Standardization of Selected Medicinal Herbs from Kisii Region Used in Treatment of Diabetes, Malaria and Pneumonia

Moses Akuma Guto Maobe

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DECLARATION

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

Signature: _____ Date: _____

Moses Akuma Guto Maobe

This thesis has been submitted for examination with our approval as University Supervisors:

Signature: _____ Date: _____

Prof. Erastus Gatebe,
JKUAT, Kenya.

Signature: _____ Date: _____

Dr. Leonard Gitu,
JKUAT, Kenya.

Signature:_____ Date:_____

Dr. Henry Rotich
Kenya Bureau of Standards, Kenya.

DEDICATION

This thesis is dedicated to my dear wife Josephine and Children Elijah, Esther, Zacharius, Christine and Justine for their patience, love and understanding during my busy schedule and for allowing me to use their time to study.

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LIST OF ACRONYMS

AIDS	acquired immunodeficiency syndrome
API	Ayurvedic Pharmacopoeia of India
BHP	British Herbal Compendium
EC	European Community
ECD	Electron capture detector
FD	Fluorescence detector
FID	Flame ionization detector
GC	gas chromatography
HPLC	High-performance liquid chromatography
ICP–MS	Inductively coupled plasma–mass spectrometry
ICP–OES	Inductively coupled plasma–optical emission spectrometry
LC–UV–MS	Liquid chromatography–ultraviolet-mass spectrometry
MAE	Microwave-assisted extraction
MS	Mass spectrometry
NMR	Nuclear magnetic resonance spectroscopy
PC	Paper chromatography
TCM	Traditional Chinese medicine
UV	Ultraviolet
MCA	Medicines Control Agency
WHO	World Health Organisation
US	United States
DDT	dichlorodiphenyltrichloroethane

LIST OF MEDICINAL HERBS

ABSTRACT

The herbs used as phytomedicines for the treatment of diabetes, malaria and pneumonia in Kisii region, Southwest Kenya are *Carissa spinarum, Urtica dioica, Warburgia ugandensis, Senna didymobotrya, Physalis Peruviana, Bidens pilosa, Leonotis nepetifolia* and *Toddalia asiatica*. The objective of this study was to standardize the selected eight herbs. Leaf samples of these herbs were obtained from Kisii region, washed, air-dried and milled. Standardization parameters namely: phytochemical screening, heavy metal analysis, antimicrobial activity and identification of functional groups were studied. The pH levels of the aqueous extract were measured using a pH meter. Results showed that the pH values ranged from 5.27 to 7.50. Phytochemical studies indicated presence of saponins, tannins, flavonoids, steroids and terpenoids in eight herbs while alkaloids in two, cardiac glycoside in five and anthraquinones in one and all were confirmed by TLC. Heavy metal analysis was done by atomic absorption spectrometry. The results showed the heavy metal present as: Fe, Cr, Cu, Zn, Co, Mn, Ni, Pb, Cd and Hg. The biological activity of these herbs by well diffusion method indicated that hexane, dichloromethane, ethyl acetate and ethanol extracts against *Staphylococcus aureus, Escherichia coli* and *Candida albicans,* showed antimicrobial and antifungal activity. The FTIR spectra analysis indicated the functional groups present as: OH, CH_2 and CH_3, C-N, C=N, C=C, C=O, C-O, C≡N and N≡C. Therefore extracts from these herbs have a potential to control *Staphylococcus aureus, Escherichia coli* and *Candida albicans*. These findings if incorporated with the existing knowledge and experiences that the traditional healers have, could improve their utilization.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the Study

The use of herbs as medicine is the oldest form of healthcare known to humanity and has been used in all cultures throughout history (Kunle *et al.,* 2012., Barnes *et al.,* 2007). Early humans recognized their dependence on nature for a healthy life by depending on the diversity of plant resources for medicine to cure many of ailments. Led by instinct, taste, and experience, primitive men and women treated illness by using herbs, animal parts, and minerals that were not part of their usual diet (Kunle *et al.,* 2012., Barnes *et al.,* 2007). Primitive people learned by trial and error to distinguish useful herbs with beneficial effects from those that were toxic or inactive, and also which combinations or processing methods had to be used to gain consistent and best results (Gisesa, 2004., Karinge, 2006., Tolo *et al.,* 2010., Kunle *et al.,* 2012). Even in ancient cultures, tribal people methodically collected information on herbs and developed well-defined herbal pharmacopeias (Gisesa, 2004., Karinge, 2006., Kunle *et al.,* 2012). Physical evidence of the use of herbal remedies some sixty thousand years ago has been found in a burial site of a Neanderthal man uncovered in 1960 in a cave in northern Iraq (Kunle *et al.,* 2012). The knowledge of plant-based drugs developed gradually and was passed on, therefore, laying the foundation for many systems of traditional medicine all over the world such as Ayurvedic herbalism, Chinese herbalism, African herbalism and Western herbalism (Gisesa, 2004., Karinge, 2006., Kunle *et al.,* 2012). In some communities herbal medicine is still a central part of their medical system (WHO, 2005). Due to low

toxicity and known pharmacological activity, herbal medicine has been popularly and extensively used for many centuries among the Gusii community, such as *Carissa spinarum, Urtica dioica, Warburgia ugandensis, Senna didymobotrya, Physalis peruviana, Bidens pilosa, Leonotis nepetifolia* and *Toddalia asiatica*, for the treatment of diabetes, malaria, pneumonia, wounds and cuts in the Kisii region, South west Kenya (Gisesa, 2004., Karinge, 2006., Tolo *et al*., 2010). Medicinal herbs are consumed worldwide for the treatment of several animal and human diseases, and such herbs are an important source of raw material for pharmaceutical industries (Kirmani *et al*., 2011). The use of herbal medicines has increased worldwide, because of the fact that the side effects and costs of these medicinal herbs are often lower than synthetic and many conventional Medicines (Calixto, 2000., Rates, 2000., Rajani and Kanaki, 2008). The assurance of safety, quality and efficacy of medicinal herbs and herbal products is becoming a crucial issue (Patil and Shettigar, 2010). There is need for the standardization of herbal raw materials and herbal formulations (Patil and Shettigar, 2010). Standardization involves adjusting the herbal medicine preparation to a defined content of a constituent or a group of substances with known therapeutic activity (Mosihuzzanman and Choudhary, 2008). Standardization method takes into consideration all aspects contributing to the quality of the herbal medicines (Patil and Shettigar, 2010). Herbal Medicines are composed of many constituents and are therefore very capable of variation. Hence it is very important to obtain reliable pharmacologically active and chemically characteristic constituents in maintaining the quality and consistency of the herbal medicine (Patil and Shettigar, 2010). Many medical herbs used can present a health risk due to the presence of toxic ingredients like heavy metals (Kirmani *et al*., 2011).

The toxicity of heavy metals depends upon the chemical form of elements. Heavy metals are dangerous in the form of their cations and are highly toxic when bonded to the short chains of carbon atoms (Hussain, 2006). Herbs may absorb heavy metals from soil, water or air. Medicinal herbs may be easily contaminated during growing and processing (Bandaranayake *et al*., 2006). Usually soil is subjected to contamination through atmospheric deposition of heavy metals from point Sources including metalliferous mining, smelting and different industrial activities (Bin, 2001., Mosihuzzanman and Choudhary, 2008). Some other sources of soil contamination involve use of fertilizers, pesticides, sewage sludge and organic manures (Kirmani *et al*., 2011). Furthermore, many dangerous and lethal side effects have been reported, including direct toxic effects, allergic reactions, effects from contaminants, and interactions with drugs and other herbs (Mosihuzzanman and Choudhary, 2008). The common effects of trace metal toxicity to living organisms include cancer, brain disorder, and gross deformities in development, carcinogenic effects and disruption of biological processes (Zevenhoven and Kilpinen, 2001). Mercury, lead and arsenic are carcinogenic and affect the central nervous system while lead, copper and cadmium affect the liver and kidneys (Zevenhoven and Kilpinen, 2001., Mustafa, *et al*, 2004., Khan *et al.*, 2008). Balammal *et al.*, 2012, reported risks associated with herbal medicine such as poisoning, hepatitis, interstitial renal fibrosis, renal failure and loss of the tubules in China, America, Korea, Belgium and Japan among others. The concern about the safety and claimed efficacy of many herbal products, toxicity and lack of proper scientific evaluation resulted in supporting the current project with the objective of standardization of medicinal herbs.

1.2 Statement of the Problem

The use of herbal medicine is complicated by numerous problems which are as follows: Lack of scientific evidence of safety and efficacy - Most herbal products has not been scientifically evaluated; thus, information is limited about their efficacy and safety (Kataria *et al.,* 2011., Tachjian and Arshad, 2010). Herbs might be contaminated with heavy metals due to lack of quality control and public misinformation such as unethical marketing techniques have led to false advertisements about the safety and efficacy of the herbs reaching the public today (Medicines Control Agency, 2002). Herbs are advocated for treatment on the basis of unproven, word-of-mouth traditions and beliefs. There is a danger to the public without a proper evaluation of the possible risks which could arise from the ingestion of medicinal herbs (Tachjian and Arshad, 2010). To achieve the desired benefit from medicinal herbs, an individual must take the required dose over a certain length of time (Bandaranayake *et al*., 2006., Mosihuzzanman and Choudhary, 2008). Herbal medicine products present a number of unique problems when quality aspects are considered (Medicines Control Agency, 2002). These arise because of the nature of the herbal ingredients, which are complex mixtures of constituents. Herbs can produce undesirable side effects and can be toxic (Bandaranayake *et al*., 2006). A particular plant part such as the leaves will have many constituents and some of them may well be toxic. The active principles are frequently unknown; and standardization, stabilization, and quality control are not easy (Mosihuzzanman and Choudhary, 2008). Furthermore, the constituents responsible for the claimed therapeutic effects are frequently unknown or only partly explained and this prevents the level of control which can routinely be achieved with synthetic drug substances

in conventional pharmaceuticals (Mosihuzzanman and Choudhary, 2008). Some of the major problems with regard to the safety of herbal medicines are related to the manufacturing practice, contamination, substitution, incorrect preparation and dosage, intentional addition of unnatural toxic substances and interaction involving herbal medicines (Mosihuzzanman and Choudhary, 2008., Balammal *et al*., 2012). The use of some medicinal herbs has been reported to be associated with ailments such as oral manifestations, including swelling, irritation, and bleeding of the tongue (Bandaranayake *et al.,* 2006*).* These potential effects of herbal medicine, in conjunction with factors related to regulation restrictions, suggest that the use of these products may be associated with various adverse reactions that can affect health (Bandaranayake *et al*., 2006). The medicinal herbs have several other problems namely, over-dosage and under dose due to lack of standardization , allergic reactions, selective analytical methods or reference compounds may not be available commercially, plant materials are chemically and naturally variable, the source and quality of the raw material are variable, the methods of harvesting, drying, storage, transportation, and processing have an effect.

1.3 Hypotheses of the Study

To achieve the objectives, the following null hypotheses were tested at 0.05 alpha level of significance.

H_{01}: There are no biological active compounds and heavy metals present in the selected medicinal herbs used in Kisii region to treat diabetes, malaria and pneumonia diseases.

H_{02}: There is no significant concentration of biologically active compounds and biological activity displayed by the medicinal herbs used in Kisii region to treat diabetes, malaria and pneumonia diseases.

1.4 Main objective

To standardize selected medicinal herbs from Kisii region used for the treatment of diabetes, malaria and pneumonia.

1.4.1 Specific objectives:

i. To measure the pH levels of the eight herbs.
ii. To test for the presence of phytochemical compounds in the eight herbs.
iii. To determine the level of heavy metals in the eight herbs.
iv. To perform thin layer chromatography on the extracts of eight herbs.
v. To study the antibacterial and antifungal activity of the eight herbs against *Escherichia coli*, *staphylococcus aureus* and *Candida albicans.*
vi. To identify the possible functional groups present in the eight herbs using infrared spectroscopy.

1.6 Justification of the Study

As with all forms of self-treatment, the use of herbal medicinal products presents a potential risk to human health (Medicines Control Agency, 2002). The patient may be exposed to potentially toxic substances present naturally in the herbal ingredients contamination (Bandaranayake *et al*., 2006., Mosihuzzanman and Choudhary, 2008., Balammal *et al*., 2012). Herbal medicinal products may in some cases compromise the efficacy of conventional medicines due to inadequate standardization and lack of quality specification, for example through herb-drug interactions (Kataria *et al.*,

2011). There are case reports of serious adverse effects after administration of herbal products (Mosihuzzanman and Choudhary, 2008). The toxic effects of herbal preparation may be attributed mainly to inherent toxicity of plant constituents and ingredients, and manufacturing malpractice and contamination (Bandaranayake *et al.,* 2006. Mosihuzzanman and Choudhary, 2008., Balammal *et al*., 2012). Therefore, there is a widespread yet false perception that herbal products are safe. However, relatively few herbal drugs have been evaluated scientifically to prove their safety, potential benefits and effectiveness (Medicines Control Agency, 2002., Kataria *et al.,* 2011). Herbal medicine has been commercializated; hence assurance of safety, quality and efficacy of medicinal plants and herbal products has become an important issue. The herbal raw material is prone to a lot of variation due to several factors, such as identity of the plants and seasonal variation, the ecotypic, genotypic and chemotypic variations, drying and storage conditions (Patil and Shettigar, 2010). In Kenya, herbal products are launched into the market without proper scientific evaluation, mandatory safety and toxicological studies. There is no effective machinery to regulate manufacturing practices and quality standards. Consumers can buy herbal products without a prescription and might not recognize the potential hazards in an inferior product (Bandaranayake *et al*., 2006., Mosihuzzanman and Choudhary, 2008., Kunle *et al.,* 2012).

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Herbal Medicine

A herb is a plant or part of a plant valued for its medicinal, aromatic, or savoury qualities (Bandaranayake *et al*., 2006). All herbs produce chemical compounds as part of their normal metabolic activities (Balammal *et al*., 2012). These include primary and secondary metabolites. The secondary metabolites include alkaloids, saponnins, tannins, flavonoids, phenolics, terpenoids, steroids, glycosides among others (Balammal *et al*., 2012). The secondary metabolites or phytochemicals have antiviral, antibacterial, antifungal, anticancer and antihelminthic properties, and can be used for treatment of animal and human diseases (Balammal *et al*., 2012). Herbal remedies or medicines consist of portions of herbs or unpurified herbs extracts containing several constituents, which often work together synergistically. Herbal medicine or herbalism is the use of herbs or herbal products for their therapeutic or medicinal value (Mosihuzzanman and Choudhary, 2008). Medicinal herbs are also referred to as botanicals, biomedicines, herbal supplements, Phytotherapeutic agents or phytomedicines (Mosihuzzanman and Choudhary, 2008). Phytotherapeutic agents or phytomedicines are standardized herbal preparations consisting of complex mixtures of one or more plants which are used in most countries for the management of various diseases (Bandaranayake *et al*., 2006., Mosihuzzanman and Choudhary, 2008). Herbal remedies or medicines may come from any part of the herb but are most commonly made from leaves, roots, bark seeds, and flowers (Mosihuzzanman and Choudhary, 2008). They are eaten, swallowed, drunk, inhaled, or applied to the skin (Mosihuzzanman and Choudhary, 2008). Herbal products often contain a variety

of naturally-occuring biochemicals from plants, many of which contribute to the plant's medicinal benefits. Chemicals known to have medicinal benefits are referred to as "active ingredients" or "active principles" or "marker compounds" or bioactive compound and their presence depends on a number of factors including the plant species, the time and season of harvest, the type of soil, the way the herb is prepared (Mosihuzzanman and Choudhary, 2008). Due to poverty and limited access to modern medicine, especially in the developing countries, uses herbal medicine as their source of primary healthcare (Kunle *et al.,* 2012., Mukherjee *et al.*, 2010). In the West, people are attracted to herbal therapies due to their balanced and moderate approach to healing, and they believe that herbal medicine will help them live healthier lives (Kunle *et al.,* 2012).

2.1.1 Selected herbs and their medicinal value:

2.1.1.1 *Carissa spinarum*

The herb *Carissa spinarum* (Family: Apocynaceae) has been used in ethno medicine for the treatment of many microbial infections such as venereal, respiratory and gastrointestinal infections (Ibrahim *et al.,* 2010). The *Carissa spinarum* has been traditionally applied to kill worms in the wounds of cattle (Rose and Prasad, 2013). It is also used in combination with the roots of some other medicinal herbs to treat rheumatism (Ibrahim *et al.*, 2010., Rose and Prasad, 2013). It is a strong purgative and is used as one of the ingredients in some purgative preparations (Rose and Prasad, 2013). The *Carissa spinarum* roots has been prescribed in the indigenous system of medicine as purgative, for the treatment of inflammation-related disorders

such as rheumatism and pain, cleaning worm infested wounds of animals and in snake bite (Hegde and Joshi, 2010). In Chinese system of medicine the roots of the plant is known for the treatment of rheumatism and hepatitis (Hegde and Joshi, 2010). The Carissa *spinarum* parts are used in ethnomedicine for wide variety of illnesses, such as epilepsy, headache, chest complaints, gonorrhoea, syphilis, rheumatism, rabies and as well as a diuretic (Ya'u *et al*., 2008). Other folkloric uses of *Carissa spinarum* include fever, sickle cell anaemia and hernia (Ya'u *et al*., 2008). Previous phytochemical investigations revealed the presence of caffeic acid, ursolic acid, naringin, various cardiac glycosides, germacrane sesquiterpene and lignans (Hegde and Joshi, 2010). Earlier studies have shown that the extract of the herb possesses cardiotonic, antibacterial and potent antioxidant activity (Hegde and Joshi, 2010). Phytochemical screening of various extract of *Carissa spinarum* leaves revealed that phytoconstituents present in the herb are alkaloids, tannins, glycosides, steroids and carbohydrates (Rose and Prasad, 2013). The leaves and fruits were reported to contain carbohydrates, tannins, flavonoids, saponins, cardiac glycosides, terpenes and steroids (Ibrahim *et al.,* 2010). The phytochemical investigation of the ethanolic extract of the roots of the plant revealed the presence of glycosides, flavonoids, saponins, triterpenoids, steroids, phenolic compounds and tannins. Furthermore, the flavonoids, tannins and triterpenoids are known to inhibit prostaglandin synthesis (Sayyah *et al.*, 2004., Hegde and Joshi, 2010). The flavonoids, triterpenoids, tannins and other chemical compounds present in the herb are responsible for the antipyretic effect.

2.1.1.2 *Urtica dioica*

Urtica dioica (Family: Urticaceae) is a herbal species which produces allergenic substances causing oedema and inflammation in humans (Khare *et al.*, 2012). It has become a source of folk medicine for the treatment of many diseases. The leaves and roots both are used internally as a blood purifier and diuretic and an infusion of the plant is used for nasal and menstrual haemorrhage, diabetes, rheumatism, eczema, anaemia, hair loss, as an expectorant and antidiarrhoeal (Khare *et al.*, 2012., Kataki *et al.*, 2012). The leaves were reported to contain tannins, saponins, flavonoids, carbohydrates and phenolic compounds (Khare *et al.*, 2012). The plant is used traditionally as emmenagogue and as anthelminthic (Kataki *et al.*, 2012). It is also used in nephritis, haematuria, jaundice and menorrhagia (Kataki *et al.*, 2012). Moreover, previous studies reported the presence of lectins, linolenic acid, lutein, lutein isomers, β-carotene and β-carotene isomers, neoxanthin, violaxanthin and lycopene in this herb. The herb is also claimed to possess diuretic and natriuretic effects along with antidiabetic and antihypertensive activity (Kataki *et al.*, 2012).

2.1.1.3 *Warburgia ugandensis*

Warburgia ugandensis (Family: Canellaceae) is one of the most highly utilized medicinal herbs in Kenya (Ngure *et al.*, 2009). The *Warburgia ugandensis* dried bark is chewed and the juice swallowed as a treatment for stomach ache, constipation, toothache, venereal diseases, cough, fever, muscle pains, weak joints and general body pains (EL-Kamali, 2009., Ngure *et al.*, 2009). The leaf decoction baths are used as a cure for skin diseases while the bark, roots or leaves can be boiled in water and drunk to treat malaria, although this causes violent vomiting (Ngure *et al.*, 2009). *Warburgia ugandensis* is also used by traditional healers to treat visceral

leishmaniasis (Ngure *et al*., 2009). The stem barks are taken orally in boiled water or soup. Previous studies on *Warburgia ugandensis* have shown good antibacterial, antifungal, antiviral activity and trypanocidal effects (EL-Kamali, 2009., Ngure *et al.*, 2009). Crude extracts and purified compounds of *Warburgia ugandensis* showed activity against *Mycobacterium tuberculosis* H37Rv and M. Bovis BCG Pasteur (Ngure *et al*., 2009), *Candida albicans* and measles virus (Ngure *et al*., 2009). Similarly, the activity of *Warburgia ugandensis* against trypanosomes has been demonstrated (EL-Kamali, 2009., Ngure *et al.*, 2009). *Warburgia ugandensis* has been widely used in folk medicine for treating malaria, Polygodial: antifeedant, antiyeast (Kokwaro, 2002., EL-Kamali, 2009).

2.1.1.4 *Senna didymobotrya*

Senna didymobotrya (Family: Fabaceae – Caesalpinioideae) is a medicinal herb which is widely used as a purgative and an anti-malaria medicine (Orwa *et al.*2009*)*. A decoction of the leaves is used against stomach complaints. Leaves and roots contain a number of anthraquinones, choline, and the trisaccharide raffinose (Orwa *et al.2009)*. It is commonly used as a stupefacient poison for fishing (Ganapaty *et al.*, 2002., Orwa *et al.2009)*. The leaves of *Senna didymobotrya* have been used for the treatment of ringworm infections, as a laxative and in the treatment of leprosy and syphilis (Ganapaty *et al.*, 2002). The leaves of *Senna didymobotrya* has been used as an antidote for snake bites (Ganapaty *et al.*, 2002). *Senna didymobotrya* is known for its anthraquinone glycosides and laxative action (Ganapaty *et al.*, 2002). The anthraquinone molecules have remarkable biological properties. The *Senna*

didymobotrya are considered to possess purgative, wound healing, anti-inflammatory and hepatoprotective properties (Ganapaty *et al.*, 2002). The anthraquinones obtained from the leaves and pods of *Senna didymobotrya* are classified as contact purgatives. The anthraquinones inhibit salt and water absorption in the colon and increase peristalsis, which results in soft and bulky faeces (Ganapaty *et al.*, 2002). The literature reveals that rhein possesses significant purgative activity while chrysophanic acid-9-anthrone exhibited a good degree of fungicidal activity (Ganapaty *et al.*, 2002). Sennosides are effective orally and are available as calcium salts (Ganapaty *et al.*, 2002). The anti-inflammatory activity of *Senna didymobotrya* may be attributed to the flavonoid molecules present in them (Ganapaty *et al.*, 2002).

2.1.1.5 *Physalis peruviana*

Physalis peruviana (Family: Solanaceae) has been widely used in folk medicine for treating malaria, asthma, cancer, leukemia, hepatitis, diabetes, liver and kidney fibrosis, rheumatism, dermatitis and other diseases (Franco *et al.*, 2007., El-Gengaihi *et al.*, 2012., Licodiedoff *et al.*, 2013), coupled to other proven medicinal remedies as a diuretic and the reduction of bad cholesterol and glucose level (Licodiedoff *et al.*, 2013). Extracts of medicinal herbs are believed to contain different chemo preventive or chemotherapeutic compounds, which possess more than one mechanism of actions (El-Gengaihi et al., 2012). Several chemical compounds may be found within the genus's diversity, such as simple flavonoids or glycosides (kaempferol, quercetin, and rutin), linear-chain fatty acids (C_6 to C_4), ascorbic acid, carotenoids, alkaloids and terpenes such as Withaesteroids, (Angelo and Jorge, 2007., Huber *et al.*, 2007). Flavonoids, hydroxybenzoic and hydroxycinnamic acids and stilbenes are phenolic

compounds which have currently been highlighted owing to their beneficent biological effect to health and because of their anti-oxidant activities that combat free radicals (Chirinos *et al.,* 2010; Licodiedoff *et al.,* 2013).

2.1.1.6 *Leonotis nepetifolia*

The *Leonotis nepetifolia* (Family: Lamiaceae) has been used in herbal medicine in many cultures. The *Leonotis nepetifolia* has been described as a multipurpose herb which is used extensively for its medicinal properties (Gungurthy *et al.*, 2013). All parts of *Leonotis nepetifolia* possess valuable medicinal properties. The different alkaloids, flavonoids, diterpenoids, polyphenolics, iridoid glycosides and other constituents of *Leonotis nepetifolia* may be involved in the anti-nociceptive, anti-inflammatory, arthritic effects (Gungurthy *et al.*, 2013). It is also having anti-asthmatic and anti-diarrhea properties (Gungurthy *et al.,* 2013). In Trinidad's traditional medicine, an infusion is used against fever, coughs, womb prolapsed, malaria and treatment of diabetes (Gungurthy *et al.*, 2013). Also Leonotis *nepetifolia* has been used in folk medicine for treating hepatitis, pneumonia, wounds (Kokwaro, 2002., EL-Kamali, 2009). Leaf juice of *Leonotis nepetifolia* is used against Malaria (Kumar and Dash, 2012). The *Leonotis nepetifolia* leaf extract has antioxidant, anticancer activities, and the leaves are brewed as a tea for fever, coughs, womb prolapse and malaria (Veerabadran *et al.*, 2013). The preliminary phytochemical screening of *Leonotis nepetifolia* has revealed the presence of phenolics, flavonoids and reducing sugar in high amounts, whereas, terpenoids, steroids, and tannins were present in moderate amount. Alkaloids, glycosides and saponins were present in trace amount (Veerabadran *et al*., 2013). *Leonotis nepetifolia* is an important herb which

has been used to treat bronchial asthma, diarrhoea, fever, analgesic, influenza and malaria (Narayan, 2012). This herb exhibited various biological activities such as antifungal and antibacterial activities, antioxidant, and anticancer activities (Narayan, 2012). The phytochemical examination of this herb parts, indicated the presence of different diterpenoids of labdone type, besides the presence of other bioactive compounds. Phytochemical examination indicated the presence of alkaloids, saponins, tannins, steroids and terpenoids (Narayan, 2012).

2.1.1.7 *Bidens pilosa.*

Bidens pilosa (Family: Asteraceae) is widely used in traditional medicine for anti-influenza, diabetic control and treatment of gastroenteritis (Ashafa and Afolayan, 2009). Extensive research have shown that *Bidens pilosa* possessed antihyperglycemic (Hsu *et al.,* 2009., Ashafa and Afolayan, 2009), anti-ulcerogenic (Ashafa and Afolayan, 2009), anti-inflammatory, vasodilative, hypertensive, antimalarial, hepato-protective, antipyretic (Sundararajan *et al.,* 2006., Ashafa and Afolayan, 2009), anticancer and antitumor (Sundararajan *et al.*, 2006; Kviencinski *et al.*, 2008), antioxidant (Ashafa and Afolayan, 2009), antiviral (Ashafa and Afolayan, 2009), antifungal (Deba *et al*., 2008) and anti-bacterial (Ashafa and Afolayan, 2009), activities. Previous studies have shown that *Bidens pilosa* contains compounds like flavonoids, phenylacetylenes, alkaloids, sterols, triterpenoids and tannins (Ashafa and Afolayan, 2009) that are responsible for the antimicrobial activity of this species. *Bidens pilosa* has been used in folk medicine for treating Furuncle, hepatitis, otitis, wounds (Kokwaro, 2002., EL-Kamali, 2009). The chemicals in the herb *Bidens pilosa* are phenylpropanoids, glucosides, polyacetylenes, diterpene flavonoids and

flavone glycoside (Patel *et al.,* 2013). Herbal extract has been reported for use in treatment of diabetes, hypertension, inflammation, immunosuppression, arthritis, cancer and malaria (Patel *et al.,* 2013). It is capable of showing its anti – arthritic activity because of its free radical scavenging and nitric oxide synthase inhibition activity (Patel *et al.,* 2013).

2.1.1.8 *Toddalia asiatica*

Toddalia asitica (Family: Rutaceae) is used as a fork medicine in Kenya (Balasubramaniam *et al*., 2011). Pharmacological studies of *Toddalia asitica* showed that the herb constitutes the tumor selective cytotoxicity, antibacterial and antifungal activity, anti-malaria activity, antiviral activity against influenza type A virus (Balasubramaniam *et al*., 2011). *Toddalia asiatica* contains coumarins, quinoline and benzophenanthridin alkaloids (Balasubramaniam, *et al*., 2011). The alkaloids of the crude extract have been shown to have anti-inflammatory, anti-malarial and anti-leukimatic properties (Balasubramaniam, *et al*., 2011). *Toddalia asiatica* is an important herb used for the treatment of a range of diseases like cough, malaria, indigestion, influenza lung diseases and rheumatism, fever, stomach ailments, cholera and diarrhea (Nabwami *et al* .,2007). Coumarine derivatives with antiplasmodial (Nabwami *et al*., 2007), and antimicrobial (Nabwami *et al*., 2007), activity have been isolated from its leaves. This plant is used traditionally in Kenya by many communities for the treatment of malaria and other ailments (Kokwaro, 2002., EL-Kamali, 2009). All parts of the plant are claimed to have medicinal value, but the roots in particular are believed to be more effective. Decoctions or infusions of the roots are drunk to treat malaria, fever and to cure stomachache (Kokwaro,

2002., EL-Kamali, 2009). For toothache the root is chewed while for the treatment of coughs the fruits are chewed (Kokwaro, 2002., EL-Kamali, 2009). The ethyl acetate extract of *Toddalia asitica* root contain a comarin derivative, as the major antimalarial principle of this extract (Kokwaro, 2002., EL-Kamali, 2009).

2.2 Quality Control and Standardization of Herbal Medicines

In indigenous and traditional systems of medicine, the herbal medicines are primarily dispensed as water decoction or ethanolic extract (Kamboj, 2000). The medicinal herb was subjected to a single solvent extraction once or repeatedly, or water decoction (Kamboj, 2000). The extract was then checked for indicated biological activity in an experimental animal model. The bioactive compound of extract was standardized on the basis of "active" principle or major compound along with fingerprints. The herbal drugs are herbs or herbs parts that have been converted into phytopharmaceuticals by means of simple processes involving harvesting, drying, and storage (Mosihuzzanman and Choudhary, 2008., Kunle *et al*., 2012). Hence they are capable of variation which is caused by differences in growth, geographical location, and time of harvesting. Standardization of herbal medicines is the process of prescribing a set of standards or inherent characteristics, constant parameters, definitive qualitative and quantitative values that carries an assurance of quality, efficacy, safety and reproducibility (Mosihuzzanman and Choudhary, 2008., Kunle *et al.*, 2012). Hence standardization is a tool in the quality control process.

Factors that influence the quality of medicinal herbs are:

1. Medicinal herbs are usually mixtures of many constituents.

2. The active principle is, in most cases unknown.

3. Selective analytical methods or reference compounds may not be available commercially.

4. Plant materials are chemically and naturally variable.

5. Chemo-varieties and chemo cultivars exist.

6. The source and quality of the raw material are variable.

7. The methods of harvesting, drying, storage, transportation, and processing (for example, mode of extraction and polarity of the extracting solvent, instability of constituents among others) also affect herbal quality.

2.2.1 Standardization of herbal medicines

This involves adjusting the medicinal herb preparation to a defined content of a constituent or a group of substances with known therapeutic activity by adding excipients or by mixing herbal medicines or herbal medicine preparations (Mosihuzzanman and Choudhary, 2008., Ismail and Siddique, 2010., Kunle *et al.*, 2012). Herbal extracts made directly from crude herb material show substantial variation in composition, quality, and therapeutic effects. Standardized extracts are high-quality extracts containing consistent levels of specified compounds, and they are subjected to rigorous quality controls during all phases of the growing, harvesting, and manufacturing processes (Mosihuzzanman and Choudhary, 2008., Ismail and Siddique, 2010., Kunle *et al.*, 2012). When the active principles are unknown, marker substances should be established for analytical purposes and standardization. Marker substances are chemically defined constituents of a herbal medicine that are important for the quality of the finished product (Mosihuzzanman and Choudhary, 2008., Ismail and Siddique, 2010., Kunle *et al.*, 2012). The chemical

markers chosen would also be the compounds that are responsible for the pharmacological effects in the body. There are two types of standardization namely, "true" standardization, which a definite phytochemical or group of constituents is known to have activity. Ginkgo with its 26% ginkgo flavones and 6% terpenes is a classic example. These products are highly concentrated and no longer represent the whole herb, and are now considered as Phytopharmaceuticals (Mosihuzzanman and Choudhary, 2008., Ismail and Siddique, 2010., Kunle *et al.,* 2012). The other type of standardization is based on the guarantee of the manufacturers for the presence of a certain percentage of marker compounds which are not indicators of therapeutic activity or quality of the herb. However, the active constituents of *Senna didymobotrya* (Gisesa, 2004), *Carissa spinarum* (Kokwaro, 2009., Tolo *et al.,* 2010), *Leonotis nepetifolia* (Kokwaro, 2009), *Bidens pilosa (*Kokwaro, 2009), *Warburgia ugandensis* (Karinge, 2006), *Urtica dioica (*Kokwaro, 2009) and *Physalis peruviana* (Kokwaro, 2009), herbs are not known. In such cases, products may be standardized on the content of certain bioactive compounds.

2.2.2 The need for standardization

The impediment in herbal medicine acceptance is the lack of standard quality control profile. The quality of herbal medicine that is, the profile of the constituents in the final product has implications in efficacy and safety (Mosihuzzanman and Choudhary, 2008., Ismail and Siddique, 2010., Kunle *et al.,* 2012). However, due to the complex nature and inherent variability of the constituents of herb-based medicines, it is difficult to establish quality control parameter. Furthermore, the constituents responsible for the claimed therapeutic effects are frequently unknown

or only partly explained (Mosihuzzanman and Choudhary, 2008., Ismail and Siddique, 2010., Kunle *et al.*, 2012). This is further complicated by the use of combination of herbal ingredients as being used in traditional practice. It is common to have as many as five different herbal ingredients in one product and any reference standard is lacking (Mosihuzzanman and Choudhary, 2008., Ismail and Siddique, 2010., Kunle *et al.*, 2012). These variations increase during storage and further processing. Hence for herbal drugs and products, standardization encompasses the entire field of study from cultivation of medicinal herbs to its clinical application.

2.2.3 Safety of herbal medicine

Herbal medicines are generally regarded as safe based on their long-standing use in various cultures (Bandaranayake *et al*, 2006). However, there are case reports of serious adverse events after administration of herbal products. In a lot of cases, the toxicity has been traced to contaminants and adulteration, but some of the plants used in herbal medicines can also be highly toxic (Mosihuzzanman and Choudhary, 2008., Balammal *et al*., 2012). As a whole, herbal medicines can have a risk of adverse effects. The toxic effects of herbal preparation may be attributed mainly to the following:

(i) Inherent toxicity of plant constituents and ingredients
(ii) Manufacturing malpractice and contamination

2.2.4 Contaminants in herbal medicine

Potential contaminants of herbal medicines include microorganisms, microbial toxins, pesticides, fumigation agents, radioactivity, and the presence of toxic compounds of toxic metals (Barnes *et al.,* 2007., Mosihuzzanman and Choudhary, 2008., Ismail and Siddique, 2010., Kunle *et al.,* 2012). Herbs may be contaminated easily during growing and processing. So it is important to have a good quality control for herbal medicines in order to protect consumers from contamination (Fuh *et al.,* 2003**)**. The heavy metals such as Hg, Pb, Cu, Cd, and As, are widely considered as potential contaminants in our environment due to their toxicities to human. Contamination by toxic metals can either be accidental or intentional. Contamination by heavy metals such as Hg, Pb, Cu, Cd, and Ar in herbal remedies can be attributed to many causes, including environmental pollution, and can pose clinically relevant dangers for the health of the user and should therefore be limited (Khan, *et al.,*2008**).** The potential intake of the toxic metal can be estimated on the basis of the level of its presence in the product and the recommended or estimated dosage of the product by atomic absorption spectrophotometry (AAS) method (Khan, *et al.,* 2008**).** After collection and transformation of herbs into dosage form the heavy metals confined in plants finally enter the human body and may disturb the normal functions of central nervous system, liver, lungs, heart, kidney and brain, leading to hypertension, abdominal pain, skin eruptions, intestinal ulcer and different types of cancers (Khan *et al.,* 2008). WHO recommends that medicinal plants which form the raw materials for the finished products may be checked for the presence of heavy metals (Jabeen *et al.,* 2010), further it regulates maximum permissible limits

of toxic metals like As, Cd and Pb, which amount to 1.0, 0.3 and 10 ppm, respectively (Jabeen *et al*., 2010).

2.2.5 Overview of standardization of herb extracts

A standard extracts is a measurable marker compound that is extracted from the herb (Barnes et al., 2007., Mosihuzzanman and Choudhary, 2008., Ismail and Siddique, 2010., Kunle *et al.*, 2012). This marker may be an active constituent or known marker compounds present in herbal product. Primarily finger print profile is best choice for standardization followed by determination of maximum number of known active constituents or determination of inactive markers. Chemical standardization often involves chemical identification by spectroscopic or chromatographic fingerprint and chemical assay with known therapeutic activity. Tools for standardization of herbal extract are UV-VS, FT-IR, HPLC, GC-MS, LC-MS and LC-UV-MS-NMR. (Ismail and Siddique, 2010). Thin layer chromatography (TLC) is frequently used for the analysis of medicinal herb raw materials. The advantages of using TLC to construct the fingerprints of medicinal herbs are its simplicity, versatility, high velocity, specific sensitivity and simple sample preparation. Thus, TLC is a convenient method of determining the quality and possible adulteration of herbal products (Balammal *et al*., 2012).

2.2.6 Factors that affect standardization of herbal products.

Ismail and Siddique (2010) provided a review of factors that affect standardization of herbs. Extracts contains complicated mixture of organic chemicals such as secondary metabolites, which creates confusion in determining the biological activity in humans. In processing of herb, heating or boiling may alter the dissolution rate or

even the pharmacological activity of organic constituents. Environment factors, such as soil, altitude, seasonal variations, atmospheric humidity, rainfall pattern, shade, dew, and frost may affect the level of components in a herbal product. Adulteration in any form causes several severe problems (Ismail and Siddique, 2010).

2.2.7 Protocols for standardization of herbs

Once the identity of a herb is established, the next step is phytochemical screening, which involves extraction, bioassays, purification, and characterization of the active constituents (Table 2.1).

Table 2.1: Protocols for standardization of herbs.

Parameters	Specifications
1. Authentication	The first stage was to identify the plant species with Latin binomial name.
2. Physical parameters	Physical tests include pH, hardness, sedimentation, and ash value.
3. Chromatographic and spectroscopic evaluation	Sophisticated modern techniques of standardization such as UV–vis spectrophotometry, TLC, HPTLC , HPLC, NMR, near infrared spectroscopy provides quantitative and semiquantitative information about the main active constituents or marker compounds present in the crude drug or herbal products. Markers play an important role in fingerprinting of herbs.
4.Microbiological parameters	Microbiological contamination can be measured according to methods described in the *Romanian Pharmacopoeia* , as well as in the *British Pharmacopoeia*. Microbiological analysis includes analysis of limits of *E. coli* and molds, total viable aerobic count, total enteriobacteria and their count, aflatoxin analysis.

5.Pesticide residue analysis	Standard limits of pesticides have been set by WHO and FAO (Food and Agricultural Organization). Some common pesticides that cause harm to human beings, such as DDT, BHC, toxaphene, and aldrin, should be analyzed.
6. Heavy metal analysis	Toxic metals such as Cu, Zn, Mn, Fe, and particularly Cd, As, Pb and Hg should be analyzed.

Protocols for standardization of herbs: (Mosihuzzanman and Choudhary, 2008).

2.3 Traditional use of herbal medicine

In comparison with modern medicine, herbal medicines cost less, are more often used to treat chronic diseases, and the occurrence of undesirable side effects seems to be less frequent (Bandaranayake *et al.*, 2006). Several important factors have contributed to the growth of the use of traditional herbs worldwide, among which the following may be mentioned: preference of consumers for natural therapies; concern regarding undesirable side effects of modern medicines and the belief that herbal drugs are free from side effects; great interest in alternative medicines; preference of populations for preventive medicine due to increasing population age; the belief that herbal medicines might be of effective benefit in the treatment of certain diseases where conventional therapies and medicines have proven to be inadequate; tendency towards self-medication; improvement in quality, proof of efficacy and safety of herbal medicines and high cost of synthetic medicines (Barnes et al., 2007., Mosihuzzanman and Choudhary, 2008., Ismail and Siddique, 2010., Kunle *et al.*, 2012). Herbal medicine is the oldest form of health care known to humanity and has

been used in all cultures throughout history (Bandaranayake *et al*., 2006). Herbal medicine can broadly be classified into a few basic systems:

(i)Ayurvedic herbalism, which originated in India more than 5000 years ago and was practiced in neighbouring countries such as Sri Lanka.

(ii) Chinese herbalism, which is a part of traditional oriental medicine.

(iii) African herbalism.

(iv) Western herbalism, which originated from Greece and Rome and then spread to Europe and North and South America.

Chinese and Ayurvedic herbalism have developed into highly sophisticated systems of diagnosis and treatment over the centuries. Both have a long and impressive history of effectiveness. Medicinal plants are distributed worldwide, but they are most abundant in tropical countries.

2. 4.0 Chemical constituents of herbs

Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties (Doss and Anand, 2012). The plants produce these chemicals to protect themselves but recent research demonstrates that they can protect humans and animals against diseases (Doss and Anand, 2012). A number of phytochemical are known, some of which include: alkaloids, saponins, flavonoids, tannins, glycosides, anthraquinones, steroids and terpenoids (Venkatesh *et al* 2011., Doss and Anand, 2012). They do not only protect the plants but have enormous physiological activities in humans and animals. These include cancer prevention, antibacterial, antifungal, antioxidative, hormonal action, enzyme stimulation and many more (Venkataswamy *et al.,* 2010**.,** Venkatesh *et al.,* 2011., Doss and Anand, 2012). The following

subsections outline some of the biochemical properties (including the physiological activities) of these phytochemicals.

2. 4 .1 Flavonoids

Flavonoids represent a very wide group of water-soluble derivatives of the basic compound (Figure 1.1). They are polymeric compounds possessing fifteen carbon atoms, with two benzene rings joined by a linear three-carbon (3-C) chain as its basic structure; the variation is the state of oxidation of the connecting 3-C moiety, which determines the properties and class of each compound. They are generally known to be physiologically active and these include: antioxidant, antimicrobial, anticancer, vasoprotective, anti-inflammatory, anti-viral, antithrombitic and antiallergenic effects activities (Shirwaikar *et al.,* 2003., Ahmad *et al.,* 2006., Venkataswamy *et al.,* 2010**.,** Venkatesh *et al.,* 2011). Flavonoid has been referred to as nature's biological response modifiers because of strong experimental evidence of their inherent ability to modify the body's reaction to allergen, virus and carcinogens (Ogbonnnia *et al.,* 2008.,). They show anti-allergic, anti-inflammatory, anti-microbial and anti-cancer activity (Donald *et al*.,2005., Nisar *et al.,* 2011).

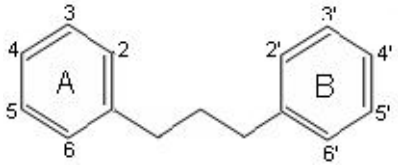


Figure 1.1: General structure of flavonoids

2. 4 .2 Alkaloids

Most of the known alkaloids contain a basic nitrogen atom (Figure 1.2). An alkaloid containing plant almost never contains one alkaloid but rather a whole range of closely related components (Donald *et al*.,2005). The term alkaloid is commonly applied to basic nitrogenous compounds that are physiologically active. They nearly contain their nitrogen as part of a heterocyclic system and are often quite complex in structure (Ahmad *et al.,* 2006). Alkaloids usually show specific pharmacological activity. Amongst the pharmacologically active ingredients found in plants, alkaloids are the most arguably important. Some alkaloids have further use; quinine (commonly found in *chinchona* spp), for example, is used in the food industry for bitter flavouring and as an antimalaria drug (Ngbede *et al*., 2008., Ogbonnia *et al.*, 2008). They are known to have antioxidant and antimicrobial activities (Ngbede *et al.,* 2008., Ogbonnia *et al.*, 2008).

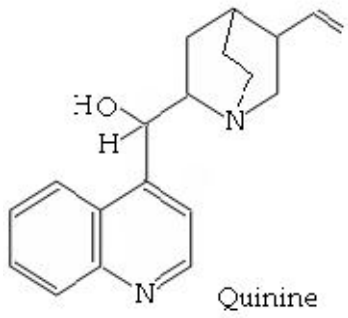


Figure 1. 2: Shows an example of alkaloid

2. 4 .3 Saponins

Saponins are special glycosides with distinctive foaming characteristics; they form froth or foam when shaken with water (Figure 1.3). They can be said to be natural detergents found in plants because they contain both water-soluble and lipid-soluble components. They consist of a lipid-soluble nucleus, having either a steroid or - 16 - triterpenoids saponins with one or more side chain of water-soluble carbohydrate (Donald *et al.*, 2005., Ahmad *et al.*, 2006). Their physiological action depends on the fact they break up the red blood cells – Haemolysis. Saponins have a bitter and acidic taste. Saponins are thought to act as resistant compounds against plants pathogens and are membrane active agents. The properties of saponins include antioxidant effect, direct and select cytotoxicity of cancer cell, immune-modulation, acid and neutral sterol metabolism and regulation of all proliferation (Doss and Anand, 2012., Ngbede *et al.*, 2008., Ogbonnia *et al.*, 2008). Their natural tendency to inhibit the growth of microbes may prove to be especially useful for treating those difficult to control fungal and yeast infections. Among the chemical properties of saponins, their polarity, hydrophobicity and nature of the reactive groups seem to be important determinants of their biological properties. They are known to have antibacterial, antitumour and cytotoxic, fungucidal and molluscicidal activities (Ngbede *et al.*, 2008., Ogbonnia *et al.*, 2008). Saponin is used as mild detergents and in intracellular histochemistry staining to allow antibody access to intracellular proteins. In medicine, it is used in hypercholestrolaemia, hyperglycaemia, antioxidant, anti-cancer, and anti-inflammatory and weight loss. It is also known to have anti-fungal properties (Ahmed *et al.*, 2007., Ngbede *et al.*, 2008).

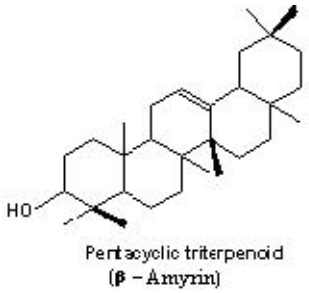


Figure 1.3: Structural type of sapogenin

2. 4 .4 Tannins

Chemically, tannins are complex substances, which usually occur as mixtures of polyphenols that are difficult to separate because they do not crystallize (Figure 1.4). Complex tannins are generally considered to have arisen from simple polyphenols by polymerization (Donald *et al.*, 2005., Ahmad *et al.*, 2006). Tannins are one of the many types of secondary compounds found in plants. They are oligomeric compounds with multiple structure units with free phenolic groups. Their molecular weight ranges from 500 to 20,000 or more. They are soluble in water, with exception of some high molecular weight structures, and are able to bind proteins forming insoluble or soluble tannin-protein complexes. They have astringent property and are used as astringents, healing agent for gonorrhoea, burns and piles (Argal and Pathak, 2006., Ngbede *et al*., 2008., Doss and Anand, 2012). Tannins solution is used in the putrefaction of animal hides by converting them to leather. Many extracts with high tannin content are used to promote wound healing (Doss and Anand, 2012). This is achieved through encouragement of the formation of new tissues under the leathery layer formed on broken mucosal surface by the action of tannins. Prolonged

utilization of certain tannins rich plant material may be hazardous owing to their carcinogenic potential (Doss and Anand, 2012). Tannins were reported to exhibited antiviral, antibacterial and anti-tumor activities (Argal and Pathak, 2006., Ngbede *et al.*, 2008., Doss and Anand, 2012). It was also reported that certain tannins are able to inhibit HIV replication selectively and is also used as diuretic (Ngbede *et al.*, 2008., Ogbonnia *et al.*, 2008., Doss and Anand, 2012). *Ellagitannins*, the phenolic groups consist of hexahydroxydiphenic acid, which spontaneously dehydrates to the lactone form, ellagic acid (Figure 1.4). Its molecular weight ranges from 2000 to 5000.

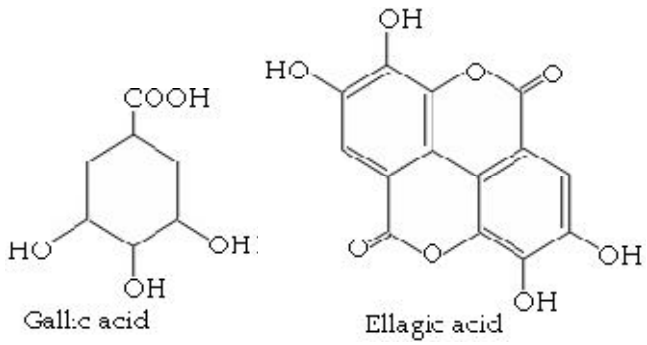


Figure 1.4: Gallotannins and Ellagitannins:

2. 4 .5 Coumarins

Coumarins are phytochemical widely distributed in several plants (Figure 1.5), including: *Carissa spinarum, Urtica dioica, Warburgia ugandensis, Senna didymobotrya, Physalis peruviana, Bidens Pilosa, Leonotis nepetifolia* and *Toddalia asiatica*. They are unsaturated aromatic lactones, which occur either in the Free State or combined with the sugar glucose (Coumarin glycoside).

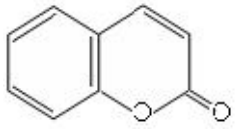


Figure 1.5: General structure of coumarins

They possess characteristic odour and have aromatic bitter taste (Donald *et al.*, 2005., Ahmad *et al.*, 2006). Coumarins have blood-thinning, anti-fungal and anti-tumour activities (Ahmed *et al.*, 2007., Anpin Raja *et al.*, 2011). They are known to increase the blood flow in the veins and to decrease capillary permeability. Coumarin can be toxic when used at high doses for a long period. Coumarin seems to work as a pesticide in the plants that produce it.

2. 4 .6 Anthraquinones

Anthraquinones occur in various types of plant materials and may occur as free anthraquinones, or as glycosides (Figure 1.6). Natural products have also been found to contain reduced derivatives of anthraquinones. They are oxanthrones, anthranols and anthrones and compounds formed by union of two conthrone molecules (Donald *et al.*, 2005., Ahmad *et al.*, 2006).

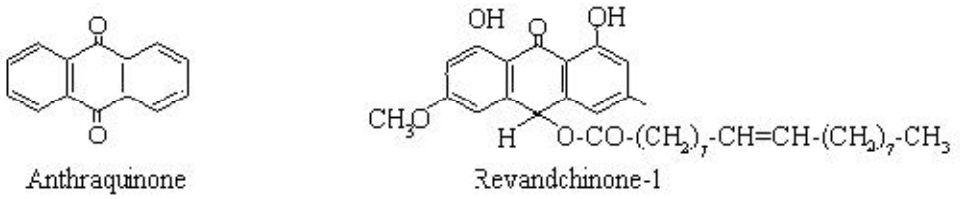


Figure 1.6: Anthraquinone and an oxanthrone

They are usually soluble in hot water and dilute alcohol and are known to have antibacterial and antifungal properties (Ngbede *et al.*, 2008., Ogbonnia *et al.*, 2008).

2. 4 .7 Glycosides

Glycosides are compounds that yield one or more sugars among the products of hydrolysis (Figure 1.7). Glycosides can be considered as sugar-ethers consisting of non-sugar and a component sugar in the same molecule (Donald *et al*., 2005., Ahmad *et al.*, 2006). The sugar and the non-sugar components are known as *aglycone* and *glycone* respectively (Figure 1.7).

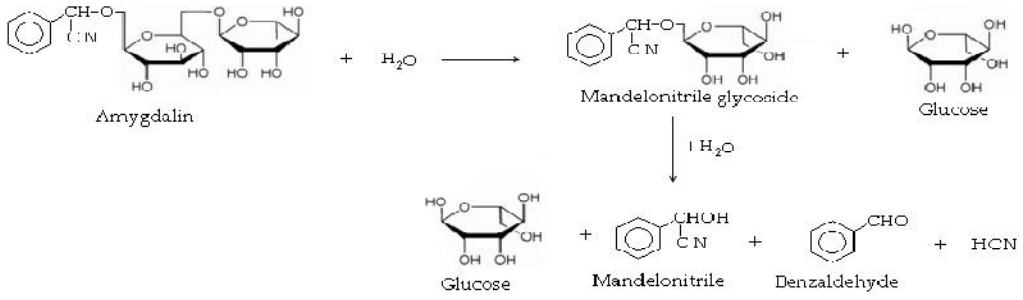


Figure 1.7: Hydrolysis of Amygdalin

2. 4 .8 Cyanogenic glycosides

The aglycone of this group contains a cyanide group, and the glycoside can release the poisonous hydrogen cyanide if acted upon by some enzyme. They yield hydrocyanic acid on hydrolysis (Donald *et al*., 2005., Ahmad *et al*., 2006). The group is mostly represented by amygdalin (Figure 1.8), found in large quantities in bitter almonds.

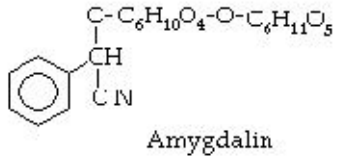


Figure 1.8: Cyanogenic glycosides

2. 4 .9 Isothiocyanate glycosides

The aglycones of this group are isothiocyanates (Figure 1. 9). These aglycones may be either aliphatic or aromatic derivatives (Donald *et al*., 2005., Ahmad *et al*., 2006). Principal among these are sinigrin, sinalbin and gluconapin (Figure 1.9). Most of these glycosides are known to have anticancer properties (Ngbede *et al*., 2008., Ogbonnia *et al*., 2008). Sinalbin, for instance, is believed to prevent cancer of the colon if foods containing it are eaten regularly (Doss and Anand, 2012).

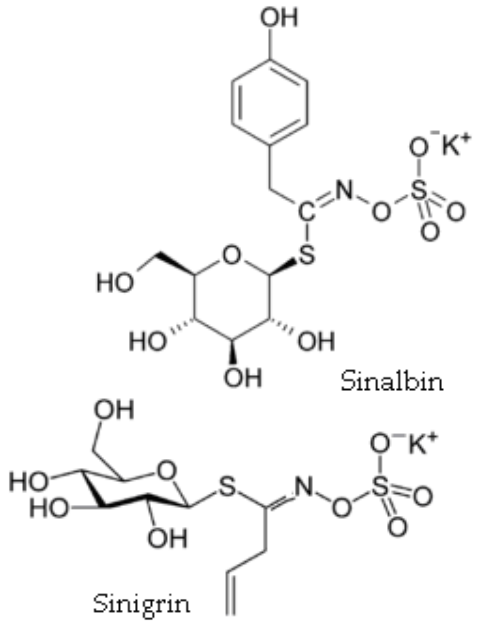
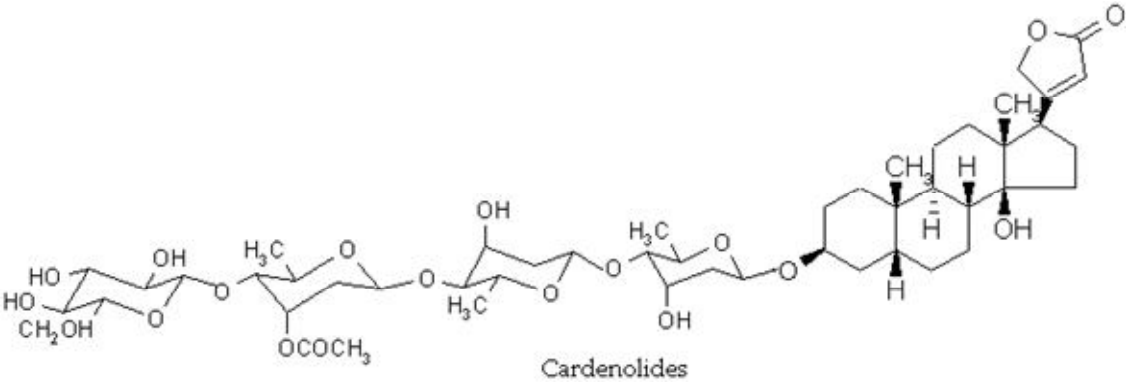


Figure 1. 9: Isothiocyanate glycosides

2. 5.0 Cardiac Glycosides

The aglycone is part of a steroidal nucleus (Figure 2.0). Two classes have been observed in nature - the cardenolides and the bufadienolides. The cardenolides have an unsaturated butyrolactone ring while the bufadienolides have a α-pyrone ring (Donald *et al.*, 2005., Ahmad *et al.*, 2006). .



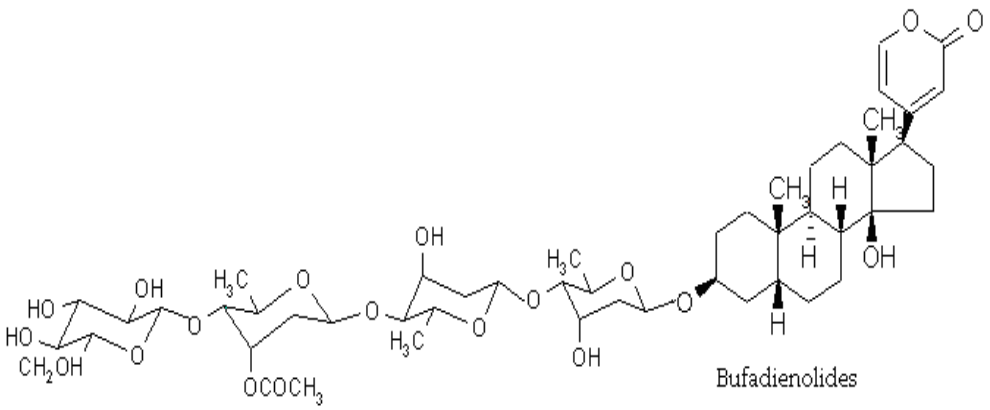


Figure 2.0: Classes of cardiac glycosides

Some common examples include: digitoxin, digoxin and gitoxin (Figure 2.1).

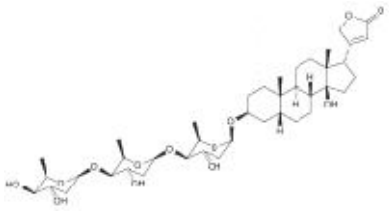


Figure 2.1: Digitoxin

The cardiac glycosides are an important class of naturally occurring drugs whose actions include both beneficial and toxic effects on the heart. Plants containing cardiac steroids have been used as poisons and heart drugs at least since 1500 B.C. Throughout history these plants or their extracts have been variously used as arrow poisons, emetics, diuretics, and heart tonics (Doss and Anand, 2012). Cardiac steroids are widely used in the modern treatment of congestive heart failure and for treatment of arterial fibrillation and flutter (Doss and Anand, 2012). Cardiac glycosides on the other hand are known to work by inhibiting the Na+ / K+ pump (Doss and Anand, 2012).. This causes an increase in the level of sodium ions in the myocytes which then lead to a rise in the level of calcium ion. This inhibition

increase the amount of Ca^{2+} ions available for contraction of the heart muscle which improves cardiac output and reduces distention of heart; thus are used in the treatment of congestive heart failure and cardiac arrhythmia (Ngbede *et al*., 2008., Ogbonnia *et al.*, 2008). .

2.5.1 Terpenoids and Steroids

Terpenes are derived biosynthetically from units of isoprene, which has a molecular formula of C_5H_8 (Ahmad *et al.*, 2006., Donald *et al*., 2005). The basic molecular formulae of terpenes, then, are multiples of it, $(C_5H_8)_n$, where n is the number of linked isoprene units (Figure 2.2).

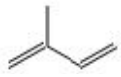


Figure 2.2: Isoprene unit

The isoprene units may be linked together "head-to-tail" to form linear chains or they may be arranged to form rings.

Terpenes are classified into – monoterpenes, sesquiterpenes, diterpenes, sesterpenes, triterpenes, tetraterpenes, and rubber – depending upon the total number of carbon atoms or isoprene units in the molecule.

Monoterpenes consist of two isoprene units and have the molecular formular $C_{10}H_{16}$. They may be linear (acyclic) or may contain rings (Donald *et al*., 2005., Ahmad *et al.*, 2006). Monoterpenes include: linalol, nerol, geraniol, myrcene, ocimene, alloocimene, menthol, limonene (Figure 2.3).

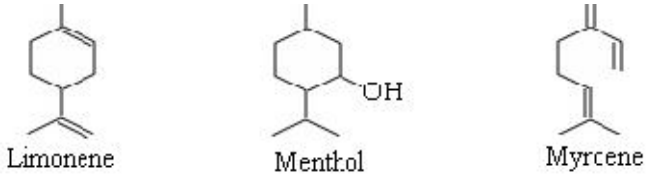


Figure 2.3: Monoterpenes

Sesquiterpenes are fifteen (15) carbon compounds derived by the assembly of three (3) isoprenoids units and are found mainly in higher plants but also in several invertebrates, with a molecular formula of $C_{15}H_{24}$. They present several acyclic, mono-. di-, tri- and tetracyclic systems. Some of the natural sesquiterpenes are: farnesoic acid, methyl farnesoate, juvenile hormone III, farnesol and nerolidol (Figure 2.4).

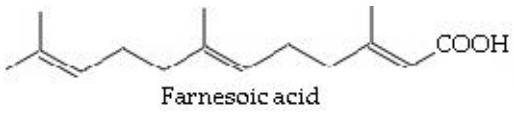


Figure 2.4: Sesquiterpene

Sesterpenes are also derived from geranyl pyrophosphate and have 25 carbon atoms. They were isolated from insect protective waxes and from fungal sources. They include gascardic acid, geranylfarnesol and pimeric acid (Figure 2.5).

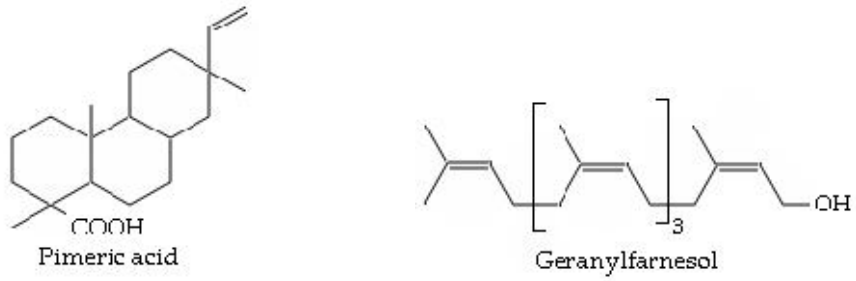


Figure 2.5: Examples of sesterpenes

Triterpenes form a large group of natural substances which include steroids and consequently sterols. They are made up of six isoprene units having 30 carbon atoms. Squalene is the immediate biological precursor of all triterpenoids (Ngbede *et al.*, 2008., Anpin Raja *et al.*, 2011). Other examples are cholesterol and lanosterol (Figure 2.6).

Figure 2.6: Triterpenes

Steroids are modified triterpenoids which are also derived from squalene by cyclisation, unsaturation and substitution. The nucleus of all steroids is the tetracyclic C_{17} hydrocarbon 1, 2 cyclopentanoperhydrophenanthrene (gonane or sterane) substituted by methyl groups at C_{10} and C_{13}, as well as alkyl side-chain at C_{17}. Steroids may possess a nucleus derived from the former one by one or more C-C

bond scissions or ring expansion or contractions (Donald *et al.*, 2005., Ahmad *et al.*, 2006). The diversity of biological activities of steroids includes the development and control of the reproductive tract in humans, the moulting in insect (ecdysis) and the induction of sexual reproduction in aquatic fungi (antheridiol). In addition steroid contributes to a varied range of therapeutic applications such as cardiotonics (digitoxin), Vitamin D precursors (ergosterol), oral contraceptive agents (some synthetic estrogens and progestins), anti-inflammatory agents (corticosteroids) and anabolic agent (androgens) (Doss and Anand, 2012). Plant steroids are known to be important for their cardiotonic activities, posses insecticidal and anti-microbial properties (Ngbede *et al.*, 2008., Anpin Raja *et al.*, 2011). They are also used in nutrition, herbal medicine and cosmetics (Anpin Raja *et al.*, 2011).

2.5.2 Carotenoids

Carotenoids are a class of yellow, red or red natural fat-soluble pigments (lipochromic pigments) found principally in plants, algae, and photosynthetic bacteria, where they play a critical role in the photosynthetic process (Donald *et al.*, 2005., Ahmad *et al.*, 2006). They also occur in some non-photosynthetic bacteria, yeasts, and molds, where they may carry out a protective function against damage by light and oxygen. In animal organism, carotenoids are either dissolved in fats or combined with protein in the aqueous. In higher plants, the carotenoids are found in leaves together with chlorophyll. Carotenoids are responsible for many of the red, orange, and yellow colour of plant leaves, fruits, and flowers, as well as the colours of some birds, insects, fish and crustaceans. The majority of carotenoids are derived

from a 40-carbon polyene chain, which could be considered the backbone of the molecule. This chain may be terminated by cyclic end-groups (rings) and may be complemented with oxygen-containing functional groups. Hydrocarbon carotenoids are known as carotenes, while oxygenated derivatives of the carotenes are known as xanthophylls. The carotenes which are soluble in petroleum ether 28 are α-carotene, β-carotene and γ-carotene (lycopene). These are shown below (Figure 2.7).

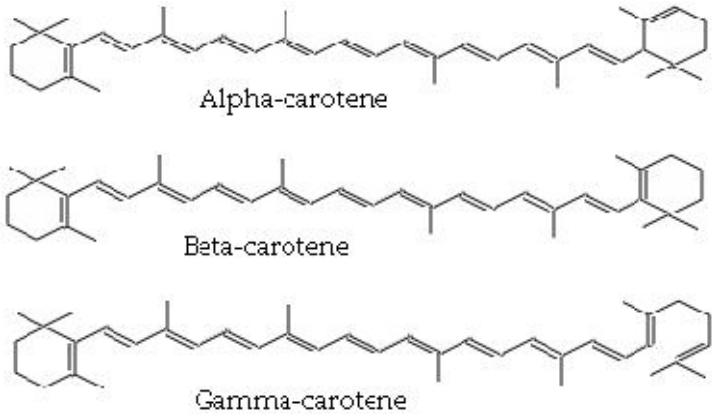


Figure 2.7: Some Carotenes

The xanthophylls, which are alcohols, aldehydes, ketones, epoxides, and acids, are soluble in ethanol. They comprise: hydroxylated Carotenoids (crytoxanthin, lutein, the major yellow pigment of marigold petals and zeaxanthin); Methoxylated Carotenoids, which are lycopene derivatives (e.g. rhodovibrin); Oxocarotenoids (capsanthin and rhodoxanthin); epoxycarotenoids, which exist in nature as 5, 6- and 5, 8-epoxides or both (examples, violaxanthin, flavoxanthin and luteochrom), and Carboxycarotenoids (bixin and crocetin) (Figure 2.8).

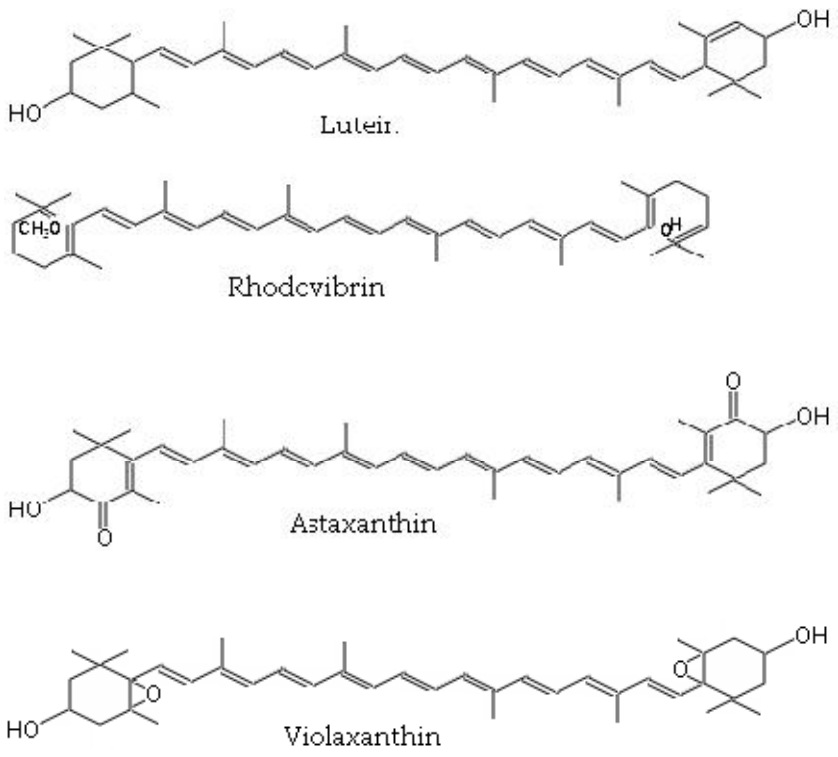


Figure 2.8: Structures of selected Xanthophylls

In human beings, carotenoids can serve several important functions. The nutritional role for carotenoids is their provitamin- A activity. Deficiency of vitamin- A is a major cause of premature death in developing nations, particularly among children. Vitamin A, which has many vital systemic functions in humans, can be produced within the body from certain carotenoids, notably beta-carotene. Carotenoids also play an important potential role in human health by acting as biological antioxidants, protecting cells and tissues from the damaging effects of free radicals and singlet oxygen (Donald *et al*., 2005., Ahmad *et al.,* 2006). Lycopene is particularly effective at quenching the destructive potential of singlet oxygen. Lutein and zeaxanthin, xanthophylls found in corn and in leafy greens such as kale and spinach, are believed

to function as protective antioxidants in the mascular region of the human retina 31 and hence improving vision. Astaxanthin, a xanthophyll found in salmon, shrimp, and other sea foods, is another naturally occurring xanthophyll with potent antioxidant properties. Other health benefits of carotenoids that may be related to their antioxidative potential include enhancement of immune system function, protection from sunburn, and inhibition of the development of certain types of cancers (Ngbede *et al*., 2008., Ogbonnia *et al*., 2008). They have also been shown to prevent colds and flu, support successful pregnancy, and ward off diabetes. Their antioxidant effects enable these compounds to play a crucial role in protecting plants against damage during photosynthesis

2.6.0 Bacterial contaminants of wounds

2.6.1 *Staphylococcus aureus*

Staphylococci are Gram-positive spherical bacteria that occur in microscopic clusters resembling grapes. They are found primarily on mammalian skin, including the anterior nares of humans but can also be found, although infrequently, on the other body sites such as mouth, throat, mammary glands and intestinal tract (Jagessar *et al.*, 2007). *Staphylococcus aureus* (yellow) are significant in their interactions with humans. *Staphylococcus aureus* causes superficial skin lesions such as boils, sties and furunculosis; more serious infections such as pneumonia, mastitis, phlebitis, meningitis, and urinary tract infections; and deep-seated infections, such causes a variety of suppurative (pus-forming) infections and toxinoses in humans as osteomyelitis and endocarditis (Jagessar *et al*., 2008). The *Staphylococcus aureus* is a major cause of hospital acquired (nosocomial) infection of surgical wounds and

infections associated with indwelling medical devices (Jagessar *et al.*, 2008., Srimathi *et al.*, 2011). The *Staphylococcus aureus* causes food poisoning by releasing enterotoxins into food, and toxic shock syndrome by release of super antigens into the blood stream (Jagessar *et al*., 2008).

2.6.2 *Escherichia coli*

Escherichia coli (*E. coli*) is one of many species of bacteria living in the lower intestines of mammals, known as gut flora (Srimathi *et al.*, 2011). When located in the large intestine, it actually assists with waste processing, vitamin K production, and food absorption (Jagessar *et al.*, 2008).

2.6.3 *Candida albicans*

Candida albicans is a diploid fungus (a form of yeast) and is a casual agent of opportunistic oral and genital infections in humans (Jagessar *et al.*, 2008).

2.6.4 Determination of microbial activities

Much of microbiology depends on the ability to grow and maintain micro organisms in the laboratory, and this is possible only if suitable culture media are available. Knowledge of a micro organisms normal habitat often is useful in selecting the appropriate culture medium because its nutrients requirements reflect its natural surroundings (Jagessar *et al*., 2008). This subsection reviews the nutritional requirements of micro organisms, the various culture media used and the antimicrobial activity tests undertaken in the laboratory.

2.6.5 Culture *media*

For any bacterium to be propagated for any purpose it is necessary to provide the appropriate biochemical and biophysical environment (Jagessar *et al*., 2008). The biochemical (nutritional) environment is made available as a culture medium, and depending upon the special needs of particular bacteria (as well as particular investigators) a large variety and types of culture media have been developed with different purposes and uses. Culture media may be classified into several categories depending on their composition or use. A *chemically-defined (synthetic) medium* is one in which the exact chemical composition is known. A *complex (undefined) medium* is one in which the exact chemical constitution of the medium is not known (Jagessar *et al*., 2008). Defined media are usually composed of pure biochemicals; complex media usually contain complex materials of biological origin such as blood or milk or yeast extract or beef extract, the exact chemical composition of which is obviously undetermined. A defined medium is a *minimal medium* if it provides only the exact nutrients (including any growth factors) needed by the organism for growth (Jagessar *et al*., 2008). Other concepts employed in the construction of culture media are the principles of selection and enrichment. A *selective medium* is one which has a component(s) added to it which will inhibit or prevent the growth of certain types or species of bacteria and/or promote the growth of desired species (Jagessar *et al*., 2008). One can also adjust the physical conditions of a culture medium, such as pH and temperature, to render it selective for organisms that are able to grow under these certain conditions.

2.6.6 Antimicrobial activity tests

Antimicrobial activity is measured in vitro in order to determine the potency of an antibacterial agent in solution, its concentration in body fluids and tissues, and to determine the sensitivity of a given microorganism to known concentration of the drug (Jagessar *et al.*, 2008). The determination of these quantities may be undertaken by one of the principal method –well diffusion (Jagessar *et al*., 2008). In the well diffusion method, a filter disk, a porous cup or a bottomless cylinder containing measured quantities of drug is placed on a solid medium which has been heavily seeded with the test organisms. After incubation, the diameter of the clear zone of inhibition surrounding the deposit of drug is taken as a measure of the inhibitory power of the drug against the particular test organism (Jagessar *et al*., 2008). This is normally referred to as minimum inhibitory concentration (MIC). An antibiotic's MIC is the smallest amount per unit of volume that will inhibit the growth of a certain organism.

2.6.7 Thin layer chromatography (TLC)

Thin layer chromatography is a simple, quick, and inexpensive procedure that gives the chemist a quick answer as to how many components are in a mixture (Patil *et al*., 2010). TLC is also used to support the identity of a compound in a mixture when the R_f of a compound is compared with the R_f of a known compound (Jagessar *et al*., 2008., Patil *et al*., 2010). However, with silica gel, the dominant interactive forces between the adsorbent and the materials to be separated are of the dipole-dipole type. Highly polar molecules interact fairly strongly with the polar Si-O bonds of these adsorbents and will tend to stick or adsorb onto the fine particles of the adsorbent

while weakly polar molecules are held less tightly (Jagessar *et al*., 2008., Patil *et al*., 2010). Weakly polar molecules thus generally tend to move through the adsorbent more rapidly than the polar species (Figure 2.9).

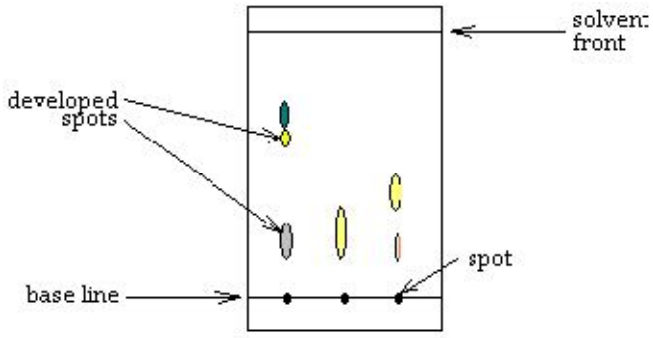


Figure 2.9: Chromatograph

The retention factor or R_f is defined as the distance travelled by the compound divided by the distance travelled by the solvent.

$$R_f = \frac{\text{distance travelled by the compound}}{\text{Distance travelled by the solvent front}}$$

"Relative R_f" means that the values are reported relative to a standard, or it means that you compare the R_f values of compounds run on the same plate at the time. The larger an R_f value of a compound, the larger the distance it travels on the TLC plate (Patil *et al*., 2010). . When comparing two different compounds run under identical chromatography conditions, the compound with the larger R_f is less polar because it interacts less strongly with the polar adsorbent on the TLC plate. The R_f can provide corroborative evidence as to the identity of a compound. If the identity

of a compound is suspected but not yet proven, an authentic sample of the compound, or standard, is spotted and run on a TLC plate side by side (or on top each other) with the same compound (Jagessar *et al.*, 2008., Patil *et al*., 2010). If two substances have different R_f values, they are definitely different compounds. This identity check must be performed on a single plate, because it is difficult to duplicate all the factors which influence R_f exactly from experiment to experiment.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

This subsection lists the chemicals, equipments and glass wares used in carrying out the various experiments

3.1.1 Chemicals

Conc. sulphuric acid(H_2SO_4), Conc. hydrochloric acid , 96% ethanol, Chloroform, Ammonia, Potassium iodide, Nutrient agar, Glacial acetic acid, Olive oil, Acetic anhydride, Acetone, Sodium hydroxide, Dragencloffs reagent, 2% H_2S0_4, Lead acetate, Potassium hydroxide 1% , 1M HCl , 1M H_2SO_4 , 50% HNO_3, Iodine , Ferric chloride (FCl_3) 5%

3.1.2 Equipments and glass wares

Bruch Rotavapour R-4000 Efficient, (Rotary evaporator), Atomic absorption spectrometry (AAS), a single beam Infrared spectrometer (Shimadzu FT-IR-8400), PH-meter (Hanna, HI8519). Filter papers (Whatman), Beakers (250ml, 600ml, 1L), Test tubes, Capillary tube , 50ml burette, 1ml pipette, 10ml dropping pipette, Petri dishes, 6 mm cork borer, Heating mantle, Electric oven, 250ml conical flask ,

3.2 Methods

3.2.1 Sample collection and preparation

The sample collection and preparation was done using standard procedures (Jagessar *et al*.,2008., Patil *et al*., 2010). Leaves of the *Carissa spinarum, Urtica dioica Warburgia ugandensis, Senna didymobotrya, Physalis peruviana, Bidens pilosa, Leonotis nepetifolia* and *Toddalia asiatica* were picked from each of the mentioned

herbs randomly and placed in labelled paper bags at the site in Kisii region, southwest Kenya. The verification of the herbal species was done by the Botanist; Egerton University. The leaves of the authenticated medicinal herbs were then transported from their site in Kisii region and air-dried at room temperature in the Chemistry Laboratory of Jomo Kenyatta University of Agriculture and Technology.

3.2.2 Extraction of the plant material

The extraction of the herb material was done using standard procedures (Patil *et al.*, 2010., Arya and Thakur, 2012). The powdered material 30g each of Carissa *spinarum, Urtica dioica, Warburgia ugandensis, Senna didymobotrya, Physalis peruviana, Bidens pilosa, Leonotis nepetifolia* and *Toddalia asiatica* was extracted with different solvent according to polarity and concentrated for further studies on rotary evaporator at $40^{\circ C}$. Solvent Polarity in ascending order: Hexane < Dichloromethane < Ethyl acetate < Ethanol

The extraction was carried out by soaking 30 g of each herb in 200 ml of each solvent, hexane, dichloromethane, ethyl acetate and ethanol at room temperature for 24 hours. The hexane extract was filtered through filter paper and a residue obtained was again soaked with hexane. The same process of extraction was repeated three times with each solvent and the combined filtrates were concentrated under vacuum at low temperature ($40^{\circ C}$) using rotary evaporator (Khan *et al.*, 2008). The concentrated extract of hexane, dichloromethane, ethyl acetate and ethanol were transferred to labelled bottles with known empty masses, sealed with aluminium foil and thin holes were made on it to allow the solvents to escape. The labelled hexane,

dichloromethane, ethyl acetate and ethanol extracts were air-dried at room temperature which allowed solvents to escape and solidified.

3.2.3 Standardization of herbal crude medicines – processes and procedures

According to (Mosihuzzanman and Choudhary, 2008., Ismail and Siddique, 2010., Kunle *et al.,* 2012), standardization and quality control of herbals is the process involved in the physicochemical evaluation of crude drug covering aspects, such as selection and handling of crude material, safety, efficacy and stability assessment of finished product, documentation of safety and risk based on experience, provision of product information to consumer and product promotion. Attention is normally paid to such quality indices such as:

1. Macro and microscopic examination: For Identification of right variety and search of adulterants.

2. Foreign organic matter: This involves removal of matter other than source plant to get the drug in pure form.

3. Ash values: These are criteria to judge the identity and purity of crude drug – Total ash, sulphated ash, water soluble ash and acid insoluble ash etc.

4. Moisture content: Checking moisture content helps reduce errors in the estimation of the actual weight of drug material. Low moisture suggests better stability against degradation of product.

5. Extractive values: These are indicative weights of the extractable chemical constituents of crude drug under different solvents environment.

6. Crude fibre: This helps to determine the woody material component, and it is a criterion for judging purity.

7. Qualitative chemical evaluation: This covers identification and characterization of crude drug with respect to phytochemical constituent. It employs different analytical technique to detect and isolate the active constituents. Phytochemical screening techniques involve botanical identification, extraction with suitable solvents, purification, and characterization of the active constituents of pharmaceutical importance.

8. Chromatographic examination: Include identification of crude drug based on the use of major chemical constituents as markers.

9. Quantitative chemical evaluation: To estimate the amount of the major classes of constituents.

10. Toxicological studies: This helps to determine the pesticide residues, potentially toxic elements, safety studies in animals and Microbial assay to establish the absence or presence of potentially harmful microorganisms.

3.3.4 Mass recovery

The mass recovery of each solid extract of hexane, dichloromethane, ethyl acetate and ethanol was obtained from the difference between the mass of bottle plus its contents and that of empty bottle. The most suitable solvent had the highest mass recovery. To determine the percentage yield of extracts from the various solvents, the averages of the masses of the various dried extracts were taken and expressed as percentages of the masses of the powdered air-dried samples taken.

3.3.5 Determination of pH levels

The powdered 1.0g of herbs was measured by an electrical balance and then transferred into 25 ml conical flask and 25 ml of distilled water was added and heated on hot plate to boiling. The aqueous herbal extracts were left to cool and then filtered. The pH levels of the herbs were measured by pH-meter (Hegazy, 2011**).**

3.3.6 Phytochemical screening

The phytochemical screening was performed for testing different chemical groups present in extracts (Arya and Thakur, 2012). Chemical tests were carried out on the aqueous leave extracts of *Carissa spinarum, Urtica dioica, Warburgia ugandensis, Senna didymobotrya, Physalis peruviana*, *Bidens Pilosa*, *Leonotis nepetifolia* and *Toddalia asiatica* using standard procedures to identify the constituents as described by (Egwaikhide and Gimba, 2007., Ngbede *et al.*, 2008., Ogbonnnia *et al.*, 2008., Arya and Thakur, 2012).

3.3.6.1Test for Cardiac glycosides

5.0 ml aqueous leave extract of each herb was added into 1ml of glacial acetic acid containing one drop of ferric chloride solution. This was then under layered with 1 ml of concentrated sulphuric acid (H_2SO_4). In this test a brown ring was observed at the interface in the aqueous extract tested for the cardiac glycoside indicating the presence of a de-oxysugar characteristic of cardenolides.

3.3.6.2 Test for Alkaloids

10 ml aqueous leave extract of each herb was warned with 2% H_2S0_4 for two minutes. It was filtered and few drops of Dragencloffs reagent were added. Orange red precipitate indicated the presence of alkaloids.

3.3.6.3 Test for Saponins

5 ml aqueous leave extract of each herb was mixed with 5ml of distilled water and then heated to boil. Frothing (appearance of creamy miss of small bubbles) shows the presence of saponins. The same aqueous leave extract sample with few drops of olive oil formed a soluble emulsion confirmed presence of saponins.

3.3.6.4 Test for Tannins

10 ml aqueous leave extract of each herb was heated on water bath. The mixture was filtered and ferric chloride was added to the filtrate. A dark green solution indicates the presence of tannins.

3.3.6.5 Test for Steroids and Terpenoids

5 ml aqueous leave extract of each herb was mixed with 2ml of chloroform ($CHCl_3$) and 3 ml concentrated H_2S0_4 was carefully added to form a layer. A reddish brown colouration of the interface was formed to indicate positive results for the presence of steroids and terpenoids.

3.3.6.6 Test for Flavonoids

5 ml aqueous leave extract of each herb was mixed with 2 ml diluted NaOH and HCl then shaken. A yellow solution that turns colourless indicated the presence of flavonoids.

3.3.6.7 Test for anthraquinones

10 ml aqueous leave extract of each herb was boiled with 2ml 10% HCl for few minutes in a water bath. It was filtered and allowed to cool. Equal volume of $CHCl_3$ was added to the filtrate. Few drops of 10% NH_3 were added to the mixture and heated. Formation of rose-pink or red colour indicated the presence of anthraquinones.

3.3.7 Determination of heavy metals

The Wet digestion and analysis was carried out on each herb of *Carissa spinarum, Urtica dioica, Warburgia ugandensis, Senna didymobotrya, Physalis peruviana, Bidens Pilosa, Leonotis nepetifolia* and *Toddalia asiatica* using standard procedures to identify the heavy metals as described by (Okalebo *et al.,* 2002., Khan *et al.,* 2008).

3.3.7.1 Wet digestion procedure

One gram of each selected medicinal herbs was digested with 5 ml of 16 M HNO_3 in the covered beakers to near dryness. It was necessary, another 5 ml portion of 16 M HNO_3 was further added each time until the sample was completely digested and a solution became clear. Five millilitres of 12 M HCl were then added to ensure complete digestion. After cooling to room temperature, the digested solutions were diluted to 100 ml with deionized water for lead, cadmium and chromium. The samples of each herb were analyzed using atomic absorption spectrometry (AAS) for, iron, chromium, copper, zinc, cobalt, manganese. nickel, lead, cadmium and mercury.

3.3. 7.2 Calibration of Equipment

Calibration of equipment was done using standard procedures to identify the heavy metals as described by (Khan *et al.,* 2008). Calibration standard of each metal under investigation was prepared by appropriate dilution of the stock solutions of Fe, Cr, Cu, Zn, Co, Mn, Ni, Pb, Cd and Hg. All chemicals used in the study were of analytical reagent grade (Khan *et al.,* 2008). The calibration standards were prepared as established for the used AAS, in the following range (Okalebo *et al.,* 2002., Khan *et al.,* 2008): Cr 0.5 and 2.5. ppm, Mn 0.5 and 5.0 ppm, Fe 0.5 and 2.5 ppm, Ni 2.0 and 10.0 ppm, Co 0.5 and 5.0 ppm, Cu 0.5 and 2.5.0 ppm, Zn 0.5 and 5.0 ppm, Cd 0.2 and 1.0 ppm, Hg 0.02 and 0.08 ppm, Pb 0.5 and 2.5 ppm.

3.3. 7.3 Preparation of blank solutions

The blank sample (H_2O) underwent the same digestion procedures as that of the samples.

3.3.7.4 Atomic absorption spectrometry (AAS) procedure for herbal sample analysis

The sample of each herb digested was analysis using atomic absorption spectrometry interfaced with computer. The samples and standards at different intervals were aspirated into an air acetylene flame and the concentration recorded in the computer (Okalebo *et al.,* 2002., Khan *et al.,* 2008).

3.3.8 Thin layer chromatography

After concentration of each extract of hexane, dichloromethane, ethyl acetate and ethanol from *Carissa spinarum, Urtica dioica, Warburgia ugandensis, Senna didymobotrya, Physalis peruviana, Bidens Pilosa, Leonotis nepetifolia* and *Toddalia asiatica*, identification of phytoconstituents was carried out by thin layer

chromatography using different detecting reagents (Patil *et al*., 2010., Arya and Thakur, 2012). The extract of each hexane, dichloromethane, ethyl acetate and ethanol was dissolved by using appropriate solvent in a concentration of 1 mg/ml and subjected for spotting. Silica gel G was used as a stationary phase and different solvent systems were used as mobile phase. Spots were detected by using both non-destructive and destructive visualisation techniques (Jagessar *et al*., 2008., Patil and Shettigar, 2010., Arya and Thakur, 2012). Non-Destructive technique involved use of UV light, iodine chamber and destructive technique involved use of spraying reagents (Anisaldehyde sulphuric acid). The extract of each hexane, dichloromethane, ethyl acetate and ethanol were applied with the help of micro capillary, just 2 cm above from the bottom. The spots were equally sized, dried and developed (Fig. 3.0).

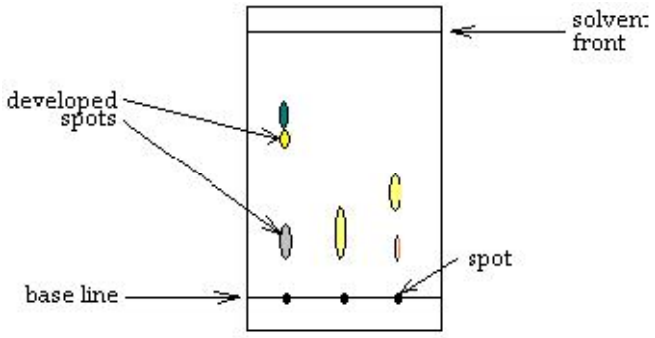


Figure 3.0: Chromatograph

The spot moved by solvents and shape of spots were also marked out with a pencil. The distances moved by the solvents and the spots were measured, in millimetres, with a rule. The retention factors of the samples were then determined and finally the R_f values were noted (Jagessar *et al*., 2008., Patil and Shettigar, 2010., Arya and Thakur, 2012).

$$\text{i.e. } R_f = \frac{\text{distance travelled by the compound}}{\text{distance travelled by the solvent}}$$

The same procedure was repeated for the crude extracts of dichloromethane, ethyl acetate and ethanol using the following solvent systems: hexane :dichloromethane, ethyl acetate: ethanol (1:1), hexane : dichloromethane (9:1), hexane: dichloromethane (3:1), dichloromethane: ethyl acetate (9:1), dichloromethane: ethyl acetate (3:1), dichloromethane: ethyl acetate (1:3), ethyl acetate: ethanol(9:1), ethyl acetate: ethanol(3:1), ethyl acetate: ethanol(1:1), ethyl acetate: ethanol(1:3).

3.3.9 Antimicrobial activity test

The crude extracts were tested against 24 hour broth cultures of *Escherichia coli, Staphylococcus aureus,* and *Candida albicans*. The various steps taken in determining the antimicrobial activities of the test samples are briefly described in the next subsections. The tests were performed at the Department of Food Science and Technology Laboratory of the JKUAT.

3.3. 9.1 Antibacterial and antifungal bioassays

Bioassay tests were performed on the herbal crude extracts in triplicate to ascertain their activity against *Escherichia coli, Staphylococcus aureus,* and *Candida albicans* (Jagessar *et al*., 2008). The pure solvents were used as control while the antibiotic disc containing compounds, erythromycin, chloramphenicol, minocyline and cotrimoazol was used as reference. In the test tube, 20ml nutrient agar was melted at 100^{o}C and stabilized at 45^{o}C for about 15 minutes. About 0.1ml inoculums were

added from culture tubes to the agar in the test tube by the use of a loop. The test tube containing the agar and the inoculums was then rolled in between the palms gently to mix the inoculums thoroughly with the agar (Jagessar *et al*., 2007). The loop was flamed before it was used each time. The content of the test tube was poured into a Petri dish and allowed to set. The Petri dishes were then labelled with the respective organism (inoculums) and date. By means of a 6 mm cork borer, four cups were bored, well separated and equidistant from each other in the agar. The cups were labelled with the three crude extracts. Each cup was filled with its corresponding 0.002mg/ml extract to about three-quarters full. The same procedure for each extract, pure solvent and antibiotic disc was repeated in triplicate. They were kept on a bench at room temperature for about 60 minutes (for the extracts to diffuse into the agar). The plates were then incubated aerobically at 37^{o}C and examined for any zone of inhibition after 24 hours. The reading was done against a dark background under reflected light. The diameters of the zones of growth of inhibition were measured with the help of Hi-Antibiotic zone scale (ranged 1cm to35 cm or 10mm to 400mm) from the underside of the covered plates for spots with inhibitions (Patil *et al*., 2010). The average of the diameters was taken (Jagessar *et al*., 2008). The actual zones were calculated by subtracting the diameter of the cups (6 mm) from the total zone of growth (Figure 3.1).

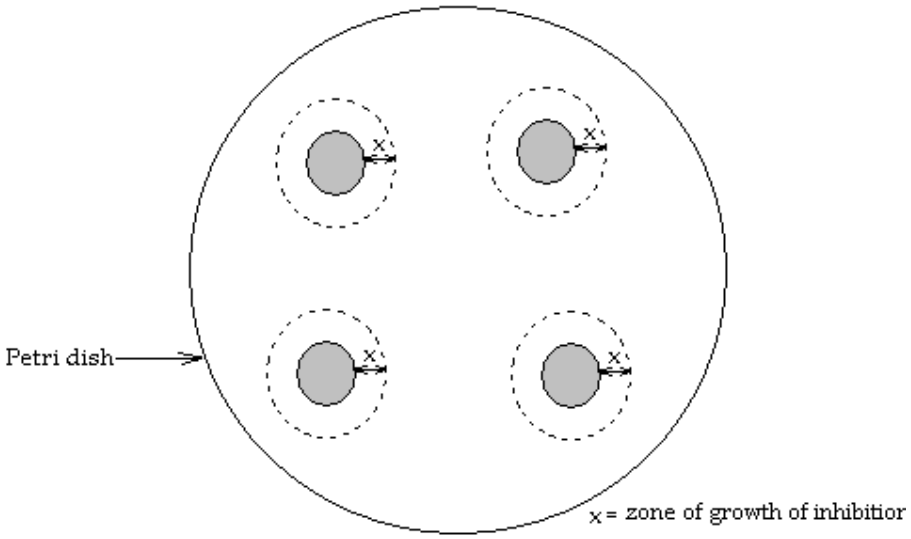


Figure 3.1: Measurement of zone of growth of inhibition

3.4.0 Infrared spectroscopy analysis

The sample extracts of each herb of *Carissa spinarum, Urtica dioica, Warburgia ugandensis, Senna didymobotrya, Physalis peruviana, Bidens Pilosa, Leonotis nepetifolia and Toddalia asiatica* were analysed using Fourier Transformed Infrared (FTIR) spectrophotometer model 8400 at the Chemistry Department of the JKUAT University. About 0.02 g of the air-dried samples was dissolved in hexane, dichloromethane, ethyl acetate and ethanol in different 10 ml volumetric flasks. A drop of each extract 0.002g/ml was applied on a sodium chloride cell to obtain a thin layer. The cell was mounted on the FTIR and scanned through the IR region. The functional groups in the herbs were determined (Skoog *et al*., 2007).

3.5 Data collected and analysis

3.5.1 Statistical analysis:

All experiments were done in triplicate. The mean and standard deviation of at least three experiments were determined and results were reported as mean values± standard deviation. Statistical analysis of the differences between mean values obtained for the experimental groups were done by analysis of variance (ANOVA) and paired t- test. P values of ≤ 0.05 were considered significant.

Null hypothesis 1: There is no bioactive compound present in the selected traditional herbs used in Kisii region to cure diabetes, malaria and pneumonia at significance level of 95%.

The statistical t-test was used for Statistical analysis of the above stated null hypothesis. The results were computed to obtain the calculated **t** -value which was compared with the critical **t**-value of the eight herbs. The null hypothesis (H_o) was retained for all eight herbs because the calculated **t** -value is greater than the

tabulated **t-**value. Therefore there is no significant phytochemical compound in the eight herbs at significance level of 95%.

Null hypothesis 2: There is no heavy metal present in the selected traditional herbs used in Kisii region to cure diabetes, malaria and pneumonia at significance level of 95%. The paired t-test was used for statistical analysis of the above null hypothesis of the following herbs: It is noted that the calculated t-value for Cr, Mn, Fe, Co, Ni, Cu, Zn, Cd, Hg and Pb is 0.39, 1.00, 0.96, 2.10, 0.04, 0.71, 2.10, 2, 34, 1.26 and 0.98 which is less than the tabulated **t** value of 2.3646. The null hypothesis (H_o) was retained. Therefore there is no significant heavy metal present in the selected herbs at 95% level of significance.

Data collected

The antibacterial activity of the selected eight herbs against gram-positive bacteria *Staphylococcus aureus* and gram-negative bacteria *Escherichia coli* were obtained by measuring the diameters of the inhibition zones and compared them with that of the control drug erythromycin, chloramphenicol, minocyline and cotrimoazol. Antibacterial activity was expressed as the mean zone of inhibition diameters (mm) produced by the herb extracts.

Data analysis

The null hypothesis being tested is that there is no biological activity displayed by the Compounds present in the selected traditional herbs used in Kisii region to treat diabetes, malaria and pneumonia diseases.

Results obtained in this study were expressed as mean inhibition diameter zone (mm) ± S.D of three replicates. The mean and the S.D of each herbal extract were

compared with that of the control drug erythromycin, chloramphenicol, minocyline and cotrimoazol, were used to compute the calculated t-value. Differences between the critical t-value and calculated t-values of the diameter of the inhibition zones of the herbal extracts on gram-positive *Staphylococcus aureus,* gram-negative bacteria *Escherichia coli* and fungus *Candida albicans* were computed. For all the eight herbs, the null hypothesis was retained because the calculated t-value was less than the critical t-value at $p \leq 0.05$.

Null hypothesis 3: There is no biological activity displayed by the compounds present in the selected traditional herbs used in Kisii region to cure diabetes, malaria and pneumonia at significance level of 95%. It was observed that the t-calculated value for biological activity displayed by selected herbs against *S. aureus, E. coli* and *C. albicans* in hexane, dichloromethane, ethyl acetate and ethanol extract was as follows: 1.5, 0 and 0 for hexane extract, 2, 2.2 and1.5 for dichloromethane extract, 0.7, 1.2 and 2 for ethyl acetate, 1.3, 1.7 and 1.5 for ethanol extract while **t** critical value was 2.37. The null hypothesis (H_o) was retained because the **t** calculated values was less than the critical t value. Therefore there is no significant biological activity displayed by the traditional herbs at 95% level of significance.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

In the following subsections, the results obtained for the pH levels, phytochemical screening, determination of heavy metal, thin layer chromatographic analyses, antibacterial and antifungal tests and infrared analyses are presented and discussed.

4.1 Percentage yield

The mass recovery of the hexane, dichloromethane, ethyl acetate and ethanol extracts was obtained and its percentage calculated (Table 3.1).

Table 3.1: Shows percentage of yield extract

plant	**Percentage of yield extract (%)**			
solvent	hexane	dichloromethane	Ethyl acetate	ethanol
Carissa spinarum	4.5	2.6	0.81	10.8
Urtica dioica	0.8	2.2	0.67	1.82
Warburgia ugandensis	1.94	3.65	1.66	3.69
Senna didmobotrya	3.2	3.32	2.1	5.0
Physalis peruviana	1.82	2.5	2.05	5.75
Bidens pilosa	3.5	0.93	2.21	10.71
Leonotis nepetifolia	1.1	2.75	0.96	5.0
Toddalia asiatica	2.33	1.74	1.67	6.22

Results obtained showed that the extract yields of ethanol recorded better yield than any other suitable solvents used. The differences in the extract yields from the extracted herbal materials in the present analysis might be attributed to the different

availability of extractable components, resulting from the varied chemical composition of plants.

4.2 pH levels of aqueous extract

Results obtained indicated that the pH levels of the aqueous extract of eight herbs ranged between 5.27 to 7.50 (Table 3.2).

Table 3.2: Shows the pH levels of herbs

Plant samples	pH
Carissa spinarum	5.90
Urtica dioica	7.50
Warburgia ugandensis	6.03
Senna didymobotrya	5.27
Physalis peruviana	5.67
Bidens pilosa	5.99
Leonotis nepetifolia	5.94
Toddalia asiatica	5.64
Distilled water	7.00

The herb *Urtica dioica* recorded highest pH level while *Senna didymobotrya* recorded lowest pH level. The pH was found to be in the acceptable range of 4.0 to 7.5 for medicinal herbs (Vaikosen and Alade, 2011., Hegazy, 2011).

4. 3 Phytochemical screening

The preliminary phytochemical screening results of *Carissa spinarum, Urtica dioica, Warburgia ugandensis, Senna didymobotrya, Physalis peruviana, Bidens Pilosa, Leonotis nepetifolia* and *Toddalia asiatica* aqueous extracts, showed the presence of various bioactive secondary metabolites constituents (Table 3.3).

Table 3.3: Phytochemical screening of aqueous extracts.

Plant	**Phytochemical constituents**						
	cardiac glycoside	**alkaloid**	**saponin**	**tannin**	**steroid**	**flavonoid**	**anthraquinone**
Carissa spinarum	+	-	+	+	+	+	+
Urtica dioica	-	-	+	+	+	+	-
Warburgia ugandensis	-	+	+	+	+	+	-
Senna didymobotrya	+	-	+	+	+	+	-
Physalis peruviana	+	+	+	+	+	+	-
Bidens pilosa	+	-	+	+	+	+	+
Leonotis nepetifolia	+	-	+	+	+	+	-
Toddalia asiatica	-	-	+	+	+	+	-

Key: (+) = indicate present, (-) = indicate absent.

Results obtained showed that the phytochemicals present were saponin, tannin, flavonoid, steroid and terpenoid, in all the eight herbs analysed (Nabwami *et al*., 2007., Ashafa and Afolayan, 2009., Raymond and Jagessar, 2010., Hegde and Joshi, 2010., Ibrahim *et al.,* 2010., Khare *et al.,* 2012., Rose and Prasad, 2013., Veerabadran *et al.,* 2013). However, alkaloid and anthraquinone was present in two herbs while cardiac glycoside was found to be present in five herbs (Rose and Prasad, 2013., Veerabadran *et al*., 2013). The selected eight herbs have bioactive compounds which are responsible for their medicinal value.

4.4 Levels of heavy metal

The analysed medicinal herbs revealed that the heavy metals present in all the herbs are iron, chromium, copper, zinc, cobalt, manganese, nickel, lead, cadmium and mercury (Table 3.4).

Table 3.4: Levels of heavy metal.

Plant	**Metal Concentration (ppm) (mean ±S.D)**									
	Cr	Mn	Fe	Co	Ni	Cu	Zn	Cd	Hg	Pb
Carissa spinarum	0.73±0.2	17.3± 0.2	3.87±0.6	3.87±0.4	0.59±0.4	1.04±0.2	1.51±0.1	0.035±0.01	0.0024±0.1	0.25±0.1
Urtica dioica	0.57±0.1	7.25±0.1	4.03±0.3	4.03±0.6	1.16±0.3	0.83±0.03	1.67±0.1	0.21±0.01	0.00453±0.1	0.27±0.0
Warburgia ugandensis	1.52±0.8	15.01±0.1	0.97±0.2	0.97±0.3	0.83±0.1	0.31±0.1	1.14±0.0	0.04±0.02	0.00265±0.1	0.38±0.1
Senna didymobotrya	0.75±0.2	3.25±0.6	4.07±0.5	4.07±0.2	0.84±0.1	0.96±0.3	1.16±0.0	0.04±0.01	0.00269±0.2	0.33±0.1
Physalis peruviana	2.04±0.7	6.06±0.1	3.17±0.2	3.17±0.1	1.03±0.4	1.44±0.2	0.99±0.1	0.06±0.0	0.00572±0.2	0.41±0.3
Bidens pilosa	0.98±0.8	11.15±0.2	2.9±0.4	2.9±0.4	1.6±0.2	1.33±0.3	1.83±0.2	0.10±0.01	0.00651±0.2	0.32±0.2
Leonotis nepetifolia	1.18±0.1	5.76±0.1	6.07±0.7	6.07±0.2	1.06±0.6	0.93±0.1	1.15±0.1	0.06±0.02	0.00838±0.1	0.17±0.1
Toddalia asiatica	0.58±0.3	6.01±0.1	3.4±0.4	3.4±0.7	0.84±0.4	1.06±0.1	0.74±0.1	0.10±0.01	0.00683±0.4	0.15±0.1
WHO	0.02	2.00	20.00	0.14	1.63	3.00	27.40	0.30	1.00	0.43

The results indicated that chromium recorded the highest concentration in *Physalis peruviana* and lowest concentration in *Carissa spinarum*. The highest concentration of manganese was recorded in *Carissa spinarum* while the lowest concentration in *Senna didymobotrya*. The highest concentration of iron was recorded in *Leonotis nepetifolia* and lowest concentration *Warburgia ugandensis*. The highest concentration of cobalt was recorded in *Leonotis nepetifolia* whereas lowest concentration was recorded in *Bidens pilosa*. The nickel recorded the highest concentration in *Bidens pilosa* and lowest concentration in *Carissa spinarum*. The highest concentration of copper was recorded in *Physalis peruviana* while the lowest concentration was in *Warburgia ugandensis*. The highest concentration of zinc was recorded in *Bidens pilosa* and lowest concentration in *Toddalia asiatica*. The highest concentration of cadmium was recorded in *Urtica dioica* and lowest concentration in *Carissa spinarum*. The highest concentration of mercury was recorded in *Leonotis nepetifolia* while the lowest concentration in *Warburgia ugandensis*. The highest concentration of lead was recorded in *Physalis peruviana* and lowest concentration in *Toddalia asiatica*.

4.4.1 Chromium

Results obtained showed that the highest concentration of Chromium was found in *Physalis peruviana* 2.035 ppm, followed by *Warburgia ugandensis* 1.517 ppm, *Leonotis nepetifolia* 1.183 ppm, *Bidens Pilosa* 0.983 ppm, *Senna didymobotrya* 0.70 ppm, *Carissa spinarum* 0.733 ppm, *Toddalia asiatica* 0.583 ppm and *Urtica dioica* 0.567 ppm (Table 3.4). The permissible limit set by FAO/WHO (1984) in edible plants was 0.02 ppm. After comparison, metal limit in the studied medicinal herbs

with those proposed by FAO/WHO (1984) it was found that all herbs accumulate Chromium above permissible limit set by FAO/WHO (1984) in edible plants. However, for medicinal plants the WHO (2005) limits not yet been established for Chromium. Although in medicinal plants, permissible limits for Chromium set by Canada, were 2 ppm in raw medicinal plant material and 0.02 mg/day in finished herbal products (WHO, 2005). It is observed that the herb *Physalis peruviana* with the concentration 2.035 ppm has equivalent concentration to the permissible limits for Chromium set by Canada. However, the other selected seven herbs have concentration within permissible limits for Chromium set by Canada. Chronic exposure to Chromium may result in liver, kidney and lung damage (Jabeen *et al.*, 2010). It was also reported by (Khan *et al.*,2012) that the toxic effects of Chromium intake is skin rash, nose irritations, bleeds, upset stomach, kidney and liver damage, nasal itch and lungs cancer, chromium deficiency is characterized by disturbance in glucose, lipids and protein metabolism.

4.4.2 Manganese

Results showed Manganese concentration is high in all plants (Table 3.4). The maximum concentration of Manganese was found in *Carissa spinarum* 17.33 ppm followed by *Warburgia ugandensis* 15.01 ppm, *Bidens Pilosa* 11.15 ppm, *Urtica dioica* 0.567 ppm, *Physalis peruviana* 6.061 ppm, *Toddalia asiatica* 6.010 ppm, *Leonotis nepetifolia* 5.761 ppm and *Senna didymobotrya* 3.254 ppm. It was observed that *Senna didymobotrya* accumulate lowest Manganese of 3.254 ppm and *Carissa spinarum* accumulate maximum of 17.33 ppm. The permissible limit set by FAO/WHO (1984) in edible plants was 2 ppm. After comparison, metal limit in the

studied medicinal plants with those proposed by FAO/WHO (1984) it was found that all herbs accumulate Manganese above this limit. However, for medicinal herbs the WHO (2005) limits not yet been established for Manganese. It was reported by (Jabeen *et al.,* 2010) that the range of Manganese in selective medicinal herbs of Egypt in the study carried out was between (ppm) 44.6 to 339. The concentration of all the selected eight herbs was in the range of (ppm) 3.254 to 17.33. However, the concentration of the selected eight studied herbs was within normal level for the element in selective medicinal herbs of Egypt. Deficiency of Manganese in human causes myocardial infection and other cardiovascular diseases, also disorder of bony cartilaginous growth in infants and children and may lead to immunodeficiency disorder and rheumatic arthritis in adults (Khan *et al.*, 2008).

4.4.3 Iron

Results revealed that maximum concentration of iron was found in *Leonotis nepetifolia* 6.067 ppm, followed by *Senna didymobotrya* 4.067 ppm, *Urtica dioica* 4.033 ppm, *Carissa spinarum* 3.867 ppm, *Toddalia asiatica* 3.4 ppm, *Physalis peruviana* 3.167 ppm, *Bidens Pilosa* 2.9 ppm and *Warburgia ugandensis* 0.967 ppm. The range of iron in the studied herbs was lowest of 0.967 ppm in *Warburgia ugandensis* and highest of 6.067 ppm in *Leonotis nepetifolia* (Table 3.4). The permissible limit set by FAO/WHO (1984) in edible plants was 20 ppm. After comparison, metal limit in the studied medicinal herbs with those proposed by FAO/WHO (1984) it is found that all herbs accumulate iron below this limit. However, for medicinal plants the WHO (2005) limits not yet been established for iron. It was reported by (Jabeen *et al.,* 2010) that the range of iron in selective

medicinal herbs of Egypt in the study carried out was between 261 ppm to 1239 ppm. However, the concentration of the selected eight studied herbs is within normal range for the element in selective medicinal herbs of Egypt. Iron is an essential element for human beings and animals and is an essential component of haemoglobin. It facilitates the oxidation of carbohydrates, protein and fat to control body weight, which is very important factor in diabetes (Ullah *et al.,* 2012). Iron is necessary for the formation of haemoglobin and also plays an important role in oxygen and electron transfer in human body (Jabeen *et al.,* 2010). Low iron content causes gastrointestinal infection, nose bleeding and myocardial infection (Ullah *et al.,* 2012).

4.4.4 Nickel

Results showed that highest concentration of nickel was found in *Bidens Pilosa* 1.6 ppm, followed by *Urtica dioica* 1.156 ppm, *Leonotis nepetifolia* 1.056 ppm, *Physalis peruviana* 1.033 ppm, *Senna didymobotrya* 0.844 ppm, *Toddalia asiatica* 0.844 ppm, *Warburgia ugandensis* 0.833 ppm and *Carissa spinarum* 0.589 ppm. It is observed that *Carissa spinarum* accumulate lowest Nickel that is, 0.589 ppm and *Bidens Pilosa* accumulate maximum that is 1.6 ppm (Table 3.4). It was reported by (Jabeen *et al.,* 2010) that the permissible limit set by FAO/WHO (1984) in edible plants was 1.63 ppm. After comparison, metal limit in the studied medicinal herbs with those proposed by FAO/WHO (1984) it was found that selected seven herbs accumulate nickel below this limit. However, with exception of *Bidens Pilosa* which accumulated nickel at equivalent permissible limit. However, for medicinal plants the WHO (2005) limits not yet been established for nickel. Nickel toxicity in human

is not a very common occurrence because its absorption by the body is very low (Jabeen *et al.,* 2010). The most common ailment arising from nickel is an allergic dermatitis known as nickel itch, which usually occurs when skin is moist, further more nickel has been identified as a suspected carcinogen and adversely affects lungs and nasal cavities. Although nickel is required in minute quantity for body as it is mostly present in the pancreas and hence plays an important role in the production of insulin. Its deficiency results in the disorder of liver (Khan *et al.,* 2008).

4.4.5 Cobalt

Results indicated that the concentration level of Cobalt in the selected medicinal herbs was highest in *Leonotis nepetifolia* 6.067 ppm, followed by *Senna didymobotrya* 4.067 ppm, *Urtica dioica* 4.033 ppm, *Carissa spinarum* 3.867 ppm, *Toddalia asiatica* 3.4 ppm, *Physalis peruviana* 3.167 ppm, *Bidens Pilosa* 2.9 ppm and *Warburgia ugandensis* 0.967 ppm. The *Leonotis nepetifolia* has higher Cobalt concentration of 6.067 ppm while *Warburgia ugandensis* recorded the lowest accumulation of 0.967 ppm (Table 3.4). There are no regulatory limits by WHO/FAO (2005) for Cobalt content in herbal plants and preparations. It was reported by (Jabeen *et al.,* 2010) that the study carried out in seven herbs in Turkey determined Cobalt concentration ranged between 0.14 ppm to 0.48 ppm. However, the selected herbs from Kisii region Southwest Kenya have high Cobalt concentration ranges between 0.967 ppm to 6.067 ppm than that recorded by seven herbs in Turkey. At low concentrations Cobalt play prominent role in the formation of cyanocobalmin – vitamin B 12, an essential vitamin in man (Khan *et al*., 2008., Ullah *et al.,* 2012). .

4.4.6 Copper

Results indicated that the high concentration of Copper was found in *Physalis peruviana* 1.44 ppm, followed by *Bidens Pilosa* 1.328 ppm, *Toddalia asiatica* 1.058 ppm, *Carissa spinarum* 1.039 ppm, *Senna didymobotrya* 0.959 ppm, *Leonotis nepetifolia* 0.933 ppm, *Urtica dioica* 0.827 ppm and *Warburgia ugandensis* 0.305 ppm(Table3.4). The lowest concentration of Copper observed is 0.305 ppm in *Warburgia ugandensis* and highest concentration was recorded as 1.44 ppm in *Physalis peruviana* (Table3.4). It was reported by (Jabeen *et al.,* 2010) that the permissible limit set by FAO/WHO (1984) in edible plants was 3.00 ppm. After comparison, metal limit in the studied medicinal herbs with those proposed by FAO/WHO (1984) it was found that all the selected herbs from Kisii region Southwest Kenya accumulate Copper below the permissible limit set by FAO/WHO (1984) in edible plants. However, for medicinal herbs the WHO (2005) limits not yet been established for Copper. Although in medicinal plants, permissible limits for Copper set by China and Singapore, were 20 ppm and 150 ppm respectively (WHO, 2005). The selected eight herbs were found to have permissible limits for Copper below that set by China and Singapore. It was reported by (Jabeen *et al.*, 2010) that the range of Copper contents in the 50 medicinally important leafy material growing in India were 17.6 ppm to 57.3 ppm. High levels of Copper may cause metal fumes fever with flue like symptoms, hair and skin decoloration, dermatitis, irritation of the upper respiratory tract, metallic taste in the mouth and nausea. Copper deficiency results in anemia and congenital inability (Khan *et al*., 2008., Ullah *et al.*, 2012).

4.4.7 Zinc

Results showed that high concentration of Zinc was found in *Bidens Pilosa* 1.833 ppm followed by *Urtica dioica* 1.661 ppm, *Carissa spinarum* 1.513 ppm, *Senna didymobotrya* 1.160 ppm, *Leonotis nepetifolia* 1.148 ppm, *Warburgia ugandensis* 1.139 ppm, *Physalis peruviana* 0.989 ppm and *Toddalia asiatica* 0.736 ppm (Table 3.4). It was reported by (Jabeen *et al.,* 2010) that the permissible limit set by FAO/WHO (1984) in edible plants was 27.4 ppm. The zinc concentration in the selected eight herbs analysed ranges between 0.989 ppb to 1.833 ppb compared to 27.4 ppm permissible limit in edible plants. Therefore the zinc concentration in the eight herbs was within permissible limits. Zinc is an essential trace element and plays an important role in various cell processes including normal growth, brain development, behavioural response, bone formation and wound healing (Khan *et al.*, 2008., Jabeen *et al.,* 2010). Zinc deficient diabetics fail to improve their power of sensitivity and cause loss of sense of touch and smell (Khan *et al*., 2008., Jabeen *et al.*, 2010). The dietary limit of Zinc is 100 ppm (Jabeen *et al.*, 2010).

4.4.8 Cadmium

Results showed that highest concentration of Cadmium was found in *Urtica dioica* 0.206 ppm, followed by *Toddalia asiatica* 0.104 ppm, *Bidens Pilosa* 0.1008 ppm, *Physalis peruviana* 0.063 ppm, *Leonotis nepetifolia* 0.061 ppm, *Warburgia ugandensis* 0.041 ppm, *Senna didymobotrya* 1.160 ppm and *Carissa spinarum* 0.035 ppm (Table 3.4). It was reported by (Jabeen *et al.*, 2010) that the permissible limit set by FAO/WHO (1984) in edible plants was 0.21 ppm. However, for medicinal herbs the permissible limit for Cadmium set by WHO, China and Thailand was 0.3 ppm. Similarly, permissible limits in medicinal plants for Cadmium set by Canada were 0.3 ppm in raw medicinal plant material and 0.006 mg/day in finished herbal products (WHO, 2005). After comparison, metal limits in the studied eight medicinal herbs with those proposed by FAO/WHO (1984) and WHO (2005), it was found that all studied eight herbs accumulate Cadmium below the permissible limit set by WHO, Canada, China and Thailand. Cadmium causes both acute and chronic poisoning, adverse effect on kidney, liver, vascular and immune system (Khan *et al.*, 2008., Jabeen *et al.*, 2010).

4.4.9 Mercury

Results indicated that the highest concentration of Mercury was found in *Leonotis nepetifolia* 0.00838 ppm, followed by *Toddalia asiatica* 0.006833ppm, *Physalis peruviana* 0.00572 ppm, *Bidens Pilosa* 0.006507 ppm, *Urtica dioica* 0.004533 ppm, *Senna didymobotrya* 0.002887 ppm, *Warburgia ugandensis* 0.002653 ppm and *Carissa spinarum* 0.0024 ppm (Table 3.4, Figure 3.10). The lowest concentration of

Mercury obtained is 0.0024 ppm in *Carissa spinarum* and maximum concentration was recorded as 0.00838 ppm in *Leonotis nepetifolia* (Table 3.4). It was reported by (Jabeen *et al.,* 2010) that the permissible limit set by FAO/WHO (1984) in edible plants was 0.02 ppm and in herbal medicine was 0.1 ppm. After comparison, metal limit in the studied medicinal herbs with those proposed by FAO/WHO (1984) it was found that selected eight herbs accumulate Mercury below permissible limit set by FAO/WHO (1984) in herbal medicine. Exposures to high levels of metallic, inorganic, or organic mercury can permanently damage the brain, kidneys and developing foetus. The effects of mercury on brain functioning result in irritability, shyness, tremors, changes in vision or hearing and memory problems. Exposure to methyl mercury is worse for young children than for adults, because more of it passes into children's brains where it interferes with normal development (Vaikosen and Alade, 2011).

4.4.10 Lead

Results showed that highest concentration of Lead was found in *Physalis Peruviana* 0.407 ppm, followed by *Warburgia ugandensis* 0.380 ppm, *Senna didymobotrya* 0.326 ppm, *Bidens Pilosa* 0.315 ppm, *Urtica dioica* 0.267 ppm, *Carissa spinarum* 0.25 ppm, *Leonotis nepetifolia* 0.166 ppm and *Toddalia asiatica* 0.148 ppm.The herb *Physalis Peruviana* exhibited higher Lead concentration of 0.407 ppm and *Toddalia asiatica* possess minimum concentration of Lead 0.148 ppm (Table 3.4). It was reported by (Jabeen *et al.,* 2010) that the permissible limit set by FAO/ WHO (1984) in edible plants was 0.43 ppm. However, for medicinal herbs limit was 10 ppm set by China, Malaysia, Thailand and WHO. Similarly plants with those proposed by WHO

(2005) it was found that all the selected eight herbs accumulate Lead below permissible limit set by China, Malaysia, Thailand and FAO/ WHO. Lead causes both acute and chronic poisoning, and also poses adverse effects on kidney, liver, vascular and immune system (Khan *et al.*, 2008., Jabeen *et al.*, 2010). Lead is non-essential trace elements having functions neither in humans body nor in plants. They induce various toxic effects in humans at low doses. The typical symptoms of lead poisoning are colic, anaemia, headache, convulsions and chronic nephritis of the kidneys, brain damage and central nervous system disorders (Khan *et al.*, 2008).

These finding showed that all the eight herbs screened have heavy metals. It has been established fact that overdoses or prolonged ingestion of medicinal plants leads to the chronic accumulation of different elements which causes various health problems (Mtunzi *et al.*, 2012). Despite the accumulation of toxic elements which were present like mercury, lead and cadmium that might cause harm to children and adults, useful elements such as chromium, copper, cobalt, manganese, nickel, zinc and iron were present in selected herbs, which help in the good health (Mtunzi *et al.*, 2012).

4. 5 Thin layer chromatography

The results showed (distances travelled by solvent front and extracts) by performing the thin layer chromatography was analysed and the R_f values are displayed and discussed (Table 3.5, 3.6, 3.7, 3.8).

Table 3.5: The TLC for hexane extracts.

Solvent system Hexane/ dichloromethane, (50:50 v/v)	**Herbs**	**No of spots visible by UV**	**R_f value = distance moved by sample/distance moved by solvent**
	Carissa spinarum	1	0.0375
	Urtica dioica	1	0.0375
	Warburgia ugandensis	4	0.025 0.0375 0.0475 0.075
	Senna didymobotrya	2	0.0375 0.5
	Physalis peruviana	7	0.025 0.0375 0.125 0.3375 0.4125 0.5 0.725
	Bidens pilosa	10	0.0375 0.0625 0.075 0.0875 0.125 0.1875 0.35 0.425 0.5 0.75
	Leonotis nepetifolia	6	0.025 0.0375 0.3375 0.4125 0.5 0.725
	Toddalia asiatica	2	0.0125 0.025

Each spot is presumably due to a pure natural product or phytochemical which has a specific R_f value. The larger the R_f value, the lower the polarity of natural products / phytochemicals. TLC analysis in various solvent systems for each solvent type revealed the presence of spots that range from one to maximum ten. The highest number of spot in hexane extract was recorded in *Bidens pilosa* (10) and least number of spots was recorded in *Urtica dioica* and *Carissa spinarum* one each. The highest number of spot in dichloromethane extract was recorded in *Warburgia ugandensis* (5) while the lowest number of spots was three. The highest number of spot in ethyl acetate was recorded in extract *Senna didymobotrya* (7) and the lowest number of spots was recorded in *Urtica dioica* (1). The highest number of spot in ethanol extract was recorded in *Senna didymobotrya* (4) and the lowest number of spots was recorded in *Carissa spinarum.* The mixture of ethyl acetate: dichloromethane (9:1) was better for the ethyl acetate crude extract, giving seven separations for *Senna didymobotrya,* four separations for *Bidens pilosa, Leonotis nepetifolia* and *Toddalia asiatica,* two separations for *Physalis peruviana* and *Carissa spinarum,* one separation for *Urtica dioica* and *Warburgia ugandensis.* The mixture of ethanol: hexane (9:1) was better for the ethanol crude extract, giving four separations for *Senna didmobotrya,*three separations for *Urtica dioica, Physalis peruviana, Bidens pilosa, Leonotis nepetifolia* and *Toddalia asiatica,* two separations for *Warburgia ugandensis,* one separation for *Carissa spinarum* (Figure 3.12).

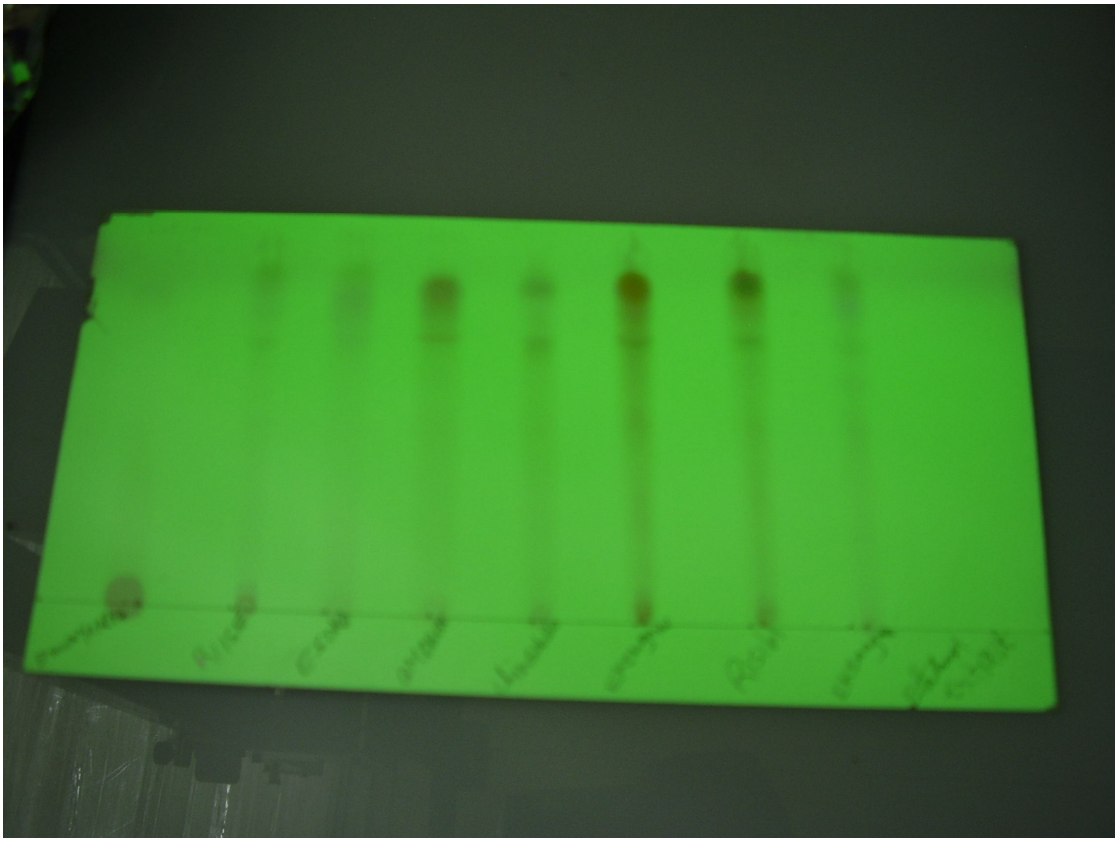


Fig. 3.12: TLC plate analyses: From left *Carissa spinarum, Urtica dioica, Warburgia ugandensis, Senna didymobotrya, Physalis peruviana, Bidens pilosa, Leonotis nepetifolia and Toddalia asiatica.*

4.6 Antimicrobial Test

The phytoconstituents present in various extracts of *Carissa spinarum, Urtica dioica, Warburgia ugandensis, Senna didymobotrya, Physalis peruviana, Bidens pilosa, Leonotis nepetifolia* and *Toddalia asiatica* ma**y** have antimicrobial activity. It was important then, to ascertain particular micro organisms for which they are active by Well diffusion method. The microbial activity of the various extracts against *Escherichia coli, Staphylococcus aureus* and *Candida albicans* was determined and the results are outlined and discussed in this section. Results obtained in this study were expressed as mean inhibition zone (mm) ± S.D of triplicate. The mean and the S.D. of each herbal extract were used to compute the calculated t-value. Differences between the critical t-value and calculated t-values of the diameter of the inhibition zones of the herbal extracts on gram-positive *Staphylococcus aureus* and gram-negative bacteria *Escherichia coli* were computed. The p value ≤ 0.05 was significant.

4.6.1 Inhibition zones of hexane extracts against *Staphylococcus aureus.*

Results showed antimicrobial activity by well diffusion method of studied herbs (Table 3.9).

Table 3.9: The antimicrobial and antifungal activity of hexane extracts

Well diffusion	Extract concentration (0.000002 µg / ml)	Diameter of zone of inhibition (mm)		
(**Hexane extract**)	Volume extract (ml) 0.2ml= (4 x10^{-7} µg)	*Staphylococcus aureus*	*Escherichia coli*	*Candida albicans*
Carissa spinarum	0.2	24±1.0	12±0.0	12±0.0
Urtica dioica	0.2	16±1.0	12±0.0	12±0.0
Warburgia ugandensis	0.2	23±1.0	12±0.0	12±0.0
Senna didymobotrya	0.2	16±1.0	12±0.0	12±0.0
Physalis peruviana	0.2	20±0.0	12±0.0	12±0.0
Bidens pilosa	0.2	24±1.0	12±0.0	12±0.0
Leonotis nepetifolia	0.2	24±1.0	12±0.0	12±0.0
Toddalia asiatica	0.2	25±1.0	12±0.0	12±0.0
Control (Hexane)	0.2	12±0.0	12±0.0	12±0.0
Reference				
Erythromycin	15 µg	31±1.0	12±0.0	12±0.0
Minocycline	30 µg	32±1.0	30±1.0	16±1.0
Chloramphenicol	30µg	33±1.0	25±1.0	12±0.0
Cotrimoazol	25µg	12±0.0	18±1.0	25±1.0

Results showed that the control experiments of pure distilled solvent alone, rather than pure plant extract induced no zone of inhibition. The zone of inhibition for the control measured 12 mm indicating lack of suppression of the bacteria involved and therefore negative results. The hexane extracts of all the herbs studied against gram-negative bacteria *Escherichia coli* and the fungus *Candida albicans* showed no antimicrobial and antifungal activity respectively. However, hexane extracts of all the eight herbs tested against gram-positive bacteria *Staphylococcus aureus* displayed antimicrobial activity (mm) of 16 to 25 (Balammal *et al*., 2012). The reference antibiotic compounds, erythromycin, chloramphenicol, minocyline and cotrimoazol, showed antimicrobial activity (mm) against *Staphylococcus aureus* (31 to 33), *Escherichia coli* (31 to 33), and antifungal activity (mm) against *Candida albicans* (16 to 25) respectively. It was observed that the hexane extracts against *Staphylococcus aureus* of *Toddalia asiatica* exhibited the highest antimicrobial activity of 25 mm while *Urtica dioica* showed the lowest antimicrobial activity of 16 mm. The antibacterial activity of hexane crude extracts of the selected eight herbs was relatively closer to that of reference antibiotic compounds, indicating that they have a potential to control Gram positive bacteria but, not Gram negative bacteria and the fungus *Candida albicans* (Hegde and Joshi, 2010).

4.6.2 Inhibition zones of hexane extracts against *Escherichia coli*.

Results of hexane extract of all the eight herbs against gram-negative bacteria *Escherichia coli* measured 12mm, which is zero zone of inhibition indicating no antimicrobial activity (Table 3.9). The reference antibiotic compounds tested against

gram-negative bacteria *Escherichia coli* namely, minocyline, chloramphenicol and cotrimoazol measured antimicrobial activity (mm) 30, 25 and 18 respectively.

4.6.3 Inhibition zones of hexane extracts against *Candida albicans*.

Results showed that the antifungal activity of the control pure solvent hexane and all eight hexane extracts measured 12 mm diameter zone of inhibition, indicating no antifungal activity (Table 3.9). The antibiotics namely minocycline, chloramphenicol and cotrimoazol showed the antifungal activity (mm) of 18 to 30 respectively(Ibrahim *et al*., 2010., Rose and Prasad, 2013**.,** Chirinos *et al*., 2010; Licodiedoff *et al.,* 2013., Veerabadran *et al*., 2013).

4.6.4 Inhibition zone of dichloromethane extracts against *Staphylococcus aureus*.

The results obtained showed antimicrobial activity (mm) in all the studied herbs (Table 4.0).

Table 4.0: antimicrobial and antifungal activity of dichloromethane extracts

Well diffusion	Extract concentration (0.000002 µg / ml)	Diameter of zone of inhibition (mm)		
Extract (Dichloromethane)	Volume extract (ml) 0.2ml= (4 x10^{-7} µg)	*Staphylococcus aureus*	*Escherichia coli*	*Candida albicans*
Carissa spinarum	0.2	20±0.0	12±0.0	12±0.0
Urtica dioica	0.2	20±0.0	12±0.0	12±0.0
Warburgia ugandensis	0.2	20±0.0	12±0.0	12±0.0
Senna didymobotrya	0.2	20±0.0	12±0.0	12±0.0
Physalis peruviana	0.2	23±1.0	12±0.0	12±0.0
Bidens pilosa	0.2	20±0.0	12±0.0	16±0.0
Leonotis nepetifolia	0.2	25±1.0	19±1.0	19±1.0
Toddalia asiatica	0.2	24±1.0	12±0.0	12±0.0
Control (CH_2Cl_2)	0.2	12±0.0	12±0.0	12±0.0
Reference				
Erythromycin	15 µg	31±1.0	12±0.0	12±0.0
Chloramphenicol	30 µg	33±1.0	25±1.0	12±0.0
Minocycline	30 µg	32±1.0	30±1.0	16±1.0
Cotrimoazol	25µg		18±1.0	25±1.0

The results showed that the control pure dichloromethane solvent recorded 12mm diameter zone of inhibition, indicating no antibacterial activity against gram-positive bacteria *Staphylococcus aureus.* However, the dichloromethane crude extract of all

the selected eight herbs displayed the antibacterial activity (mm) of 20 to 25 against gram-positive bacteria *Staphylococcus aureus.* The *Leonotis nepetifolia* showed the highest antibacterial activity of 25mm while *Carissa spinarum, Urtica dioica, Warburgia ugandensis, Senna didymobotrya* and *Bidens Pilosa,* showed the lowest antibacterial activity (mm) of 20. The reference antibiotic compounds, minocyline, chloramphenicol, and cotrimoazol displayed antibacterial activity (mm) of 16 to 25 respectively against gram-positive bacteria *Staphylococcus aureus*. These results showed that dichloromethane extracts of all selected eight herbs have a potential to control gram-positive bacteria *Staphylococcus aureus* (Hsu *et al*., 2009., Ashafa and Afolayan, 2009., Narayan, 2012).

4.6. 5 Inhibition zones of dichloromethane extracts against *Escherichia coli*

Results showed that the pure dichloromethane solvent and other seven herbs studied against gram-negative bacteria *Escherichia coli* showed no antibacterial activity (Table 4.0). However, dichloromethane extracts of *Leonotis nepetifolia* exhibited antibacterial activity of 19 mm against gram-negative bacteria *Escherichia coli.* The reference antibiotic compounds, minocyline, chloramphenicol, and cotrimoazol against gram-negative bacteria *Escherichia coli* measured antibacterial activity (mm) 18 to 30. These results indicated that the dichloromethane extracts of *Leonotis nepetifolia* has a potential to control gram-negative bacteria *Escherichia coli* (Narayan, 2012., Gungurthy *et al*., 2013., Veerabadran *et al*., 2013).

4.6.6 Inhibition zones of dichloromethane extracts against *Candida albicans*.

Results showed that the pure solvent dichloromethane and dichloromethane extracts of six other selected herbs studied against *Candida albicans* displayed no antifungal

activity (Table 4.0). However, the dichloromethane extracts of *Leonotis nepetifolia* and *Bidens pilosa* showed antifungal activity (mm) 16 to 19 against *Candida albicans* (Sundararajan *et al*., 2006., Ashafa and Afolayan, 2009., Narayan, 2012., Patel *et al.,* 2013., Veerabadran *et al*., 2013). The reference antibiotic compounds, minocycline, chloramphenicol and erythromycin measured antifungal activity (mm) 31 to 33 respectively. These results showed that dichloromethane extracts of *Leonotis nepetifolia* and *Bidens pilosa* have a potential to control the fungus *Candida albicans*.

4.6.7 Inhibition zones of ethyl acetate extracts against *Staphylococcus aureus*

The results indicated that the selected herbs showed antimicrobial activity (Table 4.1).

Table 4.1: antimicrobial and antifungal activity of ethyl acetate extracts

Well diffusion	Extract concentration (0.000002 µg / ml)	Diameter of zone of inhibition (mm)		
Extract (Ethyl acetate)	Volume extract (ml) 0.2ml= (4×10^{-7} µg)	*Staphylococcus aureus*	*Escherichia coli*	*Candida albicans*
Carissa spinarum	0.2	24±1.0	12±0.0	12±0.0
Urtica dioica	0.2	23±1.0	12±0.0	12±0.0
Warburgia ugandensis	0.2	25±1.0	12±0.0	12±0.0
Senna didymobotrya	0.2	24±1.0	12±0.0	18±1.0
Physalis peruviana	0.2	24±1.0	14±1.0	15±0.0
Bidens pilosa	0.2	25±1.0	12±0.0	18±1.0
Leonotis nepetifolia	0.2	20±1.0	12±0.0	24±1.0
Toddalia asiatica	0.2	22±1.0	12±0.0	17±1.0
Control (Ethyl acetate)	0.2	12±0.0	12±0.0	12±0.0
Reference				
Erythromycin	15 µg	31±1.0	12±0.0	12±0.0
Chloramphenicol	30 µg	33±1.0	25±1.0	12±0.0
Minocycline	30 µg	32±1.0	30±1.0	16±1.0
Cotrimoazol	25µg	18±1.0	18±1.0	25±1.0

The results showed that pure ethyl acetate solvent measured 12mm diameter zone of inhibition against gram-positive bacteria Staphylococcus *aureus,* indicating no

antimicrobial activity (Table 4.1). However, ethyl acetate extracts of all the selected eight herbs studied against gram-positive bacteria *Staphylococcus aureus* indicated antimicrobial activity (mm) 20 to 25. The herbs *Bidens pilosa* showed the highest antimicrobial activity of 25mm and *Leonotis nepetifolia* showed the least antimicrobial activity of 20mm against gram-positive bacteria *Staphylococcus aureus* (Sundararajan *et al*., 2006., Ashafa and Afolayan, 2009., Narayan, 2012., Patel *et al.*, 2013., Veerabadran *et al.*, 2013). The reference antibiotic compounds, minocyline, chloramphenicol, erythromycin and cotrimoazol against gram-positive bacteria *Staphylococcus aureus* measured antimicrobial activity (mm) 18 to 33. These results indicated that ethyl acetate extracts of the selected herbs have a potential to control gram-positive bacteria *Staphylococcus aureus.*

4.6.8 Inhibition zones of ethyl acetate extracts against *Escherichia coli*

Results showed that pure ethyl acetate solvent and ethyl acetate extracts of seven selected herbs against gram-negative bacteria *Escherichia coli* measured 12mm diameter zone of inhibition, indicating no antimicrobial activity (Table 4.1). However, ethyl acetate extracts of *Physalis peruviana* showed antimicrobial activity 14 mm (Franco *et al.*, 2007., Chirinos *et al*., 2010., El-Gengaihi *et al*., 2012., Licodiedoff *et al.*, 2013). The reference antibiotic compounds, minocyline, chloramphenicol, and cotrimoazol against gram-negative bacteria *Escherichia coli* measured antimicrobial activity (mm) 18 to 30. The ethyl acetate extracts of *Physalis peruviana* has a potential to control gram-negative bacteria *Escherichia coli*.

4.6.9 Inhibition zones of ethyl acetate extracts against *Candida albicans*.

The results showed that the pure solvent ethyl acetate measured (12 mm), indicating no antifungal activity (Table 4.1). However, the ethyl acetate extracts of the selected herbs against the fungus *Candida albicans* showed antifungal activity (mm) 15 to 24 (Franco *et al.*, 2007., Chirinos *et al*., 2010., El-Gengaihi *et al*., 2012., Licodiedoff *et al.*, 2013). The herbs *Leonotis nepetifolia* showed the highest antifungal activity 24 mm against *Candida albicans* while the *Physalis peruviana* exhibited the least antifungal activity 24 mm. The reference antibiotics compounds, minocycline and cotrimoazol showed antifungal activity (mm) 16 to 25. The ethyl acetate extracts of the selected herbs have a potential to control *Candida albicans* except for *Carissa spinarum* and *Urtica dioica.*

4.6.10 Inhibition zones of ethanol extracts against *Staphylococcus aureus*

The results showed that the extracts of the herbs have antimicrobial activity (Table 4.2).

Table 4.2: The antimicrobial and antifungal activity of ethanol extracts

Well diffusion	Extract concentration (0.000002 µg / ml)	Diameter zone of inhibition (mm)		
(Ethanol)	Volume extract (ml) 0.2ml= (4 x10^{-7} µg)	*Staphylococcus aureus*	*Escherichia coli*	*Candida albicans*
Carissa spinarum	0.2	16±1.0	12±0.0	12±0.0
Urtica dioica	0.2	26±1.0	12±0.0	12±0.0
Warburgia ugandensis	0.2	27±1.0	12±0.0	12±0.0
Senna didymobotrya	0.2	18±1.0	13±1.0	12±0.0
Physalis Peruvian	0.2	27±1.0	13±1.0	19±1.0
Bidens pilosa	0.2	27±1.0	13±1.0	12±0.0
Leonotis nepetifolia	0.2	26±1.0	17±1.0	27±1.0
Toddalia asiatica	0.2	26±1.0	12±0.0	13±1.0
Control (C_2H_5OH)	0.2	12±0.0	12±0.0	12±0.0
Reference				
Erythromycin(Ery)	15 µg	31±1.0	12±0.0	12±0.0
Chloramphenicol	30 µg	33±1.0	33±1.0	25±1.0
Minocycline	30 µg	32±1.0	30±1.0	16±1.0
Cotrimoazol	25µg	18±1.0	18±1.0	24±1.0

Results showed that pure ethanol solvent measured 12mm diameter zone of inhibition against gram-positive bacteria *Staphylococcus aureus* indicating no antibacterial activity (Table 4.2). However, ethanol extracts showed antimicrobial activity (mm) 16 to 27 against gram-positive bacteria *Staphylococcus aureus.* The highest antimicrobial activity 27 mm of the ethanol extracts against gram-positive bacteria *Staphylococcus aureus* was recorded in *Warburgia ugandensis, Physalis peruviana* and *Bidens pilosa* while the least antimicrobial activity 16mm was recorded in *Carissa spinarum* (Ganapaty *et al.*, 2002., Ngure *et al.,* 2009., Rose and Prasad, 2013., Khare *et al.,* 2012., Kataki *et al.*, 2012). The reference antibiotic compounds, minocycline and cotrimoazol showed antimicrobial activity (mm) 31 to 33.The antimicrobial activity of ethanol extracts was relatively high and closer to reference antibiotic compounds compared to that of other extracts, hexane, dichloromethane and ethyl acetate of the same herb.

4.6.11 Inhibition zones of ethanol extracts against *Escherichia coli*

Results showed that pure ethanol solvent measured 12mm diameter zone of inhibition against gram-negative bacteria *Escherichia coli,* indicating no antibacterial activity (Table 4.2). However, the ethanol extracts of the eight selected herbs against gram-negative bacteria *Escherichia coli* showed antibacterial activity (mm) 13 to 17 respectively. The herb *Leonotis nepetifolia* measured the highest antibacterial activity of 17 mm against gram-negative bacteria *Escherichia coli* while *Senna didymobotrya, Physalis peruviana and Bidens pilosa* measured the lowest antibacterial activity 13mm (Ganapaty *et al.*, 2002., Ngure *et al.*, 2009., Narayan, 2012., Khare *et al.*, 2012., Kataki *et al.*, 2012., Rose and Prasad, 2013). The

reference antibiotic compounds, minocyline, chloramphenicol and cotrimoazol against gram-negative bacteria *Escherichia coli* measured antibacterial activity (mm) 18 to 33. These findings indicated that the herb *Leonotis nepetifolia* has a potential to control gram-negative bacteria *Escherichia coli*.

4.6.12 Inhibition zones of ethanol extracts against *Candida albicans*.

Results showed that pure ethanol solvent measured 12mm diameter zone of inhibition against the fungus *Candida albicans,* indicating no antifungal activity (Table 4.2). However, the ethanol extracts of the eight selected herbs against the fungus *Candida albicans* showed antifungal activity (mm) 13 to 27 respectively. The herb *Leonotis nepetifolia* measured the highest antifungal activity 27 mm followed by *Physalis peruviana* with antifungal activity 19 mm. The lowest antifungal activity 13 mm of the ethanol extracts against the fungus *Candida albicans* was recorded in *Toddalia asiatica* (Franco *et al.,* 2007., Chirinos *et al*., 2010; El-Gengaihi *et al*., 2012., Licodiedoff *et al.,* 2013., Gungurthy *et al.,* 2013., Veerabadran *et al*., 2013). . The reference antibiotic compounds, minocyline, chloramphenicol and cotrimoazol against the fungus *Candida albicans* measured antifungal activity (mm) 16 to 25. These results indicated that the ethanol extract of *Leonotis nepetifolia, Physalis peruviana* and *Toddalia asiatica,* have a potential to control fungus *Candida albicans*.

4.7 Infrared spectroscopy analysis

Infrared spectra of crude extracts of FT- IR spectra (Figures 3.25 to 3.69) obtained from *Carissa spinarum, Urtica dioca, Warburgia ugandensis, Senna didymobotrya, Physalis peruviana, Bidens Pilosa, Leonotis nepetifolia* and *Toddalia asiatica*; are presented and discussed in the following subsections. The deductions made from the spectra are also presented and discussed.

4.7.1 *Carissa spinarum*

Results of FT- IR spectra hexane extract was recorded in Figure 3.25. The FT-IR Spectroscopic analysis of *Carissa spinarum* (Figure.3.25, 3.26, 3.27, 3.28), revealed the presence of aromatic compounds with C-H (stretch) at 3055.0 cm^{-1}, C=C (stretch) at 1531.4, 1654.8 cm^{-1}, and C-H (bend) at 732.9, 898.8 cm^{-1} in the dichloromethane extract (Ragavendran, *et al.*,2011., Muruganantham *et al*., 2009**.,** Donald *et al.*, 2005).

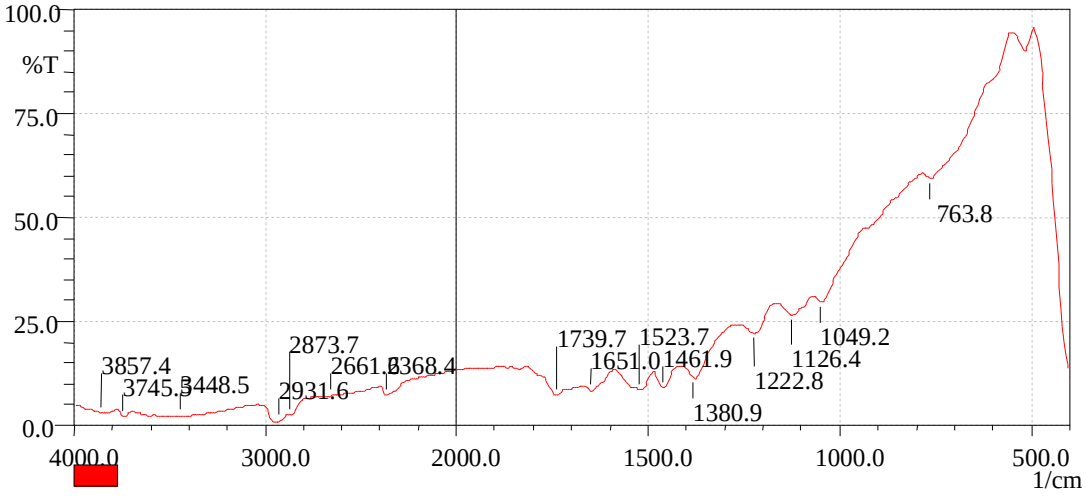


Figure 3.25: FTIR spectrum of *Carissa spinarum* (hexane crude extract)

The alkaloids were present due to N-H stretch at 3448.5, 3448.5, 3548.8, 3363.6 cm^{-1}, C-N stretch at 1049.2, 1126.4, 1265.2 cm^{-1} and N-H bend at 1551.0, 1523.7 cm^{-1} fingerprint peaks found in primary, secondary and tertiary amines of the hexane, dichloromethane, ethyl acetate and ethanol crude extract (Skoog *et al*., 2007). The saponins were found to be present due to the presence of C=O stretch at 1739.7 cm^{-1} and C-O stretch at 1245.9 cm^{-1} in the ethyl acetate crude extracts as carboxylic acid anhydrides. The unsaturated aromatic lactones with C=O stretch at 1739.7, 1654.8 cm^{-1} and C-O stretch at 1265.2, 1245.9, 1326.9 cm^{-1}, which occurred either in the Free State or combined with the sugar glucose (Coumarin glycoside) were found to be present in the hexane, dichloromethane and ethyl acetate crude extracts. The tannins were present as free phenols with O-H stretch at 3625.9 cm^{-1} and C-O stretch at 1245.9, 1377.1 cm^{-1} in the ethyl acetate extract (Egwaikhide and Gimba, 2007). The polyphenols were found to be present with O-H (stretch) at 3448.5, 3463.9, 3363.6 cm^{-1} and C=O (stretch) at 1265.2, 1245.9. 1396.4 cm^{-1} for dichloromethane, ethyl acetate and ethanol extract. Anthraquinones were present as aromatic ethers with C-O stretch at 1265.2, 1245.9 and 1049.2, 1056.9 cm^{-1} in dichloromethane, ethyl acetate and ethanol extract. The esters peak for C=O stretch at 1739.7 cm^{-1} and C-O stretch at 1245.9 cm^{-1} were due to the presence of terpenoids and steroids. The presence of quinones revealed that Flavonoids were present with O-H (stretch) at 3363.6 cm^{-1} and C=O (stretch) at 1654.8 cm^{-1} for ethyl acetate and ethanol extract. The terpenes were present with C-H (Stretch) at 3055.0cm^{-1}, C=C (Stretch) at 1654.8 cm^{-1} and =C-H (bend) at 1446.5, 902.6 cm^{-1} for dichloromethane extract (Ragavendran, *et al.*,2011., Muruganantham *et al*., 2009**.,** Donald *et al.,* 2005). The Cyanogenic glycosides were present due to the

presence of C≡N (stretch) at 2368.4, 2360.7, 2360.7, 2245.0 cm^{-1} for hexane, dichloromethane, ethyl acetate and ethanol extract while N≡C (stretch) at 2086.8, 2140.8 cm^{-1} was for Isothiocyanate glycosides for ethyl acetate and ethanol extract (Ragavendran, *et al*.,2011., Muruganantham *et al*., 2009**.,** Donald *et al.,* 2005). The presence of components C=O stretch at 1739.7 cm^{-1} and C-O stretch at 1245.9, 1265.2 of dichloromethane and ethyl acetate extract, revealed the presence of cardiac glycosides(Ragavendran, *et al*.,2011., Muruganantham *et al*., 2009**.,** Donald *et al.,* 2005). The medium absorption band occurs at 740.6, 621.0 , 667.3 cm^{-1} dichloromethane, ethyl acetate and ethanol respectively may be due to aliphatic C–Cl absorption and brominates compound. The brominates compound shows an infrared band region 667.3 cm^{-1} (Ragavendran, *et al.,*2011., Muruganantham *et al*., 2009**.,** Donald *et al.,* 2005). The more intense bands occurring at 3448.5 cm^{-1}, 2927 cm^{-1}, 2862.2 cm^{-1}, 1654.8 cm^{-1}, 1446.5 cm^{-1}, 1265.2 cm^{-1}, 1049.2 cm^{-1}, 740.6 cm^{-1} and 667.3 cm^{-1} corresponding to O–H/N–H, C–H, C–O and C–Cl/C–S stretching / bending vibrations respectively indicate the presence of amino acids, alkenes, nitrates, ethers, organic halogen compounds and carbohydrates in *Carissa spinarum* (Skoog *et al*., 2007).

4.7.2 *Urtica dioica*

Results of FT- IR spectra hexane extract was recorded in Figure 3.29.

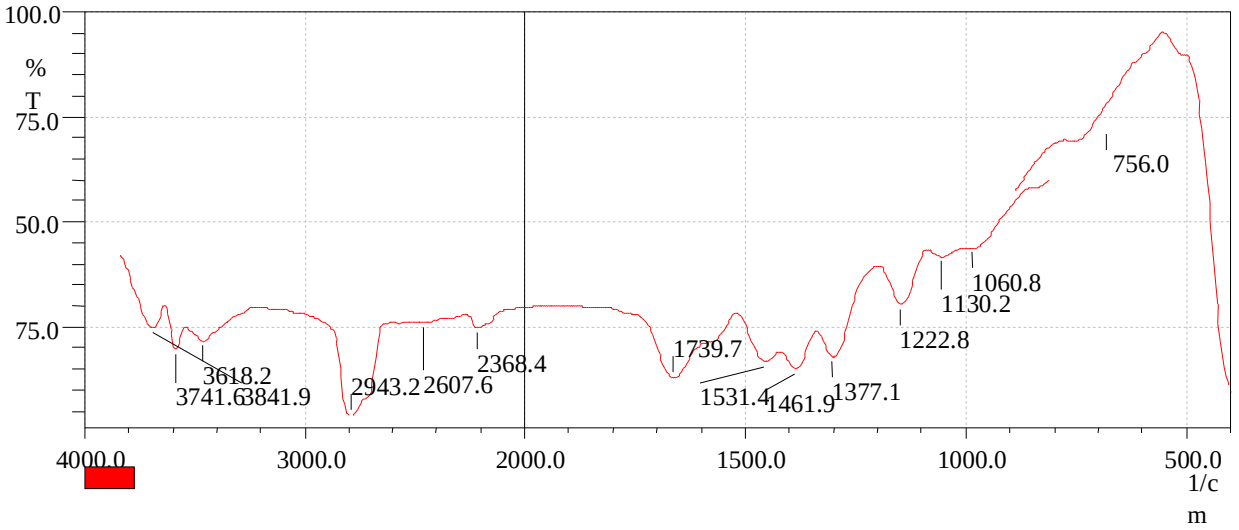


Figure 3.29: FTIR spectrum of *Urtica dioica* (hexane crude extract)

The FT-IR Spectroscopic analysis of *Urtica dioica* (Figure 3.29, 3.30, 3.31, 3.32), revealed the presence of alkaloids due to N-H stretch at 3359.8 cm^{-1}, C-N stretch at 1330.8 cm^{-1} and N-H bend at 1654.8 cm^{-1} fingerprint peaks found in primary, secondary and tertiary amines of the ethanol crude extract (Ragavendran, *et al.*,2011., Muruganantham *et al.*, 2009**.,** Donald *et al.*, 2005). The saponins were found to be present due to the presence of C=O stretch at 1735.8, 1743.5cm^{-1} and C-O stretch at 1245.9, 1238.2 cm^{-1} in the dichloromethane and ethyl acetate crude extracts as carboxylic acid anhydrides (Ragavendran, *et al.*,2011., Muruganantham *et al.*, 2009**.,** Donald *et al.*, 2005). The unsaturated aromatic lactones with C=O stretch at1753.8, 1743.5 cm^{-1} and C-O stretch at 1245.9, 1238.2 cm^{-1}, which occurred

either in the Free State or combined with the sugar glucose (Coumarin glycoside) were found to be present in the dichloromethane and ethyl acetate crude extracts. The tannins were present as free phenols with O-H stretch at 3618.2,3622.1 cm^{-1} and C-O stretch at 1245.9, 1238.2, 1377.1 cm^{-1} in the hexane, dichloromethane and ethyl acetate extract (Ragavendran, *et al.*,2011., Muruganantham *et al.*, 2009**.,** Donald *et al.*, 2005). The polyphenols were found to be present with O-H (stretch) at 3460.1, 3467.8, 3359.8 cm^{-1} and C=O (stretch) at 1377.1, 1245.9, 1238.2. 1392.5 cm^{-1} for dichloromethane, ethyl acetate and ethanol extract (Figure 4.30, 4.31, 4.32). Anthraquinones were present as aromatic ethers with C-O stretch at 1245.9, 1238.2 and 1049.2, 1053.1 cm^{-1} in dichloromethane, ethyl acetate and ethanol extract (Figure 3.30, 3.31, 3.32). The esters peak for C=O stretch at 1735.8, 1743.5 cm^{-1} and C-O stretch at 1245.9, 1238.2 cm^{-1} were due to the presence of terpenoids and steroids (Egwaikhide and Gimba, 2007). The presence of quinones revealed that Flavonoids were present with O-H (stretch) at 3363.6 cm^{-1} and C=O (stretch) at 1654.8 cm^{-1} for ethanol extract. The terpenes were absent with no C-H (Stretch), C=C (Stretch) and =C-H (bend) for the analysed extract (Ragavendran, *et al.*,2011., Muruganantham *et al.*, 2009**.,** Donald *et al.*, 2005) . The Cyanogenic glycosides were present due to the presence of C≡N (stretch) at 2368.4, 2314.4, 2376.1, 2248.8 cm^{-1} for hexane, dichloromethane, ethyl acetate and ethanol extract while N≡C (stretch) at 2086.8, 2140.8 cm^{-1} was for Isothiocyanate glycosides for dichloromethane, ethyl acetate and ethanol extract (Skoog *et al.*, 2007). The presence of components C=O stretch at 1735.8, 1243.5 cm^{-1} and C-O stretch at 1245.9, 1238.2 of dichloromethane and ethyl acetate extract, revealed the presence of cardiac glycosides.

4.7.3 *Warburgia ugandensis*

Results of FT- IR spectra hexane extract was recorded in Figure 3.33.

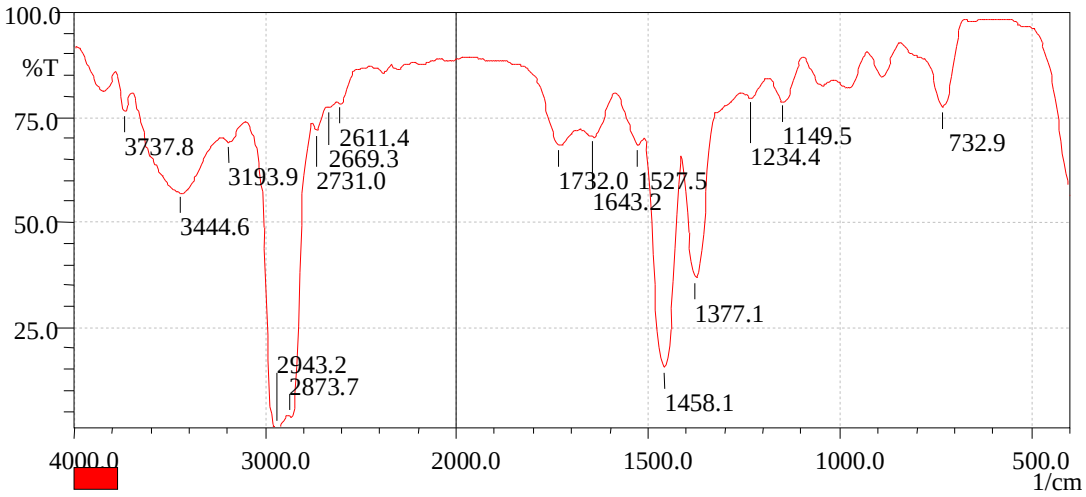


Figure 3.33: FTIR spectrum of *Warburgia ugandensis* (hexane extract)

The FT-IR Spectroscopic analysis of *Warburgia ugandensis* (Figure.3.33, 3.34, 3.35, 3.36), revealed the presence of alkaloids due to N-H stretch at 3444.6, 3440.8, 3460.1, 3359.8 cm^{-1}, C-N stretch at 1253.6, 1242.1. 1377.1, 1330.8 cm^{-1} and N-H bend at 1527.5, 1643.2, 1531.4, 1569.9, 1654.8 cm^{-1} fingerprint peaks found in primary, secondary and tertiary amines of the hexane, dichloromethane, ethyl acetate and ethanol crude extract (Ragavendran, *et al.*,2011., Murugananthan *et al*., 2009**.,** Donald *et al.*, 2005). The saponins were found to be present due to the presence of C=O stretch at 1728.1, 1751.2 cm^{-1} and C-O stretch at 1242.1, 1253.6 cm^{-1} in the dichloromethane and ethyl acetate crude extracts as carboxylic acid anhydrides (Figure 3.34, 3.35). The unsaturated aromatic lactones with C=O stretch at1728.1,

1751.2 cm^{-1} and C-O stretch at 1253.6, 1242.1cm^{-1}, which occurred either in the Free State or combined with the sugar glucose (Coumarin glycoside) were found to be present in the dichloromethane and ethyl acetate crude extracts(Figure 3.34, 3.36). The tannins were absent as free phenols with O-H stretch and C-O stretch in the hexane, dichloromethane, ethyl acetate and ethanol extract. The polyphenols were found to be present with O-H (stretch) at 3444.6, 3440.8, 3460.1, 3359.8 cm^{-1} and C=O (stretch) at 1377.1, 1253.6, 1242.1. 1384.8 cm^{-1} for hexane, dichloromethane, ethyl acetate and ethanol extract (Ragavendran, *et al.*, 2011., Muruganantham *et al.*, 2009**.,** Donald *et al.*, 2005). Anthraquinones were present as aromatic ethers with C-O stretch at 1253.6, 1242.1 and 1049.2, 1053.1 cm^{-1} in dichloromethane, ethyl acetate and ethanol extract. The esters peak for C=O stretch at 1728.1, 1751.2, 1654.8 cm^{-1} and C-O stretch at 1253.6, 1242.1, 1049.2cm^{-1} were due to the presence of terpenoids and steroids for dichloromethane, ethyl acetate and ethanol extract (Egwaikhide and Gimba, 2007). The presence of quinones revealed that Flavonoids were present with O-H (stretch) at 3440.8, 3359.8 cm^{-1} and C=O (stretch) at 1643.2, 1654.8 cm^{-1} for dichloromethane and ethanol extract. The terpenes were absent with no C-H (Stretch), C=C (Stretch) and =C-H (bend) for the analysed extract (Ragavendran, *et al.*,2011., Muruganantham *et al.*, 2009**.,** Donald *et al.*, 2005). The Cyanogenic glycosides were present due to the presence of C≡N (stretch) at 2302.8, 2356.9, 2252.7 cm^{-1} for dichloromethane, ethyl acetate and ethanol extract while N≡C (stretch) at 2090.7, 2137.0 cm^{-1} was for Isothiocyanate glycosides for dichloromethane, ethyl acetate and ethanol extract (Skoog *et al.*, 2007). The presence of components C=O stretch at 1728.1, 1751.2 cm^{-1} and C-O stretch at 1253.6,

1242.1cm^{-1} of dichloromethane and ethyl acetate extract, revealed the presence of cardiac glycosides .

4.7.4 *Senna didymobotrya*

Results of FT- IR spectra hexane extract was recorded in Figure 3.37. The FT-IR Spectroscopic analysis of *Senna didymobotrya* (Figure 3.37, 3.38, 3.39, 3.40), revealed the presence of alkaloids due to N-H stretch at 3448.5, 3618.2, 3363.6 cm^{-1}, C-N stretch at 1253.6, 1257.5, 1049.2 cm^{-1} and N-H bend at 1527.5, 1647.1, 1654.8 cm^{-1} fingerprint peaks found in primary, secondary and tertiary amines of the hexane, dichloromethane and ethanol crude extract (Ragavendran, *et al.*,2011., Muruganantham *et al.*, 2009**.,** Donald *et al.*, 2005).

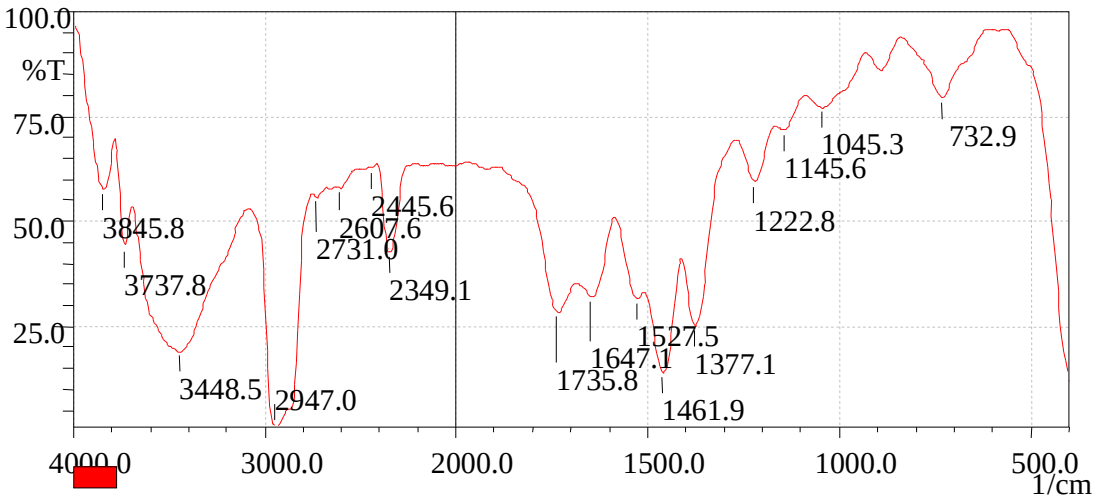


Figure 3.37: FTIR spectrum of *Senna didymobotrya* (hexane extract)

The saponins were found to be present due to the presence of C=O stretch at 1735.8, 1743.5 cm^{-1} and C-O stretch at 1257.5, 1238.2 cm^{-1} in the dichloromethane

and ethyl acetate crude extracts as carboxylic acid anhydrides (Egwaikhide and Gimba, 2007). The unsaturated aromatic lactones with C=O stretch at 1735.8, 1743.5 cm^{-1} and C-O stretch at 1257.5, 1238.2 cm^{-1}, which occurred either in the Free State or combined with the sugar glucose (Coumarin glycoside) were found to be present in the dichloromethane and ethyl acetate crude extracts (Ragavendran, *et al.*,2011., Muruganantham *et al*., 2009**.,** Donald *et al.*, 2005). The tannins were present as free phenols with O-H stretch at 3618.2, 3625.9 cm^{-1} and C-O stretch at 1257.5, 1238.2, 1377.1 cm^{-1} in the dichloromethane and ethyl acetate extract. The polyphenols were found to be present with O-H (stretch) at 3448.5, 3467.8, 3363.6 cm^{-1} and C=O (stretch) at 1377.1, 1238.2, 1388.7 cm^{-1} for hexane, ethyl acetate and ethanol extract (Ragavendran, *et al.*,2011., Muruganantham *et al*., 2009**.,** Donald *et al.*, 2005). Anthraquinones were present as aromatic ethers with C-O stretch at 1257.5, 1238.2 and 1049.2, 1053.1 cm^{-1} in dichloromethane, ethyl acetate and ethanol extract. The esters peak for C=O stretch at 1735.8, 1743.5, 1654.8 cm^{-1} and C-O stretch at 1257.5, 1238.2, 1326.9 cm^{-1} were due to the presence of terpenoids and steroids for dichloromethane, ethyl acetate and ethanol extract (Skoog *et al.*, 2007). The presence of quinones revealed that Flavonoids were present with O-H (stretch) at 3363.6 cm^{-1} and C=O (stretch) at 1654.8 cm^{-1} for ethanol extract. The terpenes were absent with no C-H (Stretch), C=C (Stretch) and =C-H (bend) for the analysed extract. The Cyanogenic glycosides were present due to the presence of C≡N (stretch) at 2349.1, 2349.1, 2376.1, 2248.8 cm^{-1} for hexane, dichloromethane, ethyl acetate and ethanol extract while N≡C (stretch) at 2090.7, 2137.0 cm^{-1} was for Isothiocyanate glycosides for ethyl acetate and ethanol extract (Ragavendran, *et al.*,2011., Muruganantham *et al*., 2009**.,** Donald *et al.*, 2005). The presence of

components C=O stretch at 1735.8, 1743.5 cm^{-1} and C-O stretch at 1257.5, 1238.2cm^{-1} of dichloromethane and ethyl acetate extract, revealed the presence of cardiac glycosides .

4.7.5 *Physalis peruviana*

Results of FT- IR spectra hexane extract was recorded in Figure 3.41.

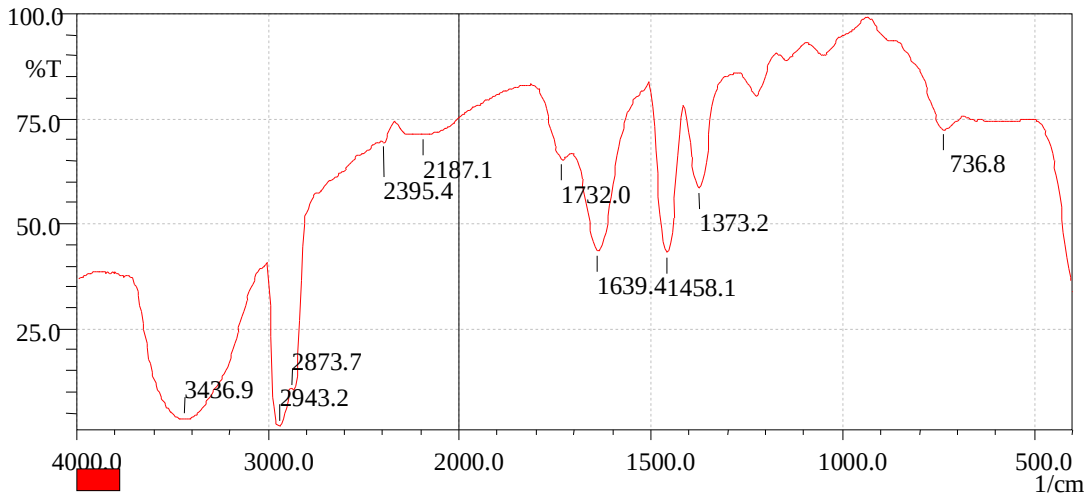


Figure 3.41: FTIR spectrum of *Physalis peruviana* (hexane crude extract)

The FT-IR Spectroscopic analysis of *Physalis peruviana*, (Figure 3.41, 3.42 3.43, 3.44). revealed the presence of aromatic compounds with C-H (stretch) at 3066.6 cm^{-1}, C=C (stretch) at 1639.4 cm^{-1}, and C-H (bend) at 732.9, 887.2 cm^{-1} in the dichloromethane extract (Ragavendran, *et al.*,2011., Muruganantham *et al.*, 2009**.,** Donald *et al.*, 2005). The alkaloids were present due to N-H stretch at 3440.8, 3541.1, 3463.9, 3363.6 cm^{-1}, C-N stretch at 1265.2, 1245.9, 1049.2,1064.6, 1326.9 cm^{-1} and N-H bend at 1639.4, 1554.5, 1654.8 cm^{-1} fingerprint peaks found in

primary, secondary and tertiary amines of the dichloromethane, ethyl acetate and ethanol crude extract(Figure 3.42, 3.43, 3.44). The saponins were found to be present due to the presence of C=O stretch at 1739.7cm^{-1} and C-O stretch at 1245.9 cm^{-1} in the ethyl acetate crude extracts as carboxylic acid anhydrides (Figure 3.43). The unsaturated aromatic lactones with C=O stretch at 1739.7 cm^{-1} and C-O stretch at 1245.9 cm $^{-1}$, which occurred either in the Free State or combined with the sugar glucose (Coumarin glycoside) were found to be present in the ethyl acetate crude extracts (Egwaikhide and Gimba, 2007). The tannins were present as free phenols with O-H stretch at 3625.9 cm^{-1} and C-O stretch at 1245.9, 1377.1 cm^{-1} in the ethyl acetate extract (Ragavendran, *et al.*,2011., Muruganantham *et al.*, 2009**.,** Donald *et al.*, 2005). The polyphenols were found to be present with O-H (stretch) at 3436.9, 3440.8, 3463.9, 3363.6 cm^{-1} and C=O (stretch) at 1373.2, 1265.2, 1245.9, 1377.1, 1326.9 cm^{-1} for hexane, dichloromethane, ethyl acetate and ethanol extract. Anthraquinones were present as aromatic ethers with C-O stretch at 1265.2, 1245.9 and 1049.2, 1064.6 cm^{-1} in dichloromethane, ethyl acetate and ethanol extract (Skoog *et al*., 2007). The esters peak for C=O stretch at 1739.7 cm^{-1} and C-O stretch at 1245.9 cm^{-1} were due to the presence of terpenoids and steroids for ethyl acetate extract. The presence of quinones revealed that Flavonoids were present with O-H (stretch) at 3436.9, 3440.8, 3363.6 cm^{-1} and C=O (stretch) at 1639.4, 1654.8 cm^{-1} for hexane, dichloromethane and ethanol extract. The terpenes were present with C-H (Stretch) at 3066.6 cm^{-1}, C=C (Stretch) at 1639.4 cm^{-1} and =C-H (bend) at 1431.1 cm^{-1}for the dichloromethane extract (Ragavendran, *et al.*,2011., Muruganantham *et al.*, 2009**.,** Donald *et al.*, 2005). The Cyanogenic glycosides were present due to the presence of C≡N (stretch) at 2395.4 cm^{-1} for hexane extract while N≡C (stretch) at

2187.1cm^{-1} was for Isothiocyanate glycosides for hexane extract (Fig.4.41). The presence of components C=O stretch at 1739.7 cm^{-1} and C-O stretch at 1245.9 cm^{-1} of ethyl acetate extract, revealed the presence of cardiac glycosides .

4.7.6 *Bidens pilosa*

Results of FT- IR spectra hexane extract was recorded in Figure 3.45.

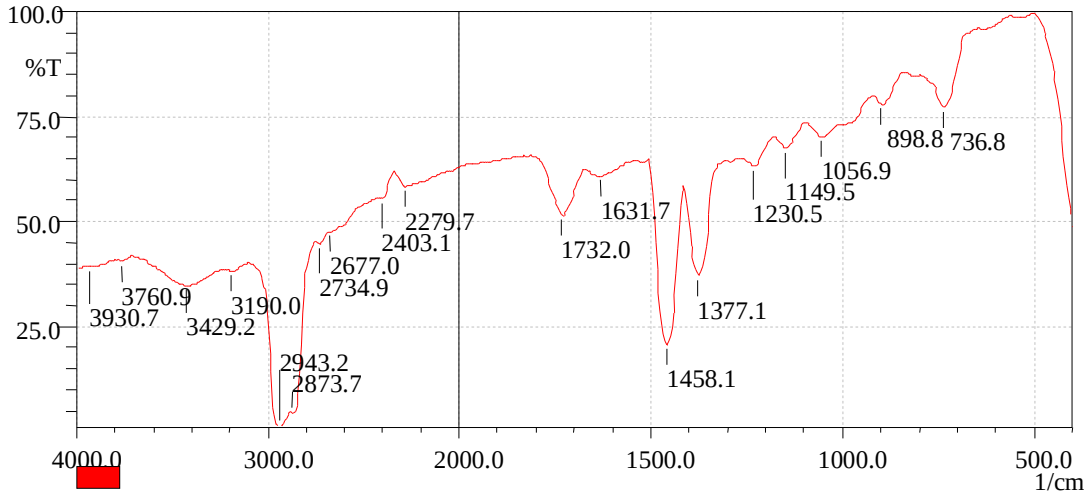


Figure 3.45: FTIR spectrum of *Bidens pilosa* (hexane crude extract)

The FT-IR Spectroscopic analysis of *Bidens pilosa* (Figure 3.45, 3.46, 3.47). , revealed the presence of aromatic compounds with C-H(stretch) at 3055.0 cm^{-1}, C=C (stretch) at 1612.4cm^{-1}, and C-H (bend) at 732.9, 898.8 cm^{-1} in the dichloromethane extract (Ragavendran, *et al.*,2011., Muruganantham *et al.*, 2009**.,** Donald *et al.*, 2005). The alkaloids were present due to N-H stretch at 3402.2, 3463.9, 3367.5 cm^{-1}, C-N stretch at 1377.1, 1265.2, 1245.9, 1330.8, 1064.6, 1049.2 cm^{-1} and N-H bend at 1612.4, 1651.0, 1558.4 cm^{-1} fingerprint peaks found in primary, secondary and

tertiary amines of the dichloromethane, ethyl acetate and ethanol crude extract (Figure 3.47, 3.46, 3.47). The saponins were found to be present due to the presence of C=O stretch at 1735.5 cm^{-1} and C-O stretch at 1245.9 cm^{-1} in the ethyl acetate crude extracts as carboxylic acid anhydrides(Figure 3.46). The unsaturated aromatic lactones with C=O stretch at1735.8 cm^{-1} and C-O stretch at 1245.9 cm^{-1}, which occurred either in the Free State or combined with the sugar glucose (Coumarin glycoside) were found to be present in the ethyl acetate crude extracts (Egwaikhide and Gimba, 2007). The tannins were absent as free phenols with O-H stretch at 3683.8 cm^{-1} and C-O stretch at1265.2 cm^{-1} in the dichloromethane extracted. The polyphenols were found to be present with O-H (stretch) at 3429.2, 3402.2, 3463.9, 3367.5 cm^{-1} and C=O (stretch) at 1377.1, 1265.2, 1245.9. 1396.8 cm^{-1} for hexane, dichloromethane, ethyl acetate and ethanol extract (Ragavendran, *et al.,*2011., Muruganantham *et al*., 2009**.,** Donald *et al*., 2005). Anthraquinones were present as aromatic ethers with C-O stretch at 1265.2, 1245.9 and 1049.2, 1064.6 cm^{-1} in dichloromethane, ethyl acetate and ethanol extract. The esters peak for C=O stretch at 1735.8cm^{-1} and C-O stretch at 1245.9 cm^{-1} were due to the presence of terpenoids and steroids for dichloromethane, ethyl acetate and ethanol extract (Skoog *et al*., 2007). The presence of quinones revealed that Flavonoids were present with O-H (stretch) at 3367.5 cm^{-1} and C=O (stretch) at 1651.0 cm^{-1} for ethanol extract. The terpenes were present with C-H (Stretch) at 3190.0, 3055.0 cm^{-1}, C=C (Stretch) at 1631.7, 1612.4 cm^{-1} and =C-H (bend) at 1458.1, 1431.1, 1319.2 cm^{-1} for hexane and dichloromethane extract (Ragavendran, *et al.,*2011., Muruganantham *et al*., 2009**.,** Donald *et al.,* 2005). The Cyanogenic glycosides were present due to the presence of C≡N (stretch) at 2303.1, 2279.7, 2302.8 cm^{-1} for hexane and

dichloromethane extract while N≡C (stretch) at 2144.7 cm^{-1} was for Isothiocyanate glycosides for dichloromethane extract (Figure 3.45). The presence of components C=O stretch at 1735.8cm^{-1} and C-O stretch at 1245.9 cm^{-1} of ethyl acetate extract, revealed the presence of cardiac glycosides.

4.7.7 *Leonotis nepetifolia*

Results of FT- IR spectra hexane extract was recorded in Figure 3.48.

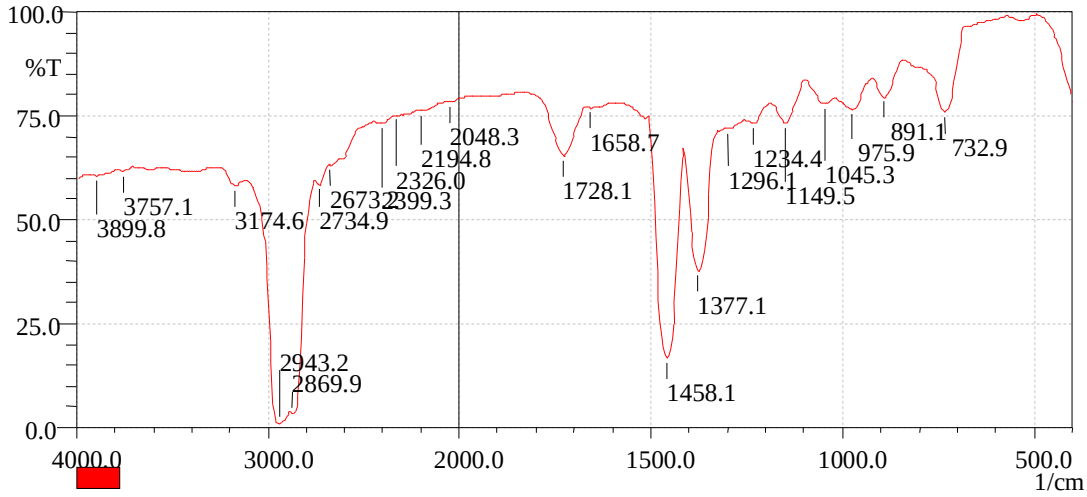


Figure 3.48: FTIR spectrum of *Leonotis nepetifolia* (hexane extract)

The FT-IR Spectroscopic analysis of *Leonotis nepetifolia* (Figure 3.48, 3.49, 3.50, 3.51)., revealed the presence of aromatic compounds with C-H stretch at 3058.9 cm^{-1}, C=C stretch at 1554.5, 1624.0 cm^{-1}, and C-H (bend) at 736.8, 898.8 cm^{-1} in the dichloromethane extract (Ragavendran, *et al.*,2011., Muruganantham *et al*., 2009**.,** Donald *et al*., 2005). The alkaloids were present due to N-H stretch at 3452.3, 3460.1, 3394.5 cm^{-1}, C-N stretch at 1265.2, 1242.1, 1377.1, 1326.9, 1049.2 cm^{-1} and

N-H bend at 1554.5, 1624.0, 1647.1 cm^{-1} fingerprint peaks found in primary, secondary and tertiary amines of the ethanol crude extract (Skoog *et al*., 2007) . The saponins were found to be present due to the presence of C=O stretch at 1743.5, 1647.1cm^{-1} and C-O stretch at 1242.1, 1326.9, 1060.8 cm^{-1} in the ethyl acetate and ethanol crude extracts as carboxylic acid anhydrides (Figure 3.50, 3.51). The unsaturated aromatic lactones with C=O stretch at1743.5 cm^{-1} and C-O stretch at 1242.1, cm^{-1}, which occurred either in the Free State or combined with the sugar glucose (Coumarin glycoside) were found to be present in the ethyl acetate crude extracts (Egwaikhide and Gimba, 2007). The tannins were present as free phenols with O-H stretch at 3679.9 cm^{-1} and C-O stretch at 1265.2 cm^{-1} in the dichloromethane extract (Ragavendran, *et al.*,2011., Muruganantham *et al*., 2009**.,** Donald *et al.*, 2005) . The polyphenols were found to be present with O-H (stretch) at 3174.6, 3452.3, 3460.1, 3394.5 cm^{-1} and C=O (stretch) at 1377.1, 1265.2, 1242.1, 1396.4 cm^{-1} for hexane, dichloromethane, ethyl acetate and ethanol extract (Ragavendran, *et al.*,2011., Muruganantham *et al.*, 2009**.,** Donald *et al.*, 2005). Anthraquinones were present as aromatic ethers with C-O stretch at 1265.2, 1242.1 and 1049.2, 1060.8 cm^{-1} in dichloromethane, ethyl acetate and ethanol extract. The esters peak for C=O stretch at 1743.5 cm^{-1} and C-O stretch at 1242.1cm^{-1} were due to the presence of terpenoids and steroids. The presence of quinones revealed that Flavonoids were present with O-H (stretch) at 3460.1, 3394.5 cm^{-1} and C=O (stretch) at 1643.2, 1647.1 cm^{-1} for ethyl acetate and ethanol extract. The terpenes were present with C-H (Stretch) at 3174.6, 3058.9 cm^{-1}, C=C (Stretch) at 1658.7, 1624.0 cm^{-1} and =C-H (bend) at 1458.1, 1431.1, 975.9, 891.1, 898.8 cm^{-1} for the hexane and dichloromethane extract (Skoog *et al*., 2007). The Cyanogenic glycosides were

present due to the presence of C≡N (stretch) at 2399.3, 2302.8, cm^{-1} for hexane and dichloromethane extract while N≡C (stretch) at 2094.8, 2144.7, 2086.8 cm^{-1} was for Isothiocyanate glycosides for hexane, dichloromethane and ethyl acetate extract (Ragavendran, *et al.*,2011., Muruganantham *et al.*, 2009**.,** Donald *et al.*, 2005). The presence of components C=O stretch at 1743.5 cm^{-1} and C-O stretch at 1242.1 $cm^{-1}$of ethyl acetate extract, revealed the presence of cardiac glycosides.

4.7.8 *Toddalia asiatica*

Results of FT- IR spectra hexane extract was recorded in Figure 3.52.

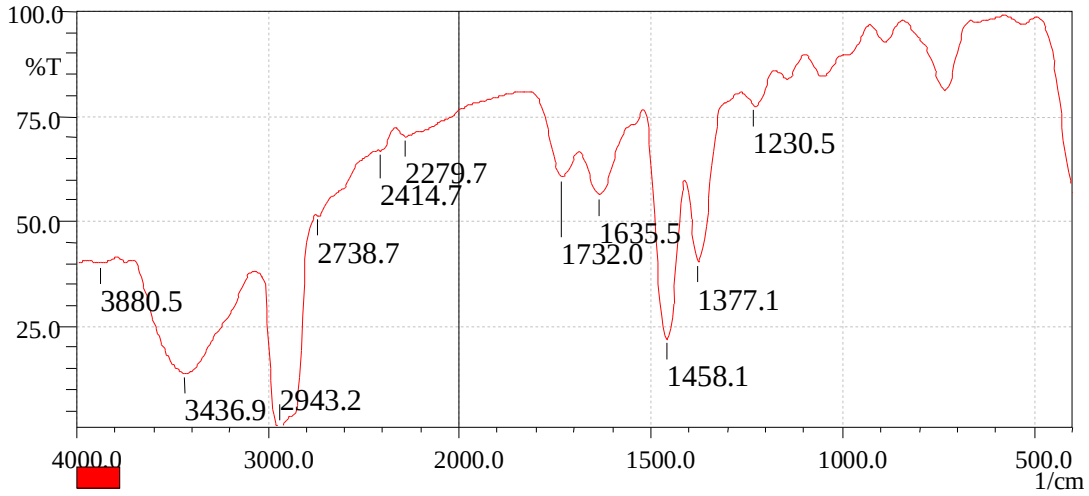


Figure 3.52: FTIR spectrum of *Toddalia asiatica* (hexane crude extract)

The FT-IR Spectroscopic analysis of *Toddalia asiatica* (Figure 3.52, 3.53, 3.54, 3.55)., revealed the presence of aromatic compounds with C-H stretch at 3058.9 cm^{-1}, C=C stretch at 1635.5 cm^{-1} and C-H (bend) at 732.9, 894.9 cm^{-1} in the

dichloromethane extract (Donald *et al.,* 2005., Muruganantham *et al.,* 2009**.,** Ragavendran, *et al.*,2011). The alkaloids were present due to N-H stretch at 3444.6, 3456.2, 3363.6 cm^{-1}, C-N stretch at 1265.2, 1245.9, 1330.8, 1049.2 , 1064.6 cm^{-1} and N-H bend at 1635.5, 1643.2, 1651.0cm^{-1} fingerprint peaks found in primary, secondary and tertiary amines of the dichloromethane, ethyl acetate and ethanol crude extract (Donald *et al.,* 2005., Muruganantham *et al.,* 2009**.,** Ragavendran, *et al.*,2011). The saponins were found to be present due to the presence of C=O stretch at 1712.7, 1735.8cm^{-1} and C-O stretch at 1265.2, 1245.9 cm^{-1} in the dichloromethane and ethyl acetate crude extracts as carboxylic acid anhydrides (Egwaikhide and Gimba, 2007). The unsaturated aromatic lactones with C=O stretch at1712.7, 1735.8 cm^{-1} and C-O stretch at 1265.2, 1245.9 cm^{-1}, which occurred either in the Free State or combined with the sugar glucose (Coumarin glycoside) were found to be present in the dichloromethane and ethyl acetate crude extracts (Figure 3.53, 3.54). The tannins were absent as free phenols with O-H stretch and C-O stretch in the hexane, dichloromethane, ethyl acetate extract (Skoog *et al*., 2007). The polyphenols were found to be present with O-H (stretch) at 3436.9, 3444.6, 3456.2, 3363.6 cm^{-1} and C=O (stretch) at 1377.1, 1365.5, 1265.2, 1245.9. 1396.4, 1064.6 cm^{-1} for hexane, dichloromethane, ethyl acetate and ethanol extract (Donald *et al.,* 2005., Muruganantham *et al*., 2009**.,** Ragavendran, *et al.*,2011). Anthraquinones were present as aromatic ethers with C-O stretch at 1265.2, 1245.9 and 1049.2, 1064.6 cm^{-1} in dichloromethane, ethyl acetate and ethanol extract. The esters peak for C=O stretch at 1712.7, 1735.8 cm^{-1} and C-O stretch at 1265.2, 1245.9 cm^{-1} were due to the presence of terpenoids and steroids in the dichloromethane and ethyl acetate extract (Skoog *et al.,* 2007). The presence of quinones revealed that

Flavonoids were present with O-H (stretch) at 3444.6, 3456.2, 3363.6 cm^{-1} and C=O (stretch) at 1635.5, 1643.2, 1651.0 cm^{-1} for dichloromethane, ethyl acetate and ethanol extract (Ragavendran, *et al.*,2011., Muruganantham *et al*., 2009**.,** Donald *et al.,* 2005). The terpenes were present with C-H (Stretch) at 3058.9 cm^{-1}, C=C (Stretch) at 1635.5 cm^{-1} and =C-H (bend) at 1431.1 cm^{-1} for the dichloromethane extract. The Cyanogenic glycosides were present due to the presence of C≡N (stretch) at 2279.7 cm^{-1} for hexane extract while N≡C (stretch) at 2086.8 cm^{-1} was for Isothiocyanate glycosides for ethyl acetate extract (Ragavendran, *et al.*,2011., Muruganantham *et al*., 2009**.,** Donald *et al.,* 2005). The presence of components C=O stretch at 1712.7, 1735.8 cm^{-1} and C-O stretch at 1265.2, 1245.9 $cm^{-1}$of dichloromethane and ethyl acetate extract, revealed the presence of cardiac glycosides.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusions

The pH level of the herbs studied was determined and found to be within the acceptable range set by other countries like Egypt, Canada, U.S.A., China, and Malaysia among others. However, the usage of these herbs for treatment is not safe because heavy metals are present and therefore overdoses or prolonged ingestion of medicinal herbs leads to the chronic accumulation of different elements which causes various health problems.

The bioactive compounds present in the selected herbs are: cardiac glycoside, Alkaloids, saponins, tannins, flavonoids, steroids, terpenoids and polyphenols. These compounds are responsible for the biological activity (antimicrobial and antifungal activity) and other medicinal values of these herbs.

The heavy metals and their level of concentration in the selected herbs were determined. The heavy metals present in the herbs are: iron, chromium, copper, zinc, cobalt, manganese. nickels, lead, cadmium and mercury. The heavy metal levels in these selected herbs were below the maximum permissible limits set by FAO/WHO.

The retention factor, R_f of some compounds present in the selected herbs was determined. Each phytochemical (pure natural compound) has a specific R_f value and their number was determined.

The selected eight herbs have biological activity against studied microorganisms. The *E. coli* was found to be the most insensitive strain of the two bacteria to the herbal extracts used. Among the tested microbial strains, gram positive bacteria was found to be more sensitive to the herbal extracts than gram negative bacteria and the

fungus. The antibacterial activity was more pronounced on gram positive bacteria (*S. aureus*) than the gram negative bacteria (*E. coli*).

The functional groups present in the herbs tested are : -OH, aliphatic CH_2 and CH_3 groups, primary, secondary and tertiary amines, amides, carboxylic anhydrides, carboxylic acids, nitriles, isonitriles, lactones, ketones, aldehydes, aromatics, ethers, phenols, quinones, conjugated C=C and C=O, C-O, C-N, C≡N, N≡C. This could confirm the presence of cardiac glycosides, alkaloids, saponins, tannins, flavonoids, steroids and terpenoids, polyphenols and anthraquinones as found in the phytochemical screening. The fingerprints of bioactive compounds present in the selected herbs were determined. The presence of C=O, C-H, C=C and C-O bond stretching compounds are responsible for the biological activity and other medicinal values of these selected herbs.

These findings indicated that the selected herbs have a pontential in controlling gram positive bacteria *Staphylococcus aureus,* gram negative bacteria *Escherichia coli* and fungus *Candida albicans.* The knowledge acquired if incorporated with the one of the traditional healers can improve the use of traditional herbs.

5.2 Recommendations

1. The crude extracts of the eight selected herbs was found to inhibit the growth of *Escherichia coli, Staphylococcus aureus,* and *Candida albicans* considerably; minimum bacterialcidal concentrations (MBC) must be performed on the herb to ascertain whether it is just inhibitory or can also kill these organisms and other organisms.
2. Further investigations on herbal extracts with the aim of isolating the active compound(s) and standardize the extracts.

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APPENDICES

Appendix I Chemicals prepared for phytochemical screening

The methods for the preparation of the various reagents used in the phytochemical screening are outlined in the section

1. Lead acetate solution 13.9g of Pb $(C_2H_3O_2)_2.3H_2O$ was dissolved to make up to 100ml of solution

2. Ferric chloride solution 13.5g of $FeCl_3.6H_2O$ was dissolved in 100ml of distilled water containing 20ml conc. HCl.

3. Potassium hydroxide solution 0.56g was dissolved in 100ml distilled water and filled to1 litre mark.

Appendix II TLC Tables of medicinal herbs

Table 3.6: The TLC for dichloromethane extracts.

Solvent system Dichloromethane/Hexane, (90:10 v/v)	**Herbs**	**No of spots visible by UV**	**R_f value = distance moved by sample/distance moved by solvent**
	Carissa spinarum	3	0.0125 0.0375 0.1125
	Urtica dioica	3	0.0375 0.05 0.1375
	Warburgia ugandensis	5	0.025 0.0625 0.125 0.15 0.2373
	Senna didymobotrya	4	0.0292 0.0588 0.07353 0.1324
	Physalis peruviana	3	0.0294 0.05882 0.1177
	Bidens pilosa	4	0.04412 0.07353 0.125 0.4265
	Leonotis nepetifolia	3	0.04412 0.0625

			0.1324
	Toddalia asiatica	3	0.0294 0.05882 0.01324

Table 3.7 The TLC for ethyl acetate extracts.

Solvent system Ethyl acetate/dichloromethane, (90:10 v/v)	**Ethyl acetate extract**	**No of spots visible by UV**	**R_f value = distance moved by sample/distance moved by solvent**
	Carissa spinarum	2	0.0294 0.1618
	Urtica dioica	1	0.0294
	Warburgia ugandensis	1	0.2294
	Senna didymobotrya	7	0.0294 0.0375 0.10294 0.1912 0.7353 0.8235 0.9265
	Physalis peruviana	2	0.0294 0.1618

	Bidens pilosa	4	0.0294 0.08824 0.20588 0.4706
	Leonotis nepetifolia	4	0.0294 0.07353 0.19118 0.8824
	Toddalia asiatica	5	0.0294 0.10294 0.22059 0.83824

Table 3.8: The TLC for ethanol extracts.

Solvent system Ethanol/Hexane, (90 :10)	**Ethanol extract**	**No of spots visible by UV**	**R_f value = distance moved by sample/distance moved by solvent**
	Carissa spinarum	1	0.058824
	Urtica dioica	3	0.0588224 0.6706 0.835294
	Warburgia ugandensis	2	0.04706 0.84706
	Senna didymobotrya	4	0.035294 0.705882 0.811765 0.82353
	Physalis peruviana	3	0.04706 0.82353 0.835294
	Bidens pilosa	3	0.04706 0.70588 0.8471
	Leonotis nepetifolia	3	0.035294 0.71765 0.87059
	Toddalia asiatica	3	0.058823 0.0705882 0.8824

Appendix III FTIR spectrum of medicinal herbs

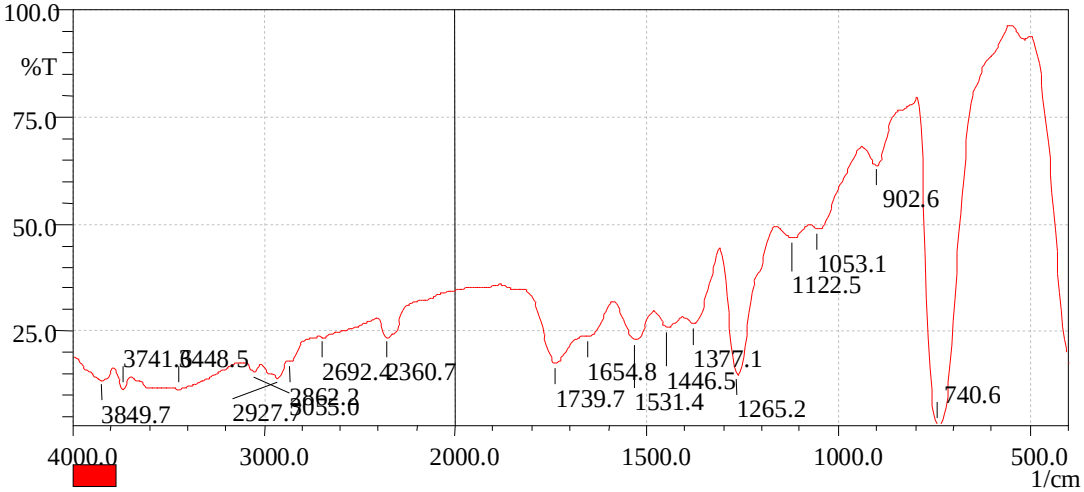


Figure 3.26: FTIR spectrum of *Carissa spinarum* (dichloromethane extract)

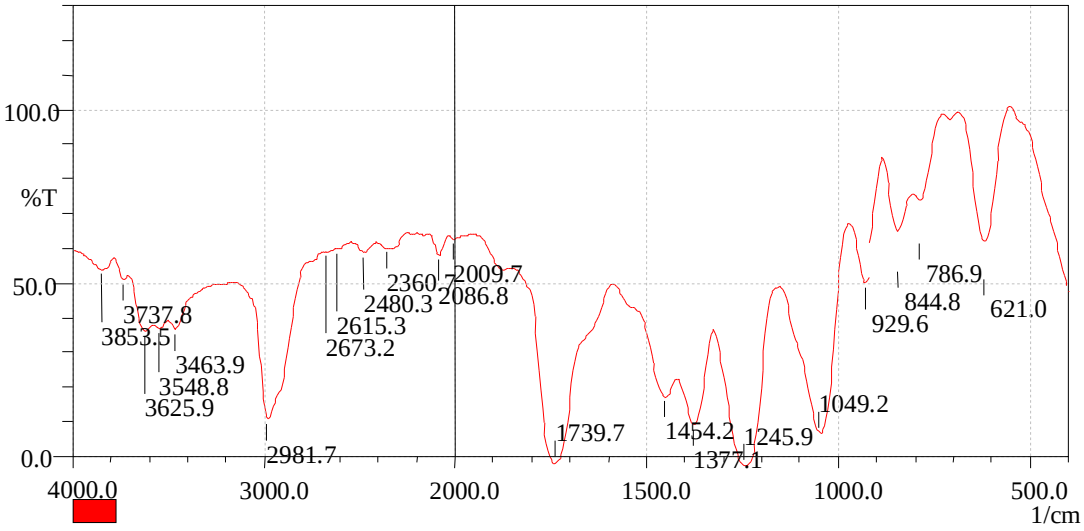
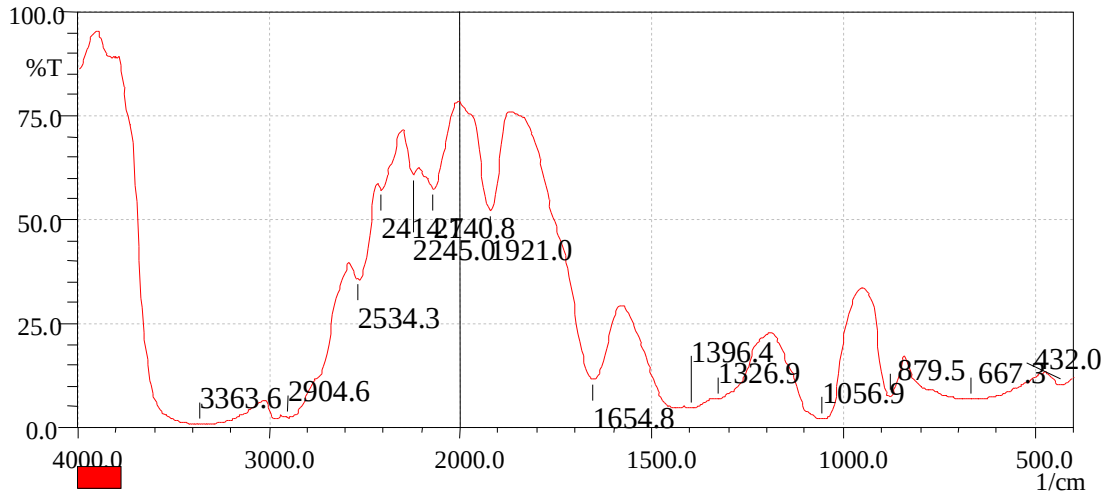


Figure 3.27: FTIR spectrum of *Carissa spinarum* (ethyl acetate crude extract)



Lsn fLine

Figure 3.28: FTIR spectrum of *Carissa spinarum* (ethanol crude extract)

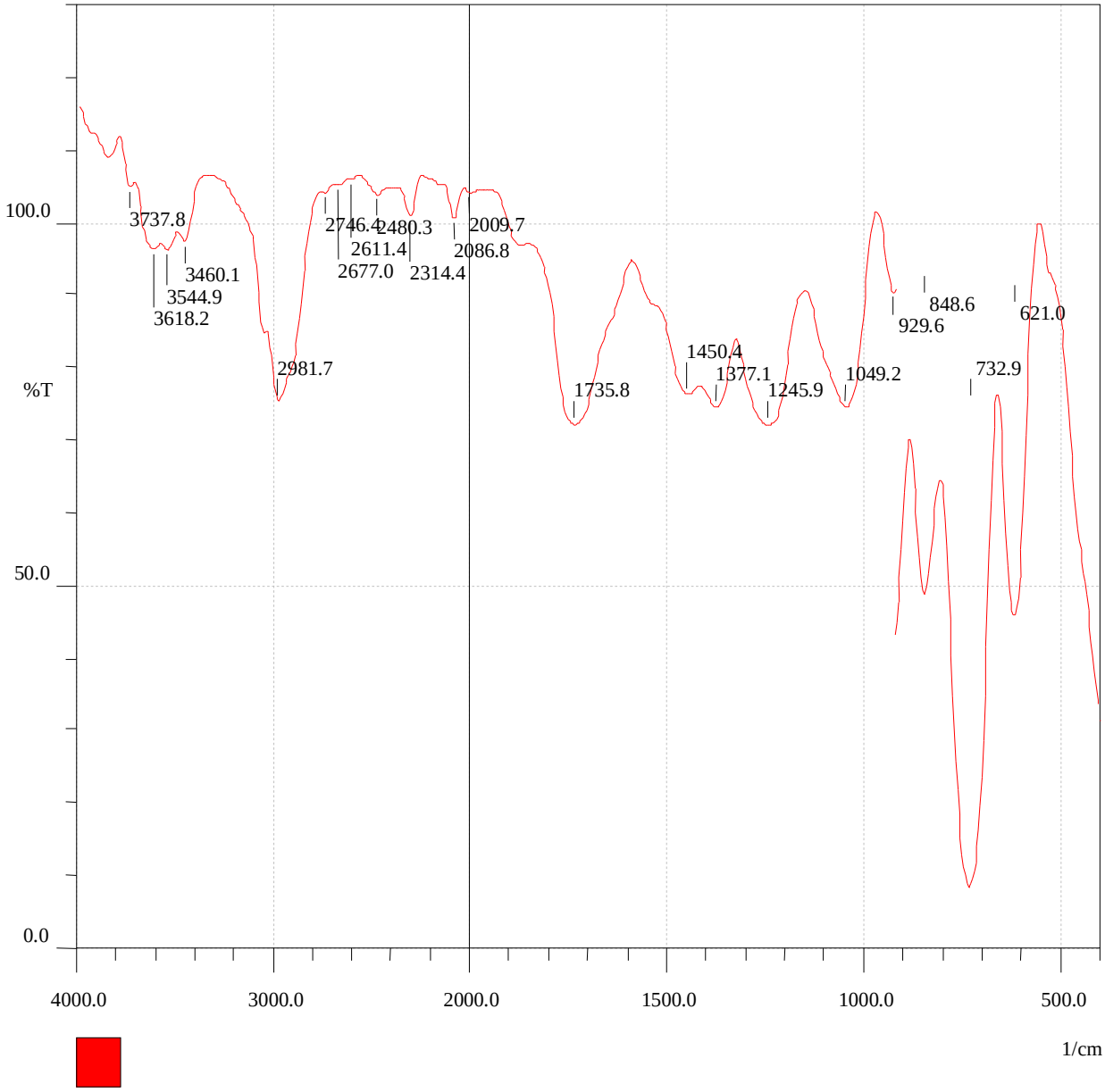

100.0
50.0
0.0
%T
3737.8
3618.2
3544.9
3460.1
2981.7
2746.4
2677.0
2611.4
2480.3
2314.4
2086.8
2009.7
1735.8
1450.4
1377.1
1245.9
1049.2
929.6
848.6
732.9
621.0
4000.0
3000.0
2000.0
1500.0
1000.0
500.0
1/cm

Figure 3.30: FTIR spectrum of *Urtica dioica* (dichloromethane extract)

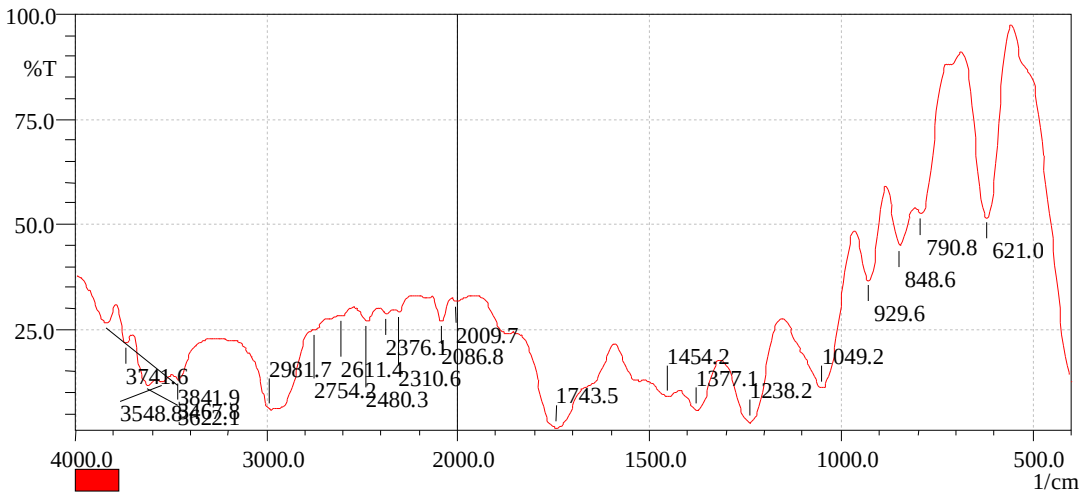


Figure 3.31: FTIR spectrum of *Urtica dioica* (ethyl acetate extract)

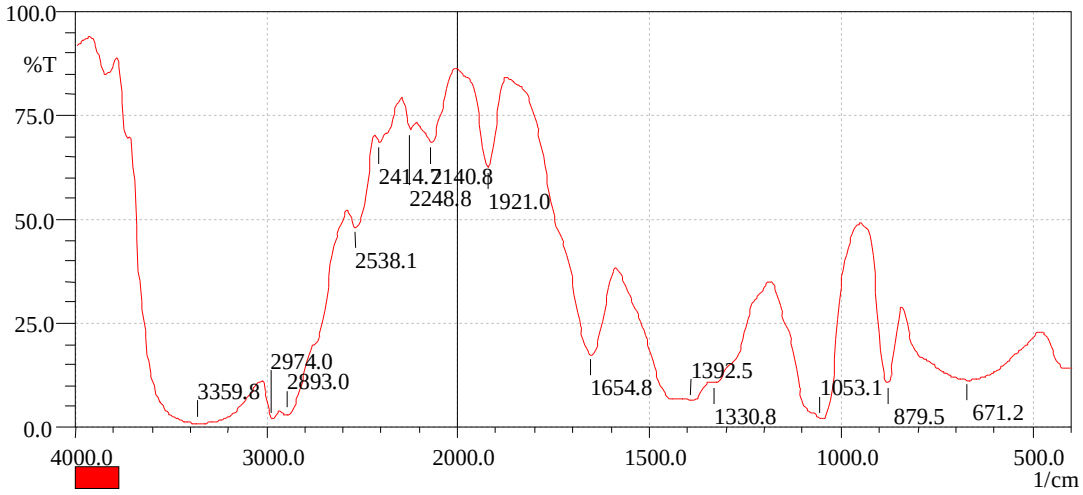


Figure 3.32: FTIR spectrum of *Urtica dioica* (ethanol crude extract)

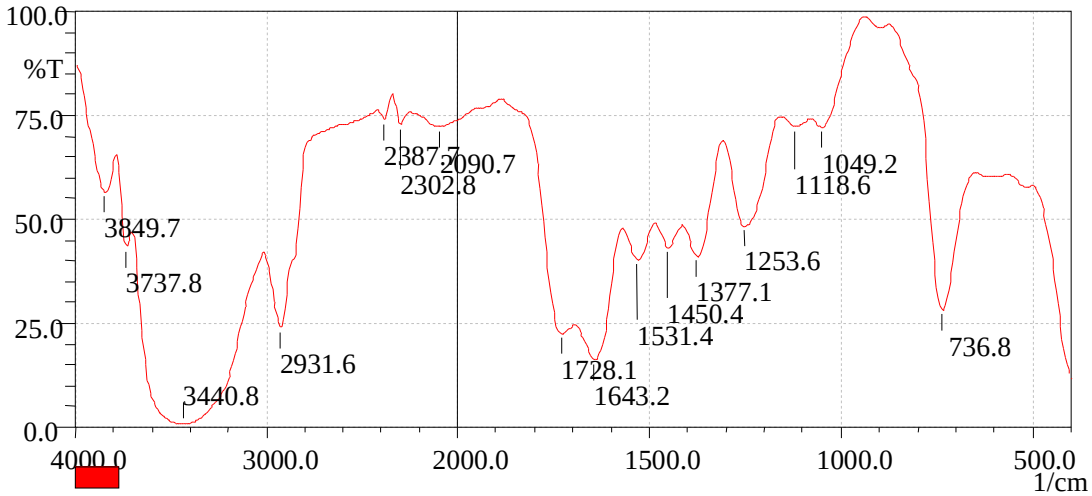


Figure 3.34: FTIR spectrum of *Warburgia ugandensis* (dichloromethane extract)

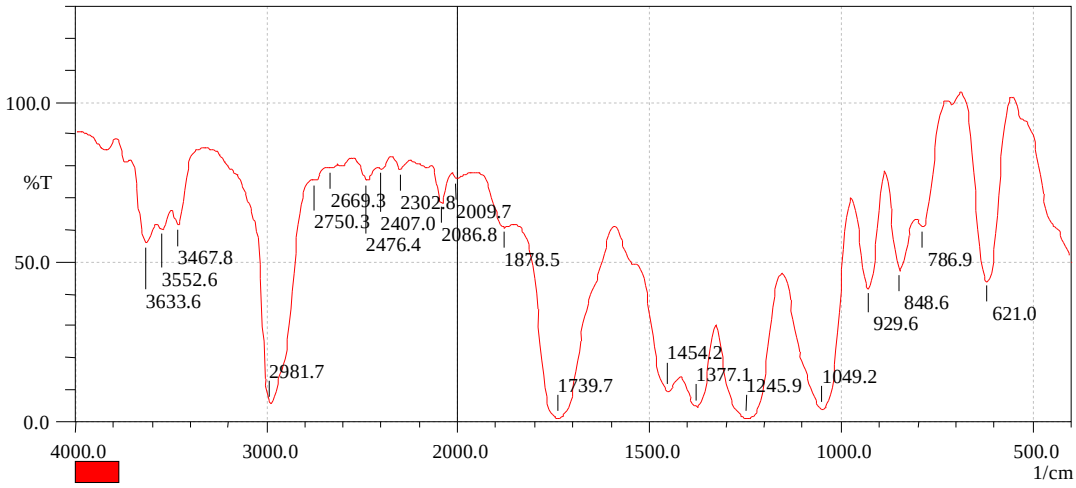


Figure 3.35: FTIR spectrum of *Warburgia ugandensis* (ethyl acetate extract)

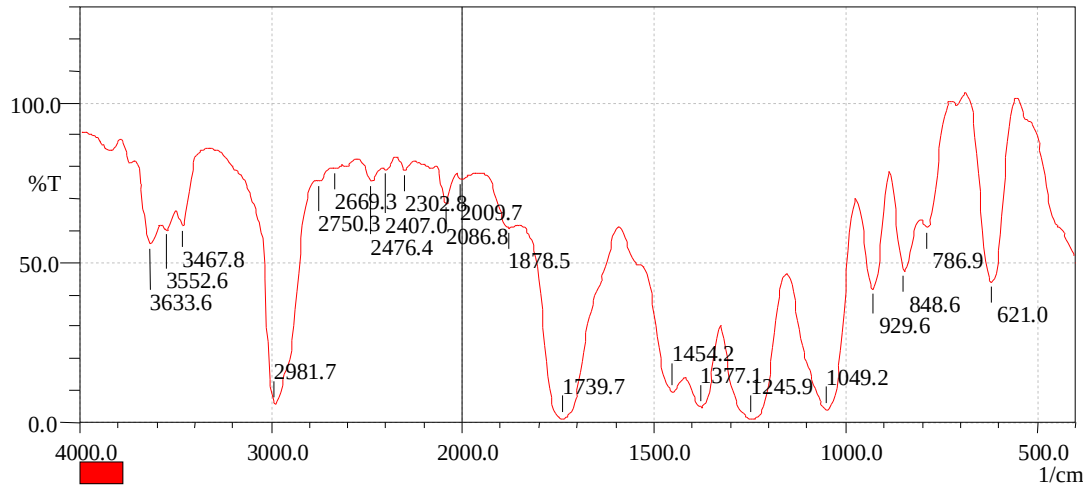


Figure 3.36: FTIR spectrum of *Warburgia ugandensis* (ethanol extract)

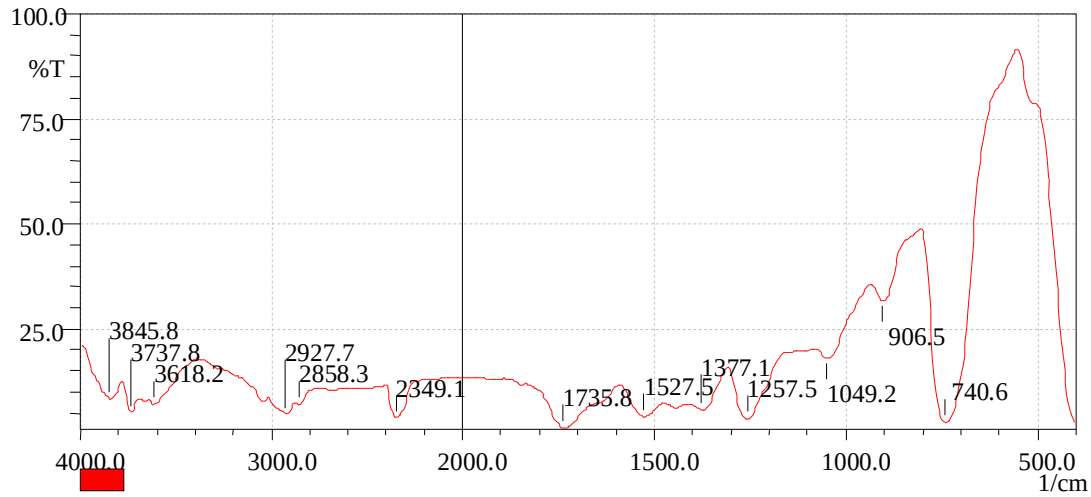


Figure 3.38: FTIR spectrum of *Senna didymobotrya* (dichloromethane extract)

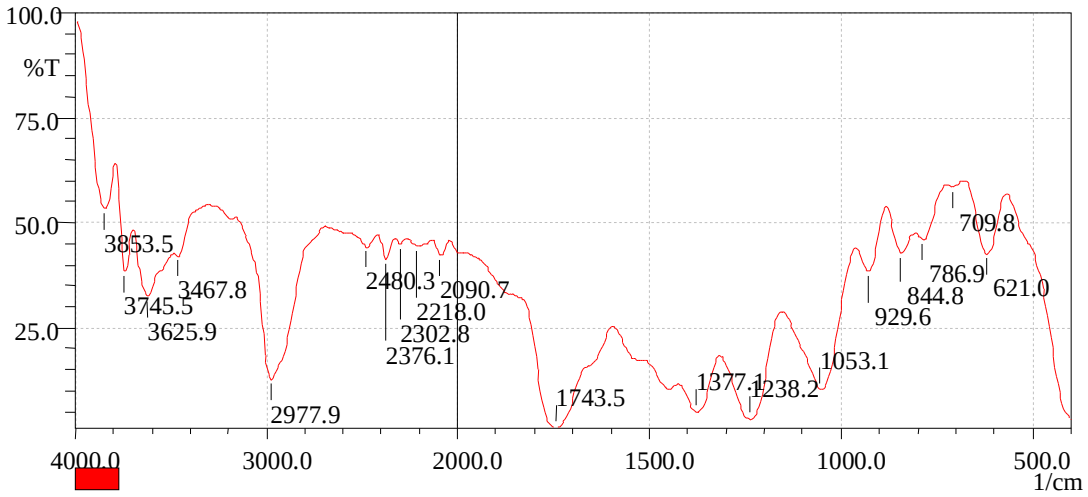


Figure 3.39: FTIR spectrum of *Senna didymobotrya* (ethyl acetate extract)

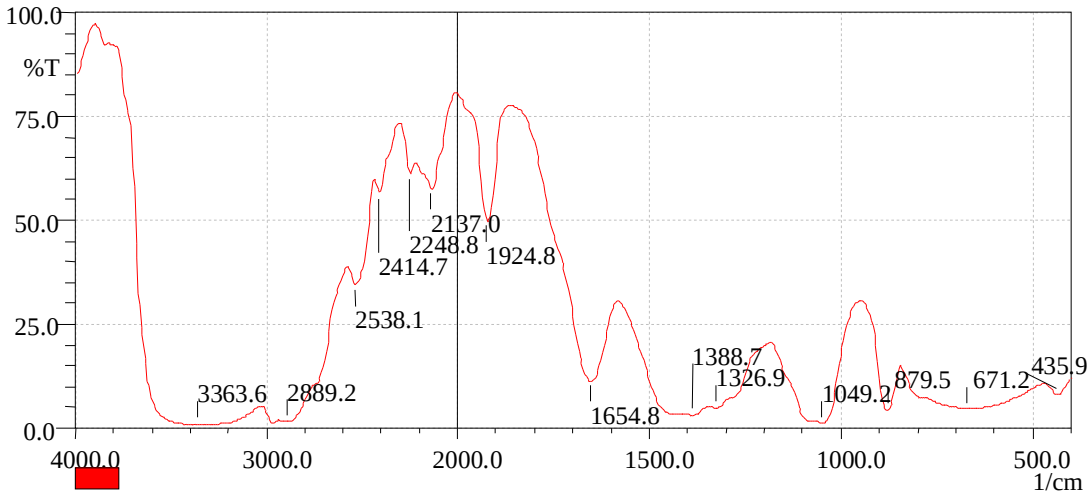


Figure 3.40: FTIR spectrum *Senna didymobotrya* (ethanol extract)

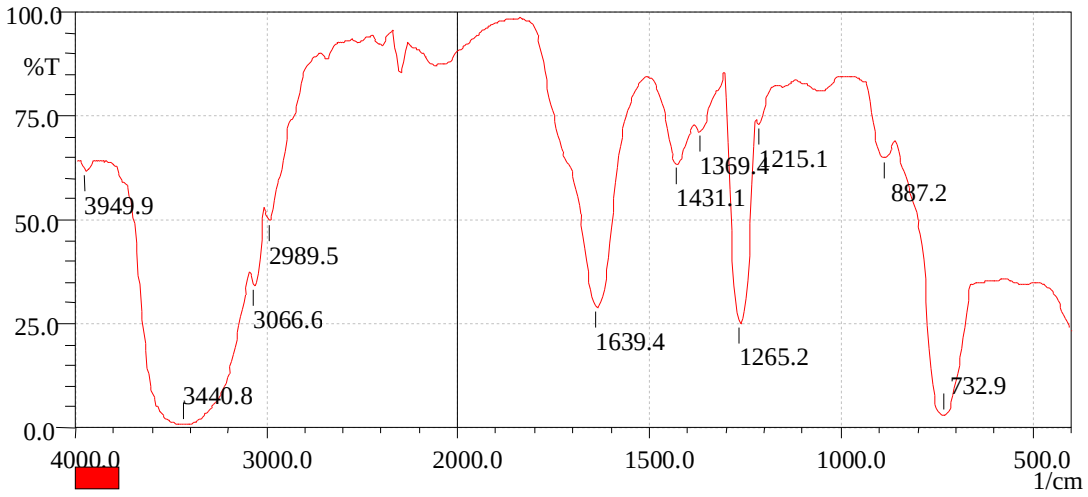


Figure 3.42: FTIR spectrum of *Physalis peruviana* (dichloromethane extract)

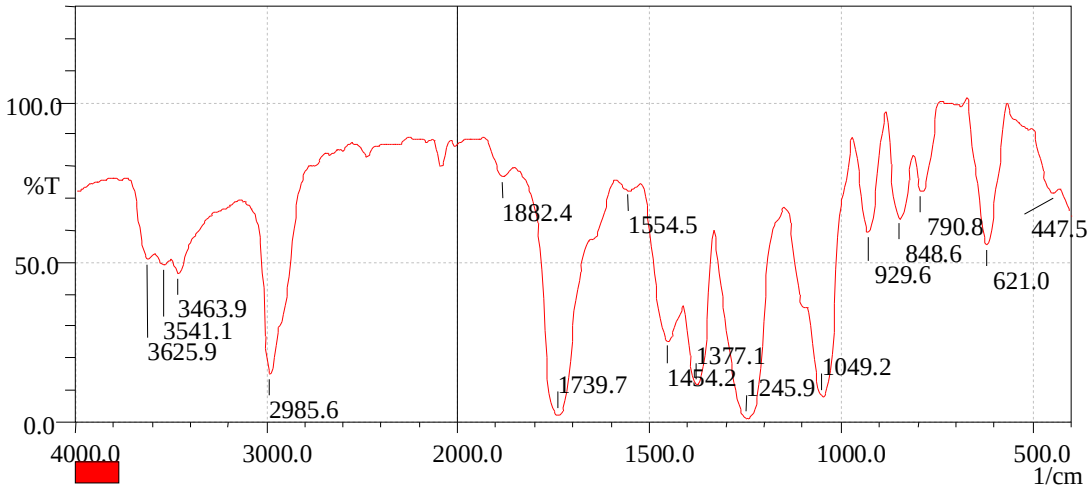


Figure 3.43: FTIR spectrum of *Physalis peruviana* (ethyl acetate extract)

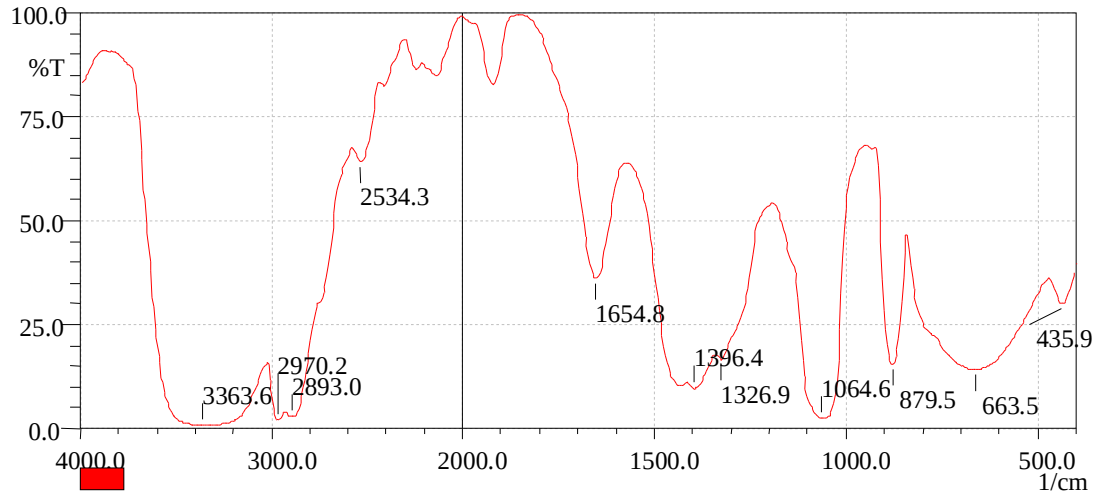


Figure 3.44: FTIR spectrum of *Physalis peruviana* (ethanol crude extract)

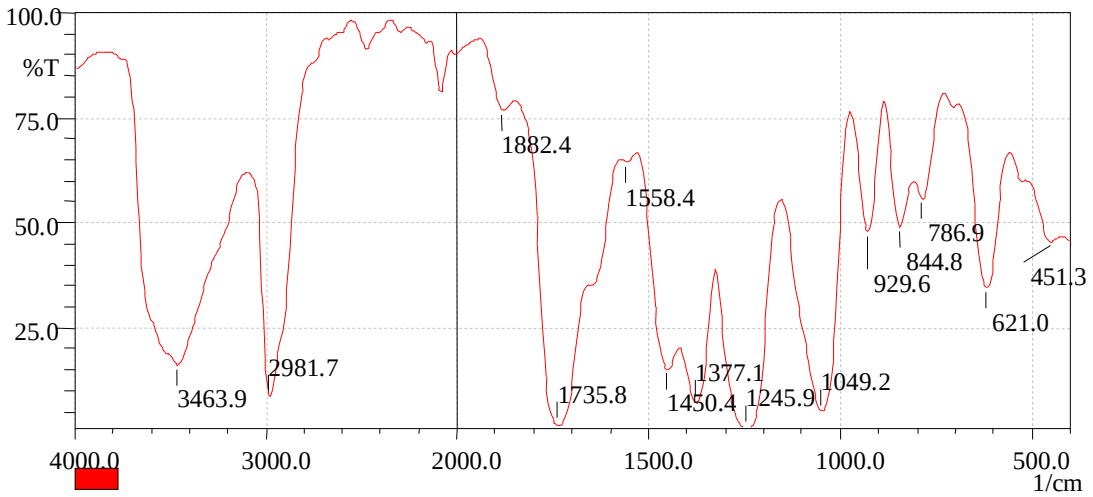


Figure 3.46: FTIR spectrum of *Bidens pilosa* (ethyl acetate crude extract)

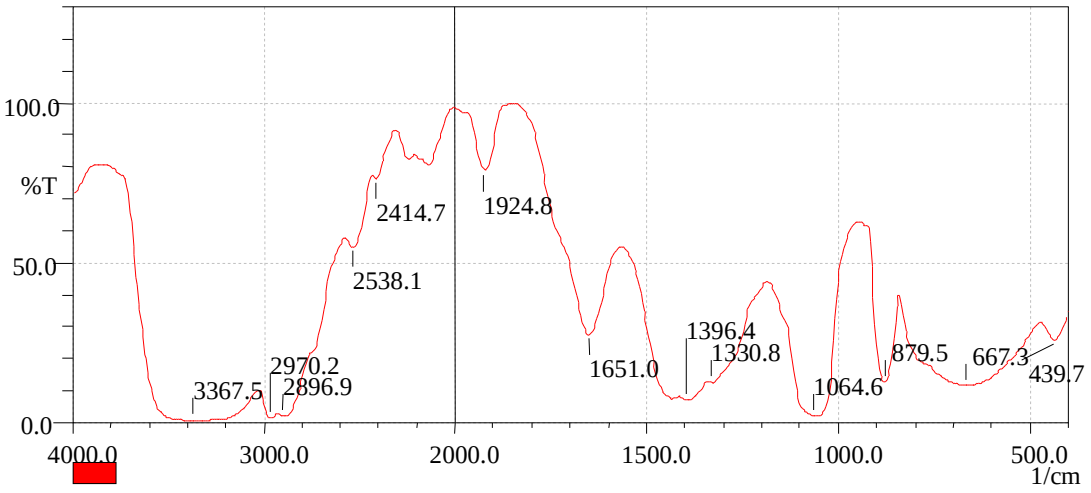


Figure 3.47: FTIR spectrum of *Bidens pilosa* (ethanol crude extract)

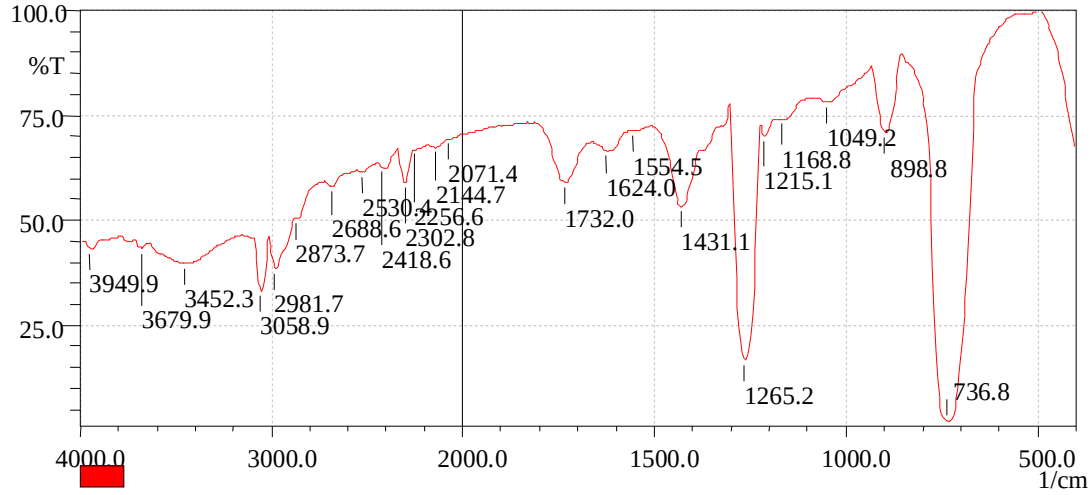


Figure 3.49: FTIR spectrum of *Leonotis nepetifolia* (dichloromethane crude extract)

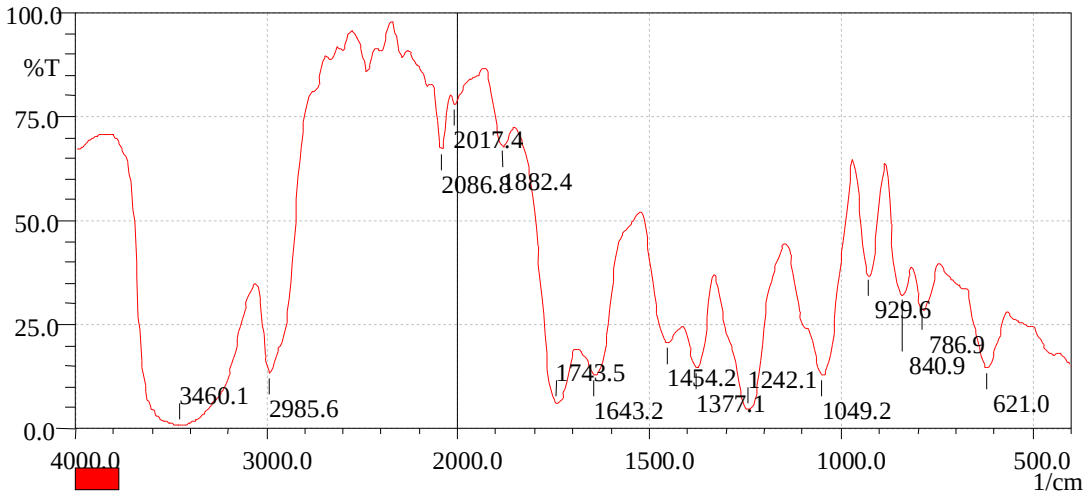


Figure 3.50: FTIR spectrum of *Leonotis nepetifolia* (ethylacetate crude extract)

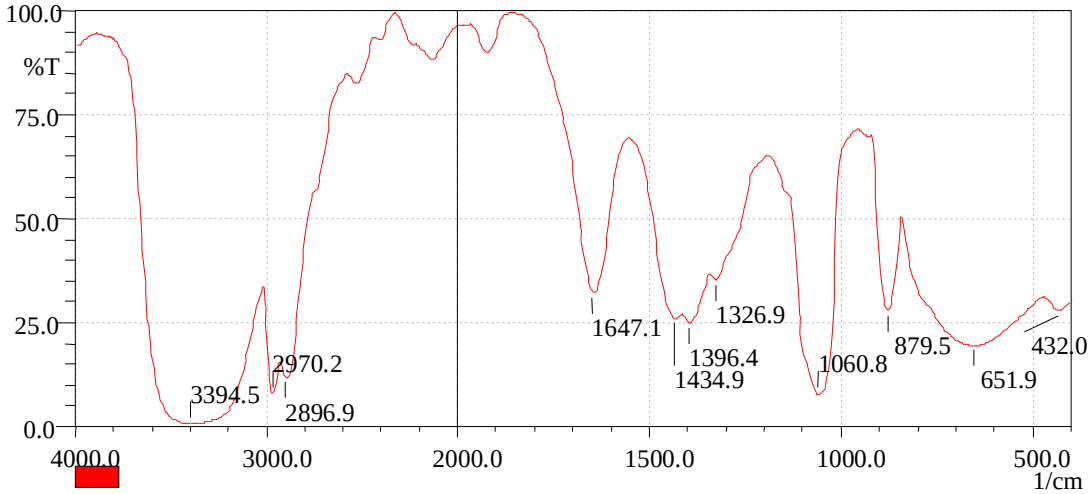


Figure 3.51: FTIR spectrum of *Leonotis nepetifolia* (ethanol crude extract)

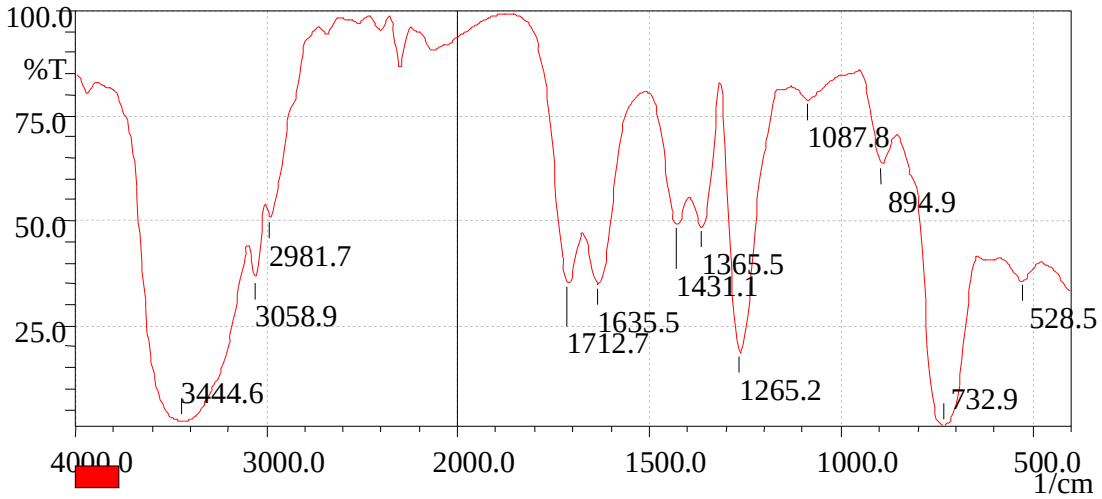


Figure 3.53: FTIR spectrum of *Toddalia asiatica* (dichloromethane crude extract)

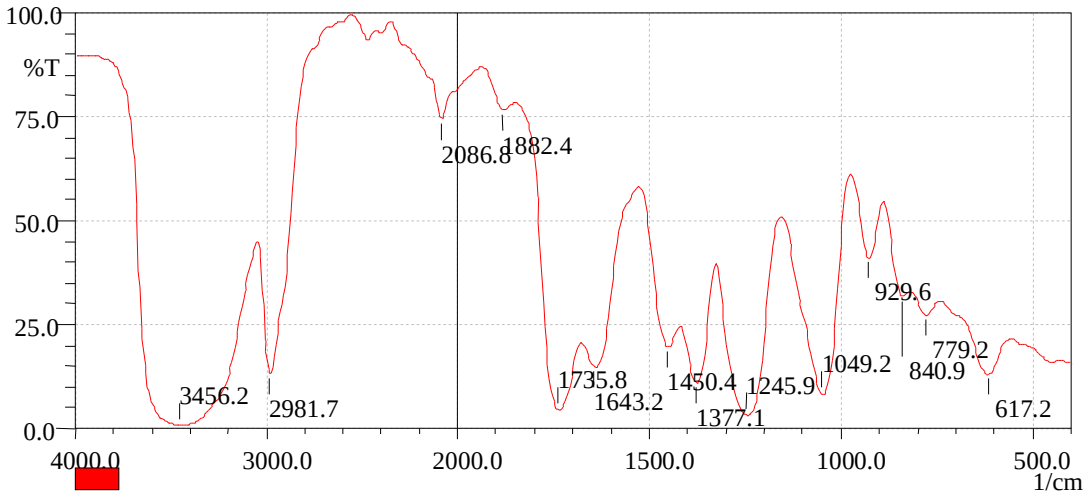


Figure 3.54: FTIR spectrum of *Toddalia asiatica* (ethyl acetate crude extract)

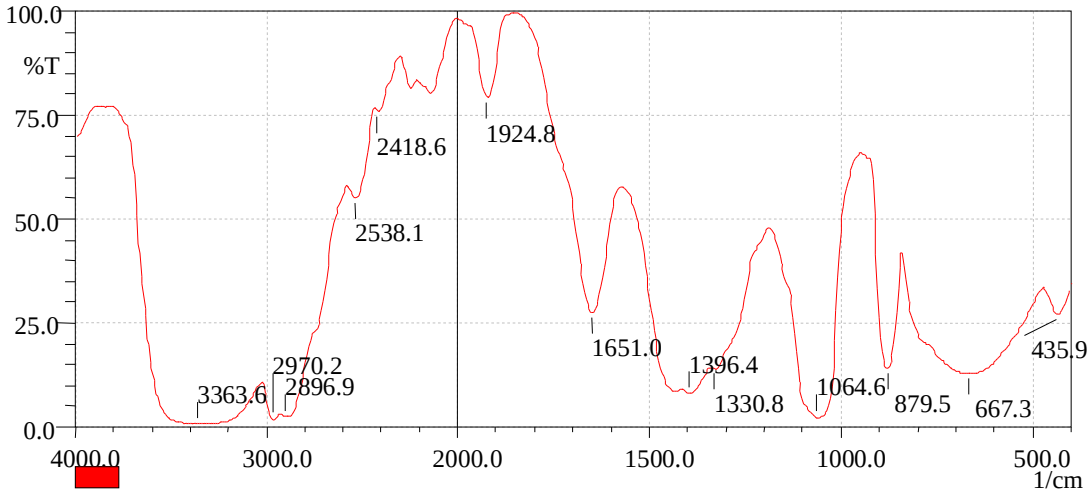


Figure 3.55: FTIR spectrum of *Toddalia asiatica* (ethanol crude extract)