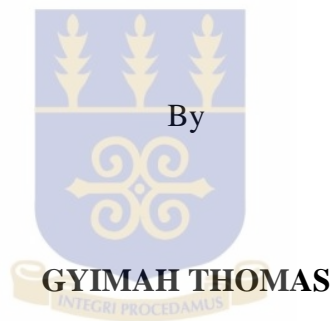


**DNA BARCODING AS A TOOL FOR SUSTAINABLE  
HARVESTING OF MEDICINAL PLANTS USED IN THE  
TREATMENT OF DIARRHOEA AND MALE SEXUAL  
WEAKNESS IN GHANA.**

A Thesis Submitted to the

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**10226236**

In partial fulfilment of the requirement for the award of

**MPHIL ENVIRONMENTAL SCIENCE DEGREE**

**JULY 2013**

## DECLARATION

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## **DEDICATION**

To the glory of God and my family most especially Mrs. Nkrumah Adutwumwaah.



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## ABSTRACT

Due to high poverty levels coupled with high patients to doctor ratio in Ghana, most people especially the urban poor and rural dwellers resort to herbal medicines which are quite cheap for their primary healthcare. Developing measures to maximize the medicinal potentials of indigenous plants will reduce the number of lives lost through diseases. A major setback is that most herbal products sold in public places lack scientific evidence for safety, quality and efficacy. As the safety of herbal medicine depends on the ability to correctly identify the plants used in their preparation, this study sought to use DNA barcoding as (i) an identification tool in the authentication of herbal medicines and (ii) their sustainable management in southern Ghana. Silica-dried leaves samples from thirty seven different medicinal tree species covering 24 families, used in the treatment of diarrhoea and male sexual weakness from three different conservation areas: Ankasa, Bia and Kakum, were sequenced at the *rbcL* gene region. A success sequence rate of 94.59% was achieved for the *rbcL* gene region. To verify the authenticity of the reference DNA database produced from the thirty five medicinal tree sequences in the proper identification of medicinal plants species, silica-dried samples of eleven tree species from farmlands within the Akuapem North district of the Eastern region (specifically around Aburi and its environs) were sequenced in the *rbcL* gene region. All the eleven tree species had their counterparts successfully sequenced in the reference DNA database. A sequence success rate of 90.91% was achieved, and each of the sequences in the verification data perfectly matched their counterparts in the reference database implying that DNA barcoding can be used in the identification of all medicinal plants species to ensure the safety of herbal medicines in Ghana. The study also sought to find out: (i) the medicinal tree species used in the treatment of diarrhoea, male sexual weakness or

both, (ii) how herbalists identify the plants species they use, (iii) their availability, distribution abundance, (iv) the quantity of medicinal plants used in the treatment of diarrhoea and male sexual weakness per month, (v) the parts and sources of the plants species used and (vi) the awareness of forest conservation in southern Ghana. Structured questionnaire and interview guides were used to seek information from a purposive group including herbalists, commercial medicinal plants collectors and sellers in two different markets; Kasoa and Nyanyano, and the communities around the three selected conservation areas; Ankasa, Bia and Kakum. The traditional method of identification was the only available method used for identification of medicinal plant species by both practicing herbalists and commercial plant collectors. Seventy three plants species were found to be effective for the treatment of diarrhoea and male sexual weakness or both. Roots were the commonly used part of the plants in herbal medicine preparation. The parts of plants used, and the rate of collection of some medicinal plants were found to affect the survival of some plants species. There was a hundred percent level of awareness of forest conservations in southern Ghana. DNA barcoding is the best identification tool for medicinal plants species which when accepted for use will completely eliminate misidentification and help in the proper documentation of medicinal plants species. This will inform on the proper conservation and management options for the protection of threatened species as well as the controlled harvesting and trade in vulnerable and important medicinal plants.

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## LIST OF ABBREVIATIONS

CBOL:	Consortium for Barcode of Life
COX 1:	Cytochrome Oxidase 1 gene
CTAB:	Cetyl trimethylammonium bromide
DNA:	Deoxyribonucleic acid
FAO:	Food and Agriculture Organization
GHS:	Ghana Health Service
GPS:	Global Position System
IUCN:	International Union for Conservation of Nature
<i>matK</i> :	Maturase K
MOH:	Ministry of Health
RAPD:	Randomly Amplified Polymorphic Deoxyribonucleic acid
<i>rbcL</i> :	ribulose-bisphosphate carboxylase gene
SPSS:	Statistical Package for Social Scientists
UNAIDS:	United Nations Programme on HIV/AIDS
USA:	United States of America
WHA:	World Health Assembly
WHO:	World Health Organization
WIPO:	World Intellectual Property Organization
WRI:	World Resource Institute
WWF:	World Wide Fund

## **CHAPTER ONE**

### **1.0 INTRODUCTION**

#### **1.1 Background**

The use of herbal medicine for the cure of diseases is known worldwide and has been in existence for centuries. The importance of herbal medicine as a source of primary health care was officially recognized by the World Health Organization (WHO) in the Primary Health Care Declaration of Alma Ata in 1978 (Rukangira, 2001).

The Traditional Medicine Practice Act 595 of Ghana defines herbal medicines as "any finished labelled medicinal products that contain as active ingredients, aerial or underground parts of plants, or other plant materials, or the combination of them whether in crude state or plant preparation" (<http://apps.int/medicinesdocs>).

According to the World Health Organization, more than 4 billion people rely on herbal plant-based systems of medicine for their primary health care and this number is continuously increasing with the increasing population growth and poverty mostly in Africa (Van Andel, 2006). According to Wambebe (2008), a World Health Organization survey in Ghana, Nigeria, Mali and Zambia in 1998, revealed that sixty percent of children with symptoms associated with malaria infection were treated with herbal medicine and an estimated eighty (80) percent (%) of the populations of most developing countries depend on herbal medicine for their primary health care (Van Andel, 2006; WHO, 2002). Furthermore, about two thirds of AIDS patients in developing countries use herbal medicines (UNAIDS, 2002).

The demand for medicinal plants is gradually increasing in both developing and developed countries, and a large market exist worldwide for such products, perhaps due to their ability to cure diseases with comparatively less side effects (Van Anandel, 2006 and Njume & Goduka, 2012). The huge demand for herbal products has put much pressure on some common plants species leading to potential cases of over exploitation and biodiversity losses.

Furthermore, when a plant in high demand becomes scarce in an area, some commercial collectors in their quest to meet the demand of their customers may cut down a whole tree to harvest the bark from the stem and branches as well, while others collect their congener species which at times result in undesirable consequences (Guo *et al.*, 2011). At times, a 'look-alike' species may be poisonous which can result in undesirable consequences such as death (Abbiw, 1990), or may compromise the quality and efficacy of the resulting herbal medicine.

The possibility of Deoxyribonucleic acid (DNA) barcoding to identify the correct species used in herbal medicine preparation can prevent the harvest and trade in red listed species whether internationally or locally, hence ensuring their sustainability and also remove all forms of mis-identifications, whether deliberately or mistakenly when accepted for use in the herbal medicine industry.

In Ghana, many diseases are treated with herbal medicines, most popular ailments amongst them include diarrhoea and dysentery, male sexual disorders, male and female infertility, piles and constipation, malaria, typhoid fever, sexually transmitted infections and skin rashes. This research is focused on barcoding some trees used in the preparation of herbal medicine for the treatment of diarrhoea and male sexual weakness for proper identification and sustainable harvesting of such plant species.

Diarrhoea is the frequent passage of unformed, loose or watery stools, more than three times in a day (Palombo, 2006 and Mandomando *et al.*, 2007). It is a common cause of death in developing countries and the second most common cause of infant mortality worldwide (O’Ryan *et al.*, 2005 and Bryce *et al.*, 2005). Over 800 million cases of diarrhoea occur every year in developing countries, causing up to 4.5 million deaths (Esrey *et al.*, 1990). According to the Ministry of Health (), Ghana has a doctor to patient ratio of about 1:10,700 which is unfairly distributed, with rural areas having unfair share of health post as well as health workers in the country (MOH, 2005). The use of plant materials which are readily available with little or no cost in the treatment of such diseases cannot be compromised (Bisi-Johnson *et al.*, 2009 and Njume & Goduka, 2012).

The second disease of concern to this research, male sexual weakness is the persistent inability to attain and maintain an erection sufficient to permit satisfactory sexual performance (Wespes *et al.*, 2009; Yakubu *et al.*, 2007 and Hatzimouratidis *et al.*, 2010). According to (Wespes *et al.*, 2009), sexual weakness is a benign disorder that affects the physical and psycho-social health with a significant impact on the quality of life of sufferers, their partners and families.

In order to achieve the main aim of marriage which is procreation and sexual fulfilment of both partners (Yakubu *et al.*, 2007), there is the need to result to aphrodisiac which is defined as any food or drug that arouses the sexual instinct, induces venereal desire and increases pleasure and performance (Neelesh *et al.*, 2011), in cases where the male sex organ and factors relating to erection does not function normally (Guay *et al.*, 2003).

Presently, there are many potent herbal aphrodisiacs available with little or very little side effects (Indurwade *et al.*, 2005). Recently, many herbal industries have emerged in Ghana with many different kinds of herbal preparations as aphrodisiacs to solve different forms of sexual disorders. The popularity of some of these herbal aphrodisiacs is due to heavy media advertisement and thus patronage is high even among the youth. Hence the need to ensure the safety of these herbal aphrodisiacs.

The vision of WHO's Department of Essential Medicines and Pharmaceutical Policies is that “people everywhere have access to the essential medicines they need. The medicines should be available within the context of functioning health systems at all times in adequate amounts, in the appropriate dosage forms, with assured quality, safety, efficacy, adequate information, and at a price the individual and the community can afford” (<http://www.who.int/medicines/en/>). Most often, accurate information on the safety, efficacy, and quality of herbal medicines are virtually not available to patients and physicians (O’Hara *et al.*, 1998; Wambebe, 2008). Hence there is the need to research into these areas of herbal medicine from selection of ingredients to administration of the final product, in order not to endanger the lives of the majority of people that depend on them.

The safety of herbal medicine depends largely on the ability to correctly identify the species of plants used in the preparation (Abbiw, 1990), and DNA barcoding is one of the ways of ensuring this. DNA barcoding is a diagnostic technique that uses short DNA sequence(s) for identification of species, compared to taxonomic identification which uses morphological features of such plants which at times are very difficult to differentiate between/among identical species, thereby resulting in mis-identification ([www.barcodinglife.org](http://www.barcodinglife.org)). The basic rationale of using the short DNA sequence called

(universal molecular yardstick), is that “it differentiates among species of a taxon under the assumption that the sequences chosen have relatively lower ‘within-taxon’ variation than that ‘between-taxa’ which can be used in distinguishing between closely related species ([www.barcodinglife.org](http://www.barcodinglife.org)). DNA barcoding, according to Hebert *et al.* (2003) is a tool that obtains species specific DNA signature based on the premise that sequence diversity within small stretches of the organism’s genome can provide a ‘biological barcode’ to enable identification of any organism at the species level.

## **1.2 Justification**

The WHO has proposed herbal medicines to be one of the means to achieve total healthcare coverage of the world (WHO, 2002). With a medical doctor to population ratio of about 1:40000 and an indigenous healer to population ratio of about 1:500 in Sub-Saharan Africa, the overflow of people to the indigenous health systems is almost always inevitable (Njume & Goduka, 2012). Developing measures to maximize the medicinal potentials of indigenous plants will reduce the number of lives lost (Njume & Goduka, 2012). Despite all these positive attributes, most herbal products sold in public places lack scientific evidence for safety, quality and efficacy, hence the need to research into these areas (Wambebe, 2008).

The safety of medicinal plants largely depends on the ability to eliminate all forms of mis-identification. Abbiw (1990) documented that a house wife died on admission at the University of Ghana Hospital-Legon in 1984 after mistakenly taking an herbal concoction of *Erythrophleom suaveolens* instead of *Khaya senegalensis*, because the two plants are morphologically similar. Van Andel *et al.*, (2012) have argued that, due

to the difficulties involved in identifying market samples of medicinal plant parts, molecular methods such as DNA barcoding should be employed to prevent mis-identification. Unlike taxonomic identification that has about six percent errors, DNA barcoding, is a perfect tool of identification that can be used by all including non-taxonomists and when employed can reduce or totally eliminate all mis-identification (Dexter *et al.*, 2010 and Marshall, 2005).

Diarrhoea in its various forms is one of the major causes of death in Ghana and is the third most common disease found in Out Patients Departments of the various hospitals in Ghana (GHS, 2004). Some forms are so deadly that in the absence of a health post, the victim is likely to lose his/her life in a couple of days. It is therefore imperative to identify and authenticate plants with the potential of curing such diseases to be used in places with no health post or even where there are, since they are seen as the last resort to healthcare due to poverty (Njume & Goduka, 2012).

The increasing number of aphrodisiacs on the Ghanaian market today requires proper research into their safety most especially in order not to endanger the lives of the youths, who in their quest to enjoy their sex life resort to them (Althof, 2002 and Hatzimouratidis *et al.*, 2010).

Due to high rate of deforestation, rampant bushfires, climate change, commercialization and high patronage in medicinal plants species, there is the fear of losing some important plant species as in the case of South Africa, where to date thirty nine (39) medicinal plant species have been exploited to the extent that they are now endangered and one species is already extinct (Van Andel, 2006). To prevent this from happening in Ghana, it is important to provide a mechanism for monitoring the rate of harvesting medicinal plants to ensure sustainable harvesting, so as to provide

policy makers with science-based evidence for decision-making that will lead to the management and conservation of plants.

### **1.3 Research questions**

The study seeks to answer the following questions:

- Which plants are commonly used for the treatment of diarrhoea and male sexual weakness?
- Which part of the plant species is used in the preparation of the herbal concoction?
- What quantity of each of these plants species is harvested per month?
- What can be done to ensure sustainability of medicinal plants species in Ghana?
- Can DNA barcoding be used to identify the plants used in herbal preparations?

### **1.4 Main Objective**

To use DNA Barcoding (i) as an identification tool in the authentication of herbal medicines and (ii) sustainable management of medicinal plants species in southern Ghana.

### **1.5 Specific objectives**

- To find out which medicinal plant species is commonly harvested for the treatment of diarrhoea and/or male sexual weakness in southern Ghana.

- To find out the part of the plants species used in the preparation of the herbal concoction and if this can affect the survival of the plants involved.
- To find out the quantity of each medicinal plant species harvested and if this is sustainable.
- To provide 37 DNA barcodes sequence of 37 medicinal plants species using ribulose-bisphosphate carboxylase gene (*rbcL*) region.

## CHAPTER TWO

### 2.0 LITERATURE REVIEW

#### 2.1 Importance of Herbal Medicines

Indigenous health systems and the use of herbal plants have been recognized as pivotal in primary health care and a system to consider in achieving one of the targets of the Millennium Development Goals on health (Bisi-Johnson *et al.*, 2009). According to the World Health Organization (WHO, 1978), herbal medicine has been described as one of the means to achieve total health care coverage of the world's population, yet this form of health care system has long been relegated to a marginal place (Bisi-Johnson *et al.*, 2009). Recently, WHO commitment to the millennium declaration has been reaffirmed by its governing bodies (WHO, 2002a; 2002b) and Ministers of Health of the WHO African Region (2007) have also made a declaration to recognize the role of herbal medicine in primary health care. Many countries including Ghana have since worked towards the incorporation of herbal medicine into their main stream of health care. The many herbal hospitals in Ghana and some district hospitals having herbal clinics or departments are all efforts toward the incorporation of herbal medicine into the main health care delivery system of the country.

Populations of developing countries continue to depend heavily on herbal medicines as their primary source of healthcare (Cunningham, 1993). The many ethnobotanical studies that have been carried out across Africa confirm that, the main constituents of Traditional African Medicine are the native plants (Adjanohoun *et al.*, 1988). It is therefore imperative to protect and maintain these plant species.

## 2.2 Regulations on Herbal Medicine

The World Health Organization (WHO) recognized the need ‘to facilitate integration of herbal medicine into the national health care system by assisting Member States to develop their own national policies on herbal medicine’ perhaps due to the increasing population and the need to achieve total healthcare coverage of the world (WHO, 2002; IUCN & WWF, 1993).

Legislative controls in respect of medicinal plants have evolved around an unstructured control model due to the different ways in which different countries define herbal medicine. Hence different countries have adopted various approaches to licensing, dispensing, manufacturing and trading herbal medicines to ensure the safety, quality and efficacy of herbal medicine (WHO 2002).

A 2003 resolution at the World Health Assembly (WHA) urged WHO member states to: ‘take measures to protect, preserve and to improve if necessary traditional medical knowledge and medicinal plant resources for sustainable development of traditional medicine. Depending on the circumstances in each country, such measures may include, where appropriate, the intellectual property rights of herbal practitioners over herbal medicine formulas and texts, as provided for under national legislation consistent with international obligations, and the engagement of World Intellectual Property Organization (WIPO) in the development of national *sui generis* protection systems’ (WHA, 2003).

To achieve sustainable harvesting of medicinal plants which are the basic ingredients for herbal medicine, proper identification is key. Ecologists need to assess the availability of such plant species in order to advice on them. Mis-identification can therefore lead to wrong judgement which can lead to the conservation of an abundant

number of species at the expense of a threatened one. Furthermore, herbal medicines must be safe. Mis-identification can cause serious health problems, worsen the plight or at worst even cause the death of those who depend on them. DNA barcoding must therefore be employed in the identification of all species in order to prevent mis-identification and its consequences.

A 2001 global survey by the WHO to document national policies on Traditional Medicine and regulation of herbal medicines of its member states revealed that, a few member states had a policy on traditional medicine (WHO, 2005). The following challenges were identified to be facing countries about herbal medicine which needed to be addressed in their policies; regulatory status, assessment of safety and efficacy, quality control, safety monitoring and lack of knowledge about traditional medicine within national drug regulatory authorities (WHO, 2005).

The national policies in traditional medicine define the role of herbal medicine in respect to national health care programmes and also ensure that, the necessary regulatory and legal mechanisms are created for promoting and maintaining good practices, assuring authenticity, safety and efficacy of herbal medicines and providing equitable access to health care resources and information about those resources (WHO, 2001).

### **2.3 Traditional Medicine Policy in Ghana**

Ghanaians are allowed to practice traditional medicine but are bound by the restrictions contained in the Poisons Order (1952) and are limited by the use of the substances listed therein. Furthermore the Medical and Dental Decree of 1972 and the

Nurses and Midwives Decree of 1972 permits indigenous inhabitants of Ghana to practice herbal medicine, provided they do not practice life-endangering procedures (WHO, 2001).

These decrees have seen many people moving into the herbal medicine industry, with the challenge of how to ensure safety of the medicine produced. Many herbal preparations on the Ghanaian market today lack safety and quality assurance certificate from the Food and Drug Authority and Ghana Standard Authority; the institutions to oversee and certify all such products in Ghana. Furthermore, there are many quack herbalists in the industry since the Ghana Association of Traditional Medicine has not been able to include all the practitioners in the field due to the informal nature of the job, the huge number and the fact that they are widely spread throughout the nation.

Mis-identification is another huge challenge in the industry, due mainly to the fact that, the same plants can have different local names and vice-versa. In Africa for example, the same plant can have different local names, like wise different plants can have the same local name (Sofowora, 2008), a situation which can result in the collection of wrong plant species. Apart from the fact that mis-identification poses threat to the users of the herbal medicine products, it also affects the sustainable harvesting of important medicinal plants species. Experienced herbalists whose attendants collect wrong plants species can surely identify and dispose of them; which affect the availability and survival of the plants involved. Ecologists make wrong decisions about a plant species due to mis-identification which in turn affects conservation and management practices of such plant species. DNA barcoding due to its ability to eliminate mis-identification is the best identification tool for both

ecologists and herbalists in their quest to develop sustainable herbal medicine industry.

## **2.4 Challenges of Herbal Medicine Industry in Ghana**

### **2.4.1 Biodiversity Loss**

Biodiversity is the variety of plants, animals, and other organisms, as well as the habitats in which they are found, and the ways that these living things interact with each other and with the environment (Rapid Assessment Program, 2007 and Dudley, 2002).

Biodiversity loss is one of the most serious environmental problems facing the world today and also a major threat, not only to the herbal medicine industry but also to the modern pharmaceutical industry. At least twenty five percent (25%) of all modern drugs originally come from rainforests (Kong *et al.*, 2003). About twenty five percent of all prescription drugs in the United States of America are derived from plants (Kumar & Kumar, 2012 and Kong *et al.*, 2003).

According to Young (1999), natural substances have long served as sources of therapeutic drugs and many substances have been derived from herbal medicine, e.g., Digitalis (from Foxglove), Ergotamine (from contaminated rye), quinine (from Cinchona), among others. More recently, many antibiotics, antifungal and anticancer agents have been derived from bacteria, fungi, plants and animal sources. These natural products remain as important sources of new drugs as statistics show that between 1983 and 1994, about five percent (5%) of newly approved drugs were natural products and about thirty five percent (35%) were either compounds derived

directly from natural products or synthetics based on natural products (Cragg *et al.*, 1997).

Natural habitats in the moist tropical regions, which harbour the majority of the world's flora and fauna, are being lost at an alarming rate. It is estimated that in tropical rain forests alone, the rate of loss of entire species is now a minimum of about twenty seven thousand (27,000) per year, or three per hour, and the rate is increasing (Wilson, 1992). Ghana is listed among some West African countries with the highest rate of deforestation of between 1.3% and 1.5% occurring annually (FAO, 1997). This is one important indicator of habitat conversion and may have implications for biodiversity loss in the country (Barbier *et al.*, 1995 and Reid, 1992). With this alarming rate of forest loss, many of our biodiversity are under threat of extinction.

Given the rate of forest loss in Ghana, 1.3% from 1981–1985 (Repetto, 1988), 1.5% from 1980–1990 and 1.3% from 1990–1995 (FAO, 1997), the biodiversity of the forest in Ghana is at high risk of extinction (Benhin & Barbier, 2004). It must be noted that conserving forest biodiversity by valuing and harnessing it as medicine is consistent with poverty reduction and local public health improvement effort (Bodeker, 2005). To ensure a sustainable herbal medicine industry, biodiversity which is an irreplaceable resource and the only source of raw materials for the industry must be available, and also the products must be safe and effective. Measures must therefore be put in place to check the rate at which Ghana's biodiversity is being lost. In an interview with some farmers, herbalists and forest guards in Kakum, the major causes of biodiversity loss were attributed to bad farming practices, wildfires, illegal logging and over exploitation and bad harvesting practices of medicinal plants.

Measures must therefore be put in place to eliminate these factors in order to maintain Ghana's biodiversity.

#### **2.4.2 Effect of herbal medicine collection on biodiversity**

According to Verma and Singh (2008), the basic uses of plants in medicine will continue in the future, as a source of therapeutic agents, and as raw material base for the extraction of semi-synthetic chemical compounds such as cosmetics, perfumes and food industries. However, over exploitation and unsustainable harvesting of medicinal plants is a major threat to many West African medicinal plants species. This is due to factors such as high population growth, lack of access to western medicine, poverty, and growing markets in herbal products (Hamilton, 2004 and Boon & Ahenkan, 2008).

Ramakrishnappa (2002), pointed out that the threat posed by over exploitation of medicinal plants has serious implications on the survival of several plant species, many of which are faced with extinction. Collection of plants for herbal medical use in Africa especially, is extremely detrimental to certain species. For example an interview with some staffs of Kasapreko Company Limited revealed that, in Ghana *Khaya senegalenses* has been exploited over the years that, they are now imported from neighbouring countries. The unsustainable methods of harvesting medicinal plants such as root excavation, back striping and felling of trees, call for concern since such methods have been reported as threat to the survival of most plant species (Akerele *et al.*, 1991 and Cunningham, 2001).

In most countries including Ghana, the prominent mode of obtaining medicinal plants on both large and small scale is wild harvesting (Lange, 1998). Though many medicinal plants are commonly available in the wild and can be freely harvested, the sale of large quantities of plant materials results in uncontrolled collection from the wild which can lead to destruction and ultimate death and probable loss of many plants species, especially the most common valuable and endemic species that have restricted geographical distributions. For example, medicinal plants like *Curcuma caesia* and, *Rauvolfia serpentina* were reported to occur abundantly in central India before 1980 (IUCN, 1994). They are now critically endangered due to their growing economic importance and rampant harvesting (Prasad & Patnaik, 1998). Two African medicinal plants, *Harpagophytum procumbens* and *Harpagophytum zeyheri*, are now threatened with extinction due to commercial extraction for their medicinal value (Van Andel, 2006).

According to Van Andel (2006), the gathering of medicinal plants in the past was restricted to only the healers and their apprentices. However due to rapid urbanization and demand by both small and big cities large quantities of plant materials are collected by commercial harvesters and sold through increasing numbers of informal sellers (mainly women) to urban traders and herbalists (Van Andel *et al.*, 2012). This shift from subsistence use to commercial trade has led to increased pressure on wild medicinal plant populations and that local herbalists are worried that their source of raw materials might become so scarce that they would have to buy from merchants in the city (Van Andel, 2006).

It came to light from interview with prominent stakeholders of the herbal industries that, most of the big herbal industries including “Kasapreko” Company limited,

import *Khaya senegalensis*, which is a basic ingredient in most of their herbal products like bitters and other alcoholic beverages from the Ivory Coast. Some other companies including “Madam Catherine” have collapsed due to the scarcity of *Kaya senegalensis* that compelled the collectors to opt for *Khaya ivorensis* .

#### **2.4.3 Emerging Markets in herbal Medicine**

The big growing markets that exist worldwide for medicinal plants have increased the pressure on collection of some medicinal plant species. According to Verma & Singh (2008), herbal medicines are in high demand and their popularity is increasing consistently. Agarwal, (2005) partly attributes this to the toxicity and side effects of allopathic or orthodox medicines. The international trade for medicinal plants according to the Export Import Bank is worth about US\$ 62 billion with an annual growth of about seven percent (7%) worldwide (Verma & Singh, 2008).

A survey of Ghana’s herbal market by Van Andel *et al.*, (2012) revealed that, an estimated nine hundred and fifty one (951) tons of crude herbal medicine were sold at Ghana’s herbal markets in 2010, worth about US\$ 7.8 million. Further emphasis was placed on the fact that scanty revenue data exist on the tons of *Griffonia simplicifolia*, *Voacanga africana* seeds and *Fadogia agrestis* bark that are exported annually Van Andel *et al.*, (2012).

The increasing trade in herbal medicine in West Africa has been attributed to high levels of unemployment, rapid urbanization and low levels of formal education (Van Andel, 2006). The significant socio-economic importance of this trade is the millions of people, especially women, generating an income by plant collection and marketing

(Cunningham, 2001; Sunderland & Ndoye, 2004 and Williams, 2007). The concern of this trade is the dangers it poses to the existence of wild population of popular West African medicinal plants (Cunningham, 1993; Blay, 2004; Ndam & Marcelin, 2004). The ripple effect is that people will be ripped off their cheap and available source of primary health care owing to the unavailability of medicinal plants (; Hamilton, 2004).

## **2.5 Ways of ensuring sustainability of the herbal medicine industry**

### **2.5.1 Cultivation of medicinal plants and its challenges as ingredients for herbal medicine**

The present rate of decline of some medicinal plants species is alarming and calls for immediate attention, not only for conservation but also for propagation of some useful plants in backyard gardens and in large plantations as well (Birdi *et al.*, 2008 and Ramakrishnappa, 2002). In view of this Rukangira (2001) suggested that *ex-situ* cultivation of the desired medicinal and aromatic plants would be necessary so as to obtain raw materials grown under the same conditions of climate and ecology.

Although cultivation of medicinal plants can be a perfect option of ensuring continuous supply, concerns have been raised about the efficacy of cultivated plants as raw materials for herbal medicine Birdi *et al.*, 2008. Medicinal properties in plants are mainly due to the combination of secondary products generally produced for defence against predators, pathogens, competitors or for protection and adaptation to environmental stress related to changes in soil conditions, temperature, water status, light levels, UV exposure, and mineral nutrients in their natural habitats (Birdi *et al.*,

2008 and Wink, 1999). These secondary metabolites may not be expressed in optimum quantities when cultivated under optimum conditions to obtain better vegetative yields. For example, Robbins (1998) reported that wild ginseng roots are five to ten times more valuable than cultivated ginseng roots. According to Ellenberger (1998), biochemical analyses of cultivated *Arnica montana* showed differences in the biochemical properties compared to that of the wild. It will therefore be necessary to produce synthetic compounds of the active ingredients of the medicinal plant species. This will not only ensure sustainable harvest, but will also enhance safety, efficacy and large production of such drugs to meet the high demand of the world.

### **2.5.2 Factors to consider in collecting herbal medicine**

Factors such as topography, geology, soil, climate and vegetation which can affect the medicinal properties of a medicinal plant must be considered when selecting plant products for herbal drug preparations in order to achieve safe and effective herbal medicine (Sofowora, 2008). This has long been demonstrated by some herbalists even though they may not be aware of the theory behind it, as at times they request for parts of specific plants in specific location either on a hill, mountain or valley to treat specific diseases. As an example, *Chromolaena odorata* growing in Cote d'Ivoire produces leaf essential oil rich in geijerene and pregeijerene which have antibacterial activity against Gram negative organisms while the same plant growing in Nigeria has none of these two terpenes and has mainly antibacterial activity against Gram positive organisms (Safowora, 2008).

It is important therefore to find the genetic variations within the same plant species growing in the wild at different places and altitude. This will inform on where to pick herbs to treat specific diseases most especially those plants with more than one medicinal uses. It will also give insight into how best medicinal plants can be cultivated in order to obtain the desired properties.

### **2.5.3 Ensuring sustainability of herbal medicine industry**

To practically sustain the herbal medicine industry, Bisi-Johnson *et al.* (2009) wrote; “it is becoming increasingly urgent to document the medicinal use of African plants because of the rapid loss of the natural habitat for some of these plants due to anthropogenic activities”. The migration factor especially among the youths to urban areas as well as the demise of most of the local practitioners with their wealth of knowledge are sources of threat to the future of most of the important cultures including knowledge on the use of plant species (Akerele *et al.*, 1991; Bodeker, 1994; Schlage *et al.*, 1999).

Countries can sustain their biodiversity in medicinal plants and their herbal industries by working hand in hand with industries towards monitoring and maintaining controlled non-destructive harvesting with habitat management and authenticating all herbs used in herbal preparation through DNA barcoding (Birdi *et al.*, 2008).

## **2.6 DNA- Barcoding and authentication of plant identities**

DNA barcoding is a diagnostic technique that uses short DNA sequence(s) for identification of species ([www.barcodinglife.org](http://www.barcodinglife.org)). It is a tool that obtains species-specific DNA signature based on the simple premise that sequence diversity within small stretches of the organism's genome can provide a 'biological barcode' to enable identification of any organism at the species level (Hebert *et al.*, 2003 and Savolainen *et al.*, 2005). As a result, scientists believe that DNA barcoding will provide a 'universal key' that will allow identification of a species by running unknown DNA sequences through a DNA barcode database ([www.barcodinglife.org](http://www.barcodinglife.org)).

DNA barcoding is a taxonomic tool which provides for use by both taxonomists and non taxonomists. It enables species identification including any life forms or stages, facilitates species discoveries based on cluster analyses of gene sequences, promotes development of handheld DNA sequencing technology that can be applied in the field of biodiversity inventories, provides insight into the diversity of life among others (Savolainen *et al.*, 2005 and Chase *et al.*, 2005).

## **2.7 DNA barcoding and morphological identification**

One of the desires of taxonomy is to identify biological specimens or fragments of biological origin, but this has been very difficult due to a shortage of natural history specialists. According to Chase *et al.*, (2005), there can be only one expert of taxonomists for a particular group of species worldwide, and this makes it very difficult, for a smooth identification of such species. Furthermore, environmental

samples of minute organisms from soil, water and other sources may have very few morphological traits making it very difficult for such species to be identified.

Dexter *et al.*, (2010) reported a total error rate of about 6.8 to 7.6% in morphological identification of all individual species. However, incorrect splitting of rare morphological variants of common species and incorrect lumping of geographically segregated, morphologically similar species are the two most common morphological identification errors committed by taxonomists (Dexter *et al.*, 2010). These forms of mis-identifications have significant and measurable impact on ecological analyses.

Plant taxonomists use both reproductive characters, i.e., the morphology of flowers, fruits, their associated structures and vegetative characters i.e., the morphology of leaves, twigs, bark and wood. Practically, only few reproductive plants are seen on the field, leaving taxonomists with no choice but to rely on only vegetative characters for identification which is at times very difficult to differentiate between congeners (Dexter *et al.*, 2010).

Unlike morphological identification, DNA barcoding uses short DNA sequence(s) for species identification ([www.barcodinglife.org](http://www.barcodinglife.org)). It obtains species specific DNA signature, based on the simple premise that sequence diversity within small stretches of an organism's genome can provide a 'biological barcode' to enable identification of any organism at the species level (Hebert *et al.*, 2003 and Marshall, 2005), and on the assumption that the sequences chosen have relatively lower 'within-taxon' variation than that 'between-taxa' ([www.barcodinglife.org](http://www.barcodinglife.org)).

To enhance accuracy in tropical tree identification, Dexter *et al.*, (2010) argued that every tree has distinct DNA which is specific to the species alone, which enables molecular approaches to be incorporated into ecological surveys. This will hasten the

slow pace and reduce the stress involved in morphological identification and also expedite species identification and discovery.

According to the Scientific Correspondence (2007), DNA barcoding is a spectacular and glamorous branch of biology that has rejuvenated taxonomy as a fashionable science and also brought back the charm of 18th century biology with a promise of cheaper and less painstaking path for identification and discovery of new species on the 'PCR-desk' by 'lay-taxonomists' who need not necessarily be 'naturalists'. DNA barcoding has many advantages over morphological identification and promises a quicker, painless and cheaper identification of all species including museum and herbarium samples and discovery of all species and organisms on earth.

## **2.8 Some applications of DNA barcoding**

DNA barcoding can be applied in various important ways including; forensic science, tracing of illegal trade in organisms, bio security, pharmaceutical and herbal industries among others.

DNA barcoding can be used to identify strains of marijuana and other illegal plant species (Coyle *et al.*, 2001). This is known as forensic botany; and its success stems from the fact that plant remains can be found almost everywhere and offer multiple sources of evidence, both macroscopic and microscopic, such as pieces of wood, (even as charcoal), seeds, fruits, leaves, twigs, plant hairs, microscopic air-borne pollen and spores, or in aquatic environments, algal cells; when these plant remains are correctly identified whether by their morphology where possible or by DNA which is appropriate. Forensic Botany can help in gathering other useful information

such as the season or geographical location in which a crime took place, whether a body has been moved following a murder; if a body is buried, how long it has been buried, and whether a suspect was present at the crime scene (Szibor *et al.*, 1998).

The ability to separate and identify individual elements in botanical mixtures using DNA barcoding has strengthened the possibility of its uses to prove cases in law courts based on forensic botany (<http://www.promega.com>). The potential of DNA barcoding to identify a plant species from minute leaf fragments and pollen grains have increased its acceptance and use by forensic botanists because often botanical trace evidence does not contain the necessary morphological features that would allow one to identify a plant at the genus or species level (Coyle *et al.*, 2001).

According to Coyle *et al.*, (2001), the first criminal case that used plant DNA typing to gain legal acceptance was a homicide that occurred in 1992 in Arizona's Maricopa County, where a few seed pods from a Palo Verde tree were linked to the source using Randomly Amplified Polymorphic (RAPD) analyses. It was later proven that the seed pods were from the same tree under which the body of the dead woman was found. The same method was used in Italy to settle a lawsuit involving the unauthorized commercialization of strawberry plant (Congiu *et al.*, 2000).

The success of amplifying DNA samples of museum specimen makes it a possible tool for tracing illegal trade in bush meat (<http://spectrum.library.concordia.ca>). Without DNA barcoding, it will be very difficult to enforce wildlife laws such as the Convention on the International Trade of Endangered Species, the United States Endangered Species Act among others; since all species reaching the markets are very difficult to identify morphologically (<http://www.colorado.edu>). Most animal species except for pets get to the market processed, either the furs or feathers are removed and

dried or frozen making morphological identification very difficult or almost impossible, but with DNA barcoding of processed and prepared meats, hides and other goods can be identified and that the offenders can be prosecuted.

DNA barcoding also has the potential of tackling illegal logging. According to Nielsen & Kjaer (2008), DNA obtained from dried wood samples can be amplified and thus with available DNA data of timber species, dried wood can be traced from the source even to the consumer. This is one bright opportunity to solve the ever challenging issue of illegal logging in the forestry industry. DNA barcoding offer tremendous promise for use in timber tracking at all levels; regional scale, concession and for tracking individual logs or wood products and can also be easily automated offering quick, cheap and high-volume processing (Lowe & Cross, 2011).

DNA barcoding has the potential to be used in maintaining and ensuring the security of a nation's biological diversity. Bio security encompasses protecting against any risk through 'biological harm', not least being the economic impact from the spread of pest insects (Armstrong & Ball, 2005). The ability to control invasive species depends much on the proper identification of the species involved and how fast this can be done. Invasive species are mostly alien species, experts are most often not available, and above all the eggs and larvae of these species are first found making morphological identification very difficult (<http://www.genematrix.net>). Most often these stages lack features that enable their morphological identification, hence they have to be reared till they grow to adult stage before identification. These slow processes give alien species ample time to establish themselves in their new environment. DNA barcoding is therefore an appropriate tool for identifying alien species due to its ability to identify the organism at any stage of its life cycle.

According to (Hebert *et al.*, 2003), the emergence of DNA barcoding as a means of species identification has the potential to address all the shortcomings in morphological and other molecular forms of identification.

In a verification of forty nine (49) insect species identified around the borders of New Zealand as important invasive pests by DNA barcoding proved with about a hundred percent certainty that, an error of eight percent existed in the previous identification. Three species out of the forty nine were completely new species that had not been thought of (Armstrong & Ball, 2005). DNA barcoding is therefore an important tool that can be relied upon in dealing with alien species.

DNA barcoding has been applied successfully in the herbal industry to unveil the activities of some quacks in the industry. The decline of *Scutellaria baicalensis*, a botanical source of a well-known herbal Chinese medicine through overexploitation has forced most herbalists to use the dried roots of its congeners, *Scutellaria amoena*, *Scutellaria rehderiana*, and *Scutellaria Viscidula* (Guo *et al.*, 2011). This adulteration of medicine if not checked can cause a series of inconsistent effects, quality control problems and serious health problems (Guo *et al.*, 2011). DNA barcoding was employed to give a proper identification of the plant species and above all has potential of identifying the plant species used for a herbal preparation from already prepared herbal medicine (Guo *et al.*, 2011).

DNA barcoding is a novel tool to authenticate herbal medicines and also remove quacks from the herbal industry. The application of DNA barcoding technology was able to reveal that about twenty five percent of cohosh black pills, a herbal medicine prepared from *Actaea racemosa* to correct the symptoms associated with menopause in USA were fake (<http://www.scientificamerica.com>). Due to the high demand for

this drug and the scarcity of *Actaea racemosa*, similar plants species were used which made the drug toxic, causing several damages to the liver of those who took them.

From an unverified source, a very popular blood tonic here in Ghana-viz: Madam Catherine faded out due to a similar incidence of mis-identification. According to the source, scarcity of the basic ingredient *Khaya senegalensis* forced the plant collectors of the company to collect its congener, *Khaya ivorenses* and the results was haemolysis. From this same source, Kasapreko Company Limited, a well known Ghanaian liquor industry now imports *Khaya senegalensis*, a basic ingredient of the company, from Ivory Coast due to the scarcity of the plant species in Ghana. It is therefore very necessary that DNA barcoding be accepted as a verification tool for all medicinal plant species to prevent the unfortunate happening of misidentification which can result to the death of people (Abbiw, 1990).

## **2.9 Challenges of DNA Barcoding**

The success of DNA barcoding is based on the assumption that the short DNA sequence chosen has relatively lower ‘within-taxon’ variation than that ‘between-taxa’ and thus can discriminate among species ([www.barcodinglife.org](http://www.barcodinglife.org)). The efforts to produce DNA barcodes, according to Chase *et al.*, (2005), have been very successful for animals and fungi using the cytochrome oxidase 1 gene (COX 1). This gene has proven to be 98%-100% successful in identifying animals and some fungi (Hajibabaei *et al.*, 2006 and Ward *et al.*, 2005).

Unlike animals, ‘land plants have had the reputation of being problematic for DNA barcoding for two general reasons: the standard DNA regions used in algae, animals

and fungi have exceedingly low levels of variability and the typically used land plant plastid phylogenetic markers (e.g. *rbcL*, *trnL-F*, etc.) appear to have too little variation' (Chase *et al.*, 2005). The challenge of barcoding land plants has been to find a plastid DNA region that shows enough variation within it, and conserved enough to be present and retrievable across the land plants diversity (Chase *et al.*, 2005). The search for a standard DNA sequence to barcode land plants has *proven* to be controversial and all proposed markers have had problems that have proven to be difficult for scientist to resolve (Science Correspondence, 2007)

Many DNA regions of land plants including a two, three-region options; *rpoC1*, *rpoB* and *matK*, or *rpoC1*, *matK* and *psbA-trnH* among others were proposed, but were not accepted as standard regions for barcoding land plants (Chase *et al.*, 2005). The Consortium for Barcode of Life (CBOL) finally accepted and approved of two gene loci, taken from chloroplast genes called *matK* and *rbcL*, as the standard region for barcoding land plants, but still proposes that the search for a more suitable region be continued (Janzon, 2009; [www.sciencemag.org](http://www.sciencemag.org) and [www.scienceamerica.org](http://www.scienceamerica.org)).

DNA barcoding is a perfect tool that can lessen the hard work and hurdles in identification and discovery of new species. It must therefore be acceptable for use in the core areas of science that deals with identification, verification and authentication of species such as the Food and Agriculture industries, Quarantine Services, Food and Drugs Authority, Standards Authority, Pharmaceutical industries, Herbal medicine industries among others.

## CHAPTER THREE

### 3.0 MATERIALS AND METHODS

#### 3.1 Desk Study

An extensive literature review was conducted to select thirty seven (37) medicinal tree species used in the treatment of diarrhoea or male sexual weakness (Abbiw, 1990; Birdi et al., 2010; Hoffman et al., 2004; Patel et al., 2011; Ralebona et al., 2012; Tajuddin et al., 2003 and Teke et al., 2010). The plants were selected based on their recorded usage for the treatment of the said diseases and were subsequently collected from three different conservation sites: Ankasa Nature Reserve, Bia Conservation Area and Kakum Conservation Area, all in southern Ghana.

DNA barcode database was developed from the thirty seven (37) tree species selected from the desk study to serve as a reference DNA database of medicinal trees for future identification of such tree species. Hence trees in their natural states as far as possible were needed to give true barcodes of the species involved. Since these three (3) forests are well protected and internationally recognised conservation areas with less or no anthropological influences, they were the best sites for the study.

To verify the authenticity of the data in the molecular identification of such medicinal trees, DNA barcode database was produced from eleven trees already in the reference database for comparison.

## 3.2 Study area

### 3.2.1 Description of the study areas.

#### 3.2.2 Ankasa Conservation Area

The Ankasa Conservation Area lies in Southwest Ghana on the border with Ivory Coast in the Jomoro District of the Western region *between latitude* 5°09' and 5°25' N and longitude 2°29' and 2°45' W. It covers a total area of about 509 km<sup>2</sup>. It is the only wet evergreen protected area in its natural state with the highest Genetic Heat Index in Ghana. It is home to over eight hundred vascular plant species including some endemic ones like the recently identified *Psychotria species*, forest elephants, leopard, bongo, chimpanzees and virtually all of the West African forest primates. It has an impressive avifauna, six hundred butterfly species and its network of streams is an important breeding ground for many of the fish species in the Eburneo-Ghanaian ichthyofauna region as well as being of immense importance for the biotic integrity of waters west and south of the Protected Area.

Ankasa is characterised by a distinct bi-modal rainfall pattern which occurs from April to July and September to November with an average annual rainfall of 1700mm to 2000mm and mean monthly temperatures ranges from 24°C to 28°C. A high relative humidity of about 90% during the night and 75% in the early afternoon exist in Ankasa all year round. Figure 3.1 presents the map of Ankasa conservation area with its adjoining settlements.

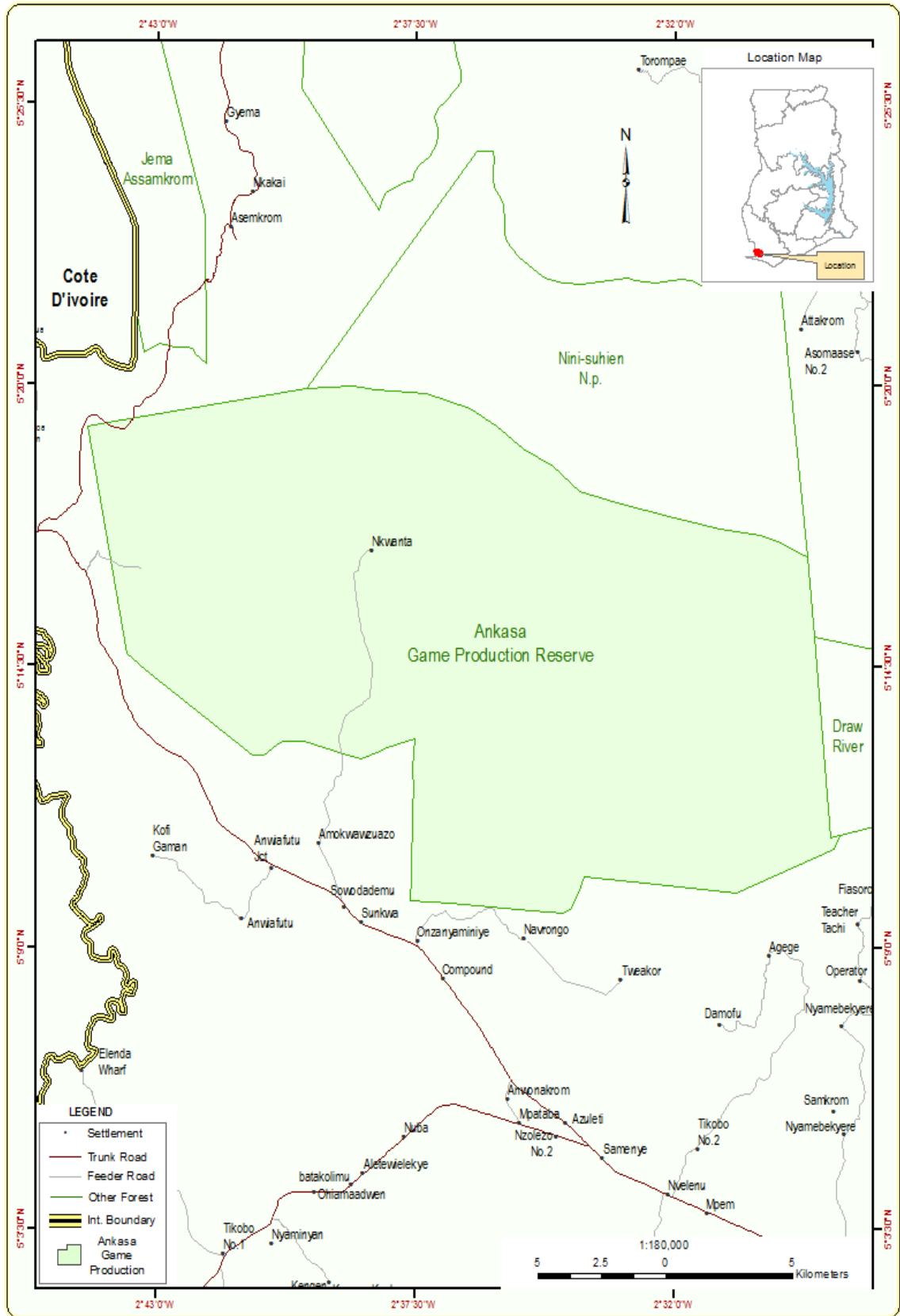


Fig 3.1 Map of Ankasa Conservation Area

### 3.2.3 Bia Conservation Area

The Bia conservation area lies in Southwest Ghana on the border with La Côte d'Ivoire in the administrative jurisdiction of Juabeso District Assembly. It lies between latitudes 6° 20' and 6° 38' N and longitude 2° 58' and 3°58' W covering a total land area of about 306km<sup>2</sup> which comprise Bia National Park in the North and the adjoining Bia Resource Reserve in the South (Forestry Commission, 2010).

According to Hall and Swaine (1981), The Bia conservation area lies in the transition zone between two vegetation categories of Ghana; Bia Biosphere Reserve lies within the Moist Evergreen Forest Vegetation Zone in the south while Bia National Park is within the Moist Semi-deciduous Vegetation. It is home to some 404 species of butterflies, 130 species of birds, African elephant, Chimpanzee, white-nosed sooty Manabey, Olive colobus, Bongo, among others (IUCN/PACO, 2010).

Rainfall peaks in June and October with mean annual total between 1500mm to 1800 mm. Mean temperatures fall between 24°C and 28°C. Relative humidity is generally high throughout the year, being about 90% during the night falling to 75% in early afternoon (Forestry Commission, 2010). Although some parts of the forest had been logged over the past years till 1974, it has some remnants of untouched forest complete with its full diversity of life (Forestry Commission, 2010). Figure 3.2 presents the map of Bia Conservation Area and the settlements around it.

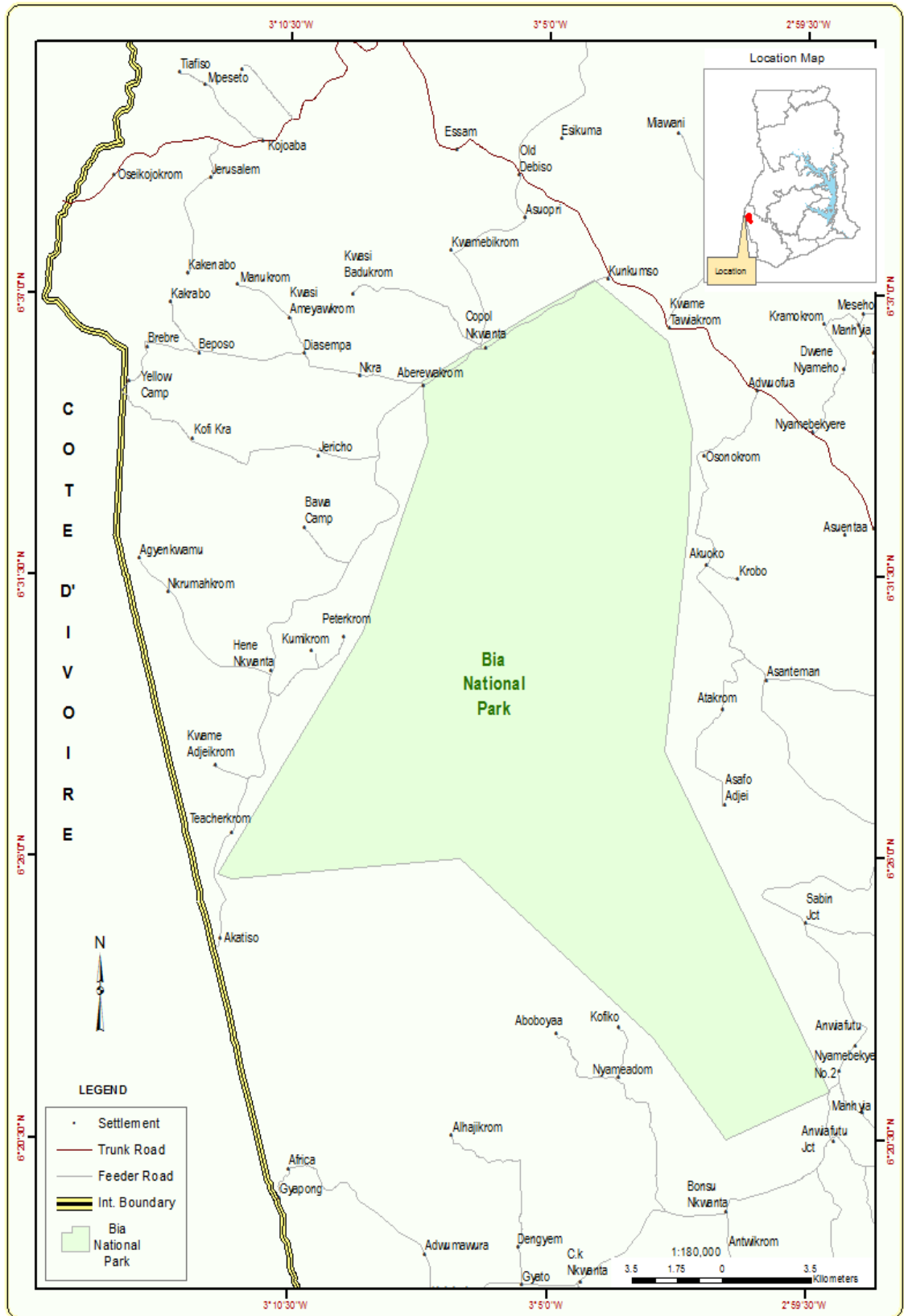


Fig. 3.2 A map of Bia conservation area.

### 3.2.4 Kakum Conservation Area

The Kakum Conservation Area comprises Kakum National Park and Assin Attandanso Resource Reserve. They are located in the Twifo Heman and Assin Atandanso Districts of the Central Region of Ghana respectively. Kakum Conservation Area is a moist evergreen forest (Hall and Swaine, 1981), made up of about 360km<sup>2</sup> of contiguous forest that lies between longitudes 1°51' and 1°30' W and latitudes 5°20' and 5°40'N (Forestry Commission, 1996).

The conservation area has a characteristic rainfall pattern which experiences a two-peak rainy season. The major peak is between May to July and the minor falling between September to December, separated by a short dry period in August (Forestry Commission, 1996). The wet season is followed by a long dry season from January to April during which most streams dry up and rivers break into pools. The mean annual rainfall is between 1,500mm and 1,750mm. The prevailing winds are south-westerly and are generally light. The average relative humidity is about 85% with temperatures fluctuating between 24.2°C and 31.6°C (Forestry Commission, 1996).

Kakum Conservation area falls into two vegetation zones, transition forest and moist semi-deciduous forest zones based of a classification of forest by Taylor (1952). One hundred and five species of vascular plants have been identified in the conservation area (Forestry commission, 1996). Thirty (30) different mammal species, four (4) reptile species, 266 bird species, 405 species of butterflies have been identified from the reserve (Forestry commission, 1996 and Larsen, 1994). Figure 3.3 presents the map of Kakum conservation area and its adjoining communities.

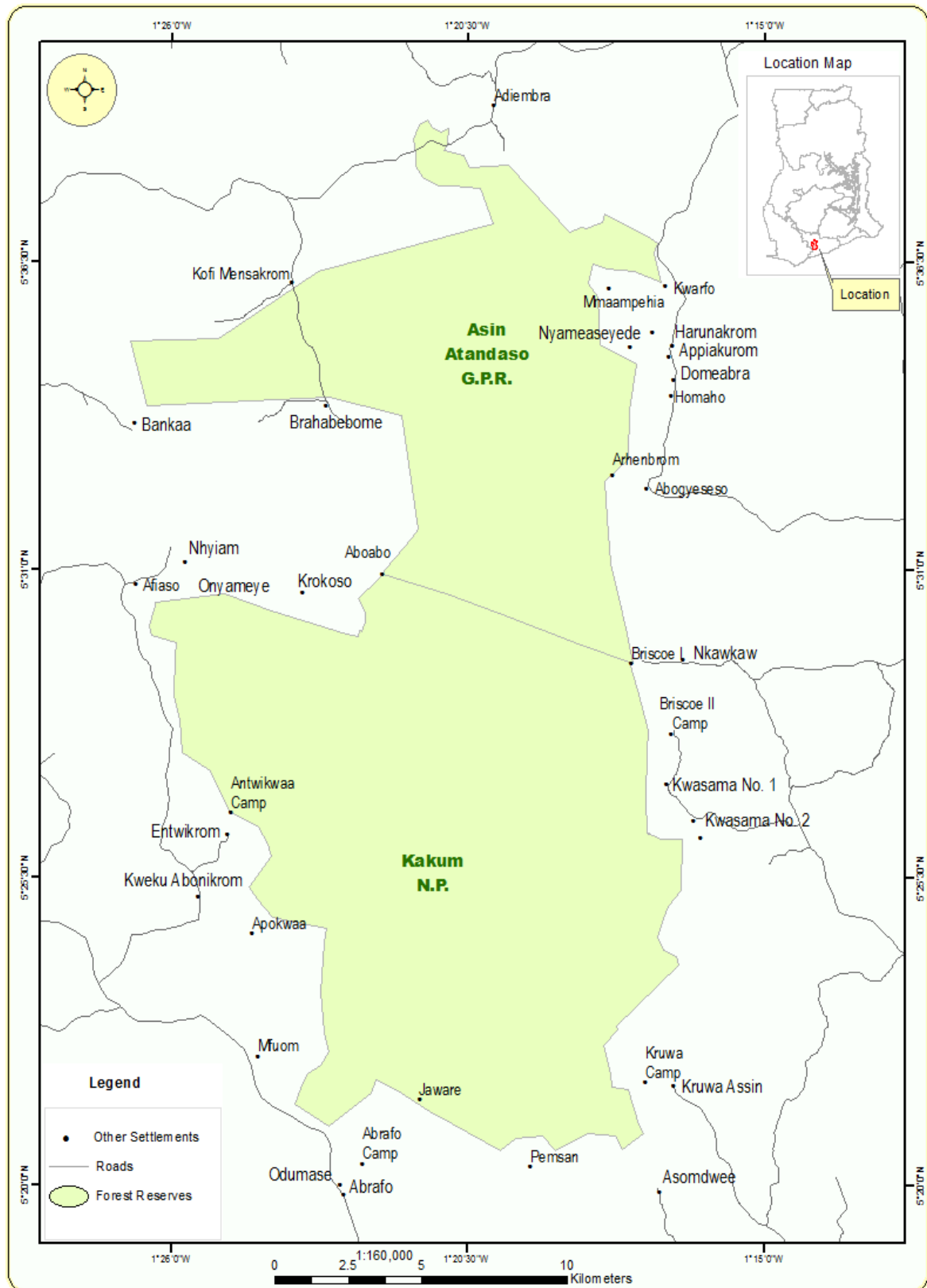


Fig 3.3 A map of Kakum conservation area.

### **3.2.5 Aburi and its environs**

Aburi is a town in the Akuapim South Municipal Assembly. Mean monthly temperature varies between 22°C and 26°C with a mean annual rainfall of about 1600mm. It is situated in the Akuapim ridge about 39 kilometres of north-east of Accra with an elevation of about 1200-1500ft above sea level. (<http://www.levoyageur.net>)

### **3.3 Taxon sampling**

Leaf samples of the thirty seven (37) medicinal tree species selected on the basis of desk study were collected from the three sites with a pole pruner. Ten of the tree species were collected from Ankasa Nature Reserve (Table 4.1), fourteen of them were collected from Bia Conservation Area (Table 4.2) while leaves of the remaining thirteen tree species were collected from Kakum Conservation Area (Table 4.3). Leaf samples of all the eleven trees for verification of the reference database were collected from farmlands in Aburi and its environs. The spotting of all the tree species in the field was done with the aid of a taxonomist from the University of Ghana Herbarium and relevant literature (Hawthorne, 1990; Hawthorne and Jongkind, 2006; Hawthorne and Gyakari, 2006).

GPS coordinates of each tree species from which samples were taken was recorded with a Garmin e Trex handheld GPS. A full picture of the life form of each of the tree species was also taken with a Samsung digital camera to help in morphological identification. Leaf samples taken were then prepared for DNA extraction and herbarium voucher specimens.

### **3.4 Preparing Samples for DNA Extraction**

About 0.5g of green healthy leaf samples of each tree species were cut into smaller pieces with a pair of scissors and put into a Ziploc bag containing silica gel (Chase & Hills, 1991). A small voucher with the following information; name of collectors, place of collection, GPS location, species name of plant, date of collection and voucher number was prepared for each sample and inserted into the Ziploc bag with the voucher number written outside of the bag with a permanent pen. The bag was then sealed with caution to eliminate air. The silica gel in each Ziploc bag was changed after absorbing enough moisture; this was evident when the silica gel changed from a blue colour to white. Total dryness of each sample was achieved within approximately twenty four hours (24hrs) and stored frozen in a refrigerator awaiting DNA extraction.

### **3.5 DNA Extraction**

Laboratory analyses were done at the Vincent Savolainen Laboratory, Imperial College, London UK. Total genomic DNA was extracted from each of the thirty six (36) silica gel-dried leaves samples using a modified cetyl trimethylammonium bromide (CTAB) technique (Doyle & Doyle, 1987).

### **3.6 Extraction process**

About 0.3g of each silica-dried leaf samples were ground with a laboratory mortar and pestle to break up the thick cell wall covering the cells of the plant in order to release

the DNA of the leaf sample. To each ground sample, one millilitre extraction buffer was added and incubated for an hour over a water bath at temperature of 60°C with occasional swirling after which samples were cooled at room temperature. The extraction buffer is a mixture of chemicals which consist of 2% CTAB, 100 mM Tris/HCl, pH 7.5, 1.4 M NaCl, 2% polyvinylpyrrolidone (PVP)-40, 20 mM ethylene diamine tetraacetic acid (EDTA), pH 8.0. and 20 µL/mL b-mercaptoethanol which is added immediately prior to use (Bafeel *et al.*, 2011 and Norris *et al.*, 2009). Six hundred micro litres of Chloroform isoanyl alcohol (CIA) solution was added to each sample and mixed gently for five minutes. The CIA which is a detergent binds to protein and lipids of cell membrane of the leaf sample, dissolves and breakup the bonds of the cell membrane resulting to a precipitate of the lipids and proteins. A fifteen (15) minute centrifugation was done to separate the DNA from the precipitates. The supernatants were then transferred into new tubes and equal volume of isopropanol added and mixed gently. The samples were then incubated at a temperature of -20°C overnight and centrifuged for fifteen minutes. The supernatants were then washed with seventy (70) percent ethanol followed by another ten minutes centrifugation. The wash was then repeated for the supernatants two more times to remove all remaining protein and lipids by dissolving in the alcohol while the DNA which is insoluble in alcohol precipitates. The thoroughly washed supernatant is the DNA which was then dried. The dried DNA was dissolved in fifty (50) micro litre TERNase solution and incubated for one hour at 37°C after which the DNA is ready for amplification. All chemicals were used according to manufacturers recommended concentrations and quantities.

### **3.7 Choice of DNA barcode regions**

Even though the consortium for the barcode of life plant working group has standardized the *rbcL* and *matK* plastid gene regions for the barcoding of land plants (Janzon, 2009; [www.sciencemag.org](http://www.sciencemag.org); [www.scienceamerica.org](http://www.scienceamerica.org)), only the *rbcL* region was used in this study due to the high amplification achieved for this region (Bafeel *et al.*, 2011; Hollingsworth *et al.*, 2009 and Norris *et al.*, 2009).

### **3.8 DNA Amplification and Sequencing**

Amplifications were performed using an Eppendorf Mastercycler EP Gradient S thermocycler and Sigma brand reagents and 0.5 units Sigma Jumpstart *Taq* polymerase with denaturation at 94°C for 45 seconds, annealing at 55°C for 45 seconds and extension at 72°C for 3 minutes (Norris *et al.*, 2009). The *rbcL* primer (*ATGTCACCACAAACAGAGACTAAAGC*) was used due to its reported high success sequence yielding rate (Kress & Erickson, 2007 and Norris *et al.*, 2009).

### **3.9 Sequence Editing**

This is a computer software program and was done using standard software program and finally the edited sample was analyzed.

### **3.10 Social Survey**

A purposive sampling method which targeted medicinal plant collectors, sellers and herbalists was used in the survey. Questionnaires and interview guides were used in

the survey to find out the current state of the medicinal plants species used in the treatment of diarrhoea and/or male sexual weakness. The methods of identification of the plants species used, availability, distribution and abundance, the quantity used/sold per month, the parts and sources of the plants used by respondents in the preparation of herbal medicine for the treatment of diarrhoea or male sexual weakness. Information on the level of awareness, the source of awareness and the contribution of forest conservation in the availability of medicinal plants species were also sought from the respondents.

In all fifty (50) people were interviewed from a total of five (5) different places. Twenty two (22) of the respondents were herbal plant sellers, eighteen (18) of them were herbalists, five (5) were herbal plant collectors and the remaining five (5) were modern herbal producers. The sample size was chosen based on the availability and the willingness of the respondents to participate. The eighteen (18) herbalists, who were either farmers, hunters or both, were interviewed from the forest fringed communities in the sampling areas (Ankasa, Bia and Kakum conservation area); and there were six (6) from each of the three (3) areas. All the five herbal plant collectors were from Kakum and sold their products to some herbal plant sellers in Kasoa and Nyanyano markets. All the five (5) modern herbal producers were interviewed from Kasoa.

A preliminary study showed that the herbal plant sellers received their stocks from the collectors once every week. The quantity of herbal products that had been collected by the each of the five (5) herbal collectors was weighed with a spring balance before they were sent to the markets. This was repeated for four (4) consecutive times. The average quantity of each species collected were put together and used in the analyses.

Ten (10) herbal plant sellers were interviewed at Nyanyano and the remaining twelve (12) in Kasoa markets. Plants used to treat male sexual weakness and/or diarrhoea according to the sellers was recorded with their respective parts used and the quantity they had was weighed with a spring balance and recorded. This was also repeated for four (4) consecutive times and the average quantity computed and used in the analyses. Each of the herbalists and modern herbal producers was interviewed but no records about the quantity of each species they use was made. The quantity of each species weighed for both the market women and herbal plant collectors were put together and used in the analyses.

A statistical tool, SPSS 17 and Microsoft windows excel were used to analyse the samples. Percentages, means, frequencies, were presented in the form of tables and charts and were used for the analyses of the data.

### **3.11 Identification of samples**

Some of the market samples were identified on the spot. For those that were very difficult to be identified on the spot, some of the parts were bought and labelled appropriately and brought to the University of Ghana Herbarium for identification. Information such as the colour of the plant and sap before drying, place of collection and local name among others that could help in the identification of the plants were recorded.

## CHAPTER FOUR

### 4.0 RESULTS

#### 4.1 DNA barcoding

DNA of thirty seven (37) different tree species used in the treatment of both diarrhoea and/or male sexual weakness was extracted for sequencing at the *rbcL* gene region. Thirty five (35) of them were successfully amplified representing a success rate of 94.59%. Two species *Bridelia ferruginea* and *Ziziphus mauritiana* were not successfully amplified at the *rbcL* gene region. Ten (10) of the species that were collected from Ankasa Conservation area, their families, authority names, GPS coordinates and elevations of the exact places they were collected from, are presented in Table 4.1. Four (4) of the nine (9) tree species; *Cinnamomum zeylanicum*, *Myristica fragrans*, *Xylopia aethiopica* and *Magnifera indica* were collected from gardens and backyard of houses since they were hardly found in the wild.

**Table 4.1 Plant species collected from Ankasa, their respective families, authority names, GPS locations and elevations of the places where plant samples were collected.**

Family	Species	Authority name	GPS Coordinates		Elevation (ft)
			Latitude	Longitude	
Clusiaceae	<i>Allanblackia parviflora</i>	A. Chev.	W002*44.184'	N02*16.789'	360
Burseraceae	<i>Canarium schweinfurthii</i>	Engl.	W002*44.184'	N02*16.789'	425
Clusiaceae	<i>Garcinia afzelii</i>	Engl.	W002*39.089'	N05*13.040'	311
Loganiaceae	<i>Strychnos afzelii</i>	Gilg.	W002*44.184'	N02*16.789'	512
Dracaenaceae	<i>Dracaenia surculosa</i>	Lindl.	W002*39.089'	N05*13.040'	544
Lauraceae	<i>Cinnamomum zeylanicum</i>	Blume	W002*45.184'	N02*17.789'	403
Annonaceae	<i>Xylophia aethiopica</i>	A. Rich.	W002*45.184'	N02*17.789'	491
Myristicaceae	<i>Myristica fragrans</i>	Houtt.	W002*45.184'	N02*17.789'	337
Anacardiaceae	<i>Magnifera indica</i>	L.	W002*39.089'	N05*13.040'	453
Euphorbiaceae	<i>Bridelia ferruginea</i>	Benth	W002*39.089'	N05*13.040'	321

Fourteen (14) medicinal plants species were collected from Bia conservation area. Of these, only *Terminalia catappa* was planted in gardens and at the backyard of houses to provide shade for relaxation (mostly in the afternoons) and also serve as a place for most indoor games like “dame” (draft), “oware” and ludu among others. Table 4.2 shows the species, their respective families, authority names, GPS coordinates and elevations of the exact place where the samples were collected.

**Table 4.2 Plants species collected from Bia, their families, authority names, GPS location and elevation of the places from where samples were collected.**

Family	Species	Authority name	GPS Coordinates		Elevation (Ft)
			Latitude	Longitude	
Leguminosae	<i>Albizia zygia</i>	Benth.	W003*05.828'	N06*36.434'	629
Apocynaceae	<i>Rauwolfia vomitoria</i>	Afzel.	W003*05.828'	N06*36.434'	628
Bignoniaceae	<i>Kigelia Africana</i>	Benth.	W002*59.168'	N06*22.121'	586
Clusiaceae	<i>Garcinia kola</i>	Heckel	W003*01.959'	N06*32.672'	747
Bignoniaceae	<i>Newbouldia laevis</i>	P. Beauv	W002*59.168'	N06*22.121'	586
Simaroubaceae	<i>Harrisonia abyssinica</i>	Oliv.	W003*01.959'	N06*32.672'	747
Euphorbiaceae	<i>Ricinodendron heudelotii</i>	Baill,	W003*05.828'	N06*36.434'	629
Combretaceae	<i>Terminalia catappa</i>	L.	W003*05.828'	N06*36.434'	629
Sterculiaceae	<i>Cola gigantea</i>	A. Chev.	W003*01.959'	N06*32.672'	747
Moraceae	<i>Trilipisium madagascariensis</i>	DC.	W002*59.168'	N06*22.121'	586
Annonaceae	<i>Monodora tenuifolia</i>	Benth.	W003*05.828'	N06*36.434'	629
Rhamnaceae	<i>Ziziphus mauritiana</i>	Lam.	W002*51.168'	N06*22.131'	398
Leguminosae	<i>Tamarindus indica</i>	L.	W003*05.828'	N06*22.131'	321
Sterculiaceae	<i>Cola nitida</i>	A. Chev.	W003*05.828'	N06*36.434'	629

Thirteen (13) different plants species were collected from Kakum Conservation Area.

Table 4.3 presents the species, their respective families, authority names, the GPS location and elevation of the exact places where the plant samples were collected from.

**Table 4.3 Plant species collected from Kakum, their families, authority names, GPS location and elevation of the places from where samples were collected.**

Family	Species	Authority name	GPS location		
			Latitude	Longitude	Elevation (ft)
Luguminosae	<i>Millettia thonningii</i>	Baker	W001*22.992'	N05* 20.846'	261
Irvingiaceae	<i>Irvingia gabonensis</i>	Baill. ex Lanen.	W001*22.992'	N05* 20.846'	261
Rubiaceae	<i>Corynanthe pachyceras</i>	K. Schum.	W000*10.488'	N05* 51.160'	579
Euphorbiaceae	<i>Margaritaria discoidea</i>	G. L. Webster	W001*22.992'	N05* 20.846'	261
Anacardiaceae	<i>Sclerocarya birrea</i>	Hochst.	W000*10.488'	N05* 51.160'	296.
Sapindaceae	<i>Blighia sapida</i>	K.D. Koenig.	W000*10.488'	N05* 51.160'	579
Rubiaceae	<i>Morinda lucida</i>	Benth.	W001*22.992'	N05* 20.846'	261
Meliaceae	<i>Trichilia monadelpha</i>	J.J. de Wilde	W000*10.488'	N05* 51.160'	579
Myrtaceae	<i>Psidium guajava</i>	L.	W000*10.488'	N05* 51.160'	579
Leguminosae	<i>Pterocarpus erinaceus</i>	Fern.-Vill.	W000*41.498'	N05* 51.160'	642
Caesalpiniaceae	<i>Piliostigma thonningii</i>	Milne-Redh.	W000*41.499'	N05* 51.161'	672
Meliaceae	<i>Pseudocedrella kotschyi</i>	Harms	W000*41.499'	N05* 51.161'	672
Moraceae	<i>Ficus carpensis</i>	Thunb.	W000*41.450'	N05* 51.163'	672

The study also sought to find out whether DNA barcoding can be used to authenticate medicinal plants species. Ten (10) out of the eleven (11) trees whose sequence were to test the validity of the reference data for identification of medicinal tree species were successfully amplified representing a success rate of 90.91%. Although *Albizia zygia* was successfully amplified in the reference database, it failed to sequence in the verification data. Table 4.4 presents the species, their respective families and authority names, GPS locations and elevations of the places from where samples were collected for authentication.

**Table 4.4 Plants species, their respective families, authority names, GPS location and elevations of the places from where samples were taken for authentication.**

Family	Species	Authority name	GPS location		Elevation
			Latitude	Longitude	(ft)
Leguminosae	<i>Albizia zygia</i>	J.F. Macbr.	W000*10.039'	N06*51.838'	1443
Sapindaceae	<i>Blighia sapida</i>	K.D. Koenig.	W000*10.009'	N05*5.819'	1464
Sterculiaceae	<i>Cola gigantea</i>	A. Chev.	W000*21.834'	N06*05.996'	618
Euphorbiaceae	<i>Margaritaria discoidea</i>	G. L. Webster	W000*10.043'	N05*51.786'	1487
Luguminosae	<i>Millettia thonningii</i>	Baker	W000*20.676'	N05*06.596'	567
Rubiaceae	<i>Morinda lucida</i>	Benth.	W000*11.106'	N05*49.620'	1297
Bignoniaceae	<i>Newbouldia laevis</i>	P. Beauv	W000*08.752'	N05*57.462'	641
Myrtaceae	<i>Psidium guajava</i>	L.	W000*11.108'	N05* 49.593'	1299
Apocynaceae	<i>Rauvolfia vomitoria</i>	Afzel.	W000*10.008'	N05*51.320'	1487
Combretaceae	<i>Terminalia catappa</i>	L.	W000*10.168'	N05* 51.272'	1540
Anacardiaceae	<i>Magnifera indica</i>	L.	W002*39.089'	N05*13.040'	453

Thirty seven (37) different medicinal tree species used in the treatment of both diarrhoea and male sexual weakness were sequenced at the *rbcL* gene region, but thirty five (35) of these produced a better sequence. Each of the tree species that were successfully sequenced were separated to the species level. Figure 4.1 shows the cladogram of the medicinal tree species that were successfully sequenced. These sequences constitute the database of DNA barcodes for the identification of the thirty five (35) tree species used in the treatment of diarrhoea and male sexual weakness in southern Ghana.

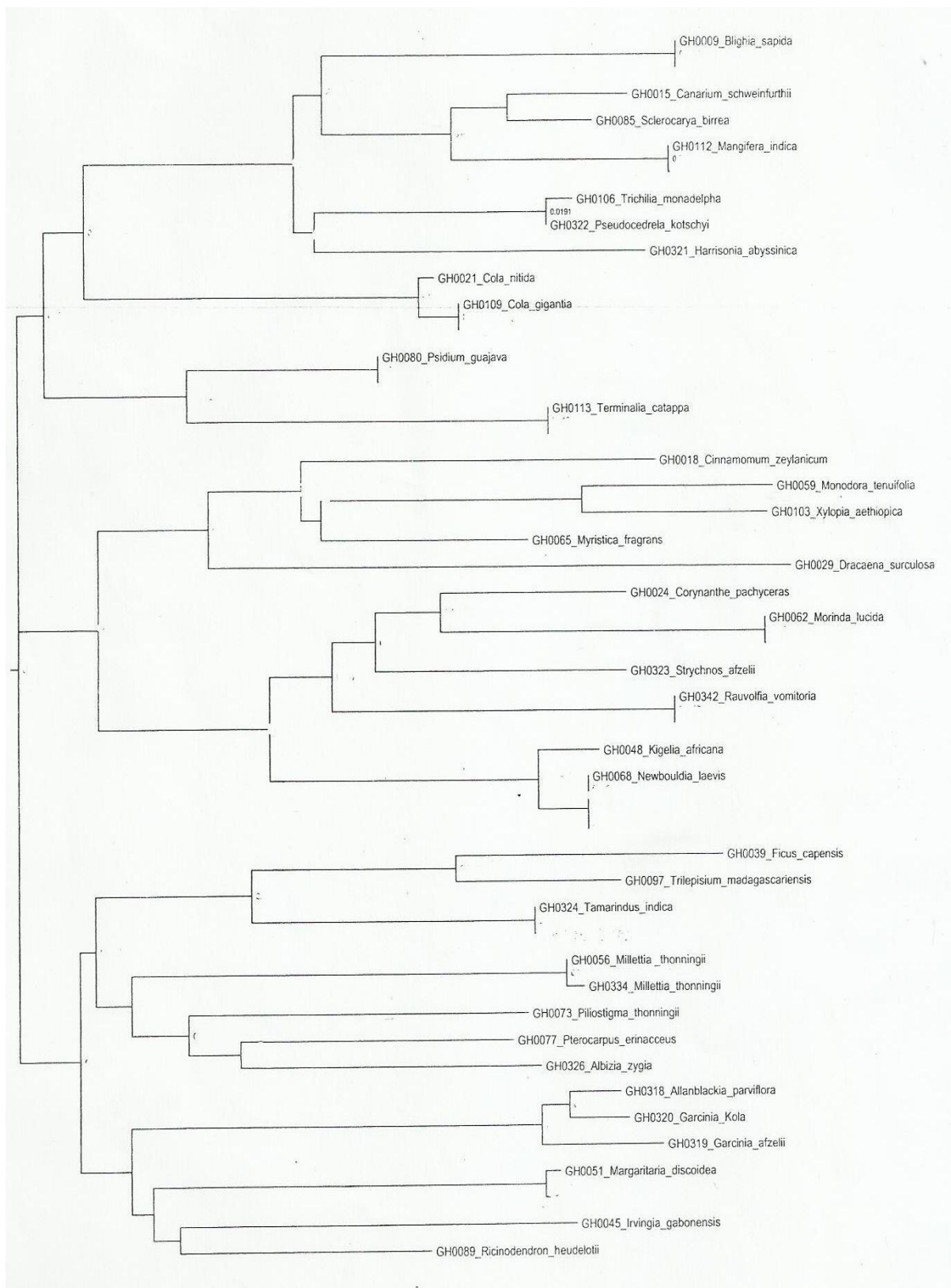


Fig. 4.1 Cladogram of trees used in the treatment of diarrhoea and male sexual weakness.

Fig 4.2 presents the superimposed cladogram of the DNA reference database produced from thirty five (35) medicinal tree species used in the treatment of both diarrhoea and male sexual weakness in southern Ghana and that of ten (10) medicinal tree species for verification. All the sequences from the ten medicinal plants perfectly matched their counterparts in the database.

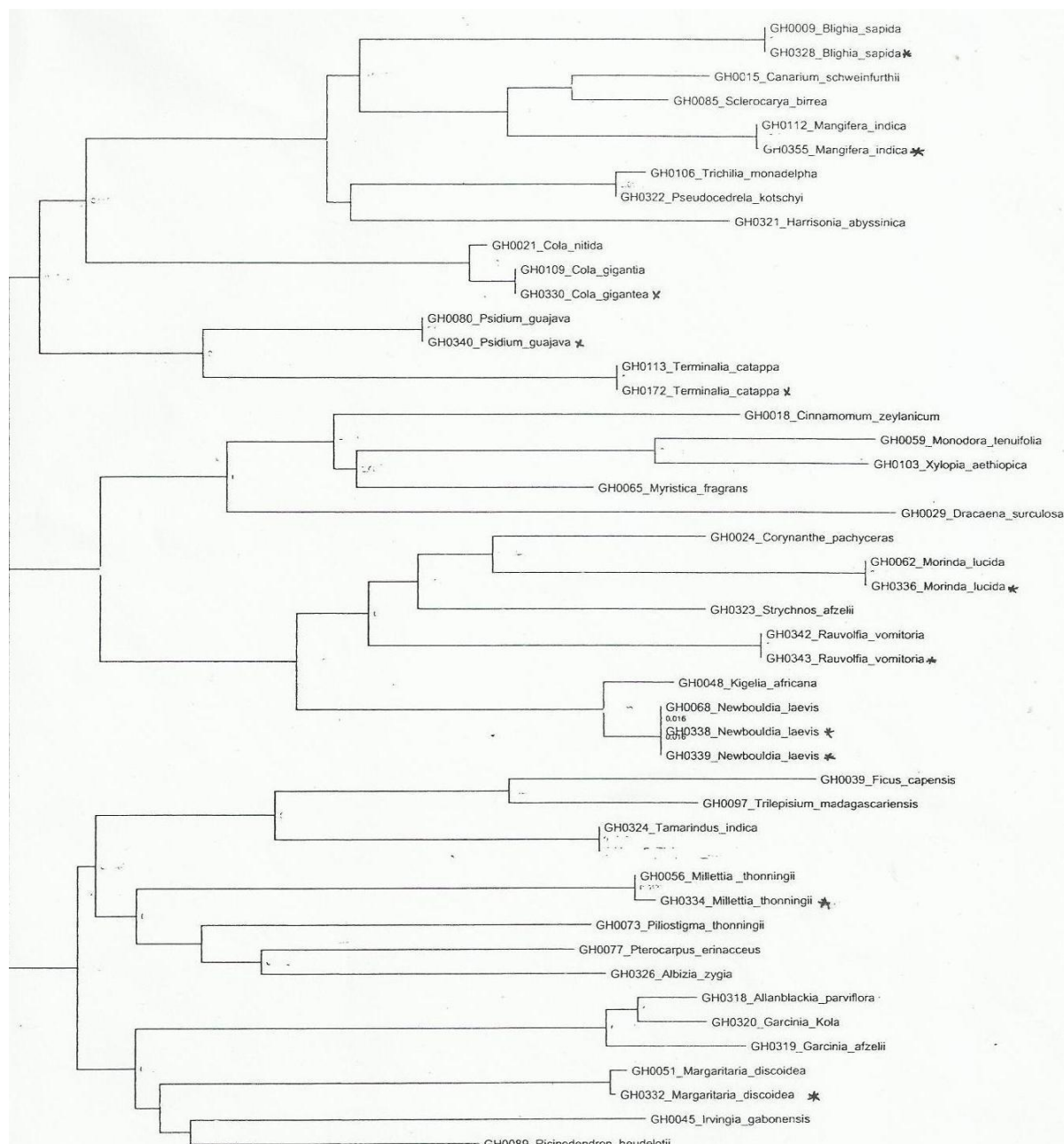
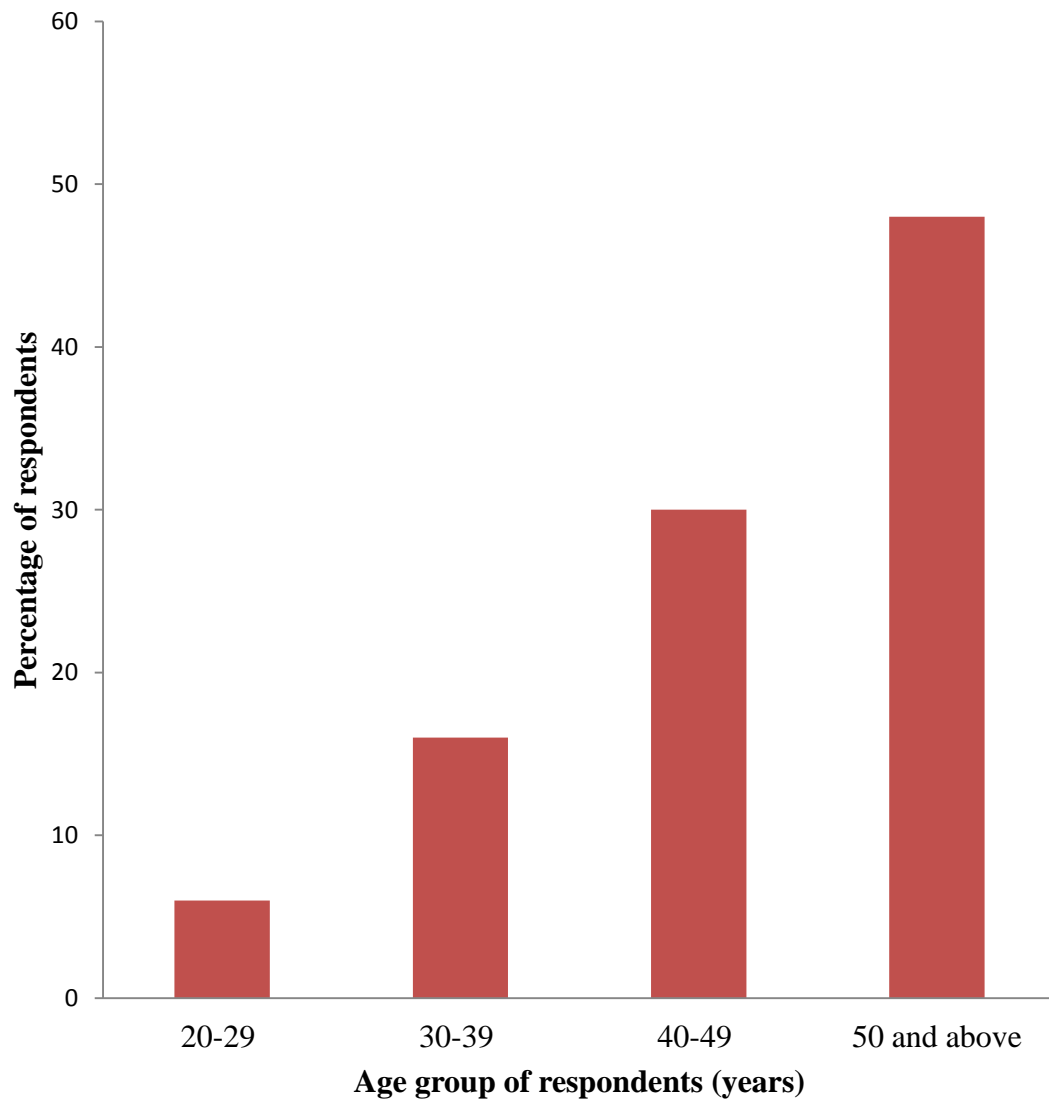


Fig. 4.2 Superimposed cladogram of both sequenced database of thirty (30) trees and ten (10) sequences of trees for database verification.

## 4.2 Social survey

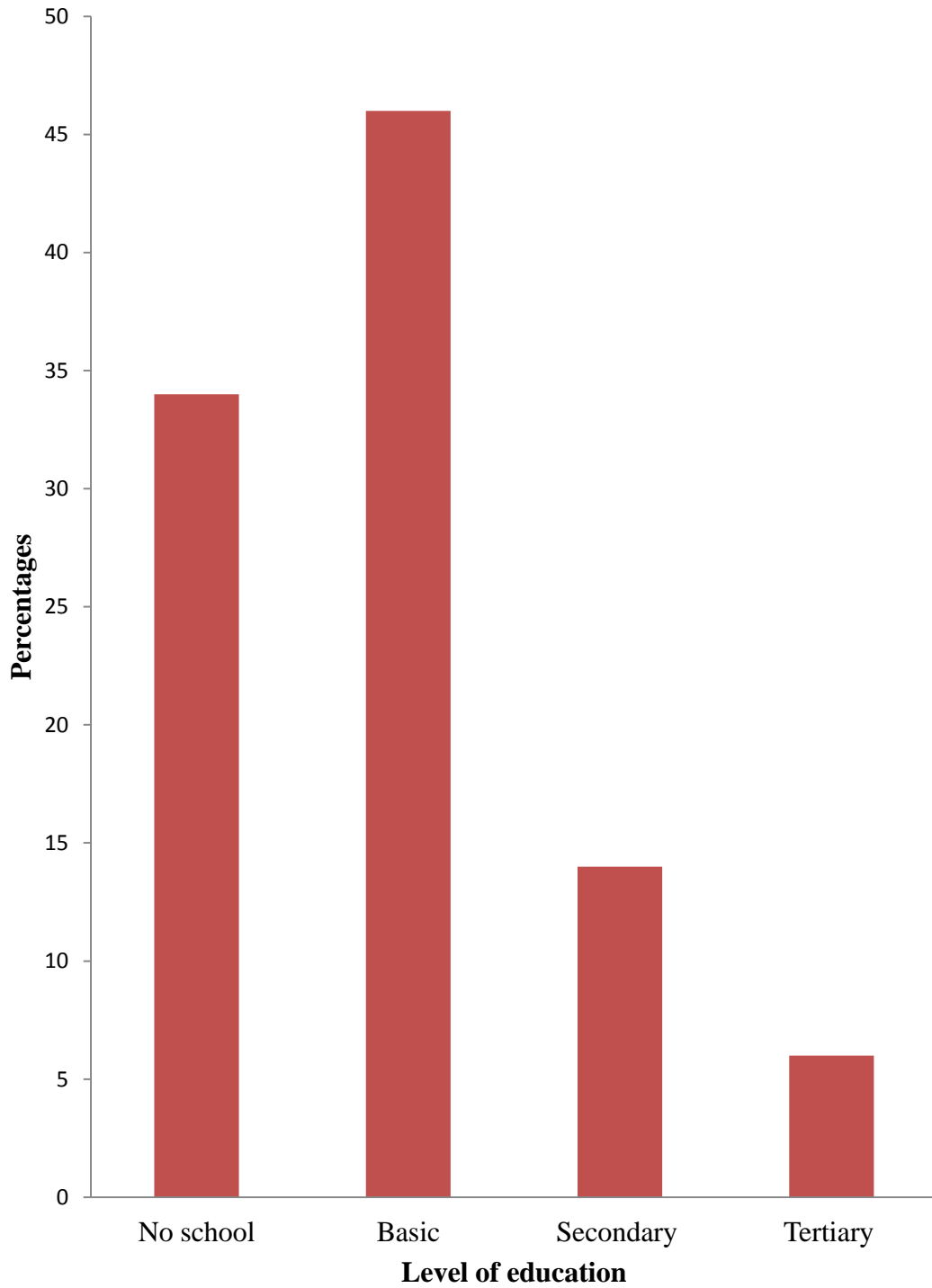
### 4.2.1 Demography of respondents

Fifty (50) individuals were interviewed out of which twenty seven (27) were females and the remaining twenty three (23) were males. The ages of the respondents were grouped into four categories; 20 to 29 years, 30 to 39 years, 40 to 49 years and 50 years and above. These age categories captured entirely everybody within the target group of the study. Fig 4.3 shows the percentage of respondents within each of the age groups.



**Fig.4.3 Percentage of respondents within each of the age groups.**

Thirty four (34) percent of the respondents had no formal education with majority of the educated [forty six percent] having the basic level of education. Fig 4.4 presents the percentage of the respondents and their level of education.



**Fig 4.4 Educational levels of respondents.**

#### **4.2.2 Methods used by collectors to identify medicinal plants species.**

According to the herbal plant collectors and herbalists, traditional identification methods which use morphological and physiological features of the plants are by far the only available identification methods for use and this approach require a lot of experience to identify plants correctly. Due to this, one has to undergo at least a three year apprenticeship training which prepares him/her for identification, collection and preparation of herbal medicines. It was also confirmed that misidentification is possible especially when the trees involved are not in flower. For the herbal plant sellers, 18 out of the 22 interviewed depended on the names given by the suppliers whereas the remaining 7 have gained a lot of experience from their long duration/period of selling such items and can identify most of them whether fresh or dried by their colour and scent.

#### **4.2.3 Plants and their various parts used in herbal medicine preparation.**

##### **4.2.3.1 Plants species used in the treatment of Diarrhoea**

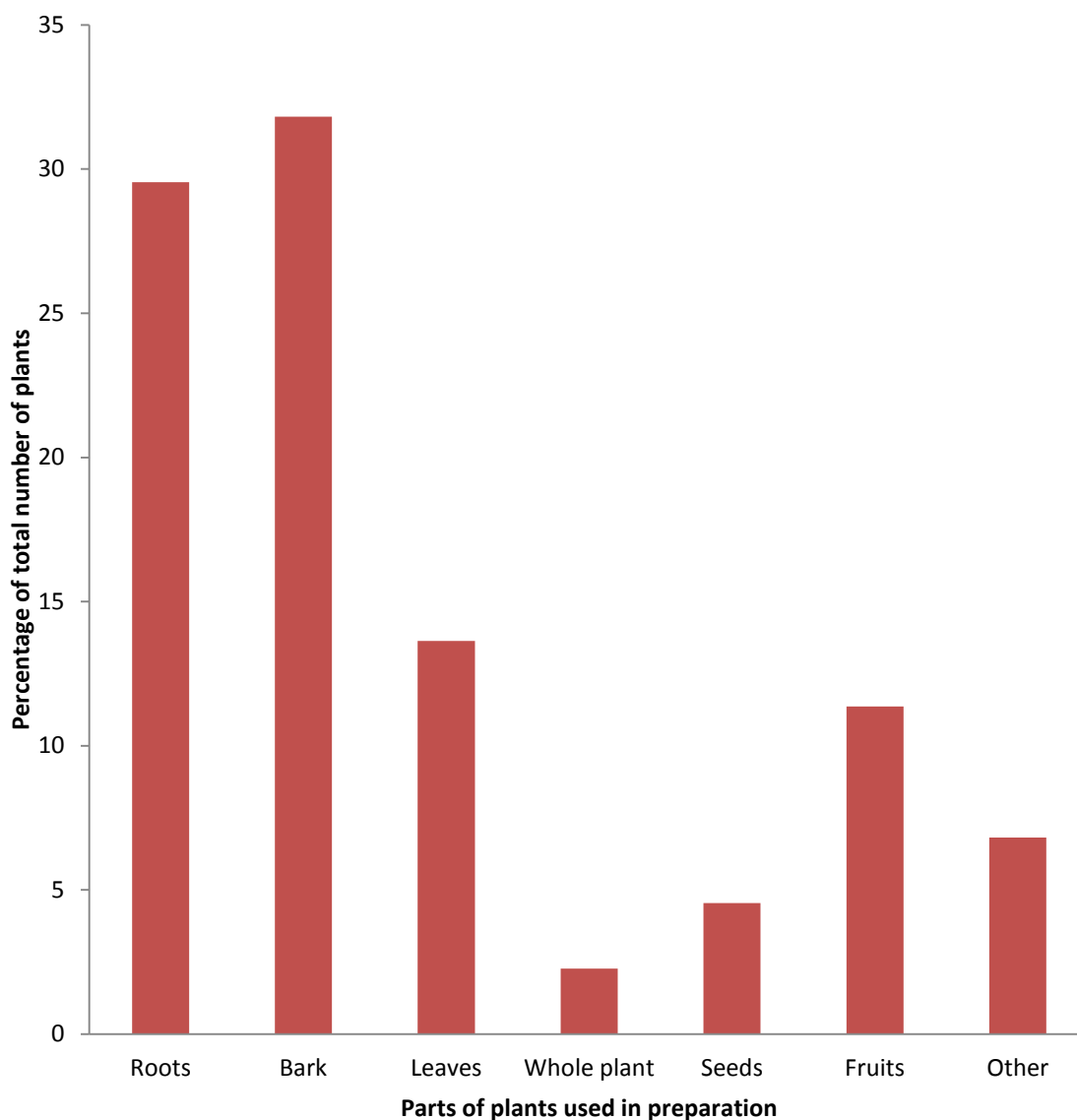
Some plant species like *Mangifera indica* and *Psidium guajava* mostly found in backyards of most houses can be used in the preparations of herbal concoction to treat diarrhoea. Although *Psidium guajava* has the highest frequency of 43, it constitutes only 1.17% while *Sclerocrya birrea* with the lowest frequency (one) also constitutes 1.17% of the total quantity of plant part sold per month. Plant species, their families, local names, parts and quantity used/sold per month by respondents are presented in Table 4.5.

**Table 4.5 Plants species used in treatment of diarrhoea, parts and quantity sold in a month by respondents.**

Family	Plant species	Akan name	Parts used	Freq.	Qty(Kgs)	%
Fabaceae	<i>Albizia feruginia</i>	Awiemfosamina	Roots	29	52	5.08
Clusiaceae	<i>Allanbackia parviflora</i>	Sonkyi	Bark	23	60	5.86
Leguminosae	<i>Amphimas pterocarpoides</i>	Yaya	Bark	6	32	3.13
Anacardiaceae	<i>Anacardium occidentale</i>	Atea	Leaves	23	4	0.39
Euphorbiaceae	<i>Bridelia ferruginia</i>	Opam-fufuo	Roots	12	39	3.71
Burseraceae	<i>Canarium schweinfurthii</i>	Bediwonua	Roots	12	39	3.71
Malvaceae	<i>Ceiba pentandra</i>	Onyina	Root	13	39	3.71
Amaranthaceae	<i>Celosia argenta</i>	Nkyewodua	Seeds	1	02	0.2
Lauraceae	<i>Cinnamomum zeylanicum</i>	True cinnamon	Whole plant	23	48	4.69
Rutaceae	<i>Citrus aurantifolia</i>	Ankaa twadee	Fruits	9	14	1.37
Rutaceae	<i>Citrus limon</i>	Ankaa dwea	Fruits	4	6	0.59
Arecaceae	<i>Cocos nucifera</i>	Kube	Fruit	23	16	1.56
Combretaceae	<i>Combretum molle</i>	Gburega	Bark	8	28	6.64
Moraceae	<i>Ficus lutea</i>	Fonto	Bark	3	20	1.95
Simaroubaceae	<i>Irvingia gabonensis</i>	Abesebuo	Fruit & bark	25	56	5.47
Meliaceae	<i>Khaya ivorensis</i>	Dubini	Roots	11	34	3.32
Anacardiaceae	<i>Magnifera indica</i>	Amango	Roots	31	6	0.59
Sapotaceae	<i>Manilkara obovata</i>	Barekankum	Roots	2	10	0.98
Fabaceae	<i>Millitia thonningii</i>	Sante	Leaf & bark	14	50	4.88
Annonaceae	<i>Monodora tenuifolia</i>	Motokuradua	Bark	19	64	6.25
Moringaceae	<i>Moringa oleifera</i>	Moringa	Gum	9	02	0.2
Cecropiaceae	<i>Myrianthus arboreus</i>	Anyankoma	Leaf & bark	12	22	3.71
Fabaceae	<i>Pentaclethra macrophyla</i>	Oil bean tree	Pods	9	14	1.37
Clusiaceae	<i>Pentadesma butyraceum</i>	Abotoasebie	Bark	11	52	5.08
Fabaceae	<i>Piliostigma thonningii</i>	Opitipataa	Bark	15	26	2.54
Myrtaceae	<i>Psidium guajava</i>	Oguawa	Roots	43	12	1.17

Family	Plant species	Akan name	Parts used	Freq.	Qty(Kgs)	%
Rubiaceae	<i>Rothmannia longiflora</i>	Saman kube	Leaves	3	4	0.39
Anacardiaceae	<i>Sclerocrya birrea</i>	Nanogba	Bark	1	12	1.17
Bignoniaceae	<i>Spathodea campanulata</i>	Kokuanisuo	Leaves	16	10	0.98
Combretaceae	<i>Strephonema pseudocola</i>	Awuruku	Leaves	6	20	1.95
Fabaceae	<i>Tamarindus indica</i>	Indian tamarid	Roots	1	8	0.78
Combretaceae	<i>Terminalia macroptera</i>	Kwatiri	Fruits	4	4	0.39
Moraceae	<i>Trilepisium madagascariensis</i>	Okure	Roots	25	46	4.49
Asteraceae	<i>Vernonia conferta</i>	Flakwa	Bark	3	10	0.98
Sapotaceae	<i>Vitellaria paradoxa</i>	Nkuto	Butter	5	8	0.78
Annonaceae	<i>Xylopiya aethiopica</i>	Hwentia	Whole plant	29	96	9.38

Fig 4.6 presents the parts of trees used in the treatment of diarrhoea. Two percent (2%) of the preparations involve the use of whole plants whereas thirty two (32%) percent utilise plant bark. Some plants parts such as flowers and latex are denoted by others on the bar graph.



**Fig. 4.5 Plants parts used in the treatment of diarrhoea as a percentage of total number of species used in treatment of diarrhoea.**

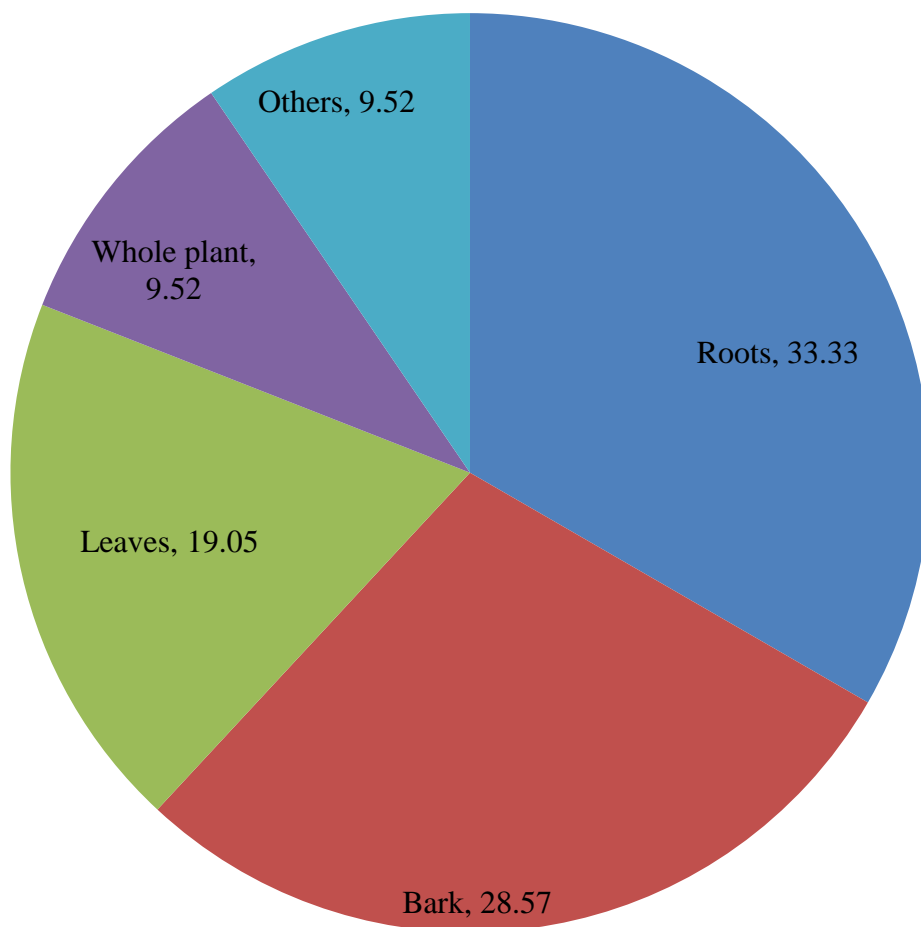
#### **4.2.3.2 Plant species used in the treatment of male sexual weakness**

A list of plant families, local names as given by respondents, parts used the quantity and percentage of each plant species for the treatment of male sexual weakness, sold per month are presented in Table 4.6. Of the eighteen (18) different plant species, *Khaya senegalensis* constituted 18.19%.

**Table 4.6 Plants species used in treatment of male sexual weakness, parts and quantity sold per month by respondents.**

Family	Plant species	Akan name	Parts used	Freq	Qty(Kg)	%
Mimosaceae	<i>Albizia zygia</i>	Okoro	leaves	15	78	9.1
Apocynaceae	<i>Alstonia boonei</i>	Nyamedua	Bark	7	32	3.73
Sterculiaceae	<i>Cola gigantean</i>	Watapuo	Roots	14	28	3.26
Sterculiaceae	<i>Cola nitida</i>	Bese	Nuts	2	8	0.93
Rubiaceae	<i>Corynanthe pachyceras</i>	Pamprama	Bark	26	104	12.13
Asparagaceae	<i>Dracaena surculosa</i>	Mobia	Roots	2	8	0.93
Sterculiaceae	<i>Heritiera utilis</i>	Nyankom	Bark & leaf	15	68	7.93
Meliaceae	<i>Khaya senegalensis</i>	Mahogany	Bark	46	157	18.19
Sterculiaceae	<i>Mansonia altissima</i>	Opronno	Roots	18	36	4.2
Myristicaceae	<i>Myristica fragrans</i>	Nut meg	Nuts	16	32	3.73
Leguminosae	<i>Piptadeniastrum africanum</i>	Dahoma	Bark	1	20	0.93
Loganiaceae	<i>Strychnos afzelii</i>	Duapepere	leaves	4	12	1.4
Combretaceae	<i>Terminalia catappa</i>	Abrofokatee	Whole plant	18	20	2.33
Malvaceae	<i>Theobroma cacao</i>	Kokoo	Root & seed	9	20	2.33
Meliaceae	<i>Trichilia monadelphia</i>	Tanduro	Bark	21	112	13.06
Phyllanthaceae	<i>Uapaca guineensis</i>	Kuntan	Roots	3	20	2.33
Solanaceae	<i>Withania somnifera</i>	Tuentini	Roots	2	96	11.90
Rutaceae	<i>Zanthoxylum leprieuri</i>	Oyaa	Leaves	5	19.7	2.29

Different parts of the same plant may have different uses. While the bark of a plant may function as a cure for malaria, its roots may be used as an aphrodisiac. Fig 4.6 shows the parts of plants used in herbal preparations for the treatment of male sexual weakness. While 33.33% of the total is roots, 9.52% of the preparations involve the use of the whole plant.



**Fig 4.6 Plants parts used in the treatment of male sexual weakness as a percentage of total number of species used in treatment of male sexual weakness.**

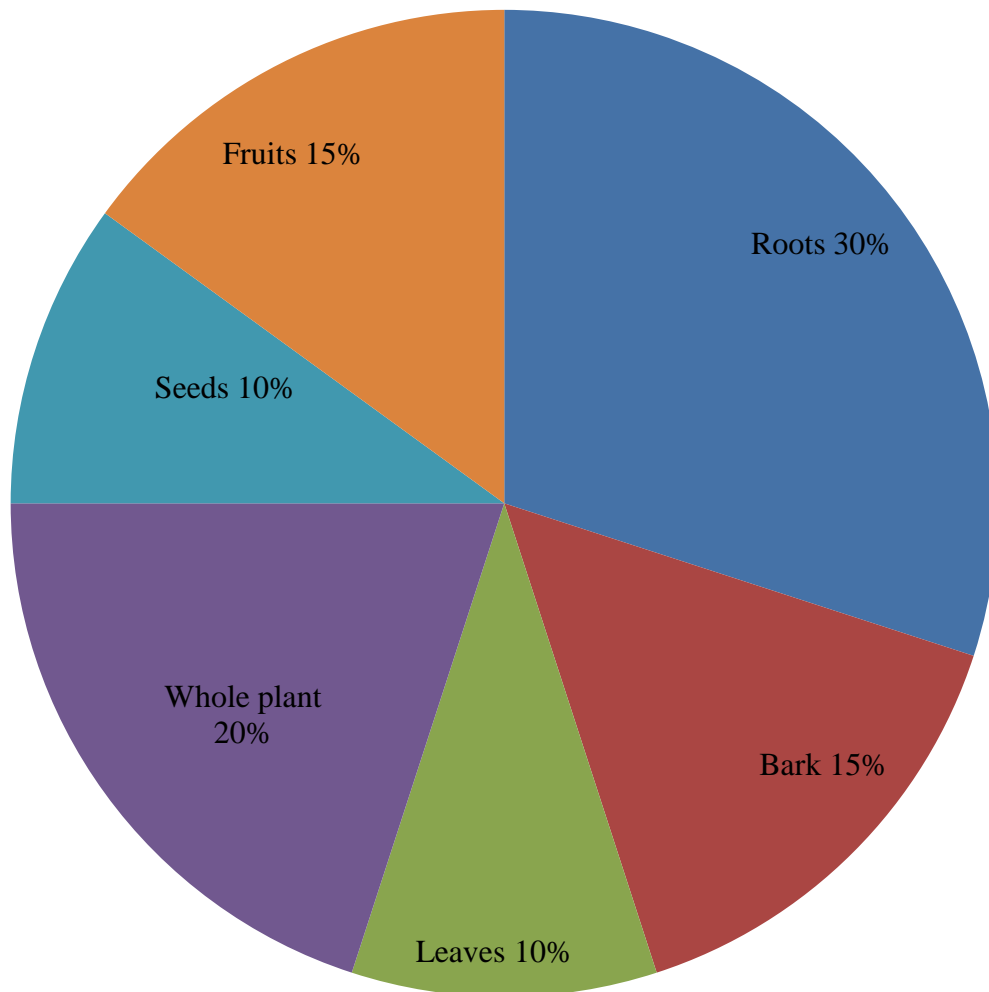
#### **4.2.3.3 Plants species used in the treatment of both diarrhoea and male sexual weakness.**

Although diarrhoea is not related in any way to male sexual weakness, some plants used in their treatment are common. In some plants, for example *Margaitaria descoidea* and *Ficus capensis* the same part (root) of the plant is used in treating both diseases while in others for example *Rauwolfia vomitoria* and *Newbouldia laevis* different parts are used. The plant species capable of treating both diseases, their families, local name, the parts used in the preparation, the frequency, quantity and percentage of each used or sold per month by the respondents are presented in Table 4.7.

**Table 4.7 Plants species used in treatment of diarrhoea and male sexual weakness, families, local names, parts used, frequency and quantity sold per month by respondents.**

Family	<i>Plant species</i>	Akan name	Part used	Freq	Qty(kg)	%
Bombacaceae	<i>Adansonia digitata</i>	Baobab	Seed & fruit	21	6	1
Cluciaceae	<i>Garcinia kola (Heckel)</i>	Tweapea	Whole plant	15	6	1
Clusiaceae	<i>Garcinia afzelii</i>	Nsokodua	Whole plant	17	8	1.3
Capparaceae	<i>Harrisonia abyssinica</i>	Penku	Fruit & leave	18	10	1.6
Bigoniaceae	<i>Kigelia africana</i>	Nufuten	Leaves	27	10	1.6
Moraceae	<i>Ficus capensis</i>	Nwadua	Bark	11	12	2
Rubiaceae	<i>Massularia acuminata</i>	Pobe	Roots	15	24	3.9
Fabaceae	<i>Erythrina senegalensis</i>		Flower & root	19	28	4.6
Phallanthaceae	<i>Margaitaria descoidea</i>	Pepea	Roots	14	36	5.8
Miliaceae	<i>Pseudocedrela kotschy</i>		Roots	6	39	6.2
Euphorbiaceae	<i>Ricinodendro heudelotii</i>	Wamma	Roots	21	44	7.1
Apocynaceae	<i>Rauwolfia vomitaria</i>	Kakapenpen	Root & bark	39	72	12
Rubiaceae	<i>Crossopteryx febrifuga</i>	Pakyisie	Bark	23	79	13
Bigoniaceae	<i>Newbouldia laevis</i>	Sasanemasa	Whole plant	31	119	19
Rubiaceae	<i>Morinda lucida</i>	Konkroma	Whole plant	35	120	19

Fig 4.8 presents the parts of plants used in the preparation of herbal concoction for the treatment of both diarrhoea and male sexual weakness. Ten percent 10% of the preparations involve the use of leaves of plants whereas 30% involves the use of plant roots.



**Fig. 4.8 Plants parts used in the treatment of both diarrhoea and male sexual weakness as a percentage of total number of species used in treatment of both diseases**

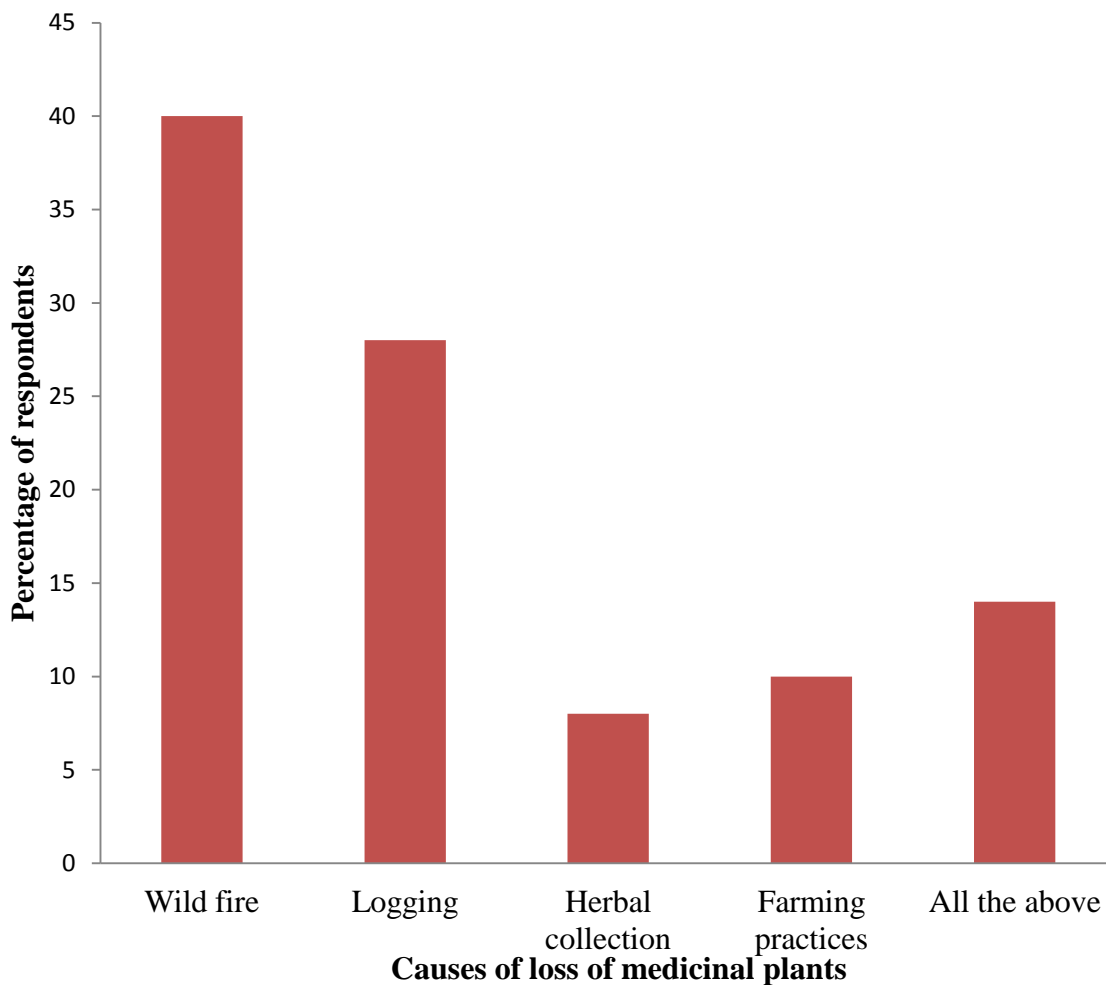
#### **4.2.3 The state of herbal medicine in southern Ghana**

The interview sought to find out how respondents obtained the plants species they use. Forty percent (40%) of the respondents collect their samples by themselves; sixteen percent (16%) send their apprentices who are mostly their children to collect

them while the remaining forty four percent (44%) buy from commercial plant collectors.

Ninety six percent (96%) of the respondents perceive changes in the availability of some medicinal plant species increasing their search time for such species whereas the remaining four percent (4%) think there are no changes in availability of any of the plants species they deal in.

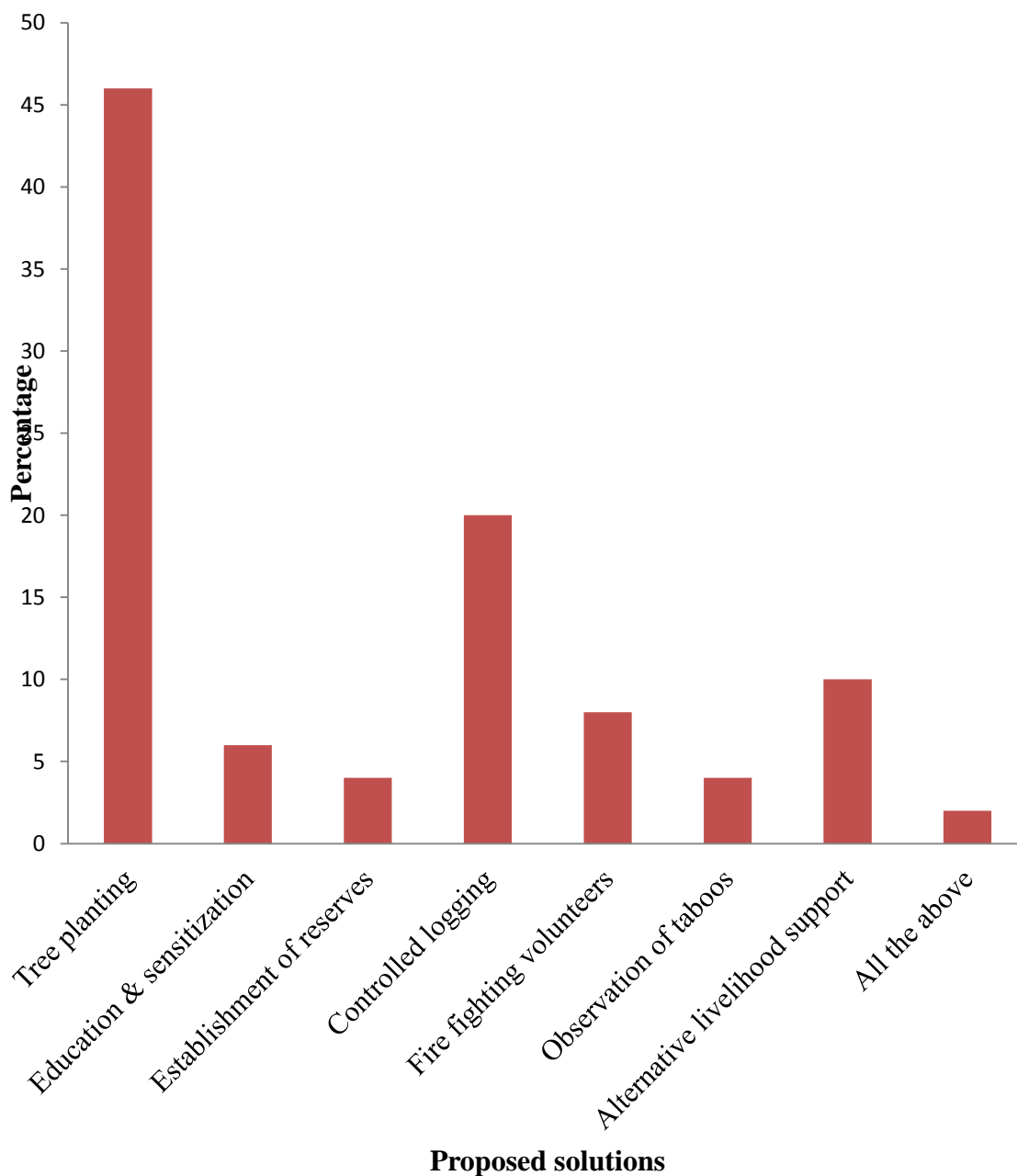
According to the respondents, the reductions in the availability of medicinal plants are mostly due to wildfires which almost consume some places virtually every year, especially in the savannah areas. While wildfire threatens the survival of savannah species, illegal logging is the leading cause of loss of medicinal plants species in the forest areas. Bad farming practices such as shifting cultivation and slash and burn also contribute to the loss of medicinal plants. The commercialisation of herbal collection is also a major threat to the survival of herbal plants. Fig 4.8 presents the causes of loss of medicinal plants species as stated by the respondents.



**Fig. 4.8. Causes of loss of medicinal plants species according to respondents.**

Although forty six percent (46%) of the respondents think tree planting can help solve the problem of reduction in the availability of medicinal plants only four percent (4%) think establishment of forest reserves can contribute to the availability of medicinal plant species. Controlled logging was noted to contribute to the survival of plants. Setting up youth volunteers to fight wild fires in most of the forest fringed communities, educating the people on the need to protect biodiversity, setting of taboos which forbid people to go to the forest certain days or kill some species of animals and plants and also providing alternative livelihood support to reduce the dependency of the forest and natural resources will go a long way to protect medicinal

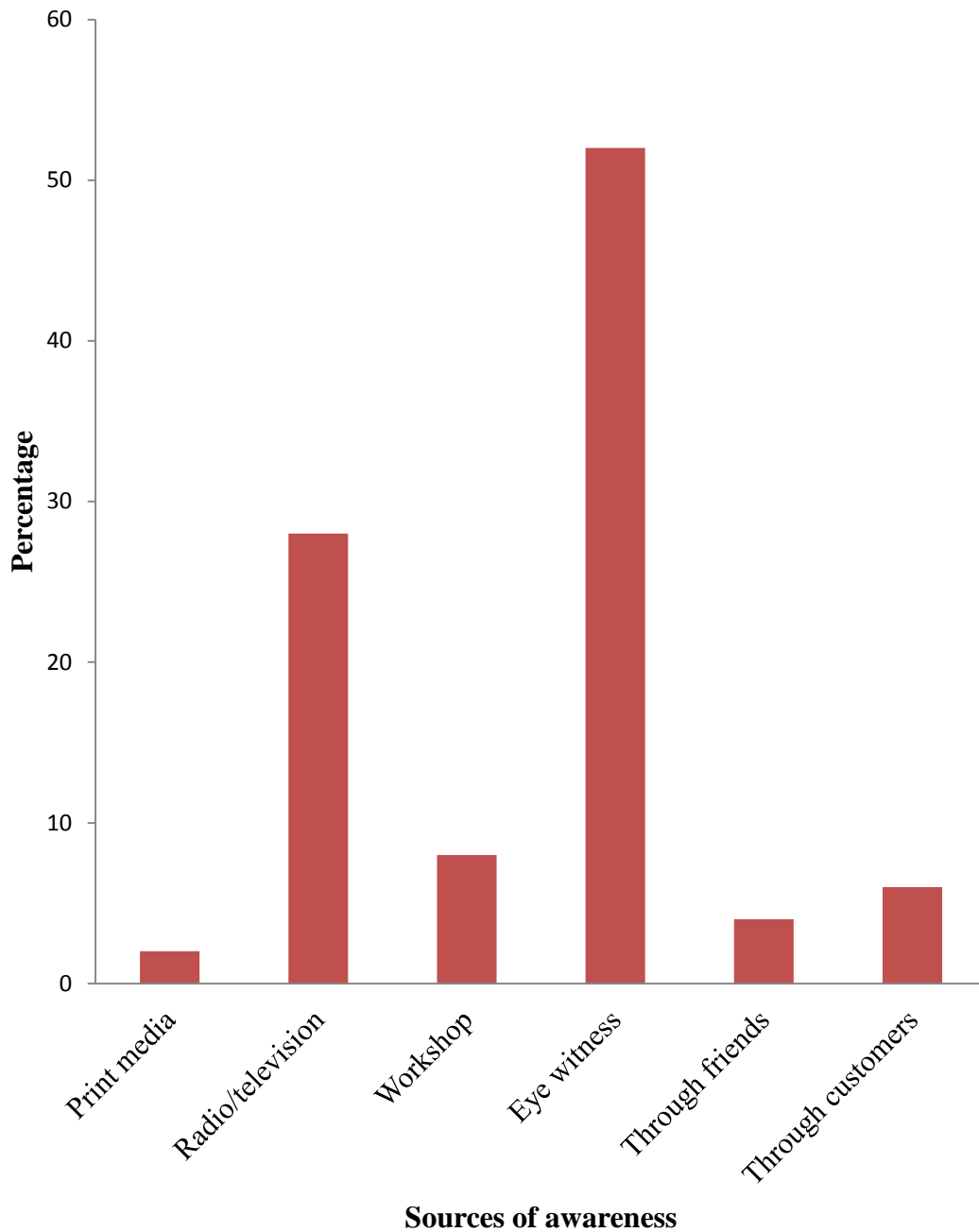
plants and the biological diversity of the nation. Fig 4.9 presents the mitigation measures proposed by respondents to solve the destruction and decrease in the availability of medicinal plants species.



**Fig. 4.9 Measures proposed by respondents to ensure sustainability of medicinal plants species.**

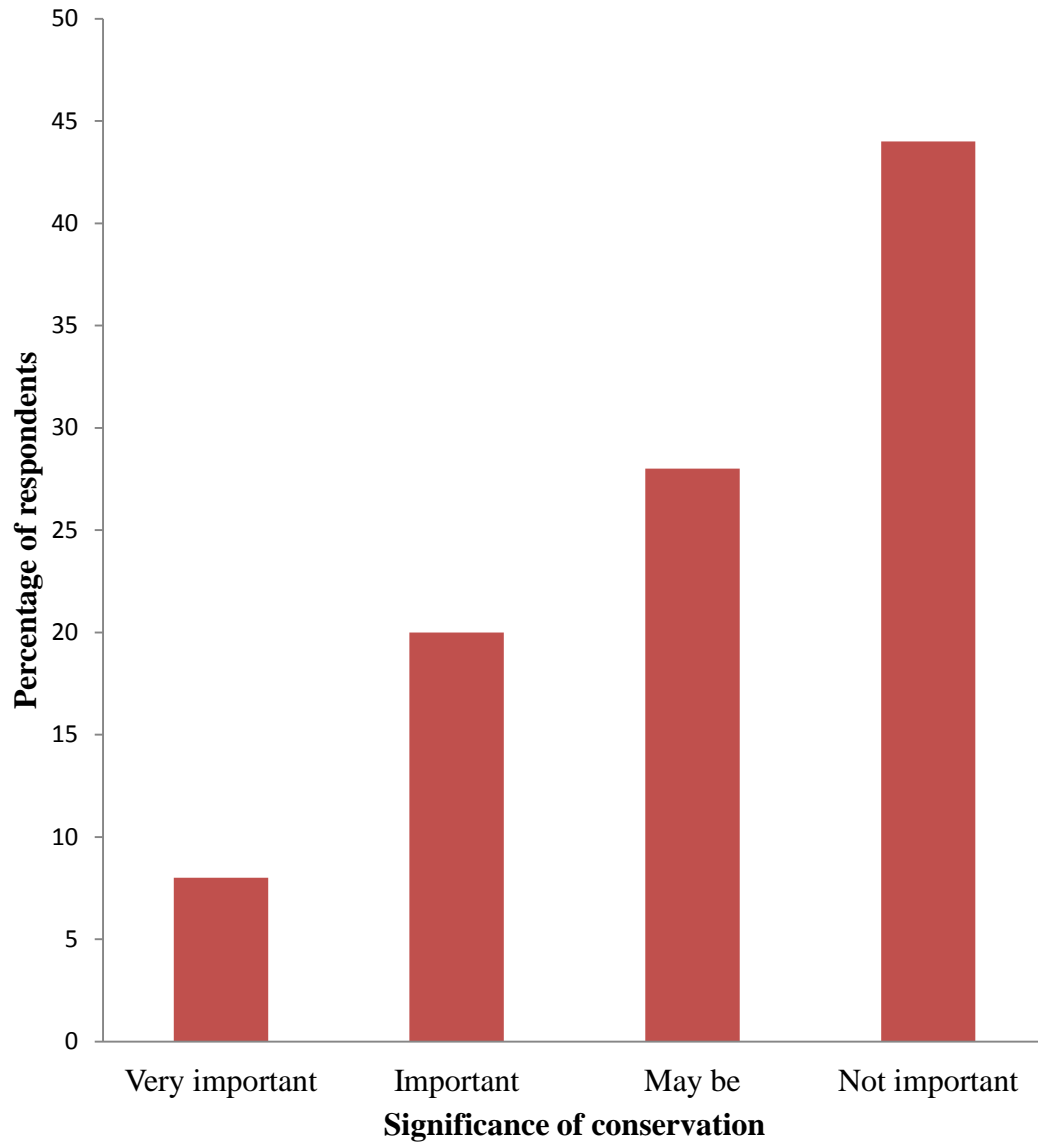
#### **4.2.4 Awareness of forest conservation**

The interview sought to find the level of awareness of respondents about forest conservation. All the respondents were aware of forest conservation. Most of the respondents were eye witness of forest conservation. The radio and television also provide some education on forest conservation to some of the respondents. Customers, friends and workshops that are organised for some of the forest fringe communities help in advertising the need for forest conservation. However few people depend on the print media for information in the survey areas. Figure 4.10 presents the sources of awareness of forest conservation by the respondents.



**Fig. 4.10 Sources of awareness of forest conservation**

Even though all the respondents were aware of forest conservation, only twenty eight (28) percent think it is of any significance to ensuring the availability of medicinal plants species while forty eight (48%) percent think it is of no significance to ensuring sustainability of medicinal plants species in Ghana. Figure 4.12 presents the opinion of respondents about the significance of forest conservation to ensuring sustainability of medicinal plants species.



**Fig. 4.11 Significance of forest conservation to ensuring the sustainability of medicinal plants as given by respondents.**

## CHAPTER FIVE

### 5.0 DISCUSSION

#### 5.1 DNA barcoding

Bafeel *et al.* (2011), recorded that DNA barcoding relies on the information encoded in the nucleotide sequence of a standard region of the genome as a tool for species identification. A 2-locus combination of the *rbcL* and *matK* has been accepted as the standard regions for barcoding (CBOL Plant Working Group, 2009), based on two main criteria: efficient recovery of good quality sequences and high levels of species discrimination (Burgess *et al.*, 2011). The *rbcL* gene region which has a high success rate was used in this study. A success rate of 94.59% was achieved; however Bafeel *et al.* (2011) achieved a success rate of 88% while 100% success rate was achieved by Norris *et al.*, (2009) at the same region. These results are explained by a report by Hollingsworth *et al.* (2009), that no single gene locus has high levels of universality and resolvability and that more than one of the seven main candidate plastid regions (*rbcL*, *matK*, *rpoCl*, *rpoB*, *trnH-psbA*, *atpF-atpH*, and *psbK-psbI*) should be used at a time. Even though *rbcL* and *matK* have been accepted as the standard regions for barcoding land plants, both can fail to successfully sequence some plants species and that the other plastid candidates will have to be employed (Roy *et al.*, 2010, Bafeel *et al.*, 2011).

Moreover, the chemotypic heterogeneity among plant species tends to interrupt plant DNA yield, hence the possibility of closely related plant species requiring different DNA isolation protocol (Khanuja *et al.*, 1999); and this may account for why success rate was not hundred percent. Better still, the failure of *Abizia zygia* to sequence in the

verification data can be attributed to errors such as primer mismatch at the annealing sites, poor quality, or little amount of DNA extracted (Soininen *et al.*, 2009).

According to Altschul *et al.* (1997), the basic concept in species identification is to match the sequence of the evidence item to a reference. This study matched a sequence from ten different plant species with the reference DNA data produced from thirty five (35) different medicinal tree species. The 100% match of the later sequences with the reference data means that DNA barcoding can be used to identify correctly and perfectly all medicinal plant species. The correct identification is key to the safety of herbal medicine since misidentification can result in fatal consequences. According to Rahman *et al.* (2009), molecular tools such as DNA barcoding have been developed to characterize congener species and also detect the origin of species which are very difficult to be done by the traditional identification which is based on the morphological and physiological traits of the species involved. Dexter *et al.*, (2010) reported an error rate of about 6.8 to 7.6% in morphological identification of all individual species which has significant and measurable impact on ecological analyses and other core areas of science that depend on identification such as the quarantine service, pharmaceutical industry, timber industry and the security industry among others.

Any medicinal plant species whether fragment, macerated, powdered or whole can be perfectly identified with the reference database since a 100% matching success was achieved (Nielsen, L.R. & Kjaer, E.D., 2008). The acceptance and use of DNA barcoding as an identification tool for medicinal plants identification will not only ensure the safety of herbal medicine but will also provide a better record on the quantity of medicinal plants that are harvested. Bisi-Johnson *et al.* (2009), Cunningham (2001) and Van Andel *et al.* (2012), have all called for proper records on

the current rate of harvest of medicinal plants in order to inform on proper measures to conserve and manage them. If the identities of all medicinal plants species are to be verified with DNA barcoding before use, there will be a single channel through which all medicinal plants will pass to qualify for use. This will enhance proper and accurate records on the exploitation rate of each plants species, the sources and the quantity among others particularly ensuring safety and prevention of poisoning of customers. This will also help to improve conservation of vulnerable and endangered species.

Even though there was a hundred percent (100) successful match of the verification sequence with the reference sequence database, there were slight variations in the genes of the individuals of the same plant species. These variations were quite conspicuous for *Millettia thonningii*, *Margaritaria descoidea* and *Magnifera indica*. According to Bokhari *et al.* (1990), Kamal *et al.* (2010), and Sofowora (2008), plants adapt to harsh climatic conditions and variety of anthropological activities that may affect their survival by developing different survival characteristics and molecular diversity. These molecular diversities are exhibited in the DNA barcodes as slight variations between the same species of plants. This explains the variations that were evident in the in the DNA barcode of the reference and verification data that were developed in this study.

This diversity also accounts for the possibility of DNA barcoding to trace species to their respective sources if enough DNA reference database exist. Plants in the same vegetation zone under the same edaphic and environmental conditions will be subjected to the same survival challenges thereby producing similar genes slightly different from their counterparts in different vegetation zones under different edaphic and ambient environmental conditions. These differences in genes will be evident in their DNA barcodes which can be linked to their possible source with available DNA

reference data. It must be noted however, that DNA barcoding operates on the assumption that the gene region which is chosen has lower 'within-taxon' variation than that 'between-taxa' ([www.barcodinglife.org](http://www.barcodinglife.org)), and that no matter the variations within the same species; they can be perfectly separated from other species even if they are of the same genus.

These molecular diversities otherwise known as secondary characteristics determine the medicinal value of a plant (Birdi *et al.*, 2008 and Wink, 1999). It is therefore evident that the genetic diversity can affect the medicinal value of a plant and also explains why the same plant species from different areas produces different compounds for the treatment of different diseases (Sofowora, 2008). To cultivate medicinal plant species, it is therefore important to take into consideration the environmental conditions, soil characteristics, topography and also reduce anthropological influences as far as possible in order to achieve the desired characteristics of the medicinal plant species needed.

From the above discussions it is clear that DNA barcoding is the best identification tool with the potential of eliminating all forms of mis-identification. It will also facilitate better records on medicinal plant species which will aid in ensuring their sustainable harvest and also help to check and regulate the harvest of each medicinal plant species.

## 5.2 Social survey

### 5.2.1 Nature of the herbal medicine industry

Herbal medicine industry is widespread and highly important for affordable healthcare in Ghana. It is a major source of livelihood and income for most of the respondents, mostly the women who engage in it as their major occupation (Ofori *et al.*, 2012). The sustainability and availability of medicinal plants is a problem to the industry since most of the plants species in trade (82.61%) found in this survey were obtained from the wild. In the same vein, Ofori *et al.* (2012), found 78.75% of the 160 different plants species coming from the wild.

All the 81% of the 69 plants species found in this survey were common to the areas where they were harvested or used. However, Agbovie *et al.* (2002) found 71.42% of 399 species of plants to be available in the areas they were used. This is explained by the fact that most medicinal plant collectors like herbalists are familiar with the plants in their vicinity and thus deal in them. However, the medicinal plant sellers buy their products from different people from different places and thus have knowledge in plants from various places on Ghana. Van Andel (2006) and Van Andel *et al.* (2012), have both recorded that common plants to an area are largely used in herbal medicine preparation. It is therefore imperative for each community to make conscious efforts to protect their own medicinal plants to continuously enjoy cheap and affordable herbal medicines.

### **5.2.2 Plants and plants parts used in herbal medicine preparation**

Same plants species can have more than one chemical constituent that functions against several disease-causing organisms, hence the ability of one plant to serve as a cure for different unrelated diseases (Birdi *et al.*, 2008, Sofowora, 2008). Furthermore, the same species of plants at different locations can function differently due to environmental, edaphic, climatic and anthropological effects which cause the development of molecular diversity which affect the medicinal value and function of the same plant species (Kamal *et al.*, 2010; Sofowora, 2008). These explain why fifteen plants species were used for the treatment of both diarrhoea and male sexual weakness. Furthermore, different preparation methods of medicinal plants may yield different active ingredients which can cure different diseases. Hence methods of herbal medicine preparation are very crucial in obtaining an effective herbal drug for the intended purpose (Birdi *et al.*, 2008).

### **5.2.3 Factors that influence the parts of plants used**

In present days, literature shows that herbal medicine is mostly sold in the form of leaves of entire plants (Bye & Linares, 1983; Macía *et al.*, 2005; Van Andel *et al.*, 2007). However literature has it that about 50% of the herbal products sold in the markets of Eastern and Southern Africa are dominated by bark and roots of medicinal plants species (Cunningham, 1997; Williams, 2007; McMillen, 2008). A market survey by Van Andel *et al.* (2012) revealed that 20% of the floristic diversity of the Ghanaian herbal market is made up of underground parts of plants and 6% of the daily volume of medicinal plants offered for sale consists of underground plant organs. The result obtained from this study is different from that of Van Andel *et al.*

(2012) and others that have been reported. While 14.5% of the floristic diversity of the study area was made up of leaves, 25.19% was made up of plant bark and 30.78% of underground plant organs. This may be due to the fact that the survey was conducted in only the dry season and this might have influenced the part of the plants available for use in herbal medicine preparation. Furthermore, the survey areas (Kasoa and Nyanyano) are coastal areas with vegetation similar to that of greater Accra whose herbal market is made up of only 17% leaves (Cunningham, 1997; [www.ghana.gov.gh](http://www.ghana.gov.gh)) and this might account for the herbal markets of the study areas also making up of 14.5% of leaves. Cunningham (1997) argued that due to local climatic conditions, leaves represent the bulk of the species diversity on the markets of Abidjan (Ivory Coast), much more than in Greater Accra Region (Ghana). According to Van Andel *et al.* (2012), the parts of plants used in herbal preparation are partly determined by climatic conditions. This may be due to the fact that, in the humid West African forest zone, leaves are abundant all year round, whereas in dryer regions, such as East and South Africa and the Dahomey Gap (which stretches from Accra to Western Nigeria), leaves are not abundant throughout the year and therefore people rely more on roots, seeds and bark which have a longer shelf-life, and can be transported over longer distances without perishing.

The part of the plant used in herbal medicine preparation is very crucial to the survival of the plant itself. Collection of leaves is less destructive compared to the collection of roots, bark and worst of all whole plant as in the case of chewing sticks (Blay, 2004). In view of this, collection of medicinal plants in southern Ghana is unsustainable due to the large composition of roots and bark (Akerle *et al.*, 1991 and Cunningham, 2001). This calls for effective management and conservation efforts and the practice of non destructive and sustainable plant collection methods such as the

collection of leaves, controlled bark and roots collection by allowing plants ample time to recover from the shock of the previous collection before revisiting the same plant for new/fresh collection.

#### **5.2.4 Problems with identifying market samples**

According to McMillen (2008), traditional healers often work with powdered medicine, which is easily made from dried materials, hence making market identification very difficult. Van Andel *et al.* (2012), argue that, in African markets, roots, bark and wood are much more common, and most vendors buy their medicinal products (mostly dried or chipped) from commercial plant collectors, hence plant identification is very difficult. At times, seeds must be cultivated before identification is possible, and in the case of bark and roots, harvesters must sometimes be followed to the source of availability before identification can be made. Therefore ethnobotanists should make an effort to collect the medicinal products (bark, roots and seeds) as well when making voucher specimen. Van Andel *et al.* (2012), proposed the use of molecular techniques such as DNA barcoding in the identification of medicinal plant products such as roots, barks, shredded or powdered materials in order to prevent possible misidentification.

In this survey, 75% of the respondents obtained their herbal products from plant collectors. These products were mostly pre-processed (dried, chipped, or at times powdered) before getting to the middlemen mostly due to the long distances over which the products have to travel. This goes to support the argument by Van Andel *et al.* (2012) that identification of such products is very difficult and thus molecular identification methods of such medicinal products is the best option.

### **5.2.5 State of the herbal medicine industry**

About nine hundred and fifty one (951) tons of crude herbal products amounting to 7.8 million US dollars were sold on the Ghanaian markets in 2012 (Van Andel *et al.*, 2012). The majority of these products came from the wild but their effects on the sustainability and survival of some of these medicinal plants species are not known. Andel *et al.*, (2012) found eleven (11) tree species listed as vulnerable by the IUCN red list of endangered species in their market survey while this survey found six species to be vulnerable and one species near threatened ([www.iucnredlist.org](http://www.iucnredlist.org)). These are all evidence that most important common medicinal plant species are getting scarce and this can have serious repercussion on the health of most people particularly the urban poor and rural dwellers who depend very much on herbal medicines. It also calls for conservation of the vulnerable and threatened species to protect them from being wiped out completely.

### **5.2.6 Conservation awareness and ensuring sustainability of medicinal plants**

With the rate of loss of medicinal plants in Ghana, cultivation of important medicinal plant species is the best option despite the concerns about the effectiveness of such plants species in herbal preparation. Rukangira (2001) calls for *ex situ* cultivation of medicinal plants to be a replacement for the wild ones and must be encouraged. However, in order to meet the demand by the growing population of the world, production of synthetic compounds of the active ingredients of herbal plants species should also be encouraged.

Although 96% of the respondents in the survey were aware of the reduction in the availability of some medicinal plant species, only fifteen percent (15) had planted some of the important species that they use in their backyard and farms. According to Ofori *et al.* (2012), 25% of the 295 people who acknowledged the fact that medicinal plants cultivation would be a profitable venture, were making efforts to cultivate or retain some species on their farms and gardens for their own use. Asase *et al.* (2005) confirmed in an ethnobotanical survey of some Ghanaian anti-malaria plants that some species with medicinal uses are not abundant and the worst of all, people in that study area were not fully aware that some of their medicinal plants species were becoming threatened or extinct and that no efforts at all were made to cultivate or conserve such plants species.

To effectively deal with the over-exploitation of medicinal plants, Cunningham (1993), called for a clear understanding of the scale and complexity of the problem. According to Cunningham (2001), information about some of these medicinal plants is not complete and that the growing demand of such plants will intensify the pressure on the wild population leading to local extinction. Bisi-Johnson *et al.* (2009) therefore called for an urgent documentation of all medicinal plants of Africa. This will inform on policies towards their conservation and management.

Controlling problems such as habitat destruction, over-exploitation, wild fires among others, which fuel the extinction of medicinal plants species, will go a long way to sustain the herbal medicine industry. Even though many people are aware of forest conservation, 28% of respondents in this study think it can have any impact on the mitigation of the declining medicinal plants population but cultivation of medicinal

plants and controlled logging can reverse the declining populations of medicinal plants species in southern Ghana.

The fear of people losing farmlands to conservation and others losing the direct and indirect benefits of forests such as game and other non-timber forest products such as firewood, snail and mushroom collection among others accounts for the strong rejection of forests conservation by the respondents. For people to accept and involve themselves in forest conservation, it is very appropriate to practice integrated conservation methods whereby non-timber forest products are sustainably harvested while the forest is being protected.

Conscientious efforts should be made to protect our medicinal plants species by practicing appropriate and sustainable harvesting methods and cultivation of some valuable and vulnerable species.

## CHAPTER SIX

### 6.0 CONCLUSION AND RECOMMENDATIONS

#### 6.1 Conclusion

Herbal medicines play important role in the health care of Ghanaians most especially the urban poor and rural dwellers. However, correct identification of the plants species used in the preparation is crucial to the safety and efficacy of the resulting herbal medicine. The problem is even worse when it comes to the identification of market samples which are mostly dried, macerated or powdered.

DNA barcoding, a diagnostic tool that uses species-specific DNA sequence(s) for identification of all species, has proven to be the best tool for identifying medicinal plants as well as all other plants species. DNA barcoding has a hundred percent ability to identify all medicinal plants to the species level. It also has the ability to identify dried, macerated and powdered species alike. DNA barcoding if accepted for use can help in the provision of better records which will inform on better conservation of vulnerable species and the sustainable harvesting of such plants.

*Khaya senegalensis* is the commonest used tree species for the treatment of male sexual weakness whereas *Psidium guajava* is highly known for the treatment of diarrhoea in southern Ghana. *Rauvoulfia vomitoria* is highly used for the treatment of both diarrhoea and male sexual weakness. The rate of harvest of medicinal plants in southern Ghana is quite alarming and most of these species will be wiped out if no efforts are made to conserve and also control their harvesting. Roots and bark of trees dominated the herbal medicine industry in southern Ghana. However the harvest of both parts (roots and bark) is highly detrimental to the survival of tree species.

Six (6) tree species were listed as vulnerable, one species near threatened, one was of least concern and the rest have not been evaluated by the IUCN red list of endangered species. About two 2500kg of parts of tree species used in the treatment of diarrhoea and/or male sexual weakness were collected within a month in the southern parts of Ghana.

There is a high level of awareness of forest conservation. However, respondents believe it has little impact on ensuring the continued survival of medicinal tree species in southern Ghana. Controlling the underlining factors such as habitat destruction over-exploitation among others that fuel the extinction of biodiversity and the cultivation of medicinal plant species will go a long way to ensure the survival of medicinal tree species in Ghana.

## **6.2 Recommendations**

All herbal plants species should be authenticated by DNA barcoding before they are used in the preparation of herbal medicine so as to prevent possible mis-identification.

Both standard DNA regions *rbcL* and *matK* should be barcoded in subsequent researches in order to achieve a hundred percent (100%) sequence success rate for all tree species.

Proper records on herbal plants collections should be kept.

Herbal market surveys should be done on seasonal bases to find out the seasonal variations in parts of medicinal plants that are used.

The effect of herbal collections on tree species should be evaluated.

The cultivation of medicinal tree species should be encouraged in order to reduce the pressure on the wild species.

Production of synthetic compounds of the active ingredients of medicinal plants should also be encouraged so as to support large scale production of such drugs to feed the growing demand of the world.

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## APPENDICES

### Appendix 1

#### Sample of interview guide used in the survey

This is completely a student research which seeks to achieve the following goals:

- To ensure the sustainability of medicinal plant species in Ghana.
- To ensure that safer herbal medicines are delivered to the local and international markets.

Respondents are however assured that information will be treated as confidential as possible. It will be a great pleasure if you can willingly respond to the following questions.

#### PART 1

##### Demography of respondents

1. Name of community.....
2. Sex                                   A. Male [    ]    B. Female [    ]
3. Age in years A. 20-29 [    ] B. 30-39 [    ] C. 40-49 [    ] D. 50 and above
4. What is your main occupation?.....
5. What other source of income activity do you have?.....
6. What is your level of education? A. Basic [    ] B. Secondary [    ]  
C. Tertiary [    ] D. Others specify.....

#### PART II

7. Can you name the plants you use to treat diarrhea? .....
- .....

8. Can you name the parts of each of the plants named above you use to prepare the herbal concoction? .....
- .....
9. Can you estimate the quantity you use/sell per week? .....
- .....
10. Can you name the plants you use to treat male sexual weakness? .....
- .....
11. Can you name the parts of each of the plants named above you use to prepare the herbal concoction? .....
- .....
12. Can you estimate the quantity you use/sell per week? .....
- .....
13. How do you identify the plant species you use?
14. Do you perceive any changes in the availability of any of the plants you use?  
A. Yes [ ] B. No [ ]
15. What in your opinion is the cause of the changes you perceive?  
.....
16. How in your opinion can medicinal plants be conserved?  
.....
17. Have you heard of forest conservation?  
A. Yes [ ] B. No [ ]
18. Where did you hear of it?  
.....
19. Is forest conservation of any importance to the conservation of medicinal plants?  
A. Important B. Very important C. May be D. Not important

## Appendix 2

Dried parts of some medicinal plants species collected from Kasoa markets for identification.



Photograph of medicinal plants parts collected for identification

### Appendix 3

Photograph of sample tree and a voucher specimen.



A photograph of the life form of a tree from which sample was taken



A photograph of sample prepared for herbarium voucher