DNA BARCODING IN SUSTAINABLE HARVESTING OF TREE SPECIES AND SAFETY OF HERBAL MEDICINES USED IN THE TREATMENT OF MALARIA IN SOUTHERN GHANA

BY

CHARLES OBENG APPIAH

THIS THESIS IS SUBMITTED TO THE UNIVERSITY OF GHANA, LEGON IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF MPHIL ENVIRONMENTAL SCIENCE DEGREE.

DECEMBER, 2014

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DECLARATION

I hereby declare that, except for references to other peoples' work which have been duly cited, this work is the result of my own research under supervision and that this thesis has neither in whole nor in part been presented for another degree elsewhere.

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ABSTRACT

Herbal medicines continue to be used in every country around the world in healthcare delivery, however, due to poverty and high doctor to patient ratio; about 75% of the population of the developing world solely rely on herbal medicine for their primary health care. It is thus imperative to develop measures to maximize the medicinal potentials of indigenous plants to reduce the number of lives lost through diseases. However, most herbal products sold in public places lack scientific evidence for safety, quality and efficacy, and 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 to authenticate the identity of medicinal plants species used in the preparations of herbal medicines used to treat malaria and their sustainable management in southern Ghana. Leaf samples of 50 different plant species were collected in duplicate from three biodiversity hotspots; Bia Biosphere Reserve, Ankasa Resource Reserve and Kakum National Park and their DNA extracted and sequenced at the rbcLa gene region to serve as a background database for identification of those species. A success rate of 80% was achieved. A cladogram was then generated from these sequences together with sequences of other tree species from Ghana in the Gene Bank of Consortium for the Barcode of Life Database for comparison. Each of the sequences correctly matched their counterpart which means that DNA barcoding can be used in the identification of all medicinal plants species. The study also sought to find out the medicinal tree species used in the treatment of malaria, and how herbalists identify the plant species they use. Structured questionnaire and interview guides were used to seek information from herbalists, commercial herbal plants collectors and sellers in two different markets; Kasoa and Nyanyano, and the communities around three biodiversity hotspots; Ankasa, Bia and Kakum. Morphological identification was the only available method used for identification of medicinal plant species. Fifty plant species were found to be effective for the treatment of

malaria in southern Ghana. Roots, bark and leaves were the commonly used part of the plants in herbal medicine preparation. The parts of plants used can affect the survival of some plants species. There was a hundred percent level of awareness of forest conservation in southern Ghana. DNA barcoding is the best identification tool for medicinal plant species which when accepted for use will completely eliminate misidentification and help in the proper documentation of medicinal plant species. This will inform on the proper conservation and management options for the protection of vulnerable species as well as the controlled harvesting and trade in vulnerable and important medicinal plants.

DEDICATION

This work is dedicated to my wife Capt. Mrs. Juliana Obeng Appiah and my children Nana Kwasi Appiah, Adeapena Maame Yaa Adutwunwaa Appiah and Aseda Ewurabena Maafoah Appiah for the sacrifices they made on my behalf while l was pursuing my Program.

I also dedicate it to my parents Mr. and Mrs. Appiah for their selfless love and inspiration that has made all this possible as well as my Former District Director of Agric Mr. P.E. Azidoku for the immersed support he rendered me through the course of my studies.

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LIST OF ABBREVIATIONS

ACT	Artemisinin-based Combination Therapy
CBOL	Consortium for Barcode of Life
CDB	Convention on Biological Diversity
CIA	Chroloformisoamyl alcohol
CITES	Convention on International Trade of Endangered Species
COI	Cytochrome oxidase-I
CTAB	Cationic surfactant hexadecyltrimethylammonium bromide
DNA	Deoxyribonuclei acid
GAP	Good Agricultural Practises
GAIT	Global Access to Information & Communication Technology
GDP	Gross Domestic Product
GMP	Good Manufacturing Practises
matK	Maturase K
NTFPs	Non-Timber Forest Products
PCR	Polymerase Chain Reaction
rbcL	Ribulose-1, 5-bisphosphate carboxylase oxygenase large subunit
rpoB	RNA polymerase, beta-subunit
UNICEF	United Nations Children's Fund

UNCTAD	United Nations Conference on Trade and Development
USAID	United States Agency for International Development
WHO	World Health Organization

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Herbal medicines continue to be used in every country around the world in healthcare delivery. The World Health Organization estimates that in most of the developing world, 75% of the populations rely on some form of herbal medicines for their primary healthcare needs (WHO, 2002).

The word herb, as used in herbal medicine or botanical medicine means a plant or its part that is used to make medicine to assist the healing process during illness and disease condition (WHO, 2000). An herb can therefore be leaf, stem, root, seed, fruit, flower, or bark with medicinal properties, which may be used in many forms for example, fresh, dried, cut, as a powder, ointment, oil extract, or made into liquid by infusion (Amenuvor, 2006).

Herbs have provided humans with medicine from the earliest beginnings of civilization. Throughout history, various cultures have handed down their accumulated knowledge of the medicinal use of herbs to generations after them. This accumulated knowledge that has been passed on from generations to generations serves as the basis for herbal medicine practice today (Amenuvor, 2006).

All over the world plants and their extracts serve as the main source of ingredients for many synthetic drugs some of these include morphine, codeine, aspirin, and ephedrine among others. The western world saw herbal medicines as an “old wives tale” and few people believed they could really work. Large pharmaceutical companies, who made lots of profits from synthetic medicines, did nothing to disprove this misconception by the western world (Amenuvor, 2006).

United Nation Agencies, United Nations Conference on Trade and Development (UNCTAD) and Global Access to Information & Communication Technology (GAIT) have indicated in various reports (WHO, 2005) that 33% of drugs produced in the developed countries come directly from higher plants; 25% of these come from tropical plants. Another 27% of drugs come from lower plants and microbes. It is estimated that about 80% of all drugs in use owe their origins either directly or indirectly to medicinal plants (Mills & Bone, 2005).

The World Health Organization (WHO, 2008) has adopted a policy of encouraging the development and utilization of herbal medicine in the Primary Health Care delivery system, in third world countries. This policy is based on the sound recognition of the role of herbal medicine in the health care programs in most developing countries especially in Africa, Asia and Latin America. The organization estimates that between 60 to 90% of the populations of these countries rely on medicinal plants totally or partially for their health care needs, and Ghana is no exception. Hence there is no doubt that the herbal medicine practitioners will be the first point of contact to treat most illness.

Before synthetic drugs came into widespread use, herbal medicine played an important role in human healthcare delivery especially in the control and cure of malaria, and other diseases since the issues of availability and affordability were critical. Malaria is caused by a single celled protozoan parasite called *Plasmodium* spp and transmitted to man through the anopheles mosquito. It is one of the major fatal diseases in the world, especially in the tropics and endemic in about 102 countries with more than half of the world population at risk (Smyth, 1994).

In Ghana 3.5 million people contract malaria every year, with approximately 20,000 people dying from the disease annually, of which 38% of the deaths are children under the age of five (Ahorlu *et al.,* 2005). Even if a child survives, the consequences from severe malaria

such as convulsions or brain dysfunction can hamper schooling and long-term development. Malaria can reduce the effectiveness of labor leading to both economic and human loses (Srisilam & Versham, 2003). The annual economic loss to malaria is estimated at 1-2 percent of the Gross Domestic Product (GDP) of Ghana (UNICEF, 2007).

Considering the poverty level of some rural Ghanaians, the unavailability of health post in some rural areas and the cost of treatment of malaria, it is imperative to identify, authenticate and ensure the availability of plant species with the potential of curing this disease to be used in remote and poor communities (Njume & Goduka, 2012).

The vision of WHO's Department of Essential Medicines and Pharmaceutical Policies is that "people everywhere have access to the essential medicines they need which 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/). However, some writers have argued that, 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).

Van Andel (2006) and Njume & Goduka, (2012) have both argued that the demand for medicinal plants is gradually increasing in both developing and developed countries, and a large market exist worldwide for such products. This demand has put much pressure on most common species of medicinal plants which can lead to potential over exploitation. In the same vein, the scarcity of a plant in high demand, for instance *Khaya senegalensis* in the case of Ghana, can compel some commercial collectors to adopt bad collection practices in order to meet the demand of their customers. Other unfaithful collectors may collect congener or surrogate species which at times are poisonous and can cause various undesirable

consequences, even death (Guo *et al.*, 2011; Abbiw, 1990). To make herbal medicine safe and sustainable, there is the need to adopt proper measures to resolve this problem.

DNA barcoding has the ability to correct possible misidentification which is a shortcoming of morphological identification. It can also prevent the trade in red listed species both locally and internationally (Coyle, 2001) by identifying all species whether processed, semi-processed, fragmented, crushed, macerated, among others which will be very difficult to identify using their morphological features. This makes it the recommended tool to address the problems that are associated with safety of herbal medicines through poor identification of plant materials. This work focuses on DNA barcoding plant species used in the treatment of malaria in southern Ghana to be used as a reference data for the identification of such plant species. DNA barcoding is a diagnostic technique that depends on short DNA sequence(s) for species identification and has the ability to differentiate between congener species. This tool works on the principle that this short DNA sequences chosen have lower "within taxon" variation than that "between taxa" and can thus be applied to distinguish between closely related species (www.barcodinglife.org) which is difficult to be done by morphological identification.

1.2 Statement of the Problem

Plant mis-identification may be due to similarities in appearance during bulk purchase, when harvested or at the time of collection (Boulata & Nace, 2000). Poor identification and the wrong use of herbal medicines have resulted in adverse effects which in some instances have caused the hospitalization and death of the consumers (Abbiw, 1990). This situation has made the consumption of herbal medicine risky and unsafe especially when its source is unreliable. Proper collection methods and identification is fundamental for sustainable and safe herbal medicine industry.

Recently people all over the world recognize the importance of herbal medicine in healthcare and this has led to the growing demand and a large market for medicinal plants. However, the continued availability of most of these plants is questionable due to over-collection, deforestation and habitat destruction, coupled with climate change and rampant bush fires (http://www.unepwcmc.org/species/plants/ghana).

1.3 Justification

According to WHO (2002), herbal medicine is one of the means to achieve total healthcare coverage of the world. Moreover with a medical doctor to population ratio of about 1:12,000 and an indigenous healer to population ratio of about 1:400 in Ghana (Busia, 2007), many people will inevitably seek treatment from the indigenous health systems (Njume & Goduka, 2012). Developing measures to maximize the medicinal potentials of indigenous plants can reduce the number of lives lost through a deadly disease such as malaria (Njume & Goduka, 2012). On the contrary Wambebe, (2008) has reported that, most herbal products sold in public places lack scientific evidence for safety, quality and efficacy, hence the need to research into these areas.

However, the safety of herbal medicine largely depends on the ability to correctly identify the plants species used in their preparation. Accounts of a woman died at the University of Ghana Hospital in 1984 after mistakenly taking an herbal concoction of *Erythrophleom suaveolens* instead of *Khaya senegalensis* and an incidence of *Erythrophleum guineense* poisoning of two men in Saltpond which also resulted from mis-identification have been given by Abbiw (1990). There is therefore the need for a more definite tool of identification that can solve the issue of mis-identification and its fatal consequences in the herbal medicine industry.

Van Andel *et al*., (2012) proposed the use of molecular methods such as DNA barcoding in the identification of market samples of species of medicinal plants parts due to the difficulties

in the morphological identification of such products. Unlike morphological identification that has been reported with about 6% error rate, DNA barcoding, is a perfect tool of identification that can be used by all including non-taxonomists and when employed can completely eliminate mis-identification from the herbal medicine industry (Dexter *et al.*, 2010; Marshall, 2005).

With the limited health posts mostly in the rural parts of the country, coupled with the attitude of some Ghanaians seeing the hospital as the final point of contact to the cure of diseases perhaps due to poverty, it is imperative to authenticate and encourage the use of herbal medicine which is comparatively cheap and available in all parts of the country in the treatment of malaria.

Furthermore, the high rate of deforestation, rampant bushfires, climate change, commercialization and high patronage in medicinal plants species, poses a huge threat to the survival of some medicinal plants species. Accordingly, thirty nine medicinal plant species have been exploited to the extent that they are now endangered and one species is already extinct in South Africa (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 scientific-based evidence for decision-making that will lead to the management and conservation of medicinal plant species.

1.4 Objective of Study

The main objective of this study was to use DNA Barcoding to authenticate the identity of species of medicinal plants used in the preparation of herbal medicines used to treat malaria and their sustainable management in southern Ghana.

1.4.1 The Specific Objectives of this work were;

(I). To assess plant species commonly used in the treatment of malaria in southern Ghana.

(II). To find out the parts of plants used in the preparation of herbal concoction for the cure of malaria and its possible effects on the survival of the plants species.

(III). To assess the utility of DNA barcoding as a tool in identification of species of medicinal plants

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 History of Herbal Medicine

Humans have used plants to treat their ailments for over tens of thousands of years now. The earliest written accounts of the use of herbs originated in China, although other civilizations from the ancient world were using plants as natural remedies for their ailments (Olurinde *et al*., 2010). Western herbal medicine dates back to ancient Greece with its famous doctors like Hippocrates and Galen (Olurinde *et al*., 2010). Herbal medicine, sometimes referred to as Herbalism, Botanical Medicine or Herbology, is the use of plants, in a wide variety of forms, for their therapeutic value (WHO, 2001). Herbs produce and contain a variety of chemical compounds that act upon the body and are used to prevent or treat disease or promote health and well-being (Olurinde *et al*., 2010).

The period covering the 15th to 17th centuries was the most popular time for herbalism in Europe (Olurinde *et al*., 2010). Herbal remedies are still relatively popular today, perhaps due to the fact that they are regarded as harmless with little or no side effects. As herbal treatments have been around for so long, and in so many cultures, there are numerous ways that they are believed to work. Many explanations lie in the mystical thinking of ancient cultures. Some believe that the appearance of the plant gives an indication of what it can be used to treat, the physical resemblance between the plant and the body part to be cured is known as the "'Doctrine of Signatures"; for example a heart shaped plant would be used to treat heart problems. From the middle ages on, many practitioners have tried to classify herbal remedies by observation of their effects. This is closer to the modern scientific approach of gathering evidence (Olurinde *et al*., 2010).

Eastern herbal medicine still adheres to some of the mystical approach in its theories whilst Western herbalists tend to use herbs for the ingredients they contain, mixing and matching them in the way that conventional medicine does with modern drugs. Remedies are produced by either taking the whole plant, or just the part of the plant required, or often mixing it with other plants. They are usually boiled in water or alcohol, and made into herbal teas, decoctions, syrups, tinctures, infused oils, salves, and ointment/creams (Lucy and Edgar, 1999).

Over three-quarters of the world population relies mainly on plants and plant extracts for healthcare needs (WHO, 2003). More than 30% of the entire plant species, at one time or other was used for medicinal purposes (Baird *et al*., 1996). The FAO in 1999 reported that in Japan, herbal medicinal preparations are more in demand than mainstream pharmaceutical products. The last three decades have seen substantial growth in herb and herbal product markets across the world. Research have shown that as a result of the combination of various plants in the production of herbal medicine to combat malaria it is difficult for the parasite to develop resistance for most of the herbal preparations on the market today (Baird *et al.*, 1996).

2.2 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 reckon with in achieving one of the targets of the Millennium Development Goals on health (Bisi-Johnson *et al*., 2009). World Health Organization (WHO, 1978), argued that, herbal medicine is one of the means to achieve total health care coverage of the world's population. However, Bisi-Johnson *et al*., (2009) opined that, this form of health care system has long been relegated to a marginal place. 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. This has seen many countries including Ghana, worked towards the incorporation of herbal medicine into their main stream of health care. The many herbal hospitals in Ghana; Agbeve herbal hospital, Amen scientific herbal hospital, Kingdom herbal hospital among others, 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), and the many ethno-botanical 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; Bever, 1987). It is therefore imperative to protect and maintain these plant species.

2.3 Treatment of malaria with herbal medicine in Ghana.

Ailments have over the years been a scourge and a threat to mankind (Gyasi *et al.,* 2011). People from different cultural backgrounds have used different herbal plants, plant extracts, animal products and mineral substances (Addae-Mensah, 1992) as the means to care, cure and treat ill-health, with disease prevention, and with health promotion (Curtis and Taket, 1996) since pre-historic times. Traditional Medicine embraces the ways of protecting and restoring health that existed before the arrival of orthodox medicine (WHO, 2001). In spite of control programmes in many countries there has been very little improvement in the control of malaria and infections can reduce the effectiveness of labour and can lead to both economic and human loses (Asase *et al.*, 2005). Control of malaria is complex because of the appearance of drug resistant strains of *Plasmodium* and with the discovering that man may become infested with species of simian (monkey) malaria (Symth, 1994). It is therefore

important to search for new anti-malarial compounds, either synthetic or natural compounds that kill either the vector or parasite.

The use of plant-derived drugs for the treatment of malaria has a long and successful tradition (Asase *et al*., 2005) and, several plant species including *Alstonei boonei* De Willd (Apocynaceae), *Azadirachta indica* A. Juss, (Meliaceae), *Cryptolepis sanguinolenta* (Lindl.) Schttr. (Asclepidaceae), *Morinda lucida* Benth. (Rubiaceae), among others are used in the treatment of malaria (Abbiw, 1990).

The popularity and wide use of herbal medicine for the treatment of malaria has been attributed to its cost effectiveness, less side-effects, efficacy and availability of the drugs.

2.4 Policies and Regulations of herbal medicines in Ghana

Though Ghanaians are allowed to practice traditional medicine, they are bound by the restrictions contained in the Poisons Order (1952) and are limited by the use of the substances listed therein. They are further permitted by the Medical and Dental Decree of 1972 and the Nurses and Midwives Decree of 1972 to practice safe herbal medicine (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.

The effort of the Ghana Association of Traditional Medicine to bring all herbal practitioners together in order to supervise their activities has not been easy due to the informal nature of the herbal medicine industry, the huge number and the fact that they are widely spread throughout the nation.

By law, medical, health and nutrient content claims may be made. The Ghana herbal pharmacopoeia was published in 1992; it is not considered to be legally binding. The national pharmacopoeia also contains monographs on herbal medicines. Regulatory requirements for manufacturing of herbal medicines include the same Good Manufacturing Practices (GMP) rules that apply to conventional pharmaceuticals and special GMP rules. While a large proportion of manufacturers of herbal medicines in Ghana are small scale industries, efforts have been made to provide training in GMP compliance. Implementation of the manufacturing requirements is ensured through annual inspections. Safety assessment requirements include traditional use without demonstrated harmful effects, reference to documented scientific research on similar products and phytochemical analysis. Compliance with these requirements is ensured through the pharmaco-vigilance centre.

The general lack of knowledge about herbal medicines within national drug authorities and the lack of appropriate evaluation methods are factors that delay the creation or updating of national policies, laws and regulations for traditional and herbal medicines. In order to meet these challenges, the WHO Traditional Medicine Strategy was developed, with its four primary objectives: framing policy; enhancing safety, efficacy and quality; ensuring access; and promoting rational use of herbal medicines. This resolution is to assist its member states to formulate policies and laws to control their herbal medicine resources

2.4.0 Challenges facing the herbal medicine industry in Ghana

2.4.1 Biodiversity Loss

Rapid Assessment Program, (2007) and Dudley, (2002) defined biodiversity as 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. 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 as twenty five percent of all modern drugs originally come from rainforests (Kong *et al.*, 2003). Kumar & Kumar, (2012) and Kong *et al.*, *(*2003), have both argued that, about twenty five percent of all prescription drugs in the United States of America are derived from plants.

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. According to Cragg *et al*., (1997), these natural products remain as important sources of new drugs as statistics show that between 1983 and 1994, about five percent of newly approved drugs were natural products and about thirty five percent were either compounds derived directly from natural products or synthetics based on natural products. Hence biodiversity must be well conserved in all regions to ensure continues inventory in the pharmaceutical sector.

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 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; Reid, 1992). With this alarming rate of forest loss, many of our biodiversity are under threat of extinction (Benhin &

Barbier, 2004). It is therefore important to put measures in place to conserve species of medicinal plants.

2.4.2 Effect of herbal medicine collection on biodiversity

Just as biodiversity loss affect the herbal industries, so does the activities of the herbal and pharmaceutical industries on biodiversity. Verma and Singh (2008) argued that, 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, lack of access to western medicine, poverty, high population growth and growing markets in herbal products have led to the over exploitation and unsustainable harvesting of medicinal plants in the West African sub region and thereby poses a major threat to the survival of some important medicinal plants (Hamilton, 2004; 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. 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; 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 people in 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 (Van Andel, 2006).

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. The market for ayurvedic medicines alone is estimated to be expanding at 20% annually (Hamilton, 2004). Sales of medicinal plants have grown by nearly 25% in India in the past ten years (1987-96), the highest rate of growth in the world (Masood, 1997) but the per capita expenditure in India on medicines per annum is amongst the lowest in the world.

Two of the largest users of medicinal plants are China and India. Traditional Chinese Medicine uses over 5000 plant species, India uses about 7000. According to Export Import Bank, the international market for medicinal plant related trade having a growth rate of 7%

per annum. China's share in world herbal market is US$ 6 billion while India's share is only US$1 billion. The annual export of medicinal plants from India is valued at Rs. 1200 million. All the major herbal-based pharmaceutical companies are showing a constant growth of about 15% (Mukherjee, 2003).

In Ghana, an estimated 60–80% of the rural communities depend primarily on herbal medicine for the treatment of different ailments. Dependence on medicines derived from indigenous plants is especially predominant in rural areas where modern western medicine is often unavailable or is simply too expensive (NEWMONT, 2007). The WHO reports that its quantitative market survey reveals that the trade in Ghana's herbal medicine is of considerable economic importance and its medicinal flora has been well documented since much research has been devoted to traditional healers and their possible cooperation with western-trained health personnel. Plants sold at the market were mostly used for malaria and other infections, women's health, rituals, aphrodisiacs and against sexually transmitted diseases (WHO, 2000). An estimated 951 tons of crude herbal medicine were sold at Ghana's herbal markets in 2010, with a total value of around US$ 7.8 million (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; 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).

2.5.0 How to ensure sustainability of the herbal medicine industry

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

Given the demand for a continuous and uniform supply of medicinal plants and the accelerating depletion of forest resources which has led to rapid loss of biodiversity, cultivation of medicinal plant species appear to be an important strategy for meeting a growing demand (Uniyal *et al.,* 2000). According to Ramakrishnappa, (2002), the present rate of decline of 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.

Although cultivation of medicinal plants can be a perfect option of ensuring continuous supply of medicinal plants, concerns have been raised about the efficacy of cultivated plants as raw materials for herbal medicine (Birdi *et al.,* 2008). According to Cunningham, (1994) cultivated herbal plant materials are abhorred by herbal medicine practitioners in Botswana on the claim that such plant materials have no medicinal powers like that of the wild.

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; Wink, 1999) which may not be expressed in optimum quantities when cultivated under optimum conditions to obtain better vegetative yields. In view of this, Robbins (1998) reported that wild ginseng roots are five to ten times more valuable in terms of medicinal properties than cultivated ginseng roots. Ellenberger (1998), found a vast difference in the

biochemical properties between cultivated *Arnica montana* and that of the wild which may affect their medicinal properties as well.

However Rukangira (2001) opined that *ex-situ* cultivation of the desired medicinal and aromatic plants should rather be encouraged so as to obtain conditions similar to that of the wild. Some authors have however argued that, while it can be presumed that cultivated plants are likely to be somewhat different in their properties from those gathered from their natural habitats, it is also clear that certain values in plants can be deliberately enhanced under controlled conditions of cultivation which can enhance their medical values (Palevitch, 1991; Uniyal *et al*., 2000).

Commercial cultivation of medicinal plants is an available option to check the continual lost of biodiversity to the herbal and pharmaceutical industries so as to conserve the rare floristic wealth and genetic diversity (Anon, 2002). In the pharmaceutical industry, where the active ingredient of a plant cannot be synthesised synthetically, a large quantity of such plant will be needed to produce the commercial scale to feed the ever growing population of the world and this can only be assured if the plants are grown on large scales for sale. We cannot only depend on the wild in such situations; hence large scale cultivation of the concerned medicinal plants is of great importance. It is also necessary to develop genetically superior planting material for assured uniformity and desired quality and resort to organised cultivation to ensure the supply of raw material at growers end.

Factors such as topography, geology, soil, climate and vegetation can affect the medicinal properties of a medicinal plant and must thus be considered when setting up medicinal plant farms (Sofowora, 2008). This should be done in consultation with traditional herbalist to identify the location of an effective plant for the cure of a specific disease. This has long been demonstrated by traditional herbalists, even though they may not be aware of the theory

behind it. Plants of specific locations are required by these herbalists to treat specific diseases. At times it demands that they go into sacred grooves where mankind is prohibited from going except by permission from the authorities in charged. Though these have long been termed spiritual, it practically signifies the potential differences in the medicinal properties of plants at different locations, topography, ecology and even type of soil. Safowora, (2008) clearly pointed out that, *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.

To cultivate a very useful and potential herbal plant, it is imperative to know the ecological conditions that prevail at the places where the herbalist prefer to take the plant in question from and try to plant them in a similar if not the same ecological area. 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 collect herbs to treat specific diseases most especially those plants with more than one medicinal uses. It will also give clear insight into how best medicinal plants can be cultivated in order to obtain the desired properties since the genetic barcodes of plants are location and ecological specific just like their medicinal properties (Bokhari *et al.*, 1990; Kamal *et al*., 2010; Sofowora, 2008).

2.6.0 DNA-Barcoding

DNA-Barcoding is a diagnostic technique/tool that uses short DNA sequence(s) for identification of species (www.barcodinglife.org). This tool according to Hebert *et al., (*2003) and Savolainen *et al., (*2005) 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. Scientists

have based on this to 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). Hence before DNA-Barcoding can be useful in the identification of species, there should be a database of known species through which the species will be compared.

The advantage of this tool over morphological identification is its ability to be used by both taxonomists and non-taxonomists alike and it also enables species identification at 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; Chase *et al.*, 2005).

2.6.1 DNA barcoding versus morphological identification

Chase *et al.*, (2005), have argued that, the shortage of natural history specialist coupled with the environmental samples of minute organisms from soil, water and other sources which may have very few morphological traits are all limitations of morphological identification. These accounts for the error rate of about 6.8 to 7.6% in morphological identification of all individual species (Dexter *et al.*, 2010). 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 plants are seen on the field with flowers or fruits,

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). According to Hebert *et al.*, (2003) and Marshall, (2005), 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, and on the assumption that the sequences chosen have relatively lower 'within-taxon' variation than that 'between-taxa' (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, and that can be used as a landmark for molecular identification of all trees. DNA barcoding when accepted for use as an identification tool will hasten the slow pace and reduce the stress involved in morphological identification and also expedite species identification and discovery. Though some scientist have argued that DNA barcoding cannot replace morphological identification it can greatly complement, enhance and expedite the work of taxonomists (Dexter *et al.*, 2010)

The Scientific Correspondence (2007) is of the view that, 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.

2.6.2 Some applications of DNA barcoding

DNA barcoding can be applied in various important ways including; pharmaceutical and herbal industries, forensic science, tracing of illegal trade in organisms, bio security among others. The increasing loss of biological diversity coupled with the current rate of unsustainable harvest of medicinal plants presents a daunting challenge to all stakeholders in the herbal industry which requires an urgent solution. DNA barcoding has been applied successfully in the herbal industry to unveil the activities of some unqualified herbalists 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).

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 severe damages to the liver of those who took them.

This is very likely to happen in Ghana, most especially as *Khaya senegalensis* which is well known for its uses as blood tonic and an aphrodisiac nationwide keeps on decreasing (Van Andel *et al.,* 2012).

From an unverified source, a very popular herbal blood tonic of all times in Ghana: 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.

Not only in the herbal industry is DNA barcoding useful, but has also been successfully used in the security industry as a tool in the forensic laboratory to fight crime and also to tract down drug peddlers. According to Coyle *et al.,* DNA barcoding can be used to identify strains of marijuana and other illegal plant species. The success of this 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 and 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 strengthen the possibility of its uses to prove cases in law courts based on forensic botany (http://www.promega.com). Coyle *et al.*, (2001) argued that, 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 most 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.

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. According to Congiu *et al.*, *(*2000), the same method was used in Italy to settle a lawsuit involving the unauthorized commercialization of strawberry plant.

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 or semi processed, making morphological identification very difficult or almost impossible.

However DNA barcoding can be successful for identifying processed and prepared meats, hides, among others and that the offenders can be prosecuted.

DNA barcoding can be applied in several areas including tackling and tracking illegal logging (Lowe & Cross, 2011), control invasive species (Armstrong & Ball, 2005) and has the potential to address all the shortcomings of morphological identification among others.

2.6.3 Challenges of DNA Barcoding

Though DNA barcoding has numerous advantages and uses, it does not come without some challenges. 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). 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, Herbet *et. al.,* 2004).

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. *rbc*L, *trn*L-F, etc.) appear to have too little variation' (Chase *et al*., 2005). According to 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. 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) Seven main candidates plastid regions (*rbcL, matK, rpoCl, rpoB,*

trnH-psbA, atpF-atpH, and psbK-psbI) have been proposed for use as the standard region for barcoding land plants, however Hollingsworth *et al. (*2009) argued that no single gene locus has high levels of universality and resolvability and that more than one of these seven main candidate plastid regions should be used at a time.

However, the Consortium for Barcode of Life (CBOL) has accepted and approved of two gene loci, taken from chloroplast genes called *matK* and *rbcL*, as the standard regions for barcoding land plants, but still proposes that the search for a more suitable region be continued (Janzon, 2009; www.sciencemag.org; 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.

2.6.4 DNA barcoding as a tool for biodiversity conservation

One of the arguments for promoting DNA barcoding initiatives is that genetic data have the potential to enhance conservation strategies, and indeed molecular tools are used in conservation biology to inform at different levels of analysis (Haig, 1998). DNA barcoding, in particular, can contribute to conservation policy in two important ways: by speeding up local biodiversity assessments to prioritise conservation areas or evaluate the success of conservation actions, and by providing information about evolutionary histories and phylogenetic diversity.

A central argument for the use of DNA barcoding is its efficiency: barcoding has the potential of identifying species quickly and cheaply in under a decade of research; Hebert *et*

al., (2010) argue that barcodes for 0.1 % of all described plant species have been collected. It is estimated that sequencing can take place in under 90 min at a cost of $2–5 per species, compared to several months of field work and a cost of at least $100 per specimen with morphological analyses. Rapid biodiversity assessment is a research priority given the ongoing species depletion, extinction, and over exploitation, the most critical contribution of barcoding to biodiversity conservation is facilitating biodiversity assessment cheaply and quickly where financial resources are limited. This is especially important because the vast majority of described biodiversity is in developing countries, where resources for comprehensive biodiversity assessments are lacking. This is also critical since there is higher rate of dependence on biodiversity for Health, Food and Shelter as well as for economic benefits in most of these developing countries.

A particular strength of barcoding is that it can potentially identify species from small or incomplete samples, including e.g. tiny leaves and stems, making the use of this type of data more effective. The most significant contribution of barcoding is in identifying species where morphology is insufficient, because morphological keys are only applicable at particular life stages or development (Hennig1976). Since conservation actions are spearheaded by the identification of species that needs protection in a locality DNA barcoding is an ideal tool to drive biodiversity inventory and also implement conservation actions. It 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 Materials and sampling sites

An extensive literature review was conducted to find out which medicinal tree species are used in the treatment of malaria in Ghana. Some of the works consulted include: Abbiw, 1990; Tajuddin *et al*., 2003; Hoffman *et al*., 2004; Asase *et al*., 2005; Birdi *et al*., 2010; Teke *et al*., 2010; Patel *et al.*, 2011 and Ralebona *et al*., 2012. Fifty tree species (Table 3.1) were selected based on their recorded usage for the treatment of malaria and their occurrence in the wild in Southern Ghana. These medicinal trees were subsequently collected from three different biodiversity hot spots in the country: Ankasa Resource Reserve, Bia Biosphere Reserve and Kakum National Park, all in southern Ghana.

3.1.1 Ankasa Resource Reserve

The Ankasa Resource Reserve, Southwest Ghana is near Ghana's border with La Cote d'ivoire in the Jomoro District of the Western region. It lies between latitudes 5°09' and 5°25' N and longitudes 2°29' and 2°45' W. It covers a total area of about 509 km² and the only wet evergreen protected area in its natural state with the highest Genetic Heat Index in Ghana (www.ghanawestcoast.com/gwc/ankasa.php).

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 (www.ghanawestcoast.com/gwc/ankasa.php). 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 Ankasa Resource Reserve. Ankasa Resource Reserve is however rated as

Ghana's most botanically diverse nature reserve and the world's second richest forest in terms of its variety of flora and fauna (Forestry Commission, 2010).

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 1700 mm to 2000 mm and mean monthly temperatures ranges from 24°C to 28°C (Forestry Commission, 2010). A high relative humidity of about 90% during the night and 75% in the early afternoon exist in Ankasa all year round (www.ghanawestcoast.com/gwc/ankasa.php). Figure 3.1 presents the map of Ankasa Resource Reserve with its adjourning settlements.

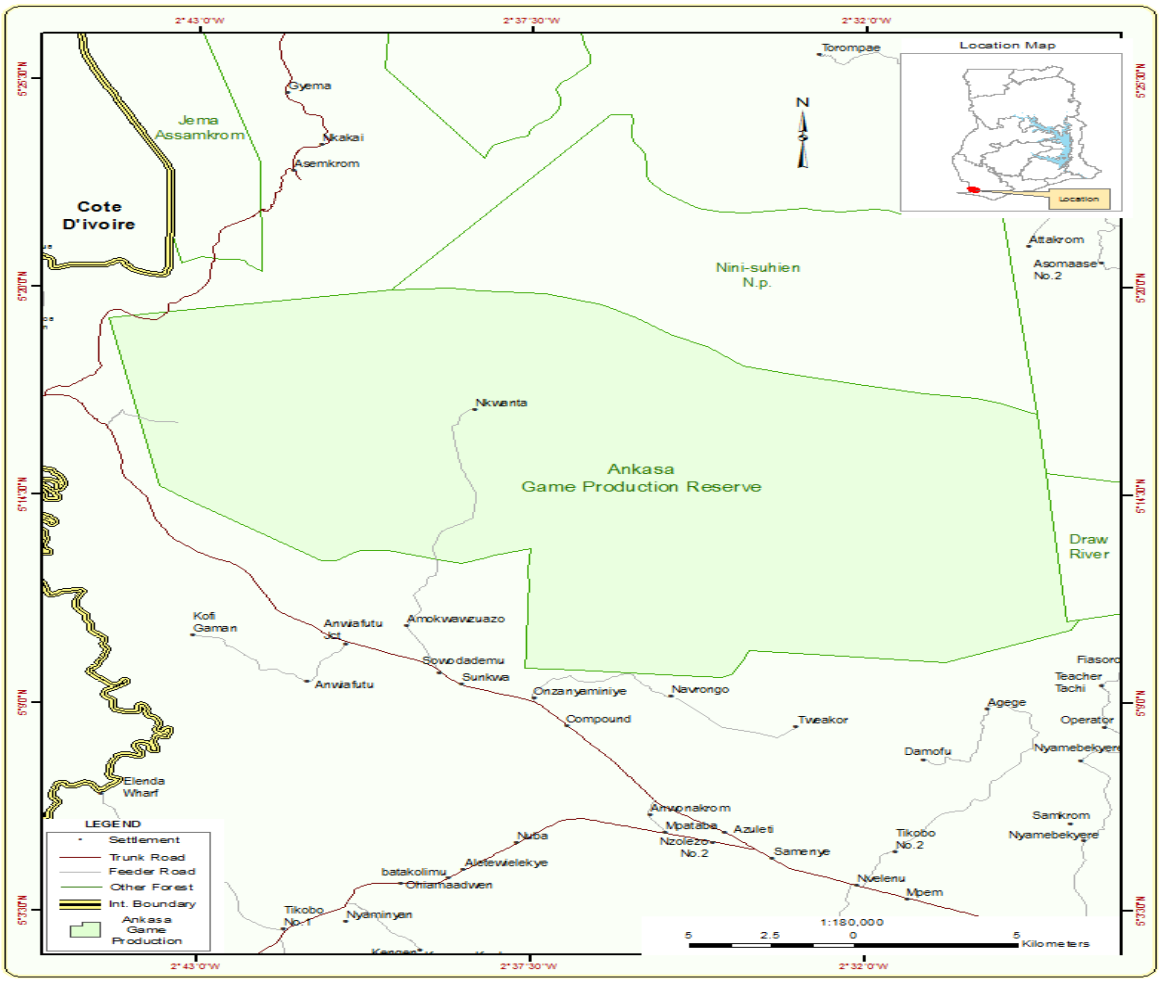


Figure 3.1: Map of Ankasa Resource Reserve

3.1.2 Bia Biosphere Reserve and National Park

The Bia Biosphere Reserve lies in Southwest Ghana near the border with La Côte d'Ivoire in the administrative district of Juabeso District Assembly. It lies between latitudes 6° 20' and 6° 38' N and longitudes 2° 58' and 3°58' W covering a total land area of about 306km² (Forestry Commission, 2010). The Bia Biosphere Reserve comprise of the Bia National Park in the North and the adjoining Bia Production Reserve in the South (Forestry Commission, 2010).

According to Hall and Swaine (1981). The Bia Biosphere Reserve is made up of two vegetation categories; Bia biosphere Reserve lies within the Moist Evergreen Forest Vegetation Zone in the South while the Moist Semi-deciduous Vegetation is in the North. 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 1500 mm 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 of Ghana's last remnants of relatively untouched forest complete with its full diversity of life. Some of the tallest trees left in West Africa are found in this national park (Forestry Commission, 2010). Figure 3.2 presents the map of Bia Biosphere Reserve and its surrounding settlement.

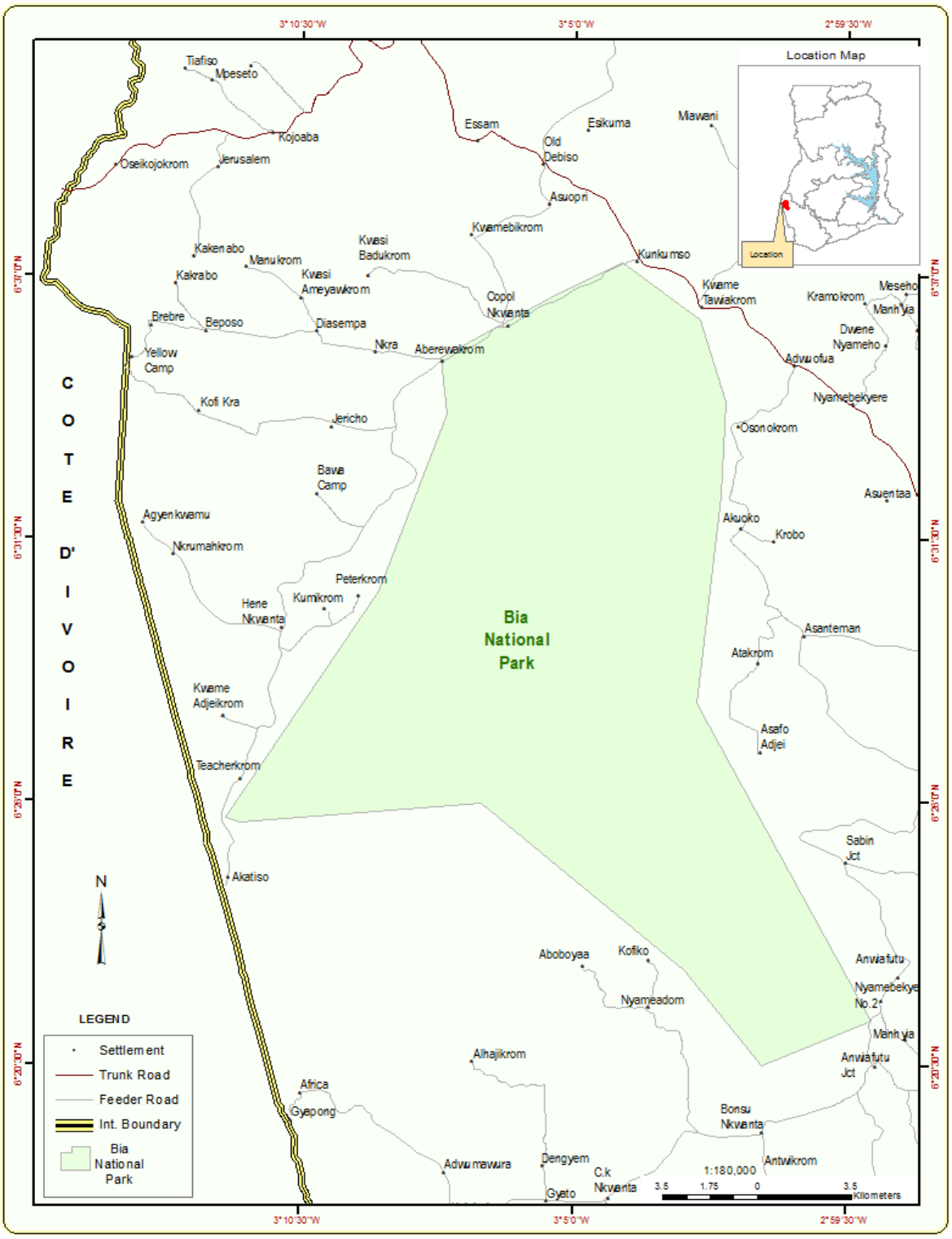


Figure 3. 2: A map of Bia Biosphere Reserve.

3.1.3 Kakum National Bark

The Kakum National Park is located in the Twifo Heman district of the Central Region of Ghana. Kakum National Park is a moist evergreen forest (Hall and Swaine, 1981), made up of about 360 km2 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 Kakum National Park has a characteristic rainfall pattern which experiences a bimodal rainy season. The major peak is between May to July and the minor falling between October to November, separated by a short dry period in August (Forestry Commission, 1996). The wet season is followed by a long dry season from December to April during which most streams dry up and rivers break into pools. The mean annual rainfall is between 1,500 mm and 1,750 mm. 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).

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). The Kakum National Park is well known for its unique canopy walk, the only of its kind in West Africa (Forestry commission, 1996). Figure 3.3 presents the map of Kakum National Park and its surrounding communities

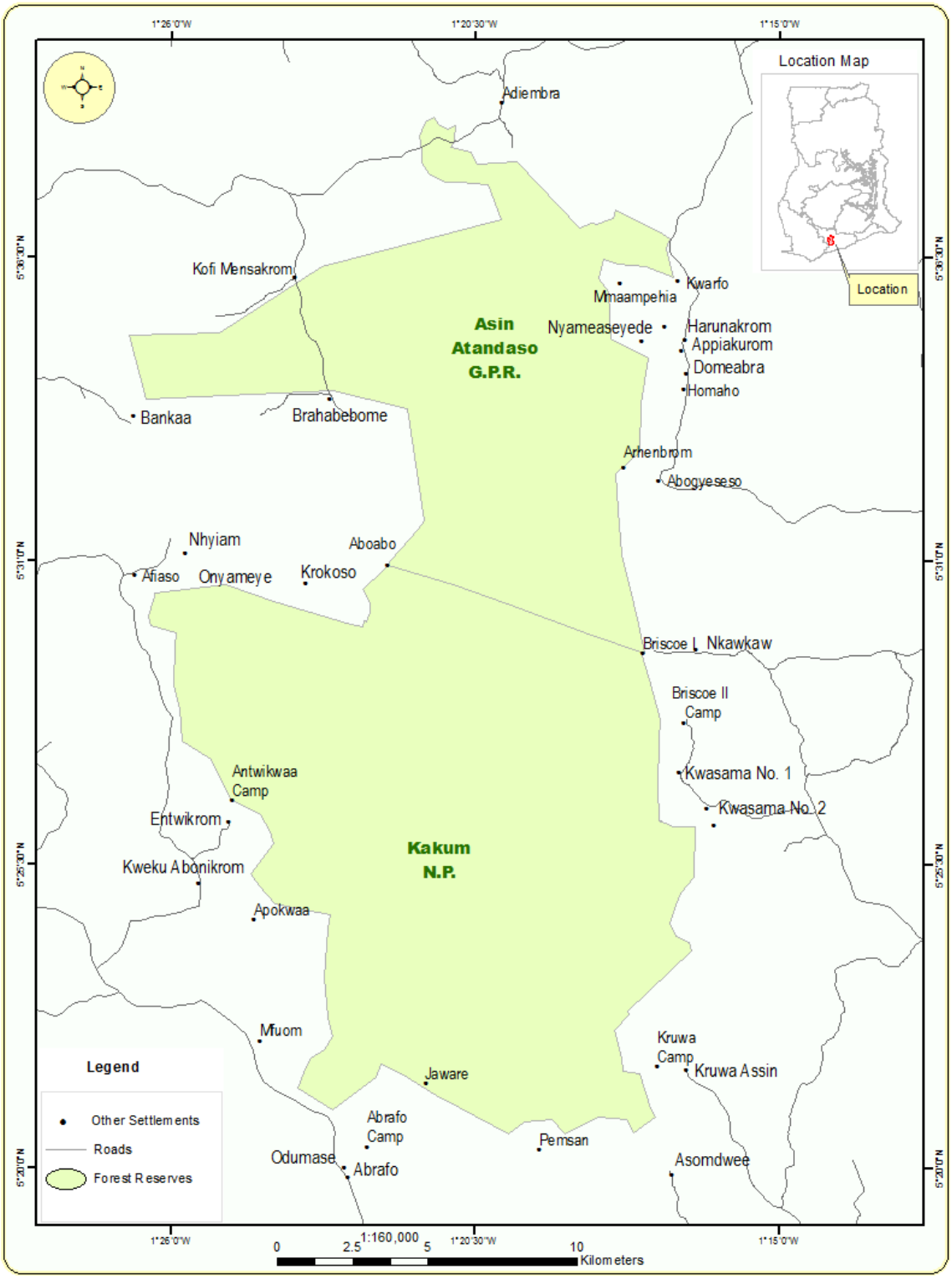


Figure 3. 3: A map of Kakum National Park.

3.2 Taxon sampling

Green healthy leaf samples were collected in duplicate or triplicate depending on spotting of the particular plant species in any of the three sampling sites during the sampling period. Fifteen of the tree species were collected from Ankasa Resource Reserve, eighteen of them were collected from Bia Biosphere Reserve while leaves of the remaining seventeen tree species were collected from Kakum National Park. To test or verify the DNA sequences generated from the leaf samples collected from Ankasa, Bia and Kakum, ten other trees were sampled from Aburi and surrounding areas in the Akuapim South Municipal Assembly. Green healthy leaf samples were collected and treated as those from Ankasa, Bia and Kakum. A pole pruner was employed to harvest the leaves when tall trees were the only source of the leaf sample. The spotting of all the tree species in the field was done with the aid of a taxonomist from the Ghana Herbarium, Department of Botany, University of Ghana. Identification of all samples was confirmed in the Ghana Herbarium.

The location of each tree species from which samples were taken was recorded with a Garmine Trex handheld GPS. A photo of the life form of each of the tree species was also taken with a Samsung digital camera. Herbarium voucher specimens were prepared and deposited in Ghana Herbarium.

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 24 hours.

3.3 DNA Extraction

Total genomic DNA was extracted from each of the 50 silica gel-dried leaves samples using a modified cetyl trimethylammonium bromide (CTAB) technique (Doyle & Doyle, 1987).

About 0.3 g of each silica-dried leaf samples were ground with a laboratory mortar and pestle. To each ground sample, one millilitre extraction buffer was added and incubated for an hour over a water bath at a temperature of 60°C with occasional swirling after which samples were cooled at room temperature. The extraction buffer was 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 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 pellets were then washed with 70 percent ethanol followed by another ten minutes centrifugation. The wash was then repeated for the pellets 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 pellets was then dried. The dried DNA was dissolved in 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.

3.4 Choice of DNA barcode regions

Even though the consortium for the barcode of life plant working group has standardized the *rbcLa* and *matK* plastid gene regions for the barcoding of land plants (Janzon, 2009; www.sciencemag.org; www.scienceamerica.org), only the *rbcLa* 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). The *rbcLa* primer *ATGTCACCACAAACAGAGACTAAAGC* was used (Kress & Erickson, 2007 and Norris *et al.*, 2009).

3.5 DNA Amplification, Sequencing and editing

Amplifications were performed using an Eppendorf Mastercycler EP Gradient S thermocycler and Sigma brand reagents and 0.5 units Sigma Jumpstart *Taq* polymerase. The DNA amplification protocol: pre-melting at 94^{o}C for 60s, denaturation at 94^{o}C for 45s, annealing at 55^{o}C for 45s and extension at 72^{o}C for 3 minutes (Norris *et al.*, 2009) for the *rbcLa region.*

The resulting PCR products were purified using QIAquick columns following the manufacturer's instructions. Cycle sequencing was done on purified PCR products using BigDye TM v.3.2 Terminator mix (Applied Biosystems, Inc., Warrington, Cheshire, UK), and the same primers used in the PCR reactions. Cycle-sequenced products were purified with EtOH-NaCl and sequenced on an ABI 3130X1 Genetic Analyser.

DNA sequences were edited and assembled using Sequencher 3.1. (Gene Code, ANN arbor, Michigan, USA) and the rbcL sequences were aligned manually in Phylogenetic Analysis Using Parsimony software program (PAUP*) v.4.0b.10 (Swofford, 2002).

The *rbcLa* matrix is composed of 119 sequences, with sequences having a minimum of 431 base pairs (bp) and a maximum of 579 bp. Finally, all the DNA sequences generated were combined to form single-locus DNA matrices. These matrices represent a DNA cladogram database for the trees used in the preparation of herbal medicine to treat malaria in southern Ghana, together with the sequences of other tree species from Ghana in the Gene Bank of CBOLD. The sequences mined from the CBOLD Gene Bank were also collected from the same forest reserves and also sequence at the same gene region which have been indicated with asterisks on the cladogram.

3.6 Social Survey

A purposive sampling method which targeted medicinal plant collectors, sellers and herbalists was used in the survey. Both closed and open ended interview guides were used in the survey to collect information from the respondents. In all 98 people were interviewed, 59 herbal plant sellers were interviewed from 2 markets, 33 and 26 from Kasoa and Nyanyano markets respectively. Eight herbal plant collectors were interviewed from the communities around Kakum. Ten and thirteen herbalists were interviewed from Bia, Kakum and Ankasa respectively. Eight herbal industries that preferred not to mention their names were also interviewed. Information about plants used in the treatment of malaria and other important information were obtained from the respondents.

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

3.7 Identification of market 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 Characteristics of species used in malaria treatment and their DNA Sequences

DNA of fifty (50) different tree species used in the treatment of malaria was extracted for sequencing at the *rbcLa* gene region. Forty three (43) of them were successfully amplified representing a success rate of 86%. Seven species namely, *Pterocarpus erinacceus, Pycnanthus angolensis, Picralima nitida, Adansonia digitata, Petersianthus macrocarpus, Afzelia africana,* and *Futumia elastica* were not successfully amplified at the *rbcLa* gene region. Characteristics of the 43 species of plants used in the treatment of malaria that was successfully sequenced and their sequences at the *rbcLa* gene region are presented below.

4.1.1 Species: *Trichilia monadelpha* (Thonn.) J.J.de Wilde

Family: Meliaceae

Specimen Number: GH0114

Location: Kakum National Park 176m N05° 20.947' W001° 23.035'

Description: *Trichilia monadelpha* is a tree up to 20m, often seen with a new flush of paler green leaves, along forest edges. Leaves are pinnate, with 4-6 (rarely 3 or 7) pairs of leaflets with single unpaired one on end. Flowers are in pinnacle <10cm long, petals pale, c.8mm long; yellowish anthers on rim of lobed staminal tube which is hairy within. Fruits are globose, leathery 3-valved capsule 15mm covered in short hairs seeds black with reddish aril (Orwa *et al.*, 2009).

Plate 1: *Trichilia_monadelpha* (Thonn.) J.J.de Wilde

DNA barcode: *rbcLa*

CGACTAAGCAAGTGTTGGATTCAAAGCCGGTGTTAAAGATTATAAATTGACTTATTATACTCCTGA
CTATGTAACCAAAGATACTGATATCTTGGCAGCATTCCGAGTAACTCCTCAACCCGGAGTTCCGCC
CGAGGAAGCAGGAGCTGCGGTAGCTGCGGAATCTTCTACTGGTACATGGACAACTGTGTGGACCG
ATGGGCTTACTAGCCTTGATCGTTACAAAGGACGATGCTACAACATTGAGCCCGTTGCTGGAGAAG
AAAATCAATATATATGTTATGTAGCTTACCCTTTAGACCTTTTTGAAGAAGGTTCTGTTACTAACAT
GTTTACGTCCATTGTGGGTAATGTATTTGGGTTCAAAGCCCTGCGCGCTCTACGTCTAGAGGATCT
ACGAATCCCTATCGCATATATTAAAACTTTCCAAGGTCCACCTCATGGTATCCAAGTTGAGAGAGA
TAAATTGAACAAGTATGGCCGTCCCCTATTGGGATGTACTATTAAACCAAAATTGGGGTTATCCGC
TAAGAATTATGGTAGAGCAGTTTATGAATGTCTACGCGGTGGACTTGACTTTACCAAAGATGATGA
GAACGTGAACTCCCAACCATTTATGCGTTGGCGAGACCGTTTCGTATTTTGTGCGGAAGCACTCTT
TAAAGCACAAGCTGAAACAGGTGAAATCAAAAGGTCATTACTTGAATGCACTG

4.1.2 Species: *Trichilia_monadelpha* (Thonn.) J.J.de Wilde

Family: Meliaceae

Specimen Number: IDMTG078-13|GH0115

Location: Ankasa Resource Reserve 158m NO5° 16.98' W002° 36.541'

Plate 2: *Trichilia_monadelpha* (Thonn.) J.J.de Wilde

DNA barcode: *rbcLa*

CGACTAAGCAAGTGTTGGATTCAAAGCCGGTGTTAAAGATTATAAATTGACTTATTATACTCCTGA
CTATGTAACCAAAGATACTGATATCTTGGCAGCATTCCGAGTAACTCCTCAACCCGGAGTTCCGCC
CGAGGAAGCAGGAGCTGCGGTAGCTGCGGAATCTTCTACTGGTACATGGACAACTGTGTGGACCG
ATGGGCTTACTAGCCTTGATCGTTACAAAGGACGATGCTACAACATTGAGCCCGTTGCTGGAGAAG
AAAATCAATATATATGTTATGTAGCTTACCCTTTAGACCTTTTTGAAGAAGGTTCTGTTACTAACAT
GTTTACGTCCATTGTGGGTAATGTATTTGGGTTCAAAGCCCTGCGCGCTCTACGTCTAGAGGATCT
ACGAATCCCTATCGCATATATTAAAACTTTCCAAGGTCCACCTCATGGTATCCAAGTTGAGAGAGA
TAAATTGAACAAGTATGGCCGTCCCCTATTGGGATGTACTATTAAACCAAAATTGGGGTTATCCGC
TAAGAATTATGGTAGAGCAGTTTATGAATGTCTACGCGGTGGACTTGACTTTACCAAAGATGATGA
GAACGTGAACTCCCAACCATTTATGCGTTGGCGAGACCGTTTCGTATTTTGTGCGGAAGCACTCTT
TAAAGCACAAGCTGAAACAGGTGAAATCAAAAGGTCATTACTTGAATGCACTG

4.1.3 Species: *Terminalia catappa* L.

Family: Combretaceae

Specimen Number: GH0171

Location: Ankasa Resource Reserve 154m NO5° 16.919' W002° 36.672'

Description: *Terminalia catappa* is a tall deciduous and erect tree reaching 15-25 m, trunk 1-1.5 m in diameter, often buttressed at the base. Leaves alternate obovate with short petioles, spirally clustered at the branch tips, 15-36 cm long, 8-24 cm wide, dark green above, paler beneath, leathery and glossy. Flowers slightly fetid, greenish-white, very small, with no petals but 10-12 conspicuous stamens, arranged in several slender spikes 15-25 cm long in the leaf axils. Fruit hard, to 7 cm, green-red, rounded and flattened, egg-shaped, with 2 ridges but no wings, 2.5 x 3-6 cm long, yellow or reddish when rip (Orwa *et al.*, 2009).

Plate 1: *Terminalia catappa* L.

DNA barcode: *rbcLa*

CAGAAACTAAAGCAAGTGTTGGATTCAAAGCTGGTGTTAAAGATTATAAACTGACTTATTATACTC
CTGACTATCAAACCAAAGATACTGATATCTTGGCAGCATTCCGAGTAACTCCTCAACCTGGAGTTC
CGCCTGAGGAAGCAGGGGCTGCAGTGGCTGCTGAATCTTCTACTGGAACATGGACAACTGTATGG
ACCGATGGGCTTACCAGCCTTGACCGTTATAAAGGAAGATGCTACCACATCGAACCTGTTGCTGGA
GAAGAAAATCAATATATATGCTATGTAGCTTATCCCTTAGACCTTTTTGAAGAAGGTTCTGTTACT
AATATGTTTACTTCCATTGTGGGTAATGTATTTGGGTTCAAAGCCCTACGCGCTCTACGTCTGGAGG
ATCTGCGAATTCCTACGTCCTATATTAAAACTTTCCAAGGCCCTCCTCACGGGATCCAAGTTGAGA
GAGATAAATTGAACAAGTATGGTCGTCCCCTATTGGGATGTACTATTAAACCTAAATTGGGGTTAT
CCGCTAAGAACTACGGTAGAGCAGTTTATGAATGTCTTCGGGGTGGACTTGATTTTACGAAGGATG
ATGAGAACGTGAACTCACAACCATTTATGCGTTGGAGAGACCGTTTCTTATTTTGTGCCGAAGCAC
TTTATAAAGCACAAGCTGAAACTGGTGAAATCAAAGGGCATTACTTGAATGCAACTG

4.1.4 Species: *Terminalia catappa* L.

Family: Combretaceae

Specimen Number: IDMTG124-13|GH0170

Location: Kakum National Park 174m N05° 20.968’ W001° 23.087’

Plate 2: *Terminalia catappa* L.

DNA barcode: *rbcLa*

CAGAAACTAAAGCAAGTGTTGGATTCAAAGCTGGTGTTAAAGATTATAAACTGACTTATTATACTC
CTGACTATCAAACCAAAGATACTGATATCTTGGCAGCATTCCGAGTAACTCCTCAACCTGGAGTTC
CGCCTGAGGAAGCAGGGGCTGCAGTGGCTGCTGAATCTTCTACTGGAACATGGACAACTGTATGG
ACCGATGGGCTTACCAGCCTTGACCGTTATAAAGGAAGATGCTACCACATCGAACCTGTTGCTGGA
GAAGAAAATCAATATATATGCTATGTAGCTTATCCCTTAGACCTTTTTGAAGAAGGTTCTGTTACT
AATATGTTTACTTCCATTGTGGGTAATGTATTTGGGTTCAAAGCCCTACGCGCTCTACGTCTGGAGG
ATCTGCGAATTCCTACGTCCTATATTAAAACTTTCCAAGGCCCTCCTCACGGGATCCAAGTTGAGA
GAGATAAATTGAACAAGTATGGTCGTCCCCTATTGGGATGTACTATTAAACCTAAATTGGGGTTAT
CCGCTAAGAACTACGGTAGAGCAGTTTATGAATGTCTTCGGGGTGGACTTGATTTTACGAAGGATG
ATGAGAACGTGAACTCACAACCATTTATGCGTTGGAGAGACCGTTTCTTATTTTGTGCCGAAGCAC
TTTATAAAGCACAAGCTGAAACTGGTGAAATCAAAGGGCATTA

4.1.5 Species: *Myrianthus_arboreus* P. Beauv.

Family: Moraceae

Specimen Number: GH0120

Location: Ankasa Resource Reserve 170m NO5° 16.104' W002° 36.501'

Description: Myrianthus arboreus is a dioecious tropical tree up to 15 m high with spreading branches from a short stem. Usually have stilt roots with very large alternately shaped leaves, 5-7 digitately compound, coarsely toothed, with hood like stipules the central leaflet is about 25 x 9 cm. Young leaves are usually red in colour. The female inflorescences are paired, stalked greenish clusters (pedunculate), each flower with a thick curled style projecting out of the fused calyx, and a basal ovule. Fruit a syncarp of basally fused, yellow false drupes up to 10 cm, with stylar remains projecting from each drupe (Orwa *et al.*, 2009).

Plate 1: *Myrianthus_arboreus* P. Beauv.

DNA barcode: (*rbcLa*) Sequencing was not successful

4.1.6 Species: Myrianthus_arboreus P. Beauv.

Family: Moraceae

Specimen Number: IDMTG088-13|GH0119

Location: Kakum National Park. 138m N05° 20.482' W001° 22.750'

Plate 2: *Myrianthus_arboreus* P. Beauv.

DNA barcode: *rbcLa*

CAGAAACTAAAGCAAGTGTTGGATTCAAAGCTGGTGTTAAAAGATTATAAATTGACTTATTACACT
CCTGAATATGAAACCAAAGATACTGATATCTTAGCAGCATTTCGAGTAACTCCTCAACCTGGAGTT
CCCCCTGAAGAAGCAGGGGCTGCGGTAGCTGCTGAATCTTCTACTGGTACATGGACAACTGTATGG
ACTGACGGGCTTACCAGTCTTGATCGCTACAAAGGTCGATGCTACCACATCGAGCCTGTTGCTGGA
GAAGAAAATCAATATATTGCTTATGTAGCTTACCCCTTAGACCTTTTTGAAGAAGGTTCTGTTACTA
ACATGTTTACTTCCATTGTGGGTAATGTATTTGGGTTCAAGGCCCTGCGCGCTCTACGTCTGGAGG
ATTTGCGAATCCCCCCTGCTTACTCTAAAACTTTCCAAGGCCCGCCTCATGGCATCCAAGTTGAGA
GAGATAAATTGAACAAGTATGGCCGCCCCCTATTGGGATGTACTATTAAACCTAAATTGGGGTTAT
CCGCTAAGAATTATGGTAGAGCGGTTTATGAATGTCTTCGTGGTGGACTTGATTTTACCAAAGATG
ATGAGAACGTGAATTCCCAACCATTTATGCGTTGGGAGAGACCGTTTCGTATTTTGTGCCGAAGCA
ATTTATAAAGCACAGGCTGAAACAGGTGAAATCAAAGGACATTTACTTGAATTGCAA

4.1.7 Species: *Greenwayodendron oliveri* (Engl.) Verdc.

Family: Annonaceae

Specimen Number: GH0211

Location: Kakum National Park. 138m N05° 20.832' W001° 22.984'

Description: *Greenwayodendron oliveri* is a tree that can be found in moist forests and grows between 5-15m tall. Leaves are asymmetric, 6-8 pairs of laterals meeting 2/3 way to the margin; petiole 1-4 mm, slender; leaves have a few hair when developed. Leaves are papery not shiny, drying black scented but not strongly.

Plate 1: *Greenwayodendron oliveri* (Engl.) Verdc.

DNA barcode: *rbcLa*

GAAACTAAAGCAAGTGTTGGATTCAAAGCTGGTGTTAAAGAGTACAAATTGACTTATTATACTCCT
GAATATGAAACCAAAGATACTGATATCTTGGCAGCATTCCGAGTAACTCCTCAACCCGGAGTTCCG
CCTGAGGAAGCAGGGGCTGCGGTAGCTGCCGAATCTTCTACTGGTACATGGACAACTGTGTGGAC
CGATGGACTTACCAGCCTTGATCGTTACAAAGGACGATGCTACCACATCGAGCCCGTTGCTGGGGA
GGAAAATCAATATATTGCTTATGTAGCTTACCCTTTAGACCTTTTTGAAGAGGGTTCTGTTACTAAT
ATGTTTACTTCCATTGTAGGTAATGTATTTGGGTTCAAAGCTCTACGAGCTCTACGTCTGGAAGACT
TGCGAATTCCTACTTCTTATATCAAGACTTTCCAGGGCCCGCCCCATGGCATCCAAGTTGAGAGAG
ATAAATTGAACAAGTATGGCCGTCCCCTATTGGGATGTACTATTAAACCAAAATTGGGGTTATCCG
CCAAGAACTACGGTAGGGCGGTTTATGAATGTCTCCGCGGTGGACTTGATTTTACCAAGGATGATG
AGAACGTGAACTCCCAACCATTTATGCGCTGGAGAGACCGTTTCTGCTTTTGTGCCGAAGCACTTT
ATAAAGCGCAGCCGAAACAGGTGAAATCAAAGGACATTACTTGAATGCACTG

4.1.8 Species: *Greenwayodendron oliveri* (Engl.) Verdc.

Family: Annonaceae

Specimen Number: IDMTG155-13|GH0212

Location: Ankasa Resource Reserve 166m NO5° 16.201' W002° 36.421'

Plate 1: *Greenwayodendron oliveri* (Engl.) Verdc.

DNA barcode: *rbcLa*

GAAACTAAAGCAAGTGTTGGATTCAAAGCTGGTGTTAAAGAGTACAAATTGACTTATTATACTCCT
GAATATGAAACCAAAGATACTGATATCTTGGCAGCATTCCGAGTAACTCCTCAACCCGGAGTTCCG
CCTGAGGAAGCAGGGGCTGCGGTAGCTGCCGAATCTTCTACTGGTACATGGACAACTGTGTGGAC
CGATGGACTTACCAGCCTTGATCGTTACAAAGGACGATGCTACCACATCGAGCCCGTTGCTGGGGA
GGAAAATCAATATATTGCTTATGTAGCTTACCCTTTAGACCTTTTTGAAGAGGGTTCTGTTACTAAT
ATGTTTACTTCCATTGTAGGTAATGTATTTGGGTTCAAAGCTCTACGAGCTCTACGTCTGGAAGACT
TGCGAATTCCTACTTCTTATATCAAGACTTTCCAGGGCCCGCCCCATGGCATCCAAGTTGAGAGAG
ATAAATTGAACAAGTATGGCCGTCCCCTATTGGGATGTACTATTAAACCAAAATTGGGGTTATCCG
CCAAGAACTACGGTAGGGCGGTTTATGAATGTCTCCGCGGTGGACTTGATTTTACCAAGGATGATG
AGAACGTGAACTCCCAACCATTTATGCGCTGGAGAGACCGTTTCTGCTTTTGTGCCGAAGCACTTT
ATAAAGCGCAGCCGAAACAGGTGAAATCAAAGGACATTACTTGAATGCACTG

4.1.9 Species: *Holarrhena_floribunda* (G.Don.) Dur.& Schinz

Family: Apocynaceae

Specimen Number: GH0121

Location: Kakum National Park. 139m N05° 20.844' W001° 22.962'

Description: *Holarrhena floribunda* is a shrub to medium sized tree, 4.5-15 m high. Leaves shining, mostly ovate-acuminate, or ovate-lanceolate, 5-18 cm long and 2-8 cm broad with 6-12 pairs of lateral nerves. Flowers white, scented and in almost umbel-like inflorescences; corolla- tube 5-9 mm long and lobes 3.5-8 mm and overlapping to the right. Anthers fertile to the base. Paired narrowly cylindrical fruiting follicles, 30-60 cm, with seeds having apical tufts of hair (Orwa *et al.*, 2009).

Plate 1: *Holarrhena_floribunda* (G.Don.) Dur.& Schinz

DNA barcodes: *rbcLa*

CGCTAAGCAAGTGTTGGATTCAAAGCCGGTGTTAAAGAGTACAAATTGACTTATTATACTCCTGAA
TACAAAACTAAAGATACTGATATCTTGGCAGCATTCCGAGTAACTCCTCAACCCGGAGTTCCACCC
GAAGAAGCGGGGGCCGCGGTAGCTGCCGAATCTTCTACTGGTACATGGACAACTGTGTGGACCGA
TGGACTTACCAGCCTTGATCGTTACAAAGGGCGATGCTACCACATCGAGCCCGTTCCTGGAGAAGA
AGATCAATATATTGCTTATGTAGCTTACCCCCTAGACCTTTTTGAAGAAGGTTCTGTTACTAACATG
CTTACTTCCATTGTAGGTAATGTATTTGGGTTCAAAGCCCTACGCGCTCTACGTCTGGAAGATTTGC
GAATCCCTACGGCTTATATTAAAACCTTCCAAGGCCCGCCTCATGGCATCCAGGTTGAGAGAGATA
AATTGAACAAATATGGTCGTCCCCTGTTGGGATGTACTATTAAACCTAAATTGGGGTTATCCGCTA
AAAACTATGGTAGGGCAGTTTATGAATGTCTTCGCGGTGGGCTTGATTTTACCAAAGATGATGAAA
ACGTGAACTCCCAACCGTTTATGCGTTGGAGAGATCGTTTCTTATTTTGTGCCGAAGCACTTTTTAA
AGCACAGGGCCGAAACCGGGCGAAATCAAAGGGCATTACTTGACGCACG

4.1.10 Species: *Holarrhena floribunda* (G.Don.) Dur.& Schinz

Family: Apocynaceae

Specimen Number: IDMTG090-13|GH0122

Location: Ankasa Resource Reserve 165m NO5° 16.101' W002° 36.521'

Plate 1: *Holarrhena_floribunda* (G.Don.) Dur.& Schinz

DNA barcodes: *rbcLa*

CGCTAAGCAAGTGTTGGATTCAAAGCCGGTGTTAAAGAGTACAAATTGACTTATTATACTCCTGAA
TACAAAACTAAAGATACTGATATCTTGGCAGCATTCCGAGTAACTCCTCAACCCGGAGTTCCACCC
GAAGAAGCGGGGGCCGCGGTAGCTGCCGAATCTTCTACTGGTACATGGACAACTGTGTGGACCGA
TGGACTTACCAGCCTTGATCGTTACAAAGGGCGATGCTACCACATCGAGCCCGTTCCTGGAGAAGA
AGATCAATATATTGCTTATGTAGCTTACCCCCTAGACCTTTTTGAAGAAGGTTCTGTTACTAACATG
CTTACTTCCATTGTAGGTAATGTATTTGGGTTCAAAGCCCTACGCGCTCTACGTCTGGAAGATTTGC
GAATCCCTACGGCTTATATTAAAACCTTCCAAGGCCCGCCTCATGGCATCCAGGTTGAGAGAGATA
AATTGAACAAATATGGTCGTCCCCTGTTGGGATGTACTATTAAACCTAAATTGGGGTTATCCGCTA
AAAACTATGGTAGGGCAGTTTATGAATGTCTTCGCGGTGGGCTTGATTTTACCAAAGATGATGAAA
ACGTGAACTCCCAACCGTTTATGCGTTGGAGAGATCGTTTCTTATTTTGTGCCGAAGCACTTTTTAA
AGCACAGGGCCGAAACCGGGCGAAATCAAAGGGCATTACTTGACGCACG

4.1.11 Species: *Parkia biglobosa* (Jacq.) R. Br. ex G. Don

Family: Mimosoideae

Specimen Number: GH0125

Location: Kakum National Park. 141m N05° 20.841' W001° 22.960'

Description: *Parkia biglobosa* is a perennial deciduous tree with a height ranging from 7 to 20 m. Leaves alternate, dark green, bipinnate to 30 cm long, pinnae up to 17 pairs with 13-60 pairs of leaflets, 8-30 mm x 1.5-8 mm, of distinctive shape and venation. Hermaphrodite flowers orange or red in colour: calyx 10-13 (16 max.) mm; corolla 10-14 (17 max.) mm long, lobes very short 1-3 mm long, connate in the middle and free or connate at base. Pods, pink brown to dark brown when mature, about 45 cm long and 2 cm wide; may contain up to 30 seeds embedded in a yellow pericarp (Orwa *et al.*, 2009).

Plate 1: *Parkia_biglobosa* (Jacq.) R. Br. ex G. Don

DNA barcode: *rbcLa*

CAGAAACTAAAGCAAGTGTTGGATTCAAAGCTGGTGTTAAAGATTATAAATTGACTTATTATACTC
CTGACTATGAAACTAAAGATGGTGATATCTTGGCAGCATTCCGAGTAACTCCTCAACCAGGAGTTC
CGCCTGAAGAAGCGGGTGCCGCGGTAGCTGCTGAATCTTCTACTGGTACATGGACAACTGTGTGG
ACCGATGGGCTTACCAGTCTTGATCGTTACAAAGGACGATGCTACCACATCGAGCCCGTTCCTGGA
GAAGAAAGTCAATTTATTGCTTATGTAGCTTATCCCTTAGACCTTTTTGAAGAAGGTTCTGTTACTA
ACATGTTTACTTCCATTGTGGGTAATGTATTTGGGTTCAAGGCCCTGCGCGCTCTACGTCTGGAGG
ATTTGCGAATCCCTCCTTCTTATTCTAAAACTTTCCAAGGTCCGCCTCACGGCATCCAAGTTGAGAG
AGATAAATTGAACAAATACGGCCGTCCCCTATTGGGATGTACTATTAAACCAAAATTGGGGTTATC
CGCTAAGAATTACGGTAGAGCGGTTTATGAATGTCTCCGTGGTGGACTTGATTTTACCAAAGATGA
TGAGAATGTGAATTCCCAACCATTTATGCGTTGGAGAGACCGTTTCTTATTTTGTGCCGAAGCACTT
TATAAAGCACAGGCCGAAACAGGTGAAATCAAAGGGCATTACTTGATGCATG

4.1.12 Species: *Parkia biglobosa* (Jacq.) R. Br. ex G. Don

Family: Mimosoideae

Specimen Number: IDMTG092-13|GH0126

Location: Ankasa Resource Reserve 171m NO5° 16.123' W002° 36.302'

Plate 2: *Parkia_biglobosa* (Jacq.) R. Br. ex G. Don

DNA barcode: *rbcLa*1

CAGAAACTAAAGCAAGTGTTGGATTCAAAGCTGGTGTTAAAGATTATAAATTGACTTATTATACTC
CTGACTATGAAACTAAAGATGGTGATATCTTGGCAGCATTCCGAGTAACTCCTCAACCAGGAGTTC
CGCCTGAAGAAGCGGGTGCCGCGGTAGCTGCTGAATCTTCTACTGGTACATGGACAACTGTGTGG
ACCGATGGGCTTACCAGTCTTGATCGTTACAAAGGACGATGCTACCACATCGAGCCCGTTCCTGGA
GAAGAAAGTCAATTTATTGCTTATGTAGCTTATCCCTTAGACCTTTTTGAAGAAGGTTCTGTTACTA
ACATGTTTACTTCCATTGTGGGTAATGTATTTGGGTTCAAGGCCCTGCGCGCTCTACGTCTGGAGG
ATTTGCGAATCCCTCCTTCTTATTCTAAAACTTTCCAAGGTCCGCCTCACGGCATCCAAGTTGAGAG
AGATAAATTGAACAAATACGGCCGTCCCCTATTGGGATGTACTATTAAACCAAAATTGGGGTTATC
CGCTAAGAATTACGGTAGAGCGGTTTATGAATGTCTCCGTGGTGGACTTGATTTTACCAAAGATGA
TGAGAATGTGAATTCCCAACCATTTATGCGTTGGAGAGACCGTTTCTTATTTTGTGCCGAAGCACTT
TATAAAGCACAGGCCGAAACAGGTGAAATCAAAGGGCATTACTTGATGCATG

4.1.13 Species: *Balanites aegyptiaca* (L.) Del

Family: Zygophyllaceae

Specimen Number: GH0129

Location: Bia Biosphere Reserve. 215m N06°32.652' W00°301.949'

Description: *Balanites aegyptiaca* is a multibranched, spiny shrub or tree up to l0 m high. Leaves compound and spirally arranged on the shoots, dark green with 2 firm coriaceous leaflets; dimensions and shapes varying widely. Petiole canaliculate, from 5 mm to 20 mm with a short rachis. Inflorescence a sessile or shortly pedunculate fascicle of a few flowers. Flower buds ovoid and tomentose. Individual flowers hermaphroditic, pentamerous an actinomorphic, 8-14 mm in diameter and generally greenish-yellow. Fruit ellipsoid, up to 4 cm long, green. Ripe fruit brown or pale brown with a brittle coat enclosing a brown or brown-green sticky pulp and a hard stone seed (Orwa *et al.*, 2009).

Plate 1: *Balanites aegyptiaca* (L.) Del

DNA barcode: *rbcLa*

TAAGCAAGTGTTGGATTCAAAGCTGGTGTTAAAGATTATAAATTGACTTATTATACTCCTGAATAT
GAAACAAAGGATACTGATATTTTGGCAGCATTCCGAGTAACTCCTCAACCTGGAGTTCCACCTGAG
GAAGCAGGGGCTGCGGTAGCAGCTGAATCTTCTACTGGTACATGGACAACTGTGTGGACTGATGG
GCTTACTAGTCTTGATCGTTACAAAGGACGATGCTACCACATCGAGCCCGTTGCTGGAGAAGAAA
ATCAATATATTGCTTATGTAGCTTACCCCTTAGACCTTTTTGAAGAAGGTTCTGTTACTAACATGTT
TACTTCCATTGTGGGTAATGTATTTGGGTTCAAAGCCCTACGCGCTCTGCGTCTGGAGGATTTGCG
AATCCCTACTTCTTATATTAAAACTTTCCAAGGGCCGCCTCACGGTATCCAAGTTGAGAGAGATAA
ATTGAATAAGTATGGCCGTCCCCTATTGGGATGTACTATTAAACCTAAATTGGGGCTATCCGCTAA
AAATTATGGTAGAGCAGTTTATGAATGTCTTCGCGGTGGGCTTGATTTTACCAAAGATGATGAGAA
CGTGAACTCCCAACCATTTATGCGTTGGAAGAGACCGTTTCTTATTTTGTGCCGAAGCACTTTATAA
AGCACAGGCTGAAACTGGGTGAAATCCAAAGGGCATTACTTGATGCACG

4.1.14 Species: *Balanites aegyptiaca* (L.) Del

Family: Balanitaceae

Specimen Number: IDMTG095-13|GH0129

Location: Kakum National Park 163m N05° 20.937' W001° 23.085'

Plate 2: *Balanites aegyptiaca* (L.) Del

DNA barcode: (*rbcLa*) Sequencing was not successful

4.1.15 Species: *Tetrapleura tetraptera* (Schum. & Thonn.) Taube

Family: Fabaceae

Specimen Number: GH0131

Location: Bia Biosphere Reserve 173m. N06°22.131'W002°59.158'

Description: *Tetrapleura tetraptera* is deciduous; it reaches 20-25 m in height, with a girth of 1.5-3 m. Leaves are sessile, glabrous or minutely hairy with a common stalk 15-30 cm long, slightly channelled on the upper surface. Flowers are pinkish-cream turning to orange and are densely crowded in spike like racemes 5-20 cm long, usually in pairs in the upper leaf axils. Fruit is very persistent, hanging at the ends of branches on stout stalks 25 cm long. It is shiny, glabrous, dark purple-brown, usually slightly curved, 15-25 cm (Orwa *et al.*, 2009).

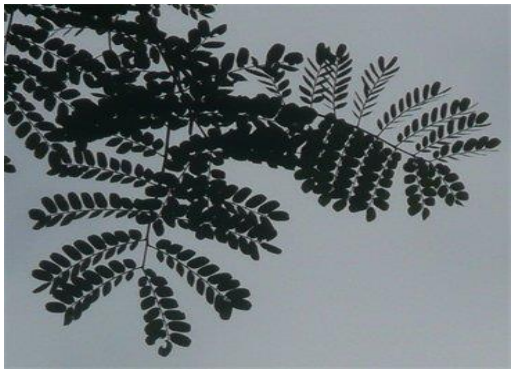


Plate 1: *Tetrapleura_tetraptera* (Schum. & Thonn.) Taube

DNA barcode: *rbcLa*

CAACTAAAGCAAGTGTTGGGTTCAAAGCTGGTGTTAAAGATTATAAATTGACTTATTATACTCCTG
ACTATGAAACCAAAGATACTGATATCTTGGCAGCATTCCGAGTAACTCCTCAACCTGGAGTTCCGC
CTGAAGAAGCAGGTGCAGCGGTAGCTGCTGAATCTTCTACTGGTACATGGACAACTGTGTGGACC
GATGGGCTTACCAGTCTTGATCGTTACAAAGGACGATGCTACCACCTTGAGCCCGTTGCTGGAGAA
GAAAATCAATATATTGCTTATGTAGCTTATCCCTTAGACCTTTTTGAAGAAGGTTCTGTTACTAACA
TGTTTACTTCCATTGTGGGTAATGTATTTGGGTTCAAGGCCCTGCGCGCTCTACGTCTGGAGGATTT
GCGAATCCCTCCTGCTTATTCTAAAACTTTCCAAGGTCCGCCTCACGGCATCCAAGTTGAGAGAGA
TAAATTGAACAAGTACGGCCGTCCCCTATTGGGATGTACTATTAAACCTAAATTGGGGTTATCCGC
TAAGAATTACGGTAGAGCAGTTTATGAATGTCTCCGCGGTGGACTTGATTTTACCAAAGATGATGA
GAATGTGAATTCCCAACCATTTATGCGTTGGAGAGACCGTTTCTTATTTTGTGCCGAAGCACTTTAT
AAAGCACAGGCCGAAACAGTGAAATCAAGGGCATTACTTGATGCACTG

4.1.16 Species: *Tetrapleura tetraptera* (Schum. & Thonn.) Taube

Family: Fabaceae – Mimosoideae

Specimen Number: IDMTG096-13|GH0131

Location: Kakum National Park 167m N05° 21.172' W001° 23.321'

Plate 2: *Tetrapleura_tetraptera* (Schum. & Thonn.) Taube

DNA barcode: *rbcLa*1

CAACTAAAGCAAGTGTTGGGTTCAAAGCTGGTGTTAAAGATTATAAATTGACTTATTATACTCCTG
ACTATGAAACCAAAGATACTGATATCTTGGCAGCATTCCGAGTAACTCCTCAACCTGGAGTTCCGC
CTGAAGAAGCAGGTGCAGCGGTAGCTGCTGAATCTTCTACTGGTACATGGACAACTGTGTGGACC
GATGGGCTTACCAGTCTTGATCGTTACAAAGGACGATGCTACCACCTTGAGCCCGTTGCTGGAGAA
GAAAATCAATATATTGCTTATGTAGCTTATCCCTTAGACCTTTTTGAAGAAGGTTCTGTTACTAACA
TGTTTACTTCCATTGTGGGTAATGTATTTGGGTTCAAGGCCCTGCGCGCTCTACGTCTGGAGGATTT
GCGAATCCCTCCTGCTTATTCTAAAACTTTCCAAGGTCCGCCTCACGGCATCCAAGTTGAGAGAGA
TAAATTGAACAAGTACGGCCGTCCCCTATTGGGATGTACTATTAAACCTAAATTGGGGTTATCCGC
TAAGAATTACGGTAGAGCAGTTTATGAATGTCTCCGCGGTGGACTTGATTTTACCAAAGATGATGA
GAATGTGAATTCCCAACCATTTATGCGTTGGAGAGACCGTTTCTTATTTTGTGCCGAAGCACTTTAT
AAAGCACAGGCCGAAACAGTGAAATCAAGGGCATTACTTGATGCACTG

4.1.17 Species: *Blighia unijugata* Baker

Family: Sapindaceae

Specimen Number: GH0137

Location: Kakum National Park 169m N05°21.181' W001° 23.347'

Description: *Blighia unijugata* is a shrub or small tree. Bark grey to brownish, generally smooth but often with small knobbly warts. Leaves paripinnate, up to 30 cm with 2-3 pairs of leaflets; leaflets ovate to elliptic, end pair largest, up to 15 cm, glossy dark green above, hairless, apex tapering into a driptip; margin entire, often wavy. Flowers unisexual, usually on different trees, white, sweetly scented. Fruit in clusters, 3-lobed, somewhat pear-shaped, with ridge-like wings, bright pink to red, splitting with the valves curling backwards (Orwa *et al.*, 2009).

Plate 1: *Blighia unijugata* Baker

DNA barcode: *rbcLa*

CAGAAACTAAAGCAAGTGTTTGGATTCAAAGCCGGTGTTAAAGATTATAAATTGAATTATTATACT
CCTGACTATGTAACCAAAGATACTGATATCTTGGCAGCATTCCGAGTAACTCCTCAACCTGGGGTT
CCGCCCGAGGAAGCAGGGGCCGCGGTAGCTGCGGAATCTTCTACTGGTACATGGACAACTGTGTG
GACTGATGGGCTTACCAGCCTTGATCGTTACAAAGGACGATGCTACAACATTGAGCCTGTTGCTGG
AGAAGAAAATCAATATATATGTTATGTAGCTTACCCTTTAGACCTTTTTGAAGAAGGTTCTGTTACT
AACATGTTTACTTCCATTGTAGGTAATGTATTTGGGTTTAAAGCCTTGCGCGCTCTACGTCTAGAGG
ATCTACGAATCCCTCCCGCGTATTCGAAAACTTTCCAAGGCCCTCCTCACGGCATCCAAGTTGAGA
GAGATAAATTGAACAAGTATGGACGTCCCCTATTGGGATGTACTATTAAACCTAAATTGGGATTAT
CTGCTAAGAACTACGGTAGAGCAGTTTATGAATGTCTACGTGGTGGACTTGACTTTACCAAAGATG
ATGAGAACGTAAACTCCCAACCATTTATGCGTTGGAGAGACCGTTTCTTGTTTTGTGCAGAAGCGC
TTTATAAAGCGCAGGCGGAAACTGGTGAAATCAAAGGTCATTACTTGAATGCAAACTG

4.1.18 Species: *Blighia unijugata* Baker

Family: Sapindaceae

Specimen Number: IDMTG100-13|GH0137

Location: Bia Biosphere Reserve. 175m. N06°22.122' W002°59.125'

Plate 2: *Blighia unijugata* Baker

DNA barcode: (*rbcLa*) Sequencing was not successful

4.1.19 Species: *Khaya grandifoliola* C.DC

Family: Meliaceae

Specimen Number: GH0140

Location: Bia Biosphere Reserve 192m N06°36.454' W00305.838'

Description: *Khaya grandifoliola* is usually deciduous, monoecious, medium-sized to large tree up to 40 m tall. Leaves arranged spirally but clustered near ends of branches, paripinnately compound with 3–5 pairs of leaflets; stipules absent; petiole and rachis together up to 50 cm long. Flowers unisexual, male and female flowers very similar in appearance, regular, usually 5-merous, whitish, sweet-scented; pedicel 1–2 mm long; calyx lobed almost to the base, with rounded lobes c. 1.5 mm long; petals free, elliptical, c. 5 mm × 2 mm, somewhat hooded; stamens fused into an urn-shaped tube c. 5 mm long, with usually 10 included anthers near apex, alternating with rounded lobes; disk cushion-shaped; ovary superior, globose to conical, 1–2 mm in diameter (Opuni-Frimpong, E., 2008)

Plate 1: *Khaya_grandifoliola* C.DC

DNA barcode: *rbcLa*

AAACAGAAACTAAAGCAAGTGTTGGATTCAAAGCCCGGTGTTAAAGATTATAAATTGACTTATTAT
ACGCCTGACTATGTAACCAAAGATACTGATATCTTGGCAGCATTCCGAGTAACTCCTCAACCCGGA
GTTCCGCCCGAGGAAGCAGGGGCTGCGGTAGCTGCGGAATCTTCTACTGGTACATGGACAACTGT
GTGGACCGATGGGCTTACTAGCCTTGATCGTTACAAAGGACGATGCTACAACATTGAGCCAGTTGC
TGGAGAAGAAAATCAATATATATGTTATGTAGCTTACCCTTTAGACCTTTTTGAAGAAGGTTCTGT
TACTAACATGTTTACATCCATTGTGGGTAATGTATTTGGGTTCAAAGCCCTGCGCGCTCTACGTCTA
GAGGATCTACGAATCCCTCCCGCGTATTCTAAAACTTTCCAAGGCCCGCCTCATGGCATCCAAGTT
GAGAGAGATAAATTGAACAAGTATGGTCGTCCCCTATTGGGATGTACAATTAAACCTAAATTGGG
GTTATCCGCTAAGAATTACGGTAGAGCAGTTTATGAATGTCTACGCGGTGGACTTGACTTTACCAA
AGATGATGAGAACGTGAACTCCCAACCATTTATGCGTTGGAGAGACCGTTTCATATTTTGTGCGGA
AGCAATCTATAAAGCGCAAGCTGAAACAGGTGAAATCAAAGGGCATTACTTGAATGC

4.1.20 Species: *Khaya grandifoliola* C.DC

Family: Meliaceae

Specimen Number: IDMTG102-13|GH0140

Location: Kakum National Park 171m N05° 20.918' W001° 23.033'

Plate 2: *Khaya_grandifoliola* C.DC

DNA barcode: (*RbcLa*) Sequencing was not successful

4.1.21 Species: *Enantia chlorantha* Oliv.

Family: Annonaceae

Specimen Number: GH0143

Location: Bia Biosphere Reserve 178m N06°22.121' W002°59.168'

Description: *Enantia chloranth* is an evergreen tree (top: 12-30 m), the yellow bark. Leaves oblong obovate to oblong elliptical (long : 5 to 9.5 cm wide : 2-7 cm) at the base to Subrounded cuneiform , the apex acuminate, pubescent in reverse, the petiole pubescent (long : 3-10 mm). Solitary and axillary flowers, sepals lanceolate and obsolete , with greenish-yellow petals , fleshy, oblanceolate (long : 2-3 cm) , with cylindrical and hairy carpels (long : 2-3 mm). Fruits composed of 20 black and fleshy mericarps (long : 25-28 mm diam : 10-13 mm) , red stipes 7-18 mm long (Orwa *et al.*, 2009).

Plate1: *Enantia chlorantha* Oliv.

DNA barcode: *rbcLa*

GAAAACTAAAGCAAGTGTTGGGATTCAAAAGCTGGTGTTAAAAGAGTACAAATTGACTTATTATA
CTCCTGAATATGAAACCAAAGATACTGATATCTTGGCAGCATTCCGAGTAACTCCTCAACCCGGAG
TTCCGCCTGAGGAAGCAGGGGCTGCGGTAGCTGCCGAATCTTCTACTGGTACATGGACAACTGTGT
GGACCGATGGACTTACCAGCCTTGATCGTTACAAAGGACGATGCTACCACATCGAGCCCGTTGCTG
GGGAGGAAAATCAATATATTGCTTATGTAGCTTACCCTTTAGACCTTTTTGAAGAGGGTTCTGTTA
CTAACATGTTTACTTCCATTGTAGGTAATGTATTTGGGTTCAAAGCTCTACGAGCTCTACGTCTGGA
GGACCTGCGAATTCCTACTTCTTATATCAAGACTTTCCAGGGCCCGCCCCATGGCATCCAAGTTGA
GAGAGATAAATTGAACAAGTATGGCCGTCCCCTATTGGGATGTACTATTAAACCAAAATTGGGGTT
ATCCGCCAAGAACTACGGTAGGGCGGTTTATGAATGTCTCCGCGGTGGACTTGATTTTACCAAGGA
TGATGAGAACGTGAACTCCCAACCATTTATGCGCTGGAGAGACCGTTTCTTATTTTGTGCCGAAGC
ACTTTTTAAAGCGCAGGCCGAAACAGGTGAAATCAAAGGGACATTACTTGAATGCAACTG

4.1.22 Species: *Enantia chlorantha* Oliv.

Family: Annonaceae

Specimen Number: IDMTG104-13|GH0144

Location: Ankasa Resource Reserve 182m NO5° 16.121' W002° 36.321'

Plate2: *Enantia chlorantha* Oliv.

DNA barcode: ***rbcla***

GAAAACTAAAGCAAGTGTTGGGATTCAAAAGCTGGTGTTAAAAGAGTACAAATTGACTTATTATA
CTCCTGAATATGAAACCAAAGATACTGATATCTTGGCAGCATTCCGAGTAACTCCTCAACCCGGAG
TTCCGCCTGAGGAAGCAGGGGCTGCGGTAGCTGCCGAATCTTCTACTGGTACATGGACAACTGTGT
GGACCGATGGACTTACCAGCCTTGATCGTTACAAAGGACGATGCTACCACATCGAGCCCGTTGCTG
GGGAGGAAAATCAATATATTGCTTATGTAGCTTACCCTTTAGACCTTTTTGAAGAGGGTTCTGTTA
CTAACATGTTTACTTCCATTGTAGGTAATGTATTTGGGTTCAAAGCTCTACGAGCTCTACGTCTGGA
GGACCTGCGAATTCCTACTTCTTATATCAAGACTTTCCAGGGCCCGCCCCATGGCATCCAAGTTGA
GAGAGATAAATTGAACAAGTATGGCCGTCCCCTATTGGGATGTACTATTAAACCAAAATTGGGGTT
ATCCGCCAAGAACTACGGTAGGGCGGTTTATGAATGTCTCCGCGGTGGACTTGATTTTACCAAGGA
TGATGAGAACGTGAACTCCCAACCATTTATGCGCTGGAGAGACCGTTTCTTATTTTGTGCCGAAGC
ACTTTTTAAAGCGCAGGCCGAAACAGGTGAAATCAAAGGGACATTACTTGAATGCAACTG

4.1.23 Species: *Cleistopholis patens* (Benth.) Engl. & Diels

Family: Annonaceae

Specimen Number: GH0147

Location: Ankasa Resource Reserve 180m NO5° 16.101' W002° 36.521'

Description: Evergreen tree, with light gray bark. Elliptical oval leaves (long: 12-25 cm, wide 3-5 cm), leathery, hairless petiole at (long: 10 mm). Flowers solitary and axillary, yellowish green, oblong or to obovaux outer petals (long: 7-10 mm), the inner being tiny (long: 2 mm). Apocarpiques fruit, composed of carpels stipitate, globular (long 2-5 cm, diameter: 1-5 cm (Orwa *et al.*, 2009).

Plate 1: *Cleistopholis patens* (Benth.) Engl. & Diels

DNA barcode: *rbcLa*

GAAACTAAAGCAAGTGTTGGATTCAAAGCTGGTGTTAAAGAGTACAAATTGACTTATTATACTCCT
GAATATGAAACCAAGGATACTGATATCTTGGCAGCATTCCGAGTAACTCCTCAACCCGGAGTTCCG
CCCGAGGAAGCAGGGGCTGCGGTAGCTGCCGAATCTTCTACTGGTACATGGACAACTGTGTGGAC
CGATGGACTTACCAGCCTTGATCGTTACAAAGGACGATGCTACCACATCGAGCCCGTTGCTGGGGA
GGAAAATCAATTTATTGCTTATGTAGCTTACCCTTTAGACCTTTTTGAAGAGGGTTCTGTTACTAAC
ATGTTTACTTCCATTGTAGGTAATGTATTTGGGTTCAAAGCTCTACGAGCTCTACGTCTGGAAGACT
TGCGAATTCCTACTTCTTATGTCAAGACTTTCCAGGGCCCGCCTCACGGCATCCAAGTTGAGAGAG
ATAAATTGAACAAGTATGGTCGTCCCCTATTGGGATGTACTATTAAACCAAAATTGGGGTTATCCG
CCAAGAACTACGGTAGGGCGGTTTATGAATGTCTCCGTGGTGGACTTGATTTTACCAAGGATGATG
AGAACGTGAACTCCCAACCATTTATGCGCTGGAGAGACCGTTTCTTATTTTTGTGCCGAAGCAATT
TTTAAAGCGCAGGCCGAAACAGGTGAAATCAAAGGGCATTACCTGAAATGCAACTGC

4.1.24 Species: *Cleistopholis patens* (Benth.) Engl. & Diels

Family: Annonaceae

Specimen Number: IDMTG170-13|GH0146

Location: Bia Biosphere Reserve 178m N062°2.221' W002°59.218'

Plate 2: *Cleistopholis_patens* (Benth.) Engl. & Diels

DNA barcode: *rabcL*

GAAACTAAAGCAAGTGTTGGATTCAAAGCTGGTGTTAAAGAGTACAAATTGACTTATTATACTCCT
GAATATGAAACCAAGGATACTGATATCTTGGCAGCATTCCGAGTAACTCCTCAACCCGGAGTTCCG
CCCGAGGAAGCAGGGGCTGCGGTAGCTGCCGAATCTTCTACTGGTACATGGACAACTGTGTGGAC
CGATGGACTTACCAGCCTTGATCGTTACAAAGGACGATGCTACCACATCGAGCCCGTTGCTGGGGA
GGAAAATCAATTTATTGCTTATGTAGCTTACCCTTTAGACCTTTTTGAAGAGGGTTCTGTTACTAAC
ATGTTTACTTCCATTGTAGGTAATGTATTTGGGTTCAAAGCTCTACGAGCTCTACGTCTGGAAGACT
TGCGAATTCCTACTTCTTATGTCAAGACTTTCCAGGGCCCGCCTCACGGCATCCAAGTTGAGAGAG
ATAAATTGAACAAGTATGGTCGTCCCCTATTGGGATGTACTATTAAACCAAAATTGGGGTTATCCG
CCAAGAACTACGGTAGGGCGGTTTATGAATGTCTCCGTGGTGGACTTGATTTTACCAAGGATGATG

AGAACGTGAACTCCCAACCATTTATGCGCTGGAGAGACCGTTTCTTATTTTTGTGCCGAAGCAATT
TTTAAAGCGCAGGCCGAAACAGGTGAAATCAAAGGGCATTACCTGAAATGCAACTGC

4.1.25 Species: *Spathodea campanulata* Beauv.

Family: Bignoniaceous

Specimen Number: GH0150

Location: Kakum National Park 178m N05° 20.947' W001° 23.035'

Description: *Spathodea campanulata* is a large tree that can reach 50 ft in height. The pinnate leaves grow to 40 cm (16 in) long and they are bronze in color when young, turning deep glossy-green at maturity. Then, the flared, funnel shaped flowers appear in 8 to 10 cm long racemes on the tips of the branches, all over the tree. The flowers are large, brilliant red-orange (yellow in some cultivars) in color and with crinkled margins. The fruit is a long pod, up to 45 cm long (18 in.) that breaks open when they fall from the tree. Inside there are very small seeds with transparent wings (Labrada *et, al.,* 2003).

Plate 1: *Spathodea campanulata* Beauv.

DNA barcode: *rbcLa*

AAAGCAAAGTGTTTGGATTCAAAAGCTGGTGTTAAAGATTATAAATTGACTTTATTACACTCCTGA
ATATCAAGTCAAAGATACTGATATCTTGGCAGGCATTTCGAGTAACTCCTCAACCAGGAGTTCCCC
CTGAAGAAGCAGGGGCTGCGGTAGCTGCTGAATCTTCTACTGGTACATGGACAACTGTATGGACT

GACGGGCTTACCAGTCTTGATCGCTACAAAGGTCGATGCTACAACATCGAGCCCGTTGCTGGAGA
AGACAATCAATATATTGCTTATGTAGCTTACCCCTTAGACCTTTTTGAAGAAGGTTCTGTTACTAAC
ATGTTTACTTCCATTGTGGGTAATGTATTTGGGTTTAAGGCCCTGCGTGCGCTACGTCTGGAAGATT
TGCGAATCCCTCCTGCTTATTCTAAAACYTTCCAAGGRCCACCTCATGGTATCCAAGTTGAGAGAG
ATAAATTGAACAAGTATGGCCGCCCCCTATTGGGATGTACTATTAAACCTAAATTGGGGTTATCCG
CTAAGAATTACGGTAGAGCMGTTTATGAATGTCTTCGCGGTGGACTTGATTTTACCAAAGATGATG
AGAACGTGAATTCCCAACCATTTATGCGTTGGAGAGACCGTTTCGTATTTTGTGCCGAAGCAATTT
ATAAAGCACAAGCTGAAACAGGTGAAATCAAAGGACATTACTTTGAATTGCCAACTG

4.1.26 Species: *Spathodea campanulata* Beauv.

Family: Bignoniaceous

Specimen Number: IDMTG109-13|GH0149

Location: Bia Biosphere Reserve 193m N06^{o} 36.354' W003^{o} 05.743'

Plate 2: *Spathodea campanulata* Beauv.

DNA barcode: *rbcLa*

AAAGCAAAGTGTTTGGATTCAAAAGCTGGTGTTAAAGATTATAAATTGACTTTATTACACTCCTGA
ATATCAAGTCAAAGATACTGATATCTTGGCAGGCATTTCGAGTAACTCCTCAACCAGGAGTTCCCC
CTGAAGAAGCAGGGGCTGCGGTAGCTGCTGAATCTTCTACTGGTACATGGACAACTGTATGGACT
GACGGGCTTACCAGTCTTGATCGCTACAAAGGTCGATGCTACAACATCGAGCCCGTTGCTGGAGA
AGACAATCAATATATTGCTTATGTAGCTTACCCCTTAGACCTTTTTGAAGAAGGTTCTGTTACTAAC
ATGTTTACTTCCATTGTGGGTAATGTATTTGGGTTTAAGGCCCTGCGTGCGCTACGTCTGGAAGATT
TGCGAATCCCTCCTGCTTATTCTAAAACYTTCCAAGGRCCACCTCATGGTATCCAAGTTGAGAGAG
ATAAATTGAACAAGTATGGCCGCCCCCTATTGGGATGTACTATTAAACCTAAATTGGGGTTATCCG
CTAAGAATTACGGTAGAGCMGTTTATGAATGTCTTCGCGGTGGACTTGATTTTACCAAAGATGATG
AGAACGTGAATTCCCAACCATTTATGCGTTGGAGAGACCGTTTCGTATTTTGTGCCGAAGCAATTT
ATAAAGCACAAGCTGAAACAGGTGAAATCAAAGGACATTACTTTGAATTGCCAACTG

4.1.27 Species: *Khaya senegalensis* (Desr.) A. Juss

Family: Meliaceae

Specimen Number: GH0157

Location: Kakum National Park 192m N05° 20.951' W001°23.062'

Description: *Khaya senegalensis* is a deciduous evergreen tree, 15-30 m high, up to 1m in diameter, with a clean bole to 8-16 m, buttresses not prominent or absent. Leaves alternate, compound, stipules absent; petiole and rachis 13-33 cm long; leaflets 3-4 (max. 7) usually opposite pairs, oblong to narrowly oblong-elliptic, 4-12 x 2-5 cm, apex acute to shortly acuminate, base rounded, margins entire, pale green, lateral nerves 8-16, petiolules about 3.5 cm long. Inflorescence a lax, much-branched axillary panicle up to 17 cm long. Fruit an upright, almost spherical, woody capsule, 4-6 cm in diameter, opening by 4 valves from the apex (a distinction from K. ivorensis, which is closely related but has 5 valves (Orwa *et al.*, 2009).

Plate 1: *Khaya senegalensis* (Desr.) A. Juss

DNA barcode: ***rbcLa***

GAAACTAAAGCAAGTGTTGGATTCAAAGCCGGTGTTAAAGATTATAAATTGACTTATTATACGCCT
GACTATGTAACCAAAGATACTGATATCTTGGCAGCATTCCGAGTAACTCCTCAACCCGGAGTTCCG
CCCGAGGAAGCAGGGGCTGCGGTAGCTGCGGAATCTTCTACTGGTACATGGACAACTGTGTGGAC
CGATGGGCTTACTAGCCTTGATCGTTACAAAGGACGATGCTACAACATTGAGCCAGTTGCTGGAGA

AGAAAATCAATATATATGTTATGTAGCTTACCCTTTAGACCTTTTTGAAGAAGGTTCTGTTACTAAC
ATGTTTACATCCATTGTGGGTAATGTATTTGGGTTCAAAGCCCTGCGCGCTCTACGTCTAGAGGAT
CTACGAATCCCTCCCGCGTATTCTAAAACTTTCCAAGGCCCGCCTCATGGCATCCAAGTTGAGAGA
GATAAATTGAACAAGTATGGTCGTCCCCTATTGGGATGTACAATTAAACCTAAATTGGGGTTATCC
GCTAAGAATTACGGTAGAGCAGTTTATGAATGTCTACGCGGTGGACTTGACTTTACCAAAGATGAT
GAGAACGTGAACTCCCAACCATTTATGCGTTGGAGAGACCGTTTCGTATTTTGTGCGGAAGCAATC
TATAAAGCGCAAGCTGAAACAGGTGAAATCAAAGGGCATTACTTGAATGCAACTG

4.1.28 Species: *Khaya_senegalensis* (Desr.) A. Juss

Family: Meliaceae

Specimen Number: IDMTG114-13|GH0158

Location: Ankasa Resource Reserve 176m NO5° 16.112' W002° 36.321'

Plate 2: *Khaya senegalensis* (Desr.) A. Juss

DNA barcode: *rbcLa*

GAAACTAAAGCAAGTGTTGGATTCAAAGCCGGTGTTAAAGATTATAAATTGACTTATTATACGCCT
GACTATGTAACCAAAGATACTGATATCTTGGCAGCATTCCGAGTAACTCCTCAACCCGGAGTTCCG
CCCGAGGAAGCAGGGGCTGCGGTAGCTGCGGAATCTTCTACTGGTACATGGACAACTGTGTGGAC
CGATGGGCTTACTAGCCTTGATCGTTACAAAGGACGATGCTACAACATTGAGCCAGTTGCTGGAGA
AGAAAATCAATATATATGTTATGTAGCTTACCCTTTAGACCTTTTTGAAGAAGGTTCTGTTACTAAC
ATGTTTACATCCATTGTGGGTAATGTATTTGGGTTCAAAGCCCTGCGCGCTCTACGTCTAGAGGAT
CTACGAATCCCTCCCGCGTATTCTAAAACTTTCCAAGGCCCGCCTCATGGCATCCAAGTTGAGAGA
GATAAATTGAACAAGTATGGTCGTCCCCTATTGGGATGTACAATTAAACCTAAATTGGGGTTATCC
GCTAAGAATTACGGTAGAGCAGTTTATGAATGTCTACGCGGTGGACTTGACTTTACCAAAGATGAT
GAGAACGTGAACTCCCAACCATTTATGCGTTGGAGAGACCGTTTCGTATTTTGTGCGGAAGCAATC
TATAAAGCGCAAGCTGAAACAGGTGAAATCAAAGGGCATTAC

4.1.29 Species: *Persea americana* Mill.

Family: Lauraceae

Specimen Number: GH0162

Location: Kakum National Park 182m N05° 20.957' W001° 23.045'

Description: *Persea americana* is a medium to large tree, 9-20 m in height. Leaves are 7-41 cm in length and variable in shape (elliptic, oval, lanceolate). They are often pubescent and reddish when young, becoming smooth, leathery, and dark green when mature. Flowers are yellowish green, and 1-1.3 cm in diameter. The many- flowered inflorescences are borne in a pseudo-terminal position. The fruit is a berry, consisting of a single large seed, surrounded by a buttery pulp (Orwa *et al.*, 2009).

Plate 1: *Persea americana* Mill**.**

DNA barcode: (*rbcLa*) Sequencing was not successful

4.1.30 Species: *Persea americana* Mill.

Family: Lauraceae

Specimen Number: IDMTG117-13|GH0161

Location: Bia Biosphere Reserve 158m. N06° 22.119' W0025° 9.158'

Plate 2: *Persea americana* Mill**.**

DNA barcode: *rbcLa*

GAAACTAAAGCAAGTGTTGGATTCAAAGCTGGTGTTAAAGATTACAAATTGACTTATTATACTCCT
GACTATGAAACCAAAAGTACTGATATTTTGGCAGCATTTCGAGTAACTCCTCAACCCGGAGTTCCA
CCTGAGGAAGCAGGGGCTGCGGTAGCTGCCGAATCTTCTACTGGTACATGGACAACTGTGTGGAC
CGATGGACTTACCAGCCTTGATCGTTACAAAGGACGATGCTACCACATCGAGCCCGTTGCTGGGGA
GGAAAGTCAATTTATTGCCTATGTAGCTTACCCTTTAGACCTTTTTGAAGAAGGTTCTGTTACGAAC
ATGTTTACTTCTATTGTGGGTAATGTATTTGGGTTCAAAGCTCTACGAGCTCTACGTCTGGAGGATC
TGCGAATTCCTCCTGCTTATTCCAAAACTTTCCAAGGCCCGCCCCATGGCATCCAAGTTGAGAGAG
ATAAATTGAACAAGTATGGTCGTCCCCTATTGGGATGTACTATTAAACCAAAATTGGGGTTATCCG
CCAAGAACTACGGTAGAGCGGTTTATGAATGTCTCCGTGGTGGACTTGATTTTACCAAGGATGATG
AGAACGTGAACTCCCAACCATTTATGCGTTGGAGAGACCGTTTCTTATTTTGTGCCGAAGCAATTT
ATAAATCGCAGCCGAAACAGGTGAAATCAAAGGACATTACTTGAATGCAACTG

4.1.31 Species: *Theobroma cacao* L.

Family: Malvaceae

Specimen Number: GH0168

Location: Bia Biosphere Reserve 158m. N06° 22.119' W002° 59.158'

Description: *Theobroma cacao* is an evergreen tree about 12-15 feet high and branching at the top. The leaves are dark green, petiolate, lanceolate shaped and smooth on both sides. The flowers are very small, clustered, white with a reddish tint and scentless. The fruits grow right out of the trunk and branches of the tree and ripen into long, orange pods. The opened pod reveals several rows of seeds, also called cocoa "beans". There are approximately 40 seeds in each pod and each of them is coated with a sweet, juicy, delicious pulp (Orwa *et al.*, 2009).

Plate 1: *Theobroma cacao* L

DNA barcode: *rbcLa* (Sequencing was not successful**)**

4.1.32 Species: *Theobroma cacao* L.

Family: Malvaceae

Specimen Number: IDMTG121-13|GH0167

Location: Kakum National Park 180m N05° 20.657' W001° 23.033'

Plate 2: *Theobroma cacao* L

DNA barcode: *rbcLa*

CAGAAACTAAAGCAAGTGTTGGATTCAAAGCTGGTGTTAAAGAGTATAAATTGACTTATTATACTC
CTGAATATGAAGTCAAAGATACTGATATCTTGGCAGCCTTCCGAGTAACTCCTCAACCCGGAGTTC
CGCCTGAGGAAGCAGGGGCCGCGGTAGCTGCTGAATCTTCTACTGGTACATGGACAACCGTGTGG
ACCGATGGGCTTACCAGCCTTGATCGTTACAAAGGGCGATGCTACCACATCGAGCCCGTTGCTGGA

GAAGAAAATCAATATATATGTTATGTAGCTTACCCCTTAGACCTTTTTGAAGAAGGTTCTGTTACT
AACATGTTTACTTCCATTGTGGGTAATGTATTTGGGTTCAAAGCCCTGCGCGCTCTACGTCTAGAG
GATCTGCGAATCCCTACTGCTTATATTAAAACTTTCCAAGGCCCGCCTCATGGCATCCAGGTTGAA
AGAGATAAATTGAACAAGTACGGTCGTCCCCTATTGGGATGTACTATTAAACCTAAATTGGGGTTA
TCCGCTAAGAACTACGGTAGAGCAGTTTATGAATGTCTACGTGGTGGACTTGATTTTACCAAAGAT
GATGAGAATGTGAACTCCCAACCATTTATGCGTTGGAGAGACCGTTTCTTATTTTGTGCCGAAGCA
ATTTATAAAGCACAGGCCTGAAACAGGTGAAATCAAAGGGCATTTACTTGAATG

4.1.33 Species: *Diospyros mespiliformis* Hochst. ex A. DC

Family: Ebenaceae

Specimen Number: GH0175

Location: Ankasa Resource Reserve 181m NO5° 16.101' W002° 36.521'

Description: *Diospyros mespiliformis* is a tall, evergreen tree 15-50 m high, with dense, rounded and buttressed stem. Leaves alternate, shiny-green above, paler beneath, 4-7 cm long, 1.5-5.5 cm wide, oblong elliptic or oblolanceolate-elliptic, rarely lanceolate-elliptic, pubescent when young later becoming glabrescent or with few persistent, appressed hairs beneath, acute or subacuminate at the apex, cuneate or rounded at base with impressed midrib above, prominent beneath. Flowers pentamerous, white and fragrant. Fruits usually globose, fleshy, up to 3 cm in diameter, greenish and pubescent when young, yellowish to orange yellow and glabrous when ripe, bell shaped, with persistent style and enlarged calyx and contain 4-6 seeds (Orwa *et al.*2009).

Plate 1: *Diospyros mespiliformis* Hochst. ex A. DC

DNA barcode: *rbcLa* (Sequencing was not successful**)**

4.1.34 Species: *Diospyros mespiliformis* Hochst. ex A. DC

Family: Ebenaceae

Specimen Number: IDMTG126-13|GH0174

Location: Kakum National Park 191m N05* 20.921' W001* 23.042'

Plate 2: *Diospyros mespiliformis* Hochst. ex A. DC

DNA barcode: *rabcL*

ACAACTAAAGCAAGTGTTGGATTCAAAGCTGGTGTTAAAGATTACAAATTGACTTATTATACTCCT
GACTATGAAACCAAAGATACTGATATCTTGGCAGCATTCCGAGTAACTCCTCAACCTGGAGTTCCA
CCGGAAGAAGCAGGGGCCGCGGTAGCTGCCGAATCTTCTACTGGTACATGGACAACTGTGTGGAC
CGATGGACTTACTAGTCTTGATCGTTACAAAGGGCGATGCTACCACATCGAGCCCGTTGCTGGAGA
AGAAAGTCAATTTATTGCTTATGTAGCTTATCCTTTAGACCTTTTTGAAGAAGGTTCTGTTACTAAC
ATGTTTACTTCCATTGTGGGTAATGTATTTGGGTTCAAAGCCCTGCGCGCTCTACGTCTGGAAGATT
TGCGAATCCCTACTTCGTATGTTAAAACTTTCCAAGGACCACCTCATGGTATCCAAGTTGAAAGAG
ATAAATTGAACAAGTATGGTCGTCCCCTGTTGGGATGTACTATTAAACCGAAATTGGGGTTATCCG
CTAAAAACTACGGTAGAGCAGTTTATGAATGTCTCCGCGGTGGACTTGATTTTACCAAAGATGATG
AGAACGTGAACTCCCAACCATTTATGCGTTGGAGAGACCGTTTCTTATTTTGTGCCGAAGCACTTT
ATAAAGCACAGGCTGAAACAGGTGAAATCAAAGGGCATTACTTGATGAC

4.1.35 Species: *Azadirachta indica* A. Juss

Family: Meliaceae

Specimen Number: GH0180

Location: Ankasa Resource Reserve 173m NO5° 16.111' W002° 36.525'

Description: *Azadirachta indica* is a small to medium-sized tree, usually evergreen, up to 15 (30 max.) m tall. Leaves alternate, crowded near the end of branches, simply pinnate, 20- 40 cm long, exstipulate, light green, with 2 pairs of glands at the base, otherwise glabrous; petiole 2-7 cm long, subglabrous; rachis channelled above; leaflets 8-19, very short petiol. nflorescence an axillary, many-flowered thyrsus, up to 30 cm long; bracts minute and caducous; flowers bisexual or male on same tree. Fruit 1 (max. 2)-seeded drupe, ellipsoidal, 1-2 cm long, greenish, greenish-yellow to yellow or purple when ripe; exocarp thin, mesocarp pulpy, endocarp cartilaginous; seed ovoid or spherical; apex pointed; testa thin, composed of a shell and a kernel (sometimes 2 or 3 kernels), each about half of the seed's weight (Orwa *et al.*, 2009)

Plate 1: *Azadirachta_iindica* A. Juss

DNA barcode: *rbcLa*

GAAACTAAAGCAAGTGTTGGATTCAAAGCTGGTGTTAAAGATTATAAACTGACTTATTATACTCCT
GACTATCAAACCAAAGATACTGATATCTTGGCAGCATTCCGAGTAACTCCTCAACCTGGAGTTCCG
CCTGAGGAAGCAGGGGCTGCAGTGGCTGCTGAATCTTCTACTGGGACATGGACAACTGTATGGAC
CGATGGGCTTACCAGCCTTGACCGTTATAAAGGAAGATGCTACCACATCGAACCTGTTGCTGGAGA
AGAAAATCAATATATATGCTATGTAGCTTACCCCTTAGACCTTTTTGAAGAAGGTTCTGTTACTAAT
ATGTTTACTTCCATTGTGGGTAATGTATTTGGGTTCAAAGCCCTACGCGCTCTACGTCTGGAGGATC
TGCGAATTCCTACGGCCTATATTAAAACTTTCCAAGGCCCTCCTCACGGGATCCAAGTTGAGAGAG
ATAAATTGAACAAGTATGGTCGTCCCCTATTGGGATGTACTATTAAACCTAAATTGGGGTTATCCG
CTAAGAACTACGGTCGAGCAGTTTATGAATGTCTTCGTGGTGGACTTGATTTTACGAAGGATGATG
AGAACGTGAACTCACAACCATTTATGCGTTGGAGAGACCGTTTCTTGTTTTGTGCCGAAGCAATTT
ATAAAGCACAAGCTGAAACTGGTGAAATCAAAAGGACATTTACTTGAATGCAACTG

4.1.36 Species: *Azadirachta_iindica* A. Juss

Family: Meliaceae

Specimen Number: IDMTG131-13|GH0180

Location: Bia Biosphere Reserve.185m N063° 7.384' W0030° 3.604'

Plate 2: *Azadirachta indica* A. Juss

DNA barcode: ***rbcLa***

GAAACTAAAGCAAGTGTTGGATTCAAAGCTGGTGTTAAAGATTATAAACTGACTTATTATACTCCT
GACTATCAAACCAAAGATACTGATATCTTGGCAGCATTCCGAGTAACTCCTCAACCTGGAGTTCCG
CCTGAGGAAGCAGGGGCTGCAGTGGCTGCTGAATCTTCTACTGGGACATGGACAACTGTATGGAC
CGATGGGCTTACCAGCCTTGACCGTTATAAAGGAAGATGCTACCACATCGAACCTGTTGCTGGAGA
AGAAAATCAATATATATGCTATGTAGCTTACCCCTTAGACCTTTTTGAAGAAGGTTCTGTTACTAAT
ATGTTTACTTCCATTGTGGGTAATGTATTTGGGTTCAAAGCCCTACGCGCTCTACGTCTGGAGGATC
TGCGAATTCCTACGGCCTATATTAAAACTTTCCAAGGCCCTCCTCACGGGATCCAAGTTGAGAGAG
ATAAATTGAACAAGTATGGTCGTCCCCTATTGGGATGTACTATTAAACCTAAATTGGGGTTATCCG
CTAAGAACTACGGTCGAGCAGTTTATGAATGTCTTCGTGGTGGACTTGATTTTACGAAGGATGATG
AGAACGTGAACTCACAACCATTTATGCGTTGGAGAGACCGTTTCTTGTTTTGTGCCGAAGCAATTT
ATAAAGCACAAGCTGAAACTGGTGAAATCAAAAGGACATTTACTTGAATGCAACTG

4.1.37 Species: *Eucalyptus sp* Cattai

Family: Myrtaceae

Specimen Number: GH0182

Location: Ankasa Resource Reserve 173m NO5° 16.121' W002° 36.542'

Description: *Eucalyptus sp*. is a tree to 4.5 m high; adult leaves disjunct, lanceolate to broad lanceolate, 4.6-11.5 cm long, 1-4.2 cm wide, dark green, glossy, discolorous, penniveined. Umbellasters 6-8-flowered; peduncle flattened or angular, 5-13 mm long; pedicels terete, 0-6

mm long. Buds fusiform to ovoid or conical, 6-10 mm long, 3.5-5 mm diam., scar present; calyptra conical to hemispherical, sometimes slightly beaked, more or less ribbed, at least as wide as hypanthium, length longer or shorter than hypanthium; fruit hemispherical or cup-shaped, 5-6 mm long, 5-7 mm long; disc flat to raised; valves exserted (Orwa *et al.*, 2009)

Plate 1: *Eucalyptus sp* Cattai

DNA barcode: *rbcLa*

AAAACTAAAGCAAGTGTTGGATTCAAAGCTGGTGTTAAAGATTATAAACTGACTTATTATACTCCT
GACTATGAAACCAAAGATACTGATATCTTGGCAGCATTCCGAGTAACTCCTCAACCTGGAGTTCCT
CCTGAGGAAGCAGGGGCTGCGGTAGCTGCTGAATCTTCTACTGGTACATGGACAACTGTGTGGAC
CGATGGGCTTACCAGCCTTGATCGTTATAAAGGAAGATGCTACCACATCGAGCCTGTTGCTGGAGA
AGAAAATCAATATATATGTTATGTAGCTTACCCTTTAGACCTTTTTGAAGAAGGTTCTGTTACTAAT
ATGTTTACTTCCATTGTGGGTAATGTATTTGGGTTCAAAGCCCTGCGCGCTCTACGTCTGGAGGATC
TGCGAATCCCTCCTTCCTATACGAAAACTTTCCAAGGCCCGCCTCATGGCATCCAAGTTGAGAGAG
ATAAATTGAACAAATATGGGCGTCCCCTATTGGGATGTACTATTAAACCGAAATTGGGGTTATCCG
CTAAGAACTACGGTAGAGCAGTTTATGAATGTCTTCGTGGTGGACTTGATTTTACGAAAGATGATG
AGAACGTGAACTCACAACCATTTATGCGTTGGAGAGACCGTTTCTTATTTTTGTGCCGAAGCCATT
TTTAAATCACAGGCTGAAACAGGTGAAATCAAAGGGCATTACTTTGAATGCAACTG

4.1.38 Species: *Eucalyptus sp* Cattai

Family: Myrtaceae

Specimen Number: IDMTG132-13|GH0182

Location: Ankasa Resource Reserve 176m NO5° 16.131' W002° 36.732'

Plate 2: *Eucalyptus sp* Cattai

DNA barcode: *rbcLa* (Sequencing was not successful**)**

4.1.39 Species: *Psydrax subcordata* (DC.) Bridson.

Family: Rubiaceae

Specimen Number: GH0186

Location: Bia Biosphere Reserve 199m N06° 37.484' W0030° 3.804'

Description: *Psydrax odorata* is a shrub or small tree ranging in height from 2 to 10 meters, but spreading only 1 to 2.5 m in width. The elliptical leaves are glossy green on the top surface and up to 9 cm long. The small, white flowers are fragrant and are born in clusters up to 3.5 cm long. The fruits of alahe`e are between 0.5 and 1 cm in diameter, more or less round, and black at maturity. The fruits generally ripen in the late fall and winter and contain one or two seeds (Orwa *et al.*, 2009)

Plate1: *Psydrax subcordata* (DC.) Bridson

DNA barcode: *rbcLa*

CGACTAAAGCAAGTGTTGGATTCAAAGCTGGTGTTAAAGAGTACAAATTGACTTATTATACTCCTG
AATACGAAACCAAAGATACTGATATCTTGGCAGCATTCCGAGTAACTCCTCAACCGGGAGTTCCAC
CTGAAGAAGCGGGGGCCGCGGTAGCTGCCGAATCTTCTACTGGTACATGGACAACTGTGTGGACC
GATGGGCTTACCAGCCTTGATCGTTACAAAGGGCGATGCTATCACATCGAGCCAGTTCCTGGAGAA
GAAGATCAATTTATTGCTTATGTAGCTTACCCCTTAGACCTTTTTGAAGAAGGTTCTGTTACTAACA
TGTTTACTTCCATTGTAGGTAATGTATTTGGGTTCAAAGCCCTGCGCGCTCTACGTCTGGAAGATTT
GCGAATCCCTACTGCTTATGTTAAAACCTTCCAAGGCCCGCCTCATGGCATCCAAGTTGAAAGAGA
TAAATTGAACAAGTATGGTCGTCCCCTGTTGGGATGTACTATTAAACCTAAATTAGGTTTATCTGCT
AAAAACTACGGTAGAGCTTGTTATGAATGTCTTCGCGGGGGACTTGATTTTACCAAAGATGATGAA
AACGTGAACTCCCAACCATTTATGCGTTGGAGAGATCGTTTCTTATTTTGTGCTGAAGCGCTTTATA
AAGCACAAGCTGAAACAGGTGAAATCAAAGGGCATTACTTGAATGCACTG

4.1.40 Species: *Psydrax subcordata* (DC.) Bridson.

Family: Rubiaceae

Specimen Number: IDMTG135-13|GH0186

Location: Ankasa Resource Reserve 162m NO5° 16.119' W002° 36.412'

Plate 2: *Psydrax subcordata* (DC.) Bridson

DNA barcode: *rbcLa* (Sequencing was not successful**)**

4.1.41 Species: *Annona muricata* L.

Family: Annonaceae

Specimen Number: GH0190

Location: Bia Biosphere Reserve. 178m N06° 22.121' W002° 59.168'

Description: *Annona muricata* is a slender, evergreen tree, 5-10 m in height and 15 cm in diameter. Leaves alternate, 7.6-15.2 cm long, 2.5-7.6 cm wide, leathery, obovate to elliptic, glossy on top, glabrous on underside, simple; stipules absent; blade oblanceolate, green on top, paler and dull on under side with fine lateral nerves; a strong, pungent odour; petioles short, 3-10 m. Flower terminal or lateral, large; stalks stout, green, 1.3-1.9 cm long; 3 sepals, minute, inconspicuous, broad, green, 3 mm long, triangular; petals yellowish-green, 6 in 2 whorls of 3, outer petals larger, ovate-acute, valvate, cordate with pointed apex. Fruit 14-40 x 10-18 cm, weighing up to 7 kg, ovoid, heart shaped, an oblong syncarp composed of numerous united pistils, pistils end in a fleshy spine or short base of spine 1.5 mm or more in length, which grows from the style (Orwa *et al.*, 2009).

Plate 1: *Annona muricata* L.

DNA barcode: *rbcLa* (Sequencing was not successful**)**

4.1.42 Species: *Annona muricata* L.

Family: Annonaceae

Specimen Number: IDMTG136-13|GH0188

Location: Ankasa Resource Reserve 182m NO5° 16.133' W002° 36.732'

Plate 2: *Annona muricata* L.

DNA barcode: *rbcLa*

GAAAACTAAAGCAAAGTGTTGGATTCAAAGCGGGTGTTAAAGAGTACAAATTGACTTATTATACT
CCTGAATATGAAACCAAAGATACTGATATCTTGGCAGCATTCCGAGTAACCCCTCAACCTGGAGTT
CCGCCCGAGGAAGCAGGGGCTGCGGTAGCTGCCGAATCTTCTACCGGTACATGGACAACCGTGTG
GACCGATGGACTTACCAGCCTTGATCGTTACAAAGGACGATGCTACCACATCGAGCCTGTTGCTGG
AGAGGAAAATCAATATATTGCTTATGTAGCTTACCCTTTAGATCTTTTTGAGGAGGGTTCTGTTACT
AACATGTTTACTTCCATTGTAGGTAATGTGTTTGGGTTCAAAGCTCTACGAGCTCTACGCCTAGAG
GACTTGCGAATTCCTACTTCTTATATCAAGACTTTCCAGGGCCCGCCCCACGGCATCCAAGTTGAG
AGAGATAAATTGAACAAGTATGGCCGTCCCCTATTGGGATGTACTATTAAACCCAAATTGGGGTTA
TCCGCCAAGAACTACGGTAGGGCGGTTTATGAATGTCTCCGCGGTGGACTTGATTTTACCAAGGAT
GATGAGAACGTGAACTCCCAACCATTTATGCGCTGGAGAGACCGTTTCTTATTTTGTGCTGAAGCC
CTTTTTAAAGCGCAGGCCGAAACGGGTGAAATCAAAGGACATTACTTGAATGCAACTG

4.1.43 Species: *Anogeissus leiocarpus* (DC.) Guill. & Perr

Family: Combretaceae

Specimen Number: GH0193

Location: Kakum National Park 179m N05° 20.947' W001° 23.035'

Description: is a tree of up to 30 m in height, typically 15-18 m with light green foliage. Leaves are alternate to subopposite, elliptic to ovate-lanceolate in shape, and 2-8 cm long and 1.5-3.5 cm across. The leaves are acuminate or mucronate at the apex and cuneate at the base. The in florescence is a spherical, axillary and terminal cluster. The yellow-green scented flowers are brown- ish-orange at the centre and with white hairs. The flowers are bisexual,

apetalous, 5-6 mm in diameter, and with 10 stamens. Fruits are yellowish to reddish brown, trape-ziform samaras, 4-7 mm long and 6-10 mm across (Orwa *et al.*, 2003).

Plate 1: *Anogeissus leiocarpus* (DC.) Guill. & Perr

DNA barcode: *rbcLa*

AAACTAAAGCAAGTGTTGGATTCAAAGCTGGTGTTAAAGATTATAAACTGACTTATTATACTCCTG
ACTATCAAACCAAAGATACTGATATCTTGGCAGCATTCCGAGTAACTCCTCAACCTGGAGTTCCGC
CTGAGGAAGCAGGGGCTGCAGTGGCTGCTGAATCTTCTACTGGGACATGGACAACTGTATGGACC
GATGGGCTTACCAGCCTTGACCGTTATAAAGGAAGATGCTACCACATCGAACCTGTTGCTGGAGA
AGAAAATCAATATATATGCTATGTAGCTTACCCCTTAGACCTTTTTGAAGAAGGTTCTGTTACTAAT
ATGTTTACTTCCATTGTGGGTAATGTATTTGGGTTCAAAGCCCTACGCGCTCTACGTCTGGAGGATC
TGCGAATTCCTACGGCCTATATTAAAACTTTCCAAGGCCCTCCTCACGGGATCCAAGTTGAGAGAG
ATAAATTGAACAAGTATGGTCGTCCCCTATTGGGATGTACTATTAAACCTAAATTGGGGTTATCCG
CTAAGAACTACGGTCGAGCAGTTTATGAATGTCTTCGTGGTGGACTTGATTTTACGAAGGATGATG
AGAACGTGAACTCACAACCATTTATGCGTTGGAGAGACCGTTTCTTGTTTTGTGCCGAAGCAATTT
ATAAAGCACAAGCTGAAACTGGTGAAATCAAAGGACATTACTTGAAATGCAACTG

4.1.44 Species: *Anogeissus leiocarpus* (DC.) Guill. & Perr

Family: Combretaceae

Specimen Number: IDMTG140-13|GH0194

Location: Ankasa Resource Reserve. 165m NO5° 16.111' W002°36.324'

Plate 2: *Anogeissus leiocarpus* (DC.) Guill. & Perr

DNA barcode: *rbcLa* (Sequencing was not successful**)**

4.1.45 Species: *Daniellia oliveri* (Rolfe) Hutch. & Dalziel

Family: Fabaceae – Caesalpinioideae

Specimen Number: GH0204

Location: Ankasa Resource Reserve. 167m NO5° 16.101' W002°36.521'

Description: Deciduous, medium-sized tree up to 25(–35) m tall; bole straight and cylindrical, up to 200 cm in diameter, without buttresses. Leaves alternate, paripinnately compound with (3–) 6–11 pairs of leaflets; stipules up to 6.5(–9) cm × 0.5 cm, soon falling. Flowers bisexual, zygomorphic, scented; pedicel 7–13 mm long, glabrous, enlarging in fruit, below the middle with 2 caducous bracteoles c. 0.5 cm long; sepals 4, oblong, c. 1.5 cm long, glabrous but margins hairy; petals 5, white to creamy, unequal, ovate-elliptical, 4 petals 1–1.5 (–3) mm long. Fruit an obliquely lanceolate, flattened pod 6–10 cm × 3–4.5 cm, with stipe c. 1 cm long, glabrous, green becoming brown, dehiscing with 2 papery valves, 1-seeded (Orwa *et al.*, 2009).

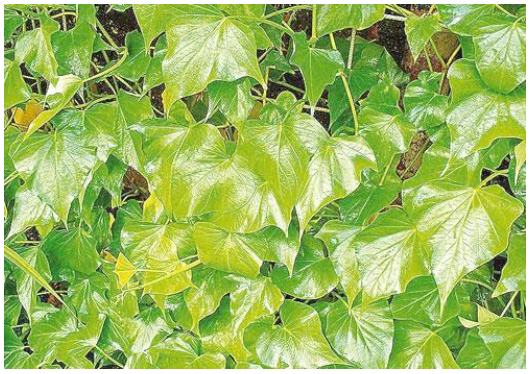


Plate 1: *Daniellia oliveri* (Rolfe) Hutch. & Dalziel

DNA barcode: *rbcLa*

GAAACTAAAGCACGTGTTGGATTCAAAGCAGGTGTTAAAGATTATAAATTGACTTATTATACTCCT
GACTATGAAACCAAGGATACTGATATCTTGGCAGCATTCCGAGTAACTCCTCAACCAGGAGTTCCG
CCCGAAGAAGCAGGTGCCGCAGTAGCTGCTGAATCTTCTACTGGTACATGGACAACTGTGTGGAC
CGACGGGCTTACCAGCCTTGATCGTTACAAAGGACGATGCTACCACATTGAGCCCGTTGCTGGAGA
AGAAAATCAATATATTGCTTATGTAGCTTATCCCTTAGACCTTTTTGAAGAAGGTTCTGTTACTAAC
ATGTTTACTTCCATTGTGGGTAATGTCTTTGGGTTCAAGGCCCTGCGCGCTCTACGTCTGGAGGATT
TGCGAATCCCTACTGCTTATATTAAAACTTTCCAGGGTCCGCCTCACGGTATCCAAGTTGAGAGAG
ATAAATTGAACAAGTATGGCCGCCCCCTATTGGGATGTACTATTAAACCTAAATTGGGGTTATCCG
CTAAGAATTACGGTAGAGCGGTTTATGAATGTCTCCGCGGTGGACTTGATTTTACCAAAGATGATG
AGAACGTGAATTCCCAACCATTTATGCGTTGGAGAGACCGTTTCTTATTTTGTGCCGAAGCAATTT
ATAAAGCACAGGCCGAAACGGGTGAAATTAAAGGGCATTACTTGAATGCAACTG

4.1.46 Species: *Daniellia oliveri* (Rolfe) Hutch. & Dalziel

Family: Fabaceae – Caesalpinioideae

Specimen Number: IDMTG148-13|GH0203

Location: Kakum National Park 174m N05° 20.921' W001° 23.015'

Plate 2: *Daniellia oliveri* (Rolfe) Hutch. & Dalziel

DNA barcode: *rbcLa*1

GAAACTAAAGCACGTGTTGGATTCAAAGCAGGTGTTAAAGATTATAAATTGACTTATTATACTCCT
GACTATGAAACCAAGGATACTGATATCTTGGCAGCATTCCGAGTAACTCCTCAACCAGGAGTTCCG
CCCGAAGAAGCAGGTGCCGCAGTAGCTGCTGAATCTTCTACTGGTACATGGACAACTGTGTGGAC
CGACGGGCTTACCAGCCTTGATCGTTACAAAGGACGATGCTACCACATTGAGCCCGTTGCTGGAGA
AGAAAATCAATATATTGCTTATGTAGCTTATCCCTTAGACCTTTTTGAAGAAGGTTCTGTTACTAAC
ATGTTTACTTCCATTGTGGGTAATGTCTTTGGGTTCAAGGCCCTGCGCGCTCTACGTCTGGAGGATT
TGCGAATCCCTACTGCTTATATTAAAACTTTCCAGGGTCCGCCTCACGGTATCCAAGTTGAGAGAG
ATAAATTGAACAAGTATGGCCGCCCCCTATTGGGATGTACTATTAAACCTAAATTGGGGTTATCCG
CTAAGAATTACGGTAGAGCGGTTTATGAATGTCTCCGCGGTGGACTTGATTTTACCAAAGATGATG
AGAACGTGAATTCCCAACCATTTATGCGTTGGAGAGACCGTTTCTTATTTTGTGCCGAAGCAATTT
ATAAAGCACAGGCCGAAACGGGTGAAATTAAAGGGCATTACTTGAATGCAACTG

4.1.47 Species: *Anthocleista nobilis* G.Don

Family: Gentianaceae

Specimen Number: GH0208

Location: Kakum National Park 164m N05° 20.567' W001° 23.053'

Description: Small to medium-sized tree up to 18(–30) m tall; bole branchless for up to 15 m long, up to 45(–90) cm in diameter. Leaves opposite, crowded at the end of branchlets, simple; petiole 1–6 cm long, auricled; blade oblong-elliptical, obovate-elliptical to oblanceolate, 7–35 cm × 4–12 cm, in young plants up to 150 cm × 25 cm, base long-decurrent, apex rounded, margin wavy and recurved. Inflorescence an erect terminal dichasial cyme 12–60 cm long, many-flowered; peduncle and branches creamy or green, thickened at the nodes. Flowers bisexual, regular; sepals 4, free, orbicular to ovate-elliptical, 7–10 mm long; corolla with cylindrical tube 30–45 mm long, fleshy, lobes 11–14, oblong-lanceolate, 9–12 mm long, obtuse to rounded, spreading, white; stamens as many as corolla lobes and alternating with them, Fruit an ellipsoid berry 3–4 cm × 2–2.5 cm, thick-walled, green, many-seeded (Orwa *et al.*, 2003).

Plate 1: *Anthocleista nobilis* G.Don

DNA barcode: *rbcLa* (Sequencing was not successful**)**

4.1.48 Species: *Anthocleista nobilis* G.Don

Family: Gentianaceae

Specimen Number: IDMTG150-13|GH0206

Location: Ankasa Resource Reserve. 172m NO5° 16.115' W002° 45.324'

Plate 2: *Anthocleista nobilis* G.Don

DNA barcode: *rbcLa*

CGAACTAAAGCAAGTGTTGGATTCAAAGCTGGTGTTAAAGAGTACAAATTGACTTATTATACTCCT
GAATACGAAACCAAAGATACTGATATCTTGGCAGCATTCCGAGTAACCCCTCAACCCGGAGTTCC
ACCTGAAGAAGCAGGGGCCGCGGTAGCTGCCGAATCTTCTACTGGTACATGGACAACTGTGTGGA
CCGATGGACTTACTAGCCTTGATCGTTACAAAGGGCGATGCTACGGCATTGAGCCCGTTCCTGGAG
AAGAAAATCAATATATTGCTTATGTAGCTTATCCCTTAGACCTTTTTGAAGAAGGTTCTGTTACTAA
CATGTTTACTTCCATTGTAGGTAATGTATTTGGGTTCAAAGCCCTACGTGCTCTACGTCTGGAAGAT
TTGCGAATCCCAACCGCTTATATTAAAACTTTCCAAGGCCCTCCTCATGGCATCCAAGTTGAGAGA
GATAAATTGAACAAGTATGGTCGTCCCCTGTTGGGATGTACTATTAAACCTAAATTGGGGTTATCT

GCTAAAAACTACGGTAGAGCAGTTTATGAATGTCTTCGTGGTGGACTTGATTTTACCAAAGATGAT
GAAAACGTGAACTCTCAACCATTTTATGCGTTGGAGAGATCGTTTCTTATTTTTGTGCCGAAGCGCT
TTATAAAGCACAGGCTGAAACCGGGTGAAAATAAAAGGGCATTACTTGAATTG

4.1.49 Species: *Rauvolfia vomitoria* Afzel.

Family: Apocynaceae

Specimen Number: GH0082

Location: Kakum National Park 178m N05° 20.947' W001° 23.035'

Description: Rauvolfia vomitoria is a shrub or small tree up to 8 m. Older parts of the plant contain no latex. The branches are whorled and the nodes enlarged and lumpy. Leaves in threes, elliptic-acuminate to broadly lanceolate. Flowers are minute, sweet-scented, branches of inflorescences are distinctly puberulous with hardly any free corolla lobes. Fruits are fleshy and red in colour (Orwa *et al.*, 2009).

Plate 1: *Rauvolfia vomitoria* Afzel.

DNA barcode: *rbcLa*

CAGAACTAAAGCAAGTGTTGGATTCAAAGCTGGTGTTAAAGAGTACAAATTGACTTATTATACTCC
TGAATACGAAACCAAAGATACTGATATCTTGGCAGCATTCCGAGTAACTCCTCAACCTGGAGTTCC
ACCCGAAGAAGCAGGGGCCGCGGTAGCTGCCGAATCTTCTACTGGTACATGGACAACTGTGTGGA
CCGATGGACTTACCAGCCTTGATCGTTACAAAGGGCGATGCTACCACATCGAGCCCGTTCCTGGAG
AAGAAGAGCAATTTATTGCTTATGTAGCTTACCCCTTAGACCTTTTTGAAGAAGGTTCCGTTACTA
ACATGTTTACTTCCATTGTAGGTAATGTATTTGGGTTCAAAGCCCTACGCGCTCTACGTCTGGAAG
ATTTGCGAATCCCTCCGGCTTATGTTAAAACTTTCCAAGGCCCGCCTCATGGCATCCAGGTTGAGA
GAGATAAATTGAACAAATATGGTCGTCCCTTCTTGGGATGTACTATTAAACCTAAATTGGGGTTAT

CCGCTAAAAACTACGGTAGGGCATGTTATGAATGTCTTCGTGGCGGACTTGATTTTACCAAAGATG
ATGAAAACGTGAACTCCCAACCGTTTATGCGTTGGAGAGATCGTTTCTTATTTTGTGCCGAAGCAC
TTTATAAAGCACAAGCTGAAACCGGTGAAATCAAAAGGGCATTACTTGAATGCACTG
GGAAATCAAAGGACATTACTTGAATGCACTG

4.1.50 Species: *Rauvolfia vomitoria* Afzel.

Family: Apocynaceae

Specimen Number: IDMTG085-13|GH0116

Location: Ankasa Resource Reserve. 181m NO5° 16.255' W002° 45.521'

Plate 2: *Rauvolfia vomitoria* Afzel.

DNA barcode: *rbcLa*1

CAGAACTAAAGCAAGTGTTGGATTCAAAGCTGGTGTTAAAGAGTACAAATTGACTTATTATACTCC
TGAATACGAAACCAAAGATACTGATATCTTGGCAGCATTCCGAGTAACTCCTCAACCTGGAGTTCC
ACCCGAAGAAGCAGGGGCCGCGGTAGCTGCCGAATCTTCTACTGGTACATGGACAACTGTGTGGA
CCGATGGACTTACCAGCCTTGATCGTTACAAAGGGCGATGCTACCACATCGAGCCCGTTCCTGGAG
AAGAAGAGCAATTTATTGCTTATGTAGCTTACCCCTTAGACCTTTTTGAAGAAGGTTCCGTTACTA
ACATGTTTACTTCCATTGTAGGTAATGTATTTGGGTTCAAAGCCCTACGCGCTCTACGTCTGGAAG
ATTTGCGAATCCCTCCGGCTTATGTTAAAACTTTCCAAGGCCCGCCTCATGGCATCCAGGTTGAGA
GAGATAAATTGAACAAATATGGTCGTCCCTTCTTGGGATGTACTATTAAACCTAAATTGGGGTTAT
CCGCTAAAAACTACGGTAGGGCATGTTATGAATGTCTTCGTGGCGGACTTGATTTTACCAAAGATG
ATGAAAACGTGAACTCCCAACCGTTTATGCGTTGGAGAGATCGTTTCTTATTTTGTGCCGAAGCAC
TTTATAAAGCACAAGCTGAAACCGGTGAAATCAAAAGGGCATTACTTGAATGCACTG
GGAAATCAAAGGACATTACTTGAATGCACTG

4.1.51 Species: *Oncoba spinosa* Forssk.

Family: Salicaceae

Specimen Number: GH0214

Location: Bia Biosphere Reserve 191m. N06°36.434' W003°05.828'

Description: *Oncoba spinosa* grows up to 5 m, but may sometimes reach a height of 8 m. The leaves are simple, ovate-elliptic in form with a somewhat pointed tip and rounded, broad base. The leaves are dark, glossy green in colour and somewhat leathery and hairless. The margins are coarsely toothed. It bears large (90 mm wide) showy, sweet-scented, white flowers with masses of yellow, overlapping stamens in the centre. The rounded fruit of up to 60 mm in diameter, consists of a hard shell that becomes dark-reddish brown when mature and small, with shiny brown seeds embedded in a dry, sour, yellowish pulp (Orwa *et al.*, 2009).

Plate 1: *Oncoba_spinosa* Forssk.

DNA barcode: *rbcLa*

CAGAACTAAAGCAAGTGTTGGATTCAAGGCTGGTGTTAAAGATTATAAATTGACTTATTATACTCC
TGACTATGAAACCAAAGATACTGATATCTTGGCAGCATTCCGAGTAACTCCTCAACCTGGAGTTCC
GCCCGAGGAAGCAGGGGCCGCGGTAGCTGCTGAATCTTCTACTGGTACATGGACAACTGTGTGGA
CCGATGGGCTTACCAGTCTTGATCGTTATAAAGGACGATGCTACGGCATCGAGCCCGTTACTGGAG
AAGAAAATCAATATATTGCTTATGTAGCTTACCCCTTAGACCTTTTTGAAGAAGGTTCTGTTACTAA
CATGTTTACTTCCATTGTGGGTAATGTATTTGGGTTCAAAGCCCTACGCGCTCTACGTCTGGAGGAT
TTGCGAATCCCCCCTTCTTATACTAAAACTTTTCAAGGCCCACCTCACGGCATCCAAGTTGAGAGA
GATAAATTGAACAAGTATGGTCGCCCCCTATTGGGCTGTACTATTAAACCTAAATTGGGGTTATCC
GCTAAGAATTACGGTAGAGCAGTTTATGAATGTCTACGCGGTGGACTTGATTTTACCAAAGATGAT
GAGAACGTGAACTCCCAACCATTTATGCGTTGGAGAGATCGTTTCTTATTTTGTGCCGAAGCACTT
TATAAAGCACAGGGCTGAAACCGGTGAAATCAAAAGGGCATTTATTTAAACGCATG

4.1.52 Species: *Oncoba spinosa* Forssk.

Family: Salicaceae

Specimen Number: IDMTG156-13|GH0324

Location: Ankasa Resource Reserve 179m NO5° 16.101' W002° 36.521'

Plate 2: *Oncoba spinosa* Forssk.

DNA barcode: ***rbcLa*** (Sequencing was not successful)

4.1.53 Species: *Allanblackia floribunda* Oliv.

Family: Clusiaceae

Specimen Number: GH0169

Location: Bia Biosphere Reserve 191m. N06°36.434' W003°05.828'

Description: Allanblackia floribunda is an evergreen forest tree confined to tropical Africa, to 30m tall. Leaves opposite, 8-22 cm long by 2-4.5 cm wide; elliptic elongated, or somewhat oblanceolate, abruptly and sharply acuminate, cuneate or rounded at the base; with many pairs of very thin lateral nerves running at a wide angle to the midrib; stalk stout, 1-2 cm long. Flower unisexual, monoecious, pink or red, very fragrant, up to 5 cm across when expanded and 1.5 cm across in bud. Fruit is an ovoid 5-lobed berry-like drupe 9-20 cm long and 7-14 cm in diameter with tough flesh, hanging at the end of a short stalk. Seeds are

brittle-shelled, 2-5 cm long by 1.5-3.2 cm in diameter, 40-50 per fruit, embedded in a pinkish gelatinous pulp (Orwa *et al.*, 2009).

Plate 1: *Allanblackia floribunda* Oliv.

DNA barcode: *rbcLa*

CAGAAACTAAAACAGGTATTTTTTTCAAGGCTGGTGTTAAAGAGTATAAATTGAATTATTATACTC
CTCAGTATGAAACAAAAGATACTGATATCTTGGCAGCATTCCGAGTAACTCCTCAACCTGGAGTTC
CACCCGAAGAAGCAGGAGCTGCAGTAGCTGCGGAATCTTCTACAGGTACATGGACAACTGTGTGG
ACCGATGGGCTTACCAGTCTTGATCGTTATAAAGGACGATGCTACCACATCGAGCCCGTTGCTGGA
GAAGAAAATCAATATATTTGTTATGTAGCTTATCCCTTAGACCTTTTTGAAGAAGGTTCTGTTACTA
ATATGTTTACTTCCATTGTCGGTAATGTATTTGGATTCAAAGCCCTACGCGCTCTACGACTGGAGG
ATTTGCGAGTCCCTCCGGCTTATATTAAAACTTTCCAAGGTCCGCCTCATGGCATCCAAGTTGAAA
GAGATAAATTGAACAAGTACGGCCGCCCCCTACTGGGCTGTACTATTAAACCTAAATTGGGGCTAT
CCGCTAAGAATTATGGTAGAGCAGTTTATGAATGTCTCCGCGGTGGACTTGATTTTACAAAAGATG
ATGAGAACGTGAACTCCCAACCATTTATGCGTTGGAGAGACCGCTTCTTATTTTGTGCCGAAGCAC
TTTATAAAGCACAGGCCGAAACAGGTGAAATCAAAGGGCATTTATTTGAATGCACT

4.1.54 Species: *Allanblackia floribunda* Oliv.

Family: Clusiaceae

Specimen Number: IDMTG122-13|GH0348

Location: Ankasa Resource Reserve 174m NO5° 16.221' W002° 36.321'

Plate 2: *Allanblackia floribunda* Oliv

DNA barcode: ***rbcLa*** (Sequencing was not successful)

4.1.55 Species: *Bombax buonopenza* P.Beauv.

Family: Malvaceae

Specimen Number: GH0196

Location: Kakum National Park 80m. N05° 20.846'W001° 22.992'

Description: Large tree up to 40 m tall; bole straight, cylindrical, branchless for 18–24 m, up to 75(–150) cm in diameter. Leaves palmately compound with 5–9 leaflets; stipules narrowly lanceolate, soon falling; petiole 22–24 cm long, petiolules absent or up to 2 cm long. Flowers solitary or 2–6 together in small axillary cymes on short branches; pedicel up to 2.5 cm long; calyx cup-shaped, 1–1.5 cm × 2.5–4.5 cm, 5-lobed; petals 5, oblong to oblong-lanceolate, 5.5–9.5 cm × 2.5–3.5 cm, red; staminal tube 7–8 mm long, stamens c. 180, in outer circle in 5 bundles of c. 30, in inner circle in 5 pairs; ovary superior, conical, 5.5–8 mm long, 5-ribbed, style 4–5 cm long, stigma 5-lobed. Fruit an oblong capsule 8–20 cm × 3.5–8 cm, 5-ridged to 5-angular, glabrous, dark brown to black, loculicidal, many seeded (Orwa *et al.,* 2009).

Plate 1: *Bombax buonopenza* P.Beauv

DNA barcode: *rbcLa*

ACTAAGCAAGTGTTGGATTCAAAGCTGGTGTTAAAGAGTATAAATTGACTTATTATACTCCTGAAT
ATGAAGTCAAAGATACTGATATCTTGGCAGCCTTCCGAGTAACTCCTCAACCCGGAGTTCCGCCTG
AGGAAGCAGGGGCCGCGGTAGCTGCTGAATCTTCTACTGGTACATGGACAACCGTGTGGACCGAT
GGGCTTACCAGCCTTGATCGTTACAAAGGGCGATGCTACCACATTGAGCCCGTTGCTGGAGAAGA
AAATCAATATATATGTTATGTAGCTTACCCCTTAGACCTTTTTGAAGAAGGTTCTGTTACTAACATG
TTTACTTCCATTGTGGGTAATGTATTTGGGTTCAAAGCCCTGCGCGCTCTACGTCTAGAGGATCTGC
GAATCCCTACTTCTTATACTAAAACTTTCCAAGGCCCGCCTCATGGCATCCAGGTTGAAAGAGATA
AATTGAACAAGTACGGTCGCCCCCTATTAGGATGTACTATTAAACCTAAATTGGGGTTATCCGCTA
AGAACTACGGTAGAGCAGTTTATGAATGTCTACGTGGCGGACTTGATTTTACCAAAGATGATGAG
AATGTGAACTCCCAACCATTTATGCGCTGGAGAGACCGTTTCTTATTTTGTGCCGAAGCAATTTAT
AAATCACAGGCTGAAACAGGTGAAATCAAAGGGCATTACTT

4.1.56 Species: *Bombax buonopenza* P.Beauv.

Family: Malvaceae

Specimen Number: IDMTG110-13|GH0197

Location: Bia National Park. 224m. N06°32.562' W003°01.659'

Plate 2: *Bombax buonopenza* P.Beauv

DNA barcode: *rbcLa*

ACTAAGCAAGTGTTGGATTCAAAGCTGGTGTTAAAGAGTATAAATTGACTTATTATACTCCTGAAT
ATGAAGTCAAAGATACTGATATCTTGGCAGCCTTCCGAGTAACTCCTCAACCCGGAGTTCCGCCTG
AGGAAGCAGGGGCCGCGGTAGCTGCTGAATCTTCTACTGGTACATGGACAACCGTGTGGACCGAT
GGGCTTACCAGCCTTGATCGTTACAAAGGGCGATGCTACCACATTGAGCCCGTTGCTGGAGAAGA
AAATCAATATATATGTTATGTAGCTTACCCCTTAGACCTTTTTGAAGAAGGTTCTGTTACTAACATG
TTTACTTCCATTGTGGGTAATGTATTTGGGTTCAAAGCCCTGCGCGCTCTACGTCTAGAGGATCTGC
GAATCCCTACTTCTTATACTAAAACTTTCCAAGGCCCGCCTCATGGCATCCAGGTTGAAAGAGATA
AATTGAACAAGTACGGTCGCCCCCTATTAGGATGTACTATTAAACCTAAATTGGGGTTATCCGCTA
AGAACTACGGTAGAGCAGTTTATGAATGTCTACGTGGCGGACTTGATTTTACCAAAGATGATGAG
AATGTGAACTCCCAACCATTTATGCGCTGGAGAGACCGTTTCTTATTTTGTGCCGAAGCAATTTAT
AAATCACAGGCTGAAACAGGTGAAATCAAAGGGCATTACTTGAATGCACC

4.1.57 Species: *Anacardium occidentale* L.

Family: Anacardiaceae

Specimen Number: GH0353

Location: Kakum National Park 176m N05° 20.947' W001° 23.035'

Description: *Anacardium occidentale* is a medium-sized tree, spreading, evergreen, much branched; grows to a height of 12m. Leaves simple, alternate, coriaceus, glabrous, obovate, rounded at ends, 10-18 x 8-15 cm, with short petiole, pale green or reddish when young and

dark green when mature. The inflorescence is a terminal panicle-like cluster commonly bearingmale and hermaphroditic flowers. The male flowers are the most numerous and usually bear 1 exserted stamen and 9 small inserted ones (Orwa *et al.*, 2009).

Plate 1: *Anacardium occidentale* L.

DNA barcode: *rbcLa* (Sequencing was not successful)

4.1.58 Species: *Anacardium occidentale* L.

Family: Anacardiaceae

Specimen Number: IDMTG110-13|GH0152

Location: Bia Biosphere Reserve 191m. N063°6.434'W003°05.828'

Plate 2: *Anacardium occidentale* L.

DNA barcode: *rbcLa*

CAGAAACTAAAGCAAGTGTTGGATTCAAAGCCCGGCGTTAAAGACTATAAATTGACTTATTATACT
CCTGACTATATAACCAAAGATACTGATATCTTGGCAGCATTCCGAGTAACTCCTCAACCTGGAGTT
CCACCCGAGGAAGCAGGGGCTGCGGTAGCTGCGGAATCTTCTACTGGTACATGGACAACTGTGTG
GACCGATGGGCTTACCAGCCTTGATCGTTACAAAGGACGATGCTACAACATTGAGCCCGTTGCTGG
AGAAGAAAATCAATATATATGTTATGTAGCTTACCCTTTAGACCTTTTTGAAGAAGGTTCTGTTACT
AACATGTTTACTTCCATTGTGGGTAATGTATTTGGGTTCAAAGCCCTGCGCGCTCTACGTCTAGAG
GATCTACGAGTCCCTATCGCGTATATAAAAACTTTCCAAGGACCACCGCATGGGATCCAAGTTGAG
AGAGATAAATTGAATAAGTATGGCCGTCCCCTATTGGGATGTACTATTAAACCAAAATTAGGTTTA
TCCGCTAAGAACTACGGTAGAGCTGTTTATGAATGTCTACGTGGTGGACTTGACTTTACCAAAGAT
GATGAGAACGTGAACTCCCAACCATTTATGCGTTGGAGAGACCGTTTCCTATTTTGTGCGGAAGCG
CTTTTTAAAGCGCAGCTGAAACAGTGAAATTAAAGGTCATTACTTGATGCACTG

4.1.59 Species: *Mangifera indica* L.

Family: Anacardiaceae

Specimen Number: GH0110

Location: Kakum National Park 178m N05° 20.965' W001° 23.067'

Description: *Mangifera indica* is a large evergreen tree to 20 m tall with a dark green, umbrella-shaped crown. Leaves alternate, simple, leathery, oblong-lanceolate, 16-30 x 3-7 cm, on flowering branches, up to 50 cm on sterile branches, curved upward from the midrib and sometimes with edges a little wavy. Inflorescence 16 cm or more in length, a much-branched panicle bearing many very small (4 mm) greenish-white or pinkish flowers. Fruit an irregularly egg-shaped and slightly compressed fleshy drupe, 8-12 (max. 30) cm long, attached at the broadest end on a pendulous stalk (Orwa *et al.*, 2009).

Plate 1: *Mangifera indica* **L.**

DNA barcode: *rbcLa*

CGCAAACTAGCAAGTGTGGGATTCAAAGCCGGCGTTAAAGACTATAAATTGACTTATTATACTCCT
GACTATATAACCAAAGATACTGATATCTTGGCAGCATTCCGAGTAACTCCTCAACCTGGAGTTCCA
CCCGAGGAAGCAGGGGCTGCGGTAGCTGCGGAATCTTCTACTGGTACATGGACAACTGTGTGGAC
CGATGGGCTTACCAGCCTTGATCGTTACAAAGGACGATGCTACAACATTGAGCCCGTTGCTGGAGA
AGAAAATCAATATATATGTTATGTAGCTTACCCTTTAGACCTTTTTGAAGAAGGTTCTGTTACTAAC
ATGTTTACTTCCATTGTGGGTAATGTATTTGGGTTCAAAGCCCTGCGCGCTCTACGTCTAGAGGATC
TACGAATCCCTACCGCGTATATAAAAACTTTCCAAGGACCACCGCATGGGATCCAAGTTGAGAGA
GATAAATTGAACAAGTATGGCCGTCCCCTATTGGGATGTACTATTAAACCGAAATTAGGTTTATCC
GCTAAGAACTACGGTAGAGCTGTTTATGAATGTCTACGTGGTGGACTTGACTTTACCAAAGACGAT
GAGAACGTGAACTCCCAACCATTTATGCGTTGGAGAGACCGTTTCCTATTTTGTGCGGAAGCTCTT
TTTAAAGCGCAGCTGAAACAGGTGAAATTAAAGGTCATTACTTGATGCGACTG

4.1.60 Species: *Mangifera indica* L.

Family: Anacardiaceae

Specimen Number: IDMTG112-13|GH0111

Location: Bia Biosphere Reserve. 189m. N063°6.312'W003°05.624'

Plate 2: *Mangifera indica* **L.**

DNA barcode: ***rbcLa*1**

CGCAAACTAGCAAGTGTGGGATTCAAAGCCGGCGTTAAAGACTATAAATTGACTTATTATACTCCT
GACTATATAACCAAAGATACTGATATCTTGGCAGCATTCCGAGTAACTCCTCAACCTGGAGTTCCA
CCCGAGGAAGCAGGGGCTGCGGTAGCTGCGGAATCTTCTACTGGTACATGGACAACTGTGTGGAC
CGATGGGCTTACCAGCCTTGATCGTTACAAAGGACGATGCTACAACATTGAGCCCGTTGCTGGAGA
AGAAAATCAATATATATGTTATGTAGCTTACCCTTTAGACCTTTTTGAAGAAGGTTCTGTTACTAAC
ATGTTTACTTCCATTGTGGGTAATGTATTTGGGTTCAAAGCCCTGCGCGCTCTACGTCTAGAGGATC
TACGAATCCCTACCGCGTATATAAAAACTTTCCAAGGACCACCGCATGGGATCCAAGTTGAGAGA
GATAAATTGAACAAGTATGGCCGTCCCCTATTGGGATGTACTATTAAACCGAAATTAGGTTTATCC
GCTAAGAACTACGGTAGAGCTGTTTATGAATGTCTACGTGGTGGACTTGACTTTACCAAAGACGAT
GAGAACGTGAACTCCCAACCATTTATGCGTTGGAGAGACCGTTTCCTATTTTGTGCGGAAGCTCTT
TTTAAAGCGCAGCTGAAACAGGTGAAATTAAAGGTCATTACTTGATGCGACTG

4.1.61 Species: *Alstonia boonei* De. Wild.

Family: Apocynaceae

Specimen Number: GH0224

Location: Bia Biosphere Reserve 227m. N06°32.672' W003°01.959'

Description: *Alstonia boonei* is a large deciduous tree, up to 45 m tall and 1.2 m in diameter; bole often deeply fluted to 7 m, small buttresses. Leaves in whorls of 5-8, simple, subsessile to petiolate, stipules absent; petiole 2-10 (max. 15) mm long, stout; blade oblanceolate to obovate, rarely elliptic, 7-26 x 3-9.3 cm; apex acute to rounded or sometimes emarginat. nflorescence terminal, compound with 2-3 tiers of pseudo-umbels; primary peduncles 0.5-7 cm long, greyish pubescent; bracts ovate- triangular, 1-1.5 mm long, pubescent; pedicels

about 5 m. Fruit formed by 2 pendent green follicles up to 60 cm long, longitudinally striate, dehiscing lengthways while on the tree; seeds numerous, flat, about 4 x 2 mm, with tufts of hair at each end 10 m (Orwa *et al.*, 2009).

Plate 1: *Alstonia boonei* De. Wild

DNA barcode: *rbcLa*

CAGAAACTAAAAGCAAAGTGTTGGATTCAAAGCTGGTGTTAAAGAGTACAAATTGACTTATTATA
CTCCTGAATACGAAACCAAAGATACTGATATCTTGGCAGCATTCCGAGTAACTCCTCAACCCGGAG
TTCCACCTGAAGAAGCAGGGGCCGCGGTAGCTGCCGAATCTTCTACTGGTACATGGACAACTGTGT
GGACCGATGGACTTACCAGCCTTGATCGTTACAAAGGGCGATGCTACCACATCGAGCCTGTTGCTG
GAGAAGAAGATCAATATATTGCTTATGTAGCTTACCCCTTAGACCTTTTTGAAGAAGGTTCTGTTA
CTAACATGTTTACTTCCATTGTAGGTAATGTATTTGGGTTCAAAGCCCTACGCGCTCTACGTCTGGA
AGATTTGCGAATCCCTCCGGCTTATATTAAAACCTTCCAAGGCCCGCCTCATGGCATCCAGGTTGA
GAGAGATAAATTGAACAAATATGGTCGTCCCCTGTTAGGATGTACTATTAAACCGAAATTGGGGTT
ATCCGCTAAAAACTACGGTAGGGCAGTTTATGAATGTCTTCGTGGTGGACTTGATTTTACCAAAGA
TGATGAAAACGTGAACTCCCAACCGTTTATGCGTTGGAGAGATCGTTTCTTATTTTGTGCCGAAGC
ACTTTATAAAGCACAGGCTGAAACCGGTGAAATCAAAGGGCATTACTTGAATGCAACTG

4.1.62 Species: *Alstonia boonei* De. Wild.

Family: Apocynaceae

Specimen Number: IDMTG112-13|GH0155

Location: Kakum National Park 78m. N05° 20.926'W001° 22.612'

Plate 2: *Alstonia boonei* De. Wild

DNA barcode: *rbcLa*

CAGAAACTAAAAGCAAAGTGTTGGATTCAAAGCTGGTGTTAAAGAGTACAAATTGACTTATTATA
CTCCTGAATACGAAACCAAAGATACTGATATCTTGGCAGCATTCCGAGTAACTCCTCAACCCGGAG
TTCCACCTGAAGAAGCAGGGGCCGCGGTAGCTGCCGAATCTTCTACTGGTACATGGACAACTGTGT
GGACCGATGGACTTACCAGCCTTGATCGTTACAAAGGGCGATGCTACCACATCGAGCCTGTTGCTG
GAGAAGAAGATCAATATATTGCTTATGTAGCTTACCCCTTAGACCTTTTTGAAGAAGGTTCTGTTA
CTAACATGTTTACTTCCATTGTAGGTAATGTATTTGGGTTCAAAGCCCTACGCGCTCTACGTCTGGA
AGATTTGCGAATCCCTCCGGCTTATATTAAAACCTTCCAAGGCCCGCCTCATGGCATCCAGGTTGA
GAGAGATAAATTGAACAAATATGGTCGTCCCCTGTTAGGATGTACTATTAAACCGAAATTGGGGTT
ATCCGCTAAAAACTACGGTAGGGCAGTTTATGAATGTCTTCGTGGTGGACTTGATTTTACCAAAGA
TGATGAAAACGTGAACTCCCAACCGTTTATGCGTTGGAGAGATCGTTTCTTATTTTGTGCCGAAGC
ACTTTATAAAGCACAGGCTGAAACCGGTGAAATCAAAGGGCATTACTTGAATGCAACTG

4.1.63 Species: *Entada Africana* Guill.

Family: Fabaceae

Specimen Number: GH0217

Location: Bia Biosphere Reserve. 247m. N06°32.652' W003°01.979'

Description: *Entada africana* is a small tree up to 4-10 m in height and 90 cm in girth; branching low down, with a wide crown. Leaves bipinnate, alternate, 3-9 pairs, with a glabrous common stalk 15-45 cm; rachis 25-30 cm long with 2-9 pairs of pinnae; 8-24 pairs of leaflets. Flowers creamy-white or reddish-yellow, about 6 mm long, slightly scented, densely clustered in spike-like racemes 5-15 cm long including the short central stalk; spikes solitary or in small clusters in the leaf axils or arranged in panicles at the ends of shoots, 1-4.

Fruit is a pod, 15-40 x 5-8 (15) cm, very persistent, hanging down untidily for many months and eventually breaking up on the tree (Orwa *et al.*, 2009).

Plate 1: *Entada Africana* Guill

DNA Barcode: ***rbcLa*** (Sequencing was not successful)

4.1.64 Species: *Entada Africana* Guill.

Family: Fabaceae – Mimosoideae

Specimen Number: IDMTG157-13|GH0215

Location: Ankasa Resource Reserve 174m NO5° 16.113' W002° 36.431'

Plate 2: *Entada Africana* Guill

DNA Barcode: ***rbcLa*1**

AGTGTTGGGTTCAAAGCTGGTGTTAAAGATTATAAATTGACTTATTATACTCCTGACTATGAAACC
AAAGATACTGATATCTTGGCAGCATTCCGAGTAACTCCTCAACCTGGAGTTCCGCCTGAAGAAGCA
GGTGCCGCGGTAGCTGCTGAATCTTCTACTGGTACATGGACAACTGTGTGGACCGATGGGCTTACC
AGTCTTGATCGTTACAAAGGACGATGCTACCACATCGAGCCCGTTGCTGGAGAAGAAAATCAATTT
ATTGCTTATGTAGCTTATCCCTTAGACCTTTTTGAAGAAGGTTCTGTTACTAACATGTTTACTTCCA

TTGTGGGTAATGTATTTGGGTTCAAGGCCCTGCGCGCTCTACGTCTGGAGGATTTGCGAATCCCTC
CTGCTTATTCTAAAACTTTCCAAGGTCCGCCTCACGGCATCCAAGTTGAGAGAGATAAATTGAACA
AGTACGGCCGTCCCCTATTGGGATGTACTATTAAAC

4.1.65 Species: *Cassia sieberiana* DC.

Family: Fabaceae

Specimen Number: GH0177

Location: Ankasa Resource Reserve 174m NO5° 16.113' W002° 36.431'

Description: *Cassia sieberiana* is a perennial legume shrub or tree, up to 20 m high. Leaves are spirally arranged with elliptical to ovate leaflets. The fruit is a cylindrical pod (up to 90 cm) containing ellipsoid, rusty to dark brown seeds (Orwa *et al.*, 2009).

Plate 1: *Cassia sieberiana* DC.

DNA barcode: ***rbcLa***

AGTGTTGGGTTCAAAGCTGGTGTTAAAGATTATAAATTGACTTATTATACTCCTGACTATGAAACC
AAAGATACTGATATCTTGGCAGCATTCCGAGTAACTCCTCAACCTGGAGTTCCGCCTGAAGAAGCA
GGTGCCGCGGTAGCTGCTGAATCTTCTACTGGTACATGGACAACTGTGTGGACCGATGGGCTTACC
AGTCTTGATCGTTACAAAGGACGATGCTACCACATCGAGCCCGTTGCTGGAGAAGAAGGTCAATA
TATTGCTTATGTAGCTTATCCCTTAGACCTTTTTGAAGAAGGTTCTGTTACTAACATGTTTACTTCC
ATTGTGGGTAATGTATTTGGGTTCAAGGCCCTGCGCGCTCTACGTCTGGAGGATTTGCGAATCCCT
ATTTCTTATATTAAAACTTTCCAAGGTCCGCCTCACGGCATCCAAGTTGAGAGAGATAAATTGAAC
AAGTACGGCCGTCCCCTATTGGGATGTACTATTAAACCTAAATTGGGGTTATCCGCTAAGAATTAC
GGTAGAGCAGTTTATGAATGTCTC

4.1.66 Species: *Cassia sieberiana* DC.

Family: Fabaceae

Specimen Number: IDMTG127-13|GH0176

Location: Bia Biosphere Reserve 243m. N06°32.542' W003°01.657'

Plate 2: *Cassia sieberiana* DC

DNA barcode: ***rbcLa*1**

AGTGTTGGGTTCAAAGCTGGTGTTAAAGATTATAAATTGACTTATTATACTCCTGACTATGAAACC
AAAGATACTGATATCTTGGCAGCATTCCGAGTAACTCCTCAACCTGGAGTTCCGCCTGAAGAAGCA
GGTGCCGCGGTAGCTGCTGAATCTTCTACTGGTACATGGACAACTGTGTGGACCGATGGGCTTACC
AGTCTTGATCGTTACAAAGGACGATGCTACCACATCGAGCCCGTTGCTGGAGAAGAAGGTCAATA
TATTGCTTATGTAGCTTATCCCTTAGACCTTTTTGAAGAAGGTTCTGTTACTAACATGTTTACTTCC
ATTGTGGGTAATGTATTTGGGTTCAAGGCCCTGCGCGCTCTACGTCTGGAGGATTTGCGAATCCCT
ATTTCTTATATTAAAACTTTCCAAGGTCCGCCTCACGGCATCCAAGTTGAGAGAGATAAATTGAAC
AAGTACGGCCGTCCCCTATTGGGATGTACTATTAAACCTAAATTGGGGTTATCCGCTAAGAATTAC
GGTAGAGCAGTTTATGAATGTCTC

4.1.67 Species: *Khaya grandifoliola* C.DC.

Family: Meliaceae

Specimen Number: GH0142

Location: Ankasa Resource Reserve 163m NO5° 16.118' W002° 36.643'

Description: Usually deciduous, monoecious, medium-sized to large tree up to 40 m tall. Leaves arranged spirally but clustered near ends of branches, paripinnately compound with 3–5 pairs of leaflets; stipules absent; petiole and rachis together up to 50 cm long. Inflorescence an axillary panicle up to 40 cm long. Flowers unisexual, male and female flowers very similar in appearance, regular, usually 5-merous, whitish, sweet-scented; pedicel 1–2 mm long. Fruit an erect, nearly globose, woody capsule 6–9 cm in diameter, greyish brown, dehiscent by 5 valves, many-seeded (Orwa *et al.*, 2009).

Plate 1: *Khaya grandifoliola* C.DC**.**

DNA barcode: ***rbcLa*** (Sequencing was not successful)

4.1.68 Species: *Khaya grandifoliola* C.DC.

Family: Meliaceae

Specimen Number: IDMTG102-13|GH0140

Location: Bia Biosphere Reserve 235m. N06°32.541' W003°01.823'

Plate 2: *Khaya grandifoliola* C.DC**.**

DNA barcode: ***rbcLa*1**

AGTGTTGGATTCAAAGCCGGTGTTAAAGRTTATAAATTGACTTATTATACGCCTGACTATGTAACC
AAAGATACTGATATCTTGGCAGCATTCCGAGTAACTCCTCAACCCGGAGTTCCGCCCGAGGAAGC
AGGGGCTGCGGTAGCTGCGGAATCTTCTACTGGTACATGGACAACTGTGTGGACCGATGGGCTTAC
TAGCCTTGATCGTTACAAAGGACGATGCTACAACATTGAGCCAGTTGCTGGAGAAGAAAATCAAT

ATATATGTTATGTAGCTTACCCTTTAGACCTTTTTGAAGAAGGTTCTGTTACTAACATGTTTACATC
CATTGTGGGTAATGTATTTGGGTTCAAAGCCCTGCGCGCTCTACGTCTAGAGGATCTACGAATCCC
TCCCGCGTATTCTAAAACTTTCCAAGGCCCGCCTCATGGCATCCAAGTTGAGAGAGATAAATTGAA
CAAGTATGGTCGTCCCCTATTGGGATGTACAATTAAACCTAAATTGGGGTTATCCGCTAAGAATTA
CGGTAGAGCAGTTTATGAATGTCTA

4.1.69 Species: *Myrianthus arboreus* P. Beauv

Family: Moraceae

Specimen Number: GH0120

Location: Ankasa Resource Reserve 161m NO5° 16.218' W002° 36.463'

Description: *Myrianthus arboreus* is a dioecious tropical tree up to 15 m high with spreading branches from a short stem. Leaves very large, alternately shaped, 5-7 digitately compound, coarsely toothed, with hood like stipules the central leaflet is about 25 x 9 cm. Young leaves are usually red in colour. Male inflorescences yellow, much branched and panicle like are produced in large axillary pairs in the latter part of the dry season. The female inflorescences are paired, stalked greenish clusters (pedunculate), Fruit a syncarp of basally fused, yellow false drupes up to 10 cm, with stylar remains projecting from each drupe (Orwa *et al.*, 2009).

Plate 1: *Myrianthus arboreus* P. Beauv

DNA barcode: *rbcLa* (Sequencing was not successful)

4.1. 70 Species: *Myrianthus arboreus* P. Beauv

Family: Moraceae

Specimen Number: IDMTG088-13|GH0119

Location: Bia Biosphere Reserve. 225m. N06°32.415' W003°01.328'

Plate 2: *Myrianthus arboreus* P. Beauv**.**

DNA barcode: *rbcLa*

AGTGTTGGATTCAAAGCTGGTGTTAAAGATTATAAATTGACTTATTACACTCCTGAATATGAAACC
AAAGATACTGATATCTTAGCAGCATTTCGAGTAACTCCTCAACCTGGAGTTCCCCCTGAAGAAGCA
GGGGCTGCGGTAGCTGCTGAATCTTCTACTGGTACATGGACAACTGTATGGACTGACGGGCTTACC
AGTCTTGATCGCTACAAAGGTCGATGCTACCACATCGAGCCTGTTGCTGGAGAAGAAAATCAATAT
ATTGCTTATGTAGCTTACCCCTTAGACCTTTTTGAAGAAGGTTCTGTTACTAACATGTTTACTTCCA
TTGTGGGTAATGTATTTGGGTTCAAGGCCCTGCGCGCTCTACGTCTGGAGGATTTGCGAATCCCCC
CTGCTTACTCTAAAACTTTCCAAGGCCCGCCTCATGGCATCCAAGTTGAGAGAGATAAATTGAACA
AGTATGGCCGCCCCCTATTGGGATGTACTATTAAACCTAAATTGGGGTTATCCGCTAAGAATTATG
GTAGAGCGGTTTATGAATGTCTT

4.1.71 Species: *Melia azedarach* L.

Family: Meliaceae

Specimen Number: GH0135

Location: Ankasa Resource Reserve 158m NO5° 16.126' W002° 36.478'

Description: *Melia azedarach* is a small to medium, deciduous tree from 6 to 35 metres in height. The bark is brown with narrow furrows which give a striped appearance. The leaves are twice-compound (bipinnate) with oval to elliptical-shaped leaflets from 20 to 70 mm long

and are dark green in colour. The pink to lilac flowers are star-shaped, about 18 mm in diameter and have a chocolate scent. They occur in conspicuous clusters from the leaf axils. The flowers are followed by yellow clusters of fruit which are poisonous if eaten by humans and animals although many birds seem partial to them and are not affected. M. azedarach is monoecious (separate male and female flowers, but on the same plant). It produces very large numbers of bird-dispersed seed (Orwa *et al.*, 2009).

Plate 1: *Melia azedarach* L.

DNA barcode: *rbcLa*

AGTGTTGGATTCAAAGCCGGTGTTAAAGATTATAAATTGACTTATTATACTCCTGATTATCCAACC
AAAGATACTGATATCTTGGCAGCATTCCGAGTAACTCCTCAACCCGGAGTTCCGCCCGAGGAAGC
AGGGGCTGCGGTAGCTGCGGAATCTTCTACTGGTACATGGACAACCGTGTGGACCGATGGGCTTA
CTAGCCTTGATCGTTACAAAGGACGATGCTACAATATTGAGCCCGTTGCTGGAGAAGAAAATCAA
TATATATGTTATGTAGCTTACCCTTTAGACCTTTTTGAAGAAGGTTCTGTTACTAACATGTTTACGT
CCATTGTGGGTAATGTATTTGGGTTCAAAGCCCTGCGTGCTCTACGTCTAGAGGATCTACGAATCC
CTCCCGCGTATTCTAAAACTTTCCAAGGACCACCTCACGGGATCCAAGTTGAAAGAGATAAATTGA
ACAAGTATGGTCGTCCTCTATTGGGATGTACTATTAAACCAAAATTGGGGTTATCCGCTAAG

4.1.72 Species: *Melia azedarach* L.

Family: Meliaceae

Specimen Number: IDMTG098-13|GH0134

Location: Kakum National Park 140m N05° 20.671' W001° 23.315'

Plate 1: *Melia azedarach* L.

DNA barcode: *rbcLa*

AGTGTTGGATTCAAAGCCGGTGTTAAAGATTATAAATTGACTTATTATACTCCTGATTATCCAACC
AAAGATACTGATATCTTGGCAGCATTCCGAGTAACTCCTCAACCCGGAGTTCCGCCCGAGGAAGC
AGGGGCTGCGGTAGCTGCGGAATCTTCTACTGGTACATGGACAACCGTGTGGACCGATGGGCTTA
CTAGCCTTGATCGTTACAAAGGACGATGCTACAATATTGAGCCCGTTGCTGGAGAAGAAAATCAA
TATATATGTTATGTAGCTTACCCTTTAGACCTTTTTGAAGAAGGTTCTGTTACTAACATGTTTACGT
CCATTGTGGGTAATGTATTTGGGTTCAAAGCCCTGCGTGCTCTACGTCTAGAGGATCTACGAATCC
CTCCCGCGTATTCTAAAACTTTCCAAGGACCACCTCACGGGATCCAAGTTGAAAGAGATAAATTGA
ACAAGTATGGTCGTCCTCTATTGGGATGTACTATTAAACCAAAATTGGGGTTATCCGCTAAGAATT
ACGGTAGAGCTGTTTATGAATGTCTA

4.1.73 Species: *Morinda lucida* Hook.

Family: Rubiaceae

Specimen Number: GH0061

Location: Bia Biosphere Reserve 225m. N06°32.352' W003°01.415'

Description: Evergreen shrub or small to medium-sized tree up to 18(–25) m tall, with bole and branches often crooked or gnarled.Leaves opposite, simple and entire; stipules ovate or triangular, 1–7 mm long, falling early; petiole up to 1.5 cm long; blade elliptical, 6–18 cm × 2–9 cm, base rounded to cuneate, apex acute to acuminate, shiny above, sometimes finely pubescent. Flowers bisexual, regular, 5-merous, heterostylous, fragrant; calyx cup-shaped, c. 2 mm long, Fruit a drupe, several together arranged into an almost globose succulent syncarp 1–2.5 cm in diameter, soft and black when mature; pyrene compressed ovoid, up to 6.5 mm × 4 mm, dark red-brown, very hard, 1-seeded (Lemmens, 2008).

Plate 1: *Morinda lucida* Hook.

DNA barcode: *rbcLa*

AGTGTTGGATTCAAAGCTGGTGTTAAAGAGTACAAATTGACTTATTATACTCCTGAATACGAAACC
AAAGATACTGATATCTTGGCAGCATTCCGAGTAACTCCCCAACCTGGAGTTCCACCGGAAGAAGC
AGGGGCCGCGGTAGCTGCCGAGTCTTCTACTGGTACATGGACAACTGTATGGACGGATGGACTTA
CCAGTCTTGATCGTTACAAAGGGCGATGCTACCACATCGAGCCAGTTCCTGGAGAAGAAGATCAA
TATATTGCTTATGTAGCTTACCCGTTAGACCTTTTTGAGGAAGGTTCTGTTACTAACATGTTTACTT
CCATTGTCGGTAATGTATTTGGGTTCAAAGCCCTGCGCGCTCTGCGTCTGGAAGATTTGCGAATTC
CCATTTCTTATATTAAAACCTTCCAAGGCCCGCCTCATGGCATTCAAGTCGAGAGAGATAAATTGA
ACAAGTATGGTCGTCCCCTGTTGGGATGTACTATTAAACCGAAATTAGGTTTATCTGCTAAAAACT
ATGGTAGAGCAGTTTATGAATGTCTT

4.1.74 Species: *Morinda lucida* Hook.

Family: Rubiaceae

Specimen Number: IDMTG043-13|GH0060

Location: Ankasa Resource Reserve 163m NO5° 16.109' W002° 36.632'

Plate 2: *Morinda lucida* Hook.

DNA barcode: *rbcL*1

AGTGTTGGATTCAAAGCTGGTGTTAAAGAGTACAAATTGACTTATTATACTCCTGAATACGAAACC
AAAGATACTGATATCTTGGCAGCATTCCGAGTAACTCCCCAACCTGGAGTTCCACCGGAAGAAGC
AGGGGCCGCGGTAGCTGCCGAGTCTTCTACTGGTACATGGACAACTGTATGGACGGATGGACTTA
CCAGTCTTGATCGTTACAAAGGGCGATGCTACCACATCGAGCCAGTTCCTGGAGAAGAAGATCAA
TATATTGCTTATGTAGCTTACCCGTTAGACCTTTTTGAGGAAGGTTCTGTTACTAACATGTTTACTT
CCATTGTCGGTAATGTATTTGGGTTCAAAGCCCTGCGCGCTCTGCGTCTGGAAGATTTGCGAATTC
CCATTTCTTATATTAAAACCTTCCAAGGCCCGCCTCATGGCATTCAAGTCGAGAGAGATAAATTGA
ACAAGTATGGTCGTCCCCTGTTGGGATGTACTATTAAACCGAAATTAGGTTTATCTGCTAAAAACT
ATGGTAGAGCAGTTTATGAATGTCTT

4.1.75 Species: *Zanthoxylum zanthoxyloides* (Lam.) Zepern. & Timler

Family: Rutaceae

Specimen Number: GH0192

Location: Kakum National Park 177m N05° 20.955’ W001° 23.042’

Description: *Zanthoxylum zanthoxyloides* is a Shrub or small tree, spiny and more or less scandent, up to 6–8(–12) m tall. Leaves alternate, glabrous, imparipinnately compound with 5–7(–11) opposite or alternate leaflets, up to 12(–20) cm long; petiole 2–5 cm long, glabrous, spiny beneath with recurved prickles; stipules absent; petiolules 2–5 mm long; leaflets obovate to elliptical, 5–10 cm × 2–4 cm, base cuneate to rounded, apex obtuse or rounded, sometimes apiculate or notched, with many glandular dots, smelling of pepper and lemon when crushed. Inflorescence a lax terminal or axillary panicle 5–25 cm long, with short branches. Flowers unisexual, regular, 5-merous, white or greenish, sessile; corolla barely open; male flowers with stamens slightly exserted; female flower with superior ovary, 1-celled, style short, lateral. Fruit an ovoid follicle, 5–6 mm in diameter, brown, with glandular dots, dehiscent, 1-seeded (Lemmens, 2008).

Plate 1: *Zanthoxylum zanthoxyloides* (Lam.) Zepern. & Timler

DNA barcode: ***rbcLa***

TTTGTTGGATTCAAAGCCGGTGTTAAAGATTATAAATTGACTTATTATACTCCTGAATATGTAACCA
AAGATACTGATATCTTGGCAGCATTCCGAGTAACTCCTCAACCCGGAGTTCCACCCGAGGAAGCG
GGGGCTGCGGTAGCTGCGGAATCTTCTACTGGTACATGGACAACTGTGTGGACCGATGGGCTTACC
AGCCTTGATCGTTACAAAGGGCGATGCTACAACATTGAGCCCGTTGCTGGAGAAGAAAATCAATA
TATATGTTATGTAGCTTACCCGTTAGACCTTTTTGAAGAAGGTTCTGTTACTAACATGTTTACTTCC
ATTGTGGGTAATGTATTTGGTTTCAAAGCCCTGCGCGCTCTACGTCTAGAGGATCTACGAATCCCT
ACCGCGTATACTAAAACTTTCCAAGGCCCGCCTCACGGCATCCAAGTTGAGAGAGATAAATTGAA
CAAGTATGGACGTCCCCTGTTGGGATGTACTATTAAACCTAAATTGGGGTTATCCGCTAAGAATTA
CGGTAGGGCAGTTTATGAATGTCTA

4.1.76 Species: *Zanthoxylum zanthoxyloides* (Lam.) Zepern. & Timler

Family: Rutaceae

Specimen Number: IDMTG138-13|GH0191

Location: Bia Biosphere Reserve 221m. N06°32.315' W003°01.418'

Plate 2: *Zanthoxylum zanthoxyloides* (Lam.) Zepern. & Timler

DNA barcode: ***rbcLa*1**

TTTGTTGGATTCAAAGCCGGTGTTAAAGATTATAAATTGACTTATTATACTCCTGAATATGTAACCA
AAGATACTGATATCTTGGCAGCATTCCGAGTAACTCCTCAACCCGGAGTTCCACCCGAGGAAGCG
GGGGCTGCGGTAGCTGCGGAATCTTCTACTGGTACATGGACAACTGTGTGGACCGATGGGCTTACC
AGCCTTGATCGTTACAAAGGGCGATGCTACAACATTGAGCCCGTTGCTGGAGAAGAAAATCAATA
TATATGTTATGTAGCTTACCCGTTAGACCTTTTTGAAGAAGGTTCTGTTACTAACATGTTTACTTCC
ATTGTGGGTAATGTATTTGGTTTCAAAGCCCTGCGCGCTCTACGTCTAGAGGATCTACGAATCCCT
ACCGCGTATACTAAAACTTTCCAAGGCCCGCCTCACGGCATCCAAGTTGAGAGAGATAAATTGAA
CAAGTATGGACGTCCCCTGTTGGGATGTACTATTAAACCTAAATTGGGGTTATCCGCTAAGAATTA
CGGTAGGGCAGTTTATGAATGTCTA

4.1.77 Species: *Pseudocedrela kotschyi* (Schweinf.) Harms

Family: Meliaceae

Specimen Number: GH0322

Location: Kakum National Park 172m N05° 20.923' W001° 23.021'

Description: *Pseudocedrela kotschyi* is a deciduous, monoecious, small tree up to 12(–20) m tall. Leaves alternate but often clustered at the ends of branchlets, paripinnately compound with 8–18 leaflets; stipules absent; petiole and rachis together up to 30(–40) cm long; petiolules 1–3 mm long. Flowers unisexual, male and female flowers very similar in appearance, regular, (4–)5-merous, whitish; pedicel 2–4 mm long; calyx lobed almost to the base, c. 1.5 mm long. Fruit a narrowly obovoid to club-shaped capsule 7–14.5 cm long, erect, brown, dehiscing with 5 woody valves, with fibres between the valves, many-seeded (Lemmens, 2008).

Plate 1: *Pseudocedrela kotschyi* (Schweinf.) Harms

DNA barcode: ***rbcLa*** (Sequencing was not successful)

4.1.78 Species: *Pseudocedrela kotschyi* (Schweinf.) Harms

Family: Meliaceae

Specimen Number: IDMTG053-13|GH0074

Location: Bia Biosphere Reserve 243m. N06°32.235' W003°01.328'

Plate 2: *Pseudocedrela kotschyi* (Schweinf.) Harms

DNA barcode: *rbcLa*

GATTATAAATTGACTTATTATACTCCTGACTATGTAACCAAAGATACTGATATCTTGGCAGCATTCC
GAGTAACTCCTCAACCCGGAGTTCCGCCCGAGGAAGCAGGAGCTGCGGTAGCTGCGGAATCTTCT
ACTGGTACATGGACAACTGTGTGGACCGATGGGCTTACTAGCCTTGATCGTTACAAAGGACGATGC
TACAACATTGAGCCCGTTGCTGGAGAAGAAAATCAATATATATGTTATGTAGCTTACCCTTTAGAC
CTTTTTGAAGAAGGTTCTGTTACTAACATGTTTACGTCCATTGTGGGTAATGTATTTGGGTTCAAAG
CCCTGCGCGCTCTACGTCTAGAGGATCTACGAATCCCTATCGCATATATTAAAACTTTCCAAGGTC
CACCTCATGGTATCCAAGTTGAGAGAGATAAATTGAACAAGTATGGCCGTCCCCTATTGGGATGTA
CTATTAAACCAAAATTGGGGTTATCCGCTAAGAATTATGGTAGAGCAGTTTATGAATGTCTA

4.1.79 Species: *Myristica fragrans* Hout

Family: Myristicaceae

Specimen Number: GH0063

Location: Kakum National Park 155m N05° 20.657' W001° 23.215'

Description: fragrans is a spreading aromatic evergreen tree usually growing to around 5 to 13 metres high, occasionally 20 metres. The pointed dark green leaves (5 to 15 cm × 2 to 7 cm) are arranged alternately along the branches and are borne on leaf stems about 1 cm long. Upper leaf surfaces are shiny. Flowers are usually single sexed; occasionally male and female flowers are found on the same tree. Female flowers arise in groups of 1 to 3; males in groups of 1 to 10. Flowers are pale yellow, waxy, fleshy and bell-shaped. Male flowers are 5 to 7 mm long; female flowers are up to 1 cm long. The fruits are fleshy, drooping, yellow,

smooth, 6 to 9 cm long with a longitudinal ridge (Lemmens, 2008)..

Plate 1: *Myristica fragrans* Hout

DNA barcode: *rbcLa*

AGTGTTGGATTCAAAGCTGGTGTTAAAGAGTACAAATTGACTTATTATACTCCTGAATATGCAACCAAAGATA
CTGATATCTTAGCAGCATTCCGAGTAACTCCTCAACCCGGAGTTCCGCCTGAGGAAGCAGGGGCTGCGGTAGC
TGCCGAATCTTCTACTGGTACATGGACAACTGTGTGGACCGATGGACTTACCAGCCTTGATCGTTACAAAGGA
CGATGCTACCACATCGAGCCCGTTGCTGGGGAGGAAAATCAATATATTGCTTATGTAGCTTACCCTTTAGACCT
TTTTGAAGAAGGTTCTGTTACTAACATGTTTACTTCCATTGTGGGTAATGTATTTGGGTTCAAAGCCCTACGAG
CTCTACGTCTGGAGGATCTGCGAATTCCCACTTCTTATATCAAAACTTTCCAAGGCCCGCCCCATGGCATCCAA
GTTGAGAGAGATAAATTGAACAAGTACGGTCGTCCCCTATTGGGATGTACTATTAAACCAAAATTGGGGTTAT
CCGCTAAGAACTACGGTAGGGCGGTTTATGAATGTCTC

4.1.80 Species: *Myristica fragrans* Hout

Family: Myristicaceae

Specimen Number: IDMTG138-13|GH0064

Location: Bia Biosphere Reserve 221m. N06°32.234' W003°01.314'

Plate 2: *Myristica fragrans* Hout

DNA barcoding: *rbcLa*

AGTGTTGGATTCAAAGCTGGTGTTAAAGAGTACAAATTGACTTATTATACTCCTGAATATGCAACCAAAGATA
CTGATATCTTAGCAGCATTCCGAGTAACTCCTCAACCCGGAGTTCCGCCTGAGGAAGCAGGGGCTGCGGTAGC
TGCCGAATCTTCTACTGGTACATGGACAACTGTGTGGACCGATGGACTTACCAGCCTTGATCGTTACAAAGGA
CGATGCTACCACATCGAGCCCGTTGCTGGGGAGGAAAATCAATATATTGCTTATGTAGCTTACCCTTTAGACCT
TTTTGAAGAAGGTTCTGTTACTAACATGTTTACTTCCATTGTGGGTAATGTATTTGGGTTCAAAGCCCTACGAG
CTCTACGTCTGGAGGATCTGCGAATTCCCACTTCTTATATCAAAACTTTCCAAGGCCCGCCCCATGGCATCCAA
GTTGAGAGAGATAAATTGAACAAGTACGGTCGTCCCCTATTGGGATGTACTATTAAACCAAAATTGGGGTTAT
CCGCTAAGAACTACGGTAGGGCGGTTTATGAATGTCTC

4.1.81 Species: *Albizia zygia* (DC.) Macbr

Family: Fabaceae

Specimen Number: GH0004

Location: Bia Biosphere Reserve 219m. N06°32.124' W003°01.244'

Description: *Albizia zygia* is a deciduous tree 9-30 m tall with a spreading crown and a graceful architectural form. Leaves pinnate, pinnae in 2-3 pairs and broadening towards the apex, obliquely rhombic or obovate with the distal pair largest, apex obtuse, 29- 72 by 16 43mm, leaves are glabrous or nearly so. Flowers subsessile; pedicels and calyx puberulous, white or pink; staminal tube exserted for 10-18 mm beyond corolla. Fruit pod oblong, flat or somewhat transversely plicate, reddish-brown in colour, 10-18 cm by 2-4 cm glabrous or nearly so (Lemmens, 2008).

Plate 1: *Albizia zygia* (DC.) Macbr

DNA barcode: *rbcLa*

AGTGTTGGATTCAAAGCTGGTGTTAAAGATTATAAATTGAATTATTATACTCCTGACTATGAAACC
AAAGATAGTGATATCTTGGCAGCATTCCGAGTAACTCCTCAACCTGGAGTTCCGCCTGAAGAAGCA
GGTGCCGCGGTAGCTGCTGAATCTTCTACTGGTACATGGACAACTGTGTGGACCGATGGGCTTACC
AGTCTTGATCGTTACAAAGGACGATGCTACCACATCGAGTCCGTTGTTGGAGAAGAAGATCAATAT
ATTGCTTATGTAGCTTATCCCTTAGACCTTTTTGAAGAAGGTTCTGTTACTAACATGTTTACTTCGA
TTGTGGGTAATGTATTTGGGTTCAAGGCCCTGCGCGCTCTACGTCTGGAAGATTTGCGAATCCCCC
CTTCTTATTCTAAAACTTTCCAAGGTCCGCCTCACGGCATCCAAGTTGAGAGAGATAAATTGAACA
AGTACGGCCGTCCCCTATTGGGATGTACTATTAAACCAAAATTGGGGTTATCCGCGAAGA

4.1.82 Species: *Albizia zygia* (DC.) Macbr

Family: Fabaceae

Specimen Number: IDMTG138-13|GH0003

Location: Ankasa Resource Reserve 157m NO5° 16.138' W002° 36.331'

Plate 2: *Albizia zygia* (DC.) Macbr

DNA barcode: *rbcLa*

AGTGTTGGATTCAAAGCTGGTGTTAAAGATTATAAATTGAATTATTATACTCCTGACTATGAAACC
AAAGATAGTGATATCTTGGCAGCATTCCGAGTAACTCCTCAACCTGGAGTTCCGCCTGAAGAAGCA
GGTGCCGCGGTAGCTGCTGAATCTTCTACTGGTACATGGACAACTGTGTGGACCGATGGGCTTACC
AGTCTTGATCGTTACAAAGGACGATGCTACCACATCGAGTCCGTTGTTGGAGAAGAAGATCAATAT
ATTGCTTATGTAGCTTATCCCTTAGACCTTTTTGAAGAAGGTTCTGTTACTAACATGTTTACTTCGA
TTGTGGGTAATGTATTTGGGTTCAAGGCCCTGCGCGCTCTACGTCTGGAAGATTTGCGAATCCCCC
CTTCTTATTCTAAAACTTTCCAAGGTCCGCCTCACGGCATCCAAGTTGAGAGAGATAAATTGAACA
AGTACGGCCGTCCCCTATTGGGATGTACTATTAAACCAAAATTGGGGTTATCCGCGAAGA

4.1.83 Species: *Kigelia africana* (Lam.) Benth.

Family: Bignoniaceae

Specimen Number: GH0046

Location: Ankasa Resource Reserve 178m NO5° 16.113' W002° 36.425'

Description: *Kigelia africana* is a large deciduous tree which grows to 20 meters or more. The unusual grey, sausage-shaped fruit that give the tree its common name (the sausage tree) hang from rope-like stalks. They can reach over a metre in length and weigh as much as 10kg. The fruit pulp within the thin skin is firm and fibrous fruit pulp and contains many small seeds (Lemmens, 2008).

Plate 1: *Kigelia africana* (Lam.) Benth.

DNA barcode: *rbcLa*

AGTGTTGGATTCAAAGCAGGCGTTAAAGAGTACAAATTGACTTATTATACTCCTGAATACGAAACC
AAGGATACTGATATCTTGGCAGCATTCCGAGTAACTCCTCAACCTGGAGTTCCACCTGAAGAAGCA
GGGGCAGCGGTAGCTGCCGAATCTTCTACCGGTACATGGACAACTGTGTGGACCGATGGACTTAC
CAGCCTTGATCGTTACAAAGGGCGATGCTACCACATCGAGCCCGTTCCTGGAGAAGCAGATCAAT
ATATCTGTTATGTAGCTTACCCTTTAGACCTTTTTGAAGAAGGTTCTGTTACTAACATGTTTACTTC
CATTGTAGGAAATGTATTTGGATTCAAAGCCCTGCGTGCTCTACGTCTGGAAGATCTGCGAATCCC
TACTGCTTATATTAAAACTTTCCAAGGCCCGCCTCATGGGATCCAAGTTGAGAGAGATAAATTGAA
CAAGTATGGTCGTCCCCTGTTGGGATGTACTATTAAACCTAAATTGGGGTTATCTGCTAAAAACTA
TGGTAGAGCAGTTTATGAATGTCTT

4.1.84 Species: *Kigelia africana* (Lam.) Benth.

Family: Bignoniaceae

Specimen Number: IDMTG138-13|GH0047

Location: Bia Biosphere Reserve 301m. N06°32.624' W003°01.543'

Plate 2: *Kigelia africana* (Lam.) Benth.

DNA barcode: *rbcLa*

AAACCAAGGATACTGATATCTTGGCAGCATTCCGAGTAACTCCTCAACCTGGAGTTCCACCTGAAG
AAGCAGGGGCAGCGGTAGCTGCCGAATCTTCTACTGGTACATGGACAACTGTGTGGACCGATGGA
CTTACCAGCCTTGATCGTTACAAAGGGCGATGCTACCACATCGAGCCCGTTCCTGGAGAAGCAGAT
CAATATATCTGTTATGTAGCTTACCCTTTAGACCTTTTTGAAGAAGGTTCTGTTACTAACATGTTTA
CTTCCATTGTAGGAAATGTATTTGGATTCAAAGCCCTGCGTGCTATACGTCTGGAAGATCTGCGAA
TCCCTCCTGCTTATATTAAAACTTTCCAAGGCCCGCCTCATGGGATCCAAGTTGAGAGAGATAAAT
TGAACAAGTATGGTCGTCCCCTGTTGGGATGTACTATTAAACCTAAATTGGGGTTATCTGCTAAA

4.1.85 Species: *Cinnamomum zeylanicum* Blume

Family: : Lauracae

Specimen Number: GH0016

Location: Ankasa Resource Reserve 151m NO5° 16.132' W002° 36.223'

Cinnamon is an evergreen tree which grows from 20 to 30 feet. the leaves petiolate, entire, leathery when mature, upper side shiny green, underside lighter; flowers small white in panicles; fruit, an oval berry like an acorn in its receptacle, bluish when ripe with white spots on it, bigger than a blackberry (Lemmens, 2008).

Plate 1: *Cinnamomum zeylanicum* Blume

DNA barcode:

AGTGTTGGATTCAAAGCTGGTGTTAAAGATTACAAATTGACTTATTATACTCCTGACTATGAAACC
AAAAGTACTGATATTTTGGCAGCATTTCGAGTAACTCCTCAACCCGGAGTTCCACCTGAGGAAGCA
GGGGCTGCGGTAGCTGCCGAATCTTCTACTGGTACATGGACAACTGTGTGGACCGATGGACTTACC
AGCCTTGATCGTTACAAAGGACGATGCTACCACATCGAGCCCGTTCCTGGGGAGGAAACTCAATTT
ATTGCCTATGTAGCTTACCCTTTAGACCTTTTTGAAGAAGGTTCTGTTACGAACATGTTTACTTCTA
TTGTGGGTAATGTATTTGGGTTCAAAGCTCTACGAGCTCTACGTCTGGAGGATCTGCGAATTCCTC
CTGCTTATTCCAAAACTTTCCAAGGCCCGCCCCATGGCATCCAAGTTGAGAGAGATAAATTGAACA
AGTATGGTCGTCCCCTATTGGGATGTACTATTAAACCAAAATTGGGGTTATCCGCCAAGAACTACG
GTAGAGCGGTTTATGAATGTCTC

4.1.86 Species: *Cinnamomum zeylanicum* Blume

Family: : Lauracae

Specimen Number: GH0017

Location: Bia Biosphere Reserve 471m. N06°32.234' W003°01.329'

Plate 2: *Cinnamomum zeylanicum* Blume

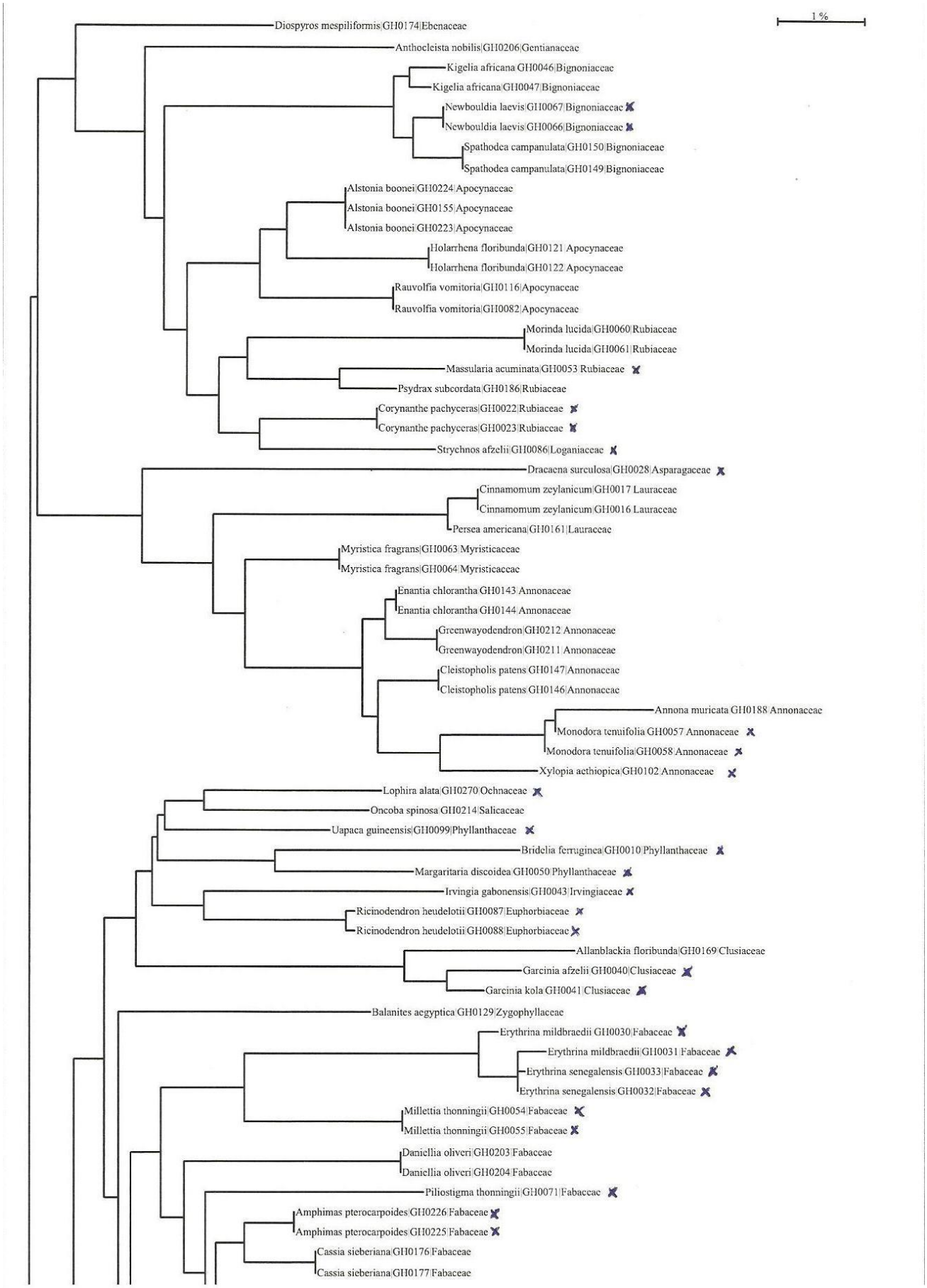
DNA barcode: *rbcLa*

AGTGTTGGATTCAAAGCTGGTGTTAAAGATTACAAATTGACTTATTATACTCCTGACTATGAAACC
AAAAGTACTGATATTTTGGCAGCATTTCGAGTAACTCCTCAACCCGGAGTTCCACCTGAGGAAGCA
GGGGCTGCGGTAGCTGCCGAATCTTCTACTGGTACATGGACAACTGTGTGGACCGATGGACTTACC
AGCCTTGATCGTTACAAAGGACGATGCTACCACATCGAGCCCGTTCCTGGGGAGGAAACTCAATTT
ATTGCCTATGTAGCTTACCCTTTAGACCTTTTTGAAGAAGGTTCTGTTACGAACATGTTTACTTCTA
TTGTGGGTAATGTATTTGGGTTCAAAGCTCTACGAGCTCTACGTCTGGAGGATCTGCGAATTCCTC
CTGCTTATTCCAAAACTTTCCAAGGCCCGCCCCATGGCATCCAAGTTGAGAGAGATAAATTGAACA
AGTATGGTCGTCCCCTATTGGGATGTACTATTAAACCAAAATTGGGGTTATCCGCCAAGAACTACG
GTAGAGCGGTTTATGAATGTCTC

4.2 Relationship between the trees species used in the treatment of malaria in Ghana

To get a robust cladogram of sequences of trees used in the treatment of malaria in southern Ghana, other sequences of trees from Ghana were mined from BOLD and used in the generation of the cladogram together with the sequences generated. The cladogram generated was used to show the relationship between the species (Fig.4.1). The cladogram shows that specimens of the same species have similar DNA sequences. Each of the tree species were separated to the species level.

(*) Shows plants that served as reference data on which others were matched



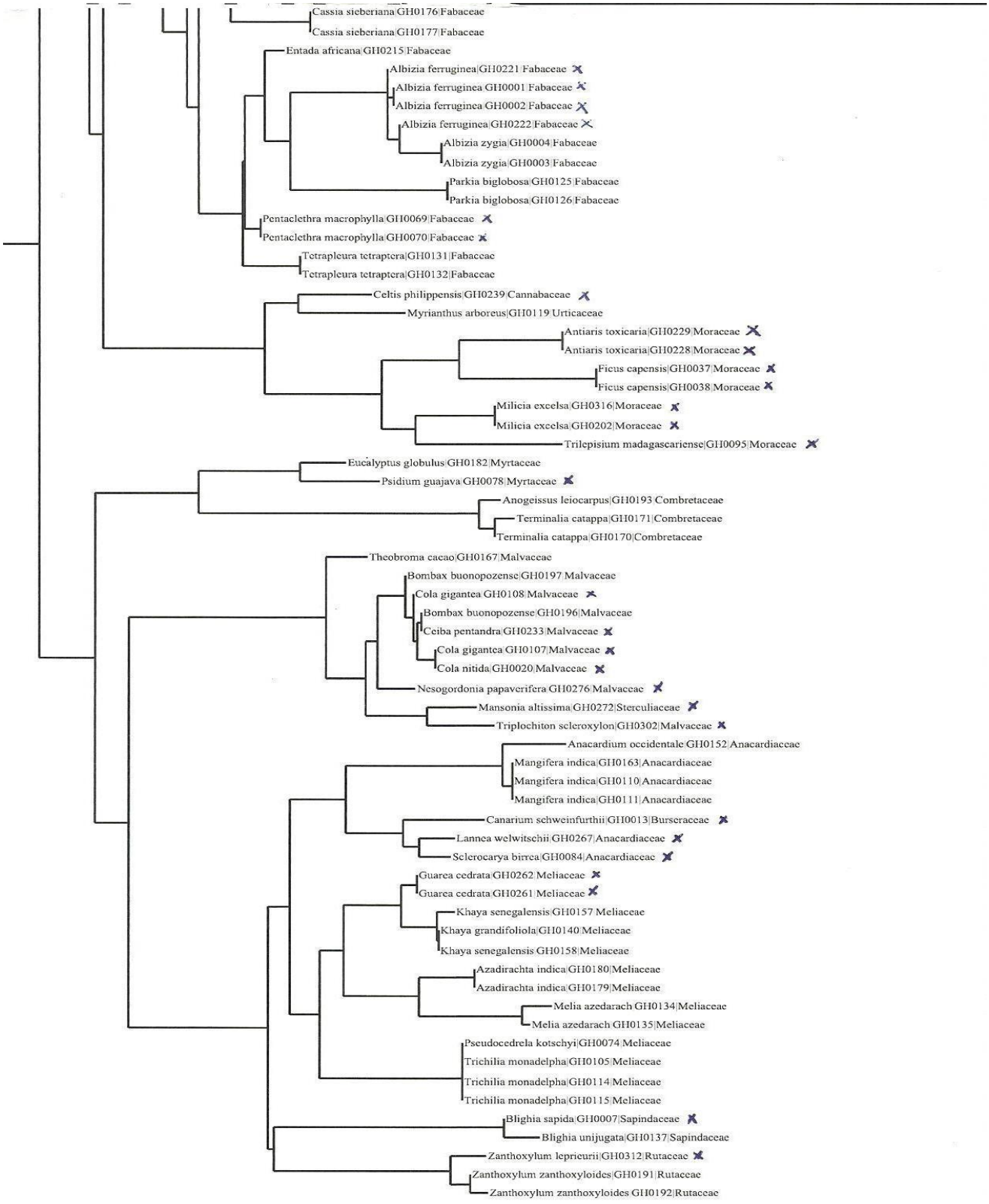


Figure 4.1: Cladogram of the sequences trees used in the treatment of malaria and that of other trees from Ghana mined from BOLD.

4.3.0 Social survey

4.3.1 Demography of respondents

Ninety eighty individuals were interviewed out of which (65) were females and the remaining thirty four (33) 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 50years and above. These age categories captured entirely everybody within the target group of the study. Table 4.1 shows the percentage of respondents within each of the age groups.

Table 4. 1: Percentage of respondents within each of the age groups.

Age Groups	20-29	30-39	40-49	50 and above
Percentage each age group	7	15	31	47

Forty six (46) percent of the respondents had basic level of education while only four percent had a tertiary level of education. Fig 4.2 presents the percentage of the respondents and their level of education.

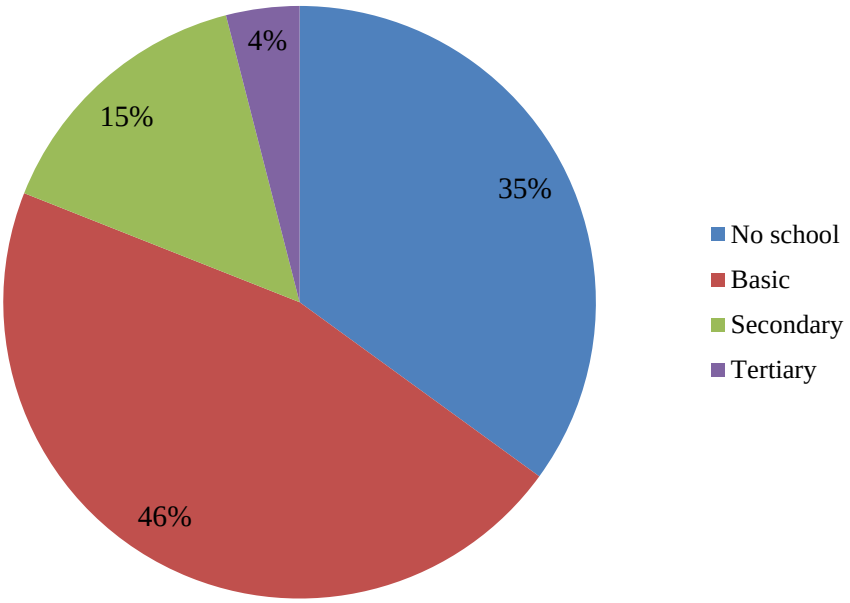


Figure 4. 2: Educational levels of respondents.

Thirty one herbalists who were farmers, hunters or herbal plant collectors were interviewed from the three conservation sites where samples were taken. None of them were full time herbalists. Fifty nine were herbal plants sellers interviewed from Kasoa and Nyanyano both in the Central region of Ghana. The remaining eight were herbal industries who preferred not to mention their names. 95% of the herbal plants sellers interviewed were females with the remaining 5% being males who sell fetish animals and aphrodisiac herbs. With the exception of the three males who collected the plants part they sell by themselves all of them bought their products from commercial herbal plants collectors. Unlike the herbalists interviewed from the conservation sites, herbal plant selling was the only source of income for the herbal plants sellers interviewed from the two markets.

4.3.2 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 was possible especially when the trees are not in flower.

The herbal plant sellers, like the herbalists depend on experience and the morphological features of the plant for its identification. The major challenge they face is how to identify the roots, chopped and macerated stems and dried parts of plants.

According to them, though they can identify the plant parts by their colour and scent they produced at times is impossible and one has to confide in the collectors for the names of such plants species. However some sellers demand that the collectors take some leaves, fruits and flowers where available along with the non-leafy parts to aid in the identification of the plants they collect. Even with this they cannot be sure they are given the right plants they demand and expressed interest in a conventional method that could solve their problem.

4.3.3 Plants and the various parts used in herbal medicine preparation for malaria

Many plants were sold on the markets which have the potential to cure malaria. Some of these; *Mangifera indica*, *Azadirachta indica*, *Anacardium occidentale*, among others have been become domesticated and that their availability on the markets was very low. Table 4.2 presents the families, local name in Akan, frequency of citation, parts of the plant used and the frequency of encounter of the plants species used in the treatment of malaria.

Table 4. 2: Plant species used in the treatment of malaria encountered in the survey, their families, local name in Akan, parts used and the frequency of encounter in the market.

Family	Name of Plant	Akan name	Parts Use	Freq of encounter
Bombacaceae	*Adansonia Digitata*	Baoba	Whole plant	30
Clusiaceae	*Allanblankia floribunda*	Bohwe	Bark	9
Apocynaceae	*Alstonia boonei*	Nyamedua	Whole plant	36
Anacardiaceae	*Anacardium occidentale*	Atea	Stem & Leaves	29
Annonaceae	*Annona muricata*	Obrofo Ntumkum	Leaves	24
Combretaceae	*Anogeissus leiocarpus*	Kane	Roots	29
Loganiaceae	*Anthocleista nobilis*	Odeefuor kete	Stem & Roots	20
Meliaceae	*Azadirachta indica*	Neem	Leaves	48
Zygophyllaceae	*Balanites aegyptiaca*	Kabawoo	Roots	15
Sapindaceae	*Blighia unijugata*	Akyibiri	Roots	12
Malvaceae	*Bombax buonopozense*	Akatabena, Akata	Bark & Stem	35
Leguminosae	*Cassia sieberiana*	Sanya	Bark & Root	20
Annonaceae	*Cleistopholis patens*	Engo ni ntsini	Bark & Leaves	15
Leguminosae	*Daniellia oliveri*	Sanya	Bark & Stem	28
Ebenaceae	*Diospyros mespiliformis*	Kusibiri	Leaves & Stem	9
Annonaceae	*Enantia chlorantia*	Yellow wood	Roots & Stem	12
Fabaceae	*Entanda Africana*	Kaboya	Bark & Stem	19
Myrtaceae	*Eucalyptus sp.*		Fruits & Leaves	13
Myrtaceae	*Greenwayo-dandrum oliveri*		Leaves	14
Apocynaceae	*Holerrhena floribunda*	Osese	Leaves & bark	0
Meliaceae	*Khaya grandifoliola*	Dubini	Root & Bark	33
Meliaceae	*Khaya senegalensis*	Mahogany	Bark, & Root	47
Anacardiaceae	*Mangifera indica*	Mango	Leaves & Stem	31
Cecropiaceae	*Myrianthus aboreus*	Anyankoma	Root & Bark	0
Meliaceae	*Melia azedarach*	Persian	Roots & Bark	11
Moraceae	*Melicia excels*	Odum	Bark	22
Salicaceae	*Oncoba spinoosa*	Asratua dua	Leaves	16
Leguminosae	*Parkia biglobosa*	dawadawa	Roots & Leaves	22
Lauraceae	*Persia Americana*	pear	Leaves	26
Apocynaceae	*Pricalina nitida*	Akuama	Whole plant	42

Rubiaceae	*Psydrax subcordatum*	Ogyapon	Bark	26
Leguminosae	*Pterocarpus erinaceus*	Rose wood	Leaves	3
Myristicaceae	*Pycnanthus angolensis*	Otie	Bark & Stem	46
Apocynaceae	*Ravolfia vomitaris*	Kakapempe-m	Whole plant	41
Bignoniaceae	*Spathodea campanulata*	Kookoo nisuo	Stem & Root	9
Combretaceae	*Terminalia catapa*	Abrofo-nkate	Leaves & Stem	44
Leguminosae	*Tetrapleura tetraptera*	Prekese abena	Fruits & Bark	35
Malvaceae	*Theombroma cacao*	Cocoa	Leaves	32
Meliaceae	*Trichillia monadelpha*	Tandro	Bark & Stem	28
Rutaceae	*Zanthoxylum zanthoxyloides*	Okanto	Bark & stem	33
Combretaceae	*Terminalia ivorensis*	Emire	Bark & Stem	29
Lecythidaceae	*Petersianthus macrocarpus*	Esia	Bark & Stem	38
Rubiaceae	*Morinda lucida*	Konkroma	Bark & Leaves	49
Musaceae	*Musa paradisiaca*	Brodie	Leaves	15
Apocynaceae	*Funtumia elastica*	Funtum	Leaves	12
Lamiaceae	*Tectona grandis*	Teak	Leaves & Bark	14
Rubiaceae	*Mitragyna inermis*	Kukyemfie	Roots	9
Meliaceae	*Pseudocedrela kotschyi*	Kurubeta	Roots	15
Fabaceae	*Afzelia Africana*	Opapao	Bark	12
Malvaceae	*Sterculia setigera*	Adodowa	Bark	16

The parts of plant used in the preparation of herbal medicines have significant impact on the survival of the plant species. While in some of the plants only the leaves are used in herbal preparation, in others any part of the plant can be used in such preparation. Fig. 4.3 presents the percentage of each part used in the treatment of malaria.

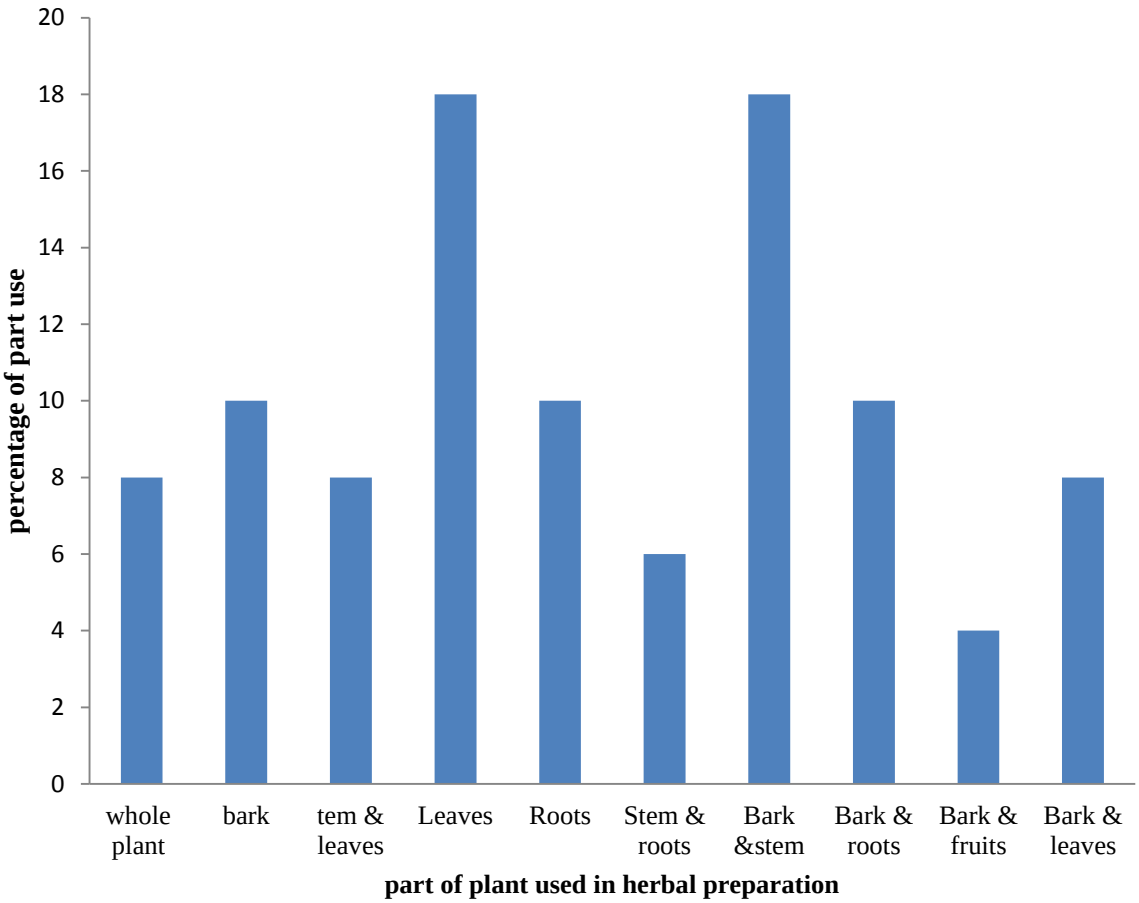


Figure 4. 3: Percentage of plant parts used to treat malaria.

4.3.4 The state of herbal medicine in southern Ghana

The interview sought to find out how respondents obtained the plant species they use. 16% of the respondents collect their samples by themselves, 27% send their apprentices who are mostly their children to collect them while the remaining 57% buy from commercial plant collectors.

96% of the respondents perceive changes in the availability of some medicinal plant species increasing their search time for such species while the remaining 4% think there are no changes in availability of any of the plant species they deal in.

Several factors including wildfires, illegal logging, farming practices among others were listed as the causes of the loss of medicinal plants species. Figure 4.4 presents the causes of loss of medicinal plants species as stated by the respondents.

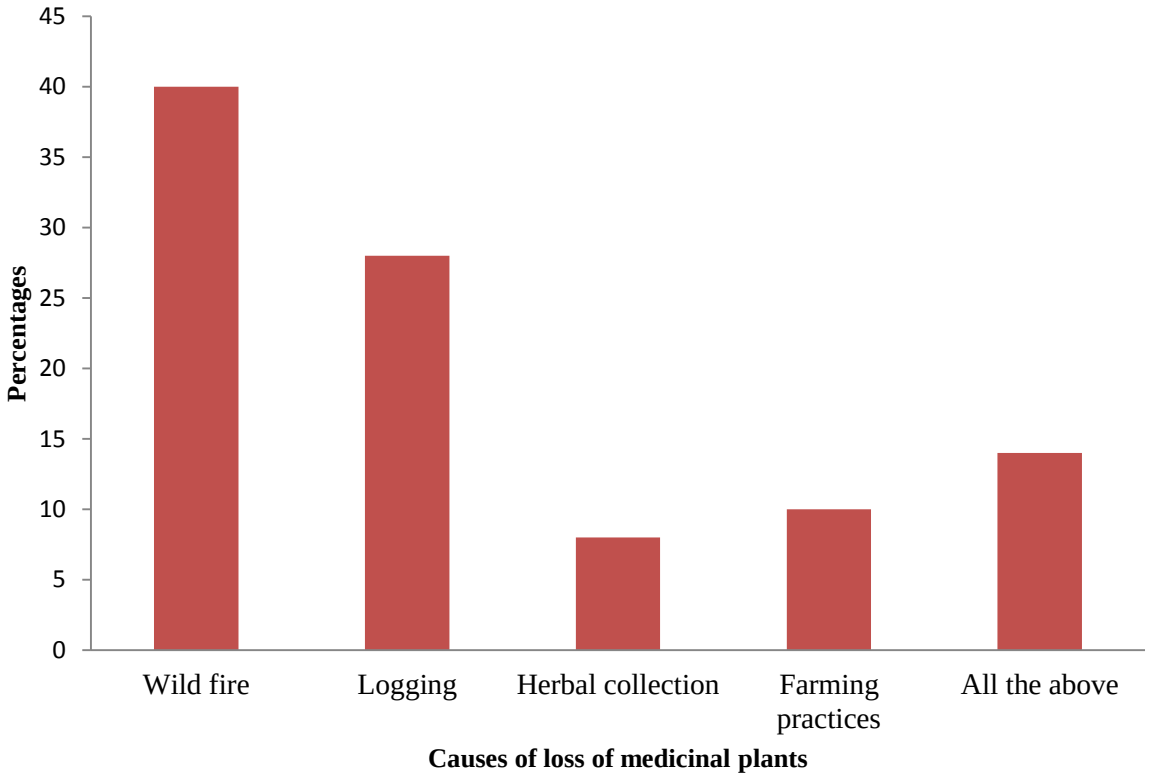


Figure 4. 4: Causes of loss of medicinal plants species according to respondents

Although 50% of the respondents think tree planting can help solve the situation, only 4% think establishment of forest reserves can contribute to the availability of medicinal plants species. Figure 4.5 presents the mitigation measures proposed by respondents to solve the destruction and decrease in the availability of medicinal plants species.

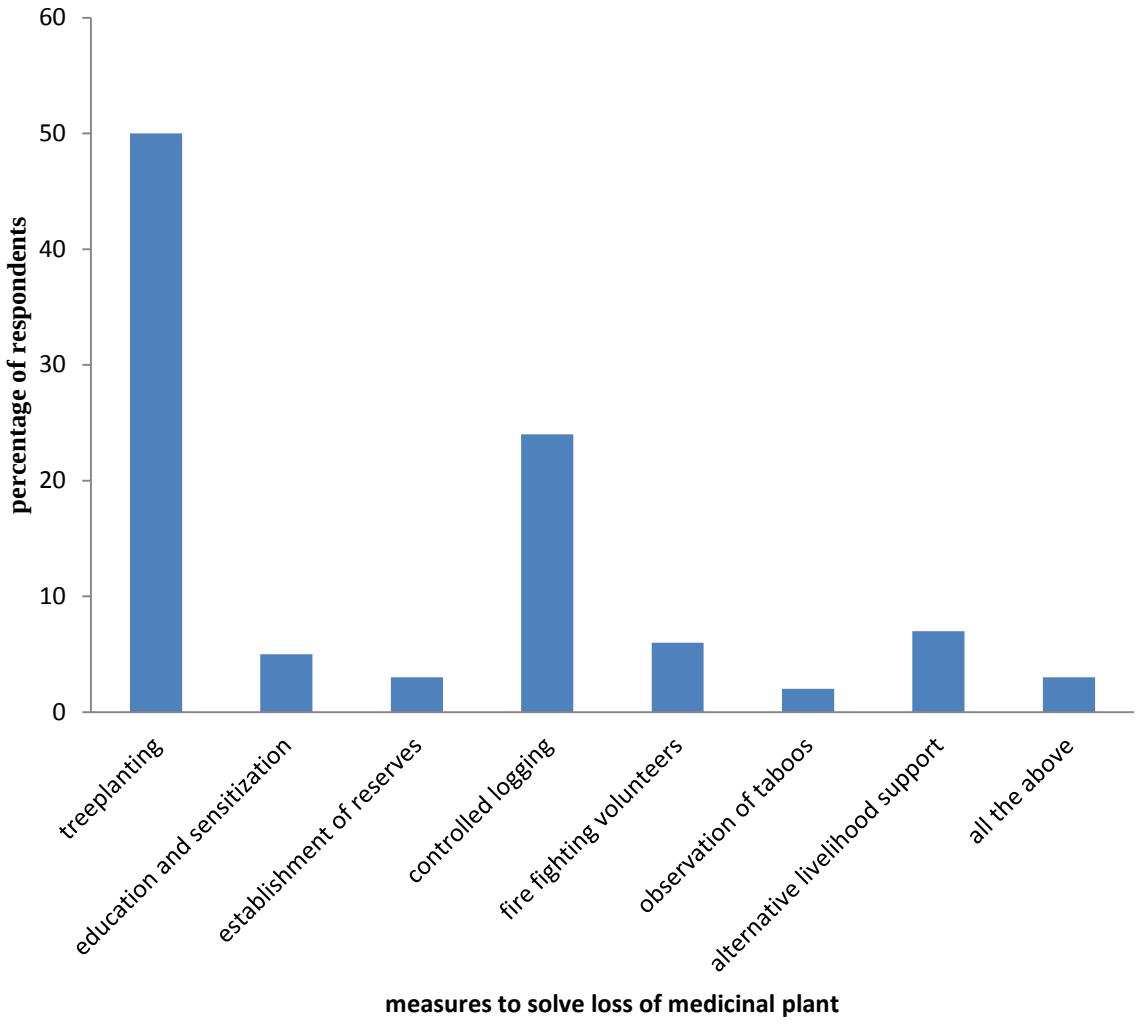


Figure 4.5: Measures proposed by respondents to ensure sustainability of medicinal plants species.

4.3.5 Awareness of forest conservation

The interview sought to find out about the level of awareness of respondents about forest conservation. All the respondents are aware of forest conservation. Most of the respondents come from areas where conservation activities are on-going. Figure 4.6 shows the sources of awareness of forest conservation by the respondents.

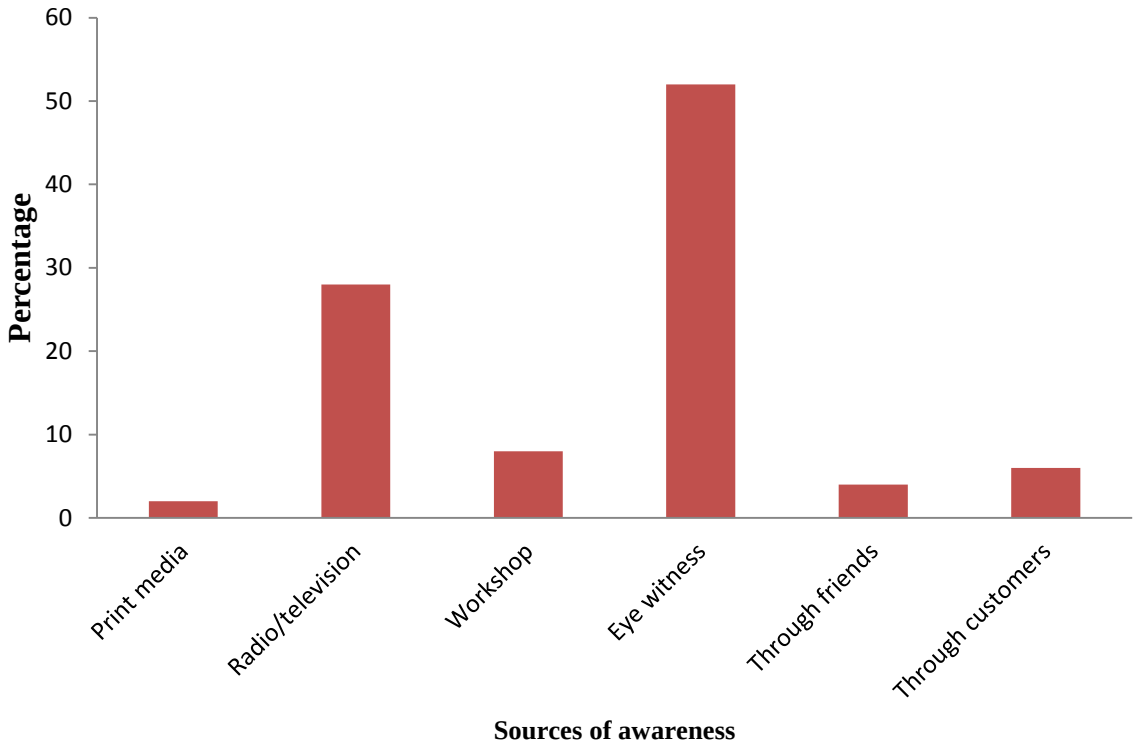


Figure 4. 6: Sources of awareness of forest conservation.

Even though all the respondents are aware of forest conservation, only twenty eight percent think it is of any significance to ensuring the availability of medicinal plant species while forty eight percent think it is of no significance to ensuring sustainability of medicinal plants species in Ghana. Figure 4.7 presents the opinion of respondents about the significance of forest conservation to ensuring sustainability of medicinal plant species.

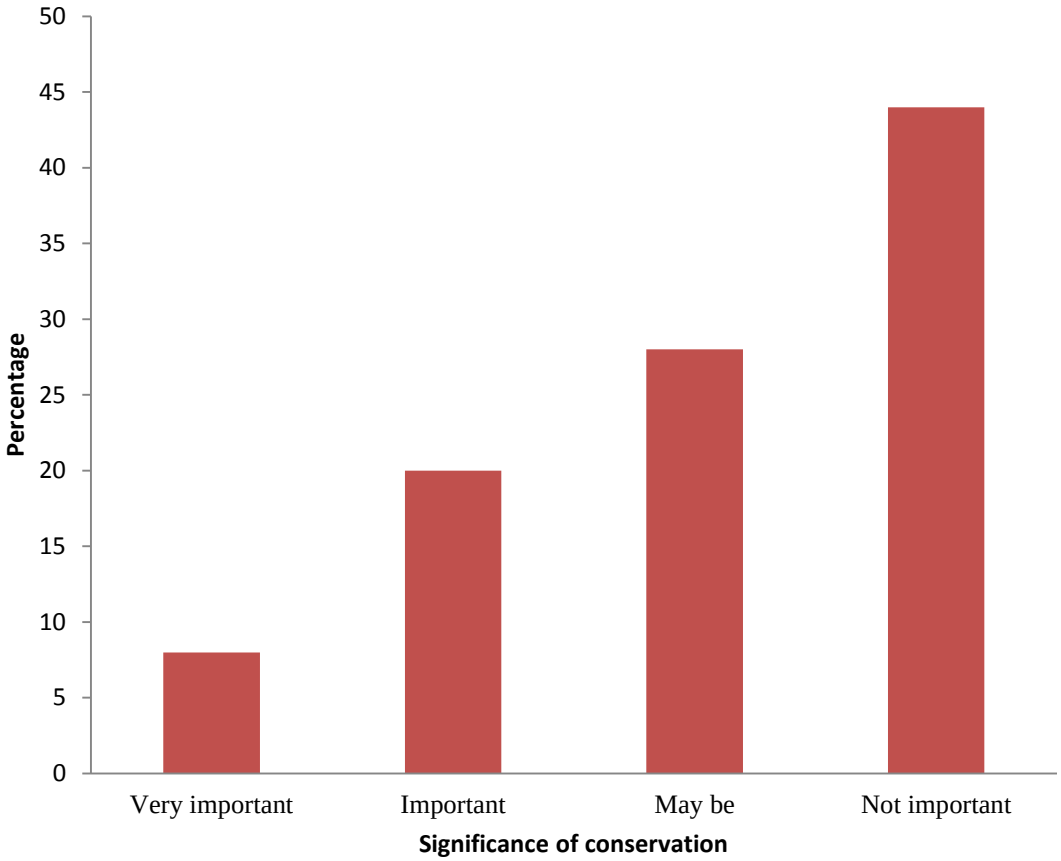


Figure 4. 7: Significance of forest conservation.

CHAPTER FIVE

5.0 DISCUSSION

5.1 DNA barcoding

According to Bafeel *et al.,* (2011), DNA barcoding depends on the information encoded in the nucleotide sequence of a standard region of the genome of a species as a tool for its identification. Traditionally, plant identification is based on morphology, with the limitation that many species must be collected at a very specific time of the year so that reproductive features can be used for accurate identification. With DNA barcoding, once a reference library is built, identification of plants is possible at all life stages, from seed to mature plant, and even a fragment of a leaf might be used to identify a species (Williams *et al.,* 2009). Based on efficient recovery of good quality sequences and high levels of species discrimination (Burgess *et al*., 2011), a 2-locus combination of *rbcL* and *matK* has been accepted as the standard regions for barcoding (CBOL Plant Working Group, 2009). The *rbcLa* gene region which has a high success rate was used in this study. A success rate of 88% and 100% was achieved by Bafeel *et al.,* (2011) and Norris *et al*., (2009) at the same gene region respectively; however, this work achieved a success rate of 80%. These results can be explained by a report of 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 candidates plastid regions (*rbcL, matK, rpoCl, rpoB, trnH-psbA, atpF-atpH, and psbK-psbI*) should be used at a time. Roy *et al*., (2010) and Bafeel *et al*., (2011) have both argued that 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 engaged. According to literature, plant species have chemotypic heterogeneity which tends to interrupt plant DNA yield, hence the possibility of closely

related plant species requiring different DNA isolation protocol (Khanuja *et al*., (1999), Aras *et al.,* (1993), Temiesak *et al*., (1993), Vanijajiva *et al*., (2005), this may also explain why success rate was not hundred percent. Soininen *et al*., (2009) mentioned errors such as primer mismatch at the annealing sites, poor quality, or little amount of DNA extracted as the possible reasons why DNA sequences may fail.

The basic concept in species identification is to match the sequence of the evidence item to a reference (Altschul *et al.,* 1997). A cladogram was generated from the 43 plants that were successfully sequenced together with the sequences of other tree species from Ghana in the Gene Bank of CBOLD (2012) for comparison.

Each of the species correctly matched their counterparts (fig. 4.1) which implies a 100% sequence matching success. Hence DNA barcoding can be used to identify correctly all medicinal plant species which is key to the safety of herbal medicine (Abbiw, 1990).

Molecular tools such as DNA barcoding has been developed to characterize similar species and also detect their origin (Rahman *et al.,* 2009) which is often difficult to be carried out by traditional identification methods. 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, the security industry, food and drugs board and the standard board 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 *et al.,* 2008). The acceptance and use of DNA barcoding as an identification tool for medicinal plants identification will ensure the safety of herbal medicine by eliminating misidentification completely. 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 regularised 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. This will also help to improve conservation of vulnerable and endangered species.

Even though there was a hundred percent successful match of the various sequences of the same species from different places, there were slight variations in the genes of the individual plants of the same species which is evident from the cladogram. According to Bokhari *et al.*, (1990), Kamal *et al*., (2010); 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 and these were slightly prominent in *Kigelia Africana,Zanthoxylum zanthoxyloides, Khaya senegalensis, Melia azedarach and Terminalia catappa*. These variations can help to link individual species to their source since the same plant at different places will experience different environmental conditions and hence produce slightly different genetic variations.

These differences in genes will be evident in their DNA barcodes which can be linked to its possible source with available DNA reference data. It must be noted however that DNA barcoding operates on the assumption that gene region chosen has lower 'within-taxon' variation than that 'between-taxa' (www.barcodinglife.org), and that no matter the variations within the same species; they can be 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, Wink, 1999, Sofowora, 2008). Hence, to cultivate medicinal plants species, it is 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 appropriate identification tool with the potential of eliminating all forms of mis-identification. It will also facilitates better records on medicinal plants species which will aid in ensuring their sustainable harvest an also help to check and regulate the harvest of each medicinal tree 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. Most of the respondents generate their source of livelihood and income from herbal medicine, mostly the women who engage in it as their major occupation (Ofori *et al*., 2012). Herbalists like herbal plant sellers have much knowledge in the use of the plants species around 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. This is in line with a work done by Agbovie *et al.*, (2002) who also concluded that herbalists use the common plants in their area. 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.

The sustainability and availability of the medicinal plants is a problem to the industry since most of the plants species in trade used for malaria treatment found in this survey were obtained from the wild and this corresponds with, Ofori *et al*., (2012).

5.2.2 Plants and plant parts used in herbal medicine preparation

According Birdi *et al.,* (2008) and Sofowora, (2008), same plant species can have more than one chemical constituent that function against several disease causing organisms, hence the ability of one plant to serve as a cure for different unrelated diseases. Furthermore, the same species at different locations can function differently due to environmental, edaphic, climate and anthropological effects which causes the development of molecular diversity which affect the medicinal value and function of the same plant species (Kamal *et al*., 2010; Sofowora, 2008). This has put much pressure on some plant species like *Khaya senegalesis, Rauvolfia vomitoris, Alstonia boonei* among others that are known to be effective for the treatment of several diseases. There is the need therefore to make conscious efforts towards the conservation and protection of such important species.

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, 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. However the results of this study deviated from that of Van Andel *et al*., (2012) and other literatures. While 18% of the floristic diversity of the study area was made up of leaves, 10% was made up of plant bark and 10% of underground plant organs. Since the survey was done in the dry

season most of the leaves of plants may be dried up and that the herbalists will have to depend on the bark and leaves which are available. 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) and this might account for the herbal markets of the study areas also making up of 18% 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). The parts of plants used in herbal preparation are influenced by the climatic conditions that prevail in the area (Van Andel *et al*., 2012).

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

5.2.3 Problems with identifying market samples

Traditional healers often work with powdered medicine, which is easily made from dried materials, hence making market identification very difficult (McMillen, 2008). According to Van Andel *et al.*, (2012), African herbal markets are made up of mainly, roots, bark and wood which are much difficult to identify. At times seeds must be cultivated before identification is possible, and in the case of bark and roots, harvesters must at times be followed to the source before identification can be done. In view of this ethno botanists

should make an effort to collect the medicinal products (bark, root, and seed) as well when making voucher specimen. To prevent possible misidentification the use of molecular techniques such as DNA barcoding in the identification of medicinal plant products such as roots, barks, shredded or powdered materials was proposed by (Van Andel *et al*., 2012).

In this survey 57% 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 the products have to travel through. This goes to support the argument by Van Andel *et al*., (2012) that identification of such products are very difficult and thus molecular identification methods of such medicinal products is the best option.

5.2.4 State of the herbal medicine industry

According to Van Andel *et al*., (2012) majority of the 950 tons of crude herbal products sold on the Ghanaian markets in 2010 were obtained from the wild. However, the effects of this large herbal collection on the sustainability and survival of some of these medicinal plants species are not known.

96% of the respondents ascertaining the fact that the herbal plants they use are getting more scarce and that they have to walk longer distances to collect them is enough evidence that most important common medicinal plant species are getting scarce and this can have serious repercussion on the health of most people mostly the urban poor and rural dwellers who depend much on herbal medicines. It also calls for conservation of these medicinal plants species to protect them from being wiped out completely.

5.2.5 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 plant 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 plant species should also be encouraged.

Although 96% of the respondents in the survey were aware of the reduction in the availability of some medicinal plants species, none had planted some of the important species they use in their backyard and farms, however, Ofori *et al.,* (2012) recorded 25% of his respondents 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 that no efforts at all were made to cultivate or conserve medicinal plants species in his study area.

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 are 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 factors like 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 the respondents think it can have any impact on the mitigation of the declining medicinal plants population but cultivation of medicinal plants and controlled logging can contribute to solving the problem.

The fear of people losing farmlands to conservation and others losing their 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.

Consensus 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 RECOMENDATION

6.1 Conclusion

Herbal medicines play important role in the health care of Ghanaians most especially the urban poor and rural dwellers. This study established that medicinal trees are used in the treatment of malaria in Ghana; however, correct identification of the plant species used in the preparation is vital to the safety and efficacy of the resulting herbal medicine. The bigger problem is with the identification of market samples which are mostly dried, macerated or powdered.

DNA barcoding, a problem-solving tool that utilises species-specific DNA sequence(s) for identification of all species, is an efficient and appropriate tool for identifying medicinal plants as well as all other plant 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 and if accepted for use will help in the provision of better records which will inform on better conservation of vulnerable species and the sustainable harvesting of such plants.

The rate of harvest of medicinal trees in southern Ghana is not sustainable and most of these species will be wiped out if no efforts are made to conserve and also control their harvest. Unsustainable collection of roots, bark, stem and even the whole plant are highly detrimental to the survival of tree species

There is a high level of awareness of forest conservation, however respondents believe it has little impact on ensuring the continue 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.

6.1 Recommendations

To ensure anti-malaria medicinal plants are not over exploited a form of monitoring and checks in harvesting would need to be implemented to enhance its conservation. Combining training in the sustainable harvesting of medicinal plants with education on its cultivation could greatly enhance our ability to conserve plants in the future.

There is the need to establish an efficient monitoring system that will establish a detail DNA barcodes database of all medicinal plants in Ghana to serve as a reference point for species identification if we aim to ensure sustainable harvesting of medicinal plants.

More detailed Ethno-botanical studies should be carried out on anti-malaria plants species all over the country using an enhanced system that combines both scientific and physical characteristic methods of identification to enable proper verification of the contents of medicinal plants use in the production of anti-malaria herbal medicines in other to regulate the threats that consumers face when they use them.

Furthermore commercial exploitation of, bark, roots and whole plants from the wild should be banned by legislation that provides a better monitoring system for its enforcement and as a deterrent defaulters should be made to pay heavy fines as is done in countries like Chad.

In order to have a focussed approach for developing the whole sector of herbal medicine both in terms of conservation, cultivation, sustainable use and legal protection, an institutional mechanism need to be evolved to ensure that useful policies are formulated that will integrate the concerns of all stakeholders.

It will be necessary, based on an understanding that where medicinal plants are currently distributed, a series of well enhanced conservation programmes need to be developed for their in-situ conservation and to designate specific genetic reserves throughout the country for their protection.

Finally, further research should be carried out on this study using two or more plastids regions in the sequencing of the DNA barcodes.

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