ANALYSIS OF PROXIMATE, MICRONUTRIENTS AND DETERMINATION OF PHYTOCHEMICALS IN SELECTED MEDICINAL PLANTS IN MBITA-HOMABAY COUNTY

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NOVEMBER, 2016

DECLARATION

I hereby declare that this is my original work and has not been presented for the award of a degree in this university or any other university.

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DEDICATION

This work is dedicated to my late parents; Mr. Alexander Oyugi and Mrs. Catherine Ojung'a Oyugi , my uncle Alfred Aloyo Malowa, my brother Nicholas Oyugi, sisters; Magdaline, Mary, Consolata and Monica, my dear wife Melvine and our loving children Mary, Catherine, David, Florence Azulene and Marene Katno for their true love, patience and unfailing support throughout my academic endeavors.

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I offer my special gratitude to my family members and relatives for their prayers, encouragement and support during the entire study program. Finally, I am greatly thankful to my parents who provided me support and put me on this track but did not survive to see me on this stage.

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ABBREVIATIONS AND ACRONYMS

AAS	Atomic Absorption Spectroscopy
AIDS	Acquired Immune Deficiency Syndrome
AOAC	Association of Official Analytical Chemists
AOCS	American Oil Chemist Society
DNA	Deoxyribonucleic Acid
FAO	Food and Agricultural Organization
FNB	Food and Nutrition Board
HIF	Hypoxia Inducible Factors
MAO	Monoamine Oxidase
NCEs	New Chemical Entities
PPM	Parts Per Million
PAs	Proanthocyanidins
RDA	Recommended Dietary Allowance
ROS	Reactive Oxygen Species
SOD	Superoxide Dismutase
WHO	World Health Organization

ABSTRACT

About 80% of low income earners in developing countries rely on traditional medicine for their primary health care, hence medicinal plants play an important role in the society. *Lannea schweinfurthii*, *Rhus natalensis* and *Euclea divinorum*, has been extensively used in treating severe headache, dermatological, gastrointestinal, gynaecological, abdominal pains, female sterility among others in several parts of East Africa. In addition, many communities in Kenya including Mbita - Homabay County residents rely on these plants for their medicinal values. Many of the studies done on these plants have focused on isolation and characterization of phytochemicals. However, there is limited information on proximate and micronutrients analysis of these plants. Hence, this study focused on both qualitative and quantitative determination of the phytochemicals, micronutrients and proximate constituents. The analyses were done using conventional standard methods. The study revealed that the plants contained flavonoids, phenols, alkaloids and saponins. Mean levels of flavonoids were found to be 19.80±0.20% in *L. schweinfurthii* root barks and 9.46±0.12% in *L. schweinfurthii* leaves. Alkaloids were found to be 7.66±0.24% in *E. divinorum* leaves and 1.40±0.00% in *E. divinorum* root barks, saponins were found to be 47.60±0.05% in *E. divinorum* root barks and 7.65±0.05% in *E. divinorum* leaves and phenols were 8.54±0.12% in *R. natalensis* leaves. Proximate analysis revealed varied proportions of moisture, ash, crude protein, crude lipid, crude fibre and carbohydrates in all the plants investigated. The highest % by mass were; moisture content 14.50±0.50% in *L. schweinfurthii* root barks, crude protein 23.61±0.80% in *R. natalensis* leaves, crude fibre 1.80±0.20% in *R. natalensis* root barks, crude lipid 14.66±0.30% in *R. natalensis* leaves, ash 11.85±0.75% in *E. divinorum* root barks and carbohydrate 63.39±0.23% in *E. divinorum*. The dried plant parts were used for the study. Micronutrients analysis revealed presence of Cu, Fe, Zn and Mn which as 1.10±0.01 of *R. natalensis* leaves, 4.90±0.01 of *L. schweinfurthii* leaves, 4.50±0.01 of *R. natalensis* leaves and 2.30±0.01 of *E. divinorum* leaves in ppm respectively. Pb was 3.10±0.02 in *E. divinorum* root barks and Cd was not detected. T-test and ANOVA were used to analyze data. The findings provided evidence that crude aqueous and organic solvent extracts of plants studied contain compounds therefore used as traditional medicines. The findings suggested that the selected medicinal plants have a promising potential to cure ailments and maintain a healthy life and should be investigated further to determine the components responsible for activity.

CHAPTER ONE

INTRODUCTION

1.1 Background information

Medicinal plants form the basis of health care throughout the world since the earliest days of humanity (Newman *et al.,* 2003). Plants are important for pharmacological research and drug development (Newman *et al.,* 2003). Most of plant-derived medicines have been developed on the basis of traditional knowledge in health care and in many cases; there is a correlation between the indications of pure substances and those of respective crude extracts used in traditional medicine (Osho *et al.*, 2007).

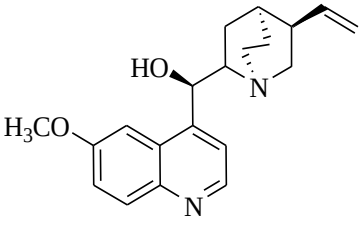
Herbal preparations constitute valuable natural resource from which chemicals of potential interest for medicine, agriculture, industry and other areas can be identified and isolated (Sneader, 1985). Besides, herbal medicine takes into account of every country's socio-cultural background. Of late, despite emphasis being put in research of synthetic drugs, interest in medicinal plants has been reborn. This is due to the fact that many synthetic drugs are potentially toxic and not free of side effects on the host for example, cisplatin which may cause hearing problem and numbness (www.doctorslounge.com), entecavir which may cause diarrhea and dizziness (www.doctorslounge.com) and zidovudine which may cause constipation, weakness and loss of appetite (www.doctorslounge.com) and, they are also costly. The use of traditional medicines is increasing and getting popularity throughout the developed and developing world (Jia and Zhang, 2005). Herbal medicines are the finished labeled medicinal product that contains active ingredients, aerial or underground parts of the plant or other plant material or combinations (Chakravarthy, 1993; Chaudhari, 1996; Ritch, 2000).

Close to 80% of the marginal (low income earners) people in developing countries rely on traditional medicine for their primary health care (Latif *et al*., 2004). With the increase in people's preference and demand, worth of herbal product industry is increasing day by day (Shinwari *et al.,* 2006). Since many of these herbal products are used orally, carrying out proximate and nutrient analysis of these products and raw material used therein plays a crucial role in assessing nutritional significance and health effects (Kochhar *et al.*, 2006; Pandey *et al.*, 2006; Taiga *et al.*, 2008). As far as herbal drug's standardization is concerned, WHO has also emphasized on the need and importance of determining proximate and micronutrients analysis. Such herbal formulations must pass through standardization processes (Niranjan and Kanaki, 2008; Ojokoh, 2008).

The active ingredients of plants that can provide effective therapeutic potential can occur in all plant structures but concentration is often higher in one part, such part is preferred. Examples include roots, flower, fruit, leaves, bark of the stem and seeds (Akinleye *et al.,* 1996). Natural products are chemical compounds obtained from plants, animals and insects as well as a plethora of other living organisms. The study on natural products encompasses the investigation into their molecular structure, biogenesis, and biological functions in the organism, therapeutic applications and other uses. Studies on natural products have become more and more important with the realization that plants provide a source of useful chemicals that may be used directly or as templates for the development of drugs useful for defense or protection against various diseases. They are also useful as nutraceuticals and health foods or supplements to promote good health and growth (Akinleye *et al.,* 1996).

The discovery of penicillin from the fungus *Penicillium notatum* by Fleming in 1928 marked a new era in medicine. It promoted the intensive investigation of nature as a source of novel

bioactive compounds. Since then plants and microorganisms have together served as a prolific source of structurally diverse and bioactive metabolites, yielding many important products one finds in the pharmaceutical industry today. This has been the result of systematic investigations carried out on just 5-15% of the total terrestrial flora, mostly of higher plants. A larger fraction is virtually untapped and still remains to be investigated. The potential wealth of discovery offered by this biological resource is just enormous. The continuing threat to biodiversity through the destruction of terrestrial and marine ecosystems lends urgency to the need to expand the systematic exploration of these biological resources in the search of new bioactive molecules. The following are examples of some drugs isolated from different plants and their therapeutic applications. They include; Quinine (**1**) an anti-malarial drug isolated from *Cinchona succirubra*, Morphine (**2**) a Pain reliever isolated from *Papaver somniferum* and Griseofulvin (**3**) an antibiotic drug isolated from *Penicillium griseofulvum* (Dewick, 2001).



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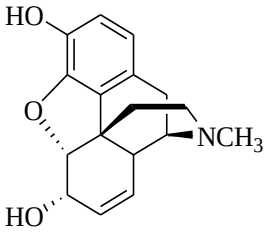

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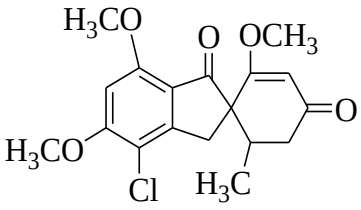

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Proximate analyses of plants play a crucial role in assessing their nutritional significance. Carbohydrates, fats, fibre and protein are the essential nutrients of life. The quality and quantity of proteins in the plants parts are basic factors and important for the selection of plants for nutritive value, systematic classification and plant improvement programs (Nisar *et al*., 2009). As various medicinal plant species are also used as food along with their medicinal benefits, evaluating their nutritional significance can help to understand the worth of these plants species (Pandey *et al*., 2006).

Micronutrients play crucial roles in human nutrition, including the prevention and treatment of various diseases and conditions as well as the optimization of physical and mental functioning (Wood and Ronnenberg, 2006). A number of micronutrients such as copper, zinc and manganese are known to serve as antioxidants or as essential cofactors for antioxidant enzymes (Gunter *et al*., 2013). Immune systems are weakened by lack of micronutrients. Iron contributes to formation of red blood cells and haemoglobin and also to normal oxygen transport in the body (Prasad, 1998; Cousins *et al*., 2006; Lee and Kim, 2008).

1.2 Statement of the problem and justification

Indigenous communities have for a long time incorporated the use of traditional medicine, mainly from plant sources in the cure or lessening of impact of common ailments. In Kenya, quite a number of plants used in folklore medicine have been identified and application of their crude extracts documented (Kokwaro, 2009). However, determining their active principles, efficacy, nutritive value and mechanism of their action have been reported only to a small extent. In fact, only some aspect of phytochemical analysis has been done which involves isolation of active compounds used as antibacterial, anti-inflammation, antiplasmodial and antioxidants (Gathirwa *et al*., 2008).

Previous studies majored on isolation, purification and characterization of specific phytochemicals, little work had been done on micronutrients and proximate constituents. Current study focused on partitioning of phytochemicals, micronutrients and proximate constituents on selected medicinal plants. It is therefore, of great importance to carry out proximate, micronutrients and phytochemical analysis on medicinal plants; *Lannea schweinfurthii*, *Rhus natalensis* and *Euclea divinorum.* These scientific data will help local herbalists in administering

treatment to their clients by following the recommended procedures in herbal drug preparation and administration (Pandey *et al*., 2006).

The determination of proximate, micronutrients and phytochemical constituents which the study focused on would enhance treatment of diseases, improve nutritional and provide scientific data for future reference (Newman *et al*., 2003; Pandey *et al*., 2006; Wood and Ronnenberg, 2006).

1.3 Hypothesis

Medicinal plants extracts of *L. schweinfurthii*, *R. natalensis* and *E. divinorum* obtained from Mbita Sub County contain proximate, micronutrients and phytochemical constituents in significant amounts.

1.4 Objectives

1.4.1 General objective

To carry out the proximate, micronutrients and phytochemical analysis of selected medicinal plants namely *L. schweinfurthii*, *R. natalensis* and *E. divinorum* that are used to treat various ailments among the Luo people in Mbita-Homabay County.

1.4.2 Specific objectives

i. To determine proximate constituents namely; moisture, ash, crude fats, proteins and carbohydrates of *L. schweinfurthii, R. natalensis* and *E. divinorum* leaves and root barks.
ii. To determine the micronutrients constituents namely Cu, Mn, Fe and Zn and levels of Pb and Cd in *L. schweinfurthii, R. natalensis* and *E. divinorum* leaves and root barks.
iii. To qualitatively and quantitatively screen the phytochemical constituents of *L. schweinfurthii, R. natalensis* and *E. divinorum* leaves and root barks.

1.5 Significance of the study

The use of medicinal plants as food alternative and medicine traces back to ancient human civilization. The study aimed to assess the nutritional significance of *L. schweinfurthii, R. natalensis* and *E. divinorum* which are economically important medicinal plants collected from Mbita in Homabay County. The determination of proximate, micronutrients and phytochemicals constituents and ascertaining their existence proved that the medicinal plants investigated could be used to improve nutrition and also used as medicine.

In addition, the study was very important, because it provided important information about the contents of the *L. schweinfurthii, R. natalensis* and *E. divinorum* leaves and root barks which were investigated in the study. The study also provided scientific data which would be of particular importance for the local practioners as well as for the local people using these herbs for treating a variety of body ailments.

CHAPTER TWO

LITERATURE REVIEW

2.1 Natural products

Natural products have played an important role as a source of drugs for thousands of years and many useful drugs have been derived from phytochemicals (Hostettmann *et al*., 1996). Plants have been considered since immemorial time, among the common sources of medicaments. Bioactive compounds from medicinal plants demonstrate their physiological activity (Holetz *et al*., 2002). It has been reported that the greater part of traditional medicines and therapy involve the use of plants extract or their active ingredients (George and Roger, 2002).

Biologically, active compounds from natural sources have always been of great interest to scientists working on drug discovery. There has been growing interest to evaluate plants possessing activity against various disease pathogens (Dhir *et al*., 2002). The healthcare delivery of the larger proportion of the rural communities in Kenya and most parts of Africa today hinged to a large extent on medicinal plants based on traditional health care delivery system (Dhir *et al*., 2002).

According to World Health Organization (WHO) estimates 80% of the world population relies on the traditional herbs, for their primary health care needs (WHO, 2005). Sub Saharan Africa accounts for 70 % of this population (WHO, 2005). The high cost of acquiring modern medicines and their inadequate supplies in most health care facilities as well as the side effects associated with their use, and the belief that plants hold cure for many disease conditions have led to a reawakening of interest in the use of plants and plant products in recent years. Medicinal plants are an important and treasured local resource for the people of East Africa (Minja, 1999).

The use of the plants in the indigenous cultures are multiple and diverse (Minja, 1999). Many commercially proven drugs used in modern medicine were initially used in crude form in traditional or folk healing practices, or for other purposes that suggested potentially useful biological activity (Minja, 1999).

2.2 Medicinal plants as a source of important drugs

Pharmacologically active compounds from plants and other natural sources, combinatorial chemistry, synthetic chemistry, and molecular modeling can be used as drugs for different diseases (Newman *et al*., 2003). Although there is a lot of research done in molecular modeling, combinatorial chemistry and synthetic chemistry, natural products remain an important source of new drugs, new chemical entities (NCEs) and new drug leads (Newman *et al*., 2003).

According to a survey done in 2001 and 2002, approximately one quarter of the best-selling drugs in the world were natural products or derived from natural products (Newman *et al*., 2003). It has also been reported that approximately 28% of NCEs between 1981 and 2002 were natural products or natural product-derived natural products while 20% of NCEs were considered natural product mimics, meaning that the synthetic compound was derived from the study of natural products (Newman *et al*., 2003). On the bases of this report it has been assumed that research on natural products accounts for approximately 48% of the NCEs reported from 1981–2002. Furthermore it has been known that natural products also provide a starting point for laboratory syntheses with diverse structures and often with multiple stereo centers that can be challenging synthetically (Clardy and Walsh, 2004). Natural products shows many structural features in common (such as aromatic rings, chiral centers, degree of molecule saturation, complex ring systems, and number ratio of hetero atoms) which have been shown to be very important to drug discovery efforts (Clardy and Walsh, 2004). Many synthetic and medicinal chemists are working

in the creation of natural product and natural-product like libraries that resembles the structural features of natural products with the compound-generating potential of combinatorial chemistry (Burke *et al*., 2004; Tan, 2004). Natural products can be a source of new drugs or as a lead for drug development.

Sometimes new chemical structures are very difficult to find during drug discovery from medicinal plants, in such cases known compounds with new biological activity can provide important drug directions. Molecular target plays an important role in drug discovery, since the sequencing of the human genome; a lot new molecular targets have been identified as important and useful in various diseases (Kramer and Cohen, 2004). The developments of high-throughput screening technique have shown the point and more selective activity directed towards these targets on the reported compounds from medicinal plants. It has also been known that the compounds isolated from traditionally used medicinal plants have been shown to act on newly validated molecular targets, one example is indirubin, which targeted and inhibit cyclin dependent kinases (Eisenbrand *et al*., 2004) and another example is kamebakaurin, which target and inhibit NF-nB (Lee *et al*., 2002).

2.3 Phytochemistry

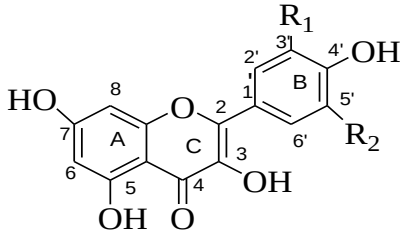
Plants secondary metabolites are not essential for life as compared to primary metabolites, that the absence of secondary metabolites results not in failure of life, but in long-term impairment of the organism's survivability/ fecundity or aesthetics, or perhaps in no significant change at all but it is useful for animal’s ailments and normalizes the physiological abnormalities produced due to different diseases in animal bodies (Dewick, 2001). Secondary metabolites are often very restricted to a particular set of species within a phylogenetic group. In broad sense secondary metabolites may be classified into; small molecules alkaloids, terpenoids, glycosides, Phenols

and Phenazene), big small molecules (Polyketides, Non ribosomal peptides etc), non small molecules (DNA, RNA, ribosome, polysaccharides) (Dewick, 2001).

Secondary metabolites play an important role in the treatment of diseases and in the ecological interaction of plants with other organisms. Each plant species, family or genus produces a characteristic mixture of secondary metabolites (Dewick, 2001). Thousands of phytochemicals have been discovered in several major classes but the health properties of only a few have been investigated. Many of the known phytochemicals that belong to several chemical classes have inhibitory effect on a wide range of microorganisms (Khan *et al*., 2011). They have been used as medicines, flavouring and colouring agents. Some of the well-known phytochemicals are: alkaloids, flavonoids, saponins, tannins polyphenols and terpenoids (Dewick, 2001).

2.3.1 Flavonoids

Flavonoids are a diverse group of secondary plant metabolites that are characterized by the presence of a C15-(C6-C3-C6) flavone nucleus that is based on a heterocyclic ring systems derived from phenylalanine (ring B) and polyketide biosynthesis (ring A) linked through an oxygen containing pyran or pyrone ring (ring C) (Maraisi *et al*., 2006). The numbering system for the flavonoid skeleton is as shown in chemical structure **(4)**. We have Kaempferol when R_1= H, R_2=H; quercetin, R_1= OH, R_2= H; myricetin, R_1=OH, R_2= OH.



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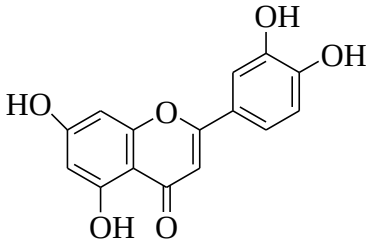
Flavonoids are pale yellow compounds that are poorly soluble in water (Maraisi *et al*., 2006). They occur in foods as O-glycosides, D-glucose being the most common sugar residue although D-galactose, L-rhamnose, L-arabinose, D-xylose and D-glucuronic acid are also found. The preferred binding site for the sugar residue is C3 with binding occurring less frequently at the C7 position (Macheix *et al*., 1990).

Flavonoids are polyphenolic compounds that are ubiquitous in nature and are categorized, according to their chemical structure into flavones, anthocyanidins, isoflavones, catechins, flavonols, chalcones and flavanones. More than 4,000 flavonoids have been recognized, many of which occur in vegetables, fruits and beverages like tea, coffee and fruit drinks. These polyphenolic compounds have strong antioxidant activity. Biological activities ascribed in this class include: antifungal, antiviral, antibacterial, anti-inflammatory, hepatoprotective anti-thrambotic and anticarcinogenic activities which are associated with their antioxidant and free radical scavenging properties (Okwu and Omodamiro, 2005; Robak and Gryglewski, 1988). Anthraquinones have laxative effect, anti-inflammatory, anti-cancer and antimalarial properties (Sakulpanich and Gritsanapan, 2009).

The antiradical property of flavonoids is directed mostly toward HO as well as peroxyl and alkoxyl radicals. Furthermore, as these compounds present a strong affinity for iron ions their antiperoxidative activity could also be ascribed to a concomitant capability of chelating iron (Morel *et al.*, 1993). One of the undeniable functions of flavonoids and related polyphenols is their role in defending plants against microbial attack. This not only comprises their presence in plants as constitutive mediators but also their accumulation as phytoalexins in response to microbial attack (Harborne and Baxter, 1999). Because of their extensive ability to prevent spore germination of plant pathogens, they have been suggested also for use against fungal pathogens

(Harborne and Baxter, 1999). There is an ever growing interest in plant flavonoids for treating human diseases and particularly for monitoring the immunodeficiency virus which is the contributing agent of AIDS (Jensen *et al*., 1998). The majority of flavonoids documented as constitutive antifungal agents in plants are flavanones, isofavonoids or flavans (Jensen *et al*., 1998). The recognition that a flavone glycoside, namely luteolin (**5**), is an antifungal component of the marine angiosperm *Thalassia testudinum* (Jensen *et al*., 1998).



(5)

For a group of compounds of relatively homogeneous structure, the flavonoids inhibit a mystifying number and variety of eukaryotic enzymes and have an extremely wide range of activities. In the event of enzyme inhibition, this has been assumed to be due to the interaction of enzymes with different parts of the flavonoid molecule such as carbohydrate, phenyl ring, phenol and benzopyrone ring (Havsteen, 1983).

2.3.2 Saponins

Saponins are a group of secondary metabolites found widely distributed in the plant kingdom as plant glycosides, characterized by a skeleton resulting of the 30-carbon precursor oxidosqualene to which glycosyl residues are attached along with it they have sturdy foaming property (Hall *et al*., 1991). Conventionally, they are subdivided into triterpenoid and steroid glycosides, or into triterpenoid which are found primarily in dicotyledonous plants but also in some monocots, spirostanol, and furostanol saponins (Hostettmann and Marston, 1995). Steroid saponins occur

chiefly in monocotyledons family such as the Lilliaceae, Agavaceae, and Droscoraceae and in certain dicotyledons, such as foxglove (Hostettmann and Marston, 1995).

Saponins containing plants are used as traditional medicines, especially in Asia, and are intensively used in food, veterinary and medical industries (Hostettmann and Marston, 1995). The pesticidal activity of saponins has long been reported (Irvine, 1961) Saponins-glycosides are very lethal to cold-blooded organisms, but deceptively not to mammals (Hostettmann and Marston, 1995; Hall *et al*., 1991). Plant extracts containing a high percentage of saponins are commonly used in Africa to treat water supplies and wells contaminated with disease vectors; after treatment, the water is safe for human drinking (Hall *et al*., 1991).

These are known to be immune boosters. They are also known to demonstrate anti-inflammatory, anti-haemolytic, cholesterol lowering and anticancer properties (Sauvaire *et al.*, 1996). Saponins have been shown to interpolate into cell membranes, from side to side by interaction with cholesterol, forming holes' or pores, their specific capability to form pores in membranes has backed to their common use in physiological research (Choi *et al*., 2001).

2.3.3 Tannins

Tannins are naturally occurring uncrystallisable colloidal substances with pronounced astringent properties and whose main characteristic is to bind and precipitate gelatin from solution. It is the ability to form insoluble compounds with gelatin yielding tissue which enables tannins to convert raw hide and skin into leather. In the presence of tannins, the dermal network of hide is consolidated into firmer and drier structures that are thermally more stable, durable and more water resistant than the original hide (Yoshida and Hatano, 2000). Tannins are responsible for the astringent taste of wine or unripe fruits. Tannin products are responsible for the enchanting colours seen in flowers and in autumn leaves. Tannins are subdivided into three groups, the

hydrolysable tannins (HT), proanthocyanidins (PAs) which are also called condensed tannins (CT) and mixed tannins (Jose *et al*., 2001).

Tannins have a characteristic of binding and precipitating proteins. They can have a large influence on the nutritive value of many foods eaten by humans and feedstuff eaten by animals. Tannins are found commonly in fruits such as grapes, persimmon, blueberry, tea, chocolate, legume forages, legume trees like *Acacia* spp., *Sesbania* spp., in grasses i.e.; sorghum, corn, etc. The characteristics of tannins are that they are oligomeric compounds with numerous structure units with free phenolic groups, molecular weight fluctuating from 500 - 20,000, soluble in water, with exception of some high molecular weight structures they are able to bind proteins and form insoluble or soluble tannin-protein complexes. Presently there is a cumulative interest in tannins as bioactive component of foods as well as biological antioxidants. Tannins are an exceptional group of water soluble phenolic metabolites of relatively high molecular weight and having the ability to complex strongly with carbohydrates and proteins (Chavan *et al*., 2001).

Abundant studies have demonstrated supposedly significant biological effects of tannins such as antioxidant or radical scavenging activity as well as inhibition of lipid peroxidation and lipoxygenases in vitro, antimicrobial and antiviral, antimutagenic (Dolara *et al.*, 2005), and antidiabetic properties (Anderson and Polansky, 2002). The antioxidant activity of tannins results from their free radical and reactive oxygen species-scavenging properties, as well as the chelation of transition metal ions that modify the oxidation process (Serrano *et al*., 2009). Antioxidants have also been reported to provide synergistic benefits for the treatment of diabetes because of their insulin enhancing potential. They also play a role in inhibiting the growth of bacteria by reacting with protein on the cell wall (Dangoggo *et al*., 2012).

2.3.4 Terpenoids

These are an abundant class of natural products that are responsible for many fragrances. They are made up of C5 isoprene units and possess strong antimicrobial properties (Talukdar *et al*., 2010). These compounds are active against fungi, bacteria, mollusks, insects, worms and termites. Derivatives and oxidative decomposition products from naphthoquinones are responsible for the dark colour in the bark and other parts. Terpenoids, benzopyrones, polyphenols and tannins are also common in this family (Wallnöfer, 2001).

The classification of terpenoids can be made according to the number of isoprene units used. Hemiterpenoids**:** consist of a single isoprene unit. The only hemiterpene is the Isoprene itself, but oxygen-containing derivatives of isoprene such as isovaleric acid and prenol is classify as hemiterpenoids. Monoterpenoids: biochemical modifications of monoterpenes such as oxidation or rearrangement produce the related monoterpenoids. Monoterpenoids have two isoprene units. Monoterpenes may be of two types namely linear (acyclic) or contain rings e.g. Geranyl pyrophosphate, Eucalyptol, Limonene and Pinene. Sesquiterpenes**:** Sesquiterpenes have three isoprene units e.g. Farnesyl pyrophosphate, Artemisinin, Bisabolol. Diterpenes: it composed of four isoprene units and has the molecular formula $C_{20}H_{32}$. They derive from geranylgeranyl pyrophosphate. There are some examples of diterpenes such as cembrene, kahweol, taxadiene and cafestol (precursor of taxol). Retinol, retinal, and phytol are the biologically important compounds while using diterpenes as the base. These three compounds are known to be antimicrobial and anti inflammatory. Sesterterpenoids**:** Terpenoids having 25 carbons and five isoprene units. Triterpenes**:** It consists of six isoprene units, squalene found in wheat germ, and olives is an example (Talukdar *et al*., 2010). Tetraterpenoids**:** It contains eight isoprene units which may be acyclic like lycopene, monocyclic like gamma-carotene, and bicyclic like alpha-

and beta-carotenes. Polyterpenoids: It consists of a larger number of isoprene units (Talukdar *et al*., 2010).

2.3.5 Alkaloids

Alkaloids are natural product that contains basic nitrogen atoms. The name of alkaloids derives from the “alkaline” and it was used to describe any nitrogen-containing base. Alkaloids are naturally synthesis by a large numbers of organisms, including animals, plants, bacteria and fungi. Alkaloids are a group of natural products (also called secondary metabolites). Alkaloids can be easily purified from various crude extracts by acid-base extraction (Okwu, 2004). There are very many alkaloids which are toxic to other organisms. They often have some pharmacological effects such as antimalaria, antitumor among others and are used for the treatment of various diseases and recreational drugs (Okwu, 2004). Some alkaloids are used as the local anesthetic and stimulant as cocaine. Alkaloids have stimulant property as caffeine and nicotine, morphine are used as the analgesic and quinine as the antimalarial drug. Almost all the alkaloids have a bitter taste (Okwu, 2004). Alkaloids are known to possess many pharmacological properties like analgesic (morphine), stimulants (caffeine), antitumor (vinblastine), antimalaria (quinine), antibacterial (berberine) and amoebicide (emetine) (Dewick, 2001).

2.3.6 Phenols

Phenolic compounds can be defined as naturally occurring organic species that possess at least one aromatic ring with one or more hydroxyl groups attached to the ring. Most naturally occurring phenolic compounds exist as conjugates with monosaccharides and polysaccharides linked to one or more of the phenolic groups (Harbone, 1998).

Phenolic compounds are some of the most widespread molecules among plant secondary metabolites. They are known to act as natural antioxidants and antinitrosating agents which are of great significance in plant development. The antioxidant properties of phenols are determined by their radical scavenging ability and consequent inhibitory action on lipid peroxidation under oxidative stress situations, which link with their substitution pattern for example Tocopherols which have a potent ability to inhibit lipid peroxidation *in vivo* by trapping peroxyl radicals (Rigobello *et al*., 2004).

2.4 Phytochemical studies of medicinal plants under investigation

2.4.1 *Lannea schweinfurthii* (Engl.) Engl.

Lannea schweinfurthii commonly known as false marula in "English" and Kuogo in "Dholuo" belongs to a family of Anacardiaceae, genus *Lannea*. It is a shrub or a tree, up to 13 m high with a grey bark, a rounded and spreading crown with drooping branchlets. Leaves 3-5 foliolate; leaflets 3-5, 2-7 cm long, and 1-6 cm broad. Racemes axillaries, simple or branched, up to 15 cm long. Flowers are creamy and strongly scented (Kokwaro, 1976; Kokwaro and Timothy, 1998). *L. schweinfurthii* has numerous uses as a medicinal plant among the Luos. Infusions or decoctions from the bark, roots or leaves are drunk to treat severe headache, dermatological problems, decoctions of roots in concoctions with *E. divinorum* treat venereal diseases, gynaecological problems, dysentery and other stomach problems and leaves are chewed to cure coughs. In addition, it is given to pregnant women to relieve abdominal pains and a poultice of the leaves is applied to the abdomen to hasten childbirth (Kokwaro, 1976; Kokwaro and Timothy, 1998). These are the ethnobotanical uses. Phytochemicals reported are flavanols and their pharmacological uses are antioxidant, antimalarial, antibacterial and anti-inflammation (Gathirwa *et al*., 2008). The photograph 1 below shows *Lannea schweinfurthii* plant.

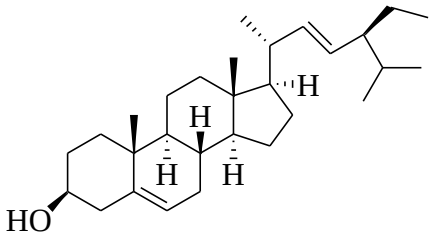
Photograph 2.1: *Lannea schweinfurthii* plant

2.4.2 Rhus natalensis (krauss)

Rhus natalensis commonly known as Natal rhus in "English" and sangala in "Dholuo", belongs to a family of *Anacardiaceae*, genus of *Rhus.* It is a small tree, up to 6.5 m high, branchlets grey-brown. Leaves are pale green, trifoliolate; petiole 1-4 cm long; leaflets sessile, obovate to oblanceolate, glabrous beneath, entire crenulate. Panicles slender, up to 15 cm long; flowers greenish-yellow, and very small. Fruit globose, about 8 mm in diameter. *R. natalensis* has various medicinal uses (Kokwaro, 1976; Kokwaro and Timothy, 1998). The roots are pounded steeped in hot or cold water and the extract drunk for influenza, abdominal pains, gastrointestinal problems and for gonorrhea. The root decoction also forms part of a medicine for hookworm. The leaves are used for cough mixtures; they are pounded and steeped in hot water and the patient drinks the extracts. Sometimes the steam from the hot leaf extract is inhaled to cure colds. Decoction of boiled leaves is a good remedy for stomach-ache and abdominal pains, especially

in young children. Leaves are further used as an inhalant in hot water for colds. Roots mixed with other plants make a valuable drug for expectant mothers: believed to make delivery easy and used to treat gastrointestinal problems (Kokwaro, 1976; Kokwaro and Timothy, 1998). *R. natalensis* leaves and roots are used for the treatment of pain and inflammation. Leaves are boiled and given to cattle as a pain killer (Schlage *et al*., 2000; Minja, 1999). It has also been found to be potential antiplasmodial agent in combination with other herbs (Gathirwa *et al*., 2008).

The isolated compounds from this plant as reported by Njoroge *et al*., (2011) are; stigmasterol (**6**), epicatechin (**7**), lupeol (**8**), sitosterol (**9**). They belong to modified triterpenoids or steroids except epicatechin (**7**) which belongs to flavonoids.



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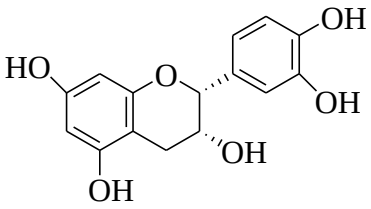

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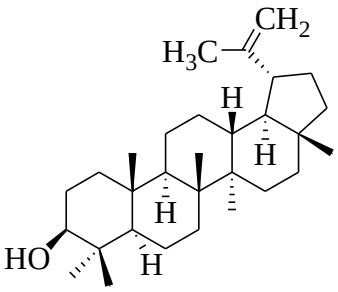

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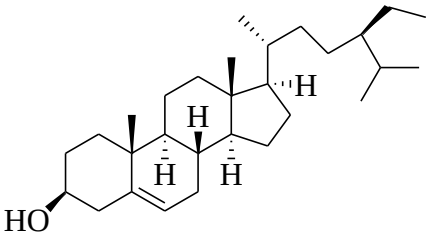


(9)

Photograph 2.2: *Rhus natalensis* plant

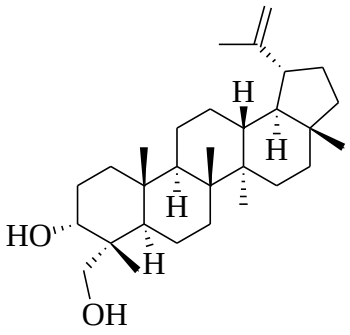
2.4.3 *Euclea divinorum* (Hiern)

E. divinorum commonly known as Magic guarri in "English" and Ochol in "Dholuo" belongs to a family of Ebenaceae and genus *Euclea.* A much branched small tree. Leaves elliptic, 5-10 cm long, 2-4 cm broad, glabrous except for some reddish or pale scales beneath. Flowers fragrant, cream, males 10 or more together in lax racemes, up to 4 cm long, females in stouter and shorter racemes up to 2 cm long; calyx lobes short.

The roots and twigs are used as toothbrushes. This plant has been used successfully in the leather production industry and offers a sustainable source of natural tannin (van Grinsven *et al*., 1999). The root is a source of traditional dye for commercial craftwork production and gives a dark brown to black colour depending on how long the roots are boiled (van Wyk and Gericke, 2000). The fruits give a red sap which is used for dyeing clothes, baskets and mats. It is known for its economic and cultural values by people in the north and northeastern regions in Namibia as it is used in traditional religious rites. The name *Euclea divinorum* and its common name magic guarri are derived from its

use in divination (van Wyk and Gericke, 2000). The Ovambo people use it to treat nose bleed where they administer it for a period of 1-4 days (Cheikhyoussef *et al*., 2011). The root bark is used in treating diarrhoea, convulsion, cancer, skin disease and gonorrhoea (Mebe *et al*., 1998; Luleka *et al*., 2008). A root infusion is dropped in the ear to treat headache and an ointment is rubbed on the body to treat convulsions. The bark is chewed to serve as a mouth wash. In the north-east of Namibia, especially the Kavango region, an infusion is made from the roots and drunk to treat ailments such as malaria, fevers and venereal diseases. The roots are also chewed and rubbed onto sores and wounds as well as to treat toothache and fungal diseases (Chinsembu *et al*., 2011).

E. divinorum has numerous uses as a medicinal plant. Decoction of roots treats female sterility and other gynaecological problems, gastrointestinal problems including constipation and dysentery (Kokwaro, 1976; Kokwaro and Timothy, 1998). A number of studies have reported the presence of naphthoquinones, triterpenes and flavonoids in the *Euclea* species. Mebe *et al*., (1998) reported the isolation of lupeol **(8)**, lupene-3, 23-diol **(10),** betulin **(11)**, and catechin **(12)** from the root bark of *E. divinorum.*



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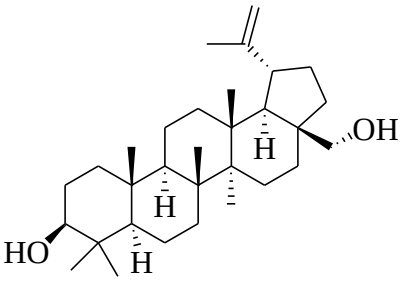

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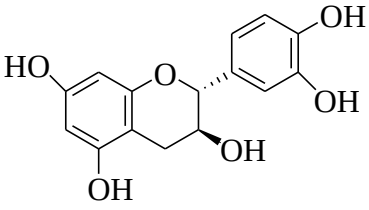

(12)

Photograph 2.3: *Euclea divinorum* plant

2.5 Analysis of selected medicinal plants

2.5.1 Proximate analysis

Proximate Analysis is the partitioning of compounds in a plant extract into six categories based on the chemical properties of the compounds. The six categories are: moisture, ash, crude protein (or Kjeldahl protein), crude lipid, crude fibre and nitrogen-free extracts (digestible carbohydrates). Proximate and nutrient analysis of medicinal plants plays a crucial role in assessing their nutritional significance. As various medicinal plant species are also used as food along with their medicinal benefits, evaluating their nutritional significance can help to understand the worth of these plants species (Pandey *et al*., 2006). Carbohydrates, proteins and fats form the key portion of the diet, whereas minerals and vitamins form somewhat a minor part. As plants form main portion of our diet; so their nutritive value is imperative (Jimoh and Oladiji,

2005). Besides these biochemicals; the moisture, fiber, ash contents and the energy values of individual vegetable species have also been reported to be important to the human health as well as for soil quality (McSweeney *et al*., 2005).

Each medicinal plant species has its own nutrient composition besides having pharmacologically important phytochemicals. These nutrients are essential for the physiological functions of human body. Such nutrients and biochemicals like carbohydrates, fats and proteins play an important role in satisfying human needs for energy and life processes (Novak and Haslberger, 2000).

Fortunately, chemical composition diversity in plants also includes many compounds that are beneficial to humans: vitamins, nutrients, antioxidants, anticarcinogens, and many other compounds with medicinal value (Novak and Haslberger, 2000). Plants are also known to have high amounts of essential nutrients, vitamins, minerals, fatty acids and fibre (Gafar and Itodo, 2011).

2.5.1.1 Kjeldahl method

The Kjeldahl method or Kjeldahl digestion in analytical chemistry is a method for the quantitative determination of nitrogen in chemical substances developed by Johan Kjeldahl in 1883 (Kjeldahl, 1883).

The method consists of heating a substance with sulphuric acid, which decomposes the organic substance by oxidation to liberate the reduced nitrogen as ammonium sulphate. In this step potassium sulphate is added to increase the boiling point of the medium (from 337 °C to 373 °C). Chemical decomposition of the sample is complete when the initially very dark-coloured medium has become clear and colourless. The solution is then distilled with a small quantity of sodium hydroxide, which converts the ammonium salt to ammonia. The amount of ammonia present, and thus the amount of nitrogen present in the sample, is determined by back titration.

The end of the condenser is dipped into a solution of boric acid. The ammonia reacts with the acid and the remainder of the acid is then titrated with a sodium carbonate solution by way of a methyl orange pH indicator.

The Kjeldahl method is summarized below;

Degradation: Sample + $H_2SO_4 \rightarrow (NH_4)_2SO_4$ (aq) + CO_2 (g) + SO_2 (g) + H_2O (g)

Neutralization (Liberation of ammonia):

$(NH_4)_2SO_4$ (aq) + 2NaOH (aq) $\rightarrow Na_2SO_4$ (aq) + $2H_2O$ (l) + $2NH_3$ (g)

Capture of ammonia: $B(OH)_3 + H_2O + NH_3 \rightarrow NH_4^+ + B(OH)_4^-$

Back-titration: $B(OH)_3 + H_2O + Na_2CO_3 \rightarrow NaHCO_3$(aq) + $NaB(OH)_4$(aq) + CO_2(g) + H_2O

In practice, this analysis is largely automated; specific catalysts accelerate the decomposition. Originally, the catalyst of choice was mercuric oxide. However, while it was very effective, health concerns resulted in it being replaced by cupric sulfate. Cupric sulfate was not as efficient as mercuric oxide, and yielded lower protein results. It was soon supplemented with titanium dioxide, which is currently the approved catalyst in all of the methods of analysis for protein in the Official Methods and Recommended Practices of AOAC International (A.O.A.C, 1990).

The Kjeldahl method's universality, precision and reproducibility have made it the internationally recognized method for estimating the protein content in foods and it is the standard method against which all other methods are judged. It is also used to assay soils, waste waters, fertilizers and other materials. It does not, however, give a measure of true protein content, as it measures non protein nitrogen in addition to the nitrogen in proteins. This is evidenced by the 2007 pet food incident and the 2008 Chinese milk powder scandal, when melamine, a nitrogen-rich chemical, was added to raw materials to fake high protein contents

(Schlein and Lisa, 2008). Also, different correction factors are needed for different proteins to account for different amino acid sequences. Additional disadvantages, such as the need to use concentrated sulfuric acid at high temperature and the relatively long testing time (an hour or more), compare unfavorably with the Dumas method for measuring crude protein content.

2.5.2 Micronutrients analysis

Metals play an important role in the metabolism. The deficiency or excess of trace elements lead to various complications and metabolic disorders in human being. The present work included quantitative determination of various elements from the leaves and root barks of selected medicinal plants. Micronutrients analysis of Cu, Mn, Fe and Zn and the toxicity levels of Pd and Cd were scrutinized. These analyses were done using AAS (Hussain *et al.,* 2009).

2.5.2.1 Iron

Iron has the longest and best described history among all the micronutrients. Iron requirements for expectant mothers are much greater than at any other stage in life. In fact, the recommended dietary intake for iron increases by an extra 10-20 mg a day during pregnancy. The main reason for this increased requirement is that the growing foetus needs to build up its own iron reserves and it does this by taking the iron from the mother's body (Yip and Dallman, 1996).

It is therefore important to eat a variety of iron-rich foods when you are expecting. In some cases, an additional iron supplement may be required. Iron plays an important role in the body. One of the main roles of iron is to help our red blood cells transport oxygen to all parts of the body. Iron also plays an important role in specific processes within the cell that produce the energy for our body. It is for this reason that one of the first symptoms of low body iron stores is tiredness and fatigue (Yip and Dallman, 1996).

It is a key element in the metabolism of almost all living organisms. In humans, iron is an essential component of hundreds of proteins and enzymes (Wood and Ronnenberg, 2006). Haeme is an iron-containing compound found in a number of biologically important molecules. Haemoglobin and myoglobin are haeme-containing proteins that are involved in the transport and storage of oxygen (Wood and Ronnenberg, 2006). Haemoglobin is the primary protein found in red blood cells and represents about two thirds of the body's iron. The vital role of hemoglobin in transporting oxygen from the lungs to the rest of the body is derived from its unique ability to acquire oxygen rapidly during the short time it spends in contact with the lungs and to release oxygen when needed during its circulation through the tissues (Wood and Ronnenberg, 2006).

Myoglobin functions in the transport and short-term storage of oxygen in muscle cells, helping to match the supply of oxygen to the demand of working muscles (Yip and Dallman, 1996; Brody, 1999). Cytochromes are haeme-containing compounds that have important roles in mitochondrial electron transport; therefore, cytochromes are critical to cellular energy production and thus life. They serve as electron carriers during the synthesis of ATP, the primary energy storage compound in cells. Cytochrome P450 is a family of enzymes that functions in the metabolism of a number of important biological molecules, as well as the detoxification and metabolism of xenobiotics. Non-haeme iron-containing enzymes, such as NADH dehydrogenase and succinate dehydrogenase, are also critical to energy metabolism (Yip and Dallman, 1996). Catalase and peroxidases are haeme-containing enzymes that protect cells against the accumulation of hydrogen peroxide, a potentially damaging reactive oxygen species (ROS), by catalyzing a reaction that converts hydrogen peroxide to water and oxygen. As part of the immune response, some white blood cells engulf bacteria and expose them to ROS in order to

kill them. The synthesis of one such ROS, hypochlorous acid, by neutrophils is catalyzed by the haeme-containing enzyme myeloperoxidase (Yip and Dallman, 1996; Brody, 1999).

Inadequate oxygen, such as that experienced by those who live at high altitudes or those with chronic lung disease, induces compensatory physiologic responses, including increased red blood cell formation, increased blood vessel growth, and increased production of enzymes utilized in anaerobic metabolism. Under hypoxic conditions, transcription factors known as hypoxia inducible factors (HIF) bind to response elements in genes that encode various proteins involved in compensatory responses to hypoxia and increase their synthesis. Research indicates that an iron dependent prolyl hydroxylase enzyme plays a critical role in regulating HIF and, consequently, physiologic responses to hypoxia (Ivan *et al.*, 2001). When cellular oxygen tension is adequate, newly synthesized HIFa subunits are modified by a prolyl hydroxylase enzyme in an iron-dependent process that targets HIFa for rapid degradation. When cellular oxygen tension drops below a critical threshold, prolyl hydroxylase can no longer target HIFa for degradation, allowing HIFa to bind to HIFb and form an active transcription factor that is able to enter the nucleus and bind to specific response elements on genes. Ribonucleotide reductase is an iron-dependent enzyme that is required for DNA synthesis (Beard and Dawson, 1997). Thus, iron is required for a number of vital functions, including growth, reproduction, healing, and immune function (Yip and Dallman, 1996).

2.5.2.2 Zinc

Zinc is an essential trace element for all forms of life. Clinical zinc deficiency in humans was first described in 1961, when the consumption of diets with low zinc bioavailability due to high phytic acid content was associated with "adolescent nutritional dwarfism" in the Middle East (Prasad, 1998). Since then, zinc insufficiency has been recognized by a number of experts as an

important public health issue, especially in developing countries (Prasad, 1998). Zinc is an important trace mineral that people need to stay healthy. This element is second only to iron in its concentration in the body. Numerous aspects of cellular metabolism are zinc-dependent. Zinc plays important roles in growth and development, the immune response, neurological function, and reproduction Zinc is found in cells throughout the body. It is needed for the body's defensive (immune) system to properly work. It plays a role in cell division, cell growth, wound healing, and the breakdown of carbohydrates. Zinc is also needed for the senses of smell and taste. During pregnancy, infancy, and childhood the body needs zinc to grow and develop properly. On the cellular level, the function of zinc can be divided into three categories namely, catalytic, structural and regulatory (Cousins *et al.*, 2006).

Nearly 100 different enzymes depend on zinc for their ability to catalyze vital chemical reactions. Zinc-dependent enzymes can be found in all known classes of enzymes (FNB, 2001). Zinc plays an important role in the structure of proteins and cell membranes. A fingerlike structure, known as a zinc finger motif, stabilizes the structure of a number of proteins. For example, copper provides the catalytic activity for the antioxidant enzyme copper-zinc superoxide dismutase (CuZnSOD), while zinc plays a critical structural role (FNB, 2001; Cousins *et al.*, 2006). The structure and function of cell membranes are also affected by zinc. Loss of zinc from biological membranes increases their susceptibility to oxidative damage and impairs their function (O'Dell, 2000).

Zinc finger proteins have also been found to regulate gene expression by acting as transcription factors. Zinc also plays a role in cell signaling and has been found to influence hormone release and nerve impulse transmission. Zinc has been found to play a role in apoptosis, a critical

cellular regulatory process with implications for growth and development, as well as a number of chronic diseases (Truong-Tran *et al.*, 2000).

2.5.2.3 Copper

Copper (Cu) is an essential trace element for humans and animals. In the body, copper shifts between the cuprous (Cu^{1+}) and cupric (Cu^{2+}) forms, though the majority of the body's copper is in the Cu^{2+} form. The ability of copper to easily accept and donate electrons explains its important role in oxidation-reduction reactions and in scavenging free radicals (Linder and Hazegh-Azam, 1996). Although Hippocrates is said to have prescribed copper compounds to treat diseases as early as 400 B.C. (Turnlund, 2006), scientists are still uncovering new information regarding the functions of copper in the human body.

Copper is a critical functional component for a number of essential enzymes known as cuproenzymes. Some of the physiologic functions known to be copper-dependent are discussed below. The copper-dependent enzyme, cytochrome *c* oxidase, plays a critical role in cellular energy production. By catalyzing the reduction of molecular oxygen to water, cytochrome *c* oxidase generates an electrical gradient used by the mitochondria to create the vital energy-storing molecule, ATP (Uauy *et al.*, 1998). Another cuproenzyme, lysyl oxidase, is required for the cross-linking of collagen and elastin, which are essential for the formation of strong and flexible connective tissue. The action of lysyl oxidase helps maintain the integrity of connective tissue in the heart and blood vessels and also plays a role in bone formation (Turnlund, 2006).

Two copper-containing enzymes, ferroxidase I and ferroxidase II have the capacity to oxidize ferrous iron (Fe^{2+}) to ferric iron (Fe^{3+}), the form of iron that can be loaded onto the protein transferring for transport to the site of red blood cell formation. Although the ferroxidase activity

of these two cuproenzymes has not yet been proven to be physiologically significant (Turnlund, 2006).

The fact that iron mobilization from storage sites is impaired in copper deficiency supports their role in iron metabolism (Turnlund, 2006). A number of reactions essential to normal function of the brain and nervous system are catalyzed by cuproenzymes. Dopamine-b-monooxygenase catalyzes the conversion of dopamine to the neurotransmitter norepinephrine. Monoamine oxidase (MAO) plays a role in the metabolism of the neurotransmitters norepinephrine, epinephrine, and dopamine. MAO also functions in the degradation of the neurotransmitter serotonin, which is the basis for the use of MAO inhibitors as antidepressants (FNB, 2001). The myelin sheath is made of phospholipids whose synthesis depends on cytochrome *c* oxidase activity (Turnlund, 2006). The cuproenzyme, tyrosinase, is required for the formation of the pigment melanin. Melanin is formed in cells called melanocytes and plays a role in the pigmentation of the hair, skin, and eyes (Turnlund, 2006).

Superoxide dismutase (SOD) functions as an antioxidant by catalyzing the conversion of superoxide radicals to hydrogen peroxide, which can subsequently be reduced to water by other antioxidant enzymes (Johnson *et al.,* 1992). Two forms of SOD contain copper: 1. copper/zinc SOD is found within most cells of the body, including red blood cells, and 2. extracellular SOD is a copper-containing enzyme found at high levels in the lungs and low levels in blood plasma (Turnlund, 2006). Ceruloplasmin is a protein found in most tissues in the human body and may function as an antioxidant in two different ways. Firstly it binds free copper and iron ions and as a result prevents free copper and iron ions from catalyzing oxidative damage. Secondly the ferroxidase activity of ceruloplasmin facilitates iron loading onto its transport protein,

transferring, and may prevent free ferrous ions (Fe^{2+}) from Participating in harmful free radical generating reactions (Johnson *et al.,* 1992).

Copper-dependent transcription factors regulate transcription of specific genes. Thus, cellular copper levels may affect the synthesis of proteins by enhancing or inhibiting the transcription of specific genes. Genes regulated by copper-dependent transcription factors include genes for copper/zinc superoxide dismutase (Cu/Zn SOD), catalase, and proteins related to the cellular storage of copper (Uauy *et al.,* 1998). A variety of indicators were used to establish the recommended dietary allowance (RDA) for copper, including plasma copper concentration, serum ceruloplasmin activity, superoxide dismutase activity in red blood cells, and platelet copper concentration (FNB, 2001).

2.5.2.4. Manganese

Manganese is a trace mineral that is present in tiny amounts in the body. It is found mostly in bones, the liver, kidneys, and pancreas. Manganese helps the body form connective tissue, bones, blood clotting factors, and sex hormones. It also plays a role in fat and carbohydrate metabolism, calcium absorption, and blood sugar regulation. Manganese is also necessary for normal brain and nerve function (Lee and Kim, 2008). Manganese also plays a role in wound healing which is a complex process that requires increased production of collagen.

Manganese is required for the activation of prolidase, an enzyme that functions to provide the amino acid, proline, for collagen formation in human skin cells. A genetic disorder known as prolidase deficiency results in abnormal wound healing among other problems, and is characterized by abnormal manganese metabolism. Glycosaminoglycan synthesis, which requires manganese-activated glycosyltransferases, may also play an important role in wound healing (Shelter and Shelter, 1994).

Manganese is also an important metal for human health, being absolutely necessary for development, metabolism, and the antioxidant system. Nevertheless, excessive exposure or intake may lead to a condition known as manganism, a neurodegenerative disorder that causes dopaminergic neuronal death and parkinsonian- like symptoms. The classes of enzymes that have manganese cofactors are very broad, and include oxidoreductases, transferases, hydrolases, lyases, isomerases and many others. The best-known manganese-containing polypeptides may be arginase, the diphtheria toxin, and Mn-containing superoxide dismutase (Mn-SOD) (Lee and Kim, 2008).

Manganese is a component of the antioxidant enzyme superoxide dismutase (SOD), which helps fight free radicals. Free radicals occur naturally in the body but can damage cell membranes and DNA. They may play a role in aging, as well as the development of a number of health conditions, including heart disease and cancer. Antioxidants, such as SOD, can help neutralize free radicals and reduce or even help prevent some of the damage they cause. Low levels of manganese in the body can contribute to infertility, bone malformation, weakness, and seizures.

It is fairly easy to get enough manganese in the diet, this nutrient is found in whole grains, nuts, and seeds, but some experts estimate that as many as 37% of Americans do not get the recommended dietary intake (RDI) of manganese in their diet. The American diet tends to contain more refined grains than whole grains, and refined grains only provide half the amount of manganese as whole grains. However, too much manganese in the diet could lead to high levels of manganese in the body tissues. Abnormal concentrations of manganese in the brain, especially in the basal ganglia, are associated with neurological disorders similar to Parkinson's disease. Early life manganese exposure at high levels, or low levels, may impact

neurodevelopment. Elevated manganese is also associated with poor cognitive performance in school children (Gunter *et al.,* 2013).

2.5.3 Determination of cadmium and lead

2.5.3.1 Cadmium

Cadmium which is one of the dangerous heavy metals occurs naturally in ores together with zinc, lead and copper. Cadmium compounds are used as stabilizers in PVC products, colour pigment, several alloys and, now most commonly, in re-chargeable nickel–cadmium batteries. Metallic cadmium has mostly been used as an anticorrosion agent (cadmiation). Cadmium is also present as a pollutant in phosphate fertilizers. Natural as well as anthropogenic sources of cadmium, including industrial emissions and the application of fertilizer and sewage sludge to farm land, may lead to contamination of soils, and to increased cadmium uptake by crops and vegetables, grown for human consumption. The uptake process of soil cadmium by plants is enhanced at low pH (Jarup *et al.,* 1998).

Cigarette smoking is a major source of cadmium exposure. Biological monitoring of cadmium in the general population has shown that cigarette smoking may cause significant increases in blood cadmium (B-Cd) levels, the concentrations in smokers being on average 4–5 times higher than those in non-smokers (Jarup *et al.,* 1998). Despite evidence of exposure from environmental tobacco smoke (Hossn *et al.,* 2001), however, this is probably contributing little to total cadmium body burden.

Food is the most important source of cadmium exposure in the general population in most countries (WHO, 1992). Cadmium is present in most foodstuffs, but concentrations vary greatly, and individual intake also varies considerably due to differences in dietary habits (Jarup *et al.,*

1998). Women usually have lower daily cadmium intakes, because of lower energy consumption than men. Gastrointestinal absorption of cadmium may be influenced by nutritional factors, such as iron status (Flanagan *et al.*, 1978).

Inhalation of cadmium fumes or particles can be life threatening, and although acute pulmonary effects and deaths are uncommon, sporadic cases still occur (Barbee and Prince, 1999). Cadmium exposure may cause kidney damage. The first sign of the renal lesion is usually a tubular dysfunction, evidenced by an increased excretion of low molecular weight proteins [such as β_2-microglobulin and α_1-microglobulin (protein HC)] or enzymes [such as N-Acetyl-β-D-glucosaminidase (NAG)] (Jarup, *et al.*, 1998*;* WHO, 1992). It has been suggested that the tubular damage is reversible, but there is overwhelming evidence that the cadmium induced tubular damage is indeed irreversible (Jarup *et al.*, 1998). WHO, 1992 estimated that a urinary excretion of 10 nmol/mmol creatinine (corresponding to *circa* 200 mg Cd/kg kidney cortex) would constitute a 'critical limit' below which kidney damage would not occur. However, WHO calculated that *circa* 10% of individuals with this kidney concentration would be affected by tubular damage. Several reports have since shown that kidney damage and/or bone effects are likely to occur at lower kidney cadmium levels.

The exposure of Cadmium was caused by cadmium-contaminated water used for irrigation of local rice fields. A few studies outside Japan have reported similar findings (Jarup *et al., 1998*). During recent years, new data have emerged suggesting that also relatively low cadmium exposure may give rise to skeletal damage, evidenced by low bone mineral density (osteoporosis) and fractures.

Animal experiments have suggested that cadmium may be a risk factor for cardiovascular disease, but studies of humans have not been able to confirm this (Jarup *et al.*, 1998). However, a

Japanese study showed an excess risk of cardiovascular mortality in cadmium-exposed persons with signs of tubular kidney damage compared to individuals without kidney damage (Jarup *et al., 1998*). It has been noted that the assessment was based on few studies of lung cancer in occupationally exposed populations, often with imperfect exposure data, and without the capability to consider possible confounding by smoking and other associated exposures (such as nickel and arsenic). Cadmium has been associated with prostate cancer, but both positive and negative studies have been published (Jarup *et al., 1998*). Early data indicated an association between cadmium exposure and kidney cancer (Jarup *et al., 1998*).

2.5.3.2 Lead

The general population is exposed to lead from air and food in roughly equal proportions. Earlier, lead in foodstuff originated from pots used for cooking and storage, and lead acetate was previously used to sweeten port wine. During the last century, lead emissions to ambient air have further polluted our environment; over 50% of lead emissions originate from petrol. Over the last few decades, however, lead emissions in developed countries have decreased markedly due to the introduction of unleaded petrol. Subsequently blood lead levels in the general population have decreased. Occupational exposure to inorganic lead occurs in mines and smelters as well as welding of lead painted metal, and in battery plants. Low or moderate exposure may take place in the glass industry (WHO, 1995).

High levels of air emissions may pollute areas near lead mines and smelters. Airborne lead can be deposited on soil and water, thus reaching humans *via* the food chain. Up to 50% of inhaled inorganic lead may be absorbed in the lungs. Adults take up 10–15% of lead in food, whereas children may absorb up to 50% *via* the gastrointestinal tract. Lead in blood is bound to erythrocytes, and elimination is slow and principally *via* urine. Lead is accumulated in the

skeleton, and is only slowly released from this body compartment. Half-life of lead in blood is about one month and in the skeleton 20–30 years (WHO, 1995).

The symptoms of acute lead poisoning are headache, irritability, abdominal pain and various symptoms related to the nervous system. Lead encephalopathy is characterized by sleeplessness and restlessness. Children may be affected by behavioral disturbances, learning and concentration difficulties. In severe cases of lead encephalopathy, the affected person may suffer from acute psychosis, confusion and reduced consciousness (WHO, 1995).

People who have been exposed to lead for a long time may suffer from memory deterioration, prolonged reaction time and reduced ability to understand. Individuals with average blood lead levels under 3 μmol/l may show signs of peripheral nerve symptoms with reduced nerve conduction velocity and reduced dermal sensibility. If the neuropathy is severe the lesion may be permanent. The classical Photograph includes a dark blue lead sulphide line at the gingival margin. In less serious cases, the most obvious sign of lead poisoning is disturbance of haemoglobin synthesis, and long-term lead exposure may lead to anaemia. Acute exposure to lead is known to cause proximal renal tubular damage (WHO, 1995).

2.5.4 Phytochemical analysis of medicinal plants

Phytochemical analysis involves the determination of the presence of phytochemical constituents in the crude samples of the medicinal plants. Phytochemical tests are carried out first to establish the presence or otherwise of some specific phytochemicals. The leaves and root-barks of medicinal plants are screened for bioactive compounds; alkaloids, saponins, tannins, flavonoids, phenols, steroids, carbohydrates, proteins and glycosides. The methods used and their corresponding inferences as described by Sofowara (1993); Trease and Evans, (1989); Harborne (1973) and AOAC (2004).

Numerals of intricate organic compounds are entailed by all human beings as added caloric prerequisites to accomplish the requirement for their muscular bustle (William, 1972). The medicinal and nutritional uses of these plants have been attributed to the phytochemical composition and nutritional content of the plants respectively.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Plant collection and identification

The plant samples of *Lannea schweinfurthii*, *Rhus natalensis* and *Euclea divinorum* root barks and leaves were collected from Mbita Sub-county, Homabay County-Kenya on December, 2013 based on ethno-botanical information. These plants were collected from their natural habitat and from identified herbalists. These plants were authenticated by a plant taxonomist in Department of Botany, Kenyatta University.

3.2 Preparation of the samples

The leaves and root- barks of plant species were cleaned using water until soil and other materials on them were removed. Thereafter, they were then air dried under shade for a week. The plants materials were then ground into fine powder using the grinding mill (QBXO190-Q4 Euro Crank Arm) then wrapped in air-tight containers and placed in the laboratory at room temperature (25^{o}C) prior to further analysis.

3.2.1 Hot water extraction

For phytochemical analysis, the preparations of plant extract samples were done using hot water extraction (Harborne, 1973). Five gm of dried finely powdered plant material were placed in a beaker and 200 ml of distilled water was added. The mixture was heated on a hot plate with continuous stirring at 30 - 40ºC for 20 minutes. Then the water extract were filtered through Whatman filter paper no. 1 and the filtrate was used for the phytochemical analysis. The water extracts were then kept in refrigerator at 4^{o}C when not in use and without freezing.

3.2.2 Solvent extraction

Crude plant extracts were prepared by Soxhlet extraction method. About 20 gm of powdered plant material were uniformly packed into a thimble and extracted with 250 ml of different solvents done through sequential extraction or separately depending on the procedure to be followed thereafter. Solvents used were; *n*-hexane, diethyl ether, dichloromethane, acetone, *n*-butanol, ethanol, methanol, and due to their increasing polarity. The process of extraction was continued for about 8 hours or till the solvent in siphon tube of an extractor becomes colourless depending on the solvent used. After that the extracts were dried using rotary evaporator at 40-60^{o}C. The dried extracts were carefully removed and kept in refrigerator at 4^{o}C awaiting phytochemical analysis.

3.3 Proximate analysis

The moisture, ash, crude fats, proteins and carbohydrates of all the samples were carried out using standard AOAC method (1990). The moisture and ash were determined using weight difference method. Crude fat were extracted by means of the Soxhlet apparatus with petroleum ether (40 to 60°C) for 8 hours. Crude fibers were done by successive digestion of the defatted samples with 1.25% sulphuric acid and 1.25% sodium hydroxide solutions. The nitrogen value, which is the precursor for protein of a substance, were determined by micro Kjeldahl method described by Pearson (1976), involving digestions, distillation and finally titration of the sample. The nitrogen value was converted to protein by multiplying a factor of 6.25. Carbohydrate was determined by difference method. The carbohydrate was calculated by difference method and as the nitrogen free extract (NFE), calculated as % NFE = 100 - % (a + b + c + d + e) where a = protein, b = fat, c = fibre, d = ash, e = moisture (Pearson, 1976 and James, 1995).

All the proximate values were reported in % (AOAC, 1990 and AOCS, 2000). The proximate analyses were done in triplicates.

3.3.1 Determination of moisture content

Two g of the fresh sample of each plant material were placed in the crucible and heated at 105˚ C until a constant weight was attained. The moisture content of each variety was calculated as loss in weight of the original sample and expressed as percentage moisture content (FAO, 1980).

3.3.2 Determination of crude protein

The crude protein was determined by the Kjeldahl method with slight modification (AOAC, 1990). The determination of crude protein involved three steps namely; digestion, distillation and titration.

Digestion: One g of ground sample was weighed into a digestion flask. Reagent blank and high purity lysine HCl was included as check of correctness of digestion parameters. 15 g potassium sulfate, 0.04 g anhydrous copper sulfate, 0.5 to 1.0 g alundum granules, 16.7 g K_2SO_4, 0.01 g anhydrous copper sulfate, 0.6 g TiO_2 and 0.3 g pumice were added. Then 20 mL sulfuric acid was added. The flask was placed on preheated burner (adjusted to bring 250 mL water at 25^{o}C to rolling boil in 5 minutes) and the mixture was heated until white fumes clear bulb of flask were seen, swirled gently, and heating continued for 90 min for copper catalyst. The mixture was then cooled and cautiously 250 mL of distilled water was added to room temperature.

Distillation: A mixture of 15 mL of hydrochloric acid and 70 mL of water (V HCl) were accurately measured to form acid standard solution then added to the titration flask. For reagent blank, 1 mL of acid and approximately 85 mL water were added followed by three to four drops of methyl red indicator solution. In addition, two to three drops of tributyl citrate, an antifoam

agent was added to digestion flask to reduce foaming. This was then followed by addition of another 0.5 to 1.0 g alundum granule. Slowly down side of flask, sufficient 45% sodium hydroxide solution (approximately 80 mL) was added to make mixture strongly alkali. The flask was connected to distillation apparatus and distilled until at least 150 mL distillate was collected in titrating flask.

Titration: Excess acid was titrated with standard 0.1M sodium hydroxide solution to orange endpoint (color changed from red to orange to yellow) and volume was recorded to nearest 0.01 mL (VNaOH). The reagent blank (B) was titrated similarly.

Calculations were done as follows:

%N (DM basis) = [(V HCl x N HCl) - (V BK x N NaOH) - (V NaOH x N NaOH)]/1.4007 x W x Lab DM/100

Where DM – dry matter; V NaOH = mL standard NaOH needed to titrate sample; V HCl = mL standard HCl pipetted into titrating flask for sample; N NaOH = Normality of NaOH; N HCl = Normality of HCl; V BK = mL standard NaOH needed to titrate 1 mL standard HCl minus B; B = mL standard NaOH needed to titrate reagent blank carried through method and distilled into 1 mL standard HCl; 1.4007 = milli equivalent weight of nitrogen x 100; W = sample weight in grams.

Calculation percent crude protein (CP):

Crude Protein (Dry Matter (DM) basis) = % N (DM basis) X F; where F = 6.25 (AOAC, 1990).

3.3.3 Determination of crude lipid

This estimation was performed using the Soxhlet extraction method. 10 g of the powdery form of each plant sample were weighed and wrapped with a filter paper and placed in a thimble. The thimble was covered with cotton wool and placed in the extraction column that was connected to a condenser. 200 ml of *n* – hexane was used to extract the lipid (AOAC, 1990).

3.3.4 Determination of crude fibre

Five grammes of the powdery form of each plant material and 200 ml of 1.25 % H_2SO_4 were heated for 30 min and filtered with a buchner funnel. The residue was washed with distilled water until it was acid free. 200 ml of 1.25% NaOH was used to boil the residue 30 minutes; it was filtered and washed several times with distilled water until it was alkaline free. It was then rinsed once with 10% HCl and twice with ethanol. Finally it was rinsed with petroleum ether three times. The residue was put in a crucible and dried at 105^{o} C in an oven overnight. After cooling in a desiccator, it was ignited in a muffle furnace at 550^{o} C for 90 minutes to obtain the weight of the ash (AOAC, 1990).

3.3.5 Determination of ash content

The total ash content of a substance is the percentage of inorganic residue remaining after the organic matter has been ignited. 2 g of the pulverized plant samples was placed in a crucible and ignited in a muffle furnace at 550^{o}C for 6 hours. It was then cooled in a desiccator and weighed at room temperature to get the weight of the ash (AOAC, 1990).

3.3.6 Determination of carbohydrate

The carbohydrate content was determined by subtracting the summed up percentage compositions of moisture, protein, lipid, fibre, and ash contents from 100 (Otitoju, 2009).

3.4 Analysis of micronutrients

The method of AOAC (1990) was employed for the determination of mineral content. 2 g of the of the pulverized plant samples was placed in a crucible and ignited in a muffle furnace at 550 $^{\circ}$C for 6 hours. The resulting ash was dissolved in 10 ml of 10 % HNO_3 and heated slowly for 20 minutes. After heating, it was filtered and the filtrate was used for the determination of mineral content. Atomic absorption spectrophotometer (AAS) was used to determine Cu, Fe, Zn and Mn and toxicity of Pb and Cd. The results were obtained while using a working standard of 1000 ppm for each of the species and replicated thrice.

3.5 Qualitative analysis of phytochemicals

The confirmatory qualitative tests on the phytochemicals were done according to the standard methods (Yadav and Agarwala, 2011; Trease and Evans, 1989; Sofowora, 1993; Harborne, 1973).

3.5.1 Test for proteins

Millon's test

Five ml of the crude plant water extract was mixed with 2 ml of Millon's reagent. Millon's reagent is prepared by dissolving 5 g each of $HgNO_3$ and $Hg(NO_3)_2$ in 100 ml of dilute HNO_3. Appearance of white precipitate which turned red upon gentle heating confirmed the presence of protein (Yadav and Agarwala, 2011; Trease and Evans, 1989; Sofowora, 1993; Harborne, 1973).

Ninhydrin test

Five ml of the crude plant water extract was boiled with 2 ml of 0.2 % solution of ninhydrin and appearance of violet colour suggested the presence of amino acids and proteins (Yadav and Agarwala, 2011; Trease and Evans, 1989; Sofowora, 1993; Harborne, 1973).

3.5.2 Test for carbohydrates

Fehling's test

Equal volume of Fehling A reagent (prepared by dissolving 7 g of $CuSO_4.5H_2O$ in 100 ml distilled water containing 2 drops of dilute sulphuric acid) and Fehling B reagent (prepared by dissolving 35g potassium tartrate and 12 g of NaOH in 100 ml distilled water) were mixed together and 2 ml of it added to 5 ml of crude water extract and gently boiled. A brick red precipitate at the bottom of the test tube indicated the presence of reducing sugars (Yadav and Agarwala, 2011; Trease and Evans, 1989; Sofowora, 1993; Harborne, 1973).

Benedict's test

Five ml of crude water extract was mixed with 2 ml of Benedict's reagent (which is prepared by dissolving a mixture of 173 g of sodium citrate, 100 g of sodium carbonate and 17.3 g of cupric sulphate pentahydrate in one litre of distilled water) and boiled. A reddish brown precipitate formed which indicated the presence of the carbohydrates (Yadav and Agarwala, 2011; Trease and Evans, 1989; Sofowora, 1993; Harborne, 1973).

3.5.3 Test for phenols and tannins

Five ml of water extract was mixed with 2 ml of 2 % solution of $FeCl_3$. A blue-green or black coloration indicated the presence of phenols and tannins (Yadav and Agarwala, 2011; Trease and Evans, 1989; Sofowora, 1993; Harborne, 1973).

3.5.4 Test for flavonoids

Shinoda test

Five ml of water extract was mixed with few fragments of magnesium ribbon and concentrated HCl was added drop wise. Pink scarlet colour appeared after few minutes which indicated the

presence of flavonoids (Yadav and Agarwala, 2011; Trease and Evans, 1989; Sofowora, 1993; Harborne, 1973).

Alkaline reagent test

Five ml of water extract was mixed with 2 ml of 2% solution of NaOH. An intense yellow colour was formed which turned colourless on addition of few drops of dilute HCl acid which indicated the presence of flavonoids (Yadav and Agarwala, 2011; Trease and Evans, 1989; Sofowora, 1993; Harborne, 1973).

3.5.5 Test for saponins

Five ml of water extract was mixed with 5 ml of distilled water in a test tube and it was shaken vigorously. The formation of stable foam was taken as an indication for the presence of saponins (Yadav and Agarwala, 2011; Trease and Evans, 1989; Sofowora, 1993; Harborne, 1973).

3.5.6 Test for glycosides

Salkowski's test

Five ml of water extract was mixed with 2 ml of chloroform. Then 2 ml of concentrated H_2SO_4 was added carefully and shaken gently. A reddish brown colour indicated the presence of steroidal ring, i.e., glycone portion of the glycoside (Yadav and Agarwala, 2011; Trease and Evans, 1989; Sofowora, 1993; Harborne, 1973).

Keller-kilani test

Five ml of water extract was mixed with 2 ml of glacial acetic acid containing 1-2 drops of 2 % solution of $FeCl_3$. The mixture was then poured into another test tube containing 2 ml of concentrated H_2SO_4. A brown ring at the interphase indicated the presence of cardiac glycosides (Yadav and Agarwala, 2011; Trease and Evans, 1989; Sofowora, 1993; Harborne, 1973).

3.5.7 Test for steroids

Five ml of water extract was mixed with 2 ml of chloroform and concentrated H_2SO_4 was added sidewise. A red colour was produced in the lower chloroform layer which indicated the presence of steroids. Another test was also performed by mixing crude extract with 2 ml of chloroform. Then 2 ml of each of concentrated H_2SO_4 and acetic acid was poured into the mixture. The development of a greenish coloration indicated the presence of steroids (Yadav and Agarwala, 2011; Trease and Evans, 1989; Sofowora, 1993; Harborne, 1973).

3.5.8 Test for terpenoids

Five ml of water extract was dissolved in 2 ml of chloroform and evaporated to dryness. To this, 2 ml of concentrated H_2SO_4 was added and heated for about 2 minutes. A grayish colour indicated the presence of terpenoids (Yadav and Agarwala, 2011; Trease and Evans, 1989; Sofowora, 1993; Harborne, 1973).

3.5.9 Test for alkaloids

Five ml of water extract was mixed with 2 ml of 1 % HCl and heated gently. Two drops of Mayer's reagent (prepared by dissolving a mixture of 1.36 g of mercuric chloride and 5 g of potassium iodide in 100 ml distilled water) and Dragendorff's reagent (which is a solution of potassium bismuth nitrate) were then added to the mixture. Turbidity of the resulting precipitate was taken as evidence for the presence of alkaloids (Yadav and Agarwala, 2011; Trease and Evans, 1989; Sofowora, 1993; Harborne, 1973).

3.6 Quantitative determination of phytochemicals

3.6.1 Determination of flavonoids

To determine flavonoids, 5 g of each plant sample was weighed in a 250 ml titration flask, and 100 ml of the 80 % aqueous methanol was added at room temperature and shaken for 4 hours in an electric shaker. The entire solution was filtered through Whatman filter paper number 42 (125 mm). To the filtrate, 100 ml of the 80 % aqueous methanol was again added at room temperature, shaken for 4 hours in an electric shaker then filtered through Whatman filter paper number 42 (125 mm). The filtrate was later transferred into a weighed crucible and evaporated to dryness over a water bath and weighed again (Boham *et al.,* 1994). The difference in weight gave the weight of flavonoids which was expressed as a percentage of the weight of sample analyzed.

$$\% \text{ Flavonoids} = \frac{w1 + w2}{\text{weight of sample}} \times 100$$

Where W1 = Weight of empty crucible; W2 = Weight of crucible + flavonoids precipitate

3.6.2 Determination of alkaloids

For alkaloids determination, 5 g of each sample was weighed into a 250 ml beaker, and 200 ml of 20 % acetic acid in ethanol was added and allowed to stand for 4 hours. This was filtered and the extract was concentrated using a water bath to evaporate about one-quarter of the original volume. The concentrated ammonium solution was added dropwise to the extract until the precipitation was completed. The entire solution was allowed to settle and washed using distilled water then filtered. The weight of the precipitate was recorded (Obadoni et al., 2001).

$$\% \text{ Alkaloids} = \frac{w1 + w2}{\text{weight of sample}} \times 100$$

Where W1 = Weight of empty crucible; W2 = Weight of crucible + alkaloids precipitate

3.6.3 Determination of saponins

20 g of each ground plant samples were dispersed in 200 ml of 20 % ethanol. The suspension was heated over a hot water bath for 4 hours with continuous stirring at about 55 ^{0}C. The mixture was filtered and the residue re-extracted with another 200 ml of 20 % ethanol. The combined extracts were reduced to 40 ml over water bath at about 90 ^{0}C. The concentrate was transferred into a 250 ml separator funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. 60 ml of *n*-butanol was added to the aqueous layer. The solution of *n*-butanol extracts were washed twice with 10 ml 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the samples were dried in the oven to a constant weight. The saponins content was calculated in percentage (Obadoni *et al*., 2001).

$$\% \text{ Saponins} = \frac{w1 + w2}{\text{weight of sample}} \times 100$$

Where W1 = Weight of empty crucible; W2 = Weight of crucible + saponins precipitate

3.6.4 Determination of phenols

To determine the total phenols, 5 g of the plant sample was weighed into a 250 ml titration flask and 100 ml *n*-hexane was added twice for 4 hours interval each; the filtrates were discarded for fat free sample preparation. Then, 100 ml diethyl ether was added, heated for 15 min cooled up to room temperature and was filtered into a separating funnel. About 100 ml of the 10 % NaOH solution was added and swirled to separate the aqueous layer from the organic layer. It was washed three times with 25 ml de-ionized water. The total aqueous layer was acidified up to pH 4.0 by adding 10 % HCl solution and 50 ml dichloromethane (CH_2Cl_2) to acidify the aqueous

layer in the separating flask. Consequently, the organic layer was collected, dried and then weighed (Iqbal *et al.*, 2011).

$$\% \text{ Phenols} = \frac{w1 + w2}{\text{weight of sample}} \times 100$$

Where W1 = Weight of empty crucible; W2 = Weight of crucible + phenols precipitate

3.7 Data Analysis

Each experiment was done in triplicates. The results were presented with their means and standard deviations. All data collected were subjected to t-test and ANOVA analysis.

CHAPTER FOUR

RESULTS AND DISCUSSIONS

4.1 Proximate analysis of crude medicinal plants extracts

Proximate analyses of crude extracts of selected medicinal plants namely *L. schweinfurthii*, *R. natalensis* and *E. divinorum* root barks and leaves were carried out using conventional methods. The determination of the proximate constituents is necessary in assessing nutritional levels of leaves and root barks of these frequently consumed species in the traditional medicine. These analyses revealed important findings and results obtained as presented in table 4.1.

Table 4. 1: Proximate analysis of medicinal plants extracts

Proximate analysis											
Sample name	**Moisture**		**Crude fibre**		**Ash**		**Protein**		**Crude lipid**		**carbohydrate**
	mean ±SD	% by mass	mean ±SD	% by mass	mean ±SD	% by mass	mean ±SD	% by mass	mean ±SD	% by mass	% by mass
L. schweinfurthii leaves	0.27± 0.01	13.65 ±0.75	0.05± 0.01	1.00± 0.20	0.22± 0.01	11.00 ±0.50	3.51± 0.27	21.97 ±0.70	0.45± 0.01	9.00± 0.20	43.38± 0.34
L. schweinfurthii root bark	0.29± 0.01	14.50 ±0.50	0.02± 0.01	0.46± 0.12	0.24± 0.02	11.85 ±0.75	2.46± 0.27	15.36 ±0.70	0.28± 0.01	5.60± 0.02	52.23± 0.17
R. natalensis leaves	0.19± 0.01	9.35± 0.77	0.01± 0.01	0.20± 0.12	0.19± 0.01	9.50± 0.50	3.78± 0.30	23.61 ±0.80	0.17± 0.01	3.46± 0.11	53.88± 0.35
R. natalensis root bark	0.22± 0.01	11.15 ±0.29	0.09± 0.01	1.80± 0.20	0.20± 0.01	10.00 ±0.50	2.45± 0.30	15.33 ±0.80	0.13± 0.01	2.60± 0.20	59.12± 0.09
E. divinorum leaves	0.21± 0.01	10.50 ±0.29	0.03± 0.01	0.60± 0.12	0.15± 0.02	7.65± 0.75	1.39± 0.30	8.66± 0.80	0.46± 0.01	9.20± 0.20	63.39± 0.23
E. divinorum root barks	0.26± 0.01	13.00 ±0.50	0.04± 0.01	0.80± 0.20	0.17± 0.01	8.50± 0.50	1.99± 0.30	12.30 ±0.80	0.73± 0.02	14.66 ±0.30	50.74± 0.44

The moisture content of medicinal plants ranged from 9.35±0.77% for *R. natalensis* leaves to 14.50±0.50% *for L. schweinfurthii* root barks. The high moisture content provides for greater activity of water soluble enzymes and co-enzymes needed for metabolic activities of these plants (Iheanacho and ubebani, 2009).

The crude fiber content was found to be present in all the medicinal plants samples investigated in varied proportions. The lowest quantity of fibre was 0.20±0.12% found in *R. natalensis* leaves. The highest crude fibre content was found to be 1.80±0.20% in *R. natalensis* root barks. Crude fibre helps to prevent constipation, bowel problems and piles. Fibers in the diet are necessary for digestion and for effective elimination of wastes, and can lower the serum cholesterol, the risk of coronary heart disease, hypertension, constipation, diabetes, colon and breast cancer (Sodipo *et al*., 2000).

The ash content of medicinal plants ranged from 7.65±0.75% for *E. divinorum* leaves to 1.85±0.75% for *L. schweinfurthii* root barks dry weight. The ash content range indicates that the medicinal plants are rich in mineral elements. This value is higher than that reported for sweet potato leaves and *Tribulus terrestris* leaves, but lower than some leafy vegetables commonly consumed in Nigeria such as *Talinum triangulare*, *Occimum graticimum* and *Hibiscus esculentum (*Akindahunsi and Salawu, 2005; FAO, 1980).

The crude protein of medicinal plants ranged from 23.61±0.80% for *R. natalensis* leaves to 8.66±0.80% for *E. divinorum leaves*. The relatively higher protein content of medicinal plants indicated their nutritional superiority over *Clerodendrum volubile* leaves which had 11.2% protein content (Erukainure *et al*., 2011). It was observed that the medicinal plants investigated revealed that their leaves registered a larger percentage of proteins than their roots except for *E. divinorum leaves*. Of the three medicinal plants studied, *E. divinorum* registered the lowest

percentage of proteins compared to *R. natalensis* and *L. schweinfurthii.* Plant foods that provide more than 12% of their calorific value from protein have been shown to be good source of protein such as cabbage, 12.8% and lettuce, 14% and also crude protein in the leafy plants would require dietary supplementation with proteins from cereals and legumes (Ali, 2009; Erukainure *et al*., 2011; Ejoh *et al*., 2007).

The crude lipid content ranged from 2.60±0.20% of *R. natalensis* root barks to 14.66±0.30% for *E. divinorum* root barks dry weight. The lipid level of the medicinal plants investigated were within range as the results obtained from *Clerodendrum volubile* leaves as was reported by Erukainure *et al*., (2011). Consumption of (crude) lipids in large amounts is a good dietary habit and recommended to individuals suffering from overweight or obesity (Sodipo *et al*., 2000; Erukainure *et al*., 2011).

Carbohydrates are one of the most important components in many foods, and the digestible carbohydrates are considered as an important source of energy. The carbohydrate content of the medicinal plant samples varied considerably, ranging from 43.38±0.34% for *L. schweinfurthii* leaves to 63.39±0.23% for *E. divinorum* leaves. These values are normally considered to be sufficient and therefore an indication that they could be an important source of dietary calories. The recommended carbohydrate daily dietary allowance values for children, adults, pregnant and lactating mothers are 130, 130, 175 and 210 g respectively (FNB, 2002).

4.2 Micronutrients analysis of crude medicinal plants extracts

Minerals in the diet are required for proper growth and good health. The absorbance of the various micronutrients and determination of lead and cadmium in the different concentrations (in ppm) in their standards were obtained and data is given in table 4.2

Table 4. 2: Mean absorbance of standards of studied micronutrients and lead and cadmium ions

Standards in ppm	Absorbance (mean±SD)					
	Copper	**Iron**	**Zinc**	**Manganese**	**Lead**	**Cadmium**
2	0.057±0.001	0.004±0.001	0.010±0.001	0.022±0.001	0.006±0.001	0.125±0.001
4	0.133±0.001	0.015±0.001	0.031±0.001	0.105±0.001	0.017±0.001	0.180±0.001
6	0.221±0.001	0.025±0.001	0.049±0.001	0.193±0.001	0.028±0.001	0.227±0.001
8	0.321±0.001	0.035±0.001	0.069±0.001	0.292±0.001	0.038±0.001	0.285±0.001
10	0.399±0.001	0.045±0.001	0.091±0.001	0.385±0.001	0.049±0.001	0.333±0.001

2; 4; 6; 8; 10; - absorbance of standards of different micronutrients and SD - standard deviation.

From the results in table 4.2, calibration graphs were then drawn as shown in figure 4.1 to figure 4.6. These graphs were used to calculate various concentrations of the micronutrients using Beer's law. There was significant different between the samples tested.

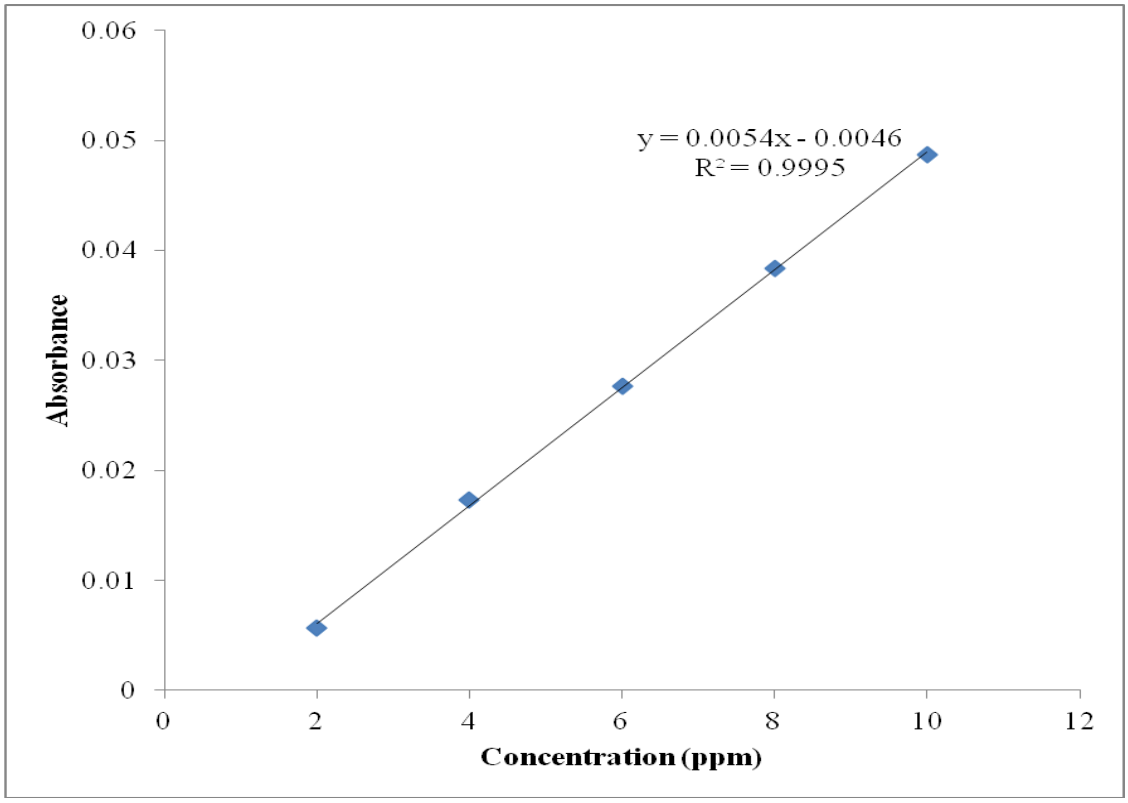

Figure 4. 1: Calibration curve of Pb ions

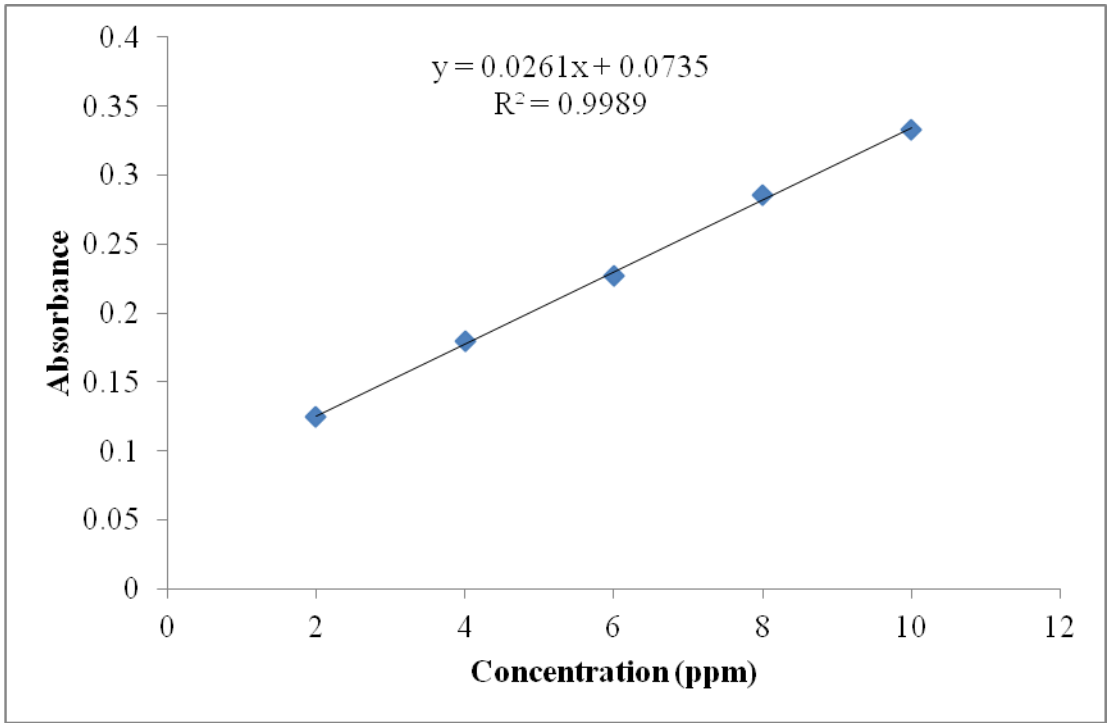

Figure 4.2: calibration curve of Cd ions

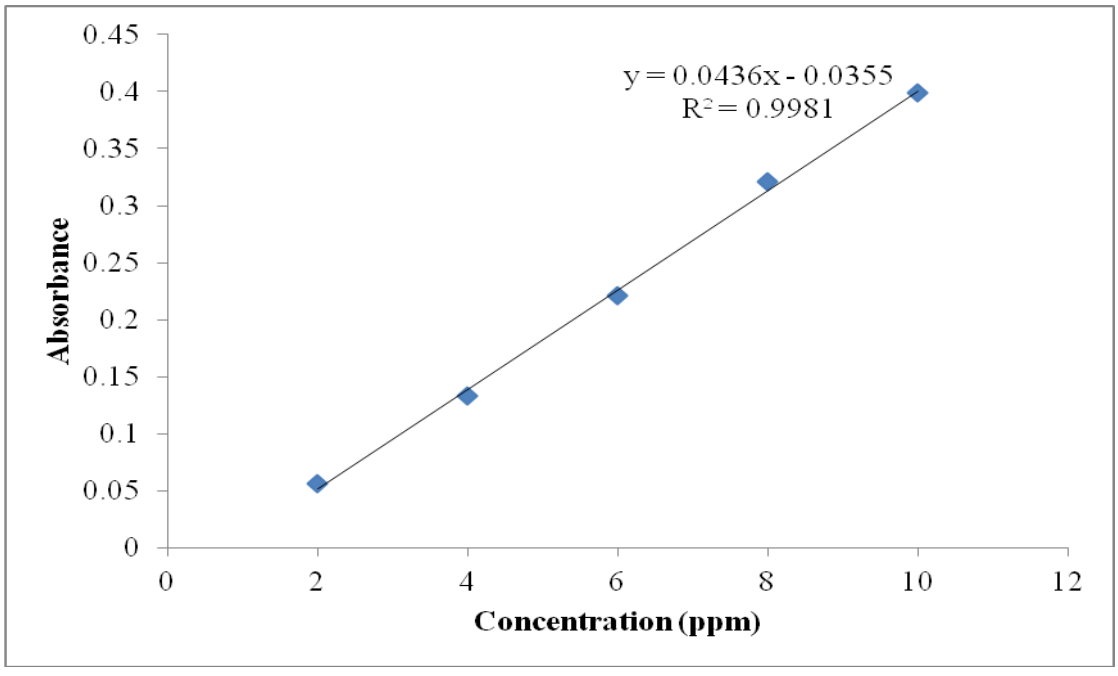

Figure 4.3: Calibration curve of Cu ions

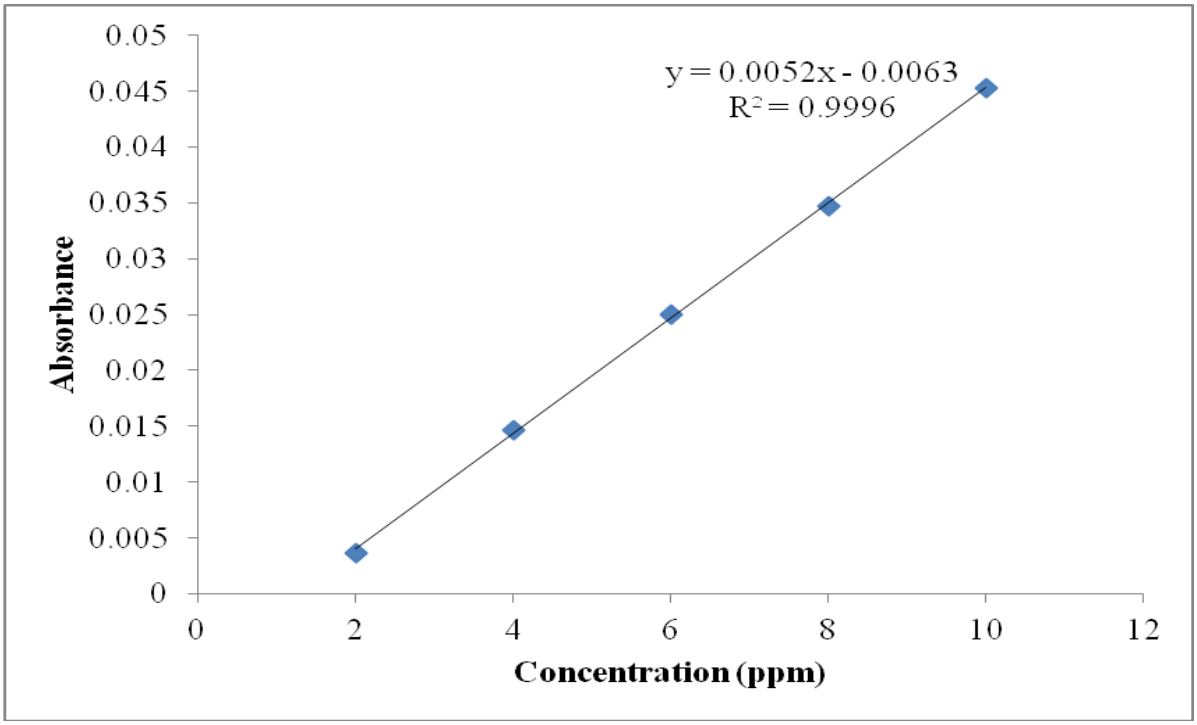

Figure 4.4: Calibration curve of Fe ions

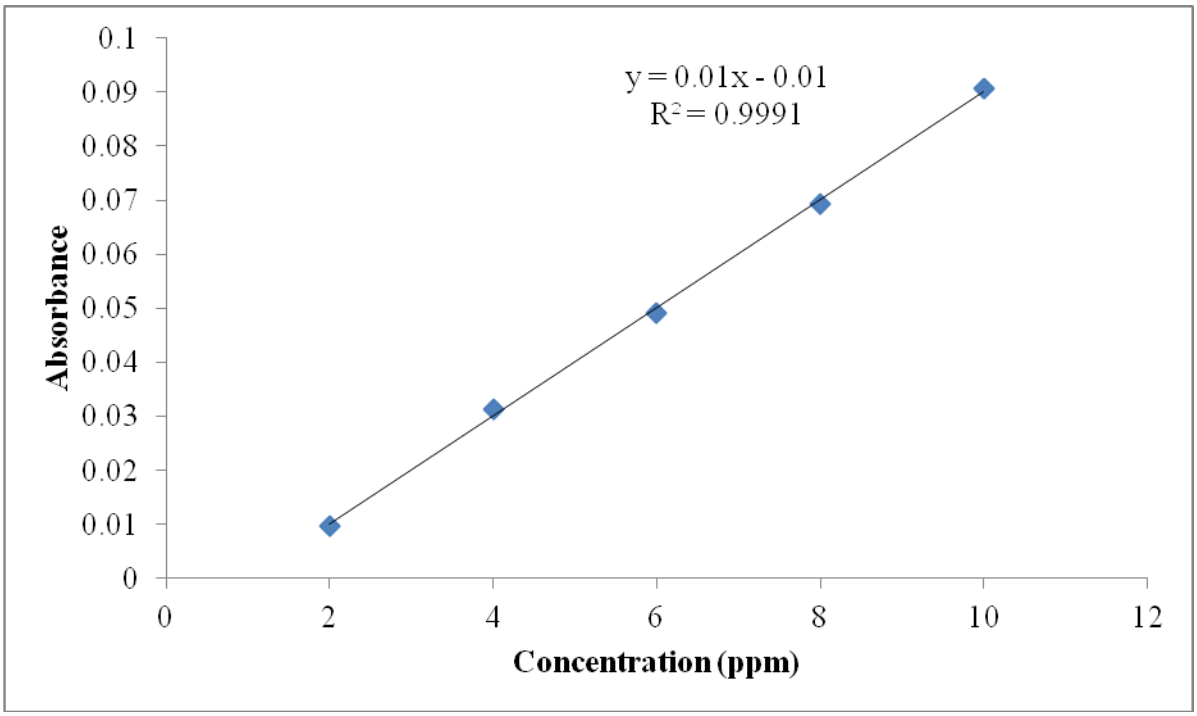

Figure 4.5: Calibration curve of Zn ions

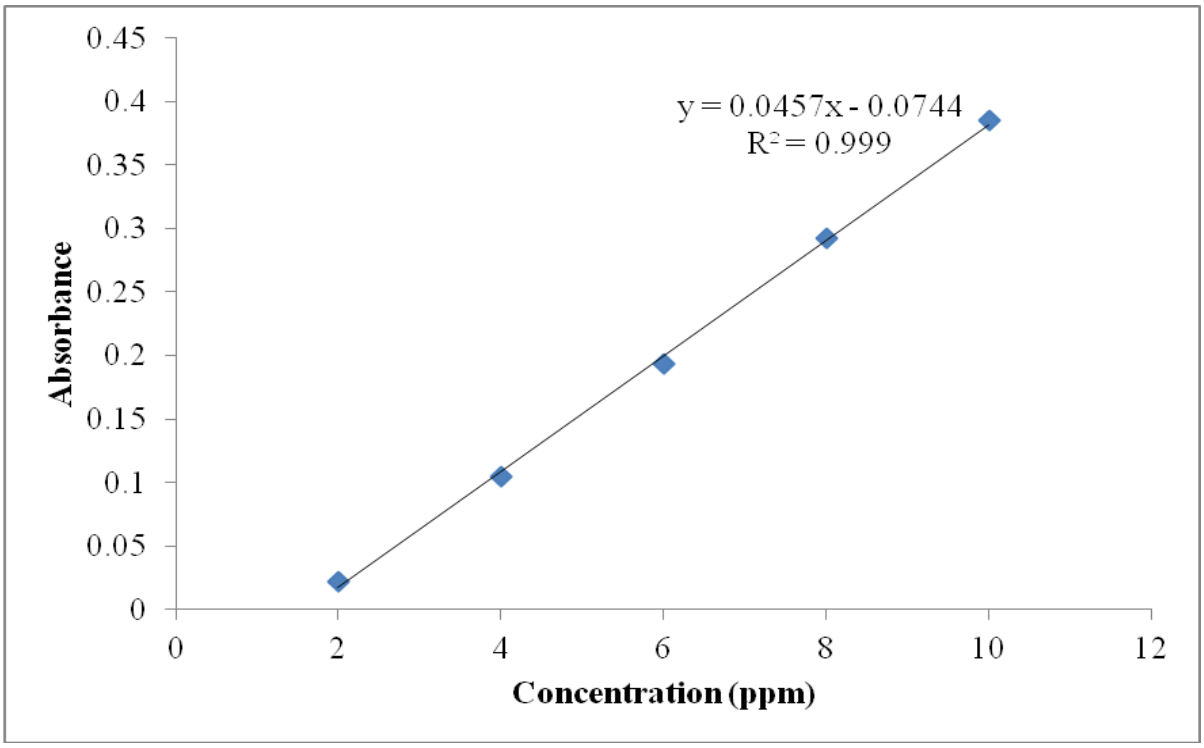

Figure 4.6: Calibration curve of Mn ions

The plant samples were then run using Atomic Absorption Spectroscopy. The analyses of copper, iron, zinc manganese, were investigated and the results obtained were shown in table 4.3. Lead and cadmium are harmful heavy metals when ingested.

Table 4. 3: Micronutrients, lead and cadmium analysis of crude plants extracts

Sample name	Absorbance (mean±SD)					
	Copper	Iron	Zinc	Manganese	Lead	Cadmium
LSL	0.004±0.000	0.001±0.000	0.016±0.001	0.016±0.000	0.001±0.001	0.003±0.001
LSRB	0.003±0.001	0.001±0.001	0.002±0.001	0.003±0.001	0.001±0.001	0.002±0.001
RNL	0.013±0.001	0.018±0.002	0.035±0.001	0.019±0.001	0.001±0.001	0.008±0.001
RNRB	0.003±0.002	0.003±0.001	0.014±0.001	0.005±0.001	0.001±0.001	0.001±0.001
EDL	0.007±0.001	0.014±0.001	0.015±0.001	0.029±0.002	0.001±0.001	0.011±0.001
EDRB	0.006±0.001	0.001±0.000	0.016±0.002	0.013±0.001	0.012±0.001	0.024±0.001

LSL - *L. schweinfurthii* leaves; LSRB - *L. schweinfurthii* root barks; RNL - *R. natalensis* leaves; RNRB - *R. natalensis* root barks; EDL - *E. divinorum* leaves; EDRB - *E. divinorum* root barks and SD - standard deviation.

The concentrations in ppm of the results of the minerals estimation of the medicinal plants were presented in table 4.4.

Table 4. 4: Concentrations of micronutrients, lead and cadmium in crude plants extracts

	Concentration in ppm (mean±SD)					
Plant sample	**Copper**	**Iron**	**Zinc**	**Manganese**	**Lead**	**Cadmium**
LSL	0.90±0.00	1.40±0.01	2.60±0.02	2.00±0.01	1.00±0.00	ND
LSRB	0.90±0.01	1.40±0.02	1.20±0.01	1.70±0.01	1.10±0.01	ND
RNL	1.10±0.01	4.90±0.01	4.50±0.01	2.10±0.02	1.10±0.01	ND
RNRB	0.90±0.02	1.90±0.01	2.40±0.02	1.80±0.01	1.00±0.00	ND
EDL	1.00±0.01	3.90±0.00	2.50±0.01	2.30±0.01	1.00±0.01	ND
EDRB	1.00±0.01	1.40±0.01	2.60±0.01	1.90±0.00	3.10±0.02	ND

LSL - *L. schweinfurthii* leaves; LSRB - *L. schweinfurthii* root barks; RNL - *R. natalensis* leaves; RNRB - *R. natalensis* root barks; EDL - *E. divinorum* leaves; EDRB - *E. divinorum* root barks; SD - standard deviation and ND - not detected.

The study showed that copper was the least abundant micronutrient investigated with a range of 0.90 to 1010 ppm in all the medicinal plants studied and iron was most abundant with a range of 1.04 to 4.90 ppm. The level of zinc in all the medicinal plant samples ranged between 1.20±0.01 ppm in *L. schweinfurthii* root barks to 4.50±0.01 ppm in *R. natalensis* leaves. Zinc is an essential micronutrient for human growth and immune functions (Black, 2003). The deficiencies of essential vitamins or minerals lead to several physiological disorders and diseases, slowed growth, and lack of deposition of proteins in tissues (Cousins, 2006). *R. natalensis* leaves contained highest micro-elements such as iron 4.90±0.01 ppm and zinc 4.50±0.01 ppm in comparison with other plants in this study; it had high nutritional value from point of view of the above trace (micro) elements. Zinc that is required for the proper functioning of the reproductive system (Hambidge, 2006) was found to be present in the medicinal plants studied.

Additionally, the non-essential heavy metals namely lead and cadmium were also analyzed. The mean concentrations of lead and cadmium, the dangerous heavy metals when ingested, were

investigated and presented in table 4.4. The analyses revealed that mean concentration of Pb ranged from 1.00±0.00 ppm (µg/litre) to 3.10±0.02 ppm (µg/litre) and found to be in marginal concentrations compared to WHO (2005) maximum allowable limits of 10 mg/kg. The results revealed that *E. divinorum* root barks registered highest concentration of Pb of 3.10±0.02 ppm but still this was marginal and below WHO (2005) maximum allowable limits of lead and cadmium as 10 mg/kg and 0.3 mg/kg respectively in herbal plants. Cd was not detected therefore was absent in all the medicinal plant samples. According to a study done by Kofi and Rita *et al*., (2013) indicated that *Ocimum gratissum* and *Alstonia boonel* registered signals below the detection limit however, in *Moringa oleifera* 0.350 µg /g was registered. The most common sources for cadmium in soil and plants are phosphate fertilizers, sewage sludge application, and combustion of fossil fuels. Thus, there is the need for a proper and efficient waste disposal system as cadmium levels above permissible values may result in irreversible kidney damage (Kofi and Rita *et al*., 2013).

4.3 Determination of phytochemicals in the crude medicinal plants extracts

4.3.1 Qualitative determination of phytochemicals in the crude plants extracts

The presence or absence of phytochemicals was determined using qualitative tests and the results are shown in table 4.5 below. The study revealed that the phytochemicals which included carbohydrates, phenols and tannins, flavonoids, glycosides, steroids, terpenoids, saponins, proteins and alkaloids were present in most parts of the medicinal plants investigated.

Glycosides were found to be present in all medicinal plants except in *R. natalensis* leaves where it was absent and steroids were absent in all medicinal plants except in *L. schweinfurthii* root barks where it was present.

Table 4. 5: Qualitative determination of phytochemicals in the crude plants extracts

Qualitative determination of phytochemicals									
Sample name	**C**	**P and T**	**F**	**G**	**S**	**T**	**S**	**P**	**A**
LSL	X	X	X	X	Y	X	X	X	X
LSRB	X	X	X	X	X	X	X	X	X
RNL	X	X	X	Y	Y	X	X	X	X
RNRB	X	X	X	X	Y	X	X	X	X
EDL	X	X	X	X	Y	X	X	X	X
EDRB	X	X	X	X	Y	X	X	X	X

LSL - *L. schweinfurthii* leaves; LSRB - *L. schweinfurthii* root barks; RNL - *R. natalensis* leaves; RNRB - *R. natalensis* root barks; EDL - *E. divinorum* leaves; EDRB - *E. divinorum* root barks, X – Presence , Y – absence, C – Carbohydrates, P and T – Phenols and Tannins, F – Flavonoids, G – Glycosides, S – Steroids, T – Terpenoids, S – Saponins, P – Proteins and A – Alkaloids

4.3.2 Quantitative determination of phytochemicals in the crude plants extracts

The quantitative determinations of phytochemicals (mean±SD and % by mass) of *L. schweinfurthii*, *R. natalensis* and *E. divinorum* plants revealed the presence of flavonoids, alkaloids, saponins and phenols in sufficient and different quantities. The results were summarized in the table 4.6. Phytochemical screening of the plant extracts revealed the presence of constituents which are known to exhibit medicinal value as well as physiological activities.

From table 4.5 and table 4.6, the analysis of the plant extracts revealed the presence of phytochemicals such as phenols and tannins, flavonoids, saponins, alkaloids and terpenoids which were present in all the plants extracts. Glycosides were present in all except from the *R. natalensis* leaves. Steroids were absent in all the plants extracts except in the *L. schweinfurthii* root barks. Flavonoids were found to be most in *L. schweinfurthii* root barks and least in *L. schweinfurthii* leaves. Phenols were sufficiently detected in all the plants studied. The three medicinal plants investigated showed relatively high total phenols and flavonoids content compared to results obtained by Ademiluyi and Oboh (2008) in African mistletoe leaves (*Viscum album*).

Table 4. 6: Quantitative determination of phytochemicals in the crude plants extracts

Sample name	Flavonoids		Alkaloids		Saponins		Phenols	
	mean± SD	% by mass	mean± SD	% by mass	mean± SD	% by mass	Mean± SD	% by mass
LSL	0.47± 0.01	9.46± 0.12	7.46± 0.30	4.66± 0.12	2.03± 0.01	10.15± 0.05	0.37± 0.02	7.46± 0.30
LSRB	0.99± 0.01	19.80± 0.20	2.00± 0.20	1.80± 0.20	3.30± 0.01	16.52± 0.03	0.10± 0.01	2.00± 0.20
RNL	0.90± 0.01	18.06± 0.12	8.54± 0.12	3.54± 0.12	3.71± 0.01	18.88± 0.05	0.43± 0.01	8.54± 0.10
RNRB	0.55± 0.01	10.94± 0.22	3.40± 0.20	2.00± 0.20	2.84± 0.01	14.22± 0.03	0.17± 0.01	3.40± 0.20
EDL	0.95± 0.01	19.00± 0.20	8.06± 0.30	7.66± 0.24	1.53± 0.01	7.65± 0.05	0.40± 0.01	8.06± 0.30
EDRB	0.61± 0.01	12.14± 0.12	2.46± 0.30	1.40± 0.00	9.52± 0.01	47.60± 0.05	0.12± 0.00	2.46± 0.30

LSL - *L. schweinfurthii* leaves; LSRB - *L. schweinfurthii* root barks; RNL - *R. natalensis* leaves; RNRB - *R. natalensis* root barks; EDL - *E. divinorum* leaves; EDRB - *E. divinorum* root barks

Flavonoids are water soluble phytochemical and an important plant phenolic. They show antioxidant activities and have the property of preventing oxidative cell damage and carcinogenesis (Naser *et al*., 2013). They have anti cancer, anti inflammatory activities and a large effect in lower intestinal tract and heart disease (Okwu, 2001). This is the reason why these plants are being used for skin diseases. Phenols and phenolic compounds are greatly used in skin infections and other wounds treatment and also for healing, when compared to other bactericides (Okwu, 2001).

The phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites (Singh *et al*., 2007). They possess biological properties such as antiapoptosis, antiaging, anticarcinogen, anti-inflammation, antiatherosclerosis, cardiovascular protection and improvement of endothelial function, as well as inhibition of angiogenesis and cell proliferation activities (Han *et al*., 2007). Several studies have described the antioxidant properties of

medicinal plants which are rich in phenolic compounds (Brown *et al*., 1998; Krings and Berger, 2001).

Natural antioxidants mainly come from plants in the form of phenolic compounds such as flavonoids, phenolic acids and tocopherols (Ali *et al*., 2009). Tannins bind toproline rich protein and interfere with protein synthesis. Flavonoids are hydroxylated phenolic substances known to be synthesized by plants in response to microbial infection and they have been found to be antimicrobial substances against wide array of microorganisms *in vitro*. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell wall (Marjorie, 1996). They also are effective antioxidant and show strong anticancer activities (Salah *et al*., 1995; Del-Rio *et al*., 1997; Okwu *et al*., 2004).

Alkaloids were found to be more in *E. divinorum* leaves and were least in *E. divinorum* root barks. Alkaloids are used in the pharmaceutical industries in the production of analgesics, owing to its analgesic properties (Okwu and Ndu, 2006). The presence of alkaloids may be responsible for the analgesic use of these medicinal plants by rural communities in Kenya particularly the people in Mbita Homabay County. The presence of these phytochemicals may be responsible for the traditional medical use of these medicinal plants in the management of the various ailments mentioned in the literature. The biological function of alkaloids and their derivatives are very important and are used in analgesic, antispasmodic and bactericidal activities. However, alkaloids are mainly observed in large amount in flowering plants and they have an important physiological effect on mankind (Stary, 1998; Naser *et al*., 2013). Morphine, quinine, ephedrine, nicotine and strychnine are the major types of alkaloids. In these types, morphine and codeine are narcotic analgesics as well as is anti-tussive agent (Stary, 1998). Alkaloids were least reported compared to flavonoids, saponins and phenols.

The saponins content in *E. divinorum* root barks were very high (47.60±0.05%) and was least in *E. divinorum* leaves (7.65±0.05). Saponins are known to have hypocholesterolemie properties and as such, consumers of saponins containing plants may enjoy chemo protection against heart diseases. The plants extracts investigated contain saponins which are known to produce inhibitory effect on inflammation (Just *et al*., 1998). Saponins have the property of precipitating and coagulating red blood cells. Some of the characteristics of saponins include formation of foams in aqueous solutions, hemolytic activity, cholesterol binding properties and bitterness (Sodipo *et al*., 2000; Okwu *et al*., 2004). Saponins have properties of precipitating and coagulating red blood cells and they also have cholesterol binding properties, formation of foams in aqueous solutions and hemolytic activity (Sodipo *et al*., 2000). Various studies have shown that saponins although non toxic can generate adverse physiological responses in animals that consume them. They exhibit cytoxic effects and growth inhibitions against a variety of cells, making them have anti-inflammatory and anticancer properties. They also show tumour inhibiting activities on animals (Akindahunsi and Salawu, 2005). Steroidal compounds are of importance in pharmacy because of their relationship with such compounds as sex hormones (Okwu, 2001).

Steroids were absent in all except in *L. schweinfurthii* root barks. Steroids have been reported to have antibacterial properties (Raquel *et al*., 2007) and they are very important compounds especially due to their relationship with compounds such as sex hormones (Okwu, 2001). Sofowora, (1993) reported the roles of these phytochemicals as analgesic, anti-inflammatory, anti-hypertensive and anti-microbial. Saponins and tannins also exhibit cytotoxic effects and growth inhibition making them suitable as tumor inhibiting agents (Akindahunsi *et al*., 2005; Asl and Hossein, 2008).

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

This study was carried out to evaluate the proximate, micronutrients and phytochemicals to ascertain their compositions in the selected medicinal plants namely *L. schweinfurthii*, *R. natalensis* and *E. divinorum.*

The proximate composition analysis showed presence of carbohydrates from 43.18±0.34% to 63.88±0.23% , crude proteins from 8.66±0.80% to 23.61±0.80%, crude lipids from 2.60±0.20% to 14.66±0.30%, crude fibre from 0.46±0.12% to 1.80±0.20%, ash from 7.65±0.75% to 11.85±0.75% and moisture content ranged from 9.35±0.77% to 14.50±0.50%. Therefore, they reveal much about the nutritional value of these medicinal plants.

The mineral elements analysed also revealed that medicinal plants studied contained micronutrients such as copper, zinc, iron and manganese in quantities within the acceptable range and toxicity of heavy metals; lead and cadmium were below the maximum permissible limits of 10 mg/kg and 0.3 mg/kg respectively according to WHO (2005). They cause danger such as kidney damage, abdominal pains and others when ingested. However, the study did not reveal the existence of cadmium in *L. schweinfurthii*, *R. natalensis* and *E. divinorum* plants which were investigated.

The presence of proximate constituents, micronutrients and phytochemicals as revealed in this study, implies that medicinal plants investigated if consumed in sufficient amounts and doses, would contribute greatly towards meeting human nutritional requirement for normal growth and adequate protection/ treatment against various diseases subject to favourable toxicity tests.

The phytochemicals identified were; flavonoids, saponins, alkaloids, phenols, tannins and terpenoids. The presence of these phytochemicals in *L. schweinfurthii, R. natalensis* and *E. divinorum* plants may be the reason for their ethnobotanical uses. Many evidences gathered in earlier studies confirmed the identified phytochemicals to be bioactive. In addition, several studies discussed earlier herein, confirmed the presence of these phytochemicals and they contributed to medicinal as well as physiological properties to the plants studied in the treatment of different ailments such as antifungal, antiviral, antibacterial, anti-inflammatory and anticarcinogenic activity.

The plants investigated were known for their ethnobotanical uses: *L. schweinfurthii* treats severe headache, dermatological, venereal diseases, gynaecological, gastrointestinal, and coughs. *E. divinorum* treats female sterility, gynaecological, and gastrointestinal. *R. natalensis* treats influenza, abdominal pains, gastrointestinal, gonorrhea, colds, abdominal pains, and pain killer.

5.2 Recommendations

From the study we recommend that further work on proximate, micronutrients and phytochemicals could be done on these medicinal plants considering age of the plants, different locations and using other parts of the plants not studied.

Standardization of herbal drugs derived from *L. schweinfurthii, R. natalensis* and *E. divinorum* medicinal plants are done.

Support in terms of financial and technical is given to herbalists to help them package their herbal drugs before they are sold to consumers in the market.

The dosage administered by herbalists to be investigated.

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