PHYTOCHEMICAL INVESTIGATION, MINERAL ANALYSIS AND PROPAGATION OF SOME WILD LAMIACEAE USED IN TRADITIONAL MEDICINE IN URIRI DISTRICT, KENYA

BY

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ABSTRACT

Plants have been used for medicinal purposes by many societies across the world, hence in Kenya. In Uriri District, Kenya, the increased use of medicinal plants for traditional medicine has led to over exploitation of tree species whereas the shrubby and herbaceous species remain underutilized. There is scarce literature related to the study, thus the need for this research. This study was carried out among two main communities of Uriri District, the Luo and Maragoli. The objective of the study was to establish the traditional medicinal use, phytochemical and mineral analysis and propagation of selected Lamiaceae herbs from Uriri District. Observation, photography and oral interviews were the methods used to collect information. Phytochemical screening involved the qualitative and calorimetric techniques to test for the presence of alkaloids, terpenoids, saponins, flavonoids, sterols, cardiac glycosides and tannins, while mineral analysis involve dry ashing technique and qualitative analysis of potassium (K), calcium (Ca), zinc (Zn), and iron (Fe) in the plants selected using atomic absorption spectrophotometer(AAS) and flame photometer. All the analysis were conducted in Maseno University Chemistry Laboratory. Propagation of the target plants was done at the Botanic Garden - Maseno University. Twelve (12) medicinal plants of Lamiaceae family were collected and identified at the East African Herbarium and it was revealed that they were; *Becium obovatum* (E.Mey.Ex Benth) N.E.Br, *Calamintha nepeta* (L) Savi, *Fuestia africana* T.C.E Fries, *Hyptis pectinata* (L) Poit, *Hoslundia opposita* Vahl , *Leonotis nepetifolia* (R,Br) Ait.F, *Leucas calostachys* Oliv, *Ocimum kilimandscharicum* Baker Ex. Gurke, *Plectranthus barbatus* Andrews, *Plectranthus longipes* Bak, *Pycnostachys speciosa* Gurke and *Satureja biflora* (Ham Ex. D.Don) Brig. The phytochemical screening revealed that all the plant species collected had a wide range of secondary metabolites which included sterols, terpenoids, tannins, saponins alkaloids, flavonoids and glycosides. Mineral analysis on some of the collected plant species revealed the presence of Ca, Fe, Zn and K. Seed propagation was successful for all the plants collected while vegetative propagation was only successful in four species of plants. In conclusion, out of the 12 plants collected 10 (83.3 %) were well known to the interviewees and are of common use in the area. The presence of secondary metabolites in the plants contributed to their medicinal value while the minerals were useful in maintaining proper body healthcare. Seed propagation was the cheapest means of conserving some of the species of Lamiaceae due to their germinability. This research has provided an insight into Lamiaceae plants used in traditional healthcare, the secondary metabolites and minerals contained, and their importance. The research recommends that the plant species collected be subjected to further phytochemical analysis; the extraction of essential oils if exploited will be useful to the cosmetic and pharmaceutical industries and institutions be set up to collect and document information on herbal medicine to prevent such knowledge from disappearing due to generational change.

CHAPTER ONE

1.0 INTRODUCTION

1.1. Background

Plants have been used to treat or prevent illnesses since before recorded history. The sacred Vedas dating back between 3500B.C. and 800 B.C. give many references of medicinal plants. One of the remotest works in the traditional herbal medicine is "Vikirshayurveda" compiled even before the beginning of the Christian era. "Rig Veda," one of the oldest available literature written around 2000B.C. mentions the use of *Cinnamonum verum* (cinnamon), *Zingiber officinale* (ginger), *Santalum album* (sandalwood), among others not only in religious ceremonies but also in medicinal preparation (Bentley and Trimen, 1980). In countries like Pakistan, about 3200 species of plants have been reported to be of use in the indigenous systems of healthcare like Unani, allopathic and Homeopathic medicines. Besides, about 300 species of plants are used in traditional medicine in Asia (Haq, 1998, Perveen and Hussain, 2007).

With 70-80% of Africa's population relying on traditional medicine, the importance of the role of medicinal plants in healthcare system is enormous. Medicinal plants are now being given serious attention as evidenced by the recommendations given by the World Health Organization in 1970 (Wondergem *et al.*, 1989). Proven traditional remedies should be incorporated within national drug policies, by recent moves towards a greater professionalism within African medicine (Last and Chavunduka, 1986) and also by increased commercialization of pharmaceutical production using traditional medicinal parts with known efficacy (Sofowara, 1981). There are considerable economic benefits in the development of indigenous medicine and in the use of, medicinal plants for the treatment of various diseases. The high cost of conventional drugs and inaccessibility to western healthcare facilities has led to over reliance on traditional medicine since it is affordable to the rural people. On the other hand, even when Western health facilities are available, traditional medicine is viewed as an efficient and acceptable system from a cultural perspective (Mangutu, 1997, Miaron *et al.*, 2008). Because of the side effects and the resistance that pathogenic microorganisms build against antibiotics that are commonly used in conventional medicine, many scientists have recently paid attention to extracts and biologically active compounds isolated from plant species used in herbal medicine (Essawi and Srour, 2000). Due to poor communication, poverty, and inaccessibility to modern health facilities, most people especially those from the rural areas are forced to practice traditional medicine for their common day to day ailments. Traditional knowledge of medicinal plants and their use by the indigenous cultures are not only useful for conservation of cultural traditions and biodiversity but also for community health and drug development at present and also in the future. Medicinal plants have an important value in the socio-cultural, spiritual and medicinal use in the lives of people of developing countries (Shinwari, 2005), hence, this study investigated how herbs have been used among the Luo and Maragoli communities of Uriri division in primary healthcare.

Herbal medicines have been used for many years dating back as 3000BC (Ayensu, 1978: WWF, 1993). Despite enormous advances in conventional medicine, traditional medicines have been encouraged by the World Health Organization (WHO, 1978), partly because some conventional drugs have failed to prove their effectiveness, have serious side effects, or cannot cure certain new illnesses such as AIDS and cancer. Medicinal and aromatic plants are reservoirs of certain curative elements used by a large population of Africans in the treatment of various diseases such as malaria, diabetes, mental disorders, cancer, hypertension and HIV/AIDS (Okigbo *et al.*, 2009). The medicinal values of these plants lie in some chemical substances that produce a definite physiological action on human body. The most important of these bioactive constituents of plants are the alkaloids, tannins, terpenoids, flavonoids and the phenolic compounds (Hill, 1952). Knowledge of organic components of plants is desirable, not only for the discovery of therapeutic agents but also because such information can be of value in disclosing new sources of such economic materials such as tannins, oils, gums, precursors for synthesis of complex chemical substances. The knowledge of chemical constituents of plants would further be valuable in discovering the actual value of folkloric remedies (Ghaderi *et al.*, 2003), hence, this study carried out phytochemical screening of bioactive compounds and minerals in plants in order to establish their importance in primary healthcare.

Traditional medicine or ethnomedicine is a set of empirical practices embedded in the knowledge of a social group often transmitted orally from generation to generation with the intent to solve health problems (Anyinam, 1987). It is an alternative to western medicine and is strongly linked by religious beliefs and practices by indigenous cultures (Hedberg *et al.*, 1982). In contrast to western medicine, which is technically and analytically based, traditional African medicine takes a holistic approach: good healthcare, disease success or misfortunes are seen as chance occurrences but are believed to arise from the actions of the individuals and ancestral spirits according to the balance or imbalance between individual and social environment (WHO,1978). Medicinal plants need more attention due to their important role in primary healthcare delivery system for improvement of people's health (Akerele, 1988). It is an essential component of human healthcare especially for the rural communities who solely rely on forest plants for food, shelter, energy and medicine (Hamayun *et al.*, 2003).

Medicinal aromatic plants play a significant role in the lives of people and are present in innumerable forms. These plants are used as raw materials for medicines, cosmetics, perfumery, and insecticides and in the food industry. Medicinal and aromatic plants can be defined as plants used in official and traditional medicine, aroma and flavour (Baricevic *et al.*, 2004). The word aromatherapy combines two words: aroma (a fragrance or sweet smell) and therapy (a treatment). Aroma and massage therapy are the practice of using essential oils for psychological and physical well-being via inhalation or massage (Edris, 2007). The pharmaceutical attributes of aromatic plants are partially attributed to essential oils. Essential oils are natural, complex multi component systems composed mainly of terpenes in addition to some other non-terpene components (Edris, 2007). The inhalation of essential oils or their individual volatile terpenes has a significant role in controlling the central nervous system, for instance the pre-inhalation of *Acorus gramineus* rhizome essential oils are used in Chinese folk medicine in the treatment of epilepsy (Koo *et al.*, 2004). *Lavandula hybrida* Reverchon Grosso (Lavender) essential oil has demonstrated an interesting analgesic activity mainly after inhalation at doses devoid of sedative side effects (Barocelli *et al.*, 2004). Aroma inhalation of *Lavandula hybrida* (lavender), *Mentha piperita* (peppermint), *Rosemarinus officinalis* (rosemary) and *Salvia sclarea* (clary-sage) essential oils can significantly decrease symptoms associated with anxiety and stress. Inhalation of essential oils can also moderate sympathetic activities in normal adults (Haze *et al.,* 2002). Inflammatory diseases such as allergy, rheumatism and arthritis are often alleviated using essential oil massage therapy (Lawless, 1997). This action is mainly due to the suppression of histamine release (Brand *et al.,* 2002) and cytokine production (Brand *et al.,* 2001). Massage therapy using essential oil can be useful in the treatment of people suffering from dementia. Essential oil of *Melissa officinalis* (lemon balm) applied cutenously in a lotion to patients with severe dementia was found to be an effective treatment (Ballard *et al.,* 2002).

Most parts of Kenya are endowed with a wide variety of indigenous medicinal plants. These plants are used by the local herbalists for the treatment of various diseases and are distributed in various families: Papilionaceae, Lamiaceae, Verbenaceae, Asteraceae, Myrsinaceae, Polygonaceae, Combretaceae, Rubiaceae among others. Plants in these families are rich in bioactive compounds that include; alkaloids, terpenoids, saponins and even phenols. Plants of Lamiaceae family are known for their essential oils (Iwalokun *et al.,* 2003). Many active essential oils have been isolated from members of this family. This family is also famous for the presence of diterpenoids among its members. The Lamiaceae species are important for their antimicrobial properties which are used in research of antimicrobial activities, for instance, *Salvia argentenea* L, *Stachys annua* L, *Ballota nigra* L, *Melisa officinalis* L among others (Sarac and Ugur, 2007). The Myrsinaceae and the Polygonaceae are the most important ethnomedical anthelminthic and antibacterial with strong cross ethnic usage (Juma *et al.*, 2001). *Terminalia brownii,* a member of the Combretaceae family is a multi purpose medicinal plant used by most herbalists in Kenya for various conditions. It can either be used alone or in combination with other plants (Gachanja *et al.*, 2007).

Various plants of Papilionaceae family from Kenya for instance; *Abrus precatorius* L, *Abrus schimperi* Bak, and *Lonchocarpus eriocalyx* Harms have been reported to be effective in the treatment of gonorrhoea, pneumonia, and blood pressure and diabetics respectively (Gachanja *et al.*, 2008). The Verbanaceae plant family member for instance *Vitex strickens* Vatke and Hiderbr is a plant whose root decoction is used for the treatment of malaria while leaf decoction of *Chlerodendron myricoides* (Hoschst) Vatke is very useful for the treatment of pneumonia (Gachanja *et al.*, 2008). The Amaranthaceae comprises plants that are commonly used as traditional vegetables for instance the Genus *Amaranthus*. However, some species like *Aerva lanata* (L) Shultes has its leaves prepared into a decoction used for bathing children suffering from malaria (Maundu *et al.*, 1999). Leaves of *Achyranthes aspera* (Amaranthaceae) are pounded and the filtrate taken for the treatment of malaria and gonorrhoea while the leaf filtrate of *Cyanthula polycephala* is taken orally to cure stomachache and also acts as an anthelmintic (Olembo *et al.*, 1995). The plants of Rubiaceae family are equally important medicinally for they have been found to be effective in the treatment of illnesses and conditions such as, fever, boils, joint pains, backache, birth pains, headache, colds, rashes, venereal diseases and even for correcting foetus presentation (Olembo *et al.*, 1995). Ethnobotanical surveys and palynological characterization of some medicinal plants in Kombewa division have been studied where the plants have been found to offer cheap remedy to the local community even to diseases whose cure have not yet been discovered in conventional medicine (Arwa, 2005).

In Migori District, research has been conducted on the value of leafy vegetables used by the Luo people living in Uriri, Karungu, Nyatike and Rongo divisions (Anyara and Owuor, 2007). Among the leafy vegetables studied were: *Solanum nigrum, Cleome gynandra, Vigna unguiculata, Asystasia schimperi, Corchorus sp, Amaranthus sp, and Crotalaria sp.* Further, aspects of ethnomedicine, ethnosystematics and ethnobotany of the Luo community of Migori district (Uriri included) revealed a rich ethnobotanical knowledge and a fascinating relationship between drug use and culture. It was found out that 272 genera of flowering plants were useful in providing medicinal remedy: from this list 4% of the plant species studied were from Lamiaceae family (Owuor, 1999). Phytochemical research conducted in the area has revealed that alkaloids, aurones, chalcones, flavones, anthaquinones, tannins, saponins, sterols and cardiac glycosides were some of the secondary metabolites present in these plants (Owuor, 1999). Terpenoids and flavonoids were not studied.

In Uriri District, very little research has been conducted and results documented on the use of medicinal plants for the provision of primary health care. Very scanty information exists on the use of plants from Lamiaceae family in traditional medicine from Uriri District. Much of that information has not been documented, though it is publicly acknowledged.

This research therefore carried out documentation and phytochemical screening of the plants of Lamiaceae family in Uriri District and came up with a compilation that will provide basic information on the plants and secondary metabolites present in various plants and their relationships in terms of the diseases they treat.

1.2. Statement of the problem

The use of medicinal plants for treatment of various diseases has been a common practice among the residents of Uriri District for quite a long time. In most cases, the use of barks and roots from trees and shrubs has been more pronounced because they are perceived to be of higher medicinal value. Due to increased harmful harvesting techniques of such plants, there is agrowing trend where a whole plant is eliminated from its ecosystem in an attempt to utilize the root part, without replacement or the whole plant dying due to the removal of its bark. The use of herbaceous plants as phytomedical remedy has been given very little attention due to their occurrence as weeds in agricultural fields, as undergrowths in a few remnant forested areas and in abandoned farms. The people are not aware that most herbaceous species especially those from Lamiaceae family possess some of the most active principles that are of greater importance in primary healthcare. This research is set to study and document the herbaceous species especially those from Lamiaceae family and their role in traditional medicine before the custodians of such knowledge die. The active principles and minerals hence chemistry of most of the plants used in Uriri District is not known since such a research study has never been conducted. This research therefore seeks to carry out phytochemical screening and mineral analysis on some wild Lamiaceae used in traditional medicine.

Due to potential misuse of overuse, there is therefore need to conserve the medicinal plant species by the propagation of target plants which have proven to contain a diverse array of active principles (secondary plant metabolites) with greater degree of effectiveness. WHO recommends that all available means can be used to improve healthcare and thus includes traditional medicine hence this research.

1.3. Justification

The knowledge on the use of plants for medication is vanishing without being passed to the next generation. Therefore this project hopes to capture this valuable information on medicinal plant use from the traditional medical practitioners and document it so that the future generation can be accessible to it. Africa in general and particularly in East Africa there has been reported a significant reduction in the use and conservation of medicinal plant species which has instead led to the loss of some treasured plant species. With regard to that, the project seeks to conserve the threatened species of medicinal plants especially those of Lamiaceae family through the propagation of selected plants that are of great medicinal significance.

The research study will help in the provision of phytochemical knowledge and information to future scholars, researchers and industrialists who may wish to carry out related research in the same area or in other areas of the country.

Through propagation the people of the area will find cheaper ways of conserving the herbaceous species most of which are threatened and risk being extinct.

1.4. Objectives of the study

1.4.1. Main Objective

To establish the traditional medicnal use, phytochemical and mineral analysis and propagation of selected Lamiaceae herbs from Uriri District.

1.4.2. Specific objectives

i. To identify and to prepare herbarium specimens of plants of Lamiaceae family used in traditional medicine in Uriri District.

ii. To carry out phytochemical screening and mineral analysis in the selected plant species from Uriri District.

iii. To investigate the possible seed and vegetative methods of propagating the medicinal plants of selected wild Lamiaceae in Uriri District.

1.5. Significance of the study

This study is intended to improve knowledge base on medicinal plants used by the locals (Kenyans) in that it will not only identify the plants but also reveal to the people the active curative principles that make the plants effective in application. The study will also contribute information related to proper conservation techniques that are intended to prevent over-exploitation of medicinal species in the wild through propagation.

The research focuses on two millennium development goals (Ministry of Planning and National Development, 2003). First, it aims to contribute towards Millenium development goal number 1 on eradication of poverty and hunger. With regard to this, the project hopes to sensitize people on the importance of the products of Lamiaceae for instance; the essential oils that can be extracted from plants and then commercialized to industries that refine such oils in order to benefit the communities that live in areas where such products are obtained. Consequently, the lifestyle of such people will be improved thereby reducing poverty level. Secondly, the research also will contribute towards Millennium development goal on combating HIV/AIDS, Malaria and other diseases. Since the cost of conventional medicine has increasingly gone up, there is need to sensitize people on the effectiveness of the medicinal plant in the provision of primary healthcare to combat the common ailments and manage some of the diseases such as Malaria and tuberculosis. This is due to the fact that these plants used for medicine are cheaply available and their usage acknowledged by a greater portion of the population. Besides, with the integration of herbal medicine into primary healthcare system, it will be possible for people to access the set up health facilities. As a result, people will receive information pertaining to plants that are of great medical importance and also how they can prepare such plants for home-based care without necessarily visiting the traditional medical practitioners in times of emergency.

By addressing the two millennium development goals, this research will also be addressing aspects of vision 2030 on healthcare and industrialization (MPND-GOK, 2003). A healthy population will be able to work and produce sufficient food thereby curbing food insecurity and maintaining a healthy labourforce for the industries.

1.6. Hypothesis

i. All wild plants of Lamiaceae family from Uriri District are of medicinal value.

ii. Secondary metabolites are present in all the plants of Lamiaceae family from Uriri District.

iii. All the wild plants of Lamiaceae family can be propagated by either seeds or vegetatively.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1.1. Traditional medicine/ medicinal plants

The World Health Organization (WHO, 1978), defines traditional medicine as the sum total of knowledge or practices whether explicable or inexplicable, used in diagnosing, preventing or eliminating a physical, mental or social disease which may rely exclusively on past experience or observations handed down from generation to generation, verbally or in writing. It also comprises therapeutic practices that have been in existence often for hundreds of years before the development of modern scientific medicine and is still in use today without any documented evidence of adverse effects. The populations of developed countries worldwide continue to rely heavily on the use of traditional medicine as their main source of primary healthcare.

Ethnobotanical surveys carried out throughout Africa confirm that native plants were the main constituents of traditional African medicines (Adjanohoun *et al.*, 1980; Hedberg *et al.*, 1985; Kokwaro, 1976; Oliver-Bever, 1987). More than 1200 Kenyan plant species are recorded as medicinal (Kokwaro, 1976). The ethnomedicinal uses of many of these plants, the majority of which have not undergone even initial phytochemical investigation has been well documented (Beentje, 1994). The World Health Organization long recognized that traditional medicinal plants could be useful in an integrated delivery healthcare system of a country. However such plants must not be dangerous, but be effective and their preparations not adulterated or made harmful by parasites or micro-organisms (WHO, 1978). Herbal medicine is a finished and labeled medicinal product that contain active ingredients, aerial or underground parts of identified and proven plant materials or combination thereof, whether in crude form or as plant preparations (WHO, 1978). They also include plant juices, gums, fatty oils, essential oils among others. The use of traditional medicine in the management of HIV/AIDS opportunistic infections has been conducted in Tanzania where the roots and stem/bark of plant families like Anacardiaceae, Asteraceae, Capparaceae, Clusiaceae, Euphorbiaceae, Lamiaceae, Mimosaceae, Myrtaceae, Papilionaceae and Rubiaceae were found to be rich in secondary metabolites for maintenance of body health and boosting of the body immune system (Kisangau *et al.*, 2007)

Renowned bodies such as the Kenya Medical Research Institute (KEMRI), African Medical Research Foundation (AMREF) and the World Health Organization (WHO) have performed extensive research in herbal medicine with encouraging findings. They recommend and refer patient to some reliable practicing herbalists. Such herbalists have used plants for treatment of diseases against magic and religious practices (Kokwaro, 1976). Besides, efficacy studies on some medicinal plants have been undertaken, and their pharmacological capability in preventing diseases have been confirmed by research bodies such as the U.S. Centre for Disease Control (CDC), Pharmaceutical companies and KEMRI (Balick, 1996). Previous similar studies led to the discovery of drugs such as quinine from *Cinchona Sp*, Aspirin from *Filependula ulmaria,* reserpine from *Rauwolfia serpentina* and digitoxin from *Digitalis purpurea* (Balick, 1996), among others.

In Kenya, comprehensive ethnobotanical information and healing methods among the local communities is not completed. However, indigenous information on medicinal plants is recorded by several authors: (Glover, 1996; Lindslay and Hepper, 1978; Kokwaro, 1993; and Kaendi, 1997). Elsewhere, herbal medicine research has been reported: (Barakat *et al*., 2003; Camejo-Rodriques *et al*., 2003 and Lucia *et al*., 2003). Many medications used to treat common ailments are found in plants (Trinkana 1998). Research conducted in Nyanza and Central provinces of Kenya revealed that while plant species in traditional medicine continue to be reliable sources for discovery of useful compounds, screening of plants growing under different environmental conditions could provide another source for compounds with antimicrobial activities (Njoroge and Newton, 1994; Muregi *et al*., 2003). A study conducted in Maseno University on phytochemical compounds and antimicrobial activity of extracts of *Aspilia mossambicensis* (*Aspilia* plant) (oliv) wild revealed that the plant had antimicrobial activity against gram negative bacterium, *Salmonella typhi* and gram positive bacterium, *Salmonella pyogenes* including the fungal pathogen *A. niger* (Musyimi *et al*., 2008). In a study conducted among the Luo and Kamba communities in Kenya, it was established that the plants used as antivenins were similar and they included *Combretum collinum, Steganotaenia araliacea, Solanum incanum, Grewia bicolor, G. fallax* and *G. truncata* (Owuor and Kisangau, 2006).

2.1.2. Lamiaceae family

The family Lamiaceae was established by De Jussieu in 1789 as the orderLabiatae. The name Labiatae alludes to the flowers typically having petals fused into an upper and lower lip (labium), the flowers thus having an open mouth. The name Labiatae is acceptable though botanists more often use Lamiaceae based on the genus *Lamium* (Elizabeth, 2005). This research study will put into use the name Lamiaceae which is in conformity with the rules of ICBN which provides that plant family names end in -aceae. The name Labiatae on the other hand is a conserved name as per the ICBN.

Cronquist (1981) describes the Lamiaceae plants as mostly herbs or shrubs comprising about 200 genera and 3200 species with aromatic properties, herbage, quadrangular stems and verticillate inflorescences. The leaves are opposite or whorled, and are simply or occassionaly pinnately compound; stipules are absent. The flowers are bisexual and zygomorphic. The calyx is synsepalous, typically 5-merous, sometimes 2-liped, and usually has 5-15 conspicuous ribs. The corolla is sympetalous and typically bilabiate with two lobes forming an upper lip and three lobes forming a lower lip. The androecium consists of either 4-digynamous stamen or only two stamens that are adnate to the corolla tube or the perigynous zone, alternate with lobes. The gynoecium consists of a single compound ovary of two carpels, a single gynobasic style and a deeply 4-lobed superior ovary with four apparent locules, each with a single basal axile ovule. A hypogynous often asymmetric nectariferous disk is generally present between the stamen and the ovary. The fruit consist of four one-seeded nutlets that rarely may be fleshy and drupaceous. In terms of distribution, about 60 genera with about 980 species of Lamiaceae occur in the sub-Saharan region (Klopper *et al.*, 2006).

Many workers (Cantino, 1992), believe the Lamiaceae to be related closely to family Verbenaceae, so much that the elements of the latter have been transferred into the former, although technically, the Verbenaceae can be said to have entire ovaries, usually rounded stems which, when crushed lack the minty scent. Early workers, because of their 4-lobed ovaries, thought that Lamiaceae was closely related to the Boraginaceae, but DNA studies (Olmstead *et al.*, 1992) suggested that the latter belonged elsewhere. Heywood (1978) gave a concise well-illustrated overview of the family Lamiaceae.

Lamiaceae family produces more essential oil than any other plant family and their main areas of distribution are in the tropical and warmer temperate regions of the earth. These plants are easy to identify by their common family characteristics: herbs and small shrubs with square stem bearing minute oil glands that dot the usually opposite (or sometimes whorled) leaves. They usually have some flowers or spikelets (called axils) towards the top or the stem. The oil glands on the leaves are the source of essential oils used in aromatherapy (Marlene, 2000). Lamiaceae species have provided important resources for the old and new world and their use in medicine and as condiment in regional cuisine is of central importance for instance in countries like Turkey, China, Middle East countries, India Brazil, Egypt among others (Essawi and Srour, 2000; Haq, 1998; Matkowski *et al., 2008* and Sarac and Ugur, 2007). Folk medicine for a long time has placed emphasis on extracts or essential oils of Lamiaceae used for antibacterial, antiviral, carminative and spasmolytic activities (Arnason *et al.*, 1995). Research conducted on some plant species of Lamiaceae family revealed that several popular medicinal plants of the family belonged to genera *Ballota, Galeopsis, Lamium, Leonurus, Marrubium, Ajuga, Ocimum* among others (Matkowski *et al.*, 2008).

Research studies in the past reveal that the family Lamiaceae is subdivided into lower taxa subfamilies and tribes which also differ in chemical properties. The largest subfamily Nepetoideae comprises mostly of the medicinal spices and aromatic herbs such as *Rosemarinus officinalis* (rosemary), *Ocimum basilicum* (basil), *Melissa officinalis* (lemon balm), *Oreganum vulgare* (oregano), and *Satureja hortensis* (savory) among others. They contain Rosmarinic acid and are frequently abundant in fragrant volatile terpenes (Wink, 2003). The rosmarinic acid has antibacterial, antiviral, antioxidant and anti-inflamatory properties (Farzaneh *et al.*, 2006). The second largest subfamily, Lamioideae contains a variety of iridoid glycosides, phenylpropanoids and phenylethanoid glycosides but lacks rosmarinic acid which distinguishes it from Nepetoideae (Zgorka and Glowniak, 2001: Pedersen, 2000). Recently, medicinal plants from subfamily Lamioideae of the Lamiaceae were tested for antioxidant activity and screened for polyphenols. The study included the aerial parts of *Ballota nigra, Lamium maculutum, Leonurus cardiaca, Marrubium vulgare* and *Galeopsis tetrahit.* The findings of the research revealed that the medicinal herbs belonging to the subfamily Lamioideae are rich sources of antioxidants (Matkowski *et al.*, 2008).

In Kenya, research studies on the plants of Lamiaceae species with medicinal values have been conducted in the past by Githinji (1990) and Kokwaro (1993) where they comprehensively illustrated and documented the role of these plants in traditional medicine. In a study conducted among the Marakwet People of the Rift Valley Province, fifteen Lamiaceae species were found to be of medicinal value and were of use in the treatment of common ailments for instance gastrointestinal infections and disorders, fever, bacterial infection among others (Lindsay and Hepper, 1978). *Hyptis pectinata* and *Plectranthus comosus* have been found to offer medicinal remedy to stomach disorders and ear problems respectively (Arwa, 2005).

The traditional healthcare system that involves medicinal plants is still not wholesomely acceptable. This is because the health practice is mostly associated with superstitious acts and witchcraft given that some practitioners not only use plant for healing but they also make use of some animal parts like claws, horns, teeth among others. Also the hygienic standards maintained by some practitioners are questionable in that some plants used are not cleaned before being administered, this can cause a lot of harm to the patient as the dust particles and other material deposits on the plant might cause further complications (Elujoba *et al.*, 2003).

In Uriri District and Migori district, the previous studies conducted on the plants of Lamiaceae family are not very clear. This research study therefore sourt to address the gap by documenting the plants of Lamiaceae family of medicinal value and provide their importance and roles on primary healthcare system that will be of much benefit to the residents of Uriri District.

2.2.1. Phytochemical screening

According to Stace (1989), phytochemistry is an area of plant taxonomy that seeks to utilize chemical information to improve the classification of plants. It relates taxonomic classification to living species and their biochemistry, the application of different taxonomic techniques to gain understanding in the relationships between taxonomic classifications, secondary metabolism of plants and to facilitate searching for sources of desired natural compounds. He further states that it attempts to classify and identify organisms (plants) according to demonstratable differences and similarities in chemical composition. Phytochemical studies often make use of accumulated ethnobotanical knowledge, particularly in regard to the use of plants in traditional medicine (Pickman, 1986). Secondary plant metabolites are a wide range of compounds from different metabolite families that can be highly inducible in response to stress. These compounds are non-essential for cell structure and maintanance of life but are often involved in plant protection against biotic and abiotic stresses (Weisshaar and Jenkins, 1998; Hattenschwiller and Vitousek, 2000). Some secondary metabolites such as carotenoids and flavonoids are also involved in cell pigmentation in flowers and in seeds, which attract pollinators and seed dispersers. They are therefore involved in plant reproduction (Winkel-Shirley, 2001). Moreover, plant secondary metabolites present chemical and pharmaceutical properties interesting for human health (Raskin *et al.*, 2002; Reddy *et al.*, 2003). Knowledge of secondary metabolites responsible for biological activity and of chemotaxonomic relationships within specific groups being studied is helpful in finding new sources of potentially, economically important products or compounds which can be transformed into active principles (Ivie *et al.*, 1983).

Tomas-Barbaren *et al.*, 1998, wrote about the occurrence of flavonoids in the Lamiaceae in which they noted that some genera are rich in the usual α- glycoside of luteolin, apigenin and chrysoeriol. Some genera produce flavone 3-glycoside almost exclusively. Other individual species produce flavonols in addition to flavone glycosides as well as flavonoid p-coumaroyl glycoside. Flavonoids also occur in the Lamiaceae in a variety of structural forms including flavones, flavonols, flavonones, dihydroflavonols and chalcones (Tomas-Barbaren *et al,* 1992). Compounds belonging to terpenoids alkaloids and flavonoids are currently used as drugs or as dietary supplements to cure or prevent various diseases and in particular some of these compounds seem to be efficient in preventing and inhibiting other kinds of cancer (Raskin *et al.*, 2002; Watson *et al.*, 2001; Reddy *et al.*, 2003; Mang'uro *et al.*, 2007). More research carried out in numerous research centres across the world show that the complex pharmacological activity of some medicinal plants of the family Lamiaceae is strictly connected to the presence of phenolics (Zgorka and Glowniak, 2001).

Many authors have reported anti-microbial, anti-fungal, anti-oxidant and anti-radiant properties of spices and essential oils as well as plant phenolics including flavonoids. They are used to enhance health and to prevent various degenerative diseases (Luisa, 2006). Phytochemical analysis on *Otostegia persica* (Labiatae) to test on the antioxidant activity of the plant and its constituents has also been conducted (Fariba *et al.*, 2003). Numerous phenolic antioxidants have been isolated and characterized from *Rosmarinus officinalis* (rosemary) leaves including caffeic acid and its most active ester, or dimmer, rosmarinic acid (Cuvelier *et al.*, 1996, Chen and Ho, 1997) which is an effective free radical scavenger (Chen and Ho, 1997). The most important abundant phenolic compound in the rosemary leaves is carnosic acid, a labile abietane diterpene that undergoes an oxidative degradation and re-arrangement cascade, producing a series of compounds, many with antioxidant activities (Richheimer *et al.*,1999) such as carnosol, rosmanol, rosmariquinone (Hall *et al.*,1998) and methyl carnosate (Cuvelier *et al.*, 1994).

A study conducted on some of the plants of Lamioidae subfamily of Lamiaceae revealed that the medicinal herbs belonging to that subfamily for instance *Lamium maculutum, Leonorus cardiaca* among others have strong antioxidant properties which are useful in the treatment of cancer (Matkowski *et al.*, 2008). Free radicals and other oxygen reactive species have been reported to cause oxidation of biomolecules like proteins, DNA, amino acids and unsaturated lipids and ultimately produce molecular alterations related to aging, arteriosclerosis and cancer (Gardner, 1997), alzheimer's disease (Butterfield and Lauderback, 2002), parkinson's disease, diabetes and asthma (Zarkovic, 2003). Early reports had indicated that essential oil components especially monoterpenes have multiple pharmacological effects on mevalonate metabolism which could account for the terpene tumor suppressive activity (Elson, 1995). Antioxidant activities cannot only be attributed to the presence of phenolics in the essential oils; monoterpene alcohols, ketones, aldehydes, hydrocarbons and ethers also contribute to the free radical scavenging activity of some essential oils (Miguel *et al.*, 2004).

In the field of complementary and alternative respiratory medicine, inhalation of peppermint essential oil vapour has been suggested as adjuncts in combined multidrug therapy in patients with disseminated and infiltrate pulmonary tuberculosis. The action of the oils is mainly due to the antimicrobial activity of its volatile constituents (Shkurupii *et al.*, 2002). Thyme and oregano essential oils can inhibit some pathogenic bacterial strains such as *Escherichia coli, Salmonella enteriditis, Salmonella choleraesuis* and *Salmonella typhimurium,* with the inhibition directly correlated to phenolic components; carvacrol and thymol (Penelver *et al.*, 2005). *Ocimum gratissimum* essential oil can inhibit extacellular protease and the expression of O-lipopolysaccaride rhamnose in virulence and multidrug-resistant strains of 22 shigellae (Iwalokun *et al.*, 2003). Thus the oils may find use in the therapeutic measure against Shigelosis. Many species of *Mentha* genera have also shown antibacterial properties which are due to the presence of monoterpenes, diterpenes and sesquiterpenes present in the essential oils of these plants (Richardson, 1992).

Experimental work on Lavender oil revealed the presence of terpenoid compounds which are of antimicrobial, antiviral, and antifungal properties (Safaei, 2004). Research shows that plant extracts, especially essential oils may afford a potential alternative to synthetic antiviral drug; they have demonstrated virucidal properties with the advantage of low toxicity compared with the synthetic antiviral drugs (Baqui *et al., 2001;* Primo *et al.*, 2001 and Schnitzer *et al.*, 2001). *Mentha piperita* (Peppermint*)* essential oil exhibits a high level of virucidal activity against HSV-1, HSV-2 and acyclovir- resistant strains of HSV-1 in viral suspension tests (Schuhmacher *et al.*, 2003).

In the Asian continent, most recent researches have been conducted on various plants of Lamiaceae family to test on their effectiveness in the provision of primary healthcare. Some of the properties tested were: antiproliferative activity (Abu-Dahab *et al.*, 2007); antimicrobial evaluation (Himal *et al.*, 2008); and antioxidant activity of subfamily Lamioidae of Lamiaceae (Matkowski *et al.*, 2008) among many others. According to Farad (2006), the phytochemistry of the genus *Lamium* of the Lamiaceae family has been extensively studied since 1967. During the past 40 years, iridoids and secoiridoids, phytoecdysteroids, phenylpropanoids, flavonoids, anthocyanins, betains, terpenes and megastimen compounds as well as essential oils have been discovered.

In East Africa, phytochemical analyses have been conducted in a smaller range of aromatic plants of Lamiaceae family that include genera *Lamium*, *Plectranthus*, and *Salvia* among others (Vagionas *et al.*, 2007). Githinji (1990), conducted chemotaxonomic study of some of Kenyan medicinal Lamiaceae species in some of the selected parts of the country and came up with a documentation pertaining to their medicinal importance. Antibacterial and cytotoxic screening on some Kenyan medicinal plants showed the presence of terpenoids, glycosides, ajugarin i and ii which were isolated from *Ajuga remota* of Lamiaceae family (Matu and Van Staden (2003), Manguro *et al.*, 2006).

The scarcity of information relating to phytochemical screening and mineral analysis of plants of Lamiaceae family in Uriri District was quite evident since a similar research had not been conducted in the past. Consequently this research study sought to come up with information based on the resulting phytochemical screening and minerals analysis of plants of Lamiaceae family in the area. This was intended to provide knowledge based on the various secondary metabolites and mineral nutrients present in the plant species of Lamiaceae family selected and their usefulness in the treatment of various ailments and disorders among the residents. Consequently, the application of such knowledge was intended to help in improving the primary healthcare system which can be made available through home-based care.

2.2.2. Mineral nutrients in plants

Minerals are inorganic substances present in all body tissues and their presence is necessary for maintenance of certain physiological processes which are essential to life (Soetan, *et al.*, 2010). They are used by the body in many ways, though they yield no energy, they play important roles in the human body (Eruvbetre, 2003). Magnesium, copper, selenium, zinc, iron, manganese and molybdenum are important co-factors found in the structure of certain enzymes and are dispensable in numerous biochemical pathways (Soetan *et al.*, 2010). Biological assays conducted in the last two decades have clarified the significance and importance of mineral elements in human nutrition. Analytical techniques have also led to the detection of trace elements as essential nutrients and are still an active area of research (Soetan *et al*., 2010). Mineral elements in plants have been estimated by calorimetric procedures, wet – ashing (using nitric acid, perchloric acid and sulphuric acid) and dry-ashing procedures (Gursuch, 1959). The atomic absorption spectrophotometer technique is a recent method employed in analyzing the pre-tested samples of dried plant extracts and it is the technique that this study has adopted in the analysis. Nutrient sufficiency is the basis of good health, productive lives and longevity for everyone (Welch, 2004). Humans require at least 50 known nutrients in adequate amounts, consistently to live happy and productive lives (WHO, 2002). The major minerals required by the body include sodium, potassium, chloride, calcium, magnesium, phosphorous and sulphur. On the other hand, the most important trace minerals are iron, zinc, copper, chromium, fluoride, iodine, selenium, manganese, molybdenum, arsenic, boron, cobalt, silicon, nickel and vanadium (Whitney and Rolfes, 1996).

Recent research indicates that minerals play a significant role against a variety of degenerative diseases and processes. They can also protect the body from the effects of toxic minerals (Schauss, 1984). The fluid balance in the body, vital for all life processes is largely by sodium potassium and chlorides and fluid balance is regulated by charged sodium and chloride ions in the extacellular fluid and potassium in the intracellular fluid and by some electrolytes across the cell membranes. Tight control is critical for muscle contraction, nerve impulse transmission, heart function and control of blood pressure (Whitney and Rolfes, 1996). Such studies further indicate that sodium plays an important role in the absorption of nutrients such as glucose, amino acids and even water. Chloride is a component of hydrochloric acid, an important part of gastric juice that aids in food digestion. Potassium and sodium act as cofactors for certain enzymes. Calcium and magnesium are essential for the structural roles as they aid in the development and maintenance of bones and teeth. They are also needed for maintaining cell membrane and connective tissues. Several hormones, enzymes and protein that regulate energy and fat metabolism require calcium and phosphorous to become active. Calcium aids in blood clotting while sulphur is a key component in various proteins and vitamins and participates in drug detoxifying pathway in the body (Soetan, *et al*., 2010).

Trace minerals have specific biological functions in the body (Witney and Rolfes, 1996). They are essential in the absorption and utilization of many nutrients and aid enzymes and hormones in activities that are vital for life. Iron plays a major role in oxygen transport and storage; it is also a component of haemoglobin in red blood cells and myoglobin in muscle cells. Cellular energy production requires many trace minerals including copper iron and zinc which act as enzyme co-factors in the synthesis of many proteins, hormones, neurotransmitters and genetic materials. Iron and zinc support immune function, while chromium and zinc aid insulin action. Zinc is also essential for many other bodily functions such as growth, development of sexual organs and reproduction (Welch, 2004). Zinc, copper and selenium prevent oxidative damage of body cells. Fluorine stabilizes bone mineral and hardens tooth enamel thus increasing resistance to tooth decay. Iodine is essential for normal thyroid function, which is critical for many aspects of growth and development particularly brain development. Thus trace minerals contribute to physical growth and mental development.

In Tanzania, the effect of a local mineral, 'kadosero' (a crude mineral used by traditional healers as supplement to plant extracts against microbial infections and it contains sulphates, chlorides and sodium ions) towards antimicrobial activity of medicinal plant extract has been studied and found to increase the activity of plant extract of *Balanites aegyptiaca* against microbes (Otieno *et al.*, 2006).

In Siaya district of Kenya, plants used by the Luo speaking people have been surveyed in the past, where wild fruits, underground portions, leaves and fungal fruiting bodies were found to be very important as dietary supplements (Johns and Kokwaro, 1990).

Aspects of ethnomedicine, ethnosystematics and ethnobotany of the Luo community of migori district revealed a rich ethnobotanical knowledge and fascinating relationship between drug use and culture. It was found out that 272 genera of Angiosperms were useful in providing medicinal remedy. Out of the plant species collected 4% were from family Lamiaceae (Owuor, 1999). The plant information gathered indicated that most plants were useful in treating complications resulting from gastrointestinal infections such as diarrhea, dysentery and flatulence. The phytochemical research on the plants collected from the area revealed that alkaloids, aurones, chalcones, flavones, anthraquinones, tannins, saponins, sterols and cardiac glycosides were some of the secondary metabolites reported in these plants (Owuor, 1999). The terpenoids and flavonoids were however not researched on, therefore the need for this research to carry out phytochemical screening on the selected plants of Lamiaceae specifically in Uriri District and document the findings.

In Uriri District research on the mineral constituents of medicinal plants is quite inadequate. Some information exists on the use of leafy vegetables such as *Vignia unguculata, Crotalaria sp, Corchurus sp, Solanum sp, Amaranthus sp and Sesamum sp* in forklore medicine among the Luo community of Uriri district (Anyara and Owuor, 2007). Since very little information exist on the mineral components of plants of Lamiaceae family, this research study sought to carry out mineral analysis on these herbs and document the findings on their importance in traditional medicine.

2.3. Propagation and conservation

Plant propagation employs various methods like stem and root cuttings, use of seeds, grafting, budding, tissue culture and layering (Hartmann *et al.*, 2001). Reearch has revealed that germination capacity of some medicinal plants is generally low due to dormancy. Any aberration or mechanical injury to the seed coat or any chemical treatment given may release the dormancy. Seed germination responses of certain cultivated medicinal plants to various physical and chemical treatments aimed at breaking dormancy and thereby improve germination percentage (Sriram, 2004). Some plant species of Lamiaceae family such as *Hemigenia exilis, Lavandula anguistifolia, Satureja Montana, Ocimum species, Oregano species, Thyme species* and *Plectranthus species* have successfully propagated under treatment with gibberelic acid and potassium nitrate (Aoyma *et al.*, 1996; Bhuse *et al.*, 2001; Cochrane, *et al.*, 1999; Gupta, 2003). The results of these studies have revealed that the germination percentages of the plant species increased significantly from 20-70% to 84-96% with an improved seedling survival. It has also been reported that germination media is very important in initiating germination by providing optimum moisture with proper oxygen supply to trigger the germination process and providing mechanical support for growth of seedlings (Lalith, 2008). It has however been revealed in other studies that the conventional methods of propagating lamiaceae species through seeds are unproductive due to low seed viability (>50%). This is attributed to seasonal variation. The low germination rate potential restricts the multiplication of the species (Heyhood, 1978; Sulistiarini, 1999).

Due to excessive human exploitation, non-regulated collection, unresolved inherent problems of seed viability and seed germination, many plant species have become endangered, thus the need for conservation (Arora and Bhojuani, 1989). The medicinal resources in Africa may be doomed to extinction by overexploitation resulting from excessive commercialization, habitat destruction and other natural and man made destructive influences unless energetic conservation measures to ensure continued availability are put in place (Rukangira, 2000). This can be done by establishing Medicinal plant gardens and farms by propagating plants (Rukangira, 2000).

In Uriri District, no serious propagation of medicinal plants especially the herbaceous species has been reported as most people only focus on a few shrubs and trees that they value their medicinal importance. This research study therefore sourt to provide the residents of Uriri District with the basic information on how cheap propagation could be conducted. This is intended to help in conserving the overexploited tree species that currently are threatened as a result of uncontrolled harvesting of their essential part.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1. Study area

Uriri District is in Migori County Nyanza Province in Kenya. Currently, it is a gazzetted district of its own (See Figure 1). It covers an area of approximately 380.6471km^2. The District comprises of seven locations (See Appendix 2) and fourteen sub- locations. The total population was 115,751 people with a density of 304.09/km^2. (2009 Population and housing census report). The district experiences an annual rainfall of 700mm-1800mm annually with short rains between March and May while long rains between Octobers to December periods. The climate is of mild inland equatorial type, modified by relief, altitude and proximity to the lake. It favours the cultivation of sugar cane which is an industrial crop, besides other crops like cassava, maize, sorghum and tobacco. It experiences a minimum temperature of 17°C and a maximum of 20°C, with high humidity and a potential evaporation of 1800- 2000mm per year (Migori District Development Plan 2002-2008).

3.2. Identification of wild Lamiaceae

3.2.1 Data collection

This study was conducted among the Luo and Maragoli communities residing in Uriri District. It emphasized on the usage of herbs and shrubs of Lamiaceae family as remedy to the various ailments and complications encountered. Observation, oral interviews and questionnaires (See Appendix 3) were the tools commonly used for data collection. Photos of the various plants collected were taken using a digital camera. Oral interviews were conducted with the aid of an interpreter. This process was guided by village elders from various areas who were well acquainted with the medicinal practitioners (herbalists). The questionnaires were not directly administered to the respondents instead they were filled later after the interviews. In most cases, they were used as guides. This was so because of the high level of illiteracy among the respondents and also for allaying all forms of suspicion on the respondents operations. Out of the 120 medicinal practitioners (aged 50 years and above) targeted, 60 were interviewed, 54 of which gave harmonized information while 6 gave conflicting information that could not be used in this study. The number 60 was arrived at through purposive sampling where a 50% number of the target population was deemed appropriate as a representative sample. The data collection process took 6 months from November, 2009 to August, 2010 (see appendix 4 and5).

Figure 1: Map of Uriri District

3.2.2. Preparation of herbarium specimen

Plant collection and the preparation of herbarium specimens was based on the standard taxonomic procedures according to Stace (1993), Bridson *et al.*, 1998 and Judd *et al.*, 2008. After collection, the plant specimen were processed in the Botany laboratory (Maseno University) where drying was done at 40° C for 72 hours. The plant specimens were then pressed, mounted and sprayed with pyrethrin based insecticides and stored as voucher specimens at the University Herbarium, Maseno. The duplicates were deposited at the East African Herbarium. Naming of the specimen was done at The East African Herbarium in The National Museums of Kenya in Nairobi. Two plants were collected per species for preparing voucher specimen, one specimen to be deposited in Maseno University Herbarium and the other at the East African Herbarium.

3.2.3. Description of plants, diseases treated and mode of drug preparation

3.2.3.1 Description of the wild Lamiaceae plants collected

Plant description was based on the morphological attributes of the leaves, stems, roots, flowers, seeds and leaf arrangement according to Bell and Brian (1993), Agnew and Agnew (1994) and Raven *et al.*, (2005).

3.2.3.2 Diseases treated by the wild Lamiaceae plants collected and the mode of preparation of plant extracts

Information relating to the diseases treated by the various plants of Lamiaceae family collected was obtained from traditional medical practitioners from the two communities investigated in Uriri district during the study period. The study involved interviews, photo taking and ocular observations. In total, 120 medicinal practitioners were targeted. Questionnaires were filled after every interview sessions to reduce incidences of the respondents suspecting the activities of the researcher. The data collected was related and only that which appeared to be common among many practitioners was selected for documentation. This was based on the number of informants who offered the same information, the recommended number being three according to Kokwaro (1976).

3.3. Phytochemical and mineral analysis

3.3.1. Procedure for phytochemical tests

Phytochemical screening was conducted to test for the presence of compounds such as alkaloids, flavonoids, terpenoids, saponins, tannins, sterols and the cardiac glycosides using stems, leaves and roots of the selected plant species of Lamiaceae collected from the study area. The chemical test was carried out on the aqueous extract and on powdered sample from the leaves, stem, and the root using standard procedures to identify the constituents as described by Sofawara (1993), Trease and Evans (2002) and Siddiqui and Ali (1997). The phytochemical screening of the various plant species was supposed to reveal the presence or absence of the various secondary metabolites to be tested.

3.3.1.1. Test for tannins

About 0.5 grams of dried powdered sample was boiled in 20 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride were then added. An observation of brownish green colouration indicated a positive test.

3.3.1.2. Test for saponins

About 2 grams of powdered sample was mixed with 20 ml of distilled water in a boiling tube and then placed in a hot water bath for 5 minutes and then filtered. 10 ml of the filtrate was mixed with 5ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with three drops of olive oil and shaken vigorously, then observed for formation of emulsion.

3.3.1.3. Test for flavonoids

About 5gm of powdered plant sample in a boiling tube was heated with 10 ml of ethyl acetate over a steam bath for three minutes. The mixture was then filtered and 4 ml of the filtrate shaken with 1ml of dilute ammonium solution. A yellow colouration observed indicated a positive test for the flavonoids.

3.3.1.4. Test for terpenoids (Salkowski test)

To each 5 ml of plant extract in a test tube, 2ml of chloroform and 3ml of 0.1M sulphuric acid was added to form a layer. A reddish brown colouration of the interface formation indicated a positive test for the terpenoids.

3.3.1.5. Test for glycosides (Keller.Killani test)

Exactly 5ml of each extract in a test tube was treated with 2 ml of glacial acetic acid containing one drop of 0.1% ferric chloride solution. This was underlayed with 1 ml of concentrated hydrochloric acid. A brown ring of the interface indicating a deoxy-sugar characteristic of cardinolides determined a positive test.

3.3.1.6. Test for sterols

About 2ml of acetic anhydride was added to 0.5 grams ethanolic extract of each sample with 2ml of 0.1 M sulphuric acid in a test tube. The colour changed from violet to blue or green in the sample will indicate the presence of sterols.

3.3.1.7. Test for alkaloid

A sample of 2 grams of dried plant extract was mixed with 40ml of 0.1 M hydrochloric acid in a boiling tube and heated in a water bath for 10 minutes. The mixture was then be cooled and filtered. To a portion of the filtrate, few drops of Mayors reagent were added. A slight turbidity of heavy precipitate indicated the presence of alkaloids.

The positive results were reflected by the colour chages shown by the resulting mixture (See table 1).

Table 1: Table of colours for positive phytochemical test

Test	Observation	Inference
Test for tannins	Brownish green colouration	Positive test
Test for saponins	Formation of an emulsion	Positive test
Test for flavonoids	Yellow colour formed	Positive test
Test for terpenoids	Reddish brown colouration	Positive test

(Salkowski test)		
Test for glycosides (Keller Killani)	Formation of a brown ring	Positive test
Test for steroids	Colour change from violet to blue	Positive test
Test for alkaloids	White precipitate formed	Positive test

3.3.2. Procedure for mineral analysis in plants

The macronutrients and micronutrients present in plants were analyzed by dry ashing. The method was appropriate for analyzing potassium, calcium, and magnesium (macronutrients) and also iron, zinc, and manganese (micronutrients). The analysis was as per the guidelines provided by Reuter and Robinson (1986, 1997) and Jones *et al.*, 1991. The analysis involved the use of qualitative techniques to determine the presence of the targeted minerals, while the concentration of the mineral constituent was determined by the use of flame photometer to analyse potassium and calcium and atomic absorption spectrophotometer to analyse zinc, and iron. The procedure involved in the analysis was that of Chapman and Pratt (1961) which has been modified as follows.

About 0.5g portion of ground plant extract was weighed in a 50 ml porcelain crucible and placed into a cool muffle furnace where the temperature was increased gradually to 550°C after which the ashing continued for five hours after attaining the 550°C. The muffle was then shut off and the door opened cautiously for rapid cooling. When cool, the porcelain crucible was taken out carefully. The cooled ash was dissolved in 5ml portion 2N hydrochloric acid and mixed using a plastic rod. After 20 minutes, the volume was made up to 50 ml using de-ionized water. Thorough mixing was done and the mixture allowed to stand for 30 minutes after which it was filtered through Whatman filter paper No. 42, discarding the first portion of the filtrate. The aliquots for potassium and calcium were analyzed by flame photometry while those of zinc and iron by atomic absorption spectrometry.

3.3.2.1. Determination of potassium

The solution was sprayed starting with standards, the sample and then the blank solution directly into the flame photometer at wavelength of 766.5 nm.

3.3.2.2. Determination of calcium

The standard, blank and the sample solution was sprayed into the flame photometer at wavelength of 422.7nm.

3.3.2.3. Determination of zinc

Zinc was measured by atomic absorption as it will absorb radiation from element specific hollow cathode lamp at wavelength of 213.9nm.

3.3.2.4. Determination of iron

Iron was measured by atomic absorption as it absorbs radiation from an element-specific hollow cathode lamp at wavelength of 248.3nm.

The results obtained were represented in graphical manner where by absorbance was plotted against sample concentration.

3.4. Seed and vegetative propagation of the wild Lamiaceae plants

3.4.1. Seed propagation

Seeds of the selected plant species were collected from mature plants after flowering and seeding in October. The fully expanded seeds were wrapped in fine nylon mesh bags and transported to the Botanic Garden-Maseno University where they were sun dried for three day before being planted. Fifteen seeds from every plant species were propagated in finely prepared soil in propagation trays. They were watered on a daily basis for a period of 3 months. They were not subjected to any kind of treatment to ascertain their possibility of growing on their own. Seed viability test (Germination probity) determined by calculating the germination percentage of the propagated seed using the fomula below:

$$\text{Germination percentage} = \frac{\text{Number of seeds that germinated}}{\text{Number of seeds planted}} \times 100$$

3.4.2. Vegetative propagation

Medial stem cuttings measuring 30cm were obtained from the entire parent plants collected and planted in plastic containers within the botanic garden. The cuttings were obtained 10 cm from the ground surface. Watering was done on a daily basis as observations were made on which species could be propagated by vegetative means. This was done by observing leaf emergence over the 3 months propagation period.

The stem cuttings were also not subjected to any kind of treatment. Leaf emergence was determined at different time within the 3 months duration, since it varied depending on the plant species. Leaf emergence rate was worked out using the following formulae:

$$\text{Leaf emergence rate} = \frac{\text{Number of stem cuttings with leaves}}{\text{Number of stem cuttings}} \times 100$$

Data analysis

Information collected from the traditional medical practitioners and those from phytochemical screening and mineral analysis of plants was subjected to qualitative data analysis. The tabulation of the results was done using Microsoft office Excel and Word while the preparation of a database of documented plants of Lamiaceae was done using Microsoft office Access. The graphs that represent the concentration of minerals and their absorbance were generated using Graphpad Prism. The relationship between absorbance and sample concentration was determined using regression analysis in order to get the coefficient of determination (r^2).

CHAPTER FOUR

4.0. RESULTS

4.1. Identification of wild Lamiaceae and the diseases treated

4.1.1. Species of plants of Lamiaceae family collected from Uriri District

From the field survey twelve plant of Lamiaceae family were collected and identified from different sites within the area of study. The plants were assigned both the Luo and Maragoli names commonly used by the local residents. Scientific names were also assigned (Agnew and Agnew, 1994; Beentje, 1994), (See table 2).

Table 2: List of plants of Lamiaceae family cited in Uriri District as used in traditional medicine

Scientific name	Luo name	Maragoli name
Becium obovatum (E.Mey.Ex Benth) N.E.Br	Ndwendwe, Makobo, Bwar	Kirungamunyu chikiguru
Calamintha nepeta (L) Savi	Mieny mar abondo, Mdhuse	Zambire, Orusara
Fuestia africana T.C.E Fries	Abunga useke/ aremo	Mkuvizanyingu
Hoslundia opposita Vahl	Ofwongo	Kifunya
Hyptis pectinata (L) Poit	Oluo chiel	Azuya
Leonotis nepetifolia (R, Br) Ait.F.	Nyanyodhi	Risivi
Leucas calostachys Oliv.	Bware	Kikubangano
Ocimum kilimandscharicum Baker Ex. Gurke	Omieny	Risanzura
Plectranthus barbatus Andrews	Okita	Ekeroka
Plectranthus longipes Bak	Okita matindo	Ekeroka
Pycnostachys speciosa Gurke	Bware,	Iridumusi

Satureja biflora Brig.	Dindi, Odote, Lweya, Kurgweno	Mataniga, Izuzi, Ubangu, Riaisi.

4.1.2. Deposition of Herbarium specimen

A total of 24 herbarium specimen of the plant species collected (Table 2) were prepared, two representing each species, 12 were deposited at the East African Herbarium in the National museums of Kenya while the other 12 specimen were deposited at the University Herbarium, Maseno University.

4.1.3.1. Description of wild Lamiaceae plants collected

The plant species collected all conformed to the standard description of Lamiaceae family. The leaves were slightly toothed and oppositely arranged on the quadriangular stems. Their flowers were bi-lipped with the upper lip having two petals fused into a hood and the lower lip having three petal lobes.

4.1.3.1.1. *Becium obovatum*

It is perennial with several stems arising annually from a woody rootstock; stem is erect or ascending, rarely spreading, slender, simple or sparingly branched. The leaves are sub sessile or shortly petiolate; the blade very variable in shape from linear-elliptic to lanceolate, lanceolate-oblong, ovate, sub-rotund or obovate, subglobrous to villous, gland dotted, apex acute to rounded, base cuneate to obtuse, margin entire or with few shallow teeth. Petiole 0-5mm long. Inflorescence often subcapitate or with 1-3 spaced venticils below the crowded apex; pedicel 1mm long. Calyx 4-5mm long at flowering, enlarging to 7-10mm long, reticulate veined, pubescent; tube campanulate. The corolla is white to pale yellow, 10-17mm long with longitudinal violet lines on the upper lip. Stamens are exserted by 14-20mm.

4.1.3.1.2. *Calamintha nepeta*

It is a perennial, robust, strongly aromatic herb having pubescent grey hair. The main stem has a square cross section, a woody base and produces several side branches. The broad oval-shaped leaves are quite small, measuring about15-18mm long and 8-10 mm wide with a quite prominent pinnate venation. The leaf outline is shallow crenate. Leaves are opposite and decussate and those at the upper part of the flowering part of the stems are found in perpendicular pairs, together with a pair of flower cymes- opposite to the leaves, hence producing an x-shaped arrangement with the leaf pair at one side and the pair of flower bunches at the other side. Leaves have a petiole measuring 2-3 mm long and the flower cymes have similar sized peduncles. The flower is composed of a vase-like tubular calyx and a lilac to violet corolla. The calyx has longitudinal pale green strips and tri-angular teeth arranged actinomorphically at the calyx rim. The corolla has 5- lilac periath lobes; three being at the lower half and the other two at the upper half (Fig. 2).

Figure 2: Photo of *Calamintha nepeta*

4.1.3.1.3. *Fuerstia africana*

It is an herb 0.55m tall. The stem is erect, quadriangular with short lateral branches, puberulent to pubescent with short eglandular hairs below in the inflorescence axis. The leaves are opposite, simple and without stipules, blade glandular-punctate beneath, broadly elliptic 20-50 mm. long 1-2mm. wide, serrate in the distal portion, apex acute or obtuse, base attenuate, with short addpressed hairs on vein, otherwise glabrous. The inflorescence is lax with verticals irregularly 2-4 flowered, depending on phylotaxy of the node, each flower subtended by a bract 2-3mm. apart; bracts green 1-3mm long, sometimes erect above forming a very small inconspicuous coma; the calyx is bilabiate, shortly tubular; upper lip broad, ovate, shortly decurrent; lower lip 4-lobed, lateral lobes deltoid, cuspidate or acuminate, median lobes lanceolate, acuminate 6-8mm long at anthesis, pubescent with patent glandular and eglandular hairs and yellow sessile glands. The corolla is pink to white 10mm. long; upper lip erect, 4-lobed with medium lobes narrow and exceeding the lateral lobes; the lower lip is horizontal, concave (Fig. 3).

Figure 3: photo of *Fuerstia Africana*

4.1.3.1.4. *Hoslundia opposita*

It is an erect herb that can grow up to 3 m height. It grows a long road sides, in fallow lands and in waste lands. The stem has nodes, with whorled branching system. It has a grayish to brownish bark embedded on a smooth on hairy stem. The leaves are green, simple, opposite and lanceolate up to 120 mm long, ovate with acute apices and serrated margins. The flowers are small with white petals. The fruits are green but upon maturity, they turn orange and the calyx become larger and fleshy (Fig. 4).

4.1.3.1.5. *Hyptis pectinata*

This is a slender erect herbaceous sub-shrub with a four-angled puberulent stem; the foliage is aromatic if rubbed ad crushed. The leaves are ovate-elliptic, cunneate to round at the base, acute or blunt at the tip, puberulent or glabrescent dorsally, crenate-serrate, and 20-90 mm long. The flowers are sub-sessile, white to pale violet, in cymules axillary to reduced leaves, subtended by linear pubescent bracts 1-3 mm long; calyx about 2mm, enlarging in fruit to 4mm; corolla 2-5mm, the lower lip is darker; the filament is puberscent; nutlets are oblong and black. It may be up to 4m tall (Fig. 5).

Figure 4: Photo of *Hoslundia opposite*

Figure 5: Photo of Hyptis pectinata

4.1.3.1.6. *Leonotis nepetifolia*

It is a small herb with four angled stems. The stem is light green and covered with short hairs. It has oppositely paired branches. The bark is light green and has short hairs. The leaves are simple and dense. The flowers are orange and form whorls. The lower leaves are up to 75-90 mm long with a stalk of about 25mm long and a coarsely toothed blade, bluntly narrowed at the tip and narrowed gradually or abruptly at the base. The upper leaves in the axil of which the flower arise are sessile and about 18.75mm inches long. There is a curved tubular, hairy corolla, divided above into two lips, the upper longer than the lower and hooded, somewhat resembling a lions ear. The calyx bears 5 long pointed teeth of equal length. There are four stamen attached to the corolla tube, the lower part longer than the upper part and the 4- lobed ovary develops into a fruit of 4 nutlets (Fig. 6).

Figure 6: Photo of *Leonotis nepetifolia*

4.1.3.1.7. *Leucas calostachys.*

It is a perennial herb with densely hairy stems, woody towards the base. The leaves are nearly sessile, oblong 250-300 mm long, crenate in the upper half, thinly hairy above, densely and softly hairy beneath. The leaves are much reduced, bracts setaceous, half as long as the calyx. The calyx is hairy 10mm long, throat oblique and the lower teeth deltoid. The corolla is usually white, bi-lipped and is half as long as the calyx with densely villous upper lip (Fig. 7).

4.1.3.1.8. *Ocimum kilimandscharicum*

It is an aromatic under shrub with pubescent quadriangular brachlets; leaves simple, opposite, ovate or oblong, narrow at the base, deeply serrated, acute, pubescent on both surfaces; flowers in 4-6 floret whorls or racemes, with a densely pubescent axis, corolla 2-3 times the length of the calyx, fruits ovoid- oblong nutlets brown-black (Fig.8).

Figure 7: Photo of *Leucas calostachys*

Figure 8 Photo of *Ocimum kilimandscharicum*

4.1.3.1.9. *Plectranthus barbatus*

This is an aromatic woody herb forming a dense bush or shrub, 1-4 metres high. The stems and roots are somewhat fleshy. The young stems are angled in cross-section.

The leaves are large, soft, and widest in the middle or towards the base, up to 120×110mm, softly hairy on both surfaces, the margins are, toothed. The flowers are blue to purple in terminal heads up to 400mm long (Fig. 9).

Figure 9: Photo of *Plectranthus barbatus*

4.1.3.1.10. *Plectranthus longipes*

It is a tall, much branched, erect herb with pubescent stems, leaves with very long petioles, ovate, 50-75mm long, deeply crenate, not cordate at the base, green and slightly pubescent on both surfaces. Whorls 4-6 flowered arranged in a long lax racemose panicle; bracts minute; pedicels 4-6 mm long. Fruit calyx pilose, 6mm long; tube campanulate; upper lip broadly ovate, as long as the tube; lower linear as long. Corolla 8mm long; the lower lip is shorter than the tube. The stamens are not exserted Fig. 10).

4.1.3.1.11. *Pycnostachys speciosa*

It is an annual herb 0.3-1.5m with erect stems, quadriangular, pubescent with short, downward pointing eglandular hairs and red sessile glands. The leaves are opposite, sessile blades, narrow elliptic to narrowly ovate to lanceolate, serrate, apex acumate, base gradually narrowing to the stems with red and yellow sessile glands and pubescent with short, stiff eglandular hairs, the hairs more plentiful on the venation below. The inflorescence is dense when in flower forming a narrowly conical shape, becoming more cylindrical in fruit. The flowers are sessile in single flowered cymes, bracts subtending entire inflorescence narrowly ovate, the apex acuminate, enclosing buds then becoming deflexed in flower. The flowering calyx has a tube 1mm. long, while the fruiting calyx has a tube of 3-7mm. long. The corolla is pale blue 14-18mm, tube with short eglandular hairs very narrow and parallel sided for 5-6mm, then curving sharply downwards and broadening before opening into two lips, posterior shorter lip and anterior longer lip (Fig. 11).

4.1.3.1.12. *Satureja biflora*

It is a perennial herb, 0.2-0.6m tall with several stems arising, often annually from a woody base. The stems are slender, softly woody, usually erect, simple or sparingly branched, tomentose, bearded below the nodes. The leaves are sessile or subsessile with elliptic to ovate blade; the apex is acute to obtuse, the base truncate with an entire margin. The flowers are usually pedunculate cymes, in the axils of the leaves for almost the entire length of the stem but mainly in the upper third; bracteoles small, linear. The calyx is 15-ribbed, hispidulous, tubular up to 4mm long, subequally toothed: The corolla is white to pale mauve 5-7mm long, the upper lip 1mm long while the lower lip 1-2 mm long. The leaves are pleasantly lemon scented (Fig. 12).

Figure 10: Photo of *Plectranthus longipes*

Figure 11: Photo of *Pycnostachys speciosa*

Figure 12: Photo of *Satureja biflora*

Seven plant species: *Ocimum kilimandscharicum, Plectranthus barbatus, hoslundia opposita, Pycnostachys specieosa, Hyptis pectinata Leonotis nepetifolia* and *Leucas* *calostachys* were all erect shrubs with their heights ranging between 1.2 metres to 2.5 metres (Table 3). The other plant species: *Becium obovatum, Calamintha nepeta, Fuerstia africana, Plectranthus longipes* and *Satureja biflora* were herbaceous in nature displaying heights below 1 metre.

Table 3: Nature of plant species

Plant name	Height	Nature
Becium obovatum	0.45 metres	Herb
Calamintha nepeta	0.65 metres	Herb
Fuestia africana	0.5 metres	Herb
Hoslundia opposita	1.7 metres	Shrub
Hyptis pectinata	2.3 metres	Shrub
Leonotis nepetifolia	2.2 metres	Shrub
Leucas calostachys	1.6 metres	Shrub
Ocimum kilimandscharicum	1.75 metres	Shrub
Plectranthus barbatus	1.2 metres	Shrub
Plectranthus longipes	0.8 metres	Herb
Pycnostachys speciosa	1.25 metres	Shrub
Satureja biflora Syn. *Micromeria imbricata*	0.4 metres	Herb

4.1.3.2. Diseases treated by the wild Lamiaceae plants collected and the mode of preparation of plant extracts.

The information collected from the traditional medical practitioners revealed that the plants collected were very useful in the treatment of common ailments. A total of nine plants were found to be useful in the treatment of gastrointestinal infections and other complications related to the digestive system such as ulcers, constipation and intestinal parasitic infections. Some five plants were found to be effective in the treatment of complications related to the nervous system such as convulsion, insomnia and nervous tension. Lung related complications that were treated by some of the plants included lung congestion and coughs. Other diseases and complications that were found to be effectively suppressed by the plants collected included: cold and flu, warts and abscesses, measles, painful menstruation, fever, snake bites, skin infections and abdominal pain. The summarized results were represented in a tabulated form (See Table 4).

Table 4: Diseases treated and the mode of preparation of the plant extracts.

PLANT NAME	MODE OF DRUG PREPARATION	DISEASE(S) TREATED OR MANAGED
Becium obovatum	Decoction and concoction	Gastrointestinal infections, anthelminthic, swellings/warts, genital stimulant/ depressant.
Calamintha nepeta	Infusion and concoction	Nervous tension, depression, insomnia, fever, cold, flu and painful menstruation.
Fuerstia africana	Maceration, concoction, infusion	Stomachache, urinary infections, ulcers and snake bites.
Hoslundia opposita	Concoction and infusion	Colds, sore throat, gonorrhea, convulsion, stomach pains, ringworms and parasitic skin infection.
Hyptis pectinata	Infusion, concoction and maceration	Gastrointestinal infection, fever, some skin infections, lung conjestion, rheumatism.
Leonotis nepetifolia	Decoction and concoction	Resizing distended stomach in young children, fever, wound prolapses, malaria, coughs
Leucas calostachys	Concoction, infusion,	Stomachache, diarrhoea

	maceration	gastrointestinal diseases and constipation and cold.
Ocimum kilimandscharicum	Decoction, tisane and concoction	Diarrhoea, cold and flu, coughs, abdominal pains, annorexia and measles.
Plectranthus barbatus	Concoction, maceration and infusion	Stomachache, dysentery, diarrhoea and intestinal infections, abscess.
Satureja biflora	Infusion, maceration and concoction	Stomachache, rheumatism, chronic diarrhea

4.2.1. Results of phytochemical tests/screening

The phytochemical tests conducted involved grinding the leaves, roots and the stem parts of the plant species and then mixing them up when on powdered form. Six of the ten plant species analysed by qualitative methods showed positive test for all the phytochemicals while four plant species lacked some phytochemicals. *Hoslundia opposita*, *Hyptis pectinata* and *Leonotis nepetifolia* lacked alkaloids. Glycosides were absent in *Calamintha nepeta* and *Hyptis pectinata* while flavonoids were only absent in *Hyptis pectinata* (See Table 5), this was evident by the colour changes that the aqueous plant extracts exhibited which did not conform to standard colouration provided, thus testing negative (See Table 6). Detection of the respective phytochemicals in plant species was determined by colour changes that were exhibited. Each phytochemical had a characteristic colour change that indicated a positive test of its presence (See table 1).

Most plants collected were used in their dried forms except for a few that had delicate parts of which when dried would lose their volatile phytochemicals. The tests conducted therefore involved the plant material that were solar dried and then subjected to the standard phytochemical procedures that revealed the presence of alkaloids, terpenes, glycosides, sterols, saponins flavonoids and tannins (see Table 5). *Hoslundia opposita* lacked alkaloids and glycosides, *Plectranthus longipes* and *Hyptis pectinata* lacked both alkaloids and flavonoids, while *Leonotis nepetifolia* glycosides and alkaloids. *Calamintha nepeta* and *Pycnostachys speciosa* only lacked the glycosides. This was determined by the colours displayed by the different plant extracts that were subjected to various qualitative techniques in the laboratory to determine either the presence or absence of the various phytochemicals (See Table 6).

All the plant extracts tested positive, a clear indication that they all contained tannins. The other phytochemicals that tested positive in all the plant extracts included the sterols, terpenoids and saponins. The saponins when tested in the plant extracts using distilled water, an emulsion was formed in all the boiling tubes used, an indication that the test was positive. For the sterols, a positive test was marked by a colour change from violet to green when the plant extract was treated using acetic anhydride and sulphuric acid. The Salkowski test which revealed the presence of terpenoids involved treating the plant extract with chloroform and sulphuric acid where a positive test was confirmed by a red-brown coloration of an interface. The presence of glycosides was determined using the Keller Killani test where the plant extract were treated with glacial acetatic acid, 0.1% Ferric chloride and hydrochloric acid. A positive test was indicated by the formation of a brown ring of deoxyribose sugar in most of the plant extracts except in *Calamintha nepeta*, *Hoslundia opposita* and *Leonotis nepetifolia*. For the flavonoids, a positive test was indicated by the formation of a yellow colour when the plant extract was reacted with ethyl acetate and ammonia. Only the extract of *Hyptis pectinata* tested negative to flavonoids due to the formation of a green precipitate.

The test for alkaloids was positive in most plants except, *Hoslundia opposita*, *Hyptis pectinata* and *Leonotis nepetifolia*. A positive test was indicated by the formation of a white precipitate when the plant extract was reacted with hydrochloric acid and Mayor's reagent.

Table 5: Results of phytochemical tests/screening

NAME OF PLANT	Sterols	terpenoids	Alkaloids	Saponins	Glycosides	Flavonoids	Tannins
Becium obovatum	√	√	√	√	√	√	√
Calamintha nepeta	√	√	√	√	X	√	√
Fuerstia africana	√	√	√	√	√	√	√

Hoslundia opposita	√	√	X	√	X	√	√
Hyptis pectinata	√	√	X	√	√	X	√
Leonotis nepetifolia	√	√	X	√	X	√	√
Leucas calostachys	√	√	√	√	√	√	√
Ocimum kilimandscharicum	√	√	√	√	√	√	√
Plectranthus barbatus	√	√	√	√	√	√	√
Satureja biflora	√	√	√	√	√	√	√

KEY

X.........Absence of

√..........Presence of

Table 6: Colour change test

Plant name	Phytochemical						
	Sterols	Terpenoids	Alkaloids	Saponins	Glycosides	Flavonoids	Tannins
Becium obovatum	Violet to blue	Red brown	White ppt	Cream emulsion	Brown ring	Cream colour	Dirty green
Calamintha nepeta	Violet to blue	Red brown	White ppt	Cream emulsion	Yellow colour	Yellow colour	Deep brown
Fuerstia africana	Violet to blue	Red brown	White ppt	White emulsion	Brown ring	Yellow colour	Deep brown
Hoslundia opposita	Violet to blue	Red brown	Green colour	White emulsion	Orange colour	Yellow colour	Brown
Hyptis pectinata	Violet to blue	Red brown	Yellow colour	White emulsion	Brown ring	Red colour	Brown green
Leonotis nepetifolia	Violet to blue	Red brown	Green colour	White emulsion	Green colour	Yellow colour	Brown green
Leucas calostachys	Violet to blue	Red brown	White ppt	White emulsion	Brown ring	Cream colour	Brown green
Ocimum kilimandscharicum	Violet to blue	Red brown	White ppt	Cream emulsion	Brown ring	Yellow colour	Brown green
Plectranthus	Violet	Red	White	White	Brown	Cream	Brown

barbatus	to blue	brown	ppt	emulsion	ring	colour	
Satureja biflora	Violet to blue	Red brown	White ppt	White emulsion	Brown ring	Yellow colour	Brown green

Key
Ppt- Precipitate

4.2.2. Results of mineral analysis

During the research period, 12 plants of wild Lamiaceae were collected from different areas within Uriri District. Out of the 12 plants collected, 5 plants were selected for mineral analysis. The number 5 was based on plants rarely found in the area and those whose usage were restricted to very few practitioners with vast knowledge on how well the doses could be administered. The selected plant were *Fuerstia Africana, Leucas calostachys, Hyptis pectinata, Satureja biflora and Plectranthus longipes* and very little ethnobotanical information was found regarding their usage (see Table 7.1). The methods used for the analysis of minerals revealed that calcium, iron, potassium and zinc were available in the plants collected in varied concentrations (See Table 7.1).

During dry ashing, a high temperature was maintained in order to vapourize water and other volatile material that were not necessary for the analysis. The organic substances were also burnt up so that only the non volatile minerals were left in form of ash. This pre treatment method was preferred because the ash could be analysed for specific mineral content as in the case of the analysis of calcium, potassium, zinc an iron. This method has also been applied in other researches where the results obtained have been compared against those of wet ashing technique and found to conform when analysed using the Atomic Absorption Spectrophotometer (Anderson, 1989 and Okalebo, 1985).

Four minerals were considered in the study and they included zinc, iron, calcium and potassium. Zinc and iron were analysed using the Atomic Absorption Spectrophotometer while potassium and calcium were done using the flame photometer. Standards were prepared at concentrations (ppm) of 0,2,4,8 and 10. The results obtained from the Atomic Absorption Spectrophotometer and the Flame photometer were tabulated and then plotted in graphs that formed standard calibration curves in all the cases (See Figures 2,3,4 and 5) and (Appendices; 6,7,8 and 9). For zinc and iron, the results obtained from the AAS were tabulated as below (see Table 7.2 and 7.3) while for calcium and potassium the flame photometer results were represented in Tables 7.4 and 7.5 respectively.

The Atomic Absorption Spectrophotometer revealed the presence of zinc and iron in the concentrations and absorbance indicated in Tables 7.2 and 7.3. The concentrations of potassium and calcium were determined using Flame photometeras indicated in Tables 7.4. and 7.5. The actual concentrations and absorbance of the plant samples analysed were shown by tables 7.6, 7.7, 7.8 and 7.9. (See appendices 6, 7, 8 and 9) The concentration of solutions were expressed in parts per million (ppm) to facilitate calculations on dilute solutions.

The standard calibration curves were used for determining the range of concentration of the elements analysed from a series of calibration standards used up. The calibration curve was plotted with the absorbance against concentration of the standards. The curves obtained from the results recorded deviations which could be attributed to unabsorbed radiation, stray light or disproportionate decomposition of molecules at high concentration. The five standards and a blank prepared were intended to produce sufficient information to fit the standard curve appropriately by establishing a relationship between the measured absorbance and the analyte concentration, a principle that relies on the Beer Lambert Law (Raghbir, 2006). The concentration of the analytes are directly proportional to their absorbance thus an increase in the absorbance leads to an increase in concentration, while a decrease in absorbance decreases the concentration (See Figures 2,3,4 and 5). The calibration of standard curve was as per the Beer's Law that the absorbance of an analyte is proportional to its concentration (Haswell, 1991).

Table 7.1: Table showing availability of minerals in selected plants

PLANT NAME	K	Ca	Fe	Zn
Fuerstia africana	√	√	√	√
Hyptis pectinata	√	√	√	√
Leucas calostachys	√	√	√	√

Plectranthus longipes	√	√	√	√
Satureja biflora	√	√	√	√

Table 7.2: Table of zinc analysis showing standard concentration (ppm) and absorbance.

Standard number	Std concentration (ppm)	Absorbance
Standard 1	0	-0.01
Standard 2	2	0.0016
Standard 3	4	0.0058
Standard 4	8	0.0056
Standard 5	10	0.0063

Table 7.3: Table of iron analysis showing standard concentration (ppm) and absorbance.

Standard number	Std concentration (ppm)	Absorbance
Standard 1	0	-0.0035
Standard 2	2	0.0019
Standard 3	4	0.0136
Standard 4	8	0.0207
Standard 5	10	0.0269

Table 7.4: Table of calcium analysis showing standard concentration (ppm) and absorbance.

Standard number	Std concentration (ppm)	Absorbance
Standard 1	0	0.001
Standard 2	2	0.123
Standard 3	4	0.263
Standard 4	6	0.350
Standard 5	8	0.516
Standard 6	10	0.600

Table 7.5: Table of potassium analysis showing standard concentration (ppm) and absorbance.

Standard number	Std concentration (ppm)	Absorbance
Standard 1	0	0.001
Standard 2	2	0.0463
Standard 3	4	0.1154
Standard 4	6	0.1350
Standard 5	8	0.1730
Standard 6	10	0.2372

Table 7.6: Table showing actual sample concentration and absorbance of zinc in the analysed plant species

Plant name	Sample ID	Actual Conc.(ppm)	Absorbance
Fuerstia africana	E	10.8859	0.0071
Leucas calostachys	I	9.2194	0.0061
Hyptis pectinata	K	13.5522	0.0087
Satureja biflora	L	8.5529	0.0033
Plectranthus longipes	M	9.0528	0.006

Table 7.7: Table showing actual sample concentration and absorbance of iron in the analysed plant species

Plant name	Sample ID	Actual Conc.(ppm)	Absorbance
Fuerstia africana	E	5.063	0.0131
Leucas calostachys	I	2.1559	0.0048
Hyptis pectinata	K	4.0823	0.0103
Satureja biflora	L	3.4518	0.0085
Plectranthus longipes	M	2.1909	0.0049

Table 7.8: Table showing actual sample concentration and absorbance of calcium in the analysed plant species

Plant name	Sample ID	Actual Conc.(ppm)	Absorbance
Fuerstia africana	E	8.4373	0.5062
Hyptis pectinata	K	2.7324	0.1639
Leucas calostachys	I	4.0968	0.2458
Plectranthus longipes	M	3.9042	0.2341
Satureja biflora	L	5.1648	0.3099

Table 7.9: Table showing actual sample concentration and absorbance of potassium in the analysed plant species

Plant name	Sample ID	Actual Conc.(ppm)	Absorbance
Fuerstia africana	E	3.6233	0.0764
Hyptis pectinata	K	4.5472	0.1096
Leucas calostachys	I	7.4688	0.2148
Plectranthus longipes	M	3.8527	0.0846
Satureja biflora	L	9.1223	0.2744

Linear regression between absorbance and standard concentrations of the mineral ions indicated that an increase in concentration increased the absorbance in all the tested samples (Figures 13-16). The relationship between absorbance and sample concentration was weakest in zinc with a coefficient of determination of r^2=0.613 and strongest in Calcium (r^2=0.994). Iron had a relationship of r^2=0.966 while potassium had a value of r^2=0.981. The values of potassium, iron and calcium deviated significantly from zero while that of zinc did not deviate significantly from zero. The former therefore had strong relationships.

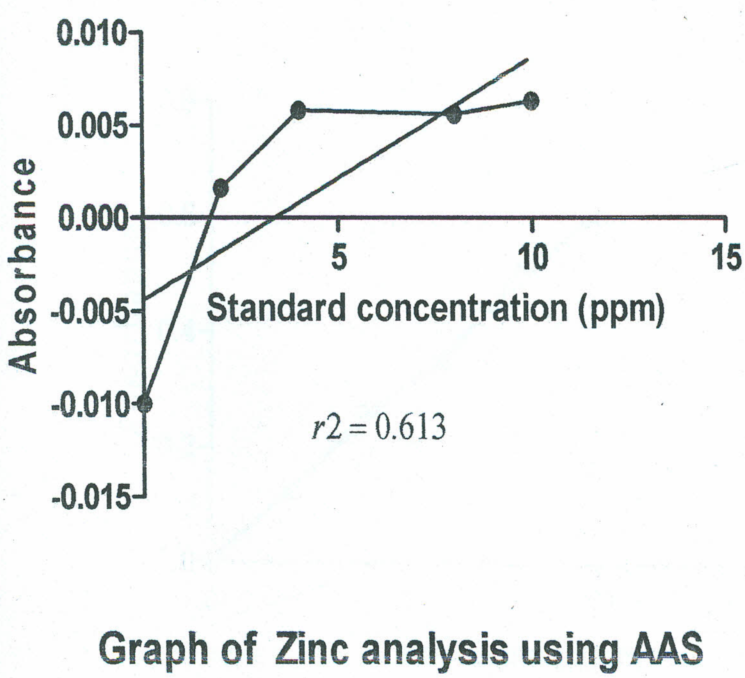
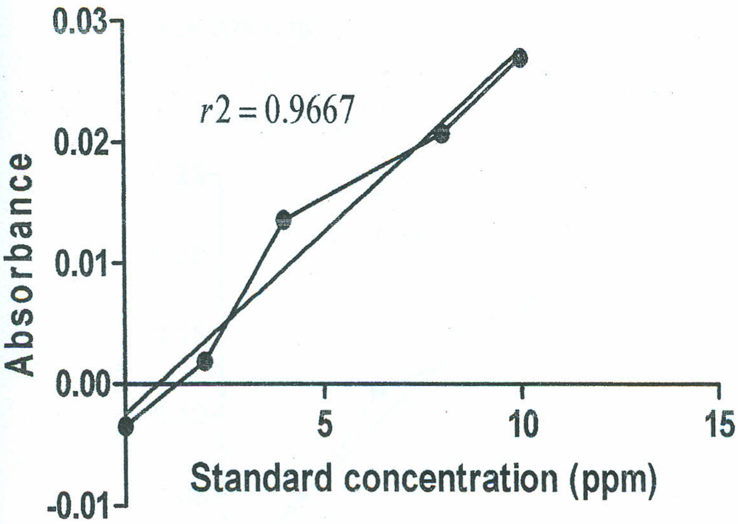
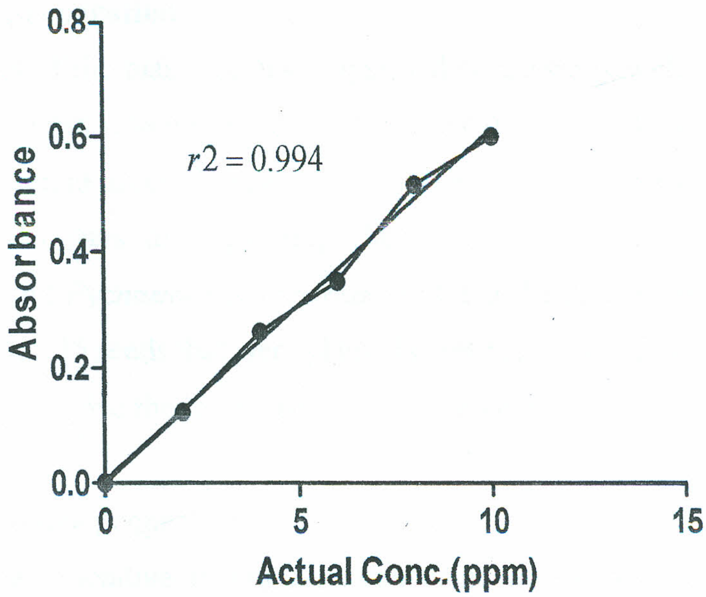


Figure 13: Graph of zinc analysis showing absorbance against standard concentration



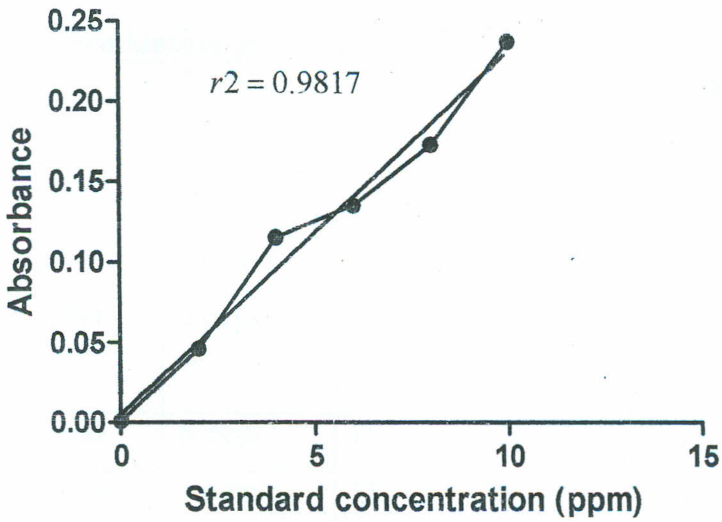
Graph on analysis of Iron analysis using AAS

Figure 14: Graph of iron analysis showing absorbance against standard concentration



Graph on analysis of calcium using flame photometer

Figure 15: Graph of calcium analysis showing absorbance against standard concentration



Graph of potassium analysis using flame photometer

Figure 16: Graph of potassium analysis showing absorbance against standard concentration

4.3.1. Seed propagation

All the seeds of the plant species propagated successfully with their leaves emerging at different times. *Pycnostachys speciosa* and *Calamintha nepeta* were the fastest species to sprout after 6 days, while *Satureja biflora* took the longest time to germinate, 16 days after planting (See Table 8). *Calamintha nepeta, Leonotis nepetifolia* and *Pycnostachys speciosa* exhibited the highest germination percentage of 80%, all the 15 seeds that were planted germinated successfully. On the other hand, *Hyptis pectinata* had the lowest germination percentage of 33.3% (See Table 8).

4.3.2. Vegetative propagation

Most of the vegetative propagation conducted on the selected plant species were unsuccessful within the three months propagation period. Only four plant species: *Becium obovatum, Hoslundia opposita, Plectranthus barbatus* and *Plectranthus longipes* showed emergence of leaves at different times. *Hoslundia opposita* and *Plectranthus barbatus* exhibited the quickest leaf emergence after 9 days, followed by *Plectranthus longipes* in 13 days and the *Becium obovatum* which took the longest time, 16 days (See Table 9). *Plectranthus barbatus* showed 100% successful propagation, Hoslundia opposita and *Plectrantus longipes* had 63.5% while *Becium obovatum* had the lowest percentage of 37.5% (See Table 9).

Table 8: Seed germination percentage of plant species

Name of Plant	Time of leaf emergence	Number of seeds planted	Number of germinated seeds	Fraction of germinated seeds	Germination percentage
Becium obovatum	14 days	15	08	08/15	53.33%
Calamintha nepeta	8 days	15	12	12/15	80%
Fuerstia Africana	6 days	15	11	11/15	73.33%
Hoslundia opposite	12 days	15	08	8/15	53.33%
Hyptis pectinata	13 days	15	05	5/15	33.33%
Leonotis nepetifolia	8 days	15	12	12/15	80%
Leucas calostachys	15 days	15	9	9/15	60%
Ocimum kilimandsharicum	10 days	15	08	08/15	53.33%
Plectranthus barbatus	7 days	15	11	11/15	73.33%
Plectranthus longipes	12 days	15	07	7/15	46.67%
Pycnostachys speciosa	6 days	15	12	12/15	80%
Satureja biflora	16 days	15	08	8/15	53.33%

Table 9: Vegetative propagation percentage of plant species

Plant name	Time for leaf emergence	Number of stem cuttings planted	Number of cuttings that produced leaves	Fraction of cuttings that leaved	Percentage of successful propagation
Becium obovatum	16 days	8	3	3/8	37.5%
Calamintha nepeta	-	8	0	-	
Fuerstia Africana	-	8	0	-	
Hoslundia opposita	9days	8	5	5/8	63.5%
Hyptis pectinata	-	8	0	-	0
Leonotis nepetifolia	-	8	0	-	0
Leucas calostachys	-	8	0	-	0
Ocimum kilimandsharicum	-	8	0	-	0
Plectranthus barbatus	9 days	8	8	8/8	100%
Plectranthus longipes	13 days	8	5	5/8	63.5%
Pycnostachys speciosa	-	8	0	-	0
Satureja biflora	-	8	0	-	0

CHAPTER FIVE

5.0. DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1. DISCUSSION

5.1.1.1. Identification of plants of Lamiaceae family collected

The general collection and assignment of local names to the 12 plants of Lamiaceae family collected were done at the initial stage of the research study. The names were provided by the local residents who either belonged to the Maragoli or Luo community. Most of the names given were correct since no contradictions were encountered however, others were contradicting for example *Plectranthus longipes* and *Fuerstia africana* and in some cases a single plant had several local names some of which were also used to refer to other common species of either the same family or different families for instance in the cases of *Pycnostachys speciosa, Satureja biflora* and *Plectranthus longipes* (See Table 2). The presentation of the prepared herbarium specimens to the East African Hebarium at the National Museums of Kenya was a move intended to ensure proper identification of plant species of Lamiaceae family collected. This was done through correct assignment of botanical names using morphological traits of leaves and flowers and it was consequently intended to authenticate the results of this research study.

5.1.1.3.1. Description of Wild Lamiaceae plants collected

All the plants collected were herbs in nature and were commonly used as whole. The leaves and roots are known to accumulate alkaloids, tannins and inulins which are active components of most herbal preparation (Mahonge *et al.*, 2006) hence when the whole plant is used then the chances are high that the active compounds will be available in larger quantities. The identification of plant species conformed to the guideline provided by Bell and Brian, (1993) and Raven *et al.*, (2005). Such a guideline was essential in providing a standardized way of identification inorder to avoid any form of wrong identification. Standard methods of identification were appropriate since the medicinal practitioners interviewed did not have the taxonomical information required to scientifically describe the plant species collected adequately. The methods were also appropriate because they used the simplest taxonomical terms possible to describe the plant species. The scientific naming of the plant species was very essential in understanding the plants and made it easier to retrieve plant related information for documentation in this research study

5.1.1.3.2. Diseases treated using wild Lamiaceae plants collected and their mode of preparation

The twelve plant species collected were all identified and assigned names at the East African Herbarium, ten were found to be commonly utilized in the treatment of several ailments and complications (See Table 2). The remaining two plant species, *Pycnostachys speciosa* and *Plectranthus longipes* were not commonly encountered by most residents and even their names could not be easily established since different respondents had different names. Most of the plant species in the study have been reported as medicinal plants elsewhere in Africa and even in Kenya through past studies although their uses have differed across various cultures (Adjanohoun *et al.*, 1988; Gachanja, 2007; Githinji and Kokwaro, 1993; Juma *et al.*, 2007; Kokwaro, 1976 and Njoroge *et al.*, 2004; Okoli *et al.*, 2007; Oliver- Bever, 1987). It was found out from the practitioners that the plants administered as decoctions (see Table 4) were characterized with tough leaves, bark and even the roots. They therefore had to be boiled longer to soften their parts before being administered. This method however, may not be most appropriate since the subjection of the plant material to high temperature is highly likely to alter the chemical composition of the plant, especially the very volatile ones as reported in past research studies (George and Pamplona, 2000; Okello *et al.*, 2010). Infusion method was used to administer the herbs that had delicate soft parts (see Table 4), where plant leaves or the whole plant were dipped in hot water and left for some time for the active ingredients to be extracted. The plants that were macerated were found also to have highly soluble chemical components that would easily dissolve in cold water when left overnight in a covered container, as has also been reported in the past (Githinji and Kokwaro, 1993; George and Pamplona, 2000; Trease and Evans, 2002).

All the plants collected were administered as concoction in different cases (see Table 4), in this case it was revealed that some plants which were considered to be of lower medicinal value were combined with other plants of the same family or different families to form a powerful mixture that was more effective than would be the case if a plant was used alone. This is also in line with some revelation by some researches on Ethnobotany among various communities in Kenya (Arwa, 2005; Gachanja, 2007; Githinji, 1990; Kisangau, 1999; Okello *et al*., 2010; Omino and Kokwaro, 1993). In some cases, concoctions were prepared when a plant was considered useful and had some poisonous ingredients in it, the other plant used alongside was meant to neutralize the effects of the poison. The administration of herbs as tisane involved adding the leaf extracts of a plant especially *Ocimum kilimandscharicum* to tea, porridge or any available beverage.

Although there has been substantial research on the phytochemical analysis of Lamiaceae plant species and their composition, most studies conducted have been limited to the locally grown cultivars. It is well known that environmental conditions and agricultural practices may significantly modify productivity, oil content and chemical composition of plant species (Daniel *et al*., 2011; Trease and Evans, 2002; Nterubanza *et al*., 1984; Suarez *et al*., 2003). The phytochemicals tests conducted revealed that all the plant species of Lamiaceae collected tested positive for sterols, terpenoids, tannins and saponins. This is a clear indication that such plant species have a powerful medicinal value especially to their users. The various medicinal properties exhibited by the various phytochemicals are useful in the treatment of most common ailments.

The commonly used plant species of Lamiaceae family collected were found to contain all the group of compounds tested in the laboratory (see Table: 5). Such plants included *Fuerstia africana*, *Becium obovatum*, *Leucas colostachys*, *Ocimum kilimandscharicum*, *Plectranthus barbatus* and *Satureja biflora*. *Hyptis pectinata* was also commonly used by the practitioners however the phytochemicals revealed that it lacked alkaloids and flavonoids. The other species which included *Leonotis nepetifolia*, *Hoslundia opposita*. and *Calamintha nepeta* lacked some phytochemicals however they were still useful in the treatment of various ailments. The colour changes detected after the screening indicating the positive tests conformed to some results obtained from past researches on phytochemical content of different plants from different areas (Abulude, 2007; Aguinaldo, *et al*., 2005 Edeoga *et al*., 2005; Nonito and Mylene, 2010 Okwu and Okwu, 2004; Sazada *et al*., 2009). The absence of certain phytochemicals such as alkaloids in *Hoslundia opposita*, and *Leonotis nepetifolia*; glycosides in *Calmintha nepeta, Hoslundia opposita*, and *Leonotis nepetifolia*; and Flavonoids in *Hyptis pectinata* suggest that they could either be present in undetectable amounts or this could be probably due to their low solubility in organic solvents. This is in line with some past studies conducted among some various plant families Apocynaceae, Lamiaceae Verbanaceae among others (Adepoju and Oyewole, 2008; Adilson *et al.*, 2004; Edeoga *et al.*, 2006; Ibrahim *et al.*, 2000). Tannins are usually associated with flavonoids which are their monometric precussors (Sale and Maji, 2006; Trease and Evans, 1989), however from this study, *Hyptis pectinata*, did not test positive for flavonoids yet it had tannins. It is therefore difficult to reconcile the different results for tannins obtained from the plant species in this research.

Most of the Lamiaceae plants collected were found to be commonly used in the treatment of gastrointestinal infection, cold, flu, skin infection and fever (see Table: 4). Data from recent research conducted reveal that *Fuerstia Africana, Leonotis nepetifolia, Ocimum kilimandscharicum* and *Plectranthus barbatus* are strong herbs for gastrointestinal infections and stomach disorders (Lukhoba, 2006; Njoroge *et al.*, 2004; Nyunja *et al.*, 2009). *Ajuga remota, Leucas calostachys, Ocimum kilimandscharicum, Hoslundia opposite, Leonotis mollisina, Plecranthus barbatus, Leucas calostachys, Ocimum latifolium and Plectranthus comosus* have been researched on and it has been documented that they are ethnobotanically important in providing a vast array of remedies on urinary, respiratory, reproductive, nervous and gastrointestinal infections (Jeruto *et al.*, 2010; Okello *et al.*, 2009). The remedy provided by the plant species could be attributed to the presence of a vast array of phytochemicals such as alkaloids, flavonoids, sterols, saponins, tannins, terpenoids and glycosides which have a curative activity against pathogens and therefore support their traditional use in various illnesses.

Alkaloids have analgesic, antispasmodic and antibacterial properties (Stray, 1998). The flavonoids are antioxidants, anti-inflammatory, anticarcinogenic and antimicrobial (Manikandan *et al.*, 2006). The properties of the tannins include anti-inflamatory, regeneration, anticatarrhal, antimicrobial and soothing effect (Okwu, 2004). Elsewhere, tannins have been reported to contain antiviral, antitumor, antiinflamatory and wound healing properties among other organs (Amakaha *et al.*, 2002; Zheljazkov *et al.*, 2008), thus plant species from this study found to contain tannins could be highly likely to contain such properties thus, being useful therapeutic aspects to cure various diseases (Okwu and Josiah, 2006). The actions of saponins include expectorant, anticatarrhal, antimicrobial and cough suppressant properties (Okigbo *et al.*, 2009). The glycosides have a laxative and caminative, effects (Scalbert *et al.*, 2005). The terpenoids possess soothing relief, antimicrobial, carminative effect and antiseptic properties (Sofowora, 1993). Lastly, the sterols have a demulscent and antimicrobial effect.

The curative properties exhibited by the phytochemicals tested were of a very wide range. Many studies have reported that phenolic compounds possess biological activities such as antiinflamatory, antiulcer, antispasmodic and anticancer properties (Carlo *et al.*, 1999). The anticatarrhal effect is a property commonly of tannins and saponins which reduces the rate at which mucus is produced by the body. Past researches show that *Sambucus nigra* and *Hydrastis canadensis* are the plant with rich anticatarrhal effect due to their ability to reduce the production of mucus, a function attributed to the presence of tannins. This research further reveals that the action of tannins from these plants is more in the respiratory system (Eldin and Dunford, 1999). This property is very essential in providing a remedy against cold and flu as evidenced by plants such as *Fuerstia africana*, *Leucas calostachys*, *Ocimum kilimandscharicum*, *Hyptis pectinata*, *Calamintha nepeta* and *Hoslundia opposita* (See Table 10). Saponins also exhibit expectorant effect which aid in the removal of mucus from the lungs, an action brought about by their ability to reduce viscosity and relax bronchial spasm. This is very common in reducing lung congestion and curing sore throat by plants such as *Hyptis pectinata* and *Hoslundia opposita* (See Table 4).

Research conducted in other plant families has revealed that the expectorant property of medicinal plants is attributed to the presence of saponins due to their ability to produce form, haemolytic effect on red blood cells and also cholesterol binding properties (Harborne, 1973; Okwu, 2004). *Plectranthus barbatus* has been frequently cited as a species used to relieve cold, flu, bronchitis, pneumonia and for general respiratory complication (Lukhoba, 2006; Neuwinger, 2000; Yogarasimhan, 2000), functions attributed to expectorant effects of saponins. Phytochemical screening on the gum of *Spondias mombin* revealed that the plant species is very rich in saponins thus its effectiveness as an expectorant in expelling tapeworms. Other plants that are rich in saponins and have recorded expectorant property include *Alchornea laxifolia, Zingiber officinalis, Saponaria officinalis* and *Glycarrhiza glabra* (Farombi *et al.*, 2003; Martinez, 2000; Farad, 2006). From this is highly likely that the presence of saponins in the plants species of Lamiaceae make them effective as expectorants.

Tannins, phenols and alkaloids have an antiinflamatory effect which is very useful in reducing inflammations in the body thereby reducing incidences of rheumatoid arthritis and also inhibiting diarrhoea. This could results from the binding effect on mucous membrane due to their action on constituent protein. Consequently, diseases such as rheumatism, chronic diarrhea, muscle tension and painful menstruation could be minimized by the administration of herbs such as *Leucas colostachys*, *Ocimum kilimandscharicum*, *Hyptis pectinata, Calamintha nepeta* and *Hoslundia opposita* and *Satureja biflora* (See Table 10). This concurs with Okigbo *et al.*, 2009 who asserts on the importance of tannins, phenols and alkaloids in suppressing the incidences of inflammations in the body. Past studies conducted on some plants of Lamiaceae family including species of *Salvia, Satureja, Thymus* and *Ocimum* have have reported their effectiveness as antiinflamatory agents (Cadirci *et al.*, 2010; Goze *et al.*, 2009; Suarez *et al.*, 2003), a property strongly attributed to the presence of phenolic compounds (Cardici *et al.*, 2010), Tannins and alkaloids (Okwu and Okwu, 2004; Stray, 1998). Other studies conducted in other plant families such as Apocynaceae, Verbanaceae, Rutaceae, Rubiaceae and asteraceae on alkaloids and tannins have shown that the presence of such phytochemicals in the respective plants is very essential for their effectiveness in treating diseases whose remedies are related to antiinflamatory properties (Jeruto *et al.*, 2011; Okwu, 2001).

The antispasmodic property relieves spasm in the smooth muscles, thereby reducing muscle tension in the gut and the myometrium of the uterus and it is exhibited by the alkaloids. This property could be attributed to the ability of *Calamintha nepeta* and *Hoslundia opposita* to provide a remedy in relieving stomach pains and painful menstruation (See Table 10). This conforms to past research studies conducted on some plant of Lamiaceae family such as *Mentha longifolia, Ocimum vulgaris, Ocimum graticimum, Mellisa officinalis, Thymus fallax* among others which revealed that the plants had strong antispasmodic due to the presence of terpenes and alkaloids (Goze *et al*., 2009; Stray, 1998). Some experimental studies show that species of *Satureja* have in the past been used in traditional medicine as antimicrobial, antispasmolytic, antibacterial and analgesic agents due to the presence of alkaloids and essential oils (Hajhashemi *et al*., 2000).

The analgesic property exhibited commonly by the alkaloids is a remedy of pain in that it relieves the body of pains associated with fever; abdominal pain and stomach pain. Some of the plant species with analgesic effect included *Fuerstia africana, Ocimum kilimandscharicum* and *Calamintha nepeta* whose activities are attributed to the presence of alkaloids (See Table 10). Past studies on some plant species of Lamiaceae family that included *Agastache, sinensis, Leucas aspera, Mentha piperita, Nepeta caesarea, Nepeta italica, Origanum onites, Roylea elegans, Salvia haematode, Sideritis mugronensis* and *Thymus vulgaris* have revealed that such plants exhibit a strong analgesic properties due to their rich alkaloid content (Aboutabl, 1996; Aydin, 1998; Aydin, 1999; Forestrieri, 1996; Reddy; 1993). This implies that there is a great possibility of the plants from this study that tested positive for the alkaloids and also were used for relieving pain related illness to exhibit analgesic properties.

The carminative effect is a property exhibited by the terpenoids and the glycosides (Okwu and Josiah, 2006). This property is important in relieving build up of trapped wind in the digestive system and it acts as a remedy to constipation, gastrointestinal infections and relieving distended stomach in children. Some of the plants collected that could probably reveal such a property contained either glycosides or terpenoids or both phytochemical and they included; *Leucas colostachys* Oliv., *Plectranthus barbatus* Andrews, *Becium obovatum* and *Leonotis nepetifolia* (See Table 10). The terpenoids, glycosides, sterols and tannins are phytochemicals with soothing or demulscent effect which initiates the production of mucilage that is useful in the digestive system to ease constipation and relieve distended stomach in children. Important plants that provided such a remedy included *Leucas colostachys* and *Leonotis nepetifolia* which all contained terpenoids, glycosides and tannins except that *Leonotis nepetifolia* lacked glycosides (See Table 10).

The antimicrobial effect of plant species collected may have resulted from the combination of secondary metabolites present which included; alkaloids, flavonoids, saponins, tannins, terpenoids, sterols and glycosides. Such a property is well correlated to the ability of the plant species to fight microorganisms such as bacteria, viruses, protozoa and fungi. It is therefore an essential property in providing a remedy to most of the ailments and complications which include gastrointestinal infections, swellings and wounds, cold and flu, diarrhoea, cough, dysentery, abscess (boils), stomachache, parasitic skin infection, ringworms and gonorrhea. This property could probably be exhibited by all the plant species collected due to their ability to act as a remedy to at least one of the diseases mentioned as being caused by a microbe (See Table 10). The leaves and roots of *Hoslundia opposita* have been reported to be effective in the treatment of gonorrhea, cold, mange and skin diseases all of which are caused by microbes (Katrina, 1992; Okwu and Josiah, 2006). This is also in line with research conducted in the past relating the antimicrobial properties of *Hoslundia opposita* to the presence of vast array of phytochemicals. It has been reported that many plant species exhibit antimicrobial effect, hence, their usage in the treatment of microbial infections. The presence of flavonoids, phenolic compounds, alkaloids, tannins and diterpenoids in these plants make them potent in providing such roles (Farombi *et al.*, 2001; Harborne *et al* 1986; Kozioc and Marcia, 1998; Sofowara, 1993; Thomas, 1989).

Antimicrobial tests conducted on some species from selected genera of Lamiaceae which included *Ajuga, Ballota, Chamaedrys, Lamium, Leucas, Marrubium, Micromeria, Ocimum, Phlomis, Salvia, Satureja and Teucrium* revealed that the herbs had antibacterial activities against several pathogenic bacteria especially the Gram positive bacteria like the Staphylococci hence their usage (Sarac and Ugur, 2007). Essential oils with high concentration of thymol and cervacrol like *Origanum vulgare* (oregano), *Satureja hortensis* (savory) and *Thymus vulgaris* (thyme) usually inhibit Gram positive more than Gram negative pathogenic bacteria (Nevas *et al.*, 2004). Strong *in vitro* evidence indicate that plants of Lamiaceae family have essential oils that act as antibacterial agent against a wide spectrum of bacterial strains including *Lysteria monocytogenes, L. innocua, salmonella typharium, Escherichia coli, Shigellia dysenteria, Bacillus aureus, Staphylococcus aureus* and *Salmonela* *choleraesuis* (Burt, 2004 and Hulin *et al., 1998;* Schmidt *et al.*, 2005). These further support the use of Labiates in Uriri. Besides, the medicinal properties of *Nepeta* and *Fuerstia* species are related to terpenoids and flavonoids, for example, compounds such as 1-8 Cineale, are very common in *Nepeta* and have expectorant, antiseptic and anthelminthic activities (Jamzad, 2001) hence their use. Chemical composition and antimicrobial activity of essential oil of *Satureja biflora* has revealed it effectiveness on both gram positive bacteria (*Staphylococcus aureus* and *Bacillus spp)* and gram negative bacteria (*Salmonela typhi* and *Klebsiella pneumonia*), and pathogenic fungi (Matasyo *et al.*, 2006). The plant was very rich in terpenoids as well as phenolic compounds which made it effective in inhibiting most of the microbes used in the experiment. This can explain that the antimicrobial property exhibited by the plant species from Uriri.

The alkaloids present in some of the plants analysed for instance *Fuerstia africana* and *Ocimum kilimandscharicum* had an effect on the nervous system by relieving nervous tension and providing a remedy in cases of anorexia, insomnia, depression and convulsion (See Table 10). This further supports the plant use in this area. Alkaloids (Stray, 1998), triterpenes, tannins, sterols, carotenoids and polysaccharides from *Croton zehutneri, Acorus calamus and Aeollanthus* have been reported to possess a strong nerve stimulating property that has made the plants effective as sedatives, hypnotic, hypothermic and anticonvulsant and also in homeopathic treatments such as cardiac complications, flatulence and hyperthyroidism (Elisabetsy *et al.*, 1995 Zanoli *et al.*, 1997;). From this it can be deduced that the effectiveness of the Lamiaceae plant species collected in treatment of complications related to the nervous system is due to the presence tannins, alkaloids terpenoids.

Terpenoids were highly likely to exhibit anticarcinogenic and antioxidant property from the plant species considered in this study. The action of such properties was by scavenging on the free radicals to minimize incidences of tumor development that can cause cancer Matkowsi *et al.*, 2008). This property is important in suppressing swollen body parts, warts and abscess and was mainly contributed by *Becium obovatum* (See Table 10). Diterpenoids and their aroma components have shown cancer suppressive activity when tested on human cancer cell lines including glioma, colon cancer, gastric cancer, human liver tumour, pulmonary tumours, breast cancer, leukemia among other (Edris, 2007) hence the plants tested and reported herein. The essential oil of *Ocimum basilicum* (basil), *Syzigium aromaticum* (clove), *Myristica fragrans* (nutmeg), *Origanum vulgare* (oregano) and *Thymus vulgaris* (thyme) have proven free radical scavenging and antioxidant properties in the DPPH radical assay at room temperature (*Tomaino et al.*, 2005). *Thymus serpyllus* showed a free radical scavenging activity close to that of synthetic butylated hydrotoluene (BHT) in a β-carotene/ linoleic acid system (Tepe *et al.*, 2005).

The antioxidant activity was attributed to high content of phenolic thymol and carvacrol (Sokmen *et al.*, 2004). Other Lamiaceae plants that have been reported to possess antioxidant activities include *Salvia cryptantha, Salvia multicaulis, Thymus caespititius Thymus mastichina, Melissa officinalis, Mentha aquatic, Mentha longiflora, and Mentha piperita* whose actions are attributed to the presence of Curcumin acid and ascorbic acid in the *Salvia* genera; 1,8-cineole and linalool in the *Thymus* genera; geranial, citronellal, isomenthone and menthone in genera *Melissa* and 1,8-cineole, menthone and isomenthone in the *Mentha* genera (Tepe *et al., 2004;* Miguel *et al 2004;* Mimica-Dikic *et al., 2004 and* Mimica-Dikic *et al., 2003).* Phenolic compound have show a strong protective factor against cancer and heart diseases because of their antioxidant potency and their ubiquity in a wide range of plant species (Gordana et al., 2007; Sokmen *et al.*, 2004). Some of the compounds include styrypyrones, flavonoids, isoflavonoids, tannins and flavonones. This clearly shows that the presence of terpenoids and flavonoids in the plant species collected for this research could be responsible for their anticarcinogenic or antioxidant properties.

Table 10: Table showing properties exhibited by the phytochemicals tested and their relationship with the diseases treated

Plant species	Diseases and complications	Phytochemicals	Medicinal property
Fuerstia africana, Leucas colostachys, Ocimum kilimandscharicum, Hyptis pectinata,	Cold and flu	Tannins and saponins (Okwu, 2004)	Anticatarrhal effect

Calamintha nepeta and *Hoslundia opposita* Vahl.			
Leucas colostachys, *Ocimum kilimandscharicum*, *Hyptis pectinata*, *Calamintha nepeta*, and *Hoslundia opposita* and *Satureja biflora*	Rheumatism, diarrhoea, muscle tension and painful menstruation	Alkaloids, Phenols and Tannins (Manikandan *et al*., 2006)	Antiinflammatory effect
Leucas colostachys, *Plectranthus barbatus*, *Becium obovatum* and *Leonotis nepetifolia.*	Constipation, gastrointestinal infection and distended stomach in children	Terpenoids and glycosides (Sofowara, 1993)	Carminative effect
Calamintha nepeta and *Hoslundia opposita*	Painful menstruation and stomach pain	Alkaloids (Okigbo *et al*., 2009)	Antispasmodic property
Leucas colostachys and *Leonotis nepetifolia*	Constipation and distended stomach in children	Terpenoids, tannins, sterols and glycosides (Sofowara, 1993)	Soothing/ demulscent effect
Hyptis pectinata and *Hoslundia opposita*	Sore throat and lung congestion	Saponins (Okigbo *et al*., 2009)	Expectorant effect
Fuerstia africana and *Ocimum kilimandscharicum*	Insomnia, depression, convulsion, anorexia and nervous tension,	Alkaloids, terpenoids and tannins (Stray, 1998; Zanoli *et al*., 1997)	Nerve stimulant
Becium obovatum	Swollen body organs and warts on the body	Flavonoids, tannins (Manikandan	Antioxidant/ anticarcinogenic effect

		et al., 2006)	
Fuerstia africana, *Ocimum kilimandscharicum* and *Calamintha nepeta*	Fever, abdominal pain and stomachache	Alkaloids (Okwu 2004; Okigbo *et al.*, 2006)	Analgesic property
Fuerstia africana, *Becium obovatum*, *Leucas colostachys*, *Leonotis nepetifolia*, *Hoslundia opposita*, *Calamintha nepeta*, *Ocimum kilimandscharicum*, *Plectranthus barbatus* Andrews, *Satureja biflora*, and *Hyptis pectinata*	Gatrointestinal infection, swellings and wounds, cold and flu, diarrhoea, cough, dysentery, abscess (boils), stomachache, parasitic skin infection, ringworms and gonorrhea.	Tannins, sterol glycosides, alkaloids, saponins, flavonoids and terpenoids (Manikandan *et al.*, 2006)	Antimicrobia l effect

The presence of iron, calcium, zinc and potassium in the plant is a clear indication of their rich value in the maintenance of proper healthcare and protection of the body against some diseases (Table 11), adopted by WHO, 2002. This is in line with past researches conducted which indicate that trace minerals such as zinc and iron are required for production of various enzymes, hormones and biochemical mediators for regulation of biological processes, energy production, RNA and DNA synthesis and for protecting the body against reactive oxygen free radicals. They are also useful in promoting physical growth, sexual maturation and boosting neuromotor development (Singh, 2004 and Onyenzili *et al.*, 2003). Research on the presence of potassium, sodium, calcium and magnesium has shown that the minerals are responsible for repair of worn out cells, strong bones and teeth, building of red blood cells and for body mechanisms. Also calcium and potassium are responsible for for disease prevention and control, therefore contributes to medicinal influence of plants (Aliyu *et al.*, 2008; WHO, 1996). Zinc, chromium, manganese, copper and selenium have been reported to exhibit strong antioxidant property and their presence in some plant species of Lamiaceae family such as *Lavandula anguistifolia, Mellisa officinalis, Rosmarinus officinalis, Salvia officinalis, Salvia tribolia* and *Thymus zygus* (Trease and Evans, 2002; Gupta and Sharma, 2006) is a clear indication on how important such minerals are important to the human body. Studies have also shown that optimal uptake of sodium, potassium, magnesium, calcium, iron, manganese, copper, zinc and iodine could reduce individual risk factors including these related to cardiovascular diseases (Imelouane *et al.*, 2011). This study further indicates that *Lavandula dentata, rosmarinus, tournofortii, Thymus vulgaris* and *Artemisia herba* have showntheir capability of accumulating the above stated minerals which make them useful for medicinal purposes in addition to their bioactive secondary metabolites.

A combination of these mineral and the phytochemicals present in the herbal extract of the plants tested could be a major contributor to the effectiveness of such herbs in traditional medicine. This is because of the wide range of diseases that they can treat when prepared and administered correctly. When herbs are taken as medicine, they not only provide a remedy to the diseases they are intended to treat but also go a long way to provide the body with the necessary minerals as dietary supplement. This is in line with studies on phytochemical screening and elemental analysis on *Ocimum basilicum, ocimum graticimum, Hymenocardia ulmoides, Vitex ferruginea, Vernonia amygdalina, Hoslundia opposita* among others drawn from different families that have revealed the presence of tannins, flavonids, alkaloids, cardiac glycosides, triterpenoids and steroids together with high concentration of sodium potassium, magnesium, calcium, phosphorous, iron, manganese, selenium and iron (Andzouana and Mombouli, 2011; Daniel *et al.*, 2011; Dike, 2010; Mariita *et al.*, 2010). This indicated that the bioactive compounds and minerals could greatly enhance the curative processes of health using such plant species.

It has also been noted that phytochemicals alongside minerals in plants are valuable are valuable sources of medicine for maintainance of human health and prevention of illnesses such as cancer, heart diseases, diabetis and high blood pressure (Aliyu *et al.*, 2008; Amin *et al.*, 2004). Deficiency of such minerals can lead to serious health problems for instance reduced resistance to infectious diseases, blindness, lethargy, mental retardation, anaemia and even poor body metabolism especially in children (Ekweugu, 2008 and Onyenzili *et al.*, 2003). This is majorly due to the existence of minerals in varying degree concentration in all plant species of Lamiaceae family.

Table 11: Table on importance of minerals

Mineral	Importance
Calcium	Building and maintaining strong bones and teeth, controlling muscle growth, controlling electrical impulses, maintaining blood pressure and aiding blood clotting.
Iron	Maintains healthy immune system, as a component of haemoglobin it helps in transporting respiratory gases and reducing incidences of anaemia.
Zinc	Growth and repair of tissues, an essential component of most enzymes, an important antioxidant, forms connective tissues such as tendons and ligaments and is critical in immune response.
Potassium	Cofactors of enzymes, main base ion of intracellular fluid, maintains electrical potential of the nervous system and aids in the functioning of muscles and nerve tissues.

Adopted from WH: 2002.

5.1.3. Seed and vegetative propagation of Wild Lamiaceae collected

The propagation tests conducted revealed that all the plants of Lameaceae family collected can be propagated through seeds (see Table 8). This explains the observation that all the plants were found in colonies in specific areas they inhabited in the wild since their modes of dispersal were by seeds. This is in line with the successful micropropagation and *in-vitro* clonal propagation of plant species such as *Ocimum kilimandscharucum, Ocimum gratissimum, Satureja biflora, Mentha piperita, Tylophora indica* and *Ocimum basilicum* (Begum, 2000; Dewir *et al.*, 2005; Faisal and Anis, 2003; Gopi *et al.*, 2006; Saha *et al.*, 2010a; Saha *et al.*, 2010b; Saha *et al.*, 2010c). This is also in line with other reports in which the seeds have recorded very high percentages when subjected to germination probity tests from different environments (Jeruto *et al.*, 2010; Saha *et al.*, 2010c; Matasyo *et al.*, 2007; Thagavel *et al.*, 2011;) meaning that the plants can be cultivated to remove pressure from the wild and thus conserved through seeds. It has been shown that aromatic plants that exhibit a germination percentage of less 65% when propagated by seeds have low seed viability hence should be well concerved (FAO, 2006).

The species that were successfully propagated by vegetative means had succulent bark, that when dipped in the soil in the presence of water easily generated the adventitious roots and grew. Most of the plants could not be propagated successfully by vegetative means (See Table 9). This was partly because they had slender stems with very thin barks that could not aid in the formation of adventitious roots. Others failed since there was no treatment with auxins conducted. Previous studies on *Calamintha nepeta, Ocimum basilicum, Fuerstia africana Mentha piperita* and *Zingiber officinale* have revealed that Butyric acid stimulates shoot formation (Begum *et al.*, 2000; Faisal *et al.*, 2007; Saha *et al.*, 2010a; Saha *et al.*, 2010c; Otroshy and Moradi, 2011; Gangare *et al.*, 1998). Elsewhere, it has been reported that the use of auxins promote rooting of stem cutting of plants, meaning that failure to use growth hormones may hinder successful propagation (Gopi *et al.*, 2008; Hartman *et al.*, 2001; Jeruto *et al.*, 2010; Saha *et al.*, 2010b). This especially explains why three plant species *Calamintha nepeta, Ocimum kilimandscharicum* and *Hyptis pectinata* failed to vegetatively propagate, yet elsewhere, they have been reported to propagate successfully under the influence of growth hormones

Most of the practitioner (80%) interviewed asserted that they saw no need of propagating most of these species since they were readily available in the wild, and most of them also pose as noxious weeds and attempts to propagate them would only worsen the situation further by jeopadising agricultural practices. The predicament here is that some of these plants are only restricted to specific areas and despite the fact that they are a threat to agricultural practices must not be justification enough for their eradication because their conservation might be beneficial elsewhere. The species propagated through vegetative means were few and only limited to some specific areas. This is attributed to the fact that most of them are not effectively dispersed through seeds, as their seeds cannot easily stand the dispersal pressures hence, only a few seeds can germinate. All the interviewees attested to this fact and it was evident that in their homesteads, there were quite a number of such plants both in Family Lamiaceae and other families that were propagated. The most commonly found plant was *Plectranthus barbatus* which the practitioners said was important because of its multipurpose medicinal role and hence they saw the need of conserving it alongside other herbs and shrubs.

5.2: CONCLUSION

The study conducted among the traditional medicinal practitioners in Uriri District revealed that of the twelve plant species collected, ten, *Becium obovatum, Calamintha nepeta, Fuerstia africana, Hoslundia opposita, Hyptis pectinata, Leonotis nepetifolia, Leucas calostachys, Ocimum kilimandscharicum, plectranthus barbatus and Satureja biflora* were commonly found and their usage well known. Two plants, *Plectranthus longipes* and *Pycnostachys speciosa* were found to be uncommon among the residents hence; their usage could not be well established. Documentation of all the twelve plants has been done on their medicinal uses

The presence of phytochemicals with various pharmacological and biological properties determines the medicinal value of the plant species of Lamiaceae family as useful sources of drugs in Ethnomedicine. These phytochemicals are the active ingredients present in plants that make them useful in traditional medicine. The study revealed the presence of minerals such as calcium, potassium, zinc and iron in the target plants of Lamiaceae family, and these are essential in maintaining proper body healthcare. Minerals are important as nutritional supplements and are mandatory for the improvement of physical growth and also for the prevention of common day to day infections. This study therefore, provides knowledge of phytochemical and phytotherapeutic potential of plants of Lamiaceae family that may be useful to scientists and pharmaceutical industries among others.

5.2.3: Propagation and conservation

From this study it can be concluded that the target species can be propagated by vegetative means and by seed and that seeds can be adopted as a cheap way of conserving such species. Important medicinal plants can be made available in future on sustainable basis through propagation.

5.3: RECOMMENDATIONS

i. Since very little ethnobotanical information exists on plants such as *Satureja biflora*, *Leucas calostachys*, *Pycnostachys speciosa* and *Plectranthus longipes*, further research is recommended on the study of such plants and their uses. Also thorough and comprehensive phytochemical analysis is recommended to establish the specific bioactive principles present in them that can be used that can be used to reveal their effectiveness. Thorough ethnobotanical surveys should also be conducted in the area on other plant families so as to establish their medicinal importance.

ii. Due to the fears related to consumption of crude extracts of plants, this research recommends that future studies should engage in the extraction of the phytochemicals present in plants and converting them into finished products of utilizable forms such as syrups and tablets.

iii. Knowledge on traditional medicine is mostly among the elderly especially those over age 50. This research study recommends that attempts should be made to engage the younger generation in adopting such knowledge so that they can pick up from the older generation. This can be made possible through the establishment of institutions where such information can be disseminated.

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