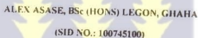
ETHNOBOTANY, BIOACTIVITY AND VARIATION STUDIES OF TRADITIONAL GHANAIAN ANTI-MALARIAL PLANTS

A THESIS SUMBMITTED

BY



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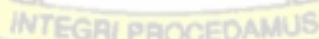

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I, the undersigned, Alex Asase do hereby declare that with the exception to other people's work that have been duly cited, this project work was done by me and that no part of the work has been presented for any other degree in this University or elsewhere.

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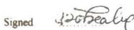
Signed 

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DEDICATION

This thesis is dedicated to my parents especially my late father who never lived to enjoy the fruits of his labour

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ABSTRACT

An investigation on traditional plants used in the treatment of malaria in Ghana was conducted. The study involved a collection of information from an indigenous group of people living in the Wechiau Community Hippopotamus Sanctuary Ghana about their knowledge on plant species used for the treatment of malaria. It also involved a study of the bioactivity and variations within selected plant species in the sanctuary. The investigation on the indigenous uses involved ethnobotanical interviews and field studies. Forty – one species of plants in 19 families were mentioned as used for the treatment of malaria in the sanctuary. Eight of the species of plants, namely, *Afraegle paniculata, Haematostaphis barteri, Indigofera pulchra, Monathotaxis* sp., *Ozoroa insignis, Strychnos innocua, Strychnos spinosa* and *Xeroderris stuhlmannii* have not been previously investigated for their anti-malarial uses. The efficacy of four of the anti-malarial species, namely, *Cassia sieberiana, Haematostaphis barteri, Mitragyna inermis* and *Pseudocedrela kotschyi* was determined by testing their extracts against several organisms including *Plasmodium*, bacteria, fungi and an insect pest. The methods of the bioassays included microdilution technique for *in vitro* antiplasmodial assay, TLC agar-overlay diffusion for microbial bioassay and a binary choice assay for insect anti-feedant testing. The extracts were moderately active against *Plasmodium* and varied in their activity against the test organisms. From the stem and root barks of *Haematostaphis barteri* three stilbene compounds were isolated for the first time from a member of the family Anacardiaceae. The identification of stilbenes was made through the use of NMR and APCI-MS experimentation. Variation study using data from a range of characters including morphology, anatomy of barks, ecology, distribution, comparative phytochemistry and molecular (AFLP markers) were conducted to understand infraspecific taxonomy of the four species of plants and for their conservation in the sanctuary. Data obtained from the

morphometric measurements, maceration, field ecological methods, specimen computerisation, phytochemical methods such as HPLC and LC-MS and AFLP methodologies were used for the variations analysis. The variations in the selected species were largely due to morphological and chemical variability. The variations in the species of plants were found to be due to plasticity of environmental factors and that the taxonomies of the four species are stable in the sanctuary

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LIST OF ABBREVIATIONS AND ACRONYMS

APCI- Atmospheric Positive Chemical Ionisation

EI- Electron spray Ionisation

EWI- Earthwatch Institute

GC / MS- Gas chromatography / Mass spectrometry

GPS - Global Positioning System

HMBC- Two Dimensional Heteronuclear Multiple Bond Correlation Spectroscopy

HPLC- High Performance Liquid Chromatography

HSQC- Two Dimensional Heteronuclear Single Quantum Coherence Spectroscopy

IC_{50} - Inhibitory Concentration at 50 %

LC / MS - Liquid Chromatography / Mass Spectrometry

NMR- Nuclei Magnetic Resonance

NOESY- One dimensional selective nuclear Overhauser Enhancement Spectroscopy

PCR- Polymerase Chain Reaction

TLC – Thin Layer Chromatography

UV- Ultraviolet

CHAPTER ONE

GENERAL INTRODUCTION AND LITERATURE REVIEW

1.1.0. General Introduction

The use of plants for medicinal purposes has existed for centuries. A medicinal plant is a species which contains substances that possess therapeutic properties that can be used as precursor for drug synthesis Medicinal plants are still used for the medical needs of the populations of countries not fully developed economically and often with limited medical resources. The efficacies of medicinal plants in treating diseases are well known. Medicinal plants also provide templates for development of new drugs through further scientific research and they are often more accessible and cost effective (Sofowora, 1987).

The World Health Organisation (WHO) has a policy of encouraging the development and utilisation of traditional medicine in Primary Health Care delivery system in developing countries In Africa, Asia and Latin America between 60-90 % of populations rely on medicinal plants either totally or partially for health care needs. In Ghana, the figure is about 70 % and it is estimated that there is one traditional doctor to every 400 people, as compared to one orthodox doctor to every 1200 (PORSPI, 1992).

Many plants have been credited with curative properties in Ghana. Some of these claim are authentic and others doubtful. The amount of knowledge on medicinal uses of plant possessed by Ghanaians is extensive, yet this knowledge is often undocumented and guarded with secrecy and when those Ghanaians die this knowledge is lost (Dokosi, 1969; Mshana et al., 2001). Knowledge of alleged medicinal values of plants is often acquired through family inheritance and apprenticeship. Other people also learn the medicinal uses of plants when treating themselves with plants. Some people also claim that they have

acquired their knowledge from particular shrines through the process of divination. There is yet another group who claim they were kidnapped by dwarfs and taught the medicinal uses of plants (Dokosi, 1969).

There is hardly any disease in Ghana which has no claims of herbal treatment. For example, some Ghanaian herbalists report that they have used plants to cure people with the HIV / AIDS (Addae-Mensah, 1999). Thus plant species are used in the treatment of a wide range of diseases and illnesses in Ghana including malaria. This study will concentrate on some of the species of plants reported to be effective for the treatment of malaria.

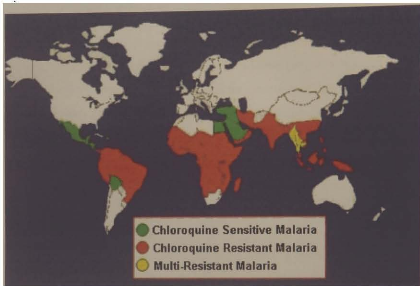
1.1.1. Malaria

Malaria is caused by single celled protozoan parasites of the genus *Plasmodium* in the phylum Apicomplexa and transmitted through Anopheles mosquito. In man, there are four species of the parasitic protozoa, which cause four kinds of malaria. The most important of these four is *Plasmodium falciparum*. It can be rapidly fatal and is responsible for the majority of malaria related deaths. *Plasmodium falciparum* occurs mostly in Africa, New Guinea and Haiti. *Plasmodium vivax* is the most widespread of the four species with infections reported from India sub-continent, Central America but less in Africa. Both *Plasmodium malaria* and *Plasmodium ovale* are found in most endemic areas in sub-Saharan Africa and also common in Africa.

The symptoms of malaria throughout the tropics include severe chills, fevers, sweating, enlarged and tender spleen, confusion and great thirst. Ultimately, a victim of malaria may die of anaemia, kidney failure or brain damage (Raven and Johnson, 1996). Malaria is one

of the most fatal diseases of the world ranking in this respect with tuberculosis, cancer and HIV / AIDS (Symth, 1994). The world number of clinical cases is estimated to be over 300-500 million cases per year with more than 1.5-2.7 million deaths each year (Sirisilam and Veeresham, 2003).The situation is more complex associated with two further problems; (1) the appearance of drug resistance strains of *Plasmodium* and (2) the discovery that man may become infested with simian (monkey) malaria (Symth, 1994). The current distribution of malaria drug-resistance in the word is presented in Figure 1.

Figure 1: Current distribution of malaria resist[illegible]



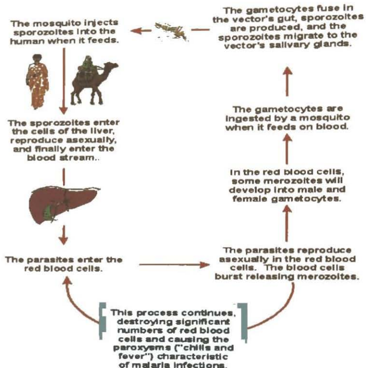
Source: http://www.traveldoctor.co.uk/malaria.htm

1.1.2. Life-cycle of malaria

Naturally acquired malaria begins with the bite of a *Plasmodium*-infected female *Anopheles* mosquito after taking blood from an infected human being. At least 65 different species of the *Anopheles* genus are involved in the transmission of malaria (Raven and Johnson, 1996). The most common species in Africa is *Anopheles gambiae*. When the mouth parts of an *Anopheles* mosquito penetrates the human skin and obtains blood, it injects saliva mixed with an anticoagulant. If the mosquito is infected with *Plasmodium* its saliva will contain elongated plasmodium cells known as sporozoites (a stage of the life cycle) and these would be injected into the bloodstream of the victim.

The parasite makes its way through the bloodstream to the liver, where it rapidly divides asexually. After the division phase, merozoites (which are the next stage of the life cycle) are formed, and these either reinvade other liver cells or enter the host's bloodstream. In the bloodstream, they invade the red blood cells dividing rapidly within them and causing cells to become enlarge and ultimately rupture. Anaemia results and toxic substances are released throughout the body of the human host bringing about the well – known cycle of fever and chill that is characteristic of malaria. The cycle repeats itself regularly every 48 hours, 72 hours or longer, depending on the species of plasmodium involved. The merozoites develop in the gut of the mosquito to sporozoites when it takes meal from the infected human and the cycle repeats itself.

Figure 2: Life cycle of Malaria



Source;

http://www.biosci.ohio-tate.edu/~parasite/lifecycles/plasmodium_lifecycle.html

1.1.3. Malaria chemotherapy

Efforts to eradicate malaria have met with limited successes in many countries. The *Anopheles* mosquito has become resistant to insecticides such as DDT due to inappropriate use of the insecticides. The development of vaccines to combat the disease is currently the order of the day. The different stages of the life cycle of *Plasmodium* produce different antigens, and they are sensitive to different control agents.

Chemotherapy has been of great importance in the treatment of malaria. Drugs developed for the treatment of malaria affect different stages of the life cycle of the parasite in man. Hence anti-malarial drugs have been grouped on this basis. The tissue schizonticide act on the asexual erythrocytic parasite form effecting only a suppression of the clinical symptoms and have no effect on the liver stages of the parasite. Drug of this group include mefloquine and the 4-aminoquinolines. Gametocidal drugs also affect the sexual form of the parasite and prevent its transmission. Primaquine is known to be a very good gametocidal drug. The other group of drug is the sporotocidal agents that inhibit the development of oocysts on the mosquito stomach wall.

The success of chemotherapy in the treatment of malaria became limited with the development of drug resistant strain of the parasite. The first chloroquine-resistant *Plasmodium falciparum* was observed in the 1960's (Warhurst, 1987). Chloroquine resistance is now found throughout Africa and China. These issues have made the treatment of malaria a big problem especially in Africa.

Malaria has been estimated to cause more than a million deaths a year in Africa. It is the leading causes of under five mortality (20 %) and cause 10 % of the continents overall disease burden. It is understood to be both a disease of poverty and the cause of poverty (WHO, 2003). The total cost of a case of fever associated with malaria in Africa was estimated to be $ 9.84 in 1987 (Shepard et al., 1991).

The disease is a major cause of morbidity and mortality in Ghana, especially among infants and children under five years of age who account for 40 % of the cases (Asenso-Okyere and Dzator, 1997). Official reports indicate that some 9 % of deaths in Ghana are attributable to malaria which accounts for 30 % of out patient visits and 9 % of hospital admissions (Asenso-Okyere, 1994). When the losses to productive activities due to malaria morbidity are taken into consideration, the total average cost of an episode of the disease is US $ 8.67 in Ghana (Asenso-Okyere and Dzator, 1997).

One of the challenges facing Africa in the fight against malaria is drug resistance. The parasite has developed resistant strains against chloroquine the most common and cheapest anti-malarial drug on the market. Today the commonest form of treatment for malaria in African including Ghana, is the use of natural remedies from plants.

1.1.5. Anti-malarial plants

Quinine, isolated from *Cinchona* spp., is the oldest and in many areas it is still an important anti-malarial drug. In the majority of cases, quinine administration results in a prompt remission of symptoms and disappearance of *Plasmodium falciparum* and *Plasmodium vivax* from the blood. A "new" anti-malarial drug called Qinghaosu is derived from the sweet wormwood (Qinghao) plant in the genus *Artemisia* within the family

Compositae. It has been used in China for more than two thousand years to treat fevers associated with malaria. The drug has been shown to be effective in the treatment of the most deadly forms of *Plasmodium falciparum* malaria and has been effective against strains of *Plasmodium falciparum* that are resistant to chloroquine. There are a large number of other plant species reported with anti-malarial properties. A list of plant species with anti-malarial compounds recently have been reviewed by Srisilam and Veersham, (2003).

Species of plants used to treat malaria in Ghana has been documented by various authors (Ayitey-Smith, 1989, Abbiw, 1990, Mshana et al., 2001). Some of the common plant species used to treat malaria in Ghana includes *Azadirachta indica* A. Juss., *Crytolepis sanguinolenta* (Lindl.) Schltr., *Nauclea latifolia* Sm., *Ocimum viride* Willd., *Alstonia boonei* De Willd., *Morinda lucida* Benth., *Vernonia amygdalina* Delile., *Khaya senegalensis* A. Juss., *Cochlospermum tinctorium* A. Rich., *Cassia podocarpa* Guill. & Perr., *Jatropha curcas* L., *Momordica charantia* L., *Ceiba pentandra* (L.) Gaertn., *Aspilia latifolia* (Pers.) C. D. Adams and *Sterculia foetida* L. But the list of the species used to treat malaria in Ghana is still far from complete.

1.1.2. Aims of study

The aim of the current study was therefore to further investigate plant species used traditionally to treat malaria in Ghana. The objectives of the study were to;

(1) conduct an ethnobotanical study to collate information from an indigenous group of people in Ghana about their current traditional uses of plants for the treatment of malaria,

(2) conduct bioactivity studies on selected plants identified from the ethnobotanical studies in order to evaluate the efficacy of the selected species by testing their extracts against several different organisms including *Plasmodium*. It also involved bioassay-guided isolation of compounds and other generally bioactive compounds in the active extracts of the plants, and

(3) study variations within populations of the selected species from the study area using data from a wide range of characters (including morphology, anatomy, ecology, distribution, phytochemistry and AFLP markers) in order to understand the infraspecific taxonomy of the species and for their conservation of the species of plants

1.2.0. Literature Review

1.2.1. Ethnobotany

Ethnobotany may be defined as the study of the inter-relationships between people and plants in their environment. It includes an appraisal of the indigenous knowledge of plants on the culture, customs, beliefs and uses of plants by the local people. The science of ethnobotany relates and draws upon various academic disciplines such as ecology, botany, plant taxonomy, economics, anthropology, sociology, linguistics, economic botany, pharmacology and conservation biology and thus making ethnobotany a multi and cross-disciplinary subject (Martin, 1995, Maheshwari, 1988).

Ethnobotany involves basic documentation of the indigenous knowledge on plants, a study of local people understanding of their changing environment and applied projects which intends to incorporate local people in the conservation of their biodiversity and generate income through ecotourism and other activities (Martin, 1995, Cunningham, 2001).

The urgent need for ethnobotanical studies has come as a result of the realization that a large amount of information on plant resources, especially their uses, is contained in oral folk knowledge of indigenous people. Most of this knowledge are lost through jealousy, acculturation, changes in life patterns, ecosystem and habitat loss (Mshana et al., 2001). The uses of plants include food, fibres, medicine, dyes, construction, contraceptives, aphrodisiacs, etc. The most popular and crucial traditional uses of the plants are for their medicinal values

The need to document and study the plant species especially medicinal plants cannot be overemphasized following the adoption of the Convention on Biological Diversity (CBD).

Articles 7 and 10 of the convention are mainly devoted to issues concerning research and training, and sustainable uses of components of biological diversity

There have been some remarkable discoveries from investigations on indigenous medicinal uses of plants. These includes development of (1) tranquillizer respersine from *Rauvolfia serpentine* Benth., (2) anti-cancer agents vinblastine and vincristine from *Catharanthus roseus* (L.) G. Don, (3) quinine for the treatment of malaria from *Cinchona ledgeriana* Moens ex Tremen, (4) protein sweetener from *Thaumatococcus danielli* Benth.,(5) michellamine that potentially inhibits HIV-Virus from *Ancistrocladus* sp, (6) L-Dopa, used to treat Parkinson's disease from *Mucuna deeringiana* (bort) Merr., (7) Ephedra, a decongestant derived from *Ephedra sinica* Stapf., (8) digitalin and digoxin, heart drugs also derived from *Digitalis purpurea* Swallen, (9) picrotoxin used as a nervous system stimulant and cases of barbiturate poison is derived from *Anamirta cocculus* Wight & Arn, and (10) aspirin was also first extracted from the leaves and bark of *Salix alba* L and *Filipendula ulmaria* L (Maxim) (Martin, 1995; Sofowora, 1987).

With such major triumphs ethnobotanical studies now receive support from many organizations including National Cancer Institute (NCI) screening plants for anti-cancer, heart properties and anti- HIV. Other organisations such as ENRECA-DANIDA are investigating plants for their anti-malarial properties. Institutions such as Darwin Initiative (UK), United Nations Educational, Scientific and Cultural Organization (UNESCO) and Worldwide Fund for Nature (WWF) have also supported various projects dealing with indigenous uses of plants

Non-governmental organisations including World Conservation Monitoring Centre (WCMC) and Botanical Gardens International and many small pharmaceutical companies such as those in USA, Europe and Japan including Phyto-Rika in Ghana have also shown interests in medicinal plants. There are other agencies and organizations involved in the documentation of food plants and other indigenous uses of plants. These agencies and organizations include International Plant Genetic Resources Institute (IPGRI) and International Institute for Tropical Agriculture (IITA).

One of the earliest works on ethnobotany in West Africa was that of Dalziel (1937). He documented the uses of plant species for food, construction, medicines etc. Burkill (1985-1999) later expanded and revised the lists of plant uses for the Flora of West Tropical Africa. Other works on the documentation of traditional uses of West African plants includes those of Ayensu (1978), Sofowora (1987), Singha (1963), Oliver-Bever (1983) and Anonymous (1985). The pioneering works of Adjanohoun et al., (1979, 1980, 1983,1984,1989,1991 and 1993) are really admirable.

The documentation of traditional uses of plants species in Ghana has received remarkable contributions from the works of Irvine (1961), Abbiw (1990) and Dokosi (1998). Irvine's book documented the uses of woody species providing their vernacular names, synonyms, homonyms, and notes on description, habitats and phenology. The work of Dokosi (1998) was a continuation of that of Irvine (1961) and treated mainly the herbaceous species.

In his work, Abbiw (1990) provided a compendium of useful plant species found in Ghana and scored them directly under their usage categories such as food, medicine, fodder, construction, industrial crops etc. Literature on medicinal uses of plants in Ghana is very diverse and includes the works of Ampofo (1983), Enti, (1988), Ayitey-Smith, (1989),

PORSPI (1992), Parry (1997) and Mshana et al. (2001). These literatures contain some amount of information on plants used for malaria treatments in Ghana. But the list is not exhaustive, there are still more plants to be identified and investigated for the treatment of malaria in Ghana.

1.2.2. Bioactivity

The species that are reported used to treat malaria in the study area will vary in their potency. To further our understanding about their efficacy and their active compounds, a series of biological test will be undertaking on the extracts from the selected plants. Several bioactive compounds have been isolated from traditionally used species of plants (Wright and Phillipson, 1990; Deharo et al., 2001). In fact, plants sampled from ethnobotanical leads are known to show better activity when compared to species randomly sampled (Khafagi and Dewedar, 2000).

1. 2.3. Variation within populations

Variations may be defined as divergence from types in certain characteristics. All organisms vary to some extent and all variations fall within individuals genetic limits. The population used by the taxonomist may be defined as any group of individuals considered together at any one time because of features that they have in common (Davis and Heywood, 1963).

The variations within populations can be considered as (1) individuals variation, that is, variations among individuals in populations and (2) collective or group variations among populations (Davis and Heywood, 1963). There are three basic types of variation, (1) genetic or environmental variation (2) continuous or discontinuous variation and (3) the

form of variation including monomorphic, dimorphic, polymorphic, and heteromorphic variations

The basic taxonomic principles on variation patterns include; (1) all taxonomic entities, including varieties or subspecies and species as well as genera and higher categories are not simple units but complex systems of populations, (2) any population will be constant for some characteristics and highly variable for others, and (3) when a series of populations are compared they may often be separated into two or more groups on the basis of discontinuities in certain characters (Oteng-Yeboah, 1992).

The causes of variations within individuals of a natural population include external environmental modification, mutation and genetic recombination (Davis and Heywood, 1963; Lawrence, 1966).Phenotypic plasticity in response to environmental factors contribute to the differences among individuals in any populations. The environmentally caused variations such as in the quantitative features like stem height, girth size, leaf dimension and pollen size etc. tend to be continues and follows a normal distribution curve. Genetic variations are transmitted from one generation to another by gene segregation and recombination. It is the recognition of kinds of organisms which is the result of variations that are described by the taxonomist. There are however limitations such as self fertilization, asexual reproduction, stabilizing selection that are placed on variation in natural populations.

Species and lower taxa are formulated around natural populations. Davis and Heywood (1963) indicated that the inherent variability of species is accepted as a phenomenon

requiring study and appropriate treatment, not just as an inconvenience in that individuals deviate from a single pattern or type.

1.2.4.0. Sources of data for the variations study

Plant taxonomy is dependent on other sciences for its sources of data. The other sciences are equally dependent on plant taxonomy through its involvement in identification and nomenclature. Sources of data used in taxonomic studies include morphology, anatomy, cytology, genetics, ecology, palynology, physiology, phytochemistry, embryology, geology, geography, paleobotany and molecular data. The sources of data used for the variation studies included;

12.4.1. Structural studies (morphological and anatomical studies)

Traditionally, morphological and anatomical characters were used for taxonomic studies (Davis and Heywood, 1963; Stace, 2000). Thus the early classifications of the angiosperms such as Bentham and Hooker (1862-1883) and Hutchinson (1926-1934) were based on structural data.

In spite of the fact that most modern systems of classification are based on molecular studies, data from morphology and anatomy are still important. Besides, information on the morphology of plants is important for identification and to assist us understand evolutionary development of plants The development of scanning electron microscopy and transmission electron microscopy has reinforced the use of structural data for taxonomic studies Morphological studies have been used to understand the variations in many species including *Taraxacum* spp. (Taylor, 1987) and *Justicia betonica* S L (Balkwill et al , 1994).

1.2.4. 2. Ecological and distributional studies

Ecology is the study of the inter-relationships among organisms and their environment. The environment of an organism consists of both living (biotic) and non-living (abiotic) components. Ecology is one of the traditional subject areas that continue to provide information for systematic uses (Davis and Heywood, 1963, Stace, 2000). Ecological areas of systematic value include habitat, phenology, adaptations, climatic factors, species association, plant-animal interactions, pollination, germination and soil (Davis and Heywood, 1963).

The distribution of taxa is important in interpreting the origin, migration and evolution of species and floras. The local distribution of species is also important in the conservation of the species. The sources of data used for the variations study were;

1.2.4.3. Comparative phytochemical studies

Chemical taxonomy often known as chemotaxonomy, chemosystematics, or comparative phytochemistry is the use of chemical methods to obtain information about the occurrence of different groups of compounds within a species or in species from different families and genera. The rapid developments in comparative chemistry may be partly due to the development of new techniques such as HPLC, LC-MS, GC-MS and electrophoresis for rapid analysis of chemical compounds.

The group of compounds known as secondary metabolites are usually used as characters in chemosystematic studies. The most well known secondary metabolites used for

chemosystematic studies included phenolics especially flavonoids, as well as alkaloids and terpenoids

Classifications produced from both chemical and molecular characters have often been congruent (Grayer et al., 1999). Chemical data can be used at all levels of the taxonomic hierarchy to study the relationship among taxa. However, different types of compounds have to be used depending on the taxonomic level (Grayer et al., 1999). For example, flavonoids can be used to study the relationship among species and genera within a family. There are a number of other groups of compounds such as betalins, glucosinolates, ellagic acid and ellagitannins, polyacetylenes, anthraquinones, sequiterpenes lactones, various types of alkaloids, cyanogenic glycosides, limonoids and iridoids that can be taxonomically important at the family level (Dalgren et al., 1981; Grayer et al., 1999).

Chemical characters have also been used to understand variability in species. Flavonoids and essential oils have been the most important group of compounds in the assessment of infraspecific variability in plants. For example, chemical variability in flavonoids has been successfully studied in *Ocimum americanum* (Vieria et al., 2003). Leaf flavonoids have also been used for infraspecific taxonomic studies (Chaves et al., 1997; Grayer et al., 2000; Grayer et al., 2002). Essential oil has also been used to study infraspecific variations in a number of species including *Ocimum basilicum* (Grayer et al., 1996), *Cunila galioides* Benth. (Echeverrigaray et al., 2003), and *Ocimum gratissimum* L (Vieria et al., 2001).

1.2.4. 4. Molecular studies

Molecular methodologies have been used to investigate taxonomic problems at all levels; including evolutionary relationships and origin of island species (Richardson et al., 2003),

., 1998) and populations (Soltis and Soltis, 1998). DNA

sequence data have been used at the population level to investigate variations and genetic relationships in populations of a species for assessment of their taxonomic and evolutionary affinities, and for conservation programmes. Random amplified polymorphic DNAs (RAPDs) has been the most extensive DNA fingerprinting technique used to investigate problems at the population level (Adams et al., 1990; Demeke and Adams, 1994). However, the RAPD technique has quite a large number of limitations including non-reproducibility and difficulty for homology assessment.

Amplified fragment length polymorphism (AFLP) has been found to be a very useful technique in investigations at the population level (Sigh, 1999; Zhu etal., 1989). AFLP has been patented by Vos et al., (1995). AFLP has been used to study genetic diversity in *Azadirachata indica* (Sigh, 1999) and sunflower (Hongtrakul et al, 1997). Biodiversity in rice, hops and grapevine were also successfully studied using AFLP (Zhu et al., 1998). Species delimitation and origin of island representatives of *Phylica* has been studied using AFLP markers (Richardson et al., 2003). The advantages of the AFLP techniques include (1) no prior sequence information is needed, (2) produce a large number of polymorphic bands and (3) the technique is highly reproducible. On the other hand, the AFLP technique has the limitation of being expensive and require high quality DNA sample. However, the advantages of AFLP are enormous and exceed the limitations.

CHAPTER TWO

MATERIALS AND METHODS

2.1.0. Study area

2.1.1. Selection of study area

The Wechiau Community Hippopotamus Sanctuary was selected for the study. The selection of the area for the study was based on the preliminary studies by Prof. A. A. Oteng-Yeboah and the author. They found that the people of the sanctuary area have a very rich botanical heritage and the people depend on plants for the treatment of most of their ailments including malaria.

The sanctuary was also found ideal for the study because it is one of the first truly community-based conservation sites in Ghana where local people are taking full control of the uses and management of their own biological resources. The chiefs and people of the area have decided to protect one of the two remaining populations of *Hippopotamus amphibius* in Ghana and other biological resources in a 40 km stretch of land along the Black Volta River. They hope to develop the ecotourism potentials for the area. The other remaining population of *Hippopotamus amphibius* in Ghana is found in the Bui National Park which is also under threat as the government plans to develop a dam on the river for hydroelectrical power generation.

The people of the sanctuary area manage their biological resources with recommendations from scientists who use the area as their ecological laboratory. In order to provide more information to those in the sanctuary area this study was undertaken to investigate the uses of the plants in the sanctuary. The aim was to provide recommendations for the sustainable management of the plant resources in the sanctuary. There was also an urgent need to

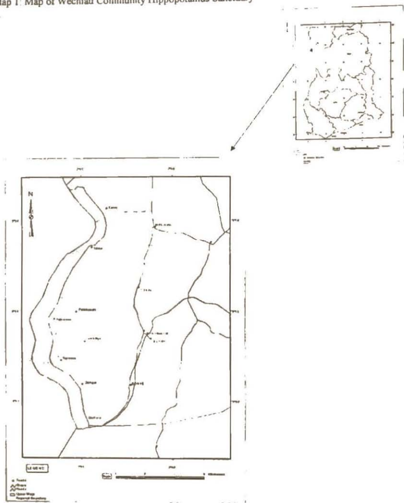
document the indigenous knowledge on plants in the area, which is likely to become adulterated through acculturation when the area is fully opened to tourist.

Articles, 6, 8 and 10 of the Convention on Biological Diversity (CBD) refer to the conservation and sustainable uses of Biological diversity. The CBD also highlights the need to support local populations in the conservation and sustainable uses of their biological resources to enable them earn the full benefit of it. It was with all these ideas in mind that the Wechiau Community Hippopotamus Sanctuary area was selected for study.

2.1.2. Geographical location of sanctuary area

The study area is at Wechiau about 40 km southwest of Wa in the Upper West Region of Ghana. The study area is positioned on latitude 09°49'762 N and longitude 02°40'965W (Map 1). The core of the sanctuary occupies an area of about 40 km^2 along the bank of the Black Volta River. The lodges and research centres in the sanctuary are situated in three communities namely Kantu in the northern part, Talewona in the middle portion and Tankara in the southern part of the sanctuary.

Map 1: Map of Wechiau Community Hippopotamus Sanctuary



2.1.3. Vegetation of the sanctuary area

The vegetation of the sanctuary area is primarily Guinea savanna. Oteng-Yeboah & Asase (2001) in their ecological baseline studies of the flora categorised the vegetation of the sanctuary area into 3 types namely, the riverine forest, flood plain and higher ground / Guinea savanna

The riverine forest vegetation is located on the bank of the Black Volta River. It forms a closed canopy with loose undergrowth with tree species such as *Celtis integrifolia* Lam., *Cola laurifolia* Mast., *Pterocarpus santalinoides* L' Her. Ex Dc., and *Kigelia africana* (Lam.) Benth. Occasionally, there are breaks in the canopy in areas with human interferences such as bushfires, farming and grazing by domesticated animals. Herbaceous species and climbers were not very common in the Riverine forest and species found included *Andropogon gayanus* Kunth., *Passiflora foetida* L and *Gloriosa superba* L.

The flood plain vegetation is located next to riverine forest and is usually flooded during the rainy season but dry and patchy during the dry season. The dominant plant species are *Mitragyna inermis* (Willd.) Oktze and *Vetiveria fulvibarbis* (Trin.) Stapf. Other common species of the area are *Acacia nilotica* (L) Willd. ex Delile, *Acacia gourmaensis* A. Chev. and *Terminalia macroptera* Guill. & Perr. The herbaceous plants include *Cassia mimosoides* L and *Crotalaria goorensis* Guill. & Perr.

The higher ground / Guinea savanna vegetation of the sanctuary area is the main vegetation of the sanctuary area and dominated by typical savanna plants such as *Afzelia africana* Sm, *Cochlospermum planchonii* Hook, *Cochlospermum tinctorium* A. Rich., *Parkia biglobosa* (Jacq.) Don, *Daniellia oliveria* (Rolfe) Hutch. & Dalziel and *Vitellaria paradoxa* G. F.

Gaertn. Other species included *Terminalia avicenoides* Guill. & Perr., *Ozoroa insignis* Delile, *Securindaca longepedunculata* Fresen, *Hymenocardia acida* Tul., *Tamarindus indica* L and *Parinari polyandra* (Benth) Dandy. The herbaceous species consisted of plants such as *Amorphophallus dracontiodes* (Engl.) N. E. Br., *Kaempferia aethiopica* Solms ex. Engl., *Andropogon* spp., *Hyperthelia* spp., and *Curculigo pilosa* Engl.

2.1.4. Climate of sanctuary area

The sanctuary area experiences one rainy season between June and October each year with a peak period in August. There is a long dry season from November to May. In the dry season, the Northeast Trade Winds (Harmattan) blowing from the Sahara Desert brings in the hot and dusty weather. The average annual rainfall and temperature ranges are 1034.1 mm / year and 11 °C respectively. The highest daily temperature ranges occur in March / April with the day's temperature reaching 42 °C and the night temperatures can drop to between 15 °C - to - 20 °C.

2.1.5. Hydrology, geology and soils of sanctuary area

The Black Volta River flows southwards from Burkina Faso and its main tributaries drain the entire sanctuary area. The river forms the international boundary between Ghana in the west and Burkina Faso in the east flowing downstream to Bui area, it joins with other bigger tributaries such as the Volta Rivers and eventually enters into the Volta Lake. There are a number of islands on the Black Volta River, which are well vegetated forming a microhabitat for wildlife and places for the hippos to bask

The terrain of the sanctuary area is generally flat with a few isolated rocky hills, these areas are very common in the Talewona area. The rocky hills consist of large boulders of

granite / quartz with few herbaceous species at the summits and provide habitats for most reptiles. The soils of the sanctuary area are generally gravely and sandy in hilly areas.

2.1.6. Biodiversity of the sanctuary area

Hippopotamus (*Hippopotamus amphibious*) is the keystone or prime species in the sanctuary area both from the point of conservation and ecotourism. However, a large number of plant species and other wildlife abound in the sanctuary area. In an ecological baseline survey conducted between 1999-2002, over 210 species of plants belonging to 51 families, about 200 species of birds, 16 species of bats belonging to five families, 26 species of rodents, 13 species of snakes, 6 species of amphibians were encountered (EWI, 2002). Species of fish, molluscs and arthropods have not yet been studied.

2.1.7. Local politics and population of sanctuary area

The chief of Wechiau (Wechiau Naa) is the Paramount chief of the traditional area and all other chiefs are divisional chiefs under his authority. There are approximately 22 villages and settlements in the sanctuary area. Thes include 2 Wale villages, 6 fishing camps, and 14 Brifo villages. The estimated population of the communities of the sanctuary area is about 8,700 people. The two Wale villages, namely, Wechiau and Tokali, are very important to the sanctuary because they both own the entire sanctuary land and make up about 50 % of the Sanctuary Management Board.

The Sanctuary Management Board is the decision making body of the sanctuary and implements many of the decisions as well.

2.1.8. Ethnic groups in sanctuary area

There are two main ethnic groups in the sanctuary area; Wale, and Birifor. Other ethnic groups such as Ewe, Dagaare and Hausa can be found in the sanctuary area. The Wale people are believed to be the landowners of the sanctuary. The Birifos migrated to the present land in the beginning of the 19th century across the Black Volta River from Burkina Faso in order to escape the rigid laws implemented by the French military. There are many Birifo people still living in Tempare and Gao in Burkina Faso today. They are traditionally great warriors as well as hunters and farmers. The Birifo people speak the Lobi language.

2.1.9. Religion in sanctuary area

Three main religious groups namely, Muslims, Christians and traditional believers are found in the sanctuary area. The Wale people are predominately Muslims with a few Christians and traditional believers. The Birifo people are mainly traditional believers, but have been recently influenced by Christianity and Islam.

2.1.10. Health facilities in sanctuary area

The nearest hospital to the sanctuary area is at Wa. The hospital has a surgeon, medical assistants and nurses, and is situated about 40 km from the sanctuary. Transportation to the hospital is limited by fewer vehicles and poor roads. There is also a medical clinic at Wechiau. This clinic is powered by solar energy and equipped with only basic facilities for first aids for snakebites, diarrhoea and fevers.

2.1.11. Occupation, agriculture and commerce in sanctuary area

The people living close to the sanctuary area are largely farmers, hunters, cattle herdsmen [illegible] ne famous Lobi sculpture and herbal practice is also

common. Other sources of livelihood very common among women are local beer (pito) brewing and pottery

Crops cultivated are mostly food crops such as Bambara beans, corn, millet, guinea corn, groundnuts and yams, and cash crops such as cotton and tobacco. Non-Timber or non-wood Forest Products (NTFPs) are principally shea nuts (*Vitellaria paradoxa*) and dawadawa (*Parkia biglobosa*), which are usually collected by the women and processed into oil, cream and spices, respectively, for the local markets. Domesticated animals include cattle, sheep and goats.

There are markets in the big villages including Wechiau, Tokali and Donye, but most of the other sanctuary villages have small markets where pito, akpeteshie (local gin), tobacco and fried bean cakes (kosae) are sold. A variety of items such as meat, herbal preparations, bread, candies, eggs, vegetables, groceries, clothing, fish are sold at these markets.

2.2.0. Methods

2.2.1. Ethnobotanical Studies

2. 2.1. 1. Ethnobotanical survey

The following three techniques were used to obtain information about the species of plants used in the treatment of malaria in the study area:

1. Field interviews; involved walking with local people in the areas where they normally collected their medicinal plants whiles interviewing them. After picking a plant, they consulted among themselves on the anti-malarial uses of the plant. The three local people involved in this study were those selected by the sanctuary management board as having the greatest knowledge about the traditional uses of plants in the area.
2. House- to - house interviews; fourteen local people were interviewed using a questionnaire. Locally trained guides served as interpreters during the conduct of the interviews. The questionnaire had prompts for the source of information, identity of the plant species and methods of preparation, prescription and administration (Appendix 1).
3. Interviews based on anti-malarial species collected by herbalist; four herbalists in the area were identified with the assistance of the sanctuary management board. It was arranged for them to collect plants they used in the treatment of malaria upon which they were interviewed. They were then interviewed about how they used the plants.

In addition to the above techniques, samples of the species identified as having anti-malarial uses were collected and further informal interviews were conducted in order to

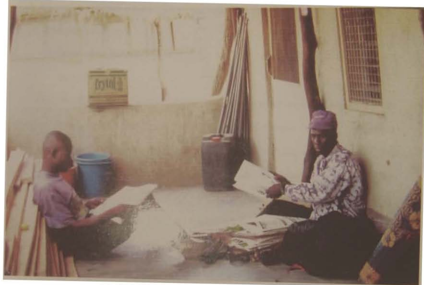
collect more information on other uses of these species in the sanctuary. Vouchers of the species collected during the study were deposited at the Ghana Herbarium (GC), Legon, Ghana (Plate 1). The identification of the species was authenticated by comparison with herbarium vouchers and with the Flora of West Tropical Africa (Hutchinson, and Dalziel, 1954-1972).

Twenty-two people 17 from the Brefo and 5 from Wale areas were interviewed over a period of six months. Information on the people interviewed on species used to treat malaria in the sanctuary is presented in Appendix 2. In most cases they were interviewed on more than one occasion. Twelve out of the 22 (54.5 %) people interviewed were males and all of them were older than 20 years. The people interviewed included Christians, Muslims and believers of local spiritual tradition and other non-religious people; only two of the people had some level of formal education.

2. 2. 1.2. Distributional ranges of anti- malarial species in sanctuary

To determine the distributional ranges of the anti-malarial plants identified through the ethnobotanical surveys, quadrats of sizes 25 m x 25 m, 5 m x 5 m and 1 m x 1 m were randomly taken in the study area. Forty-one quadrats of each size were studied. The first two sizes (25 m x 25 m and 5 m x 5 m) were used to assess the tree - shrub species and the 1 m x 1 m size was used to asses the herbaceous or ground cover species.

Plate 1: Pressing specimens of anti-malarial plants identified from the field with a local guide at the camp.



2. 2.1.3. Analyses of ethnobotanical data

The information obtained through the ethnobotanical interviews was analysed with regard to the following parameters:

(i) Taxonomic diversity, growth forms and the parts of the plant used to treat malaria.

(ii) The percentage of people who have knowledge about the use of a species in the treatment of malaria was evaluated by the formula; (number of people interviewed citing species / number of people interviewed) x 100.

(iii) Preference Ranking (PR) –The protocol of preference ranking is similar to that used by Martin (1995). In this case the plants were ranked according to their level of effectiveness in the treatment of malaria by the local people. Each rank is given an integer (1, 2 or 3) with the most effective plants assigned a value of 3. For example, if a plant was thought to be very effective in the treatment of malaria it was given a value 3.

The data from the ecological sampling were used to determine the distribution and density of the anti-malarial plants from the sanctuary area. The distribution of the plants was determined from the number of times a species occurred in the total number of quadrats examined. The density of a species was also evaluated from the mean number of individuals of the species per unit area.

2.2.1.4. Selection of species for further studies

The plant species used by the people living in the sanctuary will vary in their efficacy in the treatment of malaria. In order to investigate the efficacy of plants used to treat malaria

in the sanctuary and their variabilities, four species of plant were selected. The following parameters were considered in the selection of plant species for further investigation of efficacy;

(1) The percentage of people who have knowledge on a species being used to treat malaria in the sanctuary; plants which a large number of the people have knowledge about their use to treat malaria will continually be used by the people for their treatment of malaria,

(2) Preference ranking of species; plants which were indicated by the people as very effective for the treatment of malaria in the sanctuary will serve as leads in the search of active compounds against *Plasmodium*,

(3) Distributional ranges of species in the sanctuary; plants which are abundant and widely distributed in the sanctuary will most often be collected for their treatment of malaria in the sanctuary, and

(4) Species used alone to treat malaria; the anti-malarial properties of plants which are used in combination with other species might be due to synergism. The selection of species used alone to treat malaria was also to avoid the screening of the so-called 'blind species' which might often be added to herbal preparations to hide the identity of the actual plant responsible for the active of the preparation.

None of the above parameters was given prominence in the selection of species for further studies. Four species of plants, namely *Cassia sieberiana* DC, *Haematostaphis barteri* Hook f., *Mitragyna inermis* (Willd.) O. Kuntze and *Pseudocedrela kotschyi* (Schwein.f) Harms. which are used by the people to treat malaria were selected.

2.2.2.0. Bioactivity Studies

2.2.2.1 Initial preparation of plant samples for bioactivity studies

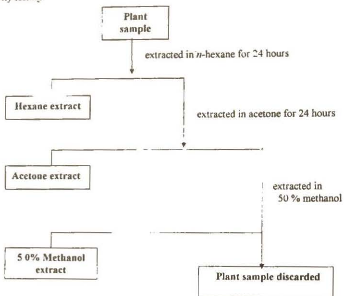
Plant samples collected from the field were immediately air-dried after collection from the field. The woody plant samples of roots and stem barks were freeze dried before milling. The dried leave samples were ground using a benchtop mill (Pulverisette 14 Rotor Speed Mill, Fritsch). The root and stem bark samples were ground using pestle and mortar and manual cutting mill (Glen Creston SM 2000).

2.2.2.2. Sequential extraction of plant samples for bioactivity studies

The powdered plant samples were sequentially extracted in *n*-hexane, acetone and 50% methanol (Figure 3). The aim of the sequential extraction was to selectively extract compounds that vary in their polarity. This can simplify the chromatographic process. The samples were first extracted in *n*-hexane for 24 hours. The filtrates were evaporated to dryness *in vacuo* using a rotary evaporator (Buchi, RE 111 Rotavapor; Plate 2), concentrated and their weights determined. The residual plant material was also rotary evaporated and extracted in a similar manner in acetone for 24 hours. Both the filtrates and the residue plant material were rotary evaporated (Buchi, R 111 Rotavapor). The filtrates were dried concentrated and their weights determined. The residual plant material were further extracted in 50 % aqueous methanol for 48 hours, rotary evaporated (Buchi, R 114 Rotavapor) to dryness and their weights determined. The remaining residue after the 50% aqueous methanol extraction was discarded.

Plate 2 Drying filtrates *in vacuo* using a rotary evaporator **(Buchi RE 111 Rotavapor)**

Figure 3: Flow chart showing the sequential extraction of plant samples used for bioactivity testing



2.2.2.3. *In vitro* antiplasmodial bioassay

Extracts from the sequential extraction of the plant samples were used for the anti-malarial testing. About 1mg of extracts was submitted to the Danish University of Pharmaceutical Sciences for *in vitro* antiplasmodial testing. The *in vitro* antiplasmodial assay was performed with modification according to the microdilution technique of Desjardins et al. (1979). The sensitivity of the chloroquine - sensitive strain of *Plasmodium falciparum* (3D7) to the extracts from the four species was tested. The samples were dissolved in dimethysulphoxoide (DMSO) with a final maximum concentration of 0.5 %.

However, all of the samples did not dissolve fully in DMSO. Each extract was tested in duplicates (n = 2). The detail of the technique for the *in vitro* antiplasmodial assay is as described in Ziegler et al. (2002). The inhibitory concentrations at 50 percent (IC_{50}) were evaluated using regression equations of plots of parasite counts against logarithms of extract concentration. Microsoft Excel 2003 was used to calculate the IC_{50} values.

2.2.2.4. Anti-microbial bioassay

The antimicrobial bioassays were based on a TLC - autobiographic procedure (Homans and Fucha, 1970; Rahalison, et al., 1991) using species of fungi and bacteria.

Fungal species and strain; *Cladosporium herbarium* IMI 300461 ex. Wall contaminant obtained from CABI Bioscience (Egham, Surrey, UK). Fungi was kept on malt extract (2%)-agar (1.5%) medium at 4 °C.

Bacterial species and strain; Gram negative bacteria. *Bacillus subtilis* IMI 347329 ex *Solanum tuberosum*. Gram negative bacteria; *Pseudomonas syringae* pv. *Spyringae* IMI 34748 (ACTCC19310) ex. *Spyringae vulgarise.*

Media; MEPG; Malt extract (Oxiod, L39) 2%, Peptone (Oxoid, L39) 2% Glocuse 1% (product No. L39) obtained from OXOID (Basingstoke, Hampshire, England) was used in culturing the fungal species.

Positive controls; Nystatin (mycostatin, Sigma St. Louis, USA product No. N-3503) was used as a positive control for the anti-fungal bioassay. Chloramphenicol (Sigma St. Louis, USA product No. C0378) was used as a positive control for the anti-bacterial bioassays.

Preparation of samples for testing

The extracts from sequential extraction of the plant samples were re-dissolved in the appropriate solvents to make a concentration of 1mg of extract per 100 µl of solvent. Approximately 10 µl of the extracts were applied in strips (1m length) onto the TLC plates (1.05554 Aluminium sheets, 20 x 20 cm, Silica gel $60F_{254}$ MERCK). The TLC plates were developed using the following solvent systems; hexane extract in 100 % chloroform, acetone extract in chloroform: acetone 9:1 and 50 % methanol extract in chloroform: methanol: water 64: 36: 4. After the TLC plates had been developed, they were left in the fume cupboard to dry the solvents. The TLC plates were then observed under UV light (250nm and 300nm).

Nystatin (1mg per 100 µl for anti-fungal bioassay only) and chloramphenicol (for anti-bacterial bioassay only) was then applied onto the TLC plates at three different concentrations of 1µl, 5 µl and 10 µl above the solvent front. The TLC plates were then affixed to polystrene dishes with the aid of sellotape.

Testing for anti-fungal activity

A suspension of the cultured fungi (*Cladosporium herbarium* IMI3000461 ex. Wall contaminant) was made in MEPG. An inoculum of the fungus was then sprayed onto the prepared TLC plates in a fume cupboard. The TLC plates covered with polystrene were incubated at 25-30 ^{0}C for three day.

Detection of anti-fungal activity

The incubated TLC plates were visually examined after three days. Inhibition of fungal growth was observed as colourless spots against a general grey background as evident from the positive control spots.

Testing for anti-bacterial activity

A sample (5.6 g) of nutrient agar was dissolved in 200 ml of distilled water. The agar solution was heated whiles stirring occasionally until it was colourless and was allowed to cool to about 60 ^{0}C. A smear of the cultured bacteria was suspended in about 1ml of distilled water and sonicated. A small colony of the bacterial was taken into a 100 ml beaker and the agar solution added to make up 50 ml. The resulting solution was poured onto the TLC plates, spread evenly by shaking and excess solution was poured off. This was done very quickly before the agar solidified. The TLC plates were covered with polystrene and incubated at 37 ^{0}C overnight.

Detection of anti-bacterial activity

The stain ρ-Iodonitrotrtrazolium violet was made up by dissolving 1mg ρ-Iodonitrotrtrazolium in 1 ml of ethanol. A 1000 ppm solution of this was added to 19 ml of distilled water and poured onto and spread evenly on 2 TLC plates (20 cm x 10 cm in

Time (minutes)	Flow (μl)	% A	% D
Initial	4.50	25.0	75.0
20 00	4.50	100.0	0.0
60.0	4.50	100.0	0.0
61.00	4.50	25.0	75.0

2.2.2.6.2. Isolation and identification of stilbene compounds from *Haematostaphis barteri* Hook. f.

Initial HPLC analyses of 80 % aqueous methanol root bark and acetone of stem extracts of *Haematostaphis barteri* showed the presence of stilbenoid compounds, based on their characteristic UV spectrum (UV absorbance at approximately 300 nm). The acetone extract of stem bark was redissolved in 90 % methanol for the HPLC analysis. The HPLC system consisted of Waters LC 600 pump, 996 photodiode array detector and autosampler. Merck Lichrospher 100RP-18 (5μm) columns were used; 4.0 mm (i.d.) x 250 mm columns were used; 4.0 mm (i.d) x 250 for analytical work and 10.0 mm (i.d.) x 250 mm for semi-preparative isolations. A gradient profile of solvent system consisting of two solvents denoted with A (2% HOAc) and B (MeOH-HOAc-H_2O; 18:1:1) was used.

The solvent systems for semi-preparative isolations were denoted with A (H_2O) and B (MeOH). The autosampler made 40 μl injections. Initial conditions were 75 % A, 25 % B, with a linear gradient reaching B = 100 % at t = 20 minutes. This was followed by isocratic elution (B =100 %) at t = 24 minutes, after which the programme returned to the initial solvent composition. Column temperature was maintained at 30 °C and flow rates of 1.0 ml $mins^{-1}$ and 4 5 ml min^{-1} were used for analytical and semi-preparative HPLC respectively.

A delay of 13 minutes between injections was used in order to enable the system to equilibrate.

Plate 3: HPLC-PAD system used for the analysis and isolation of compounds

(1) Controller (2) Photodiode array detector (3) Pump (4) Autosampler (5) solvents
(6) Computer (7) Carrier gas

2.2.3.0. Variability Studies

2.2.3.1. Sampling procedure

The study area was divided into three populations for sampling purposes. These populations were namely: Kantu area, Talewona area and Tankara area. These populations were situated both near the extreme ends as well as in the middle portion of the sanctuary and therefore represent a large proportion of the environmental variations within the sanctuary. The choice of the sites was also associated with the availability of research and lodging facilities in the three areas for fieldwork.

2.2.3.2. Morphological studies

Morphometric studies were carried out on 150 specimens of each of the four species. These specimens were selected from 15 individuals from the three populations in the sanctuary. The height of individuals of plants was taken with Suunto Clinometer. The girth at breast height (1.3 m above the ground level) of the individuals of the plant species was also taken with a tape measure. Measurements were taken with a transparent ruler and eye-piece graticule.

One-way analysis of variance (ANOVA) and Multiple range tests analyses were carried using Stagraphics package (Stagraphics Plus 3.0) and multivariate analysis namely Pincipal component analysis (PCA) was executed using PC-ORD version 3.04 (McCune and Mefford, 1997).

2.2.3.3. Maceration preparation

A total of 27 specimens were examined from 9 individuals of the populations in the sanctuary. Selected stem and root barks of individuals were cut into longitudinal pieces (about the size of a matchstick) and placed into a beaker. A solution of 50:50 by volume of hydrogen peroxide and acetic acid was then added (just enough to cover the pieces of bark) and the preparation was kept overnight under a mild heat. The beaker, with its, content was heated the next day on a water bath until the sticks appeared whitish / pale in colour. The sticks were removed and gently washed in a sieve with water.

The macerated pieces were collected, stained in dilute glycerine and examined under the light microscope. Measurements of the fibres under the Leica Galen III microscope were taken with the ocular micrometer in the same way as for the measurements of some floral parts. ANOVA and Multiple range test analysis of anatomical data were analysed with the same procedures used for the morphological studies.

2.2.3.4.0. Ecological and distributional studies

2.2.3.4.1. Species habitat in sanctuary

A study was made of the general habitat conditions in which the individuals of the anti-malarial species were found in the sanctuary. The habitat of the species were studied using transects. The transects were footpaths which cross each other at various irregular intervals in the sanctuary. The footpaths varied in lengths and were used by the local people when collecting plants to treat malaria. The anti-malarial species were searched for within a radius of 20 m radius from transects. When an anti-malarial plant was identified, data was collected on the topography, canopy cover, nature of anthropogenic disturbance, elevation

and geographical position. The geographical position and elevation of the individual anti-malarial species were recorded with Global Positioning System (GPS) Magellan 3.5.

2.2.3.4 .2. Phenological studies

Five individuals of the four anti-malarial species were selected and tagged in each population for phenological studies. Only matured individuals at about 1 km apart were selected from each population for observations. The selection of individuals for observations was also based on convenience for a local trained person receiving no wages to visit the plants in between the author's field visits. The individual species were observed and notes taken on leaf (flushing and shedding), flower (flushing) and fruit (formation and shedding) activities during visits. A general observation of all individuals in each locality was also undertaken during visits. The numbers of individuals of the anti-malarial species counted in a particular phenophase in a month was used to determine the peak period of that activity in a year.

2.2.3.4.3. Species association studies

In order to provide basis for association of anti-malarial species with other plants within the sanctuary flora quadrats studies was undertaken. Quadrats of size 5 m x 5 m were demarcated around at least four randomly selected individuals of each anti-malarial species per population. All species found rooted in a quadrat were listed and identified. Specimens of species that could not be identified in the field were collected and later determined at the Ghana Herbarium (GC) of the Department of Botany at Legon. The study was carried out during the rainy season (June/ July) when the vegetation was lush and not burnt.

Estimate of inter-population similarity (IPS) of species associated with anti-malarial species in the sanctuary was obtained using the formula;

$$IPS = \frac{2S_{xy}}{(Sn_x + Sn_y)},$$

Where; $2S_{xy}$ is number of shared species between populations x and y, and $Sn_x + Sn_y$ is number of species in populations x and y, respectively.

An estimate of the inter-population diversity (IPD) of species associated with the four anti-malarial plants was then evaluated as IPD = 1- IPS.

2.2.3.4. 4. Soil particle size analysis

Soil samples were collected from the top soil (0-10cm depth) from five randomly selected spots in each of the three vegetation types of the sanctuary area namely, Riverine forest, Flood plain and higher ground flora / Guinea Savanna (Oteng-Yeboah & Asase, 2001). In each of the three vegetation types of the three locality areas the soil samples collected were bulked together The soil samples were dried and then used for the particle size analysis. The protocol of soil particle size analysis is after Bouyocous (1967).

2.2.3.4.6. Distribution of species in Ghana

The distribution of the anti-malarial species in Ghana was studied by examining herbarium specimens. Herbarium specimen data of collections of the four anti-malarial species in the Ghana Herbarium (GC) were pooled and computerised. The BG-Base Software (Walter et [illegible] ne herbarium specimen data. The lists of specimen

examined and computerised are presented in Appendix 3. The gazetteer of plant collecting localities in Ghana by Hall (1980) was used to supplement specimen label data where coordinates of collections were not available. ESRI ArcView GIS and ArcIMS packages were used to generate the distribution maps of the anti-malarial plants in Ghana.

2.2.3.5. Comparative phytochemical studies

For phytochemical studies, leaves, stem and root barks of three individuals of the selected species were collected from the same three populations in the sanctuary. The same plant parts of a species from the same population were bulked together and used for analysis. The collection of plant parts from a large number of individuals from each population in large quantities for analysis was found not suitable due to problems with handling, storage and immigration. This procedure was also adopted because it was the usual way the local people collected the species to treat malaria.

2.2.3.5 .1. Extraction and preparation of plant samples for analysis of flavonoids and other phenolics

Pulverised plant samples were weighed and extracted in 80 % methanol for 24 hours. The filtrates were taken to dryness using the rotary evaporator (Buchi R114 Rotavapor), concentrated and their weights determined. The dried extracts were redissolved in 80 % methanol (HPLC grade), filtered through nylon membranes (Nylon Acrodisc 13 0.45 µm Gelman) into HPLC vials.

The HPLC system consisted of Waters LC 600 pump, 996 photodiode array detector and autosampler. Merck Lichrospher 100RP-18 (5 µm) columns were used; 4.0 mm (i.d.) x 250

mm (Plate xx). A gradient profile of solvent system consisting of two solvents denoted with A (2 % HOAc) and B (MeOH-HOAc-H_2O; 18:1:1) was used. Injections of 40 µl were made by the autosampler. The flavonoid and phenolic components in the plant samples were determined by their characteristic UV spectra and retention times. Liquid Chromatography – Mass spectrometry (LC-MS) in positive mode was used to identify the main flavonoid compounds in the four species.

The identification of flavonoids was similar to that of Grayer et al. (2000). The LC-MS identification of the flavonoid compounds was done with Dr Renée J. Grayer (RBG, Kew).

2.2.3.5. 2. Extraction and preparation of plant samples for alkaloid analysis

Powdered plant samples of known weights were extracted in 100 % aqueous methanol for a minimum of 24 hours. The filtrates were dried in a fume chamber and their weights were determined. The dried extracts were redissolved in 1ml of methanol, suspended in 50 ml of 0.5 M HCl in a separating funnel and then partitioned with an equal volume of chloroform ($CHCl_3$) three times to give acidic chloroform layer (A). The aqueous layers were basified with ammonia (NH_3) to a pH of 10 and then partitioned with 50 ml $CHCl_3$ three times to give a basic chloroform layer (B). The remaining aqueous phases were concentrated and stored. The acidic and basic chloroform layers were evaporated to dryness and redissolved in acetone.

The acetone dissolved chloroform layers A and B were applied onto TLC plates (1.05553, 20 x 20 cm, Silica gel 60, Merck) and developed using the solvent system chloroform - acetone (9:1). Galanthamine was used as a positive standard. The developed TLC plates were kept in the fume chamber to completely dry off solvent and the plates were then

quently sprayed with Dragendorff's reagent.

One sample of each of the acidic chloroform layer (A) and basic chloroform layer (B) extract per plant part for each species was used for LC-MS analysis The LC-MS system consisted of a Waters Model LC 600 pump (Water, Watford), UK) coupled with a model 996 photodiode array detector. A Phenomenex Polar RP (5μm) 150 mm x 4.6 mm column was used. A linear gradient of three solvents was used; water (solvent A), methanol (solvent B) and 5 % acetic acid in methanol (solvent C).

Initial conditions were 80 % A and 20 % C (t = 0 min), changing to 80 % B and 20 % C at t = 20 min. This was followed by isocratic elution at t = 25 min with 80 % B and 20 % C, changing to 80 % A and 20% B at t = 27 min for 10 min after which the programme returned to the initial solvent composition Mass- spectra were recorded using quadropole ion trap MS (Finnigin LCQ) fitted with an Atmospheric Chemical Ionisation (APCI) using vaporizer temperature, 550 °C, sheath and auxiliary nitrogen gas flows pressures of 80 and 10 psi, respectively, a capillary temperature of 150 °C and a needle voltage (automatically adjusted by instrument), ca. ± 4kV. The mass spectrometer was controlled by Xcalibur 1.1 Software (Finnigin).

The identification of the alkaloid compounds was by comparing the molecular masses of the compounds with an online alkaloid library and standards. Identification of the alkaloid compounds was done with Dr. G. Kite (RBG, Kew).

2.2.3.5.3. Extraction and preparation of plant samples for HPLC-DAD analysis to determine quantitative variations in flavonoids and other phenolics

Ground plant samples of 500 mg weight were extracted in acetone for 24 hours. The [illegible] ermined. Prior to HPLC analysis, the filtrates were

redissolved in 100µl 90 % methanol, filtered through nylon membrane (Nylon Acrodisc 13 0.45 µm Gelman) into HPLC vials. Each plant sample was prepared in triplicate. The HPLC system and conditions were the same as previously used in the analysis of flavonoid and phenolic components in the samples.

The result was analysed by using Millennium[32] software which automatically scored the peak areas of the different compounds. However, Millennium[32], did not automatically score the peak areas of all the different compounds in the different samples of a species. Since no qualitative variability was observed in the previous study, the results were manually edited to include only compounds with peaks areas in all the different samples of a species. Multivariate analyses of the peak areas were done with Mintab.

2.2.3.6.0. Molecular studies (Amplified Fragment Length Polymorphism markers)

2.2.3.6.1. Extraction of total genomic DNA

Leaf samples were collected from nine individuals of each species. The leaf samples were collected from 3 individuals of each of the three populations in the sanctuary and stored in silica-gel. DNA was extracted from 0.13-0.32 g silica-gel dried individual leaf samples. Total DNA extracts were made using a 2X CTAB protocol modified from Doyle and Doyle (1987). Most of the samples were extracted using 2X CTAB methods in which DNA was purified using QIAquick columns following the manufacturer's protocols (QIAGEN, Crawley, West Sussex, UK).

DNA from one sample of each species was purified on caesium chloride / ethidium bromide gradients and these are stored at Kew in the DNA Bank. The DNA samples stored included samples of *Cassia sieberiana*, *Haematostaphis barteri*, *Mitragyna inermis* and

Pseudocedrela kotschyi with reference numbers 17539, 17541, 1742 and 17540, respectively. DNA levels were checked by using electrophoretic agarose gel analysis and photographs were taken under UV light for later reference.

2.2.3.6.2. Quantification of DNA

DNA was quantified using Helena Biosciences Eppendorf Biophotometer following the protocol of the manufacturer (Applied Biosystems). Data recorded on the concentration, absorbance at wavelength 320 nm, and wavelength ratios 260 nm / 280 nm and 260 nm / 230 nm. The concentration of the DNA in a sample was measured in nanograms per microlitre of the sample. Absorbance at 320 nm was the background reading and indicates a dirty curvette or significant matter in the sample when the value is high. The 260 nm / 280 nm ratio measures the purity of the DNA sample. Pure DNA samples should have values between 1.8 and 1.9 and a lower ratio indicated protein contamination (proteins absorb at 280 nm). The 260 nm / 230 nm also measure the purity of the DNA. Proteins and reagent contaminates absorbs at 230 nm and pure DNA samples have 260 nm / 230 nm ratio greater than 2.

2.2.3.6.3. Amplified fragment length polymorphisms (AFLPs) analysis

The protocols supplied by Applied Biosystems (Warrington, Cheshire, UK) and after Vos *et al.*, (1995) were used for the amplified fragment length polymorphisms. DNA was restricted with endonucleases *MseI* and *EcoRI*, and fragments were ligated to double stranded adaptors. DNA amplification process consisted of both preselective amplification using primers with one base pair (bp) and selective amplification using dye labelled oligonucleotides primers with three base pair extension. The selective primers pairs were

CAT / ACT and CAA / AGC for *Cassia sieberiana*, and CAT / ACT, for *Pseudocedrela kotschyi*. These primer pairs were selected after an initial study of a range of primer pairs on three individuals from two populations of the sanctuary. The two populations selected were those separated with the largest geographically distance (also largest genetic distance) between them. The chosen primer pairs shared some fragments between the two individuals from the same population as well as from the other population to allow for the recognition of the species, and at the same time gave sufficient variation for distinction of closely related individuals

AFLPs were separated and visualised using an ABI 377 automatic sequencer following the protocols of the manufacture (Applied Biosystems; Plate 4).The fragments were sized by running dye-labelled standards in each lane. The AFLPs profiles were edited using Genscan version 2.02 and Genotyper version 1.1 (Applied Biosystems). Genscan automatically scored fragments ranging between 50-500 bp in length but fragments below 50 bp are not automatically scored. Fragments were therefore manually edited because some were just below the threshold permitted by the software in some individuals. The fragments were then tabulated as a binary matrix.

2.2.3.6. 4. Analysis of AFLP markers

Fragments were analysed using PAUP version 4.0d64 for Macintosh (Swofford 1998). Algorithm was distance neighbour joining/ UPGMA and trees were produced using Jaccard's coefficient of similarity. The binary matrix was converted into a similarity matrix using MacClade (Maddison and Maddison 1992). Fragments unique to a particular group of individuals in the species were identified by visual inspection using MacClade. Principal coordinate analysis was then executed on the data using Le Progiciel R and Excel (Version

Plate 4 PCR system a... used for AFLPs

(a) C

(b) ABI 377 automatic sequencer

CHAPTER THREE

RESULTS

3.1.0. Ethnobotanical studies

3.1.1. Anti-malarial plants

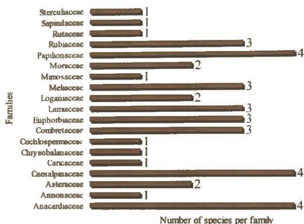
A total of 41 species from 36 genera in 19 plant families were identified during the ethnobotanical surveys as being used to treat malaria The species, their families, survey method used to identify their uses, percentage of people interviewed with knowledge about their use to treat malaria and the preference ranking of the species are presented in Table 1.

The greatest number of species used to treat malaria in the sanctuary were identified during the house-to-house and field interviews which identified 29 (70.7%) and 14 (36.6%) of the species, respectively. Six (85.7%) of the seven species collected by persons being interviewed were also encountered during the house-to-house interviews. *Mitragyna inermis* (Willd) O. Ktze. was the only species identify by all the three methods of survey. The other species frequently identified included *Indigofera pulchra* Linn. *Azadirachta indica*, and *Mangifera indica* Linn. However, the species reported most effective for the treatment of malaria in the sanctuary included the most frequently cited, *Mitragyna inermis*, as well as *Cassia sieberiana* DC., *Cochlospermum tinctorium* A. Rich., *Haematostaphis barteri* Hook. f., *Indigofera pulchra*, *Pseudocedrela kostchyi* (Schweinf.) Harms., *Strychnos innocua* Del., and *Vernonia amygdalina* Del.

Of the 19 families containing anti-malarial species the most predominant in terms of number of species were Anacardiaceae (4), Caesalpiniaceae (4) and Papilionaceae (4)

followed by Combretaceae (3), Euphorbiaceae (3), Lamiaceae (3) and Rubiaceae (3) (Figure 4).

Figure 4: Number of species of families with plants used for the treatment of malaria in Wechiau Community Hippopotamus Sanctuary Ghana



le 1: Methods of identification and percentage of people with knowledge about their anti-malarial use (PPK) and Preference Ranking (PR) of ies used to treat malaria in the Wechiau Community Hippopotamus Sanctuary Ghana.

ies (Voucher numbers)	Family	Method of interviews			PPK	PR
		Field	House-to-house	Plants collected by people		
thospermum hispidum Schrank (GC 47761)	Asteraceae		√		9.8	1
gle paniculata (S..m & Thonn.) Engl.(GC 47780)	Rutaceae				4.8	1
Afzelia africana Sm. (GC 47762)	Caesalpinaceae		√		9.8	1
Anogeissus leiocarpus (Dc.) Guill & Perr. (GC 47763)	Combretaceae	√	√		4.8	2
Azadirachta indica A. Juss. (GC 47764)	Meliaceae		√		29.3	2
Carica papaya Linn. (GC 47765)	Caricaceae		√		9.8	2
Cassia sieberiana DC. (GC 47799, AA)	Caesalpinaceae	√			9.8	3
Cochlospermum tinctorium A. Rich (GC 47766)	Cochlospermaceae			√	9.8	3
Combretum ghasalense Engl. & Diel (GC47767)	Combretaceae		√	√	9.8	2
Combretum sp. Loef. (GC 47768)	Combretaceae				4.8	1

'able 1 (continued)

Species (Voucher numbers)	Family	Method of interviews			PPK	PR
		Field	House-to-house	Plants collected by people		
Ficus graphalocarpa (Miq) Steud ex A. Rich.(GC 47769)	Moraceae	√			14.6	1
Ficus platyphylla Del. (GC4770)	Moraceae		√		4.8	1
Gardenia ternifolia Schum & Thonn. (GC 47771)	Rubiaceae	√	√		9.8	2
Haematostaphis barteri Hook. f. (GC 47772)	Anacardiaceae	√			9.8	3
Hyptis spicigera Lam. (GC 47773)	Lamiaceae		√		4.8	1
Indigofera pulchra Willd. (GC 47774)	Papilionaceae		√	√	9.8	3
Jatropha curcas Linn. (GC 47775)	Euphorbiaceae		√		9.8	1
Jatropha gossypiifolia Linn. (GC 47776)	Euphorbiaceae		√		9.8	1
Khaya senegalensis (Desv.) A.Juss. (GC 47777)	Meliaceae	√	√		4.8	2
Lannea acida A. Rich. (GC 47778)	Anacardiaceae		√		9.8	1
Leucas martinicensis (Jacq.) Ait. f. (GC 47779)	Lamiaceae		√		9.8	1

le 1 (continued)

Species (Voucher numbers)	Family	Method of interviews			PPK	PR
		Field	House-to-house	Plants collected by people		
Mangifera indica Linn. (GC 47780)	Anacardiacae		√		24.4	1
Mitragyna inermis (Willd). O. Ktze. (GC 47799, AA	Rubiaceae	√	√	√	24.4	3
Monanthotaxis sp. (Bail) Verdc. (GC 47781)	Annonaceae	√			19.5	2
Nauclea latifolia Sm. (GC 47782)	Rubiaceae	√	√		4.8	2
Ocimum canum Linn. (GC 47800)	Lamiaceae		√		4.8	1
Ozoroa insignis Del. (GC 47783)	Anacardiaceae	√			4.8	2
Parinari polyandra Benth. (GC 47784)	Chrysobalanaceae	√			4.8	2
Parkia biglobosa (Jacq.) R Br. ex Don (GC 47785)	Mimosaceae		√		4.8	2
Paullinia pinnata Linn. (GC 47786)	Sapindaceae			√	4.8	1
Pericopsis laxiflora (Benth). Van Meeuwen.(GC 47787)	Papilionaceae		√		9.8	1
Pseudocedrela kotschyi (Shweinf.) Harms. (GC 47798)	Meliaceae	√			14.6	3

le I (continued)

Species (Voucher numbers)	Family	Method of interviews			PPK	PR
		Field	House-to-house	Plants collected by people		
Pterocarpus erinaceus Poir (GC 47789)	Papilionaceae		√		4.8	1
Ricinus communis Linn. (GC 47790)	Euphorbiaceae		√		4.8	1
Senna occidentalis Linn. (GC 47791)	Caesalpinaceae		√		4.8	2
Sterculia setigera Del. (GC 47792)	Sterculiaceae		√		4.8	1
Strychnos innocua Del. (GC 47793)	Loganiaceae		√	√	4.8	3
Strychnos spinosa Lam. (GC 47794)	Loganiaceae		√		4.8	2
Tamarindus indica Linn. (GC 47795)	Mimosaceae	√			4.8	2
Vernonia amygdalina Del. (GC 47796)	Asteraceae		√	√	14.6	3
Xeroderris stuhlmannii (Taub.) Mendonca. (GC 47797)	Papilioanaceae	√	√		4.8	1

A list of the local names, habits and how the plants are used in the treatment of malaria in the area are presented in Table 2. Trees constituted 62 % of the total plant species used by the communities in the treatment of malaria in the study area and only two (5 %) species were climbers. A total of 45 herbal preparations were recorded as some preparations included the use of more than one species. For example, *Azadirachta indica* and *Ocimum canum* were often used in more than one of the preparations. Of the 45 herbal preparations reported for the species used to treat malaria in the sanctuary, the leaves (including twigs), roots, stem barks and whole plants accounted for 76.6 %, 9.4 %, 7.8 % and 6.3 %, respectively. Most of the preparations involved boiling the leaves and then drinking the infusion.

2: The growth forms and local names of species of plants that are used to treat malaria in the Wechiau Community Hippopotamus uary along with information on how the plants are used.

Species	Growth form	Lobi name	Wale name	How used
Acanthospermum hispidum	Herb	Bongore		Grind whole plant with hot pepper, sieve and drink as required.
Afraegle paniculata	Tree			Boil roots and drink as required.
Afzelia africana	Tree			Boil leaves and drink as required.
Anogeissus leiocarpus	Tree	Sisinrah	Surah	Boil leaves and twigs, and massage body with decoction for 3 days.
Azadirachta indica	Tree	Akagyatia		(1) Pound leaves, sieve and use for enema. (2) Boil leaves with leaves of *Jatropha gossypifolia* and *Combretum* sp., drink and use for steam baths.
Carica papaya	Tree	Kwalentia		Boil leaves with leaves of *Azadirachta indica*. Drink as desired and use for steam baths.
Cassia sieberiana	Shrub		Vabine	Boil chopped roots and drink as desired. Add sugar to taste

2 (continued)

Species	Growth form	Lobi name	Wale name	How used
Cochlospermum tinctorium	Herb		Gbelonbile	Boil chopped roots and drink as desired
Combretum ghasalense	Shrub	Popal	Kpamara	Boil leaves with that of *Jatropha gossypifolia* and whole plant of *Ocimum canum*, and drink. Use also for steam baths
Combretum sp.	Shrub	Kpekakra		Boil leaves with stem bark of *Mangifera indica*, leaves of *Azadirachta indica* and drink as desired.
Ficus gnaphalocarpa	Tree	Konkon		Pound roots with roots of *Gardenia ternifolia* and *Anogessius leiocarpus*. Mould into ball and dry. Mash in water and drink
Ficus platyphylla	Tree		Selinge	Boil leaves and stems barks, drink as desired. Massage body with decoction.

: 2 (continued)

:cies	Growth form	Lobi name	Wale name	How used
Gardenia tenifolia	Shrub	Dajeda	Dajugo	Boil leaves and twigs, and drink as desired.
r::ematostaphis barteri	Tree	Dole	Genbereni	Boil leaves with leaves of *Pseudocedrela kotschyi* and *Ficus ghaphalocarpa*. Drink mornings and evenings and massage body.
Hyptis spicigera	Herb	Donbeleva		Boil leaves and drink.
Indigofera pulchra	Herb	Balesama		Boil whole plants, drink and massage body.
Jatropha curcas	Shrub	Nato		Boils leaves with leaves of *Azadirachta indica* and *Carica papaya*. Drink and use for bathing.
Jatropha gossypiifolia	Shrub	Natogyere		Boil leaves with leaves of *Combretum ghaselensis* and whole plant of *Ocimum canum*, and drink. Use also for steam baths.
Khaya senegalensis	Tree		Koke	Boil stem bark and drink.
Lannea acida	Tree	Manvora/ Vaaworo	Gbent ore	Boil leaves with leaves of *Azadirachta indica* and *Mangifera indica*. Drink and use for steam baths.

: 2 (continued)

Species	Growth form	Lobi name	Wale name	How used
cas martinicensis	Herb	Dadeleva		Boil whole plant with *Hyptis spicigeria* and drink as required
fera indica	Tree	Mango	Mango	Boil stems barks and drink as required.
yna inerm	Tree	Yiela	Yiele	(1) Boil leaves and twigs, and drinks. (2) Boil twigs with whole plant of *Indigofera pulchra*. Drink 3 times daily.
Monanthotaxis sp	Climber	Woretia		Boil leaves and drink 3 times daily. Massage body with decoction.
Nauclea latifolia	Shrub	Gongan	Gounge	(1) Pound roots, add lemon juice and palm wine, and drink as desired. (2) Boil leaves and drink as desired.
Ocimum canum	Herb	Worobagnui		(1) Boil whole plant with leaves of *Azadirachta indica, Combretum ghaselensis* and *Mitrgyana inermis* and drink. (2) Boil leaves with that of *Mangifera indica, Mitragyna inermis* and whole plants of *Indigofera indica* and drink. Use also for steam baths.

le 2 (continued)

Species	Growth form	Lobi name	Wale name	How used
Ozoroa insignis	Shrub		Dato	Boils leaves and twigs, and drink.
Parinari polyandra	Shrub		Bongekapala	Boil leaves, drink and use for bathing
Parkia [illegible]	Tree	Dowa	Dowa	Boil leaves and stem barks, and drink as required
Paullinia pinnata	Shrub	Chiau		Boil leaves and drink. Bath mornings and evenings.
Pericopsis laxiflora	Tree			Boil leaves with that of *Combretum* sp and *Pericopsis laxiflora*, and drink as required.
Pseudocedrela kotschyi	Tree	Kpela	Kpale	Boil twigs and leaves, and drink as required.
Pterocarpus erinaceus	Tree		Pulinyie	Boils leaves with leaves of *Afzelia africana*. Drink and use for steam baths.
Ricinus communis	Shrub	Beton		Squeeze leaves in a pot to ferment. Bath with fermented solution.
Senna occidentalis	Herb	Bontore		Boil leaves with leaves of *Mangifera indica* and *Carica papaya*. Drink and bath decoction.

2 (continued)

Species	Growth form	Lobi name	Wale name	How used
lia setigera	Tree	Bulinyanie		Boil leaves and drink.
nos innocua	Tree	Kolan	Polea	Boil leaves and drink as required.
spinosa	Tree	Dajekokora	Polane	Boil leaves and drink. Grind twigs, add to pomade and smear on body.
Tamarindus indica	Tree			Boil leaves and stem bark, and drink.
Vernonia amygdalina	Shrub	Jankpantire		Boil leaves in a maize dough solution ('Konbire' in Lobi) and drink.
Xeroderris stuhlmannii	Tree			Boil leaves with that of *Combretum* sp and *Pericopsis laxiflora*, and drink as required.

Information about the other uses of the anti-malarial plants species in the sanctuary was outlined in Table 3. Infusions of some of the species have other medicinal uses. For example, boiled roots of *Cassia sieberiana* were used to treat stomach aches and also as an aphrodisiac The boiled stem bark and leaves of *Khaya senegalensis* was also drunk as a blood tonic and the boiled roots of *Monathotaxis* sp. (Bail.) Verdc. was also used to treat stomach aches. In contrast, many of the species including *Mitragyna inermis*, *Lannea acida*, *Khaya senegalensis* and *Xerroderis stuhlmannii* were used as sources of timber. Some species were used as domestic source of energy. These species included *Combretum ghaselensis*, *Ozoroa insignis* and *Pterocarpus erinaceus*. Some of the species such as *Combretum* sp and *Pterocarpus erinaceus* were also used for feeding livestock and *Afzelia africana* was used in carving the popular lobi sculpture.

Table 3: Other uses of anti-malarial plants in the Wechiau Community Hippopotamus Sanctuary Ghana

Species	Part(s) used	Use(s)
Afraegle paniculata	Roots	Boil and drink decoctions for stomach aches.
Afzelia Africana	Leaves, Stem	Leaves used to feed livestock. Stems are used in carving Lobi sculpture.
Anogeissus leiocarpus	Stem bark	Boil and drink for stomach aches.
Azadirachta indica	Whole plant, Leaves	Trees usually provide shade in front of houses. The leave decoctions are drunk for fevers.
Carica papaya	Fruits	The peeled fruits are eaten raw.
Cassia sieberiana	Roots	Boil and drink decoctions for stomach aches and as aphrodisia.
Cochlospermum tinctorium	Roots	Boil and drink decoction for yellow fever.
Combretum ghasalense	Branches	Fuel wood.
Combretum sp.	Leaves	Leaves used to feed livestock.

Table 3 (continued)

Species	Part(s) used	Use(s)
Ficus platyphylla	Leaves	Boil and drink decoction for body building.
Gardenia ternifolia Schum.	Whole plant	Planted around houses and gardens as a hedge.
Haematostaphis barteri	Fruits	Fruits eaten raw.
Hyptis spicigera	Whole plant	Insect repellent (mosquitoes).
Jatropha curcas	Whole plant, latex	Planted around houses and gardens as a hedge. The latex from twigs are used to treat mouth sores.
Jatropha gossypiifolia	Whole plant	Planted around houses and gardens as a hedge.
Khaya senegalensis	Stem bark & leaves	Boil and drink as a blood tonic. Stem used for building boat.
Lannea acida	Stem and branches	Stems are used as beams and the branches for roofing houses.
Mangifera indica	Fruits	Fruits eaten raw.
Mitragyna inermis	Branches	Branches for roofing houses.

Species	Part(s) used	Use(s)
Monathotaxis sp.	Roots	Boil and drink decoctions for stomach aches.
Nauclea latifolia	Fruits	Fruits eaten raw.
Ocimum canum	Seeds	Soak seeds and apply in infusion to eyes to treat eye troubles.
Ozoroa insignis	Branches	Fuel wood
Parkia biglobosa	Stem bark, fruits, seeds	Boil and drink decoctions for stomach aches. Fruit pulp eaten raw and the seed use as spice.
Pericopsis laxiflora	Branches	For roofing houses.
Pseudocedrela kotschyi	Stem bark, branches	Stomach aches, building
Pterocarpus erinaceus	Branches, leaves	Leaves used to feed livestock and branches for fuel wood.
Ricinus communis	Whole plant	Planted around houses and gardens as a hedge.
Senna occidentalis	Whole plant	Boil and drink decoctions with a swollen body.
Sterculia setegera	Branches	For roofing houses.
Strychnos spinosa	Fruits	Fruit pulp eaten.

Species	Part(s) used	Use(s)
Tamarindus indica	Leaves, fruits	Leaves cooked with porridge to make it taste sour and the fruit eaten.
Xeroderris stuhlmannii	Branches	For roofing houses.

3.1. 2. Distributional ranges of anti-malarial plants in study area

The species reported to be used for the treatment of malaria were found around the vicinity of their habitation and in the wild, which is in the core area of the sanctuary. Thirteen of the 41 species used in the treatment of malaria were found around the vicinity of their habitation. These species were often those frequently found by their resident, such as *Azadirachta indica* and *Indigofera pulchra*, as well as *Carica papaya*, *Mangifera indica*, *Acanthospermum hispidum*, *Jatropha curcas*, *Jatropha gossypiifolia*, *Hyptis spicigera*, *Leucas martinicensis*, *Ocimum canum*, *Ricinus communis*, *Senna occidentalis*, and occasionally *Parkia biglobosa*.

The remaining 28 species were found in the wild. The distribution and density of the reported anti-malarial species are presented in Table 4. Of these species, the most widely distributed species were *Combretum ghasalense*, *Pterocarpus erinaceous*, *Mitragyna inermis* and *Anogeissus leiocarpus*. However, the most abundant anti-malarial plants were *Pseudocedrela kotschyi*, *Combretum ghasalense* Engl. & Diel., *Combretum* sp. and *Mitragyna inermis*. The least dominant species used to treat malaria in the sanctuary included *Cochlospermum tinctorium*, *Gardenia ternifolia*, *Nauclea latifolia*., *Ozoroa insignis* and *Sterculia setigeria*.

Some of the species found in the wild area of the sanctuary and used to treat malaria were not found during the quadrat studies. Such species were flagged as 'rare' in the sanctuary and included *Afraegle paniculata*, *Ficus gnaphalocarpa*, *Ficus platyphylla*, *Khaya senegalensis*, *Parinari polyandra*, *Strychnos innocua*, *Strychnos spinosa*, *Xeroderris stuhlmannii*, *Monathotaxis* sp. and *Vernonia amygdalina*.

Table 4: Distribution and density of species used to treat malaria in the core area of Wechiau Community Hippopotamus Sanctuary Ghana

Species	Numbers of quadrats included / total	Relative frequency	Density / m^2	Relative density
Afzelia africana	6	5.2	0.0061	4.2
Anogeissus leiocarpus	12	10.3	0.0061	4.2
Cassia sieberenia	5	4.3	0.0072	5.0
Cochlospermum tinctorium	2	1.7	0.0016	1.1
Combretum ghasalense	25	22.6	0.034	23.4
Combretum sp.	11	9.5	0.012	8.3
Gardenia ternifolia	2	1.7	0.0016	1.1
Haematostaphis barteri	2	1.7	0.0032	2.2
Lannea acida	5	4.3	0.0048	3.3
Mitragyna inermis	15	12.9	0.012	8.3
Nauclea latifolia	2	1.7	0.0016	1.1
Ozoroa insignis	1	0.9	0.0016	1.1
Parkia biglobosa	2	1.7	0.0016	1.1
Pseudocedrela kotschyi	3	2.6	0.042	28.9
Pterocarpus erinaceus	17	14.7	0.0057	3.9
Sterculia setegera	2	1.7	0.0016	1.1
Tamarindus indica	4	3.4	0.0024	1.7

3.2.0. Bioactivity studies

3.2.1. Initial extraction of plant samples

Results of initial extraction of plant samples are presented in Table 5 -8. Generally, the leaves of the four anti-malarial species produced higher percentage yields when compared with stem barks and roots. Also, the percentage yields of the 50 % aqueous methanol extracts was higher than the acetone and hexane extracts. Thus, results of the sequential extraction indicate that the four anti-malarial species contained more polar compounds with little non-polar components.

Table 5: Results of sequential extraction of *Cassia sieberiana* samples

Plant part	Solvent used	Weight extracted (g)	Weight of extract (mg)	% yield of material extracted
Leaf	Hexane	100.07	2.32	2.32
	Acetone	100.07	4.2	4.20
	50% MeOH	100.07	12.3	12.29
Stem bark	Hexane	9.28	0.014	0.15
	Acetone	9.28	0.90	9.70
	50% MeOH	9.28	1.21	13.03
Root	Hexane	3.41	0.01	0.18
	Acetone	3.41	0.01	0.24
	50% MeOH	3.41	0.0099	0.29

Table 6: Result of sequential extraction of *Haematostaphis barteri* samples

Plant part	Solvent used	Weight extracted (g)	Weight of extract (mg)	% yield of material extracted
Leaf	Hexane	100.83	1.16	1.15
	Acetone	100.83	2.46	2.44
	50% MeOH	100.83	11.46	11.37
Stem bark	Hexane	20.04	0.13	0.65
	Acetone	20.04	0.17	0.85
	50% MeOH	20.04	1.08	5.39
Root	Hexane	3.05	0.01	0.92
	Acetone	3.05	0.01	0.27
	50% MeOH	3.05	0.04	1.31

Table 7: Result of sequential extraction of *Mitragyna inermis* samples

Plant part	Solvent used	Weight extracted (g)	Weight of extract (mg)	% yield of material extracted
Leaf	Hexane	100.07	2.32	2.32
	Acetone	100.07	5.63	5.63
	50% MeOH	100.07	15.37	15.37
Stem bark	Hexane	26.52	0.11	0.041
	Acetone	26.52	0.12	0.45
	50% MeOH	26.52	1.24	4.68
Root	Hexane	2.74	0.001	0.026
	Acetone	2.74	0.001	0.033
	50% MeOH	2.74	0.094	3.4

Table 8: Result of sequential extraction of *Pseudocedrela kotschyi* samples

Plant part	Solvent used	Weight extracted (g)	Weight of extract (mg)	% yield of material extracted
Leaf	Hexane	100.53	2.30	2.29
	Acetone	100.53	9.41	9.36
	50% MeOH	100.53	25.99	25.85
Stem bark	Hexane	16.01	0.38	2.37
	Acetone	16.01	0.42	2.62
	50% MeOH	16.01	0.82	5.12
Root	Hexane	3.26	0.00027	0.083
	Acetone	3.26	0.0020	0.061
	50% MeOH	3.26	0.017	0.52

3.2.2. *In vitro* antiplasmodial bioassay

Anti-malarial bioassay was carried out on 21 randomly selected extracts from the four anti-malarial species. The IC_{50} for the positive control chloroquine was 8.8 ± 0.23 ng / ml. The IC_{50} of extracts from the four anti-malarial species ranged from 4.75 µg / ml to values > 500 µg / ml. Only one of the 21 plant extracts tested for *in vitro* antiplasmodial activity was active. This was the hexane extract of leaf of *Pseudocedrela kotschyi* that had an IC_{50} value less than 5 µg / ml. Eight of the extracts also exhibited some inhibition of growth of *Plasmodium falciparum* (3D7). These extracts had IC_{50} > 5 µg / ml and < 50 µg / ml. These were extracts from stem bark of *Cassia sieberiana*, leaf and stem bark of *Haematostaphis barteri*, leaf of *Mitragyna inermis* and stem bark of *Pseudocedrela kotschyi*. The results of the antiplamodial testing are presented in Table 9.

Table 9. Result of *in vitro* antiplasmodial (IC_{50} + S>EM>) testing of plant extracts

Species	Plant part	Extract type	IC_{50} (± S.E.M)
Cassia sieberiana	Leaf	not known	26.42 ± 1.59
	stem bark	hexane	7.92 ± 0.54
	stem bark	acetone	9.65 ± 2.55
	root	hexane	35.84 ± 3.32
Haematostaphis barteri	Leaf	not known	8.47 ± 1.01
	stem bark	hexane	>500
	stem bark	acetone	12.81 ± 1.81
	stem bark	50% MeOH	72.50 ± 1.11
Mitragyna inermis	Leaf	acetone	7.50 ± 0.35
	stem bark	hexane	69.40 ± 69.86
	stem bark	acetone	85.8 ± 41.43
	stem bark	50% MeOH	> 500
	root	acetone	74.50 ± 36.70
	root	50% MeOH	54.64 ± 4.68
Pseudocedrela kotschyi	Leaf	hexane	4.56 ± 0.11
	Leaf	acetone	44.35 ± 4.92
	Leaf	50% MeOH	54.04 ± 19.35
	stem bark	hexane	5.96 ± 0.30
	stem bark	acetone	83.55 ± 25.46
	stem bark	not known	17.16 ± 0.028

3.2.3. Anti-microbial bioassay

Anti-microbial screening was carried out on 36 plant extracts from the four anti-malarial species. Each extract was tested on gram positive and negative bacteria, and fungal species Out of the 36 extracts tested, 23 of them inhibited growth of the micro-organisms (Table 10). Seven (30.4 %) of the 23 extracts inhibited the growth of *Cladosporium herbarum* IMI 300461 ex. Wall contaminant These extracts were mainly from 50 % methanol extracts of the four species. However, of all the extracts tested, the acetone extract of root of *Mitragyna inermis* produced the most significant inhibition on the growth of *Cladosporium herbarum* (Plate 5)

Plate 5 Bio-autographic plate showing inhibition of fungal growth

(a) Acetone extracts

(b) 50 % methanol extracts

[From left to right: *Cassia sieberiana, Haematostaphis barteri, Mitragyna inermis* and *Pseudocedrela kotschyi*. First 4 samples were leaves, next 4 root bark and last batch of 4 stem barks]

Altogether, 16 extracts inhibited growth of the bacterial species tested (Plate 6). Twelve of these extracts inhibited the growth of *Bacillus subtillis* IMI 347329 ex. *Solanum tuberosum* (gram - positive bacteria) and 4 extracts inhibited growth of *Pseudomonas syringae* pv. *Spyringae* IMI 34748 (ACTCC19310) ex *Spyringae vulgarise* (gram-negative bacteria). Thus 25 % of the extracts screened for anti-microbial bioassay and 75 % of the extracts which inhibited the growth of the microbes also inhibited the growth of the gram-positive bacteria.

The acetone extracts from all four species inhibited the growth of *Bacillus subtillis*. The activity of 50 % methanol extracts against *Bacillus subtillis* was in either the stem bark or root of all four species. The hexane extracts from roots of *Mitragyna inermis* was the only hexane extract to exhibit activity against *Bacillus subtillis*. The extracts which inhibited the growth of *Bacillus subtillis* were also obtained from stem barks and roots.

Hexane extracts from the leaves of *Cassia sieberiana*, *Haematostaphis barteri* and *Mitragyna inermis* inhibited the growth of *Pseudomonas syringae*. Only one 50 % methanol, leaf extract of *Haematostaphis barteri* inhibited growth of *Pseudomonas syringae* and no acetone extracts were active.

Plate 6: Bio-autographic plate showing inhibition of growth of bacteria

(a) Acetone extracts

(b) 50 % methanol extract

[From left to right: *Cassia sieberiana*, *Haematostaphis barteri*, *Mitragyna inermis* and *Pseudocedrela kotschyi*. First 4 samples were leaves, next 4 root bark and last batch of 4 stem barks].

Table 10: Extract of anti-malarial species anti-microbial activity

Species	Plant part	Extract type	Gram + bacteria	Gram − bacteria	Fungal species
Cassia sieberiana	Leaf	Hexane	−	+	−
		Acetone	+	−	−
		50 % methanol	−	−	+
	Stem bark	Acetone	+	−	−
	Root	50 % methanol	+	−	−
Haematostaphis barteri	Leaf	50 % methanol	−	+	−
	Stem bark	50 % methanol	+	−	+
	Root	Acetone	+	−	−
Mitragyna inermis	Leaf	Hexane	−	+	−
		Acetone	+	−	−
		50% methanol	−	−	+
	Stem bark	50% methanol	+	−	+
	Root	Hexane	+	−	−
		Acetone	+	−	+
Pseudocedrela kotschyi	Leaf	Hexane	−	+	−
	Stem bark	Acetone	+	−	−
		50% methanol	−	−	+
	Root	Acetone	+	−	−
		50% methanol	+	−	+

Key: + means inhibited microbial growth and − means no inhibition of microbial growth.

3.2.4. Insect anti-feedant bioassay

The results of the insect anti-feedant bioassays are presented in Table 11. Of the 36 plant extracts tested, five showed significant anti-feedant activity, whereas four stimulated feeding. All the extracts with significant anti-feedant properties were from hexane extracts. The hexane extract of roots of *Cassia sieberiana* was the most potent of the anti-feedant extracts (Feeding index, % mean ± SEM; 96 ± 2.5). The other extracts with anti-feedant properties were from stem bark (*Cassia sieberiana* and *Pseudocedrela kotschyi*) and leaves (*Mitragyna inermis* and *Haematostaphis barteri*).

The four extracts that significantly stimulated feeding were acetone extracts from leaves of *Cassia sieberiana* (Feeding index, % mean ± SEM; -72. ±14.6) and *Mitragyna inermis* (Feeding index, % mean ± SEM; -51± 9.7) and stem bark of *Pseudocedrela kotschyi* (Feeding index, % mean ± SEM; -46 ± 4.7). The 50 % methanol extract of root of *Cassia sieberiana* (Feeding index, % mean ± SEM,-66± 7.9) also stimulated feeding. Thus, the anti-feedant properties of extracts from *Cassia sieberiana* and *Mitragyna inermis* varied depending on the kind of extract and plant part. For example, the hexane extract of root of *Cassia sieberiana* showed a potent anti-feedant property whiles the 50% methanol extract of root material stimulated feeding

The anti-feedant activity of materials from a species collected from plants from three different populations varied (Table 12). For instance, the acetone extract of *Mitragyna inermis* from Tankara did not significantly stimulate feeding whereas the extracts from plants sampled from other two populations of the sanctuary did stimulate feeding.

The anti-feedant activity of hexane extracts of *Cassia sieberiana* also varied significantly among populations. However, the hexane extract of stem bark of *Pseudocedrela kotschyi* seems to have retained its potent anti-feedant property.

11: Summary of result of insect feeding bioassay

part	Extract type / solvent used for extraction	Insect Feeding index (± S.E.M)			
		Cassia sieberiana	*Haematostaphis barteri*	*Mitragyna inermis*	*Pseudocedrela kotschyi*
	Hexane	33 ±19.1	62 ± 19.8*	63 ± 12.3*	46 ± 15.3
s	Acetone	- 72 ± 14.6*	23 ± 12.1	- 16 ± 16 5	-29 ± 1 5
	50 % methanol	-31 ± 3.6	-37 ± 4 6	-7 ± 17 4	4 ± 22.5
Stem bark	Hexane	49 ± 3.48*	11 ±11.4	36 ± 13.7	71 ± 5.33*
	Acetone	-37 ± 4	-7 ± 11.7	-39 ± 9.1	-46 ± 4.7*
	50 % methanol	-1 ± 9.9	-35 ± 4.5	4 ± 23.6	-31 ± 7.9
Roots	Hexane	96 ± 2.5*	10 ±11.2	-15 ± 12.3	19 ± 11.3
	Acetone	-1 ± 11.6	-7 ±17.9	-51 ± 9.7*	- 15 ± 10.8
	50% methanol	-66 ± 7.9*	9 ± 10.4	-2 ± 16.9	-22 ± 16 3

$P < 0.05$: positive values = antifeedant, negative values = phygostimulant, Wilcoxon matched pair test (n = 10-15), *: significant antifeedant or stimulatory activity *

Table 12: Comparison of anti-feedant activity of species sampled from three different populations from Wechiau Community Hippopotamus Sanctuary Ghana

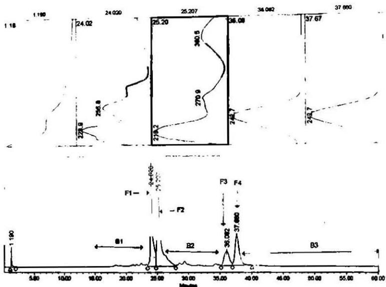
Species	Plant part	Extract type	Population	Anti-feedant index (± SEM)	Date tested
Cassia sieberiana	root	hexane	Kantu	-18.5 ± 10.46	22 / 10 / 03
			Talewona	96 ± 2.50	12 / 09 / 03
			Tankara	-18.6 ± 8.28	22 / 10 / 03
		50% MeOH	Kantu	-66 ± 7.9	12 / 09 / 03
			Talewona	-44.9 ± 17.63	22 / 10 / 03
			Tankara	3.2 ± 9.62	22 / 10 / 03
Mitragyna inermis	root	acetone	Kantu	-51 ± 9.70	12 / 09 / 03
			Talewona	-18.2 12.8	22 / 10 / 03
			Tankara	38.3 12.02	22 / 10 / 03
Pseudocedrela kotschyi	stem bark	hexane	Kantu	44.5 19.06	22 / 10 / 03
			Talewona	71 ± 5.33	12 / 09 / 03
			Tankara	51.5 10.60	22 / 10 / 03

3. 2.5. Isolation and identification of peaks from *Cassia sieberiana*

From the hexane extract of *Cassia sieberiana*, four major non-polar components were identified at UV maximum at 254 nm (Figure 5). Repeated semi-preparative HPLC collections of the peaks produced **F1** (3.3 mg), **F2** (8.2 mg), **F3** (0.9 mg) and **F4** (1.7 mg). The backgrounds between peaks (B1, B2 and B3) were also collected for insect testing.

An attempt was made to elucidate the structures of peaks **F1** and **F4** using available NMR spectroscopic methods. The structure of the peaks could not be determined. Mass spectra of the peaks were studied by Atmospheric Pressure Chemical Ionisation (APCI) and Electron Spray (ES) LC-MS in positive and negative modes. However, the peaks could not fully ionise using the two LC-MS methods. The phytochemical study on *Cassia sieberiana* was therefore dropped.

Figure 4: HPLC profile of *Cassia sieberiana* root (hexane extract re-dissolved in 100 % methanol) at PDA 254nm and 200µ l injection volume



3.2.5. Isolation and identification of compounds from *Haematostaphis barteri*

The acetone extract of the stem bark (3.0 mg) of *Haematostaphis barteri* was redissolved in 90 % methanol and yielded compounds **(A)** 1.8 mg at t_R 9.3 min, **(B)** 2.4 mg at t_R 12.5 min, **(C)** 0.5 mg at t_R 13.9 min and **(D)** 0.5 mg at t_R 15.5 min on semi-preparative HPLC isolation. From the 80 % methanol extract of the root bark also were obtained **0**, **1** and **2**. Analytical HPLC showed that during isolation the compounds partly isomerised from *E*-form to *Z*- form (compound in the *E*-form have a UV maximum of approximately 300 nm, whereas the *Z*-form have their UV maximum at 280 nm).

The HPLC profiles of stem and root barks are shown in Figures 6 and 7. The structures of compounds from the root bark were determined from positive ion APCI-MS. The positive ion APCI-MS first order of **2** showed an MS $[M + H]^+$ at *m / z* 243.1 (Figure 10).

The structure of **2** was identified as the aglycone of **KEW 775**. The 1D ^1H NMR spectra data of **2** **(KEW 791)** confirmed that the sample was a trisubstituted aglycone with one methoxy substituent and only the (*E*) isomer was present. 1D ^1H NMR spectra data of **0** (**KEW 793**) indicated that the sample was the same as **KEW 791**. However, no further NMR data could be recorded on the compounds due to the same amount of sample available. ^1H, ^{13}C HSQC and HMBS NMR experiments on a Bruker Avance 400 NMR spectrometer. Compound **B (KEW 775)** was identified as a mixture of 5 – methoxy (*E*) and 5 - methoxy (*Z*) reverastrol 3- *O* – rutinoside (approximately 1:1). NMR spectra data of **KEW 775** are presented in Table 13. The spectra data are similar to those in literature (Wanjala and Majinda, 2001), however, the $^{\delta}H$ (3', 5') of 6.86 in the previous report is probably incorrect as are $^{\delta}C$ C-3 + C-5 which should be interchanged. Not all the ^{13}C NMR assignment for KEW 775 can be determined because of the small amount of sample

available. This resulted in only assignments from indirectly - detected ^{13}C experiments (HSQC and HMBC) rather than the direct observe 1D ^{13}C NMR spectrum.

From APCI-MS in positive mode, A gave a first order MS of $[M+H]^+$ 537.0 (Figure 8). Compound A was identified as resveratrol rutinoside. The 1D ^{1}H NMR data of sample C (KEW 792) indicates that the sample contains the same mixture of stilbenes as KEW 775 and an additional compound which does not appear to be a stilbene. Positive ion APCI-MS supported that C contained KEW 775 since the first order MS showed an $[M+H]^+$ m/z at 551.0 (Figure 9).

Figure 5 HPLC profile of stem bark of *Haematostaphis barteri* (acetone extract re-dissolved in 100% MeOH) at PDA 300nm and Injection volume 50μ l.

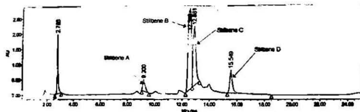


Figure 6: HPLC profile of root bark of *Haematostaphis barteri* (80 % MeOH extract) at PDA 300nm

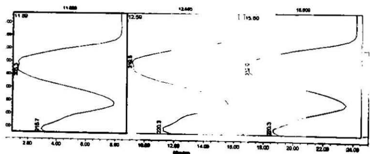


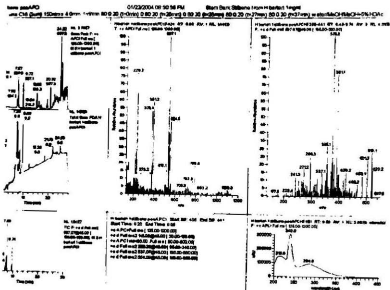
Table 13 NMR data of KEW 775 in MeOH-*d4*, on Bruker Avance 400 NMR spectrometer at 30 °C

POSITION	$^{\delta}$H (*E*)- ISOMER	$^{\delta}$C (*E*)- ISOMER
1		141.6
2	6.82 M	108.7
3		N.D.
4	6.58 M	103.3
5		162.5
6	6.76 M	106.6
A	6.89, *D* (16.3)	126.7
B	7.05, *D* (16.3)	130.4
1'		130.4
2' 6'	7.39, *D* (8.6)	129.0
3' 5'	6.78, *D* (8.6)	116.6
4'		158.8
5-OCH_3	3.82 *S*	56.0
GLUCOSE		
1	4.88 *D* (7.3)	102.6
RHAMNOSE		
1	4.71 *D* (1.5)	102.1

[Internal chemical shifts reference for sample: tetramethylsilane (TMS), with chemical shifts values of 0.00ppmm in both ^{1}H and ^{13}C NMR spectra. Standard pulse sequences and parameters were used for all experiments]

Figure 7: APCI-MS analysis of A (initially compound O) from stem bark of *Haematostaphis barteri*

Figure 8: APCI-MS analysis of C (initially compound 3, KEW 792) from stem bark of *Haematostaphis barteri*



The structures of the compounds from the root bark were determined from positive ion APCI-MS. The positive ion APCI-MS first order of 2 showed an MS $[M+H]^+$ at *m/z* 243.1 (Figure 10). The structure of 2 was identified as the aglycone of KEW 775. The ID 1H NMR spectra of 2 (KEW 791) confimed that the sample was a trisubstituted aglycone with methoxy substituent and only the *(E)* isome was present. It was identified as 3, 4^1 dihydroxyl-5-methoxystilbene and 1D ^{1}H NMR spectra of 0 (KEW 793) indicated that the sample was the same as KEW 791. However, no further NMR data could be recorded on the compounds due to the small amount of sanple available.

3.3.0. Variation studies

3. 3.1. Morphological studies

The morphological characters of individuals of the four selected species varied within and among the populations in the sanctuary. The variations were mainly quantitative in nature. Statistical analyses of morphological characters of the four selected species are presented in Appendix 3. Some morphological features of the four plants are presented in Plates 7-10.

In *Cassia sieberiana*, the vegetative characters were the most variable characters. Four out of the fifteen morphological characters examined varied significantly ($p > 0.05$ %) among the three populations in the sanctuary (Table 14). These characters were; number of leaflets per leaf, leaflet length, leaflet size and petiolule length. The PCA analysis of morphological characters in *Cassia sieberiana* is presented in Figure 11. The first three principal components with eigenvalues 8.9, 0.020 and 0.012 explained 99.52 %, 0.23 % and 0.13 % respectively of the total variation. The variation in the morphological characters was well partitioned among the three populations of the sanctuary. However, deviant individuals were found among all the three populations in the sanctuary.

Figure 10: PCA analysis of 15 morphological characters in *Cassia sieberiana* from Wechiau Community Hippopotamus Sanctuary Ghana

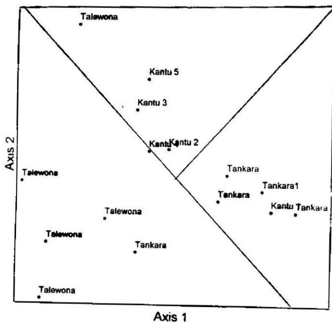
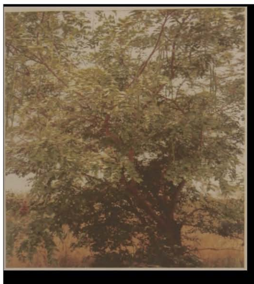


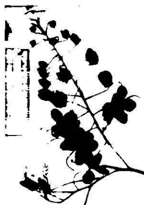
Table 14: Comparison of morphometric characters (mean ± S. E. M.) of *Cassia sieberiana* from three populations in the Wechiau Community Hippopotamus Sanctuary Ghana

...racters	Populations		
	Kantu area	Talewona area	Tankara area
...lant height (m)	4.7 ± 0.20	5.6 ± 0.96	4.3 ± 0.40
...tem girth (m)	0.5 ± 0.099	0.4 ± 0.065	0.4 ± 0.036
...umber of leaflets per leaf*	14.8 ± 0.12	17.7 ± 0.85	13.4 ± 0.75
...eaflet length (cm)*	6.4 ± 0.26	5.3 ± 0.33	5.9 ± 0.36
...eaflet width (cm)	2.5 ± 0.21	2.4 ± 0.20	3.1 ± 0.12
...eaflet size (cm^2)*	15.1 ± 1.01	12.7 ± 0.50	7.3 ± 1.03
...eaflet shape	2.81 ± 0.033	2.32 ± 0.34	2.6 ± 0.41
...achis length (cm)	13.0 ± 0.57	11.9 ± 0.85	12.7 ± 0.52
...etiolule length (cm)*	0.4 ± 0.020	0.5 ± 0.022	0.4 ± 0.16
Calyx length (cm)	2.1 ± 0.05	2.0 ± 0.047	2.04 ± 0.053
Calyx width (cm)	1.1 ± 0.041	1.1 ± 0.05	1.1 ± 0.075
Longest filament length (cm)	2.9 ± 0.13	2.9 ± 0.058	2.9 ± 0.067
Longest filament anther length (mm)	3.9 ± 0.04	4.0 ± 0.037	4.0 ± 0.051
Fruit length (cm)	64.5 ± 4.10	56.4 ± 4.30	60.5 ± 0.016
Fruit width (cm)	1.4 ± 0.014	1.4± 0.050	1.7 ± 0.13

*Characters showing statistically significant variation ($p \geq 0.05$).

Plate 7 Morphological features of *Cassia sieberiana*





b) Close-up picture of inflorescence

With *Haematostaphis barteri*, four out of the seventeen morphological characters examined including the height of the plant, stem girth, leaflet shape and rachis length showed statistically significant difference at $p > 0.05$ % among populations (Table 15). The first three PCA accounted for 93.55 % of the total variation. The first principal component (PC1) with eigenvalue 6.60 explained 73.35 % of variation, PC 2 with eigenvalue 1.23 also explained 13.67 % of the variation and PC3 with eigenvalue 0.59 accounted for 6.53 % of variation. The spatial relationships among the morphological characters of *Haematostaphus barteri* are presented in Figure 12. There were no however groupings in the morphological characters from the different populations in the sanctuary as observed for *Cassia sieberiana*.

Figure 11: PCA analysis of 17 morphological characters from *Haematostaphis barteri*

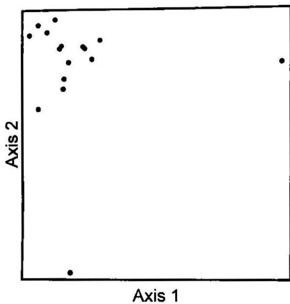


Table 15: Comparison of morphometric characters (mean ± S. E. M) of *Haematostaphis barteri* from three populations of the Wechiau Community Hippopotamus Sanctuary Ghana

Characters	Populations		
	Kantu area	Talewona area	Tankara area
1 Plant height (m)*	3.4 ± 0.24	4.1 ± 0.29	2.8 ± 0.15
2 Stem girth (m)*	0.5 ± 0.079	0.8 ± 0.13	0.5 ± 0.037
3 Number of leaflets per leaf	18.0 ± 0.25	18.0 ± 0.85	16.3 ± 0.35
4 Leaflet length (cm)	6.9 ± 0.30	6.6± 0.22	5.9 ± 0.57
5 Leaflet width (cm)	2.4 ± 0.11	1.1 ± 0.084	2.4 ± 0.098
6. Leaflet size (cm^2)	17.1 ± 2.02	16.9 ± 0.96	15.0 ± 2.04
7 Leaflet shape*	3.1 ± 0.084	2.7 ± 0.069	2.9 ± 0.13
8 Rachis length (cm) *	24.7 ± 0.22	16.3 ± 0.96	17.3 ± 0.91
9 Petiolule length (cm)	0.4 ± 0.028	0.3 ± 0.026	0.4 ± 0.018
10 Male flower length (mm)	3.2 ± 0.042	3.0 ± 0.27	2.7 ± 0.27
11 Male flower calyx length (mm)	2.1 ± 0.80	2.1 ± 0.090	2.1 ± 0.065
12 Male flower calyx width (mm)	1.3 ± 0.057	1.2 ± 0.071	1.0 ± 0.11
13 Stamen length (mm)	1.7 ± 0.16	1.8 ± 0.11	1.8 ± 0.18
14 Female flower length (cm)	3.2 ± 0.12	3.2 ± 0.14	3.1 ± 0.28
15 Female flower calyx length (mm)	2.5 ± 0.082	2.4 ± 0.068	2.3 ± 0.047
16 Female flower calyx width (mm)	1.8 ± 0.052	1.5 ± 0.15	1.1 ± 0.11
17 Fruit length (cm)	2.0 ± 0.076	2.2 ± 0.67	2.1 ± 0.68

*Characters showing statistically significant variation ($p \geq 0.05$).

Plate 8. Morphological features of *Haematostaphis barteri*

(a) Habit x 5

(b) Close-up picture of fruits and leaves x 5

In *Mitragyna inermis*, both vegetative and floral characters were variable. From the ANOVA and multiple range test analysis the characters of the leaf width, stipule length, stipule size, anther width and stigma width showed significant difference ($p > 0.05$ %) among the populations in the sanctuary The PCA analysis of the morphological characters is presented in Figure 13. The PC1 with eigenvale 2.9 explained 99.9%, PC2 explained 0.53% and PC3 explained 0.034 of the total variation. The PCA analysis of the morphological characters did not show any particular pattern of variation

Figure 12: PCA analysis of 20 morphological characters of *Mitragyna inermis* from Wechiua Community Hippopotamus Sanctuary Ghana

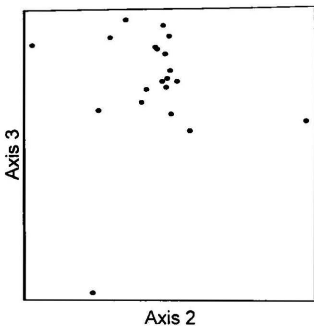


Table 16: Comparison of morphometric characters (mean ± S. E. M.) of *Mitragyna inermis* from three populations in the Wechiau Community Hippopotamus Sanctuary Ghana

Characters	Populations		
	Kantu area	Talewona area	Tankara area
1 Plant height (m)	4.7 ± 0.52	5.6 ± 0.96	4.2 ± 0.35
2. Stem girth (m)	0.5 ± 0 023	0.4± 0.065	0.4 ± 0.034
3. Leaf length (cm)	8.1 ± 0 2	8.8 ± 0.44	8.2 ± 0.37
4. Leaf width (cm)*	4.6 ± 0.32	3.3 ± 0.57	4.2 ±0.086
5. Leaf size (cm^2)	40.1 ± 4.55	38 5 ± 2 52	34.4 ± 2.04
6. Leaf shape	1.9 ± 0.054	2.1 ± 0.14	2.0 ± 0.080
7. Stipule length (cm) *	1.2 ± 0.060	1.5 ± 0.091	1.0 ± 0.061
8. Stipule width (cm)	0.5 ± 0 034	0.5 ± 0.037	0.4 ± 0.069
9. Stipule size (cm^2)*	0.6 ± 0 077	0.8 ± 0.1	0.4 ± 0.036
10 Stipule shape	2.8 ± 0.17	3 3 ± 0.28	2.8 ± 0.42
11. Flower length (cm)	1.1 ± 0.034	1.1 ± 0.033	1.2 ± 0.045
12. Corolla length (cm)	0.7 ± 0.026	0.7 ± 0.019	0.8 ± 0.04
13 Corolla lobe length (cm)	0.3 ± 0.015	0 3 ± 0.016	0.3 ± 0.012
14 Anther length (mm)	1.6 ± 0 046	1.6 ± 0.049	1.7 ± 0.099
15. Anther width (mm)*	0.6 ± 0 026	0.6 ± 0.023	0.7 ± 0.018
16 Style length (cm)	1.0 ± 0.042	0.9 ± 0.028	1.0 ± 0.056
17 Style width (mm)	0.4 ± 0.013	0 3 ± 0.019	0.4 ± 0.045
18 Stigma length (mm)	1.7 ± 0.095	1.5 ± 0.065	1.9 ± 0.10
19 Stigma width (mm)*	0.7 ± 0.05	0.6 ± 0.068	0.8 ± 0.052
20. Style + stigma length (cm)	1.0 ± 0.06	1.2 ±0.035	1.2 ± 0.086

... ant variation ($p \geq 0.05$).

Plate 9: Morphological features of *Mitragyna inermis*

(a) Habit

(b) Close- up picture of leaves

'he largest number of characters showing significant morphological difference among opulations in the sanctuary was observed in *Pseudocedrela kotschyi*. Six out of the 23 haracters examined in this plant were statistically different. These characters included :aflet length, leaflet size, corolla length, corolla size, calyx size and staminal column :ngth. The spatial relationships among the morphological characters are presented in igure 14. The first three PCA explained 99 % of the total variation. The PC1 with genvalue 2.93 explained 97.5 % of variation, PC2 with eigenvalue 0,057 explained 1.91 › of variation and PC3 with eigenvalue 0.017 explained 0.58 % of variation. The high ifference among the eigenvalues indicated that the character variations are not correlated ithin the populations in the sanctuary.

Figure 13: PCA analysis of 23 morphological characters in *Pseudocedrela kotschyi* from Wechiau Community Hippopotamus Sanctuary Ghana

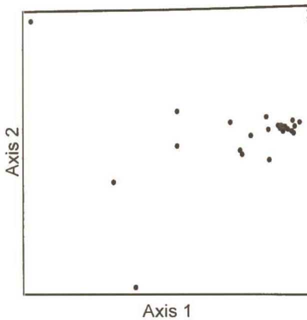


Table 17: Comparison of morphometric characters of *Pseudocedrela kotschyi* from three populations of the Wechiau Community Hippopotamus Sanctuary Ghana

Character	Populations		
	Kantu area	Talewona area	Tankara area
1. Plant height (m)	5.0 ± 0.78	5.7 ± 0.32	5.0 ± 0.29
2. Stem girth (m)	1.2 ± 0.051	1.1 ± 0.22	0.9 ± 0.089
3. Number of leaflets per leaf	13.4 ± 0.58	12.7 ± 0.70	12.4 ± 0.71
4. Leaflet length (cm)*	9.1 ± 0.51	6.3 ± 0.35	7.1 ± 0.39
5. Leaflet width (cm)	4.2 ± 0.37	3.6 ± 0.40	3.2 ± 0.20
6. Leaflet size (cm^2)*	37.4 ± 4.67	24.3 ± 3.63	24.0 ± 2.69
7. Leaflet shape	2.3 ± 0.22	2.1 ± 0.066	2.2 ± 0.34
8. Rachis length (cm)	21.4 ± 0.86	16.8 ± 1.46	20.3 ± 0.90
9. Petiolule length (cm)	0.3 ± 0.076	0.3 ± 0.054	0.3 ± 0.046
10. Flower length (mm)	5.6 ± 0.17	6.2 ± 0.22	6.5 ± 0.21
11. Pedicel length (mm)	2.4 ± 0.19	3.3 ± 0.099	4.1 ± 0.87
12. Corolla length (mm)*	1.0 ± 0.056	1.0 ± 0.11	1.4 ± 0.11
13. Corolla width (mm)	0.7 ± 0.060	0.7 ± 0.038	0.9 ± 0.088
14. Corolla size (mm^2)	0.6 ± 0.10	0.9 ± 0.19	1.2 ± 0.22
15. Corolla shape	1.5 ± 0.076	1.4 ± 0.15	1.7 ± 0.082
16. Calyx length (mm)	3.4 ± 0.13	3.5 ± 0.094	3.3 ± 0.031
17. Calyx width (mm)	1.8 ± 0.040	1.8 ± 0.065	1.8 ± 0.076
18. Calyx size (mm^2) *	6.2 ± 0.35	6.2 ± 0.37	6.6 ± 0.047
19. Calyx shape	1.9 ± 0.080	2.0 ± 0.046	2.1 ± 0.066
20. Staminal column length (mm)*	2.4 ± 0.046	2.7 ± 0.13	2.3 ± 0.11
21. Fruit length (cm)	13.4 ± 0.20	13.8 ± 0.38	11.0 ± 0.23
22. Fruit width (cm)	2.3 ± 0.34	2.2 ± 0.057	2.3 ± 0.025
23. Number of seeds per fruit	15.9 ± 0.66	13.2 ± 3.02	21.4 ± 0.35

...riation ($p \geq 0.05$).

Plate 10: Morphological features of *Pseudocedrela kotschyi*

(a) Habit

[illegible] ... re of fruit and leaves.

3.3.2. Anatomical studies of barks

The features of fibres of the four plants are presented in Plate 11. The macerates of the species also contained sclerids. Result of the measurements of fibres of stem and root barks of the four species are presented in Tables 8 and 9.The characters of stem and root bark fibres were less variable. Only characters of fibres from *Haematostaphis barteri* and *Pseudocedrela kotschyi* varied significantly at $p \geq 0.5$ % among the three populations in the sanctuary. These characters included stem bark fibre length: width ratio and root bark fibre length times width in *Haematostaphis barteri* and root bark fibre length: width ratio in *Pseudocedrela kotschyi* Statistical analyses of the stem and root bark fibres of the four plants are presented in Appendix 4

18: Comparison of root and stem bark fibres (mean ± S.E. M) of *Cassia sieberiana* and *Haematostaphis barteri* in three populations of the

iau Community Hippopotamus Sanctuary Ghana

acters	*Cassia sieberiana*			*Haematostaphis barteri*		
	Kantu area	Tankara area	Talewona area	Kantu area	Tankara area	Talewona area
[illegible] (mm)	0.97 ± 0.13	0.83 ± 0.044	0.87 ± 0.026	0.97 ± 0.064	1.04 ± 0.026	1.08 ± 0.021
[illegible] (mm)	0.021 ± 0.00088	0.036 ± 0.018	0.016 ± 0.0013	0.023 ± 0.0088	0.025 ± 0.00033	0.023 ± 0.0012
[illegible] x RBFW (mm^2)	0.022 ± 0.0017	0.020 ± 0.005	0.015 ± 0.0015	0.027 ± 0.0067	0.027 ± 0.0012	0.024 ± 0.0023
RBFL: RBFW ratio	42.2 ± 2.79	52.6 ± 5.47	56.0 ± 5.9	43.87 ± 2.17	38.18 ± 2.41	51.67 ± 0.09
SBFL (mm)	1.00 ± 0.032	1.03 ± 0.018	0.93 ± 0.082	1.06 ± 0.021	1.20 ± 0.022	0.89 ± 0.094
SBFW (mm)	0.030 ± 0.0065	0.015 ± 0.0067	0.018 ± 0.0023	0.026 ± 0.00012	0.029 ± 0.00058	0.037 ± 0.0087
SBFL x SBFW (mm^2)	0.023 ± 0.00058	0.02 ± 0.0012	0.023 ± 0.00033	0.026 ± 0.0017	0.075 ± 0.002	0.025 ± 0.0015
SBFL: SBFW ratio	45.4 ± 2.87	49.4 ± 1.55	46.0 ± 2.5	33.18 ± 1.31	37.69 ± 1.05	34.13 ± 4.56

Key to table; RBFL; root bark fibre length, RBFW; root bark fibre width, RBFL x RBFW, root bark fibre length times width, RBFL: RBFW root bark fibre length: ratio

SBFL; stem bark fibre length, SBFW, stem bark fibre width, SBFL x SBFW; stem bark fibre length times width, RBFL: RBFW stem bark fibre length: width ratio

19: Comparison of root and stem bark fibres (mean ± S.E M) of *Mitragyna inermis* and *Pseudocedrela kotschyi* in three populations from

iau Community Hippopotamus Sanctuary in Ghana

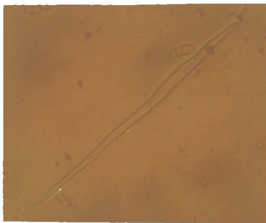
acters	*Mitragyna inermis*			*Pseudocedrela kotschyi*		
	Kantu area	**Tankara area**	**Talewona area**	**Kantu area**	**Tankara area**	**Talewona area**
[illegible] (mm)	1 04 ± 0.089	1 18 ± 0.047	1.10 ± 0.026	0 98± 0.044	1.17 ± 0.048	1.10 ± 0.026
[illegible] W (mm)	0.022 ± 0.0037	0.031 ± 0.015	0.029 ± 0.0055	0.024 ± 0.0033	0.020 ± 0.0023	0.019± 0.026
[illegible] L x RBFW (mm^2)	0.020 ± 0.0029	0.023 ± 0.0035	0.039 ± 0.0086	0.023 ±0.0021	0.023 ± 0.0036	0.021 ± 0.025
[illegible]L: RBFW ratio	58 5 ± 5.76	67.2 ± 6.24	66 2 ± 4.78	42.07 ± 3.87	66.14 ± 6 14	64.95 ± 4.7
SBFL (mm)	1.07 ± 0.083	1.26 ± 0.053	1.21 ± 0 0088	1.07 ±0.085	1.20 ±0.087	1.06 ± 0.068
SBFW (mm)	0.021 ± 0.001	0.022 ± 0.00067	0.022 ± 0.0018	0.021 ± 0.0017	0.022 ± 0.0017	0.022 ± 0.0013
SBFL x SBFW (mm^2)	0.022 ± 0.0032	0.027 ± 0.0015	0.046 ± 0.022	0.023 ± 0.004	0.025 ± 0.0029	0.026 ± 0.0035
SBFL: SBFW ratio	57 5 ± 5 01	61.9 ± 1.88	56.4 ± 4.26	58.55 ± 5 77	52.33 ± 3.67	50.97 ± 5.88

Key to table; RBFL, root bark fibre length, RBFW; root bark fibre width, RBFL x RBFW, root bark fibre length times width, RBFL RBFW root bark fibre length: ratio

SBFL; stem bark fibre length, SBFW, stem bark fibre width, SBFL x SBFW; stem bark fibre length times width, RBFL: RBFW stem bark fibre length:width ratio.

Plate 11 Micrograph of stem and root bark fibres of species.

(a) Stem bark fibre of *Cassia sieberiana*



(b) Root bark fibre of *Haematostaphis barteri*

Scale: 1 cm = 30μm

(c) Stem bark fibre of *Mitragyna inermis*.

(d) Root bark fibre of *Pseudocedrela kotschyi*

Scale: 1 cm = 30μm

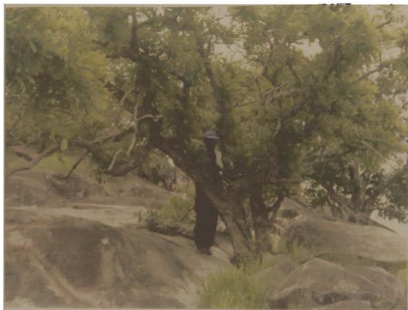
3.3.3.0 Ecological and distributional studies

3.3.3.1. Habitats of species in sanctuary

The habitats of the four species varied in the sanctuary. *Cassia sieberiana* was found in the Riverine forest vegetation, *Haematostaphis barteri* in rocky areas, *Mitragyna inermis* in floodplain and *Pseudocedrela kotschyi* in the gravely parts of the higher ground vegetation. A typical habitat of the *Haematostaphis barteri* in the sanctuary is presented in Plate 12.

The distribution of the four species also varied in the three populations of the sanctuary. In particular, *Cassia sieberiana* and *Haematostaphis barteri* were widely distributed in Talewona area of the sanctuary. The GPS data on geographical distribution and elevation of individual plants of the four species in the sanctuary are presented in Appendix 5. From the study of the individuals in the different populations in the sanctuary the habitats of each species did not varied significantly among the three populations in the sanctuary

Plate 12: A typical habitat of *Haematostaphis barteri* in the Wechiau Community Hippopotamus Sanctuary in Ghana



3.3.3.2. Phenological studies

The phenological stages of the four species including formation of new leaves, leaf shedding, flowering period and fruiting period were studied. *Cassia sieberiana* flowered during February - March, May - June and September - October. However, fruiting in *Cassia sieberiana* begun in late October and continued until December when the fruits started to drop. Fruits were continuously dispersed until February. New leaf formation usually starts after flower development. This plant was defoliated during flowering periods and new leaf formation starts soon after flowering.

With *Haematostaphis barteri*, flowering was observed between February and May when the entire plant was defoliated. Fruits started to develop in late May. The fruiting period continued through to August. It was difficult to determine the period when the fruits were shed because fruits were plucked and eaten by the local people before they were shed by the plants. The development of new leaves in *Haematostaphis barteri* started in late February after flowers have developed and continued until later July. The leaves were shed in October until late February.

Flower development in *Mitragyna inermis* was observed twice in a year. The development of new flowers begun in May and flowers developed into fruits in July. The next flowering period started in September and fruits were formed in late October or November. These fruits fell between December and January. Formation of leaves in *Mitragyna inermis* begun in March after the species was defoliated. Leaf activity continued until July or August. The leaves were shed in late November and shedding continued until late February.

ı *Pseudocedrela kotschyi* flowering was observed between February and March. The owers developed into fruits in May or June and fruits were shed in July. New leaves arted forming on this plant in February and continued until July. The leaves were shed in ctober until February.

he time of flowering varied in *Cassia sieberiana*, *Haematostaphis barteri* and *seudocedrela kotschyi* among the three populations in the sanctuary. In particular *Cassia eberiana* and *Pseudocedrela kotschyi* flowered earlier in Kantu area of the sanctuary. *eamatostaphis barteri* was also observed to have a longer flowering period in Talewona ea of the sanctuary. There were however no observed variability in both new leaf rmation and shedding among populations of the sanctuary.

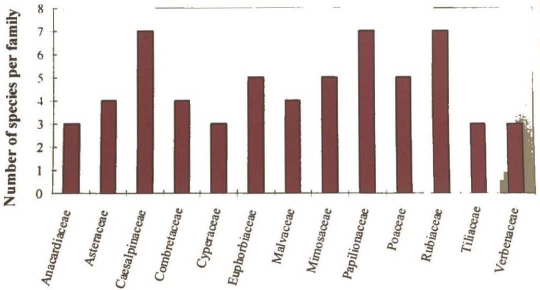
3.3.3. Species association studies

pecies associated with the four selected species of plant in the sanctuary are presented in ppendix 6. The associated species belong to 31 families. The most predominated families ere Caesalpinaceae, Papilionaceae and Rubiaceae (Figure 15). Out of the 85 species entified, 8 (9.41%) of them were found associated with all the four anti-malarial species. nese species included *Amorphophallus drancontoides*, *Andropogon contortus*, *Cassia ımmosoides*, *Hibiscus asper*, *Stylochiton lancifolia*, *Synedrella nodiflora* and *Tephrosia atycarpa*.

owever, species associated with only each of the four species were also observed. For ample, *Cola laurifolia*, *Paullinia pinnata* and *Cardiospermum grandiflorum* were only sociated with *Cassia sieberiana*. The species associated with the four selected pants in [illegible] hree populations in the sanctuary and

percentage occurrence of the total number of quadrats studied are presented in Appendix 7-10

the four anti-malarial plants in Wechiau Community Hippopotamus Sanctuary Ghana.



The diversity of species associated with the four species of plant among the three populations of the sanctuary is presented in Table 20. The kinds of species associated with the four species of plant varied among the three populations in the sanctuary. In particular, there was a large inter-population diversity of species associated with *Haematostaphis barteri* and *Pseudocedrela kotschyi* among the three populations. The least diversity of associated species among populations was found with *Mitragyna inermis*.

Table 20: Diversity of species associated with anti-malarial species in Wechiau Community Hippopotamus Sanctuary Ghana

Species	Inter-population diversity (IPD)			Mean
	Kantu-Talewona	Kantu-Tankara	Talewona-Tankara	
Cassia sieberiana	0.71	0.56	0.77	0.68 ± 0.12
Haematostaphis barteri	0.87	0.79	0.92	0.86 ± 0.066
Mitragyna inermis	0.49	0.55	0.12	0.39 ± 0.23
Pseudocedrela kotschyi	0.45	0.83	0.94	0.75 ± 0.26

.3.3.4. **Soil particle size composition**

he composition of soil particle size varied among the three vegetation types as well as mong the three different populations of the sanctuary. The soils of the Riverine forest egetation contained the largest amounts of clay and silt, and the least amount of sand. In ontrary, the higher ground vegetation contained the largest amount of sand and least mounts of silt and clay. The soils of the flood plain vegetation also contained more sand articles than the Riverine forest vegetation but less clay and silt particles

he largest amounts of clay and silt particles in the three populations of the sanctuary were ound in soils from Kantu area. In contrast, the largest amount of sand particles in the anctuary was found in the soils of Talewona area. The soils of Tankara also contained nore clay and sand particles than that of the Talewona area. Details of the result of the soil article size analysis are presented in Table 21.

Table 21 Soil particle size composition of three vegetation types from three populations in the Wechiau Community Hippopotamus Sanctuary Ghana

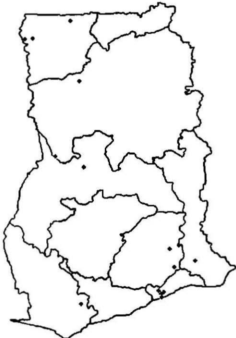
Vegetation type	Population	Sand (%)	Clay (%)	Silt (%)
Riverine Forest	Kantu area	16.11	58.93	23 21
	Talewona area	14.32	64.29	19.64
	Tankara area	7.62	70.69	20.69
Flood plain	Kantu area	11.71	62.50	23 21
	Talewona area	44 24	46.00	6.00
	Tankara area	40.65	48.08	9.62
Guinea Savanna	Kantu area	54 25	39 06	6.25
	Talewona area	66.55	32.76	0.20
	Tankara area	63 43	30.36	5.36

.3.3.5. Distribution of anti-malarial species in Ghana

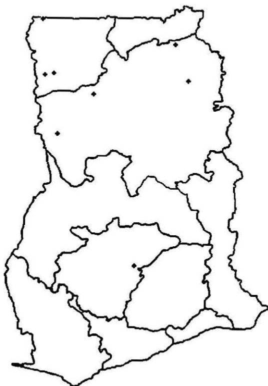
'he distributions of the four species of plant in Ghana are presented in Maps 2- 5. In eneral, *Cassia sieberiana* and *Mitragyna inermis* of the four anti-malarial species were ridespread in Ghana occurring in all parts of the country except in the forest. The two ther anti-malarial species, namely *Haematostaphis barteri* and *Pseudocedrela kotschyi* vere restricted to the northern parts of Ghana. The only specimen of *Pseudocedrela otchyi* found in the coastal area of Ghana was a specimen collected by Akpabla, G. K., at Achimota with collection number 1865. The specimen of *Haematostaphis barteri* collected y Adams, C. D at Agogo Ashanti with collection number 2560 was found to be seedling nd might be a misidentification

Details on the distribution of the four anti-malarial species in Ghana in relation to protected reas, habits, protected areas and habitats, elevation and rainfall could be located at http: www.unep-wcmc.org/species/plants/ghana/map.htm.

Map 2: Current distribution of *Cassia sieberiana* in Ghana



Map 3: Current distribution of *Haematostaphis barteri* in Ghana



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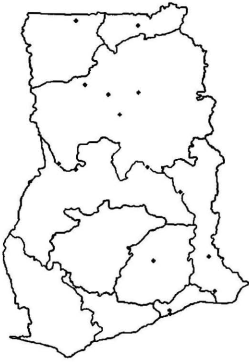
Map 4: Current distribution of *Mitragyna inermis* in Ghana



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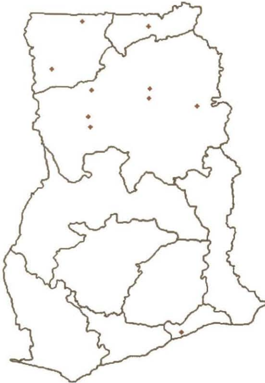
Map 5 Current distribution of *Pseudocedrela kotschyi* in Ghana



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3.5.0. Comparative phytochemical studies

3.5.1. Phenolic compounds in species

The results from 80 % methanol extraction of samples of the four species of plant are presented in Tables 22- 23. The average percentage yield varied among the plant parts of the same species. The extracts from the leaves on average produced the highest yield of the material extracted.

Typical HPLC profiles of leaf, stem bark and root bark of the four plants are presented in Appendix 12-15. No qualitative variability was observed in the HPLC profiles of *Cassia sieberiana*, *Haematostaphis barteri* and *Pseudocedrela kotschyi* among the different populations of the sanctuary. However, in *Mitragyna inermis*, the HPLC profiles of the root from Tankara area was different to that of the two other populations.

The four species of plant contained various phenolic compounds especially flavonoids. In *Cassia sieberiana*, *Mitragyna inermis* and *Pseudocedrela kotschyi*, the leaves, stem bark and roots contained flavonol glycosides. In addition, the stem bark and roots of *Mitragyna inermis* contained caffeic acid derivates. The leaf of *Haematostaphis barteri* also contained both flavone-*C*-glycosides and flavone-*O*-glycosides, and ellagi-tannins. The stem and root bark in addition to flavonoids contained stilbene compounds. The main flavonoid compounds identified from the leaves of the four plants are presented in Table 24. Positive ion APCI-MS data of the compounds are presented in Appendix 16-19

...le 22. Summary of results of extraction of *Cassia sieberiana* and *Haematostaphis barteri* samples in 80% methanol for HPLC analysis of ...vonoids and other phenolics

...aracters	Population	*Cassia sieberiana*			*Haematostaphis barteri*			
		Weight extracted (g)	Weight of extract (g)	% of weight extracted	Weight extracted (g)	Weight of extract (g)	% of weight extracted	Weight extracted (...
...f	Kantu	9.96	0.9115	9.15	10.19	0.33	3.61	10.19
	Talewona	9.47	0.7885	8.33	10.74	0.91	8.45	10.74
	Tankara	9.65	1.2537	12.99	9.92	0.32	3.22	9.92
...n bark	Kantu	4.90	0.4994	10.19	-	-	-	-
	Talewona	4.62	0.7936	17.18	5.58	0.16	2.92	5.58
	Tankara	5.38	0.5160	9.59	4.74	0.33	7.06	4.74
Root bark	Kantu	3.73	0.4759	12.76	3.05	0.057	1.89	3.05
	Talewona	3.03	0.4521	14.92	3.07	0.038	1.20	3.07
	Tankara	3.41	0.2805	8.23	3.49	0.036	1.03	3.49

ıe 23 Summary of result of extraction of *Mitragyna inermis* and *Pseudocedrela kotschyi* samples in 80% methanol for HPLC analysis of

ɔnoids and phenolics

racters	Population	*Mitragyna inermis*			*Pseudocedrela kotschyi*			
		Weight extracted (g)	Weight of extract (g)	% of weight extracted	Weight extracted (g)	Weight of extract (g)	% of weight extracted	Weight extracted (g
	Kantu	9 72	1 76	18.13	10 44	2 53	24.19	10 44
	Talewona	10.24	1.30	12.67	9.61	2.38	24.76	9.61
	Tankara	9.89	1.16	11.71	10.04	1.95	19.40	10.04
bark	Kantu	3.48	0.13	3.77	4.16	0.10	2 50	4 16
	Talewona	4.06	0.43	10.51	4.05	0.34	8 48	4.05
	Tankara	4.13	0.19	4.67	4 91	0.43	8.82	4.91
Root bark	Kantu	3 18	0.078	2.44	3.20	0 10	3.15	3.20
	Talewona	3 10	0.42	13.38	3.14	0.098	3 11	3.14
	Tankara	2.74	1.87	68.14	3.26	0 15	4.65	3.26

... flavonoid compounds identified from *Cassia sieberiana*, *Haematostaphis barteri*, *Mitragyna inermis* and *Pseudocedrela kotschyi*.

...t	Compound number	Rt (min) HPLC	UV HPLC	Rt (min) LC-MS	[M + H]⁺	[I + H]⁺	[A + H]⁺	Identification
...sia sieberiana	1	12.61	261, 352	9.01	465	-	319	Myricetin 3- rhamnosi...
(... 12237)	2	14.75	256, 351	10.48	449	-	303	Quercertin 3- rhamnosi...
...matostaphis barteri	1	11.79	268, 338	8 37	433	n.d	n.d	Vitexin
(... 2241)	2	12 66	270, 339	8.72	579	433, 415, 313	-	Vitexin or isovetixin O-glucoside
...gyna inermis	1	4.80	300sh, 326	5.70	355	-	163	Chlorogenic acid
(... 12245)	2 (mixture of 2 flavonoids	13.19	256, 355	9.34	465 611	- 465	303 303	Quercetin 3- glucoside ... galatoside / quercetin 3-rhamnosylglucoside
Pseudocedrela kotschyi (BI 12247)	1	10.20	259, 356	8.15	481	-	319	Myrecetin 3- glucoside galatoside
	2	11.63	260, 322	8 96	465	-	319	Myrecetin -rhamnosi...
	3	13.95	257, 350	10.48	449	-	303	Quercetin 3-rhamnosi...

4.5.2 Detection and identification of alkaloids in species

The result of detection of alkaloids in species using Dragendorff spray indicated the presences of alkaloid in only the basic chloroform layer of leaf of *Mitragyna inermis* from Talewona area of the sanctuary. The LC-MS analysis did show the mass of any known alkaloids in samples of *Cassia sieberiana*, *Haematostaphis barteri* and *Pseudocedrela kotschyi*. However, alkaloid compounds were identified from samples of *Mitragyna inermis* by comparing their molecular mass with standards.

Four major alkaloid compounds were identified from *Mitragyna inermis*. These compounds included isorchyncophylline (*m/z* 84), ciliaphylline (*m/z* 414), rhyncophylline N-oxide (*m/z* 400) and rotundifoline N-oxide (*m/z* 9416). The distribution of the alkaloids in different parts of *Mitragyna inermis* are presented in Table 25.

Table 25: Distribution of alkaloids in leaf, stem bark and root of *Mitragyna inermis*

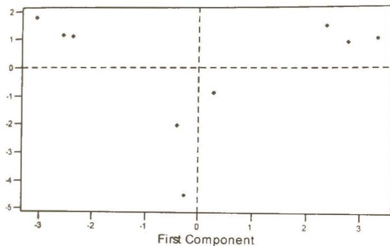
Compounds	Molecular weight	Plant parts		
		leaf	Stem bark	root
Isorchyncophylline [$C_{22}H_{28}N_2O_4$]	384	+	+	+
Ciliaphylline [$C_{23}H_{30}N_2O_5$]	414	+	–	+
Rhyncophylline N-oxide [$C_{22}H_{28}N_2O_5$]	400	+	–	–
Rotundifoline N-oxide [$C_{22}H_{28}N_2O_6$]	416	+	–	–

3.5.3. Chemical variability in species among populations in sanctuary

The HPLC analysis of peaks (compounds) from the four species showed variations in the relative proportion of peaks among the three populations of the sanctuary In order to understand the peaks responsible for most of the variability and the patterns of variations, peaks areas were subjected to Principal Component analysis.

In *Cassia sieberiana*, seventeen peaks were examined (Appendix 15). These peaks were selected from the leaf (peaks 1-6), stem bark (peaks 7-12) and roots (peaks 13-17). The PCA analysis of the peaks partitioned the variation into three distinct groups corresponding to the three populations of the sanctuary (Figure 16). The eigen analysis of correlation matrix and factor loading of component are presented in Table 26. The First Principal Component (PCI) was weighted by peaks 2, 3, 8 and 11. The PC2 also correlated with peaks 5, 9, 13, 14 and 17 and PC3 was explained by peaks 4 and 10.

Figure 14 PCA plot of peak areas of phenolics in *Cassia sieberiana* from three populations in Wechiau Community Hippopotamus Sanctuary.



atrix and factor loading of 17 peaks areas of phenolics in *Cassia sieberiana*.

Eigenvalue	5.7639	4.3729	2.5450
Proportion	0.339	0.257	0.150
Cumulative	0.339	0.596	0.746
Peaks	**PC1**	**PC2**	**PC3**
1	0.256	0.067	-0.108
2	0.396	-0.060	-0.023
3	0.398	-0.086	0.060
4	-0.189	0.086	-0.292
5	0.016	-0.386	-0.345
6	0.144	-0.306	-0.389
7	0.203	0.094	0.018
8	0.363	0.215	-0.081
9	0.369	0.205	-0.082
10	-0.144	0.032	-0.524
11	0.381	0.166	-0.050
12	0.055	-0.378	-0.355
13	0.123	-0.399	0.226
14	-0.140	-0.023	0.176
15	0.100	-0.386	0.237
16	0.183	0.072	-0.059
17	0.082	-0.390	0.264

with *Haematostaphis barteri*, eight peaks selected from leaf (peaks 1- 6) and root (peaks 7- 8) was examined for patterns of quantitative among populations in the sanctuary (Appendix 16). The spatial relationship of the peak areas are presented in Figure 17. Three distinct grouping were observed but were not as tight as observed in *Cassia sieberiana*. The PC1 was correlated with peaks 1- 5 which where selected from the leaf. The PC2 correlated with peak 7 from root bark. The PC3 with was explained by peak 6 and 7 (Table 27). Thus the peaks from leaf were responsible for the variability among populations in the sanctuary in *Haematostaphis barteri*.

Figure 15: PCA plot of peak areas of phenolic from *Haematostaphis barteri* in three populations in Wechiau Community Hippopotamus Sanctuary.

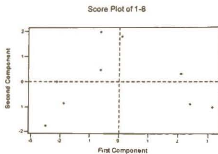


Table 27: Eigen analysis of correlation matrix and factor loading of 8 peak areas of phenolics in *Haematostaphis barteri*.

Eigenvalue	5 9072	1 6365	1 2129
Proportion	0 656	0.182	0.135
Cumulative	0.656	0.838	0.973
Peaks	**PC1**	PC2	**PC3**
1	0.454	-0.212	-0.099
2	0.439	-0.274	-0.153
3	0.294	0.146	0.220
4	0.434	0.025	0.396
5	0.462	-0.177	0.040
6	-0 174	0 493	0.677
7	-0.068	-0.644	-0.505
8	-0.278	-0.411	0.214

Ten peaks were from HPLC profiles of *Mitragyna inermis* were used for investigations The peaks their retention time (min) and UV max (nm) examined in this plants are presented in Appendix 17. The variability in the peaks areas was also partitioned among the three populations of the sanctuary The spatial relationships of the peak areas revealed by PCA are presented in Figure 18. The PCI was explained by peaks 1-3, and 5. The PC2 correlated with peaks 4 and 5, and PC3 also correlated with peaks 9 and 10. The eigenvalue and correlation matrix of the peaks are presented in Table 18. Thus the peaks from the leaf and stem bark explained much of the variability in *Mitragyna inermis*

Figure 10 PCA plot of peak areas of phenolics from *Mitragyna inermis* in three populations in the Wechiau Community Hippopotamus Sanctuary Ghana

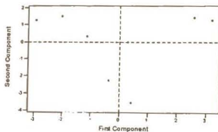


Table 28. Eigen analysis of correlation matrix and factor loading of 10 peak areas of phenolics in *Mitragyna inermis*.

Eigenvalue	4.4517	3.6319	0.8056
Proportion	0.445	0.363	0.081
Cumulative	0.445	0.808	0.889
Peaks	PC1	PC2	PC3
1	0.384	-0.265	-0.184
2	0.412	-0.200	-0.299
3	0.375	-0.291	-0.225
4	0.096	-0.403	-0.038
5	-0.417	-0.216	-0.006
6	-0.367	-0.282	-0.159
7	-0.374	-0.291	-0.127
8	-0.284	-0.062	-0.701
9	-0.063	-0.424	0.496
10	0.030	-0.497	0.212

In *Pseudocedrela kotschyi*, 12 peaks were studied. The information on the peaks is presented in Appendix 18. The spatial relationships of the peak areas showed little partitioning with samples from Talewona area most widely dispersed in both PCA and cluster analysis (not shown). The PCA plot of the peak areas is presented in Figure 19. The PC1 was weighted by peaks 8-10, PC2 was explained by peak 3, 6, 10, 11 and PC3 correlated with peaks 3-5 and 10-12 (Table 29)

Figure 17. PCA plot of peak areas of phenolics from *Pseudocedrela kotschyi* in three populations in Wechiau Community Hippopotamus Sanctuary Ghana.

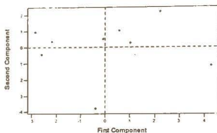


Table 29. Eigen analysis of correlation matrix and factor loading of 12 peak areas of phenolics in *Pseudocedrela kotschyi*

Eigenvalue	5.4942	2.9004	1.8885
Proportion	0.458	0.242	0.157
Cumulative	0.458	0.700	0.857
Peaks	**PC1**	**PC2**	**PC3**
1	-0.270	-0.133	-0.534
2	-0.274	0.019	-0.540
3	-0.164	-0.428	0.181
4	-0.380	-0.151	0.145
5	-0.379	-0.237	0.090
6	-0.213	0.310	0.230
7	-0.413	0.062	-0.126
8	0.351	-0.039	-0.073
9	0.188	-0.484	0.182
10	0.068	0.469	0.066
11	-0.262	0.398	0.205
12	-0.299	-0.069	0.456

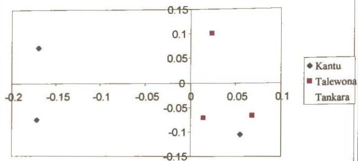
3.6. Molecular studies (AFLP Markers)

The AFLP technique requires high quality DNA samples. The poor quality of DNA extracted from *Heamatostaphis barteri* and *Mitragyna inermis* was not suitable for AFLP analysis. Only DNA samples extracted from individuals of *Cassia sieberiana* and *Mitragyna inermis* could be used for complete AFLP analysis.

In *Cassia siebreina*, a total of 135 fragments were obtained of which 23.53 % were polymorphic, that is, varied between two or more individuals. The number of unique bands for each population was 6 for Kantu area, 1 for Talewona area and 3 for Tankara area. The four vectors of Principal Coordinate (PCO) explained 90.36 % of variation (Figure 20). The eigenvalues and their percentage of variance are presented in Table 30.

There was low diversity in individual within Tankara area than the other two populations Individuals from Kantu area were generally distantly isolated from Talewona and Tankara area. The neighbour joining analysis revealed similar partitioning as PCO. Deviant individuals were found among populations (Figure 20).

Figure 18: Principal coordinate (PCO) plot of *Cassia sieberiana*



With *Pseudocedrela kotschyi*, 111 fragments were obtained and 38.74% of the fragments were polymorphic. The number of unique fragment to each population ranged 10 for Kantu area, 1 for Talewona area and 4 for Tankara area. The result of PCO analysis is summarised in Figure 21 Much of the variation (93.18 %) was explained by the first five vectors. Eigenvalues and their percentage variance are presented in Table 30. The variation in individuals within populations was generally higher than among populations. In particular, there was a high genetic diversity in individuals of Kantu area. The result of unrooted phylogram produce from neighbour analysis revealed similar pattern to that of PCO.

Figure 19: Principal Coordinate plot of *Pseudocedrela kotschyi*

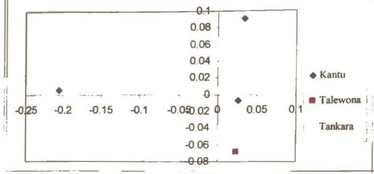


Table 30 Eigen values and percentages of variance for PCO analysis of AFLP data

Cassia sieberiana		*Pseudocedrela kotschyi*	
Eigenvalues	**% of variance**	**Eigenvalues**	**% of variance**
0.078	43.36	0.050	56.70
0.050	27.99	0.014	15.57
0.016	10.14	0.011	12.69
0.011	8.89	0.0056	8.42

Figure 20. Unrooted phylogram of *Cassia sieberiana* from AFLP data

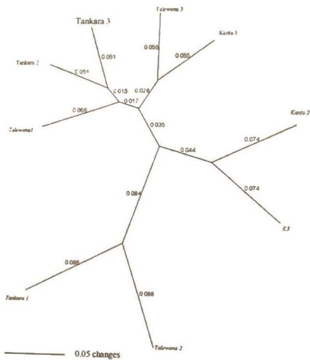
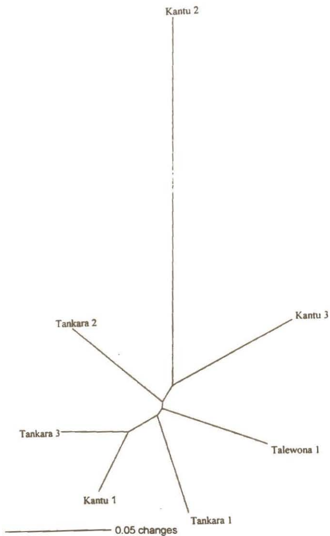


Figure 21. Unrooted phylogram of *Pseudocedrela kotschyi* from AFLP data



PTER FOUR

DISCUSSION

4.1. Ethnobotanical studies

The present survey has provided information about the range of species of plants used in the treatment of malaria in the Wechiau Community Hippopotamus Sanctuary area. The species used in the treatment of malaria represent 19.5 % of the 210 species reported for the sanctuary (Oteng-Yeboah and Asase, 2001).

The use of a combination of different methods of interviews has led to the identification of 41 species of plants used in the sanctuary to treat malaria. The study has also shown how the method of survey can influence the scope of information obtained about the uses of species. Interviews based on plants collected by persons being interviewed identified the least number of species whereas the house - to - house interviews identified the most species. The field interviews were the most effective time limited method because of the fewer number of people involved in the interviews when compared to the house - to- house interviews. Field interviews have proved very successful in other ethnobotanical studies in Ghana (Oteng-Yeboah, 1999).

Most of the species used to treat malaria in the sanctuary are known to be anti-malarial plants and thus corroborate data from many other sources including Irvine (1961), PORSPI (1982), Ampofo (1983), Dokosi (1998), Ayitey-Smith (1989), Abbiw (1990), ITC (1990), and Mshana et al., (2001). The study has, however, also identified and documented for the first time eight plant species namely, *Afraegle paniculata, Haematostaphis barteri, Indigofera pulchra, Monathotaxis* sp., *Ozoroa insignis, Strychnos innocua, Strychnos spinosa* and *Xeroderris stuhlmannii* which are used in the treatment of malaria in Ghana.

Plant species used in the treatment of malaria in the sanctuary area were derived from very diverse plant groups. However, taxonomically important families containing plants used to treat malaria in the sanctuary included Anacardiaceae, Caesalpinaceae, Papilionaceae, Asteraceae and Rubiaceae The contribution of species from these plant families in the treatment of various illnesses and diseases is well known in Ghana (PORSPI, 1982, Mshana et al., 2001) Many species from Rubiaceae and Asteraceae including *Cinchona* and *Artemisia annua* have been investigated for their antiplasmodial activity (Srisilam and Veersham, 2003). The families with many species used for the treatment of malaria have very little phylogenetic relationships (Chase et al., 1998; Takhtajan, 2001).

The method of preparation and mode of administration of the plant species for the treatment of malaria were mostly done by boiling and drinking. However, none of the people interviewed provided any information about how they might "standardize" treatments and the amounts used were generally vague. Thus the quality could vary greatly among prescriptions. This lack of standardization and quality control has been reported as one of the main disadvantages of traditional medicine (Evans-Anfom, 1986, Sofowora, 1987) Most of the species were also reported to be used as mixtures, which makes it more complex to standardize and investigate the active compounds in the mixtures.

The fact that the most common parts of the plant species used in the treatment of malaria were leaves and twigs together is very encouraging for sustainable harvesting of the plants. Root and bark harvesting could easily threaten local populations of plants (Cunningham, 2001). If a conservation strategy is to be developed for the sanctuary then, priority should be given to those anti-malarial plants with others uses and high preference ranking in the sanctuary. These species included *Cassia sieberiana, Mitragyna inermis, Haematostaphis*

barteri, *Pseudocedrla kostcnyi*, and *Indigofera pulchra*. These species will be among the most exploited species in the sanctuary area and are thus likely to be the most threatened. Plants earmarked as 'rare' in the present study could be cultivated as part of their home gardening strategy. The result of this study highlights the importance of identifying species that are used but are not abundant. This can assist with priotizing the development of sustainable harvesting strategies for species within the communities. Developing this is urgent since present observations had shown that the conservation strategy is very important as the people living in the sanctuary area were not fully aware of the fact that some of their medicinal plant species were becoming threatened or extinct.

Further work should be done on the comparative anti- malarial activity of the different species. Virtually, all groups of people in the sanctuary area rely on medicinal plants for the treatment of their illnesses and diseases since the nearest hospital is at Wa, about 42 km away accentuated by poor roads and means of transport. Plants species are used in many aspects of their lives including shelter, food, sculpture and medicine. The people in the sanctuary maximize the use of their flora in their day - to - day activities. It is important that the entire ethnoflora of the sanctuary is documented so that information about sustainable uses of plants is conserved.

4.2.0. Bioactivity Studies

4.2.1. *In vitro* antiplasmodial bioassay

The results of the *in vitro* antiplasmodial bioassay show that most of the extracts from the four species were not very active against the *Plasmodium* parasite. The poor activity of the extracts tested might be due to the fact that the samples did not dissolve fully in DMSO (the testing solvent). The most active extract was from the hexane extract of stem bark of *P. kotschyi*, a member of the Meliaceae. The anti-plasmodial activity of methanolic extract of leaves of *P. kotschyi* in a previous study showed modest inhibition of growth of Dd2 and 3D7 strains of *P. falciparium* with IC_{50} of 15 and 50 ug / ml respectively (Tahir et al, 1999). However, the hexane extract of *Pseudocedrela kotschyi* could contain the non-polar limonoids, which are the major active components in Meliaceae and have been reported to possess *in vitro* anti-malarial activity (Bray et al., 1990; Tahir et al., 1999; Bickii et al., 2000).

The various extracts from different parts of the three other species traditionally used to malaria only moderately inhibited the growth of *Plasmodium falciparum* (3D7). This lack of activity could be because the active compounds in the plant had not been extracted or the bioassay is not relevant to record the mode of actions of 'active' compounds in the extracts. The moderate activity of *Haematostaphis barteri* might be due to stilbenes which were the major compounds present in the roots and stem bark. Stilbenes have been found to possess anti-malarial properties (Boonlaksiri et al., 2000)

The IC_{50} values of *Mitragyna inermis* in the present study is generally similar to that obtained in previous studies (Traore-Keita et al., 2000; Traore et al., 2000, Mustofa et al.,

2000, Azas et al., [illegible] e, Traore et al. (2000) and Mustofa et al. (2000) reported IC_{50} values of 5 ug / ml and 45 ug / ml respectively for *M. inermis*.

The parts of the species used traditionally to treat malaria corroborated with the extracts with modest activity against *Plasmodium* parasite. For example, the most active parts of *P. kotschyi* and *M. inermis* were leaves which are the parts used to treat malaria in the sanctuary.

The species are traditionally used to treat malaria, however, they might be more active against symptoms associated with malaria rather than having anti-plasmodium activity. There are many reasons why the extracts might not have activity including the fact that the concentration of active compounds might be very low in the extracts or compounds are not active in the bioassay. Another reason for lack of activity could be associated with the fact that most of the species used to treat malaria in the sanctuary were prepared as mixtures and their activity might be dependent on the synergy among groups of compounds.

4.2.2. Anti-microbial bioassay

The four selected species where found to inhibit growth of micro-organisms. The anti-microbial activity against *Bacillus subtillis* and *Cladosporium herbarium* was mostly associated with the acetone and 50 % methanol extracts of stem barks and roots of the four species. In contrast, active extracts against *Pseudomonas syringae* were hexane extracts from leaves. This means that the anti-microbial properties of the species against *Bacillus subtillis* and *Cladosporium herbarium* were polar compounds found in the stem and roots whiles the active compounds against *Pseudomonas syringae* were non-polar compounds

located in the leaves of the species. Thus different types of compounds from different parts of the four species were responsible for the anti-microbial properties of the four species.

Most of the extract from the four species, however, inhibited the growth of *Bacillus subtilis*. Most plant extracts are known to inhibit growth of gram-positive bacteria compared to gram-negative and fungal growth inhibition (Testuo, *pers. com*). There were however, no previous report on the anti-microbial properties of *Cassia sieberiana Haematostaphis barteri* and *Pseudocedrela kotschyi*.

The potent anti-microbial activity of the four species used traditionally to treat malaria in the sanctuary might illustrate the fact that these species might rather be active against other human pathogenic micro-organisms which produce symptoms similar to malaria and not *Plasmodium*. This is because of the poor inhibition of growth against *Plasmodium* observed in the present study. In fact most people attribute all kinds of fevers to malaria infestations.

4.2.3. Insect anti-feedant bioassay

The four species were also found to have anti-feedant properties. The anti-feedant properties were observed with hexane extracts and this means that the compounds responsible for the anti-feedant properties of the species are non-polar compounds. These might include compounds such as quinones from *Cassia sieberiana* and limonoids from *Pseudocedrela kotschyi*. The anti-feedant properties of limonoids are well investigated (Ley al., 1988, Ley et al., 1989; Abdelgalcil et al., 2001). The anti-feedant activity of quinones has also been researched by Morimoto et al., (1999 and 2002) and Krishanakunari et al., (2001).

It thus also follows [illegible] responsible for the anti-feedant properties of the four species are better extracted in hexane. The acetone and 50 % methanol extracts on the other hand, contained compounds that stimulated feeding of the insect larvae.

The most significant insect anti-feedant activity was found in hexane extract of root of *Cassia sieberiana*. Species from the genus *Cassia* are known to possess biological activity against various insect pests (Lienard et al., 2003; Belmain et al., 2002). The variability in efficacy of *Cassia sieberiana* from the three different populations might be due to lost of activity since the extracts were not tested on the same day. Lost of anti-feedant activity with time has been observed in extracts of a related species, *Cassia tora* (Simmonds, *pers com*). In a previous study, Belmain et al. (2002) has also indicated that *Mitragyna inermis* used as post-harvest protectants in Ghana has some ability to control storage pest. This is, however, to the best of my knowledge the first report on the biological activity of extracts of *Haematostaphis barteri* and *Pseudocedrela kotschyi* against insects.

The anti-feedant properties of the four species varied depending on extract type and plant part. Only certain parts of each plant species have potential anti-feedant properties whiles other parts stimulated feeding. The anti-feedant properties of the species also varied among the three different populations of the sanctuary. Some of the extracts might have also lost their activity with time. This information is important when considering using botanicals for control of insect pest.

4.2.4. Isolation and identification of compounds from plants

It was not possible to determine the structures of peaks isolated from *Cassia sieberiana*. This could be due to the solvent composition used for the isolation which was more polar

for the non-polar hexane extract from the leaf of *Cassia sieberiana*. The peaks isolated could therefore contain several particles on the HPLC column that had the same retention time as the peaks. Thus the NMR spectrum (not shown in this report) of peak **1** (**KEW 785**) contained approximately 95 % contaminants and there was a trace of phenolic compound with two exchangeable OH protons at δ_H 12.02 and 12.12 ($CDCl_3$). The 1D ^1H NMR of **2** (**KEW 786**) indicates that the sample is most probably a common terpenoids.

The stem and root barks of *Haematotaphis barteri* contained stilbenes as the major compounds. The (*E*) isomer of 5- methoxy (*E*) reverastrol 3- *O* – rutinoside has only been previously identified by Wanjala and Majinda (2001) as a constituent of the root bark of *Elephantorrhiza goetzei* (Fabaceae). The (Z) isomer of the present study is an artefact. The other stilbenes from *Haematostaphis barteri* were (A) reverastrol rutinoside and the aglycone of stilbene **B**. The activity of *Haematostphis barteri* in the biological testing could therefore be due to stilbenes which are the main constituents of the stem and root bark. Stilbenes have been reported to be active against several organisms including *Plasmodium*, bacterial, fungi (Gorham, 1989; Boonslaksiri, et al., 2000).

4.3.0. Variation Studies

4.3.1. Morphological variations

The present study has shown morphological variability in the four species. The variabilities in the characters were quantitative and the variations were continuous. This kind of variation is not useful for delimitation of infraspecific groupings (Davis and Heywood, 1960; Pollard and Briggs., 1982). Of the four selected species, *P. kotschyi* and *M. inermis* were morphologically most variable.

Much of variability associated with morphological characters such as stem girth size, leaf length and plant height have been shown to be phenotypically plastic (Davis and Heywood, 1963, Pollard and Briggs, 1982). Given the fact that the present study was based on samples collected from populations of a small geographical area it is likely that a range of morphotypes will be recognised in the plants when specimens from their entire geographic range are examined.

The study has however shown diagnostic characters which will be useful for investigations of infraspecific taxonomy of the four species. For example, the characters of the leaf of *Cassia sieberiana* were more variable compared to the characters of the fruit as initially thought.

4.3. 2. Anatomical variations

The characters of fibres were less significantly variable compared to the morphological characters. This was not surprising since morphological characters are phenotypically more plastic as compared to anatomical characters (Essilfie, 2002). However, some levels of [illegible] morphologically more variable *Pseudocedrela kotschyi* as

well as *Haemalostaphis barteri*. Since features of fibres are generally stable, the micrographs and measurements of fibres of the four species could be used with other data for authentication of samples of the four species in the sanctuary.

4.3.3. Species habitat in sanctuary

The habitats of the four anti-malarial species in the sanctuary were different and similar to that described by Hutchinson and Dalziel (1954-72) and Irvine (1961) for each of the species. The variations in the habitats of a species among populations have been used to study the infraspecific taxonomy of species (Morton, 1967; Balkwill et al., 1994; Philips, 1994). In the present study, the habitat of the four species was not significantly different among the populations in the sanctuary. Thus the habitats of the selected species in the different populations in the sanctuary will therefore not contribute to biological separation of the plants, for instance, through attraction of different pollinators.

4.3.4. Phenological studies

Phenological variations are very important in the study of variation within populations of a species. For instance, Balkwill et al. (1994) used phenological variation to assign in infraspecific categories to *Justicia betonica* S. L. Differences in flowering time would significantly reduce gene flow among individuals within populations. The phenological variations of the four selected taxa were not significantly different among the three populations in the sanctuary. It is therefore possible for gene exchange among individuals of *C. sieberiana*, *H. barteri*, *M. inermis* and *P. kotschyi* within the populations in sanctuary. This possibility of exchange of genetic material among different populations will not lead to the recognition of distinct 'types' of the four species in the sanctuary.

The times of phenological stages observed in the four species were similar to that of previous reports (Hutchinson and Dalziel, 1954-1972; Irvine 1961, Keay et al., 1964). In contrary to the present observation for *Cassia sieberiana*, Ewusie (1968) reported that this plant fruited and produced new leaves throughout the year in the coastal plains of Ghana. It is possible that this species have patterns of phenological variation influenced by different environments. This could confirm the phenological plasticity of *C. sieberiana* in the sanctuary.

4.3.5. Species association studies

The association of plant with other species in its flora is important in understanding relationships such as symbiosis and competition which might exist among them. The relationship among plants could be structural, functional or chemical. The release of chemicals by one plant species that affect other species in its vicinity, usually to their detriment is termed allelopathy (Min An et al., 2003). Allelopathic substances are usually secondary plant metabolites such as gaseous compounds and terpenoids and they are release either through the air on soil

The present study showed a high diversity of species associated with the four selected plants varied among the populations in the sanctuary. In particular, there was a high inter-population diversity of species associated with *H. barteri* and *P. Kotschyi* among populations in the sanctuary. This high diversity of species associated with the four selected plant among populations could be responsible for some of the differences in the species among the populations. The allelopathic effect of plant growing in the vicinity of another plant has been reported. However, further ecological-biochemical studies are

required to verify this fact since none of the associated species are known exhibit allelopathy.

4.3.6. Soil particle size analysis

The composition of soil particles size differed among the three vegetations as well as populations of the sanctuary. Thus *Cassia sieberiana, Haematostaphis barteri, Mitragyna inermis* and *Pseudocedrela kotschyi* derived on different soil conditions in the sanctuary. Through contact with soil, plants may have to cope with toxic heavy metal or excess salinity. Equally plants have to be subjected to biochemical stress due to mineral deficiency in soil.

Lawson (1985) has also shown that soil factors are important in giving local variations to the savannas. Thus, differences in soil particle size composition (condition) among different populations of the sanctuary could affect the growth, functioning and chemistry of the four plants in the sanctuary. This difference in the soil conditions among populations of the sanctuary could also be responsible for some of the variations in the four plants

4.3.7. Distribution of anti-malarial species in Ghana

The current distribution of species in Ghana was based mainly on herbarium material. The production of species distribution maps based on herbarium material alone does not present a true picture of their distribution due to the way collections could be targeted. However, it does provide the some information needed to assess the conservation status and plan for future collections and conservation of the species. The distribution maps in the present study of the four anti-malarial species have shown that the four species could be found in

some of the protected areas in Ghana. Most of the species could be found in the Mole National Park which is the most developed Wildlife Reserve in Ghana. None of the four anti-malarial species is thus threatened in Ghana. The creation of the Wechiau Community Hippopotamus Sanctuary will be an additional refuge for these species in Ghana.

4.3.8. Comparative phytochemical studies of phenolics and alkaloids in species

The phytochemical studies have indicated the main phenolic constituents in the four species. Within a species, the diversity of compounds in the leaf, stem and root barks differed. This is could reflect the ecological role the compounds have in the plants or adaptation in the plants. The different parts of the plant will therefore not have the same efficacy in biological test as observed during the bioactivity investigations recorded in this study.

The HPLC profiles of the same organ from a species did not vary greatly among populations. However, the chemistry of the roots of *Mitragyna inermis* did vary. Takayama et al. (1998) has indicated that species of *Mitragyna* could show chemotypes in different areas. However, a larger number of individuals would have to be sampled in order to confirm the existence of chemotypes in *Mitragyna inermis* from the sanctuary.

The most common flavonoid compounds in the leaves of *Cassia sieberiana, Mitragyna inermis* and *Pseudocedrela kotschyi* were quercetin and myricetin glycosides. From *Cassia sieberiana*, previous phytochemical reports have only indicated the presences of condensed tannins, flavonoids and anthracene derivates in the leaf and root (Paris et al., 1967; Duquenois and Anton, 1968). From a petrol ether extract of roots of this plant, small amount of [illegible] were isolated (Waterman and Faulkner, 1979). No previous

phytochemical report on flavonoids ... *Mitragyna inermis* and *Pseudocedrela kotschyi* were found. In contrary, to the leaf constituent found in those three species, the leaves of *Haematostaphis barteri* contained vitexin and its glycosides.

The stem and root barks of *Haematostaphis barteri* contain stilbene compounds. Stilbenes have limited distribution in the plant kingdom (Gorham, 1989). This group of compounds are known to occur in the Orchidaceae, Combretaceae, Discoraceae, Hydranageaceae and Hepaticae. There are also reports of stilbenes in the families Cyperaceae and Moraceae. This is, however, the first report of stilbene compounds in the family Anacardiaceae. The distribution of stilbene compounds in the family Anacardiaceae could throw more light into the taxonomy of the group. Perhaps, further chemical studies on different species of Anacardiaceae could clarify the position of *Haematostaphis barteri* within the family Anacardiaceae

The detection of alkaloids in *Mitragyna inermis* was expected since they are common in the family Rubiaceae. Many phytochemical studies have been carried out on indole alkaloids from this species (Shellard and Sarpong, 1969, 1970; Shellard et al, 1971; Bishay, 1988, Takayama et al., 2000). Recently, Cheng et al. (2002) isolated two 27-nortriterpenoid glycosides, named inermside I and II, from *Mitragyna inermis*. Indole and oxindole alkaloids especially, rhynchophylline, rhyncholine, ciliaphylline, isorchynchophylline, rotundifoline, and isortundifoline have been identified from many species of *Mitragyna*. This group of alkaloids have also been identified from species of *Uncaria* and *Nauclea* (Philipson et al., 1973). Thus the presence of this group of alkaloid compounds in the three genera supports their taxonomic affinity in the sub-family Cinchoindeae of the family Rubiaceae. Although, there are many reports on the anti-

plasmodial activity of species of *Mitragyna* and *Nauclea* there is no previous report on anti-malarial properties of species of *Uncaria*. It must be worth investigating species of the genus *Uncaria* for their anti-malarial properties.

The only published phytochemical investigation on *Pseudocedrela kotschyi* dealt with limonoids (Niven and Taylor, 1987) and essential oils (Boyom et al., 2004). This group of triterpenoid compounds are common in the Meliaceae. The *in-vitro* plasmodial activity of *Pseudocedrela kotschyi* is mostly likely to be limonoids which are the active compounds in other species in the family Meliaceae. However further phytochemistry as they needs to be undertaken on the extracts to confirm this hypothesis.

4.3.9. Chemical variability in species among populations in sanctuary

The result of this study has shown variability in the relative proportions of the main peaks in HPLC traces of the four species. The relative proportions of the peaks (compounds) differed significantly among populations from the different areas of the sanctuary. The variability in the peaks was well partitioned during PCA analysis and correlated with the three populations of the sanctuary. Each sample of a species used for the analysis represents a typical prescription used by herbalist for the treatment of malaria in the sanctuary. If the differences in peaks (compounds) are associated with with differences in active compounds then the efficacy of prescriptions for the treatment of malaria will vary significantly depending on where the plant sample was collected within the sanctuary.

The variability in the relative proportions of the peaks among populations in the sanctuary could also indicate possible chemotypes in the sanctuary. However, a larger sampling intensity is required to profile the quantitative variations in the four plants from the

sanctuary. The peaks from leaves of the four species were responsible for most of the chemical variability among populations of the sanctuary

The physiological developmental stage of a plant can influence the proportion of secondary metabolism in different tissues within a plant. The variability observed among the populations could be because the sample contained individuals in different stages of their development, although attempts were made to collect only from mature plants.

Medicinal plants may be wittingly or unwittingly substituted by other samples, some of which may be toxic (Kite et al., 2002). Crude samples of medicinal plant might also be mixed with plants that look similar in appearance. For the authentication of herbal species their chemical infraspecific variability should be known (Vieira, et al., 2003). The HPLC profiles of the four species studied here could be used to authenticate herbal products made from the four species in the sanctuary. However, more information is required to correlate the profile of compounds in the plant with the activity of a plant before we can use this type of method to asses the quality of a species

4.3.10. Molecular studies (AFLP markers)

The result of AFLP showed high genetic diversity within population compared to among populations in the sanctuary in both *Cassia sieberiana* and *Pseudocedrela kotschyi*. Deviant individuals were always observed among groups in both the PCO and neighbour joining analysis. This could be due to extensive gene flow among the three populations of the sanctuary (Zawko et al., 2001). Thus the three populations of *Cassia sieberiana* and

Pseudocedrela kotschyi in the sanctuary area are not genetically isolated and individuals of plants in different populations exchange genetic material.

Generally, there were a high genetic diversity within individuals from Kantu area and this population was also genetically distant from the other two populations in the sanctuary. This could be because Kantu area is geographically farther from the other two populations in the sanctuary

4.3.11. Patterns and correlations of variability from all data sources

The morphological and phytochemical analysis of variability in individuals of *Cassia sieberiana* showed three groups for each species. These groups corresponded to the three populations in the sanctuary. However, only the *H. barteri*, *M. inermis* and *P. Kotchyi* showed as revealed by the PCA analysis. The use of morphological characters as descriptors separate to plants among the three populations in the sanctuary is difficult due to their continuous variations. Although, a limited number of samples were for the phytochemical analysis, the patterns of the variability as observed from PCA suggest that the samples collected from the three populations in the sanctuary are well differentiated. While the phytochemical analysis showed a good discrimination among population in the sanctuary, the use of secondary metabolites as infraspecific markers is influenced by factors such as environment, plant development and method used for extraction (Vieira et al., 2001). In order to use the variations in secondary metabolites as infraspecific markers in the sanctuary, a large number of individuals at the same stage of their development will have to be used for analysis

The AFLP markers were obtained randomly from the total DNA of *C. sieberiana* and *P. kotschyi*. However, the patterns of variability from the AFLP markers were correlated to

that of the morphological and phytochemical characters. Genetic variations among individuals within the same population were generally higher than intrapopulation genetic variations. Hence deviant individuals were observed among populations from both the PCO and neighbour joining analysis of the AFLP markers. Deviant individuals were also observed among populations during the PCA analysis of morphological characters in all the four plants. With this sampling in the present study, the variability among populations suggests that it is possible for gene exchange among the three populations in the sanctuary.

Thus the variability in morphological and chemical characters could be due to phenotypic plasticity. This is also confirmed by the fact that individuals from the three populations of the sanctuary flower at the same time. Phenotypic variability among populations of species was not surprising due to the differences in soil conditions and species associated with the four plants in the three populations in the sanctuary. It is possible that further sampling will confirm the phenotypic variability of the four species from different areas of the sanctuary

..APTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

The present investigation has identified a range of species used by an indigenous group of people in Ghana to treat malaria. The species of plants used for the treatment of malaria formed ca. 20 % of their entire flora and eight of these plants have not previously been investigated for their anti-malarial activity. This study has shown that there are still plants used by indigenous people to treat malaria which have not yet been investigated. This study has also shown that the scope of knowledge obtained about traditional uses of plants will depend on the methods used to interview people. The results have also indicated that species that are used to treat malaria are not the most abundant. Thus if their use was to increase, it is important that a strategy is drawn up for the conservation of these plants.

In this study, the biological activities of extracts of the four species used traditionally to treat malaria were not very active in the bioassay used to evaluate activity against the parasite. This means that further experiments using different bioassays should be undertaken to evaluate the use of *Cassia sieberiana, Haematostaphis barteri, Mitragyna inermis* and *Pseudocedrela kotschyi* for the treatment of malaria. However, it could be that the compounds in the extracts do not cure the disease but might have effects on symptoms associated with malaria

The extracts of these species were active against some micro-organisms and an insect pest. The people in the sanctuary had not reported that these plants had anti-insect and anti-microbial activity. This finding highlights the need to screen extracts of traditionally used medicinal plant species in different assays in order to fully evaluate their biological

activity. It is recommended that *in-vitro* antiplasmodial bioassay should be conducted on the other species of plant used to treat malaria in the sanctuary.

The result of the variations studies has shown that the four species selected for this study varied among different areas of the sanctuary. The variability in the four species was mainly quantitative and included a range of characters largely from morphology and chemistry. The differences in chemistry could mean that the efficacy of plant collected from different parts of the sanctuary to treat malaria will differ. The seasonal variability in the efficacy of the four species was not investigated in the present study. It is important that future studies should look at the seasonal variations in efficacy of the four plants in order to determine the period of optimal yield of the active compounds.

The variability in the species, at least, in *Cassia sieberiana* and *Pseudocedrela kotschyi* was not genetic as extensive gene flow among the different populations was observed. The variability in morphology, chemistry and efficacy of *Cassia sieberiana, Haematostaphis barteri, Mitragyna inermis* and *Pseudocedrela kotschyi* in the sanctuary was therefore due to differences in environmental factors. It is important that the entire sanctuary area is conserved in order to maintain the genetic diversity of the four species in the sanctuary. The present study has also demonstrated that the taxonomy of the four species in the sanctuary is stable.

It is hoped that the communities living in the sanctuary area as well as the Sanctuary Management Board (SMB), Non-governmental organisations (NGOs) especially Nature Conservation Research Centre (NCRC) and Earthwatch Institute, and institutions such as the Council for Scientific and Industrial Research (CSIR, Ghana) currently working in the

seful reference for the conservation and sustainable uses of plants in the sanctuary area. It is also believed that this thesis will serve as a general reference for people interested in the investigation of traditional uses of plants, their conservation and sustainable uses.

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Appendix 1: Sample of ethnobotanical data recording sheet

1 Sheet number. 2. Date

[A]. Source of information

1 Name of informant. 2. Sex

3 Age 4 Occupation

5 Ethnic background 6. Level of education

7 Location

8 Address / House number

[B] Medicinal Plant

1. Scientific name of plant (if known).

2 Local names of plant;

Local name	Ethnic group	Locality

3. Plant part(s) used

4. Other use(s) of plant

[C]. Methods of preparation and prescriptions

1. Methods of preparation

2. Quantity used

3. Methods of administration

4. Efficacy of preparation (if known)

5. Side effects (if known)

[D]. Conservation status.

1. Methods used in collecting plant ......

2. Areas where collections take place ..

3. Frequency of collection. .

4. Awareness of conservation

5. Attempts made to conserve

[E] Others.

[Appendix 3: One-way analysis of variance (ANOVA) and multiple range test analysis (where variations were significant only) of morphometric data]

3.1. *Cassia sieberiana*

Plant height:

ANOVA Table

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	2.73628	2	1.36814	0.80	0.4737
Within groups	20.6352	12	1.7196		
Total (Corr.)	23.3714	14			

Stem girth size:

ANOVA Table

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	0.63156	2	0.31578	2.32	0.1411
Within groups	1.63644	12	0.13637		
Total (Corr.)	2.268	14			

Number of leaflets per leaf:

ANOVA Table

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	48.6436	2	24.3218	11.22	0.0018
Within groups	26.0102	12	2.16752		
Total (Corr.)	74.6538	14			

Multiple Range Tests

Method 95.0 percent LSD

	Count	Mean	Homogeneous Groups
Tankara area	5	13.384	X
Kantu area	5	14.83	X
Talewona area	5	17.716	X

Contrast	Difference	+/- Limits
Kantu area - Talewona area	*-2.886	2.02877
Kantu area - Tankara area	1.446	2.02877
Talewona area - Tankara area	*4.332	2.02877

* Denotes a statistically significant difference

Leaflet length:

ANOVA Table

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	3.25737	2	1.62869	3.22	0.0757
Within groups	6.06136	12	0.505113		
Total (Corr.)	9.31873	14			

Multiple Range Tests

Method 95.0 percent LSD

	Count	Mean	Homogeneous Groups
Talewona area	5	5.282	X
Tankara area	5	5.928	XX
Kantu area	5	6.42	X

Contrast	Difference	+/- Limits
Kantu area - Talewona area	*1.138	0.979367
Kantu area - Tankara area	0.492	0.979367
Talewona area - Tankara area	-0.646	0.979367

* Denotes a statistically significant difference

Leaflet width:

ANOVA Table

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	0.79572	2	0.39786	2.25	0.1481
Within groups	2.12268	12	0.17689		
Total (Corr.)	2.9184	14			

Leaflet size

ANOVA Table

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	51.3191	2	25.6595	6.63	0.0115
Within groups	46.462	12	3.87184		
Total (Corr.)	97.7811	14			

Multiple Range Tests

Method: 95.0 percent LSD

	Count	Mean	Homogeneous Groups
Talewona area	5	12.722	X
Kantu area	5	15.058	XX
Tankara area	5	17.252	X

Contrast	Difference	+/- Limits
Kantu area - Talewona area	2.336	2.7115
Kantu area - Tankara area	-2.194	2.7115
Talewona area - Tankara area	*-4.53	2.7115

* Denotes a statistically significant difference.

Leaflet shape:

ANOVA Table

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	0.199773	2	0.0998867	0.75	0.4939
Within groups	1.6014	12	0.13345		
Total (Corr.)	1.80117	14			

Rachis length:

ANOVA Table

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	3.19545	2	1.59773	0.70	0.5146
Within groups	27.2884	12	2.27404		
Total (Corr.)	30.4839	14			

Petiolule length:

ANOVA Table

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	0.0177733	2	0.00888667	2.56	0.1189
Within groups	0.04172	12	0.00347667		
Total (Corr.)	0.0594933	14			

Multiple Range Tests

Method: 95.0 percent LSD

	Count	Mean	Homogeneous Groups
Tankara area	5	0.364	X
Kantu area	5	0.388	XX
Talewona area	5	0.446	X

Contrast	Difference	+/- Limits
Tankara area - Talewona area	*-0.082	0.0812517
Tankara area - Talewona area	-0.024	0.0812517
Talewona area - Tankara area	0.058	0.0812517

* Denotes a statistically significant difference

Calyx length:

ANOVA Table

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	0.0253333	2	0.0126667	0.27	0.7697
Within groups	0.568	12	0.0473333		
Total (Corr.)	0.593333	14			

Calyx width:

ANOVA Table

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	0.03052	2	0.01526	1.17	0.3430
Within groups	0.15632	12	0.0130267		
Total (Corr.)	0.18684	14			

Longest filament length:

ANOVA Table

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	0.0292133	2	0.0146067	0.04	0.9585
Within groups	4.12568	12	0.343807		
Total (Corr.)	4.15489	14			

Longest filament anther length:

ANOVA Table

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	0.004	2	0.002	0.33	0.7230
Within groups	0.072	12	0.006		
Total (Corr.)	0.076	14			

Fruit length:

ANOVA Table

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	470.729	2	235.365	1.08	0.3700
Within groups	2612.14	12	217.678		
Total (Corr.)	3082.87	14			

Fruit width:

ANOVA Table

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	0.442013	2	0.221007	6.86	0.0103
Within groups	0.38656	12	0.0322133		
Total (Corr.)	0.828573	14			

Multiple Range Tests

Method: 95.0 percent LSD

	Count	Mean	Homogeneous Groups
Kantu area	5	1.364	X
Talewona area	5	1.376	X
Tankara area	5	1.734	X

Contrast	Difference	+/-Limits
Kantu area - Talewona area	-0.012	0.247325
Kantu area - Tankara area	*-0.37	0.247325
Talewona area - Tankara area	*-0.358	0.247325

* Denotes a statistically significant difference

3.2. *Haematostaphis barteri*

Plant height:

ANOVA Table

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	3.17529	2	1.58765	5.71	0.0181
Within groups	33748	12	0.278123		
Total (Corr.)	77	14			

Multiple Range Tests

Method: 95.0 percent LSD

	Count	Mean	Homogeneous Groups
Tankara area	5	2.972	X
Kantu area	5	3.394	XX
Talewona area	5	4.088	X

Contrast	Difference	+/- Limits
Kantu area - Talewona area	-0.694	0.726724
Kantu area - Tankara area	0.422	0.726724
Talewona area - Tankara area	*1.116	0.726724

* denotes a statistically significant difference.

Stem girth size:

ANOVA Table

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	0.429956	2	0.214978	7.62	0.0084
Within groups	0.31028	11	0.0282073		
Total (Corr.)	0.740236	13			

Multiple Range Tests

Method: 95.0 percent LSD

	Count	Mean	Homogeneous Groups
Tankara area	5	0.48	X
Kantu area	5	0.558	X
Talewona area	5	0.9	X

Contrast	Difference	+/- Limits
Kantu area - Talewona area	*-0.342	0.247973
Kantu area- Tankara area	0.078	0.233791
Talewona area- Tankara area	*0.42	0.247973

* Denotes a statistically significant difference

Number of leaflets per leaf:

ANOVA Table

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	9.54292	2	4.77146	3.12	0.0810
Within groups	18.3431	12	1.52859		
Total (Corr.)	27.886	14			

Leaflet length:

ANOVA Table

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	2.45249	2	1.22625	1.58	0.2452
Within groups	9.29024	12	0.774187		
Total (Corr.)	11.7427	14			

Leaflet width:

ANOVA Table

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	0.00709333	2	0.00354667	0.07	0.9295
Within groups	0.57864	12	0.04822		
Total (Corr.)	0.585733	14			

Leaflet size:

ANOVA Table

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	17.1221	2	8.56105	0.62	0.5536
Within groups	165.34	12	13.7784		
Total (Corr.)	182.462	14			

Leaflet shape:

ANOVA Table

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	0.98752	2	0.49376	10.55	0.0023
Within groups	0.56144	12	0.0467867		
Total (Corr.)	1.54896	14			

Multiple Range Tests

Method: 95.0 percent LSD

	Count	Mean	Homogeneous Groups
Tankara area	5	2.484	X
Talewona area	5	2.684	X
Kantu area	5	3.1	X

Contrast	Difference	+/- Limits
Kantu area- Talewona area	*0.416	0.298066
Kantu area - Tankara area	*0.616	0.298066
Talewona area - Tankara area	0.2	0.298066

* Denotes a statistically significant difference.

Rachis length:

ANOVA Table

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	213.617	2	106.808	35.77	0.0000
Within groups	35.8313	12	2.98594		
Total (Corr.)	249.448	14			

Multiple Range Tests

Method: 95.0 percent LSD

	Count	Mean	Homogeneous Groups
Talewona area	5	16.262	X
Tankara area	5	17.276	X
Kantu area	5	24.726	X

Contrast	Difference	+/- Limits
Kantu area - Talewona area	*8.464	2.38118
Kantu area - Tankara area	*7.45	2.38118
Talewona area - Tankara area	-1.014	2.38118

* Denotes a statistically significant difference.

Leaflet stalk:

ANOVA Table

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	0.0113733	2	0.00568667	1.90	0.1923
Within groups	0.03596	12	0.00299667		
Total (Corr.)	0.0473333	14			

Male flower length:

ANOVA Table

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	0.77236	2	0.38618	2.75	0.1039
Within groups	1.68428	12	0.140357		
Total (Corr.)	2.45664	14			

Male flower calyx length:

ANOVA Table

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	1.13321	2	0.566607	1.72	0.2201
Within groups	3.94916	12	0.329097		
Total (Corr.)	5.08237	14			

Male flower calyx width:

ANOVA Table

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	0.17836	2	0.08918	2.68	0.1091
Within groups	0.3994	12	0.0332833		
Total (Corr.)	0.57776	14			

Stamen length:

ANOVA Table

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	0.733693	2	0.366847	1.34	0.2987
Within groups	3.28848	12	0.27404		
Total (Corr.)	4.02217	14			

Female flower length:

ANOVA Table

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	0.40036	2	0.20018	1.19	0.3368
Within groups	2.013	12	0.16775		
Total (Corr.)	2.41336	14			

ANOVA Table

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	0 87184	2	0 43592	3.13	0.0807
Within groups	1.673	12	0.139417		
Total (Corr.)	2 54484	14			

Female calyx width

ANOVA Table

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	1 76481	2	0.882407	23.54	0.0001
Within groups	0 44976	12	0.03748		
Total (Corr.)	2.21457	14			

Fruit length:

ANOVA Table

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	0.0880533	2	0.0440267	1.92	0 1889
Within groups	0.27504	12	0.02292		
Total (Corr.)	0 363093	14			

3.3. *Mitragyna inermis*

Plant height:

ANOVA Table

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	0 288413	2	0.144207	0.28	0.7625
Within groups	6.23996	12	0.519997		
Total (Corr.)	6.52837	14			

Stem girth size

ANOVA Table

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	0.31972	2	0.15986	0.90	0.4338
Within groups	2.14104	12	0.17842		
Total (Corr.)	2.46076	14			

Leaf length:

ANOVA Table

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	1.80521	2	0.902607	1.49	0.2634
Within groups	7.24948	12	0.604123		
Total (Corr.)	9.05469	14			

Leaf width:

ANOVA Table

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	4.23745	2	2.11873	2.94	0.0914
Within groups	8.64884	12	0.720737		
Total (Corr.)	12.8863	14			

Multiple Range Tests

Method: 95.0 percent LSD

	Count	Mean	Homogeneous Groups
Talewona area	5	3.322	X
Tankara area	5	4.168	XX
Kantu area	5	4.602	X

Contrast	Difference	+/- Limits
Kantu area – Talewona area	*1.28	1.16987
Kantu area - Tankara area	0.434	1.16987
Talewona area – Tankara area	-0.846	1.16987

* Denotes a statistically significant difference

Leaf size:

ANOVA Table

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	86.2718	2	43.1359	0.83	0.4599
Within groups	624.216	12	52.018		
Total (Corr.)	710.488	14			

Leaf index:

ANOVA Table

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	0.145613	2	0.0728067	1.48	0.2671
Within groups	0.59176	12	0.0493133		
Total (Corr.)	0.737373	14			

Stipule length:

ANOVA Table

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	0.603893	2	0.301947	11.60	0.0016
Within groups	0.31228	12	0.0260233		
Total (Corr.)	0.916173	14			

Multiple Range Tests

Method: 95.0 percent LSD

	Count	Mean	Homogeneous Groups
Tankara area	5	1.044	X
Kantu area	5	1.148	X
Talewona area	5	1.512	X

Contrast	Difference	+/- Limits
Kantu area - Talewona area	*-0.364	0.222296
Kantu area - Tankara area	0.104	0.222296
Talewona area - Tankara area	*0.468	0.222296

* Denotes a statistically significant difference.

Stipule width:

ANOVA Table

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	0.00292	2	0.00146	0.12	0.8887
Within groups	0.14708	12	0.0122567		
Total (Corr.)	0.15	14			

Stipule size:

ANOVA Table

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	0.298573	2	0.149287	5.15	0.0242
Within groups	0.34756	12	0.0289633		
Total (Corr.)	0.646133	14			

Multiple Range Tests

Method: 95.0 percent LSD

	Count	Mean	Homogeneous Groups
Tankara area	5	0.42	X
Kantu area	5	0.548	XX
Talewona area	5	0.762	X

Contrast	Difference	+/- Limits
Kantu area-Talewona area	-0.214	0.234517
Kantu area -Tankara area	0.128	0.234517
Talewona area -Tankara area	*0.342	0.234517

* Denotes a statistically significant difference.

Stipule index:

ANOVA Table

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	0.933613	2	0.466807	0.97	0.4052
Within groups	5.74608	12	0.47884		
Total (Corr.)	6.67969	14			

Flower length:

ANOVA Table

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	0.0223333	2	0 0111667	1.56	0.2494
Within groups	0.08576	12	0 00714667		
Total (Corr.)	0.108093	14			

Corolla length:

ANOVA Table

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	0 02644	2	0.01322	2.94	0.0915
Within groups	0.054	12	0.0045		
Total (Corr.)	0.08044	14			

Multiple Range Tests

Method: 95.0 percent LSD

	Count	Mean	Homogeneous Groups
Kantu area	5	0.722	X
Talewona area	5	0.744	XX
Tankara area	5	0.82	X

Contrast	Difference	+/- Limits
Kantu area-Talewona area	-0.022	0.0924394
Kantu area - Tankara area	*-0.098	0.0924394
Talewona area - Tankara area	-0.076	0.0924394

* Denotes a statistically significant difference.

Corolla lobe length:

ANOVA Table

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	0.000653333	2	0.000326667	0.30	0.7471
Within groups	0.01312	12	0.00109333		
Total (Corr.)	0.0137733	14			

Anther length:

ANOVA Table

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	0.0642533	2	0.0321267	1.35	0.2958
Within groups	0.28544	12	0.0237867		
Total (Corr.)	0.349693	14			

Anther width:

ANOVA Table

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	0.03508	2	0.01754	6.69	0.0112
Within groups	0.03148	12	0.00262333		
Total (Corr.)	0.06656	14			

Multiple Range Tests

Method: 95.0 percent LSD

	Count	Mean	Homogeneous Groups
Talewona area	5	0.552	X
Kantu area	5	0.62	XX
Tankara area	5	0.67	X

Contrast	Difference	+/- Limits
Kantu area - Talewona area	0.068	0.0705793
Kantu area - Tankara area	-0.05	0.0705793
Talewona area - Tankara area	*-0.118	0.0705793

* Denotes a statistically significant difference

Style length:

ANOVA Table

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	0.01524	2	0.00762	0.80	0.4699
Within groups	0.1136	12	0.00946667		
Total (Corr.)	0.12884	14			

Style width:

ANOVA Table

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	0.0100933	2	0.00504667	1.19	0.3374
Within groups	0.05084	12	0.00423667		
Total (Corr.)	0.0609333	14			

Stigma length:

ANOVA Table

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	0.262453	2	0.131227	3.25	0.0747
Within groups	0.48524	12	0.0404367		
Total (Corr.)	0.747693	14			

Multiple Range Tests

Method: 95.0 percent LSD

	Count	Mean	Homogeneous Groups
Tankara area	5	1.54	X
Kantu area	5	1.704	XX
Talewona area	5	1.864	X

Contrast	Difference	+/- Limits
Kantu area – Talewona area	0.164	0.277101
Kantu area – Talewona area	-0.16	0.277101
Talewona area – Tankara area	*-0.324	0.277101

* Denotes a statistically significant difference

Stigma width:

ANOVA Table

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	0.08572	2	0.04286	2.63	0.1131
Within groups	0.19568	12	0.0163067		
Total (Corr.)	0.2814	14			

Multiple Range Tests

Method: 95.0 percent LSD

	Count	Mean	Homogeneous Groups
Talewona area	5	0.632	X
Kantu area	5	0.742	XX
Tankara area	5	0.816	X

Contrast	Difference	+/- Limits
Kantu area – Talewona area	0.11	0.175968
Kantu area – Tankara area	-0.074	0.175968
Talewona area- Tankara area	*-0.184	0.175968

* Denotes a statistically significant difference

Style + Stigma length:

ANOVA Table

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	0.0513733	2	0.0256867	1.33	0.3020
Within groups	0.2326	12	0.0193833		
Total (Corr.)	0.283973	14			

3.4. *Pseudocedrela kotschyi*

Plant height:

ANOVA Table

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	1.48741	2	0.743707	1.46	0.2701
Within groups	6.10212	12	0.50851		
Total (Corr.)	7.58953	14			

Stem girth size.

ANOVA Table

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	0 114813	2	0.0574067	0.50	0.6164
Within groups	1.36712	12	0 113927		
Total (Corr)	1 48193	14			

Number of leaflets per leaf:

ANOVA Table

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	3.10192	2	1 55096	0 66	0.5359
Within groups	28 3085	12	2 35904		
Total (Corr.)	31.4104	14			

Leaflet length:

ANOVA Table

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	20.5477	2	10.2738	10.42	0 0024
Within groups	11 8345	12	0.986207		
Total (Corr)	32.3822	14			

Multiple Range Tests

Method. 95.0 percent LSD

	Count	Mean	Homogeneous Groups
Talewona area	5	6.248	X
Tankara area	5	7.142	X
Kantu area	5	9.054	X

Contrast	Difference	+/- Limits
Kantu area - Talewona area	*2.806	1 36847
Kantu area - Tankara area	*1.912	1.36847
Talewona area- Tankara area	-0.894	1 36847

* Denotes a statistically significant difference.

Leaflet width:

ANOVA Table

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	2.39116	2	1.19558	2.09	0.1663
Within groups	6.861	12	0.57175		
Total (Corr.)	9.25216	14			

Leaflet size:

ANOVA Table

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	584.904	2	292.452	4.15	0.0426
Within groups	844.842	12	70.4035		
Total (Corr.)	1429.75	14			

Multiple Range Tests

Method. 95.0 percent LSD

	Count	Mean	Homogeneous Groups
Tankara area	5	24.036	X
Talewona area	5	24.27	X
Kantu area	5	37.398	X

Contrast	Difference	+/- Limits
Kantu area - Talewona area	*13.128	11.5624
Kantu area - Tankara area	*13.362	11.5624
Talewona area - Tanakra area	0.234	11.5624

* Denotes a statistically significant difference

Leaflet shape:

ANOVA Table

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	0.199773	2	0.0998867	0.75	0.4939
Within groups	1.6014	12	0.13345		
Total (Corr.)	1.80117	14			

Leaflet width:

ANOVA Table

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	2.39116	2	1.19558	2.09	0.1663
Within groups	6.861	12	0.57175		
Total (Corr.)	9.25216	14			

Leaflet size:

ANOVA Table

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	584.904	2	292.452	4.15	0.0426
Within groups	844.842	12	70.4035		
Total (Corr.)	1429.75	14			

Multiple Range Tests

Method: 95.0 percent LSD

	Count	Mean	Homogeneous Groups
Tankara area	5	24.036	X
Talewona area	5	24.27	X
Kantu area	5	37.398	X

Contrast	Difference	+/- Limits
Kantu area - Talewona area	*13.128	11.5624
Kantu area - Tankara area	*13.362	11.5624
Talewona area - Tanakra area	0.234	11.5624

* Denotes a statistically significant difference

Leaflet shape:

ANOVA Table

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	0.199773	2	0.0998867	0.75	0.4939
Within groups	1.6014	12	0.13345		
Total (Corr.)	1.80117	14			

Rachis length:

ANOVA Table

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	56.8192	2	28.4096	4.55	0.0339
Within groups	74.9631	12	6.24692		
Total (Corr.)	131.782	14			

Multiple Range Tests

Method: 95.0 percent LSD

	Count	Mean	Homogeneous Groups
Talewona area	5	16.832	X
Tankara area	5	20.258	XX
Kantu area	5	21.416	X

Contrast	Difference	+/- Limits
Kantu area - Talewona area	*4.584	3.44417
Kantu area - Tankara area	1.158	3.44417
Talewona area - Tankara area	-3.426	3.44417

* Denotes a statistically significant difference.

Leaflet stalk:

ANOVA Table

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	0.00517333	2	0.00258667	0.14	0.8682
Within groups	0.217	12	0.0180833		
Total (Corr.)	0.222173	14			

Flower length:

ANOVA Table

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	1.90533	2	0.952667	4.75	0.0303
Within groups	2.408	12	0.200667		
Total (Corr.)	4.31333	14			

Multiple Range Tests

Method 95.0 percent LSD

	Count	Mean	Homogeneous Groups
Kantu area	5	5.66	X
Talewona area	5	6.22	XX
Tankara area	5	6.52	X

Contrast	Difference	+/- Limits
Kantu area - Talewona area	* -0.56	0.617289
Kantu area - Tankara area	-0.86	0.617289
Talewona- Tankara area	-0.3	0.617289

* Denotes a statistically significant difference

Pedicel length:

ANOVA Table

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	7.42672	2	3.71336	2.23	0.1507
Within groups	20.0266	12	1.66889		
Total (Corr.)	27.4534	14			

Corolla length:

ANOVA Table

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	0.435413	2	0.217707	4.67	0.0317
Within groups	0.55976	12	0.0466467		
Total (Corr.)	0.995173	14			

Multiple Range Tests

Method 95.0 percent LSD

	Count	Mean	Homogeneous Groups
Kantu area	5	0.97	X
Talewona area	5	1.042	X
Tankara area	5	1.362	X

Contrast	Difference	+/- Limits
Kantu area - Talewona area	-0.072	0.297619
Kantu area - Tankara area	*-0.392	0.297619
Talewona area -Tankara area	*-0.32	0.297619

* Denotes a statistically significant difference.

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	0.0744133	2	0.0372067	1.64	0.2347
Within groups	0.27236	12	0.0226967		
Total (Corr.)	0.346773	14			

Corolla size:

ANOVA Table

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	0.86032	2	0.43016	3.10	0.0824
Within groups	1.66724	12	0.138937		
Total (Corr.)	2.52756	14			

Multiple Range Tests

Method: 95.0 percent LSD

	Count	Mean	Homogeneous Groups
Kantu area	5	0.628	X
Talewona area	5	0.872	XX
Tankara area	5	1.212	X

Contrast	Difference	+/- Limits
Kantu area - Talewona area	-0.244	0.513641
Kantu area - Tankara area	*-0.584	0.513641
Talewona area- Tankara area	-0.34	0.513641

* Denotes a statistically significant difference.

Corolla index:

ANOVA Table

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	0.10468	2	0.05234	0.90	0.4327
Within groups	0.69856	12	0.0582133		
Total (Corr.)	0.80324	14			

Calyx length:

ANOVA Table

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	0.155373	2	0.0776867	0.31	0.7401
Within groups	3.021	12	0.25175		
Total (Corr.)	3.17637	14			

Calyx width:

ANOVA Table

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	0.00121333	2	0.000606667	0.03	0.9694
Within groups	0.23388	12	0.01949		
Total (Corr.)	0.235093	14			

Calyx size:

ANOVA Table

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	0.755613	2	0.377807	0.45	0.6502
Within groups	10.1574	12	0.846447		
Total (Corr.)	10.913	14			

Calyx index:

ANOVA Table

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	0.11052	2	0.05526	2.57	0.1177
Within groups	0.25792	12	0.0214933		
Total (Corr.)	0.36844	14			

Multiple Range Tests

Method: 95.0 percent LSD

	Count	Mean	Homogeneous Groups
Kantu area	5	1.906	X
Talewona area	5	2.002	XX
Tankara area	5	2.116	X

Contrast	Difference	+/- Limits
Kantu area - Talewona area	-0.096	0.202024
Kantu area - Tankara area	*-0.21	0.202024
Talewona area - Tankara area	-0.114	0.202024

* Denotes a statistically significant difference.

Staminal column length:

ANOVA Table

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	0.45012	2	0.22506	3.99	0.0470
Within groups	0.67744	12	0.0564533		
Total (Corr.)	1.12756	14			

Multiple Range Tests

Method: 95.0 percent LSD

	Count	Mean	Homogeneous Groups
Tankara area	5	2.32	X
Kantu area	5	2.386	X
Talewona area	5	2.716	X

Contrast	Difference	+/- Limits
kantu area - Talewona area	*-0.33	0.327413
Kantu area - Tankara area	0.066	0.327413
Talewona area - Tankara area	*0.396	0.327413

* Denotes a statistically significant difference

Fruit length:

ANOVA Table

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	0.466253	2	0.233127	0.41	0.6698
Within groups	6.74948	12	0.562457		
Total (Corr.)	7.21573	14			

Fruit width:

ANOVA Table

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	0.0246533	2	0.0123267	0.39	0.6823
Within groups	0.37468	12	0.0312233		
Total (Corr.)	0.399333	14			

Number of seed per fruit:

ANOVA Table

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	13.36	2	6.68001	1.25	0.3202
Within groups	63.9123	12	5.32602		
Total (Corr.)	77.2723	14			

Appendix 4. One-way analysis of variance (ANOVA) and multiple range test analysis (where variations were significant only) of anatomical data]

4.1. *Cassia sieberiana*

Root bark fibre length:

ANOVA Table

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	0.00962222	2	0.00481111	0.85	0.4741
Within groups	0.0340667	6	0.00567778		
Total (Corr.)	0.0436889	8			

Root bark fibre width:

ANOVA Table

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	0.000582889	2	0.000291444	0.93	0.4461
Within groups	0.001888	6	0.000314667		
Total (Corr.)	0.00247089	8			

Root bark fibre size:

ANOVA Table

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	0.0000842222	2	0.0000421111	1.40	0.3173
Within groups	0.000180667	6	0.0000301111		
Total (Corr.)	0.000264889	8			

Root bark fibre shape

ANOVA Table

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	308.681	2	154.34	2.13	0.2002
Within groups	435.165	6	72.5275		
Total (Corr.)	743.846	8			

Stem bark fibre length:

ANOVA Table

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	0.0158	2	0.0079	0.98	0.4272
Within groups	0.0482	6	0.00803333		
Total (Corr.)	0.064	8			

Stem bark fibre width:

ANOVA Table

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	0.000360222	2	0.000180111	1.95	0.2232
Within groups	0.000555333	6	0.0000925556		
Total (Corr.)	0.000915556	8			

Stem bark fibre size:

ANOVA Table

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	0.00000155556	2	7.77778E-7	0.41	0.6799
Within groups	0.0000113333	6	0.00000188889		
Total (Corr.)	0.0000128889	8			

Root bark fibre shape:

ANOVA Table

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	276.477	2	138.239	12.02	0.0080
Within groups	68.9778	6	11.4963		
Total (Corr.)	345.455	8			

Multiple Range Tests

Method: 95.0 percent LSD

	Count	Mean	Homogeneous Groups
Talewona area	3	38.1433	X
Kantu area	3	43.8667	X
Tankara area	3	51.6667	X

Contrast	Difference	+/- Limits
Kantu area - Talewona area	5.72333	6.77412
Kantu area - Tankara area	*-7.8	6.77412
Talewona area - Tankara area	*-13.5233	6.77412

* Denotes a statistically significant difference

Stem bark fibre length:

ANOVA Table

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	0.0704667	2	0.0352333	3.62	0.0929
Within groups	0.0583333	6	0.00972222		
Total (Corr.)	0.1288	8			

Stem bark fibre width:

ANOVA Table

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	0.000181556	2	0.0000907778	1.16	0.3745
Within groups	0.000468667	6	0.0000781111		
Total (Corr.)	0.000650222	8			

ANOVA Table

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	0.00500267	2	0.00250133	271.23	0.0000
Within groups	0.0000553333	6	0.00000922222		
Total (Corr.)	0.005058	8			

Multiple Range Tests

Method: 95.0 percent LSD

	Count	Mean	Homogeneous Groups
Tankara area	3	0.0246667	X
Kantu area	3	0.026	X
Talewona area	3	0.0753333	X

Contrast	Difference	+/- Limits
Kantu area - Talewona area	*-0.0493333	0.00606725
Kantu area - Tankara area	0.00133333	0.00606725
Talewona area - Tankara area	*0.0506667	0.00606725

* Denotes a statistically significant difference.

Stem bark fibre shape:

ANOVA Table

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	33.8988	2	16.9494	0.72	0.5256
Within groups	141.771	6	23.6285		
Total (Corr.)	175.67	8			

4.3. *Mitragyna inermis*:

Root bark fibre length:

ANOVA Table

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	0.0184889	2	0.00924444	0.87	0.4654
Within groups	0.0636667	6	0.0106111		
Total (Corr.)	0.0821556	8			

Root bark fibre width:

ANOVA Table

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	0.000120667	2	0.0000603333	0.24	0.7956
Within groups	0.00152333	6	0.000253889		
Total (Corr.)	0.001644	8			

Root bark fibre size:

ANOVA Table

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	0.000609556	2	0.000304778	3.19	0.1141
Within groups	0.000574	6	0.0000956667		
Total (Corr.)	0.00118356	8			

Root bark fibre shape:

ANOVA Table

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	133.837	2	66.9185	0.70	0.5311
Within groups	569.883	6	94.9805		
Total (Corr.)	703.72	8			

Stem bark fibre length:

ANOVA Table

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	0.0598222	2	0.0299111	3.09	0.1197
Within groups	0.0581333	6	0.00968889		
Total (Corr.)	0.117956	8			

Stem bark fibre width:

ANOVA Table

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	0.00000266667	2	0.00000133333	0.29	0.7563
Within groups	0.0000273333	6	0.00000455556		
Total (Corr.)	0.00003	8			

Stem bark fibre size:

ANOVA Table

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	0.000942889	2	0.000471444	0.99	0.4241
Within groups	0.00284867	6	0.000474778		
Total (Corr.)	0.00379156	8			

Stem bark fibre shape:

ANOVA Table

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	50.8256	2	25.4128	0.54	0.6067
Within groups	280.466	6	46.7443		
Total (Corr.)	331.291	8			

4.4. *Pseudocedrela kostchyi*:

Root bark fibre length:

ANOVA Table

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	0.0571556	2	0.0285778	6.02	0.0367
Within groups	0.0284667	6	0.00474444		
Total (Corr.)	0.0856222	8			

Multiple Range Tests

Method: 95.0 percent LSD

	Count	Mean	Homogeneous Groups
Kantu area	3	0.98	X
Tankara area	3	1.1	XX
Talewona area	3	1.17333	X

Contrast	Difference	+/- Limits
Kantu area- Talewona area	*-0.193333	0.137615
Kantu area - Tankara area	-0.12	0.137615
talewona area - Tankara area	0.0733333	0.137615

* Denotes a statistically significant difference

Root bark fibre width:

ANOVA Table

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	0.000042	2	0.000021	1.70	0.2596
Within groups	0.000074	6	0.0000123333		
Total (Corr.)	**0.000116**	**8**			

Root bark fibre size:

ANOVA Table

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	0.000008	2	0.000004	0.17	0.8484
Within groups	0.000142	6	0.0000236667		
Total (Corr.)	0.00015	8			

Root bark fibre shape:

ANOVA Table

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	1099.38	2	549.692	7.28	0.0249
Within groups	453.051	6	75.5086		
Total (Corr.)	1552.44	8			

Multiple Range Tests

Method: 95.0 percent LSD

	Count	Mean	Homogeneous Groups	
Kantu area	3	42.0667	X	
Tankara area	3	64.8333		X
talewona area	3	66.1367		X

Contrast	Difference	+/- Limits
Kantu area - Talewona area	*-24.07	17.3609
Kantu area - Tankara area	*-22.7667	17.3609
Talewona area - Tankara area	1.30333	17.3609

* Denotes a statistically significant difference

Stem bark fibre length:

ANOVA Table

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	0 00248889	2	0 00124444	0.06	0.9387
Within groups	0 116733	6	0.0194556		
Total (Corr.)	0 119222	8			

Stem bark fibre width:

ANOVA Table

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	0.00000422222	2	0.00000211111	0.29	0.7597
Within groups	0.000044	6	0.00000733333		
Total (Corr.)	0.0000482222	8			

Stem bark fibre size:

ANOVA Table

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	0.0000135556	2	0.00000677778	0 18	0.8362
Within groups	0.000220667	6	0.0000367778		
Total (Corr.)	0.000234222	8			

Stem bark fibre shape:

ANOVA Table

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	97 8105	2	48.9052	0 61	0 5749
Within groups	482.659	6	80 4432		
Total (Corr.)	580.47	8			

Appendix 5. Geo-data on distribution of anti-malarial species in the Wechiau Community Hippopotamus Sanctuary Ghana

5. 1. *Cassia sieberiana*

09° 43 815 N, 002° 47 058 W, 227m; 09° 43 66N, 002°46 927 W, 225m; 09° 451 N, 002° 46 787 W, 235m. 09° 43 491 N, 0026° 46 802 W, 243m, 09° 43 882 N, 002° 47 063 W, 240m; 09° 43 453 N, 002° 46 480 W, 209m, 09° 42 288 N, 002° 46 964 N, 229m; 09° 43 343 N, 002° 46 900 W, 225m; 09° 42 762 N, 002° 47103 W, 226m; 09° 45 104 N, 002° 47 043 W, 218m; 09° 44 706 N, 002° 47 347 W, 227m; 09° 45 104 N, 002° 47 043 N, 002° 47 043 W, 218m; 09° 41 122N, 002° 46 238 W, 229m; 09° 45 037 N, 002° 47 028 W, 228m

5.2. *Haematostaphis barteri*

09° 41 805N, 002° 45 670W, 251m; 09° 41, 912N, 002° 45 644W 259m; 090 01 818N, 002° 46 978W, 241m; 09° 41 812N, 002° 45 700W, 256m; 09° 43 975N, 002° 46 841W, 250m, 09° 42 226N, 002° 46 824W, 242m

5.3. *Mitragyna inermis*

09°. 43. 975N, 002°. 47. 037W, 220m, 09° 44. 563N, 002°. 47. 108W, 226m; 09° 43. 483N, 002° 46 484W, 252m; 09°. 45. 725N, 002° 46. 350W, 249m, 09° 43 259N, 002° 46 868W 255m, 09° 43 490N, 002° 46 570W, 240m; 09° 43 445N, 002° 46 450W, 244m; 09° 43 429N, 002°, 46, 247W, 247m; 09°. 43. 430N, 002°. 46. 888W, 09°. 43. 430N, 002°. 46. 895W, 227m; 09°. 43 406N, 002° 46 908W, 198m, 09° 45 153N, 002° 46 796W 233m, 09° 45 040N, 002° 47. 026W, 234m; 09° 41 385N, 002° 48 980W, 236m, 09°. 41. 535N, 002° 45 700W, 256m, 09° 41 195N, 002° 46 103W, 232m, 09° 41 295N, 002° 45 915N, 231m, 09° 01 18N, 002° 45 876W, 228; 09° 41 812N, 002° 45 700W, 256m, 09° 45 153N, 002°, 46 26 296W, 233m

5.4 *Pseudocedrela kostchyi*

09^{0} 44 786N, 002^{0} 46 838W, 232m, 090 44661N, 002^{0} 46 978W, 241m; 09^{0} 41 405N, 002^{0} 45 744W, 237m, 09^{0} 41 387N, 002^{0} 45 893W, 229m; 09^{0} 43 887N, 002^{0} 46 229W, 229m; 09^{0} 43 948N, 002^{0} 46 570W, 228m; 09^{0} 41 353N, 002^{0} 45 786W, 264m, 09^{0} 41 343N, 002^{0} 45 721W, 243m, 09^{0} 44 081N, 002^{0} 46 956W, 223m; 09^{0} 43 611N, 0020 46 047W, 240, 09^{0} 43 490N, 002^{0} 46 570W, 240m.

Appendix 6: Species associated with anti-malarial plants in Wechiau Community Hippopotamus Sanctuary Ghana.

Species	Growth form	Family
Abrus precatorius Linn	Herb / Vine	Papilionaceae
Acacia hockii De Willd	Small tree	Mimosaceae
Acacia nilotica (Linn.) Willd Ex Del	Small tree	Mimosaceae
Acanthospermum hispidum DC	Herb	Asteraceae
Aeschynomene indica Linn.	Herb	Papilionaceae
Amorphophallus dracontoides (Engl) N. E. Br	Herb	Araceae
Andropogon contortus Linn.	Grass	Poaceae
Annona senegalensis Pers.	Shrub	Annonaceae
Asparagus flagellaris (Kunth) Bak	Climber	Liliaceae
Aspilia africana (Pers) C D. Adams	Shrub	Asteraceae
Boerhavia diffusa Linn	Herb	Nyctaginaceae
Borreria radiata DC	Herb	Rubiaceae
Brachiaria alta (Schumach) C E Hubbard	Grass	Poaceae
Bridelia ferruginea Benth	Small tree	Euphorbiaceae
Burkea africana Hook	Small tree	Caesalpinaceae
Cardiospermum grandiflorum	Climber / vine	Convolvolaceae
Cassia mimmosoides Linn	Herb	Mimosaceae
Cassia tora Linn	Herb	Caesalpinaceae
Cissus populnes Guill & Perr.	Climber / shrub	Vitaceae
Cissus quadrancularis Linn	Climber / shrub	Vitaceae
Cleome viscosa Linn	Herb	Cappandaceae

Species	Growth form	Family
Cola laurifolia	Tree	Sterculiaceae
Combretum ghaselense Engl. & Diels	Shrub	Combretaceae
Combretum hypolinum	Shrub	Combretaceae
Combretum paniculata Vent	Shrub	Combretaceae
Commelina benghalensis Linn	Herb	Commelinaceae
Corchorus aestuans Linn	Herb	Malvaceae
Crinum humile A. Chev.	Herb	Amaryllidaceae
Crossopteryx febrifuga (Afzel. Ex G. Don.) Benth	Tree	Rubiaceae
Crotalaria goorensis Guill & Perr	Herb	Papilionaceae
Curculigo pilosa (Schum & Thonn) Engl	Herb	Hypoxidaceae
Cyperus spacelatus Rottb	Herb	Cyperaceae
Daniellia oliveri (Rolfe) Hutch & Dal	Tree	Caesalpinaceae
Demodium sp	Herb	Mimosaceae
Detarium microcarpum Guill & Perr	Small tree	Caesalpinaceae
Dichrostachys cineria	Shrub	Mimosaceae
Diospyros mespiliformis Hotchst ex Benth	Tree	Ebenaceae
Entada africana Guill. & Perr.	Small tree	Mimosaceae
Euphorbia convolvuloides Hotchst ex Benth	Herb	Euphorbiaceae
Fadogia agrestis Schweinf. ex Hiern	Shrub	Rubiaceae
Feretia apondanthera Del.	Shrub	Rubiaceae
Floscopa africana (P Beauv.) C. E. Cl	Herb	Commelinaceae
Gardenia ternifolia Schum. & Thonn.	Shrub	Rubiaceae

Species	Growth form	Family
Grewia carpinifolia Juss.	Shrub	Tiliaceae
Grewia villosum	Herb / vine	Tiliaceae
Haemanthus ruspestris Bak.	Herb	Amaryllidaceae
Hibiscus aspera Hook f	Herb	Malvaceae
Indigofera pulchra Willd	Herb	Papilionaceae
Kaempferia aethiopica (Schweinf) Solms-Laub	Herb	Zingiberaceae
Kyllinga sp	Herb	Cyperaceae
Lannea kerstingii Engl. & K Krasusc	Tree	Anacardiaceae
Lantana trifolia	Herb	Verbanaceae
Lippia multiflora Moldenke	Shrub	Verbanaceae
Millettia zechiana Harms.	Shrub	Papilionaceae
Nauclea latifolia Sm.	Shrub	Rubiaceae
Oldelandia corymbosa Linn	Herb	Rubiaceae
Ozoroa insignis Linn	Small tree	Anacardiaceae
Panicum maximum Jacq.	Herb	Poaceae
Paullinia pinnata Linn.	Herb / vine	Sapindaceae
Phyllanthus niruri	Herb	Euphorbiaceae
Piliostigma thonningii (Shum. & Thonn.) Leandri	Shrub	Caesalpinaceae
Pteleopsis suberosa Engl.& Diels	Shrub	Combretaceae
Pterocarpus erinaceus Poir	Tree	Papilionaceae
Pupalia lappacea Linn (Juss)	Herb	Amaranthaceae
Ritchiea reflexa (Thonn) Gilg&Benedict	Herb / vine	Cappandaceae

Species	Growth form	Family
Sapium grahamii (stapt) prain	Herb	Euphorbiaceae
Securinega virosa (Roxb. Ex Willd.) Baill.	Shrub	Euphorbiaceae
Sedge	Herb	Cyperaceae
Sida alba Linn.	Herb	Malvaceae
Sida cordifolia	Herb	Malvaceae
Spondias mombin Linn	Small tree	Anacardiaceae
Sterculia setigera Del	Small tree	Sterculiaceae
Stereospermum kunthianum cham	Tree	Bignoniaceae
Striga hermontheca (Dc) Benth.	Herb	Scrophulariaceae
Strychnos spinosa Lam	Small tree	Loganiaceae
Stylochiton lancifolius Kotschyi & Peyr	Herb	Araceae
Synedrella nodiflora Gaertn.	Herb	Asteraceae
Tamarindus indica Linn.	Tree	Caesalpinaceae
Tephrosia platycarpa Guill & perr.	Herb	Papilionaceae
Trichilia roka (Forsk) chiov	Shrub	Meliaceae
Tridax procunbens Linn.	Herb	Asteraceae
Triumfetta cordifolias A. Rich.	Herb	Tiliaceae
Vetiveria fulvibarvis (Trin.) Stapft.	Herb	Poaceae
Vitellaria paradoxa Gaertn. f	Tree	Sapotaceae
Vitex chrysocarpa Planch.Ex Benth.	Shrub	Verbanaceae
Ziziphus mauritianum Lam.	Small tree	Rhamanaceae

Appendix 7. Species associated … *Cassia sieberiana* in 17 plots studied in Wechiau Community Hippopotamus Sanctuary Ghana.

Species	Populations			% of total
	Kantu area	Talewona area	Tankara area	
Abrus precatorius	–	1	–	5.88
Aeschymone indica	–	1	–	5.88
Amorphophallus dracontoides	–	–	2	11.76
Andropogon contortus	2	–	1	17.65
Borreria radiate	2	2	–	23.53
Brachiaria radiate	3	1	–	23.53
Cardiospermum grandiflorum	3	–	1	23.53
Cassia mimmosoides	–	–	1	5.88
Cassia tora	–	2		11.76
Cissus quadrancularis	–	–	2	11.76
Cola laurifolia	1	–	2	17.65
Combretum ghaselensis	–	2	–	11.76
Combretum hypolinum	–	2	–	17.65
Combretum paniculata	–	–	1	5.88
Commelina benghalensis	–	2	–	11.76
Corchorus aestuans	–	–	2	11.76
Crossopteryx febrifuga	–	–	1	11.76
Cyperus spacelatus	–	1	–	5.88
Grewia carpinifolia	–	1	–	5.88

Species	Populations			% of
	Kantu area	Talewona area	Tankara area	total
Hibiscus aspera	1	1	–	11.76
Milletia zechiana	–	1	–	5 88
Mitragyna inermis	–	–	1	5 88
Paullinia pinnata	–	–	2	11 76
Phyllanthus niruri	1	–	2	17.65
Piliostigma thonningii	–	1	–	5 88
Ritchiea reflexa	–	1	1	11 76
Securinega virosa	3	–	2	29.41
Sedge	–	–	3	17.65
Setaria barbata	–	–	1	5 88
Sida alba	–	1	–	5 88
Sida cordifolia	–	3	–	17.65
Stylochiton lancifolia	–	1	–	5 88
Synedrella nodiflora	3	2	2	41.18
Tephrosia platycarpa	–	1	1	11 76
Vetiveria fulvibarvis	–	2	–	11 76
Vitex chrysocarpa	–	–	3	17.65

Appendix 8: Species associated with *Haematostaphis barteri* in 15 plots studies in Wechiau Community Hippopotamus Sanctuary Ghana.

Species	Populations			% of
	Kantu area	Talewona area	Tankara area	total
Acacia hockii	1	–	–	5.88
Acanthospermum hispidum	–	–	2	11 76
Amorphophallus dracontoides	–	1	2	17.65
Andropogon gayanus	3	5	2	58 82
Asparagus flagellaris	2	1	2	29.41
Annona senegalensis	–	2	2	23.53
Borreia radiata	–	3	5	47.06
Brachiaria lata	–	–	1	5 88
Bridellia februginea	–	2	3	29.41
Burkea africana	–	–	2	11.76
Cassia mimmosoides	–	–	3	17.65
Cissus quadrancularis	3	2	2	41.18
Combretum ghaselensis	–	1	2	17.65
Combretum hypopilinum	–	–	2	11.76
Commelina benghalensis	2	–	1	17.65
Crotalaria goorensis	–	–	2	11 76
Cyperus spacelatus	–	–	2	11 76
Daniellia oliveri	–	–	1	5 88
Detarium microcarpum	1	1	2	23 53

Species	Populations			% of
	Kantu area	Talewona area	Tankara area	total
Dichryostachys cineria	–	–	1	5.88
Diospyros mesiliformis		–	1	5.88
Feretia apondanthera	2	–	–	11.76
Gardenia ternifolia	1	–	–	5.88
Grewia carpinifolia	–	2	3	29.41
Grewia villosum	–	–	2	11.76
Hibiscus aspera	–	–	1	5.88
Indigofera pulchra	–	–	1	5.88
Kaempferia aethiopica	–	2	5	29.41
Lannea kerstingii	–	–	1	5.88
Lantana trifolia	–	–	1	5.88
Nauclea latifolia	–	–	1	5.88
Ozoroa insignis	–	2	1	17.65
Panicum maximum	1	–	–	5.88
Pteleopsis suberosa	–	–	1	5.88
Pupalia lapacu	–	–	2	11.76
Sapium grahamii	–	1	2	17.65
Sterculia setigeria	–	–	1	5.88
Stereospermum kunthianum	–	–	2	11.76
Strychnos spinosa	2	–	–	11.76

Appendix 6 (continued)

Species	Populations			% of total
	Kantu area	Talewona area	Tankara area	
Stylochiton lancifolia	–	–	3	17.65
Synedrella nodiflora	2	–	–	11.76
Tephrosia platycarpa	–	–	2	11.76
Trichillia emetica	3	–	1	23.53
Tridax procumbens	1	–	–	5.88
Trieumfetta sp	–	1	2	17.65
Vitellaria paradoxa	–	–	1	5.88

Appendix 9: Species associated with *Mitragyna inermis* in 16 plots studied in Wechiau Community Hippopotamus Sanctuary Ghana.

Species	Populations			% of
	Kantu area	Talewona area	Tankara area	total
Acacia hockii	1	–	–	6.25
Amorphophallus dracontoides		–	2	12.50
Andropogon gayanus	2	–	1	18.75
Aspilia africana	–	2	2	25.00
Annona senegalensis	1	–	–	6.25
Boeharvia diffusa	2	–	1	18.75
Borreria radiata	2	1	3	37.50
Brachiaria alata	1	1	4	37.50
Cassia mimmosoides	2	2	–	25.00
Cassia tora	1	–	–	6.25
Combretum paniculatum	–	–	1	6.25
Corchorus aestuans	–	2	3	31.25
Crinum humile	–	–	1	6.25
Crotalaria goorensis	1	3	1	25.00
Fadogia agrestis	3	–	–	18.75
Haemanthus ruspestris	1	1	–	6.25
Hibiscus aspera	–	–	2	12.50
Indigofera pulchra	–	2	–	12.50

Species	Populations			% of total
	Kantu area	Talewona area	Tankara area	
Oldelandia corymbosa	–	1	3	25.00
Pennisetum sp	1	–	–	6.25
Securinega virosa	–	1	–	6.25
Setaria barbata	1	2	–	18.75
Sida cordifolia	–	1	–	6.25
Stylochiton lancifolia	5	1	–	37.50
Synedrella nodiflora	5	3	–	50.00
Tephrosia platycarpa	–	2	–	12.50
Triumfetta sp	–	1	–	6.25
Vetiveria fulvibarvis	2	4	1	43.75

Appendix 10: Species associated with *Pseudocedrela kotschyi* in 17 plots studied

Wechiau Community Hippopotamus Sanctuary Ghana.

Species	Populations			% of
	Kantu area	Talewona area	Tankara area	total
Acacia gourmaensis	2	–	–	11.76
Amorphophallus dracontoides	–	2	–	11.76
Andropogon sp	3	1	–	23.53
Borreia radiata		2	–	11.76
Cassia mimmosoides	2	2	–	23 53
Cissus populnea	–	2	–	11.76
Combretum ghaselensis	–	–	3	17.65
Combretum paniculatum	–	1	–	5 88
Crossopteryx fefriguga	–	1	–	5.88
Euphorbia convolvuloides	–	1	–	5 88
Feretia apodanthera	1	–	1	11.76
Floscopa africana	–	1	–	5 88
Grewia carpinifolia	1	2	1	23.53
Haemanthus ruspestris	–	1	–	5 88
Hibiscus aspera	4	4	–	47.06
[illegible] pulchra	–	1		
Kaempferia aethiopica	–	2	–	[illegible]
Lippia multiflora	–	–	1	5 88
Oldelandia corymbosa	1	–	–	5 88

Appendix 10. (continued)

Species	Populations			% of
	Kantu area	Talewona area	Tankara area	total
Pennisetum sp	–	1	–	5.88
Phyllanthus niruri	–	3	–	17.65
Piliostigma thonningii	–	–	2	11.76
Pterocarpus erinaceus	–	–	1	5.88
Sapium grahamii	–	–	2	11.76
Striga hermonthetica	1	–	–	5.88
Scilla picta	–	–	2	11.76
Securinega virosa	2	1	–	17.65
Stylochiton lancifolia	4	1	–	29.41
Synedrella nodiflora	4	5	–	52.94
Tamarindus indica	–	–	1	5.88
Tephrosia platycarpa	1	–	–	5.88
Vertiveria fulvibarvis	2	3	–	29.41
Vitellaria paradoxa	–	–	1	5.88
Vitex chrysocarpa	–	1	–	5.88
Ziziphus mauritiana	1	–	–	5.88

Appendix 11: Specimens of anti-malarial species examined at the Ghana herbarium (GC)

11. 1. *Cassia sieberiana*

Schmidt, H. H *et al* 1841! Mole National Park (November; fruiting); Morton, J. K & Dokosi A. A.145! Fume road (March, flowering); Kumi, J. A. GC43034! Legon Botanical Garden (March, flowering), Enti, A. A FE2620! Pokuase (September), Enti, A A. & Agyakwa GC 35139! Bui Village (August); Sir Albert Kitson! Samdre River (March, flowering). Andoh, J E 5698! Jema, Kintampo (March, flowering); Hall & Enti. GC 35870! Mole Reserve (November); Irvine, F R 1503! Krobo Odumase (February, flowering), Johnson, W H. 652! Afram plains (March; flowering), Asamany, V T K. 221! Achimota school Avenue (February, flowering); Bucknor 2! Accra (December, fruiting), Morton, J. K 8829! Tumu(March, flowering & fruiting)

11. 2. *Haematostaphis barteri*

Morton, J. K. 9001! Gambaga (April; flowering); Hall & Houston GC 45043! Mole Game Reserve (June), Morton, J K 7319! Gambage Scarp (May); Akpabla, G. K 704! Gambaga (June; fruiting), Adams, C D. 846B! Wa (June; flowering); Adams, C D 616! Boro (June, fruiting), Darko, K W 285! Bole (April, flowering), Easterly, N. W. M. 246! Gambaga (April; flowering); Enti, A. A. F H.8208! (June), Hall, J B. GC36796! Mole Game Reserve (April); Adams, C D 2560! Agogo Ashanti (April)

11. 3. *Mitragyna inermis*

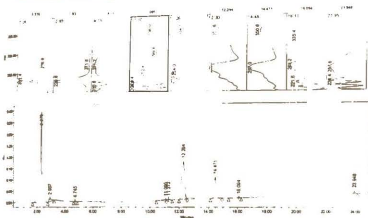
Schmidt, H H. et al.1792! Mole National Park (November; fruting); Hossain & Agyakwa GC37865! Bolgatanga (August, flowering), Dokosi, O. W. B! Kete Krachi (December, fruits); Harris, B. J! Ho (November, fruits). Boughey, A. S! Kpotame (November, flowers & fruits); Enti, A. A. 601! Sodankope -River Volta (November, flowers and fruits), Morton, J. K. GC9271! Ada (June; flowering & fruits); Goodall, D. W. 15894! Daboya (September, fruits), Brown, T. W. 929! Accra (February; flowers); Anderson, J. 1151! Krachi (flowering); Irvine, F. R. 816! Achimota (October, flowers); Enti, A.A. & Agyakwa, C. W. GC35136! Bui Dam site (October, flowers), Enti, A. A. 1057! Korni Forest Reserve (August), Morton, J. K. 7510! Tumu (May, flowering); Hall & Enti GC42715! Agoe, Volta region (May), Enti, A. A. & Agyakwa, C. W. VBS482! Yapei (January); Enti, A. A. & Agyakwa, C. W! VB423! Mile 221 Kumasi-Tamale road (January); Morton, J. K. A2063! Tefle (May, flowering); Morton, J. K. 9840! Tamale (December), Adams, C. D. 4597! Kete Krachi (December).

11. 4. *Pseudocedrela kotschyi*

Schmidt, H. H. *et al.* 1826! Mole National Park (November); Bona, S.G. GC 462! Mole National Park! (October); Goodall, D.W. 15450! Bolgatanga (April), Goodall, D. W 15179! Damongo (March); Akpabla, G. K. 415! Tamale-Yendi road (December), Adams, C. D. 830! Wa (June), Adams, C. D. 745! Wa (June), Akpabla, G. K. 1865! Achimota (February, flowering), Goodall, D. W. 151813! Damongo (March), Morton, J. K. GC7500! Tumu! (May); Enti, A. A. GC35177! Mole Game Reserve (February), Akpabla, G. K. 415! Tamale-Yendi road (December).

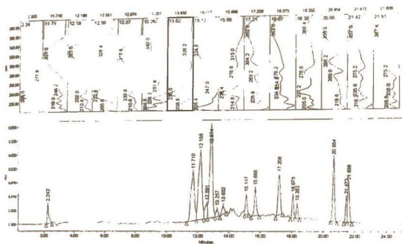
Appendix 12: HPLC profiles of *Cassia sieberiana* extracted in 80 % methanol

12. 1. HPLC chromatogram of leaf at PDA 254 nm



12.2. HPLC chromatogram of stem bark at PDA 335 nm

12. 3. HPLC chromatogram of root bark at PDA 335 nm

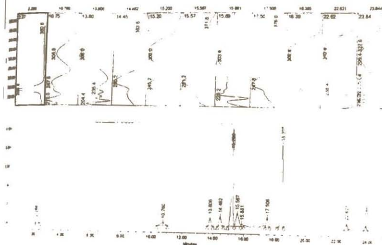


Appendix 13: HPLC profiles of *Haematostaphis barteri* extracted in 80 % methanol

13.1. HPLC chromatogram of leaf at PDA 254 nm

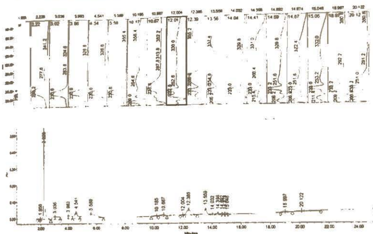
10.2. HPLC chromatogram of stem bark at PDA335 nm

13.3. HPLC chromatogram of root bark at PDA 335 nm

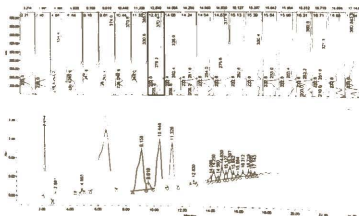


Appendix 14: HPLC profiles of *Mitragyna inermis* extracted in 80 % methanol

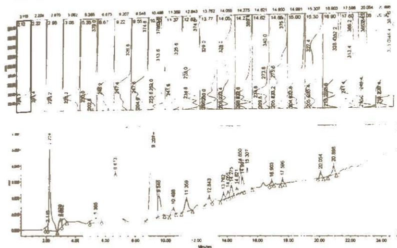
11.1. HPLC chromatogram of leaf at PDA 254 nm



14. 2. HPLC chromatogram of stem bark at PDA 254 nm



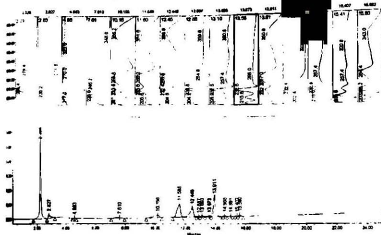
14.3. HPLC chromatogram of root bark of samples from Kantu and Talewona at PDA 254 nm



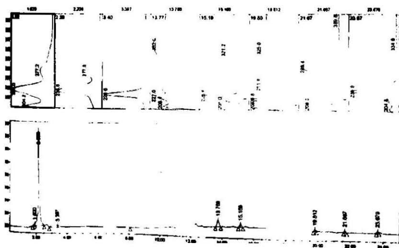
14.4. HPLC chromatogram of root bark sample from Tankara at PDA 254 nm

Appendix 15: HPLC profiles of *Pseudocedrela kotschyi* extracted in 80 % methanol

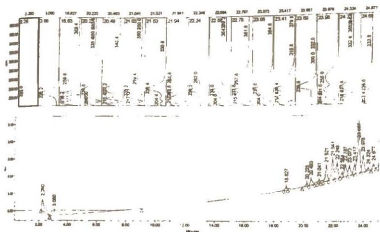
15.1. HPLC chromatogram of leaf at PDA 254 nm



15. 2. HPLC chromatogram of stem bark at PDA 254 nm

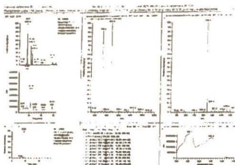


15. 3. HPLC chromatogram of root bark at PDA 254 nm



Appendix 16: APCI- MS data of analysis of leaf samples of *Cassia sieberiana*

16.1. Myricetin 3- rhamnoside



16. 2. Quercetin 3- rhamnoside

Appendix 20: Peaks used for PCA analysis of variation, their retention time and UV max, and the parts of *Cassia sieberiana* where they were identified

Peak No / compound	Plant part	Rt.(Min)	UV max (nm)
1	leaf	2.2	239, 274
2	leaf	2.7	235, 273
3	leaf	11.5	229, 324
4 (Myricetin 3- rhamnoside)	leaf	12.0	260, 351
5 (Quercetin - 3 rhamnoside)	leaf	14.2	256, 350
6	leaf	23.7	207, 257
7	stem bark	11.3	236, 279
8	stem bark	11.7	236, 278
9	stem bark	12.3	237, 278
10	stem bark	12.5	237, 276
11	stem bark	13.5	236, 277
12	stem bark	14.5	236, 278
13	root bark	12.2	237, 276, 316
14	root bark	13.0	237, 277
15	root bark	13.3	238, 277
16	root bark	14.5	238, 278
17	root bark	15.3	238, 277

Appendix 21: Peaks used for PCA analysis of variation, their retention time and UV max, and the parts of *Haematostaphis barteri* where they were identified

Peak No / compound	Plant part	Rt.(Min)	UV max (nm)
1	leaf	8.0	218, 314,
2 (Vitexin)	leaf	11.4	268, 337
3	leaf	12.3	269, 337
4 (Vitexin or isovitexin *O*- glycoside)	leaf	12.7	269, 336
5	leaf	14.0	264, 323
6	leaf	18.0	265, 296, 349
7 (Stilbene)	root	18.2	207, 237, 307
8	root	23.7	236, 288

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Appendix 22: Peaks used for PCA analysis of variation, their retention time and UV max, and the parts of *Mitragyna inermis* where they were identified

Peak No	Plant part	Rt.(Min)	UV max (nm)
1	leaf	3.9	235.8, 278.8
2	leaf	4.5	238.5, 326.4
3	leaf	12.7	229.6, 255.8, 355.8
4	leaf	13.8	230.2, 253.4,
5	root bark	8.4	246.6, 327.0
6	root bark	13.4	243.0, 328.2
7	root bark	15.0	205.8, 239.0, 326.6
8	stem bark	6.0	244.0
9	stem bark	8.5	204.8, 238.4
10	stem bark	14.6	275.2, 377.0

Appendix 23: Peaks used for PCA analysis of variation, their retention time and UV max, and the parts of Pseudocedrela kotschyi where they were identified

Peak No	Plant part	Rt.(Min)	UV max (nm)
1	leaf	10.7	233.0, 263 2, 356 2
2	leaf	12.0	261.2, 351.8
3	leaf	12.6	230.6, 260.6, 355.2
4	leaf	12.8	256 4, 354.4
5	leaf	13.8	201 6, 231.8, 284 8
6	leaf	14.2	256 2, 350 6
7	leaf	15.9	231.2, 263.8, 342 6
8	stem bark	5 0	218 8, 233.6
9	stem bark	15 0	206 6, 229.6, 321 8
10	stem bark	22.0	206.6, 228.0, 354
11	stem bark	23.3	218 2, 241.4, 290 2
12	root bark	21.9	210.4, 270.2