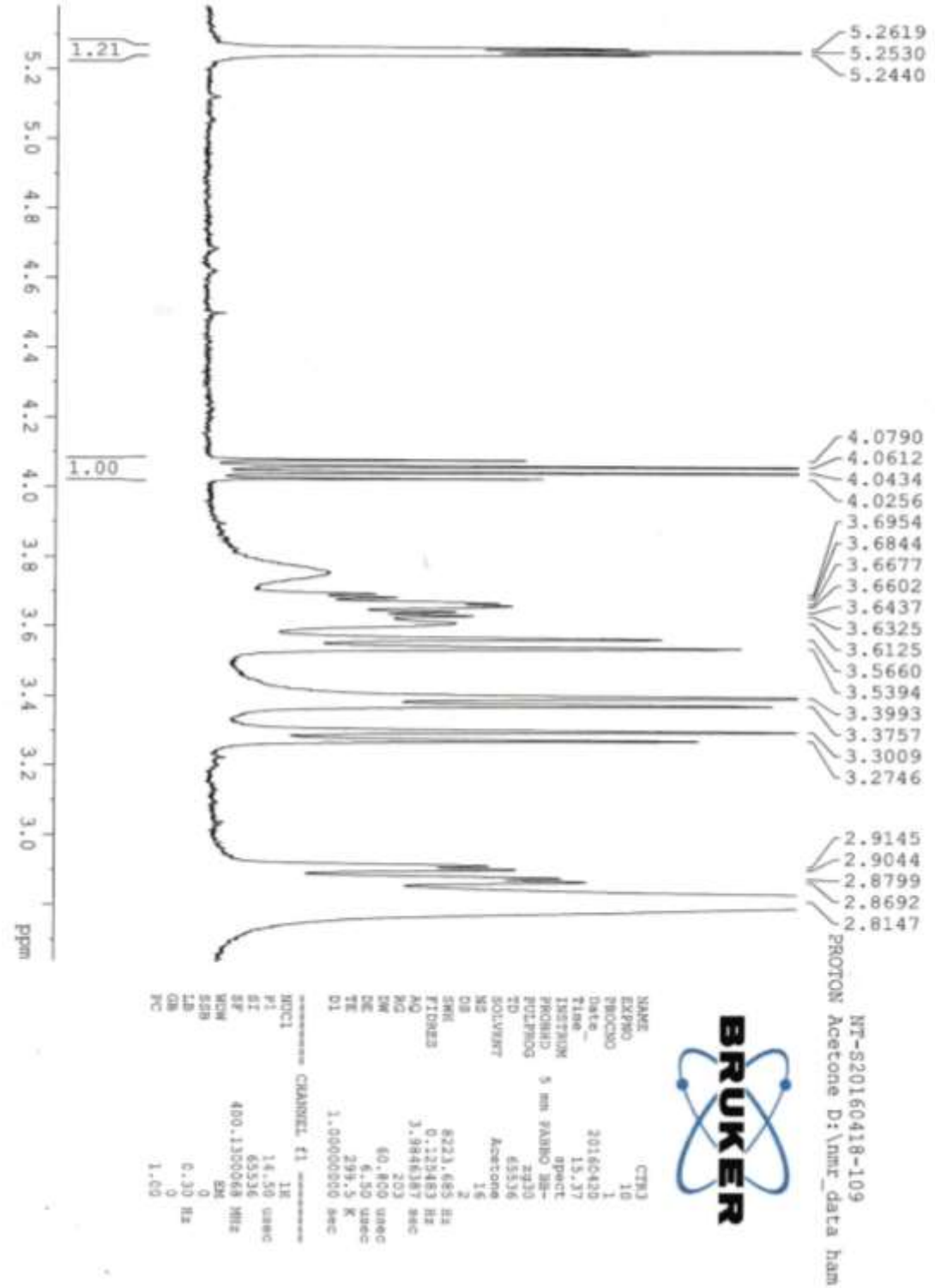


**Appendix 38: MS spectrum of compound 42**

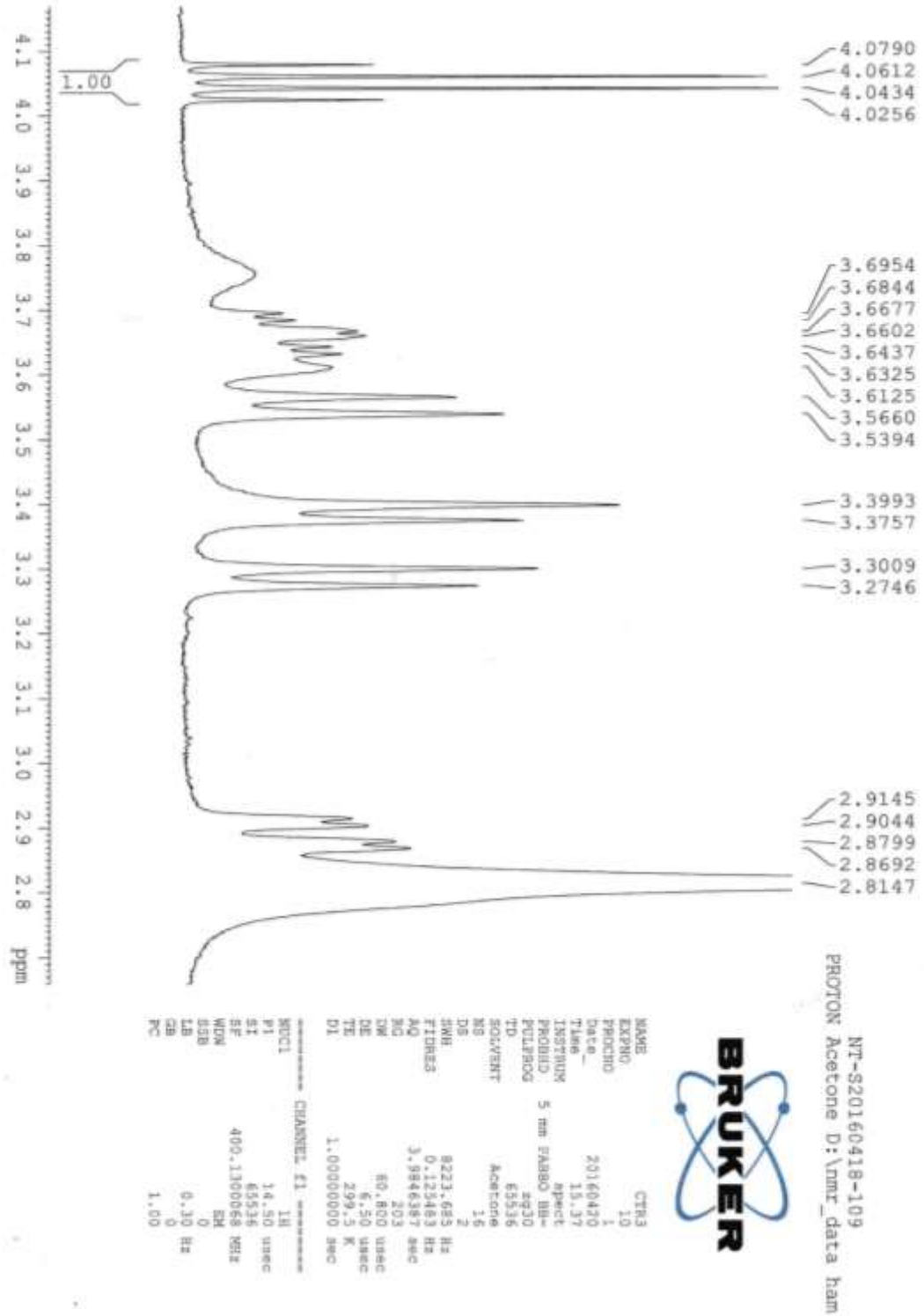
**Appendix 39: $^{1}$H NMR spectrum of compound 42 (δH 0.73 to 5.26)**



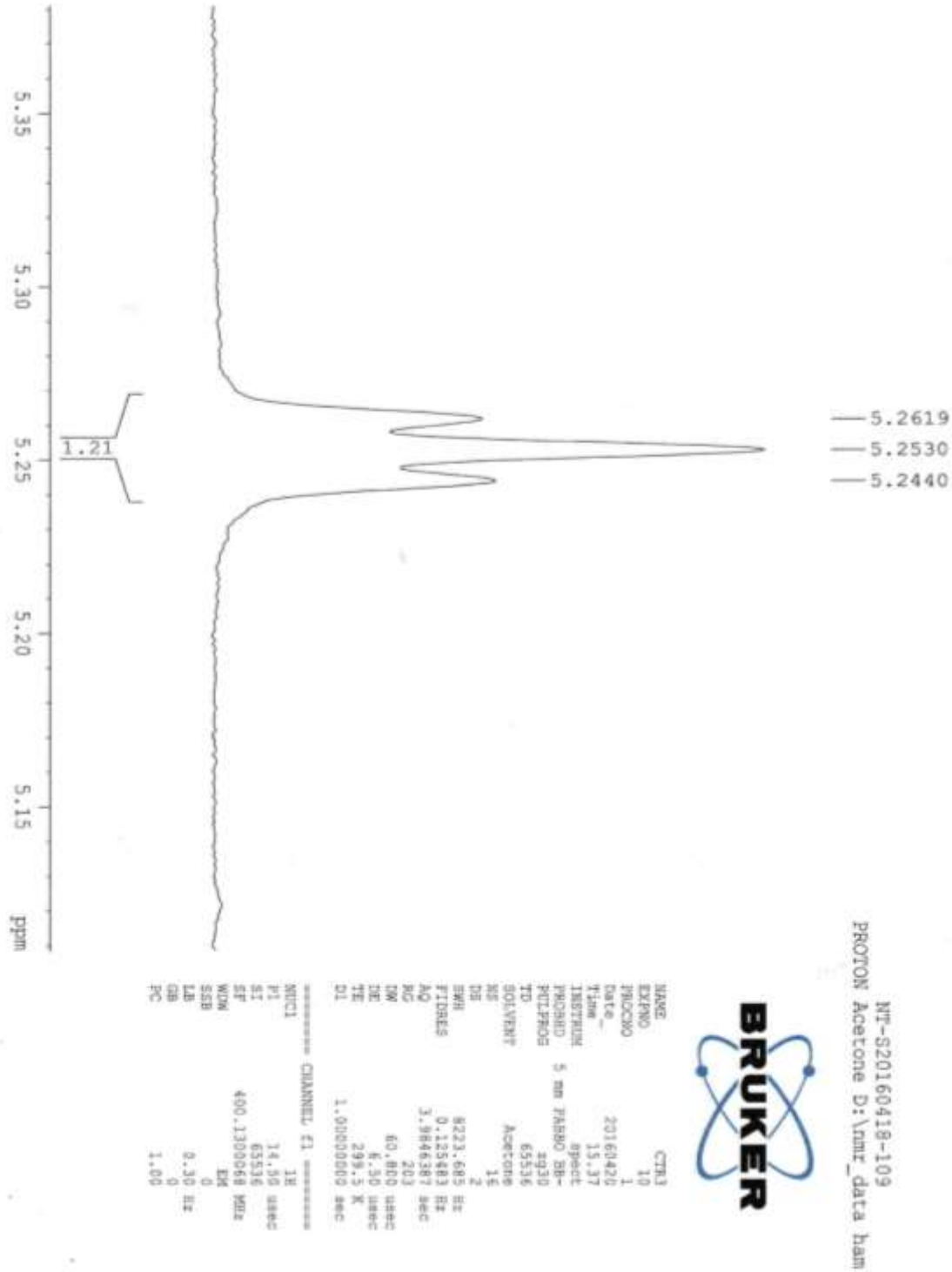
**Appendix 40: $^{1}$H NMR spectrum of compound 42 (Expanded δH 2.81 to 5.26)**



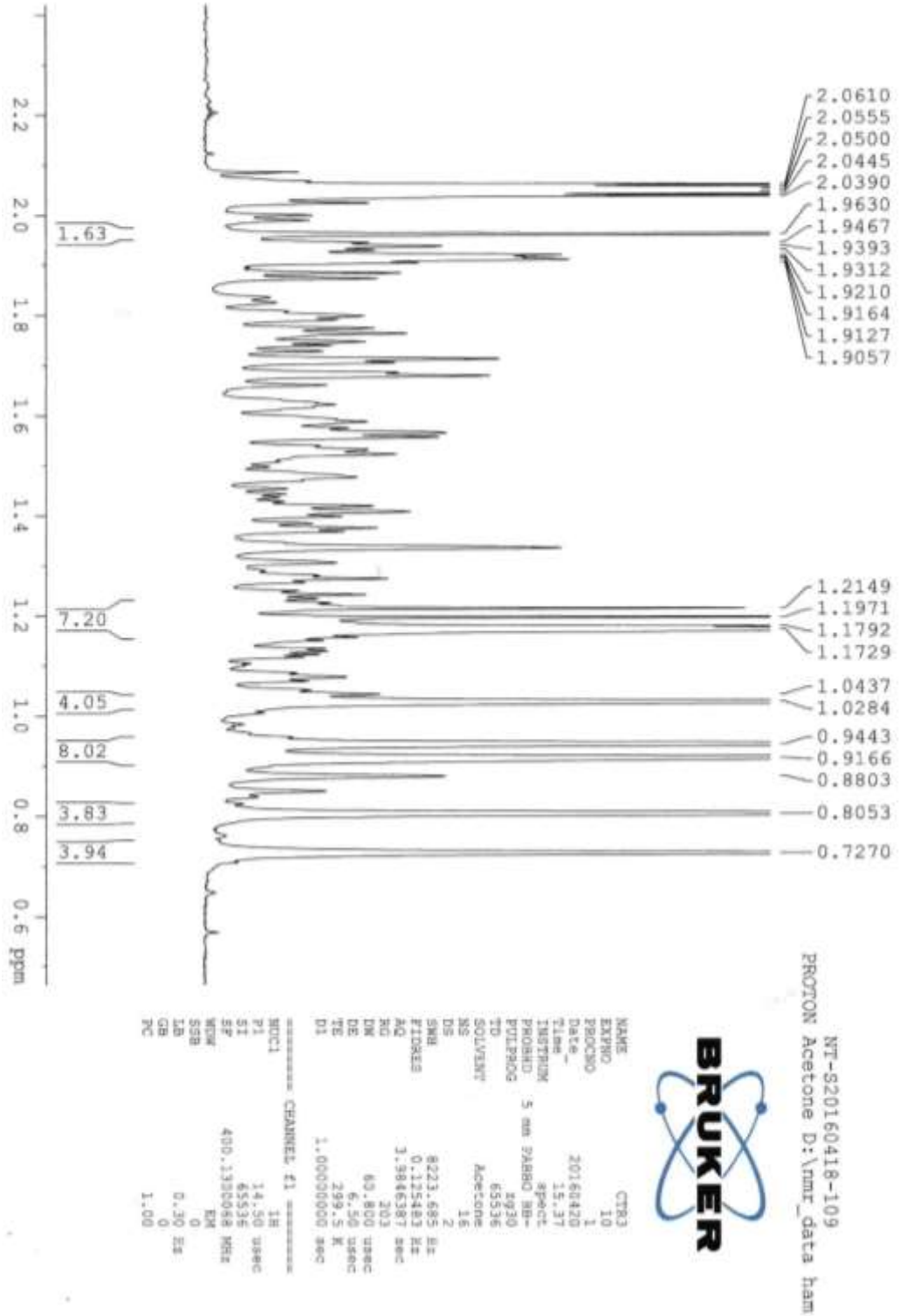
**Appendix 41: $^{1}$H NMR spectrum of compound 42 (Expanded δ H 2.81 to 4.08)**



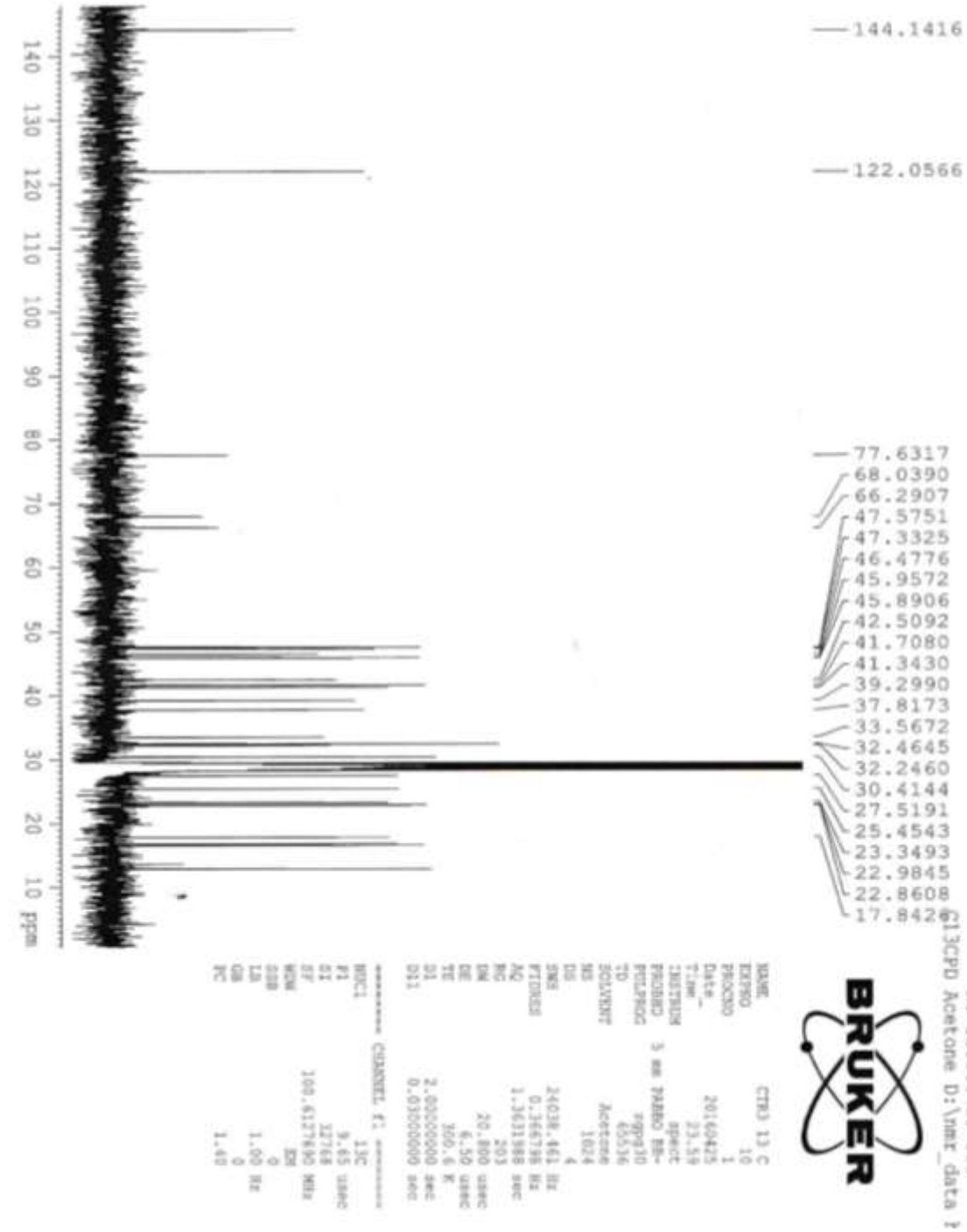
**Appendix 42: $^{1}$H NMR spectra of compound 42 (Expanded δH 5.24 to 5.26)**



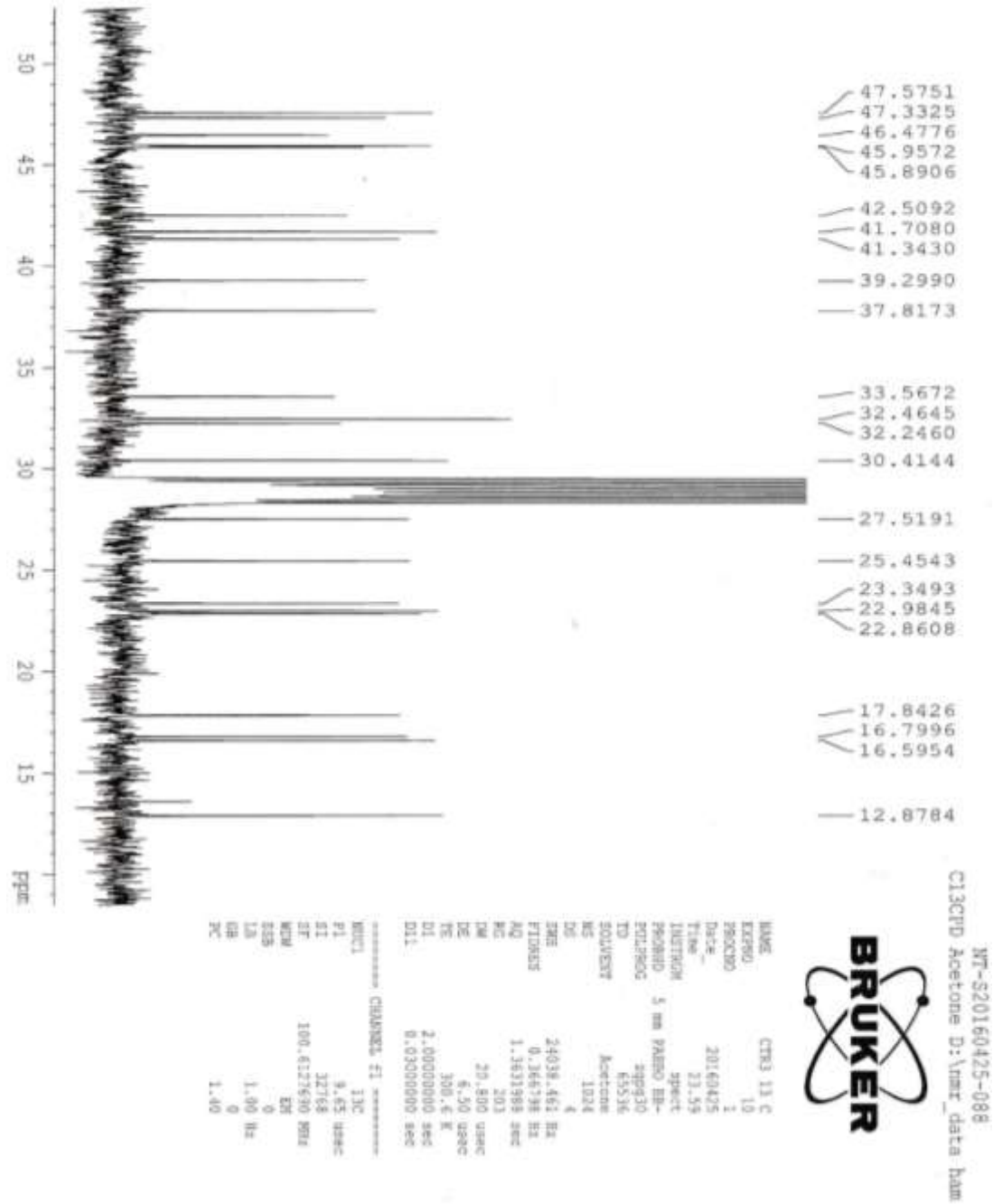
**Appendix 43: $^{1}$H NMR spectrum of compound 42 (Expanded δH 0.73 to 2.06)**



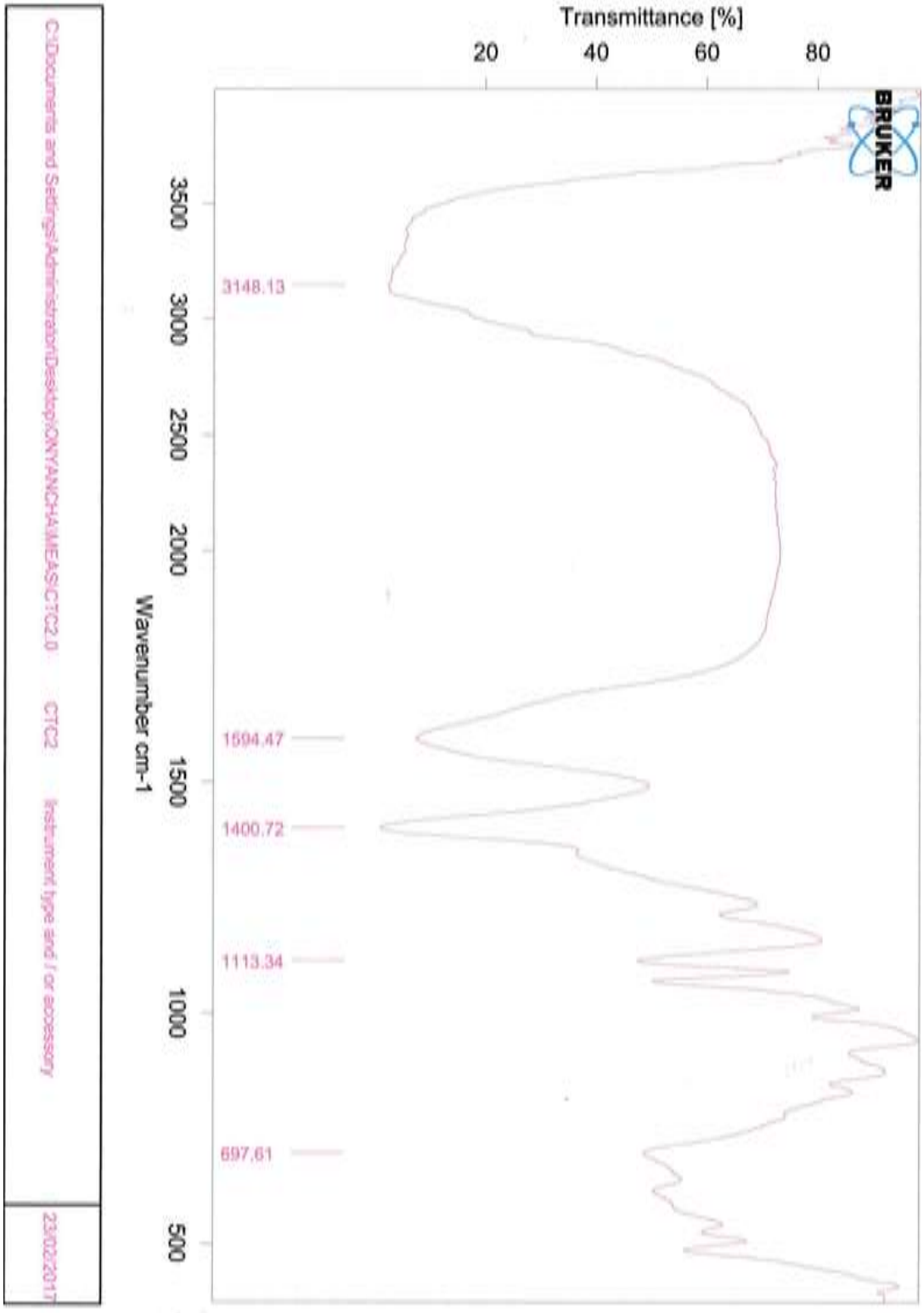
**Appendix 44: $^{13}$C NMR spectrum of compound 42 (δC 17.84 to 144.14)**



**Appendix 45: $^{13}$C NMR spectrum of compound 42 (Expanded δC 12.88 to 47.58)**

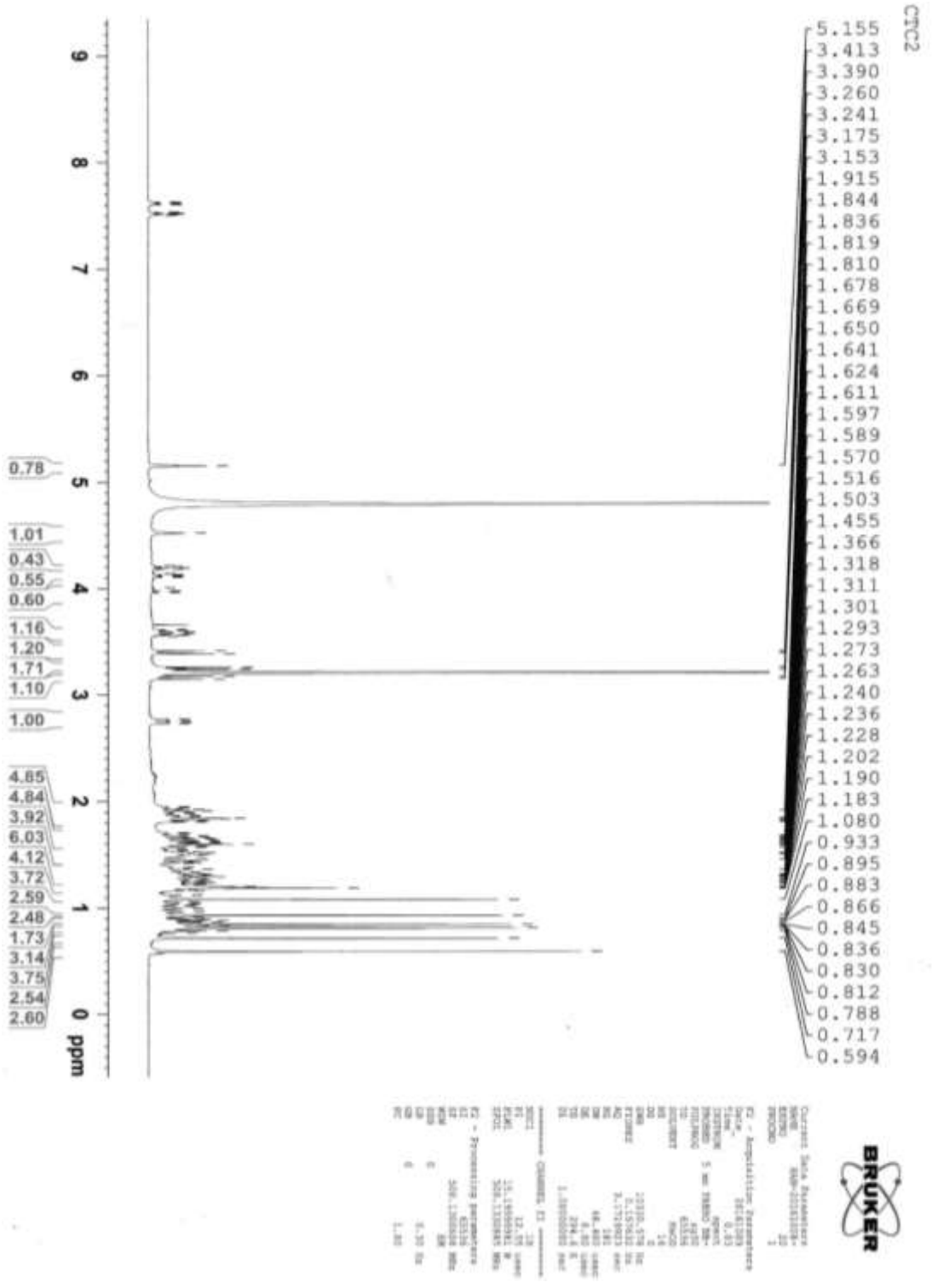


**Appendix 46: FTIR spectrum of compound 43**

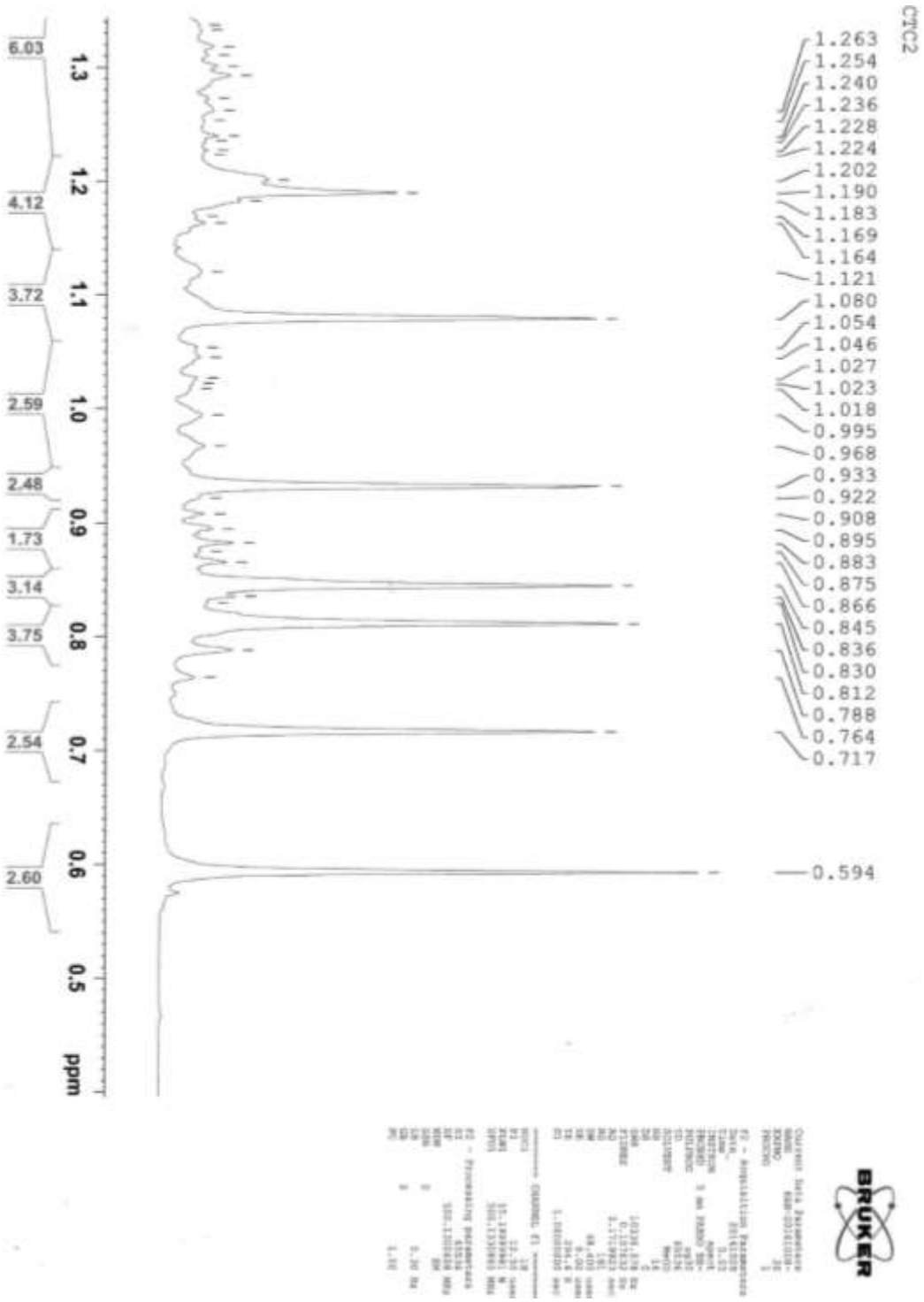


**Appendix 47: MS spectrum of compound 43**

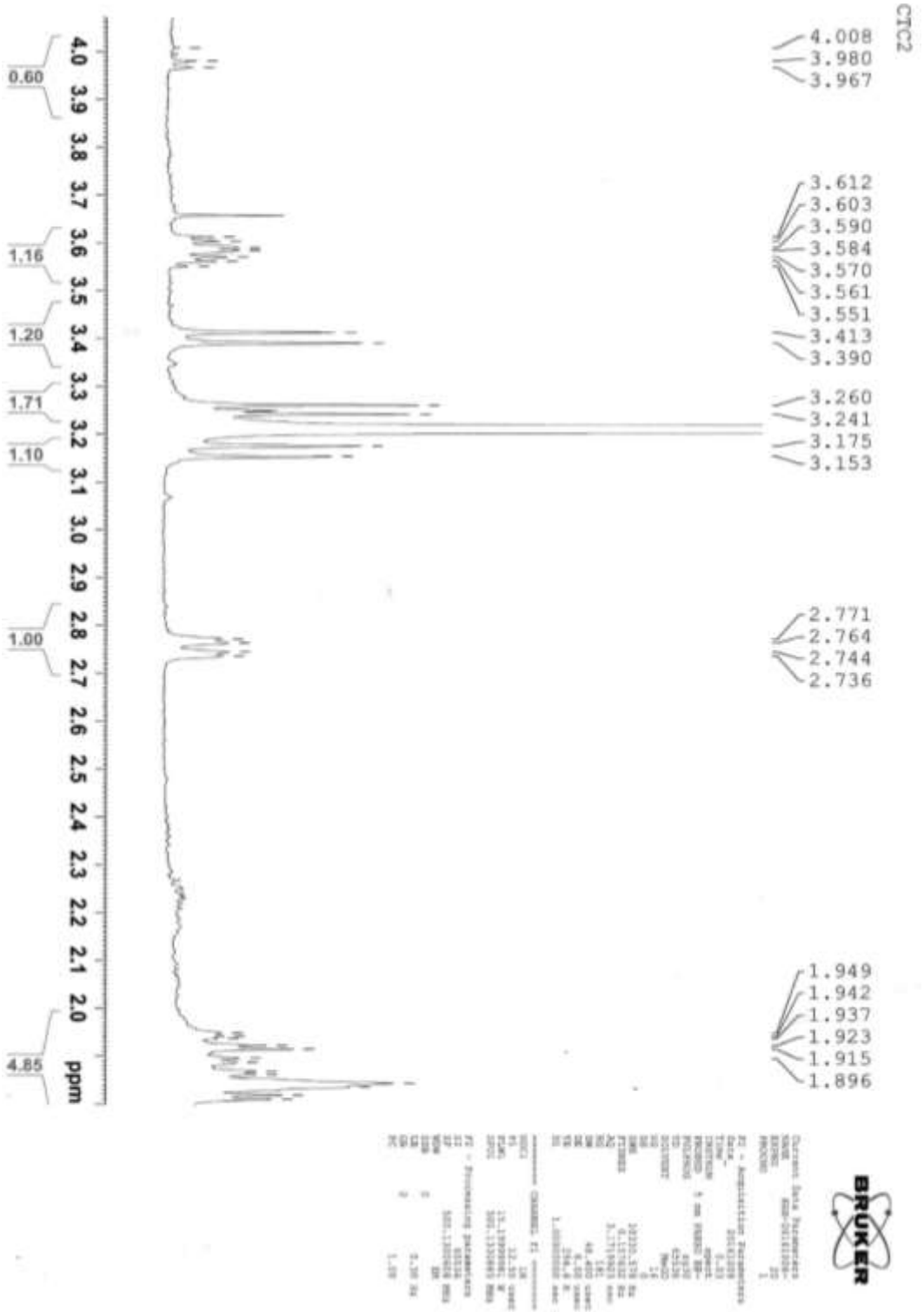
**Appendix 48: $^{1}$H NMR spectrum of compound 43 (δH 0.59 to 5.16)**



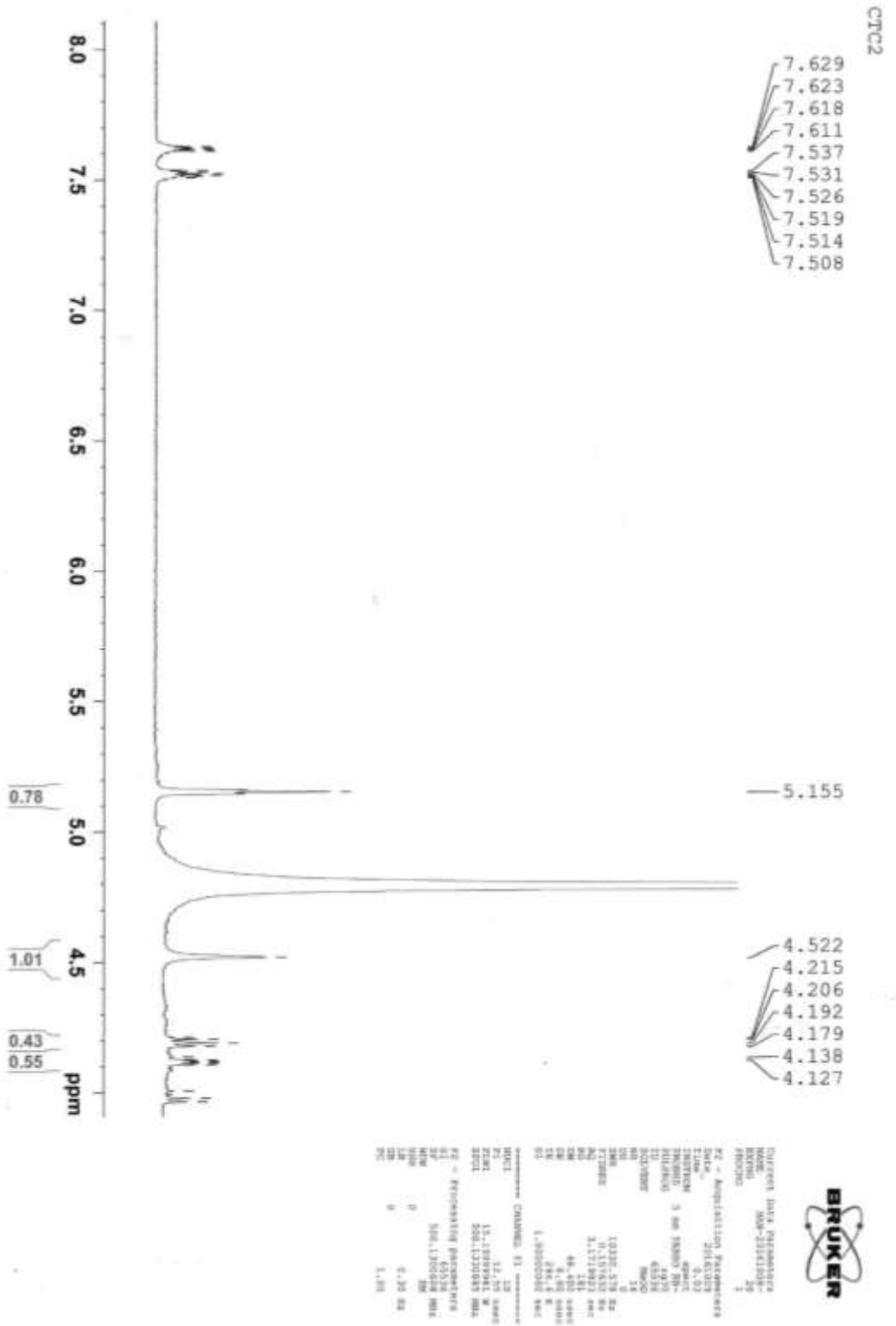
**Appendix 49: $^{1}$H NMR spectrum of compound 42 (Expanded δH 0.59 to 1.26)**



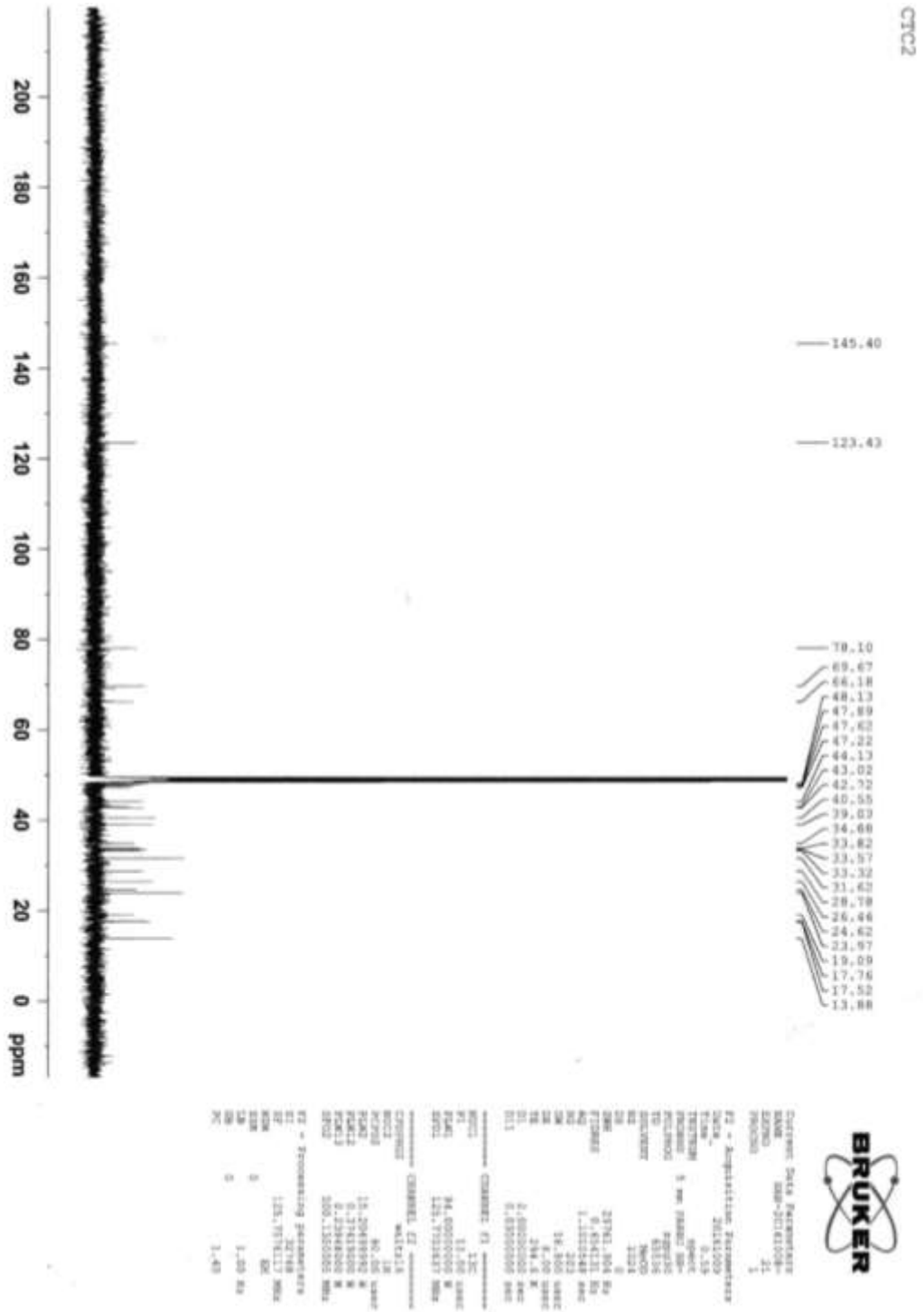
**Appendix 50: $^{1}$H NMR spectrum of compound 43 (Expanded δH 1.90 to 4.01)**



**Appendix 51: $^{1}$H NMR spectrum of compound 43 (δH 4.13 to 7.63)**



**Appendix 52: $^{13}$C NMR spectrum of compound 43 (δC 13.88 to 145.40)**



**Appendix 53: Approval letter for Research obtained from School of postgraduate Studies, Kenyatta University**

KENYATTA UNIVERSITY
GRADUATE SCHOOL

E-mail: dean-graduate@ku.ac.ke
Website: www.ku.ac.ke

P.O. Box 43844, 00100
NAIROBI, KENYA
Tel. 8710901 Ext. 57530

Our Ref: P97/24524/2011

DATE: 15th March 2015

The Principal Secretary,
Higher Education, Science & Technology,
P.O. Box 30040,
NAIROBI

Dear Sir/Madam,

RE: RESEARCH AUTHORIZATION FOR MISONGE JARED ONYANCHA– REG. NO. P97/24524/2011

I write to introduce Mr. Misonge who is a Postgraduate Student of this University. He is registered for Ph.D degree programme in the **Department of Pharmacy/Alternative Complementary Medicine.**

Mr. Misonge intends to conduct research for a Ph.D Proposal entitled, **"Evaluation of Anticancer Activity of Selected Medicinal Plants used for Management of Cancer by Traditional Medicine Practitioners in Kenya".**

Any assistance given will be highly appreciated.

Yours faithfully,

for MRS. LUCY N. MBAABU
FOR: DEAN, GRADUATE SCHOOL

**Appendix 54: Mean $IC_{50}$ values for *F. angolensis* extracts against breast cancer cell lines**

(a) *F. angolensis* stem bark methanol extract

| Well | Conc. (µg/ml) | Absorbance and % Cytotoxicity against HCC1395 | | | | | | Mean $IC_{50}$ n =3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$ = 65 | | $IC_{50}$ = 48.3 | | $IC_{50}$ = 48.3 | | |
| **A** | 0.00 | 0.932 | 0.00 | 0.946 | 0.946 | 1.041 | 0.00 | 53.9 ± 5.6 |
| **B** | 1.37 | 0.836 | 10.30 | 0.881 | 0.881 | 1.024 | 1.63 | |
| **C** | 4.12 | 0.772 | 17.17 | 0.8 | 0.8 | 0.759 | 27.09 | |
| **D** | 12.35 | 0.677 | 27.36 | 0.55 | 0.55 | 0.62 | 40.44 | |
| **E** | 37.04 | 0.524 | 40.8 | 0.52 | 0.52 | 0.598 | 42.56 | |
| **F** | 111.11 | 0.338 | 63.73 | 0.13 | 0.13 | 0.414 | 60.23 | |
| **G** | 333.33 | 0.19 | 79.61 | 0.102 | 0.102 | 0.298 | 71.37 | |
| **H** | 1000 | 0.104 | 88.84 | 0.009 | 0.009 | 0.112 | 89.24 | |

(b) *F. angolensis* stem bark water extract

| Well | Conc. (µg/ml) | Absorbance and % Cytotoxicity against HCC1395 | | | | | | Mean $IC_{50}$ n = 3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$ = 558 | | $IC_{50}$ = 525 | | $IC_{50}$ = 578 | | |
| **A** | 0.00 | 0.928 | 0.00 | 0.457 | 0.00 | 0.831 | 0.00 | 553.6 ± 15.5 |
| **B** | 1.37 | 0.828 | 10.78 | 0.456 | 0.22 | 0.797 | 4.09 | |
| **C** | 4.12 | 0.816 | 12.07 | 0.43 | 5.91 | 0.789 | 5.05 | |
| **D** | 12.35 | 0.753 | 18.86 | 0.428 | 6.35 | 0.76 | 8.54 | |
| **E** | 37.04 | 0.736 | 20.69 | 0.416 | 8.97 | 0.752 | 9.51 | |
| **F** | 111.11 | 0.694 | 25.22 | 0.247 | 45.95 | 0.711 | 14.44 | |
| **G** | 333.33 | 0.543 | 41.49 | 0.242 | 47.05 | 0.453 | 45.49 | |
| **H** | 1000 | 0.305 | 67.13 | 0.195 | 57.33 | 0.351 | 57.76 | |

(c) *F. angolensis* stem bark methanol extract

| Well | Conc. (µg/ml) | Absorbance and % Cytotoxicity against 4TI | | | | | | Mean $IC_{50}$ n = 3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$ = 15.4 | | $IC_{50}$ = 11.18 | | $IC_{50}$ = 11.5 | | |
| **A** | 0.00 | 0.489 | 0.00 | 0.368 | 0.00 | 0.349 | 0.00 | 12.9 ± 1.4 |
| **B** | 1.37 | 0.446 | 8.79 | 0.364 | 1.09 | 0.311 | 10.09 | |
| **C** | 4.12 | 0.426 | 12.88 | 0.305 | 17.12 | 0.256 | 26.65 | |
| **D** | 12.35 | 0.285 | 41.72 | 0.178 | 51.63 | 0.165 | 52.72 | |
| **E** | 37.04 | 0.006 | 98.77 | 0.009 | 97.55 | 0.007 | 97.99 | |

*F. angolensis* stem bark water extract

| Well | Conc. (µg/ml) | Absorbance and % Cytotoxicity against 4TI | | | | | | Mean $IC_{50}$ n = 3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$ = 78.8 | | $IC_{50}$ = 82.2 | | $IC_{50}$ = 104 | | |
| **A** | 0.00 | 0.499 | 0.00 | 0.508 | 0.00 | 0.512 | 0.00 | 88.3 ± 7.9 |
| **B** | 1.37 | 0.493 | 1.20 | 0.485 | 4.53 | 0.508 | 0.78 | |
| **C** | 4.12 | 0.471 | 5.61 | 0.469 | 7.68 | 0.49 | 4.30 | |
| **D** | 12.35 | 0.447 | 10.42 | 0.433 | 14.76 | 0.452 | 11.72 | |
| **E** | 37.04 | 0.396 | 20.64 | 0.397 | 21.85 | 0.398 | 22.27 | |
| **F** | 111.11 | 0.139 | 72.14 | 0.16 | 68.50 | 0.242 | 52.73 | |

**Appendix 55: Mean $IC_{50}$ values for *C. tanaense* extracts against breast cancer cell lines**

(a) *C. tanaense* root methanol extract

| Well | Conc. (µg/ml) | Absorbance and % Cytotoxicity against HCC1395 | | | | | | Mean $IC_{50}$ n = 3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$ = 188 | | $IC_{50}$ = 183 | | $IC_{50}$ = 208 | | |
| **A** | 0.00 | 1.137 | 0.00 | 0.972 | 0.00 | 0.974 | 0.00 | 193.0 ± 13.2 |
| **B** | 1.37 | 0.89 | 21.72 | 0.886 | 8.85 | 0.965 | 0.92 | |
| **C** | 4.12 | 0.878 | 22.78 | 0.832 | 14.40 | 0.913 | 6.26 | |
| **D** | 12.35 | 0.826 | 27.35 | 0.761 | 21.71 | 0.892 | 8.42 | |
| **E** | 37.04 | 0.812 | 28.58 | 0.741 | 23.77 | 0.89 | 8.62 | |
| **F** | 111.11 | 0.636 | 44.06 | 0.606 | 37.65 | 0.644 | 33.88 | |
| **G** | 333.33 | 0.446 | 60.77 | 0.25 | 74.28 | 0.289 | 70.32 | |
| **H** | 1000 | 0.307 | 73.00 | 0.119 | 87.76 | 0.092 | 90.55 | |

(b) *C. tanaense* root water extract

| Well | Conc. (µg/ml) | Absorbance and % Cytotoxicity against HCC1395 | | | | | | Mean $IC_{50}$ n = 3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$ > 1000 | | $IC_{50}$ > 1000 | | $IC_{50}$ > 1000 | | |
| **A** | 0.00 | 1.137 | 0.00 | 1.056 | 0.00 | 1.113 | 0.00 | > 1000 |
| **B** | 1.37 | 1.11 | 2.34 | 1.023 | 3.13 | 0.932 | 16.26 | |
| **C** | 4.12 | 1.108 | 2.55 | 1.008 | 4.55 | 0.909 | 18.33 | |
| **D** | 12.35 | 1.09 | 4.13 | 0.992 | 6.06 | 0.882 | 20.75 | |
| **E** | 37.04 | 1.049 | 7.74 | 0.88 | 16.67 | 0.867 | 22.10 | |
| **F** | 111.11 | 1.034 | 9.06 | 0.852 | 19.32 | 0.867 | 22.10 | |
| **G** | 333.33 | 0.897 | 21.11 | 0.764 | 27.65 | 0.807 | 30.60 | |
| **H** | 1000 | 0.751 | 33.42 | 0.756 | 28.41 | 0.753 | 32.35 | |

(c) *C. tanaense* root methanol extract

| Well | Conc. (µg/ml) | Absorbance and % Cytotoxicity against 4TI | | | | | | Mean $IC_{50}$ n = 3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$ = 19.5 | | $IC_{50}$ = 19.5 | | $IC_{50}$ = 19.5 | | |
| **A** | 0.00 | 0.516 | 0.00 | 0.5 | 0.00 | 0.495 | 0.00 | 19.5 ± 0.0 |
| **B** | 1.37 | 0.5 | 3.10 | 0.491 | 1.80 | 0.494 | 0.20 | |
| **C** | 4.12 | 0.475 | 7.95 | 0.47 | 6.00 | 0.47 | 4.85 | |
| **D** | 12.35 | 0.332 | 35.66 | 0.35 | 30.00 | 0.353 | 28.69 | |
| **E** | 37.04 | 0.155 | 69.96 | 0.14 | 72.00 | 0.112 | 77.37 | |
| **F** | 111.11 | 0.079 | 84.69 | 0.112 | 77.6 | 0.068 | 86.26 | |

(d) *C. tanaense* root water extract

| Well | Conc. (µg/ml) | Absorbance/ % Cytotoxicity against 4TI | | | | | | Mean $IC_{50}$ n=3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$ = 288 | | $IC_{50}$ = 288 | | $IC_{50}$ = 293 | | |
| **A** | 0.00 | 0.562 | 0.00 | 0.541 | 0.00 | 0.545 | 0.00 | 289.7 ± 2.9 |
| **B** | 1.37 | 0.521 | 7.30 | 0.537 | 0.74 | 0.527 | 3.30 | |
| **C** | 4.12 | 0.517 | 8.00 | 0.524 | 3.14 | 0.527 | 3.30 | |
| **D** | 12.35 | 0.516 | 8.19 | 0.518 | 4.25 | 0.525 | 3.67 | |
| **E** | 37.04 | 0.494 | 12.10 | 0.495 | 8.50 | 0.514 | 5.69 | |
| **F** | 111.11 | 0.484 | 13.88 | 0.46 | 14.97 | 0.455 | 16.51 | |
| **G** | 333.33 | 0.238 | 57.65 | 0.216 | 60.07 | 0.238 | 56.33 | |
| **H** | 1000 | 0.09 | 83.99 | 0.066 | 87.80 | 0.038 | 93.03 | |

**Appendix 56: Mean $IC_{50}$ values for *U. anisatum* extracts against breast cancer cell lines**

(a) *U. anisatum* root methanol extract

| Well | Conc. (µg/ml) | Absorbance/ % Cytotoxicity against HCC 1395 | | | | | | Mean $IC_{50}$ ± SEM n = 3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$ = 45.00 | | $IC_{50}$ = 51.7 | | $IC_{50}$ = 55 | | |
| **A** | 0.00 | 0.911 | 0.00 | 0.955 | 0.00 | 0.917 | 0.00 | 50.6 ± 2.9 |
| **B** | 1.37 | 0.856 | 6.04 | 0.698 | 26.91 | 0.777 | 15.27 | |
| **C** | 4.12 | 0.631 | 30.74 | 0.696 | 27.12 | 0.714 | 22.14 | |
| **D** | 12.35 | 0.539 | 40.83 | 0.603 | 36.86 | 0.592 | 35.44 | |
| **E** | 37.04 | 0.456 | 49.95 | 0.489 | 48.80 | 0.46 | 49.84 | |
| **F** | 111.11 | 0.438 | 51.92 | 0.455 | 52.36 | 0.45 | 50.93 | |
| **G** | 333.33 | 0.225 | 75.30 | 0.409 | 57.17 | 0.379 | 58.67 | |
| **H** | 1000 | | | 0.045 | 95.29 | 0.039 | 95.75 | |

(b) *U. anisatum* root water extract

| Well | Conc. (µg/ml) | Absorbance/ % Cytotoxicity against HCC 1395 | | | | | | Mean $IC_{50}$ ± SEM n=3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$ = 258 | | $IC_{50}$ = 238 | | $IC_{50}$ = 248 | | |
| **A** | 0.00 | 1.08 | 0.00 | 0.955 | 0.00 | 1.099 | 0.00 | 248 ± 5.8 |
| **B** | 1.37 | 1.026 | 5.00 | 0.912 | 4.50 | 0.963 | 12.37 | |
| **C** | 4.12 | 0.99 | 8.33 | 0.858 | 10.16 | 0.958 | 12.83 | |
| **D** | 12.35 | 0.967 | 10.46 | 0.806 | 15.60 | 0.908 | 17.38 | |
| **E** | 37.04 | 0.963 | 10.83 | 0.784 | 17.91 | 0.887 | 19.29 | |
| **F** | 111.11 | 0.777 | 28.06 | 0.716 | 25.03 | 0.807 | 26.57 | |
| **G** | 333.33 | 0.419 | 61.20 | 0.312 | 67.33 | 0.379 | 65.51 | |
| **H** | 1000 | 0.342 | 68.33 | 0.146 | 84.71 | 0.183 | 83.35 | |

(c) *U. anisatum* root methanol extract

| Well | Conc. (µg/ml) | Absorbance/ % Cytotoxicity against 4TI | | | | | | Mean $IC_{50}$ ± SEM n = 3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$ = 1.75 | | $IC_{50}$ = 1.88 | | $IC_{50}$ = 1.68 | | |
| **A** | 0.00 | 0.542 | 0.00 | 0.519 | 0.00 | 0.493 | 0.00 | 1.8 ± 0.06 |
| **B** | 1.37 | 0.287 | 47.05 | 0.279 | 46.24 | 0.264 | 46.45 | |
| **C** | 4.12 | 0.194 | 64.21 | 0.18 | 65.32 | 0.112 | 77.28 | |
| **D** | 12.35 | 0.109 | 79.89 | 0.146 | 71.87 | 0.084 | 82.96 | |
| **E** | 37.04 | 0.083 | 84.79 | 0.074 | 85.74 | 0.002 | 99.59 | |
| **F** | 111.11 | 0.006 | 98.89 | 0.001 | 99.81 | | | |

(d) *U. anisatum* root water extract

| Well | Conc. (µg/ml) | Absorbance/ % Cytotoxicity against 4TI | | | | | | Mean $IC_{50}$ ± SEM n = 3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$ = 141 | | $IC_{50}$ = 157 | | $IC_{50}$ = 154 | | |
| **A** | 0.00 | 0.628 | 0.00 | 0.68 | 0.00 | 0.69 | 0.00 | 150.7 ± 4.9 |
| **B** | 1.37 | 0.599 | 4.62 | 0.651 | 4.26 | 0.646 | 6.38 | |
| **C** | 4.12 | 0.576 | 8.28 | 0.618 | 9.12 | 0.616 | 10.72 | |
| **D** | 12.35 | 0.573 | 8.76 | 0.601 | 11.62 | 0.582 | 15.65 | |
| **E** | 37.04 | 0.54 | 14.02 | 0.589 | 13.38 | 0.568 | 17.68 | |
| **F** | 111.11 | 0.348 | 44.59 | 0.391 | 42.50 | 0.394 | 42.90 | |
| **G** | 333.33 | 0.106 | 83.12 | 0.163 | 76.03 | 0.184 | 73.33 | |

**Appendix 57: Mean $IC_{50}$ values for *H. abyssinica* extracts against breast cancer cell lines**

(a) *H. abyssinica* rhizome methanol extract

| Well | Conc. (µg/ml) | Absorbance and % Cytotoxicity against HCC 1395 | | | | | | Mean $IC_{50}$ ± SEM n = 3 |
|---|---|---|---|---|---|---|---|---|
| | | i $IC_{50}$ = 28.3 | | ii $IC_{50}$ = 28.3 | | iii $IC_{50}$ = 25.0 | | |
| **A** | 0.00 | 0.439 | 0.439 | 0.494 | 0.00 | 0.482 | 0.00 | 27.2 ±1.1 |
| **B** | 1.37 | 0.368 | 0.368 | 0.463 | 6.28 | 0.379 | 21.36 | |
| **C** | 4.12 | 0.349 | 0.349 | 0.449 | 9.11 | 0.355 | 26.35 | |
| **D** | 12.35 | 0.342 | 0.342 | 0.436 | 11.74 | 0.348 | 27.80 | |
| **E** | 37.04 | 0.131 | 0.131 | 0.215 | 56.48 | 0.13 | 73.03 | |
| **F** | 111.11 | 0.075 | 0.075 | 0.091 | 81.58 | 0.013 | 97.3 | |
| **G** | 333.33 | 0.051 | 0.051 | 0.075 | 84.48 | 0.011 | 97.72 | |
| **H** | 1000 | 0.013 | 0.013 | 0.045 | 90.89 | 0.01 | 97.93 | |

(b) *H. abyssinica* rhizome water extract

| Well | Conc. (µg/ml) | Absorbance and % Cytotoxicity against HCC 1395 | | | | | | Mean $IC_{50}$ ± SEM n = 3 |
|---|---|---|---|---|---|---|---|---|
| | | i $IC_{50}$ = 498 | | ii $IC_{50}$ = 498 | | iii $IC_{50}$ = 502 | | |
| **A** | 0.00 | 1.127 | 0.00 | 1.065 | 0.00 | 1.082 | 0.00 | 499.3 ± 1.3 |
| **B** | 1.37 | 0.974 | 13.58 | 0.907 | 14.84 | 0.971 | 10.26 | |
| **C** | 4.12 | 0.968 | 14.11 | 0.877 | 17.65 | 0.941 | 13.03 | |
| **D** | 12.35 | 0.941 | 16.50 | 0.877 | 17.65 | 0.909 | 15.99 | |
| **E** | 37.04 | 0.9 | 20.14 | 0.814 | 23.57 | 0.873 | 19.32 | |
| **F** | 111.11 | 0.858 | 23.87 | 0.784 | 26.38 | 0.842 | 22.18 | |
| **G** | 333.33 | 0.714 | 36.65 | 0.689 | 35.31 | 0.678 | 37.34 | |
| **H** | 1000 | 0.101 | 91.04 | 0.054 | 94.93 | 0.16 | 85.21 | |

(c) *H. abyssinica* flower methanol extract

| Well | Conc. (µg/ml) | Absorbance and % Cytotoxicity against HCC 1395 | | | | | | Mean $IC_{50}$ ± SEM n=3 |
|---|---|---|---|---|---|---|---|---|
| | | i $IC_{50}$ = 79.1 | | ii $IC_{50}$ = 78.3 | | iii $IC_{50}$ = 81.7 | | |
| **A** | 0.00 | 1.778 | 0.00 | 1.471 | 0.00 | 1.455 | 0.00 | 79.7 ± 1.0 |
| **B** | 1.37 | 1.431 | 19.52 | 1.398 | 4.96 | 1.355 | 6.87 | |
| **C** | 4.12 | 1.394 | 21.60 | 1.207 | 17.95 | 1.323 | 9.07 | |
| **D** | 12.35 | 1.285 | 27.73 | 1.174 | 20.19 | 1.176 | 19.18 | |
| **E** | 37.04 | 1.231 | 30.76 | 1.102 | 25.08 | 1.166 | 19.86 | |
| **F** | 111.11 | 1.12 | 37.00 | 0.328 | 77.70 | 0.753 | 70.20 | |
| **G** | 333.33 | 0.649 | 63.50 | 0.317 | 78.45 | 0.517 | 93.80 | |
| **H** | 1000 | 0.037 | 97.97 | 0.052 | 96.46 | 0.096 | 93.40 | |

(d) *H. abyssinica* flower water extract

| Well | Conc. (µg/ml) | Absorbance and% Cytotoxicity against HCC 1395 | | | | | | Mean $IC_{50}$ ± SEM n = 3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$ = 45.0 | | $IC_{50}$ = 35.0 | | $IC_{50}$ = 31.7 | | |
| **A** | 0.00 | 1.648 | 0.00 | 1.317 | 0.00 | 1.409 | 0.00 | 37.2 ± 4.0 |
| **B** | 1.37 | 1.46 | 11.41 | 1.278 | 2.96 | 1.231 | 12.63 | |
| **C** | 4.12 | 1.426 | 13.47 | 1.184 | 10.10 | 1.185 | 15.90 | |
| **D** | 12.35 | 1.355 | 17.78 | 0.864 | 34.40 | 0.911 | 35.34 | |
| **E** | 37.04 | 0.912 | 44.66 | 0.684 | 48.06 | 0.517 | 63.31 | |
| **F** | 111.11 | 0.539 | 67.29 | 0.302 | 77.07 | 0.272 | 80.70 | |
| **G** | 333.33 | 0.194 | 88.23 | 0.051 | 96.13 | 0.008 | 99.43 | |
| **H** | 1000 | 0.074 | 95.51 | 0.001 | 99.92 | 0.001 | 99.93 | |

(e) *H. abyssinica* rhizome water extract

| Well | Conc. (µg/ml) | Absorbance and % Cytotoxicity against 4TI | | | | Mean $IC_{50}$ ± SEM n = 3 |
|---|---|---|---|---|---|---|
| | | i | | ii | | |
| | | $IC_{50}$ =78.8 | | $IC_{50}$ = 80.8 | | |
| **A** | 0.00 | 0.499 | 0.00 | 0.508 | 0.00 | 79.8 ± 1.0 |
| **B** | 1.37 | 0.493 | 1.20 | 0.485 | 4.53 | |
| **C** | 4.12 | 0.471 | 5.61 | 0.469 | 7.68 | |
| **D** | 12.35 | 0.447 | 10.42 | 0.433 | 14.76 | |
| **E** | 37.04 | 0.396 | 20.64 | 0.397 | 21.85 | |
| **F** | 111.11 | 0.139 | 72.14 | 0.16 | 68.50 | |

(f) *H. abyssinica* flower methanol extract

| Well | Conc. (µg/ml) | Absorbance and % Cytotoxicity against 4TI | | | | | | Mean $IC_{50}$ ± SEM n = 3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$ = 45.0 | | $IC_{50}$ =78.3 | | $IC_{50}$ = 45.0 | | |
| **A** | 0.00 | 0.587 | 0.00 | 0.575 | 0.00 | 0.536 | 0.00 | 56.1 ± 11.1 |
| **B** | 1.37 | 0.572 | 2.56 | 0.529 | 8.00 | 0.53 | 1.10 | |
| **C** | 4.12 | 0.541 | 7.84 | 0.48 | 16.52 | 0.463 | 13.62 | |
| **D** | 12.35 | 0.444 | 24.36 | 0.396 | 31.13 | 0.384 | 28.36 | |
| **E** | 37.04 | 0.319 | 45.66 | 0.34 | 40.86 | 0.264 | 50.75 | |
| **F** | 111.11 | 0.122 | 79.22 | 0.243 | 57.74 | 0.233 | 56.53 | |
| **G** | 333.33 | 0.051 | 91.31 | 0.109 | 81.04 | 0.134 | 75.00 | |
| **H** | 1000 | 0.013 | 97.79 | 0.059 | 89.74 | 0.116 | 78.36 | |

(g) *H. abyssinica* flower water extract

| Well | Conc. (µg/ml) | Absorbance and % Cytotoxicity against 4TI | | | | | | Mean $IC_{50}$ ± SEM n=3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$ = 28.3 | | $IC_{50}$ = 98.3 | | $IC_{50}$ = 65.0 | | |
| **A** | 0.00 | 0.582 | 0.00 | 0.709 | 0.00 | 0.608 | 0.00 | 81.6 ± 16.6 |
| **B** | 1.37 | 0.567 | 2.58 | 0.61 | 13.96 | 0.578 | 4.93 | |
| **C** | 4.12 | 0.462 | 20.62 | 0.578 | 18.48 | 0.423 | 30.42 | |
| **D** | 12.35 | 0.445 | 23.54 | 0.439 | 38.08 | 0.422 | 30.59 | |
| **E** | 37.04 | 0.244 | 58.08 | 0.423 | 40.34 | 0.344 | 43.42 | |
| **F** | 111.11 | 0.236 | 59.45 | 0.343 | 51.62 | 0.231 | 62.01 | |
| **G** | 333.33 | 0.124 | 78.69 | 0.329 | 53.60 | 0.156 | 74.34 | |
| **H** | 1000 | 0.095 | 83.68 | 0.193 | 72.78 | 0.063 | 89.64 | |

**Appendix 58: Mean $IC_{50}$ values for *L. cornuta* extracts against breast cancer cell lines**

(a) *L. cornuta* leaf methanol extract

| Well | Conc. (µg/ml) | Absorbance and % Cytotoxicity against HCC 1395 | | | | | | Mean $IC_{50}$ ± SEM n = 3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$ = 228 | | $IC_{50}$ = 232 | | $IC_{50}$ = 235 | | |
| **A** | 0.00 | 1.027 | 0.00 | 0.976 | 0.00 | 1.01 | 0.00 | 231.7 ± 2 |
| **B** | 1.37 | 1.02 | 0.68 | 0.958 | 1.84 | 0.909 | 10.00 | |
| **C** | 4.12 | 0.951 | 7.40 | 0.925 | 5.23 | 0.903 | 10.69 | |
| **D** | 12.35 | 0.941 | 8.37 | 0.89 | 8.81 | 0.89 | 11.88 | |
| **E** | 37.04 | 0.856 | 16.65 | 0.875 | 9.10 | 0.851 | 15.74 | |
| **F** | 111.11 | 0.849 | 17.33 | 0.774 | 20.70 | 0.796 | 21.19 | |
| **G** | 333.33 | 0.223 | 78.29 | 0.245 | 74.90 | 0.259 | 74.36 | |
| **H** | 1000 | 0.074 | 92.79 | 0.078 | 92.01 | 0.047 | 95.35 | |

(b) *L. cornuta* leaf water extract

| Well | Conc. (µg/ml) | Absorbance and % Cytotoxicity against HCC 1395 | | | | | | Mean $IC_{50}$ ± SEM n=3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$ = 375 | | $IC_{50}$ =385 | | $IC_{50}$ =385 | | |
| **A** | 0.00 | 1.121 | 0.00 | 0.929 | 0.00 | 1.023 | 0.00 | 381 ± 15.3 |
| **B** | 1.37 | 0.901 | 19.63 | 0.863 | 7.10 | 1.01 | 1.27 | |
| **C** | 4.12 | 0.89 | 20.60 | 0.83 | 10.66 | 0.996 | 2.64 | |
| **D** | 12.35 | 0.844 | 24.71 | 0.802 | 13.67 | 0.974 | 4.79 | |
| **E** | 37.04 | 0.835 | 25.51 | 0.78 | 16.04 | 0.962 | 5.96 | |
| **F** | 111.11 | 0.75 | 33.10 | 0.772 | 16.90 | 0.815 | 20.33 | |
| **G** | 333.33 | 0.741 | 33.90 | 0.696 | 25.08 | 0.701 | 31.48 | |
| **H** | 1000 | 0.328 | 70.74 | 0.456 | 50.91 | 0.433 | 57.67 | |

(c) *L. cornuta* leaf methanol extract

| Well | Conc. (µg/ml) | Absorbance and % Cytotoxicity against 4TI | | | | | | Mean $IC_{50}$ ± SEM n = 3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$ = 228 | | $IC_{50}$ = 295 | | $IC_{50}$ = 306 | | |
| **A** | 0.00 | 0.603 | 0.00 | 0.559 | 0.00 | 0.594 | 0.00 | 276.3 ± 24.4 |
| **B** | 1.37 | 0.534 | 11.44 | 0.544 | 2.68 | 0.545 | 8.25 | |
| **C** | 4.12 | 0.533 | 11.61 | 0.531 | 5.01 | 0.526 | 11.45 | |
| **D** | 12.35 | 0.527 | 12.60 | 0.522 | 6.62 | 0.526 | 11.45 | |
| **E** | 37.04 | 0.467 | 22.55 | 0.49 | 12.34 | 0.48 | 19.19 | |
| **F** | 111.11 | 0.441 | 26.87 | 0.477 | 14.67 | 0.437 | 26.43 | |
| **G** | 333.33 | 0.173 | 71.31 | 0.233 | 58.32 | 0.278 | 53.20 | |
| **H** | 1000 | 0.023 | 96.19 | 0.003 | 99.46 | 0.00 | 100.0 | |

(d) *L. cornuta* leaf water extract

| Well | Conc. (µg/ml) | Absorbance and % Cytotoxicity against 4TI | | | | Mean $IC_{50}$ ± SEM n = 2 |
|---|---|---|---|---|---|---|
| | | i | | ii | | |
| | | $IC_{50}$ = 686 | | $IC_{50}$ = 715 | | |
| **A** | 0.00 | 0.592 | 0.00 | 0.589 | 0.00 | 700.5 ± 14.5 |
| **B** | 1.37 | 0.572 | 3.38 | 0.556 | 5.60 | |
| **C** | 4.12 | 0.547 | 7.60 | 0.551 | 6.45 | |
| **D** | 12.35 | 0.54 | 8.78 | 0.535 | 9.17 | |
| **E** | 37.04 | 0.54 | 8.78 | 0.496 | 15.79 | |
| **F** | 111.11 | 0.48 | 18.92 | 0.478 | 18.85 | |
| **G** | 333.33 | 0.468 | 20.95 | 0.467 | 20.71 | |
| **H** | 1000 | 0.144 | 75.68 | 0.159 | 73.01 | |

**Appendix 59: Mean $IC_{50}$ values for *S. princeae* extracts against breast cancer cell lines**

(a) *S. princeae* aerial methanol extract

| Well | Conc. (µg/ml) | Absorbance and % Cytotoxicity against HCC 1395 | | | | | | Mean $IC_{50}$ ± SEM n = 3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$ = 642 | | $IC_{50}$ = 452 | | $IC_{50}$ = 505 | | |
| **A** | 0.00 | 1.431 | 0.00 | 1.292 | 0.00 | 1.453 | 0.00 | 533 ± 56.6 |
| **B** | 1.37 | 1.388 | 3.00 | 1.269 | 1.78 | 1.451 | 0.13 | |
| **C** | 4.12 | 1.375 | 3.91 | 1.238 | 4.18 | 1.442 | 2.13 | |
| **D** | 12.35 | 1.348 | 8.1 | 1.217 | 5.80 | 1.413 | 2.75 | |
| **E** | 37.04 | 1.279 | 10.62 | 1.176 | 8.98 | 1.401 | 3.58 | |
| **F** | 111.11 | 1.114 | 22.15 | 1.045 | 19.11 | 1.342 | 7.64 | |
| **G** | 333.33 | 1.067 | 25.44 | 0.767 | 40.63 | 0.9 | 38.04 | |
| **H** | 1000 | 0.293 | 79.52 | 0.164 | 87.73 | 0.171 | 88.23 | |

(b) *S. princeae* aerial water extract

| Well | Conc. (µg/ml) | Absorbance and % Cytotoxicity against HCC 1395 | | | | | | Mean $IC_{50}$ ± SEM n = 3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$ > 1000 | | $IC_{50}$ = 835 | | $IC_{50}$ = 988 | | |
| **A** | 0.00 | 1.737 | 0.00 | 1.479 | 0.00 | 1.478 | 0.00 | 911.5 ± 76.5 |
| **B** | 1.37 | 1.613 | 7.17 | 1.463 | 1.08 | 1.423 | 3.72 | |
| **C** | 4.12 | 1.602 | 7.78 | 1.185 | 19.88 | 1.411 | 4.53 | |
| **D** | 12.35 | 1.598 | 8.00 | 1.165 | 21.23 | 1.257 | 14.95 | |
| **E** | 37.04 | 1.356 | 21.93 | 1.16 | 21.58 | 1.281 | 13.33 | |
| **F** | 111.11 | 1.239 | 28.67 | 1.159 | 21.63 | 1.164 | 21.24 | |
| **G** | 333.33 | 1.231 | 29.13 | 1.142 | 22.78 | 1.135 | 23.21 | |
| **H** | 1000 | 1.079 | 37.88 | 0.613 | 58.55 | 0.736 | 50.20 | |

(c) *S. princeae* aerial methanol extract

| Well | Conc. (µg/ml) | Absorbance/ % Cytotoxicity against 4TI | | | | | | Mean $IC_{50}$ ± SEM n = 3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$ = 215 | | $IC_{50}$ = 192 | | $IC_{50}$ = 205 | | |
| **A** | 0.00 | 0.615 | 0.00 | 0.61 | 0.00 | 0.597 | 0.00 | 204 ± 6.7 |
| **B** | 1.37 | 0.597 | 2.93 | 0.599 | 1.8 | 0.595 | 0.34 | |
| **C** | 4.12 | 0.575 | 6.50 | 0.579 | 5.08 | 0.589 | 1.34 | |
| **D** | 12.35 | 0.573 | 6.83 | 0.534 | 12.46 | 0.506 | 15.24 | |
| **E** | 37.04 | 0.559 | 9.11 | 0.463 | 24.10 | 0.455 | 23.79 | |
| **F** | 111.11 | 0.451 | 26.67 | 0.399 | 34.59 | 0.406 | 31.99 | |
| **G** | 333.33 | 0.133 | 78.37 | 0.132 | 78.36 | 0.134 | 77.55 | |
| **H** | 1000 | 0.053 | 91.38 | 0.115 | 81.15 | 0.132 | 77.89 | |

(d) *S. princeae* aerial water extract

| Well | Conc. (µg/ml) | Absorbance/ % Cytotoxicity against 4TI | | | | | | Mean $IC_{50}$ ± SEM n = 3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$ = 582 | | $IC_{50}$ = 552 | | $IC_{50}$ = 552 | | |
| **A** | 0.00 | 0.571 | 0.00 | 0.608 | 0.00 | 0.606 | 0.00 | 562 ± 10 |
| **B** | 1.37 | 0.568 | 0.53 | 0.599 | 1.48 | 0.592 | 2.31 | |
| **C** | 4.12 | 0.568 | 0.53 | 0.592 | 2.64 | 0.59 | 2.64 | |
| **D** | 12.35 | 0.557 | 2.45 | 0.584 | 3.95 | 0.579 | 4.46 | |
| **E** | 37.04 | 0.557 | 2.45 | 0.551 | 9.38 | 0.579 | 4.46 | |
| **F** | 111.11 | 0.542 | 5.07 | 0.448 | 26.32 | 0.569 | 6.11 | |
| **G** | 333.33 | 0.472 | 17.34 | 0.416 | 31.58 | 0.407 | 32.84 | |
| **H** | 1000 | 0.084 | 85.29 | 0.073 | 87.99 | 0.077 | 87.29 | |

**Appendix 60: Mean $IC_{50}$ values for *P. africana* extracts against breast cancer cell lines**

(a) *P. africana* stem bark methanol extract

| Well | Conc. (µg/ml) | Absorbance/ % Cytotoxicity against HCC 1395 | | | | | | Mean $IC_{50}$ ± SEM n=3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$ = 11.2 | | $IC_{50}$ = 11.5 | | $IC_{50}$ = 9.17 | | |
| **A** | 0.00 | 1.366 | 0.00 | 1.372 | 0.00 | 1.54 | 0.00 | 10.6 ± 0.7 |
| **B** | 1.37 | 1.334 | 2.34 | 1.365 | 0.51 | 1.507 | 2.14 | |
| **C** | 4.12 | 1.107 | 18.96 | 1.144 | 16.61 | 1.064 | 30.91 | |
| **D** | 12.35 | 0.632 | 53.73 | 0.631 | 54.01 | 0.58 | 62.34 | |
| **E** | 37.04 | 0.58 | 57.54 | 0.391 | 71.50 | 0.54 | 64.94 | |

(b) *P. africana* stem bark methanol extract

| Well | Conc. (µg/ml) | Absorbance and % Cytotoxicity against HCC 1395 | | | | | | Mean $IC_{50}$ ± SEM n = 3 |
|---|---|---|---|---|---|---|---|---|
| | | ii | | iii | | iii | | |
| | | $IC_{50}$ = 92.8 | | $IC_{50}$ = 86.7 | | $IC_{50}$ = 66.2 | | |
| **A** | 0.00 | 1.354 | 0.00 | 1.297 | 0.00 | 1.29 | 0.00 | 81.9 ± 8.05 |
| **B** | 1.37 | 1.301 | 3.91 | 1.25 | 2.39 | 1.269 | 1.63 | |
| **C** | 4.12 | 1.299 | 4.06 | 1.218 | 6.09 | 1.211 | 6.12 | |
| **D** | 12.35 | 1.102 | 18.61 | 1.194 | 7.94 | 1.158 | 10.23 | |
| **E** | 37.04 | 1.086 | 19.79 | 0.839 | 37.03 | 0.965 | 25.19 | |
| **F** | 111.11 | 0.548 | 59.53 | 0.575 | 55.67 | 0.174 | 86.51 | |
| **G** | 333.33 | 0.00 | 100.00 | 0.227 | 82.50 | 0.00 | 100.00 | |

(c) *P. africana* stem bark methanol extract

| Well | Conc. (µg/ml) | Absorbance and % Cytotoxicity against 4TI | | | | | | Mean $IC_{50}$ ± SEM n = 3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$ = 6.72 | | $IC_{50}$ = 3.18 | | $IC_{50}$ = 6.42 | | |
| **A** | 0.00 | 0.435 | 0.00 | 0.522 | 0.00 | 0.541 | 0.00 | 5.4 ± 1.1 |
| **B** | 1.37 | 0.41 | 5.75 | 0.383 | 26.63 | 0.361 | 33.27 | |
| **C** | 4.12 | 0.298 | 31.49 | 0.195 | 62.64 | 0.309 | 42.88 | |
| **D** | 12.35 | 0.036 | 91.72 | 0.184 | 64.75 | 0.163 | 69.87 | |

(d) *P. africana* stem bark water extract

| Well | Conc. (µg/ml) | Absorbance and % Cytotoxicity against 4TI | | | | | | Mean $IC_{50}$ ± SEM n = 3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$ = 53.8 | | $IC_{50}$ = 26.8 | | $IC_{50}$ = 29.7 | | |
| **A** | 0.00 | 0.626 | 0.00 | 0.587 | 0.00 | 0.558 | 0.00 | 36.7 ± 8.6 |
| **B** | 1.37 | 0.584 | 6.71 | 0.527 | 10.22 | 0.499 | 10.57 | |
| **C** | 4.12 | 0.573 | 8.47 | 0.517 | 11.93 | 0.487 | 12.72 | |
| **D** | 12.35 | 0.42 | 32.91 | 0.335 | 42.93 | 0.317 | 43.19 | |
| **E** | 37.04 | 0.378 | 39.61 | 0.262 | 55.37 | 0.263 | 52.87 | |
| **F** | 111.11 | 0.097 | 84.50 | 0.00 | 100.00 | 0.00 | 100.00 | |

**Appendix 61: Mean $IC_{50}$ values for standard against breast cancer cell lines**

**(a)** Cyclophosphamide

| Well | Conc. (µg/ml) | Absorbance and % Cytotoxicity against HCC 1395 | | | | | | Mean $IC_{50}$ ± SEM n=3 |
|---|---|---|---|---|---|---|---|---|
| | | i $IC_{50}$ = 35.0 | | ii $IC_{50}$ = 31.7 | | iii $IC_{50}$ = 31.7 | | |
| **A** | 0.00 | 0.418 | 0.00 | 0.42 | 0.00 | 0.495 | 0.00 | 32.8 ± 1.1 |
| **B** | 1.37 | 0.307 | 26.56 | 0.338 | 19.52 | 0.339 | 31.52 | |
| **C** | 4.12 | 0.291 | 30.38 | 0.305 | 27.38 | 0.337 | 31.92 | |
| **D** | 12.35 | 0.258 | 38.28 | 0.244 | 41.90 | 0.313 | 36.77 | |
| **E** | 37.04 | 0.191 | 54.31 | 0.202 | 51.90 | 0.214 | 56.77 | |
| **F** | 111.11 | 0.015 | 96.41 | 0.016 | 96.19 | 0.018 | 96.36 | |
| **G** | 333.33 | 0.006 | 98.56 | 0.006 | 98.57 | 0.009 | 98.18 | |
| **H** | 1000 | 0.005 | 98.80 | 0.004 | 99.05 | 0.007 | 98.59 | |

**(b)** Cyclophosphamide

| Well | Conc. (µg/ml) | Absorbance and % Cytotoxicity against 4TI | | | | | | Mean $IC_{50}$ ± SEM n=3 |
|---|---|---|---|---|---|---|---|---|
| | | i $IC_{50}$ = 25.0 | | ii $IC_{50}$ = 21.7 | | iii $IC_{50}$ = 21.7 | | |
| **A** | 0.00 | 0.392 | 0.00 | 0.36 | 0.00 | 0.368 | 0.00 | 22.8 ± 1.1 |
| **B** | 1.37 | 0.322 | 17.86 | 0.316 | 12.22 | 0.322 | 12.50 | |
| **C** | 4.12 | 0.265 | 32.40 | 0.274 | 23.89 | 0.277 | 24.73 | |
| **D** | 12.35 | 0.248 | 36.73 | 0.21 | 41.67 | 0.212 | 42.39 | |
| **E** | 37.04 | 0.138 | 64.80 | 0.109 | 69.72 | 0.106 | 71.19 | |
| **F** | 111.11 | 0.058 | 85.20 | 0.075 | 79.17 | 0.057 | 84.51 | |
| **G** | 333.33 | 0.003 | 99.23 | 0.008 | 97.78 | 0.004 | 98.91 | |
| **H** | 1000 | 0 | 100.0 | 0.001 | 99.72 | 0 | 100.0 | |

**(c)** Fluorouracil

| Well | Conc. (µg/ml) | Absorbance and % Cytotoxicity against HCC 1395 | | | | | | Mean $IC_{50}$ ± SEM n=3 |
|---|---|---|---|---|---|---|---|---|
| | | i $IC_{50}$ = 53.5 | | ii $IC_{50}$ = 34.5 | | Iii $IC_{50}$ = 28.4 | | |
| **A** | 0.00 | 0.474 | 0.00 | 0.483 | 0.00 | 0.497 | 0.00 | 38.8 ± 7.6 |
| **B** | 1.37 | 0.442 | 6.75 | 0.47 | 2.69 | 0.44 | 11.47 | |
| **C** | 4.12 | 0.433 | 8.65 | 0.449 | 7.04 | 0.421 | 15.29 | |
| **D** | 12.35 | 0.421 | 11.18 | 0.448 | 7.25 | 0.418 | 15.90 | |
| **E** | 37.04 | 0.302 | 36.29 | 0.215 | 55.49 | 0.158 | 68.21 | |
| **F** | 111.11 | 0.016 | 96.62 | 0.007 | 98.55 | 0.005 | 98.99 | |
| **G** | 333.33 | 0.003 | 99.37 | 0.003 | 99.38 | 0.003 | 99.40 | |
| **H** | 1000 | 0.001 | 99.79 | 0.001 | 99.79 | 0.003 | 99.40 | |

**Appendix 62: Mean $IC_{50}$ values for *F. angolensis* extracts against prostate cancer cell lines**

(a) *F. angolensis* stem bark methanol extract

| Well | Conc. (µg/ml) | Absorbance and % Cytotoxicity against DU-145 | | | | | | Mean $IC_{50}$ ± SEM n = 3 |
|---|---|---|---|---|---|---|---|---|
| | | i $IC_{50}$ = 11.7 | | ii $IC_{50}$ = 15.00 | | iii $IC_{50}$ = 11.7 | | |
| **A** | 0.00 | 0.639 | 0.00 | 0.652 | 0.00 | 0.61 | 0.00 | 12.8 ± 1.1 |
| **B** | 1.37 | 0.602 | 5.79 | 0.619 | 5.06 | 0.397 | 34.92 | |
| **C** | 4.12 | 0.483 | 24.41 | 0.41 | 37.12 | 0.372 | 39.02 | |
| **D** | 12.35 | 0.293 | 54.15 | 0.353 | 45.86 | 0.19 | 68.85 | |
| **E** | 37.04 | 0.128 | 79.97 | 0.136 | 79.14 | 0.028 | 95.41 | |
| **F** | 111.11 | 0.007 | 98.90 | 0.013 | 98.01 | 0.021 | 96.56 | |
| **G** | 333.33 | 0.002 | 99.69 | 0.013 | 98.01 | 0.01 | 98.36 | |
| **H** | 1000 | 0.001 | 99.84 | 0.003 | 99.54 | 0.004 | 99.34 | |

(b) *F. angolensis* stem bark water extract

| Well | Conc. (µg/ml) | Absorbance and % Cytotoxicity against DU-145 | | | | | | Mean $IC_{50}$ ± SEM n = 3 |
|---|---|---|---|---|---|---|---|---|
| | | i $IC_{50}$ = 122 | | ii $IC_{50}$ = 422 | | iii $IC_{50}$ = 398 | | |
| **A** | 0.00 | 0.598 | 0.00 | 0.574 | 0.00 | 0.563 | 0.00 | 314 ± 96.3 |
| **B** | 1.37 | 0.56 | 6.35 | 0.534 | 6.97 | 0.551 | 2.13 | |
| **C** | 4.12 | 0.557 | 6.87 | 0.512 | 10.80 | 0.548 | 2.67 | |
| **D** | 12.35 | 0.439 | 26.59 | 0.486 | 15.33 | 0.463 | 17.76 | |
| **E** | 37.04 | 0.423 | 29.26 | 0.462 | 19.51 | 0.429 | 23.80 | |
| **F** | 111.11 | 0.313 | 47.66 | 0.434 | 24.39 | 0.413 | 26.64 | |
| **G** | 333.33 | 0.137 | 77.09 | 0.317 | 44.77 | 0.3 | 46.71 | |
| **H** | 1000 | 0.018 | 96.99 | 0.106 | 81.53 | 0.061 | 89.19 | |

(c) *F. angolensis* stem bark methanol extract

| Well | Conc. (µg/ml) | Absorbance and % Cytotoxicity against 22Rv1 | | | | | | Mean $IC_{50}$ ± SEM n = 3 |
|---|---|---|---|---|---|---|---|---|
| | | i $IC_{50}$ = 75 | | ii $IC_{50}$ = 51.7 | | iii $IC_{50}$ = 58.3 | | |
| **A** | 0.00 | 0.427 | 0.00 | 0.475 | 0.00 | 0.417 | 0.00 | 61.7 ± 6.9 |
| **B** | 1.37 | 0.408 | 4.45 | 0.383 | 19.37 | 0.404 | 3.12 | |
| **C** | 4.12 | 0.402 | 5.85 | 0.373 | 21.47 | 0.377 | 9.59 | |
| **D** | 12.35 | 0.393 | 7.96 | 0.341 | 28.21 | 0.357 | 14.39 | |
| **E** | 37.04 | 0.341 | 20.14 | 0.242 | 49.05 | 0.304 | 27.10 | |
| **F** | 111.11 | 0.13 | 65.56 | 0.105 | 77.89 | 0.079 | 81.06 | |
| **G** | 333.33 | 0.119 | 74.95 | 0.082 | 82.74 | 0.026 | 93.76 | |
| **H** | 1000 | 0.11 | 76.84 | 0.062 | 86.95 | 0.002 | 99.52 | |

(d) *F. angolensis* stem bark water extract

| Well | Conc. (µg/ml) | Absorbance and % Cytotoxicity against 22Rv1 | | | | | | Mean $IC_{50}$ ± SEM n = 3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$ = 35 | | $IC_{50}$ = 35 | | $IC_{50}$ = 35 | | |
| **A** | 0.00 | 0.39 | 0.00 | 0.366 | 0.00 | 0.318 | 0.00 | 35 ± 0.0 |
| **B** | 1.37 | 0.335 | 14.10 | 0.306 | 16.39 | 0.252 | 20.75 | |
| **C** | 4.12 | 0.317 | 18.72 | 0.263 | 28.14 | 0.244 | 23.27 | |
| **D** | 12.35 | 0.258 | 33.85 | 0.261 | 28.69 | 0.212 | 33.33 | |
| **E** | 37.04 | 0.188 | 51.79 | 0.175 | 52.18 | 0.147 | 53.77 | |
| **F** | 111.11 | 0.168 | 56.92 | 0.167 | 54.37 | 0.12 | 62.26 | |
| **G** | 333.33 | 0.106 | 72.42 | 0.122 | 66.67 | 0.103 | 67.61 | |
| **H** | 1000 | 0.099 | 74.62 | 0.107 | 70.77 | 0.005 | 98.43 | |

**Appendix 63: Mean $IC_{50}$ values for *C. tanaense* extracts against prostate cancer cell lines**

(a) *C. tanaense* root methanol extract

| Well | Conc. (µg/ml) | Absorbance/ % Cytotoxicity against DU-145 | | | | | | Mean $IC_{50}$ ± SEM n = 3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$ = 72.5 | | $IC_{50}$ = 77.5 | | $IC_{50}$ = 71.7 | | |
| **A** | 0.00 | 0.453 | 0.00 | 0.471 | 0.00 | 0.766 | 0.00 | 73.9 ± 3.1 |
| **B** | 1.37 | 0.411 | 9.27 | 0.449 | 4.67 | 0.747 | 2.48 | |
| **C** | 4.12 | 0.4 | 11.70 | 0.428 | 9.13 | 0.661 | 13.71 | |
| **D** | 12.35 | 0.39 | 13.91 | 0.415 | 11.89 | 0.601 | 21.54 | |
| **E** | 37.04 | 0.341 | 24.72 | 0.369 | 21.66 | 0.586 | 23.50 | |
| **F** | 111.11 | 0.044 | 90.03 | 0.082 | 82.59 | 0.142 | 81.46 | |
| **G** | 333.33 | 0.039 | 91.39 | 0.042 | 91.08 | 0.064 | 91.64 | |
| **H** | 1000 | 0.005 | 98.90 | 0.003 | 99.36 | 0.005 | 99.35 | |

(b) *C. tanaense* root water extract

| Well | Conc. (µg/ml) | Absorbance/ % Cytotoxicity against DU-145 | | | | | | Mean $IC_{50}$ ± SEM n = 3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$ > 1000 | | $IC_{50}$ > 1000 | | $IC_{50}$ > 1000 | | |
| **A** | 0.00 | 0.794 | 0.00 | 0.702 | 0.00 | 0.697 | 0.00 | > 1000 |
| **B** | 1.37 | 0.749 | 5.67 | 0.664 | 5.41 | 0.64 | 8.18 | |
| **C** | 4.12 | 0.736 | 7.30 | 0.647 | 7.83 | 0.634 | 9.04 | |
| **D** | 12.35 | 0.729 | 8.19 | 0.612 | 12.82 | 0.596 | 14.49 | |
| **E** | 37.04 | 0.717 | 9.70 | 0.589 | 16.10 | 0.574 | 17.65 | |
| **F** | 111.11 | 0.674 | 15.11 | 0.546 | 22.22 | 0.569 | 18.36 | |
| **G** | 333.33 | 0.646 | 18.64 | 0.534 | 23.93 | 0.558 | 19.94 | |
| **H** | 1000 | 0.645 | 17.77 | 0.529 | 24.64 | 0.468 | 32.86 | |

(c) *C. tanaense* root water extract

| Well | Conc. (µg/ml) | Absorbance/ % Cytotoxicity against 22Rv1 | | | | | | Mean $IC_{50}$ ± SEM n = 3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$ = 198 | | $IC_{50}$ = 183 | | $IC_{50}$ = 228 | | |
| **A** | 0.00 | 0.454 | 0.00 | 0.503 | 0.00 | 0.477 | 0.00 | 203 ± 22.9 |
| **B** | 1.37 | 0.451 | 0.66 | 0.48 | 4.57 | 0.464 | 2.73 | |
| **C** | 4.12 | 0.389 | 14.32 | 0.435 | 13.52 | 0.413 | 13.42 | |
| **D** | 12.35 | 0.365 | 19.60 | 0.393 | 21.87 | 0.407 | 14.68 | |
| **E** | 37.04 | 0.307 | 32.38 | 0.37 | 26.44 | 0.357 | 25.16 | |
| **F** | 111.11 | 0.289 | 36.34 | 0.325 | 35.39 | 0.313 | 34.38 | |
| **G** | 333.33 | 0.148 | 67.40 | 0.13 | 74.16 | 0.174 | 63.52 | |
| **H** | 1000 | 0.141 | 68.94 | 0.105 | 79.13 | 0.121 | 74.63 | |

**Appendix 64: Mean $IC_{50}$ values for *U. anisatum* extracts against prostate cancer cell lines**

(a) *U. anisatum* root methanol extract

| Well | Conc. (µg/ml) | Absorbance/ % Cytotoxicity against DU-145 | | | | | | Mean $IC_{50}$ ± SEM n=3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$ = 81.7 | | $IC_{50}$ = 88.3 | | $IC_{50}$ =75 | | |
| **A** | 0.00 | 0.502 | 0.00 | 0.503 | 0.00 | 0.586 | 0.00 | 81.7 ± 3.9 |
| **B** | 1.37 | 0.452 | 10.16 | 0.466 | 7.36 | 0.512 | 12.63 | |
| **C** | 4.12 | 0.427 | 14.94 | 0.437 | 13.12 | 0.467 | 20.31 | |
| **D** | 12.35 | 0.394 | 21.51 | 0.414 | 17.69 | 0.455 | 22.35 | |
| **E** | 37.04 | 0.301 | 40.04 | 0.349 | 30.62 | 0.353 | 39.76 | |
| **F** | 111.11 | 0.209 | 58.36 | 0.219 | 56.46 | 0.249 | 57.50 | |
| **G** | 333.33 | 0.154 | 69.32 | 0.161 | 67.99 | 0.151 | 74.23 | |
| **H** | 1000 | 0.089 | 82.27 | 0.127 | 74.75 | 0.091 | 84.47 | |

(b) *U. anisatum* root water extract

| Well | Conc. (µg/ml) | Absorbance/ % Cytotoxicity against DU-145 | | | | Mean $IC_{50}$ ± SEM n = 2 |
|---|---|---|---|---|---|---|
| | | i | | ii | | |
| | | $IC_{50}$ = 645 | | $IC_{50}$ = 698 | | |
| **A** | 0.00 | 0.653 | 0.00 | 0.653 | 0.00 | 671.5 ± 26.5 |
| **B** | 1.37 | 0.61 | 6.58 | 0.637 | 2.45 | |
| **C** | 4.12 | 0.607 | 7.04 | 0.612 | 6.28 | |
| **D** | 12.35 | 0.574 | 12.10 | 0.594 | 9.04 | |
| **E** | 37.04 | 0.568 | 13.02 | 0.589 | 9.80 | |
| **F** | 111.11 | 0.518 | 20.67 | 0.535 | 18.07 | |
| **G** | 333.33 | 0.476 | 27.11 | 0.493 | 24.50 | |
| **H** | 1000 | 0.157 | 75.96 | 0.196 | 69.98 | |

(c) *U. anisatum* root methanol extract

| Well | Conc. (µg/ml) | Absorbance/ % Cytotoxicity against 22Rv1 | | | | | | Mean $IC_{50}$ ± SEM n = 3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$ = 30 | | $IC_{50}$ = 65 | | $IC_{50}$ = 12.3 | | |
| **A** | 0.00 | 0.37 | 0.00 | 0.365 | 0.00 | 0.562 | 0.00 | |
| **B** | 1.37 | 0.298 | 19.46 | 0.362 | 0.82 | 0.395 | 29.72 | 35.8 ± 15.5 |
| **C** | 4.12 | 0.283 | 23.51 | 0.352 | 3.56 | 0.34 | 39.50 | |
| **D** | 12.35 | 0.277 | 25.14 | 0.346 | 5.21 | 0.28 | 50.18 | |
| **E** | 37.04 | 0.127 | 65.68 | 0.221 | 39.45 | 0.197 | 64.95 | |
| **F** | 111.11 | 0.031 | 91.62 | 0.11 | 69.86 | 0.069 | 87.72 | |
| **G** | 333.33 | 0.03 | 91.89 | 0.095 | 73.97 | 0.047 | 91.64 | |
| **H** | 1000 | 0.002 | 99.46 | 0.09 | 75.34 | 0.016 | 97.15 | |

(d) *U. anisatum* root water extract

| Well | Conc. (µg/ml) | Absorbance/ % Cytotoxicity against 22Rv1 | | | | | | Mean $IC_{50}$ ± SEM n = 3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$= 648 | | $IC_{50}$ = 902 | | $IC_{50}$ = 922 | | |
| **A** | 0.00 | 0.475 | 0.00 | 0.501 | 0.00 | 0.501 | 0.00 | 824 ± 88.2 |
| **B** | 1.37 | 0.458 | 3.58 | 0.451 | 9.98 | 0.492 | 1.80 | |
| **C** | 4.12 | 0.455 | 4.21 | 0.436 | 12.97 | 0.457 | 8.78 | |
| **D** | 12.35 | 0.417 | 12.21 | 0.407 | 18.76 | 0.419 | 16.37 | |
| **E** | 37.04 | 0.334 | 29.68 | 0.383 | 23.55 | 0.407 | 18.76 | |
| **F** | 111.11 | 0.313 | 34.11 | 0.32 | 36.13 | 0.27 | 46.11 | |
| **G** | 333.33 | 0.299 | 37.05 | 0.26 | 48.10 | 0.26 | 48.10 | |
| **H** | 1000 | 0.475 | 0.00 | 0.248 | 50.50 | 0.197 | 50.10 | |

**Appendix 65: Mean $IC_{50}$ values for *H. abyssinica* extracts against prostate cancer cell lines**

(a) *H. abyssinica* rhizome methanol extract

| Well | Conc. (µg/ml) | Absorbance and % Cytotoxicity against DU-145 | | | | | | Mean $IC_{50}$ ± SEM n = 3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$ = 65 | | $IC_{50}$ =77.5 | | $IC_{50}$ = 57 | | |
| **A** | 0.00 | 0.219 | 0.00 | 0.216 | 0.00 | 0.218 | 0.00 | 66.5 ± 56 |
| **B** | 1.37 | 0.207 | 5.48 | 0.216 | 0.00 | 0.217 | 0.46 | |
| **C** | 4.12 | 0.197 | 10.05 | 0.211 | 2.31 | 0.19 | 12.84 | |
| **D** | 12.35 | 0.187 | 14.61 | 0.172 | 20.37 | 0.144 | 33.94 | |
| **E** | 37.04 | 0.158 | 27.85 | 0.166 | 23.15 | 0.141 | 35.32 | |
| **F** | 111.11 | 0.029 | 86.76 | 0.065 | 69.91 | 0.033 | 84.86 | |

(b) *H. abyssinica* rhizome water extract

| Well | Conc. (µg/ml) | Absorbance and % Cytotoxicity against DU-145 | | | | | | Mean $IC_{50}$ n=3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$ = 525 | | $IC_{50}$ = 565 | | $IC_{50}$ = 475 | | |
| **A** | 0.00 | 0.705 | 0.00 | 0.702 | 0.00 | 0.664 | 0.00 | 521.7 ± 26 |
| **B** | 1.37 | 0.671 | 4.82 | 0.69 | 1.71 | 0.635 | 4.94 | |
| **C** | 4.12 | 0.661 | 6.24 | 0.648 | 7.69 | 0.621 | 7.07 | |
| **D** | 12.35 | 0.618 | 12.34 | 0.642 | 8.55 | 0.587 | 12.13 | |
| **E** | 37.04 | 0.586 | 16.88 | 0.576 | 17.95 | 0.47 | 29.64 | |
| **F** | 111.11 | 0.476 | 32.48 | 0.45 | 35.90 | 0.444 | 33.53 | |
| **G** | 333.33 | 0.414 | 41.28 | 0.43 | 38.75 | 0.425 | 35.78 | |
| **H** | 1000 | 0.295 | 58.16 | 0.219 | 68.80 | 0.004 | 99.40 | |

(c) *H. abyssinica* flower methanol extract

| Well | Conc. (µg/ml) | Absorbance and % Cytotoxicity against DU 145 | | | | Mean $IC_{50}$± SEM n=2 |
|---|---|---|---|---|---|---|
| | | i | | ii | | |
| | | $IC_{50}$ = 150 | | $IC_{50}$ = 595 | | |
| **A** | 0.00 | 0.158 | 0.00 | 0.168 | 0.00 | 372.5 ± 222.5 |
| **B** | 1.37 | 0.147 | 6.96 | 0.153 | 8.93 | |
| **C** | 4.12 | 0.146 | 7.59 | 0.143 | 14.88 | |
| **D** | 12.35 | 0.133 | 15.82 | 0.142 | 15.48 | |
| **E** | 37.04 | 0.129 | 18.35 | 0.123 | 26.79 | |
| **F** | 111.11 | 0.083 | 47.47 | 0.122 | 18.45 | |
| **G** | 333.33 | 0.061 | 61.39 | 0.12 | 28.57 | |
| **H** | 1000 | 0.037 | 76.58 | 0.031 | 81.55 | |

(d) *H. abyssinica* flower water extract

| Well | Conc. (µg/ml) | Absorbance and % Cytotoxicity against DU 145 | | | | | | Mean $IC_{50}$ ± SEM n = 3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$ =730 | | $IC_{50}$ = 883 | | $IC_{50}$ = 678 | | |
| **A** | 0.00 | 0.2 | 0.00 | 0.178 | 0.00 | 0.163 | 0.00 | 763.7 ± 61.5 |
| **B** | 1.37 | 0.2 | 0.00 | 0.161 | 9.55 | 0.151 | 7.36 | |
| **C** | 4.12 | 0.174 | 13.00 | 0.156 | 12.36 | 0.149 | 8.59 | |
| **D** | 12.35 | 0.161 | 19.50 | 0.136 | 23.60 | 0.123 | 24.54 | |
| **E** | 37.04 | 0.156 | 22.00 | 0.134 | 24.72 | 0.119 | 26.99 | |
| **F** | 111.11 | 0.116 | 42.00 | 0.133 | 25.28 | 0.115 | 26.45 | |
| **G** | 333.33 | 0.14 | 30.00 | 0.132 | 25.84 | 0.1 | 38.65 | |
| **H** | 1000 | 0.071 | 64.50 | 0.081 | 54.49 | 0.064 | 60.74 | |

(e) *H. abyssinica* rhizome methanol extract

| Well | Conc. (µg/ml) | Absorbance and % Cytotoxicity against 22Rv1 | | | | | | Mean $IC_{50}$ ± SEM n=3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$ =120 | | $IC_{50}$ =135 | | $IC_{50}$ = 170 | | |
| **A** | 0.00 | 0.348 | 0.00 | 0.398 | 0.00 | 0.35 | 0.00 | 141.7 ± 14.8 |
| **B** | 1.37 | 0.319 | 8.33 | 0.381 | 4.27 | 0.342 | 2.29 | |
| **C** | 4.12 | 0.304 | 12.64 | 0.35 | 12.06 | 0.339 | 3.14 | |
| **D** | 12.35 | 0.285 | 18.10 | 0.349 | 12.31 | 0.331 | 5.43 | |
| **E** | 37.04 | 0.25 | 28.16 | 0.279 | 29.90 | 0.267 | 23.71 | |
| **F** | 111.11 | 0.179 | 48.56 | 0.218 | 45.23 | 0.225 | 35.71 | |
| **G** | 333.33 | 0.076 | 78.16 | 0.052 | 86.93 | 0.046 | 86.86 | |
| **H** | 1000 | 0.024 | 93.10 | 0.008 | 97.99 | 0.001 | 99.71 | |

(f) *H. abyssinica* rhizome water extract

| Well | Conc. (µg/ml) | Absorbance and % Cytotoxicity against 22Rv1 | | | | | | Mean $IC_{50}$ ± SEM n=3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$ =125 | | $IC_{50}$ =135 | | $IC_{50}$ =115 | | |
| **A** | 0.00 | 0.37 | 0.00 | 0.383 | 0.00 | 0.515 | 0.00 | 125 ± 5.8 |
| **B** | 1.37 | 0.341 | 7.84 | 0.349 | 8.88 | 0.457 | 11.26 | |
| **C** | 4.12 | 0.329 | 11.08 | 0.333 | 13.05 | 0.421 | 18.25 | |
| **D** | 12.35 | 0.31 | 16.22 | 0.314 | 18.02 | 0.418 | 18.83 | |
| **E** | 37.04 | 0.262 | 29.19 | 0.275 | 28.20 | 0.371 | 27.96 | |
| **F** | 111.11 | 0.195 | 47.29 | 0.214 | 44.13 | 0.27 | 47.57 | |
| **G** | 333.33 | 0.067 | 81.89 | 0.036 | 90.60 | 0.02 | 96.12 | |
| **H** | 1000 | 0.058 | 84.32 | 0.031 | 91.91 | 0.515 | 0.00 | |

(g) *H. abyssinica* flower methanol extract

| Well | Conc. (µg/ml) | Absorbance and % Cytotoxicity against 22Rv1 | | | | | | Mean $IC_{50}$ ± SEM n = 3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$ = 64 | | $IC_{50}$ = 83 | | $IC_{50}$ = 48 | | |
| **A** | 0.00 | 0.633 | 0.00 | 0.612 | 0.00 | 0.605 | 0.00 | 65±10.1 |
| **B** | 1.37 | 0.573 | 9.48 | 0.596 | 2.61 | 0.594 | 1.82 | |
| **C** | 4.12 | 0.504 | 20.38 | 0.589 | 3.76 | 0.502 | 17.02 | |
| **D** | 12.35 | 0.474 | 25.12 | 0.569 | 7.03 | 0.496 | 18.02 | |
| **E** | 37.04 | 0.399 | 36.97 | 0.527 | 13.89 | 0.358 | 40.83 | |
| **F** | 111.11 | 0.174 | 72.51 | 0.174 | 71.57 | 0.072 | 88.10 | |
| **G** | 333.33 | 0.00 | 100.00 | 0.00 | 100.00 | 0.004 | 99.34 | |

(h) *H. abyssinica* flower water extract

| Well | Conc. (µg/ml) | Absorbance and % Cytotoxicity against 22Rv1 | | | | | | Mean $IC_{50}$ ± SEM n = 3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$ = 21.7 | | $IC_{50}$ = 48.3 | | $IC_{50}$ = 88.3 | | |
| **A** | 0.00 | 1.18 | 0.00 | 0.609 | 0.00 | 0.631 | 0.00 | 52.8 ± 19.4 |
| **B** | 1.37 | 0.629 | 46.69 | 0.441 | 27.59 | 0.609 | 3.49 | |
| **C** | 4.12 | 0.622 | 47.28 | 0.424 | 30.38 | 0.567 | 10.14 | |
| **D** | 12.35 | 0.6 | 49.15 | 0.313 | 48.44 | 0.521 | 17.43 | |
| **E** | 37.04 | 0.567 | 51.95 | 0.307 | 49.59 | 0.413 | 34.55 | |
| **F** | 111.11 | 0.56 | 52.54 | 0.085 | 86.04 | 0.266 | 57.84 | |
| **G** | 333.33 | 0.366 | 68.98 | 0.035 | 94.25 | 0.226 | 64.18 | |
| **H** | 1000 | 0.001 | 99.91 | 0.001 | 99.84 | 0.001 | 99.84 | |

**Appendix 66: Mean $IC_{50}$ values for *L. cornuta* extracts against prostate cancer cell lines**

(a) *L. cornuta* leaf methanol extract

| Well | Conc. (µg/ml) | Absorbance and % Cytotoxicity against DU-145 | | | | | | Mean $IC_{50}$± SEM n=3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$=292 | | $IC_{50}$=285 | | $IC_{50}$= 292 | | |
| **A** | 0.00 | 0.697 | 0.00 | 0.743 | 0.00 | 0.401 | 0.00 | 289.7±2.3 |
| **B** | 1.37 | 0.67 | 3.87 | 0.71 | 4.44 | 0.4 | 0.25 | |
| **C** | 4.12 | 0.667 | 4.30 | 0.683 | 8.08 | 0.396 | 1.25 | |
| **D** | 12.35 | 0.643 | 7.75 | 0.673 | 9.42 | 0.385 | 4.00 | |
| **E** | 37.04 | 0.593 | 14.92 | 0.636 | 14.40 | 0.36 | 12.47 | |
| **F** | 111.11 | 0.578 | 17.07 | 0.603 | 18.84 | 0.333 | 16.96 | |
| **G** | 333.33 | 0.31 | 55.52 | 0.311 | 58.14 | 0.174 | 56.61 | |
| **H** | 1000 | 0.032 | 95.41 | 0.049 | 93.41 | 0.014 | 96.51 | |

(b) *L. cornuta* leaf water extract

| Well | Conc. (µg/ml) | Absorbance and % Cytotoxicity against DU-145 | | | | | | Mean $IC_{50}$± SEM n=3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$ = 65.0 | | $IC_{50}$ = 155 | | $IC_{50}$ = 208 | | |
| **A** | 0.00 | 0.677 | 0.00 | 0.655 | 0.00 | 0.657 | 0.00 | 142.7 ± 41.7 |
| **B** | 1.37 | 0.547 | 19.20 | 0.548 | 16.34 | 0.62 | 5.63 | |
| **C** | 4.12 | 0.479 | 29.25 | 0.544 | 16.95 | 0.608 | 7.56 | |
| **D** | 12.35 | 0.476 | 29.69 | 0.505 | 22.90 | 0.57 | 13.24 | |
| **E** | 37.04 | 0.447 | 33.97 | 0.442 | 32.52 | 0.461 | 29.83 | |
| **F** | 111.11 | 0.159 | 76.51 | 0.345 | 47.32 | 0.419 | 36.23 | |
| **G** | 333.33 | 0.025 | 96.31 | 0.216 | 67.02 | 0.193 | 70.62 | |
| **H** | 1000 | 0.003 | 99.56 | 0.006 | 99.08 | 0.01 | 98.48 | |

(c) *L. cornuta* leaf methanol extract

| Well | Conc. (µg/ml) | Absorbance and % Cytotoxicity against 22Rv1 | | | | | | Mean $IC_{50}$ ± SEM n = 3 |
|---|---|---|---|---|---|---|---|---|
| | | i $IC_{50}$ = 555 | | ii $IC_{50}$ = 475 | | iii $IC_{50}$ = 758 | | |
| **A** | 0.00 | 0.541 | 0.00 | 0.637 | 0.00 | 0.629 | 0.00 | 596 ± 84.2 |
| **B** | 1.37 | 0.528 | 2.40 | 0.538 | 15.54 | 0.575 | 8.59 | |
| **C** | 4.12 | 0.519 | 4.07 | 0.51 | 19.94 | 0.475 | 24.48 | |
| **D** | 12.35 | 0.426 | 21.26 | 0.482 | 24.33 | 0.469 | 25.44 | |
| **E** | 37.04 | 0.424 | 21.63 | 0.469 | 26.37 | 0.396 | 37.04 | |
| **F** | 111.11 | 0.419 | 22.55 | 0.422 | 33.75 | 0.394 | 37.36 | |
| **G** | 333.33 | 0.317 | 41.40 | 0.377 | 40.82 | 0.369 | 41.34 | |
| **H** | 1000 | 0.172 | 68.21 | 0.098 | 84.62 | 0.072 | 55.7 | |

(d) *L. cornuta* leaf water extract

| Well | Conc. (µg/ml) | Absorbance and % Cytotoxicity against 22Rv1 | | | | | | Mean $IC_{50}$ ± SEM n = 3 |
|---|---|---|---|---|---|---|---|---|
| | | i $IC_{50}$ = 605 | | ii $IC_{50}$ = 532 | | iii $IC_{50}$ = 768 | | |
| **A** | 0.00 | 0.478 | 0.00 | 0.507 | 0.00 | 0.465 | 0.00 | 635 ± 69.7 |
| **B** | 1.37 | 0.456 | 4.82 | 0.479 | 5.52 | 0.461 | 0.86 | |
| **C** | 4.12 | 0.449 | 6.07 | 0.448 | 11.64 | 0.439 | 5.59 | |
| **D** | 12.35 | 0.428 | 10.46 | 0.442 | 12.82 | 0.429 | 7.74 | |
| **E** | 37.04 | 0.407 | 14.85 | 0.439 | 13.41 | 0.424 | 8.82 | |
| **F** | 111.11 | 0.32 | 33.05 | 0.416 | 17.95 | 0.417 | 10.32 | |
| **G** | 333.33 | 0.288 | 39.75 | 0.296 | 41.61 | 0.389 | 16.34 | |
| **H** | 1000 | 0.172 | 64.02 | 0.149 | 70.61 | 0.152 | 67.31 | |

**Appendix 67: Mean $IC_{50}$ values for *S. princeae* extracts against prostate cancer cell lines**

(e) *S. princeae* aerial methanol extract

| Well | Conc. (µg/ml) | Absorbance/ % Cytotoxicity against DU-145 | | | | | | Mean $IC_{50}$ ± SEM n = 3 |
|---|---|---|---|---|---|---|---|---|
| | | i $IC_{50}$ = 185 | | ii $IC_{50}$ = 135 | | iii $IC_{50}$ = 135 | | |
| **A** | 0.00 | 0.474 | 0.00 | 0.374 | 0.00 | 0.418 | 0.00 | 151.7 ± 16.7 |
| **B** | 1.37 | 0.456 | 3.80 | 0.359 | 4.01 | 0.341 | 18.42 | |
| **C** | 4.12 | 0.401 | 15.40 | 0.238 | 36.36 | 0.308 | 26.32 | |
| **D** | 12.35 | 0.386 | 18.57 | 0.199 | 46.79 | 0.281 | 32.76 | |
| **E** | 37.04 | 0.317 | 33.12 | 0.192 | 48.66 | 0.253 | 39.47 | |
| **F** | 111.11 | 0.245 | 48.31 | 0.189 | 49.47 | 0.215 | 48.56 | |
| **G** | 333.33 | 0.217 | 54.22 | 0.181 | 51.60 | 0.155 | 62.92 | |
| **H** | 1000 | 0.155 | 67.30 | 0.087 | 76.74 | 0.091 | 78.23 | |

(f) *S. princeae* aerial water extract

| Well | Conc. (µg/ml) | Absorbance/ % Cytotoxicity against DU-145 | | | | | | Mean $IC_{50}$ ± SEM n = 3 |
|---|---|---|---|---|---|---|---|---|
| | | i $IC_{50}$ = 388 | | ii $IC_{50}$ = 258 | | iii $IC_{50}$ = 338 | | |
| **A** | 0.00 | 0.402 | 0.00 | 0.447 | 0.00 | 0.409 | 0.00 | 328.7 ± 37.9 |
| **B** | 1.37 | 0.36 | 10.45 | 0.401 | 10.29 | 0.382 | 6.60 | |
| **C** | 4.12 | 0.335 | 16.67 | 0.384 | 14.09 | 0.381 | 6.85 | |
| **D** | 12.35 | 0.333 | 17.16 | 0.345 | 22.82 | 0.351 | 14.18 | |
| **E** | 37.04 | 0.314 | 21.89 | 0.278 | 37.81 | 0.348 | 14.91 | |
| **F** | 111.11 | 0.305 | 24.13 | 0.259 | 42.06 | 0.283 | 30.81 | |
| **G** | 333.33 | 0.205 | 49.75 | 0.207 | 53.69 | 0.204 | 50.12 | |
| **H** | 1000 | 0.205 | 49.75 | 0.167 | 62.64 | 0.16 | 61.33 | |

(g) *S. princeae* aerial methanol extract

| Well | Conc. (µg/ml) | Absorbance/ % Cytotoxicity against 22Rv1 | | | | | | Mean $IC_{50}$ ± SEM n = 3 |
|---|---|---|---|---|---|---|---|---|
| | | i $IC_{50}$ = 280 | | ii $IC_{50}$ = 475 | | iii $IC_{50}$ = 265 | | |
| **A** | 0.00 | 0.327 | 0.00 | 0.336 | 0.00 | 0.296 | 0.00 | 340 ± 67.6 |
| **B** | 1.37 | 0.299 | 8.56 | 0.306 | 8.93 | 0.286 | 3.38 | |
| **C** | 4.12 | 0.298 | 8.87 | 0.276 | 17.86 | 0.273 | 7.77 | |
| **D** | 12.35 | 0.248 | 24.16 | 0.247 | 26.49 | 0.266 | 10.14 | |
| **E** | 37.04 | 0.245 | 25.08 | 0.228 | 32.14 | 0.218 | 26.35 | |
| **F** | 111.11 | 0.219 | 33.03 | 0.212 | 36.90 | 0.194 | 34.46 | |
| **G** | 333.33 | 0.145 | 55.66 | 0.208 | 38.10 | 0.13 | 56.08 | |
| **H** | 1000 | 0.069 | 78.90 | 0.021 | 93.75 | 0.008 | 97.30 | |

(h) *S. princeae* aerial water extract

| Well | Conc. (µg/ml) | Absorbance/ % Cytotoxicity against 22Rv1 | | | | | | Mean $IC_{50}$ ± SEM n = 3 |
|---|---|---|---|---|---|---|---|---|
| | | i $IC_{50}$ = 525 | | ii $IC_{50}$ = 450 | | iii $IC_{50}$ = 615 | | |
| **A** | 0.00 | 0.422 | 0.00 | 0.457 | 0.00 | 0.435 | 0.00 | 530 ± 47.7 |
| **B** | 1.37 | 0.332 | 21.33 | 0.343 | 24.95 | 0.35 | 19.54 | |
| **C** | 4.12 | 0.303 | 28.20 | 0.308 | 32.60 | 0.303 | 30.34 | |
| **D** | 12.35 | 0.274 | 35.07 | 0.259 | 43.33 | 0.295 | 32.18 | |
| **E** | 37.04 | 0.266 | 36.97 | 0.258 | 43.54 | 0.282 | 35.17 | |
| **F** | 111.11 | 0.265 | 37.20 | 0.254 | 44.42 | 0.281 | 35.40 | |
| **G** | 333.33 | 0.228 | 45.97 | 0.24 | 47.48 | 0.263 | 39.54 | |
| **H** | 1000 | 0.169 | 59.95 | 0.182 | 60.18 | 0.155 | 64.37 | |

**Appendix 68: Mean $IC_{50}$ values for *P. africana* extracts against prostate cancer cell lines**

(a) *P. africana* stem bark methanol extract

| Well | Conc. (µg/ml) | Absorbance and % Cytotoxicity against DU 145 | | | | | | Mean $IC_{50}$ ± SEM n = 3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$ = 20.00 | | $IC_{50}$ = 21.7 | | $IC_{50}$ = 31.5 | | |
| **A** | 0.00 | 0.613 | 0.00 | 0.614 | 0.00 | 0.585 | 0.00 | 24.4 ± 3.6 |
| **B** | 1.37 | 0.469 | 23.49 | 0.452 | 26.38 | 0.455 | 22.22 | |
| **C** | 4.12 | 0.453 | 26.10 | 0.297 | 35.57 | 0.406 | 30.60 | |
| **D** | 12.35 | 0.367 | 40.13 | 0.351 | 42.83 | 0.383 | 32.53 | |
| **E** | 37.04 | 0.152 | 75.20 | 0.166 | 72.96 | 0.263 | 55.04 | |
| **F2** | 111.11 | 0.057 | 90.70 | 0.086 | 85.99 | 0.176 | 69.91 | |
| **G** | 333.33 | 0.034 | 94.45 | 0.011 | 98.21 | 0.00 | 0.00 | |
| **H** | 1000 | 0.00 | 100.00 | 0.001 | 99.84 | 0.00 | 100.00 | |

(b) *P. africana* stem bark water extract

| Well | Conc. (µg/ml) | Absorbance and % Cytotoxicity against DU 145 | | | | | | Mean $IC_{50}$ ± SEM n = 3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$ =18.2 | | $IC_{50}$ = 20.8 | | $IC_{50}$ = 20.8 | | |
| **A** | 0.00 | 0.648 | 0.00 | 0.446 | 0.00 | 0.605 | 0.00 | 19.9 ± 0.9 |
| **B** | 1.37 | 0.485 | 25.15 | 0.31 | 30.91 | 0.511 | 15.70 | |
| **C** | 4.12 | 0.361 | 44.29 | 0.293 | 34.30 | 0.412 | 31.90 | |
| **D** | 12.35 | 0.332 | 48.77 | 0.263 | 41.03 | 0.353 | 41.65 | |
| **E** | 37.04 | 0.304 | 53.09 | 0.149 | 66.59 | 0.222 | 63.31 | |
| **F** | 111.11 | 0.228 | 64.81 | 0.144 | 67.71 | 0.074 | 87.77 | |
| **G** | 333.33 | 0.014 | 97.84 | 0.00 | 100.00 | 0.00 | 100.00 | |

(c) *P. africana* stem bark methanol extract

| Well | Conc. (µg/ml) | Absorbance and % Cytotoxicity against 22Rv1 | | | | | | Mean $IC_{50}$ ± SEM n = 3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$ = 18.5 | | $IC_{50}$ = 10.1 | | $IC_{50}$ = 30.2 | | |
| **A** | 0.00 | 0.613 | 0.00 | 0.614 | 0.00 | 0.585 | 0.00 | 19.6 ± 5.8 |
| **B** | 1.37 | 0.469 | 23.49 | 0.452 | 26.38 | 0.455 | 22.22 | |
| **C** | 4.12 | 0.453 | 26.10 | 0.351 | 42.83 | 0.406 | 30.60 | |
| **D** | 12.35 | 0.367 | 40.13 | 0.297 | 52.77 | 0.383 | 34.52 | |
| **E** | 37.04 | 0.152 | 75.20 | 0.166 | 72.96 | 0.263 | 55.04 | |
| **F** | 111.11 | 0.057 | 90.70 | 0.086 | 85.99 | 0.176 | 69.91 | |
| **G** | 333.33 | 0.034 | 94.45 | 0.011 | 98.21 | 0.00 | 100.00 | |
| **H** | 1000 | 0.00 | 100.00 | 0.001 | 99.84 | 0.00 | 100.00 | |

(d) *P. africana* stem bark water extract

| Well | Conc. (µg/ml) | Absorbance and % Cytotoxicity against 22Rv1 | | | | | | Mean $IC_{50}$ ± SEM n = 3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$ =19.1 | | $IC_{50}$ = 21.5 | | $IC_{50}$ = 21.5 | | |
| **A** | 0.00 | 0.648 | 0.00 | 0.446 | 0.00 | 0.605 | 0.00 | 20.7 ± 0.8 |
| **B** | 1.37 | 0.485 | 25.15 | 0.31 | 30.91 | 0.511 | 15.70 | |
| **C** | 4.12 | 0.361 | 44.29 | 0.293 | 34.30 | 0.412 | 31.90 | |
| **D** | 12.35 | 0.332 | 48.77 | 0.263 | 41.03 | 0.353 | 41.65 | |
| **E** | 37.04 | 0.324 | 50.00 | 0.149 | 66.59 | 0.222 | 63.31 | |
| **F** | 111.11 | 0.228 | 64.81 | 0.144 | 67.71 | 0.074 | 87.77 | |
| **G** | 333.33 | 0.014 | 97.84 | 0.00 | 100.00 | 0.00 | 100.00 | |

**Appendix 69: Mean $IC_{50}$ values for standards against prostate cancer cell lines**

(a) Cyclophosphamide

| Well | Conc. (µg/ml) | Absorbance and % Cytotoxicity against DU-145 | | | | | | Mean $IC_{50}$ ± SEM n = 3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$ = 28.3 | | $IC_{50}$ = 25.0 | | $IC_{50}$ =18.3 | | |
| **A** | 0.00 | 0.45 | 0.00 | 0.435 | 0.00 | 0.382 | 0.00 | 23.9 ± 2.9 |
| **B** | 1.37 | 0.414 | 8.00 | 0.382 | 12.18 | 0.337 | 11.78 | |
| **C** | 4.12 | 0.411 | 8.67 | 0.374 | 14.02 | 0.318 | 16.75 | |
| **D** | 12.35 | 0.354 | 21.33 | 0.267 | 38.62 | 0.182 | 52.36 | |
| **E** | 37.04 | 0.199 | 55.78 | 0.16 | 63.22 | 0.131 | 65.71 | |
| **F** | 111.11 | 0.066 | 85.33 | 0.049 | 88.73 | 0.023 | 93.98 | |
| **G** | 333.33 | 0.00 | 100.0 | 0.003 | 99.31 | 0.00 | 100.0 | |
| **H** | 1000 | 0.00 | 100.0 | 0.002 | 99.54 | 0.00 | 100.0 | |

(b) Fluorouracil

| Well | Conc. (µg/ml) | Absorbance and % Cytotoxicity against DU-145 | | | | | | Mean $IC_{50}$ ± SEM n = 3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$ = 11.9 | | $IC_{50}$ = 12.5 | | $IC_{50}$ = 30.5 | | |
| **A** | 0.00 | 0.231 | 0.00 | 0.22 | 0.00 | 0.219 | 0.00 | 18.3 ± 6.1 |
| **B** | 1.37 | 0.176 | 23.81 | 0.132 | 40.00 | 0.152 | 30.59 | |
| **C** | 4.12 | 0.152 | 34.20 | 0.129 | 41.36 | 0.131 | 40.18 | |
| **D** | 12.35 | 0.113 | 51.08 | 0.11 | 50.00 | 0.119 | 45.66 | |
| **E** | 37.04 | 0.088 | 61.9 | 0.094 | 57.27 | 0.106 | 51.60 | |
| **F** | 111.11 | 0.075 | 67.53 | 0.089 | 59.55 | 0.083 | 62.00 | |
| **G** | 333.33 | 0.069 | 70.13 | 0.078 | 64.55 | 0.083 | 62.00 | |
| **H** | 1000 | 0.042 | 81.82 | 0.046 | 79.09 | 0.044 | 79.91 | |

(c) Fluorouracil

| Well | Conc. (µg/ml) | Absorbance and % Cytotoxicity against 22Rv1 | | | | | | Mean $IC_{50}$ ± SEM n = 3 |
|---|---|---|---|---|---|---|---|---|
| | | i $IC_{50}$ = 49.03 | | ii $IC_{50}$ =15 | | iii $IC_{50}$ = 11 | | |
| **A** | 0.00 | 0.452 | 0.00 | 0.456 | 0.00 | 0.448 | 0.00 | 25 ± 12.07 |
| **B** | 1.37 | 0.269 | 40.49 | 0.275 | 39.69 | 0.252 | 43.75 | |
| **C** | 4.12 | 0.263 | 41.81 | 0.254 | 44.30 | 0.245 | 41.60 | |
| **D** | 12.35 | 0.248 | 45.13 | 0.231 | 49.34 | 0.237 | 51.43 | |
| **E** | 37.04 | 0.233 | 48.45 | 0.211 | 53.73 | 0.199 | 59.22 | |
| **F** | 111.11 | 0.193 | 57.30 | 0.195 | 57.24 | 0.176 | 63.39 | |
| **G** | 333.33 | 0.168 | 62.83 | 0.158 | 65.35 | 0.171 | 64.96 | |
| **H** | 1000 | 0.157 | 65.27 | 0.151 | 66.89 | 0.16 | 67.21 | |

**Appendix 70: Mean $IC_{50}$ values for *F. angolensis* fractions against breast cancer cell lines**

(a) Petroleum ether fraction from *F. angolensis* stem bark methanol extract

| Well | Conc. (µg/ml) | Absorbance and % Cytotoxicity against HCC 1395 | | | | | | Mean $IC_{50}$± SEM n=3 |
|---|---|---|---|---|---|---|---|---|
| | | i $IC_{50}$ = 60 | | ii $IC_{50}$ = 90 | | iii $IC_{50}$ = 40 | | |
| **A** | 0.00 | 0.456 | 0.00 | 0.523 | 0.00 | 0.576 | 0.00 | 63.3 ± 14.5 |
| **B** | 1.37 | 0.456 | 0.00 | 0.433 | 17.21 | 0.464 | 19.44 | |
| **C** | 4.12 | 0.373 | 18.20 | 0.409 | 21.80 | 0.459 | 20.31 | |
| **D** | 12.35 | 0.343 | 24.78 | 0.381 | 24.36 | 0.429 | 25.52 | |
| **E** | 37.04 | 0.319 | 30.04 | 0.331 | 36.71 | 0.286 | 50.35 | |
| **F** | 111.11 | 0.23 | 94.97 | 0.239 | 54.30 | 0.226 | 60.76 | |
| **G** | 333.33 | 0.008 | 98.25 | 0.007 | 98.66 | 0.026 | 95.49 | |
| **H** | 1000 | 0 | 100.00 | 0.005 | 99.04 | 0.018 | 96.88 | |

(b) Dichloromethane fraction from *F. angolensis* stem bark methanol extract

| Well | Conc. (µg/ml) | Absorbance/ and % Cytotoxicity against HCC 1395 | | | | | | Mean $IC_{50}$± SEM n=3 |
|---|---|---|---|---|---|---|---|---|
| | | i $IC_{50}$ = 10 | | ii $IC_{50}$ = 10 | | iii $IC_{50}$ =15 | | |
| **A** | 0.00 | 0.509 | 0.00 | 0.556 | 0.00 | 0.596 | 0.00 | 11.7 ± 1.7 |
| **B** | 1.37 | 0.402 | 21.02 | 0.346 | 37.76 | 0.408 | 31.54 | |
| **C** | 4.12 | 0.306 | 39.88 | 0.285 | 48.74 | 0.362 | 39.26 | |
| **D** | 12.35 | 0.261 | 48.72 | 0.267 | 51.98 | 0.333 | 44.13 | |
| **E** | 37.04 | 0.104 | 79.57 | 0.112 | 79.86 | 0.059 | 90.10 | |
| **F** | 111.11 | 0.004 | 99.21 | 0.02 | 96.40 | 0.052 | 91.28 | |
| **G** | 333.33 | 0.002 | 99.61 | 0.011 | 98.02 | 0.04 | 93.29 | |
| **H** | 1000 | 0 | 100.00 | 0.006 | 98.98 | 0.039 | 93.45 | |

(c) Ethyl acetate fraction from *F. angolensis* stem bark methanol extract

| Well | Conc. (µg/ml) | Absorbance and % Cytotoxicity against HCC 1395 | | | | | | Mean $IC_{50}$± SEM n=3 |
|---|---|---|---|---|---|---|---|---|
| | | i $IC_{50}$ = 220 | | ii $IC_{50}$ = 215 | | iii $IC_{50}$ = 175 | | |
| **A** | 0.00 | 0.311 | 0.00 | 0.554 | 0.00 | 0.528 | 0.00 | 203.3 ± |
| **B** | 1.37 | 0.285 | 8.36 | 0.498 | 10.11 | 0.496 | 6.06 | 14.2 |
| **C** | 4.12 | 0.342 | 9.97 | 0.491 | 11.37 | 0.513 | 2.84 | |
| **D** | 12.35 | 0.275 | 11.58 | 0.474 | 14.44 | 0.497 | 5.87 | |
| **E** | 37.04 | 0.369 | 18.65 | 0.402 | 27.44 | 0.399 | 24.43 | |
| **F** | 111.11 | 0.398 | 27.97 | 0.359 | 35.20 | 0.309 | 41.48 | |
| **G** | 333.33 | 0.089 | 71.38 | 0.191 | 65.52 | 0.143 | 72.92 | |
| **H** | 1000 | 0 | 100.0 | 0.017 | 96.93 | 0.001 | 99.81 | |

(d) Acetone fraction from *F. angolensis* stem bark methanol extract

| Well | Conc. (µg/ml) | Absorbance and % Cytotoxicity against HCC 1395 | | | | | | Mean $IC_{50}$ ± SEM n = 3 |
|---|---|---|---|---|---|---|---|---|
| | | i $IC_{50}$ = 70 | | ii $IC_{50}$ = 100 | | iii $IC_{50}$ = 95 | | |
| **A** | 0.00 | 0.454 | 0.00 | 0.389 | 0.00 | 0.424 | 0.00 | 88.9 ± 9.3 |
| **B** | 1.37 | 0.432 | 4.85 | 0.383 | 4.11 | 0.42 | 0.01 | |
| **C** | 4.12 | 0.374 | 17.62 | 0.373 | 6.68 | 0.404 | 0.05 | |
| **D** | 12.35 | 0.342 | 24.67 | 0.368 | 7.97 | 0.384 | 0.09 | |
| **E** | 37.04 | 0.279 | 38.54 | 0.291 | 25.19 | 0.302 | 28.77 | |
| **F** | 111.11 | 0.164 | 63.88 | 0.18 | 53.73 | 0.185 | 56.36 | |
| **G** | 333.33 | 0.042 | 90.75 | 0.096 | 75.32 | 0.085 | 79.95 | |
| **H** | 1000 | 0.021 | 95.37 | 0.096 | 75.32 | 0.076 | 82.08 | |

(e) Methanol fraction from *F. angolensis* stem bark methanol extract

| Well | Conc. (µg/ml) | Absorbance and % Cytotoxicity against HCC 1395 | | | | | | Mean $IC_{50}$± SEM n=3 |
|---|---|---|---|---|---|---|---|---|
| | | i $IC_{50}$ = 290 | | ii $IC_{50}$ = 200 | | iii $IC_{50}$ = 220 | | |
| **A** | 0.00 | 0.377 | 0.00 | 0.497 | 0.00 | 0.435 | 0.00 | 236.7 ± 27.3 |
| **B** | 1.37 | 0.347 | 7.96 | 0.384 | 22.74 | 0.43 | 1.15 | |
| **C** | 4.12 | 0.347 | 7.96 | 0.382 | 23.14 | 0.416 | 4.36 | |
| **D** | 12.35 | 0.341 | 9.55 | 0.361 | 27.36 | 0.406 | 6.67 | |
| **E** | 37.04 | 0.336 | 10.88 | 0.351 | 29.38 | 0.364 | 16.32 | |
| **F** | 111.11 | 0.325 | 13.79 | 0.331 | 33.40 | 0.31 | 28.74 | |
| **G** | 333.33 | 0.158 | 58.09 | 0.134 | 73.04 | 0.119 | 72.64 | |
| **H** | 1000 | 0.052 | 86.21 | 0.106 | 78.67 | 0.078 | 82.07 | |

**Appendix 71: Mean $IC_{50}$ values for *F. angolensis* fractions against prostate cancer cell lines**

(a) Petroleum ether fraction from *F. angolensis* stem bark methanol extract

| Well | Conc. (µg/ml) | Absorbance and % Cytotoxicity against DU-145 | | | | | | Mean $IC_{50}$ ± SEM n = 3 |
|---|---|---|---|---|---|---|---|---|
| | | i $IC_{50}$ = 190 | | ii $IC_{50}$ = 180 | | iii $IC_{50}$ = 175 | | |
| **A** | 0.00 | 0.345 | 0.00 | 0.337 | 0.00 | 0.326 | 0.00 | 181.7 ± 4.4 |
| **B** | 1.37 | 0.29 | 15.94 | 0.327 | 2.97 | 0.264 | 19.02 | |
| **C** | 4.12 | 0.281 | 18.55 | 0.28 | 16.91 | 0.251 | 23.01 | |
| **D** | 12.35 | 0.257 | 25.50 | 0.265 | 21.36 | 0.25 | 23.31 | |
| **E** | 37.04 | 0.246 | 28.70 | 0.26 | 22.85 | 0.223 | 31.60 | |
| **F** | 111.11 | 0.246 | 28.70 | 0.239 | 29.08 | 0.221 | 32.21 | |
| **G** | 333.33 | 0.037 | 89.28 | 0.018 | 94.66 | 0.028 | 91.41 | |
| **H** | 1000 | 0.021 | 93.91 | 0.003 | 99.11 | 0.001 | 99.69 | |

(b) Dichloromethane fraction from *F. angolensis* stem bark methanol extract

| Well | Conc. (µg/ml) | Absorbance and % Cytotoxicity against DU-145 | | | | | | Mean $IC_{50}$ ± SEM n=3 |
|---|---|---|---|---|---|---|---|---|
| | | i IC50 = 37.5 | | ii IC50 = 29.3 | | iii IC50 = 26.7 | | |
| **A** | 0.00 | 0.444 | 0.00 | 0.383 | 0.00 | 0.426 | 0.00 | 31.2 ± 3.3 |
| **B** | 1.37 | 0.417 | 6.08 | 0.332 | 13.32 | 0.401 | 6.23 | |
| **C** | 4.12 | 0.338 | 23.87 | 0.304 | 20.63 | 0.361 | 15.26 | |
| **D** | 12.35 | 0.304 | 31.53 | 0.286 | 25.33 | 0.333 | 21.83 | |
| **E** | 37.04 | 0.224 | 49.55 | 0.156 | 59.27 | 0.082 | 80.75 | |
| **F** | 111.11 | 0.029 | 93.47 | 0.016 | 95.82 | 0.048 | 88.73 | |
| **G** | 333.33 | 0.017 | 96.17 | 0.009 | 97.65 | 0.019 | 95.54 | |
| **H** | 1000 | 0.005 | 98.87 | | | 0.01 | 97.65 | |

(c) Ethyl acetate fraction from *F. angolensis* stem bark methanol extract

| Well | Conc. (µg/ml) | Absorbance and % Cytotoxicity against DU 145 | | | | | | Mean $IC_{50}$± SEM n=3 |
|---|---|---|---|---|---|---|---|---|
| | | i $IC_{50}$ =80 | | ii $IC_{50}$=110 | | iii $IC_{50}$=130 | | |
| **A** | 0.00 | 0.374 | 0.00 | 0.356 | 0.00 | 0.339 | 0.00 | 106.7±14.5 |
| **B** | 1.37 | 0.279 | 25.40 | 0.328 | 7.87 | 0.329 | 2.95 | |
| **C** | 4.12 | 0.279 | 25.40 | 0.319 | 10.39 | 0.31 | 8.55 | |
| **D** | 12.35 | 0.24 | 35.83 | 0.312 | 12.36 | 0.301 | 11.21 | |
| **E** | 37.04 | 0.218 | 41.71 | 0.284 | 20.22 | 0.214 | 36.87 | |
| **F** | 111.11 | 0.166 | 55.61 | 0.179 | 49.72 | 0.182 | 46.31 | |
| **G** | 333.33 | 0.001 | 99.73 | 0.01 | 97.19 | 0.045 | 86.73 | |
| **H** | 1000 | 0 | 100.00 | 0.004 | 98.88 | 0.028 | 91.74 | |

(d) Acetone fraction from *F. angolensis* stem bark methanol extract

| Well | Conc. (µg/ml) | Absorbance and % Cytotoxicity against DU -145 | | | | | | Mean $IC_{50}$ ± SEM n = 3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$ = 265 | | $IC_{50}$ = 275 | | $IC_{50}$ =105 | | |
| **A** | 0.00 | 0.328 | 0.00 | 0.321 | 0.00 | 0.375 | 0.00 | 215 ± 55.1 |
| **B** | 1.37 | 0.303 | 7.62 | 0.307 | 4.36 | 0.337 | 10.13 | |
| **C** | 4.12 | 0.301 | 8.23 | 0.301 | 6.23 | 0.31 | 17.33 | |
| **D** | 12.35 | 0.283 | 13.72 | 0.254 | 20.87 | 0.261 | 30.40 | |
| **E** | 37.04 | 0.23 | 29.88 | 0.236 | 26.48 | 0.23 | 38.67 | |
| **F** | 111.11 | 0.224 | 31.70 | 0.219 | 31.78 | 0.184 | 51.07 | |
| **G** | 333.33 | 0.14 | 57.32 | 0.143 | 55.45 | 0.01 | 97.33 | |
| **H** | 1000 | 0.08 | 75.61 | 0.063 | 80.37 | 0.004 | 98.93 | |

(e) Methanol fraction from *F. angolensis* stem bark methanol extract

| Well | Conc. (µg/ml) | Absorbance and % Cytotoxicity against DU-145 | | | | | | Mean $IC_{50}$ ± SEM n = 3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$ = 30 | | $IC_{50}$ = 30.7 | | $IC_{50}$ = 35 | | |
| **A** | 0.00 | 0.426 | 0.00 | 0.383 | 0.00 | 0.444 | 0.00 | 31.9 ± 1.6 |
| **B** | 1.37 | 0.401 | 5.87 | 0.332 | 13.32 | 0.417 | 6.08 | |
| **C** | 4.12 | 0.361 | 15.26 | 0.304 | 20.62 | 0.338 | 23.87 | |
| **D** | 12.35 | 0.333 | 21.83 | 0.286 | 25.33 | 0.304 | 31.53 | |
| **E** | 37.04 | 0.082 | 80.75 | 0.156 | 59.27 | 0.224 | 49.55 | |
| **F** | 111.11 | 0.048 | 88.73 | 0.016 | 95.82 | 0.029 | 93.47 | |
| **G** | 333.33 | 0.019 | 95.54 | 0.009 | 97.65 | 0.017 | 96.17 | |
| **H** | 1000 | 0.01 | 97.65 | | | 0.005 | 98.87 | |

**Appendix 72: Mean $IC_{50}$ values for *C. tanaense* fractions against breast cancer cell lines**

(a) Petroleum ether and dichloromethane mixture fraction from *C. tanaense* root methanol extract

| Well | Conc. (µg/ml) | Absorbance and % Cytotoxicity against HCC 1395 | | | | | | Mean $IC_{50}$ ± SEM n = 3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$ =125 | | $IC_{50}$ =148 | | $IC_{50}$ =168 | | |
| **A** | 0.00 | 0.573 | 0.00 | 0.578 | 0.00 | 0.527 | 0.00 | 147 ± 12.4 |
| **B** | 1.37 | 0.568 | 0.87 | 0.546 | 5.54 | 0.488 | 7.40 | |
| **C** | 4.12 | 0.554 | 3.32 | 0.542 | 6.23 | 0.485 | 7.97 | |
| **D** | 12.35 | 0.397 | 30.71 | 0.479 | 17.12 | 0.473 | 10.25 | |
| **E** | 37.04 | 0.321 | 43.97 | 0.439 | 24.05 | 0.411 | 22.01 | |
| **F** | 111.11 | 0.304 | 46.95 | 0.34 | 41.18 | 0.348 | 33.96 | |
| **G** | 333.33 | 0.031 | 94.59 | 0.02 | 96.54 | 0.004 | 99.24 | |
| **H** | 1000 | 0.023 | 95.99 | 0.013 | 97.75 | 0.003 | 99.43 | |

(b) Ethyl acetate fraction from *C. tanaense* root methanol extract

| Well | Conc. (µg/ml) | Absorbance and % Cytotoxicity against HCC 1395 | | | | | | Mean $IC_{50}$ ± SEM n=3 |
|---|---|---|---|---|---|---|---|---|
| | | i $IC_{50}$ = 47.5 | | ii $IC_{50}$ = 50 | | iii $IC_{50}$ = 57.5 | | |
| **A** | 0.00 | 0.573 | 0.00 | 0.522 | 0.00 | 0.523 | 0.00 | 51.7 ± 3 |
| **B** | 1.37 | 0.482 | 15.88 | 0.491 | 3.1 | 0.512 | 2.10 | |
| **C** | 4.12 | 0.48 | 16.23 | 0.471 | 9.77 | 0.498 | 4,78 | |
| **D** | 12.35 | 0.38 | 33.68 | 0.424 | 17.10 | 0.481 | 8.03 | |
| **E** | 37.04 | 0.331 | 42.23 | 0.309 | 40.80 | 0.365 | 30.21 | |
| **F** | 111.11 | 0.005 | 99.13 | 0.041 | 92.15 | 0.009 | 98.28 | |
| **G** | 333.33 | 0.003 | 99.48 | 0.031 | 94.06 | 0.005 | 99.04 | |
| **H** | 1000 | 0.002 | 99.65 | 0.027 | 94.3 | 0.003 | 99.43 | |

(c) Acetone fraction from *C. tanaense* root methanol extract

| Well | Conc. (µg/ml) | Absorbance and % Cytotoxicity against HCC 1395 | | | | | | Mean $IC_{50}$ ± SEM n = 3 |
|---|---|---|---|---|---|---|---|---|
| | | i $IC_{50}$ = 35 | | ii $IC_{50}$ = 37.5 | | iii $IC_{50}$ = 27.5 | | |
| **A** | 0.00 | 0.497 | 0.00 | 0.513 | 0.00 | 0.514 | 0.00 | 33.3 ± 3 |
| **B** | 1.37 | 0.486 | 2.21 | 0.469 | 8.58 | 0.469 | 8.75 | |
| **C** | 4.12 | 0.453 | 8.85 | 0.455 | 11.31 | 0.457 | 11.08 | |
| **D** | 12.35 | 0.431 | 13.28 | 0.395 | 23.00 | 0.451 | 12.26 | |
| **E** | 37.04 | 0.236 | 52.52 | 0.262 | 48.92 | 0.12 | 76.65 | |
| **F** | 111.11 | 0.005 | 98.99 | 0.011 | 97.86 | 0.022 | 95.72 | |
| **G** | 333.33 | 0.001 | 99.80 | 0.009 | 98.25 | 0.005 | 99.03 | |
| **H** | 1000 | 0 | 100 | 0.004 | 99.22 | 0.001 | 99.80 | |

(d) Methanol fraction from *C. tanaense* root methanol extract

| Well | Conc. (µg/ml) | Absorbance and % Cytotoxicity against HCC 1395 | | | | | | Mean $IC_{50}$ ± SEM n=3 |
|---|---|---|---|---|---|---|---|---|
| | | i $IC_{50}$ = 20 | | ii $IC_{50}$ = 20 | | iii $IC_{50}$ = 22.5 | | |
| **A** | 0.00 | 0.473 | 0.00 | 0.48 | 0.00 | 0.559 | 0.00 | 20.8 ± 0.8 |
| **B** | 1.37 | 0.435 | 8.03 | 0.424 | 11.67 | 0.498 | 10.91 | |
| **C** | 4.12 | 0.421 | 10.99 | 0.347 | 27.70 | 0.445 | 20.39 | |
| **D** | 12.35 | 0.325 | 31.29 | 0.278 | 42.08 | 0.355 | 36.49 | |
| **E** | 37.04 | 0.092 | 80.55 | 0.082 | 82.92 | 0.176 | 68.52 | |
| **F** | 111.11 | 0.078 | 83.51 | 0.077 | 83.96 | 0.096 | 82.83 | |
| **G** | 333.33 | 0.002 | 99.58 | 0.063 | 86.88 | 0.093 | 83.36 | |
| **H** | 1000 | 0.001 | 99.79 | 0.009 | 98.13 | 0.078 | 86.05 | |

**Appendix 73: Mean $IC_{50}$ values for *C. tanaense* fractions against prostate cancer cell lines**

(a) Petroleum ether and dichloromethane fraction from *C. tanaense* root methanol extract

| Well | Conc. (µg/ml) | Absorbance and % Cytotoxicity against DU-145 | | | | | | Mean $IC_{50}$ ± SEM n=3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$ = 60 | | $IC_{50}$ = 57.5 | | $IC_{50}$ = 62.5 | | |
| **A** | 0.00 | 0.418 | 0.00 | 0.405 | 0.00 | 0.409 | 0.00 | 60 ± 1.4 |
| **B** | 1.37 | 0.39 | 6.67 | 0.368 | 9.14 | 0.382 | 6.60 | |
| **C** | 4.12 | 0.379 | 9.33 | 0.356 | 12.10 | 0.367 | 10.27 | |
| **D** | 12.35 | 0.306 | 26.80 | 0.293 | 27.65 | 0.326 | 20.30 | |
| **E** | 37.04 | 0.275 | 34.21 | 0.277 | 31.60 | 0.288 | 29.58 | |
| **F** | 111.11 | 0.051 | 87.80 | 0.015 | 96.30 | 0.04 | 90.22 | |
| **G** | 333.33 | 0.005 | 98.80 | 0.011 | 97.28 | 0.022 | 94.62 | |
| **H** | 1000 | 0.001 | 100.23 | 0.01 | 97.53 | 0.011 | 97.31 | |

(b) Ethyl acetate fraction from *C. tanaense* root methanol extract

| Well | Conc. (µg/ml) | Absorbance/ % Cytotoxicity against DU-145 | | | | | | Mean $IC_{50}$ ± SEM n = 3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$ = 60 | | $IC_{50}$ = 72.5 | | $IC_{50}$= 77.5 | | |
| **A** | 0.00 | 0.436 | 0.00 | 0.4 | 0.00 | 0.362 | 0.00 | 69.2 ± 6 |
| **B** | 1.37 | 0.394 | 9.63 | 0.398 | 0.50 | 0.343 | 5.22 | |
| **C** | 4.12 | 0.373 | 14.45 | 0.398 | 0.50 | 0.342 | 5.52 | |
| **D** | 12.35 | 0.351 | 19.50 | 0.35 | 12.50 | 0.339 | 6.35 | |
| **E** | 37.04 | 0.282 | 35.32 | 0.32 | 20.00 | 0.311 | 14.09 | |
| **F** | 111.11 | 0.065 | 85.10 | 0.076 | 81.00 | 0.073 | 79.83 | |
| **G** | 333.33 | 0.036 | 91.74 | 0.034 | 91.50 | 0.044 | 87.84 | |
| **H** | 1000 | 0.01 | 97.70 | 0.002 | 99.5 | 0.03 | 91.71 | |

(c) Acetone fraction from *C. tanaense* root methanol extract

| Well | Conc. (µg/ml) | Absorbance/ % Cytotoxicity against DU-145 | | | | | | Mean $IC_{50}$ ± SEM n = 3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$ = 27.5 | | $IC_{50}$ = 15 | | $IC_{50}$ = 25 | | |
| **A** | 0.00 | 0.507 | 0.00 | 0.446 | 0.00 | 0.495 | 0.00 | **22.5 ± 3.8** |
| **B** | 1.37 | 0.451 | 11.04 | 0.43 | 3.59 | 0.486 | 1.82 | |
| **C** | 4.12 | 0.413 | 18.54 | 0.416 | 6.73 | 0.416 | 15.95 | |
| **D** | 12.35 | 0.337 | 33.53 | 0.239 | 46.41 | 0.403 | 18.59 | |
| **E** | 37.04 | 0.206 | 59.37 | 0.154 | 65.47 | 0.096 | 80.61 | |
| **F** | 111.11 | 0.029 | 94.28 | 0.051 | 88.57 | 0.063 | 87.27 | |
| **G** | 333.33 | 0.009 | 98.22 | 0.023 | 94.84 | 0.042 | 91.51 | |
| **H** | 1000 | 0.005 | 99.01 | 0.013 | 97.08 | 0.018 | 96.36 | |

(d) Methanol fraction from *C. tanaense* root methanol extract

| Well | Conc. (µg/ml) | Absorbance and % Cytotoxicity against DU-145 | | | | | | Mean $IC_{50}$ ± SEM n = 3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$ = 62.5 | | $IC_{50}$ = 97.5 | | $IC_{50}$ = 62.5 | | |
| **A** | 0.00 | 0.327 | 0.00 | 0.394 | 0.00 | 0.387 | 0.00 | 74.2 ± 11.7 |
| **B** | 1.37 | 0.306 | 6.42 | 0.356 | 9.64 | 0.346 | 10.59 | |
| **C** | 4.12 | 0.286 | 12.54 | 0.336 | 14.72 | 0.342 | 11.62 | |
| **D** | 12.35 | 0.278 | 14.98 | 0.334 | 15.22 | 0.329 | 14.99 | |
| **E** | 37.04 | 0.185 | 43.42 | 0.242 | 3.57 | 0.229 | 40.83 | |
| **F** | 111.11 | 0.123 | 62.38 | 0.156 | 60.41 | 0.129 | 66.67 | |
| **G** | 333.33 | 0.012 | 96.33 | 0.04 | 89.85 | 0.078 | 70.84 | |
| **H** | 1000 | 0.003 | 99.08 | 0.033 | 91.62 | 0.021 | 94.57 | |

**Appendix 74: Mean $IC_{50}$ values for *U. anisatum* fractions against breast cancer cell lines**

(a) Petroleum ether fraction from *U. anisatum* root methanol extract

| Well | Conc. (µg/ml) | Absorbance/ % Cytotoxicity against HCC 1395 | | | | | | Mean $IC_{50}$ ± SEM n = 3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$ > 1000 | | $IC_{50}$ > 1000 | | $IC_{50}$ > 1000 | | |
| **A** | 0.00 | 0.945 | 0.00 | 0.944 | 0.00 | 0.96 | 0.00 | > 1000 |
| **B** | 1.37 | 0.902 | 4.55 | 0.921 | 2.44 | 0.929 | 3.23 | |
| **C** | 4.12 | 0.898 | 4.97 | 0.919 | 2.65 | 0.901 | 6.15 | |
| **D** | 12.35 | 0.885 | 6.35 | 0.894 | 5.30 | 0.9 | 6.25 | |
| **E** | 37.04 | 0.879 | 6.98 | 0.881 | 6.67 | 0.864 | 10.00 | |
| **F** | 111.11 | 0.819 | 13.33 | 0.872 | 7.63 | 0.783 | 18.44 | |
| **G** | 333.33 | 0.783 | 17.14 | 0.779 | 17.48 | 0.76 | 20.83 | |
| **H** | 1000 | 0.558 | 40.95 | 0.505 | 46.50 | 0.535 | 44.27 | |

(b) Dichloromethane fraction from *U. anisatum* root methanol extract

| Well | Conc. (µg/ml) | Absorbance/ % Cytotoxicity against HCC 1395 | | | | | | Mean $IC_{50}$ ± SEM n = 3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$ = 813 | | $IC_{50}$ = 815 | | $IC_{50}$ = 908 | | |
| **A** | 0.00 | 1.028 | 0.00 | 1.004 | 0.00 | 0.917 | 0.00 | 845.3 ± 31.3 |
| **B** | 1.37 | 0.92 | 10.51 | 0.923 | 8.08 | 0.881 | 3.93 | |
| **C** | 4.12 | 0.86 | 16.34 | 0.92 | 8.37 | 0.857 | 6.54 | |
| **D** | 12.35 | 0.845 | 17.80 | 0.878 | 12.55 | 0.768 | 16.25 | |
| **E** | 37.04 | 0.791 | 23.05 | 0.869 | 13.45 | 0.767 | 16.36 | |
| **F** | 111.11 | 0.762 | 25.88 | 0.803 | 20.02 | 0.705 | 23.12 | |
| **G** | 333.33 | 0.681 | 33.75 | 0.67 | 33.27 | 0.696 | 24.10 | |
| **H** | 1000 | 0.452 | 56.03 | 0.437 | 56.47 | 0.422 | 53.98 | |

(c) Ethyl acetate fraction from *U. anisatum* root methanol extract

| Well | Conc. (µg/ml) | Absorbance/ % Cytotoxicity against HCC 1395 | | | | | | Mean $IC_{50}$ ± SEM n = 3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$ = 328 | | $IC_{50}$ = 353 | | $IC_{50}$ = 208 | | |
| **A** | 0.00 | 0.472 | 0.00 | 0.452 | 0.00 | 0.496 | 0.00 | 296.3 ± 44.8 |
| **B** | 1.37 | 0.417 | 11.65 | 0.437 | 3.32 | 0.492 | 0.81 | |
| **C** | 4.12 | 0.41 | 13.14 | 0.428 | 5.31 | 0.477 | 3.83 | |
| **D** | 12.35 | 0.391 | 17.16 | 0.394 | 12.83 | 0.468 | 5.65 | |
| **E** | 37.04 | 0.385 | 18.43 | 0.38 | 15.93 | 0.404 | 18.55 | |
| **F** | 111.11 | 0.359 | 23.94 | 0.364 | 19.47 | 0.339 | 31.65 | |
| **G** | 333.33 | 0.234 | 50.42 | 0.234 | 48.23 | 0.117 | 76.41 | |
| **H** | 1000 | 0.011 | 97.67 | 0.007 | 98.45 | 0.042 | 91.53 | |

(d) Acetone mixture fraction from *U. anisatum* root methanol extract

| Well | Conc. (µg/ml) | Absorbance and % Cytotoxicity against HCC 1395 | | | | | | Mean $IC_{50}$ ± SEM n = 3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$ = 103 | | $IC_{50}$ = 82.5 | | $IC_{50}$ = 77.5 | | |
| **A** | 0.00 | 0.984 | 0.00 | 0.951 | 0.00 | 0.899 | 0.00 | 87.7 ± 7.8 |
| **B** | 1.37 | 0.922 | 6.30 | 0.869 | 8.62 | 0.898 | 0.11 | |
| **C** | 4.12 | 0.911 | 7.42 | 0.864 | 9.15 | 0.896 | 0.33 | |
| **D** | 12.35 | 0.901 | 8.43 | 0.781 | 17.88 | 0.805 | 10.46 | |
| **E** | 37.04 | 0.865 | 12.09 | 0.774 | 18.61 | 0.796 | 11.46 | |
| **F** | 111.11 | 0.444 | 54.88 | 0.289 | 69.61 | 0.156 | 82.65 | |
| **G** | 333.33 | 0.094 | 90.45 | 0.094 | 90.12 | 0.129 | 85.65 | |
| **H** | 1000 | 0.051 | 94.82 | 0.085 | 91.06 | 0.114 | 87.32 | |

(e) Methanol fraction from *U. anisatum* root methanol extract

| Well | Conc. (µg/ml) | Absorbance and % Cytotoxicity against HCC 1395 | | | | | | Mean $IC_{50}$± SEM n=3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$=72.5 | | $IC_{50}$=75 | | $IC_{50}$=75 | | |
| **A** | 0.00 | 1.002 | 0.00 | 0.939 | 0.00 | 0.945 | 0.00 | 74.2±0.8 |
| **B** | 1.37 | 0.899 | 10.28 | 0.877 | 6.60 | 0.921 | 2.54 | |
| **C** | 4.12 | 0.865 | 13.67 | 0.863 | 8.09 | 0.851 | 9.95 | |
| **D** | 12.35 | 0.828 | 17.37 | 0.86 | 8.41 | 0.802 | 15.13 | |
| **E** | 37.04 | 0.706 | 29.54 | 0.716 | 23.75 | 0.731 | 22.65 | |
| **F** | 111.11 | 0.252 | 74.85 | 0.26 | 72.31 | 0.258 | 72.70 | |
| **G** | 333.33 | 0.151 | 84.93 | 0.153 | 83.71 | 0.152 | 83.92 | |
| **H** | 1000 | 0.105 | 89.52 | 0.099 | 89.46 | 0.033 | 96.51 | |

**Appendix 75: Mean $IC_{50}$ values for *U. anisatum* fractions against prostate cancer cell lines**

(a) Petroleum ether fraction from *U. anisatum* root methanol extract

| Well | Conc. (µg/ml) | Absorbance and % Cytotoxicity against DU-145 | | | | | | Mean $IC_{50}$ ± SEM n = 3 |
|---|---|---|---|---|---|---|---|---|
| | | i $IC_{50}$ > 1000 | | ii $IC_{50}$ > 1000 | | iii $IC_{50}$ >1000 | | |
| **A** | 0.00 | 0.624 | 0.00 | 0.609 | 0.00 | 0.534 | 0.00 | **> 1000** |
| **B** | 1.37 | 0.584 | 6.41 | 0.593 | 2.63 | 0.51 | 4.49 | |
| **C** | 4.12 | 0.568 | 8.97 | 0.587 | 3.61 | 0.509 | 4.68 | |
| **D** | 12.35 | 0.552 | 11.54 | 0.578 | 5.09 | 0.496 | 7.12 | |
| **E** | 37.04 | 0.495 | 20.67 | 0.561 | 7.88 | 0.479 | 10.30 | |
| **F** | 111.11 | 0.486 | 22.12 | 0.552 | 9.36 | 0.468 | 12.36 | |
| **G** | 333.33 | 0.472 | 24.36 | 0.467 | 23.32 | 0.461 | 13.67 | |
| **H** | 1000 | 0.362 | 41.99 | 0.418 | 31.36 | 0.385 | 27.90 | |

(b) Dichloromethane fraction from *U. anisatum* root methanol extract

| Well | Conc. (µg/ml) | Absorbance and % Cytotoxicity against DU-145 | | | | | | Mean $IC_{50}$ ± SEM n = 3 |
|---|---|---|---|---|---|---|---|---|
| | | i $IC_{50}$ = 523 | | ii $IC_{50}$= 563 | | iii $IC_{50}$ = 513 | | |
| **A** | 0.00 | 0.626 | 0.00 | 0.599 | 0.00 | 0.643 | 0.00 | 533.0 ± 15.3 |
| **B** | 1.37 | 0.624 | 0.32 | 0.593 | 1.00 | 0.628 | 2.33 | |
| **C** | 4.12 | 0.615 | 1.76 | 0.569 | 5.01 | 0.619 | 3.73 | |
| **D** | 12.35 | 0.6 | 4.15 | 0.566 | 5.51 | 0.588 | 8.55 | |
| **E** | 37.04 | 0.578 | 7.67 | 0.509 | 15.03 | 0.54 | 16.02 | |
| **F** | 111.11 | 0.495 | 20.93 | 0.508 | 15.19 | 0.496 | 22.86 | |
| **G** | 333.33 | 0.433 | 30.83 | 0.43 | 28.21 | 0.428 | 33.44 | |
| **H** | 1000 | 0.009 | 98.56 | 0.051 | 91.49 | 0.021 | 96.73 | |

(c) Ethyl acetate fraction from *U. anisatum* root methanol extract

| Well | Conc. (µg/ml) | Absorbance and % Cytotoxicity against DU-145 | | | | | | Mean $IC_{50}$ ± SEM n = 3 |
|---|---|---|---|---|---|---|---|---|
| | | i $IC_{50}$ = 495 | | ii $IC_{50}$ = 420 | | iii $IC_{50}$ = 490 | | |
| **A** | 0.00 | 0.252 | 0.00 | 0.221 | 0.00 | 0.232 | 0.00 | 468.3 ± 24.2 |
| **B** | 1.37 | 0.247 | 1.98 | 0.221 | 0.00 | 0.228 | 1.72 | |
| **C** | 4.12 | 0.245 | 2.78 | 0.207 | 6.33 | 0.228 | 1.72 | |
| **D** | 12.35 | 0.237 | 5.95 | 0.206 | 6.79 | 0.227 | 2.16 | |
| **E** | 37.04 | 0.231 | 8.33 | 0.205 | 7.24 | 0.225 | 3.02 | |
| **F** | 111.11 | 0.191 | 24.21 | 0.147 | 33.48 | 0.223 | 3.88 | |
| **G** | 333.33 | 0.164 | 34.92 | 0.118 | 46.61 | 0.142 | 38.79 | |
| **H** | 1000 | 0.007 | 97.22 | 0.06 | 72.85 | 0.03 | 87.07 | |

(d) Acetone mixture fraction from *U. anisatum* root methanol extract

| Well | Conc. (µg/ml) | Absorbance and % Cytotoxicity against DU-145 | | | | | | Mean $IC_{50}$ ± SEM n = 3 |
|---|---|---|---|---|---|---|---|---|
| | | i $IC_{50}$ = 32.5 | | ii $IC_{50}$ = 72.5 | | iii $IC_{50}$ = 95 | | |
| **A** | 0.00 | 0.222 | 0.00 | 0.219 | 0.00 | 0.256 | 0.00 | 66.7 ± 18.3 |
| **B** | 1.37 | 0.219 | 1.35 | 0.204 | 6.85 | 0.243 | 5.08 | |
| **C** | 4.12 | 0.209 | 5.86 | 0.202 | 7.76 | 0.237 | 7.42 | |
| **D** | 12.35 | 0.189 | 14.86 | 0.199 | 9.13 | 0.209 | 18.36 | |
| **E** | 37.04 | 0.101 | 54.50 | 0.148 | 32.42 | 0.199 | 22.27 | |
| **F** | 111.11 | 0.088 | 60.36 | 0.067 | 69.41 | 0.108 | 57.81 | |
| **G** | 333.33 | 0.056 | 74.77 | 0.061 | 72.15 | 0.004 | 98.44 | |
| **H** | 1000 | 0.032 | 85.59 | 0.014 | 93.61 | 0 | 100 | |

(e) Methanol fraction from *U. anisatum* root methanol extract

| Well | Conc. (µg/ml) | Absorbance and % Cytotoxicity against DU-145 | | | | | | Mean $IC_{50}$± SEM n=3 |
|---|---|---|---|---|---|---|---|---|
| | | i $IC_{50}$ = 77.5 | | ii $IC_{50}$ = 37.04 | | iii $IC_{50}$ = 20 | | |
| **A** | 0.00 | 0.244 | 0.00 | 0.25 | 0.00 | 0.197 | 0.00 | 44.9±17.1 |
| **B** | 1.37 | 0.241 | 1.23 | 0.249 | 0.40 | 0.193 | 2.03 | |
| **C** | 4.12 | 0.211 | 13.52 | 0.236 | 5.60 | 0.147 | 25.38 | |
| **D** | 12.35 | 0.17 | 30.33 | 0.227 | 9.20 | 0.13 | 34.01 | |
| **E** | 37.04 | 0.17 | 30.33 | 0.125 | 50.00 | 0.082 | 58.38 | |
| **F** | 111.11 | 0.088 | 63.93 | 0.124 | 50.40 | 0.055 | 72.08 | |
| **G** | 333.33 | 0.059 | 75.82 | 0.024 | 90.40 | 0.051 | 74.11 | |
| **H** | 1000 | 0.049 | 79.92 | 0.016 | 93.60 | 0.029 | 85.28 | |

**Appendix 76: Mean $IC_{50}$ values for *H. abyssinica* fractions against breast cancer cell lines**

(a) Petroleum ether fraction from *H. abyssinica* rhizome methanol extract

| Well | Conc. (µg/ml) | Absorbance and % Cytotoxicity against HCC 1395 | | | | Mean $IC_{50}$ ± SEM n = 2 |
|---|---|---|---|---|---|---|
| | | i $IC_{50}$ > 1000 | | ii $IC_{50}$ > 1000 | | |
| **A** | 0.00 | 0.898 | 0.00 | 1.012 | 0.00 | > 1000 |
| **B** | 1.37 | 0.896 | 0.22 | 0.882 | 12.85 | |
| **C** | 4.12 | 0.892 | 0.67 | 0.838 | 17.19 | |
| **D** | 12.35 | 0.883 | 1.67 | 0.825 | 18.48 | |
| **E** | 37.04 | 0.838 | 6.68 | 0.798 | 21.15 | |
| **F** | 111.11 | 0.749 | 16.59 | 0.77 | 23.91 | |
| **G** | 333.33 | 0.678 | 24.50 | 0.76 | 24.90 | |
| **H** | 1000 | 0.609 | 32.18 | 0.672 | 33.60 | |

(b) Dichloromethane fraction from *H. abyssinica* rhizome methanol extract

| Well | Conc. (µg/ml) | Absorbance and % Cytotoxicity against HCC 1395 | | | | | | Mean $IC_{50}$ ± SEM n = 3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$ = 623 | | $IC_{50}$ = 605 | | $IC_{50}$ = 603 | | |
| **A** | 0.00 | 0.99 | 0.00 | 1.073 | 0.00 | 1.078 | 0.00 | 610.3 ± 6.4 |
| **B** | 1.37 | 0.92 | 7.07 | 1.054 | 1.80 | 1.075 | 0.28 | |
| **C** | 4.12 | 0.89 | 10.10 | 1.051 | 2.05 | 1.037 | 3.80 | |
| **D** | 12.35 | 0.846 | 14.55 | 1.005 | 6.34 | 1.027 | 4.73 | |
| **E** | 37.04 | 0.832 | 15.96 | 0.98 | 8.67 | 0.971 | 9.93 | |
| **F** | 111.11 | 0.783 | 20.91 | 0.886 | 17.43 | 0.946 | 12.24 | |
| **G** | 333.33 | 0.783 | 20.91 | 0.843 | 21.44 | 0.883 | 18.09 | |
| **H** | 1000 | 0.117 | 88.18 | 0.089 | 91.71 | 0.023 | 97.87 | |

(c) Ethyl acetate fraction from *H. abyssinica* rhizome methanol extract

| Well | Conc. (µg/ml) | Absorbance and % Cytotoxicity against HCC 1395 | | | | | | Mean $IC_{50}$ ± SEM n = 3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$ = 628 | | $IC_{50}$ = 640 | | $IC_{50}$ = 630 | | |
| **A** | 0.00 | 0.89 | 0.00 | 0.931 | 0.00 | 1.041 | 0.000 | 632.7 ± 3.7 |
| **B** | 1.37 | 0.887 | 0.34 | 0.909 | 2.36 | 1.04 | 0.10 | |
| **C** | 4.12 | 0.88 | 1.12 | 0.906 | 2.69 | 0.972 | 6.63 | |
| **D** | 12.35 | 0.833 | 6.40 | 0.896 | 3.76 | 0.955 | 8.26 | |
| **E** | 37.04 | 0.83 | 6.74 | 0.841 | 9.67 | 0.948 | 8.93 | |
| **F** | 111.11 | 0.799 | 10.22 | 0.839 | 9.88 | 0.947 | 9.03 | |
| **G** | 333.33 | 0.701 | 21.24 | 0.767 | 17.62 | 0.76 | 26.99 | |
| **H** | 1000 | 0.123 | 86.18 | 0.106 | 88.61 | 0.224 | 78.48 | |

(d) Acetone fraction from *H. abyssinica* root methanol extract

| Well | Conc. (µg/ml) | Absorbance and % Cytotoxicity against HCC 1395 | | | | | | Mean $IC_{50}$ ± SEM n = 3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$ = 198 | | $IC_{50}$ = 168 | | $IC_{50}$ = 168 | | |
| **A** | 0.00 | 1.074 | 0.00 | 1.1 | 0.00 | 1.194 | 0.00 | 178 ± 10 |
| **B** | 1.37 | 1.072 | 0.19 | 1.077 | 2.09 | 1.146 | 4.02 | |
| **C** | 4.12 | 1.062 | 1.12 | 1.076 | 2.18 | 1.101 | 7.79 | |
| **D** | 12.35 | 1.017 | 5.31 | 1.049 | 4.63 | 1.098 | 8.04 | |
| **E** | 37.04 | 0.921 | 14.25 | 0.927 | 15.72 | 1.049 | 12.14 | |
| **F** | 111.11 | 0.795 | 25.98 | 0.683 | 37.91 | 0.747 | 37.44 | |
| **G** | 333.33 | 0.124 | 88.45 | 0.161 | 85.36 | 0.152 | 87.27 | |
| **H** | 1000 | 0.025 | 97.67 | 0.12 | 89.09 | 0.012 | 98.99 | |

(e) Methanol fraction from *H. abyssinica* root methanol extract

| Well | Conc. (µg/ml) | Absorbance and % Cytotoxicity against HCC 1395 | | | | | | Mean $IC_{50}$ ± SEM n = 3 |
|---|---|---|---|---|---|---|---|---|
| | | i $IC_{50}$ = 52.5 | | ii $IC_{50}$ = 35 | | iii $IC_{50}$ = 35 | | |
| **A** | 0.00 | 0.945 | 0.00 | 0.964 | 0.00 | 0.974 | 0.00 | 40.8 ± 5.8 |
| **B** | 1.37 | 0.902 | 4.55 | 0.893 | 7.37 | 0.913 | 6.26 | |
| **C** | 4.12 | 0.896 | 5.19 | 0.876 | 9.13 | 0.907 | 6.88 | |
| **D** | 12.35 | 0.859 | 9.10 | 0.838 | 13.07 | 0.844 | 13.35 | |
| **E** | 37.04 | 0.51 | 46.03 | 0.436 | 54.77 | 0.473 | 51.44 | |
| **F** | 111.11 | 0.328 | 65.29 | 0.28 | 70.95 | 0.172 | 82.34 | |
| **G** | 333.33 | 0.253 | 73.23 | 0.132 | 86.31 | 0.134 | 86.24 | |
| **H** | 1000 | 0.166 | 82.43 | 0.108 | 88.80 | 0.052 | 94.66 | |

**Appendix 77: Mean $IC_{50}$ values for *H. abyssinica* fractions against prostate cancer cell lines**

(a) Petroleum ether fraction from *H. abyssinica* rhizome methanol extract

| Well | Conc. (µg/ml) | Absorbance and % Cytotoxicity against DU 145 | | | | | | Mean $IC_{50}$± SEM n=3 |
|---|---|---|---|---|---|---|---|---|
| | | i $IC_{50}$=625 | | ii $IC_{50}$=640 | | iii $IC_{50}$=590 | | |
| **A** | 0.00 | 0.254 | 0.00 | 0.249 | 0.00 | 0.26 | 0.00 | 618.3±14.8 |
| **B** | 1.37 | 0.253 | 0.39 | 0.241 | 3.21 | 0.249 | 4.23 | |
| **C** | 4.12 | 0.24 | 5.51 | 0.232 | 6.83 | 0.236 | 9.23 | |
| **D** | 12.35 | 0.235 | 7.48 | 0.22 | 11.65 | 0.235 | 9.62 | |
| **E** | 37.04 | 0.234 | 7.87 | 0.216 | 13.25 | 0.226 | 13.08 | |
| **F** | 111.11 | 0.204 | 19.69 | 0.205 | 17.67 | 0.218 | 16.15 | |
| **G** | 333.33 | 0.197 | 22.44 | 0.188 | 24.50 | 0.174 | 33.08 | |
| **H** | 1000 | 0.037 | 85.43 | 0.05 | 79.92 | 0.058 | 77.69 | |

(b) Dichloromethane fraction from *H. abyssinica* rhizome methanol extract

| Well | Conc. (µg/ml) | Absorbance and % Cytotoxicity against DU 145 | | | | | | Mean $IC_{50}$± SEM n=3 |
|---|---|---|---|---|---|---|---|---|
| | | i $IC_{50}$=378 | | ii $IC_{50}$=298 | | iii $IC_{50}$=320 | | |
| **A** | 0.00 | 0.247 | 0.00 | 0.24 | 0.00 | 0.258 | 0.00 | 332±23.9 |
| **B** | 1.37 | 0.235 | 4.86 | 0.234 | 2.50 | 0.238 | 7.75 | |
| **C** | 4.12 | 0.233 | 5.67 | 0.234 | 2.50 | 0.232 | 10.08 | |
| **D** | 12.35 | 0.226 | 8.50 | 0.232 | 3.33 | 0.224 | 13.18 | |
| **E** | 37.04 | 0.19 | 23.08 | 0.214 | 10.83 | 0.208 | 19.38 | |
| **F** | 111.11 | 0.135 | 45.34 | 0.202 | 15.83 | 0.201 | 22.09 | |
| **G** | 333.33 | 0.129 | 47.77 | 0.105 | 56.25 | 0.125 | 51.55 | |
| **H** | 1000 | 0.032 | 87.04 | 0.1 | 58.33 | 0.11 | 57.36 | |

(c) Ethyl acetate fraction from *H. abyssinica* rhizome methanol extract

| Well | Conc. (µg/ml) | Absorbance and % Cytotoxicity against DU-145 | | | | | | Mean $IC_{50}$ ± SEM n = 3 |
|---|---|---|---|---|---|---|---|---|
| | | i $IC_{50}$ = 178 | | ii $IC_{50}$ = 283 | | iii $IC_{50}$ = 253 | | |
| **A** | 0.00 | 0.219 | 0.00 | 0.235 | 0.00 | 0.244 | 0.00 | |
| **B** | 1.37 | 0.216 | 1.37 | 0.21 | 10.64 | 0.23 | 5.74 | 238 ± 31.2 |
| **C** | 4.12 | 0.211 | 3.65 | 0.198 | 15.74 | 0.217 | 11.07 | |
| **D** | 12.35 | 0.208 | 5.02 | 0.187 | 20.43 | 0.21 | 13.93 | |
| **E** | 37.04 | 0.191 | 12.79 | 0.186 | 20.85 | 0.206 | 15.57 | |
| **F** | 111.11 | 0.139 | 36.53 | 0.182 | 22.55 | 0.19 | 22.13 | |
| **G** | 333.33 | 0.041 | 81.28 | 0.099 | 57.87 | 0.082 | 66.39 | |
| **H** | 1000 | 0.01 | 95.43 | 0.026 | 88.94 | 0.014 | 94.26 | |

(d) Acetone fraction from *H. abyssinica* root methanol extract

| Well | Conc. (µg/ml) | Absorbance and % Cytotoxicity against DU-145 | | | | | | Mean $IC_{50}$ ± SEM n=3 |
|---|---|---|---|---|---|---|---|---|
| | | i $IC_{50}$ = 488 | | ii $IC_{50}$ = 408 | | iii $IC_{50}$ = 623 | | |
| **A** | 0.00 | 0.245 | 0.00 | 0.241 | 0.00 | 0.259 | 0.00 | 506.3 ± 62.7 |
| **B** | 1.37 | 0.241 | 1.63 | 0.192 | 20.33 | 0.248 | 4.25 | |
| **C** | 4.12 | 0.228 | 6.94 | 0.189 | 21.58 | 0.235 | 9.27 | |
| **D** | 12.35 | 0.22 | 10.20 | 0.188 | 21.99 | 0.235 | 9.27 | |
| **E** | 37.04 | 0.218 | 11.02 | 0.183 | 24.07 | 0.213 | 17.76 | |
| **F** | 111.11 | 0.199 | 18.78 | 0.167 | 30.71 | 0.209 | 19.31 | |
| **G** | 333.33 | 0.158 | 35.51 | 0.133 | 44.81 | 0.194 | 25.10 | |
| **H** | 1000 | 0.003 | 98.78 | 0.017 | 92.95 | 0.044 | 83.01 | |

(e) Methanol fraction from *H. abyssinica* root methanol extract

| Well | Conc. (µg/ml) | Absorbance and % Cytotoxicity against DU-145 | | | | | | Mean $IC_{50}$ ± SEM n=3 |
|---|---|---|---|---|---|---|---|---|
| | | i $IC_{50}$ = 97.5 | | ii $IC_{50}$ = 85 | | iii $IC_{50}$ = 87.5 | | |
| **A** | 0.00 | 0.234 | 0.00 | 0.247 | 0.00 | 0.22 | 0.00 | 90 ± 3.8 |
| **B** | 1.37 | 0.208 | 11.11 | 0.225 | 8.91 | 0.203 | 7.73 | |
| **C** | 4.12 | 0.205 | 12.39 | 0.205 | 17.00 | 0.203 | 7.73 | |
| **D** | 12.35 | 0.168 | 28.21 | 0.201 | 18.62 | 0.191 | 13.18 | |
| **E** | 37.04 | 0.142 | 39.32 | 0.176 | 28.74 | 0.175 | 20.45 | |
| **F** | 111.11 | 0.111 | 52.56 | 0.095 | 61.54 | 0.079 | 64.09 | |
| **G** | 333.33 | 0.101 | 56.84 | 0.067 | 72.87 | 0.055 | 75.00 | |
| **H** | 1000 | 0.09 | 61.54 | 0.033 | 86.64 | 0.043 | 80.45 | |

**Appendix 78: Mean $IC_{50}$ values of isolated compounds against breast cancer cell lines**

**(a)** Compound **37**

| Well | Conc. (µg/ml) | Absorbance and % Cytotoxicity against HCC 1395 | | | | | | Mean $IC_{50}$ ± SEM n = 3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$ = 3.69 | | $IC_{50}$ = 3.51 | | $IC_{50}$ = 3.51 | | |
| **A** | 0.00 | 0.72 | 0.00 | 1.345 | 0.00 | 1.595 | 0.00 | 3.6 ± 0.1 |
| **B** | 1.37 | 0.456 | 36.67 | 0.868 | 35.46 | 1.141 | 28.46 | |
| **C** | 4.12 | 0.341 | 52.64 | 0.636 | 52.71 | 0.7 | 56.11 | |
| **D** | 12.35 | 0.279 | 61.25 | 0.568 | 57.77 | 0.674 | 57.74 | |
| **E** | 37.04 | 0.145 | 79.86 | 0.353 | 73.75 | 0.52 | 67.40 | |

**(b)** Compound **37**

| Well | Conc. (µg/ml) | Absorbance/ % Cytotoxicity against 4TI | | | | | | Mean $IC_{50}$ ± SEM n = 3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$ = 3.72 | | $IC_{50}$ = 3.52 | | $IC_{50}$ = 3.98 | | |
| **A** | 0.00 | 0.466 | 0.00 | 0.524 | 0.00 | 0.506 | 0.00 | 3.7 ± 0.1 |
| **B** | 1.37 | 0.385 | 17.38 | 0.357 | 31.87 | 0.501 | 9.88 | |
| **C** | 4.12 | 0.21 | 54.94 | 0.238 | 54.58 | 0.249 | 50.79 | |
| **D** | 12.35 | 0.187 | 58.58 | 0.195 | 62.79 | 0.181 | 64.23 | |
| **E** | 37.04 | 0.062 | 86.70 | 0.106 | 79.77 | 0.137 | 72.92 | |
| **F** | 111.11 | 0.04 | 91.42 | 0.086 | 84.73 | 0.072 | 85.77 | |
| **G** | 333.33 | 0.033 | 92.92 | 0.064 | 87.79 | 0.045 | 91.11 | |
| **H** | 1000 | 0.031 | 93.35 | 0.019 | 96.37 | 0.037 | 92.69 | |

**(c)** Compound **38**

| Well | Conc. (µg/ml) | Absorbance and % Cytotoxicity against HCC 1395 | | | | | | Mean $IC_{50}$ ± SEM n = 3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$ = 1.67 | | $IC_{50}$ = 8.33 | | $IC_{50}$ = 1.67 | | |
| **A** | 0.00 | 1.6 | 0.00 | 1.29 | 0.00 | 1.345 | 0.00 | 3.9 ± 2.2 |
| **B** | 1.37 | 1.146 | 28.38 | 0.896 | 30.54 | 0.868 | 35.46 | |
| **C** | 4.12 | 0.699 | 56.31 | 0.759 | 41.16 | 0.636 | 52.71 | |
| **D** | 12.35 | 0.682 | 57.38 | 0.494 | 61.71 | 0.568 | 60.08 | |
| **E** | 37.04 | 0.531 | 66.81 | 0.364 | 71.78 | 0.353 | 73.75 | |
| **F** | 111.11 | 0.204 | 87.25 | 0.173 | 86.59 | 0.103 | 92.34 | |
| **G** | 333.33 | 0.063 | 96.06 | 0.036 | 97.21 | 0.04 | 97.02 | |
| **H** | 1000 | 0.046 | 97.13 | 0.015 | 98.83 | 0.011 | 99.18 | |

**(d)** Compound **38**

| Well | Conc. (µg/ml) | Absorbance and % Cytotoxicity against 4TI | | | | | | Mean $IC_{50}$ ± SEM n = 3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$ = 5.00 | | $IC_{50}$ = 8.33 | | $IC_{50}$ = 8.33 | | |
| **A** | 0.00 | 0.466 | 0.00 | 0.506 | 0.00 | 0.523 | 0.00 | 7.2 ± 1.11 |
| **B** | 1.37 | 0.385 | 17.38 | 0.501 | 0.99 | 0.514 | 1.72 | |
| **C** | 4.12 | 0.235 | 49.57 | 0.349 | 31.03 | 0.259 | 50.48 | |
| **D** | 12.35 | 0.187 | 59.87 | 0.181 | 64.23 | 0.245 | 53.15 | |
| **E** | 37.04 | 0.062 | 86.70 | 0.137 | 72.92 | 0.184 | 64.82 | |
| **F** | 111.11 | 0.04 | 91.42 | 0.072 | 85.77 | 0.163 | 68.83 | |
| **G** | 333.33 | 0.033 | 92.92 | 0.045 | 91.11 | 0.066 | 87.38 | |
| **H** | 1000 | 0.031 | 93.35 | 0.037 | 92.69 | 0.036 | 93.12 | |

**(e)** Compound **39**

| Well | Conc. (µg/ml) | Absorbance and % Cytotoxicity against HCC1395 | | | | | | Mean $IC_{50}$ ± SEM n = 3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$ = 8.10 | | $IC_{50}$ = 6.60 | | $IC_{50}$ = 5.55 | | |
| **A** | 0.00 | 0.199 | 0.00 | 0.204 | 0.00 | 0.485 | 0.00 | 6.8 ± 0.7 |
| **B** | 1.37 | 0.198 | 0.5 | 0.201 | 1.47 | 0.413 | 14.85 | |
| **C** | 4.12 | 0.127 | 9.36 | 0.137 | 32.87 | 0.285 | 41.24 | |
| **D** | 12.35 | 0.015 | 92.46 | 0.025 | 87.75 | 0.033 | 93.20 | |
| **E** | 37.04 | 0.009 | 95.48 | 0.02 | 90.20 | 0.03 | 93.81 | |
| **F** | 111.11 | 0.006 | 96.98 | 0.004 | 98.04 | 0.025 | 94.85 | |

**(f)** Compound **39**

| Well | Conc. (µg/ml) | Absorbance and % Cytotoxicity against 4TI | | | | | | Mean $IC_{50}$ ± SEM n = 3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$ = 5.00 | | $IC_{50}$ = 5.35 | | $IC_{50}$ = 6.55 | | |
| **A** | 0.00 | 0.464 | 0.00 | 0.466 | 0.00 | 0.446 | 0.00 | 5.6 ± 0.5 |
| **B** | 1.37 | 0.418 | 9.91 | 0.39 | 16.31 | 0.394 | 11.66 | |
| **C** | 4.12 | 0.25 | 46.12 | 0.259 | 44.42 | 0.285 | 36.10 | |
| **D** | 12.35 | 0.083 | 82.11 | 0.069 | 85.19 | 0.073 | 83.63 | |
| **E** | 37.04 | 0.02 | 95.69 | 0.01 | 97.85 | 0.02 | 95.52 | |
| **F** | 111.11 | 0.02 | 95.69 | 0.001 | 99.79 | 0.003 | 99.33 | |

**(g)** Compound **42**

| Well | Conc. (µg/ml) | Absorbance and % Cytotoxicity against HCC 1395 | | | | | | Mean $IC_{50}$ ± SEM n = 3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$ = 21.9 | | $IC_{50}$ = 22.6 | | $IC_{50}$ = 22.4 | | |
| **A** | 0.00 | 1.413 | 0.00 | 1.34 | 0.00 | 1.368 | 0.00 | 22.3 ± 0.2 |
| **B** | 1.37 | 1.37 | 3.04 | 1.319 | 1.57 | 1.338 | 2.19 | |
| **C** | 4.12 | 1.344 | 4.88 | 1.289 | 3.81 | 1.154 | 15.64 | |
| **D** | 12.35 | 1.183 | 16.28 | 1.166 | 12.99 | 1.124 | 17.84 | |
| **E** | 37.04 | 0.031 | 97.81 | 0.038 | 97.16 | 0.052 | 96.20 | |

**(h)** Compound **42**

| Well | Conc. (µg/ml) | Absorbance and % Cytotoxicity against 4TI | | | | | | Mean $IC_{50}$ ± SEM n=3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$=19.2 | | $IC_{50}$=19.2 | | $IC_{50}$=19.2 | | |
| **A** | 0.00 | 0.509 | 0.00 | 0.594 | 0.00 | 0.504 | 0.00 | 19.2±0.0 |
| **B** | 1.37 | 0.409 | 19.65 | 0.533 | 10.27 | 0.475 | 5.75 | |
| **C** | 4.12 | 0.394 | 22.59 | 0.484 | 18.52 | 0.451 | 10.52 | |
| **D** | 12.35 | 0.353 | 30.65 | 0.388 | 34.68 | 0.343 | 34.52 | |
| **E** | 37.04 | 0.012 | 97.64 | 0.071 | 88.05 | 0.072 | 85.71 | |

**(i)** Compound **43**

| Well | Conc. (µg/ml) | Absorbance and % Cytotoxicity against HCC1395 | | | | | | Mean $IC_{50}$ ± SEM n = 3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$ = 23.2 | | $IC_{50}$ = 23.6 | | $IC_{50}$ = 22.7 | | |
| **A** | 0.00 | 1.342 | 0.00 | 1.373 | 0.00 | 1.342 | 0.00 | 23.2 ± 0.3 |
| **B** | 1.37 | 1.333 | 0.67 | 1.337 | 2.62 | 1.341 | 0.07 | |
| **C** | 4.12 | 1.272 | 5.50 | 1.33 | 3.13 | 1.278 | 4.77 | |
| **D** | 12.35 | 1.236 | 7.90 | 1.296 | 5.61 | 1.207 | 10.06 | |
| **E** | 37.04 | 0.037 | 97.42 | 0.038 | 97.23 | 0.003 | 99.78 | |

**(j)** Compound **43**

| Well | Conc. (µg/ml) | Absorbance and% Cytotoxicity against 4TI | | | | | | Mean $IC_{50}$ ± SEM n = 3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$ =25.8 | | $IC_{50}$=25.8 | | $IC_{50}$=25.2 | | |
| **A** | 0.00 | 0.538 | 0.00 | 0.593 | 0.00 | 0.547 | 0.00 | 25.6±0.2 |
| **B** | 1.37 | 0.498 | 7.43 | 0.547 | 7.76 | 0.542 | 0.91 | |
| **C** | 4.12 | 0.485 | 9.85 | 0.523 | 11.80 | 0.511 | 6.58 | |
| **D** | 12.35 | 0.471 | 12.45 | 0.471 | 20.57 | 0.471 | 13.89 | |
| **E** | 37.04 | 0.112 | 79.18 | 0.133 | 77.57 | 0.085 | 84.46 | |
| **F** | 111.11 | 0.005 | 99.07 | 0.007 | 98.82 | 0.024 | 95.61 | |

**(k)** Compound **40**

| Well | Conc. (µg/ml) | Absorbance/ % Cytotoxicity against HCC 1395 | | | | | | Mean $IC_{50}$ ± SEM n = 3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$ > 1000 | | $IC_{50}$ > 1000 | | $IC_{50}$ > 1000 | | |
| **A** | 0.00 | 1.261 | 0.00 | 1.219 | 0.00 | 1.359 | 0.00 | >1000 |
| **B** | 1.37 | 1.231 | 2.38 | 1.197 | 1.8 | 1.13 | 16.89 | |
| **C** | 4.12 | 1.212 | 3.89 | 1.173 | 3.77 | 1.13 | 16.89 | |
| **D** | 12.35 | 1.162 | 7.85 | 1.14 | 6.48 | 1.122 | 17.44 | |
| **E** | 37.04 | 1.122 | 11.02 | 1.129 | 7.38 | 1.102 | 18.91 | |
| **F** | 111.11 | 1.107 | 12.21 | 1.103 | 9.52 | 1.09 | 19.79 | |
| **G** | 333.33 | 1.036 | 17.84 | 1.067 | 12.47 | 1.048 | 22.88 | |
| **H** | 1000 | 0.873 | 30.77 | 0.77 | 36.83 | 0.882 | 35.10 | |

**(l)** Compound **40**

| Well | Conc. (µg/ml) | Absorbance/ % Cytotoxicity against 4TI | | | | | | Mean $IC_{50}$ ± SEM n=3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$=777 | | $IC_{50}$=762 | | $IC_{50}$=768 | | |
| **A** | 0.00 | 0.662 | 0.00 | 0.668 | 0.00 | 0.629 | 0.00 | 769±4.4 |
| **B** | 1.37 | 0.638 | 3.63 | 0.664 | 0.60 | 0.601 | 4.45 | |
| **C** | 4.12 | 0.624 | 5.74 | 0.64 | 4.19 | 0.597 | 5.09 | |
| **D** | 12.35 | 0.612 | 7.55 | 0.617 | 7.63 | 0.582 | 7.47 | |
| **E** | 37.04 | 0.549 | 17.07 | 0.581 | 13.02 | 0.568 | 9.70 | |
| **F** | 111.11 | 0.529 | 20.09 | 0.547 | 18.11 | 0.549 | 12.72 | |
| **G** | 333.33 | 0.517 | 21.90 | 0.515 | 22.90 | 0.499 | 20.67 | |
| **H** | 1000 | 0.242 | 63.44 | 0.251 | 62.43 | 0.215 | 65.82 | |

**Appendix 79: Mean $IC_{50}$ values of isolated compounds against prostate cancer cell lines**

(a) Compound **37**

| Well | Conc. (µg/ml) | Absorbance/ % Cytotoxicity against DU-145 | | | | | | Mean $IC_{50}$ ± SEM n = 3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$ = 3.42 | | $IC_{50}$ = 4.98 | | $IC_{50}$ = 2.78 | | |
| **A** | 0.00 | 0.257 | 0.00 | 0.19 | 0.00 | 0.195 | 0.00 | 3.7 ± 0.7 |
| **B** | 1.37 | 0.226 | 11.70 | 0.121 | 36.32 | 0.115 | 41.03 | |
| **C** | 4.12 | 0.106 | 58.75 | 0.101 | 46.84 | 0.084 | 56.92 | |
| **D** | 12.35 | 0.066 | 74.32 | 0.04 | 78.95 | 0.041 | 78.97 | |

**(b)** Compound **38**

| Well | Conc. (µg/ml) | Absorbance/ % Cytotoxicity against 22Rv1 | | | | | | Mean $IC_{50}$ ± SEM n = 3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$ = 7.00 | | $IC_{50}$ = 6.50 | | $IC_{50}$ =7.50 | | |
| **A** | 0.00 | 0.66 | 0.00 | 0.561 | 0.00 | 0.552 | 0.00 | 7 ± 0.3 |
| **B** | 1.37 | 0.493 | 25.30 | 0.409 | 27.09 | 0.356 | 35.51 | |
| **C** | 4.12 | 0.379 | 42.58 | 0.309 | 44.92 | 0.312 | 43.48 | |
| **D** | 12.35 | 0.249 | 62.27 | 0.22 | 60.78 | 0.215 | 61.05 | |
| **E** | 37.04 | 0.204 | 69.09 | 0.166 | 70.41 | 0.194 | 64.86 | |
| **F** | 111.11 | 0.175 | 73.48 | 0.153 | 72.73 | 0.168 | 69.57 | |
| **G** | 333.33 | 0.14 | 78.79 | 0.105 | 81.28 | 0.093 | 83.15 | |
| **H** | 1000 | 0.08 | 87.88 | 0.04 | 92.87 | 0.017 | 96.92 | |

**(c)** Compound **38**

| Well | Conc. (µg/ml) | Absorbance/ % Cytotoxicity against DU 145 | | | | | | Mean $IC_{50}$ ± SEM n = 3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$ =7.83 | | $IC_{50}$ = 6.80 | | $IC_{50}$ = 7.68 | | |
| **A** | 0.00 | 0.455 | 0.00 | 0.371 | 0.00 | 0.43 | 0.00 | 7.4 ± 0.3 |
| **B** | 1.37 | 0.332 | 27.03 | 0.309 | 16.71 | 0.339 | 21.16 | |
| **C** | 4.12 | 0.293 | 35.60 | 0.203 | 45.28 | 0.27 | 37.21 | |
| **D** | 12.35 | 0.16 | 64.84 | 0.151 | 59.30 | 0.147 | 65.81 | |
| **E** | 37.04 | 0.01 | 97.80 | 0.00 | 100.00 | 0.00 | 100.0 | |

**(d)** Compound **39**

| Well | Conc. (µg/ml) | Absorbance and % Cytotoxicity against DU-145 | | | | | | Mean $IC_{50}$ ± SEM n = 3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$ = 21.3 | | $IC_{50}$ = 8.20 | | $IC_{50}$ = 8.00 | | |
| **A** | 0.00 | 0.21 | 0.00 | 0.319 | 0.00 | 0.198 | 0.00 | 12.5 ± 4.4 |
| **B** | 1.37 | 0.2 | 4.76 | 0.215 | 32.60 | 0.191 | 3.54 | |
| **C** | 4.12 | 0.174 | 17.14 | 0.194 | 39.18 | 0.172 | 13.13 | |
| **D** | 12.35 | 0.152 | 27.62 | 0.125 | 60.82 | 0.017 | 91.41 | |
| **E** | 37.04 | 0.017 | 91.90 | 0.058 | 81.82 | 0.014 | 92.93 | |
| **F** | 111.11 | 0.005 | 97.62 | 0.025 | 92.16 | 0.006 | 96.97 | |

**(e)** Compound **42**

| Well | Conc. (µg/ml) | Absorbance and % Cytotoxicity against DU-145 | | | | Mean $IC_{50}$ ± SEM n = 3 |
|---|---|---|---|---|---|---|
| | | i | | ii | | |
| | | $IC_{50}$ = 23.1 | | $IC_{50}$ = 22.8 | | |
| **A** | 0.00 | 0.524 | 0.00 | 0.531 | 0.00 | 22.9 ± 0.1 |
| **B** | 1.37 | 0.519 | 0.95 | 0.523 | 1.51 | |
| **C** | 4.12 | 0.474 | 9.54 | 0.515 | 3.01 | |
| **D** | 12.35 | 0.404 | 22.90 | 0.412 | 22.41 | |
| **E** | 37.04 | 0.089 | 83.02 | 0.076 | 85.69 | |

**(f)** Compound **42**

| Well | Conc. (µg/ml) | Absorbance and % Cytotoxicity against 22rv1 | | | | | | Mean $IC_{50}$ ± SEM n = 3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$ = 20 | | $IC_{50}$ = 21.5 | | $IC_{50}$ = 25.3 | | |
| **A** | 0.00 | 0.577 | 0.00 | 0.495 | 0.00 | 0.361 | 0.00 | 22.3 ± 4.4 |
| **B** | 1.37 | 0.473 | 18.02 | 0.476 | 3.84 | 0.349 | 3.32 | |
| **C** | 4.12 | 0.464 | 19.58 | 0.451 | 8.89 | 0.342 | 5.26 | |
| **D** | 12.35 | 0.384 | 33.45 | 0.384 | 22.42 | 0.328 | 9.14 | |
| **E** | 37.04 | 0.114 | 80.24 | 0.005 | 98.99 | 0.027 | 92.52 | |
| **F** | 111.11 | 0.017 | 97.05 | 0.003 | 99.39 | 0.012 | 96.68 | |
| **G** | 333.33 | 0.007 | 98.79 | 0.003 | 99.39 | 0.002 | 99.45 | |
| **H** | 1000 | 0.001 | 99.83 | 0.001 | 99.80 | 0.001 | 99.72 | |

**(g)** Compound **43**

| Well | Conc. (µg/ml) | Absorbance and % Cytotoxicity against DU-145 | | | | | | Mean $IC_{50}$ ± SEM n = 3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$ = 15.8 | | $IC_{50}$ = 14.15 | | $IC_{50}$ = 31.8 | | |
| **A** | 0.00 | 0.335 | 0.00 | 0.372 | 0.00 | 0.396 | 0.00 | 20.6 ± 5.6 |
| **B** | 1.37 | 0.309 | 7.76 | 0.312 | 16.13 | 0.274 | 30.81 | |
| **C** | 4.12 | 0.249 | 25.67 | 0.265 | 28.76 | 0.226 | 42.93 | |
| **D** | 12.35 | 0.193 | 42.39 | 0.2 | 46.24 | 0.202 | 47.98 | |
| **E** | 37.04 | 0.035 | 89.55 | 0.011 | 97.04 | 0.193 | 50.51 | |
| **F** | 111.11 | 0.018 | 94.63 | 0.007 | 98.12 | 0.099 | 75.00 | |
| **G** | 333.33 | 0.014 | 95.82 | 0.004 | 98.92 | 0.012 | 96.99 | |
| **H** | 1000 | 0.01 | 97.01 | 0.002 | 99.46 | | | |

**(h)** Compound **43**

| Well | Conc. (µg/ml) | Absorbance and % Cytotoxicity against 22rv1 | | | | | | Mean $IC_{50}$ ± SEM n = 3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$ = 61.3 | | $IC_{50}$ = 66.7 | | $IC_{50}$ = 62.7 | | |
| **A** | 0.00 | 0.484 | 0.00 | 0.388 | 0.00 | 0.417 | 0.00 | 64.7 ± 1.6 |
| **B** | 1.37 | 0.41 | 15.29 | 0.373 | 3.87 | 0.382 | 8.39 | |
| **C** | 4.12 | 0.386 | 20.25 | 0.353 | 9.02 | 0.349 | 16.31 | |
| **D** | 12.35 | 0.371 | 23.35 | 0.342 | 11.86 | 0.342 | 17.99 | |
| **E** | 37.04 | 0.311 | 35.74 | 0.321 | 17.27 | 0.311 | 25.42 | |
| **F** | 111.11 | 0.108 | 77.69 | 0.01 | 97.42 | 0.018 | 95.68 | |
| **G** | 333.33 | 0.012 | 97.52 | 0.009 | 97.68 | 0.016 | 96.16 | |
| **H** | 1000 | 0 | 100 | 0.002 | 99.48 | 0.004 | 99.04 | |

**(i)** Compound **40**

| Well | Conc. (µg/ml) | Absorbance/ % Cytotoxicity against DU-145 | | | | | | Mean $IC_{50}$ ± SEM n = 3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$ = 828 | | $IC_{50}$ > 1000 | | $IC_{50}$ = 668 | | |
| **A** | 0.00 | 0.442 | 0.00 | 0.34 | 0.00 | 0.305 | 0.00 | 748 ± 80 |
| **B** | 1.37 | 0.352 | 20.36 | 0.333 | 2.06 | 0.286 | 6.23 | |
| **C** | 4.12 | 0.331 | 25.11 | 0.278 | 18.24 | 0.263 | 13.77 | |
| **D** | 12.35 | 0.276 | 37.56 | 0.276 | 18.82 | 0.258 | 15.41 | |
| **E** | 37.04 | 0.271 | 38.69 | 0.263 | 22.65 | 0.25 | 18.03 | |
| **F** | 111.11 | 0.269 | 39.14 | 0.246 | 27.65 | 0.238 | 22.95 | |
| **G** | 333.33 | 0.266 | 39.82 | 0.243 | 28.53 | 0.226 | 25.91 | |
| **H** | 1000 | 0.204 | 53.85 | 0.196 | 42.35 | 0.077 | 74.75 | |

**(j)** Compound **40**

| Well | Conc. (µg/ml) | Absorbance/ % Cytotoxicity against 22rv1 | | | | | | Mean $IC_{50}$ ± SEM n = 3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50c}$>c1000 | | $IC_{50}$ > 1000 | | $IC_{50}$ > 1000 | | |
| **A** | 0.00 | 0.564 | 0.00 | 0.572 | 0.00 | 0.575 | 0.00 | > 1000 |
| **B** | 1.37 | 0.528 | 6.38 | 0.563 | 1.57 | 0.551 | 4.17 | |
| **C** | 4.12 | 0.517 | 8.33 | 0.553 | 3.32 | 0.545 | 5.22 | |
| **D** | 12.35 | 0.497 | 11.88 | 0.528 | 7.92 | 0.54 | 6.09 | |
| **E** | 37.04 | 0.496 | 12.06 | 0.497 | 13.11 | 0.537 | 6.61 | |
| **F** | 111.11 | 0.421 | 25.35 | 0.482 | 15.73 | 0.457 | 20.52 | |
| **G** | 333.33 | 0.416 | 26.24 | 0.479 | 16.26 | 0.453 | 21.22 | |
| **H** | 1000 | 0.364 | 35.46 | 0.374 | 34.62 | 0.326 | 43.30 | |

**Appendix 80: Mean $IC_{50}$ values for the extract against normal cell lines (vero)**

(a) *F. angolensis* stem bark methanol extract

| Well | Conc. (µg/ml) | Absorbance and % Cytotoxicity against vero | | | | | | Mean $IC_{50}$ ± SEM n = 3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$ = 15 | | $IC_{50}$=21.7 | | $IC_{50}$ = 28.3 | | |
| **A** | 0.00 | 0.431 | 0.00 | 0.463 | 0.00 | 0.427 | 0.00 | 21.7 ± 3.8 |
| **B** | 1.37 | 0.431 | 0.00 | 0.368 | 20.53 | 0.418 | 2.11 | |
| **C** | 4.12 | 0.351 | 18.56 | 0.339 | 26.78 | 0.417 | 2.34 | |
| **D** | 12.35 | 0.221 | 48.72 | 0.293 | 36.72 | 0.402 | 5.85 | |
| **E** | 37.04 | 0.159 | 63.11 | 0.161 | 65.23 | 0.16 | 62.53 | |
| **F** | 111.11 | 0.124 | 71.23 | 0.032 | 93.09 | 0.04 | 90.63 | |
| **G** | 333.33 | 0.031 | 92.81 | 0.031 | 93.30 | 0.022 | 94.85 | |
| **H** | 1000 | 0.01 | 97.68 | 0.014 | 96.98 | 0.014 | 96.72 | |

(b) *F. angolensis* stem bark water extract

| Well | Conc. (µg/ml) | Absorbance and % Cytotoxicity against vero | | | | | | Mean $IC_{50}$ ± SEM n = 3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$ = 285 | | $IC_{50}$ = 285 | | $IC_{50}$ = 318 | | |
| **A** | 0.00 | 0.572 | 0.572 | 0.437 | 0.00 | 0.589 | 0.00 | 296 ± 11 |
| **B** | 1.37 | 0.557 | 0.557 | 0.433 | 0.92 | 0.578 | 1.87 | |
| **C** | 4.12 | 0.541 | 0.541 | 0.41 | 6.18 | 0.54 | 8.32 | |
| **D** | 12.35 | 0.499 | 0.499 | 0.403 | 7.78 | 0.524 | 11.04 | |
| **E** | 37.04 | 0.493 | 0.493 | 0.385 | 11.90 | 0.5 | 15.11 | |
| **F** | 111.11 | 0.369 | 0.369 | 0.327 | 25.17 | 0.39 | 33.79 | |
| **G** | 333.33 | 0.263 | 0.263 | 0.207 | 52.63 | 0.292 | 50.52 | |
| **H** | 1000 | 0.029 | 0.029 | 0.102 | 76.66 | 0.019 | 96.77 | |

(c) *C. tanaense* root methanol extract

| **Well** | **Conc. (µg/ml)** | **Absorbance and % Cytotoxicity against** vero | | | | | | **Mean $IC_{50}$** ± SEM **n = 3** |
|---|---|---|---|---|---|---|---|---|
| | | **i** | | **ii** | | **iii** | | |
| | | **$IC_{50}$ = 38.5** | | **$IC_{50}$ = 41.7** | | **$IC_{50}$ = 28.3** | | |
| **A** | 0.00 | 0.454 | 0.00 | 0.431 | 0.00 | 0.468 | 0.00 | 36.2 ± 4 |
| **B** | 1.37 | 0.446 | 1.76 | 0.378 | 12.30 | 0.429 | 8.33 | |
| **C** | 4.12 | 0.414 | 8.81 | 0.362 | 16.01 | 0.415 | 11.33 | |
| **D** | 12.35 | 0.336 | 25.99 | 0.327 | 24.13 | 0.381 | 18.59 | |
| **E** | 37.04 | 0.194 | 57.27 | 0.223 | 48.26 | 0.178 | 61.97 | |
| **F** | 111.11 | 0.141 | 68.94 | 0.098 | 77.26 | 0.135 | 71.15 | |
| **G** | 333.33 | 0.044 | 90.31 | 0.07 | 83.76 | 0.05 | 89.32 | |
| **H** | 1000 | 0.014 | 96.92 | 0.044 | 89.79 | 0.044 | 90.60 | |

(d) *C. tanaense* root water extract

| **Well** | **Conc. (µg/ml)** | **Absorbance and % Cytotoxicity against** vero | | | | **Mean $IC_{50}$** ± SEM **n = 3** |
|---|---|---|---|---|---|---|
| | | **i** | | **ii** | | |
| | | **$IC_{50}$ > 1000** | | **$IC_{50}$ > 1000** | | |
| **A** | 0.00 | 0.63 | 0.00 | 0.63 | 0.00 | > 1000 |
| **B** | 1.37 | 0.584 | 7.30 | 0.584 | 7.30 | |
| **C** | 4.12 | 0.566 | 10.16 | 0.566 | 10.16 | |
| **D** | 12.35 | 0.565 | 10.32 | 0.565 | 10.32 | |
| **E** | 37.04 | 0.558 | 11.43 | 0.558 | 11.43 | |
| **F2** | 111.11 | 0.551 | 12.54 | 0.551 | 12.54 | |
| **G** | 333.33 | 0.542 | 13.97 | 0.542 | 13.97 | |
| **H** | 1000 | 0.484 | 23.18 | 0.484 | 23.18 | |

(e) *U. anisatum* root methanol extract

| **Well** | **Conc. (µg/ml)** | **Absorbance and % Cytotoxicity against** vero | | | | | | **Mean $IC_{50}$** ± SEM **n=3** |
|---|---|---|---|---|---|---|---|---|
| | | **i** | | **ii** | | **iii** | | |
| | | **$IC_{50}$=3.68** | | **$IC_{50}$=3.38** | | **$IC_{50}$=2.85** | | |
| **A** | 0.00 | 0.456 | 0.00 | 0.53 | 0.00 | 0.68 | 0.00 | 3.3±0.2 |
| **B** | 1.37 | 0.264 | 42.11 | 0.452 | 14.72 | 0.442 | 35.00 | |
| **C** | 4.12 | 0.223 | 51.10 | 0.2 | 62.26 | 0.247 | 63.68 | |
| **D** | 12.35 | 0.163 | 64.25 | 0.146 | 72.45 | 0.041 | 93.97 | |
| **E** | 37.04 | 0.143 | 68.64 | 0.135 | 74.53 | 0.028 | 95.88 | |
| **F** | 111.11 | 0.079 | 82.68 | 0.133 | 74.91 | 0.026 | 96.18 | |
| **G** | 333.33 | 0.065 | 85.75 | 0.051 | 90.38 | 0.015 | 97.79 | |
| **H** | 1000 | 0.02 | 95.61 | 0.004 | 99.25 | 0.003 | 99.56 | |

(f) *U. anisatum* root water extract

| Well | Conc. (µg/ml) | Absorbance and % Cytotoxicity against vero | | | | Mean $IC_{50}$ ± SEM n=2 |
|---|---|---|---|---|---|---|
| | | i | | ii | | |
| | | $IC_{50}$ = 155 | | $IC_{50}$ = 152 | | |
| **A** | 0.00 | 0.413 | 0.00 | 0.395 | 0.00 | 153.5 ± 1.5 |
| **B** | 1.37 | 0.396 | 4.12 | 0.376 | 4.81 | |
| **C** | 4.12 | 0.385 | 6.78 | 0.375 | 5.06 | |
| **D** | 12.35 | 0.368 | 10.90 | 0.365 | 7.59 | |
| **E** | 37.04 | 0.33 | 20.10 | 0.342 | 13.42 | |
| **F** | 111.11 | 0.258 | 37.53 | 0.25 | 36.71 | |
| **G** | 333.33 | 0.026 | 93.79 | 0.035 | 91.14 | |
| **H** | 1000 | 0.025 | 93.95 | 0.013 | 96.71 | |

(g) *H. abyssinica* rhizome methanol extract

| Well | Conc. (µg/ml) | Absorbance and % Cytotoxicity against vero | | | | | | Mean $IC_{50}$ ± SEM n = 3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$ = 91.7 | | $IC_{50}$ = 89.3 | | $IC_{50}$ = 71.7 | | |
| **A** | 0.00 | 0.462 | 0.00 | 0.446 | 0.00 | 0.72 | 0.00 | 84.2 ± 6.3 |
| **B** | 1.37 | 0.448 | 3.03 | 0.432 | 3.14 | 0.716 | 0.56 | |
| **C** | 4.12 | 0.447 | 3.25 | 0.427 | 4.26 | 0.707 | 1.81 | |
| **D** | 12.35 | 0.44 | 4.76 | 0.409 | 8.30 | 0.695 | 3.47 | |
| **E** | 37.04 | 0.38 | 17.75 | 0.378 | 15.25 | 0.654 | 9.17 | |
| **F** | 111.11 | 0.184 | 60.17 | 0.161 | 63.90 | 0.588 | 18.33 | |
| **G** | 333.33 | 0.175 | 62.12 | 0.116 | 73.99 | 0.413 | 42.64 | |
| **H** | 1000 | 0.074 | 83.98 | 0.116 | 73.99 | 0.133 | 81.53 | |

(h) *H. abyssinica* rhizome water extract

| Well | Conc. (µg/ml) | Absorbance and % Cytotoxicity against vero | | | | | | Mean $IC_{50}$ ± SEM n = 3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$ = 160 | | $IC_{50}$ = 197 | | $IC_{50}$ = 195 | | |
| **A** | 0.00 | 0.471 | 0.00 | 0.465 | 0.00 | 0.507 | 0.00 | 184 ± 12 |
| **B** | 1.37 | 0.465 | 1.27 | 0.446 | 4.09 | 0.472 | 6.90 | |
| **C** | 4.12 | 0.45 | 2.1 | 0.445 | 4.30 | 0.463 | 8.68 | |
| **D** | 12.35 | 0.43 | 8.70 | 0.429 | 7.74 | 0.459 | 9.47 | |
| **E** | 37.04 | 0.371 | 21.23 | 0.426 | 8.39 | 0.443 | 12.62 | |
| **F** | 111.11 | 0.272 | 42.25 | 0.345 | 25.81 | 0.362 | 28.60 | |
| **G** | 333.33 | 0.121 | 74.31 | 0.053 | 88.60 | 0.084 | 83.43 | |
| **H** | 1000 | 0.00 | 100.0 | 0.00 | 100.0 | 0.046 | 90.93 | |

(i) *H. abyssinica* flower methanol extract

| Well | Conc. (µg/ml) | Absorbance and % Cytotoxicity against vero | | | | | | Mean $IC_{50}$ ± SEM n = 3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$ = 78.2 | | $IC_{50}$ = 78.3 | | $IC_{50}$ =75.0 | | |
| **A** | 0.00 | 0.758 | 0.00 | 0.398 | 0.00 | 0.422 | 0.00 | 77.2 ± 1.1 |
| **B** | 1.37 | 0.418 | 44.85 | 0.39 | 2.01 | 0.389 | 7.82 | |
| **C** | 4.12 | 0.415 | 45.25 | 0.37 | 7.04 | 0.375 | 11.14 | |
| **D** | 12.35 | 0.383 | 49.47 | 0.343 | 13.82 | 0.371 | 12.09 | |
| **E** | 37.04 | 0.375 | 50.52 | 0.247 | 37.94 | 0.266 | 36.97 | |
| **F** | 111.11 | 0.292 | 61.48 | 0.167 | 58.04 | 0.18 | 57.35 | |
| **G** | 333.33 | 0.144 | 81.00 | 0.094 | 76.38 | 0.13 | 69.19 | |
| **H** | 1000 | 0.034 | 95.51 | 0.02 | 94.97 | 0.002 | 99.53 | |

(j) *H. abyssinica* flower water extract

| Well | Conc. (µg/ml) | Absorbance and % Cytotoxicity against vero | | | | | | Mean $IC_{50}$ ± SEM n = 3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$ = 145.0 | | $IC_{50}$ = 732.0 | | $IC_{50}$ = 452 | | |
| **A** | 0.00 | 0.719 | 0.00 | 0.43 | 0.00 | 0.479 | 0.00 | 443 ± 169.5 |
| **B** | 1.37 | 0.472 | 34.35 | 0.428 | 0.5 | 0.448 | 6.47 | |
| **C** | 4.12 | 0.418 | 41.86 | 0.426 | 0.9 | 0.44 | 8.14 | |
| **D** | 12.35 | 0.415 | 42.28 | 0.423 | 1.63 | 0.414 | 13.57 | |
| **E** | 37.04 | 0.412 | 42.70 | 0.385 | 10.47 | 0.389 | 18.79 | |
| **F** | 111.11 | 0.387 | 46.18 | 0.346 | 19.53 | 0.357 | 25.47 | |
| **G** | 333.33 | 0.186 | 74.13 | 0.284 | 33.95 | 0.282 | 41.13 | |
| **H** | 1000 | 0.18 | 74.97 | 0.166 | 61.40 | 0.087 | 81.84 | |

(k) *L. cornuta* leaf methanol extract

| Well | Conc. (µg/ml) | Absorbance and % Cytotoxicity against vero | | | | | | Mean $IC_{50}$ ± SEM n = 3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$ = 362 | | $IC_{50}$ = 362 | | $IC_{50}$ = 342 | | |
| **A** | 0.00 | 0.515 | 0.00 | 0.458 | 0.00 | 0.458 | 0.00 | 355.3 ± 6.7 |
| **B** | 1.37 | 0.488 | 5.24 | 0.431 | 5.89 | 0.431 | 5.90 | |
| **C** | 4.12 | 0.457 | 11.26 | 0.427 | 6.77 | 0.427 | 6.77 | |
| **D** | 12.35 | 0.449 | 12.81 | 0.399 | 12.88 | 0.399 | 12.88 | |
| **E** | 37.04 | 0.445 | 13.59 | 0.374 | 18.34 | 0.374 | 18.34 | |
| **F** | 111.11 | 0.359 | 30.29 | 0.32 | 30.13 | 0.32 | 30.13 | |
| **G** | 333.33 | 0.27 | 47.57 | 0.235 | 48.69 | 0.235 | 48.69 | |
| **H** | 1000 | 0.045 | 91.26 | 0.06 | 86.90 | 0.06 | 86.90 | |

(l) *S. princeae* aerial methanol extract

| Well | Conc. (µg/ml) | Absorbance and % Cytotoxicity against vero | | | | | | Mean $IC_{50}$ ± SEM n=3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$ = 212 | | $IC_{50}$ = 202 | | $IC_{50}$ = 195 | | |
| **A** | 0.00 | 0.615 | 0.00 | 0.597 | 0.00 | 0.61 | 0.00 | 203 ± 4.9 |
| **B** | 1.37 | 0.597 | 2.93 | 0.595 | 0.34 | 0.599 | 1.8 | |
| **C** | 4.12 | 0.575 | 6.50 | 0.589 | 1.34 | 0.579 | 5.08 | |
| **D** | 12.35 | 0.573 | 6.83 | 0.506 | 15.24 | 0.534 | 12.46 | |
| **E** | 37.04 | 0.519 | 15.61 | 0.455 | 23.79 | 0.463 | 24.10 | |
| **F** | 111.11 | 0.451 | 26.67 | 0.406 | 31.99 | 0.399 | 34.59 | |
| **G** | 333.33 | 0.133 | 78.37 | 0.134 | 77.55 | 0.132 | 78.36 | |
| **H** | 1000 | 0.053 | 91.38 | 0.057 | 90.45 | 0.04 | 93.44 | |

(m) *S. princeae* aerial methanol extract

| Well | Conc. (µg/ml) | Absorbance and % Cytotoxicity against vero | | | | | | Mean $IC_{50}$ ± SEM n = 3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$ = 552 | | $IC_{50}$ = 648 | | $IC_{50}$ = 528 | | |
| **A** | 0.00 | 0.608 | 0.00 | 0.571 | 0.00 | 0.606 | 0.00 | 576 ± 36.7 |
| **B** | 1.37 | 0.599 | 1.48 | 0.568 | 0.53 | 0.592 | 2.31 | |
| **C** | 4.12 | 0.592 | 2.63 | 0.568 | 0.53 | 0.59 | 2.64 | |
| **D** | 12.35 | 0.584 | 3.95 | 0.557 | 2.45 | 0.579 | 4.46 | |
| **E** | 37.04 | 0.551 | 9.38 | 0.557 | 2.45 | 0.579 | 4.46 | |
| **F** | 111.11 | 0.448 | 26.32 | 0.542 | 5.08 | 0.569 | 6.11 | |
| **G** | 333.33 | 0.416 | 31.58 | 0.472 | 17.34 | 0.407 | 32.84 | |
| **H** | 1000 | 0.073 | 87.99 | 0.084 | 85.29 | 0.077 | 87.29 | |

(n) *P. africana* stem bark methanol extract

| Well | Conc. (µg/ml) | Absorbance and % Cytotoxicity against vero | | | | | | Mean $IC_{50}$ ± SEM n = 3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$ = 27.1 | | $IC_{50}$ = 20.3 | | $IC_{50}$ = 19.5 | | |
| **A** | 0.00 | 0.423 | 0.00 | 0.374 | 0.00 | 0.378 | 0.00 | 20.5±0.6 |
| **B** | 1.37 | 0.412 | 2.60 | 0.373 | 0.27 | 0.371 | 1.85 | |
| **C** | 4.12 | 0.354 | 16.31 | 0.279 | 25.40 | 0.249 | 34.13 | |
| **D** | 12.35 | 0.283 | 33.10 | 0.253 | 32.35 | 0.243 | 35.71 | |
| **E** | 37.04 | 0.164 | 61.23 | 0.05 | 86.63 | 0.053 | 85.98 | |

(o) *P. africana* stem bark methanol extract

| Well | Conc. (µg/ml) | Absorbance and % Cytotoxicity against vero | | | | | | Mean $IC_{50}$ ± SEM n = 2 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | | | |
| | | $IC_{50}$ = 202 | | $IC_{50}$ = 190 | | | | |
| **A** | 0.00 | 0.46 | 0.00 | 0.46 | 0.00 | | | 196 ± 6 |
| **B** | 1.37 | 0.454 | 1.30 | 0.454 | 1.30 | | | |
| **C** | 4.12 | 0.443 | 3.70 | 0.443 | 3.70 | | | |
| **D** | 12.35 | 0.398 | 13.48 | 0.398 | 13.48 | | | |
| **E** | 37.04 | 0.394 | 14.35 | 0.394 | 14.35 | | | |
| **F** | 111.11 | 0.362 | 21.30 | 0.362 | 21.30 | | | |
| **G** | 333.33 | 0.035 | 92.39 | 0.035 | 92.39 | | | |

**Appendix 81: Mean $IC_{50}$ values for the active fractions against normal cell lines (vero)**

(a) Petroleum ether fraction from *F. angolensis* stem bark methanol extract

| Well | Conc. (µg/ml) | Absorbance and % Cytotoxicity against vero | | | | | | Mean $IC_{50}$ ± SEM n = 3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$ = 55 | | $IC_{50}$ = 50 | | $IC_{50}$ = 65 | | |
| **A** | 0.00 | 0.76 | 0.00 | 0.747 | 0.00 | 0.728 | 0.00 | 56.7 ± 4.4 |
| **B** | 1.37 | 0.72 | 5.26 | 0.737 | 1.34 | 0.686 | 5.77 | |
| **C** | 4.12 | 0.718 | 5.53 | 0.648 | 13.25 | 0.649 | 10.85 | |
| **D** | 12.35 | 0.626 | 17.63 | 0.586 | 21.55 | 0.596 | 18.13 | |
| **E** | 37.04 | 0.498 | 34.47 | 0.423 | 43.37 | 0.484 | 33.52 | |
| **F** | 111.11 | 0.022 | 97.11 | 0.04 | 94.64 | 0.114 | 82.20 | |
| **G** | 333.33 | 0.004 | 99.47 | 0.013 | 98.26 | 0.002 | 99.73 | |
| **H** | 1000 | 0.004 | 99.47 | 0.01 | 98.66 | 0.002 | 99.73 | |

(b) Dichloromethane fraction from *F. angolensis* stem bark methanol extract

| Well | Conc. (µg/ml) | Absorbance and % Cytotoxicity against vero | | | | | | Mean $IC_{50}$ ± SEM n = 3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$ = 75 | | $IC_{50}$ = 72.5 | | $IC_{50}$ = 75 | | |
| **A** | 0.00 | 0.515 | 0.00 | 0.475 | 0.00 | 0.478 | 0.00 | 74.2 ± 0.8 |
| **B** | 1.37 | 0.499 | 3.11 | 0.469 | 1.26 | 0.472 | 1.26 | |
| **C** | 4.12 | 0.494 | 4.08 | 0.454 | 4.42 | 0.463 | 3.14 | |
| **D** | 12.35 | 0.47 | 8.74 | 0.435 | 8.42 | 0.453 | 5.23 | |
| **E** | 37.04 | 0.463 | 10.10 | 0.422 | 11.16 | 0.439 | 8.16 | |
| **F** | 111.11 | 0.004 | 99.22 | 0.004 | 99.16 | 0.007 | 98.53 | |
| **G** | 333.33 | 0.004 | 99.22 | 0.004 | 99.16 | 0.001 | 99.79 | |
| **H** | 1000 | 0.001 | 99.80 | 0.002 | 99.58 | 0.001 | 99.79 | |

(c) Ethyl acetate fraction from *F. angolensis* stem bark methanol extract

| Well | Conc. (µg/ml) | Absorbance and % Cytotoxicity against vero | | | | | | Mean $IC_{50}$ ± SEM n=3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$ = 82.5 | | $IC_{50}$ = 105 | | $IC_{50}$ = 65 | | |
| **A** | 0.00 | 0.816 | 0.00 | 0.799 | 0.00 | 0.542 | 0.00 | 84.2 ± 11.6 |
| **B** | 1.37 | 0.766 | 6.13 | 0.798 | 0.00 | 0.515 | 4.98 | |
| **C** | 4.12 | 0.697 | 14.58 | 0.757 | 0.06 | 0.453 | 16.42 | |
| **D** | 12.35 | 0.587 | 28.06 | 0.669 | 16.27 | 0.41 | 24.35 | |
| **E** | 37.04 | 0.516 | 36.76 | 0.618 | 22.65 | 0.353 | 34.87 | |
| **F** | 111.11 | 0.324 | 60.29 | 0.374 | 53.19 | 0.094 | 82.66 | |
| **G** | 333.33 | 0.053 | 93.50 | 0.008 | 99.00 | 0.003 | 99.45 | |
| **H** | 1000 | 0.042 | 94.85 | 0.006 | 99.25 | 0.542 | 100.0 | |

(d) Acetone fraction from *F. angolensis* stem bark methanol extract

| Well | Conc. (µg/ml) | Absorbance and % Cytotoxicity against vero | | | | | | Mean $IC_{50}$± SEM n=3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$= 130 | | $IC_{50}$=75 | | $IC_{50}$=10 | | |
| **A** | 0.00 | 0.747 | 0.00 | 0.774 | 0.00 | 0.691 | 0.00 | 71.6±34.7 |
| **B** | 1.37 | 0.659 | 11.78 | 0.729 | 5.81 | 0.569 | 17.66 | |
| **C** | 4.12 | 0.461 | 38.29 | 0.639 | 17.44 | 0.452 | 34.59 | |
| **D** | 12.35 | 0.435 | 41.77 | 0.444 | 42.63 | 0.34 | 50.80 | |
| **E** | 37.04 | 0.43 | 42.44 | 0.437 | 43.54 | 0.325 | 52.97 | |
| **F** | 111.11 | 0.396 | 46.99 | 0.33 | 57.36 | 0.322 | 53.40 | |
| **G** | 333.33 | 0.11 | 85.27 | 0.101 | 86.95 | 0.078 | 88.71 | |
| **H** | 1000 | 0.083 | 88.89 | 0.011 | 98.58 | 0.025 | 96.38 | |

(e) Methanol fraction from *F. angolensis* stem bark methanol extract

| Well | Conc. (µg/ml) | Absorbance/ % Cytotoxicity against vero | | | | | | Mean $IC_{50}$ ± SEM n=3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$ =168 | | $IC_{50}$ = 158 | | $IC_{50}$ = 175 | | |
| **A** | 0.00 | 0.749 | 0.00 | 0.723 | 0.00 | 0.815 | 0.00 | 167 ± 4.9 |
| **B** | 1.37 | 0.7 | 6.54 | 0.714 | 1.24 | 0.68 | 16.56 | |
| **C** | 4.12 | 0.696 | 7.08 | 0.676 | 6.50 | 0.582 | 28.59 | |
| **D** | 12.35 | 0.655 | 12.55 | 0.651 | 9.96 | 0.546 | 33.00 | |
| **E** | 37.04 | 0.614 | 18.02 | 0.632 | 12.59 | 0.519 | 36.32 | |
| **F** | 111.11 | 0.442 | 40.98 | 0.414 | 42.74 | 0.514 | 36.93 | |
| **G** | 333.33 | 0.161 | 78.50 | 0.139 | 80.77 | 0.141 | 82.70 | |
| **H** | 1000 | 0.044 | 94.13 | 0.053 | 92.67 | 0.103 | 87.36 | |

(f) Petroleum ether and dichloromethane mixture fraction from *C. tanaense* root methanol extract

| **Well** | **Conc. (µg/ml)** | **Absorbance/ % Cytotoxicity against** vero | | | | | | **Mean $IC_{50}$** ± SEM |
|---|---|---|---|---|---|---|---|---|
| | | **i** | | **ii** | | **iii** | | |
| | | **$IC_{50}$ = 37.5** | | **$IC_{50}$ = 35** | | **$IC_{50}$ = 35** | | **n = 3** |
| **A** | 0.00 | 0.651 | 0.00 | 0.668 | 0.00 | 0.607 | 0.00 | 35.8±0.8 |
| **B** | 1.37 | 0.584 | 10.29 | 0.645 | 3.44 | 0.602 | 0.82 | |
| **C** | 4.12 | 0.554 | 14.90 | 0.601 | 10.02 | 0.572 | 5.77 | |
| **D** | 12.35 | 0.496 | 23.81 | 0.547 | 18.11 | 0.563 | 7.25 | |
| **E** | 37.04 | 0.326 | 49.92 | 0.323 | 51.65 | 0.294 | 51.57 | |
| **F** | 111.11 | 0.032 | 95.08 | 0.127 | 80.99 | 0.019 | 96.98 | |
| **G** | 333.33 | 0.013 | 98.00 | 0.016 | 97.60 | 0.005 | 99.18 | |
| **H** | 1000 | 0.00 | 100 | 0.012 | 98.20 | 0.001 | 99.83 | |

(g) Ethyl acetate fraction from *C. tanaense* root methanol extract

| **Well** | **Conc. (µg/ml)** | **Absorbance/ % Cytotoxicity against vero** | | | | | | **Mean $IC_{50}$** ± SEM |
|---|---|---|---|---|---|---|---|---|
| | | **i** | | **ii** | | **iii** | | |
| | | **$IC_{50}$ = 22.5** | | **$IC_{50}$ = 20** | | **$IC_{50}$ = 17.5** | | **n = 3** |
| **A** | 0.00 | 0.561 | 0.00 | 0.526 | 0.00 | 0.602 | 0.00 | 20 ± 1.4 |
| **B** | 1.37 | 0.5 | 10.87 | 0.47 | 10.65 | 0.491 | 18.44 | |
| **C** | 4.12 | 0.451 | 19.61 | 0.432 | 17.87 | 0.44 | 26.91 | |
| **D** | 12.35 | 0.373 | 33.51 | 0.376 | 28.52 | 0.367 | 38.87 | |
| **E** | 37.04 | 0.132 | 76.47 | 0.021 | 96.00 | 0.088 | 85.38 | |
| **F** | 111.11 | 0.043 | 92.34 | 0.013 | 97.53 | 0.011 | 98.17 | |
| **G** | 333.33 | 0.009 | 98.40 | 0.004 | 99.23 | 0.004 | 99.34 | |
| **H** | 1000 | 0.002 | 99.69 | 0.001 | 99.81 | 0.003 | 99.50 | |

(h) Acetone fraction from *C. tanaense* root methanol extract

| **Well** | **Conc. (µg/ml)** | **Absorbance/ % Cytotoxicity against vero** | | | | | | **Mean $IC_{50}$** ± SEM |
|---|---|---|---|---|---|---|---|---|
| | | **i** | | **ii** | | **iii** | | |
| | | **$IC_{50}$ = 15** | | **$IC_{50}$ = 15** | | **$IC_{50}$ = 17.5** | | **n=3** |
| **A** | 0.00 | 0.604 | 0.00 | 0.645 | 0.00 | 0.575 | 0.00 | 15.8 ± 0.8 |
| **B** | 1.37 | 0.539 | 10.76 | 0.64 | 0.78 | 0.514 | 10.61 | |
| **C** | 4.12 | 0.479 | 20.70 | 0.524 | 18.76 | 0.512 | 10.96 | |
| **D** | 12.35 | 0.325 | 46.19 | 0.35 | 45.74 | 0.326 | 43.30 | |
| **E** | 37.04 | 0.154 | 74.50 | 0.113 | 82.48 | 0.113 | 80.34 | |
| **F** | 111.11 | 0.055 | 90.89 | 0.021 | 96.74 | 0.045 | 92.17 | |
| **G** | 333.33 | 0.014 | 97.68 | 0.013 | 97.98 | 0.025 | 95.65 | |
| **H** | 1000 | 0.001 | 99.83 | 0.001 | 99.84 | 0.025 | 95.65 | |

(i) Methanol fraction from *C. tanaense* root methanol extract

| Well | Conc. (µg/ml) | Absorbance/ % Cytotoxicity against vero | | | | | | Mean $IC_{50}$ ± SEM n = 3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$ = 30 | | $IC_{50}$ = 10 | | $IC_{50}$ = 10 | | |
| **A** | 0.00 | 0.576 | 0.00 | 0.559 | 0.00 | 0.586 | 0.00 | 16.7 ± 6.7 |
| **B** | 1.37 | 0.549 | 4.69 | 0.472 | 15.56 | 0.526 | 10.24 | |
| **C** | 4.12 | 0.478 | 17.01 | 0.439 | 21.47 | 0.432 | 26.28 | |
| **D** | 12.35 | 0.302 | 47.57 | 0.263 | 52.95 | 0.231 | 60.58 | |
| **E** | 37.04 | 0.28 | 51.39 | 0.151 | 72.99 | 0.189 | 67.75 | |
| **F** | 111.11 | 0.16 | 72.22 | 0.099 | 82.29 | 0.116 | 80.20 | |
| **G** | 333.33 | 0.065 | 88.72 | 0.066 | 95.35 | 0.088 | 84.98 | |
| **H** | 1000 | 0.023 | 96.00 | 0.056 | 83.97 | 0.009 | 98.46 | |

(j) Petroleum ether fraction from *H. abyssinica* rhizome methanol extract

| Well | Conc. (µg/ml) | Absorbance/ % Cytotoxicity against vero | | | | | | Mean $IC_{50}$± SEM n=3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$ = 407 | | $IC_{50}$ = 384 | | $IC_{50}$ = 354 | | |
| **A** | 0.00 | 0.719 | 0.00 | 0.738 | 0.00 | 0.765 | 0.00 | 381.7 ± 15.3 |
| **B** | 1.37 | 0.708 | 1.53 | 0.726 | 1.63 | 0.761 | 0.52 | |
| **C** | 4.12 | 0.699 | 2.78 | 0.709 | 3.93 | 0.748 | 2.22 | |
| **D** | 12.35 | 0.621 | 13.63 | 0.62 | 15.99 | 0.677 | 11.50 | |
| **E** | 37.04 | 0.577 | 19.75 | 0.595 | 19.38 | 0.579 | 24.31 | |
| **F** | 111.11 | 0.438 | 39.08 | 0.571 | 22.63 | 0.401 | 47.58 | |
| **G** | 333.33 | 0.4 | 44.37 | 0.398 | 46.07 | 0.393 | 48.62 | |
| **H** | 1000 | 0.02 | 97.22 | 0.006 | 99.19 | 0.017 | 97.78 | |

(k) Dichloromethane fraction from *H. abyssinica* rhizome methanol extract

| Well | Conc. (µg/ml) | Absorbance/ % Cytotoxicity against vero | | | | | | Mean $IC_{50}$ ± SEM n=3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$ = 239 | | $IC_{50}$ = 222 | | $IC_{50}$ = 234 | | |
| **A** | 0.00 | 0.724 | 0.00 | 0.74 | 0.00 | 0.776 | 0.00 | 231.7 ± 5 |
| **B** | 1.37 | 0.701 | 3.18 | 0.713 | 3.65 | 0.771 | 0.64 | |
| **C** | 4.12 | 0.698 | 3.59 | 0.713 | 3.65 | 0.766 | 1.29 | |
| **D** | 12.35 | 0.646 | 10.77 | 0.621 | 16.08 | 0.623 | 19.72 | |
| **E** | 37.04 | 0.559 | 22.79 | 0.572 | 22.70 | 0.6 | 22.68 | |
| **F** | 111.11 | 0.548 | 24.31 | 0.526 | 28.92 | 0.58 | 25.26 | |
| **G** | 333.33 | 0.22 | 69.61 | 0.207 | 72.03 | 0.234 | 69.85 | |
| **H** | 1000 | 0.007 | 99.03 | 0.048 | 93.51 | 0.12 | 84.54 | |

(l) Ethyl acetate fraction from *H. abyssinica* rhizome methanol extract

| Well | Conc. (µg/ml) | Absorbance/ % Cytotoxicity against vero | | | | | | Mean $IC_{50}$ ± SEM n = 3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$ = 304 | | $IC_{50}$ = 297 | | $IC_{50}$ = 314 | | |
| **A** | 0.00 | 0.709 | 0.00 | 0.701 | 0.00 | 0.647 | 0.00 | 305 ± 4.9 |
| **B** | 1.37 | 0.696 | 1.83 | 0.701 | 0.00 | 0.642 | 0.77 | |
| **C** | 4.12 | 0.684 | 3.53 | 0.695 | 0.86 | 0.637 | 1.55 | |
| **D** | 12.35 | 0.66 | 6.91 | 0.632 | 9.84 | 0.595 | 8.04 | |
| **E** | 37.04 | 0.641 | 9.59 | 0.588 | 16.12 | 0.566 | 12.52 | |
| **F** | 111.11 | 0.541 | 23.70 | 0.555 | 20.83 | 0.476 | 26.43 | |
| **G** | 333.33 | 0.327 | 53.88 | 0.311 | 55.63 | 0.307 | 52.55 | |
| **H** | 1000 | 0.138 | 80.54 | 0.072 | 89.73 | 0.017 | 97.37 | |

(m)Acetone fraction from *H. abyssinica* root methanol extract

| Well | Conc. (µg/ml) | Absorbance/ % Cytotoxicity against vero | | | | | | Mean $IC_{50}$ ± SEM n=3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$ =794 | | $IC_{50}$ = 829 | | $IC_{50}$ = 744 | | |
| **A** | 0.00 | 0.726 | 0.00 | 0.727 | 0.00 | 0.782 | 0.00 | 789 ± 24.7 |
| **B** | 1.37 | 0.715 | 1.52 | 0.704 | 3.16 | 0.744 | 4.86 | |
| **C** | 4.12 | 0.706 | 2.75 | 0.693 | 4.68 | 0.716 | 8.44 | |
| **D** | 12.35 | 0.701 | 3.44 | 0.663 | 8.80 | 0.672 | 14.07 | |
| **E** | 37.04 | 0.676 | 6.89 | 0.647 | 11.00 | 0.661 | 15.47 | |
| **F** | 111.11 | 0.665 | 8.40 | 0.571 | 21.46 | 0.657 | 15.98 | |
| **G** | 333.33 | 0.563 | 22.45 | 0.537 | 26.13 | 0.539 | 31.07 | |
| **H** | 1000 | 0.252 | 62.29 | 0.304 | 58.18 | 0.3 | 61.63 | |

(n) Methanol fraction from *H. abyssinica* root methanol extract

| Well | Conc. (µg/ml) | Absorbance/ % Cytotoxicity against vero | | | | | | Mean $IC_{50}$ ± SEM n=3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$ = 140 | | $IC_{50}$ = 128 | | $IC_{50}$ = 539 | | |
| **A** | 0.00 | 0.665 | 0.00 | 0.739 | 0.00 | 0.903 | 0.00 | 269 ± 135 |
| **B** | 1.37 | 0.657 | 1.20 | 0.718 | 2.84 | 0.726 | 19.60 | |
| **C** | 4.12 | 0.644 | 3.16 | 0.717 | 2.98 | 0.72 | 20.27 | |
| **D** | 12.35 | 0.511 | 23.16 | 0.573 | 22.46 | 0.708 | 21.59 | |
| **E** | 37.04 | 0.459 | 30.98 | 0.524 | 29.09 | 0.696 | 22.92 | |
| **F** | 111.11 | 0.379 | 43.01 | 0.388 | 47.50 | 0.692 | 23.37 | |
| **G** | 333.33 | 0.026 | 96.09 | 0.107 | 85.52 | 0.623 | 31.01 | |
| **H** | 1000 | 0.012 | 98.20 | 0.031 | 95.81 | 0.066 | 92.69 | |

(o) Petroleum ether fraction from *U. anisatum* root methanol extract

| Well | Conc. (µg/ml) | Absorbance/ % Cytotoxicity against vero | | | | | | Mean $IC_{50}$ ± SEM n=3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$ =760 | | $IC_{50}$ = 600 | | $IC_{50}$ = 600 | | |
| **A** | 0.00 | 0.718 | 0.00 | 0.701 | 0.00 | 1.011 | 0.00 | 653.3 ± 53.3 |
| **B** | 1.37 | 0.714 | 0.56 | 0.691 | 1.43 | 0.908 | 10.19 | |
| **C** | 4.12 | 0.686 | 4.46 | 0.675 | 3.71 | 0.74 | 26.81 | |
| **D** | 12.35 | 0.673 | 6.27 | 0.662 | 5.56 | 0.727 | 28.10 | |
| **E** | 37.04 | 0.633 | 11.84 | 0.615 | 12.27 | 0.725 | 28.29 | |
| **F** | 111.11 | 0.583 | 18.80 | 0.6 | 14.41 | 0.72 | 28.78 | |
| **G** | 333.33 | 0.502 | 30.08 | 0.499 | 28.82 | 0.633 | 37.39 | |
| **H** | 1000 | 0.279 | 61.14 | 0.13 | 81.46 | 0.32 | 68.35 | |

(p) Dichloromethane fraction from *U. anisatum* root methanol extract

| Well | Conc. (µg/ml) | Absorbance/ % Cytotoxicity against vero | | | | | | Mean $IC_{50}$ ± SEM n=3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$ =793 | | $IC_{50}$ = 820 | | $IC_{50}$ =740 | | |
| **A** | 0.00 | 0.71 | 0.00 | 0.74 | 0.00 | 0.651 | 0.00 | 784.3 ± 23.5 |
| **B** | 1.37 | 0.684 | 3.66 | 0.718 | 2.97 | 0.65 | 0.15 | |
| **C** | 4.12 | 0.675 | 4.93 | 0.704 | 4.86 | 0.594 | 5.70 | |
| **D** | 12.35 | 0.653 | 8.03 | 0.703 | 5.00 | 0.588 | 9.68 | |
| **E** | 37.04 | 0.624 | 12.11 | 0.665 | 10.14 | 0.54 | 16.97 | |
| **F** | 111.11 | 0.62 | 12.68 | 0.657 | 11.22 | 0.529 | 18.74 | |
| **G** | 333.33 | 0.583 | 17.89 | 0.587 | 20.68 | 0.483 | 25.81 | |
| **H** | 1000 | 0.258 | 63.66 | 0.293 | 60.41 | 0.229 | 64.82 | |

(q) Dichloromethane and ethyl acetate mixture fraction from *U. anisatum* root methanol extract

| Well | Conc. (µg/ml) | Absorbance/ % Cytotoxicity against vero | | | | | | Mean $IC_{50}$ ± SEM n = 3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$ = 823 | | $IC5_0$ = 760 | | $IC_{50}$ = 743 | | |
| **A** | 0.00 | 0.711 | 0.00 | 0.726 | 0.00 | 0.782 | 0.00 | 775.3 ± 24.3 |
| **B** | 1.37 | 0.707 | 0.56 | 0.715 | 1.52 | 0.744 | 4.86 | |
| **C** | 4.12 | 0.698 | 1.83 | 0.706 | 2.75 | 0.716 | 8.44 | |
| **D** | 12.35 | 0.663 | 6.75 | 0.701 | 3.44 | 0.672 | 14.07 | |
| **E** | 37.04 | 0.609 | 14.35 | 0.676 | 6.89 | 0.661 | 15.47 | |
| **F** | 111.11 | 0.591 | 16.89 | 0.665 | 8.40 | 0.657 | 15.98 | |
| **G** | 333.33 | 0.536 | 24.61 | 0.563 | 22.45 | 0.539 | 31.07 | |
| **H** | 1000 | 0.293 | 58.79 | 0.252 | 65.29 | 0.3 | 61.64 | |

(r) Ethyl acetate fraction from *U. anisatum* root methanol extract

| Well | Conc. (µg/ml) | Absorbance/ % Cytotoxicity against vero | | | | | | Mean $IC_{50}$± SEM n=3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$=413 | | $IC_{50}$=335 | | $IC_{50}$=360 | | |
| **A** | 0.00 | 1.222 | 0.00 | 1.266 | 0.00 | 1.264 | 0.00 | 369.3±23.0 |
| **B** | 1.37 | 1.216 | 0.49 | 1.251 | 1.18 | 1.255 | 0.71 | |
| **C** | 4.12 | 1.205 | 1.39 | 1.193 | 5.77 | 1.081 | 14.48 | |
| **D** | 12.35 | 1.195 | 2.21 | 1.167 | 7.82 | 1.07 | 15.35 | |
| **E** | 37.04 | 1.105 | 9.57 | 1.166 | 7.90 | 1.024 | 18.99 | |
| **F** | 111.11 | 1.057 | 13.50 | 1.133 | 10.51 | 0.95 | 24.84 | |
| **G** | 333.33 | 0.691 | 43.45 | 0.635 | 49.84 | 0.661 | 47.71 | |
| **H** | 1000 | 0.022 | 98.20 | 0.109 | 91.39 | 0.09 | 92.88 | |

(s) Ethyl acetate and acetone mixture fraction from *U. anisatum* root methanol extract

| Well | Conc. (µg/ml) | Absorbance/ % Cytotoxicity against vero | | | | | | Mean $IC_{50}$ ± SEM n=3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$ =740 | | $IC_{50}$ = 910 | | $IC_{50}$ = 835 | | |
| **A** | 0.00 | 0.752 | 0.00 | 0.942 | 0.00 | 0.84 | 0.00 | 828.3 ± 49.2 |
| **B** | 1.37 | 0.724 | 3.72 | 0.923 | 2.02 | 0.827 | 1.55 | |
| **C** | 4.12 | 0.697 | 7.31 | 0.906 | 3.82 | 0.809 | 3.69 | |
| **D** | 12.35 | 0.665 | 11.37 | 0.867 | 7.96 | 0.765 | 8.93 | |
| **E** | 37.04 | 0.64 | 14.89 | 0.806 | 14.44 | 0.74 | 11.90 | |
| **F** | 111.11 | 0.629 | 16.36 | 0.739 | 21.55 | 0.711 | 15.36 | |
| **G** | 333.33 | 0.614 | 18.35 | 0.696 | 26.11 | 0.65 | 22.62 | |
| **H** | 1000 | 0.216 | 71.28 | 0.437 | 53.61 | 0.345 | 58.93 | |

(t) Acetone fraction from *U. anisatum* root methanol extract

| Well | Conc. (µg/ml) | Absorbance/ % Cytotoxicity against vero | | | | | | Mean $IC_{50}$ ± SEM n=3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$ = 485 | | $IC_{50}$ = 255 | | $IC_{50}$ = 458 | | |
| **A** | 0.00 | 0.884 | 0.00 | 0.809 | 0.00 | 0.836 | 0.00 | 399.3 ± 72.6 |
| **B** | 1.37 | 0.745 | 15.72 | 0.766 | 5.32 | 0.697 | 16.63 | |
| **C** | 4.12 | 0.738 | 16.52 | 0.689 | 14.83 | 0.627 | 25.00 | |
| **D** | 12.35 | 0.729 | 17.53 | 0.688 | 14.96 | 0.596 | 28.71 | |
| **E** | 37.04 | 0.725 | 17.98 | 0.602 | 25.59 | 0.594 | 28.95 | |
| **F** | 111.11 | 0.658 | 25.57 | 0.526 | 34.98 | 0.576 | 31.10 | |
| **G** | 333.33 | 0.502 | 43.21 | 0.337 | 58.34 | 0.443 | 47.00 | |
| **H** | 1000 | 0.216 | 75.57 | 0.039 | 95.18 | 0.298 | 64.35 | |

(u) Acetone fraction from *U. anisatum* root methanol extract

| Well | Conc. (µg/ml) | Absorbance/ % Cytotoxicity against vero | | | | | | Mean $IC_{50}$ ± SEM n = 3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$ >1000 | | $IC_{50}$ > 1000 | | $IC_{50}$ > 1000 | | |
| **A** | 0.00 | 1.083 | 0.00 | 0.818 | 0.00 | 0.878 | 0.00 | > 1000 |
| **B** | 1.37 | 0.812 | 25.02 | 0.802 | 1.96 | 0.874 | 0.46 | |
| **C** | 4.12 | 0.798 | 26.32 | 0.79 | 3.42 | 0.861 | 1.94 | |
| **D** | 12.35 | 0.753 | 30.47 | 0.774 | 5.38 | 0.836 | 4.78 | |
| **E** | 37.04 | 0.7 | 35.36 | 0.764 | 6.60 | 0.816 | 7.06 | |
| **F** | 111.11 | 0.668 | 38.32 | 0.701 | 14.30 | 0.765 | 12.87 | |
| **G** | 333.33 | 0.582 | 46.26 | 0.608 | 25.67 | 0.618 | 29.61 | |
| **H** | 1000 | 0.578 | 46.63 | 0.517 | 36.80 | 0.49 | 44.19 | |

(v) Methanol fraction from *U. anisatum* root methanol extract

| Well | Conc. (µg/ml) | Absorbance/ % Cytotoxicity against vero | | | | | | Mean $IC_{50}$ ± SEM n = 3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$ = 178 | | $IC_{50}$ = 173 | | $IC_{50}$ = 150 | | |
| **A** | 0.00 | 0.786 | 0.00 | 0.815 | 0.00 | 0.841 | 0.00 | 167 ± 8.6 |
| **B** | 1.37 | 0.75 | 4.58 | 0.807 | 0.98 | 0.805 | 4.28 | |
| **C** | 4.12 | 0.742 | 5.60 | 0.751 | 7.85 | 0.797 | 5.23 | |
| **D** | 12.35 | 0.679 | 13.61 | 0.718 | 11.90 | 0.676 | 19.62 | |
| **E** | 37.04 | 0.565 | 28.12 | 0.642 | 21.23 | 0.643 | 23.54 | |
| **F** | 111.11 | 0.476 | 39.44 | 0.501 | 38.53 | 0.475 | 43.52 | |
| **G** | 333.33 | 0.189 | 75.95 | 0.161 | 80.25 | 0.147 | 82.52 | |
| **H** | 1000 | 0.122 | 84.48 | 0.083 | 89.82 | 0.113 | 86.56 | |

**Appendix 82: Mean $IC_{50}$ values for the isolated compounds against normal cell lines (vero)**

**(a)** Compound **37**

| Well | Conc. (µg/ml) | Absorbance/ % Cytotoxicity against vero | | | | | | Mean $IC_{50}$ ± SEM n = 3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$ = 21.7 | | $IC_{50}$ = 21.7 | | $IC_{50}$ = 21.7 | | |
| **A** | 0.00 | 0.54 | 0.00 | 0.633 | 0.00 | 0.601 | 0.00 | 21.7 ± 0.0 |
| **B** | 1.37 | 0.441 | 18.33 | 0.495 | 21.80 | 0.515 | 14.31 | |
| **C** | 4.12 | 0.421 | 22.04 | 0.446 | 29.54 | 0.472 | 21.46 | |
| **D** | 12.35 | 0.419 | 22.41 | 0.413 | 34.76 | 0.428 | 28.79 | |
| **E** | 37.04 | 0.058 | 89.26 | 0.017 | 97.31 | 0.022 | 96.34 | |
| **F** | 111.11 | 0.027 | 95.00 | 0.025 | 96.05 | 0.016 | 97.34 | |
| **G** | 333.33 | 0.02 | 96.30 | 0.007 | 98.89 | 0.014 | 97.67 | |
| **H** | 1000 | 0.015 | 97.22 | 0.015 | 97.63 | 0.013 | 97.84 | |

**(b)** Compound **38**

| Well | Conc. (µg/ml) | Absorbance/ % Cytotoxicity against vero | | | | | | Mean $IC_{50}$ ± SEM n=3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$ = 50 | | $IC_{50}$ = 5 | | $IC_{50}$ = 1.02 | | |
| **A** | 0.00 | 0.017 | 0.00 | 0.039 | 0.00 | 0.029 | 0.00 | 18.7 ± 15.7 |
| **B** | 1.37 | 0.016 | 5.88 | 0.039 | 0.00 | 0.012 | 58.62 | |
| **C** | 4.12 | 0.015 | 11.76 | 0.023 | 41.03 | 0.012 | 58.62 | |
| **D** | 12.35 | 0.012 | 29.41 | 0.017 | 56.41 | 0.012 | 58.62 | |
| **E** | 37.04 | 0.009 | 47.06 | 0.014 | 64.10 | 0.011 | 62.07 | |
| **F** | 111.11 | 0.007 | 58.82 | 0.014 | 64.10 | 0.01 | 65.52 | |
| **G** | 333.33 | 0.006 | 64.71 | 0.006 | 84.62 | 0.002 | 93.10 | |
| **H** | 1000 | 0.001 | 94.11 | 0.003 | 92.31 | 0.001 | 96.55 | |

**(c)** Compound **42**

| Well | Conc. (µg/ml) | Absorbance/ % Cytotoxicity against vero | | | | | | Mean $IC_{50}$ ± SEM n = 3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$ =16.2 | | $IC_{50}$ =15.9 | | $IC_{50}$ =14.4 | | |
| **A** | 0.00 | 0.912 | 0.00 | 0.835 | 0.00 | 0.962 | 0.00 | 15.5±0.6 |
| **B** | 1.37 | 0.846 | 7.24 | 0.794 | 4.91 | 0.79 | 17.88 | |
| **C** | 4.12 | 0.827 | 9.32 | 0.736 | 11.86 | 0.721 | 25.05 | |
| **D** | 12.35 | 0.521 | 42.87 | 0.475 | 43.11 | 0.523 | 45.63 | |
| **E** | 37.04 | 0.074 | 91.89 | 0.071 | 91.50 | 0.034 | 96.47 | |
| **F** | 111.11 | 0.042 | 95.39 | 0.042 | 94.97 | 0.00 | 100.0 | |

**(d)** Compound **43**

| Well | Conc. (µg/ml) | Absorbance/ % Cytotoxicity against vero | | | | | | Mean $IC_{50}$ ± SEM n = 3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$ =53 | | $IC_{50}$ = 61 | | $IC_{50}$ =62 | | |
| **A** | 0.00 | 0.444 | 0.00 | 0.383 | 0.00 | 0.38 | 0.00 | 58.7± 2.8 |
| **B** | 1.37 | 0.443 | 0.23 | 0.381 | 0.52 | 0.38 | 0.00 | |
| **C** | 4.12 | 0.395 | 11.04 | 0.379 | 1.04 | 0.372 | 2.11 | |
| **D** | 12.35 | 0.39 | 12.16 | 0.424 | 10.70 | 0.35 | 7.89 | |
| **E** | 37.04 | 0.284 | 36.04 | 0.272 | 28.98 | 0.286 | 24.74 | |
| **F** | 111.11 | 0.011 | 97.52 | 0.025 | 93.47 | 0.003 | 99.21 | |
| **G** | 333.33 | 0.011 | 97.52 | 0.025 | 93.47 | 0.002 | 99.47 | |
| **H** | 1000 | 0.01 | 97.75 | 0.019 | 95.04 | 0.001 | 99.74 | |

**(e)** Compound **40**

| Well | Conc. (µg/ml) | Absorbance/ % Cytotoxicity against vero | | | | | | Mean $IC_{50}$ ± SEM n = 3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$ = 21.7 | | $IC_{50}$ = 21.7 | | $IC_{50}$ = 18.3 | | |
| **A** | 0.00 | 0.527 | 0.00 | 0.533 | 0.00 | 0.562 | 0.00 | 20.6 ± 1.1 |
| **B** | 1.37 | 0.45 | 14.61 | 0.478 | 10.32 | 0.537 | 4.45 | |
| **C** | 4.12 | 0.439 | 16.70 | 0.424 | 20.45 | 0.473 | 15.84 | |
| **D** | 12.35 | 0.4 | 21.1 | 0.312 | 41.46 | 0.388 | 30.96 | |
| **E** | 37.04 | 0.027 | 94.88 | 0.016 | 97.00 | 0.046 | 91.81 | |
| **F** | 111.11 | 0.014 | 97.51 | 0.016 | 97.00 | 0.02 | 96.44 | |
| **G** | 333.33 | 0.01 | 98.10 | 0.014 | 97.51 | 0.02 | 96.44 | |
| **H** | 1000 | 0.001 | 99.81 | 0.004 | 99.25 | 0.014 | 97.51 | |

**Appendix 83: Mean $IC_{50}$ values for standards against normal cell lines**

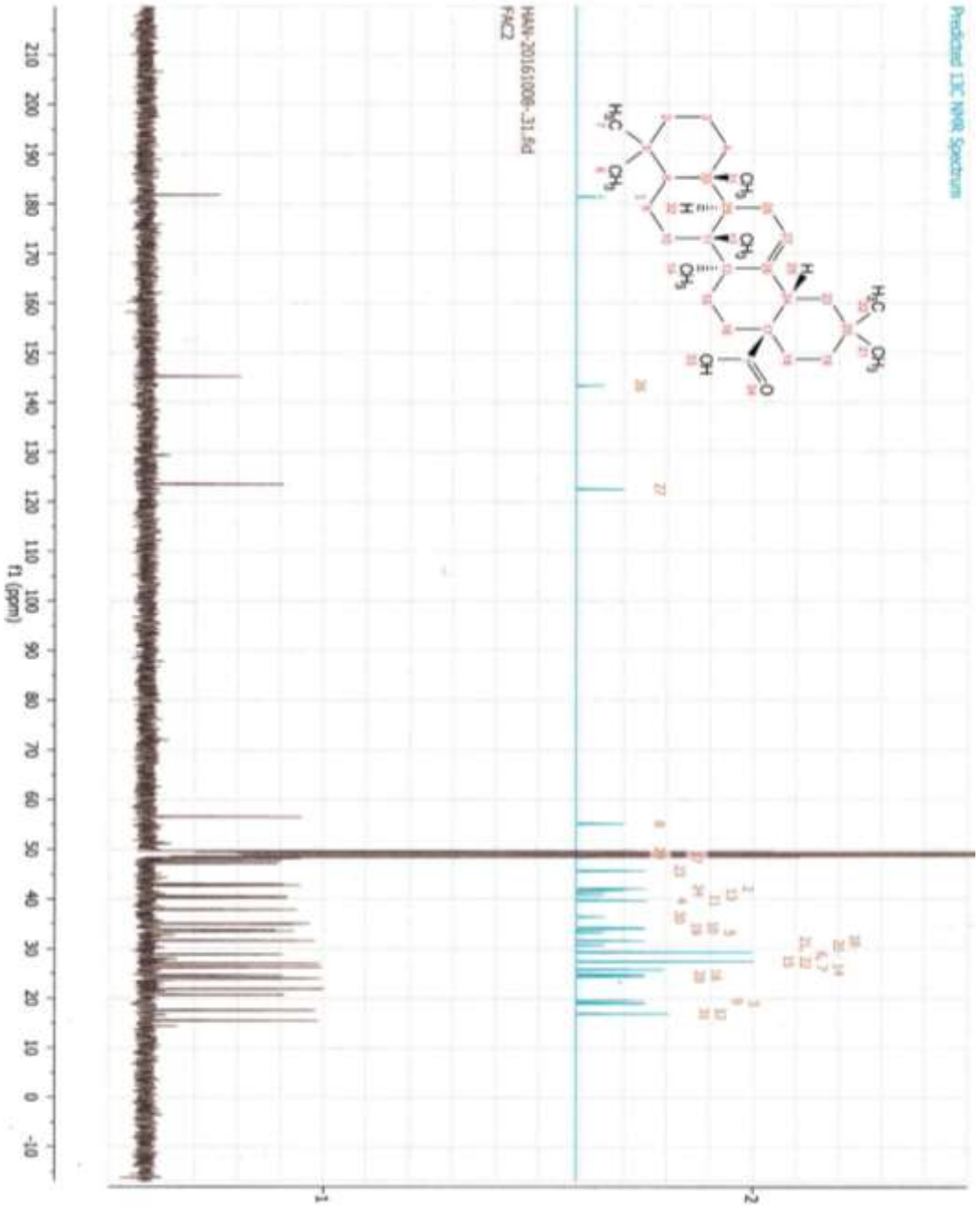
(a) Cyclophosphamide

| Well | Conc. (µg/ml) | Absorbance and % Cytotoxicity against vero | | | | | | Mean $IC_{50}$ ± SEM n=3 |
|---|---|---|---|---|---|---|---|---|
| | | i | | ii | | iii | | |
| | | $IC_{50}$ = 1.67 | | $IC_{50}$ = 5.00 | | $IC_{50}$ = 1.67 | | |
| **A** | 0.00 | 2.041 | 0.00 | 1.325 | 0.00 | 1.538 | 0.00 | 2.8 ± 1.1 |
| **B** | 1.37 | 1.699 | 16.76 | 1.09 | 17.73 | 0.869 | 43.50 | |
| **C** | 4.12 | 0.93 | 54.43 | 0.549 | 58.57 | 0.748 | 51.37 | |
| **D** | 12.35 | 0.699 | 65.75 | 0.521 | 60.68 | 0.702 | 54.36 | |
| **E** | 37.04 | 0.535 | 73.79 | 0.508 | 61.66 | 0.676 | 56.05 | |
| **F** | 111.11 | 0.534 | 73.84 | 0.503 | 62.04 | 0.621 | 59.62 | |
| **G** | 333.33 | 0.151 | 92.01 | 0.2 | 84.91 | 0.557 | 63.78 | |
| **H** | 1000 | 0.071 | 96.52 | 0.022 | 98.34 | 0.072 | 95.32 | |

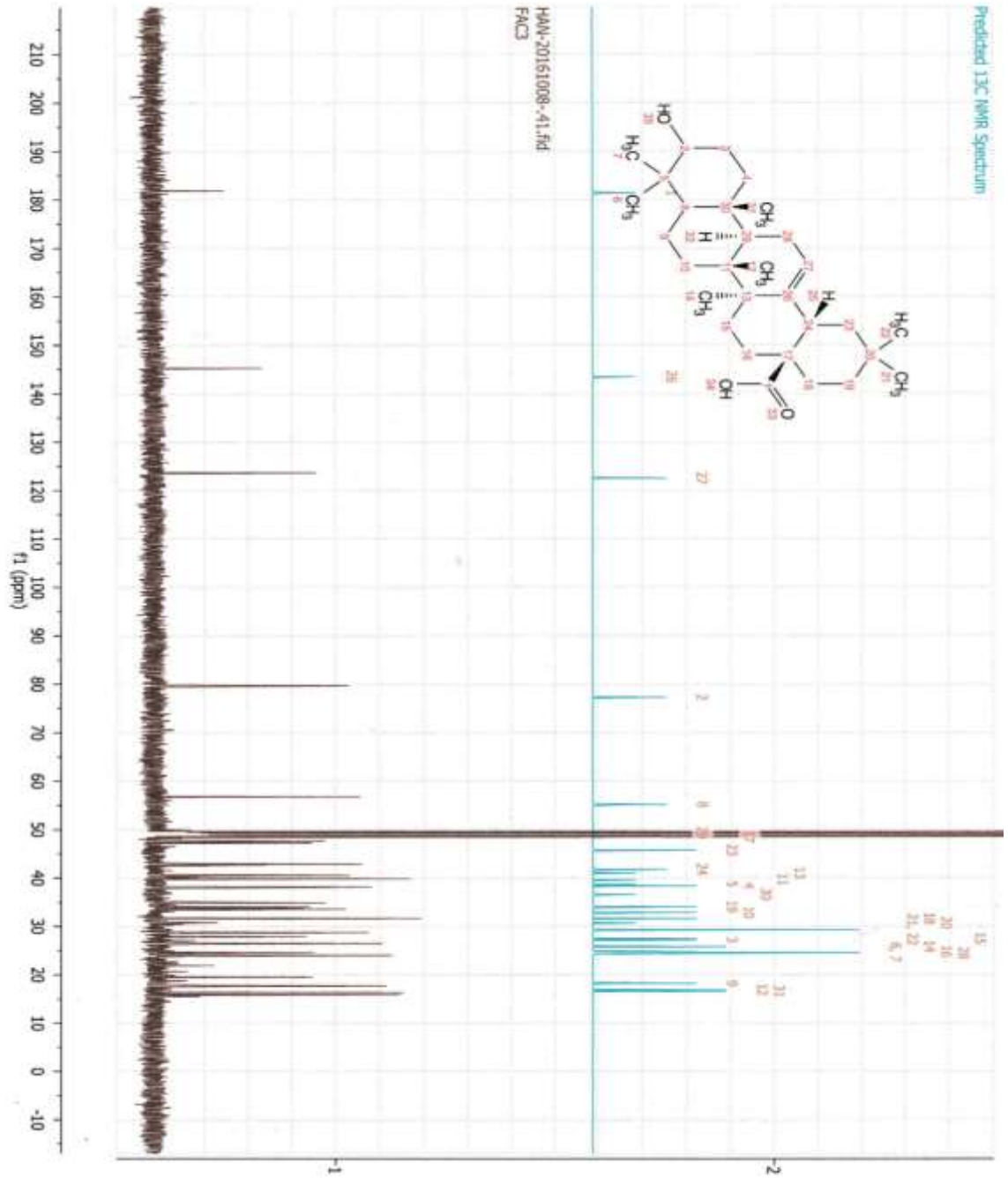
(b) Fluorouracil

| Well | Conc. (µg/ml) | Absorbance and % Cytotoxicity against vero | | | | Mean $IC_{50}$ ± SEM n = 2 |
|---|---|---|---|---|---|---|
| | | i | | ii | | |
| | | $IC_{50}$ = 110 | | IC 50 = 260 | | |
| **A** | 0.00 | 0.395 | 0.00 | 0.41 | 0.00 | 185 ± 75 |
| **B** | 1.37 | 0.247 | 37.47 | 0.262 | 36.09 | |
| **C** | 4.12 | 0.235 | 40.51 | 0.245 | 40.24 | |
| **D** | 12.35 | 0.233 | 41.01 | 0.225 | 45.12 | |
| **E** | 37.04 | 0.214 | 45.82 | 0.217 | 47.07 | |
| **F** | 111.11 | 0.195 | 50.06 | 0.215 | 47.57 | |
| **G** | 333.33 | 0.191 | 51.65 | 0.201 | 50.98 | |
| **H** | 1000 | 0.188 | 52.41 | 0.2 | 51.22 | |

**Appendix 84: Graphical superimposition of $^{13}C$ NMR spectrum of compound 38 (1) and (2)**



**Appendix 85: Graphical superimposition of $^{13}C$ NMR spectrum of compound 39 (1) and (2)**



**Appendix 86: Graphical superimposition of $^{13}C$ NMR spectrum of compound 40 (1) and (2)**