A survey on the biological activities of selected plants used to manage diarrhoea and cancer in Vumba, Zimbabwe

By

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DEDICATION

I dedicate this research to the Marekerah family for their love, prayers and support. Special dedication to my late grandmother for the hope and motivation that made me reach this far.

ACKNOWLEDGEMENTS

My outmost gratitude goes to my supervisor Dr Muleya for her time, patience and devotion. I would also like to thank Dr Guyo and Dr Mehlana, the MSU lab technicians, colleagues, members of staff at PSMAS for their facilities, equipment and knowledge. Special thanks go to my family and friends. Above all, l thank the Almighty God.

ABSTRACT

Biological and antioxidant activities of *Dicoma anomala, Kigelia pinnata, Pseudolachnostylis maprounelifolia* and *Flacourtia indica* are reported. The medicinal plant extracts of different polarities were prepared and tested for antimicrobial activity using the disc diffusion method. The acetone crude extracts showed the highest activity as compared to the chloroform and petroleum ether extracts. The qualitative analysis of the extracts showed presence of tannins, saponins, phenolics, sterols, coumarins, flavonoids, reducing sugars, terpenoids, glycosides and alkaloids. *Kigelia pinnata* acetone crude extract had highest activity followed by chloroform and lastly petroleum ether. *Flacourtia indica* showed the highest antimicrobial activity in the petroleum ether extract with a zone of inhibition of 11.67 mm. The FTIR spectra showed functional groups of hydroxyls, amides, amines, ketones, aldehydes, arene rings and carboxylic derivatives. The Uv-vis showed that *Kigelia pinnata* and *Pseudolachnostylis maprounelifolia* had the highest antioxidant activity in the crude acetone extract with values of 5.9 and 5.5 µg Ascorbic Acid Equivalence AAE/10 µg/mL respectively. *Flacourtia indica* showed the highest antioxidant activity in the petroleum extract with a value of 3.66 µg AAE/10 µg/mL.

DECLARATION

I, **Lynette Marekerah**, hereby declare that l am the sole author of this dissertation. I authorize Midlands State University to lend this dissertation to other institutions or individuals for the purpose of scholarly research

Signature……

Date……

APPROVAL

This dissertation entitled **"A survey for the biological activities of medicinal plants used to manage diarrhoea and cancer in Vumba, Zimbabwe"** by Lynette Marekerah meets the regulations governing the award of the degree of bachelor of Science in Chemical Technology Honors of the Midland State University and is approved for its contribution to knowledge and literal presentation.

Supervisor…….

Date……...

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LIST OF ABBREVIATIONS

FTIR……Fourier Transfer Infrared Spectroscopy

Uv-Vis…….Ultra violet- visible spectroscopy

E.coli……*Escherechia coli*

S.aureas……..*Staphylococcus aureas*

MIC……..Minimum Inhibitory Concentration

AAE……..Ascorbic Acid Equivalence

V.cholerae…….*Vibrio cholerae*

WHO……World Health Organization

NMR…….Nuclear Magnetic Resonance

GC-MS…….Gas Chromatograph – Mass Spectrometry

ROS……..Reactive Oxygen Species

DPPH……1-diphenyl-2-picrylhydrazyl

HVS……..Human Vaginal Swab

CHAPTER 1

BACKGROUND

1.0 Introduction

This chapter gives an outline on the background of study, the aim, objectives, justification and problem statement of the research.

1.1 Background

Medicinal plants have found their use in health delivery systems of many communities, either as pure compounds or standardized extracts thereby providing opportunities for new drug leads [1]. Traditional herbs are a mode of healthcare in Africa which is known for its richness in flora. Many plants are claimed to have antibiotic properties and are being used by tribal people worldwide [2]. Medicinal plants have been regarded as safe and non-toxic due to their natural origin and use in traditional medicine to treat various forms of diseases [3]. Such plants are commonly used in the rural parts of Zimbabwe especially where medical facilities are far from reach. These herbs have been a source of medication for relieving pain, wound healing, chronic diseases, colds, insomnia, diabetes, heart diseases, diarrhoea depression, inflammation, liver diseases just to mention a few [4]. Epidemiological studies have proven that intake of natural antioxidant is associated with reduced risk of cardiovascular disease, cancer, diabetes and other diseases associated with aging [5]. In traditional health care systems, crude plant extracts in the form of decoction, infusion, ointments tincture or herbal extract are used for treatment of infectious diseases [6]. The herbs are usually first dried then crushed into powder using a mortar and pestle. The powder can then be taken in porridge, water or even beer. Decoctions are

prepared by boiling the plant's part that is the root, stem, leaves or bark in water for a short period of time and the resulting liquid is used immediately or stored in a container for future use. Ointments are prepared by mixing the powder with castor oil and applying on the affected area for treatment of sores, ring worms or any other skin diseases [3]. An infusion is prepared by putting the plant portions in water for a short period of time and then using the resulting liquid. The herb powder is boiled in water and the vapours are inhaled and are usually used in the treatment of asthma or bad cold [7]. Most plant-derived products have a diversity of phytochemicals such as flavonoids, phenolic acids, tannins, lignin and other compounds which have been proven antimutagenic, antibacterial, antithrombotic, anticarcinogenic [8]. Many phytochemicals present in plants have been proven to inhibit bacterial pathogens [9]. The determination of biologically active compounds from the medicinal plants is dependent on type of solvent used during the extraction process. Ethanol, methanol and acetone are some of the organic solvents used to extract the bioactive compounds [10].

Vumba mountains is a place known for its flora and fauna. Many traditional healers and ordinary people from surrounding communities seek medicinal plants from these mountains. The major plants of interest in the area are *Dicoma Anomala subsp gerradii, Kigelia Africana k pinnata, Pseudolachnostylis maprounelifolia Pax* and *Flacourtia indica Burm Mer*

1.2 Diarrhoea treating plants

Diarrhoea has become one of the causes of death in developing countries. It is a major cause of mortality and morbidity in all age groups. For children among the age group of 0-3 years, diarrhoea is responsible for 1.236 million deaths per year and rating itself as the second leading cause of death in this age group [11].Diarrhoea is a gastrointestinal disorder which results in an increase in stool frequency and charge consistency [12]. Diarrhea results from an imbalance

between the absorptive and secretory mechanism in the intestinal tract accompanied by hypermortility and usually results in excess loss of fluids and electrolytes in feaces [13]. The minimal changes in the normal intestinal fluid and electrolyte balance can also result in diarrhoea [14]. These changes can be due to infectious agents, toxins and other noxious agents that might be present in the gut causing disruption of the normal fluid secretions and stimulating the gut to expel its contents [14]

Diarrhea usually arises from consumption of contaminated food, water or intolerance to certain food. The causative agents include bacteria like *Vibrio cholera, Escherichia coli, Shigellia species, Clostridium difficile* or viruses like *Rotavirus, Astrovirus, Cytomegalovirus* or parasites like *Enterocytozoon intestinales, Isospora belli, Dientamoeba fragilis* and *Encephalitozoon bieneusi* just to mention a few [15]. It can be categorized into acute diarrhea, bloody diarrhoea and persistent diarrhoea. Acute diarrhoea includes cholera and is associated with fluid loss and rapid dehydration in an affected individual [16]. It usually lasts for several hours or days. The pathogens that generally cause acute watery diarrhoea are *V. cholera*, *E. coli* bacteria as well as *rotavirus*[17]. Bloody diarrhoea is referred to as dysentery with visible blood in stools and is associated with intestinal damage and nutrient losses in the affected individual [14]. The most common cause of bloody diarrhoea is *Shigellia*. Persistent diarrhoea is an episode of diarrhoea with or without blood that lasts at least 14 days. It is susceptible normally to undernourished children and those with AIDS [18].

There has been a search for new antibacterial agents from medicinal plants due to drug resistance and undesirable side effects from antibiotics [19,20]. Traditional healers and other local residents in the Vumba area depend on medicinal plants for their traditional health delivery system. The

plants of interest were *Dicoma Anomala subsp gerradii, and Flacoutia indica Burm Merr* since they are the most commonly used.

Figure 1:*Flacourtia indica Burm Merr*

Common name: Munhunguru

Family: Salicaceae

Description: It is a shrub or small tree with leaves are elliptic, ovate or almost circular and has a scalloped or toothed margin. The flowers are inconspicuous, greenish yellow in short axillary or terminal sprays and sexes separate on different trees [21]. The fruit is fleshy, near-spherical and are purple when ripe. The flowering time is from September to December. *Flacourtia indica Burm Merr* is a deciduous thorn forest which is drought resistant. The leaves are browsed by wild and domestic animals.

Uses: The fruits are used for jaundice and enlarged spleens whilst the leaves and roots are taken for malaria and diarrhea. The roots are used for intestinal worms and pain relief.

Figure 2: *Dicoma anomala subsp gerradi*

Family: Asteraceae

Description: it is a low lying bushy perennial plant with leaves which are alternate, subsessile, leathery and are dull green on the upper side and gray hairy below [21].

Uses: This plant's root, over the years, has been used to treat stomach upsets showing the existence of antidiarrheal ingredients in the herb. Traditional healers use this herb to treat abdominal pains, malaria, wasting in infants, skin sores, gonorrhea, syphilis, diarrhoea and also as a remedy for dysentery. In other rural parts of Zimbabwe like Chipinge, the plant is dried and used to induce vomiting by chewing a small piece of the root.

Known research: It was proven to show antibacterial activity and in 1989, Zdero showed that the herb contain compounds known as germacranolides which are closely related to lactones [7,22]. The isolation of the plant extracts showed small amounts of volatile oil, armophous alkaloids, colourless crystalline glycoside and phytosterol [7,23].

1.3 Cancer treating medicinal plants

Pseudolachnostylis maprounelifolia and *Kigelia pinnata* are the most commonly used herbs by the traditional healers in the Vumba area to handle cancer. *Kigelia pinnata* leaves are dried, crushed to a powder, mixed with castor oil and applied on the affected area. *Kigelia pinnata* is commonly used for skin cancer and other skin related problems like eczema. *Pseudolachnostylis maprounelifolia* is used to manage eye melanoma and lymphoma by boiling the plant leaves and applying the resulting liquid in the affected eye.

Cancer is the second killer disease in the world which is a result of exposure to the body to carcinogens [24]. It results from uncontrolled growth and spread of abnormal cells in the body. Carcinogenesis is a process involving mutation and selective clonal expansion of mutated cells [25,26]. Eye cancer is a type of cancer which starts in the eye. The eye is made up of the orbit, the eyeball and adnexal structures. The types of cancers which can be found in the eye are primary intraocular cancer and secondary intraocular cancer[27]. The most common primary intraocular tumour is choroidal melanoma, which is caused by pigmented cells of the choroid of the eye and is malignant meaning it metastasizes and spreads to other parts [28].

1.3.1 Signs and symptoms of eye melanoma

- Eye bulging
- Vision problems
- Dark spot growing in iris
- Pupil changes shape or size
- Position of eyeball changes position with the socket

1.3.2 Signs and symptoms of eye lymphoma

- Loss of vision or blurred vision
- Sensitivity to light
- Pain in eyes
- Floaters
- Redness or swelling in eye

Eye cancer treatments include surgery, laser therapy, radiation therapy, chemotherapy, targeted therapy. These methods have proven to have serious implications as shown in the table below

Table 1:The effects of treatment

Type of treatment	Side effects
Surgery	Blood clots, bleeding, infections, complications from anesthesia, loss of vision of eye, partial blindness [29]
Radiation therapy	Causes cataracts, glaucoma, bleeding, retinal detachments, loss of vision
Laser therapy	Can damage other parts of the eye, cause vision loss, can result in partial blindness
Chemotherapy	Mouth sores, hair loss, loss of appetite, nausea, vomiting, diarrhoea, fatigue, easy bleeding, bruising, increased chance of infection [29,30]

Figure 3: *Kigelia Africana k pinnata*

Common name: Mubveve

Family: Bignoniaceae

Description: It is a medium to large tree. The bark is gray, smooth and usually flaking in larger specimen. The leaves are opposite, crowded near the ends of branches, imparipinnate with three to 5 pairs of leaflets and a terminal leaflet. The leaflets are oblong, leathery with rough hairs on both surfaces. The flowers are large and are dark maroon with yellow veining, in pendulous sprays up to 12 flowers [31]. The fruit is huge and sausage shaped up to 60 cm long and weighing up to 7 kilograms. The flowering time is from Aug-Oct.

Uses: Traditional healers have used this plant to treat a wide range of skin ailments from boils, fungal infections, acne and diseases like leprosy, syphilis and skin cancer. The Tonga women of Zambezi valley apply cosmetic preparations of the plant to their faces to ensure a blemish-free complexion.

Known research: Research has shown that this plant contains coumarins, norvibutinals, flavonoids, fatty acids, sterols, glycosides and napthaquinones [31,32]. Norvibutinal has shown tumour reducing cytotoxic activity while steroids help a range of skin conditions like eczema. Flavonoids have anticancer properties and also fungicidal properties.

Figure 4: *Pseudolachnostylis maprounelifolia Pax*

Common name: Mutsonzwa, Mukuvazviyo

Family: Phyllanthaceae

Description: It is a single-stemmed which grows up to 12 metres. It has a greyish to dark brown bark tree with leaves which are alternate and are broadly ovate to rounded. The leaves display bright autumn colours of yellow or red and are lost in winter. Flowers are in few axillary clusters and are small or inconspicuous. The fruit is slightly ribbed, spherical, split into six segments and is about 20mm in diameter. The flowering time is from July to November.

1.4 Aim

- To evaluate the biological activities of selected plant extracts of different polarities.

1.5 Objectives

- To test extracts for presence of phytochemicals using qualitative analysis.4
- To isolate medicinal fractions of plant tissues by using different solvent systems.
- To carry out quantitative determination of functional groups present using FTIR.
- To test extracts of different polarities for antimicrobial activity using the disc diffusion method.
- To find the minimum inhibitory concentration of the plant extracts.
- To determine the total phenolic content and antioxidant capacity of the plant extracts using the Phosphomolybdate assay.

1.7 Justification

Due to the high cost of synthetic drugs, most people especially in the rural areas of Zimbabwe depend on medicinal plants for their traditional health delivery system. Treatment options for cancer are typically expensive and unavailable in most rural parts of Zimbabwe hence new and widely available drugs are needed to provide treatment options. Natural products have provided important phytochemicals which might give a solution to this problem [33,34].

Most people have turned to enthnopharmacognosy due to resistance to chemically synthesized drugs and also their adverse effects because phytochemicals from plants have been proven to be safe and effective with lesser adverse effects. Most antibiotics can be associated with adverse effects such as hypersensitivity, immune suppression and also allergic reactions [35,36]. Tramadol is a synthetic drug used in the treatment of cancer and has been reported to have many

side effects which include hallucinations, swelling, seizures, diarrhoea and drowsiness just to mention a few [37]

An increase in demand for medicinal plants has led to a reduction in the quality of the products being offered. This is due to lack of information amongst those who produce and sell the herbs about the care needed during each stage of production that is from harvesting, processing up to the marketing conditions. Hence quality control of raw material is essential for obtaining herbal medicines with acceptable degree of quality for the consumers.

Development of anticancer agents like vincristine and vinblastine from *Catharanthus roseus L.S Don* has given evidence that plants can be a source of cancer chemotherapeutic agents. Vincristine and vinblastine are antileukemic alkaloids which act by disrupting microtubules thereby inhibiting cell growth. Vinblastine has since been developed into a commercial drug and is used in the treatment patients with Hodgkin's disease, testicular and renal cancer. Vincristine is combined with other anticancer agents in the treatment of lymphomas, lymphatic leukemia in children and sarcomas [23].

1.8 Problem Statement

A large number of people across the Vumba region rely on herbal medicines for their medical care. From a survey conducted, the conditions of preparation and selling of these herbs do not meet the standards of Good Manufacturing Practices. Adverse effects can arise from insufficient quality assurance and poor quality control, inherent poisonous phytochemicals and contamination. This can also be due to shortage of trained personnel in most traditional delivery systems hence it contributes to low quality products that have poor quality and might affect the users in the long run [14]. Most herbs are being sold on the streets and are exposed to harsh

conditions like excessive ultraviolet light from the sun, dust particles and toxic gases from vehicles and industries.

Other methods used to treat cancer are very expensive and unavailable in Zimbabwe hence there is need to come up with affordable ways to address this problem. Cancer treatment methods include chemotherapy, radiation therapy, laser therapy or surgery. It has been noted that these methods have negative impact on normal tissues causing bruising, hair loss, increased chance of infection or even bleeding and in fatal situation, death. During treatment, patients also experience adverse effects like loss of appetite, vomiting, diarrhoea, nausea and fatigue which is not a healthy condition for the patient. Due to these effects, there is need to develop bioactive compounds from medicinal plants which have proven to have lesser adverse effects and are cost effective.

CHAPTER 2

LITERATURE REVIEW

2.0 Introduction

This chapter gives detail on the use of the plants as antibacterial, anti-inflammatory, antioxidants and antimicrobial. It also focuses on the different phytochemicals which are present in plants and the functions of these phytochemicals. It also covers the different methods used to extract these phytochemicals and finally the analytical techniques used in characterization of plant extracts.

2.1 Phytochemicals in plants

Most plant extracts have provided unlimited opportunities for new drug discoveries due to the availability of chemical diversity [38]. The World Health Organization has found that 80% of the world's population relies on herbs for most of their primary health care needs [17]. Prevalence of drug resistance has resulted in a deeper search of natural products. Phytochemicals extracted from medicinal plants have proven to possess antioxidant, antimicrobial, anticancer and anti-inflammatory activities [39]. This has given hope since phytochemicals have the potential of filling the place of synthetic drugs. These herbs contain a wide range of substances which people use to treat chronic and some infectious diseases [40]. Most people are turning to enthnopharmacognosy due to adverse effects and microbial resistance to chemically synthesized drugs. Over the years, phytochemicals from plants have been seen safe, seen to have lesser adverse effects and contain beneficial biological activity like antimicrobial, anticancer, analgesic, antidiarrheal and wound healing activity [31,41].

Extraction is the separation of bioactive compounds of plants using solvents which diffuse into the solid plant material thereby solubilizing compounds with similar polarity. The plant constituents can be found from any part of the plant like leaves, barks, roots, fruits or seeds [42].

Phytochemistry is the study of plant chemistry and chemical structure of various organic substances found in plants [43]. Phytochemicals are plant chemicals which are non-nutritive to the plant but protect the plants from diseases, damage and contribute to the plant's colour, aroma and flavor [23]. These phytochemicals are found in different parts of the plant which include flowers, roots, leaves, stems, fruits or seeds. Phytochemicals are secondary metabolites with biological properties like antimicrobial effects, antioxidant activity, modulation of detoxification of enzymes, stimulation of the immune system, modulation of hormone metabolism, decrease of platelet aggregation and anticancer property [40]. These phytochemicals can detoxify substances that cause cancer by inhibiting the initiation, progression and spread of cancers in cells in vitro and in animals in vivo [23]. The cellular mechanisms that phenolics modulate to elicit these anticancer effects are multi-faceted and include regulation of growth factor-receptor interactions and cell signaling cascades, including kinases and transcription factors, that determine expression of genes involved in cell cycle arrest, survival and apoptosis or programmed cell death [28,44]. Phenolics can enhance the body's immune system to recognize and destroy cancer cells as well as inhibiting the development of new blood vessels that is necessary for tumour growth. They also attenuate adhesiveness and invasiveness of cancer cells thereby reducing their metastatic potential [27,45]. Phenolics tend to neutralize radicals, inhibit enzymes that activate carcinogens and activate enzymes that detoxify carcinogens for example genistern prevents formation of new capillaries that are needed for tumor growth and metastasis [23,27]. The most

important of the bioactive compounds are alkaloids, tannins, flavonoids, terpenoids and phenolic compounds.

2.2 Extraction

The soluble components of ground medicinal herbs are extracted by different solvents depending on their solubility. Solid-liquid extraction is influenced by the nature of the plant, the selectivity of solubility capacity, the level of agitation, the viscosity of leaching solvent and the volume or mass of the solvent [46].

2.2.1Choice of solvent

The type of solvent used determines the type of compounds to be extracted. Good solvents should be of low toxicity, evaporate easily, should not dissociate the extract and should have a preservative action [10]. The choice of solvent is affected by

i) Inhibitory compounds extracted

ii) Rate of extraction

iii) Ease of handling of the extracts

iv) Potential health hazards of extractants

v) Toxicity of solvent

2.3 Methods of extraction

2.3.1 Maceration

It involves the ground dry powdered herb being placed in a stoppered container with a selected solvent and allowed to stand at room temperature for a period of at least 3 days. The mixture will be frequently agitated until the soluble matter of the plant is dissolved. The mixture is strained and clarified by filtration or decanting after standing. The most frequently used solvents are

water, ethanol, acetone, hexane and methanol. Maceration in water should not be prolonged as this can result in fungal contamination which does not occur in hydroalcoholic solutions and alcohol [6,47].

2.3.2 Percolation

This is used to extract bioactive compounds in the preparation of fluid extracts or tinctures. A percolator is used and the powdered plant is moistened with a measured amount of suitable solvent and the mixture is allowed to stand for a period of 4 hours in the closed container. The mass is packed and the top of the container closed. More solvent is added to form a layer above the mass and the mixture is allowed to stand in the percolator for 24 hours. The percolator outlet is then opened and the liquid drips out. The mixed liquid is clarified by allowing it to stand for decanting or by filtration [43,48].

2.3.3 Decoction

It involves simmering the herbs in water and is the best method for extraction bioactive compounds from coarse plant material like stems, roots, bark, rhizomes and heavy leaves. The method of decoction can also involve pressing or mashing the plant sample before, during or after its preparation. The basics of decoction is that after the plant is harvested that is the roots, leaves or barks, they are dried, crushed or mashed to break down the plant material so that it releases the essential oils and the bioactive compounds. Depending on the decoction prepared, the plant can be simmered or boiled in water for a period of 10 minutes to 1 hour. Once the plant material has boiled, the liquid is strained to remove the solid part. This process is used for malt beverages since it releases the full flavor of grains used. It is a suitable method for teas, coffee and tinctures [4].

2.3.4 Soxhlet extraction

The ground plant powder is placed in a thimble and placed in a chamber. The selected extracting solvent is poured in a flask. It is heated and vapourises and the vapours condense in a condenser. The extractant then drips in the thimble which contains the plant sample. When level of the liquid rises to the top of siphon tube, the liquid contents of chamber siphon into the flask. It is a continuous process and the cycle continues until a drop of solvent from the siphon tube does not leave residue when evaporated [23]. This method has a disadvantage of requiring heat, which might change the chemical constituents of some metabolites.

2.3.5 Digestion

Digestion is a type of maceration but constitutes of slightly warming during the extraction process. This process is used provided that the temperature will not affect the bioactivity of the plant material. The temperatures used are between 35 ^{0}C - 40 ^{0}C but cannot go beyond 50 ^{0}C since high temperatures change chemical constituents of some metabolites. It is used for tougher plant parts that have poorly soluble substances. The plant parts are placed in a container and the liquid preheated to the set temperature and maintained for a period of half an hour to 24 hours. The container has to be agitated regularly [10].

2.3.6 Infusion

An infusion is a dilute solution of easily soluble bioactive compounds of the medicinal plants. The process is carried out by immersing the plant parts in an amount of boiling water and allowed to stand for 15 minutes. The mixture is then filtered. For example the infusion of the basil leaves is used for treatment for gonorrhea or for stopping nausea and vomiting [23,49].

2.4Plant Antioxidants

Natural antioxidants either in the form of raw extracts or their chemical constituents are very effective to prevent the destructive process caused by oxidative stress. Substantial evidence has accumulated and indicated key roles of reactive oxygen species (ROS) and their oxidants in causing numerous disorders and cancerous activity[86,87]. The body has an inherent antioxidative mechanism and many biological functions such as antimutagenic, anti-carcinogenic and anti-aging responses originate from this property [9,88]. Free radicals cause all damage through mechanisms of covalent bonding and lipid peroxidase with subsequent tissue injury. Antioxidant agents of natural origin have attracted special interest because of their free radical scavenging abilities[89]. Phytochemicals which poses antioxidant activity are carotenoids from fruits and carrots, allyl sulfides from leeks, garlic and onions, flavonoids from fruits and vegetables and polyphenols from tea and grapes[7,72]. Antioxidant activity can be assayed through invitro radical scavenging activity using 4-2,2 Diphenyl-1-picrylhydrazyl (DPPH) assay, 2.2azinobis(3-ethylbenzothiazoline-6-sulfonic acid) ABTS, Cupric assay or the Phosphomolybdate assay just to mention a few[58,90]. The molybdate is reduced from the +6 to the +5 valence state.

Mo^{6+}(yellow) + e^-(from antioxidant) $\longrightarrow$ Mo^{5+}(blue)

2.4.1 4-2,2 Diphenyl-1-picrylhydrazyl DPPH assay

DPPH is a rapid, simple and inexpensive method to measure antioxidant capacity of compounds. DPPH is widely used to test the ability of compounds like flavonoids, phenolic compounds to act as free radical scavengers or hydrogen donors and to evaluate antioxidant activity of compounds. DPPH has also been used to quantify antioxidants in complex biological systems [86,52]. The

DPPH assay can be used for solid or liquid samples and is not specific to the overall antioxidant component but helps in understanding the functional properties of compounds [86].

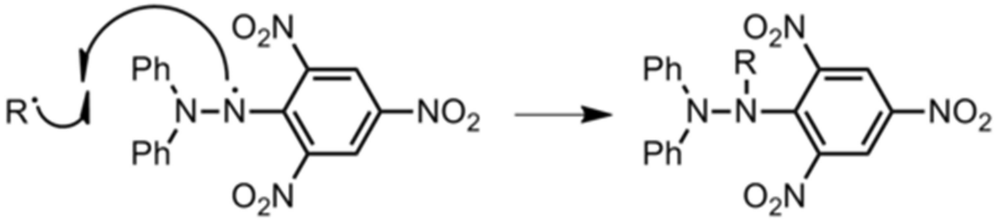


Figure 5: DPPH reaction

2.4.2 Cupric assay

Cu(II) may act as a catalyst in the presence of excessive antioxidants and the antioxidants may act as pro-oxidants. Cu(II) is an initiator for assaying the radical chain break for antioxidants. An example is the reaction of cupric ion with antioxidant compound [AOH] [86].

$Cu(II) + AOH \rightarrow Cu(I) + AO^{\cdot} + H^{+}$

$AO^{\cdot} + L\text{-}H \rightarrow AOH + L^{\cdot}$

$L^{\cdot} + O_2 \rightarrow LOO^{\cdot}$

$LOO^{\cdot} + L\text{-}H \rightarrow LOOH + L^{\cdot}$

$Cu(I) + LOOH \rightarrow Cu(II) + LO^{\cdot} + HO^{-}$

2.4.3 ABTS [2,2 azinobis (3-ethylbenzothiazoline-6-sulfonic acid)]

The ABTS assay is used to screen the relative radical-scavenging abilities of flavonoids and phenolics. ABTS can be used at different pH levels and is useful when studying the effect of pH on antioxidant activity of various compounds [47]. ABTS is soluble in aqueous and organic solvents and is useful in assessing antioxidant activity of samples in different media and is

currently most commonly used in simulated serum ionic potential solution (pH 7.4 phosphate buffer solution containing 150mM NaCl) PBS. An advantage of ABTS assay is that samples react rapidly with ABTS in the aqueous buffer solution (PBS), reaching a steady state within 30 minutes [86].

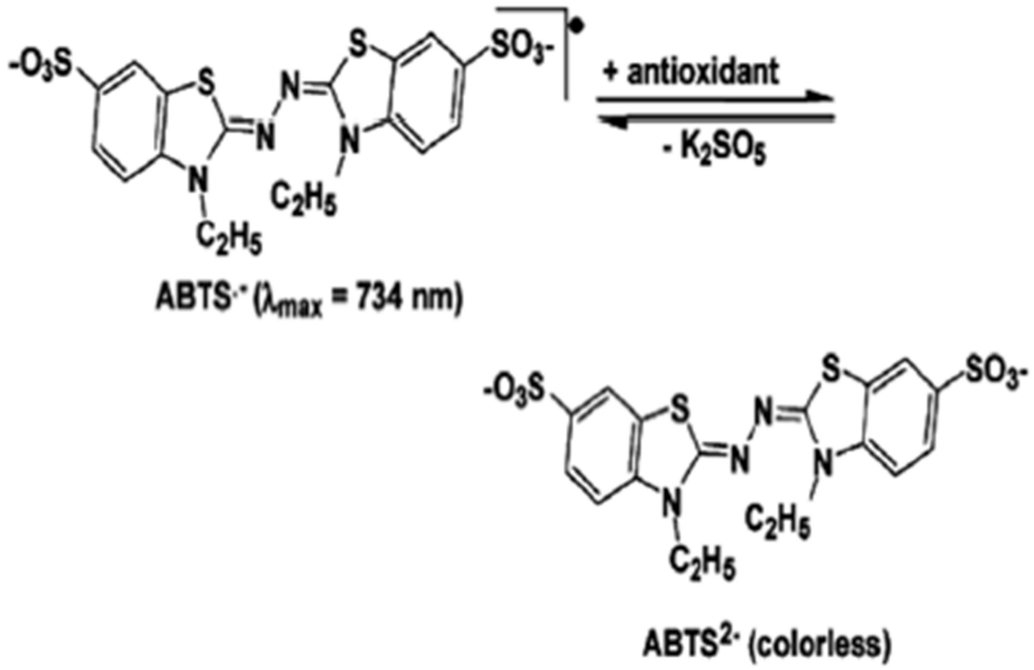


Figure 6: ABTS reaction

2.5 How ROS and RNS are formed in the body

During mitochondrial oxidative metabolism, most of the oxygen consumed is reduced to water although estimated 4% to 5% is converted to ROS which is usually the superoxide ion O_2^-[90]. This anion can be produced enzymatically by NADPH, Xanthine oxidase, cyclooxygenases and lipoxygenases [87]. Dismutation by superoxide dismutase (SOD) reduces O_2^- to H_2O_2. The H_2O_2 generated can be converted to hydroxyl radicals via Fenton reaction [91]. These hydroxyl radicals and superoxide anions can further react with other biological molecules in the system

hence resulting in the formation of other free radicals. The nitrogen derived include ntric oxide, peroxy nitrate, nitrogen dioxide and dinitrogen trioxide.

$O_2 + e^- \rightarrow O_2^{\cdot}$ superoxide anion

$O_2^{\cdot} + H_2O \rightarrow HO_2^{\cdot}$ hydroperoxyl peroxide

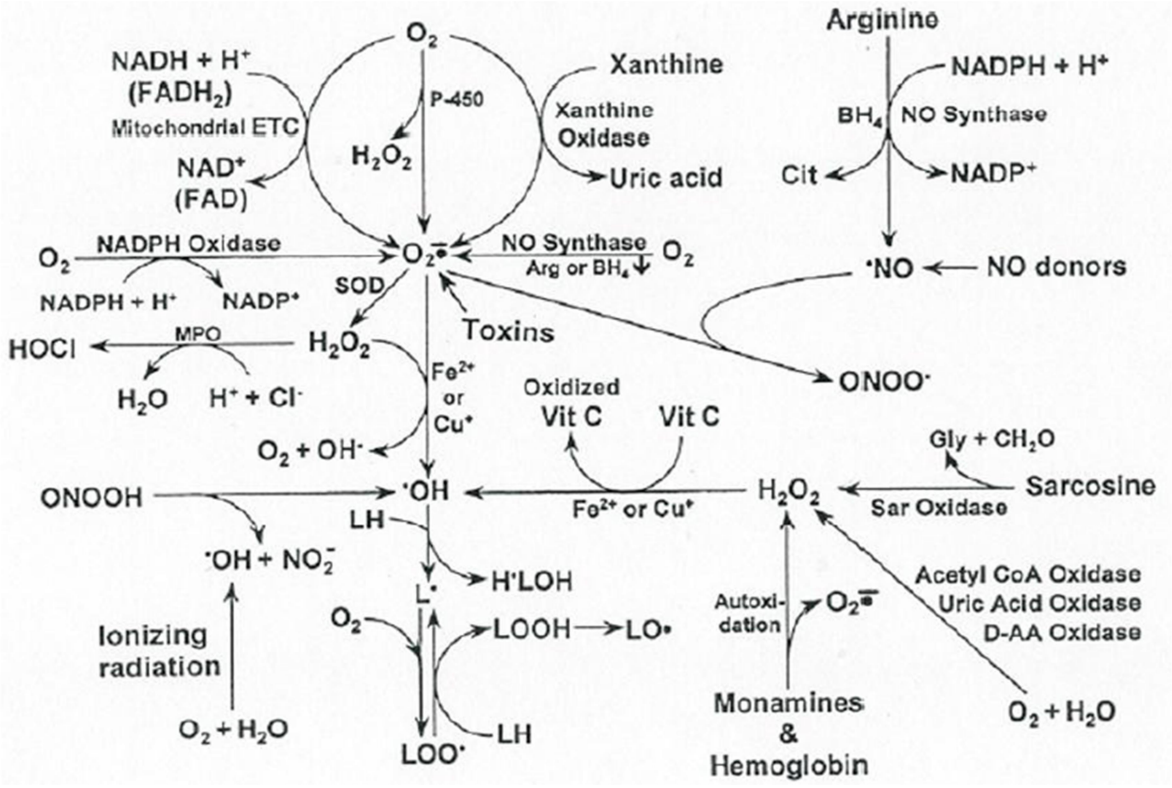
$HO_2^{\cdot} + e^- \rightarrow H_2O_2$ hydrogen peroxide

$H_2O_2 + e^- \rightarrow OH^- + {}^{\cdot}OH$ hydroxyl radical

Fenton Reaction

$H_2O_2 + Fe^{2+} \rightarrow OH + {}^{\cdot}OH + Fe^{2+}$

Oxygen radicals are produced by the reduction of molecular oxygen. Of the radicals produced, the hydroxyl radical and hydroperoxyl radical and the superoxide anion are sufficiently reactive and many interact with biomolecules. The Fenton reaction produces hydroxyl radicals and is catalyzed by transition metals such as Cu^{2+}, Cr(v) and Ni [54].



SOD – Superoxide dismutase $NO^{\cdot}$ – Nitrogen oxide radical

$H^{\cdot}$ – Hydrogen radical $LO^{\cdot}$ – Reactive oxygen specie

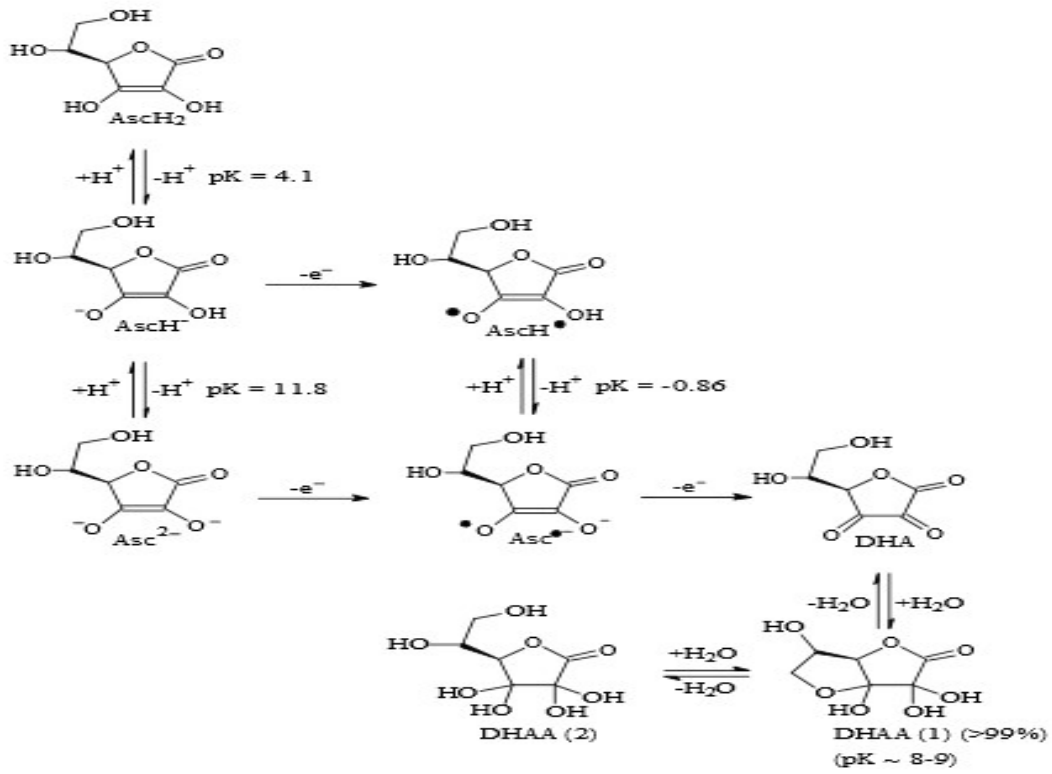
$OH^{\cdot}$ – Hydroxyl radical

Figure 7: Formation of reactive species in the body [87]

Enzymatic antioxidants include catalyse, glutathione peroxidase, glutathione reductase, glucose 6-phosphate dehydrogenase [56]. Non-enzymatic antioxidants include minerals like zinc and selenium, vitamins like Vitamin, A, C, E and K, Carotenoids B-carotene, lycopene, lutein and zeaxanthin, phenolic acids like ferulic p-coumaric, gallic acid and ellagic acid and flavonoids like quercetin kaemperol, catechin, hesperitin, genistein, cyadin, pelagonidin and chysin [56].

2.6. Vitamin C as an antioxidant

Vitamin C (ascorbic acid) is a water-soluble micronutrient required for multiple biological functions which include post-translational hydroxylation of collagen, in the biosynthesis of carnitine, conversion of the neurotransmitter dopamine to norepinephrine, peptide amidation and tyrosine metabolism [92]. A deficiency of Vitamin C in humans results in prolonged wound healing and failure of fractures to repair. Vitamin C is a six-carbon lactone that is synthesized from glucose in the liver of most mammalian species but not humans [93]. Vitamin C is an electron donor and therefore a reducing agent. Ascorbic acid donates two electrons from a double bond between the second third carbons of the 6-carbon molecule. Ascorbic acid is called an antioxidant because by donating its electrons, it prevents other compounds from being oxidised [91]. The species formed after the loss of one electron is a free radical, semi-dehydroascorbic acid or ascorbyl radical. As compared to other free radicals, ascorbyl radical is relatively stable and fairly unreactive [27]. Reactive free radicals can be reduced and the ascorbyl radical formed in its place is less reactive. Reduction of a reactive free radical with formation of a less reactive compound is free radical scavenging or quenching [93]. Vitamin C acts as a scavenger for oxidizing free radicals and harmful oxygen-derived species, such as the hydroxyl radical, hydrogen peroxide and singlet oxygen [94].



DHAA – Dehydroascorbic acid

Asc – Ascobic acid

Asc$^{\cdot}$ – Ascobyl radical

Figure 8: Reaction of Vitamin C

2.7 Anti-inflammatory activity of plant metabolites

Inflammation is a biological response of vascular tissues to harmful stimuli like pathogens, irritants or damaged cells. It can be defined as a protective response to tissue injury which can be due to physical trauma, microbiological agents and noxious chemicals [70,95]. The primary indicators of inflammation are redness, pain, heat and swelling. In the case of injury to any part of the body, the arterioles which encircle tissues dilate giving raised blood circulation towards

the affected area [32]. Medicinal plants can act as anti-inflammatory agents e.g *Achillea millefolium Linn* which is externally used for treatment of wounds, swollen and irritated skin or even burns [63]. Isoprenoids and phenolics contribute mainly to the anti-inflammatory properties [91]. Some herbs have anti-inflammatory properties and possess the ability to reduce both internal and external swelling and inflammation. *Mesua nagassariua* was studied and the plants proved to show anti-inflammatory properties both in vivo and invitro assays [32].

2.8 Antimicrobial activity of plant metabolites

Medicinal plants have shown to have a rich source of antimicrobial agents [19]. There are several reports on antimicrobial activity of different herbal extracts in different parts of the world [89]. Aqueous extract *of Psidium guava* and *Terminalia arjua* were tested and found to have antimicrobial activity using the modified agar well diffusion method of Cappucino and Sherman of 1999 [14]. Antimicrobial agents are bacteriostatic meaning they only inhibit the growth or multiplication of bacteria or bactericidal meaning they kill the bacteria [96]. The mechanism of antimicrobial agents can be categorized into

i) inhibition of ribosome function
ii) inhibition of cell wall synthesis
iii) inhibition of nucleic acid synthesis
iv) inhibition of folate metabolism
v) inhibition of cell membrane function

Bacteriostatic antibiotics include Tetracylines, Sulphonamides, Chloramphenicol, Macrolides, Spectinomycin, Trimethoprim.

Bactericidal antibiotic include Penicillins, Monobactams, Carbapenems, Glycopeptides, Fluoroquinolones and Cephalosporins [97].

2.9 Types of phytochemicals

2.9.1 Alkaloids

Alkaloids are natural products that contain heterocyclic nitrogen atoms and are basic in character. Alkaloids are colourless, crystalline solids with sharp melting points, taste bitter, are optically active and insoluble in water [50]. They are naturally synthesized by some animals, plants, bacteria and fungi. Due to their basic nature, alkaloids can be extracted with water or mild acid and then recovered as crystalline material by treatment with base. They have pharmacological effects hence used as medications, recreational drugs or in entheogenic rituals [23]. Since alkaloids are organic bases, they form salts with mineral acids or maleic acid. Their basicity depends on the position of the functional groups [51]. They fall into class of specific modulators and have been modified during evolution in such a way that they mimic endogenous ligands, hormones or substrates. Since they are derived from amino acids, several have structural similarities to neurotransmitters that can bind to neuroreceptors and either activate or inactivate them. Alkaloids can also target ion channels such as Na^+, K^+, and Ca^{2+} and can inhibit and activate these channels [52]. Morphine is a drug which was extracted from *Papaver somniferum* and is used as a narcotic analgesic. The morphine was further used to synthesize the drug Pethidine. Colchicine and codein are also examples of alkaloid derived drug which are used for the treatment of gut and coughs respectively [23,53]. The roots of *Coix lachryma jobi* have been used for the treatment of rheumatism and neuralgia. Two benzoxazinoid compounds isolated from the roots of *C.lachryma jobi L.* (Graminae) showed 85.5% inhibitory activity of histamine release [30].

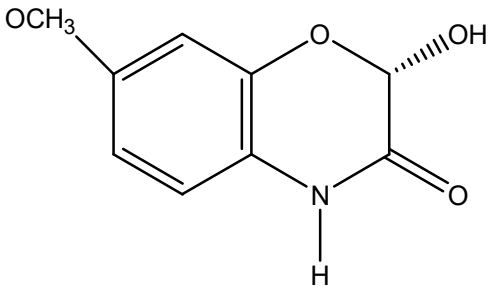


Figure 9: Benzoxazinoid for treatment of rheumatism and neuralgia

2.9.2 Phenolic acids

Phenolic acids are the most abundant secondary metabolites of plants and protect the plants against ultraviolet radiation, aggression by pathogens, parasites or predators and contribute to the colour of the plant. Phenolics react with oxygen and are critical to the preservation, maturation and aging of wine [3,27]. They are divided into two classes which are derivatives of benzoic acid such as gallic acid and derivatives of cinnamic acid such as coumaric, caffeic and ferulic acid [27,42]. They are characterized by hydroxylated aromatic rings and possess one carboxylic acid functional group [10,22]. Plant phenolic are drawing attention due to their antioxidant properties and marked effects in the prevention of various oxidative stress associated diseases such as cancer [27,54]. Tumour cells including leukemia cells have higher levels of reactive oxygen species than normal cells. Total phenolic content can be determined by Folin Ciocalteu method or Phosphomolybdate assay [55,56].

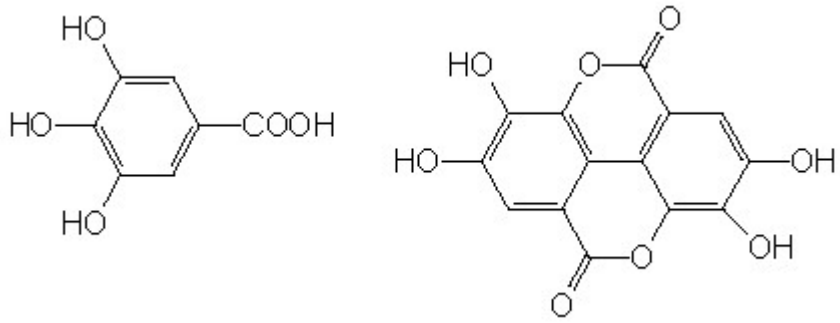
2.9.3 Tannins

Tannins are naturally occurring polyphenolic compound with high molecular weight and are classified into hydrolysable tannins which are hydrolysed by acids or enzymes or condensed tannins[57]. Hydrolysed tannins are polyesters of gallic acid and hexahydroxy-diphenic acid with a central polyols such as glucose and phenolics such as catechin [27,58]. Condensed tannins have

no carbohydrates core and have a group of polyhydroxy-flavon-3-ol oligomer and polymers linked by C-C bonds between flavanol subunits [56,59]. They can be referred to as procinthocyanidins and are resistant to decomposition. Examples are epicatechin and catechin [37]. In Zimbabwe, tannin containing herbs are used for treating different diseases like

i) Intestinal disorders such as dysentery, diarrhoea, rectal prolapse, hemorrhoids and intestinal parasites
ii) Bleeding including functional bleeding, bleeding hemorrhoids, ulcerations and bleeding wounds
iii) Excessive discharge like frequent urination, hyperhidrosis and involuntary seminal disharge [60]

Tannins are used to protect the plants against insects, infections and animal herbivores. They are light yellow or white amorphous powders and have an astringent taste. They act as a defense mechanism in plants against pathogens, hostile conditions and herbivores. Tannins are traditionally used for diarrhea, treatment of catarrh and haemorrhoids [7,61]. In Asian natural healing, tannin-containing extracts are used as astringents against diarrhea, as diuretic, against stomach and duodenal tumours [62]. This is due to a great variety in structures of tannins giving them many possibilities in forming oxidative linkages. They are strong antioxidant against lipid peroxidation when phospholipid bilayers are exposed to aqueous oxygen radical [60,63]. Studies have shown *Cedrela sinensis A. Juss* possesses various antioxidant constituent due to presence of tannins [64]. Tannins are divided into two groups which are the hydrolysable and condensed types. Hydrolysable tannins include for example gallic acid and ellargic acid.



Gallic acid Ellagic acid

Figure 10: Gallic acid and Ellagic acid

2.9.4 Flavonoids

Flavonoids are water-soluble compounds and change colour when heated with a base or ammonia since they are phenolic. They are abundant in the Rutacea, Polygonaceae, Composiate and Umbelliferae families [7]. They are polyphenolic compounds which occur as aglyconesm, glucoside and methylated derivatives [23,58]. They have been reported to have antimicrobial, cytotoxicity, anti-inflammatory as well as anti-tumour activities. Flavonoids act as powerful antioxidants which protect the human body from free radicals and reactive oxygen species and also scavenging properties [39,65]. They have also shown a role in the defense mechanism against oxidative stress from oxidizing agents and free radicals [60,66].The methanol extract of *Lippia nodiflora L* was screened by spectrophotometric DPPH assay and revealed to have significant scavenging effects with increasing concentrations in the range of 10-50 ug/mL. The compound was isolated and found to be 5-hydroxy-3,4, 7-trimethoxyflavone which is a type of flavonoid [37]. Their capacity to act as antioxidants depends on the molecular structure. The position of the hydroxyl groups gives the antioxidant and free radical scavenging activities [22,58]. Flavonoids have been reported to protect the body from various cancerous diseases by neutralizing Reactive Oxygen Species and also possess antimicrobial activities. Antimicrobial

assays were carried out on the extract and results showed that the extract inhibited the growth of *S. aureus* and *E. faecalis* at concentrations of 0.06 and 0.125mg/mL respectively. Flavonoids are divided into 6 subgroups which are, flavones, flavanols, flavonols, isoflavones, flavanones and anthocyanins[27,56]. The basic structure is the flavan nucleus which has 15 carbon atoms arranged in three rings. Quercetin is a flavonoid which was proven to relieve eczema, hay fever, asthma and sinusitis [37].

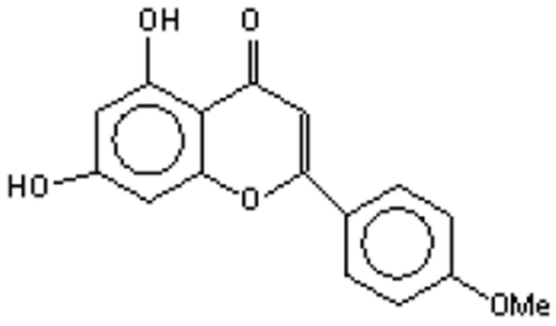


Figure 11: Acacetin used as an anti-inflammatory agent

2.9.5 Saponins

Saponins are glycosides which consist of sugar units which are linked to a steroid aglycone or a triterpene [19]. Saponins lower surface tension of aqueous solutions thereby giving stable foam when they are in contact with water. The triterpenoid saponins contain aglycones with 30 carbon atoms. Two triterpenoid saponins were isolated from the stem bark of *Kalopanaxpictus* and they both showed anti-inflammatory activity. The activity was also documented by Kwak etal who isolated a triterpenoid saponin loniceroside from *Lonicera japonica* which is a medicinal plant [27,67]. Saponins are responsible for permeabilizing the cell membrane, stimulizing lutenizing hormone release leading to abortifacient properties cytostatic and cytotoxic effects on malignant

tumour cells, lowering serum cholesterol levels [68]. Studies in vivo of mice provided evidence that saponins serve as an enhancer of targeted toxins in treatment of cancer [19,23]. Chemotherapeutic agents can be combined with saponins to enhance membrane transportation [69]. Saponins for anticancer agents have been proven to have lesser adverse effects [47]. The biological activities of saponins include antileshmanial, antibacterial, antiviral, antimalarial, antifungal and antitumoral [70,71].

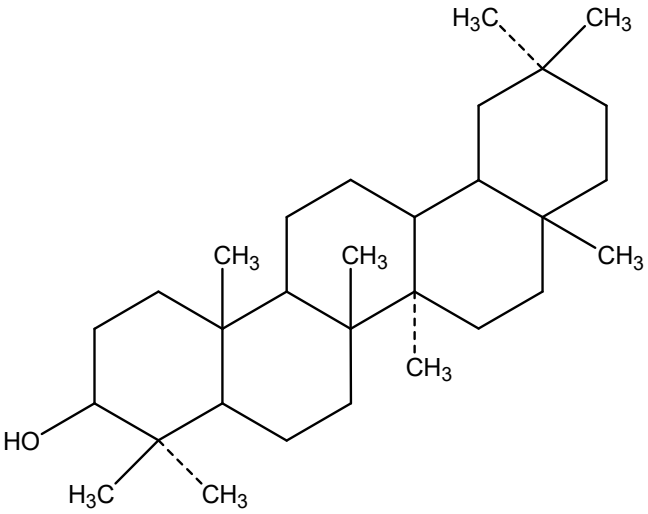


Figure 12: Triterpene

2.9.6 Terpenoids

Terpenoids have been found to have anticancer, antimalarial and anti-inflammatory properties [19,70]. The most common monocyclic monoterpene, Limonene, is found in various trees and herbs, is alsoa constituent of peel oil from lemons and oranges [44,72]. Limonene was proven to have chemopreventitive and therapeutic properties against tumour cell [44]. The caraway seed oil was found to have a monoterpene, carvone, which prevents forestomach carcinoma development and lung cancer [72]. The extracts of *Gorgonian Pseudopterogorgia*

*elisabethae*showed anti-inflammatory activity due to the presence of a diterpene, glycosidesm (pseudopterosins). It was also found to have analgestic characteristics and promote wound healing. Cis-communic acid is found in *Cryptomeria japonica Don (Taxodiaceae)* and has anti-inflammatory activity. The activity testing was done using the carrageenan-induced paw edema method in rats. The leaves of *C.japonica* have been used traditionally for the treatment of eczema, eruption and swelling injury by topical application. Cis- communic acid showed anti-inflammatory effect when applied topically to rats and an inhibitory effect on histamine-induced ileum contractions.

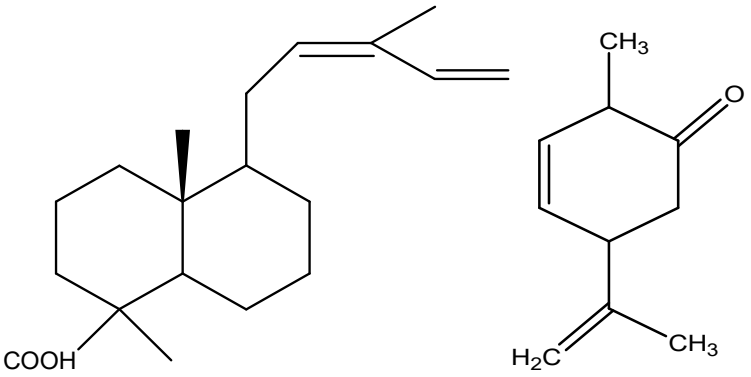
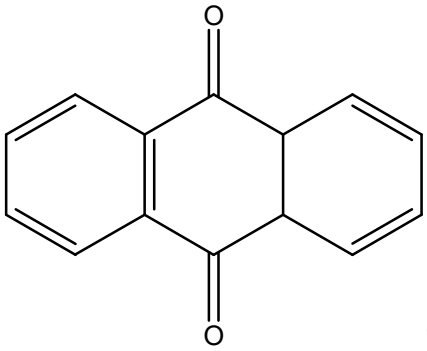


Figure 13: Cis-communic acidand Carvone for treatment of forestomach carcinoma

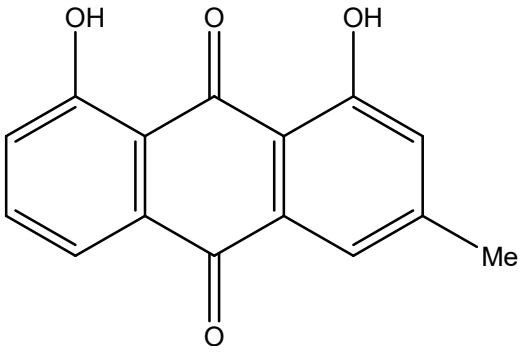
2.9.7 Anthraquinones

Anthraquinones are divided into two types which are emodin and alizarin. Alizarin types are found in the Rubiacea family while emodin types are synthesized by acetate-malonate pathway and both rings are substituted [23]. Anthraquinones have found their use in the food, medicinal and dye industry. Derivatives of 9,10 anthraquinone have found their use in humans and mammals since they display antitrypanosomal, antibacterial and antineoplastic activities [3,46]. Some anthraquinone derivatives have been reported to show antioxidant activity like alizarin 93%, anthrone 95% and rhein 71%, just to mention a few[23]. Anthraquinones are structurally

similar to anthracene and have an appearance of a yellow, light gray to gray green solid crystal. Most vegetables like lettuce, beans, peas and cabbages are rich in anthraquinones. Anthraquinone compounds can be used as laxatives and also in treatment of fungal skin diseases[73]. Plant extracts which contain anthraquinones are used for dyes, food, cosmetics and pharmaceuticals due to pharmacological and therapeutic properties[7]. Congeners of anthraquinone have found their use in Chinese medicine. The roots of *Morinda parvifolia* and *Damnacanthus subspinosus* are used for treatment in cancer and research has shown that subspinosin, rubiadin and morindaparsin which are hydroxyanthraquinone derivatives, are the active substances of the plant [28]. Glycoside derivatives of anthraquinone like Doxorubicin have been used to treat tumours in the mammary gland and even hematological malignancies [1,28]. Many anthraquinones have been reported to have antitumour, insecticidal, anticongestive, antimicrobial, sedative and hypotensive properties [73]. Structural elucidation of bioactive compounds depends on techniques like Nuclear Magnetic Resonance, Infrared Spectroscopy, Mass Spectrometry and X Ray analysis [23,74]. The anthraquinone chrysophanol has proven to have antibacterial, anti-inflammatory and hepatoprotective activity[7,23]. Napththoquinones give plants the yellow or orange pigments. Napthoquinones are common in families like Ebenaceae, Bignoniaceae, Boraginaceae, Proteaceae, Lythraceae, Droseraceae etc. pharmaceutical significance of napthoquinones is very limited[67,75].



9,10 anthraquinone



Chrysophanol

Figure 14: 9,10 Anthraquinone and Chrysophanol

2.9.8 Coumarins

Coumarins are benzo-alpha-pyrone derivatives present in plants as glycosides or in their free state. However, in larger amounts, coumarins become bitter tasting and relatively odorless. In the pharmaceutical industry, coumarines are used as precursors in the production of a number of anticoagulants[76]. Coumarins are found in plant families like Rutacea, Apiaceae, Asteracea and Leguminosea. Derivatives of coumarins include Herniarin, Umbelliferone and Chicorin, just to mention a few[77]. A nonsteroidal anti-inflammatory agent found to be effective in reducing the increased capillary permeability induced in mice by various chemical mediators involved in the inflammatory process histamine, 5-hydroxytryptamine and bradykinin and caused a decrease of 60.7% in the rat paw carrageenan edema at a dose of 40mg/kg. Its activity was compared with that of hydrocortisone, which at a dose of 10mg/kg inhibited the edema by 44% showed the greatest anticonvulsant activity in rodents. The coumarin was isolated from Kernel of *Calophyllum mophyllum Linn (Guttiferae).*

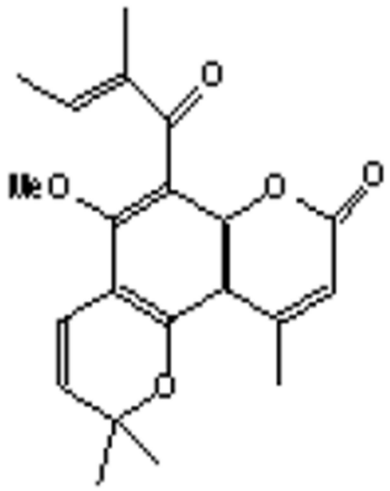


Figure 15: Calophyllolide used as an anti-inflammatory agent

Quinones

A quinone is any benzene derivative formed from the conversion of any even number of -CH= groups into –C=O- groups with necessary and probable rearrangement of double bonds This results in formation of a fully conjugated cyclic dione structure. By artificial synthesis, any identified quinone can be produced by reacting aromatic compounds such as benzene with electron donating substituents like phenols and catechols. Quinones are conjugated but not necessarily aromatic and depending on site of reaction, they may either re-aromatise or break conjugation. Most natural and even synthetic quinones have been studied to show a variety of medicinal uses. These include anti-tumor activity, anti-micro bacteria, anti-cardiovascular and also anti malaria. Anti-inflammatory and analgesic principles were isolated from the methanol extract of the whole herb of *Pyrola rofundifolia L. (Pyrolacea)* and were identified as ursolic acid and chimaphillin.

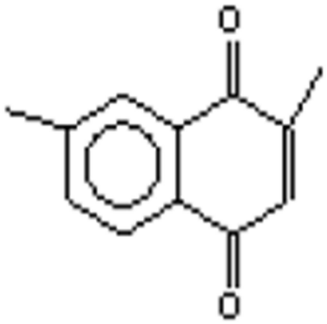


Figure 16: Chimaphillin as an analgesic and anti-inflammatory

Table 2: Biological activities of other medicinal plants

Name of plant	Plant part used	Assay used	Results	Phytochemicals present
Peumus boldus	Leaves	Micro-broth dilution	1/4>1/256 dilution levels, Anti-inflammatory	Flavonoids, alkaloids, saponins, quinones [9]
Ocimum gratissimum	Leaves	DPPH scavenging activity	84.6% at 250µg/mL	
Asparagus racemosus	Leaves	Disc diffusion assay	Inhibition zone of 22mm at 25µg/mL against *Bacillussubtilis*, 20mm for *S. aureus*	Steroids, tannins, alkaloids, phenolic compounds, carbohydrates [3]
Nitraria schoberi L	Fruits	Micro-broth dilution Protein denaturation method	anti-inflammatory,antifungal MIC against *S. aureus*95µg/mL, *C.albicans* 342 µg/mL *A.niger* 342 µg/mL 36.12% at 100µg/mL. 88.33% at 500µg/mL	xanthones, alkaloids, lectins, quinones, coumarins, flavoids,phenols [38]
Azima tetracantha	Leaves	Castor oil-induced diarrhoea	100 mg/kg inhibition of 78% ($p<0.001$)	Alkaloids, tannins, flavoids, saponins [41]

		assay		
Bridelia retusa spreng	stem bark	Agar well diffusion method DPPH scavenging activity	MIC for antimicrobial activity *S.aureus* 3.5 mg/mL, E.coli 4 mg/mL IC50 value 70.46% for 50% methanolic extract	Isoflavone, decanoic acid, Stigmasterol dehydrostigmasterol, beta-sirosterol [42]
P.aeuginosa	Leaves	Modified Kirby-Bauer disc diffusion method	MIC and MFC antimicrobial activity ranged from 31-50 µg/mL against *S.aureus*	saponins, tannins, anthraquinones, steroids, terpenoids, flavonoids [19]

2.10 Characterization

Medicinal plants have been found to be a bioresource of drugs hence people have been using the plant extracts for their healthcare delivery systems. These plants have phytochemicals and the analysis of these phytochemicals help in determining the biological activity of the medicinal plant[74,78]. Different techniques can be used to determine the presence of the phytochemicals. Spectroscopic and chromatographic techniques are the most popular and useful instrumentation of this research. Techniques like HPLC with UVCDAD, GC-MS, HPTLC-desitometry, NIR, NMR or a combination of those techniques can be used to elucidate the structure of the phytochemicals[79].

2.10.1 Fourier Transform Infrared Spectroscopy

FTIR technique is one of the widely used methods to identify different constituents of medicinal plants and also helps in elucidating the compounds structure. The FTIR helps in analyzing the structural information and composition of the medicinal plants[74]. This technique identifies

functional groups basing on the peak values which are in the region of the infrared radiation[74,80]. When the extract is passed in the FTIR, the functional groups of the plant are separated basing on the peaks ratio. The FTIR can confirm the presence of secondary alcohols, aldehydes, alkanes, alkenes, benzene rings, phenols, aromatic amines and halogen compounds just to mention a few[74,80].

2.10.2 UV-Vis Spectroscopy

The UV-Vis spectroscopy is a cheap, simple and easy-to-use technique which helps in identifying and quantifying main phytochemicals by discriminating between lipophilic phytochemicals in relation with the polarity of the extraction solvent. It makes use of light which is in the visible ranges. Therefore colour of the chemicals available will affect the absorption which occurs in the visible ranges[74]. This technique has been used to determine the total phenolic compounds of the medicinal plants[79].

2.10.3 Nuclear Magnetic Resonance (NMR)

NMR spectroscopic data provides chemists with information that is indispensable for unravelling the structure of organic compounds[81]. The utility of NMR to elucidate the structure of both known and unknown products is of vital importance. The technique can make use of one dimensional and two-dimensional nuclear magnetic resonance for structure elucidation for both natural and synthetic products[82]. NMR has been extensively used to evaluate ligand binding with an obvious utility in structure based drug discovery and design. In NMR, a sample is placed in a magnetic field and is subjected to radio frequency (RF) at the appropriate frequency. The nuclei in the sample absorb the energy[83]. The frequency of the radiation necessary for absorption depends on three things:

i) It is characteristic of the type of nucleus eg (^{1}H or ^{13}C)

ii) Frequency depends on chemical environment of the nucleus e.g the methyl and hydroxyl proton of methanol absorb at different frequencies.

iii) Depends on spatial location in the magnetic field is not everywhere uniform[83].

2.10.4 Mass Spectrometry (MS)

Mass Spectrometry is an analytical technique that involves generating charged particles (ions) from molecules of the analyte. The generated ions are analyzed to provide information about the molecular weight of the compound and elucidate its chemical structure[84]. There are many types of mass spectrometers and different samples introduction techniques which allow a wide range of samples to be analyzed. The widely utilized practise of coupling Gas Chromatography (GC) and MS is used for the analysis of the compositions of various essential oils[83]. All mass spectrometers consist of an ionizer, ion analyzer and detector. Mass spectrometry can be considered a three step procedure.

i) Creation of ions from neutral ions or molecules.

ii) Ion separation with respect to their masses using electric and magnetic fields.

iii) Electronic detection of the intensity of the separated ions

The deflection in the static separation system depends on the mass of the ion, charge to mass ratio and the speed when the ions enter the deflection system. MS is a powerful technique that can be used at every stage of natural product studies, from discovery and structural characterization of new compounds to biosynthetic enzyme identification and manipulation[85].

2.11 Bacteria

Bacteria are single-celled organisms which are microscopic which can survive in numerous environments and may have beneficial or harmful activity [45]. Bacteria which cause diseases are regarded as pathogenic. It exists in 3 forms which are coccus, bacillus and spirilus [98]. They are prokaryotic unicellular organisms which are reproduced by binary fission and contain both DNA and RNA. Bacteria is grouped into strict anaerobes which grow in absence of oxygen, strict aerobes which grow in molecular oxygen or facultative anaerobes which grow either in the presence or absence of oxygen like *E.Coli*[99]. Gram positive bacteria include Staphylococcus for skin diseases, Clostridium for tetanus of Listeria for meningitis and gram negative bacteria include Shigella for enterocolitis, *Escherichia* for diarrhoea or urinary tract infection just to mention a few [100].

2.11.1 Types of bacteria

2.11.2 *Escherichia coli*

Escherichia coli is a subgroup of fecal coliform bacteria [101]. The coliform bacteria are part of the Enterobacteriacea family. *E.coli* is a fecal coliform bacteria meaning that the bacteria lives in the intestinal tract of warm blooded animals and human waste [16]. It produces toxin and causes intestinal diseases in people which can last to a week and diarrhoea is typical. If the cases are severe, they can cause kidney problems. *E.coli* is common in animals like deer, pigs, cattle, dogs, sheep and poultry. Infection from *E.coli* can occur from eating contaminated food especially undercooked or raw beef and exposure to feaces of infected animals [62]. The signs and symptoms usually occur from 1 to 2 days after exposure and results in cramping, abdominal pain, nausea and watery diarrhoea with blood. *E.coli* can grow with or without air and can survive freezing temperatures. It is gram negative and is a facultative anaerobe which ferments

simple sugars like glucose to lactic, formic and acetic acid [27]. The four categories which causes diarrheal illness or diseases are *EnteropathogenicE.coli* which causes severe diarrhoea in infants, *EnteroinvasiveE.coli* which is similar to shigellosis, *Enterotoxigenic E.coli* which produces enterotoxins when the organism multiplies in the intestine and *EnterohemorrhagicE.coli* which usually causes abdominal cramps [101].

2.11.3 *Staphylococcus aureus*

Staphylococcus aureus is a commensal bacterium found on anterior nares and human skin but causes severe infections in humans [96,102]. Staphylococci are gram positive bacteria and are non-motile facultative anaerobes which grow by fermentation or anaerobic respiration. *S. aureus* has a tough protective cell wall which is amorphous in appearance [103]. The cytoplasm is beneath the cell wall and is made up of peptidoglycan. The survival and growth of the bacteria usually depend on the ability of the cell to adapt to changes in the environment. *S. aureus* causes hospital-acquired infections which usually result in serious complications. Infections from Nosocomial *S. aureus* can affect the skin, soft tissues, bloodstream and can even lower the respiratory tract [53].

2.11.4 *Candida albicans*

The yeast *Candida albicans* is a member of the microflora in most healthy people where it predominantly colonizes the mucosal surfaces of the gastrointestinal tract[104]. *C.albicans* possesses a gene family encoding secreted aspartic proteinase and those enzymes have been linked with the virulence of the fungus[105]. *Vulvovaginal candidiasis (candidosis)* is the most common cause of vaginitis. Most cases of acute *valvovaginal candidiasis* are caused by *Candida albicans*. The yeast *C.albicans* is a harmless commensal in most healthy people but it causes superficial as well as life-threatening systemic infections in immuno-compromised patients.

C.albicans can colonize or infect virtually all body sites because of its high adaptability to different niches[106].

2.11.5 *Chlamydia trachomatis*

Chlamydia is a common sexually transmitted disease caused by bacterium, *Chlamydia trachomatis,* which can damage a woman's reproductive organs[105]. The bacteria initially infect the cervix and the urethra. Women who have been affected have symptom[106]s like abnormal vaginal discharge or a burning sensation when urinating. Chlamydia can be treated and cured with antibiotics. A single dose of azithromycin or a week of doxycycline (twice a day) is the most commonly used treatment[106]. If untreated, the bacteria can spread into the uterus or fallopian tubes and cause pelvic inflammatory diseases.

2.11.6 *Gardnerella vaginalis*

Gardnerella vaginalis is one of the agents of bacterial vaginosis (BV) and bacterial vaginosis has been implicated in many perinatal conditions like early pregnancy loss, premature rupture of membrane, chorioamnionotis and endometritis. Other agents of BV include *Mycoplasma tominis, Ureaplasma urealyticum* etc[107].

2.11.7 High Vaginal Swab (HVS)

The genital tracts of women consists of residents microfloras which are made of a wide variety of species some of which play useful roles to the healthy state of the vagina while others reside there as commensals but may become pathogenic if opportunity arises[104]. Other researchers have isolated *Candida albicans, Trichomonus vaginalis, Eschericia coli, Staphylococcus aureus* and *Gardnerella vaginalis* from different vaginal swabs and results showed that the composition

of these pathogens differed from individuals and also non-pregnant women were found to have more organisms compared to pregnant women[106].

2.12 Why discover new drugs

It has been noted that around 25% of all drugs prescribed today come from plants. This estimate suggests that plant-derived drugs make up a significant segment of natural product-based pharmaceuticals[85]. Alkaloids have contributed the largest number of drugs to the modern pharmacopoeia ranging in effects from anticholinergic (atropine) to analgesics (opium alkaloids) and from antiparasitics (quinine) to antineoplastics (vinblastine/vincristine)[108]. Plants can be used to isolate bioactive compound for direct use as drugs for examplemorphine, taxol, digoxin and vinblastine[108]. There is also need to produce bioactive compounds of novel or known structures as lead compounds for semi-synthesis to produce patentable entities of higher activity and or lower toxicity e.g metformin and taxotere. Galegine was isolated as an active antihyperglycemic agent from the plant *Galega officinals L* used ethnomedically for the treatment of diabetes. Galegine provided the template for the synthesis of metformin and opened up interest in the synthesis of other antidiabetic drugs[108].

CHAPTER 3

METHODOLOGY

3.0 Introduction

The chapter outlines the procedure which was used to carry out in the research. This includes the sample collection, extraction of phytochemicals, and identification of functional groups,

antioxidant capacity and the antimicrobial activity of different plant extracts of different polarities.

3.1 Sample collection

Healthy fresh plant samples without traces of fungi or mould attack were randomly collected in the Vumba mountains and surrounding areas. The plants were then identified by experts of Vumba Botanical Gardens.

3.2 Sample preparation

The roots of *Dicoma anomala* and the leaves of *Kigelia pinnata*, *Pseudolachnostylis maprounelifolia,* and *Flacourtia indica* were prewashed with tapwater first then with distilled water. The roots of *Dicoma anomala* were cut into smaller pieces for drying. The plant samples were dried in the shade at an ambient temperature of around 25 ^{0}C and ground to a fine powder using a blender to obtain a homogenous sample. The ground powder was kept in labeled glass jars awaiting use.

3.3 Preparation of acetone crude extracts

A mass of 200 g of *Flacourtia indica*, *Dicoma anomala, Pseudolachnostylis maprounelifolia* and *Kigelia Africana* was placed in 4 different stoppered 2000 mL volumetric flasks. A volume of 600 mL of acetone was added in each flask and the mixture was allowed to stand at room temperature for 24 hours using the maceration process. The mixtures were then placed on a shaker for 2 hours to maximize the extraction [109]. The extracts were filtered by vacuum filtration and the filtrate was placed in labeled glass jars. The filtrate was then concentrated using a vacuum rotavapour and the resulting filtrate was placed in weighed petri dishes for drying. The mass of the extract was continuously weighed until there was a near constant mass showing that

the extract had dried. The masses were recorded and the percentage yield calculated. The extracts were stored in a fridge for future use.

3.4 Preparation of extracts of different polarities

Petroleum ether and chloroform were used to extract different phytochemicals based on their polarities. A weighed amount of the crude acetone extract was dissolved in chloroform. The mixture was shaken, and petroleum ether was added and shaken again. The fractions were separated by a separatory funnel and concentrated using a rotary vapour. The concentrated fractions were dried and the percentage yield was calculated. The extracts were then characterized by FTIR, taken for antimicrobial activity and the total phenolic content was determined by UV-VIS.

3.5 Characterization by FTIR

The plant extract was mixed with KBr in a ratio of 1:10. The plant extracts and the bonding agent (KBr) were mixed using a mortar and pestle into a homogenous sample. Discs were made by pressing the mixture between bolts and screws. The discs were put in the FTIR for analysis.

3.6 Phytochemical analysis

3.6.1 Test for glycosides

Glacial acetic acid was added to 2 mL of extract followed by one drop of 5% ferric chloride and 2 drops of concentrated H_2SO_4. Appearance of a reddish brown colour at the junction of the 2 liquids shows presence of glycosides [73].

3.6.2 Test for phytosterols

A mass of 5 mg of extract was dissolved in a few drops of acetic acid and 3 mL of acetic anhydride was added followed by 3 drops of concentrated H_2SO_4. Appearance of bluish green colour shows presence of phytosterols [110].

3.6.3 Test for proteins

Equal volumes 5% solution of NaOH and 1% $CuSO_4$ were added to 10 mg of extract. Appearance of a pink or purple colour indicates presence of proteins [110].

3.6.4 Test for coumarins

To 10 mg of extract, 1 mL of 10% NaOH was added. Presence of coumarins was indicated by formation of a yellow colour.

3.6.5 Test for terpenoids

A volume of 1 mL of chloroform was added to 10 mg of the extract followed by a few drops of concentrated H_2SO_4. A reddish brown precipitate showed presence of terpenoids.

3.6.6 Test for quinones

A mass of 10 mg of extract was treated with concentrate HCl and observed for the formation of a yellow precipitate or colouration [46].

3.6.7 Test for flavonoids

a) A mass of 10 mg of extract was suspended in 100 mL of distilled water. 5 mL of 25% ammonia as added to the filtrate followed by 3 drops of concentrated H_2SO_4. Formation of a yellow colouration shows presence of flavonoids.

b) To 10 mg of extract, 4 drops of NaOH was added. An intense yellow colour which becomes colourless on addition of few drops of dilute acid show presence of flavonoids.

3.6.8 Test for tannins

A mass of 10 mg of the plant extract was boiled in 5 mL of water in a boiling tube. 4 drops of 5% $FeCl_3$ was added. Formation of a black colouration shows presence of tannins [48].

3.6.9 Test for alkaloids

Wagner's test was used and a mass of 10 mg of the plant extract was mixed with iodine in potassium iodide. Formation of a brown or reddish precipitate indicates presence of alkaloids.

3.6.10 Test for carbohydrates

A mass of 10 mg of the plant extract was mixed with 4 mL of Benedicts reagent and boiled. Formation of a brick red precipitate shows presence of reducing sugars.

3.6.11 Test for anthraquinones

A mass of 5 mg of the plant extract was mixed with 4 mL of 3.2% HCl and boiled. The solution was filtered and the filtrate was shaken with 3 mL of benzene and filtered. 2 mL of 10% ammonia solution was added. Formation of a brick red colouration shows presence of anthraquinones [110].

3.7 Antimicrobial studies

3.7.1 Preparation of culture media

The bacteria were grown in two different nutrient agars which were Mac Conkey and PCA. A mass of 40 g of the powdered broth was weighed and suspended in 100 mL of distilled water.

The mixture was heated gently until the broth dissolved and became clear. The dissolved agar was poured in bottles and closed. The bottles were sterilized first by autoclaving at 121 ^{0}C, 15 P for 15 minutes then stored in an oven at 40 ^{0}C until use [7].

3.7.2 Culturing of bacteria

The petri dishes were first flamed to kill any organism and the workplace was first cleaned with methylated spirit. A volume of 10 mL of the prepared agar was poured into petri dishes making sure the base was totally covered. The mixture was swirled and left to dry. The HVS bacteria were transferred onto the solid agar by a swab. The petri dishes were instantly closed to avoid contamination. The petri dishes with the bacteria were incubated for 24 hours at 37 ^{0}C [7].

3.7.3 Preparation of discs

The discs were cut in a circular form with a diameter of 6 mm each. A concentration of the extracts was prepared by dissolving 50 mg of the extract in 10 mL of solvent thereby preparing a concentration of 5 mg/mL. The standard drug had 500 mg of active ingredient per tablet hence the tablet was dissolved in 100 mL to prepare a similar concentration with the extracts. The discs were inoculated in extracts and standard drug for 24 hours.

3.7.4 Transferring discs onto the cultured bacteria

After the bacteria had grown, paper discs impregnated with the standard drug and extracts were placed using forceps on the medium which was uniformly inoculated with the HVS bacteria. The petri dishes were sealed and placed in the incubator at 37 ^{0}C for 24 hours. The inhibition of the bacteria was indicated by a clear zone around the discs. The diameter of the zone of inhibition indicated the sensitivity of the bacteria on the drug and extracts and also the solubility properties

of the extracts. The study was done in triplicates and the zones of inhibition were recorded using the average values calculated as follows:

Mean inhibition diameter = Sum of all inhibition diameters/ Number of discs used

3.7.5 Minimum Inhibitory Concentration

Serial dilutions of plant extracts of different polarities which ranged from 0.9 mg/mL to 0.1 mg/ mL were prepared and the disks were inoculated in the different solutions for 24 hours. The discs were placed in the petri dishes containing the HVS bacteria and incubated for 24 hours at 37 ^{0}C. The zones of inhibition were noted to check the minimum concentration which showed activity.

3.8 Phosphomolybdate assay (total antioxidant capacity)

The total antioxidant capacity of the extracts was determined by phosphomolybdate method using ascorbic acid as a standard.The reagent solution contained 0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate. An aliquot of 1 mL of sample solution was mixed with 10 mL of reagent solution. The tubes were capped and incubated in a water bath at 95 ^{0}C for 90 minutes. The samples were cooled to room temperature. The absorbance of the mixture was measured at 695 nm against a blank. The blank contained 1 mL of the reagent solution and appropriate volume of the solvent and incubated under the same conditions. The ascorbic acid was used as a standard [58].

3.8.1 Preparation of ascorbic acid and plant concentrations

A series of 5 different concentrations of the standard was prepared starting from 0, 1.25, 2.5, 5.0 and 7.5 µg/mL. The concentration of the standard was prepared by dissolving the ascorbic acid tablet with active ingredient of 100 mg in 100 mL there by preparing a concentration of 1 mg/ml.

It was further diluted to prepare the above concentrations. The plant extracts had a concentration of 10 µg/mL which was prepared by dissolving 1 mg of extract in 100 mL of solvent.

These concentrations were used in the phosphomolybdate assay and the absorbance measured at 695 nm. The results of the absorbance and concentration of the standard were used to plot a calibration curve. The calibration curve's straight line equation was used to determine the total phenolic content of the plant extracts using the ascorbic acid equivalence concentration.

CHAPTER 4

RESULTS AND DISCUSSION

4.0 Introduction

This chapter gives detail on the obtained results, their analysis, interpretation and discussion of these results.

Table 3: Percentage yields of different extracts

Plant extract	*Kigelia pinnata*	*Dicoma anomala*	*Pseudolachnostylis maprounelifolia*	*Flacourtia indica*

Acetone	1.2	1.79	5.39	4.83
Chloroform	58.32	52.32	42.57	39.62
Petroleum ether	17.77	21.2	23.13	24.84

The acetone crude extract percentage yield obtained was 1.20% for *Kigelia pinnata*, 4.83% for *Flacourtia indica*, 5.39% for *Pseudolachnostylis maprounelifolia* and 1.79% for *Dicoma anomala*. *Pseudolachnostylis maprounelifolia* recorded the highest yield while *Kigelia pinnata* had the least percentage. In the petroleum ether extract, *Kigelia pinnata* had a percentage yield of 17.77%, *Flacourtia indica* had 24.84%, *Dicomaanomala* had 21.20 and *Pseudolachnostylis maprounelifolia* had 23.13%. Flacourtia indica recorded the highest yield and *Dicoma anomala* had the least percentage yield. In the chloroform extract, *Dicoma anomala* had a percentage yield of 52.32%, *Kigelia pinnata* had 58.32%, *Pseudolachnostylis maprounelifolia* had 42.57% and *Flacourtia indica* had 39.62%. *Flacourtia indica* had the least percentage yield and *Kigelia pinnata* recorded the highest percentage yield.

Table 4: Phytochemical results of extracts of different polarities

Phytochemical	**F(a)**	**F (c)**	**F(p)**	**K(a)**	**K(c)**	**K(p)**	**D(a)**	**D (c)**	**P(a)**	**P(c)**	**P(p)**
Tannins	+	+	-	+	+	-	-	-	-	-	-
Flavanoids	+	+	+	+	+	-	-	-	+	+	-
Steroids	-	-	-	-	-	-	-	-	-	-	-
Phenolics	+	-	-	+	+	-	-	-	+	+	-
Alkaloids	+	+	-	-	-	-	+	+	-	-	-

Quinones	-	-	-	-	-	-	-	-	-	-	-
Glycosides	-	+	-	+	+	-	+	-	-	-	-
Saponins	+	+	+	-	-	-	+	+	+	+	+
Anthraquinones	-	-	-	-	-	-	-	-	-	-	-
Coumarins	+	+	-	+	+	+	-	-	-	-	-
Terpenoids	-	-	-	+	+	-	-	-	+	-	-
Reducing sugars	+	+	+	+	+	-	+	+	-	-	-
Sterols	-	-	-	+	+	+	+	+	-	-	-
Proteins	-	-	-	-	-	-	-	-	-	-	-

Key: F – *Flacourtia indica*, **P** – *Pseudolachnostylis maprounelifolia*, **D** – *Dicoma anomala*, **K** - *Kigelia pinnata*, **(a)** – *acetone extract*, **(c)** – *chloroform extract***(p)** – *petroleum ether extract*

The crude acetone extracts of *Flacourtia indica* showed presence of tannins, flavonoids, phenolics, alkaloids, saponins, coumarins, reducing sugars and sterols. Tyagi etal in 2010 conducted phytochemical tests screening of methanolic extract of *Flacourtia indica* leaves and found presence of alkaloids, saponins, tannins, glycosides, phenolic compounds, steroids and terpenoids. Mohamed Kaou in 2010 conducted phytochemical studies in the ariel parts of *Flacourtia Indica* and isolated pyrocatechol, homaloside D and poliothrysoside. *Kigelia pinnata* had tannins, flavonoids, glycosides, coumarins, terpenoids, reducing sugars and sterols. *Pseudolachnostylis maprounelifolia* contained flavonoids, phenolics and saponins which have a defense mechanism against oxidative stress from oxidizing agents and free radicals [58]. Tannins have been reported to possess potent antimicrobial, antioxidant and anti-inflammatory activity.

Dicoma anomala tested positive for alkaloids, saponins and reducing sugars. Flavonoids have been reported to have chemical and biological activity and also scavenging properties against free radicals[3]. Terpenoids have been found to possess antimicrobial, antioxidant and anti-inflammatory properties and some terpenoid derivatives have been used in anticancer agents. An example of a terpenoid is limonene which was found to have chemopreventive and therapeutic properties against tumour cells [23]. This also confirms the use of *Kigelia Pinnata* for skin cancer since it contains terpenoids.

Petroleum ether extracts contained the least phytochemicals indicating that most of the phytochemicals present in the plant extracts were polar since petroleum ether is non-polar with a polarity index of 0.1 [47]. The petroleum ether extract of *Flacourtia indica* showed a positive result for the test of flavonoids, tannins and terpenoids. In organic chemistry, like dissolves like hence the presence of these phytochemicals postulate that the compounds are non-polar. What determines the polarity of the flavonoids are variation of the pattern and scale of prenylation, hydroxylation, glycosylation and alkalinization reactions that can alter the basic molecule [22].

Alkaloids are basic products occurring naturally in plants and can occur as one or more heterocyclic nitrogen atoms that are generally found in form of salts with organic acids. Diarrhoea treating plants, *Flacourtia indica* and *Dicoma anomala* tested positive for alkaloids. Pure isolated alkaloids and their synthetic derivatives are used as basic medicinal agents due to their analgesic, antibacterial and antiplasmodic properties. Diarrhoea results from imbalance between the absorptive and secretory mechanism in the intestinal tract accompanied by hypermotality [15]. Alkaloids on the other hand fall into a class of modulators and mimic endogenous ligands, hormones and several have structural similarities to neurotransmitters and can bind to neureceptors and either activate or inactivate them. Alkaloids also target ion channels

such as K^+,Na^+ and Ca^{2+} and can inhibit or activate them [41]. Their ability to do so is critical in the treatment of diarrhoea.Saponins have been observed to kill molluscs and protozoans, possess antioxidant activity and act as antifungal and antiviral agents [111]. Saponins were present in *Flacourtia indica, Dicoma anomala* and *Pseudolachnostylis maprounelifolia* accounting for their use as medicinal plants

4.1 FTIR Spectra Results

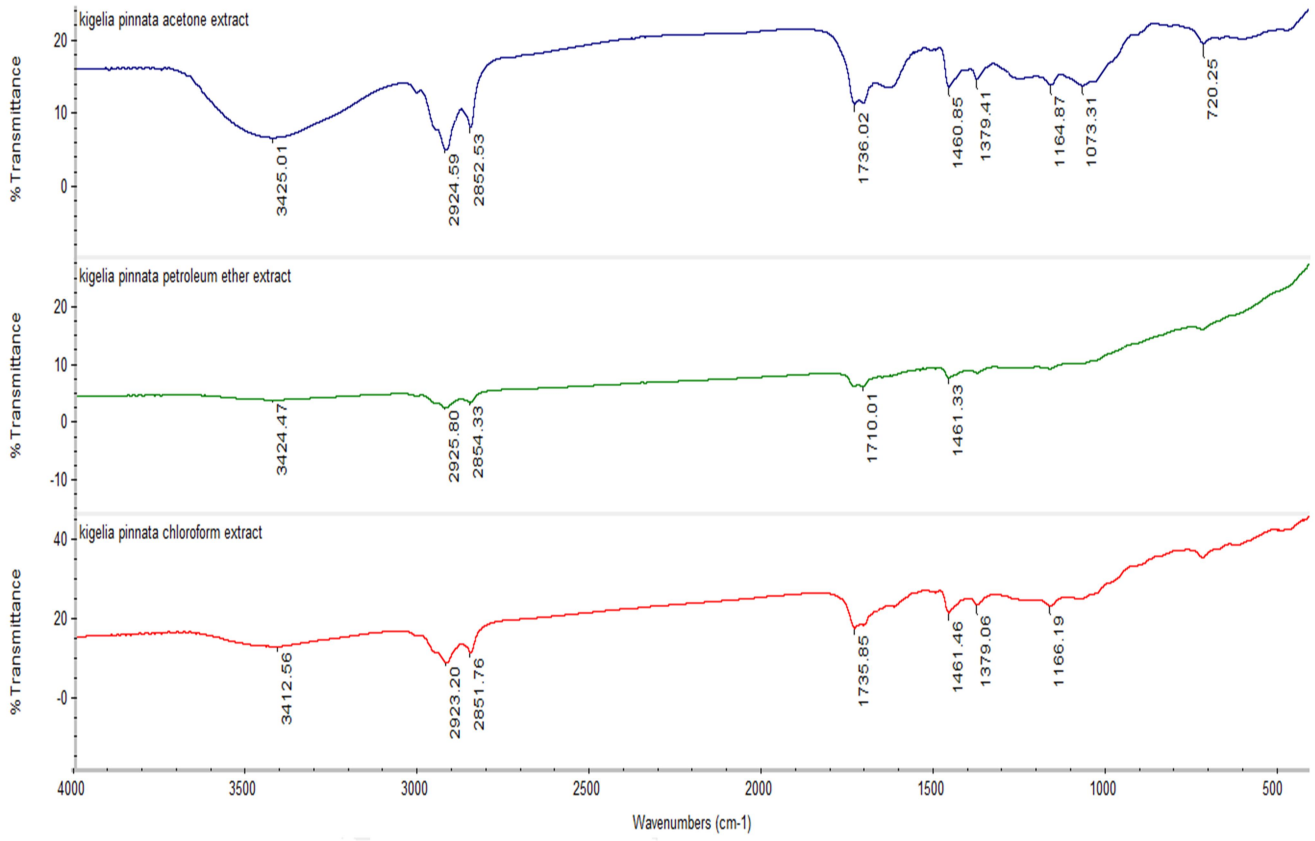


Figure 17: FTIR spectra for *Kigelia pinnata*

The peaks at 3425.01 cm^{-1}, 3424.47 cm^{-1} and 3412.56 cm^{-1} denote the O-H stretch of phenols and alcohols [112]. These peaks show the presence of phenolic compounds in the extracts and alcohol groups like those found in tannins or saponins. The peaks at 2924.59 cm^{-1}, 2925.80 cm^{-1}, 2923.20 cm^{-1}, 2852.53 cm^{-1}, 2854.33 cm^{-1} and 2851.76 cm^{-1} represent the O-H stretch of carboxylic acids and also the C-H stretch of alkanes. These groups can determine the presence of phytochemicals like tannins which possess carboxylic acid groups in their structure [113]. Peaks at 1736.02 cm^{-1} and 1735.85 cm^{-1} are representative of C=O stretch of an aliphatic aldehyde or aliphatic C=O stretching of esters while that at 1710.01 cm^{-1} denotes the aliphatic ketone C=O stretching or aromatic C=O stretching of esters. These groups can be found in saponins, terpenoids and tannins which correspond with the phytochemical results. The peaks at 1450.87

cm^{-1}, 1451.33 cm^{-1} and 1461.46 cm^{-1} show the presence of C-C stretch in ring of aromatics and C-H bend of alkanes. These groups are the typical structure of most phytochemicals since they do have benzene rings within their structure. The peaks at 1164.87 cm^{-1}, 1166.19 cm^{-1} and 1073.31 cm^{-1} show a C-N stretch of aliphatic amines [80]. The last peak at 720.25 cm^{-1} in the acetone crude extract can be due to the C-H oop of the aromatics.

The FTIR intensity is related to dipole change that occurs during vibration and number of specific bonds present. An increase in peak intensity of a particular functional group means there are more groups of the same nature. The number of specific bonds determines the intensity of a peak and the FTIR of the crude acetone extract showed a higher intensity compared to the chloroform and petroleum ether extract. The petroleum ether extract of *Kigelia pinnata* recorded the least peaks with a low intensity showing that the phytochemicals present had decreased and the extract did not contain much of non-polar phytochemicals.

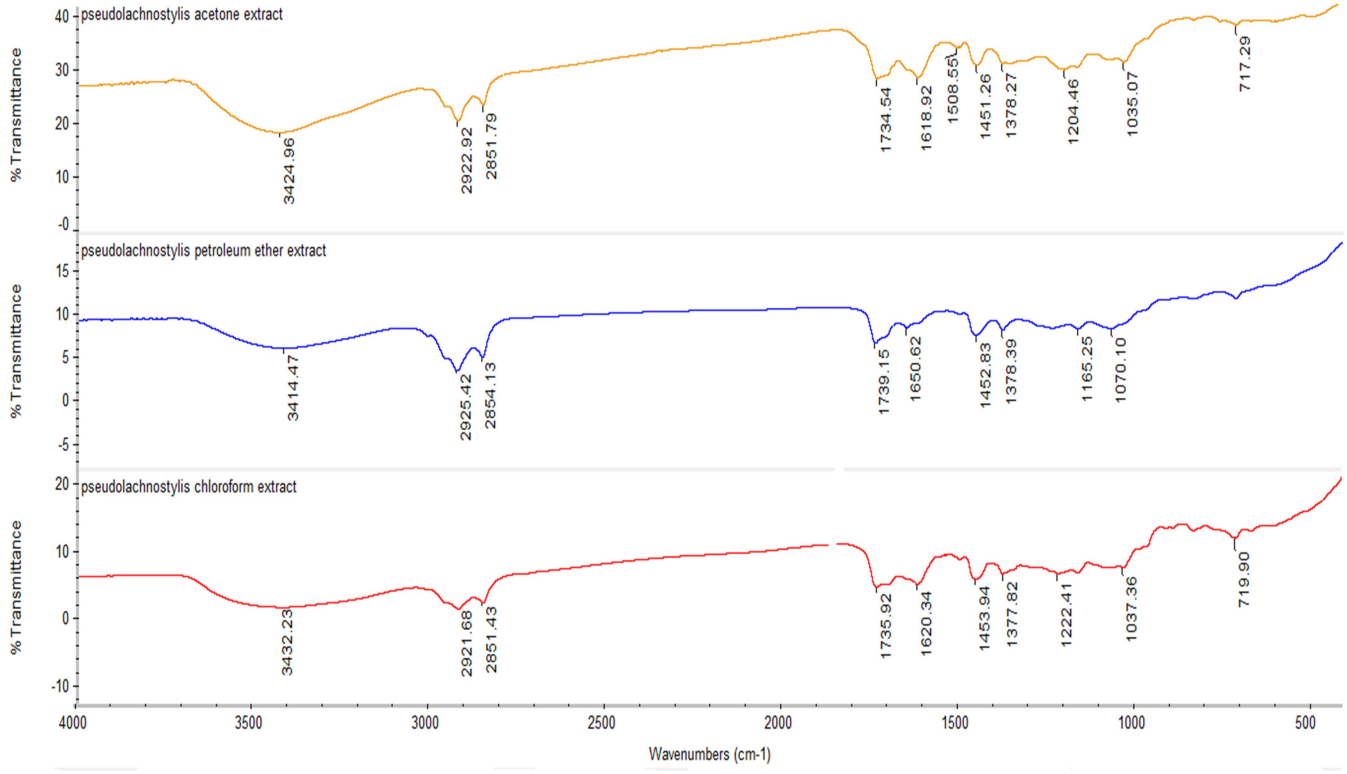


Figure 18: FTIR spectra for *Pseudolachnostylis maprounelifolia extracts*

The acetone crude extract showed peaks from 3424.96 cm^{-1} to 717.29 cm^{-1}. The peak at 3424.96 cm^{-1} denotes a stretching bonded O-H group present in alcohols and phenols. The peaks at 2922.92 cm^{-1} and 2851.79 cm^{-1} showed the presence of a C-H stretching. The peak at 1734.54 cm^{-1} denotes a stretching C=O characteristic of a carbonyl group [114]. The peak at 1618.92 cm^{-1} is due to C=C stretching in alkenes and that at 1378.27 cm^{-1} denotes a C-H bending and that at 1204.46 cm^{-1} is due to C-O stretching of the C-O bond of an ester. The 1035.07 cm^{-1} peak represents a C-O stretching of an ester or ether. The chloroform extract had almost similar peaks in the same range as that of the crude acetone extract. The peaks of the acetone crude extract and the chloroform extract had an increased peak intensity compared to the petroleum ether extract. These results were backed up by the phytochemical results which showed that the petroleum extract had the least number of phytochemicals. The peaks confirm the presence of sterols, saponins, glycosides and alkaloids.

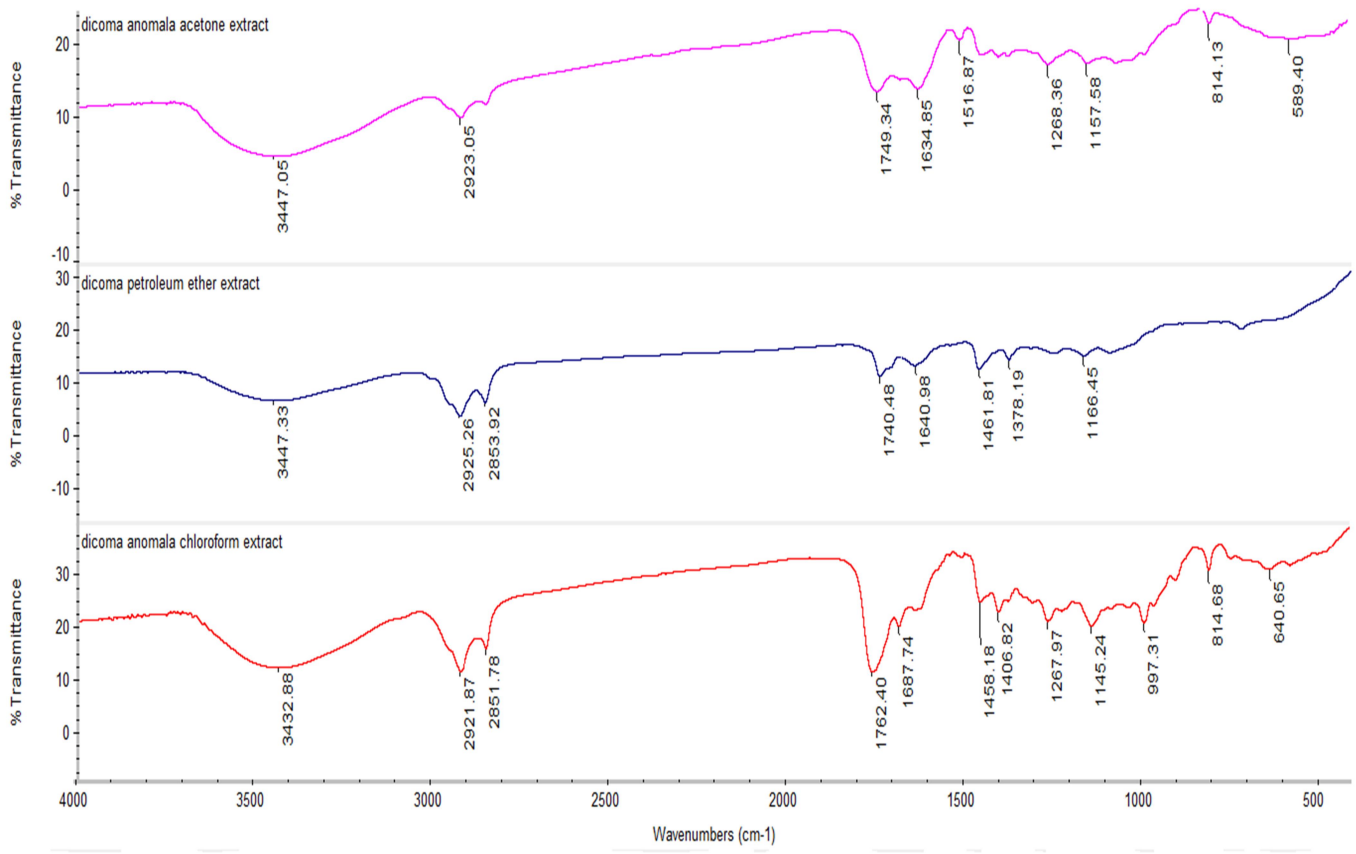


Figure 19: FTIR spectra for *Dicoma Anomala*

Figure 4.3 shows the peaks of the acetone crude extract which ranged from 3447.05 cm^{-1} to 589.40 cm^{-1}, the petroleum ether extract ranged from 3447.33 to 1166.45 cm^{-1} while the chloroform extract was from 3432.88 cm^{-1} to 640.65 cm^{-1}. The peaks at 3447.05, 3447.33 and 3432.88 cm^{-1} show the presence of an O-H stretch found in alcohols and phenols or an N-H stretch for primary or secondary amines. Alkaloids contain heterocyclic nitrogen atoms and the peaks can confirm the presence of alkaloids which is also supported by the phytochemical results. The peaks at 2923.05 cm^{-1}, 2925.26 cm^{-1} and 2921.87 cm^{-1} represent the O-H stretch of carboxylic acids and those at 2853.92 cm^{-1} and 2851.78 cm^{-1} are characteristic of an aldehyde C-H stretching [1]. The peaks at 1740.48 and 1749.34 cm^{-1} can be due aliphatic C=O stretching of esters. The peaks at 1458.18 and 1461.81 cm^{-1} represent the C-C in stretch of aromatics which

are one of the major groups of phytochemicals. The peaks at 1267.97 and 1268.36 cm^{-1} denote aromatic C-N stretching which is typical of alkaloids. The peak intensity of the petroleum ether extract was lower than the other two extract showing that a few groups were of the same nature were present.

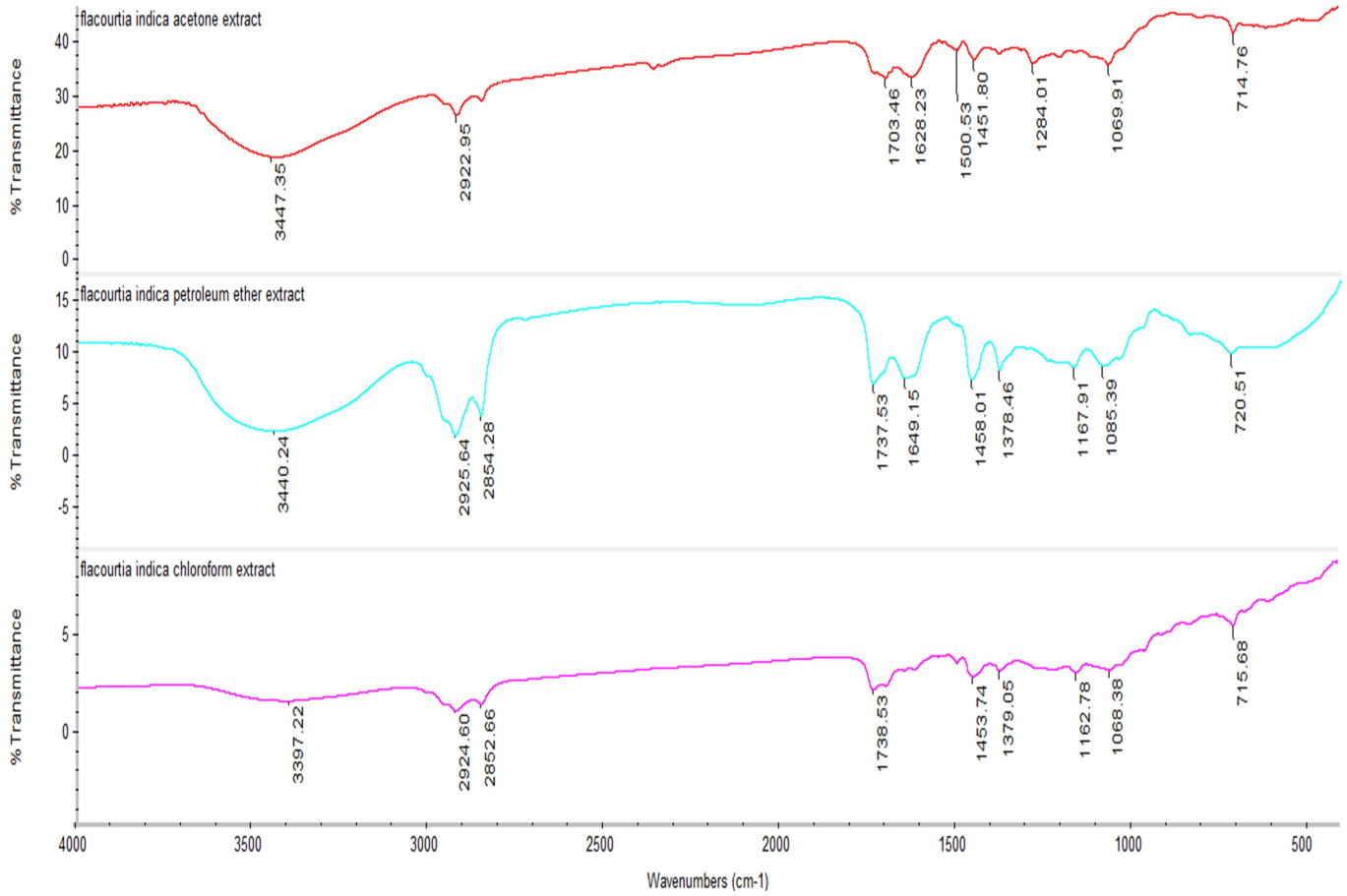


Figure 20: FTIR spectra for *Flacourtia indica* extract and fractions

The peaks at 3447.35 cm^{-1}, 3440.24 cm^{-1} and 3397.22 cm^{-1} of the acetone, petroleum ether and chloroform extracts respectively are representative of the O-H stretch of phenols or alcohols. This is backed up by the phytochemical results which showed presence of phenolics in the chloroform and crude acetone extract. The peak at 3440.24 cm^{-1} for the petroleum ether extract

and that at 3397.22 cm^{-1} for the chloroform extract can be due to the N-H stretch of primary or secondary amines or amides. This can also confirm the presence of alkaloids since they do possess that functional group. The peaks at 2922.95 cm^{-1}, 2925.64 cm^{-1}, 2854.28 cm^{-1}, 2924.60 cm^{-1} and 2852.66 cm^{-1} denote the C-H stretch of alkanes. Those at 1703.46 cm^{-1}, 1737.53 cm^{-1} and 1738.53 cm^{-1} represent C=O stretch of carbonyls, carboxylic acids, esters or saturated aliphatic which can support the presence of tannins in the extracts since tannins possess these groups. The peak at 1703.46 cm^{-1} represents an aromatic C=O stretching of an ester [112]. The peaks at 1628.23cm^{-1} and 1649.15 cm^{-1} represent the –C=C- stretch of alkenes or an N-H bend of primary amines. The peaks at 1500.53 cm^{-1}, 1451.80 cm^{-1}, 1458.01 cm^{-1} and 1453.74 cm^{-1} are representative of the C-C stretch in ring of aromatics. This collaborates with the fact that most of the phytochemicals have benzene rings within their structure for example tannins, saponins and terpenoids. The peak at 1284.01 cm^{-1} of the crude acetone extract denotes the C-N stretch of aromatic amines which confirms the presence of alkaloids. The peaks at 1069.91 cm^{-1}, 1167.91 cm^{-1}, 1085.39 cm^{-1} and 1058.38 cm^{-1} are characteristic of the C-N stretch of aliphatic amines. Finally, the peaks at 714.76 cm^{-1}, 720.51 cm^{-1} and 715.58 cm^{-1} can be due to the C-H oop of aromatics. These results show that *Flacourtia indica* extracts have both polar and non-polar phytochemicals due to the presence of almost similar groups in all the three extracts of different polarities [115]. The non-polar phytochemicals are also shown by the peak intensities of the petroleum ether extracts which is more pronounced as compared to the chloroform and the crude acetone extract. The intensity of the peaks of the petroleum ether shows that there are more groups of the same nature.

4.2 Antimicrobial Studies

The disk diffusion method was used to evaluate the antibacterial properties of the plant extracts of different polarities against a standard drug. The bacteria used was HVS (High Vaginal Swab). HVS is a combination of different bacteria and fungi like *Escherichia coli,Staphylococcus aureas*, *Klebsiella spp* or *Proteus spp* [116]. Up to date, the composition of this swab is not specific since it depends on the person swabbed. The zone of inhibition indicated the activity of the extracts on the bacteria. The standard drug showed the highest activity with a zone of inhibition and all the extracts showed activity on the HVS bacteria and *Kigelia pinnata* showed the highest activity in the acetone and chloroform extracts.

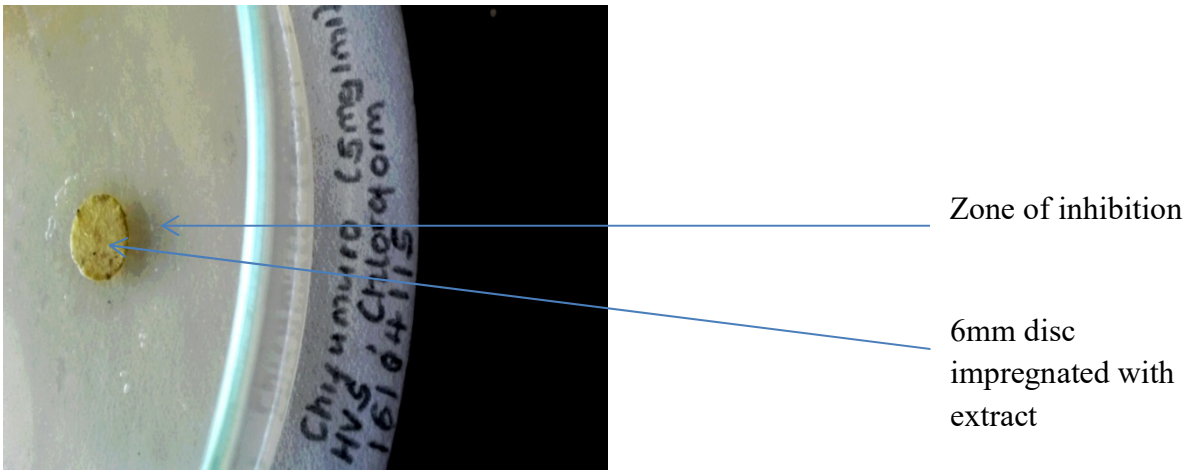


Figure 21: *Dicoma anomala* 5 mg/ml chloroform extract

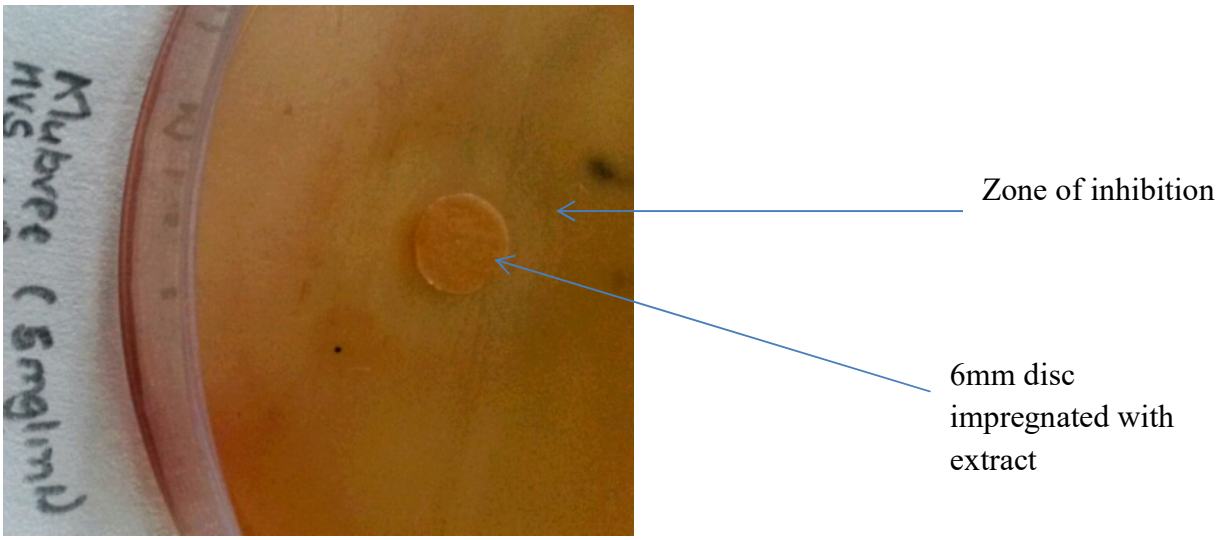


Figure 22: *Kigelia pinnata* 5 mg/ml acetone crude extract

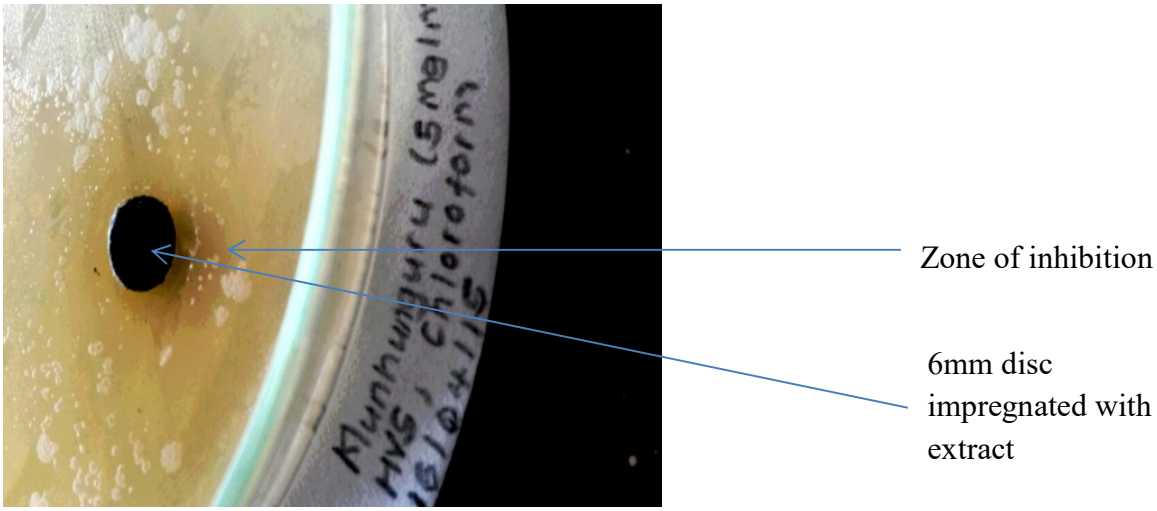
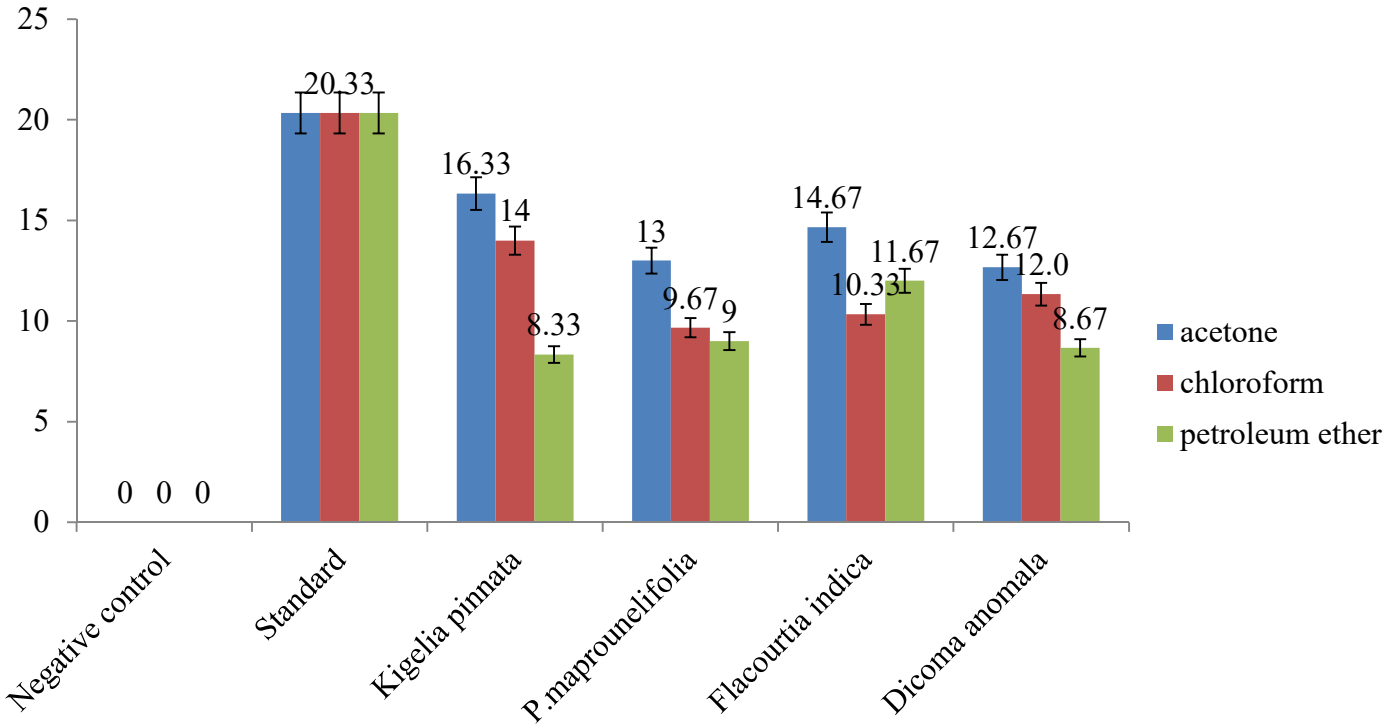


Figure 23: *Flacourtia indica* chloroform extract 5 mg/ mL



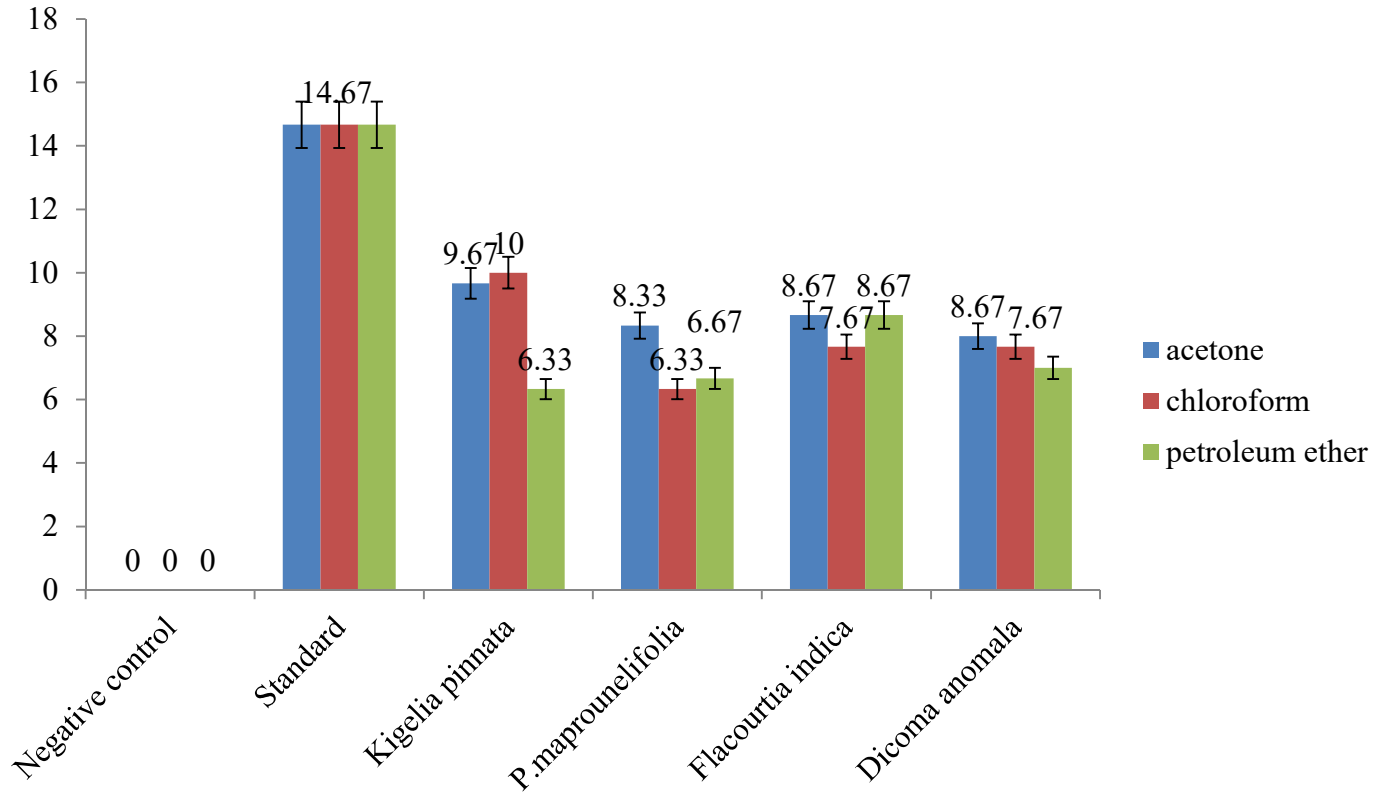
Standard – Ciproflaxin

Figure 24: Antimicrobial activity of 5 mg/mL extract

The results showed that the plant extracts were active against the HVS bacteria as represented by the zones of inhibition which ranged between 12.67 mm and 16.33 mm for the acetone extract. *Kigelia pinnata* showed the highest activity with a zone of inhibition of 16.33 mm followed by *Dicoma anomala* which had 14.67 mm, then *Pseudolachnostylismaprounelifolia* with 13.0 mm and lastly *Flacourtia indica* recorded 12.67 mm. The activity of the extracts was comparable with the standard since *Kigelia pinnata* recorded almost a 72% activity comparing it against the standard drug. The activity of the extracts was lower compared to that of the standard drug due to some factors like impurities and inert ingredients like chlorophyll which lowered the mass of the

phytochemicals [24]. The results show that all the plant extracts contain polar phytochemicals due to the activity noticed on the HVS bacteria.

The activity of the petroleum ether extracts was lower than the chloroform and the acetone crude extract. *Flacourtia indica* showed the highest activity with a zone of inhibition of 9.33 mm, then followed by *Pseudolachnostylis maprounelifolia* which had a zone of 9mm followed by *Dicoma anomala* which had a diameter of 8.67 mm and lastly *Kigelia pinnata* which had 8.33 mm. The activity of the extracts decreased significantly due to the polarity of the petroleum ether solvent. Petroleum ether does not extract phytochemicals like phenols and flavonoids which are responsible for the antibacterial activity of most extracts [117]. This was confirmed by the phosphomolybdate assay which showed that the petroleum ether extract had the least concentration of vitamin C equivalence which in turn means that it has the lowest total antioxidant capacity. *Flacourtia indica* was an exception since it recorded a higher zone of inhibition bringing to the probability of the presence of non-polar phenolics in the extract. This is also confirmed by the FTIR results which showed presence of functional groups which were similar with a more polar solvent which was acetone.



Standard - Ciproflaxin

Figure 25: Antimicrobial activity of 1 mg/mL extract

The crude acetone extract with concentration of 1 mg/mL showed activity against the HVS bacteria with the highest zone of inhibition recorded by *Kigelia pinnata* which had a diameter of 9.67 mm. This activity is confirmed by the presence of tannins, flavonoids, glycosides, coumarins, terpenoids and sterols which have been reported to possess antibacterial and antimicrobial activity. *Flacourtia indica* was the second highest with an inhibition zone of 8.67 mm since it contains active phytochemicals which are tannins, flavonoids, alkaloids, saponins, coumarins and terpenoids [118]. *Pseudolachnostylis maprounelifolia* recorded the lowest zone of inhibition. The petroleum ether extract showed the least activity with a maximum zone of inhibition of 8.7 mm shown by the *Flacourtia indica* extract. *Kigelia pinnata* showed the least

activity with a zone of inhibition of 6.3 mm. This activity was backed up by the FTIR results which showed that the petroleum ether extract had the least peaks with the lowest intensity which meant that extract contained the least active compounds. In terms of polarity, petroleum ether is non-polar with an index of 0.1, followed by chloroform which has 4.1 and finally acetone which has 5.1. *Flacourtia indica* recorded the highest activity in the petroleum ether extract which shows that the extract contained both polar and non-polar phytochemicals. These results were backed up by the FTIR results which show presence of phenols, carboxylic acids and other functional groups in all the extracts despite of the difference in polarities.

The activity of the extracts was comparable with the standard drug although the zones of inhibition of the extracts were lower. The activity of the extracts on the bacteria confirmed the presence of phytochemicals which have been proven to possess antibacterial activity. The effect of the acetone and chloroform extracts on the bacteria was bactericidal meaning it killed the bacteria surrounding the disks. This was shown by the presence of the zone of inhibition after 4 days in the incubator. The petroleum ether extract showed a bacteriostatic effect since there was growth of bacteria 4 days after incubation [69].

The activity of the plant extracts on the zone of inhibition confirms the presence of phytochemicals. Phytochemicals like saponins, terpenoids, flavonoids and alkaloids have been reported to possess antibacterial activity. The zone of inhibition of the extracts were lower than the standard drug since the drug contained 5 mg of active compound unlike extracts which contain 5 mg of both active and inactive compounds. The inactive compounds can include chlorophyll which is responsible for giving plants their green colour and for the manufacture of food in plants [10,24]. The overall order of the extract activeness was chloroform extract, acetone crude extract and lastly petroleum ether extract. The difference was due to the

composition of the plant extract since organic solvents dissolve different phytochemicals depending on the polarity of the solvent.

4.3 Minimum Inhibitory Concentration in mg/mL

MIC is the lowest concentration of an antimicrobial agent which completely inhibits the growth of organisms. The MIC value was determined as the lowest concentration of the plant extracts that inhibited the visible growth of HVS.

Table 5: MIC results of different plant extracts

Plant extract l	*Pseudolachnostyls maprounelifolia*	*Flacourtia indica*	*Kigelia pinnata*	*Dicoma anomaa*
Acetone	0.7	0.5	0.4	0.8
Chloroform	0.8	0.7	0.6	0.8
Petroleum ether	0.9	0.7	0.9	0.9

The MIC results showed that *Kigelia pinnata* had the highest antibacterial activity due to the least MIC of 0.4 and 0.6 mg/mL in the acetone crude extract and the chloroform extract respectively. The activity is due to the presence of phytochemicals like flavonoids, tannins, phenolics, alkaloids and saponins which have been recorded to possess antibacterial activity [3]. The standard Ciprofloxacin recorded an MIC of 0.125 mg/mL showing that the activity of the plant extracts was comparable with the standard. Although *Dicoma anomala* did not test positive for phenolic compounds, the activity was due to the presence of alkaloids and saponins although it was lower than the rest. Munodawafa did a research on *Dicoma Anomala* using methanol extract and recorded an MIC of 1.25 mg/mL against *Candida albicans*. Steenkamp found out that

Dicoma Anomala is active against several bacterial strains and genera like *Staphyloccocus aureus* and *Streptococcus pyogenes.*

The petroleum ether showed the highest MIC values which indicate that the extracts were not as active as the other two extracts. This was due to the low polarity of the petroleum ether solvent and since like dissolves like, only non-polar phytochemicals was present in the extract [64]. *Flacourtia indica* was an exception since it recorded an MIC value which was lower than the other extracts. This was due to the presence of non-polar flavonoids in the extract. Gopal in 2011 carried out antibacterial activity of two chloroform extract of different species *Flacourtia*plants which were *Flacourtia jangonas* and *Flacourtia sepiaria*. *Flacourtia jangonas* showed better activity against *Bacillus cereus* with MIC value range of 0.325 to 5 mg/mL [76]. The results show that activity of plants of same family and generacan differ.

The results were comparable with others found in literature with *Microsporum canis* having an MIC of 0.50 mg/mL against *Propionibacterium acnes*. Previous studies have revealed that antimicrobial activity is caused by secondary metabolites which are phytochemicals.

4.4 Phosphomolybdate assay for measurement of antioxidant capacity

Phenolics are the largest group of phytochemicals that have been touted as accounting for most of the antioxidant activity of plants. Flavanoids, tannins and terpenoids tend to have phenolic groups in their structure thus giving them antioxidant capacity. The reductive potentials of the plant extracts were dose-dependent and it was represented by an increase in intensity and absorbance as the concentration increased. The phosphomolybdate assay is a method which involves the reduction of molybdenum (VI) to molybdenum (V) by an antioxidant forming a blue complex whose formation can be spectrophotometrically monitored using a uv-vis. In this

research, the total antioxidant activities of the plant extracts was determined by this method and the results were expressed in term of µg of Ascorbic Acid Equivalents per 10 µg of the dried extracts. The acetone extracts and fractions of different polarities were evaluated for their reducing capability by comparing with the standard Ascorbic Acid.

Table 6: Ascorbic Acid Equivalence of different plant extracts from the phosphomolybdate assay

Plant extract	***Pseudolachnostylis maprounelifolia***	***Flacourtia indica***	***Kigelia pinnata***	***Dicoma anomala***
Acetone	5.5	5.0	5.9	3.49
Chloroform	4.67	3.94	5.11	2.89
Petroleum ether	0.69	3.66	0.468	0.95

In the acetone crude extract, *Kigelia pinnata* showed the absorbance of 0.732 which in turn gave a high equivalence of 5.9 µg AAE/10 µg. This shows that *Kigelia pinnata* has a high antioxidant activity which confirms its use in the treatment of skin cancer due to the fact that antioxidants have reducing ability[119,120]. Antioxidants help in reducing the metastasis process which causes tumours to grow uncontrollably. *Pseudolachnostylis maprounelifolia* and *Flacourtia indica* extract also showed an equivalence of 5.5 µg AAE/10 µg and 5.0 µg AAE/10 µg respectively. This high equivalence is supported by the phytochemical results which showed that *Kigelia pinnata, Pseudolachnostylis maprounelifolia* and *Flacourtia indica* tested positive for phenolic compounds and flavonoids. Tannins also possess antioxidant activity which results in the fact why *Kigelia pinnata* and *Flacourtia indica* had the most outstanding activity[14]. Tannins are polyphenolic secondary metabolites of plants that have been reported to be more

effective in quenching proxy radicals than simple phenolics[59,121]. *Dicoma anomala* had the least activity since the phytochemical results did not show any presence of phenolics, tannins and flavonoids which are usually responsible for the antioxidant property of plant extracts[26,54]. The chloroform extract showed a moderate AAE showing presence of antioxidants although the activity was lower than the acetone crude extract. Petroleum ether showed the least activity due to the low polarity of the solvent. The intensity of the blue complex was proportional to the antioxidant capacity of the plant extracts. Most phenolic compounds, flavonoids and tannins are polar hence non polar solvents like petroleum ether cannot extract them. This is represented by the least antioxidant activity of the plant extracts with *Kigelia pinnata* having 0.468 µg AAE/10 µg, *Dicoma anomala* had 0.95 µg AAE/10 µg and *Pseudolachnostylis maprounelifolia* had 0.69 µg AAE/10 µg. *Flacourtia indica* was unique since it showed a high antioxidant activity compared to the other three extracts.

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The research has proven that medicinal plants have phytochemicals which act as a prototype to develop effective medicine which is less toxic in controlling microorganism growth. These fractions have been seen to have therapeutic application against pathogens from humans which include fungi, bacteria or virus. *Kigelia pinnata* showed presence of flavonoids, tannins, glycosides, coumarins, terpenoids, reducing sugars and sterols. *Dicoma anomala* had alkaloids, saponins, reducing sugars, glycosides and photosterols, *Flacourtia indica* consisted of tannins, flavonoids, phenolics, alkaloids, saponins, coumarins, reducing sugars and sterols. *Kigelia pinnata* was found to have tannins, flavonoids, glycosides, reducing sugars and sterols while *Pseodolachnostylis maprounelifolia* had flavonoids and saponins.

The FTIR results showed functional groups oh hydroxyls, amines, amides, ketones, aldehydes, arene rings and carboxylic acid derivatives. Antimicrobial studies revealed that *Kigelia pinnata* had the highest activity recording a zone of inhibition of 16.33 mm for the acetone extract and 14.0 mm for the chloroform fraction. The results were comparable with the standard Ciprofloxacin which had a zone of inhibition of 20.33 mm hence the acetone crude extract had an 80.3% activity in regard to the standard. *Flacourtia indica* showed highest activity in the petroleum ether fraction with a zone of inhibition of 11.67 mm having a 57.4% activity comparing with the standard. *Kigelia pinnata* also showed the lowest MIC of 0.4 mg/mL for the acetone crude extract and *Dicoma anomala* showed the highest MIC of 0.9 mg/mL for the petroleum ether extract. The study revealed that petroleum ether fractions showed the least

activity whilst the acetone crude extract and chloroform fraction showed significant antimicrobial activity.

The phosphomolybdate assay showed that *Kigelia pinnata* had the highest reducing power with with a 5.9 µg AAE/ 10 µg followed by *Pseudolachnostylis maprounelifolia* which had 5.5 µg AAE/ 10 µg. *Flacourtia indica* showed the highest reducing power in the petroleum extract showing presence of non-polar reducing agents. The results show that Kigelia pinnata, *Flacourtiaindica*, *Pseudolachnostylis maprounelifolia* and *Dicoma anomala* have secondary metabolites which have biological activities.

5.2 Recommendations

- Further research to be done to determine the free radical scavenging power for the anticancer plant extracts using better methods like the DPPH assay.
- Isolation of the active compounds and further characterization using NMR or other techniques like GC-MS in order to identify the structure of the phytochemicals.
- Mixtures of alcohols and water have been shown to be efficient in extracting phenolic compared to mono-component systems since addition of water creates a more polar medium which facilitates extraction of most polyphenols which have been seen to possess biological activities.
- Pharmacodynamics and pharmacokinetic studies on human subjects to test the activity of the plant extracts to understand the body's response and toxicology of the plant extracts.

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APPENDIX

APPENDIX A

A1: Apparatus

Spatula, polyethene bag, glass jars, 100mL volumetric flasks, weighing crucibles, Whatman filter papers, cotton wool, 1000mL measuring cylinder, vacuum filtration flasks, retort stand, wash bottle, Erlenmeyer flasks, beakers, Buchner funnels, test tubes, boiling tubes

A2: Reagents used

Name	Chemical formula	Manufacturer	Concentration
Sodium hydroxide	NaOH	Associated Chemical Enterprise	10%
Sulphuric acid	H_2SO_4	Glass world	98%
Hydrochloric acid	HCl	Scientific Masters	2M
Lead acetate	$(CHCOO)_4PbII$	Cosmo chemicals	1%
Potassium bromide	KBr	Buck Scientific	111.03gmol
Chloroform	$CHCl_3$	Sky labs	55%
Benzene	C_6H_{14}	Sky labs	60%
Ethanol	CH_3CH_2OH	Associated Chemical Enterprise	98%
Acetone	CH_3COCH_3	Associated Chemical Enterprise	99.50%

Ammonia	NH_3	Associated Chemical Enterprise	35%
Distilled water	H_2O	MSU lab	2000mL
Potassium iodide	KI	Skylabs	0.01gmol
Mercuric chloride	HgCl	MSU lab	50mL
Glacial acetic acid		Skylabs	0
Ammonium molybdate		Associated Chemical Enterprise	4mM
Sodium phosphate	Na_4P	Skylabs	28mM
Ascobic acid	AS	Buck Scientific	5
Mackonkey agar	-	Biochemicals	80g
PCA agar	-	Biochemicals	80g

A3: Instrumentation

Table A2: Instrumentation used

Instrument name	**Model**	**Manufacturer**	**Use in practical**
Digital analytical balance	GA110	OHAUSE	Weighing samples
Oven	DGH-9070A	Labcon	Drying of samples
Mechanical shaker	HY-4	Griffin	Shaking of samples

Rotavapour	RE200	Birby	Distillation of solvent mixtures
Fourier Transform Infrared Spectrometer	Nicolet 6700	Thermoscientific	Functional group identification
Water bath	RE200B	Bibby Sterling	Heating samples
UV-Vis	UV1700	Shimadzu	Absorbance measurement

A4: Preparation of solutions

(a) 5% $FeCl_3$

$FeCl_3$ (0.5263g) was dissolved in 10mL of distilled water.

(a) Mayer's reagent

Mercuric chloride (0.1376 g) and KI (0.5175 g) was dissolved in 10 mL of distilled water.

(b) 2% NaOH

NaOH (0.1145 g) was dissolved in 5 mLof distilled water.

(c) 10% NaOH

NaOH (0.5513 g) was dissolved in 5.5 mL of distilled water.

(d) 10% lead acetate

Lead acetate (0.1022 g) was dissolved in a 100 mL volumetric flask and filled to the mark with distilled water.

APPENDIX B

Table 1: Mass of acetone crude extract

Extract	Mass of extract + container	Mass of empty container	Mass of extract
Kigelia pinnata	14.8381	12.4337	2.4044
Flacourtia indica	64.9066	55.2506	9.656
Pseudolachnostylis maprounelifolia	92.8852	82.1044	10.7808
Dicoma anomala	85.6836	82.1028	3.5798

Table 2: Mass of Chloroform extract

Extract	Mass of extract + container	Mass of empty container	Mass of extract
Kigelia pinnata	13.6054	12.4371	1.1681
Flacourtia indica	58.4173	55.2499	3.1674
Pseudolachnostylis maprounelifolia	58.6577	55.2496	3.4081
Dicoma anomala	14.0008	12.4287	1.5721

Table 3: Mass of Petroleum ether extract

Extract	Mass of extract + container	Mass of empty container	Mass of extract
Kigelia pinnata	55.3315	55.1536	0.1779
Flacourtia indica	13.0687	12.4283	0.6404
Pseudolachnostylis maprounelifolia	55.9439	55.2508	0.6931
Dicoma anomala	57.7938	57.5781	0.2157

Equation B1: % yield extract

$$= \frac{mass\ of\ extract}{mass\ of\ plant\ powder\ used} * 100\%$$

Percentage yields for acetone extract

(i) *Pseudolachnostylis* = $\frac{10.7808}{200} * 100\% = 5.39\%$

(ii) *Kigelia pinnata* =$\frac{2.4044}{200} * 100\% = 1.2\%$

(iii)*Dicoma anomala* =$\frac{3.5798}{200} * 100\% = 1.79\%$

(iv)*Flacourtia indica* = $\frac{9.6560}{200} * 100\% = 4.83\%$

Percentage yield for chloroform extract

(i) *Pseudolachnostylis* = $\frac{3.4081}{8.0054} * 100\% = 42.57\%$

(ii) *Kigelia pinnata* =$\frac{1.1683}{2.0032} * 100\% = 58.32\%$

(iii) *Dicoma anomala* $= \frac{1.5721}{3.0056} * 100\% = 52.32\%$

(iv) *Flacourtia indica* $= \frac{3.1674}{8.0008} * 100\% = 39.62\%$

Percentage yield for petroleum extract

(v) *Pseudolachnostylis* $= \frac{0.6931}{2.9967} * 100\% = 23.13\%$

(vi) *Kigelia pinnata* $= \frac{0.1779}{1.0014} * 100\% = 17.77\%$

(vii) *Dicoma anomala* $= \frac{0.2157}{1.0177} * 100\% = 21.2\%$

(viii) *Flacourtia indica* $= \frac{0.6404}{2.5783} * 100\% = 24.84\%$

APPENDIX C

Table 4: Inhibition zone for 5 mg/mL acetone crude extract

	Disc number 1-3			Average	Std Dev
Negative control	0	0	0	0	0
Standard	21	21	19	20.33333	1.154701
Kigelia pinnata	16	17	16	16.33333	0.57735
P.maprounelifolia	13	12	14	13	1
Flacourtia indica	15	15	14	14.66667	0.57735
Dicoma anomala	13	12	13	12.66667	0.57735

Table 5: Inhibition zone for 5 mg/mL chloroform extract

	Disc number 1-3			Average	Std Dev
Negative control	0	0	0	0	0
Standard	21	21	19	20.33333	1.154701
Kigelia pinnata	13	15	14	14	1
P.maprounelifolia	10	10	9	9.666667	0.57735
Flacoutia indica	10	11	10	10.33333	0.57735
Dicoma anomala	11	11	12	11.33333	0.57735

Table 6: Inhibition zone for 5 mg/mL petroleum ether extract

	Disc number 1-3			Average	Std Dev
Negative control	0	0	0	0	0
Standard	21	19	21	20.33333	1.154701
Kigelia pinnata	8	9	8	8.333333	0.57735
P.maprounelifolia	9	10	8	9	1
Flacourtia indica	13	11	12	12	1
Dicoma anomala	9	9	8	8.666667	0.57735

Table 7: Inhibition zones for acetone crude extract 1 mg/mL

	Disc number 1-3			Average	Std Dev
Negative control	0	0	0	0	0
Standard	15	15	14	14.66667	0.57735
Kigelia pinnata	9	10	10	9.666667	0.57735
P.maprounelifolia	8	8	9	8.333333	0.57735
Flacourtia indica	9	8	9	8.66667	0.57735
Dicoma anomala	7	8	9	8	1

Table 8: Inhibition zones for chloroform extract 1 mg/mL

	Disc numbers 1-3			Average	Std Dev
Negative control	0	0	0	0	0
Standard	15	14	15	14.66667	0.57735
Kigelia pinnata	9	10	11	10	1
P.maprounelifolia	6	7	6	6.333333	0.57735
Flacoutia indica	7	8	8	7.666667	0.57735
Dicoma anomala	8	8	7	7.666667	0.57735

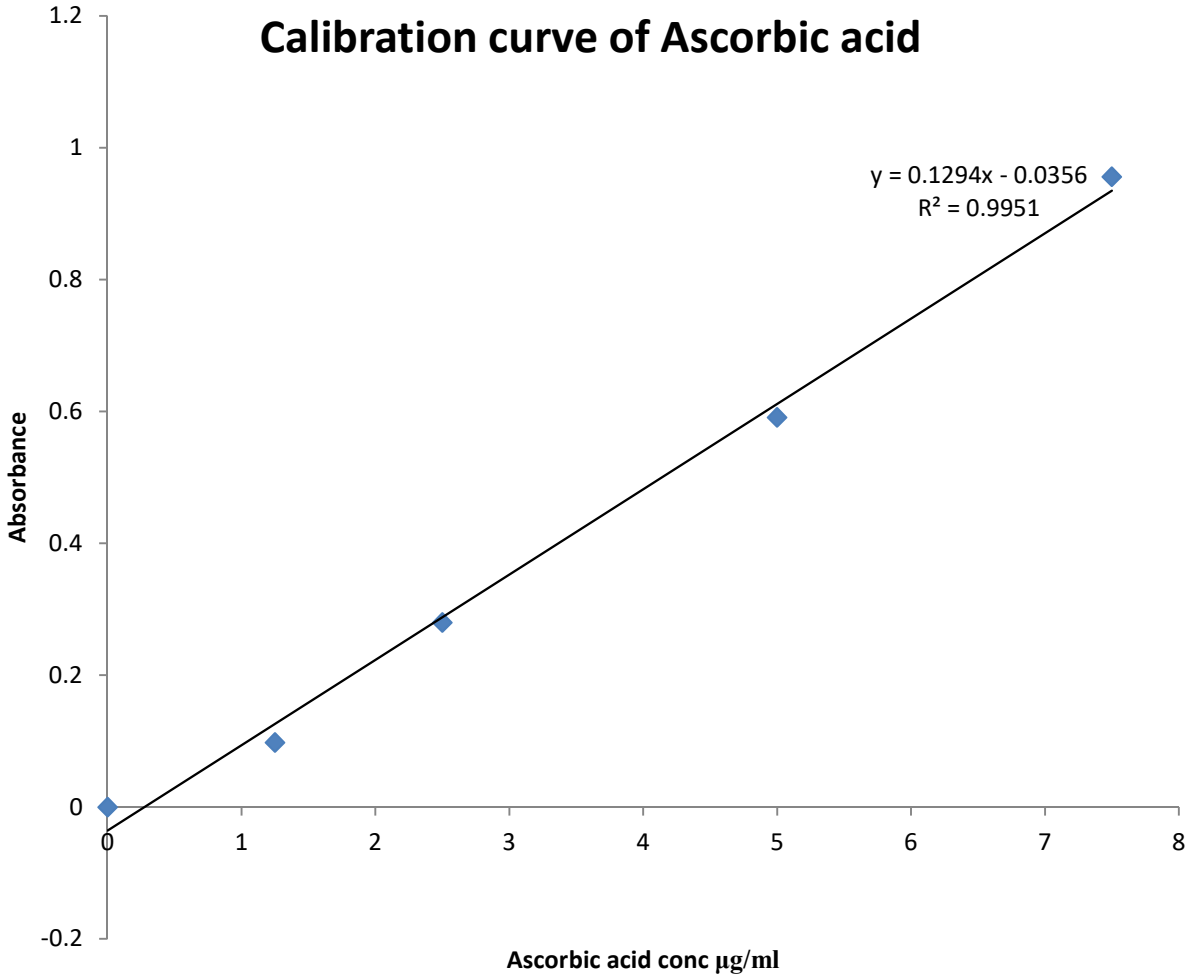
Table 9: Inhibition zone for petroleum extract 1 mg/mL

	Disc number 1-3			Average	Std Dev
Negative control	0	0	0	0	0
Standard	14	15	15	14.66667	0.57735
Kigelia pinnata	6	6	7	6.333333	0.57735
P.maprounelifolia	7	7	6	6.666667	0.57735
Flacourtia indica	9	9	8	8.666667	0.57735
Dicoma anomala	7	8	6	7	1

Equation C1

$$\text{Mean diameter} = \frac{summation\ of\ inhibition\ diameter}{Discs\ used}$$

Phosphomolybdate Assay



Absorbance results of plant extracts

Plant extract	Pseudolachnostylis*maprounelif olia*	*Flacourtia indica*	*Kigelia pinnata*	*Dicoma anomala*
Acetone	0.683	0.611	0.732	0.416
Chloroform	0.569	0.474	0.626	0.338
Petroleum ether	0.054	0.439	0.025	0.087

Using the equation of the straight line y = 0.1294x-0.0356, the x values were determined by substituting the y value with the absorbance. The x value obtained was the Ascorbic Acid Equivalence in µg/mL AAE/ 10µg/mL.

$$x = \frac{y + 0.0356}{0.1294}$$

For acetone crude extracts

(i) *Flacourtia indica* $= \frac{0.611 + 0.0356}{0.1294} = 5.0$

(ii) *Kigelia pinnata* $= \frac{0.732 + 0.0356}{0.1294} = 5.90$

(iii) *Dicoma anomala* $= \frac{0.416 + 0.0356}{0.1294} = 3.49$

(iv) *Pseudolachnostylis* $= \frac{0.416 + 0.0356}{0.1294} = 5.55$

For chloroform fraction

(i) *Flacourtia indica* $= \frac{0.474 + 0.0356}{0.1294} = 3.94$

(ii) *Kigelia pinnata* $= \frac{0.626 + 0.0356}{0.1294} = 5.11$

(iii) *Dicoma anomala* $= \frac{0.338 + 0.0356}{0.1294} = 2.89$

(iv) *Pseudolachnostylis* $= \frac{0.569 + 0.0356}{0.1294} = 4.67$

For petroleum ether fraction

(i) *Flacourtia indica* $= \frac{0.439 + 0.0356}{0.1294} = 3.66$

(ii) *Kigelia pinnata* $= \frac{0.025 + 0.0356}{0.1294} = 0.468$

(iii) *Dicoma anomala* $= \frac{0.087 + 0.0356}{0.1294} = 0.95$

(iv) *Pseudolachnostylis* $= \frac{0.054+\ 0.0356}{0.1294} = 0.69$