ETHNOPHARMACOLOGY AND TOXICOLOGY OF ANTIMALARIAL PLANTS USED TRADITIONALLY IN MSAMBWENI, KENYA.

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A thesis submitted in fulfillment of requirements for the degree of Doctor of Philosophy in Pharmacology and Toxicology of the University of Nairobi

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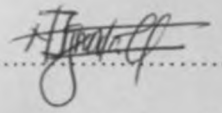
2011

Declaration

This thesis is my original work and has not been presented for a degree in any other university.



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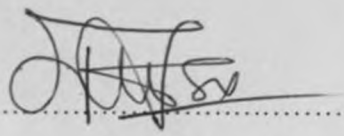

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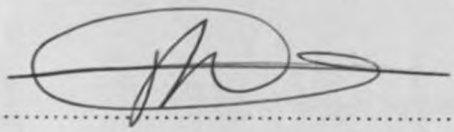

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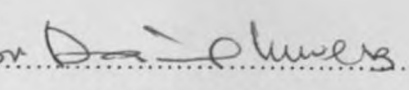

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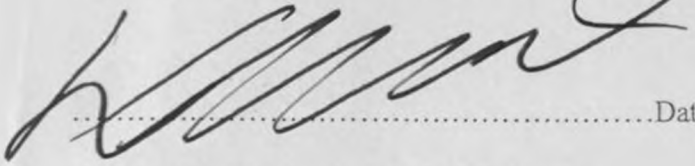

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Dedication

To our dear families whose support and encouragement make all things seem possible

Acknowledgements

Were it not for the Almighty God, I would have given up. Praise and honour be to him! For me to have arrived in my current level in academia has been a journey characterised by all kinds of twists and turns. I can say with absolute certainty that there have been thrills and pitfalls on the way.

This thesis was carried out at the Department of Public Health, Pharmacology and Toxicology (PHPT), Faculty of Veterinary Medicine, College of Agriculture and Veterinary Sciences, University of Nairobi, during the years 2008 to 2011. I am grateful to my supervisors Dr. James M.Mbaria, Dr. Daniel W. Gakuya, Dr. Stephen Gitahi Kiama and Professor Peter Karuri Gathumbi who inspired me to study ethnopharmacology, pharmacology and toxicology of natural products especially plants used in malaria treatment in a malaria endemic zone, Msambweni district, South-Coast Kenya. I would like to express my gratitude to Professor Jackson Nyarongi Ombui, chairman, department of Public Health, Pharmacology and Toxicology (PHPT) for his interest, enthusiasm and support during my work.

I am grateful to my colleagues at the RISE-AFNNET programme, Dr. Stanley Wambugu and Dr. Irene Kamanja for their friendship and help during the beginning of this thesis work. Sincere thanks are due to all my colleagues at the Department of Public Health, Pharmacology and Toxicology for creating a nice place to work in during my study period. The friendship and support of laboratory technician Moses Ndotono Njahira of BecA–ILRI hub laboratory and the whole RISE-AFNNET team is greatly acknowledged. I am grateful to my dear mother Tabitha Mukulu Nguta, for her love and support. My deep and warm thanks are due to my dear wife,

Salome, for her endless love, support and encouragement during all the years of this thesis work. Thank you for everything you are to me.

I do sincerely acknowledge the Carnegie Corporation of New York through Regional Initiative in Science and Education (Carnegie-RISE) under the African Natural Products Network (RISE-AFNNET), for financing both my study and dissemination of my novel findings in international meetings. All the traditional healers and community members in Msambweni district, South Coast Kenya, who participated in the interviews during field work, and shared their valuable information on medicinal plants with us are gratefully acknowledged. I do express my gratitude to others who have, with such good grace, given me their time and energy to supply valuable facts and opinions for this thesis. Much of any merit this thesis may have is due to the generosity of those named above as well as others too numerous to mention who have put their knowledge, resourses and practical experience at my disposal. I do hope that this collective acknowledgement will be acceptable.

Everything that came to pass in and around this work was enabled by the **LORD JESUS** who has been given all authority in heaven and on earth **(MATTHEW 28:18). PRAISE BE UNTO HIM!**

Table of contents

List of Tables

Description **Page**

List of figures

Description **Page**

List of Appendices

List of Abbreviations

ACT:	Artemisinin-based combination therapy
AL:	Artemether-lumefantrine combination
AS:	Artesunate
AS+MQ:	Artesunate + mefloquine combination
AS+SP:	Artesunate + sulfadoxine-pyrimethamine combination
ATPase:	Adenosine triphosphate
CI:	Confidence interval
Commission E:	An independent committee on herbal remedies of German Federal Institute for Drugs and Medical Devices
CQ:	Chloroquine
CYTED:	*Ciencia y tecnología para el Desarrollo*
DMSO:	Dimethyl sulphoxide
ESCOP:	European Scientific Co-operation of Phytotherapy
G6PD:	Glucose-6-phosphate dehydrogenase
HIV/AIDS	Human immunodeficiency virus/acquired immunodeficiency syndrome
HMs:	Herbal medicines
HRP_2:	Histidine-rich protein 2
IAP:	Ibero American Program
IC_{50}:	Concentration providing 50% inhibition

ICH:	International conference on harmonization of technical requirements for the registration of pharmaceuticals for human use.
LC_{50}	Concentation killing 50% of *Artemia* larvae
MQ:	Mefloquine
NaHCO3:	Sodium bicarbonate
NCE:	Novel Chemical Entities
NCI:	National Cancer Institute
NCP-tazopsine:	N-cyclopentyl-tazopsine
OECD:	Organisation for the economic co-operation and development
PAF:	Platelet aggregation factor
PCR	Polymerase chain reaction
PLDH:	*Parasite*-lactate dehydrogenase
RDP:	Reconstruction and Development Plan
RISE-AFFNET:	Regional Initiative in Science and Education African Natural Product Training Network
SP:	Sulfadoxine–pyrimethamine
SRB:	Sulforhodamine B
TMP:	Traditional Medical Practitioner
WHO	World Health Organization

Abstract

Historically, compounds containing novel structure from natural origin represent a major alternative source for the discovery and development of new drugs for several diseases. This study was undertaken in order to compose detailed documentation on wild medicinal flora used against malaria, existing knowledge, attitudes and practices related to malaria recognition, control and treatment; ethnodiagnostic skill used by the Msambweni community as a lead to traditional bioprospecting and to evaluate the toxicological activity of the crude extracts in brine shrimp bioassay using *Artemia salina* Leach (Artemiidae).

Study I was conducted with herbalists (Traditional Medical Practitioners) to document medicinal plants that are traditionally used by the Msambweni community of Kenyan South Coast to treat malaria, where the disease is endemic. Herbalists were interviewed by administration of semi structured questionnaires in order to obtain information on medicinal plants traditionally used for the treatment of malaria. Focused group discussions held with the herbalists supplemented the interview and questionnaire survey. Twenty six species of plants in twenty four genera distributed in 20 families were reported to be used in this region for the treatment of malaria. Labiatae, Rutaceae and Liliaceae families had each eleven percent of the plant species reported and represented the species that are most commonly used. Thirteen plant species, namely; *Aloe deserti* Berger (Liliaceae), *Launea cornuta* (Oliv and Hiern) C. Jeffrey (Compositae), *Ocimum bacilicum* L. (Labiatae), *Teclea simplicifolia* (Eng) Verdoon (Rutaceae), *Gerranthus lobatus* (Cogn.) Jeffrey (Cucurbitaceae), *Grewia hexaminta* Burret. (Tiliaceae), *Canthium glaucum* Hiern. (Rubiaceae), *Amaranthus hybridus* L. (Amaranthaceae), *Combretum padoides* Engl and

Diels. (Combretaceae), *Senecio syringitolius* O. Hoffman. (Compositae), *Ocimum suave* Willd (Labiatae), *Aloe macrosiphon* Bak. (Liliaceae) and *Laudolphia buchananii* (Hall.f) Stapf. (Apocynaceae) are documented from this region for the first time for the treatment of malaria.

Study II was conducted with community members to document herbal medicines used in the treatment of malaria as well as the existing knowledge, attitudes and practices related to malaria recognition, control and treatment in South Coast, Kenya. Data was collected using semi structured questionnaires and interviews. A focused group discussion held with the community members, one in each of the study villages supplemented the interview and questionnaire survey. The respondents were found to have a good understanding of malaria and could distinguish it from other disease conditions characterized by increased body temperature. They were also aware that malaria was spread by mosquitoes. Malaria prevalence was high, and affected individuals at an average of four times a year. Community members avoided mosquito bites by using mosquito nets, clearing bushes around their homesteads and burning plant parts to generate smoke. They prevented and treated malaria by taking decoctions or concoctions of traditional herbal remedies. Forty plant species in thirty five genera distributed in twenty four families were used as antimalarials in the study area. Five plant species, namely; *Heeria insignis* Del. (Anacardiaceae), *Rottboelia exaltata* L.F (Gramineae), *Pentanisia ouranogyne* S. Moore (Rubiaceae), *Agathisanthenum globosum* (A. Rich) Hiern (Rubiaceae), and *Grewia trichocarpa* Hochst ex A. Rich (Tiliaceae) are documented for the first time in South Coast, Kenya, for the treatment of malaria.

Study III was conducted with community members to systematically document ethnophytotherapeutic remedies, ethnodiagnostic skills and related traditional knowledge utilized by the Digo community of the Kenyan Coast to diagnose malaria as a lead to traditional bioprospecting. The study was carried out in three Digo villages of Diani sub-location between May 2009 and December 2009. Data was collected using semi-structured interviews, and open and close-ended questionnaires. A total of sixty (60) respondents (34 men and 26 women) provided the targeted information. The results showed that the indigenous knowledge of Digo community on malaria encompasses not only the symptoms of malaria but also the factors that are responsible for causing malaria, attributes favoring the breeding of mosquitoes and practices employed to guard against mosquito bites or to protect households against malaria. This knowledge is closely in harmony with scientific approaches to the treatment and control of the disease. The Digo community uses sixty (60) medicinal plants distributed in fifty two (52) genera and thirty one (31) families to treat malaria. The most frequently mentioned symptoms were fever, joint pains and vomiting while the most frequently mentioned practices employed to guard against mosquito bites and/or to protect households against malaria was burning of herbal plants such as *Ocimum suave* and ingestion of herbal decoctions and concoctions. The Digo community has abundant ethnodiagnostic skills for malaria which forms the basis of their traditional bioprospecting techniques. They also have abundant traditional knowledge about the causes of malaria and ethnophytotherapeutic remedies.

Artemia salina, the brine shrimp larva, is an invertebrate used in the alternative test to determine toxicity of chemicals and natural products. In study IV, the Medium Lethal Concentrations (LC_{50}

values) of 170 crude plant extracts and positive controls, cyclophosphamide and etoposide were determined using *Artemia salina.* Out of the 85 organic extracts (Chloroform/Methanol, 1:1) screened for activity against *Artemia salina* larvae, 46 (54%) of the crude extracts demonstrated activity at or below 100µg/ml, and were categorized as having strong cytotoxic activity, 35 (41.2%) of the crude extracts had LC_{50} values between 100µg/ml and 500µg/ml, and were categorized as having moderate cytotoxicity, 2 (2.4%) of the crude extracts had LC_{50} values between 500µg/ml and 1000µg/ml, and were considered to have weak cytotoxic activity, while 2 (2.4%) of the crude extracts had LC_{50} values greater than 1000µg/ml and were considered to be non toxic. Approximately 19% (16) of the aqueous extracts demonstrated activity at or below 100 µg/ml and were considered to have strong cytotoxic activity, 39% (33) of the screened aqueous crude extracts had LC_{50} values between 100µg/ml and 500µg/ml and were considered to be moderately cytotoxic, 15% (13) of the crude extracts had LC_{50} values between 500µg/ml and 1000µg/ml and were considered to have weak cytotoxic activity while 27% (23) of the aqueous extracts had LC_{50} values greater than 1000µg/ml and were categorized as non toxic.The positive controls, cyclophosphamide and etoposide exhibited strong cytotoxicity with LC_{50} values of 95µg/ml and 6µg/ml respectively in a 24 hour lethality study, validating their use as anticancer agents. In the current study, 97.6% of all the screened organic extracts and 73% of the investigated aqueous extracts demonstrated LC_{50} values <1000 µg/ml, indicating the presence of bioactive compounds responsible for the observed toxicity. This calls for in depth *in vivo* toxicological studies and chemical investigation for isolation of bioactive compounds responsible

for the observed toxicologic activity. It is concluded that some of the plants used would not make safe antimalarial drugs, and instead could be a source of novel scaffolds againt cancer.

In summary the studies above indicate that many species of antimalarial plants are used by the Msambweni community to prevent and treat malaria. The good knowledge on the disease by the study community can be utilized as a lead to bioprospecting of novel remedies accessible to the rural poor. Majority of the species identified have strong cytotoxic activity in brine shrimp (*Artemia salina)* assay, indicating that they could not make safe antimalarial remedies. In depth studies would now be needed to find the active compounds behind these toxic activities that could be used as biomarkers in development of anticancerous drugs.

CHAPTER ONE

INTRODUCTION

1.1. Background Information

The use of natural products with therapeutic properties is as ancient as human civilisation and, for a long time, plant and animal products were the main sources of drugs, De Pasquale (1984). The industrial revolution and the development of organic chemistry resulted in a preference for synthetic products for pharmacological treatment. The reasons for this were that pure compounds were easily obtained, structural modifications to produce potentially more active and safer drugs could be easily performed and the economic power of the pharmaceutical companies was increasing. Furthermore, throughout the development of human culture, the use of natural products has had medical significance and concepts of health and disease existed within each culture. However, even if we only consider the impact of the discovery of the penicillin, obtained from micro-organisms, on the development of anti-infection therapy, the importance of natural products is clearly enormous. About 25% of the drugs prescribed worldwide come from plants, 121 such active compounds being in current use. Of the 252 drugs considered as basic and essential by the World Health Organisation (WHO), 11% are exclusively of plant origin and a significant number are synthetic drugs obtained from natural precursors. Examples of important drugs obtained from plants are digoxin from *Digitalis* spp., quinine and quinidine from *Cinchona* spp., vincristrine and vinblastine from *Catharanthus roseus*, atropine from *Atropa belladonna* and morphine and codeine from *Papaver somniferum*. It is estimated that 60% of anti-tumour and

anti-infectious drugs already on the market or under clinical trial are of natural origin, Yue-Zhong Shu (1998). The vast majority of these cannot yet be synthesised economically and are still obtained from wild or cultivated plants. Natural compounds can be lead compounds, allowing the design and rational planning of new drugs, biomimetic synthesis development and the discovery of new therapeutic properties not yet attributed to known compounds, Hamburger and Hostettmann (1991). In addition, compounds such as muscarine, physostigmine, cannabinoids, yohimbine, forskolin, colchicines and phorbol esters, all obtained from plants, are important tools used in pharmacological, physiological and biochemical studies, Williamson *et al* (1996).

In recent years, there has been growing interest in alternative therapies and the therapeutic use of natural products, especially those derived from plants, Godfrank *et al* (1982); Vulto and Smet (1988); Mentz and Schenkel (1989). This interest in drugs of plant origin is mainly due to the fact that, a large percentage of the world's population does not have access to conventional pharmacological treatment, and folk medicine and ecological awareness suggest that "natural" products are harmless. However, the use of these substances is not always authorised by legal authorities dealing with efficacy and safety procedures, and review of literature point to the lack of quality in the production, trade and prescription of phytomedicinal products.

It is estimated that, in 1997, the world market for over the-counter phytomedicinal products was US$ 10 billion, with an annual growth of 6.5%, Soldati (1997). The WHO considers phytotherapy in its health programs and suggests basic procedures for the validation of drugs from plant origin in developing countries, Vulto and Smet (1998); OMS (1991). Eastern countries, such as China and India, have a well-established herbal medicines industry and Latin

American countries have been investing in research programs in medicinal plants and the standardization and regulation of phytomedicinal products, following the example of European countries, such as France and Germany. In Germany, 50% of phytomedicinal products are sold on medical prescription, the cost being refunded by health insurance, Gruenwald (1997). In North America, where phytomedicinal products are sold as "health foods", Brevoort (1997); Calixto (2000), consumers and professionals have struggled to change this by gathering information about the efficacy and safety of these products, and new guidelines for their registration are now part of FDA policy, Israelsen (1997). In 1997, the North American market for products of plant origin reached US$ 2 billion, (Brevoort, 1997). Thus, the modern social context and economic view of health services, the needs of the pharmaceutical market and the recognition that research on medicinal plants used in folk medicine represents a suitable approach for the development of new drugs, Elisabetsky (1987a); Calixto (1996) have led to an increase in research in this field, and private and governmental institutions are now financially supporting natural product research programmes worldwide.

The NCI (National Cancer Institute, USA) has tested more than 50,000 plant samples for anti-HIV activity and 33,000 samples for anti-tumour activity. In 1993, the International Program of Co-operation for Biodiversity (IPCB) was launched in order to promote natural products in Latin America and Africa, linking universities, industries and governments in a multidisciplinary programme for the sustained development and preservation of the environment, Rouhi (1997). Large pharmaceutical companies, such as Merck, CIBA, Glaxo, Boehringer and Syntex, now have specific departments dedicated to the study of new drugs from natural sources, Reid *et al*

(1993). However, the potential use of higher plants as a source of new drugs is still poorly explored. Of the estimated 250,000–500,000 plant species, only a small percentage has been investigated phytochemically and even a smaller percentage has been properly studied in terms of their pharmacological properties; in most cases, only pharmacological screening or preliminary studies have been carried out. It is estimated that 5000 species have been studied for medical use, Payne *et al* (1991). Between the years 1957 and 1981, the NCI screened around 20,000 plant species from Latin America and Asia for anti-tumour activity, but even these were not screened for other pharmacological activities, Hamburger and Hostettman (1991).

People have used plants for millennia and vast information of the medicinal uses of plants has therefore accumulated especially in the tropical parts of the world. According to the World Health Organization (WHO), about 80 % of the people in developing countries rely primarily on medicinal plants for their primary health care, Wood-Sheldon *et al* (1997). In many remote areas in African countries people consult the traditional healer of the village in case of illness. Western hospitals and medicines are often beyond the reach and Western medicines many times too expensive for the people to afford. In Africa the ethnopharmacological and ethnobotanical knowledge on the uses of medicinal plants is often orally passed down from generation to generation. This abundance of information is in danger of disappearing since it is often kept secret until the last minutes of death of the traditional healer when they eventually call on somebody to inherit the information, Kokwaro (1976). Although traditional medicine has been recognized as a part of primary health care programmes in many African countries (WHO, 1978),

there is a need to evaluate scientifically the crude extracts of plants for their medicinal and pharmacodynamic properties, clinical usefulness and toxicological potential, Kyerematen & Ogunlana (1987).

Higher plants are still poorly explored as sources of new drugs, Hostettman & Terreaux (2000). There are several ways in selecting plant materials when searching for new medicinal plants/active compounds. Ethnopharmacological information on medicinal plants is often of substantial importance for the finding of new potential medicinal plants/new ways of using an already known plant. It has been estimated that 74% of the pharmacologically active, plant derived components were discovered after the ethnomedical uses of the plants started to be investigated, Farnsworth & Soejarto (1991); Wood-Sheldon *et al* (1997). Another important way of discovering new medicinal plants and lead compounds is the phylogenetic approach in which a number of closely related species of plants, assumed to contain related chemical compounds (chemotaxonomy), are screened for their biological effects, Cotton (1996); Vuorela *et al* (2004). Random sampling of plant samples from certain habitats with high species diversity (for example tropical rainforests) can be beneficial for finding novel chemical entities (NCEs), but is somewhat time-consuming and requires hard work, Vuorela *et al* (2004). This kind of sampling is likely to be the industrial approach and most likely to be used for evaluating plants for bioactive compounds, Fabricant & Farnsworth (2001).

Malaria constitutes one of the biggest health problems in tropical Africa and is slowly spreading to hitherto non-malaria areas, Trape (2002). The emergence of resistant parasites, changes in climatic conditions over a large part of Africa, changes in land use and population migration,

Foster (1991); Ridley (1997) are suggested to have extended the areas of malaria transmission, which requires innovative strategies for malaria and the mosquito vector control. Malaria is also endemic in South-east Asia, in Central and South America and Oceania. After the African countries, India and Brazil are presently the regions of highest endemicity in the World, WHO (1997). It is estimated that the malaria incidence range between 350 and 500 million cases with 90% of these being in tropical Africa, WHO (2005).

In Kenya, more than 90% of malaria is caused by *Plasmodium falciparum,* Khaemba *et al* (1994) and is transmitted by *Anopheles gambiae* which is the most widespread in Africa and difficult to control. Each year, there are over 8.2 million malaria infections in Kenya, Jean-Marie (2002) mostly due to failure to use insecticide treated nets and increased resistance of the parasites to drugs. The disease accounts for 30% of all the outpatient cases and 19% of all admissions, 5.1% of whom die, and 72 children below the age of 5 years die daily, DMS (2006); WHO (1996); Mouchet (1999). The disease is endemic in the lowlands, particularly the coastal strip and Lake Victoria basin where transmission is sufficiently intense. In these areas, both incidence and prevalence of infection reach more than 90% of the population within 10–12 weeks after the beginning of the rainy season, Hoffman *et al* (1996).

Malaria transmission patterns in Kenya are influenced by several factors such as rainfall, relative humidity, vector species, and intensity of biting, altitude and presence of susceptible new human hosts. Patterns of endemicity are described in terms of stable, unstable, epidemic and malaria-free zones, MOH (1992). Stable malaria occurs in zones which have continuously high transmission rate throughout the year and includes the coastal strip and western parts of Kenya. Unstable

malaria occurs where there is seasonal endemicity with one or two annual transmission peaks and includes parts of Eastern Province and some parts of the Rift Valley. Epidemic malaria occurs in highland areas bordering endemic zones. Since 1988 there has been sporadic highland epidemics and considerable child mortality reported in Uasin Gishu, Nandi, Kericho, Kisii and Nyamira districts. Malaria free zones include all land that lies at attitudes of 1600 m above sea level, but due to the critical epidemiological situation of this disease, very few areas are safe, MOH (1992). A major impact of the disease was documented in the highlands of East Africa, where the spread of chloroquine (CQ) resistance was probably the only factor likely to explain the changing epidemiology of malaria in areas of low and unstable transmission, despite initial claims that it could be attributed to global warming, Shanks *et al* (2000).

1.2. Statement of the problem

Malaria has continued to be a major global public health problem and a health concern in most African countries, Nguta *et al* (2010). It is thought that malaria is by far the most serious tropical disease causing one to two million deaths per year in Africa, Nguta *et al* (2010a). The World Health Organization (WHO) has estimated that about 2 billion people in over 100 countries are exposed to malaria, Milliken (1997). The worsening economic situation of the Sub-Saharan African countries makes it difficult to expand modern health services hence effective low-cost delivery medical system is urgently needed. In Kenya, malaria continues to be a national concern as it plays a major role in the high mortality seen in infants and children. It is also responsible for abortion, premature deliveries, growth retardation, low birth weight and anemia, Nguta *et al*

(2010a). In Kenya, malaria is responsible for 30-50% of outpatient treatments, 19% of admissions and accounts for 8-10 million treatments per year, Ochola (2003).

The increasing prevalence of strains of *Plasmodium falciparum* resistance to current antimalarial drugs poses a serious problem for malaria control, Trape (2002) and leaves Africa with unprecedented situation in which the only affordable treatment options are rapidly losing therapeutic efficacy, Fidock *et al* (2004). These developments and the difficulty of creating efficient vaccines coupled with adverse side effects of the existing antimalarial drugs underline the urgent need for novel, well tolerated and more efficient antimalarial drugs affordable to the poor, living in malaria endemic tropical countries, Bickii *et al* (2000). In endemic countries, accessible treatments against malaria are mainly based on the use of traditional herbal remedies. Indeed, indigenous plants play an important role in the treatment of many diseases and 80% of the people worldwide are estimated to use herbal remedies, Geoffrey and Kirby (1996). However, few ethnobotanical studies have been conducted while few data is available on their efficacy and safety, despite the fact that validation of traditional practices could lead to innovative strategies in malaria control. The current study is designed to document anti malarial plants used by the Msambweni community, South Coast, and also to investigate their toxicity.

1.3. Objectives

1.3.1. Broad objective

To conduct ethnopharmacological and toxicological study on the commonly used antimalarial herbal plants in Msambweni District, South Coast, Kenya.

1.3.2. Specific objectives

1. To study ethnopharmacology of antimalarial phytotherapy remedies in Msambweni district, Kenya.
2. To study attitudes and traditional practices related to malaria recognition, control and treatment in Msambweni district, Kenya.
3. To evaluate the ethnodiagnostic skills of the Digo community as a lead to traditional bioprospecting
4. To investigate the acute toxicity of antimalarial crude plant extracts in brine shrimp bioassay using *Artemia salina*.

CHAPTER TWO

LITERATURE REVIEW

2.1. Introduction

In antimalarial drug discovery programs, routine screening for anti-pre-erythrocytic stage activity is seldom carried out, partly because these stages are clinically silent, but also because access to pre-erythrocytic parasites and purified infected hepatocytes is costly and restricted to a few laboratories. However, demonstration of efficacy for the treatment of acute infections has been straightforward because plant extracts or isolated compounds can be tested *in vitro* or *in vivo* using laboratory experimental models, which have been easier to develop, Hart (2005). Traditional medicines are a potential rich source of new drugs against malaria and other infectious diseases and given the remarkable antimalarial properties of *Cinchona* bark that have been known for more than 300 years, resulting in the discovery of quinine, Camacho *et al* (2000) and the more recent development of artemisinin derivatives has re-affirmed the potential of plant species to provide effective drugs for the treatment of malaria. Artemisinin, a sesquiterpene lactone was isolated from the herb *Artemisia annua* in China in 1971 and was highly unusual as it contained an endoperoxide moiety in contrast to known antimalarial drugs, Wright (2005). Its discovery heralded a new era in antimalarial drug development as the compound and its synthetic derivatives such as artemether and artesunate were rapidly effective against parasites resistant to other antimalarials. The compounds had gametocyticidal activity, thus were able to reduce transmission to the mosquito vector, Wright and Warhurst (2002).

Despite the cost and adverse effects, a standard treatment for severe malaria in Africa is the intravenous administration of quinine, WHO (2001) and resistance against the drug in Africa has not been reported, Le Bras *et al* (2006). Resistance of *P. falciparum* to current antimalarial drugs, Trape (2002), coupled with unavailability and unaffordability of these agents, Bathurst and Hentschel (2006), in addition to lack of new therapeutic agents, Benoit-Vical (2005); Mutabingwa (2005) has led the Government of Kenya to provide free and subsidized Artemisinin based combination therapy (ACT) in public health facilities and private pharmacies, respectively, as first line of treatment for uncomplicated malaria. However, hopes that artemisinins will have a major impact on malaria have been tempered by a recent study in which a number of clinical isolates of *P. falciparum* from malaria patients showed resistance to artemether, Jambou *et al* (2005). Thus the World Health Organization have called for an immediate halt to the marketing and sale of malaria medicines that contain only artemisinin or one of its derivatives, WHO (2006) and recommended the use of ACTs to minimize the risk of resistance development. Even so, there is evidence that resistance to lumefantrine–artemether may have developed in Zanzibar after a short period of use (Sisowath et al., 2005). This underlines the urgent need for continued search of new antimalarial drugs from medicinal plants.

It has long been recognized that natural product structures have the characteristics of high chemical diversity, biochemical specificity and other molecular properties that make them favorable candidates for drug discovery, and which serve to differentiate them from libraries of synthetic and combinatorial compounds, Clardy and Walsh (2004). The new drug discovery approaches need to take into account some specific concerns, in particular, the requirement for

new therapies to be inexpensive and simple to use, as well as the need to limit the cost of drug research in as much as the new therapy would be a drug of mass treatment, affordable to the poor who are most vulnerable to the disease, Bathurst and Hentschel (2006).

2.2. Plants as therapeutic resources and various phytochemicals found in medicinal plants used in treatment of malaria

Plants can be used as therapeutic resources in several ways. They can be used as herbal teas or other home-made remedies, when they are considered as medicinal plants. They can also be used as crude extracts or "standard enriched fractions" in pharmaceutical preparations, such as tinctures, fluid extracts, powder, pills and capsules, when they are considered as phytopharmaceutical preparations or herbal medicines. Finally, plants can be subjected to successive extraction and purification procedures to isolate the compounds of interest, which can themselves be active and used directly as a drug, examples being quinine, digoxin and ergotamine, or they can be used as precursors (e.g. diosgenin) in hemisynthetic processes or as models for total synthesis, with well-defined pharmacological activity or structure–activity relationship studies determining a prototype drug e.g. morphine, Rates (2001). Phytochemicals found in medicinal plants used in treatment of malaria include: alkaloids (naphthylisoquinolines, bisbenzylisoquinolines, protoberberines and aporphines, indoles, manzamines, and miscellaneous alkaloids) terpenes (sesquiterpenes, triterpenes, diterpenes, and miscellaneous terpenes) quassinoids, flavonoids, limonoids, chalcones, peptides, xanthones, quinones and coumarines.

2.2.1. Drug development from medicinal plants

According to Arias (1999) a *medicinal plant* is (1) any plant used in order to relieve prevent or cure a disease or to alter physiological and pathological process, or (2) any plant employed as a source of drugs or their precursors. A *phytopharmaceutical preparation* or *herbal medicine* is any manufactured medicine obtained exclusively from plants (aerial and non-aerial parts, juices, resins and oil), either in the crude state or as a pharmaceutical formulation. A *medicine* is a product prepared according to legal and technical procedures that is used for the diagnosis, prevention and treatment of disease and has been scientifically characterized in terms of its efficacy, safety and quality, WHO (1992). A *drug* is a pharmacologically active compound, which is a component of a medicine, irrespective of its natural, biotechnological or synthetic origin. The approach for drug development from plant resources depends on the aim. Different strategies will result in a herbal medicine or in an isolated active compound. However, apart from this consideration, the selection of a suitable plant for a pharmacological study is a very important and decisive step. There are several ways in which this can be done, including traditional use, chemical content, toxicity, randomised selection or a combination of several criteria, Ferry and Baltassat-Millet (1977); Soejarto (1996); Williamson *et al* (1996).

The most common strategy is careful observation of the use of natural resources in folk medicine in different cultures; this is known as ethnobotany or ethnopharmacology. Information on how the plant is used by an ethnic group is extremely important. The preparation procedure may give an indication of the best extraction method. The formulation used will provide information about pharmacological activity, oral versus non-oral intake and the doses to be tested. However, certain

considerations must be taken into account when the ethnopharmacological approach of plant selection is chosen. For instance, each ethnic group has its own concepts of health or illness, as well as different healthcare systems, Elisabetsky and Posey (1986). The signs and symptoms should be translated, interpreted and related to western biomedical concepts, thus allowing a focused study of a particular therapeutic property. Selection based on chemical composition uses phylogenetic or chemotaxonomic information in the search, mainly in certain genera and families, for compounds from a defined chemical class with known pharmacological activity, Gottlieb and Kaplan (1993); Souza Brito (1996). The search for highly specific potent drugs for therapeutic use and, more precisely, as an investigation tool in biological research has been quite productive in toxic plants. A number of important compounds now used in research came from toxic plants and several examples have been mentioned, Williamson *et al* (1996).

Another method of selecting a plant is that the investigator decides on a well-defined pharmacological activity and performs a randomised search, resulting in active species to be considered for further study, Harmburger and Hostettman (1991). The search for antitumor drugs is a good example of the use of this strategy. Finally, it is possible, and often desirable and inevitable, to use a combination of several criteria. Furthermore, apart from the chosen strategy, searching databanks and the scientific literature is crucial in finding active and/or toxic compounds that have already been identified, and can also be used as a criterion for choosing plants, e.g. if the purpose is to find a new source. However, the choice of a biological material to be screened for active compounds and the subsequent development of a drug must take into

account that the exploration of natural resources should meet global and regional needs for new efficient and safe drugs, while preserving natural diversity and the environment.

The present situation of exploitation of the world's vegetation may lead to the extinction of some species, which means not only the loss of interesting chemical compounds as potential drugs, but also the loss of genes, which could be of use in plant improvement or in the biosynthesis of new compounds. It is, therefore, crucial; both for the development of areas with rich flora, such as Asia and Latin America, and for the pharmaceutical industry, to protect and promote the rational exploitation of biodiversity as a source of chemical compounds that have direct biological activity or can be used for the rational planning of new drugs. By following this principle, a new understanding of sustained development emerges, involving preservation of the environment while searching for new drugs, especially in developing countries which, by coincidence, have the largest natural resources on the planet, Soejarto (1996); Brito and Nunes (1997); Rouhi (1997). Sensible use of these resources must be based on the amounts available, ease of access, the possibility of preservation and replanting and the establishment of priorities in relation to a desirable pharmacological activity. If possible, consideration should be given to the use of cultivated plants, which allows the production of homogeneous material, thus guaranteeing chemical homogeneity, and the use of plants from genetic enhancement projects, which preserve species threatened with extinction, Labadie (1986).

The search for drugs active against tumours, viruses and cardiovascular and tropical diseases is a priority. The largest research fields, as defined by the number of publications describing bioactive plant-derived compounds in the last few years, are anti-tumour drugs, antibiotics, drugs active

against tropical diseases, contraceptive drugs, anti-inflammatory drugs, immunomodulators, kidney protectors and drugs for psychiatric use, Hamburger and Hostettman (1991). Taxol is both an example of the importance of natural products and of the complexity and necessity of finding alternative routes by which it can be obtained. It is the most important natural product-derived diterpene with anti-tumour activity found in recent years. Taxol is isolated from *Taxus* (*T. brevifolia* and *T. bacata*). However, the biggest obstacle to its clinical use is obtaining the material. In order to produce 2.5 kg of taxol, 27,000 tons of *T. brevifolia* bark are required and 12,000 trees must be cut down. Due to the high demand, this species of *Taxus* will soon be extinct if no alternative source of taxol can be developed. An economically possible and technically realistic alternative is its partial synthesis, in considerable yield, from an analogue found in other species of *Taxus*, as well as the production of other hemi-synthetic analogues, Hamburger and Hostettman (1991); Wall and Wani (1996).

2.2.2. Ethnopharmacology and drug discovery

Numerous molecules have come out of ethnopharmacological experiential base, including *Rauwolfia* alkaloids for hypertension, psoralens for vitiligo, *Holarrhena* alkaloids in amoebiasis, guggulsterons as hypolipidemic agents, Mucuna pruriens for Parkinson's disease, piperidines as bioavailability enhancers, baccosides for mental retardation, picrosides for hepatic protection, phyllanthins as antivirals, curcumines for inflammation, withanolides and many other steroidal lactones and their glycosides as immunomodulators, Patwardhan (2005). Indeed today many pharmacological classes of drugs include a natural product prototype, Gilani *et al* (1992).

Aspirin, atropine, artimesinin, colchicine, digoxin, ephedrine, morphine, physostigmine, pilocarpine, quinine, quinidine, reserpine, taxol, tubocurarine, vincristine, and vinblastine are a few examples of what medicinal plants have given us in the past. Most of these plant-derived drugs were originally discovered through the study of traditional cures and folk knowledge of indigenous people and some of these could not be substituted despite the enormous advancement in synthetic chemistry. Morphine isolated from the opium poppy (*Papaver somniferum)* is one of the early molecules entered into conventional medicine and is the humanity's finest painkiller. Indeed, the isolation of morphine from crude opium by Serturner in 1806 stimulated so much wide-spread research on the vegetable drugs that Megendie was able to publish a medical formulary in 1821, which contained only pure chemical agents, hence laid the foundation for the use of pure chemicals as the alternative to the botanicals.

One of the important areas in which compounds from plant sources have contributed successfully is cardiovascular research, Gilani (1998). Digitalis and the cardiac glycoside derived from the foxglove (*Digitalis purpurea*) are perhaps the classic example. They represent a widely used group of clinically effective compounds which produce positive inotropic effect on the failing heart as well as having value in the treatment of atrial fibrillation. As a group they are unrivalled to date by any synthetic or semi-synthetic substitutes even though they are among the most toxic group of clinically useful drugs and have unique mode of action with selective cardiotonic activity, without accompanying tachycardia, Rietbrock and Woodcock (1985). A second discovery of cardiovascular activity in natural products led to the isolation of reserpine over five decades ago. Reserpine, obtained from the roots of the Indian plant *Rauwolfia serpentine*, was

brought to the attention of the modern Western world in 1949 by Vakil who described its use in hypertension; in rapid succession between 1952 and 1958, reserpine was isolated from *Rauwolfia*, its structure determined and its total synthesis achieved, Dohadwalla (1985). The indiscriminate use of reserpine as an antihypertensive agent and tranquilizer led to reports of depression and Parkinsonism effects. These findings stimulated further investigation and evidence was found that reserpine depleted not only brain serotonin but also nor-epinephrine and dopamine, Curzon (1990). This was a major stimulus for continued research on transmitter amine defects in depression and Parkinson's disease. This in part laid the foundation for the development of many of the modern psychoactive drugs and stimulated a significant interaction between researchers and drug industry.

As the adverse effects of reserpine continued to be revealed through clinical research, interest in the product gradually diminished, particularly when safer antihypertensive drugs were made available, though reserpine is still used in clinical medicine, particularly in low-income population. Indeed, there is a revival of interest in its use based on some recent clinical trials, which showed that lower doses of reserpine (0.05–0.1 mg) combined with low doses of thiazide diuretic and hydralazine provides highly effective blood pressure lowering regimen along with renal protective effect; relatively free from conventional side-effects and is perhaps the most cost-effective antihypertensive treatment available today Pavan *et al* (2003); Milne and Pinkney-Atkinson (2004). This development of reserpine clearly illustrates the fundamental scientific principle that drugs, in addition to being therapeutic agents, become tools for further understanding of disease and hence design of new drugs.

Other compounds, which are considered invaluable pharmacological "tools" for evaluating the mode of action of other drugs or investigation of basic physiological function, include muscarine and nicotine (pioneer selective agonists for muscarinic and nicotinic receptors respectively), cocaine (catecholamine uptake inhibitor) yohimbine (selective alpha-2 blocker) and himbacine, a prototype of cardio-selective antimuscarinic agents, Gilani (1998). Aspirin, an acetyl salt of salicylic acid (an active principle from *Willow* bark) is considered one of the most effective analgesic, antipyretic and anti-inflammatory agents commonly used in modern medicine. With the passage of time multiple therapeutic uses of aspirin have emerged, with most prevalent use as the antiplatelet/anticoagulant observed at the low dose to prevent further problems in patients who have already suffered from one heart attack, Saeed *et al* (2002).

The major antithrombotic drugs used today all derived from veterinary practice in Canada in the 1920s when cattle were noticed to be developing stomach haemorrhage from eating mouldy hay containing sweet clover (*Melilotus officinalis*). Dicoumarol was the major drug synthesized as a result of these observations. It was first marketed by Abbot and Lilly in 1942. Warfarin (from *Melilotus officinalis*) has been known to most people since the 1940s as a rat poison that acts through its anticoagulant action. The unsuccessful suicide attempt by a US Army recruit showed it to be a less toxic anticoagulant than the dicoumarol and therefore for human use, Minter (2001). Warfarin so named from the Wiscounsin Alumni Research Foundation who received the royalty from the drug sales is the world's most successful anticoagulant drug, used in cardiology, stroke, and in general post-operative recovery when a patient is at risk from clotting during bed rest.

2.2.3. Bioassay and isolation of the active compounds

Bioassays can be performed using microorganisms, molluscs, insects, cellular systems (enzymes, receptors, etc), cell culture (animal and human), and isolated organs or in vivo (mammals, amphibians, birds, etc), Hamburger and Hostettman (1991); Souza Brito (1996). All these methods have advantages and disadvantages and the appropriate method must be carefully selected at each step of any biological study aimed at the development of a drug or the understanding of the biological basis of a particular pathology or even the discovery of the mechanism of action of already known drugs. In general, a plant extract contains low concentrations of active compounds and a large number of promising compounds, requiring the use of sensitive bioassays suitable for the wide chemical variety and small amounts of the tested samples. Tests must be simple, reproducible, fast and cheap, Souza Brito (1996); Brito and Nunes (1997). Furthermore, new techniques that can fulfil different needs and be adjusted to the classical pharmacological study of natural compounds should be sought.

There is also a need for the improvement and establishment of experimental models not yet extensively used in the evaluation of natural products. After verifying the purity of an isolated active compound, the structure is determined by spectroscopic methods (UV, IR, mass spectrum or NMR), Verpoorte (1989). Once the chemical structure is defined, total or partial synthesis and preparation of derivatives and/or analogues can be considered, and modulation of the biological activity and definition of the structure–activity relationship can be carried out. After completing all these steps, large-scale isolation (it may necessary to collect the plant again) or partial or total synthesis is required for pharmacological evaluation in pre-clinical, clinical and toxicological

trials aimed at future therapeutic use, Hamburger and Hostettman (1991); Borris (1996). As mentioned above, the final result of this strategy, the drug, is expensive. However, the study of medicinal plants also allows their use "*in natura*" and/or in pharmaceutical formulations obtained from them, called *phytomedicines* or *herbal remedies*. This approach also requires efficacy and toxicity studies, but these are less time-consuming, as the steps of fractionation, purification and bioassay are basically not required or are far less complex, Elisabetsky (1987b).

The Traditional Medicine Division of the WHO recognises that the centuries-old use of certain plants as therapeutic resources should be taken into account as proof of their efficacy, Gilbert *et al* (1997). However, the total acceptance of plant-derived drugs and phytotherapy in scientific medicine and western health systems can only occur if these products fulfil the same criteria of efficacy, safety and quality control as synthetic products, Ca´ceres and Giron (1997); Wagner (1997). Moreover, knowledge of the main pharmacologically active plant compounds is an essential requirement for the standardisation and analysis of formulations.

In the last decade, considerable effort, e.g. the Ibero-American Program, CYTED, ESCOP (European Scientific Cooperative of Phytotherapy) and Commission E (an independent committee on herbal remedies of German Federal Institute for Drugs and Medical Devices), has been made in trying to obtain clinical proof of efficacy, to standardise procedures for obtaining herbal remedies and to define chemical composition in order to replace crude products with modern pharmacological formulations. However, lack of knowledge of chemical composition, geographical distribution and environmental impact on chemical biodiversity and plant variability makes it difficult to obtain a consistent quality. Furthermore, knowledge of the effect of

production methods and adjuvant compounds on the pharmacological properties of products derived from medicinal plants is still a huge research field, Petrovick (1997).

On the other hand, bioactivity-guided fractionation, essential when trying to isolate an active substance, may exclude plants or compounds with relevant pharmacological activities. This can occur when the effect is not caused by a single compound, but by a combination, as a result of pharmacodynamic synergism or pharmacokinetic influences. A good example of this is *Panax ginseng* in which the whole plant or its saponin fractions are more active than the isolated compounds, Hamburger and Hostettman (1991). In addition, when only one activity is considered in pharmacological screens, it is not possible to detect other potentially useful activities. *Catharanthus roseus* was initially studied for its anti-diabetic activity described in folk medicine, but it also contains a powerful anti-tumour compound, currently in clinical use, Williamson *et al* (1996). Ginkgolides are another example of the difficulties encountered in determining an active compound, Hamburger and Hostettman (1991). *Ginkgo biloba* has been used for centuries in Chinese medicine to treat asthma and cough. The clinical efficacy of *Ginkgo biloba* extract was, for many years, attributed to its phenolic compounds (flavonoids and biflavonoids).

The first pharmaceutical formulations of *Ginkgo* extracts were marketed in 1960, but, only a few years ago, it was found that the "standardised extract" inhibits platelet aggregation factor (PAF)-induced platelet aggregation. The compounds responsible for this effect were later isolated and identified as gingkolides A, B, C and M, Gilani (1998). Interestingly, these compounds were already known, their isolation having been described in 1932 and their chemical structure determined in 1967, but they were considered not to have any activity, Gilani (1998). The low

yield of material, the physico-chemical characteristics of the final compound and subsequent problems, such as solubilisation of extracts and fractions in solvents compatible with the animal system, are difficulties which must be resolved in the pharmacological evaluation of natural products. These problems, in fact, can invalidate the entire study because of false negative results, interference from compounds with unspecific or cytotoxic activity, poor absorption through natural biological barriers and poor bioavailability of the products. The limitation on the amount of material that can be obtained has been gradually overcome by the use of modern extraction, purification and isolation methods, and the development of highly specific sensitive bioassays. Auxiliary substances, such as alcohol, Tween 80, NaHCO3, carboxymethyl cellulose, citric acid, DMSO, propylene glycol, polyethylene glycol and preparation of salt derivatives, are currently used to dissolve extracted materials and isolate compounds. There is an urgent need for the development and improvement of technologies for the extraction and preparation of "enriched fractions" of suitable solubility in biological fluids, Willianson *et al* (1996).

2.2.4. Trends in ethnopharmacology: need for a paradigm shift

Research into, and development of, therapeutic materials from plant origin is a hard and expensive task, Borris (1996); Turner (1996); Willianson *et al* (1996). Each new drug requires an investment of around US$ 100–360 million and a minimum of 10 years of work, with only 1 in 10,000 tested compounds being considered promising and only 1 in 4 of these being approved as a new drug. Up to 1992, the NCI had only found 3 plant extracts active against HIV out of 50,000

tested, and only 3 out of 33,000 plant extracts tested were found to have anti-tumour activity, Williamson *et al* (1996).

Quantitative considerations regarding the average yield of active compounds and the amount of starting crude plant material required for the discovery, development and launch of a new drug on the market have been presented by McChesney (1995): 50 kg of raw material are necessary to provide 500 mg of pure compound for bioassays, toxicology, and "*in vivo*" evaluation; full pre-clinical and clinical studies can require 2 kg of pure compounds obtained from 200 ton of raw material. Plants in their crude form show interesting combination of activities in the form of botanicals both in developing countries and the industrialized world. Ethnopharmacology has already played important role in the development of conventional medicine and is likely to play more significant role in the years to come. It would not be surprising to see that the use of botanicals will be gradually accepted in the main stream of conventional medicine particularly if some mechanism of royalty comes into practice.

2.3. Traditional Medicine in Africa

African traditional medicine is the oldest and perhaps the most diverse of all medicine systems, Gurib-Fakim (2006). Africa is considered to be the cradle of mankind with a rich biological and cultural diversity, and there are marked differences between different regions of this continent when it comes to healing practices, Gurib-Fakim (2006). Medicinal and poisonous plants, including a diverse array of woody plants, have always played an important role in African life.

The traditions of collecting plants as well as processing herbal remedies and applying them have been handed down from generation to generation, Von Maydell (1990).

2.3.1. Documentation of African traditional medicinal knowledge

Most of the African cultures have a verbal tradition, and therefore written information on cultural features in the past are not so readily available from Africa as from many other parts of the world, Hedberg & Staugård (1989). There exists, however some documents on African traditional medicine, and the oldest of them is written by the famous Arab doctor and polyhistor, Avicenna, who lived 980-1037 A.D. With the colonization of Africa, European botanists started to explore the flora of various parts of the continent. The ethnobotanical information on the uses of the plants were sometimes documented on herbarium labels, and in this way ethnobotanical information on a number of plants began to accumulate, Hedberg & Staugård (1989).

Systematic accounts in written form dealing with medicinal plants in Africa are of fairly recent date, reports dealing with ethnopharmacological aspects being even more recent. An extensive review on African traditional medicine and on the use of plants for medicine is written by Maurice Iwu, a Nigerian pharmacognosist and ethnopharmacologist, Iwu (1993). A number of traditional national pharmacopeias have appeared, starting with Madagascar in 1957, and research in the field of ethnobotany and ethnopharmacology has developed rapidly in many African countries, Hedberg & Staugård (1989). The *African Pharmacopoeia*, covering traditional medicine of many African countries, has been published by the Scientific Technical Research

Commission of the Organization of African Unity, starting with volume 1 in 1985 (African Pharmacopoeia, Vol.1, 1985).

2.3.2. The importance of traditional medicine in different African countries

Traditional medicine is an important part of the health-care system in most of the African countries. About 80 – 90 % of the populations in African countries are dependent on traditional medicine for their primary health care, Hostettman *et al* (2000). For example in Sudan, traditional medicine plays an important role for health care, since access to hospitals and other medical facilities is limited and a high percentage of the population are nomads, Elegami *et al* (2002). In Tanzania, over 60 % of the health seeking population has a traditional healer as their first point of contact, Hedberg *et al* (1982). In spite of an extensive programme to create health centers and to train Rural Medical Aids and Medical Assistants, the traditional healer is still the only medical practitioner available, within reasonable distance, to many Tanzanians living in the rural parts of the country, Hedberg *et al* (1982).

Traditional medicine is also important in the big cities of Tanzania, such as Dar-es-Salaam, Swantz (1974). The number of registered traditional healers in Tanzania has been estimated to about 30,000 – 60,000, currently even 75,000, Mhame (2000), in comparison with about 600 Western-trained doctors, Weenen *et al* (1990). In South Africa it is estimated that about 27 million people depend on traditional herbal medicines for their primary health care, Meyer *et al* (1996); Mander (1998). In Nigeria traditional medicine is well acknowledged and established as a viable profession, Kafaru (1994), and almost all plants seem to have some kind of application in

traditional medicine, Babayi *et al* (2004). Traditional medicine seems to have certain advances over imported systems of medicine because it is an integral part of the people's culture and is particularly effective in solving certain cultural health problems, Von Maydell (1996). The African Union and the O.A.U. Scientific Council for Africa work for the promotion of systematic research on traditional medicine in different African countries, Swantz (1974).

The rising cost of Western medicine means that the people in African countries are increasingly turning to traditional medicine as an affordable alternative. The current policy in many African countries has been to incorporate traditional medicine into the formal health care sectors, Tsey (1997). In some African countries it is thought that traditional medicine should be taught and practiced as part of formal health care sector, Tamakloe (1995). Gradually, traditional medicinal practitioners (TMP) are being officially accepted as part of African health services and their medical knowledge is finding its place in hospitals and clinics, Neuwinger (2000). In South Africa, traditional medicinal practices have only recently been officially recognized as a legitimate form of health care and traditional medicine is now being integrated in the official health care system under the Reconstruction and Development Plan (RDP), Pick (1992). In many countries in Africa traditional methods are now being used for the treatment of HIV infection, Morris (2002) and malaria, Nguta *et al* (2010)), including the use of medicinal plants that help alleviate the symptoms of these diseases. These methods are sometimes claimed to give fewer side effects than conventional antiretroviral therapy, Morris (2002).

2.3.3. The practice of traditional medicine in the African community

The holders of traditional medicinal knowledge differ in different indigenous groups. In some cases *all* members of the community may know how to treat a wide range of common diseases and only seek the advice of a traditional healer for the treatment of specific diseases when their own treatments have failed. For example in Msambweni district, Kenya, common plant treatments are known and used by the majority of rural people, as well as by many people in the cities, although these people are not recognized as herbalists since they are not selling their services to others, Nguta *et al* (2010).

In some indigenous groups TMP hold most of the medicinal knowledge, and in these cases the knowledge is often passed down through certain families/tribes from generation to generation under a system of apprenticeship, Swantz (1974); Neuwinger (2000). The healer typically diagnoses and treats the psychological basis of the illness before prescribing medicines to treat the symptoms. Most healers believe that the body requires treatment with several different plants, and it is the combination of these which produces a healing effect, either through complementary benefits or synergy and potentiation, Neuwinger (2000). Many recipes consist of several plants, or different parts of the same plant, since different plant parts often cause different effects.

2.3.4. Information on the medicinal uses of plants in Africa is in danger of disappearing

The abundance of information on the traditional medicinal uses of plants in Africa is in danger of disappearing since the knowledge of how to use medicinal plants is mostly passed down orally and even to date is poorly documented, Gurib-Fakim (2006), although written information has

been produced for some specific regions. The large volume written by Iwu (1993) about medicinal plants and their uses in several African countries is extensive and covers much, but Africa is large and there is still large areas which have not been explored for their traditional medicine. It is rare to find healers with written documents, apart from minor memory aids as to plant characteristics, which help to find medicinal plants similar in appearance but different in healing effects. Oral transfer of knowledge is vulnerable to disruption and interference and may result in the loss and distortion of valuable ethnomedical information. In indigenous groups where traditional healers hold most of the knowledge this is likely to be even more a problem. This is further made more problematic by the wish of keeping information secret and a refusal to reveal information, an issue of particular relevance to medicinal plants, Hedberg & Staugård (1989).

Transfer of knowledge between the generations is also a problem since the younger generation understands traditional medicine as being a profession mainly conducted by members of an older generation. Still, young people are using traditional medicine for their health problems, and believe in it. Sustainability of the use of medicinal plants is an actual and important issue. The demand for medicinal plants is increasing in Africa as the population grows and pressure on medicinal plant resources will become greater than ever. The interest in plant derived medicines has also increased in the West, among the pharmaceutical companies, and thus extensive gathering of plants from tropical habitats are made in order to find novel chemical entities (NCE). Most medicinal plants are currently obtained from the wild, and there are already reports of some medicinal plant species becoming extremely rare, such as *Warburgia salutaris* (Canellaceae),

Cassine transvaalensis (Celastraceae) and *Erythrophleum lasianthum* (Leguminosae), Fennel *et al* (2004). The major factor contributing to the depletion of natural resources is the loss of habitats due to anthropogenic activity, Iwu (1995); Rukangira (2001).

Africa is estimated to have 216,634 ha of closed forest areas and with a calculated annual loss of about 1 % due to deforestation, one of the highest rates of deforestation in the world, many of the medicinal plants and other genetic materials become extinct before they are even documented. Habitat conversion threatens not only the loss of plant resources, but also traditional community life, cultural diversity, and the accompanying knowledge of the medicinal value of several endemic plant species, Iwu (1995).

A large proportion of the plants found in Africa are endemic to the continent, the republic of Madagascar having the highest rate of endemism (82 %), Gurib-Fakim (2006). Different solutions for the sustainable use of medicinal plants have been presented: Medicinal plants can, for example be grown as crops through small-scale farming in their natural habitats, Iwu (1995); Van Staden (1999). On the other hand, there are traditional healers who believe that plants grown under agricultural conditions will not have the same medicinal properties as those harvested from the wild. Cultivated material is believed to lack the "power" of wild medicinal plants, Cunningham (1993).

2.4. The flora and fauna of Kenyan biodiversity

The vegetation and animal life of Kenya reflect the variety of its topography and climate, which also leads to a wide variation in malaria and subsequent disease epidemiology, Ochola (2003).

From the coastal (Msambweni and Kilifi disticts) mangrove swamps and rain forest to the vast plains of the hinterland covered with grass giving way to typical tropical savanna and mountain forest. The highland areas to the west of the country are densely forested with bamboo, dense undergrowth and timber. The equatorial alpine climate around Mount Kenya (3000–5200 m above sea level), the second highest mountain in Africa, borders Meru district and influences the natural conditions in the district leading to a wide variety of microclimates and agroecological zones with a wide range of flora and fauna. Wildlife of great variety is to be found in Kenya, both in the sparsely populated areas and in the National Parks and Reserves that have been created for its protection. Forests occupy about 2–3% of Kenya's land area and yet, they are reservoirs of biological diversity (genes, species and ecosystems). Many of the plant species have medicinal value, with an estimate of over 400 plant species used for management of common diseases in East Africa, Kokwaro (1993); Gachathi (1989).

The forests like Kakamega, Aberdares, Mount Kenya and Arabuko Sokoke have exemplary display of flora and fauna. The biological diversity they carry is important because it contributes directly to the well being of Kenyans, especially those in the rural areas, and indirectly to the mainly agricultural economy. Harversting of medicinal plants and other forest products is strictly controlled by the Kenya Wildlife Service that oversees the conservation of the forests and its wildlife. However, human expansionist demands can be expected to wreak environmental deterioration and biotic destruction well into the next century. Kenya's strategy for conservation of forests involves intensification of timber and other non-wood products outside forest areas, Njuguna *et al* (2000).

The Taita Hills, in the coastal region of Kenya, is the northernmost extreme of the Eastern Arc Mountains, a biodiversity hotspot chain of mountains that run from south-eastern Kenya to southern Tanzania and boosts an extremely high diversity of flora and fauna. Indigenous cloud forest in the Taita Hills currently covers an area of 430 ha, reflecting 98% forest reduction over the last 200 years, mainly due to clearance for agricultural purposes, Myers *et al* (2000). Forest clearance is less widespread at present, but despite the small size of the 12 remaining indigenous forest fragments, they are of global conservation importance, holding numerous rare and endemic plants and animals. Conservation effort is being put in place through a multi-disciplinary approach that seeks better legal and law enforcement co-ordination between the Government, Local Authorities, scientists and the local community, Githiru and Lens (2004).

2.5. The role of herbal medicine for treatment of malaria

The use of traditional and herbal remedies seems to be the alternative choice of treatment in countries where malaria is endemic, Sofowora (1982); Rasoanaivo *et al* (1992); Gessler *et al* (1995). In the Third World, 80% of people are thought to rely on herbal remedies, Zirihi *et al* (2005); WHO (2002). Local medicinal plants continue to be used in the treatment of malaria and the evaluation of antimalarial activity of medicinal plants against *P. falciparum* has been extensively studied, O'Neill *et al* (1985); Carvalho *et al* (1991); Gakunju *et al* (1995); Gessler *et al* (1994); Basco *et al* (1994); Andrade-Neto *et al* (2003); Tran *et al* (2003); Simonsen *et al* (2001). Reviews for those studies have been undertaken in many countries: in South Africa, Pillay

et al (2008), in West Africa, Soh and Benoit-Vical (2007), in Brazil, Krettli *et al* (2001) and elsewhere, Kaur *et al* (2009).

In Asia, Latin America and Africa, the extensive use of natural plants as primary health remedies, due to their pharmacological properties, is quite common, Phillipson *et al* (1987). The Brazilian flora, just like the Kenyan which is transverse by the equator and rich in biodiversity offers a wide variety of bioactive substances, Brandão *et al* (1992). Many people in these areas rely on traditional medicine for the treatment of many infectious diseases, including malaria, de Mesquita *et al* (2007). South- East Asia is home to the most drug resistant parasites in the world, both *P. falciparum* and *Plasmodium vivax*, WHO (2001) and traditional medicine is also widely practiced. Vietnamese traditional medicine is similar in many respects to the traditional Chinese school having incorporated Chinese and Indian teaching, Nghiem (2002).

In Africa herbal medicines are an important part of the culture and traditions of its people and its biodiversity has played major specific roles in the cultural evolution of human societies, Mugabe and Clark (1998). Apart from their cultural significance, traditional medicines have been accessible and affordable and most people in Kenya especially in rural areas use traditional medicine and medicinal plants to treat many diseases including malaria, Njoroge and Bussmann (2006). It is estimated that there is one traditional healer for every 200–400 people in Uganda, one of East African countries. This contrasts sharply with the availability of trained medical personnel for which the ratio is 1:20,000 or less, WHO (2002a).

The role of ethnopharmacology is to give direction on the plant species for selection as well as data for plant preparation, posology, effects and side effects which could provide specific targets

for isolation of active compounds and pharmacological investigation in the quest for development of new pharmaceuticals, Cox and Balick (1994). Recent work on African plants used in the treatment of malaria is very encouraging. It is striking how many different plants are reported by herbalists to cure malaria. When we compare antimalarial species being used in Kenya with those recently reported to be used in Ivory Coast, Ghana, Madagascar, and Sudan, only Azadirachta indica (Neem tree) is shared, Benoit-Vical *et al* (1998); Addae-Kyereme *et al* (2001); Rasoanaivo *et al* (1999); El Tahir *et al* (1999). However, the chemical composition of the various plant species is affected by the climatic conditions and the locality under which the plant species are growing, Gessler *et al* (1995). The challenge will be to translate herbal medicine practice with these plants into an evidence-based monotherapy or combined therapy as suggested by Rasoanaivo *et al* (1999). There is need therefore, to collaborate with traditional healers and clinicians for observational retrospective treatment-outcome and prospective clinical study of a traditional medicine.

The administration of a traditional treatment (e.g. a plant preparation) as a decoction/ concoction, and the systematic follow up of the outcome in a clinical study with the effect of a rapid and complete cure, without failure and or serious side effects, would lead to further research of the product with a view to isolating active constituents that would form the basis of a monotherapy or combination therapy.

The use of medicinal herbs in traditional human societies has primarily been for treatment of active or acute conditions whereas in most instances, the reported use of medicinal herbs by animals appears to be prophylactic, Hart (2005). During epidemics some medicinal preparations

may be used as preventive and other preparations used as tonics to enhance better health. The traditional culture of the Maasai pastoralists of Kenya has outlived the present day development. They are known to ingest wild plant materials as foods, as regular ingredients of milk and meat based soups and as herbal medicines or as flavours and tonics for prevention or alleviation of a range of common ailments, Johns *et al* (1999). Perhaps the most widespread prophylactic use of medicinal herbs in humans is the use of spices in food preparation. Evidence for the antimicrobial effects of spices in reducing the growth of ingested food borne pathogens and in the preservation of meat, has been well documented. There are no available causal prophylaxis drugs to prevent malaria in endemic areas. Primaquine, the only drug specifically developed to inhibit the liver infection, has been curtailed by the associated toxicity, poor compliance, and increased risk of hemolysis when administered to persons with glucose-6-phosphate dehydrogenase deficiency, Carraz *et al* (2006).

The existence of medicinal plants used for prophylaxis also seems to be rare. *Strychnopsis thouarsii*, an endemic plant in Madagascar has been used for malaria prophylaxis, Boiteau (1986). Carraz *et al* (2006) isolated a compound tazopsine from the plant that had activity against the erythrocytic and the initial pre-erythrocytic phase of malaria infection. However, on derivatization of tazopsine to achieve N-cyclopentyl-tazopsine (NCP-tazopsine), the latter was shown to be specifically active against the *in vitro* liver stage but inactive against the blood forms of the malaria parasite, placing this lead molecule into a novel category of antimalarial compounds with true causal prophylactic activity. *Ampelozyziphus amazonicus* is another plant used in some regions of the Amazon to prevent malaria infection, Brandão *et al* (1992). Using

newly established methods to screen for drugs that inhibit sporozoites and/or liver stage parasites, Andrade-Neto *et al* (2008) demonstrated evident activity of its ethanolic root bark extract against pre-erythrocytic forms of *Plasmodium berghei* sporozoite cultures in hepatoma cells *in vitro* and *in vivo*.

Demonstration of efficacy for prophylactic use has been problematic because the behaviour is based on reducing the severity or likelihood of adverse events in the future. In antimalarial drug discovery programs, routine screening for anti-pre-erythrocytic stage activity is seldom carried out, partly because these stages are clinically silent, but also because access to pre-erythrocytic parasites and purified infected hepatocytes is costly and restricted to a few laboratories. However, demonstration of efficacy for the treatment of acute infections has been straightforward because plant extracts or isolated compounds can be tested *in vitro* or *in vivo* using laboratory experimental models, which have been easier to develop, Hart (2005).

Traditional medicines are a potential rich source of new drugs against malaria and other infectious diseases and given the remarkable antimalarial properties of Cinchona bark that have been known for more than 300 years, resulting in the discovery of quinine, Camacho *et al* (2000) and the more recent development of artemisinin derivatives has re-affirmed the potential of plant species to provide effective drugs for the treatment of malaria. Artemisinin, a sesquiterpene lactone was isolated from the herb *Artemisia annua* in China in 1971 and was highly unusual as it contained an endoperoxide moiety in contrast to known antimalarial drugs, Wright (2005). Its discovery heralded a new era in antimalarial drug development as the compound and its synthetic derivatives such as artemether and artesunate were rapidly effective against parasites resistant to

other antimalarials. The compounds had gametocyticidal activity, thus were able to reduce transmission to the mosquito vector, Wright and Warhurst (2002).

Despite the cost and adverse effects, a standard treatment for severe malaria in Africa is the intravenous administration of quinine, WHO (2001) and resistance against the drug in Africa has not been reported, Le *Bras et al* (2006). Resistance of *P. falciparum* to current antimalarial drugs, Trape (2002), coupled with unavailability and unaffordability of these agents, Bathurst and Hentschel (2006), in addition to lack of new therapeutic agents, Benoit-Vical (2005); Mutabingwa (2005) has led the Government of Kenya to provide free and subsidized Artemisinin based combination therapy (ACT) in public health facilities and private pharmacies, respectively, as first line of treatment for uncomplicated malaria. However, hopes that artemisinins will have a major impact on malaria have been tempered by a recent study in which a number of clinical isolates of *P. falciparum* from malaria patients showed resistance to artemether, Jambou *et al* (2005).

It has long been recognized that natural product structures have the characteristics of high chemical diversity, biochemical specificity, and other molecular properties that make them favourable structures for drug discovery, and which serve to differentiate them from libraries of synthetic and combinatorial compounds, Clardy and Walsh (2004). The new drug discovery approaches need to take into account some specific concerns, in particular, the requirement for new therapies to be inexpensive and simple to use, as well as the need to limit the cost of drug research in as much as the new therapy would be a drug of mass treatment, affordable to the poor who are most vulnerable to the disease, Bathurst and Hentschel (2006). A number of

ethnobotanical surveys have described several plant species traditionally used for the management of malaria in Kenya, Muthaura *et al* (2007); Nguta *et al* (2010). However, only about 20% of the plants with claimed bioactivities have been subjected to bioassay screening. Among the currently ongoing efforts is the discovery of new antimalarial leads from natural products, Rukunga *et al* (2007); Murata *et al* (2008); Yenesew *et al* (2003; 2004); Oketch-Rabah *et al* (2000). Kenya is rich in green tropical vegetation cover and its biodiversity nature and long history of traditional plant uses are claimed to possess medicinal value. The search for new bioactive plant products can follow three different ways of approach for the selection of medicinal plants: random, chemotaxonomical and ethnopharmacological where the chances for research success are greater, Trotter *et al* (1982); Elisabetsky and Wannmacher (1993).

2.6. Pharmacology of antimalarial drugs

2.6.1. Chloroquine (Molecular weight: 436.0)

Chloroquine is a 4-aminoquinoline that has been used extensively for the treatment and prevention of malaria. Widespread resistance has now rendered it virtually useless against *P. falciparum* infections in most parts of the world, although it still maintains considerable efficacy for the treatment of *P. vivax*, *P. ovale* and *P. malariae* infections. As with other 4-aminoquinolines, it does not produce radical cure. Chloroquine interferes with parasite haem detoxification, Krugliak and Ginsburg (1991); Bray (1998). Resistance is related to genetic changes in transporters (PfCRT, PfMDR), which reduce the concentrations of chloroquine at its site of action, the parasite food vacuole.

Pharmacokinetics

Chloroquine is rapidly and almost completely absorbed from the gastrointestinal tract when taken orally, although peak plasma concentrations can vary considerably. Absorption is also very rapid following intramuscular and subcutaneous administration, White (1988). Chloroquine is extensively distributed into body tissues, including the placenta and breast milk, and has an enormous total apparent volume of distribution.

The relatively small volume of distribution of the central compartment means that transiently cardiotoxic levels may occur following intravenous administration unless the rate of parenteral delivery is strictly controlled. Some 60% of chloroquine is bound to plasma proteins, and the drug is eliminated slowly from the body via the kidneys, with an estimated terminal elimination half-life of 1–2 months. Chloroquine is metabolized in the liver, mainly to monodesethylchloroquine, which has similar activity against *P. falciparum.*

Toxicity

Chloroquine has a low safety margin and is very dangerous in over dosage. Larger doses of chloroquine are used for the treatment of rheumatoid arthritis than for malaria, so adverse effects are seen more frequently in patients with arthritis. The drug is generally well tolerated. The principle limiting adverse effects in practice are the unpleasant taste, which may upset children, and pruritus, which may be severe in dark-skinned patients, Mnyika and Kihamia (1991). Other less common side effects include headache, various skin eruptions and gastrointestinal disturbances, such as nausea, vomiting and diarrhoea. More rarely central nervous system toxicity including, convulsions and mental changes may occur. Chronic use (>5 years continuous use as

prophylaxis) may lead to eye disorders, including keratopathy and retinopathy. Other uncommon effects include myopathy, reduced hearing, photosensitivity and loss of hair. Blood disorders, such as aplastic anaemia, are extremely uncommon, Taylor and White (2004). Acute over dosage is extremely dangerous and death can occur within a few hours. The patient may progress from feeling dizzy and drowsy with headache and gastrointestinal upset, to developing sudden visual disturbance, convulsions, hypokalaemia, hypotension and cardiac arrhythmias. There is no specific treatment, although diazepam and epinephrine (adrenaline) administered together are beneficial, Clemessy (1996).

Drug interactions

Major interactions are very unusual. There is a theoretical increased risk of arrhythmias when chloroquine is given with halofantrine or other drugs that prolong the electrocardiograph QT interval; a possible increased risk of convulsions with mefloquine; reduced absorption with antacids; reduced metabolism and clearance with cimetidine; an increased risk of acute dystonic reactions with metronidazole; reduced bioavailability of ampicillin and praziquantel; reduced therapeutic effect of thyroxine; a possible antagonistic effect on the antiepileptic effects of carbamazepine and sodium valproate and increased plasma concentrations of cyclosporine.

2.6.2. Amodiaquine (Molecular weight: 355.9)

Amodiaquine is a Mannich base 4-aminoquinoline with a mode of action similar to that of chloroquine. It is effective against some chloroquine-resistant strains of *P. falciparum*, although there is cross-resistance.

Pharmacokinetics

Amodiaquine hydrochloride is readily absorbed from the gastrointestinal tract. It is rapidly converted in the liver to the active metabolite desethylamodiaquine, which contributes nearly all of the antimalarial effect, Winstanley (1990). There are insufficient data on the terminal plasma elimination half-life of desethylamodiaquine. Both amodiaquine and desethylamodiaquine have been detected in the urine several months after administration.

Toxicity

The adverse effects of amodiaquine are similar to those of chloroquine. Amodiaquine is associated with less pruritus and is more palatable than chloroquine, but is associated with a much higher risk of agranulocytosis and, to a lesser degree, of hepatitis when used for prophylaxis, Hatton (1986). The risk of a serious adverse reaction with prophylactic use (which is no longer recommended) appears to be between 1 in 1000 and 1 in 5000. It is not clear whether the risks are lower when amodiaquine is used to treat malaria. Following overdose cardiotoxicity appears to be less frequent than with chloroquine. Large doses of amodiaquine have been reported to cause syncope, spasticity, convulsions and involuntary movements.

2.6.3. Sulfadoxine (Molecular weight: 310.3)

Sulfadoxine is a slowly eliminated sulfonamide. It is very slightly soluble in water. Sulfonamides are structural analogues and competitive antagonists of *p*-aminobenzoic acid. They are competitive inhibitors of dihydropteroate synthase, the bacterial enzyme responsible for the incorporation of *p*aminobenzoic acid in the synthesis of folic acid.

Pharmacokinetics

Sulfadoxine is readily absorbed from the gastrointestinal tract. Peak blood concentrations occur about 4 h after an oral dose. The terminal elimination half-life is 4–9 days. Around 90–95% is bound to plasma proteins. It is widely distributed to body tissues and fluids, passes into the fetal circulation and is detectable in breast milk. The drug is excreted in urine, primarily unchanged.

Toxicity

Sulfadoxine shares the adverse effect profile of other sulfonamides, although allergic reactions can be severe because of its slow elimination. Nausea, vomiting, anorexia and diarrhoea may occur. Crystalluria causing lumbar pain, haematuria and oliguria is rare compared with more rapidly eliminated sulphonamides. Hypersensitivity reactions may affect different organ systems. Cutaneous manifestations can be severe and include pruritus, photosensitivity reactions, exfoliative dermatitis, erythema nodosum, toxic epidermal necrolysis and Stevens-Johnson syndrome, Miller (1986).

Treatment with sulfadoxine should be stopped in any patient developing a rash because of the risk of severe allergic reactions, Bjorkman and Phillips-Howard (1991). Hypersensitivity to sulfadoxine may also cause interstitial nephritis, lumbar pain, haematuria and oliguria. This is due to crystal formation in the urine (crystalluria) and may be avoided by keeping the patient well hydrated to maintain a high urine output. Alkalinization of the urine will also make the crystals more soluble. Blood disorders that have been reported include agranulocytosis, aplastic anaemia, thrombocytopenia, leucopenia and hypoprothrombinaemia. Acute haemolytic anaemia is a rare complication, which may be antibody mediated or associated with glucose-6-phosphate

dehydrogenase (G6PD) deficiency. Other adverse effects, which may be manifestations of a generalized hypersensitivity reaction, include fever, interstitial nephritis, a syndrome resembling serum sickness, hepatitis, myocarditis, pulmonary eosinophilia, fibrosing alveolitis, peripheral neuropathy and systemic vasculitis, including polyarteritis nodosa. Anaphylaxis has been reported only rarely. Other adverse reactions that have been reported include hypoglycaemia, jaundice in neonates, aseptic meningitis, drowsiness, fatigue, headache, ataxia, dizziness, drowsiness, convulsions, neuropathies, psychosis and pseudomembranous colitis.

2.6.4. Pyrimethamine (Molecular weight: 248.7)

Pyrimethamine is a diaminopyrimidine used in combination with a sulfonamide, usually sulfadoxine or dapsone. It exerts its antimalarial activity by inhibiting plasmodial dihydrofolate reductase thus indirectly blocking the synthesis of nucleic acids in the malaria parasite. It is a slow-acting blood schizontocide and is also possibly active against pre-erythrocytic forms of the malaria parasite and inhibits sporozoite development in the mosquito vector. It is effective against all four human malarials, although resistance has emerged rapidly. Pyrimethamine is also used in the treatment of toxoplasmosis, and isosporiasis and as prophylaxis against *Pneumocystis carinii* pneumonia. Pyrimethamine is no longer used alone as an antimalarial, only in synergistic combination with slowly eliminated sulfonamides for treatment (sulfadoxine, sulfalene) or with dapsone for prophylaxis.

Pharmacokinetics

Pyrimethamine is almost completely absorbed from the gastrointestinal tract and peak plasma concentrations occur 2–6 h after an oral dose. It is mainly concentrated in the kidneys, lungs, liver and spleen, and about 80–90% is bound to plasma proteins. It is metabolized in the liver and slowly excreted via the kidneys. The plasma half-life is around 4 days. Pyrimethamine crosses the blood-brain barrier and the placenta and is detectable in breast milk. Absorption of the intramuscular preparation is incomplete and insufficiently reliable for this formulation to be recommended, Winstanley (1992).

Toxicity

Pyrimethamine is generally very well tolerated. Administration for prolonged periods may cause depression of haematopoiesis due to interference with folic acid metabolism. Skin rashes and hypersensitivity reactions also occur. Larger doses may cause gastrointestinal symptoms such as atrophic glossitis, abdominal pain and vomiting, haematological effects including megaloblastic anaemia, leukopenia, thrombocytopenia and pancytopenia, and central nervous system effects such as headache and dizziness. Acute overdosage of pyrimethamine can cause gastrointestinal effects and stimulation of the central nervous system with vomiting, excitability and convulsions. Tachycardia, respiratory depression, circulatory collapse and death may follow. Treatment of overdosage is supportive.

Drug interactions

Administration of pyrimethamine with other folate antagonists such as cotrimoxazole, trimethoprim, methotrexate or with phenytoin may exacerbate bone marrow depression. Given with some benzodiazepines, there is a risk of hepatotoxicity.

2.6.5. Mefloquine (Molecular weight: 378.3)

Mefloquine is a 4-methanolquinoline and is related to quinine. It is soluble in alcohol but only very slightly soluble in water. It should be protected from light. The drug is effective against all forms of malaria.

Pharmacokinetics

Mefloquine is reasonably well absorbed from the gastrointestinal tract but there is marked inter-individual variation in the time required to achieve peak plasma concentrations. Splitting the 25 mg/kg dose into two parts given at an interval of 6–24 h augments absorption and improves tolerability, Price (1999). Mefloquine undergoes enterohepatic recycling. It is approximately 98% bound to plasma proteins and is widely distributed throughout the body. The pharmacokinetics of mefloquine may be altered by malaria infection with reduced absorption and accelerated clearance, Krishna and White (1996). When administered with artesunate, blood concentrations are increased, probably as an indirect effect of increased absorption resulting from more rapid resolution of symptoms, Price (1999). Mefloquine is excreted in small amounts in breast milk. It has a long elimination half-life of around 21 days, which is shortened in malaria to about 14 days, possibly because of interrupted enterohepatic cycling, Nosten (1991). Mefloquine is metabolized

in the liver and excreted mainly in the bile and faeces. Its pharmacokinetics show enantioselectivity after administration of the racemic mixture, with higher peak plasma concentrations and area under the curve values, and lower volume of distribution and total clearance of the SR enantiomer than its RS antipode, Svensson (2002).

Toxicity

Minor adverse effects are common following mefloquine treatment, most frequently nausea, vomiting, abdominal pain, anorexia, diarrhoea, headache, dizziness, loss of balance, dysphoria, somnolence and sleep disorders, notably insomnia and abnormal dreams. Neuropsychiatric disturbances (seizures, encephalopathy, psychosis) occur in approximately 1 in 10 000 travellers receiving mefloquine prophylaxis, 1 in 1000 patients treated in Asia, 1 in 200 patients treated in Africa, and 1 in 20 patients following severe malaria, Phillips-Howard and ter Kuile (1995). Other side effects reported rarely include skin rashes, pruritus and urticaria, hair loss, muscle weakness, liver function disturbances and very rarely thrombocytopenia and leukopenia. Cardiovascular effects have included postural hypotension, bradycardia and, rarely, hypertension, tachycardia or palpitations and minor changes in the electrocardiogram. Fatalities have not been reported following overdosage, although cardiac, hepatic and neurological symptoms may be seen. Mefloquine should not be given with halofantrine because it exacerbates QT prolongation. There is no evidence of an adverse interaction with quinine, Supanaranond (1997).

Drug interactions

There is a possible increase in the risk of arrhythmias if mefloquine is given together with beta blockers, calcium channel blockers, amiodarone, pimozide, digoxin or antidepressants; there is

also a possible increase in the risk of convulsions with chloroquine and quinine. Mefloquine concentrations are increased when given with ampicillin, tetracycline and metoclopramide. Caution should be observed with alcohol.

2.6.6. Artemisinin (Molecular weight: 282.3)

Artemisinin, also known as qinghaosu, is a sesquiterpene lactone extracted from the leaves of *Artemisia annua* (sweet wormwood). It has been used in China for the treatment of fever for over a thousand years. It is a potent and rapidly acting blood schizontocide and is active against all *Plasmodium* species. It has an unusually broad activity against asexual parasites, killing all stages from young rings to schizonts. In *P. falciparum* malaria, artemisinin also kills the gametocytes, including the stage 4 gametocytes, which are otherwise sensitive only to primaquine. Artemisinin and its derivatives inhibit an essential calcium adenosine triphosphatase, PfATPase 6, Eckstein-Ludwig (2003). Artemisinin has now largely given way to the more potent dihydroartemisinin and its derivatives, artemether, artemotil and artesunate. The three latter derivatives are converted back *in vivo* to dihydroartemisinin. These drugs should be given as combination therapy to protect them from resistance.

Pharmacokinetics

Peak plasma concentrations occur around 3 h and 11 h following oral and rectal administration respectively, Navaratnam (2000). Artemisinin is converted to inactive metabolites via the cytochrome P450 enzyme CYP2B6 and other enzymes. Artemisinin is a potent inducer of its own metabolism. The elimination half-life is approximately 1 h, Ashton *et al* (1998).

Toxicity

Artemisinin and its derivatives are safe and remarkably well tolerated, Ribeiro and Olliaro (1988). There have been reports of mild gastrointestinal disturbances, dizziness, tinnitus, reticulocytopenia, neutropenia, elevated liver enzyme values, and electrocardiographic abnormalities, including bradycardia and prolongation of the QT interval, although most studies have not found any electrocardiographic abnormalities. The only potentially serious adverse effect reported with this class of drugs is type 1 hypersensitivity reactions in approximately 1 in 3000 patients, Leonardi (2001). Neurotoxicity has been reported in animal studies, particularly with very high doses of intramuscular artemotil and artemether, but has not been substantiated in humans, Hien (2003). Similarly, evidence of death of embryos and morphological abnormalities in early pregnancy have been demonstrated in animal studies. Artemisinin has not been evaluated in the first trimester of pregnancy so should be avoided in first trimester patients with uncomplicated malaria until more information is available.

2.6.7. Artemether (Molecular weight: 298.4)

Artemether is the methyl ether of dihydroartemisinin. It is more lipid soluble than artemisinin or artesunate. It can be given as an oil-based intramuscular injection or orally. It is also co-formulated with lumefantrine (previously referred to as benflumetol) for combination therapy.

Pharmacokinetics

Peak plasma concentrations occur around 2–3 h after oral administration, Ezzet *et al* (1998). Following intramuscular injection, absorption is very variable, especially in children with poor

peripheral perfusion: peak plasma concentrations generally occur after around 6 h but absorption is slow and erratic and times to peak can be 18 h or longer in some cases, Hien (2004). Artemether is metabolized to dihydroartemisinin, the active metabolite. After intramuscular administration, artemether predominates, whereas after oral administration dihydroartemisinin predominates. Biotransformation is mediated via the cytochrome P450 enzyme CYP3A4. Auto induction of metabolism is less than with artemisinin. Artemether is 95% bound to plasma proteins. The elimination half-life is approximately 1 h, but following intramuscular administration the elimination phase is prolonged because of continued absorption. No dose modifications are necessary in renal or hepatic impairment.

Toxicity

In all species of animals tested, intramuscular artemether and artemotil cause an unusual selective pattern of neuronal damage to certain brain stem nuclei. Neurotoxicity in experimental animals is related to the sustained blood concentrations that follow intramuscular administration, Brewer (1994), since it is much less frequent when the same doses are given orally or with similar doses of water-soluble drugs such as artesunate. Clinical, neurophysiological and pathological studies in humans have not shown similar findings with therapeutic use of these compounds, Hien (2004). Toxicity is otherwise similar to that of artemisinin.

2.6.8. Artesunate (Molecular weight: 384.4)

Artesunate is the sodium salt of the hemisuccinate ester of artemisinin. It is soluble in water but has poor stability in aqueous solutions at neutral or acid pH. In the injectable form, artesunic acid

is drawn up in sodium bicarbonate to form sodium artesunate immediately before injection. Artesunate can be given orally, rectally or by the intramuscular or intravenous routes. There are no co formulations currently available.

Pharmacokinetics

Artesunate is rapidly absorbed, with peak plasma levels occurring 1.5 h and 2 h and 0.5 h after oral, rectal and intramuscular administration, respectively, Ilett (2002). It is almost entirely converted to dihydroartemisinin, the active metabolite, Novaratnam (2000). Elimination of artesunate is very rapid, and antimalarial activity is determined by dihydroartemisinin elimination (half-life approximately 45 min), Hien (2004). The extent of protein binding is unknown. No dose modifications are necessary in renal or hepatic impairment.

2.6.9. Dihydroartemisinin (Molecular weight: 284.4)

Dihydroartemisinin is the main active metabolite of the artemisinin derivatives, but can also be given orally and rectally as a drug in its own right. It is relatively insoluble in water, and requires formulation with suitable excipients to ensure adequate absorption. It achieves cure rates similar to those of oral artesunate. A fixed-dose formulation with piperaquine is currently undergoing evaluation as a promising new artemisinin-based combination therapy (ACT).

Pharmacokinetics

Dihydroartemisinin is rapidly absorbed following oral administration, reaching peak levels after around 2.5 h. Absorption via the rectal route is somewhat slower, with peak levels occurring

around 4 h after administration. Plasma protein binding is around 55%. Elimination half-life is approximately 45 min via intestinal and hepatic glucuronidation, Newton (2002).

2.7. Artemotil (Molecular weight: 312.4)

Artemotil, previously known as arteether, is the ethyl ether of artemisinin, and is closely related to the more widely used artemether. It is oil-based so water insoluble. It is given by intramuscular injection only.

Pharmacokinetics

There is less published information on artemotil than for artemether. Absorption is slower and more erratic, with some patients having undetectable plasma artemotil until more than 24 h after administration.

2.7.1. Lumefantrine (benflumetol) (Molecular weight: 528.9)

Lumefantrine belongs to the aryl amino-alcohol group of antimalarials, which also includes quinine, mefloquine and halofantrine. It has a similar mechanism of action. Lumefantrine is a racemic fluorine derivative developed in China. It is only available in an oral preparation co formulated with artemether. This ACT is highly effective against multidrug-resistant *P. falciparum.*

Pharmacokinetics

Oral bioavailability is variable and is highly dependent on administration with fatty foods, White *et al* (1999). Absorption increases by 108% after a meal and is lower in patients with acute

malaria than in convalescing patients. Peak plasma levels occur approximately 10 h after administration. The terminal elimination half-life is around 3 days.

Toxicity

Despite similarities with the structure and pharmacokinetic properties of halofantrine, lumefantrine does not significantly prolong the electrocardiographic QT interval, and has no other significant toxicity, Van Vugt (1999). In fact the drug seems to be remarkably well tolerated. Reported side effects are generally mild – nausea, abdominal discomfort, headache and dizziness, and cannot be distinguished from symptoms of acute malaria.

Drug interactions

The manufacturer of artemether-lumefantrine recommends avoiding the following: grapefruit juice; antiarrhythmics such as amiodarone, disopyramide, flecainide, procainamide and quinidine; antibacterials, such as macrolides and quinolones; all antidepressants; antifungals such as imidazoles and triazoles; terfenadine; other antimalarials; all antipsychotic drugs; and beta blockers, such as metoprolol and sotalol. However, there is no evidence that co-administration with these drugs would be harmful.

2.7.2. Primaquine (Molecular weight: 259.4)

Primaquine is an 8-aminoquinoline and is effective against intra-hepatic forms of all types of malaria parasite. It is used to provide radical cure of *P. vivax* and *P. ovale* malaria, in combination with a blood schizontocide for the erythrocytic parasites. Primaquine is also

gametocytocidal against *P. falciparum* and has significant blood stage activity against *P. vivax* (and some against asexual stages of *P. falciparum*). The mechanism of action is unknown.

Pharmacokinetics

Primaquine is readily absorbed from the gastrointestinal tract. Peak plasma concentrations occur around 1–2 h after administration and then decline, with a reported elimination half-life of 3–6 h, Mihaly (1984). Primaquine is widely distributed into body tissues. It is rapidly metabolized in the liver. The major metabolite is carboxyprimaquine, which may accumulate in the plasma with repeated administration.

Toxicity

The most important adverse effects are haemolytic anaemia in patients with G6PD deficiency, other defects of the erythrocytic pentose phosphate pathway of glucose metabolism, or some other types of haemoglobinopathy, Chan *et al* (1976). In patients with the African variant of G6PD deficiency, the standard course of primaquine generally produces a benign self-limiting anaemia. In the Mediterranean and Asian variants, haemolysis may be much more severe. Therapeutic doses may also cause abdominal pain if administered on an empty stomach. Larger doses can cause nausea and vomiting. Methaemoglobinaemia may occur. Other uncommon effects include mild anaemia and leukocytosis. Overdosage may result in leukopenia, agranulocytosis, gastrointestinal symptoms, haemolytic anaemia and methaemoglobinaemia with cyanosis.

Drug interactions

Drugs liable to increase the risk of haemolysis or bone marrow suppression should be avoided.

2.7.3. Atovaquone (Molecular weight: 366.8)

Atovaquone is a hydroxynaphthoquinone antiparasitic drug active against all *Plasmodium* species. It also inhibits pre-erythrocytic development in the liver, and oocyst development in the mosquito. It is combined with proguanil for the treatment of malaria, with which it is synergistic. Atovaquone interferes with cytochrome electron transport.

Pharmacokinetics

Atovaquone is poorly absorbed from the gastrointestinal tract but bioavailability following oral administration can be improved by taking the drug with fatty foods. Bioavailabillity is reduced in patients with AIDS. Atovaquone is 99% bound to plasma proteins and has a plasma half-life of around 66–70 h due to enterohepatic recycling. It is excreted almost exclusively in the faeces as unchanged drug. Plasma concentrations are significantly reduced in late pregnancy, McGready (2003).

Toxicity

Atovaquone is generally very well tolerated, Sabchareon (1998). Skin rashes, headache, fever, insomnia, nausea, diarrhoea, vomiting, raised liver enzymes, hyponatraemia and, very rarely, haematological disturbances, such as anaemia and neutropenia, have all been reported.

Drug interactions

Reduced plasma concentrations may occur with concomitant administration of metoclopramide, tetracycline and possibly also acyclovir, antidiarrhoeal drugs, benzodiazepines, cephalosporins, laxatives, opioids and paracetamol. Atovaquone decreases the metabolism of zidovudine and

cotrimoxazole. Theoretically, it may displace other highly protein-bound drugs from plasma-protein binding sites.

2.7.4. Proguanil (Molecular weight: 253.7)

Proguanil is a biguanide compound that is metabolized in the body via the polymorphic cytochrome P450 enzyme CYP2C19 to the active metabolite, cycloguanil. Approximately 3% of Caucasian and African populations and 20% of Oriental people are "poor metabolizers" and have considerably reduced biotransformation of proguanil to cycloguanil, Kaneko (1999). Cycloguanil inhibits plasmodial dihydrofolate reductase. The parent compound has weak intrinsic antimalarial activity through an unknown mechanism. It is possibly active against pre erythrocytic forms of the parasite and is a slow blood schizontocide. Proguanil also has sporontocidal activity, rendering the gametocytes non-infective to the mosquito vector. Proguanil is given as the hydrochloride salt in combination with atovaquone. It is not used alone for treatment as resistance to proguanil develops very quickly. Cycloguanil was formerly administered as an oily suspension of the embonate by intramuscular injection.

Pharmacokinetics

Proguanil is readily absorbed from the gastrointestinal tract following oral administration. Peak plasma levels occur at about 4 h, and are reduced in the third trimester of pregnancy. Around 75% is bound to plasma proteins. Proguanil is metabolized in the liver to the active antifolate metabolite, cycloguanil, and peak plasma levels of cycloguanil occur 1 h after those of the parent drug. The elimination half-lives of both proguanil and cycloguanil is approximately 20 h, Hussein

(1996). Elimination is about 50% in the urine, of which 60% is unchanged drug and 30% cycloguanil, and a further amount is excreted in the faeces. Small amounts are present in breast milk. The elimination of cycloguanil is determined by that of the parent compound. The biotransformation of proguanil to cycloguanil via CYP2C19 is reduced in pregnancy and women taking the oral contraceptive pill, McGready (2003).

Toxicity

Apart from mild gastric intolerance, diarrhoea, occasional aphthous ulceration and hair loss, there are few adverse effects associated with usual doses of proguanil hydrochloride. Haematological changes (megaloblastic anaemia and pancytopenia) have been reported in patients with severe renal impairment. Overdosage may produce epigastric discomfort, vomiting and haematuria. Proguanil should be used cautiously in patients with renal impairment and the dose reduced according to the degree of impairment.

Drug interactions

Interactions may occur with concomitant administration of warfarin. Absorption of proguanil is reduced with concomitant administration of magnesium trisilicate.

2.7.5. Chlorproguanil (Molecular weight: 288.2)

Chlorproguanil is a biguanide and is given as the hydrochloride salt. Its actions and properties are very similar to those of proguanil. It is available only in combination with a sulfone such as dapsone (co-formulated as Lapdap).

2.7.6. Dapsone (Molecular weight: 248.3)

Dapsone is a sulfone widely used for the treatment of leprosy, and sometimes also for treatment or prophylaxis of *Pneumocystis carinii* pneumonia, and treatment of toxoplasmosis, cutaneous leishmaniasis, actinomycetoma and dermatitis herpetiformis. For malaria, dapsone is given in combination with another antimalarial. It is co formulated with chlorproguanil (as Lapdap™). Dapsone inhibits plasmodial dihydropteroate synthase.

Pharmacokinetics

Dapsone is almost completely absorbed from the gastrointestinal tract, with peak plasma concentrations occurring 2–8 h after an oral dose. Dapsone is 50–80% bound to plasma proteins, as is almost 100% of mono-acetyldapsone, its major metabolite. Dapsone undergoes enterohepatic recycling. It is widely distributed to body tissues, including breast milk and saliva. Its elimination half-life is 10–50 h. Dapsone is metabolized by acetylation, which exhibits genetic polymorphism. Hydroxylation is the other metabolic pathway, resulting in hydroxylamine dapsone, which may be responsible for dapsone-associated methaemoglobinaemia and haemolysis. Dapsone is mainly excreted in the urine, only 20% as unchanged drug.

Toxicity

Varying degrees of haemolysis and methaemoglobinaemia are the most frequently reported adverse effects and occur in most patients given more than 200 mg of dapsone daily. Doses of up to 100mg daily do not cause significant haemolysis but patients deficient in G6PD are affected by doses of >50 mg daily. Haemolytic anaemia has been reported following ingestion of dapsone in breast milk. Agranulocytosis has been reported following use of dapsone and pyrimethamine

together as malaria prophylaxis, particularly when used twice weekly. Aplastic anaemia has also been reported. Rashes, including pruritus and fixed-drug reactions may occur but serious cutaneous hypersensitivity is rare. "Dapsone syndrome" consists of rash, fever, jaundice and eosinophilia, and has been reported in a few patients using dapsone as malaria prophylaxis, but mainly in leprosy patients on long treatment courses. Other rare adverse effects include anorexia, nausea, vomiting, headache, hepatitis, hypoalbuminaemia and psychosis.

Drug interactions

There is an increased risk of dapsone toxicity with concomitant administration of probenecid, trimethoprim and amprenovir. Levels of dapsone are reduced with rifampicin.

2.7.7. Quinine (Molecular weight: 324.4)

Quinine is an alkaloid derived from the bark of the Cinchona tree. Four antimalarial alkaloids can be derived from the bark: quinine (the main alkaloid), quinidine, cinchonine and cinchonidine. Quinine is the L-stereoisomer of quinidine. Quinine acts principally on the mature trophozoite stage of parasite development and does not prevent sequestration or further development of circulating ring stages of *P. falciparum*. Like other structurally similar antimalarials, quinine also kills the sexual stages of *P. vivax*, *P. malariae* and *P. ovale*, but not mature gametocytes of *P. falciparum*. It does not kill the pre-erythrocytic stages of malaria parasites. The mechanisms of its antimalarial actions are thought to involve inhibition of parasite haem detoxification in the food vacuole, but are not well understood.

Pharmacokinetics

The pharmacokinetic properties of quinine are altered significantly by malaria infection, with reductions in apparent volume of distribution and clearance in proportion to disease severity, White (1982). In children under 2 years of age with severe malaria, concentrations are slightly higher than in older children and adults, Van Hensbroek (1996). There is no evidence for dose-dependent kinetics. Quinine is rapidly and almost completely absorbed from the gastrointestinal tract and peak plasma concentrations occur 1–3 h after oral administration of the sulfate or bisulfate, Supanaranond (1991). It is well absorbed after intramuscular injection in severe malaria, White (1995). Plasma-protein binding, mainly to alpha 1-acid glycoprotein, is 80% in healthy subjects but rises to around 90% in patients with malaria, Mansor (1991). Quinine is widely distributed throughout the body including the cerebrospinal fluid (2–7% of plasma values), breast milk (approximate 30% of maternal plasma concentrations) and the placenta, Phillips (1986). Extensive metabolism via the cytochrome P450 enzyme CYP3A4 occurs in the liver and elimination of more polar metabolites is mainly renal, Pukrittayakamee (1997). The initial metabolite 3-hydroxyquinine contributes approximately 10% of the antimalarial activity of the parent compound, but may accumulate in renal failure, Newton (1999). Excretion is increased in acid urine. The mean elimination half-life is around 11 h in healthy subjects, 16 h in uncomplicated malaria and 18 h in severe malaria, White (1982). Small amounts appear in the bile and saliva.

Toxicity

Administration of quinine or its salts regularly causes a complex of symptoms known as cinchonism, which is characterized in its mild form by tinnitus, impaired high tone hearing, headache, nausea, dizziness and dysphoria, and sometimes disturbed vision, Taylor and White (2004). More severe manifestations include vomiting, abdominal pain, diarrhoea and severe vertigo. Hypersensitivity reactions to quinine range from urticaria, bronchospasm, flushing of the skin and fever, through antibody-mediated thrombocytopenia and haemolytic anaemia, to life-threatening haemolytic-uraemic syndrome. Massive haemolysis with renal failure ("black water fever") has been linked epidemiologically and historically to quinine, but its etiology remains uncertain, Bruce-Chwatt (1987).

The most important adverse effect in the treatment of severe malaria is hyperinsulinaemic hypoglycaemia, White *et al* (1983). This is particularly common in pregnancy (50% of quinine-treated women with severe malaria in late pregnancy). Intramuscular injections of quinine dihydrochloride are acidic (pH 2) and cause pain, focal necrosis and in some cases abscess formation, and in endemic areas are a common cause of sciatic nerve palsy. Hypotension and cardiac arrest may result from rapid intravenous injection. Intravenous quinine should be given only by infusion, never injection. Quinine causes an approximately 10% prolongation of the electrocardiograph QT interval, mainly as a result of slight QRS widening, White *et al* (1983). The effect on ventricular repolarization is much less than that with quinidine. Quinine has been used as an abortifacient, but there is no evidence that it causes abortion, premature labour or fetal abnormalities in therapeutic use. Overdosage of quinine may cause oculotoxicity, including

blindness from direct retinal toxicity, and cardiotoxicity, and can be fatal, Boland *et al* (1985). Cardiotoxic effects are less frequent than those of quinidine and include conduction disturbances, arrhythmias, angina, hypotension leading to cardiac arrest and circulatory failure. Treatment is largely supportive, with attention being given to maintenance of blood pressure, glucose and renal function, and to treating arrhythmias.

Drug interactions

There is a theoretical concern that drugs that may prolong the QT interval should not be given with quinine, although whether or not quinine increases the risk of iatrogenic ventricular tachyarrhythmia has not been established. Antiarrhythmics, such as flecainide and amiodarone, should probably be avoided. There might be an increased risk of ventricular arrhythmias with antihistamines such as terfenadine, and with antipsychotic drugs such as pimozide and thioridazine. Halofantrine, which causes marked QT prolongation, should be avoided but combination with other antimalarials, such as lumefantrine and mefloquine is safe. Quinine increases the plasma concentration of digoxin. Cimetidine inhibits quinine metabolism, causing increased quinine levels and rifampicin increases metabolic clearance leading to low plasma concentrations and an increased therapeutic failure rate, Pukrittayakamee (2003).

2.7.8. Tetracycline (Molecular weight: 444.4)

The tetracyclines are a group of antibiotics originally derived from certain *Streptomyces* species, but used mostly in synthetic form. Tetracycline itself may be administered orally or intravenously as the hydrochloride salt or phosphate complex. Both are water soluble, although the intravenous

preparation is only stable for a few hours. Tetracyclines are inhibitors of aminoacyl-tRNA binding during protein synthesis. They have a broad range of uses, including treatment of some bacterial infections: *Chlamydia*, *Rickettsia*, *Mycoplasma*, Lyme disease, *Brucella*, tularaemia, plague and cholera. Doxycycline is a synthetic tetracycline with a longer half-life, which makes dosing schedules easier.

Pharmacokinetics

Some 60–80% of tetracycline is absorbed from the gastrointestinal tract following oral administration. Absorption is reduced by the presence of divalent and trivalent metal ions with which it forms stable, insoluble complexes. Thus absorption may be impaired with food or milk. Formulation with phosphate may improve absorption. Peak plasma concentrations occur 1–3 h after ingestion. Tetracycline is 20–65% bound to plasma proteins. It is widely distributed throughout the body, although less so than the more lipophilic doxycycline. High concentrations are present in breast milk (around 60% of plasma levels), and also diffuse readily across the placenta, and are retained in sites of new bone formation and teeth development. The half-life of tetracycline is around 8 h; 40–70% is excreted in the urine via glomerular filtration. The remainder is excreted in the faeces and bile. Enterohepatic recycling slows down elimination.

Toxicity

All the tetracyclines have similar adverse effect profiles. Gastrointestinal effects, such as nausea, vomiting and diarrhoea, are common, especially with higher doses, and are due to mucosal irritation. Dry mouth, glossitis, stomatitis, dysphagia and oesophageal ulceration have also been reported. Overgrowth of *Candida* and other bacteria occurs, presumably due to disturbances in

gastrointestinal flora as a result of incomplete absorption of the drug. This effect is seen less frequently with doxycycline, which is better absorbed. Pseudomembranous colitis, hepatotoxicity and pancreatitis have also been reported.

Tetracyclines accumulate in patients with renal impairment and this may cause renal failure. In contrast doxycycline accumulates less and is preferred in patients with renal impairment. The use of out-of-date tetracycline can result in the development of a reversible Fanconi-type syndrome characterized by polyuria and polydipsia with nausea, glycosuria, aminoaciduria, hypophosphataemia, hypokalaemia, and hyperuricaemia with acidosis and proteinuria. These effects have been attributed to the presence of degradation products, in particular anhydroepitetracycline. Tetracyclines are deposited in deciduous and permanent teeth during their formation and cause discoloration and enamel hypoplasia. They are also deposited in calcifying areas in bone and the nails and interfere with bone growth in young infants or pregnant women. Raised intracranial pressure in adults and infants has also been documented. Tetracyclines use in pregnancy has also been associated with acute fatty liver.

Tetracyclines should therefore not be given to pregnant or lactating women, or children of less than 8 years of age. Hypersensitivity reactions occur, although they are less common than for ß-lactam antibiotics. Rashes, fixed drug reactions, drug fever, angioedema, urticaria, pericarditis and asthma have all been reported. Photosensitivity may develop and, rarely, haemolytic anaemia, eosinophilia, neutropenia and thrombocytopenia. Pre-existing systemic lupus erythematosus may be worsened and tetracyclines are contraindicated in patients with the established disease.

Drug interactions

There is reduced absorption of tetracyclines with concomitant administration of cations, such as aluminium, bismuth, calcium, iron, zinc and magnesium. Administration with antacids, iron preparations, dairy products and some other foods should therefore be avoided. Nephrotoxicity may be exacerbated with diuretics, methoxyflurane or other potentially nephrotoxic drugs. Potentially hepatotoxic drugs should be avoided. Tetracyclines produce increased concentrations of digoxin, lithium and theophylline, and decrease plasma atovaquone concentrations and also the effectiveness of oral contraceptives. They may antagonize the actions of penicillins so should not be given concomitantly.

2.7.9. Doxycycline (Molecular weight: 444.4)

Doxycycline is a tetracycline derivative with uses similar to those of tetracycline. It may be preferred to tetracycline because of its longer half-life, more reliable absorption and better safety profile in patients with renal insufficiency, where it may be used with caution. It is relatively water insoluble but very lipid soluble. It may be given orally or intravenously. It is available as the hydrochloride salt or phosphate complex, or as a complex prepared from the hydrochloride and calcium chloride.

Pharmacokinetics

Doxycycline is readily and almost completely absorbed from the gastrointestinal tract and absorption is not affected significantly by the presence of food. Peak plasma concentrations occur 2 h after administration. Some 80–95% is protein-bound and half-life is 10–24 h, Newton (2005).

It is widely distributed in body tissues and fluids. In patients with normal renal function, 40% of doxycycline is excreted in the urine, although more if the urine is alkalinized. It may accumulate in renal failure. However, the majority of the dose is excreted in the faeces.

Toxicity

As for tetracycline. Gastro-intestinal effects are fewer than with tetracycline, although oesophageal ulceration can still be a problem if insufficient water is taken with tablets or capsules. There is less accumulation in patients with renal impairment. Doxycycline should not be given to pregnant or lactating women, or children aged up to 8 years.

Drug interactions

Doxycycline has a lower affinity for binding with calcium than other tetracyclines, so may be taken with food or milk. However, antacids and iron may still affect absorption. Metabolism may be accelerated by drugs that induce hepatic enzymes, such as carbamazepine, phenytoin, phenobarbital and rifampicin, and by chronic alcohol use.

2.8. Clindamycin (Molecular weight: 425.0)

Clindamycin is a lincosamide antibiotic, i.e. a chlorinated derivative of lincomycin. It is very soluble in water. It inhibits the early stages of protein synthesis by a mechanism similar to that of the macrolides. It may be administered by mouth as capsules containing the hydrochloride or as oral liquid preparations containing the palmitate hydrochloride. Clindamycin is given parenterally as the phosphate either by the intramuscular or the intravenous route. It is used for the treatment

of anaerobic and Gram-positive bacterial infections, babesiosis, toxoplasmosis and *Pneumocystis carinii* pneumonia.

Pharmacokinetics

About 90% of a dose is absorbed following oral administration. Food does not impede absorption but may delay it. Clindamycin phosphate and palmitate hydrochloride are rapidly hydrolysed to form the free drug. Peak concentrations may be reached within 1 h in children and 3 h in adults. It is widely distributed, although not into the cerebrospinal fluid. It crosses the placenta and appears in breast milk. It is 90% bound to plasma proteins and accumulates in leukocytes, macrophages and bile. The half-life is 2–3 h but this may be prolonged in neonates and patients with renal impairment. Clindamycin undergoes metabolism to the active *N*-demethyl and sulfoxide metabolites, and also some inactive metabolites. About 10% of a dose is excreted in the urine as active drug or metabolites and about 4% in the faeces. The remainder is excreted as inactive metabolites. Excretion is slow and takes place over many days. Clindamycin is not effectively removed from the body by dialysis.

Toxicity

Diarrhoea occurs in 2–20% of patients. In some, pseudomembranous colitis may develop during or after treatment, which can be fatal. Other reported gastrointestinal effects include nausea, vomiting, abdominal pain and an unpleasant taste in the mouth. Around 10% of patients develop a hypersensitivity reaction. This may take the form of skin rash, urticaria or anaphylaxis. Other adverse effects include leukopenia, agranulocytosis, eosinophilia, thrombocytopenia, erythema

multiforme, polyarthritis, jaundice and hepatic damage. Some parenteral formulations contain benzyl alcohol, which may cause fatal "gasping syndrome" in neonates.

Drug interactions

Clindamycin may enhance the effects of drugs with neuromuscular blocking activity and there is a potential danger of respiratory depression. Additive respiratory depressant effects may also occur with opioids. Clindamycin may antagonize the activity of parasympathomimetics.

2.9. Toxicological testing methods for crude plant extracts

2.9.1. *In vivo* evaluation of toxicity

In vivo evaluation of the toxicological profile of crude plant extracts is carried out in accordance with ICH (www.ich.org) and OECD (www.oecd.org) guidelines.

2.9.2. MTT (3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) Assay:

MTT is a yellow water-soluble tetrazolium dye that is reduced by live cells to a water-insoluble purple formazan. The amount of formazan can be determined by solubilizing it in DMSO and measuring it spectrophotometrically. Comparisons between the spectra of treated and untreated cells can give a relative estimation of cytotoxicity, Alley *et al* (1988).

2.9.3. LDH (Lactate dehydrogenase) Assay:

Cell death can occur either by apoptosis, a highly regulated pathway involving signal transduction cascades, or by necrosis. Necrosis is accompanied by mitochondrial swelling and

increased plasma membrane permeability, while apoptosis involves an articulated breakdown of the cell into membrane bound apoptotic bodies, Bonfoco *et al* (1995). There are a number of screening techniques available that detect cytotoxicity and cell death, independent of mechanism. Most of these assays assess cell viability by measuring plasma membrane permeability, Bonfoco *et al* (1995); Haslam *et al* (2000). Lactate dehydrogenase (LDH) is a soluble enzyme located in the cytosol. The enzyme is released into the surrounding culture medium upon cell damage or lysis, processes that occur during both apoptosis and necrosis. LDH activity in the culture medium can, therefore, be used as an indicator of cell membrane integrity, and thus a measurement of cytotoxicity. Since the activity of intracellular LDH corresponds to the number of cells in the culture, quantification of LDH in cell lysates can be used as a measurement of cell growth, Haslam *et al* (2000); Wolterbeek and Van Deer Meer (2005).

LDH cytotoxicity assay measures cell death in response to chemical compounds or environmental factors using a coupled two-step reaction. In the first step, LDH catalyses the reduction of NAD^+ to NADH and H^+ by oxidation of lactate to pyruvate. In the second step of reaction, diaphorase uses the newly formed NADH and H^+ to catalyze the reduction of a tetrazolium salt (INT) to highly colored formazan which absorbs strongly at 490-520nm. The amount of formazan produced is proportional to the amount of LDH released into the culture medium as a result of cytotoxicity. Hence the basis of the LDH assay: (1) LDH oxidizes lactate to pyruvate, (2) Pyruvate reacts with the tetrazolium salt (INT) to form formazan, and (3) the water-soluble formazan dye is detected spectrophotometrically.

2.9.4. Sulforhodamine B (SRB) assay:

SRB cytotoxicity assay is an accurate and reproducible assay based upon the quantitative staining of cellular proteins by sulforhodamine B (SRB). This assay has been used for high-throughput drug screening, Perez (1993). Sulforhodamine B is an anionic aminoxanthene dye that forms an electrostatic complex with the basic amino acid residues of proteins under moderately acid conditions, which provides a sensitive linear response. The color development is rapid and stable and is readily measured at absorbances between 560 and 580nm.

2.9.5. Brine shrimp assay:

Many pharmaceutical industries currently use batteries of specific bioassays to screen plant extracts for biological activities. Mostly, these tests are expensive and also require sophisticated equipments, special reagents (e.g. human serum, culture media) and aseptic conditions (e.g.antimalarial, cytotoxicity and antibacterial/antifungal tests). Thus they are beyond of research funds available in most developing countries. To alleviate this, the development of cheap and simple bioassays is needed. Since most bioactive plant constituents are toxic at higher doses, a possible approach to developing a useful general bioassay is to screen for plant extracts that are toxic to zoologic systems. For this purpose, the brine shrimp lethality test was originally proposed by Meyer *et al* (1982) and later refined by McLaughlin *et al* (1998; 1991). It represents an easy way to detect general bioactivity in plant extracts and also useful tool to monitor the isolation of bioactive constituents. The eggs of brine shrimp (*Artemia salina Leach*) available in pet shops hatch within 24 hours upon being placed in a brine solution, yielding large number of

larvae (nauplii). Toxicity of plant extracts against larvae is evaluated in the brine shrimp assay. Test extracts at concentrations of 0µg/ml (control), 10µg/ml, 100µg/ml and 1000µg/ml are introduced into vials. Ten shrimps are then transferred into each vial and the brine solution is added. Five replicates are prepared for each dose level. Survivors are counted after 24 hours and the percentage of deaths at each dose is recorded. The lethal concentration fifty (LC_{50}) is then calculated using Finney computer program.

CHAPTER THREE

ETHNOPHARMACOLOGY OF ANTIMALARIAL PHYTOTHERAPY REMEDIES IN MSAMBWENI DISTRICT, KENYA.

3.1. Introduction

Medicinal plants are important in ethnomedical practices with malaria ranking as the most important disease treated with herbal remedies. Due to either limited availability or affordability of conventional medicines in tropical countries, about 80% of the rural population depends on traditional herbal remedies, Zirihi *et al* (2005). Although there is widespread use of traditional herbal remedies in the management of malaria, Gessler *et al* (1995a), scientific understanding of the plants is however largely unexplored, WHO (2002) and therefore, there is a need to collect ethnobotanical information on antimalarial plants as a first step prior to evaluation of their efficacy and safety.

Some ethnobotanical studies have been accomplished in Kenya targeting the different communities and localities, Njoroge and Bussman (2006). These studies cover various aspects of utilization of traditional herbal remedies by local communities in Kenya. Studies, however, to document traditional antimalarial herbal remedies in Msambweni district have not been done, hence the objective of the current study. The current study was therefore conducted to document traditional antimalarial herbal remedies in Msambweni district, Kenya. Effort was made in this study to indicate the frequency of mention of each plant species traditionally used to treat malaria as an estimation of agreement on use in the study area. The results provide data for further pharmacological, toxicological and phytochemical studies. Since the plant parts utilized in

preparation of antimalarial herbal remedies are reported in this study, it serves as an indication of species that may need further ecological assessment on their regeneration status.

3.2. Materials and Methods

3.2.1. Study area.

In Msambweni, the study area centered around 04^0 28' 59.2'' S latitude and 039^0 13' 48.9'' E longitude in Diani, Pongwe Kidimu and Dzombo/Kikoneni locations of Msambweni district (Fig. 1). The vegetation consists of thickets, woodlands and grasslands. The area is hot and humid all year round with annual mean temperatures ranging between 23^0C and 34 ^{0}C and the average relative humidity ranges between 60% and 80%. The soils are made of sandstone and grit and are fairly fertile for cultivation. The type of climate is monsoon, hot and dry from January to April while June to August is the coolest period. Rainfall comes in two seasons with short rains from October to December and long rains from April to July. The total precipitation varies from 900 mm to 1500 mm per annum along the coastal belt to 500-600 mm in the hinterland, which comprises 92% of the land whose agricultural potential is low, Muthaura *et al* (2007a).

The population of Msambweni approximates 225,000 (1999 National population Census), 90% of who are Muslim and are concentrated on the southern coastal strip of Kenya between Kwale district and the border of Tanzania (Msambweni district). The community is rural and depends on crop agriculture as its major source of livelihood. The people belong predominantly to three ethnic groups, with the Digos being the majority followed by Durumas while Kambas are the minority. The main language spoken is Kidigo. The medicinal knowledge of the communities is

considered communal; however there is individually held knowledge by the herbalists. The indigenous knowledge held by the herbalists on the use of medicinal plants was transferred to them by their fathers orally. More than half of the Kenya's rare plants grow in the coastal region, most of which have been identified within the *Kaya* forest patches which comprise about 10% of the Kenya's coastal forests, Muthaura *et al* (2007). The traditional medicinal knowledge from the resources of these forests, in possession of a few herbalists, needs to be documented for the benefit of the present and future generations.

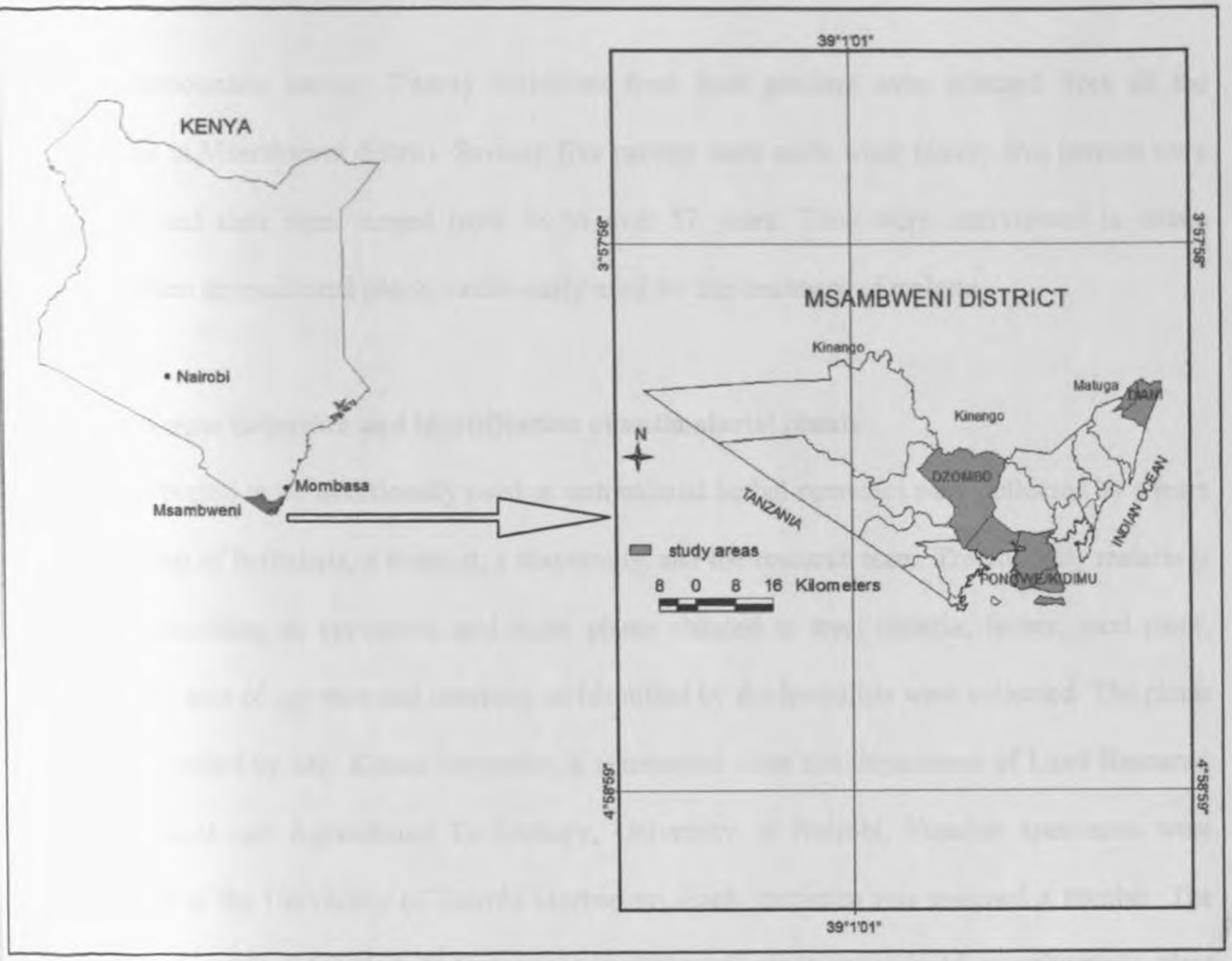

Figure 1: Map of Kenya showing Msambweni district with study areas Diani, Pongwe Kidimu and Dzombo/Kikoneni locations

3.2.2. Data collection

This study was conducted between April and July, 2009. Data on medicinal plants traditionally used to treat malaria was collected through a survey employing standardized questionnaires and interviews. Focused group discussions were held with the herbalists to supplement the interview

and questionnaire survey. Twenty herbalists from both genders were selected from all the locations in Msambweni district. Seventy five percent were male while twenty five percent were females and their ages ranged from 38 to over 57 years. They were interviewed to obtain information on medicinal plants traditionally used for the treatment of malaria.

3.2.3. Sample collection and identification of antimalarial plants

Plants reported to be traditionally used as antimalarial herbal remedies were collected by a team comprising of herbalists, a botanist, a taxonomist and the research team. Traditionally malaria is treated according to symptoms and those plants claimed to treat malaria, fevers, joint pains, headache, loss of appetite and vomiting as identified by the herbalists were collected. The plants were identified by Mr. Kimeu Musembi, a taxonomist from the department of Land Resourse, Management and Agricultural Technology, University of Nairobi. Voucher specimens were deposited at the University of Nairobi Herbarium. Each specimen was assigned a number. The information gathered included plant species, parts used, plant status (wild or cultivated), plant habit, plant habitat, method of preparation, route of administration, dosage and vernacular names.

3.3. Results

Twenty herbalists, whose experience in the use of traditional medicine ranged from 38-57 years, were interviewed on the plants that they used for treatment of malaria. Twenty six species distributed between twenty four genera and twenty families were reportedly used in herbal preparations for the treatment of malaria (Table 1).

Table 1: An inventory of plants traditionally used for the treatment of malaria in Msambweni, Kenya

Family/Species/ (Voucher specimen no.)	Vernacular name	Habit	Status	Habitat	Pu[1]/P[2]/Ra[3]	No.[e]
Amaranthanceae, *Amaranthus hybridus* L. (JN 430)	Mchicha (Swahili)	Herb	Wild	Bush	L/D/O	1
Apocynaceae, *Laudolphia buchananii* (Hall.f) Stapf. (JN 427)	Mhonga (Swahili)	Liana	Wild	Bush	L/D/O	1
Bombacacea, *Adansonia digitata* Linn. (JN 414; 415)	Mbamburi (Swahili)	Tree	Wild	Bush	L/D/O	2
Caesalpiniaceae, *Cassia occidentalis* L. (JN 425)	Mnuka uvundo (Swahili)	Herb	Wild	Bush	R, L/D/O	1
Combretaceae, *Combretum padoides* Engl and Diels. (JN 434)	Phozo (Digo)	Shrub	Wild	Bush	L/D/O	1
Compositae, *Senecio syringitolius* O. Hoffman. (JN 432)	Reisa (Digo)	Herb	Wild	Bush	L/D/O	1

Table 1 (Continued)

Family/Species/ (Voucher specimen no.)	Vernacular name	Habit	Status	Habitat	Pu[1]/P[2]/Ra[3]	No.[e]
Cucurbitaceae, *Gerranthus lobatus* (Cogn.) Jeffrey (JN 405;406; 407)	Mgore manga (Digo)	Liana	Wild	Bush	R/D/O	1
Euphorbiaceae, *Ricinus communis* L.(JN 431)	Mbono/Mbonombono (Digo)	Shrub	Cultivated, Wild	Bush,Crop field	R, L/D,C,B/O	2
Flacourtiaceae, *Flacourtia indica* (Burm.f) Merr. (JN 436)	Mtondo mbare (Digo)	Shrub	Cultivated	Crop field	R, Sb,L/D/O	1
Labiatae, *Plectranthus barbatus* Andr. (JN 418)	Kizimwilo/Mumbu (Digo)	Shrub	Wild	Bush	L/D/O	1
Labiatae, *Ocimum bacilicum* L. (JN 428)	Kivumbani (Digo)	Herb	Wild	Bush	L/D/O	2
Labiatae, *Ocimum suave* Willd (JN 408; 409)	Murihani (Giriama)	Herb	Wild	Bush	L/D/O	1
Liliacea, *Aloe deserti* Berger. (JN 424)	Ngolonje (Digo)	Herb	Cultivated, Wild	Bush, Boundary marker	L/We/O	5

Table 1 (Continued)

Family/Species/ (Voucher specimen no.)	Vernacular name	Habit	Status	Habitat	Pu[1]/P[2]/Ra[3]	No.[e]
Liliaceae, *Aloe macrosiphon* Bak. (JN 435)	Golonje (Giriama)	Herb	Wild	Bush	L/We/O	1
Liliaceae, *Aloe vera* (L) Webb.(JN 421)	Alvera (Digo)	Herb	Wild, Cultivated	Crop field, Bush	L/We/O	2
Meliaceae, *Azadirachta indica* (L) Burm. (JN 412; 422)	Mkilifi (Digo)	Tree	Cultivated, Wild	Crop field, Bush	Rb, Sb, L/D,C,B/O	19
Moracea, *Ficus bussei* Warp ex Mildbr and Burret.(JN 403)	Mgandi (Digo)	Tree	Wild	Bush	R, L/D,C/O	1
Papilionaceae, *Securidaca longepedunculata* Fres. (JN 423)	Mzigi (Digo)	Shrub	Wild	Bush	R, Sb, L/D/O	1
Rubiaceae, *Canthium glaucum* Hiern. (JN 426)	Mhonga/Mronga (Digo)	Shrub	Wild	Bush	Fr/D/O	1

Table 1 (Continued)

Family/Species/ (Voucher specimen no.)	Vernacular name	Habit	Status	Habitat	Pu[1]/P[2]/Ra[3]	No.[e]
Rutaceae, *Fagaropsis angolensis* (Engl) Del. (JN 437)	Muangani (Digo)	Tree	Wild	Bush	L/D/O	1
Rutacea, *Teclea simplicifolia* (Eng) Verdoon (JN 413)	Mulaga dare (Duruma)	Shrub	Wild	Bush	R/D/O	1
Rutaceae, *Zanthoxylum chalybeum* (Eng) Engl. (JN 433)	Mjafari /Mporojo(Giriama)	Tree	Wild	Bush	Rb/D,C/O	5
Simaroubaceae, *Harrisonia abyssinica* Oliv. (JN 438)	Mdungu /Chidore(Digo/Giriama)	Shrub	Wild	Bush	Rb, L/D/O	3
Solanacea, *Solanum incanum* L. (JN 416; 417)	Mtugudza koma (Digo)	Shrub	Wild	Bush	R, L/D/O	2
Tiliaceae, *Grewia hexaminta* Burret. (JN 401; 402)	Mkone (Digo)	Shrub	Wild	Bush	R, L/D/O	1

Table 1 (Continued)

Family/Species/ (Voucher specimen no.)	Vernacular name	Habit	Status	Habitat	Pu[1]/P[2]/Ra[3]	No.[e]
Verbenaceae, *Lantana camara* L. (JN 429)	Mjasasa (Digo)	Shrub	Wild	Bush, Boundary marker	L/D/O	2

[1]Part used

[2]Method of preparation

[3]Route of administration

[e] Number of herbalists mentioning use of the species for malaria treatment

Rb: root bark, Sb: stem bark, L: leaves, O: oral, B: bathed, R: roots, Fr: fruit

D: decoction (remedy is prepared by boiling a plant part in water)

C: concoction (remedy is prepared by boiling parts from different plants in water)

We: water extract (remedy is prepared by dissolving a plant part in water for sometime)

Shrubs comprised of 41% of all the species traditionally used for the treatment of malaria while lianas constituted only 4% of all the plant species reported in the study. Mature leaves were commonly used in the preparation of herbal remedies. Herbalists reported that the appropriate plant parts were collected as and when they were needed, and that there was no specific time to collect. They did not perform any rituals during collection or processing of herbal remedies. The herbal drugs were prepared mostly as decoctions, concoctions, water extracts usually prepared just before use or as steam baths. The water extracts and decoctions were prepared as mono-preparations from single species (Figure 2).

Figure 2: A decoction prepared from *Azadirachta indica* leaves.

Some concoctions were prepared as mixtures of *Azadirachta indica* (Meliaceae) and *Ricinus communis* (Euphorbiaceae); *Teclea simplicifolia* (Rutaceae) and *Flacourtia indica* (Flacourtiaceae) or *Grewia hexaminta* (Tiliaceae), *Solanum incanum* (Solanaceae) and *Azadirachta indica* (Meliaceae). The plant material was used fresh or dried and most plant parts to be used as remedies were stored for later use in the dry state, which allowed their utilization throughout the year. Oral doses were variable and were administered according to the age of the patient. They varied between 100 and 500mls for adults; 100 and 250mls for older children (more than 5 years) and 1-3 tablespoons for children younger than 5 years. The herbal preparations were taken 1-3 times a day for a period of 4-5 days or until the patient's condition improved. Prepared herbal medicines were never stored and remnants were discarded. There was no need to keep any since the plants from which they were prepared from were readily available. Plant species were mainly collected from Kaya Diani forest (Figure 3). They also reported that their remedies had no side effects.

There are species, which were reported in this study that are also known to be used as sources of antimalarial remedies in other countries (Table 2). They have also been investigated for their phytoconstituents and antimalarial activities (Table 2), the latter being in agreement with ethnomedical uses reported in this study. A total of 52% of the plants collected have been reported in literature as having been used for malaria or fever, an indication that the herbalists could be trusted with the information they imparted about the plants they traditionally use for preparation of antimalarial herbal remedies.

Figure 3: Kaya Diani forest. Some of the antimalarial species mentioned were collected from this forest.

Figure 4 shows the frequency of traditional antimalarial plant species in the 20 families used in Msambweni, Kenya.

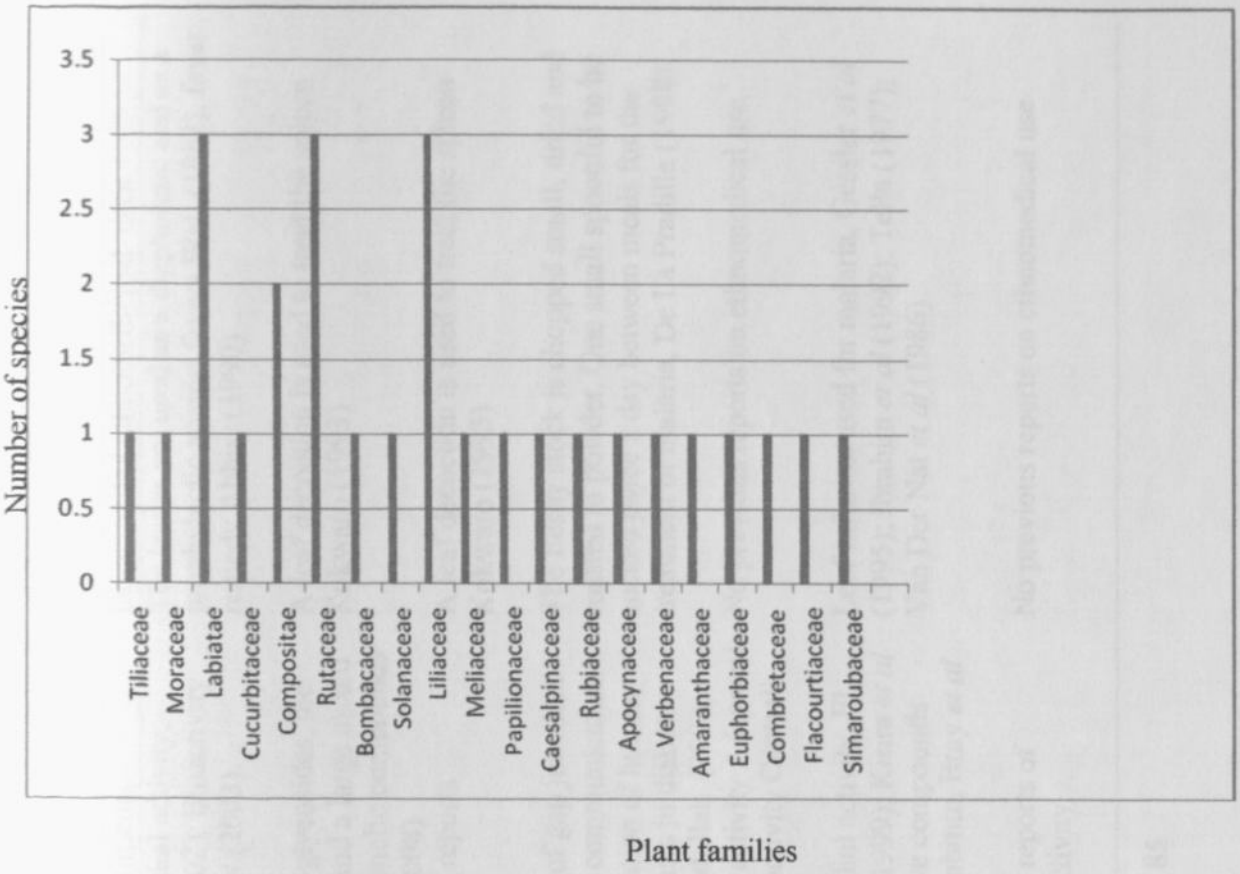

Figure 4: Frequency of traditional antimalarial plant species in the 20 families used in Msambweni, Kenya.

Table 2: Plant species collected from Msambweni district based on traditional knowledge on their use as antimalarials

Plant species	Method of administration	Active constituents	Documented ethnomedicinal uses
Adansonia digitata	Oral, one cup is taken three times daily for three to four days or until the patient recovers.	Antiplasmodial activity, Kristina (2002); Bioactivity Cantrell *et al* (2003).	The leaves are used as a diaphoretic and as a prophylactic against fevers Watt (1962), fever remedy Abbiw (1990)
Aloe deserti	Oral, a quarter of a glass is taken three times for a day.	Anthrone C-glycosides, the chromones and a large mixed group of phenolic compounds Reynolds (2008)	A leaf decoction is used to treat the spleen Kokwaro (1993)
Aloe macrosiphon	Oral, a quarter of a cup is taken three times daily for two to three days.	No previous reports	A leaf decoction is used to treat the spleen Kokwaro (1993)
Aloe vera	Oral, a quarter of a cup is taken three times daily for two to three days.	Stimulation of gap junctional intercellular communication and proliferation of human skin fibroblasts in diabetes mellitus, Abdullah (2002).	The fleshy stock is chopped small, dried and roasted to powder. One small spoonful to be sucked twice a day between meals for the treatment of malaria, De La Pradilla (1988)
Amaranthus hybridus	Oral, one cup is taken three times daily for four to five days.	Antioxidant activity Adewumi (2005); Bioactivity Cantrell (2003)	No previous reports on ethnomedical use
Azadirachta indica	Oral, a quarter of a glass is taken three times per day for two to three days.	Antiplasmodial activity, El Tahir *et al* (1999); Kirira *et al* (2006), active compounds gedunin, nimbinin, Bray *et al* (1990).	Leaf infusion used for malaria, Gessler *et al* (1995); Ibrahim *et al* (1992); Tella (1977); Van Der Nat *et al* (1986)
Canthium glaucum	Oral, one cup is taken three times daily for four days.	No previous reports of biological activity	No previous reports on ethnomedical use

Table 2 (Cont.)

Plant species	Method of administration	Active constituents	Ethnomedicinal uses
Cassia occidentalis	Oral, one cup is taken three times daily for three to four days.	Antiplasmodial activity Cimanga (2004); Tona (1999). Terpenes, steroids, coumarins, flavonoids, phenolic acids, lignans, xanthones, anthraquinones, Cimanga (2004).	It has a special reputation as an excellent oxytocin, cholagogue, anti-fever medicine, anti-worm medicine and remedy for swellings. As a cholagogue, 15g of leaves boiled in 1 liter of water and 1 glass drunk daily; as a diuretic: 4g of leaves in 180g of water each day as an infusion, Neuwinger (1994).
Combretum padoides	Oral, one cup is taken three times daily for three to five days.	Mono and bi-desmosidic triterpenoids from leaves (Rodgers and Coombes, 1999); Acetone extracts of leaves have antimicrobial effects (fresh leaves more effective than dried) MIC 0.8µg/ml against *E.coli* and *Enterobacter faecalis* Eloff (1999)	Leaves for snakebites and the roots for eliminating hookworms, Neuwinger (2000)
Fagaropsis angolensis	Oral, one cup is taken three times daily for three to four days.	Bioactivity and antiplasmodial activity, Kirira *et al* (2006)	Used for management of malaria, Njoroge and Bussmann (2006).
Ficus bussei	Oral, one cup is taken three times daily for four to five days.	Steroidal sapogenins, Wall (2006)	A decoction of leafy twigs is used as a remedy for fever pains associated with malaria, Kerharo and Bouquet (1950).
Flacourtia indica	Oral, one cup is taken three times daily for three to four days.	Antiplasmodial activity, Clarkson *et al* (2004)	The leaf sap is mixed with a root decoction as a malaria cure, Burkill (1994)
Gerranthus lobatus	Oral, one cup is taken three times daily for three days.	Flavonoid compounds, Imperato (2005).	No previous reports on ethnomedical use

Table 2 (Cont.)

Plant species	Method of administration	Active constituents	Ethnomedicinal uses
Grewia hexaminta	Oral, one cup is taken three times daily for three to four days.	Triterpenoid compounds, Raghunathaiyar (1996)	No previous reports on ethnomedical use
Harrisonia abyssinica	Oral, one cup is taken three times daily for two to three days.	Antimalarial activity, El Tahir *et al* (1999), Antiplasmodial activity Kirira *et al* (2006); Maregesi *et al* (2010). Methanol extract of dried root bark exhibited activity against *Trichophyton mentagrophytes* and *Candida albicans*, Sawhney *et al* (1978b). Chloroform extract of the stem bark exhibited antifungal activity against *Aspergillus niger*, *Microsporum canis*, *Trichophyton mentagrophytes* and *Aspergillus fumigates*, Balde *et al* (1995).	Root decoction used for fever, Kokwaro (1993), Venereal diseases (Beentje, 1994); hot water extract of fresh and dried root bark is used in Tanzania to treat skin diseases Sawhney *et al* (1978a,b)
Lantana camara	Oral, one cup is taken three times daily for three to four days.	Antiplasmodial activity Clarkson *et al* (2004). Quinine like alkaloid, *lantanine*, is present in the leaves Burkill (2000).	The infused leaves are used as a diaphoretic and febrifuge, Burkill (2000), the roots are used for malaria, and said to be effective in cases which are not responsive to quinine Burkill (2000). Tea of the leaves is believed to prevent weakness of memory and enhances intellect and cognition, Muller-Ebeling and Ratsch (1989).
Laudolphia buchananii	Oral, one cup is taken three times daily for three days.	No previous reports	No previous reports

Table 2 (Cont.)

Plant species	Method of administration	Active constituents	Ethnomedicinal uses
Launea cornuta	Oral, one cup is taken three times daily for four to five days.	Tannins and astringents Burkill (1985)	The roots are pounded and infused or decocted, the liquid being drunk as a remedy for typhoid Kokwaro (1993).
Ocimum bacilicum	Oral, one cup is taken three times daily for three to four days.	Antifungal activity Dambolena (2007), linalool, geranical, camphor compounds Dambolena (2007)	For abdominal cramps, upset stomach, nervous migraine, memory "strengthens the heart and the head" loss and forgetfulness Fuchs (1543); Sfikas (1980).
Ocimum suave	Oral, one cup is taken three times daily for three to five days.	The essential oil isolated from the aerial structures of the plant was reported active against a number of microorganisms, Janssen *et al* (1989). The ethanol extract of the leaves of Rwandese plants were found to be active against *Bacillus subtilis* and *Microsporum canis*, Vlietinck (2000).Triterpenes, Tan (1997); anti-ulcerogenic activity, Tan (1997).	In Tanzania, the scrappings of the roots mixed with *Zingiber officinalis* are used for inflamed tonsils, Hedberg *et al* (1983a) and the dried twigs are used as a chewing stick, Khan *et al* (2000).Used for treatment of Candida infections including oral candidiasis Runyoro *et al* (2006)
Plectranthus barbatus	Oral, one cup is taken three times daily for three to four days.	Antiplasmodial activity, Meyer (2002)	The plant is used as a mosquito repellant, Watt (1962).
Ricinus communis	Oral, one cup is taken three times daily for three to four days.	Antiplasmodial activity, Clarkson *et al* (2004).	Leaves are used as a remedy for fever Burkill (1994), dried root is used as a febrifuge, Watt and Breyer-Bandwisk (1962), the oil is added to paraffin based spray as an antimalarial agent, Burkill (1935)

Table 2 (Cont.)

Plant species	Method of administration	Active constituents	Ethnomedicinal uses
Securidaca longepedunculata	Oral, one cup is taken three times daily for three to four days.	The roots contain steroids, saponosides and monotropitoside, De La Pradilla (1988); aqueous, dichloromethane and ethanol extracts are reported to have activity against *Candida albicans*, Desta (1993); Taniguchi *et al* (1978).	The roots are used against malaria, Williamson (1975).
Senecio syringitolius	Oral, one cup is taken three times daily for three to four days.	No previous reports	No reports
Solanum incanum	Oral, one cup is taken three times daily for three to four days.	Antiulcerogenic effect, Farina *et al* (1998), active triterpenoid compounds-Ursolic acid (3a) Hirota *et al* (1990).	A root decoction is used against fever, Kokwaro (1993)
Teclea simplicifolia	Oral, a quarter of a cup is taken three times daily for two to three days.	Quinoline compounds Wondimu (1998).	In Kenya, the roots are regarded as poisonous, Neuwinger (1996). The maasai use a root infusion for gonorrhea, Neuwinger (2000)

Table 2 (Cont.)

Plant species	Method of administration	Active constituents	Ethnomedicinal uses
Zanthoxylum chalybeum	Oral, a half of a glass is taken three times daily for three to four days.	Antiplasmodial activity, Gessler *et al* (1994), quinoline alkaloids, Kato *et al* (1996). The bark of the Kenyan plant was reported active against *Bacillus subtilis*, *Penicillium crustosum* and *Saccharomyces cerevisiae*, Taniguchi *et al* (1978)	Stem, root bark and leaves used for malaria, Beentje (1994); Gessler *et al* (1994); Hedberg *et al* (1983), the fresh leaves of the plant from Tanzania are pounded with leaves of *Acalypha fruticosa* and *Surigada zanzibariensis* and the resulting juice is used for skin infections, Hedberg *et al* (1983a). The fresh twigs of the plant from East Africa are used as tooth brush, air fresheners and for skin infections, Hedberg *et al* (1983a); Johns et al (1990).

3.4. Discussion

The objective of this ethnomedical survey was to document the plants used traditionally at Msambweni, Kenya, against malaria. Similarity of information on the use of plant species for treatment of malaria was reported by several herbalists. Decoction of *Azadirachta indica* stem bark was used by 95% of respondents. *Azadirachta indica* has been reported to have antiplasmodial activity, Kirira *et al* (2006) and active compounds isolated such as gedunin and nimbinin, Bray *et al* (1990) which are responsible for the antimalarial activity of the plant species. Some of the plants identified by the herbalists have been reported in the literature as having been used for treatment of malaria related symptoms in other parts of the world, an indication that the herbalists could be trusted for the information they gave about the plants they use. They have also been investigated for their phytoconstituents and antimalarial activities (Table 2).

However, to the best of our knowledge, thirteen plant species, namely *Aloe deserti* Berger (Liliaceae), *Launea cornuta* (Oliv and Hiern) C. Jeffrey (Compositae), *Ocimum bacilicum* L. (Labiatae), *Teclea simplicifolia* (Eng) Verdoon (Rutaceae), *Gerranthus lobatus* (Cogn.) Jeffrey (Cucurbitaceae), *Grewia hexaminta* Burret. (Tiliaceae), *Canthium glaucum* Hiern. (Rubiaceae), *Amaranthus hybridus* L. (Amaranthaceae), *Combretum padoides* Engl and Diels. (Combretaceae), *Senecio syringitolius* O. Hoffman. (Compositae), *Ocimum suave* Willd (Labiatae), *Aloe macrosiphon* Bak. (Liliaceae) and *Laudolphia buchananii* (Hall.f) Stapf. (Apocynaceae) are ethnobotanically documented for the first time for the treatment of malaria.

The results of this study show that a large number of medicinal plants are traditionally used for treatment of malaria among the Msambweni community of Kenyan Coast. Twenty six species in twenty four genera and twenty families were documented. Labiatae, Rutaceae and Liliaceae families represented the species most commonly cited, which would indicate the importance of these families as possible sources of antimalarial drugs.

The information on the frequently utilized antimalarial plant species is an important lead to the species that can be targeted for pharmacological, toxicological and phytochemical tests. *Azadirachta indica* (Meliaceae), *Zanthoxylum chalybeum* (Rutaceae), *Aloe deserti* (Liliaceae), *Harrisonia abyssinica* (Simaroubaceae), *Launea cornuta* (Compositae), *Ricinus communis* (Euphorbiaceae) and *Lantana camara* (Verbenaceae) represented the species that were most commonly cited for traditional treatment of malaria. Plant species have been assigned numbers according to the extent of their use in treating malaria (Important value for the treatment of Malaria or IV mal, Willcox and Bodeker (2004). The use of *Ricinus communis* (Euphorbiaceae) in treatment of malaria has been reported in three continents and has an IV mal of 8, while *Lantana camara* (Verbenaceae) has been reported in two continents and has an IV mal of 7 Fowler (2006). This was consistent with our results as *Ricinus communis* (Euphorbiaceae) and *Lantana camara* (Verbenaceae) were reported as some of the commonly used species in preparation of antimalarial remedies and would indicate the importance of these plants as possible sources of antimalarial agents. Due to development of resistance to the commonly used agents, development of new antimalarial drugs from plant sources may be the way forward in dealing with global drug resistance problems of malaria, Gessler (1995). Natural products and

their derivatives represent over 50% of all drugs in clinical use in the world, Van Wyk *et al* (2002).

The roots were the second commonly used part of the plant after leaves and this was found to be destructive where in some cases the whole plant had to be uprooted. This calls for conservation and good harvesting strategies to facilitate sustainable utilization of these plant resources, Cunningham (2001). The stem bark or the leaves may be alternative parts, if the chemical composition is not significantly different from that in the roots, Muthaura *et al* (2007). Herbal medicine, in several developing countries, is still the mainstay of healthcare, DaSilva (1999). Among African medicines, indigenous plants play an important role in the treatment of a variety of diseases, Phillipson (1995) and are often used by herbalists to treat diseases identified as malaria, Omulokoli *et al* (1997). Indigenous plants are commonly used in East Africa, Chhabra *et al* (1993); Kokwaro (1993), South Africa, Watt and Breyer-Brandwijk (1962) and West Africa, Oliver-Bever (1986).

Some genera reported in this study represent plant species that are also known to be used as sources of antimalarial remedies in other parts of Africa. They have also been reported to contain antiplasmodial activity against *Plasmodium falciparum*. Those from Western Uganda include *Lantana trifolia* L., screened against wild strains of *Plasmodium falciparum* using the parasite lactate dehydrogenase (pLDH) assay. The petroleum, chloroformic and ethanolic extracts from the aerial parts of the plant had an IC_{50} (µg/ml) of 13.2, >50 and >50 respectively, Katuura (2007). Those from South Africa included plants screened against *Plasmodium falciparum* strain D10 using the parasite lactate dehydrogenase (pLDH) assay such as *Aloe forex* leaves (IC_{50} of 21

μg/ml) and *Ricinus communis* leaves (IC_{50} of 11.4 μg/ml). Others were *Ricinus communis* stems (IC_{50} of 8 μg/ml), *Ricinus communis* fruits (IC_{50} of 90 μg/ml) and *Lantana camara* leaves (IC_{50} of 11μg/ml), Clarkson *et al* (2004). Gessler *et al* (1994), while screening chloroquine resistant *Plasmodium falciparum* strain K1 against plant extracts from Tanzania found *Zanthoxylum chalybeum* root bark (IC_{50} of 4.2 μg/ml) to be one of the species with the strongest antiplasmodial activity among the antimalarial plants tested.

Kirira *et al* (2006) while screening chloroquine sensitive *Plasmodium falciparum* strain NF54 and chloroquine resistant strain ENT30 against plant extracts from Meru and Kilifi districts found *Azadirachta indica* leaves (IC_{50} >250 μg/ml) to be inactive. It is interesting to note that the most commonly used antimalarial plant species reported in this study, *Azadirachta indica*, which has also been cited severally as a potent traditional antimalarial remedy, was reported as having insignificant antimalarial activity whereas other studies have reported good antiplasmodial activity. El Tahir *et al* (1999) while screening some medicinal plants from Sudan against chloroquine sensitive *Plasmodium falciparum* strain 3D7 and resistant strain Dd2 found *Azadirachta indica* leaves (IC_{50} of 5.8 and 1.7 μg/ml, for strains 3D7 and Dd2, respectively) with highly potent antiplasmodial activity. The observed antiplasmodial activity from extracts of *Harrisonia abyssinica* against chloroquine sensitive *Plasmodium falciparum* strain NF54 and chloroquine resistant strain ENT30, Kirira *et al* (2006) has recently been confirmed by Maregesi *et al* (2010) while screening Tanzanian medicinal plants for activity against *Plasmodium falciparum* and human immunodeficiency virus, Maregesi *et al* (2010). This makes the plant quite promising as a lead for further studies.

It is noted that the plants used by the Msambweni community to treat malaria have been used in many other countries in the world for the treatment of fever frequently associated with malaria. Omino and Kokwaro (1993) reports widespread use of Apocynaceae in traditional medicine in Africa. Fowler (2006) reports the use of *Ricinus communis* (Euphorbiaceae) and *Lantana camara* (Verbenaceae) in three and two continents respectively. The potency of the extracts may also be affected by solvent of extraction, georeference, time and season of harvesting or other environmental factors, Prance (1994).

It is important to note that phytochemical compounds in traditionally used antimalarial herbal remedies are responsible for antiplasmodial activity. The most important and diverse biopotency has been observed in alkaloids, quassinoids, sesquiterpene lactones, coumarins, triterpenoids, limonoids and quinoline alkaloids, Saxena *et al* (2003). Quinoline alkaloids isolated from *Zanthoxylum chalybeum*, Kato *et al* (1996), steroidal sapogenins from *Ficus bussei*, Wall (2006), coumarins from *Cassia occidentalis* Cimanga (2004), gedunin and nimbinin, triterpenoids from *Azadirachta indica*, Bray *et al* (1990); Mackinnon *et al* (1997) are some of the specific examples. Other components responsible for antiplasmodial activity as in *Securidaca longepedunculata* roots are steroids, saponosides and monotropitosides, De La Pradilla (1988) and the leaves of *Lantana camara* have been reported to contain quinine like alkaloid, *lantanine*, Burkill (2000).

Azadirachta indica, the most commonly used species to treat malaria by the Msambweni community, South coast, is the third most commonly used herbal medicine to treat malaria in Kenya after *Ajuga remota* and *Caesalpinia volkensii*, Kuria *et al* (2001). As Sofowora (1982) noted, many people in several African countries take a decoction of *Azadirachta indica* (neem tree) for malaria fever. Their reasons for doing so include reaction to chloroquine, a dislike for

synthetic drugs, and the cost and unavailability of synthetic antimalarials, Muthaura *et al* (2007). The lack of standardization and quality control is one of the main disadvantages of traditional herbal remedies, Evans-Anfom (1986); Sofowora (1982). Isolation and characterization of active constituents need to be undertaken for use as markers in standardization of the extracts, thus minimizing the risk of over dosage and also for identification of possible lead structures that could be used for the development of novel antimalarial drugs.

Some of the species documented in this study for the treatment of malaria have been used similarly in other continents of the world. This convergence in the use of the same species in different cultures over a long period suggests strongly that these species may be effective in the treatment of malaria, Orwa (2002); Van wyk and Wink (2004). It is important, however, to validate all claims of therapeutic efficacy and safety by undertaking pharmacological, toxicological and good quality clinical studies. The literature reviewed in this study indicates that few toxicological studies have been conducted (Table 2)

Majority of the plants documented in this study were collected from community land, which is facing great pressure due to overutilization of indigenous trees and hence medicinal plants may disappear before their uses are documented. Most of the inhabitants of Msambweni district are in the low social economic bracket and very often the medicinal plant use is the only affordable treatment option. Medicinal plant use will therefore remain an integral part of the health care system to the community for a long time to come. Consequently, ethnobotanical exploration should not only be a cost effective means of locating new and useful tropical plant compounds but also be linked to the urgent need for sustainable conservation strategies for medicinal plants,

since human expansionist demands can be expected to cause environmental deterioration and biotic destruction well into the next century, Muthaura *et al* (2007). Kenya's strategy for conservation of forests involves intensification of timber and other non wood products outside forest areas, Njuguna *et al* (2000). Some plant resource users in other developing countries have realized that community forestry is not a question of trees but should include on-farm non-timber forest products for subsistence as well as for commercial purposes, Bryon (1995).

3.5. Conclusions

Many plant species reported in this study have been investigated for their phytoconstituents and pharmacological activities, the latter being in agreement with ethnomedical uses reported in this study. Thirteen plant species are documented for the first time for treatment of malaria. In Msambweni district, traditional methods of treatments based on medicinal plants are still an important part of social life and culture and the acceptability of these plants as claimed effective remedies is quite high among the population of this area. The claimed therapeutic value of the reported species call for modern scientific studies to establish their safety and efficacy and to preserve and document this flora which may otherwise be lost due to erosion of age old traditional methods of biodiversity conservation and medicinal knowledge as had been practiced in the *Kayas,* Muthaura *et al* (2007). There is general consensus that traditional knowledge on the use of medicinal plants must be conserved because of its vital role for human wellbeing. It is often argued, that if traditional knowledge which has been generated over a long period of time is lost, exploitation of plants among other things will become difficult if not impossible.

Among the reasons traditional knowledge is considered reliable for the exploitation of herbal remedies is that indigenous communities through a period of long experimentation with herbal medicines are likely to have retained those that are effective and tolerably safe while discarding preparations with low efficacy or acute toxicity, Balick (1990); Cox (1990); Van Wyk and Wink (2004).

CHAPTER FOUR

STUDY OF ATTITUDES AND TRADITIONAL PRACTICES RELATED TO RECOGNITION, CONTROL AND TREATMENT OF MALARIA IN MSAMBWENI DISTRICT, KENYA.

4.1. Introduction

Kenyan communities have unique and rich traditional practices for prophylaxis and treatment of malaria. The traditional ethnophytotherapeutic knowledge owned by the various communities is passed from generation to generation by oral means. Kenya is currently undergoing a rapid and traumatic change in its forest cover, which implies a rapid loss of this type of knowledge. There is a need therefore to package knowledge on medicinal plants used as antimalarial herbal remedies in a format that can be passed to the future generation. Some ethnobotanical studies have been accomplished in Kenya targeting the different cultures and localities among others, Johns *et al* (1990). These studies cover various aspects of plant utilization by local communities in Kenya. Studies on specialized knowledge on antimalarial herbal remedies in Msambweni, one of the malaria endemic districts in South Coast Kenya have been accomplished, Nguta *et al* (2010a). In the neighbouring country of Uganda, Tabuti (2008), has studied the common knowledge on herbal medicines (HMs) used in the treatment of malaria in Budiope county as well as the existing knowledge, attitudes and practices related to malaria recognition, control and treatment. The purpose of the present study was to utilize the existing knowledge in order to document useful medicinal plants with a clearly defined therapeutic and prophylactic context of

being used to treat and prevent malaria in a locality where malaria is endemic and with the most diverse flora and vegetation-South Coast, Kenya.

This study also addresses the following questions: (1) which medicinal plant does the South Coast community in Kenya use to treat malaria and how is the taxonomic richness? Here we are working with the supposition that the traditional medicinal knowledge of the study community is firmly rooted in the past, and is considered to be both cumulative and dynamic, building on the experiences of earlier generations and adapting to the new technological and social-economic changes of the present. Traditional knowledge is also assumed to be communal and herbal remedies are mainly used to treat malaria which is endemic in this region. Also traditional methods of treatments based on medicinal plants are still an important part of social life and culture and the acceptability of medicinal plants as claimed effective remedies is still quite high among the population of the study area; (2) what is the existing knowledge, attitudes and practices in regard to recognition, control and treatment of malaria amongst the South Coast community, Kenya? We believe that the people of South Coast, Kenya have rich ethnodiagnostic skills developed over time to positively diagnose malaria and have well developed methods utilized in malaria treatment and prevention; (3) considering that malaria is endemic in this region, and that medicinal plants are widely used as putative antimalarial remedies, are there differences in the relative importance of the different species used? In the same way that we suspect that there will be a taxonomic richness of the species utilized to treat malaria, the influence of modern communication and informal information exchange between people might result in new uses being ascribed to a given plant, de Albuquerque *et al* (2007).

Effort was made in this study to indicate the frequency of mention of each antimalarial plant species, factors responsible for causing malaria, practices employed to guard against mosquitoes bites and or to protect households against malaria, malaria treatment practices and attributes that favor the breeding of mosquitoes in the entire survey. Since the plant parts utilized in preparation of antimalarial herbal remedies are reported in this study, this may add information to the valuation of biodiversity and to forward suggestions for its sustainable use and conservation.

4.2. Materials and Methods

4.2.1. Study area

In South Coast, the study area centered on 04^0 28' 59.2''S latitude and 039^0 33' 36.2''E longitude in and around Mwaroni, Mwamambi A and Mwamambi B villages of Ngombato sub location, Diani location found in Diani division, Msambweni district in Coast province of Kenya (Fig.5).

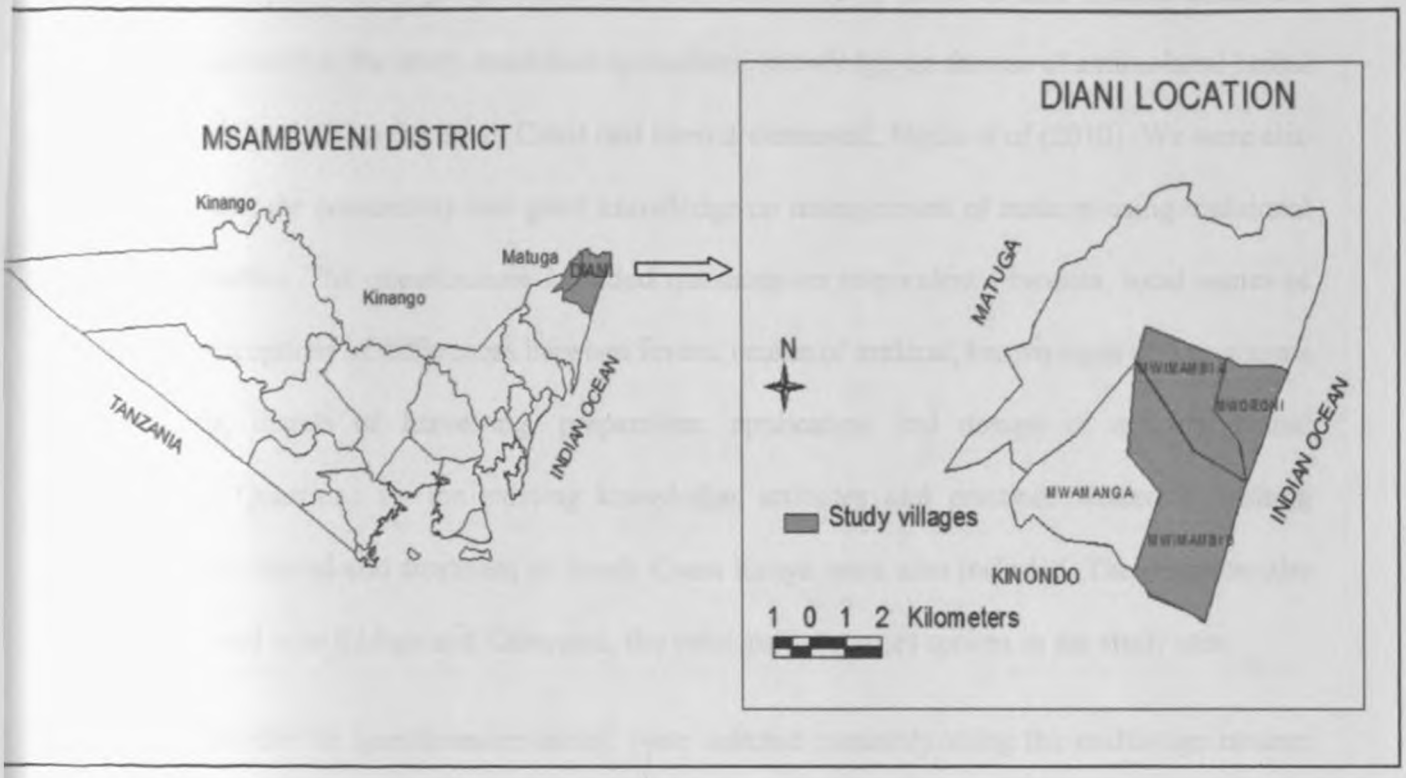

Figure 5: Map of Msambweni district showing Diani location with study villages Mwamambi A, Mwamambi B and Mwaroni

4. 2.2. Data collection

This study was conducted between May and November, 2009. Data was collected through a survey employing semi structured interviews and a guided open and closed ended questionnaire. The semi structured interviews were conducted using a checklist of questions and were held with individuals and local area leaders. Three group discussions that were held with community members, one in each of the study villages, complemented the interview and questionnaire

survey. Participants in the group discussions were identified by the local area leaders. Herbalists were not included in the study since their specialized knowledge on the use of antimalarial herbal remedies along the Kenyan South Coast had been documented, Nguta *et al* (2010). We were also informed that the community had good knowledge on management of malaria using traditional herbal remedies. The questionnaire included questions on respondent's biodata, local names of malaria, perceptions of differences between fevers, causes of malaria, known signs and symptoms of malaria, details of harvesting, preparation, application and dosage of malarial herbal medicines. Questions on the existing knowledge, attitudes and practices related to malaria recognition, control and treatment in South Coast Kenya were also included. The questionnaire was translated in to Kidigo and Kiduruma, the principal languages spoken in the study area.

Respondents for the questionnaire survey were selected randomly using the multistage random sampling method as follows: Diani location was randomly selected from among the eleven locations of Msambweni district and was considered the primary sampling unit. From within Diani location, one sub location (Ngombato) was selected. In turn, three villages; Mwaroni, Mwamambi A and Mwamambi B were selected from Ngombato sub location. The desired sample size was fixed at 65 respondents by assuming that 80% of the community had good knowledge regarding malaria and its treatment; a desired confidence interval of 95% and a relative error of estimation of 10%.

Thirty two households were randomly selected from each village by consulting the village household registers. From among the selected households, a random sample of sixteen households was picked from which men were to be interviewed while the remainder constituted

women respondents. In this way, 44 respondents were interviewed in Mwaroni and Mwamamambi A villages and 21 from Mwamamambi B village. The sample consisted of 34 male and 31 female respondents. Two guides identified with the help of the local leader were hired in each village to help locate the selected respondents and to introduce the team members to the respondents. Direct observations were made on issues relevant to the study objectives, such as plant harvesting, drug preparation and vegetation types.

All antimalarial plants mentioned by respondents in the study were identified during ethnobotanical walks with informants in the field. A voucher specimen of each plant species was collected for identification and is deposited both at the National Museums of Kenya, and also at the department of Land Resourse Management, University of Nairobi Herbaria. Species nomenclature follows the flora for tropical East Africa. A written informed consent was obtained from all the respondents in the study. The research objectives and methods were explained to respondents before each interview. The information gathered included plant species, part used, plant habit, method of preparation, dosage, vernacular names and the existing knowledge, attitudes and practices related to malaria recognition, control and treatment.

4.2.3. Statistical analysis

Questionnaire survey data was entered in Excel spreadsheets. It was checked and edited for errors. Thereafter it was summarized using SPSS and reported in figures and tables as described by Tabuti (2008). Semi structured interview data was studied and the responses grouped into classes expressing similar ideas, Tabuti (2008).

4.3. Results

4.3.1. Respondents' social-economic characteristics

Most respondents interviewed in this study lived in male headed households, belonged to the Digo ethnic group and had attained little (primary or secondary level) or no formal education (Table 3). All the respondents belonged to the Muslim religion. The main source of income of the respondents interviewed was peasant crop agriculture, while some served as traders, artisans and village elders as a way to earn secondary income. Respondents had, on average, four young dependants (1-15 years) and one elderly dependant (>60 years). Most households had *Makuti* (palm leaves) thatched huts constructed using mud. Many of the houses owned by the respondents lacked windows while some had a hole provided for a window but without a shutter. At night, the hole was covered by a piece of cloth or woven mat.

4.3.2. Traditional knowledge about malaria

Respondents had good knowledge about malaria and could readily distinguish it from other fever types on the basis of accepted signs and symptoms such as raised body temperature, chills, joint pains, weakness, headache, lethargy, abdominal pain, sneezing and flu like symptoms, loss of appetite, coughing and vomiting (Table 4). The respondents knew that mosquitoes were involved in transmission of malaria (Table 5). They also reported that young children, pregnant mothers, individuals with malnutrition and those with diseases such as AIDS and tuberculosis were most commonly affected by malaria. However, some people thought that keeping a dirty homestead or drinking dirty water caused malaria, while some believed that it was caused by dense bush or pools of stagnant water close to their homesteads.

Conditions likely to favor the breeding of mosquitoes were observed in all homesteads. Garbage, empty tins, tall grass, cattle sheds and uncleared bushes were within five meters of most homes (Table 6). All homesteads had tall plants within 3-5m of the house as well as untreated stagnant water in the compound. Furthermore, a good number of homesteads were in close proximity to wetlands and or open wells. A variety of strategies were employed by respondents to stop mosquito bites. These included the use of mosquito nets and mosquito repellants such as mosquito coils, cleaning the environment, burning the leaves of fresh *Azadirachta indica* (L) Burm, burning the ripe seeds *of Plectranthus barbatus* Andr., burning logs of plants such as *Ocimum bacilicum* L., burning the leaves of *Ocimum suave* Willd. and also removing materials likely to promote the breeding of mosquitoes such as draining stagnant water and treating water ponds with old engine oil (Table 7). Respondents also reported that they cleared bushes around their homesteads. However, this was not observed during the study, and instead, bushes were always observed close to households (Figure 5).

Figure 6: Bushes grew close to homesteads.

Table 3: Socio-economic characteristics of respondents (n=65) in a study of attitudes, traditional ethnodiagnosis, prophylaxis and therapy of malaria in Msambweni, Kenya.

Characteristic	(%)
Household head	
Male	75
Female	25
Tribe	
Digo	85
Duruma	15
Formal education	
None	40
Primary	10
Secondary	45
College	5
Religion	
Muslim	100
Primary job	
Peasant crop agriculture	85
Village elder	10
Animation	5
Secondary job	
Peasant crop agriculture	70
Trader	10
Artisan	5
Village elder	15

Table 4: Symptoms of malaria mentioned by respondents (n=65) in Msambweni district

Symptom	(%)
Fever	65
Joint pains	50
Vomiting	50
Headache	45
High temperature	40
Chills	35
Shivering	35
Loss of appetite/anorexia	30
Diarrhea	25
Abdominal pain	25
Fatigue/Lethargy	20
Sweating	20
Diagnosis from hospital	15
Confusion	10
Yellow eyes	10
Red eyes	10
Backache	10
Yellow vomitus	5
Extreme Coldness	5
Flu like symptoms/sneezing	5
Abdominal disturbances	5

Table 4 (Continued)

Symptom	(%)
Dizziness	5
Tiredness	5
Coughing	5
Scratching/itching	5
Pulsation of blood vessels	5
Weakness	5
Inability to stand	5
Nasal discharge	5

Table 5: Factors reported by traditional healers (n=65) to be responsible for causing malaria in Msambweni, Kenya

Factor	(%)
Mosquitoes	100
Age , especially young children	85
Pregnancy	45
Low immunity	40
Rainy season	35
Other diseases e.g. AIDS; TB	25
Dirty environment	20
Climatic changes e.g. beginning of rains	15
Uncleanliness	15
Cold season	15
Dirty water	15
Stagnant water	15
Illiteracy	10
Inadequate knowledge on prevention	10
New season foods e.g. mangoes	10
Drinking unclean water	10
Malnutrition	10
Drinking untreated water	5
Lack of latrines	5
Lack of mosquito nets	5
Stagnant water	5
Time of harvesting	5
Feacal waste	5

Table 6: Attributes likely to favor the breeding of mosquitoes around homesteads (n=65) in Msambweni, Kenya

Attribute	(%)
Stagnant water	70
Dirty environment	65
Bushes	55
Dirty water	40
Rainy season	30
Tall grass near the homestead	15
Cattle sheds near homesteads	15
Pit latrines	15
Garbage	15
Uncleared bushes	10
Untreated water ponds	10
Empty tins	10
Coldness	10
Piped water near homestead	5
Uncleanliness	5
Swamps	5
Flowering season	5
Untreated stagnant water	5

Table 7: Practices employed to guard against mosquito bites and/or to protect households against malaria (n=65) in Msambweni, Kenya

Practice	(%)
Taking herbal remedies	90
Burning plants to repel mosquitoes e.g. *Ocimum bacilicum* L.	55
Clearing bushes around homesteads	35
Use of mosquito nets	35
Cleaning the environment	30
Draining stagnant water	30
Burning the ripe seeds or fruits of *Plectranthus barbatus* Andr.	25
Burning the fresh leaves of *Azadirachta indica* (L) Burm	20
Garbage collection	15
Treating stagnant water with old engine oil	10
Cutting tall grass around homesteads	10
Treating drinking water	5
Boiling drinking water	5
Burning mosquito coil	5
Burning garbage/bushes	5
Cleanliness	5
Planting mosquito repellant trees around the homestead	5
Constructing cattle sheds far from homesteads	5

Table 7 (Continued)

Practice	(%)
Burning the leaves of *Ocimum suave* Willd.	5
Treating drinking water with water guard	5

4.3.3. Malaria treatment practices

The respondents reported suffering between 1 and 6 malaria episodes a year with a mean of four attacks (Figure 7). At the time the current study was conducted, 14% of the respondents reported that they were currently suffering from the disease while 22% had suffered an attack in the past one month (Figure 8). The respondents stated a preference for herbal remedies for the treatment of suspected malaria (Figure 9). They commonly self medicated with a decoction of the stem bark of *Azadirachta indica,* where approximately 125 mls was taken three times a day for four to five days. They shared information on malaria treatment amongst themselves. A variety of reasons were given why they preferred self medication using herbal remedies over self medication with allopathic medicine or even visiting a medical practitioner. The principle reason was that the herbal remedies cured suspected malaria more effectively than allopathic medicine. They also reported that herbal remedies were free, readily accessible and were also more effective than allopathic medicines. Respondents also mentioned that herbal remedies had no toxic effects if the correct dosage was taken. Majority of the respondents reported that they did not seek the services of herbalists since they knew how to prepare the necessary herbal preparations themselves.

Some community members, however, preferred to self medicate themselves with allopathic medicine. They commonly self medicated using ibuprofen, chloroquine, metakelfin, Coartem (ACTs), amodiaquine and fansidar.

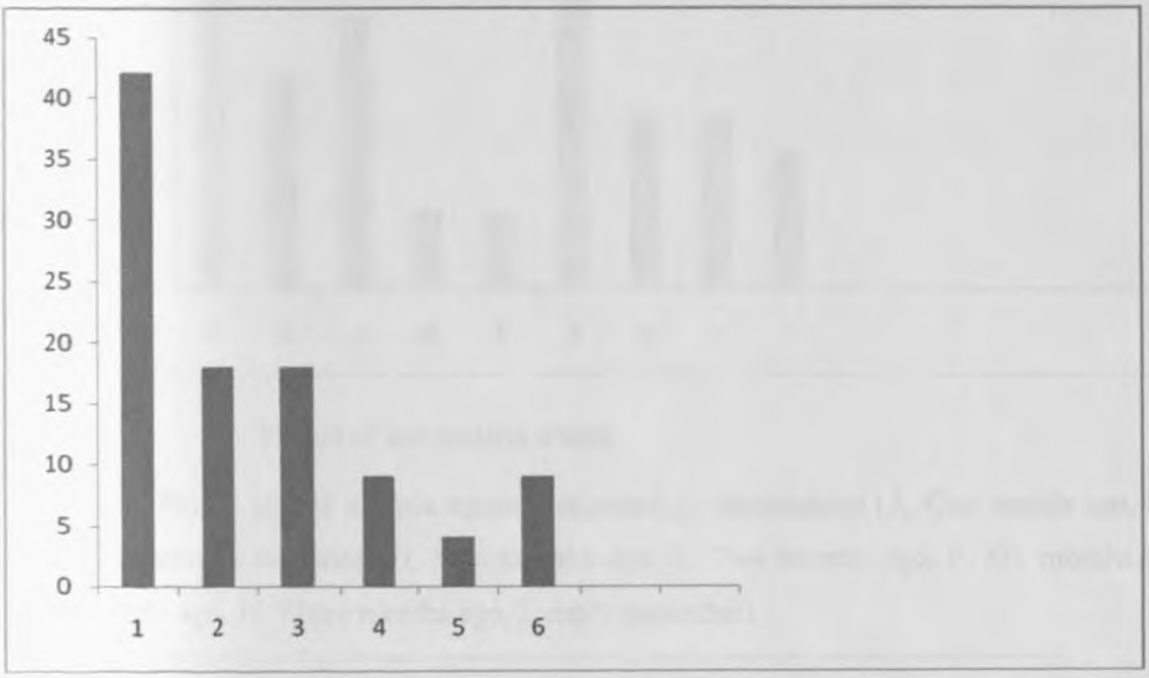

Figure 7: Number of malaria episodes suffered by respondents in Msambweni, Kenya

The main reason for self medication with conventional drugs was that they lacked the relevant traditional knowledge necessary to exploit herbal medicines for the treatment of malaria. Another frequently mentioned reason was that they believed allopathic medicine was more effective than herbal remedies. Respondents also consulted western trained medical practitioners. They preferred doctors since they believed they will correctly diagnose the disease before treatment is initiated. They also reported that they were likely to get correct treatment from the hospital.

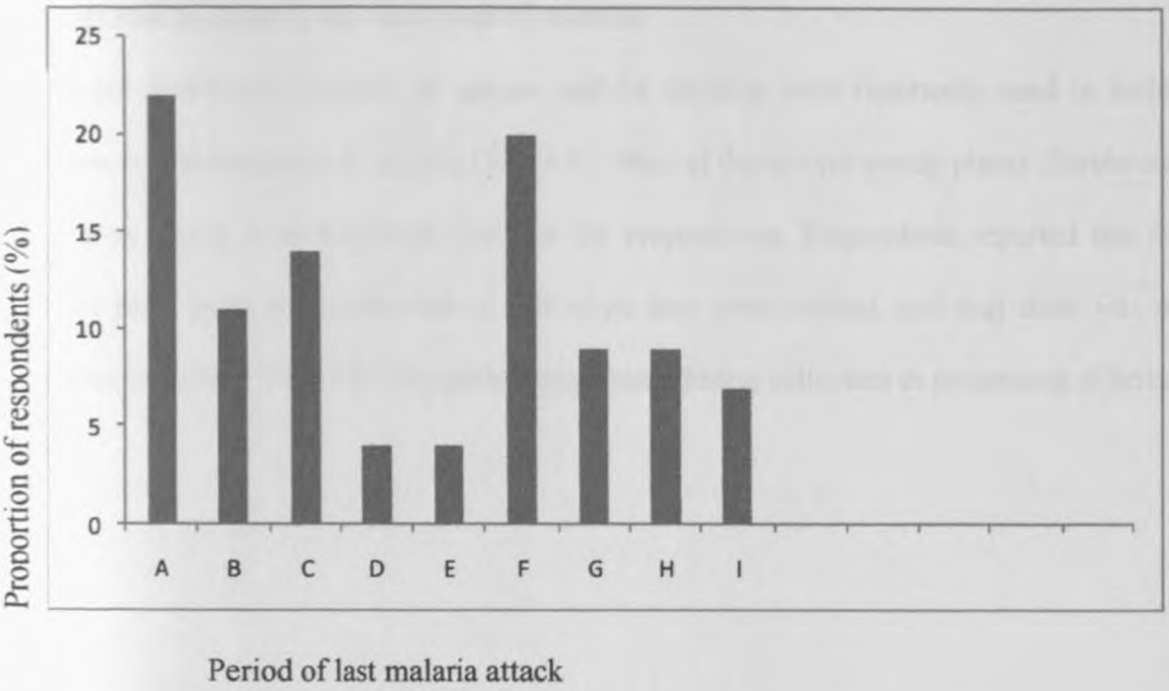

Figure 8: Period of last malaria episode reported by respondents (A, One month ago, B, One year, C, Currently suffering, D, Five months ago, E, Two months ago, F, Six months ago, G, Four months ago, H, Three months ago, I, can't remember).

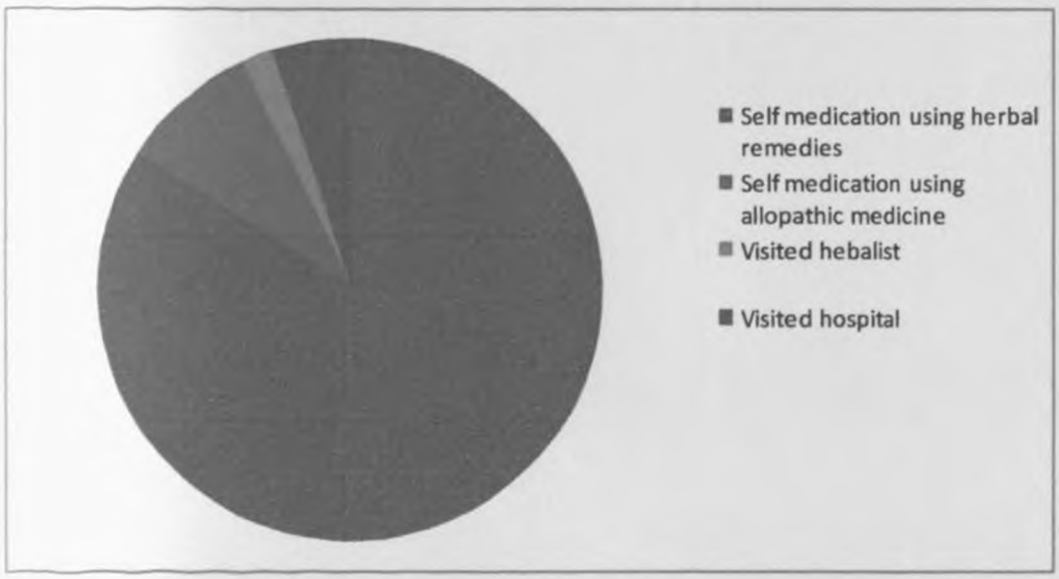

Figure 9: Treatment administered by traditional healers during the last malaria attacks in Msambweni, Kenya

4.3.4. Plant species used in the treatment of malaria

Forty species distributed between 35 genera and 24 families were reportedly used in herbal preparations for the treatment of malaria (Table 8). Most of these were woody plants (Shrubs and trees). Mature leaves were commonly used in the preparations. Respondents reported that the appropriate plant parts were collected as and when they were needed, and that there was no specific time to collect. They did not perform any rituals during collection or processing of herbal remedies.

Table 8: Plants used in the treatment of malaria by the South Coast Community, Kenya (n=58):

Family/Species/ (Voucher specimen no.)	Vernacular name	Habit[a]	Status[b]	Habitat[c]	Part used[d]	No.[e]
Amaranthanceae *Amaranthus hybridus* L. (JN 120)	Mchicha (Swa)	H	Wi	Bu	L	1
Anacardiaceae *Heeria insignis* Del. (JN 127)	Mwamadzi (D)	Tree	Wi	Bu	SB	1
Apocynaceae *Laudolphia buchananii* (Hall.f) Stapf. (JN 126)	Mhonga (Swa)	L	Wi	Bu	L	1
Bombacaceae *Adansonia digitata* Linn. (JN 109)	Mbamburi (Swa)	T	Wi	Bu	L	5
Bursaraceae *Commiphora schimperi* (Berg) Engl. (JN 129)	Dzongodzongo (D)	Tree	Wi	Bu	R, SB, L	1
Caesalpiniaceae *Cassia occidentalis* L. (JN 119)	Mnuka uvundo (Swa)	H	Wi	Bu	R,L	1
Caesalpiniaceae *Tamarindus indica* L. (JN 139)	Mkwadzu (Swa)	T	Wi	Bu	SB	1
Combretaceae *Combretum padoides* Engl and Diels. (JN 121)	Phozo (D)	S	Wi	Bu	L	1
Compositae *Launea cornuta* (Oliv and Hiern) C. Jeffrey (JN 106)	Mtsunga wa utsungu (D)	H	Wi	Bu	L	8

Table 8 (Continued)

Family/Species/ (Voucher specimen no.)	Vernacular name	Habit[a]	Status[b]	Habitat[c]	Part used[d]	No.[e]
Compositae *Senecio syringitolius* O. Hoffman. (JN 122)	Reisa (D)	H	Wi	Bu	L	1
Cucurbitaceae *Gerranthus lobatus* (Cogn.) Jeffrey (JN 114)	Mgore manga (D)	L	Wi	Bu	R	3
Euphorbiaceae *Bridelia micrantha* Baill. (Hochst). JN 137)	Mdungu (D)	Tree	Wi	Bu	SB, L	1
Euphorbiaceae *Ricinus communis* L.(JN 104)	Mbono/Mbo nombono (D)	S	Cv, Wi	Bu,Cf,Rs	R,L	22
Flacourtiaceae *Flacourtia indica* (Burm.f) Merr. (JN 112)	Mtondo mbare (D)	S	Cv	Cf	R,SB	3
Gramineae *Rottboelia exaltata* L.F (JN 128)	Mpunga (D)	Grass/ Herb	Wi	Bu	L	1
Labiatae *Hoslundia opposita* Vabl. (JN 132)	Mtserere (D)	Shrub	Wi	Bu	R	1
Labiatae *Ocimum bacilicum* L. (JN 108)	Kivumbani (D)	H	Wi	Bu	L	6
Labiatae *Ocimum suave* Willd (JN 124)	Murihani (G)	H	Wi	Bu	L	1

Table 8 (Continued)

Family/Species/ (Voucher specimen no.)	Vernacular name	Habit[a]	Status[b]	Habitat[c]	Part used[d]	No.[e]
Labiatae *Plectranthus barbatus* Andr. (JN 115)	Kizimwilo/Mumbu (D)	S	Wi	Bu	L	2
Liliaceae *Aloe deserti* Berger. (JN 102)	Ngolonje (D)	H	Wi, Cv	Bu, Bm	L	30
Liliaceae *Aloe secundiflora* Engl.(JN 136)	Mshubiri (D)	Herb	Wi	Bu	L	1
Liliaceae *Aloe macrosiphon* Bak. (JN 125)	Golonje (G)	H	Wi	Bu	L	1
Liliaceae *Aloe vera* (L) Webb. (JN 113)	Alvera (D)	H	Wi, Cv	Cf, Bu	L	3
Meliaceae *Azadirachta indica* (L) Burm.(JN 100)	Mkilifi (D)	T	Cv, Wi	Cp, Bu, Cf	RB,SB, L	57
Mimosaceae *Acacia seyal* Del. (JN 133)	Mgunga (D)	Tree	Wi	Bu	R	1
Mimosaceae *Dichrostachys cinerea* (L) Wight and Arn. (JN 134)	Chinjiri (D)	Shrub	Wi	Bu	R	1
Moraceae *Ficus bussei Warp ex Mildbr and Burret.*(JN 116)	Mgandi (D)	T	Wi	Bu	R,L	2

Table 8 (Continued)

Family/Species/ (Voucher specimen no.)	Vernacular name	Habit[a]	Status[b]	Habitat[c]	Part used[d]	No.[e]
Papilionaceae *Securidaca longepedunculata* Fres. (JN 110)	Mzigi (D)	S	Wi	Bu	R,SB,L	5
Rubiaceae *Agathisanthenum globosum* (A.Rich) Hiern (JN 131)	Chivuma nyuchi (D)	Herb	Wi	Bu	R	1
Rubiaceae *Pentanisia ouranogyne* S.moore (JN 130)	Chungu (D)	Herb	Wi	Bu	R	1
Rubiaceae *Canthium glaucum* Hiern. (JN 118)	Mhonga/Mronga (D)	S	Wi	Bu	F	1
Rutaceae *Clausena anisata* (Willd) Hook.f ex. Benth. (JN 135)	Mtondombare (D)	Tree	Wi	Bu	L, SB	1
Rutaceae *Teclea simplicifolia* (Eng) Verdoon (JN 111)	Mulaga dare (DR)	S	Wi	Bu	R	3
Rutaceae *Fagaropsis angolensis* (Engl) Del. (JN 123)	Muangani (D)	T	Wi	Bu	L	1
Rutaceae *Zanthoxylum chalybeum* (Eng) Engl. (JN 101)	Mjafari /Mporojo(G)	T	Wi	Bu	RB	32
Simaroubaceae *Harrisonia abyssinica* Oliv. (JN 103)	Mdungu/ Chidore(D/)	S	Wi	Bu	RB, L	30

Table 8 (Continued)

Family/Species/ (Voucher specimen no.)	Vernacular name	Habit[a]	Status[b]	Habitat[c]	Part used[d]	No.[e]
Solanaceae *Solanum incanum* L. (JN 107)	Mtugudza koma (D)	S	Wi	Bu	R,L	8
Tiliaceae *Grewia trichocarpa* Hochst ex A.Rich. (JN 138)	Cone (D)	S	Wi	Bu	R	1
Tiliaceae *Grewia hexaminta Burret.* (JN 117)	Mkone (D)	S	Wi	Bu	R,L	1
Verbenaceae *Lantana camara* L. (JN 105)	Mjasasa (D)	S	Wi	Bu	L	10

[a]C: climber; H: herb; S: shrub; T: tree; W: woody herb.

[b]Cv: cultivated; We: weed; Wi: wild; Sw: semi wild

[c]Bm: boundary marker; Bu: bush; Cf: crop field; Cp: compound; Rs: roadside

[d]Bk: bark; Rb: root bark; F: fruit; R: root; L: leaves; SB: stem bark; Wp: whole plant

[e]Percentage number of participants mentioning use of the species for malaria treatment

DR: Duruma; D: Digo; G: Giriama; Swa: Swahili

The herbal remedies were prepared mostly as decoctions. The infusions and decoctions were prepared as mono-preparations from single plant species such as *Zanthoxylum chalybeum* (Figure 10).

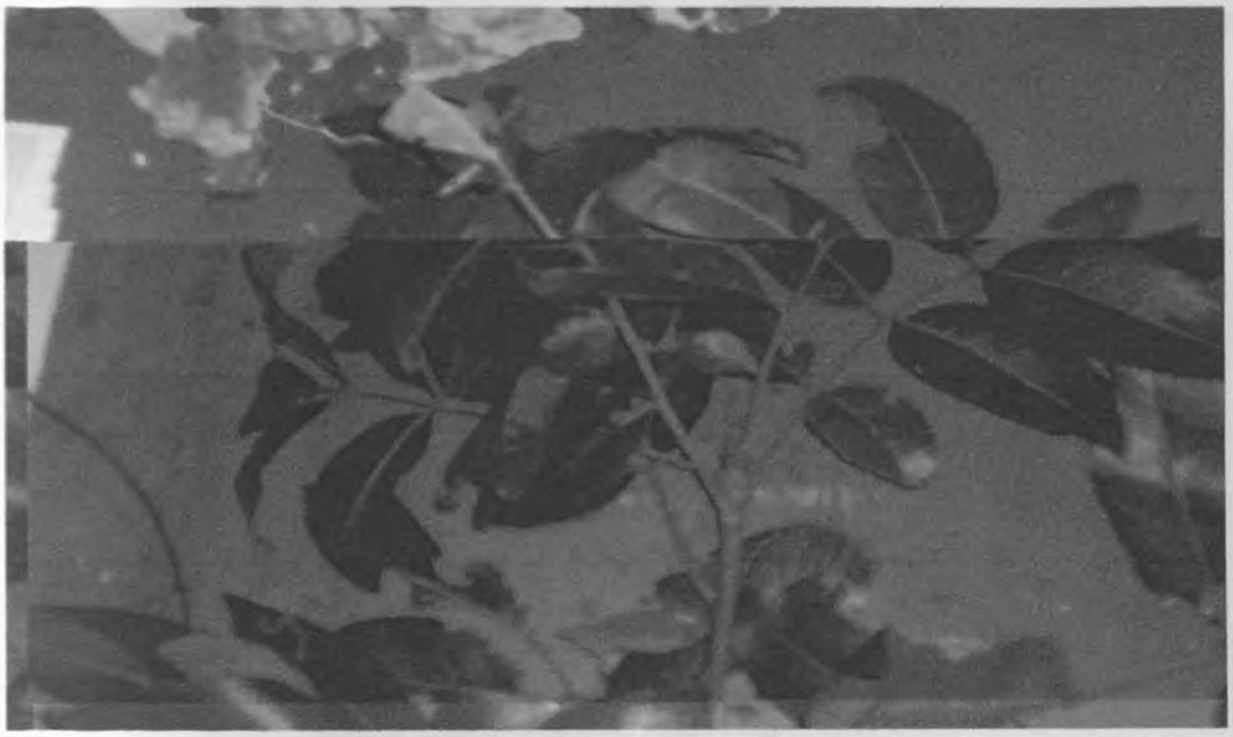

Figure 10: Leaves of *Zanthoxylum chalybeum*.

4.3.5. Reports from literature supporting use of plant species for the treatment of malaria

Information from the literature shows that 86% of the plant species reported by the Msambweni community are also used by people in other countries for the treatment of malaria (Table 9).

Table 9: Published scientific validation of the animalarial activity of the plant species identified by Msambweni community.

Species	Reported ethnomedical uses	Bioactivity/Chemical constituents
Acacia seyal	The bark is used as a febrifuge in treatment for malaria Burkill (1985)	No previous reports
Adansonia digitata	The leaves are used as a diaphoretic and as a prophylactic against fevers Watt (1962), fever remedy Abbiw (1990); leaf decoction used for malaria, Nguta *et al* (2010b)	Antiplasmodial activity Kristina (2002); Bioactivity, Cantrell *et al* (2003).
Agathisanthenum globosum	No previous reports	No previous reports
Aloe deserti	A leaf decoction is used to treat the spleen Kokwaro (1993); Leaf infusion used for malaria, Nguta *et al* (2010a).	Anthrone C-glycosides, the chromones and a large mixed group of phenolic compounds, Reynolds (2008)
Aloe macrosiphon	A leaf decoction is used to treat the spleen, Kokwaro (1993); leaf infusion used to treat malaria, Nguta *et al* (2010a).	No previous reports
Aloe secundiflora	Leaf decoction is used to treat the spleen, Kokwaro (1993)	Antimalarial activity, Oketch-Rabah *et al* (1999).
Aloe vera	The fleshy stock is chopped small, dried and roasted to powder. One small spoonful to be sucked twice a day between meals for the treatment of malaria, De La Pradilla (1988); leaf infusion used for malaria, Nguta *et al* (2010a).	Stimulation of gap junctional intercellular communication and proliferation of human skin fibroblasts in diabetes mellitus Abdullah (2002).

Table 9 (Continued)

Species	Reported ethnomedical uses	Bioactivity/Chemical constituents
Amaranthus hybridus	Leaf decoction used for malaria, Nguta *et al* (2010a)	Antioxidant activity Adewumi (2005); Bioactivity, Cantrell (2003)
Azadirachta indica	Leaf infusion used for malaria, Gessler et al (1995); Ibrahim *et al* (1992); Tella (1977); Van Der Nat *et al* (1986); Infusion prepared from roots, stem bark and leaves, Gessler *et al* (1995a); Root bark, stem bark and leaf decoction used for malaria, Nguta et al (2010a)	Antiplasmodial activity, El Tahir et al (1999); Kirira *et al* (2006), antimalarial activity has been demonstrated clinically and experimentally, Sofowora (1993), active compounds gedunin, nimbinin, Bray *et al* (1990), compounds meldenin, isomeldenin, nimocinolnimbandiol, Bray *et al* (1990)
Bridelia micrantha	No previous reports	Antiplasmodial activity, Edith *et al* (2005).
Canthium glaucum	Fruits are boiled and drunk for malaria, Nguta *et al* (2010b).	No previous reports of biological activity
Cassia occidentalis	It has a special reputation as an excellent oxytocin, cholagogue, anti-fever medicine, anti-worm medicine and remedy for swellings. As a cholagogue, 15g of leaves boiled in 1 liter of water and 1 glass drunk daily; as a diuretic: 4g of leaves in 180g of water each day as an infusion, Neuwinger (1994); root and leaf decoction used for malaria, Nguta et al (2010a).	Antiplasmodial activity, Cimanga (2004); Tona (1999). Terpenes, steroids, coumarins, flavonoids, phenolic acids, lignans, xanthones, anthraquinones, Cimanga (2004).

Table 9 (Continued)

Species	Reported ethnomedical uses	Bioactivity/Chemical constituents
Clausena anisata	Pounded roots are put into soup , as a cure for malaria, Kokwaro (1993); Roots and leaves used to treat malaria, Weenen *et al* (1990)	Antiplasmodial activity observed, Clarkson *et al* (2004).
Combretum padoides	Leaves for snakebites and the roots for eliminating hookworms, Neuwinger (2000); leaf decoction used for malaria, Nguta et al (2010a).	Mono and bi-desmosidic triterpenoids from leaves (Rodgers, 1999); Acetone extracts of leaves have antimicrobial effects (fresh leaves more effective than dried) MIC 0.8 µg/ml against *E.coli* and *Enterobacter faecalis*, Eloff (1999)
Commiphora schimperi	The inner bark is boiled in water. Strain, add milk, drink for malaria and constipation, Koch *et al* (2005).	In vitro antimalarial and cytotoxic activity, Koch *et al* (2005).
Dichrostachys cinerea	In Burkina Faso, a handful of fruits is boiled for 15 minutes in 5 litres of water; used to drink and as a wash twice daily for three days for malaria, De La Pradilla (1988).	No previous reports
Fagaropsis angolensis	Used for management of malaria, Njoroge and Bussmann (2006); leaf decoction used for malaria, Nguta *et al* (2010a).	Bioactivity and antiplasmodial activity, Kirira *et al* (2006)
Ficus bussei	A decoction of leafy twigs is used as a remedy for fever Kerharo (1950); root and leaf decoction used for malaria, Nguta *et al* (2010a).	Steroidal sapogenins, Wall (2006)

Table 9 (Continued)

Species	Reported ethnomedical uses	BioactivityChemical constituents
Flacourtia indica	The leaf sap is mixed with a root decoction as a malaria cure, Burkill (1994); decoction from leaves and roots used for malaria, Nguta *et al* (2010).	Antiplasmodial activity (Clarkson et al., 2004)
Gerranthus lobatus	Fresh leaf juice mixed with 10 ml of brandy given twice in a week is used to treat heart diseases and hypertension; hot water extract from the leaves used for malaria (Nguta et al., 2010a).	Flavonoid compounds, Imperato (2005).
Grewia hexaminta	Hot water extract from the leaves used for malaria, Nguta et al (2010)	Triterpenoid compounds, Raghunathaiyar (1996)
Grewia trichocarpa	No previous reports	No previous reports
Harrisonia abyssinica	Root decoction used for fever, Kokwaro (1993), Venereal diseases, Beentje (1994); hot water extract of fresh and dried root bark is used in Tanzania to treat skin diseases, Sawhney *et al* (1978a,b); decoction of fresh and dried root bark and leaves used for malaria, Nguta *et al* (2010a).	Antimalarial activity, El Tahir *et al* (1999), Antiplasmodial activity Kirira *et al* (2006); Maregesi *et al* (2010); Methanol extract of dried root bark exhibited activity against *Trichophyton mentagrophytes* and *Candida albicans*, Sawhney *et al* (1978b). Chloroform extract of the stem bark exhibited antifungal activity against *Aspergillus niger*, *Microsporum canis*, *Trichophyton mentagrophytes* and *Aspergillus fumigates*, Balde *et al* (1995).
Heeria insignis	A root decoction is used to treat epilepsy, Moshi *et al* (2005)	Myrcene, β-pinene, α- pinene, Ayedoun *et al* (1998)

Table 9 (Continued)

Species	Reported ethnomedical uses	Bioactivity/Chemical constituents
Hoslundia opposita	Used for mental disorders, malaria, convulsions in children, Hedberg *et al* (1983).	Antimalarial activity confirmed, Gessler *et al* (1994).
Lantana camara	The infused leaves are used as a diaphoretic and febrifuge, Burkill (2000), the roots are used for malaria, and said to be effective in cases which are not responsive to quinine, Burkill (2000). Tea of the leaves is believed to prevent weakness of memory and enhances intellect and cognition, Muller-Ebeling and Ratsch (1989); decoction from leaves used as antimalarial, Nguta *et al* (2010a).	Antiplasmodial activity, Clarkson *et al* (2004). Quinine like alkaloid, *lantanine*, is present in the leaves, Burkill (2000).
Laudolphia buchananii	Leaf decoction used for malaria, Nguta *et al* (2010a.	No previous reports
Launea cornuta	The roots are pounded and infused or decocted, the liquid being drunk as a remedy for typhoid, Kokwaro (1993); leaves are boiled and drunk for malaria, Nguta *et al* (2010).	Tannins and astringents, Burkill (1985)
Ocimum bacilicum	For abdominal cramps, upset stomach, nervous migraine, memory "strengthens the heart and the head" loss and forgetfulness, Fuchs (1543); Sfikas (1980); leaf decoction used for malaria, Nguta *et al* (2010b).	Antifungal activity, Dambolena (2007), linalool, geranical, camphor compounds, Dambolena (2007)

Table 9 (Continued)

Species	Reported ethnomedical uses	Bioactivity/Chemical constituents
Ocimum suave	In Tanzania, the scrappings of the roots mixed with *Zingiber officinalis* are used for inflamed tonsils, Hedberg *et al* (1983a) and the dried twigs are used as a chewing stick, Khan *et al* (2000). Used for treatment of Candida infections including oral candidiasis, Runyoro (2006); hot water extract from the leaves used for malaria, Nguta *et al* (2010a).	The essential oil isolated from the aerial structures of the plant was reported active against a number of microorganisms, Janssen *et al* (1989). The ethanol extract of the leaves of Rwandese plants were found to be active against *Bacillus subtilis* and *Microsporum canis,* Vlietinck (2000). Triterpenes, Tan (1997); anti-ulcerogenic activity, Tan (1997).
Pentanisia ouranogyne	No previous reports	No previous reports
Plectranthus barbatus	The plant is used as a mosquito repellant, Watt (1962); leaf decoction used for malaria, Nguta *et al* (2010a)	Antiplasmodial activity, Meyer (2002)
Ricinus communis	Leaves are used as a remedy for fever, Burkill (1994), dried root is used as a febrifuge, Watt and Breyer-Brandwijk (1962), the oil is added to paraffin based spray as an antimalarial agent, Burkill (1935); hot water extract from leaves and roots used as antimalarial, Nguta *et al* (2010a)	Antiplasmodial activity (Clarkson et al., 2004).
Rottboelia exaltata	Powdered roots are mixed with oil and left for 2 days. The mixture is applied topically. The patient is made to shave the head and rub it with the mixture once or twice a day for epilepsy (Moshi et al., 2005).	No previous reports

Table 9 (Continued)

Species	Reported ethnomedical uses	Bioactivity/Chemical constituents
Securidaca longepedunculata	The roots are used against malaria, Williamson (1975); hot water extract from the roots, stembark and leaves used for malaria, Nguta *et al* (2010a)	The roots contain steroids, saponosides and monotropitoside De La Pradilla (1988); aqueous, dichloromethane and ethanol extracts are reported to have activity against *Candida albicans*, Desta (1993); Taniguchi *et al* (1978).
Senecio syringitolius	Leaf decoction used for malaria, Nguta *et al* (2010a).	No previous reports
Solanum incanum	A root decoction is used against fever, Kokwaro (1993); root decoction used for malaria, Nguta *et al* (2010a)	Antiulcerogenic effect, Farina et al (1998), active triterpenoid compounds-Ursolic acid (3a) Hirota (1990).
Tamarindus indica	In Burkina Faso, 4 bunches of leafy twigs are boiled in 10 litres of water for 15 minutes. Bathe the body twice daily, and drink a little, for 4 days for malaria, De La Pradilla (1988).	The leaves contain luteoline, apigenine, orientine, isorientine, vitexine and pinitol, De La Pradilla (1988).
Teclea simplicifolia	In Kenya, the roots are regarded as poisonous, Neuwinger (1996). The maasai use a root infusion for gonorrhea, Neuwinger (2000); in Kenya, the Digo use a decoction of the roots for malaria, Nguta *et al* (2010a).	Quinoline compounds, Wondimu (1988).

Table 9 (Continued)

Species	Reported ethnomedical uses	Bioactivity/Chemical constituents
Zanthoxylum chalybeum	Stem, root bark and leaves used for malaria, Beentje (1994); Gessler *et al* (1994); Hedberg *et al* (1983), in Kenya, a decoction is prepared from stem bark, Kokwaro (1993), in Uganda, a decoction is prepared from roots, the fresh leaves of the plant from Tanzania are pounded with leaves of *Acalypha fruticosa* and *Surigada zanzibariensis* and the resulting juice is used for skin infections, Hedberg *et al* (1983a). The fresh twigs of the plant from East Africa are used as tooth brush, air fresheners and for skin infections, Hedberg *et al* (1983a); Johns *et al* (1990); Root bark used for malaria, Nguta *et al* (2010a).	Antiplasmodial activity, Gessler *et al* (1994), Antimalarial activity detected, Neuwinger (1996), quinoline alkaloids, Kato *et al* (1996). The bark of the Kenyan plant was reported active against *Bacillus subtilis*, *Penicillium crustosum* and *Saccharomyces cerevisiae*, Taniguchi *et al* (1978).

4.5. Discussion

The respondents interviewed in this study had good knowledge about malaria and readily distinguished it from other illnesses on the basis of widely accepted malaria signs and symptoms Gessler *et al* (1995b); Ahorlu *et al* (1997); Purcell (2004); Tabuti (2008). The community recognized the clinical features of malaria such as chills, profuse sweating, joint pains, abdominal pain, diarrhea, vomiting, anorexia and inability to stand, Ministry of Health (2006). Malaria continues to be a major health challenge in Kenya especially due to the emergence of parasite

resistance to commonly used and relatively cheap antimalarials such as chloroquine. From the current study, it is worthy noting that malaria knowledge amongst Kenyan communities has steadily improved, but some misconceptions still remain about the causes and symptoms of severe malaria, and these were also documented in this study. However, it was observed that majority of the respondents knew that malaria was spread by mosquitoes and one of the major symptoms of the disease was fever. This relatively good understanding of the causes and signs of the disease may help in the implementation of intervention measures aimed at reducing its incidence and prevalence, Ahorlu *et al* (1997); Nuwaha (2002), as opposed to some communities in developing countries that associate the disease with witchcraft, Nuwaha (2002).

Malaria is recognized by the study community as a leading cause of morbidity and mortality, especially in young children and pregnant women. This observation from the current study is in agreement with the fact that it accounts for 30% of outpatient attendants and 19% of admissions to health facilities, MOH (2006). This compares well with the prevalence observed in this study of four episodes a year. In Kenya, the disease is the most important cause of death in children under 5 years of age and is estimated to cause 20% of all deaths in this age group, MOH (2006). Parasite prevalence amongst childhood communities has been reported to often exceed 50% in high malaria risk areas such as the coastal endemic zone, MOH (2006). This was not observed in this study since the study community mainly comprised of adults who have developed immunity to the disease. High malaria prevalence as the one observed in this study may have a significant impact on the well-being and economic potential of the community. A single malaria episode can result in the loss of 5-20 days of productive labor per year. This means, therefore, that 10-40 days

are lost every year for an average sized family (six members) with two adults, Tabuti (2008). This translates to lowered income earnings.

It was observed that among suffering from malaria, there is increased absence from school and lethargy when in class leading to poorer academic performance, which may, in turn, lead to long term social consequences. In addition to the above indirect social and economic costs, there is the direct cost of treating malaria or purchasing material to stop mosquito bites such as mosquito nets, Tabuti (2008). The estimated cost for treating a single malaria episode in Kenya is put at USD 0.8, MOH (2006). For a family of six people, suffering an average of four episodes a year, this translates into a total cost of USD 19.2 every year.

The suffering from malaria and its contribution to poverty is likely to continue in the foreseeable future because the disease is resistant to the most affordable, available and safe antimalarial drugs, Kilama (2005); Sendagire *et al* (2005); Tabuti (2008). Kenya, formally abandoned the use of chloroquine in 1998 as its first line therapy in favor of an easier to use drug, sulphadoxine-pyrimethamine (SP). There has, however, been a precipitous decline in the efficacy of SP and at the same time, there is evidence of declining efficacy of amodiaquine, the current second line treatment, MOH (2006). Out of concern for this resistance, the Ministry of Health of Kenya adopted the Artemisinin-based Combination Therapy (ACTs) as the first line medicine for the treatment of uncomplicated malaria in 2006 following recommendation by the World Health Organization, Malaria Control Programme (2005).

The efficacy and performance of ACTs remains to be evaluated, Tabuti (2008). Secondly, the conditions in Diani Location as elsewhere in high malaria risk areas in Kenya are ideal for the

breeding and survival of mosquitoes. Homesteads were surrounded by dense bush and the landscape had numerous logs lying within the flight range of the mosquito *Plasmodium falciparum*, estimated at 3 km, Ghebreyesus *et al* (1999). The government of Kenya intends to start indoor residual spraying using the controversial pesticide DDT which was sanctioned in September 2006 for use by the World Health Organization, World Health Organization (2006). The government of Kenya clearly indicates that chemoprophylaxis and other preventive measures are not 100% effective. It therefore recommends early medical care if fever develops within three months of travel to an endemic area, even if adequate prophylaxis has been taken. Lastly, the infrastructure for managing malaria in Kenya as elsewhere in Africa is still weak, World Health Organization (2003). According to the respondents, Diani location had few and poorly manned health care centres.

Malaria continues to be a major health challenge in Kenya especially due to resistance of *Plasmodium* to the drugs in use currently, Njoroge and Bussman (2006). The results of this study show both indigenous and introduced species are in use for malaria treatment. This indicates that traditional medicinal practices in this region are dynamic. The information on frequently utilized antimalarial plant species is an important lead to the species that can be targeted for further pharmacological, toxicological and phytochemical analysis. Since there is no safer, effective and cheaper antimalarial remedies than chloroquine, Gessler (1995) in the treatment of malaria, development of new antimalarial drugs especially from plant sources may be the way forward in dealing with global drug resistant problems of malaria.

Malaria prevalence observed in the study community and which has direct implications on the people's health and economic well-being, calls for extensive research and development of

effective and safe antimalarials, Tabuti (2008). Within the context of growing antimalarial resistance and the difficulties for households to afford and access effective antimalarials, the development and promotion of phytomedicines may be the sustainable solution to malaria treatment, Tabuti (2008). This focus is justified because herbal medicines are widely accepted as safe and efficacious remedies by the study community. Indeed many drugs used in malaria treatment have been derived from higher plants using leads from traditional knowledge, Farnsworth (1990); Fabricant and Farnsworth (2001); Van Wyk and Wink (2004); Tabuti (2008). These include the quinoline based antimalarials as well as artemisinin and its derivatives, Orwa (2002); Waako *et al* (2005).

It is notable that some species reported in the current study are also sources of antimalarial remedies in other countries (Table 9). This correspondence in use of the same species in different cultures over a long period suggests strongly that these species may be effective in the treatment of malaria, Orwa (2002); Van Wyk and Wink (2004). It is however, important to validate all claims of therapeutic efficacy and safety by undertaking pharmacological and toxicological studies, Tabuti (2008). Lliterature data reviewed in the current study suggests that few toxicological studies have been conducted (Table 9). These calls for detailed toxicological analysis of the documented species so as to better understand their short term and long term effects amongst the Msambweni community.

It is important to validate traditional medical practices because it may generate higher confidence and hence wider use of such species, World Health Organization (2000), and hence the need for controlled efficacy and safety studies. Wider acceptance of traditional herbal remedies can yield

significant benefits for primary health care and also help create a herbal medicine market, with possibilities of adding value to medicinal plants, Tabuti (2008). Validations may proceed from observations of the treatment responses among patients taking the herbal medicines, Diallo and Paulsen (2000). Promising herbal medicines identified in this way can therefore be subjected to pharmacological screening, toxicological screening, phytochemical analysis and clinical trials to confirm their efficacy and safety, and also determine administration doses, World Health Organization (2000).

Most knowledge on medicinal plants is transferred orally in many communities, Fratkin (1996) and there is therefore the danger of losing this precious cultural heritage, Muthaura *et al* (2007). In view of the rapid loss of natural habitats, traditional community life, cultural diversity and knowledge of medicinal plants, an increasing number of ethnobotanical inventories need to be established, Van Wyk *et al* (2002). The exploitation of traditional herbal practices depends to a large extent on local traditional knowledge, Tabuti (2008). Traditional knowledge relevant to the treatment of malaria was found to be high amongst the study community.

It was observed that traditional knowledge must be conserved because of its vital role for human wellbeing. It is often argued that if traditional knowledge which has been generated over a long period of time is lost, exploitation of plants among other things will become difficult if not impossible, Tabuti (2008). Among the reasons traditional knowledge relevant for the exploitation of herbal medicines is considered reliable is that indigenous communities through a period of long experimentation with herbal medicines are likely to have retained those that are effective and tolerably safe while discarding preparations with low efficacy or acute toxicity, Balick (1990); Cox (1990); Van Wyk and Wink (2004); Tabuti (2008).

4.6. Conclusions

Majority of plant species documented in the current study have been reported in literature to have antimalrial activity, and as such could be sources of novel compounds or new source principles against malaria. Five plant species are documented for the first time for the treatment of malaria. Respondents also mentioned some plant species that have already been investigated for their phytoconstituents and pharmacological activities, the latter being in agreement with ethnomedical uses reported in this study. This study calls for rational investigation of indigenous plants along South Coast Kenya for antiplasmodial properties. Considering that most antimalarial plant species reported in this study have not been investigated pharmacologically, toxicologically or phytochemically, they remain a potential source of leads for antimalarial drug development. The claimed therapeutic value of the species reported in this study call for scientific evaluation so as to establish their safety and efficacy. Ecological studies on regeneration of plant species reported in this study are recommended since they could provide data on management of these species for sustainable utilization.

CHAPTER FIVE

ETHNODIAGNOSTIC SKILLS OF THE DIGO COMMUNITY FOR MALARIA: A LEAD TO TRADITIONAL BIOPROSPECTING?

5.1. Introduction

Malaria kills 1–2 million people each year globally and 300–500 million new clinical cases of the disease are reported annually, Snow *et al* (2005). Malaria constitutes one of the biggest health problems in tropical Africa and is slowly spreading to hitherto non-malaria areas, Trape (2002). The emergence of resistant parasites, changes in climatic conditions over a large part of Africa, changes in land use and population migration, Foster (1991) are extending the areas of malaria transmission, which requires innovative strategies for malaria and the mosquito vector control. It is estimated that the malaria incidence range between 350 and 500 million cases globally, with 90% of these being in tropical Africa, WHO (2005). In Kenya, more than 90% of malaria is caused by *Plasmodium falciparum*, Khaemba *et al* (1994) transmitted by *Anopheles gambiae* which is the most widespread in Africa and difficult to control. Each year, there are over 8.2 million malaria infections in Kenya, Jean-Marie (2002) mostly due to inadequate medical care, unavailability of insecticide treated nets and increased resistance of the parasites to drugs. The disease accounts for 30% of all the outpatient cases and 19% of all admissions, 5.1% of whom die, and 72 children below the age of 5 years die daily, DMS (2006); WHO (1996); Mouchet (1999). The disease is endemic in the lowlands, particularly the coastal strip where transmission is sufficiently intense, Muthaura *et al* (2011). Both incidence and prevalence of infection reach

more than 90% of the population within 10–12 weeks after the beginning of the rainy season, Hoffman *et al* (1996).

Human malaria transmitted by female Anopheles mosquitoes is caused by four species of *Plasmodium*, which are, *P. falciparum*, *P. vivax*, *P. ovale* and *P. malariae*. Most cases of malaria and deaths are caused by *P. falciparum*. The development of resistance to mainstay drugs like chloroquine and controlled use of new artemisinin analogs have created an urgent need to discover new antimalarial agents. The life cycle, immunological defense mechanisms, and clinical development of malaria in humans are complex processes, Kumar *et al* (2002) and successful chemotherapeutic intervention is essential in control of the disease. Nature remains an ever evolving source for compounds of medicinal importance. The use of medicinal plants for the treatment of parasitic diseases is well known and documented since ancient times. For example, use of *Cinchona succiruba* (Rubiaceae) for the treatment of malaria infection is known for centuries. Several compounds isolated from nature also form a rich source of diverse structures for optimization to obtain improved therapeutics. A number of natural products having antimalarial activities have been documented, Sharma and Sharma (2001).

The Digo community is one of the nine deeply traditional ethnic groups that form the Mijikenda community of the Kenyan coast. They inhabit a malaria endemic zone and have developed impressive traditional procedures to diagnose, prevent and treat malaria. In addition, they have a well established ethnomedical practice to cure and control the disease. This knowledge acquired through history taking, observation and palpation of sick members of the society has evolved into an ethnodiagnostic procedure, which is a major contributor to the Digo traditional bioprospecting skills. Ethnopharmacological studies on antimalarial herbal remedies in the Digo inhabited

regions of Kenya have been conducted, Nguta *et al* (2010a). Studies utilizing specialized knowledge to document plants traditionally used by the Digo community to treat malaria have also been accomplished, Muthaura *et al* (2007). These activities are focused on the discovering of new antimalarial drugs of plant origin to combat antimalarial drug resistance. In the neighboring country of Uganda, herbal medicines used in the treatment of malaria as well as the existing knowledge, attitudes and practices related to malaria recognition, control and treatment in Budiope county have also been documented, Tabuti (2008). In Tanzania, medicinal plants have been screened against malarial causal agent, *Plasmodium falciparum*, Maregesi *et al* (2010). The Digo people occupy a high incidence area for malaria at the Kenyan coast, DMS (2006) and have a great variety of unique traditional knowledge about malaria recognition and they widely use natural resources in treatment of the disease. However, the ethnodiagnostic skills utilized by the Digo community to treat malaria have not been documented.

The main objectives of the current study were to explore the traditional knowledge of malaria diagnosis and ethnophytotherapeutic practices in three Digo villages of Mwamambi A, Mwamambi B and Mwaroni. The study also addresses the questions: (1) which ethnodiagnostic skills do the Digo community utilize as a lead to traditional bioprospecting? (2) which plants do the Digo community use to treat malaria?

5.2. Materials and methods

5.2.1. Study area

In South Coast, the study area centered around 04^0 28' 59.2''S latitude and 039^0 33' 36.2''E longitude in and around Mwaroni, Mwamambi A and Mwamambi B villages of Ngombato sub

location, Diani location found in Diani division, Msambweni district in Coast province of Kenya, as previously described in chapter four.

5.2.2. Methods

Data on traditional knowledge of malaria diagnosis and ethnophytotherapeutic practices in three Digo villages was collected through survey employing semi-structured interviews and guided open and closed ended questionnaires, Huntington (2000), as cited in chapter four.

5.2.3. Data analysis

The comparative relative importance of each plant species and the collected ethnobotanical data was analyzed according to the method of Friedman, Friedman *et al* (1986) and this was used to determine the rank-order priority (ROP) depending on the proposed effectiveness of each plant. To reach this goal, the Fidelity Level (FL) of each plant was calculated as follows: FL= (lp/lu) x 100, where lp is the number of respondents who cited a given species and lu is the total number of respondents. Questionnaire survey data was entered in Excel spreadsheets. It was checked and edited for errors, and coded as described in Sarantakos, Nguta *et al* (2010b). Thereafter, it was summarized using SPSS and reported in tables. Semi-structured interview data was studied and the responses grouped into classes expressing similar ideas.

5.3. Results

5.3.1. Digo ethnodiagnostic skills

Symptoms of disease, knowledge of known vectors for malaria, season effects of disease outbreak and the age groups affected are important tools of the Digo traditional disease diagnostic procedures. Fifty percent of the respondents confirmed that they detect the first symptoms of illness. Table 10 shows the symptoms the Digo community associate with malaria.

Table 10: The symptoms the Digo community associate with malaria as a percentage of respondents (n=20) in Diani location, Msambweni district, Kenya

Symptom	(%) of respondents citing the symptom
Fever	70
Coughing	65
Vomiting	65
Headache	60
High temperature	56
Piloerection	55
Diarrhea	50
Loss of appetite	45
Swollen glands	40

The Digo community does not base their disease diagnosis on the symptoms alone but also on known vectors of disease, season of disease outbreak and the various groups most commonly affected (Table 11).

Table 11: Digo knowledge of known vectors of malaria, seasonal effects and groups of people affected as a percentage of the respondents (n=20) in Diani location, Msambweni district, Kenya

Vector/carrier	**(%)**
Mosquitoes	100
Ticks	NR
Wildlife	NR
Dust/soil	NR
Air	NR
Unknown	NR
Biting flies	25
Season effect	
Dry season	15
Wet season	90
Both seasons	5
Groups affected	
Pregnant women	90
Children (Below five years)	95
Adults	5
People with other illnesses	60

5.3.2. Herbal therapy

Sixty (60) species distributed between fifty two (52) genera and thirty one (31) families were reportedly used in herbal preparations for the treatment of malaria (Table 12). The mode of preparation, voucher specimen number and the part of the medicinal plant used for preparation of antimalarial herbal remedy was documented (Table 12).

Table 12: Plant species commonly reported by Digo people for the treatment of malaria in Diani location (n=60), Msambweni district

Scientific name/Voucher specimen Number	Family	FL	Part used	Method of preparation	Route of administration	lp /growth characteristic
Acacia seyal Del. (JN01)	Mimosaceae	16	Roots	Decoction	Oral	10/Tree
Adansonia digitata Linn.(JN02)	Bombaceae	25	Leaves	Decoction	Oral	15/Tree
Agathisanthenum globosum (A.Rich) Hiern (JN03)	Rubiaceae	16	Roots	Decoction	Oral	10/Herb
Albizia anthelmintica Brongn(JN046)	Leguminosae	10	Stem bark; root bark	Decoction	Oral	6/Shrub
Aloe deserti Berger.(JN04)	Liliaceae	20	Leaves	Infusion	Oral	12/Herb
Aloe macrosiphon Bak.(JN05)	Liliaceae	20	Leaves	Infusion	Oral	12/Herb
Aloe secundiflora Engl.(JN06)	Liliaceae	10	Leaves	Infusion	Oral	6/Herb
Aloe vera (L) Webb.(JN07)	Liliaceae	23	Leaves	Infusion	Oral	14/Herb
Amaranthus hybridus L.(JN08)	Amaranthaceae	33	Leaves	Decoction	Oral	20/Herb

Table 12 (Continued)

Scientific name/Voucher specimen Number	Family	FL	Part used	Method of preparation	Route of administration	lp /growth characteristic
Azadirachta indica(L) Burm.(JN09)	Meliaceae	98	Roots, Stem bark, Leaves	Concoction	Oral; Inhalation; Topical	59/Tree
Bridelia micrantha Baill. (Hochst).(JN010)	Euphorbiaceae	67	Stem bark; Leaves	Concoction	Oral	40/Tree
Canthium glaucum Hiern. (JN011)	Rubiaceae	33	Fruits	Decoction	Oral	20/Tree
Carissa edulis Forrsk.(JN042)	Apocynaceae	16	Root bark	Decoction	Oral	10/Shrub
Cassia occidentalis L.(JN012)	Caesalpiniaceae	37	Roots; Leaves	Decoction	Oral	22/Shrub
Centella asiatica (L.)Urban (JN043)	Asclepiadaceae	07	Leaves	Decoction	Oral	4/Herb
Cissampelos mucronata A.Rich (JN047)	Menispermaceae	07	Root bark	Decoction	Oral	4/Liana
Clausena anisata (Willd) Hook.f ex. Benth. (JN013)	Rutaceae	42	Leaves	Decoction	Oral	25/Herb
Clerodendrum myricoides (Hochst.)Vatke (JN050)	Verbenaceae	10	Root bark	Decoction	Oral	8/shrub
Combretum molle G. Don (JN 059)	Combretaceae	67	Leaves	Decoction	Oral	40/Tree
Combretum padoides Engl and Diels.(JN014)	Combretaceae	50	Leaves	Decoction	Oral	30/Tree

Table 12 (Continued)

Scientific name/Voucher specimen Number	Family	FL	Part used	Method of preparation	Route of administration	lp /growth characteristic
Commiphora schimperi (Berg) Engl.(JN015)	Bursaraceae	40	Roots; Stem bark	Decoction	Oral	24/Tree
Dichrostachys cinerea (L) Wight and AM.(JN016)	Mimosaceae	33	Roots	Decoction	Oral	20/Tree
Fagaropsis angolensis (Engl) Del. (JN017)	Rutaceae	40	Leaves	Decoction	Oral	24/Tree
Ficus bussei Warp ex Mildbr and Burret.(JN018)	Moraceae	43	Roots	Decoction	Oral	26/Tree
Flacourtia indica (Burm.f) Merr. (JN019)	Flacourtiaceae	50	Roots	Decoction	Oral	30/Tree
Flueggea virosa (Willd) Voigt. (JN049)	Euphorbiaceae	10	Root bark	Decoction	Oral	6/Herb
Gerranthus lobatus (Cogn.) Jeffrey (JN020)	Cucurbitaceae	50	Roots	Decoction	Oral	30/Climber
Grewia hexaminta Burret. (JN021)	Tiliaceae	33	Leaves	Decoction	Oral	20/Shrub
Grewia trichocarpa Hochst ex A.Rich.(JN022)	Tiliaceae	33	Roots	Decoction	Oral	20/Shrub
Harrisonia abyssinica Oliv.(JN023)	Simaroubaceae	40	Root bark	Decoction	Oral	24/Shrub
Harungana madagascariensis Poir (JN 053)	Guttiferae	73	Root bark; Stem bark	Decoction	Oral	44/Tree
Heeria insignis Del.(JN024)	Anacardiaceae	33	Stem bark	Decoction	Oral	20/Shrub

Table 12 (Continued)

Scientific name/Voucher specimen Number	Family	FL	Part used	Method of preparation	Route of administration	lp /growth characteristic
Hoslundia opposita Vabl.(JN025)	Labiatae	43	Roots	Decoction	Oral	26/Shrub
Lantana camara L.(JN026)	Verbenaceae	50	Leaves	Decoction	Oral	30/shrub
Landolphia buchananii (Hall.f) Stapf. (JN027)	Apocynaceae	33	Leaves	Decoction	Oral	20/climber
Launea cornuta (Oliv and Hiern) C. Jeffrey(JN028)	Compositae	63	Leaves	Decoction	Oral	38/Herb
Momordica foetida Schumach. (JN060)	Cucurbitaceae	80	Leaves	Decoction		48/Climber
Ocimum bacilicum L.(JN029)	Labiatae	43	Leaves	Decoction	Oral	26/Shrub
Ocimum gratissimum L.(JN058)	Lamiaceae	55	Leaves	Decoction	Oral	33/Herb
Ocimum suave Willd(JN030)	Labiatae	33	Leaves	Decoction	Oral	20/Shrub
Pentanisia ouranogyne S.moore (JN031)	Rubiaceae	40	Roots	Decoction	Oral	24/Herb
Pentas bussei K.Krause (JN048)	Rubiaceae	16	Root bark	Decoction	Oral	10/Herb
Pentas longiflora Oliv. (JN 056)	Rubiaceae	70	Root bark	Decoction	Oral	42/Herb
Plectranthus barbatus Andr. (JN032)	Labiatae	33	Leaves	Decoction	Oral	20/shrub
Rauwolfia mombasiana Stapf(JN 051)	Apocynaceae	50	Root bark	Decoction	Oral	30/Shrub

Table 12 (Continued)

Scientific name/Voucher specimen Number	Family	FL	Part used	Method of preparation	Route of administration	lp /growth characteristic
Ricinus communis L(JN033)	Euphorbiaceae	50	Roots, Leaves	Concoction	Oral; Topical	30/Herb
Rottboelia exaltata L.F(JN034)	Gramineae	37	Leaves	Decoction	Oral	22/grass
Securidaca longepedunculata Fres. (JN035)	Papilionaceae	42	Roots	Decoction	Oral	25/Tree
Senecio syringitolius O. Hoffman.(JN036)	Compositae	33	Leaves	Decoction	Oral	20/Climber
Solanum incanum L.(JN037)	Solanaceae	47	Roots; Leaves	Decoction	Oral	28/Shrub
Suregeda zanzibarensis Baill(JN045)	Euphorbiaceae	13	Root bark	Decoction	Oral	8/Shrub
Tamarindus indica L.(JN038)	Caesalpiniaceae	33	Roots; Leaves	Decoction	Oral	20/Tree
Teclea simplicifolia (Eng) Verdoon (JN039)	Rutaceae	43	Roots	Decoction	Oral	26/Shrub
Terminalia spinosa Engl. (JN 052)	Combretaceae	66	Stem bark	Cold water infusion	Oral	40/Tree
Toddalia asiatica (L.) Lam. (JN 055)	Rutaceae	58	Root bark	Decoction	Oral	35/Shrub
Tridax procumbens L. (JN 054)	Compositae	47	Whole plant	Cold water infusion	Oral	28/Herb

Table 12 (Continued)

Scientific name/Voucher specimen Number	Family	FL	Part used	Method of preparation	Route of administration	Ip /growth characteristic
Uvaria scheffleri Diels (JN041)	Annonaceae	16	Leaves	Decoction	Oral	10/Liana
Vernonia amygdalina Delile (JN057)	Asteraceae	43	Leaves	Decoction	Oral	26/Shrub
Warbugia stuhlmannii Engl.(JN044)	Canellaceae	20	Stem bark	Decoction	Oral	12/Tree
Zanthoxylum chalybeum (Eng) Engl.(JN040)	Rutaceae	53	Root bark	Decoction	Oral	32/Tree

FL is the fidelity level

Ip is the number of respondents citing each species

Lu is the total number of respondents (60)

Decoction is a method of preparation in which the plant part is boiled in water

Concoction is a method of preparation in which more than one plant part is boiled in water

Infusion is a method of preparation that involves soaking of a plant part in water

Table 13: Plants used by the Digo community to treat malaria and the published evidence of their activities and / or other uses

Family	Species/Voucher specimen Number	Traditional treatment	Plant part used	Bioactive or potentially active compounds	Screened activity
Amaranthanceae	*Amaranthus hybridus* L.(JN08)	Malaria, Nguta *et al*(2010a,b)	Leaves	Not identified	Bioactivity, Cantrell (2003)
Anacardiaceae	*Heeria insignis* Del.(JN024)	Epilepsy, Moshi *et al* (2005)	Stem bark	Myrcene,β-pinene,α-pinene, Ayedoun *et al* (1998)	Not screened
Annonaceae	*Uvaria scheffleri* Diels (JN041)	Malaria, Beentje (1994); Kokwaro (1993)	Leaves	Indole alkaloid-(DL)-schefflone, Nkunya *et al* (2004).	Antiplasmodial activity Nkunya *et al* (1991).
Apocynaceae	*Carissa edulis* Forrsk.(JN042)	Malaria, Kirira *et al* (2006); Kokwaro(1993).	Root bark	Saponins, Reed (1986), Sesquiterpenes,Achenbach *et al* (1985)	Antiplasmodial activity, Clarkson *et al(* 2004); Koch *et al* (2005).
Apocynaceae	*Landolphia buchananii* (Hall.f) Stapf. (JN027)	Malaria, Nguta et al (2010a,b)	Leaves	Not identified	Not screened
Apocynaceae	*Rauwolfia mombasiana Stapf* (JN 051)	Malaria, Beentje (1994); Kokwaro (1993)	Root bark	Yohimbine-an indole alkaloid, Iwu and Court (1979)	Antiplasmodial activity, Weenen *et al* (1990)
Asclepiadaceae	*Centella asiatica* (L.)Urban (JN043)	Fever, Manadhar (1993).	Leaves	Alkaloids, Sesquiterpenes , Holeman *et al* (1994)	Antiplasmodial activity, Clarkson *et al* (2004).

Table 13 (Continued)

Family	Species/Voucher specimen Number	Traditional treatment	Plant part used	Bioactive or potentially active compounds	Screened activity
Asteraceae	*Vernonia amygdalina Delile* (JN057)	Malaria, Asase *et al* (2005).	Leaves	Not identified	Antiplasmodial activity, Tona *et al* (2004).
Bombaceae	*Adansonia digitata* Linn.(JN02)	Malaria ,Nguta *et al* (2010a); Fevers, Watt and Breyer-Brandwijk (1962; Abbiw (1990);	Leaves	Not identified	Antiplasmodial activity, Kristina (2002); bioactivity, Cantrell (2003)
Caesalpiniaceae	*Cassia occidentalis* L.(JN012)	oxytocin, cholagogue, anti-fever medicine, anti-worm medicine and remedy for swellings, Neuwinger(1994).	Roots; Leaves	Terpenes, steroids, coumarins, flavonoids, phenolic acids, lignans, xanthones, anthraquinones, Cimanga (2004)	Antiplasmodial activity, Cimanga (2004); Tona (1999).
Caesalpiniaceae	*Tamarindus indica* L.(JN038)	Malaria, Asase *et al* (2005); De La Pradilla (1988).	Roots; Leaves	luteoline, apigenine, orientine, isorientine, vitexine and pinitol, De La Pradilla (1988).	Not screened

Table 13 (Continued)

Family	Species/Voucher specimen Number	Traditional treatment	Plant part used	Bioactive or potentially active compounds	Screened activity
Canellaceae	*Warbugia stuhlmannii* Engl.(JN044)	Tooth ache and rheumatism, Beentje (1994)	Stem bark	Sesquiterpenes, Manguro *et al* (2003)	Antibacterial, *Bacillus subtilis*, Taniguchi *et al* (1978).
Combretaceae	*Combretum padoides* Engl and Diels.(JN014)	Hookworms, Neuwinger(2000)	Leaves	Mono and bi-desmosidic triterpenoids from leaves, Rodgers (1999)	antimicrobial effects, Eloff (1999)
Combretaceae	*Commiphora schimperi* (Berg) Engl.(JN015)	Malaria and constipation, Koch *et al* (2005)	Roots; Stem bark	Not identified	In vitro antimalarial and cytotoxic activity, Koch *et al* (2005)
Combretaceae	*Terminalia spinosa Engl.* (JN 052)	Jaundice, Beentje (1994)	Stem bark	Not identified	Antiplasmodial activity, Omulokoli *et al* (1997)
Combretaceae	*Combretum molle G.* Don (JN 059)	Malaria, Tabuti J.R.S (2008).	Leaves	Not identified	Not screened
Compositae	*Launea cornuta* (Oliv and Hiern) C. Jeffrey(JN028)	Typhoid, Kokwaro (1993)	Leaves	Tannins and astringents, Burkill(1985)	Not screened

Table 13 (Continued)

Family	Species/Voucher specimen Number	Traditional treatment	Plant part used	Bioactive or potentially active compounds	Screened activity
Compositae	*Senecio syringitolius* O. Hoffman.(JN036)	No previous reports	Leaves	Not identified	Not screened
Compositae	*Tridax procumbens L. (JN 054)*	Malaria and stomachache ,(Kokwaro(1993)	Whole plant	Cpd-bergenin, Clarkson *et al* (2004);	Antimalarial activity, Clarkson *et al* (2004); Weenen *et al* (1990).
Cucurbitaceae	*Gerranthus lobatus* (Cogn.) Jeffrey (JN020)	Malaria, Nguta *et al*(2010a)	Roots	Flavonoid, Imperato (2005)	Not screened
Cucurbitaceae	*Momordica foetida Schumach. (JN060)*	Malaria, Gessler *et al* (1995a)	Leaves	Not identified	Antimalarial activity,Waako *et al* (2005)
Euphorbiaceae	*Bridelia micrantha* Baill. (Hochst).(JN010)	No previous reports	Stem bark; Leaves	Not identified	Antiplasmodial activity, Edith *et al* (2005)
Euphorbiaceae	*Ricinus communis* L(JN033)	Antimalarial agent, Burkill (1935); Fever, Burkill (1994)	Roots; Leaves	Not identified	Antiplasmodial activity, Clarkson *et al* (2004)
Euphorbiaceae	*Suregeda zanzibarensis* Baill(JN045)	Malaria, Chhabra *et al* (1990a)	Root bark	Alkaloids, Smolenski *et al* (1975)	Antiplasmodial activity Omulokoli *et al* (1997)

Table 13 (Continued)

Family	Species/Voucher specimen Number	Traditional treatment	Plant part used	Bioactive or potentially active compounds	Screened activity
Euphorbiaceae	*Flueggea virosa* (Willd) Voigt. (JN049)	Chest pains, Beentje (1994)	Root bark	Cpd-bergenin, Nyasse *et al* (2004);alkaloids, Gan *et al* (2006)	Antiplasmodial activity, Clarkson *et al* (2004)
Flacourtiaceae	*Flacourtia indica* (Burm.f) Merr. (JN019)	Malaria cure, Burkill (1994)	Roots	Not identified	Antiplasmodial activity, Clarkson *et al* (2004)
Gramineae	*Rottboelia exaltata* L.F(JN034)	Epilepsy, Moshi *et al* (2005)	Leaves	Not identified	Not screened
Guttiferae	*Harungana madagascariensis Poir* (JN 053)	Malaria, Gessler (1994)	Root bark; Stem bark	Antraquinones, saponins, steroids, Tona *et al*(1998)	Antiplasmodial activity, Gessler *et al* (1994)
Labiatae	*Hoslundia opposita* Vabl.(JN025)	Malaria, Hedberg *et al* (1983a)	Roots	Not identified	Antimalarial activity, Gessler *et al* (1994)
Labiatae	*Ocimum bacilicum* L.(JN029)	Abdominal cramps, Fuchs (1543); Sfikas (1980)	Leaves	linalool, geranical, compounds, Dambolena (2007)	Antifungal activity, Dambolena (2007)
Labiatae	*Ocimum suave* Willd(JN030)	Candida infections, Runyoro *et al* (2006)	Leaves	Triterpenes, Tan (1997)	Anti-ulcerogenic Activity, Tan (1997)

Table 13 (Continued)

Family	Species/Voucher specimen Number	Traditional treatment	Plant part used	Bioactive or potentially active compounds	Screened activity
Labiatae	*Plectranthus barbatus* Andr. (JN032)	Mosquito repellant,Watt and Breyer-Brandwijk (1962)	Leaves	Not identified	Antiplasmodial activity, Meyer (2002)
Lamiaceae	*Ocimum gratissimum L.*(JN058)	Malaria, Tor-anyiin *et al* (2003)	Leaves	Not identified	Not screened
Leguminosae	*Albizia anthelmintica* Brongn(JN046)	Malaria, fever and as emetic, Johns *et al* (1994).	Stem bark	Triterpenes, El-Hamidi (1970)	Antiparasitic activity, Gathuma *et al* (2004).
Liliaceae	*Aloe deserti* Berger.(JN04)	A leaf decoction is used to treat the spleen,Kokwaro (1993)	Leaves	Anthrone C-glycosides, chromones and phenolic compounds, Reynolds (2008)	Not screened
Liliaceae	*Aloe macrosiphon* Bak.(JN05)	A leaf decoction is used to treat the spleen, Kokwaro (1993)	Leaves	Not identified	Not screened
Liliaceae	*Aloe secundiflora* Engl.(JN06)	Leaf decoction is used to treat the spleen, Kokwaro (1993)	Leaves	Not identified	Antimalarial activity, Oketch-rabah *et al* (1999)

Table 13 (Continued)

Family	Species/Voucher specimen Number	Traditional treatment	Plant part used	Bioactive or potentially active compounds	Screened activity
Liliaceae	*Aloe vera* (L) Webb.(JN07)	Malaria, De La Pradilla (1988)	Leaves	Not identified	Stimulation of gap junctional intercellular communication and proliferation of human skin fibroblasts in diabetes mellitus, Abdullah (2002)
Meliaceae	*Azadirachta indica*(L) Burm.(JN09)	Malaria, Gessler *et al* (1995a)	Roots, Stem bark, Leaves	Gedunin, Nimbinin, Bray *et al* (1990)	Antiplasmodial activity, El Tahir *et al* (1999); Kirira *et al* (2006), antimalarial activity, Sofowora (1993)
Menispermaceae	*Cissampelos mucronata* A.Rich (JN047)	Malaria ,Gessler *et al* (1994)	Root bark	bisbenzylisoquinone alkaloids, Tshibangu *et al* (2003)	Antiplasmodial activity, Gessler *et al* (1994)
Mimosaceae	*Acacia seyal* Del. (JN01)	Malaria,Nguta *et al* (2010b)	Roots	Not identified	Not screened
Mimosaceae	*Dichrostachys cinerea* (L) Wight and AM.(JN016)	Malaria, De La Pradilla (1988)	Roots	Not identified	Not screened

Table 13 (Continued)

Family	Species/Voucher specimen Number	Traditional treatment	Plant part used	Bioactive or potentially active compounds	Screened activity
Moraceae	*Ficus bussei* Warp ex Mildbr and Burret.(JN018)	Malaria, Kerharo and Bouquet (1950)	Roots	Steroidal sapogenins Wall (2006)	Not screened
Papilionaceae	*Securidaca longepedunculata* Fres. (JN035)	Malaria ,Williamson (1975)	Roots	Steroids, saponosides and monotropitos ide, De La Pradilla (1988)	Activity against *Candida albicans*,De sta (1993); Taniguchi *et al* (1978).
Rubiaceae	*Agathisanthenum globosum* (A.Rich) Hiern (JN03)	No previous reports	Roots	Not identified	Not screened
Rubiaceae	*Canthium glaucum* Hiern. (JN011)	Malaria,Nguta *et al* (2010a)	Fruits	Not identified	Not screened
Rubiaceae	*Pentanisia ouranogyne* S.moore (JN031)	No previous reports	Roots	Not identified	Not screened
Rubiaceae	*Pentas bussei* K.Krause (JN048)	Venereal diseases, Beentje (1994)	Root bark	Oxygen heterocycles, Taniguchi *et al* (1978).	Not screened
Rubiaceae	*Pentas longiflora Oliv.* (JN 056)	Malaria, Kokwaro(1993)	Root bark	Quinoid cpds El-Hady *et al* (2002).	Antiplasmodial activity, Wanyoike *et al* (2004)
Rutaceae	*Clausena anisata* (Willd) Hook.f ex. Benth. (JN013)	Malaria,Weenen *et al* (1990)	Leaves	Not identified	Antiplasmodial activity observed, Clarkson *et al* (2004)

Table 13 (Continued)

Family	Species/Voucher specimen Number	Traditional treatment	Plant part used	Bioactive or potentially active compounds	Screened activity
Rutaceae	*Fagaropsis angolensis* (Engl) Del. (JN017)	Malaria, Njoroge and Bussman (2006)	Leaves	Not identified	Antiplasmodial activity, Kirira *et al* (2006)
Rutaceae	*Teclea simplicifolia* (Eng) Verdoon (JN039)	Malaria, Nguta *et al* (2010)	Roots	Quinoline compounds, Wondimu (1998)	Not screened
Rutaceae	*Zanthoxylum chalybeum* (Eng) Engl.(JN040)	Malaria, Beentje (1994)	Root bark	Quinoline alkaloids, Kato *et al* (1996).	Antiplasmodial activity, Gessler *et al* (1994)
Rutaceae	*Toddalia asiatica (L.) Lam.* (JN 055)	Malaria, Chhabra (1991)	Root bark	Quinoline alkaloids, Ishii et al (1991)	Antiplasmodial activity (Kuria *et al* (2001)
Simaroubaceae	*Harrisonia abyssinica* Oliv.(JN023)	Fever ,Kokwaro(1993)	Root bark	Not identified	Antimalarial activity, El Tahir *et al* (1999)
Solanaceae	*Solanum incanum* L.(JN037)	Fever, Kokwaro(1993)	Roots; Leaves	Triterpenoids, Hirota *et al* (1990)	Antiulcerogenic effect, Farina *et al* (1998)
Tiliaceae	*Grewia hexaminta* Burret. (JN021)	Malaria, Nguta *et al* (2010a)	Leaves	Triterpenoids, Raghunathaiyar (1996)	Not screened
Tiliaceae	*Grewia trichocarpa* Hochst ex A.Rich.(JN022)	Malaria, Nguta *et al* (2010a)	Roots	Not identified	Not screened

Table 13 (Continued)

Family	Species/Voucher specimen Number	Traditional treatment	Plant part used	Bioactive or potentially active compounds	Screened activity
Verbenaceae	*Clerodendrum myricoides* (Hochst.)Vatke (JN050)	Malaria, Kokwaro (1993)	Root bark	Spermidine alkaloids, Bashwira and Hootele (1988)	Antimalarial activity, El Tahir *et al* (1999)
Verbenaceae	*Lantana camara L.*(JN026)	Malaria, Burkill (2000)	Leaves	Lantanine ,Burkill (2000)	Antiplasmo dial activity, Clarkson *et al* (2004).

5.4. Discussion.

The objective of the current study was to document the ethnodiagnostic skills utilized by the Digo community to diagnose malaria. Indeed, researchers need to document how people describe the signs (or symptoms) of illnesses, Heinrich *et al* (2009). The study community has developed abundant ethnodiagnostic skills for malaria which forms the basis of their traditional bioprospecting techniques. The obvious lack of agreement between the Digo diagnosis and modern medicine in some cases such as lack of fever is probably due to alck of traditional instruments to quantify raise in body temperature. The respondents interviewed in the current study had good knowledge about malaria and readily distinguished it from other illnesses on the basis of widely accepted malaria signs and symptoms, Tabuti (2008). The community recognized

that the clinical features of uncomplicated and severe malaria included fever, vomiting and loss of appetite. The Digo community does not rely on the disease symptoms alone but also on other factors such as known disease vectors, season of disease outbreak and the various community groups commonly affected by the disease. This observation is in agreement with the conventionally accepted close relationship between malaria and the rainy season, children below the age of five years and the immunocompromised people. The Digo knowledge on the most commonly affected members of the society does not greatly deviate from the published information.

The Digo ethnomedical practice is deeply rooted in their disease diagnostic skills which form the basis for the treatment of malaria. The indigenous knowledge on malaria diagnosis is orally passed on from one generation to the next and especially from the elders to the young. Despite a rather poor knowledge on differential diagnosis, the Digo ability to diagnose malaria compares favorably with that of a modern medical doctor. From the current study, it is evident that the Digo community utilizes various techniques for disease diagnostic purposes. The Digo community lacks the conventional knowledge equivalent to classify disease causative agents into protozoas, viruses, bacteria or fungi. This limitation did not stop them from developing relatively effective herbal remedies to cure malaria. This relatively good understanding of the causes and signs of the disease may help in the implementation of intervention measures aimed at reducing its incidence and prevalence since the Digo knowledge about the transmission and major symptoms of disease are congruent with science and they do not associate it with witchcraft, as do some communities elsewhere, Nuwaha (2002).

The Digo community posess the necessary indigenous knowledge on herbal therapy used to treat malaria. Due to the deep environmental knowledge of their ecosystem, the Digo community is a self-made ethnobotanist who can name virtuially every plant found on their land. The ethnodiagnostic skills have enabled the Digo community to couple malaria to herbal remedies leading to the development of a reliable traditional bioprospecting system. Traditional bioprospecting which requires no scientific analysis but the indigenous knowledge of a community is often a lead to new herbal preparation development by local communities.

Antimalarial plant species in the study area are the dominant commercial element as they are sought by a wider spectrum of the society. Most of the plants collected have been reported in the literature, as having been used for malaria or fever (Table 13), an indication that the community could be trusted for the information they imparted about the plants they use. The results of the current study show that a large number of medicinal plants are traditionally used for treatment of malaria among the Digo community. Sixty species in fifty two genera and thirty one families were documented. Rubiaceae, Rutaceae, Liliaceae, Labiatae, Euphorbiaceae and Combretaceae families represented the species most commonly cited. Studies from other regions of Africa indicate Rubiaceae to have many species used in the management of malaria in different countries, Iwu (1994). This was consistent with our results but Rutaceae had a similar frequency on the number of species cited as sources of antimalarial remedies as Rubiaceae (Table 13), which would indicate the importance of this family as a possible source of antimalarial plants. The information on frequently utilized antimalarial plant species is also an important lead to the species that can be targeted for antiplasmodial tests, toxicological tests and phytochemical analysis. Since there is no safer, effective and cheaper antimalarial remedy than chloroquine in

the treatment of malaria, development of new antimalarial drugs from plant sources may be the way forward in dealing with global drug resistant problems of malaria, Gessler (1995a). Natural products and their derivatives represent over 50% of all the drugs in clinical use in the world, Van Wyk *et al* (2002). The common method of extracting medicine is boiling. Apparently the active principles of most of these herbal plants though not identified are thermostable.

5.5. Conclusions

There is a very high probability of discovering new medicines from bioprospecting activities because the Digo ethnomedical practice is well developed and compares favorably with modern medical practice. The Digo ethnomedicine depends on an elaborate indigenous knowledge of malaria diagnostic procedure and medicinal plants used to treat the disease which is endemic in South Coast, Kenya. It is concluded that, the Digo ethnodiagnostic skill is the basis of their traditional bioprospecting techniques.

CHAPTER SIX

EVALUATION OF ACUTE TOXICITY OF CRUDE PLANT EXTRACTS IN BRINE SHRIMP (*ARTEMIA SALINA* LEACH) ASSAY

6.1. Introduction

Since ancient times people have used plants as medicines, Parra *et al* (2001). This use has great importance, because plants can provide drugs to widen the therapeutic arsenal, Jaramillo (1989). However, during the past decade, traditional systems of medicine have become increasingly important in view of their safety, Krishnaraju *et al* (2006) and for this reason, research is carried out in order to determine the pharmacological action and toxicity of medicinal plants. Current estimates suggest that, in many developing countries, a large proportion of the population relies heavily on traditional practitioners and medicinal plants to meet primary health care needs. Indeed indigenous plants play an important role in the treatment of many diseases, Phillipson and Wright (1991) and 80% of the people worldwide are estimated to use herbal remedies, Nguta *et al* (2010); Geoffrey and Kirby (1996); Phillipson (1994). However, few data are available on their efficacy and safety, despite the fact that validation of traditional practices could lead to innovative strategies in malaria control.

Natural products represent a virtually inexhaustible reservoir of molecules, most of which are hardly explored and could constitute lead molecules for new antimalarial drugs, such as artemisinin, isolated from *Artemisia annua,* Kayser *et al* (2003). Although modern medicine may be available in developing countries, phytomedicines have often maintained popularity for

historical and cultural reasons. Concurrently, many people in developed countries have begun to turn to alternative or complementary therapies, including medicinal herbs, Farnsworth and Soejarto (1996). Kenya possesses rich floristic wealth and diversified genetic resources of medicinal plants. It has a widely ranging tropical and the agro climatic conditions, which are conducive for introducing and domesticating new and exotic plant varieties. The country boasts rich cultural traditions of plant use. Scientific understanding of medicinal plants is however, largely unexplored and toxicological investigation of the Kenyan flora only gained momentum recently. The use of the plants, plant extracts and pure compounds isolated from natural sources provided the foundation to modern pharmaceutical compounds.

The preclinical toxicological evaluation, carried out routinely in mice, is of great importance for validation of the traditional use of medicinal plants. There is currently a tendency to call for substituting the use of laboratory animals in toxicological tests, due to the high cost and the animals' suffering caused by these experiments. Alternative methods include procedures that could replace experiments carried out with animals; reduce the number of animals used in every test or refine the existing methodology in order to reduce pain and stress, according to the "3 R principle", Johnston and Rusche (1997); Yajes (1997). *Artemia salina* L. (Artemiidae), the brine shrimp, is an invertebrate component of the fauna of saline aquatic and marine ecosystems. It plays an important role in the energy flow of the food chain, Sanchez Fortun *et al* (1995) and can be used in a laboratory bioassay in order to determine toxicity through the estimation of the medium lethal concentration (LC_{50} values), Lewan *et al* (1992), which have been reported for a series of toxins and plant extracts, Meyer *et al* (1982). This method, which determines the LC_{50} value of the active compounds and extracts in saline medium in µg/ml, Massele *et al* (1995), has

been used in research on medicinal plants carried out in different countries in order to evaluate toxicity, gastro-protective action, and other biological actions, which in some cases have been related to pharmacological studies carried out for different chemical compounds, Mathews (1995); Fumaral and Garchitorena (1996), as a screening method mainly for products of plant origin.

The brine shrimp lethality assay consists of exposing larvae to test sample in saline solution and lethality is evaluated after 24 h. The commercial availability of inexpensive brine shrimp eggs, the low cost and ease of performing the assay make brine shrimp lethality assay, a very useful bench-top method, McLaughlin *et al* (1991). A number of studies have demonstrated the use of the brine shrimp assay to screen plant extracts, Sleet and Brendel (1983); Harwing and Scott (1971); Pelka *et al* (2000). Lethality assay has been used successfully to biomonitor the isolation of cytotoxic, Siqueira *et al* (1998), antimalarial, Perez *et al* (1997), insecticidal, Oberlies *et al* (1998), and antifeedant, Labbe *et al* (1993) compounds from plant extracts. The brine shrimp lethality bioassay is an efficient, rapid and inexpensive test that requires only a relatively small amount of sample (2–20 mg).

The assay is based on the premise that bioactive compounds are often toxic in high doses and that *in vivo* lethality in a simple organism can be used as a convenient monitor for screening and fractionation in the discovery of new cytotoxic natural products, McLaughlin *et al* (1991). Literature data suggest a good correlation between the activity in the brine shrimp assay and the cytotoxicity against some human solid tumor cell lines, Anderson *et al* (1991), as well as hepatotoxic activity, Kiviranta *et al* (1991). Brine shrimp bioassay has led to the discovery of the annonaceous acetogenins as a new class of natural pesticides and active antitumor agents,

McLaughlin *et al* (1998). In the current study, results of a screening of water and organic ($CHCL_3$/MeOH, 1:1) extracts of some important antimalarial plants used by the Msambweni community of Kenyan coast for lethality towards *Artemia salina* larvae are presented. The current study also seeks to use brine shrimp (*Artemia salina*) bioassay to compare the cytotoxicity of crude plant extracts and that of positive controls, cyclophospamide (Alkylating agent) and etoposide (Epipodophyllotoxin).

6.2. Materials and methods

6.2.1. Plant materials

The plant samples used in the current study were collected in August 2009 from Msambweni district of Kenya based on ethnopharmacological use through interviews with local communities and traditional health practitioners. The information gathered included part of the plant used and the method of preparation of the herbal antimalarial remedies. The plants were identified by taxonomists at the University of Nairobi and the National Museums of Kenya herbaria, Nairobi, where voucher specimens were deposited. The plant parts were chopped into small pieces; air dried at room temperature (25^0C) under shade and pulverized using a laboratory mill (Christy & Norris Ltd., England).

6.2.2. Cytotoxic drugs

Cyclophospamide, Mfg. Lic. No.: DD/140 and batch number KB 791001, was purchased from Biochem Pharmaceutical Industries Limited (Mumbai, India). Etoposide (Etosid), batch number

J8 05 26, a semi synthetic derivative of podophyllotoxin, was purchased from CIPLA Limited, plot No.S-103 Verna.

6.2.3. Preparation of crude plant extracts

Considering that people in Msambweni usually use hot water to prepare their herbal remedies as decoctions and sometimes concoctions, aqueous hot infusions of each plant part was prepared (50 grams of powdered material in 500 ml of distilled water) in a water bath at 60^0C for 1 hour. The extracts that were obtained were filtered through muslin gauze and the filtrate kept in deep freezer for 24 hours, which was then lyophilized. The lyophilized dry powder was collected in stoppered sample vials, weighed and kept at -20^0C until used. Organic extracts (Chloroform (CHCL3): Methanol (MeOH)) (1:1) (50 grams of powdered material in 500 ml of solvent) were prepared by maceration of the dried and powdered plant material with the organic solvent for 48 hours. The extract was then filtered through Whatman filter paper No.1. The filtrate was concentrated to dryness in vacuo by rotary evaporation and weighed. The dry solid extracts were stored at -20^0C in airtight containers until used.

6.2.4. Product identification and description (*Artemia salina*)

Artemia cysts, batch number DE RP 33801, were purchased from JBL GmbH & Co.KG (Neuhofen, Germany) and the product was labeled as JBL Artemio Pur Brand. The *Artemia* cysts had been harvested from Great Salt Lake, Utah, USA and were identified *as Artemia salina,* based on zoogeography, Van Stappen (2002). *Artemia saina* is endemic to North and Central America, Bowen *et al* (1985). It has been labeled as a super species (a set of ecologically isolated

and physiologically distinct semi species and species), Bowen *et al* (1985); this is important as it is indicative of intraspecies variation. This species is of great economic importance, as its commercial harvest from Great Salt Lake (Utah, USA) is estimated to represent 90% of the global trade in brine shrimp cysts, Treece (2000). This is a substantial volume of cysts when one considers that annually over 2000 metric tons of dry *Artemia* cysts are marketed worldwide, Van Stappen (1996). *A. salina* is the best studied of the *Artemia* species, Gajardo *et al* (2002), estimated to represent over 90% of studies in which *Artemia* is used as an experimental test organism (very often using material sourced from Great Salt Lake, Utah, USA), Sorgeloos and Beardmore (1995).

6.2.5. Culture and harvesting of *Artemia salina.*

Artemia salina eggs were stored at -20^{0}C before use. *A. salina* eggs were incubated for hatching in a shallow rectangular dish (14 cm x 9 cm x 5 cm) filled with 225 mls of a 3.3% w/v solution of artificial sea water. A plastic divider with several 2 mm holes was clamped in the dish to make two unequal compartments. The cysts (1.11 grams) and yeast (0.0827 grams) were sprinkled into the larger compartment which was darkened. The smaller compartment was illuminated by a tungsten filament light and gently sparged with air. After 24 hours, hatched *A. salina* larvae were transferred to fresh artificial seawater and incubated for a further 24h under artificial light with air sparging, Campbell *et al* (1994). The phototropic nauplii were collected by pipette from the lighted side, having been separated by the divider from the shells.

6.2.6. Preparation of test extracts

Stock solutions of aqueous extracts (10,000 µg/ml) were made in distilled deionized water and filter sterilized using 0.22 µm membrane filters in a laminar flow hood. The organic extracts were dissolved in dimethyl sulphoxide, $CH_3.SO.CH_3$ M.W 78.13 (DMSO); batch number PJ/25/3496/709-05/6/16, (THOMAS BAKER CHEMICALS, PVT. LIMITED, MUMBAI, INDIA) followed by subsequent dilution to lower concentration of DMSO, to <1% to avoid carry over (solvent) effect, Dorin *et al* (2001). Test extracts at appropriate amounts (5 µl, 50 µl, and 500 µl for 10 µg/ml, 100 µg/ml, and 1000 µg/ml, respectively) were transferred into 10 ml vials (5 vials for each dose and 1 for control). Five replicates were prepared for each dose level.

6.2.7. Preparation of cytotoxic drugs

Stock solutions of the positive controls, cyclophospamide and etoposide (10,000 µg/ml) were prepared in distilled deionized water and filter sterilized using 0.22 µm membrane filters in a laminar flow hood. Test solutions at appropriate amounts (5 µl, 50 µl, and 500 µl for 10 µg/ml, 100 µg/ml, and 1000 µg/ml, respectively) were transferred into 10 ml vials (5 vials for each dose and 1 for control). Five replicates were prepared for each dose level.

6.2.8. Bioassay of Artemia salina

For toxicity tests, ten *A. salina* nauplii were transferred into each sample vial using 230 mm disposable glass Pasteur pipettes (Ref. D812) (Poulten & Graf Ltd, Barking, UK) and filtered brine solution was added to make 5 ml. The nauplii were counted macroscopically in the stem of the pipette against a lighted background. A drop of dry yeast suspension (Red star) (3 mg in 5 ml

artificial sea water) was added as food to each vial. All the vials were maintained under illumination. The surviving nauplii were counted with the aid of a 3x magnifying glass, after 24 hours, and the percentage of deaths at the three dose levels and control were determined. In cases where control deaths occurred, the data was corrected using Abbott's formula, Abbott W.S (1925) as follows: % deaths = [(Test-control)/control x 100. The surviving nauplii were killed by the addition of 100µl of 5% (v/v) phenol to each vial.

6.2.9. LC50 determinations

The lethal concentration fifty (LC_{50}), 95% confidence interval and slope were determined from the 24 hour counts using the probit analysis method described by Finney, Finney D.J (1971). In cases where data was insufficient for this technique, the dose response data was transformed into a straight line by means of a logit transformation, Hafner *et al* (1977), and the LC_{50} value was derived from the best fit line obtained by linear regression analysis. The cytotoxic activity was considered weak when the LC_{50} was between 500 and 1000 µg/ml, moderate when the LC_{50} was between 100 and 500 µg/ml, as strong when the LC_{50} ranged from 0 to 100 µg/ml, Padmaja *et al* (2002) and designated as non toxic when the LC_{50} was greater than 1000 µg/ml, Meyer *et al* (1982). LC_{50} is indicative of the bioactivity level of a given plant extract or a cytotoxic drug.

6.3. Results

Brine shrimp lethality is a simple bioassay useful for screening toxicity of a large number of extracts in the drug discovery process. Crude plant extracts and positive controls, (cyclophospamide and etoposide) were evaluated by *Artemia salina* bioassay for their

toxicological activity. One hundred and seventy (170) crude extracts belonging to sixty (60) species in fifty two (52) genera and thirty two (32) families were evaluated in the current study (Table 14). The yields of the water extracts ranged between 1.06 and 21.24 % w/w, while those of organic extracts were between 0.76 and 22.4% w/w (Table 14).

Table 14: Plant extracts used in the study (quantity obtained from dried plant material, % dry weight, w/w).

Family	Plant species/ Voucher specimen number	Plant part	Solvent	%Yield (w/w)
Amaranthanceae	*Amaranthus hybridus* L.(JN08)	Leaves	$CHCL_3$/MeOH	4.68
			Water	5.8
Anacardiaceae	*Heeria insignis* Del.(JN024)	Stem	$CHCL_3$/MeOH	4.78
			Water	10.4
Annonaceae	*Uvaria scheffleri* Diels (JN041)	Stem	$CHCL_3$/MeOH	6.6
			Water	8.2
	Uvaria scheffleri Diels (JN041)	Roots	$CHCL_3$/MeOH	3.2
			Water	4.6
Apocynaceae	*Carissa edulis* Forrsk.(JN042)	Root bark	$CHCL_3$/MeOH	9.6
			Water	10.2
	Landolphia buchananii (Hall.f) Stapf. (JN027)	Leaves	$CHCL_3$/MeOH	5.4
			Water	7.8
Apocynaceae	*Rauwolfia mombasiana Stapf* (JN 051)	Root bark	$CHCL_3$/MeOH	8.8
			Water	11.4
Asclepiadaceae	*Centella asiatica* (L.)Urban (JN043)	Leaves	$CHCL_3$/MeOH	4.5
			Water	6.2
Asteraceae	*Vernonia amygdalina Delile* (JN057)	Leaves	$CHCL_3$/MeOH	5.6
			Water	6.8
Bombaceae	*Adansonia digitata* Linn.(JN02)	Leaves	$CHCL_3$/MeOH	6.96
			Water	4.84

Table 14 (Cont.)

Family	Plant species/ Voucher specimen number	Plant part	Solvent	%Yield (w/w)
Bombaceae	*Adansonia digitata* Linn.(JN02)	Stem bark	$CHCL_3$/MeOH	6.4
			Water	7.6
Bursaraceae	*Commiphora schimperi* (Berg) Engl. (JN015)	Stem bark	$CHCL_3$/MeOH	4.4
			Water	6.5
	Commiphora schimperi (Berg) Engl.(JN015)	Roots	$CHCL_3$/MeOH	8.0
			Water	12.2
Caesalpiniaceae	*Cassia occidentalis* L.(JN012)	Leaves	$CHCL_3$/MeOH	9.3
			Water	14.1
	Cassia occidentalis L.(JN012)	Roots	$CHCL_3$/MeOH	13.98
			Water	1.64
	Tamarindus indica L.(JN038)	Stem bark	$CHCL_3$/MeOH	3.32
			Water	3.48
Canellaceae	*Warbugia stuhlmannii* Engl.(JN044)	Stem bark	$CHCL_3$/MeOH	6.6
			Water	7.8
Combretaceae	*Combretum padoides* Engl and Diels.(JN014)	Leaves	$CHCL_3$/MeOH	3.4
			Water	10
	Terminalia spinosa Engl. (JN 052)	Stem bark	$CHCL_3$/MeOH	3.6
			Water	4.8
	Combretum molle G. Don (JN 059)	Leaves	$CHCL_3$/MeOH	10
			Water	12.4
Compositae	*Launea cornuta* (Oliv and Hiern) C. Jeffrey(JN028)	Leaves	$CHCL_3$/MeOH	5.6
			Water	8.12

Table 14 (Cont.)

Family	Plant species/ Voucher specimen number	Plant part	Solvent	%Yield (w/w)
Compositae	*Launea cornuta* (Oliv and Hiern) C. Jeffrey(JN028)	Roots	$CHCL_3$/MeOH	6.72
			Water	4.84
	Senecio syringitolius O. Hoffman.(JN036)	Leaves	$CHCL_3$/MeOH	2.08
			Water	2.66
	Tridax procumbens L. (JN 054)	Whole plant	$CHCL_3$/MeOH	5.4
			Water	6.6
Cucurbitaceae	*Gerranthus lobatus* (Cogn.) Jeffrey (JN020)	Stem bark	$CHCL_3$/MeOH	4.5
			Water	3.75
	Gerranthus lobatus (Cogn.) Jeffrey (JN020)	Roots	$CHCL_3$/MeOH	6.4
			Water	2.56
	Momordica foetida Schumach. (JN060)	Leaves	$CHCL_3$/MeOH	3.6
			Water	4.8
Euphorbiaceae	*Bridelia micrantha* Baill. (Hochst).(JN010)	Leaves	$CHCL_3$/MeOH	4.7
			Water	4.44
Euphorbiaceae	*Ricinus communis* L(JN033)	Leaves	$CHCL_3$/MeOH	6.1
			Water	16.66
	Ricinus communis L(JN033)	Roots	$CHCL_3$/MeOH	1.3
			Water	2.4
	Suregeda zanzibarensis Baill(JN045)	Root bark	$CHCL_3$/MeOH	13.4
			Water	16.2
	Flueggea virosa (Willd) Voigt. (JN049)	Root bark	$CHCL_3$/MeOH	3.8
			Water	5.6

Table 14 (Cont.)

Family	Plant species/ Voucher specimen number	Plant part	Solvent	%Yield (w/w)
Flacourtiaceae	*Flacourtia indica* (Burm.f) Merr. (JN019)	Leaves	$CHCL_3$/MeOH	13.16
			Water	17.02
	Flacourtia indica (Burm.f) Merr. (JN019)	Stem bark	$CHCL_3$/MeOH	8
			Water	1.54
Gramineae	*Rottboelia exaltata* L.F(JN034)	Leaves	$CHCL_3$/MeOH	4.2
			Water	8.01
Guttiferae	*Harungana madagascariensis Poir* (JN 053)	Stem bark	$CHCL_3$/MeOH	6.8
			Water	8.9
Labiatae	*Hoslundia opposita* Vabl.(JN025)	Roots	$CHCL_3$/MeOH	2.12
			Water	1.06
	Ocimum bacilicum L.(JN029)	Leaves	$CHCL_3$/MeOH	10.82
			Water	3.58
	Ocimum bacilicum L.(JN029)	Roots	$CHCL_3$/MeOH	0.76
			Water	4.80
Labiatae	*Ocimum suave* Willd(JN030)	Leaves	$CHCL_3$/MeOH	4.36
			Water	7.58
	Ocimum suave Willd(JN030)	Stem bark	$CHCL_3$/MeOH	3.28
			Water	3.75
	Plectranthus barbatus Andr. (JN032)	Leaves	$CHCL_3$/MeOH	7.46
			Water	16.6
	Plectranthus barbatus Andr. (JN032)	Stem bark	$CHCL_3$/MeOH	8
			Water	10

Table 14 (Cont.)

Family	Plant species/ Voucher specimen number	Plant part	Solvent	%Yield (w/w)
Labiatae	*Plectranthus barbatus* Andr. (JN032)	Roots	$CHCL_3$/MeOH	6.4
			Water	8.8
Lamiaceae	*Ocimum gratissimum L.*(JN058)	Leaves	$CHCL_3$/MeOH	5.6
			Water	6.8
Leguminosae	*Albizia anthelmintica* Brongn(JN046)	Stem bark	$CHCL_3$/MeOH	8.8
			Water	9.16
Liliaceae	*Aloe deserti* Berger.(JN04)	Leaves	$CHCL_3$/MeOH	3.68
			Water	3.8
	Aloe macrosiphon Bak.(JN05)	Leaves	$CHCL_3$/MeOH	5.72
			Water	4.06
	Aloe secundiflora Engl.(JN06)	Leaves	$CHCL_3$/MeOH	5.42
			Water	4.44
	Aloe vera (L) Webb.(JN07)	Leaves	$CHCL_3$/MeOH	4.26
			Water	6.0
Meliaceae	*Azadirachta indica*(L) Burm.(JN09)	Leaves	$CHCL_3$/MeOH	6.84
			Water	15.16
	Azadirachta indica(L) Burm.(JN09)	Stem bark	$CHCL_3$/MeOH	6.4
			Water	9.86
	Azadirachta indica(L) Burm.(JN09)	Stem	$CHCL_3$/MeOH	2.18
			Water	2.62
	Azadirachta indica(L) Burm.(JN09)	Root bark	$CHCL_3$/MeOH	5.64
			Water	4.98

Table 14 (Cont.)

Family	Plant species/ Voucher specimen number	Plant part	Solvent	%Yield (w/w)
Meliaceae	*Azadirachta indica*(L) Burm.(JN09)	Roots	$CHCL_3$/MeOH	4.82
			Water	2.4
Menispermaceae	*Cissampelos mucronata* A.Rich (JN047)	Root bark	$CHCL_3$/MeOH	3.6
			Water	4.8
Mimosaceae	*Acacia seyal* Del. (JN01)	Roots	$CHCL_3$/MeOH	2.96
			Water	2.94
	Dichrostachys cinerea (L) Wight and AM.(JN016)	Roots	$CHCL_3$/MeOH	3.44
			Water	1.65
	Dichrostachys cinerea (L) Wight and AM.(JN016)	Stem bark	$CHCL_3$/MeOH	4.04
			Water	1.74
Moraceae	*Ficus bussei* Warp ex Mildbr and Burret.(JN018)	Leaves	$CHCL_3$/MeOH	3.78
			Water	5.64
	Ficus bussei Warp ex Mildbr and Burret.(JN018)	Stem bark	$CHCL_3$/MeOH	2.4
			Water	7.8
Moraceae	*Ficus bussei* Warp ex Mildbr and Burret.(JN018)	Roots	$CHCL_3$/MeOH	1.72
			Water	7.64
Papilionaceae	*Securidaca longepedunculata* Fres. (JN035)	Leaves	$CHCL_3$/MeOH	22.4
			Water	3.95
	Securidaca longepedunculata Fres. (JN035)	Roots	$CHCL_3$/MeOH	22
			Water	21.24
Rubiaceae	*Agathisanthenum globosum* (A.Rich) Hiern (JN03)	Roots	$CHCL_3$/MeOH	3.64
			Water	2.11

Table 14 (Cont.)

Family	Plant species/ Voucher specimen number	Plant part	Solvent	%Yield (w/w)
Rubiaceae	*Canthium glaucum* Hiern. (JN011)	Leaves	$CHCL_3$/MeOH	5.96
			Water	5.23
	Canthium glaucum Hiern. (JN011)	Stem bark	$CHCL_3$/MeOH	7.0
			Water	8.4
	Canthium glaucum Hiern. (JN011)	Roots	$CHCL_3$/MeOH	6.8
			Water	5.88
	Pentanisia ouranogyne S.moore (JN031)	Roots	$CHCL_3$/MeOH	12.24
			Water	4.56
	Pentas bussei K.Krause (JN048)	Root bark	$CHCL_3$/MeOH	8.8
			Water	9.6
	Pentas longiflora Oliv. (JN 056)	Root bark	$CHCL_3$/MeOH	6.2
			Water	9.6
Rutaceae	*Clausena anisata* (Willd) Hook.f ex. Benth. (JN013)	Roots	$CHCL_3$/MeOH	4.32
			Water	5.20
Rutaceae	*Fagaropsis angolensis* (Engl) Del. (JN017)	Leaves	$CHCL_3$/MeOH	5.5
			Water	6.2
	Teclea simplicifolia (Eng) Verdoon (JN039)	Leaves	$CHCL_3$/MeOH	10.96
			Water	6.06
	Teclea simplicifolia (Eng) Verdoon (JN039)	Roots	$CHCL_3$/MeOH	8.08
			Water	4.62
	Zanthoxylum chalybeum (Eng) Engl.(JN040)	Leaves	$CHCL_3$/MeOH	6.48
			Water	16.02

Table 14 (Cont.)

Family	Plant species/ Voucher specimen number	Plant part	Solvent	%Yield (w/w)
Rutaceae	*Zanthoxylum chalybeum* (Eng) Engl.(JN040)	Stem bark	$CHCL_3$/MeOH	13.6
			Water	3.14
	Zanthoxylum chalybeum (Eng) Engl.(JN040)	Root bark	$CHCL_3$/MeOH	12.64
			Water	6.38
	Toddalia asiatica (L.) Lam. (JN 055)	Root bark	$CHCL_3$/MeOH	9.2
			Water	3.4
Simaroubaceae	*Harrisonia abyssinica* Oliv.(JN023)	Root bark	$CHCL_3$/MeOH	3.6
			Water	6.2
Solanaceae	*Solanum incanum* L.(JN037)	Leaves	$CHCL_3$/MeOH	5.26
			Water	10.86
	Solanum incanum L.(JN037)	Roots	$CHCL_3$/MeOH	1.96
			Water	2.32
Tiliaceae	*Grewia hexaminta* Burret. (JN021)	Leaves	$CHCL_3$/MeOH	4.78
			Water	6.06
Tiliaceae	*Grewia trichocarpa* Hochst ex A.Rich.(JN022)	Roots	$CHCL_3$/MeOH	3.44
			Water	3.14
Verbenaceae	*Clerodendrum myricoides* (Hochst.)Vatke (JN050)	Root bark	$CHCL_3$/MeOH	4.6
			Water	3.2
	Lantana camara L.(JN026)	Leaves	$CHCL_3$/MeOH	9.28
			Water	19.72

Mortality (percentage) for eighty five (85) organic ($CHCL_3$/MeOH, 1:1) crude extracts and cytotoxic drugs, after testing the different extracts and cytotoxic agents in brine shrimp (*A.salina Leach*) assay, is shown in Table 15. Increase in mortality was observed to be proportional to increase in concentration, which provided linearity in the dose-effect relationship of every extract and determination of the LC_{50} value.

Table 15: Toxicity of organic ($CHCL_3$/MeOH, 1:1) crude plant extracts and cytotoxic drugs against brine shrimp *Artemia salina*

		Percent deaths at 24 hours					
Plant species	**Plant part**	**10 µg/ml**	**100 µg/ml**	**1000 µg/ml**	**LC_{50} value (µg/ml) (Organic)[a]**	**Limits 95 % Confidence (µg/ml)**	**Slope**
Acacia seyal Del.	Roots	**54**	**96**	**100**	**8**	**0-24**	**0.8774**
Adansonia digitata Linn.	Leaves	**12**	**42**	**50**	**++**	**ND**	**1.1773**
Adansonia digitata Linn.	Stem bark	**12**	**92**	**100**	**30**	**12-75**	**0.3791**
Agathisanthenum globosum (A.Rich) Hiern	Roots	**10**	**44**	**100**	**88**	**29-240**	**0.3332**
Albizia anthelmintica Brongn	Stem bark	**14**	**36**	**94**	**110**	**33-358**	**0.3385**
Aloe deserti Berger.	Leaves	**20**	**46**	**98**	**68**	**18-209**	**0.3516**
Aloe macrosiphon Bak.	Leaves	**6**	**22**	**52**	**++**	**ND**	**0.8101**
Aloe secundiflora Engl.	Leaves	**14**	**34**	**74**	**217**	**48-3373**	**0.5540**
Aloe vera (L) Webb.	Leaves	**12**	**32**	**90**	**141**	**42-527**	**0.3686**
Amaranthus hybridus L.	Leaves	**16**	**30**	**78**	**200**	**45-2293**	**0.5257**

Table 15 (Continued)

		Percent deaths at 24 hours					
Plant species	**Plant part**	**10 µg/ml**	**100 µg/ml**	**1000 µg/ml**	**LC_{50} value (µg/ml) (Organic)[a]**	**Limits 95 % Confidence (µg/ml)**	**Slope**
Azadirachta indica (L) Burm.	Leaves	**8**	**68**	**98**	**58**	**20-161**	**0.3742**
	Stem bark	**40**	**76**	**94**	**18**	**0.1-72**	**0.6438**
	Stem	**14**	**32**	**100**	**103**	**33-307**	**0.3300**
	Root bark	**42**	**94**	**100**	**13**	**0-36**	**0.6578**
	Roots	**54**	**86**	**100**	**8.3**	**0-30**	**0.7793**
Bridelia micrantha Baill. (Hochst).	Leaves	**10**	**28**	**88**	**171**	**52-671**	**0.3807**
Canthium glaucum Hiern.	Leaves	**22**	**34**	**92**	**101**	**23-420**	**0.4038**
Canthium glaucum Hiern.	Stem bark	**28**	**36**	**92**	**81**	**12-390**	**0.4802**
Canthium glaucum Hiern.	Roots	**20**	**40**	**80**	**131**	**21-1233**	**0.5557**
Carissa edulis Forrsk.	Root bark	**14**	**36**	**94**	**110**	**33-358**	**0.3385**

Table 15 (Continued)

Plant species	Plant part	Percent deaths at 24 hours			LC_{50} value (µg/ml) (Organic)[a]	Limits 95 % Confidence (µg/ml)	Slope
		10 µg/ml	100 µg/ml	1000 µg/ml			
Cassia occidentalis L.	Leaves	20	40	80	131	22-1234	0.5557
	Roots	8	32	74	253	69-2143	0.4756
Centella asiatica (L.)Urban	Leaves	12	38	90	123	36-452	0.3695
Cissampelos mucronata A.Rich	Root bark	6	54	86	114	35-394	0.3492
Clausena anisata (Willd) Hook.f ex. Benth.	Roots	20	36	92	101	25-397	0.3891
Clerodendrum myricoides (Hochst.)Vatke	Root bark	20	92	100	25	8-65	0.4270
Combretum molle G. Don	Leaves	10	44	100	88	29-240	0.3332
Combretum padoides Engl and Diels.	Leaves	18	56	100	56	16-156	0.3355
Commiphora schimperi (Berg) Engl.	Stem bark	20	40	80	131	22-1234	0.5557
	Roots	8	84	94	47	15-133	0.3700

Table 15 (Continued)

Plant species	Plant part	Percent deaths at 24 hours: 10 µg/ml	100 µg/ml	1000 µg/ml	LC_{50} value (µg/ml) (Organic)[a]	Limits 95 % Confidence (µg/ml)	Slope
Dichrostachys cinerea (L) Wight and AM.	Stem bark	20	62	100	47	13-129	0.3360
Dichrostachys cinerea (L) Wight and AM.	Roots	30	36	94	72	7-327	0.4872
Fagaropsis angolensis (Engl) Del.	Leaves	14	40	94	101	30-326	0.3423
Ficus bussei Warp ex Mildbr and Burret.	Leaves	14	50	98	73	23-203	0.3222
Ficus bussei Warp ex Mildbr and Burret.	Stem bark	12	42	80	151	37-879	0.4481
Ficus bussei Warp ex Mildbr and Burret.	Roots	50	82	100	11	0.02-38	0.6655
Flacourtia indica (Burm.f) Merr.	Leaves	20	26	38	+	ND	7.9506
Flacourtia indica (Burm.f) Merr.	Stem bark	16	32	46	+	ND	1.8468

Table 15 (Continued)

Plant species	Plant part	Percent deaths at 24 hours			LC_{50} value (µg/ml) (Organic)[a]	Limits 95 % Confidence (µg/ml)	Slope
		10 µg/ml	100 µg/ml	1000 µg/ml			
Flueggea virosa (Willd) Voigt.	Root bark	32	90	100	17	3-52	0.5212
Gerranthus lobatus (Cogn.) Jeffrey	Stem bark	30	34	88	91	10-667	0.5729
Gerranthus lobatus (Cogn.) Jeffrey	Roots	60	100	100	6	0.35-18	1.0894
Grewia hexaminta Burret.	Leaves	4	26	74	309	98-1864	0.4359
Grewia trichocarpa Hochst ex A.Rich.	Roots	28	44	94	63	10-249	0.4451
Harrisonia abyssinica Oliv.	Root bark	60	100	100	6	0.35-18	1.0894
Harungana madagascariensis Poir	Stem bark	30	36	94	72	7-327	0.4872
Heeria insignis Del.	Stem bark	10	26	74	283	75-3275	0.5058
Hoslundia opposita Vabl.	Roots	12	38	90	123	36-452	0.3695
Lantana camara L.	Leaves	8	68	100	56	20-152	0.3845
Landolphia buchananii (Hall.f) Stapf.	Leaves	20	36	92	101	25-397	0.3891

Table 15 (Continued)

Plant species	Plant part	Percent deaths at 24 hours: 10 µg/ml	100 µg/ml	1000 µg/ml	LC_{50} value (µg/ml) (Organic)[a]	Limits 95 % Confidence (µg/ml)	Slope
Launea cornuta (Oliv and Hiern) C. Jeffrey	Leaves	26	34	100	74	16-258	0.3910
Launea cornuta (Oliv and Hiern) C. Jeffrey	Roots	12	34	84	161	44-793	0.4162
Momordica foetida Schumach.	Leaves	54	86	100	8	0-30	0.7793
Ocimum bacilicum L.	Leaves	16	26	92	140	41-537	0.3600
Ocimum bacilicum L.	Roots	14	40	94	101	30-326	0.3423
Ocimum gratissimum L.	Leaves	26	34	100	74	16-258	0.3910
Ocimum suave Willd	Leaves	14	36	100	99	33-284	0.3254
Ocimum suave Willd	Stem bark	22	28	64	382	ND	1.0661
Pentanisia ouranogyne S.moore	Roots	20	44	80	118	17-1000	0.5555
Pentas bussei K.Krause	Root bark	28	44	94	63	10-249	0.4451
Pentas longiflora Oliv.	Root bark	8	68	98	58	20-161	0.3742
Plectranthus barbatus Andr.	Leaves	14	36	94	110	33-358	0.3385
Plectranthus barbatus Andr.	Stem bark	6	60	96	77	27-219	0.3520

Table 15 (Continued)

Plant species	Plant part	Percent deaths at 24 hours: 10 µg/ml	100 µg/ml	1000 µg/ml	LC_{50} value (µg/ml) (Organic)[a]	Limits 95 % Confidence (µg/ml)	Slope
Plectranthus barbatus Andr.	Roots	18	36	98	88	25-276	0.3456
Rauwolfia mombasiana Stapf	Root bark	36	62	94	31	1-118	0.5411
Ricinus communis L	Leaves	10	28	88	171	52-671	0.3807
	Roots	6	54	86	114	35-394	0.3492
Rottboelia exaltata L.F	Leaves	14	34	74	217	48-3373	0.5540
Securidaca longepedunculata Fres.	Leaves	12	26	74	275	69-4067	0.5284
Securidaca longepedunculata Fres.	Roots	14	36	90	123	34-472	0.3759
Senecio syringitolius O. Hoffman.	Leaves	14	70	100	141	42-527	0.3686
Solanum incanum L.	Leaves	36	62	94	31	1-118	0.5411
	Roots	24	38	90	91	17-433	0.4531

Table 15 (Continued)

Plant species	Plant part	Percent deaths at 24 hours 10 µg/ml	100 µg/ml	1000 µg/ml	LC_{50} value (µg/ml) (Organic)[a]	Limits 95 % Confidence (µg/ml)	Slope
Suregeda zanzibarensis Baill	Root bark	14	42	100	83	26-234	0.3212
Tamarindus indica L.	Stem bark	16	26	65	398	ND	0.7849
Teclea simplicifolia (Eng) Verdoon	Leaves	20	92	100	25	8-65	0.4270
Teclea simplicifolia (Eng) Verdoon	Roots	20	46	98	68	18-209	0.3516
Terminalia spinosa Engl.	Stem bark	36	62	94	31	1-118	0.5411
Toddalia asiatica (L.) Lam.	Root bark	30	34	88	91	10-667	0.5729
Tridax procumbens L.	Whole plant	30	36	94	72	7-327	0.4872
Uvaria scheffleri Diels	Leaves	26	34	100	74	16-258	0.3910
Vernonia amygdalina Delile	Leaves	20	40	80	131	21-1233	0.5557
Warbugia stuhlmannii Engl.	Stem bark	54	86	100	8	0-30	0.7793
Zanthoxylum chalybeum (Eng) Engl.	Leaves	20	50	98	62	16-185	0.3508

Table 15 (Continued)

Plant species	Plant part	Percent deaths at 24 hours			LC_{50} value (µg/ml) (Organic)[a]	Limits 95 % Confidence (µg/ml)	Slope
		10 µg/ml	100 µg/ml	1000 µg/ml			
Zanthoxylum chalybeum (Eng) Engl.	Stem bark	32	90	100	19	3-52	0.5212
Zanthoxylum chalybeum (Eng) Engl.	Root bark	44	100	100	11	0-28	0.6782
[b]Cyclophosphamide		20	52	80	95	12-672	0.5554
[b]Etoposide		60	90	100	6	0-22	0.9269

[a]$CHCL_3$: MeOH (1:1)

[b]Cytotoxic drugs

ND: Not detectable

+ (non-toxic)

++(Weakly-cytotoxic)

Out of the 85 organic extracts screened for activity against *Artemia salina* larvae, 46 (54%) of the crude extracts demonstrated activity at or below 100 µg/ml, and were categorized as having strong cytotoxic activity, 35 (41.2%) of the crude extracts had LC_{50} values between 100 µg/ml and 500 µg/ml, and were categorized as having moderate cytotoxicity, 2 (2.4%) of the crude extracts had LC_{50} values between 500µg/ml and 1000µg/ml, and were considered to have weak cytotoxic activity, while 2 (2.4%) of the crude extracts had LC_{50} values greater than 1000 µg/ml and were considered to be non toxic in brine shrimp (*A.salina*) assay (Figure 11).

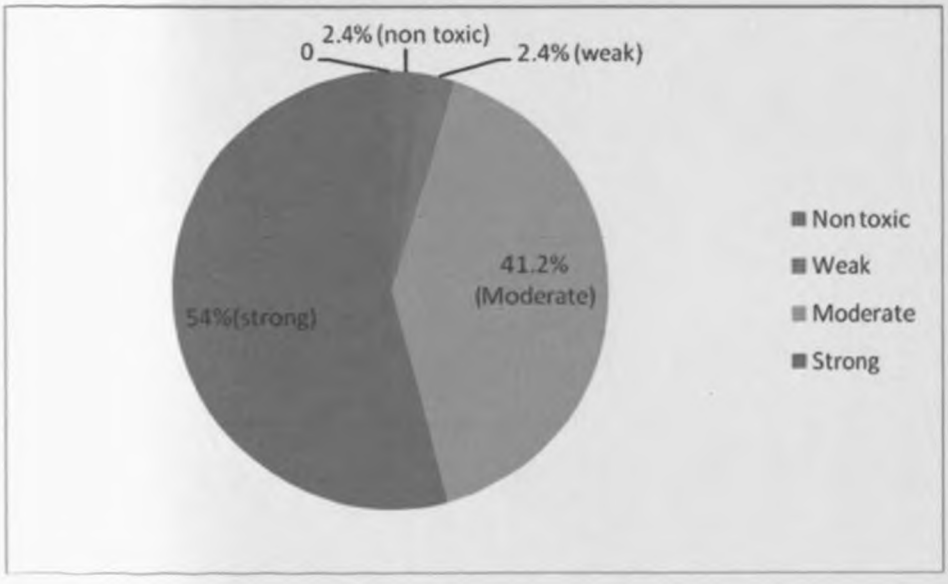

Figure 11: Lethality of organic ($CHCL_3$/MeOH, 1:1) crude plant extracts against *Artemia salina* (n=85)

The results obtained from screening 85 aqueous crude extracts from sixty (60) different plant species against *A. salina* larvae are shown in Table 16. The LC_{50} values from the brine shrimp bioassay obtained for crude extracts of the medicinal plants and that of the cytotoxic drugs,

cyclophosphamide and etoposide, are presented in Table 16. The degree of lethality was found to be directly proportional to the concentration of the extract. In the evaluation for general toxicity using brine shrimp, maximum mortalities took place at a concentration of 1000 μg/ml whereas least mortalities were at 10 μg/ml concentration. Aqueous extractions from all of the species were screened and the corresponding LC_{50} values are given.

Table 16: Toxicity of aqueous crude plant extracts and cytotoxic drugs against *Artemia salina*

Plant species	Plant part	Percent deaths at 24 hours			LC_{50} value (µg/ml)[a]	Limits 95 % Confidence (µg/ml)	Slope
		10 µg/ml	100 µg/ml	1000 µg/ml			
Acacia seyal Del.	Roots	2	26	60	++	ND	0.5215
Adansonia digitata Linn.	Leaves	18	32	98	96	28-316	0.3508
Adansonia digitata Linn.	Stem bark	26	60	90	50	6-202	0.4888
Agathisanthenum globosum (A.Rich) Hiern	Roots	18	28	56	++	ND	1.2394
Albizia anthelmintica Brongn	Stem bark	22	28	58	++	ND	1.4108
Aloe deserti Berger.	Leaves	22	42	84	104	16-679	0.5209
Aloe macrosiphon Bak.	Leaves	18	32	98	96	28-316	0.3508
Aloe secundiflora Engl.	Leaves	22	34	94	95	22-364	0.3961
Aloe vera (L) Webb.	Leaves	22	46	50	++	ND	2.3098
Amaranthus hybridus L.	Leaves	24	30	52	+	ND	2.2833
Azadirachta indica (L) Burm.	Leaves	20	26	78	207	41-4391	0.5889

Table 16 (Continued)

Plant species	Plant part	Percent deaths at 24 hours					
		10 µg/ml	100 µg/ml	1000 µg/ml	LC_{50} value (µg/ml)[a]	Limits 95 % Confidence (µg/ml)	Slope
	Stem bark	22	42	84	104	16-679	0.5209
	Stem	12	20	38	+	ND	2.1419
Azadirachta indica (L) Burm.	Root bark	8	28	82	214	66-1011	0.3990
	Roots	22	28	62	437	ND	1.1650
Bridelia micrantha Baill. (Hochst).	Leaves	16	40	96	90	26-282	0.3411
Canthium glaucum Hiern.	Leaves	10	26	48	+	ND	1.1255
Canthium glaucum Hiern.	Stem bark	20	32	46	+	ND	2.5170
Canthium glaucum Hiern.	Roots	32	60	98	35	5-113	0.4263
Carissa edulis Forrsk.	Root bark	6	40	60	368	ND	0.5977
Cassia occidentalis L.	Leaves	20	76	92	40	7-131	0.4179
	Roots	18	38	86	120	27-619	0.4447

Table 16 (Continued)

Plant species	Plant part	Percent deaths at 24 hours			LC_{50} value (µg/ml)[a]	Limits 95 % Confidence (µg/ml)	Slope
		10 µg/ml	100 µg/ml	1000 µg/ml			
Centella asiatica (L.)Urban	Leaves	12	26	68	353	ND	0.6205
Cissampelos mucronata A.Rich	Root bark	2	28	100	152	59-382	0.4255
Clausena anisata (Willd) Hook.f ex. Benth.	Roots	6	22	52	++	ND	0.8101
Clerodendrum myricoides (Hochst.)Vatke	Root bark	8	24	72	328	91-4072	0.5129
Combretum molle G. Don	Leaves	10	20	72	353	95-6289	0.5341
Combretum padoides Engl and Diels.	Leaves	18	38	86	120	27-619	0.4447
Commiphora schimperi (Berg) Engl.	Stem bark	22	40	60	318	ND	1.2966
Commiphora schimperi (Berg) Engl.	Roots	0	28	54	++	ND	0.5602
Dichrostachys cinerea (L) Wight and AM.	Stem bark	16	40	96	90	26-282	0.3411

Table 16 (Continued)

Plant species	Plant part	Percent deaths at 24 hours			LC_{50} value (µg/ml)[a]	Limits 95 % Confidence (µg/ml)	Slope
		10 µg/ml	100 µg/ml	1000 µg/ml			
Dichrostachys cinerea (L) Wight and AM.	Roots	20	24	74	259	ND	0.6686
Fagaropsis angolensis (Engl) Del.	Leaves	6	20	40	+	ND	1.2493
Ficus bussei Warp ex Mildbr and Burret.	Leaves	6	10	38	+	ND	1.3023
Ficus bussei Warp ex Mildbr and Burret.	Stem bark	20	32	46	+	ND	2.5170
Ficus bussei Warp ex Mildbr and Burret.	Roots	10	20	40	+	ND	1.6348
Flacourtia indica (Burm.f) Merr.	Leaves	22	60	84	65	7-323	0.5240
Flacourtia indica (Burm.f) Merr.	Stem bark	24	44	96	67	14-232	0.3958
Flueggea virosa (Willd) Voigt.	Root bark	14	20	76	288	74-4538	0.5238
Gerranthus lobatus (Cogn.) Jeffrey	Stem bark	4	12	40	+	ND	1.0845

Table 16 (Continued)

Plant species	Plant part	Percent deaths at 24 hours			LC_{50} value (µg/ml)[a]	Limits 95 % Confidence (µg/ml)	Slope
		10 µg/ml	100 µg/ml	1000 µg/ml			
Gerranthus lobatus (Cogn.) Jeffrey	Roots	2	6	10	+	ND	7.0999
Grewia hexaminta Burret.	Leaves	28	72	100	30	6-86	0.3914
Grewia trichocarpa Hochst ex A.Rich.	Roots	22	28	58	++	ND	1.4108
Harrisonia abyssinica Oliv.	Root bark	18	28	86	153	38-894	0.4428
Harungana madagascariensis Poir	Stem bark	24	28	84	143	26-1464	0.5493
Heeria insignis Del.	Stem bark	10	20	70	383	ND	0.5610
Hoslundia opposita Vabl.	Roots	24	34	50	+	ND	2.6410
Lantana camara L.	Leaves	4	24	58	++	ND	0.6269
Landolphia buchananii (Hall.f) Stapf.	Leaves	8	24	80	249	76-1360	0.4179
Launea cornuta (Oliv and Hiern) C. Jeffrey	Leaves	22	24	56	++	ND	1.5520

Table 16 (Continued)

Plant species	Plant part	Percent deaths at 24 hours			LC_{50} value (µg/ml)[a]	Limits 95 % Confidence (µg/ml)	Slope
		10 µg/ml	100 µg/ml	1000 µg/ml			
Launea cornuta (Oliv and Hiern) C. Jeffrey	Roots	24	60	100	44	10-126	0.3588
Momordica foetida Schumach.	Leaves	18	32	98	96	28-316	0.3508
Ocimum bacilicum L.	Leaves	26	36	96	76	15-294	0.4201
Ocimum bacilicum L.	Roots	2	28	100	152	59-382	0.4255
Ocimum gratissimum L.	Leaves	22	46	50	++	ND	2.3098
Ocimum suave Willd	Leaves	28	74	94	31	4-105	0.4692
Ocimum suave Willd	Stem bark	22	28	62	437	ND	1.1650
Pentanisia ouranogyne S.moore	Roots	8	12	62	++	ND	0.6567
Pentas bussei K.Krause	Root bark	8	20	76	311	91-2561	0.4778
Pentas longiflora Oliv.	Root bark	10	18	52	+	ND	0.9649
Plectranthus barbatus Andr.	Leaves	22	30	64	356	ND	1.0686

Table 16 (Continued)

Plant species	Plant part	Percent deaths at 24 hours			LC_{50} value (µg/ml)[a]	Limits 95 % Confidence (µg/ml)	Slope
		10 µg/ml	100 µg/ml	1000 µg/ml			
Plectranthus barbatus Andr.	Stem bark	8	22	40	+	ND	1.4205
Plectranthus barbatus Andr.	Roots	16	22	88	173	49-862	0.4003
Rauwolfia mombasiana Stapf	Root bark	32	40	44	+	ND	12.6112
Ricinus communis L	Leaves	18	26	50	+	ND	1.6704
	Roots	24	30	52	+	ND	2.2833
Rottboelia exaltata L.F	Leaves	10	24	54	++	ND	0.8962
Securidaca longepedunculata Fres.	Leaves	10	24	72	321	84-5240	0.5321
Securidaca longepedunculata Fres.	Roots	8	18	42	+	ND	1.3047
Senecio syringitolius O. Hoffman.	Leaves	20	28	80	181	36-2410	0.5544
Solanum incanum L.	Leaves	12	14	82	273	85-1854	0.4258
	Roots	4	24	62	499	ND	0.5767

Table 16 (Continued)

Plant species	Plant part	Percent deaths at 24 hours			LC_{50} value (µg/ml)[a]	Limits 95 % Confidence (µg/ml)	Slope
		10 µg/ml	100 µg/ml	1000 µg/ml			
Suregeda zanzibarensis Baill	Root bark	8	22	50	+	ND	0.9445
Tamarindus indica L.	Stem bark	16	78	94	42	10-126	0.3873
Teclea simplicifolia (Eng) Verdoon	Leaves	16	36	84	140	33-790	0.4483
Teclea simplicifolia (Eng) Verdoon	Roots	10	28	70	315	79-6706	0.5541
Terminalia spinosa Engl.	Stem bark	18	22	40	+	ND	3.2053
Toddalia asiatica (L.) Lam.	Root bark	6	10	38	+	ND	1.3023
Tridax procumbens L.	Whole plant	14	30	78	208	50-1984	0.4996
Uvaria scheffleri Diels	Leaves	12	26	42	+	ND	1.7097
Vernonia amygdalina Delile	Leaves	22	28	58	596	ND	1.4108
Warbugia stuhlmannii Engl.	Stem bark	24	30	52	+	ND	2.2833

Table 16 (Continued)

Plant species	Plant part	Percent deaths at 24 hours			LC_{50} value (µg/ml)[a]	Limits 95 % Confidence (µg/ml)	Slope
		10 µg/ml	100 µg/ml	1000 µg/ml			
Zanthoxylum chalybeum (Eng) Engl.	Leaves	28	74	94	31	4-105	0.4692
Zanthoxylum chalybeum (Eng) Engl.	Stem bark	14	20	76	288	74-4538	0.5238
Zanthoxylum chalybeum (Eng) Engl.	Root bark	16	60	98	56	17-157	0.3381
[b]Cyclophosphamide		20	52	80	95	12-672	0.5554
[b]Etoposide		60	90	100	6	0-22	0.9269

ND: Not detectable

[a]Aqueous extracts

[b]Cytotoxic drugs

+ (non toxic)

++ (Weakly cytotoxic)

Approximately 19% (16) of the 85 aqueous extracts demonstrated activity at or below 100 µg/ml and were considered to have strong cytotoxic activity, 39% (33) of the screened crude extracts had LC_{50} values between 100 µg/ml and 500 µg/ml and were considered to be moderately cytotoxic, 15% (13) of the crude extracts had LC_{50} values between 500 µg/ml and 1000 µg/ml and were considered to have weak cytotoxic activity while 27% (23) of the aqueous extracts had LC_{50} values greater than 1000µg/ml and were categorized as non toxic (Figure 12).

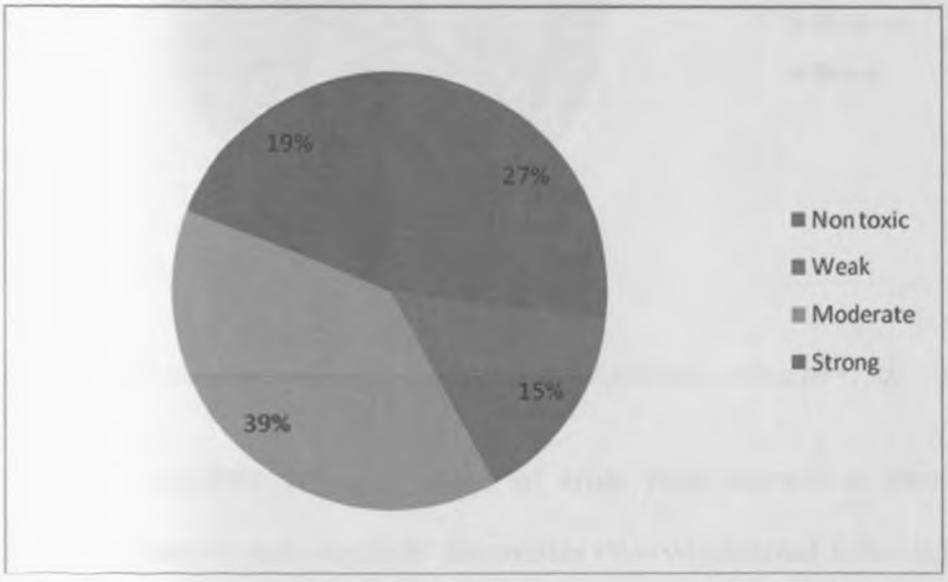

Figure 12: Bioactivity of aqueous crude plant extracts against *A. salina (n=85)*

Of all the 170 crude extracts evaluated for bioactivity in brine shrimp (*A.salina*) assay, 54% (46) of the organic and 19% (16) of the aqueous extracts demonstrated activity strong enough (LC_{50} = <100 µg/ml) to merit chemical investigation. A total of 62 (36%) of all the crude extracts assayed (170 extracts) had strong activity against *A. salina* (LC_{50} = <100 µg/ml), 68(40%) of all the assayed crude extracts had moderate activity (LC_{50} values ranged between 100-500 µg/ml), 15

(9%) of the crude extracts exhibited weak activity (LC_{50} values ranged between 500-1000 µg/ml) while 25 crude extracts (15%) were non toxic (LC_{50} > 1000 µg/ml) to *A. salina* larvae (Fig. 13).

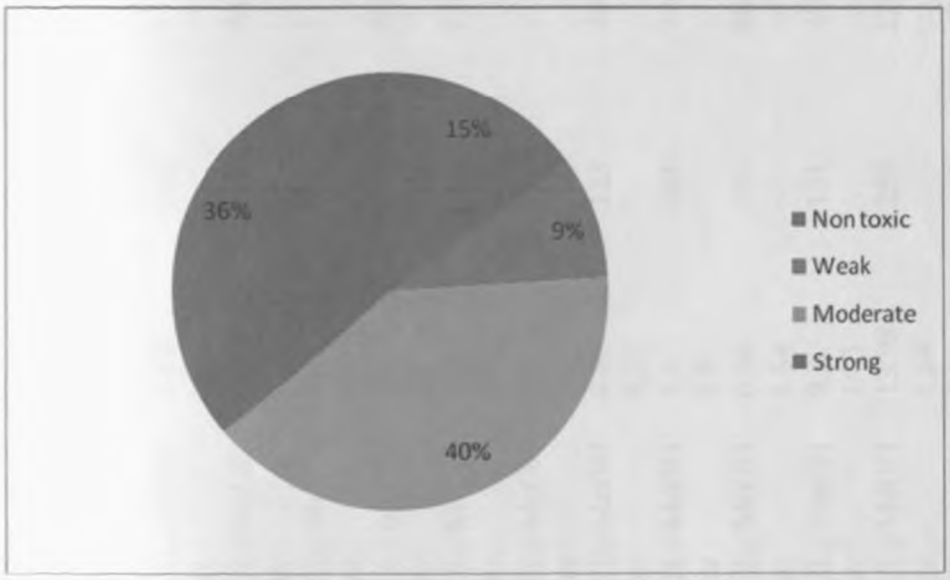

Figure 13: Bioactivity of crude extracts against *Artemia salina (n=170)*

Table 17 compares the LC_{50} values of crude plant extracts to those of cytotoxic drugs, cyclophosphamide and etoposide. Recoveries (%w/w) obtained following organic and aqueous extraction of 170 different plant parts of sixty antimalarial plant species used by the Msambweni community are also compared.

Table 17: *Comparative cytotoxicity of crude plant extracts against Artemia salina*

Family	Plant species/ Voucher specimen number	Plant part	Solvent	%Yield (w/w)	LC_{50} (µg/ml) Organic[a]	LC_{50} (µg/ml) Aqueous
Amaranthanceae	*Amaranthus hybridus* L.(JN08)	Leaves	$CHCL_3$/MeOH	4.68	**200**	+
			Water	5.8		
Anacardiaceae	*Heeria insignis* Del.(JN024)	Stem	$CHCL_3$/MeOH	4.78	**283**	**383**
			Water	10.4		
Annonaceae	*Uvaria scheffleri* Diels (JN041)	Leaves	$CHCL_3$/MeOH	4.4	**74**	+
			Water	5.6		
Apocynaceae	*Carissa edulis* Forrsk.(JN042)	Root bark	$CHCL_3$/MeOH	9.6	**110**	**368**
			Water	10.2		
	Landolphia buchananii (Hall.f) Stapf. (JN027)	Leaves	$CHCL_3$/MeOH	5.4	**101**	**249**
			Water	7.8		
	Rauwolfia mombasiana Stapf (JN 051)	Root bark	$CHCL_3$/MeOH	8.8	**31**	+
			Water	11.4		
Asclepiadaceae	*Centella asiatica* (L.)Urban (JN043)	Leaves	$CHCL_3$/MeOH	4.5	**123**	**353**
			Water	6.2		
Asteraceae	*Vernonia amygdalina Delile* (JN057)	Leaves	$CHCL_3$/MeOH	5.6	**131**	++
			Water	6.8		
Bombaceae	*Adansonia digitata* Linn.(JN02)	Leaves	$CHCL_3$/MeOH	6.96	++	**96**
			Water	4.84		
Caesalpiniaceae	*Cassia occidentalis* L.(JN012)	Leaves	$CHCL_3$/MeOH	9.3	**131**	**40**
			Water	14.1		
	Cassia occidentalis L.(JN012)	Roots	$CHCL_3$/MeOH	13.98	**253**	**120**
			Water	1.64		
	Tamarindus indica L.(JN038)	Stem bark	$CHCL_3$/MeOH	3.32	**398**	**42**
			Water	3.48		
Canellaceae	*Warbugia stuhlmannii* Engl.(JN044)	Stem bark	$CHCL_3$/MeOH	6.6	**8**	+
			Water	7.8		

Table 17 (Continued)

Family	Plant species/ Voucher specimen number	Plant part	Solvent	%Yield (w/w)	LC_{50} (µg/ml) Organic[a]	LC_{50} (µg/ml)[b] Aqueous
Combretaceae	*Combretum padoides* Engl and Diels.(JN014)	Leaves	$CHCL_3$/MeOH Water	3.4 10	**56**	**120**
	Commiphora schimperi (Berg) Engl.(JN015)	Stem bark	$CHCL_3$/MeOH Water	25.98 6.74	**131**	**318**
	Terminalia spinosa Engl. (JN 052)	Stem bark	$CHCL_3$/MeOH Water	3.6 4.8	**31**	+
	Combretum molle G. Don (JN 059)	Leaves	$CHCL_3$/MeOH Water	10 12.4	**88**	**353**
Compositae	*Launea cornuta* (Oliv and Hiern) C. Jeffrey(JN028)	Leaves	$CHCL_3$/MeOH Water	5.6 8.12	**74**	++
	Launea cornuta (Oliv and Hiern) C. Jeffrey(JN028)	Roots	$CHCL_3$/MeOH Water	6.72 4.84	**161**	**44**
	Senecio syringitolius O. Hoffman.(JN036)	Leaves	$CHCL_3$/MeOH Water	2.08 2.66	**141**	**181**
	Tridax procumbens L. (JN 054)	Whole plant	$CHCL_3$/MeOH Water	5.4 6.6	**72**	**208**
Cucurbitaceae	*Gerranthus lobatus* (Cogn.) Jeffrey (JN020)	Stem bark	$CHCL_3$/MeOH Water	4.5 3.75	**91**	+
	Gerranthus lobatus (Cogn.) Jeffrey (JN020)	Roots	$CHCL_3$/MeOH Water	6.4 2.56	**6**	+
	Momordica foetida Schumach. (JN060)	Leaves	$CHCL_3$/MeOH Water	3.6 4.8	**8**	**96**
Euphorbiaceae	*Bridelia micrantha* Baill. (Hochst).(JN010)	Leaves	$CHCL_3$/MeOH Water	4.7 4.44	**171**	**90**
	Ricinus communis L(JN033)	Leaves	$CHCL_3$/MeOH Water	6.1 16.66	**171**	+

Table 17 (Continued)

Family	Plant species/ Voucher specimen number	Plant part	Solvent	%Yield (w/w)	LC_{50} (µg/ml) Organic[a]	LC_{50} (µg/ml)[b] Aqueous
Euphorbiaceae	*Ricinus communis* L(JN033)	Roots	$CHCL_3$/MeOH	1.3	**114**	+
			Water	2.4		
	Suregeda zanzibarensis Baill(JN045)	Root bark	$CHCL_3$/MeOH	13.4	**83**	+
			Water	16.2		
	Flueggea virosa (Willd) Voigt. (JN049)	Root bark	$CHCL_3$/MeOH	3.8	**17**	**288**
			Water	5.6		
Flacourtiaceae	*Flacourtia indica* (Burm.f) Merr. (JN019)	Leaves	$CHCL_3$/MeOH	13.16	+	**65**
			Water	17.02		
	Flacourtia indica (Burm.f) Merr. (JN019)	Stem bark	$CHCL_3$/MeOH	8	+	**67**
			Water	1.54		
Gramineae	*Rottboelia exaltata* L.F(JN034)	Leaves	$CHCL_3$/MeOH	4.2	**217**	++
			Water	8.01		
Guttiferae	*Harungana madagascariensis Poir* (JN 053)	Stem bark	$CHCL_3$/MeOH	6.8	**72**	**143**
			Water	8.9		
Labiatae	*Hoslundia opposita* Vabl.(JN025)	Roots	$CHCL_3$/MeOH	2.12	**123**	+
			Water	1.06		
	Ocimum bacilicum L.(JN029)	Leaves	$CHCL_3$/MeOH	10.82	**140**	**76**
			Water	3.58		
	Ocimum bacilicum L.(JN029)	Roots	$CHCL_3$/MeOH	0.76	**101**	**152**
			Water	4.80		
	Ocimum suave Willd(JN030)	Leaves	$CHCL_3$/MeOH	4.36	**99**	**31**
			Water	7.58		
	Ocimum suave Willd(JN030)	Stem bark	$CHCL_3$/MeOH	3.28	**382**	**437**
			Water	3.75		
	Plectranthus barbatus Andr. (JN032)	Leaves	$CHCL_3$/MeOH	7.46	**110**	**356**
			Water	16.6		

Table 17 (Continued)

Family	Plant species/ Voucher specimen number	Plant part	Solvent	% Yield (w/w)	LC_{50} (µg/ml) Organic[a]	LC_{50} (µg/ml) Aqueous
Labiatae	*Plectranthus barbatus* Andr. (JN032)	Stem bark	$CHCL_3$/MeOH	6	**77**	+
			Water	10		
	Plectranthus barbatus Andr. (JN032)	Roots	$CHCL_3$/MeOH	8	**88**	**173**
			Water	12		
Lamiaceae	*Ocimum gratissimum L.*(JN058)	Leaves	$CHCL_3$/MeOH	5.6	**74**	++
			Water	6.8		
Leguminosae	*Albizia anthelmintica* Brongn(JN046)	Stem bark	$CHCL_3$/MeOH	8.8	**110**	++
			Water	9.16		
Liliaceae	*Aloe deserti* Berger.(JN04)	Leaves	$CHCL_3$/MeOH	3.68	**68**	**104**
			Water	3.8		
	Aloe macrosiphon Bak.(JN05)	Leaves	$CHCL_3$/MeOH	5.72	++	**96**
			Water	4.06		
	Aloe secundiflora Engl.(JN06)	Leaves	$CHCL_3$/MeOH	5.42	**217**	**95**
			Water	4.44		
	Aloe vera (L) Webb.(JN07)	Leaves	$CHCL_3$/MeOH	4.26	**141**	++
			Water	6.0		
Meliaceae	*Azadirachta indica*(L) Burm.(JN09)	Leaves	$CHCL_3$/MeOH	6.84	**58**	**207**
			Water	15.16		
	Azadirachta indica(L) Burm.(JN09)	Stem bark	$CHCL_3$/MeOH	6.4	**18**	**104**
			Water	9.86		
	Azadirachta indica(L) Burm.(JN09)	Stem	$CHCL_3$/MeOH	2.18	**103**	+
			Water	2.62		
	Azadirachta indica(L) Burm.(JN09)	Root bark	$CHCL_3$/MeOH	5.64	**13**	**214**
			Water	4.98		
	Azadirachta indica(L) Burm.(JN09)	Roots	$CHCL_3$/MeOH	4.82	**8.3**	**437**
			Water	2.4		
Menispermaceae	*Cissampelos mucronata* A.Rich (JN047)	Root bark	$CHCL_3$/MeOH	3.6	**114**	**152**
			Water	4.8		

Table 17 (Continued)

Family	Plant species/ Voucher specimen number	Plant part	Solvent	%Yield (w/w)	LC_{50} (µg/ml) Organic[a]	LC_{50} (µg/ml) Aqueous
Mimosaceae	*Acacia seyal* Del. (JN01)	Roots	$CHCL_3$/MeOH	2.96	**8**	++
			Water	2.94		
	Dichrostachys cinerea (L) Wight and AM.(JN016)	Roots	$CHCL_3$/MeOH	3.44	**72**	**90**
			Water	1.65		
	Dichrostachys cinerea (L) Wight and AM.(JN016)	Stem bark	$CHCL_3$/MeOH	4.04	**47**	**259**
			Water	1.74		
Moraceae	*Ficus bussei* Warp ex Mildbr and Burret.(JN018)	Leaves	$CHCL_3$/MeOH	3.78	**73**	+
			Water	5.64		
	Ficus bussei Warp ex Mildbr and Burret.(JN018)	Stem bark	$CHCL_3$/MeOH	2.6	**151**	+
			Water	4.4		
	Ficus bussei Warp ex Mildbr and Burret.(JN018)	Roots	$CHCL_3$/MeOH	1.72	**11**	+
			Water	7.64		
Papilionaceae	*Securidaca longepedunculata* Fres. (JN035)	Leaves	$CHCL_3$/MeOH	22.4	**275**	**321**
			Water	3.95		
	Securidaca longepedunculata Fres. (JN035)	Roots	$CHCL_3$/MeOH	22	**123**	+
			Water	21.24		
Rubiaceae	*Agathisanthenum globosum* (A.Rich) Hiern (JN03)	Roots	$CHCL_3$/MeOH	3.64	**88**	++
			Water	2.11		
	Canthium glaucum Hiern. (JN011)	Leaves	$CHCL_3$/MeOH	5.96	**101**	+
			Water	5.23		
	Canthium glaucum Hiern. (JN011)	Stem bark	$CHCL_3$/MeOH	5.6	**81**	+
			Water	8.4		

Table 17 (Continued)

Family	Plant species/ Voucher specimen number	Plant part	Solvent	%Yield (w/w)	LC_{50} (µg/ml) Organic[a]	LC_{50} (µg/ml) Aqueous
Rubiaceae	*Canthium glaucum* Hiern. (JN011)	Roots	$CHCL_3$/MeOH	6.8	**131**	+
			Water	5.88		
	Pentanisia ouranogyne S.moore (JN031)	Roots	$CHCL_3$/MeOH	12.24	**118**	++
			Water	4.56		
	Pentas bussei K.Krause (JN048)	Root bark	$CHCL_3$/MeOH	8.8	**63**	**311**
			Water	9.6		
	Pentas longiflora Oliv. (JN 056)	Root bark	$CHCL_3$/MeOH	6.2	**58**	+
			Water	9.6		
Rutaceae	*Clausena anisata* (Willd) Hook.f ex. Benth. (JN013)	Roots	$CHCL_3$/MeOH	4.32	**101**	++
			Water	5.20		
	Fagaropsis angolensis (Engl) Del. (JN017)	Leaves	$CHCL_3$/MeOH	5.5	**101**	+
			Water	6.2		
	Teclea simplicifolia (Eng) Verdoon (JN039)	Leaves	$CHCL_3$/MeOH	10.96	**25**	**315**
			Water	6.06		
	Teclea simplicifolia (Eng) Verdoon (JN039)	Roots	$CHCL_3$/MeOH	8.08	**68**	+
			Water	4.62		
	Zanthoxylum chalybeum (Eng) Engl.(JN040)	Leaves	$CHCL_3$/MeOH	6.48	**62**	**31**
			Water	16.02		
	Zanthoxylum chalybeum (Eng) Engl.(JN040)	Stem bark	$CHCL_3$/MeOH	13.6	**19**	**288**
			Water	3.14		
	Zanthoxylum chalybeum (Eng) Engl.(JN040)	Root bark	$CHCL_3$/MeOH	12.64	**11**	**56**
			Water	6.38		
	Toddalia asiatica (L.) Lam. (JN 055)	Root bark	$CHCL_3$/MeOH	9.2	**91**	+
			Water	3.4		

Table 17 (Continued)

Family	Plant species/ Voucher specimen number	Plant part	Solvent	%Yield (w/w)	LC_{50} (µg/ml) Organic[a]	LC_{50} (µg/ml) Aqueous
Simaroubaceae	*Harrisonia abyssinica* Oliv.(JN023)	Root bark	$CHCL_3$/MeOH	3.6	**6**	**153**
			Water	6.2		
Solanaceae	*Solanum incanum* L.(JN037)	Leaves	$CHCL_3$/MeOH	5.26	**31**	**273**
			Water	10.86		
	Solanum incanum L.(JN037)	Roots	$CHCL_3$/MeOH	1.96	**91**	**499**
			Water	2.32		
Tiliaceae	*Grewia hexaminta* Burret. (JN021)	Leaves	$CHCL_3$/MeOH	4.78	**309**	**30**
			Water	6.06		
	Grewia trichocarpa Hochst ex A.Rich.(JN022)	Roots	$CHCL_3$/MeOH	3.44	**63**	++
			Water	3.14		
Verbenaceae	*Clerodendrum myricoides* (Hochst.)Vatke (JN050)	Root bark	$CHCL_3$/MeOH	4.6	**25**	**328**
			Water	3.2		
	Lantana camara L.(JN026)	Leaves	$CHCL_3$/MeOH	9.28	**56**	++
			Water	19.72		

+ (non toxic)

++ (Weakly cytotoxic)

[a]$CHCL_3$: MeOH (1:1)
Cyclophospamide (Positive control) (LC_{50} =95 µg/ml)

Etoposide (Positive control) (LC_{50} =6 µg/ml)

W/w, weight by weight

6.4. Discussion

The evaluation of the toxic action of plant extracts is indispensable in order to consider a treatment safe; it enables the definition of the intrinsic toxicity of the plant and the effects of acute overdose, Cáceres (1996); Parra *et al* (2001). The current study aimed at screening the lethality of cytotoxic drugs, cyclophosphamide and etoposide and crude plant extracts commonly used as antimalarial phytomedicines in Msambweni district, Kenya against brine shrimp, *Artemia salina*, as an indication of their toxicity when used by the Msambweni community. Laboratory mice are sensitive to toxic substances occurring in plants. The administration of the extracts in increasing amounts enables the evaluation of the toxicity limits, and the test should be carried out in two ways, for three doses, and for both sexes, taking into account such factors as age, sex, weight, species, diet, and environmental conditions, Cáceres (1996). Because there is currently a tendency to limit the use of laboratory animals in toxicological tests, Yajes (1997), and the brine shrimp is a crustacean whose larvae are sensitive to a variety of substances, the brine shrimp bioassay can be useful as a quick and simple test for predicting the toxicity of plant extracts and guiding their phytochemical fractionation, Cáceres (1996) in the discovery of new cytotoxic natural products, McLaughlin *et al* (1991). This test is used particularly in developing countries, where 85% of the population uses medicinal plants in traditional therapy, Feroze and Chadially (1969); Nguta *et al* (2010a).

Literature data suggest a good correlation between the activity of crude plant extracts in the brine shrimp assay and the cytotoxicity against human solid tumor cell lines, Anderson *et al* (1991), *in vivo* cytotoxicity, Parra *et al* (2001) as well as hepatotoxic activity, Kiviranta *et al* (1991), suggesting that the brine shrimp bioassay is a useful alternative model. The LC_{50} values of the

brine shrimp obtained for extracts of the screened medicinal plants and that of the cytotoxic drugs, cyclophosphamide and etoposide have been presented in Tables 15, 16 and 17. The degree of lethality was found to be directly proportional to the concentration of the extract.

In bioactivity evaluation of plant extracts by brine shrimp bioassay, an LC_{50} value lower than 1000 µg/ml is considered cytotoxic, Meyer *et al* (1982). In the current study, 97.6% (98) of all the screened organic extracts and 73% (85) of the investigated aqueous extracts demonstrated LC_{50} values < 1000 µg/ml, indicating the presence of cytotoxic compounds responsible for the observed toxicological activity. The current observation indicates that some of the antimalarial plants could not make safe herbal remedies. This calls for dose adjustment amongst the community using the plant extracts for the treatment of malaria..

The most toxic aqueous extracts (LC_{50} <100 µg/ml) were the leaves and stem bark of *Adansonia digitata* Linn., leaves of *Bridelia micrantha* Baill. (Hochst)., leaves and stem bark of *Flacourtia indica* (Burm.f) Merr., leaves of *Aloe macrosiphon* Bak., leaves of *Aloe secundiflora* Engl., roots of *Dichrostachys cinerea* (L) Wight and AM. and the leaves of *Grewia hexaminta* Burret. Pharmacological properties of these plants have been demonstrated in preclinical studies, including those of *Adansonia digitata* Linn. as an antipyretic, Abbiw (1990), bioactive, Cantrell (2003) and antiplasmodial activity, Kristina (2002); *Bridelia micrantha* Baill. (Hochst) as an antiplasmodial agent, Clarkson *et al* (2004); *Flacourtia indica* (Burm.f) Merr. as an antiplasmodial agent, Clarkson *et al* (2004); *Aloe secundiflora* Engl. as an antimalarial agent, Oketch-Rabah *et al* (1999) and *Grewia hexaminta* Burret.where triterpenoids have been isolated, Raghunathaivar (1996). These plant species have demonstrated cytotoxicity low enough

(LC_{50}<100 µg/ml) to merit chemical investigation for isolation of cytotoxic compounds responsible for the observed toxicity.

Approximately 98% of all the organic extracts were toxic (LC_{50} values < 1000 µg/ml), while 54% of the organic crude extracts exhibited strong cytotoxic activity ((LC_{50} <100 µg/ml). These results indicate that majority of the cytotoxic constituents in the screened plant species are non polar, and merit further phytochemical analysis for the isolation of the cytotoxic compounds, which could serve as novel scaffolds in search for cytotoxic compounds. The current observation is in agreement with the findings of Cantrell *et al* (2003), who found organic extracts to be more toxic than aqueous extracts in a brine shrimp bioassay. The organic extract of the root bark of *Harrisonia abyssinica* Oliv. was highly toxic (LC_{50} = 6 µg/ml) against *A. salina,* and comparable to that of the anticancer agent, etoposide. Literature suggests that the species had been previously investigated for antimalarial activity, El Tahir *et al* (1999) but cytotoxic compounds have not been isolated, hence more phytochemical investigation is required for isolation of cytotoxic compounds.

6.5. Conclusions

The current study evaluated the cytotoxicity of crude plant extracts and cytotoxic drugs against *A. salina*. The standard *A.salina* bioassay is a useful screen for the toxicity based detection of plant extracts and could replace the more ethically challenged mouse bioassay for this purpose. It is also a useful screen for cytotoxic compounds in natural products, Colombo *et al* (2001); Favilla *et al* (2006) and cytotoxic drugs. *Artemia* can be maintained indefinitely in the laboratory in their cyst form, and are easily induced to hatch. As such *Artemia* provides a constantly available

bioassay species to screen for phytotoxins and evaluation of cytotoxic status of antitumor drugs. Furthermore, the *A.salina* bioassay is more sensitive than the mouse bioassay and the unit costs much lower compared to *in vitro* protein synthesis assays. Finally, while the *A. salina* bioassay provides a simple method for toxicity assessment of crude plant extracts, this should continue to be complemented by appropriate phytochemical analytical methods, Eaglesham *et al* (1999).

CHAPTER SEVEN

GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

The results indicate that finding medicinal plants with good biological activities is enhanced when plants are chosen on the basis of ethnopharmacological use. The chemotaxonomic approach on choosing a group of taxonomically related species, in our case species of Combretaceae, seems to be successful when trying to find plant species with new/improved biological activities. Although, some of the antimalarial species reported in this study have been investigated for their traditional medicinal uses before by various authors, we have, in some cases reported on different or additional ethnomedical uses of certain species. This work has also elucidated the ways in which antimalarial phytotherapeutic remedies are prepared in Msambweni district, South coast Kenya and also in different countries and regions of Africa and globally, differing from each other in terms of methods for preparing herbal medicines from the reported species. Since ethnopharmacological knowledge is often handed down orally from generation to generation and usually no written documents are available, it is important to documentate this valuable information. Also, in many countries in Africa over 80 % of the population is entirely dependent on traditional medicine for their health care, and thus written information might aid and improve the health care of the local people, as well as help to preserve the information.

The objective of the ethnomedical survey was to document the plants used traditionally at Msambweni, Kenya, against malaria. Considerable similarity of information on the use of plant species for treatment of malaria was reported by several herbalists. The use of a decoction of *Azadirachta indica* stem bark had 95% frequency among the respondents. *Azadirachta indica* has

been reported to have antiplasmodial activity, Kirira *et al* (2006) and active compounds isolated such as gedunin and nimbinin, Bray *et al* (1990) which are responsible for the antimalarial activity of the plant species. Some of the plants identified by the herbalists have been reported in the literature as having been used for treatment of malaria related symptoms in other parts of the world, an indication that the herbalists could be trusted for the information they gave about the plants they use. They have also been investigated for their phytoconstituents and antimalarial activities. However, to the best of our knowledge, thirteen plant species, namely *Aloe deserti* Berger (Liliaceae), *Launea cornuta* (Oliv and Hiern) C. Jeffrey (Compositae), *Ocimum bacilicum* L. (Labiatae), *Teclea simplicifolia* (Eng) Verdoon (Rutaceae), *Gerranthus lobatus* (Cogn.) Jeffrey (Cucurbitaceae), *Grewia hexaminta* Burret. (Tiliaceae), *Canthium glaucum* Hiern. (Rubiaceae), *Amaranthus hybridus* L. (Amaranthaceae), *Combretum padoides* Engl and Diels. (Combretaceae), *Senecio syringitolius* O. Hoffman. (Compositae), *Ocimum suave* Willd (Labiatae), *Aloe macrosiphon* Bak. (Liliaceae) and *Laudolphia buchananii* (Hall.f) Stapf. (Apocynaceae) are documented for the first time for the treatment of malaria.

The results of this study show that a large number of medicinal plants are traditionally used for treatment of malaria among the Msambweni community of Kenyan Coast. Labiatae, Rutaceae and Liliaceae families represented the species most commonly cited which would indicate the importance of these families as possible sources of antimalarial drugs. The information on the frequently utilized antimalarial plant species is an important lead to the species that can be targeted for pharmacological, toxicological and phytochemical tests. *Azadirachta indica* (Meliaceae), *Zanthoxylum chalybeum* (Rutaceae), *Aloe deserti* (Liliaceae), *Harrisonia abyssinica* (Simaroubaceae), *Launea cornuta* (Compositae), *Ricinus communis* (Euphorbiaceae) and

Lantana camara (Verbenaceae) represented the species most commonly cited. Plant species have been assigned numbers according to the extent of their use in treating malaria (Important value for the treatment of Malaria or IV mal, Willcox *et al* (2004). The use of *Ricinus communis* (Euphorbiaceae) in treatment of malaria has been reported in three continents and has an IV mal of 8, while *Lantana camara* (Verbenaceae) has been reported in two continents and has an IV mal of 7 Fowler (2006). This was consistent with our results as *Ricinus communis* (Euphorbiaceae) and *Lantana camara* (Verbenaceae) were reported as some of the commonly used species in preparation of antimalarial remedies and would indicate the importance of these plants as possible sources of antimalarial agents.

There are genera, which were reported in this study with species that are also known to be used as sources of antimalarial remedies in other parts of Africa. They have also been reported to contain antiplasmodial activity against *Plasmodium falciparum*. Those from Western Uganda include *Lantana trifolia* L., screened against wild strains of *Plasmodium falciparum* using the parasite lactate dehydrogenase (pLDH) assay. The petroleum, chloroformic and ethanolic extracts from the aerial parts of the plant had an IC_{50} (µg/ml) of 13.2, >50 and >50 respectively, Katuura (2007). Those from South Africa included plants screened against *Plasmodium falciparum* strain D10 using the parasite lactate dehydrogenase (pLDH) assay such as *Aloe forex* leaves (IC_{50} of 21 µg/ml) and *Ricinus communis* leaves (IC_{50} of 11.4 µg/ml). Others were *Ricinus communis* stems (IC_{50} of 8µg/ml), *Ricinus communis* fruits (IC_{50} of 90µg/ml) and *Lantana camara* leaves (IC_{50} of 11µg/ml), Clarkson *et al* (2004). Gessler *et al* (1994), while screening chloroquine resistant *Plasmodium falciparum* strain K1 against plant extracts from Tanzania found *Zanthoxylum*

chalybeum root bark (IC_{50} of 4.2µg/ml) to be one of the species with the strongest antiplasmodial activity among the antimalarial plants tested.

Kirira *et al* (2006) while screening chloroquine sensitive *Plasmodium falciparum* strain NF54 and chloroquine resistant strain ENT30 against plant extracts from Meru and Kilifi districts found *Azadirachta indica* leaves (IC_{50} >250 µg/ml) to be inactive. It is interesting to note that the most commonly used antimalarial plant species reported in this study, *Azadirachta indica*, which has also been cited severally as a potent traditional antimalarial remedy, was reported as having insignificant antimalarial activity whereas other studies have reported good antiplasmodial activity. El Tahir *et al* (1999) while screening some medicinal plants from Sudan against chloroquine sensitive *Plasmodium falciparum* strain 3D7 and resistant strain Dd2 found *Azadirachta indica* leaves (IC_{50} of 5.8 and 1.7µg/ml, for 3D7 and Dd2, respectively) with highly potent antiplasmodial activity.

The observed antiplasmodial activity from extracts of *Harrisonia abyssinica* against chloroquine sensitive *Plasmodium falciparum* strain NF54 and chloroquine resistant strain ENT30, Kirira *et al* (2006) has recently been confirmed by Maregesi *et al* (2010) while screening Tanzanian medicinal plants for activity against *Plasmodium falciparum* and human immunodeficiency virus, Maregesi *et al* (2010). This makes the plant quite promising as a lead for further studies. It is noted that the plants used by the Msambweni community to treat malaria have been used in many other countries in the world for the treatment of fever frequently associated with malaria. Omino and Kokwaro (1993) reports widespread use of Apocynaceae in traditional medicine in Africa. Fowler (2006) reports the use of *Ricinus communis* (Euphorbiaceae) and *Lantana camara*

(Verbenaceae) in three and two continents respectively. The potency of the extracts may also be affected by solvent of extraction, georeference, time and season of harvesting or other environmental factors, Prance (1994).

It is important to note that phytochemical compounds in traditionally used antimalarial herbal remedies are responsible for antiplasmodial activity. The most important and diverse biopotency has been observed in alkaloids, quassinoids, sesquiterpene lactones, coumarins, triterpenoids, limonoids and quinoline alkaloids, Saxena *et al* (2003). Quinoline alkaloids isolated from *Zanthoxylum chalybeum*, Kato *et al* (1996), steroidal sapogenins from *Ficus bussei*, Wall (2006), Coumarins from *Cassia occidentalis* Cimanga (2004), gedunin and nimbinin, triterpenoids from *Azadirachta indica*, Bray *et al* (1990); Mackinnon *et al* (1997) are some of the specific examples. Other components responsible for antiplasmodial activity as in *Securidaca longepedunculata* roots are steroids, saponosides and monotropitosides, De La Pradilla (1988) and the leaves of *Lantana camara* have been reported to contain quinine like alkaloid, *lantanine*, Burkill (2000). *Azadirachta indica*, the most commonly used species to treat malaria by the Msambweni community, South coast, is the third most commonly used herbal medicine to treat malaria in Kenya after *Ajuga remota* and *Caesalpinia volkensii*, Kuria *et al* (2001).

As Sofowora (1982) noted, many people in several African countries take a decoction of *Azadirachta indica* (neem tree) for malaria fever. Their reasons for doing so include reaction to chloroquine, a dislike for synthetic drugs, and the cost and unavailability of synthetic antimalarials, Muthaura *et al* (2007). The lack of standardization and quality control is one of the main disadvantages of traditional herbal remedies, Evans-Anfom (1986); Sofowora (1982).

Isolation and characterization of active constituents need to be undertaken for use as markers in standardization of the extracts, thus minimizing the risk of over dosage and also for identification of possible lead structures that could be used for the development of novel antimalarial drugs.

Some of the species documented in this study for the treatment of malaria have been used similarly in other continents of the world. This correspondence in the use of the same species in different cultures over a long period suggests strongly that these species may be effective in the treatment of malaria, Orwa (2002); Van wyk and Wink (2004). It is important, however, to validate all claims of therapeutic efficacy and safety by undertaking pharmacological, toxicological and good quality clinical studies. The literature reviewed in this study indicates that few toxicological studies have been conducted. Many plant species reported in this study have been investigated for their phytoconstituents and pharmacological activities, the latter being in agreement with ethnomedical uses reported in this study.

The respondents interviewed in this study had good knowledge about malaria and readily distinguished it from other illnesses on the basis of widely accepted malaria signs and symptoms Gessler *et al* (1995b); Ahorlu *et al* (1997); Purcell (2004); Tabuti (2008). The community recognized the clinical features of malaria such as chills, profuse sweating, joint pains, abdominal pain, diarrhea, vomiting, anorexia and inability to stand, Ministry of Health (2006). Malaria continues to be a major health challenge in Kenya especially due to the emergence of parasite resistance to commonly used and relatively cheap antimalarials. Knowledge about malaria has steadily improved in Kenya, but some misconceptions still remain about the causes and symptoms of severe malaria, and this were also documented in this study. However, majority of

the respondents knew that malaria was spread by mosquitoes and one of the major symptoms of the disease was fever. This relatively good understanding of the causes and signs of the disease may help in the implementation of intervention measures aimed at reducing its incidence and prevalence, Ahorlu *et al* (1997); Nuwaha (2002), as opposed to some communities in developing countries that associate the disease with witchcraft, Nuwaha (2002).

Malaria remains a leading cause of morbidity and mortality in Kenya, especially in young children and pregnant women. It accounts for 30% of outpatient attendants and 19% of admissions to health facilities, MOH (2006). This compares well with the prevalence observed in this study of four episodes a year. Malaria is the most important cause of death in children under 5 years of age and is estimated to cause 20% of all deaths in this age group, MOH (2006). Parasite prevalence amongst childhood communities often exceeds 50% in high malaria risk areas such as the coastal endemic zone, MOH (2006). This was not observed in this study since the study community mainly comprised of adults who have developed immunity to the disease. High malaria prevalence as the one observed in this study may have a significant impact on the well-being and economic potential of the community. A single malaria episode can result in the loss of 5-20 days of productive labor per year. This means, therefore, that 10-40 days are lost every year for an average sized family (six members) with two adults, Tabuti (2008). This translates to lowered income earnings. Among children sufferers, malaria causes absence from school and lethargy when in class leading to poorer academic performance, which may, in turn, lead to long term social consequences. In addition to the above indirect social and economic costs, there is the direct cost of treating malaria or purchasing material to stop mosquito bites such as mosquito nets, Tabuti (2008). The estimated cost for treating a single malaria episode in

Kenya is put at USD 0.8, MOH (2006). For a family of six people, suffering an average of four episodes a year, this translates into a total cost of USD 19.2 every year.

Most knowledge on medicinal plants is transferred orally in many communities, Fratkin (1996) and there is therefore the danger of losing this precious cultural heritage, Muthaura *et al* (2007). In view of the rapid loss of natural habitats, traditional community life, cultural diversity and knowledge of medicinal plants, an increasing number of ethnobotanical inventories need to be established, Van Wyk *et al* (2002). The exploitation of traditional herbal practices depends to a large extent on local traditional knowledge, Tabuti (2008). Traditional knowledge relevant to the treatment of malaria was found to be high amongst the study community.

There is general consensus that traditional knowledge must be conserved because of its vital role for human wellbeing. It is often argued that if traditional knowledge which has been generated over a long period of time is lost, exploitation of plants among other things will become difficult if not impossible, Tabuti (2008). Among the reasons traditional knowledge relevant for the exploitation of herbal medicines is considered reliable is that indigenous communities through a period of long experimentation with herbal medicines are likely to have retained those that are effective and tolerably safe while discarding preparations with low efficacy or acute toxicity, Balick (1990); Cox (1990); Van Wyk and Wink (2004); Tabuti (2008).

One of the objectives of the current study was to document the ethnodiagnostic skills utilized by the Digo community to diagnose malaria. Indeed, researchers need to document how people describe the signs (or symptoms) of illnesses, Heinrich *et al* (2009). The study community has developed abundant ethnodiagnostic skills for malaria which forms the basis of their traditional

bioprospecting techniques. The respondents interviewed in the current study had good knowledge about malaria and readily distinguished it from other illnesses on the basis of widely accepted malaria signs and symptoms, Tabuti (2008).

The community recognized that the clinical features of uncomplicated and severe malaria included chills, profuse sweating, joint pains, abdominal pain, diarrhea, vomiting, anorexia and inability to stand. Malaria continues to be a major health challenge in Kenya especially due to the emergence of parasite resistance to the commonly used and relatively cheap antimalarials. Knowledge about malaria has steadily improved in Kenya, but some misconceptions still remain about the causes and symptoms of severe malaria, and this were also documented in this study. However, majority of the respondents knew that malaria was spread by mosquitoes and one of the major symptoms of the disease was fever. This relatively good understanding of the causes and signs of the disease may help in the implementation of intervention measures aimed at reducing its incidence and prevalence since the Digo knowledge about the transmission and major symptoms of disease are congruent with science and they do not associate it with witchcraft, as do some communities elsewhere, Nuwaha (2002).

The procedure of Meyer *et al* (1982) was adopted to determine the lethality of cytotoxic drugs and crude plant extracts to brine shrimp. The *Artemia* bioassay is attractive for a variety of reasons, including (1) the commercial availability of the cysts, Togulga (1998), (2) *Artemia* can be maintained indefinitely in the laboratory in their cyst form and are easily induced to hatch Caldwell *et al* (2003), (3) the assay is quick, simple and performed at low cost, Parra *et al* (2001), (4) it requires small sample volume and can be performed with high sample throughput

(microplates), Pelka *et al* (2000); Molina Salinas and Said Fernandez (2006), and (5) it complies with animal ethics guidelines in many countries, for example, the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes, NHMRC (2004). For the detection of toxicity of plant extracts, Parra *et al* (2001) and cyanobacterial toxins, Lee *et al* (1999); Metcalf *et al* (2002); Akin-Oriola and Lawton (2006), *Artemia* bioassay has been demonstrated to provide a viable alternative to the mouse bioassay, which is expensive and associated with ethical constraints.

In the current study, maximum mortalities took place at a concentration of 1000 µg/ml whereas least mortalities were at 10 µg/ml concentration. The cytotoxic activity was considered weak when the LC_{50} was between 500 and 1000 µg/ml, moderate when the LC_{50} was between 100 and 500 µg/ml, as strong when the LC_{50} ranged from 0 to 100 µg/ml, Padmaja *et al* (2002) and designated as non toxic when the LC_{50} value was greater than 1000 µg/ml, Meyer *et al* (1982). Accordingly, 36% (62) of all the investigated crude extracts demonstrated strong toxicity while 40% (68) of all the 170 crude extracts evaluated for cytotoxicity in brine shrimp (*A.salina*) assay exhibited moderate activity, indicating that they could not make safe antimalarial remedies. Only 15% (25) of all the crude extracts assayed were shown to be non toxic to brine shrimp larvae, Meyer *et al* (1982), validating their use as safe antimalarial herbal remedies.

The current study has concluded that in Msambweni district, traditional methods of treatments based on medicinal plants are still an important part of social life and culture and the acceptability of these plants as claimed effective remedies is quite high among the population of this area. The claimed therapeutic value of the reported species call for modern scientific studies

to establish their safety and efficacy and to preserve and document this flora which may otherwise be lost due to erosion of age old traditional methods of biodiversity conservation and medicinal knowledge as had been practiced in the *Kayas*, Muthaura *et al* (2007). There is general consensus that traditional knowledge on the use of medicinal plants must be conserved because of its vital role for human wellbeing. It is often argued, that if traditional knowledge which have been generated over a long period of time is lost, exploitation of plants among other things will become difficult if not impossible. Among the reasons traditional knowledge is considered reliable for the exploitation of herbal remedies is that indigenous communities through a period of long experimentation with herbal medicines are likely to have retained those that are effective and tolerably safe while discarding preparations with low efficacy or acute toxicity, Balick (1990); Cox (1990); Van Wyk and Wink (2004).

Eighteen (18) plant species, namely; *Aloe deserti* Berger (Liliaceae), *Launea cornuta* (Oliv and Hiern) C. Jeffrey (Compositae), *Ocimum bacilicum* L. (Labiatae), *Teclea simplicifolia* (Eng) Verdoon (Rutaceae), *Gerranthus lobatus* (Cogn.) Jeffrey (Cucurbitaceae), *Grewia hexaminta* Burret. (Tiliaceae), *Canthium glaucum* Hiern. (Rubiaceae), *Amaranthus hybridus* L. (Amaranthaceae), *Combretum padoides* Engl and Diels. (Combretaceae), *Senecio syringitolius* O. Hoffman. (Compositae), *Ocimum suave* Willd (Labiatae), *Aloe macrosiphon* Bak. (Liliaceae), *Laudolphia buchananii* (Hall.f) Stapf. (Apocynaceae), *Heeria insignis* Del. (Anacardiaceae), *Rottboelia exaltata* L.F (Gramineae), *Pentanisia ouranogyne* S. Moore (Rubiaceae), *Agathisanthenum globosum* (A. Rich) Hiern (Rubiaceae), and *Grewia trichocarpa* Hochst ex A. Rich (Tiliaceae) are documented from this region for the first time for the treatment of malaria.

The current study has also demonstrated for the first time that majority of plant species used as antimalarial remedies by the Msambweni community possess strong cytotoxic effects(LC_{50} <100 µg/ml) against brine shrimp (*Artemia selina*) marvae, and could not make safe antimalarial herbal remedies. The documented`families s□em to include many medicin ▪ lly interesting geŮera and species and there is still much to elucidaŴg the biological activity of active compounds from these plants. New techniques for bioactivity guided isolation of active compounds, such as microfractionation, may enhance the finding of novel scaffolds/new source antimalarial compounds from the reported species.

The current study concludes the following:

- Herbalists from Msambweni district have a good understanding on treatment and management of malaria using phytomedicines
- The Msambweni community have a good knowledge on malaria treatment as well as its recognition and control
- The Msambweni community have developed an ethnodiagnostic skill for malaria which their lead to traditional bioprospecting
- Upto 36% of antimalarial plants used by the Msambweni community have strong cytotoxic activity against *Artemia salina* larvae and could not make safe antimalarial herbal drugs. This extracts could provide novel scaffolds for anticancerous drugs.

The current study recommends the following:

- Selection of antimalarial plant species for pharmacological, toxicological and phytochemical studies
- Characterisation of novel cytotoxic compounds from extracts exhibiting strong toxicity against *Artemia salina* larvae
- Collection and preservation of the valuable popular knowledge concerning antimalarial plant use
- Addition of information to the valuation of biodiversity and to forward suggestions for its sustainable use and conservation
- Establishing comparisons with other territories sharing similar characteristics
- Selection of plants for isolation of new and novel molecules for development as antimalarials and
- Setting up health policies in regard to prevention and treatment of malaria.

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APPENDICES

Appendix 1: List of original publications

This thesis is based on the following original publications which are referred to in the text; and on unpublished results presented in the text.

1. **J.M. Nguta J.M. Mbaria D.W.Gakuya P.K.Gathumbi.** and **S.G.Kiama. (2010b):** Traditional antimalarial phytotherapy remedies used by the South Coast community, Kenya. Journal of Ethnopharmacology **131:** 256-267.

Journal of Ethnopharmacology 131 (2010) 256–267

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jethpharm

Traditional antimalarial phytotherapy remedies used by the South Coast community, Kenya

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ARTICLE INFO

Article history:
Received 26 February 2010
Received in revised form 14 June 2010
Accepted 17 June 2010
Available online 30 June 2010

ABSTRACT

Aim of the study: This study was conducted to document herbal medicines used in the treatment of malaria as well as the existing knowledge, attitudes and practices related to malaria recognition, control and treatment in South Coast, Kenya.
Methods: Data was collected using semistructured questionnaires and interviews. A focused group dis-

2. **J.M. Nguta J.M. Mbaria D.W.Gakuya P.K.Gathumbi.** and **S.G.Kiama. (2010a):** Antimalarial herbal remedies of Msambweni, Kenya. Journal of Ethnopharmacology **128**: 424-432

Journal of Ethnopharmacology 128 (2010) 424–432

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jethpharm

Antimalarial herbal remedies of Msambweni, Kenya

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ARTICLE INFO

Article history:
Received 4 October 2009
Received in revised form 7 January 2010
Accepted 10 January 2010
Available online 22 January 2010

Keywords:
Medicinal plants
Traditional treatments
Antimalarial
Msambweni
Ethnobotany

ABSTRACT

Malaria is a serious cause of mortality globally. The disease is of regional concern in Africa and of national interest in Kenya due to its high morbidity and mortality as a result of development of resistant strains of *Plasmodium falciparum* to many existing drugs such as chloroquine. Alternative medicine using herbal remedies are commonly used to treat malaria in Kenya. However, plants used in some rural areas in Kenya are not documented. Many antimalarial drugs have been derived from plants. This study was conducted to document medicinal plants that are traditionally used by the Msambweni community of Kenyan South Coast to treat malaria, where the disease is endemic. Herbalists were interviewed by administration of semistructured questionnaires in order to obtain information on medicinal plants traditionally used for the treatment of malaria. Focused group discussions held with the herbalists supplemented the interview and questionnaire survey. Twenty-seven species of plants in 24 genera distributed in 20 families were reported to be used in this region for the treatment of malaria. Labiatae, Rutaceae and

3. **J.M. Nguta J.M. Mbaria D.W.Gakuya P.K.Gathumbi J.D.Kabasa** and **S.G.Kiama. (2011):** Ethnodiagnostic skills of the Digo community for malaria: a lead to traditional bioprospecting. Frontiers in Pharmacology doi: 10.3389/fphar.2011.00030 (**INPRESS**).

Appendix 2: List of conference proceedings

1. **J.M. Nguta., J.M. Mbaria., D.W.Gakuya., P.K.Gathumbi.** and **S.G.Kiama (2010):** Natural products from plant biodiversity and malaria: In: **The proceedings** of the 12TH International Symposium on Natural Product Chemistry and Related Biological Sciences, International Centre for Chemical and Biological Sciences (ICCBS), University of Karachi, Pakistan during November 22-25, 2010.

2. **J.M. Nguta., J.M. Mbaria., D.W.Gakuya., P.K.Gathumbi.** and **S.G.Kiama (2010):** Development of ethnomedicines for management of malaria in Msambweni district, Kenya: In: **The Proceedings** of a Workshop on Regional Networks in Africa, held on October 5-9th, 2010 at the Kopanong Hotel and Conference Centre in Benoni, South Africa, near Johannesburg.

3. **J.M. Nguta., J.M. Mbaria., D.W.Gakuya., P.K.Gathumbi.** and **S.G.Kiama (2010):** Ethnodiagnostic Skills of the Digo Community for malaria: A lead to traditional bioprospecting?: In: **The Proceedings** of the **Ist** East and Central Africa Regional Symposium for the Carnegie-RISE Fellows held on September 15th, 2010 at Royale Imperial Hotel, Kampala-Uganda, East Africa

Appendix 3: Questionnaire administered to Msambweni community

UNIVERSITY OF NAIROBI

COLLEGE OF AGRICULTURE AND VETERINARY SCIENCES

FACULTY OF VETERINARY MEDICINE

DEPARTMENT OF PUBLIC HEALTH, PHARMACOLOGY AND TOXICOLOGY

P.O Box 29053, 00625, Kangemi, Nairobi Kenya, Telephone 020-2453621

Email: dept-publichealth@uonbi.ac.ke

RE: QUESTIONNAIRE FOR ETNOMEDICAL STUDY ON PLANTS USED TO TREAT MALARIA IN MSAMBWENI DISTRICT, SOUTH COAST, KENYA

The bearer of the above mentioned questionnaire, Dr. Joseph Mwanzia Nguta, is a member of staff at the Department of Public Health, Pharmacology and Toxicology, Faculty of Veterinary Medicine, College of Agriculture and Veterinary Sciences, University of Nairobi. Please offer him the necessary co-operation.

Dr. William O. Ogara,

Chairman,

Department of Public Health, Pharmacology and Toxicology.

RESPONDENTS CONSENT AGREEMENT

I----------hereby agree to participate in this study/project with my full consent and declare that to the best of my knowledge, the information that I am going to provide on the use of medicinal plants used to treat malaria in Msambweni District is true, accurate and complete.

Signature/Thumb print.....Date.....

RESEARCHERS DECLARATION

1. The following research will be undertaken with respect to the indigenous knowledge and intellectual property rights of the herbal practitioners/local community
2. The team will at no given time initiate or conduct practices that are deemed to obtain information from the respondents by intimidation, coercion or false pretence.
3. That the respondent will be informed of the objectives of the project prior to questionnaire administration and in confidence to eliminate any degree of conspiracy
4. That the research team will be under no obligation to edit or tamper the information provided by the respondent

5. That the herbalists and the local community of Msambweni District are the owners of the traditional knowledge that will be presented in this questionnaire. Consequently, any benefits that may accrue from the use of this knowledge will be shared with them.
6. That the information to be collected will be used for the described research purpose (for documentation of plants used by the Msambweni community to treat malaria and their related toxic effects) and not for any other undisclosed intention.

Signatory Researchers:

Dr. J.M.Mbaria----------

Dr. J.M.Nguta----------

NATURAL PRODUCTS PROJECT THROUGH THE REGIONAL INITIATIVE IN SCIENCE AND EDUCATION (Carnigie-AIS-RISE) NATURAL PRODUCT NETWORK (UON/MUU/SUA)

NATURAL PRODUCTS RESEARCH: INVESTIGATING THE BIOACTIVITY AND SAFETY OF ANTIMALARIAL PHYTOMEDICINES USED BY THE MSAMBWENI COMMUNITY, SOUTH COAST, KENYA.

A collaborative Carnigie-AIS-RISE Natural Product Project

By

The University of Nairobi in Kenya

Makerere University in Uganda and

Sokoine University of Agriculture in Tanzania

A QUESTIONNAIRE FOR HERBAL PLANTS USED IN THE TREATMENT OF MALARIA IN MSAMBWENI DISTRICT, KENYAN SOUTH COAST.

This questionnaire is designed to document traditional knowledge about malaria including traditional treatments, existing malaria treatment and control practices with emphasis on the traditional treatment methods using indigenous plants, plants used, method of preparation and attitudes about antimalarial herbal remedies. The study contributes to Millennium Development Goal "6" of combating HIV/AIDS, malaria and other diseases. All the information given will be treated with confidence.

SECTION A: BIODATA.

Enumerator (Name)..... Date of interview.....

Serial No.....Name of respondent.....

Division.....Location.....Sublocation.....

Village.....

Telephone.....Gender.....

(Answer by ticking {√} in the appropriate box).

1). What is your age?

a). below 18 years { } b). 18-27 years { } c). 28-37 years { } d). 38-47 years { } e). 48-57 years { } g) over 57 years

2). What is your highest level of education?

a).Primary { } b). Secondary { } c). College { }

d). University { } e). Other (Please specify)……

3).What is your religion? ----------

4). What is your professional training? ----------

5). Are you employed? a).Yes { } b). No { }

6). If yes, what is the nature of employment? ----------

7).What is your major source of income?--- ----------

SECTION B: TO BE ADMINISTERED TO THE HERBALIST

EXPERIENCE IN TRADITIONAL MEDICINE/PRACTICE

8). For how long have you practiced as a traditional herbalist?.....

9). Where do you practice as a traditional herbalist (Location)?.....

11). How did you acquire your skills as an herbalist? .....

.....

12). Do you belong to any form of group owned by herbalists? **a)** { } **b).** No { }

13). If yes, what is the name of your group?----------

---

14).List in order of priority five diseases that you commonly treat with herbs?

a).....

c)……

d)……

e)……

f)……

g)……

h)……

i)……

j)……

k)……

TRADITIONAL KNOWLEDGE ABOUT MALARIA

A). MALARIA MORBIDITY

15) .Does malaria occur in this area (**Msambweni District**) **a)** Yes { } **b).** No { }

16). What is the local name of malaria in this area?……

17).Have you ever had a malaria attack for the last one year? **a)** Yes { } **b).** No { }

18). If yes, how many malaria attacks did you have for the last one year?……

19). When is the last time you had a malaria attack .....

20). When did you treat the last case of malaria?.....

21). How do you know that someone has malaria (**What are the signs and symptoms of malaria**).....

.....

.....

.....

.....

.....

.....

.....

.....

22).What causes malaria.....

.....

.....

.....

23). What age group is commonly affected by malaria?.....

24). Which gender is most commonly affected and why?.....

.....

.....

25). Is there a particular condition that increases an individuals susceptibility to malaria (**e.g. pregnancy or any other disease or condition**).....

---

26). Other than age and gender, are there other conditions that are likely to increase the chances of getting malaria?.....

.....

.....

27). In your own opinion, how does malaria spread in this area.....

.....

.....

28). Do you associate malaria with mosquitoes**? a).** Yes { } **b).**No { }

29). Are there any other factors that are responsible for the spread of malaria? **a).** Yes { }

b). No { }

Please list them.....

---

30).How common are malaria cases in this area of Msambweni District (**tick as necessary**).

a). Very common **b).** Common **c).** Not common

31). Do you associate malaria with mosquitoes? **a).** Yes **b).** No.

32). What other factors do you associate with malaria? **Please list**

them.....

---

33). Have you treated a case of malaria referred to you by a doctor from hospital? **a).** Yes **b).** No

FACTORS RESPONSIBLE FOR CAUSING OR SPREAD OF MALARIA

34).In your opinion what factors are responsible for the development and spread of malaria.....

.....

.....

.....

.....

MALARIA CONTROL AND TREATMENT

35). In your opinion what methods would you use to protect yourself against malaria?.....

.....

.....

.....

.....

.....

36). Please tell me some of the traditional methods you use to control malaria?.....

.....

.....

.....

.....

.....

.....

37). What do you do when you suffer from malaria?----------

---

38). If self medication with herbal medicine, which plants do you use?.....

.....

.....

.....

.....

39). Do you prefer herbal medicine to drugs from hospitals? **a).** Yes { } **b).** No { }

40). If yes, how do you treat a case of malaria?.....

.....

.....

.....

.....

HARVESTING, PROCESSING AND ADMINISTRATION OF HERBAL MEDICINES FROM MEDICINAL PLANTS.

41). Which medicinal plants do you use to treat malaria and how do you use them? Please fill in the table below.

Plant(Vernacular Name)	Status e.g. Cv=cultivated; We=weed; Wi=Wild; SW=Semi wild, or both	Habitat e.g. Bm=boundary marker; Bu=bush; Cf=crop field; Cp=compound	Part used e.g. R=root; Bk=bark; L=leaves; T=tuber, F=fruit	How is it prepared

DOSAGE, PREPARATION AND ADMINISTRATION

42).Among all the plants that you have just mentioned, which plants do you commonly use to prepare herbal remedies for the treatment of malaria in order of their efficacy. Please fill the table below.

Name of plant(Vernacular)	**Part used(Roots, Tubers, Bark, Leaves, fruits, Stem, Bulb, Other(specify)**	**Preparation (powder, boil single, mixture, Soaked in water-How much of plant part in how much water?**	**Administration(Oral,Rectal,inhalation, bathed, other(Please specify)**	**Dosage (How much is given, after how long and for how long?**	**Side effects reported/remedy**

43).Are the doses different for different groups of people. **a)**. Yes { } **b)**. No { }

44). If yes, how are they different...

.....

45). How long does your medicine take before being spoiled.....

47).How many patients out of ten recover fully.....

TOXICITY

48).Can some plants cause problems if the dosage is exceeded? **a).** Yes { } **b).** No { }

49).Can you tell me the risks associated with too much dosage in the mixture you consider most risky. Please fill in the table below.

Names of most risky mixture(Name plants used in the mixture)	**Plants associated with the risk**	**Risk of overdose**	**Antidote**

50).Have you ever experienced a case of over dosage**? a**). Yes { } **b).** No { }

51).If yes, what happened and how did you respond to the side effects?.....

.....

.....

.....

52).Do you use some plants to reduce toxicity (**if at all**) in the preparation of herbal medicine to treat a malaria case? a). Yes { } **b)**. No { }

53). If yes, please fill the table below.

Name of plant used to reduce toxicity of antimalarial medicine	**Part used**	**Amount used in the mixture for malaria treatment e.g handful, half kg. etc.....**	**What is done to the plant part before mixing**

TOXIC PLANTS

54). Which plants do you consider to be toxic to both animals and humans. Please fill the table below.

Name of toxic plant (Vernacular Name)	Toxic effects/system affected

SECTION C: TO THE ADMINISTERED TO THE COMMUNITY

EXPERIENCE IN TRADITIONAL MEDICINE/PRACTICE

8). How did you acquire your skills to treat diseases using herbs? ----------

.....

.....

9).List in order of priority five diseases that you commonly treat with herbs?

a).....

b).....

c)……

d)……

e)……

f)……

g)……

h)……

i)……

j)……

k)……

TRADITIONAL KNOWLEDGE ABOUT MALARIA

A). MALARIA MORBIDITY

10) .Does malaria occur in this area (**Msambweni District**)? **a)** Yes { } **b).** No { }

11). What is the local name of malaria in this area?.....

12).Have you ever had a malaria attack for the last one year? **a)** Yes { } **b).** No { }

13). If yes, how many malaria attacks did you have for the last one year?.....

14). When is the last time you had a malaria attack .....

15). When did you treat the last case of malaria?.....

16). How do you know that you are suffering from malaria (**What are the signs and symptoms of malaria**).....

.....

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17). What causes malaria.....

.....

.....

.....

18). What age group is commonly affected by malaria?.....

19). Which gender is most commonly affected and why?.....

.....

.....

.....

20). Is there a particular condition that increases an individuals susceptibility to malaria (**e.g. pregnancy or any other disease or condition**).....

.....

.....

.....

21). Other than age and gender, are there other conditions that are likely to increase the chances of getting malaria?.....

22). In your own opinion, how does malaria spread in this area (**e.g. are mosquitoes involved**).....

.....

.....

23). Do you associate malaria with mosquitoes**? a).** Yes { } **b).**No { }

24). Are there any other factors that are responsible for the spread of malaria? **a).** Yes { }

b).No { }

If yes, Please list them.....

---

25). Have you been referred by a doctor to an herbalist? **a).** Yes { } **b).** No { }

26). When do you go to seek the services of an herbalist?----------

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27). Do you seek the services of an herbalist when you are sick? **a**) Yes { } **b)**. No { }

28). If yes, why?----------

---

29). Do you know how to treat malaria using herbal plants? **a)**. Yes { } **b)**. No { }

30). If yes, which plants do you use, inorder of priority? ----------

---

---

31). How do you prepare the plants to treat a case of malaria?----------

---

---

32). If you do not go to herbalists, why is it so?----------

---

33). Do you then use hospital drugs if you are sick from malaria? **a)**. Yes { } **b)**. No { }

34).If yes, which hospital drugs do you commonly use? **Please list them**.----------

---

---

35). If you have a malaria attack, what is your first choice? **a).** Herbalist **b).** Doctor

c). Self medication d). Do nothing

36). Are there cases where the doctor has failed and you have been cured by an herbalist?

a). Yes { } **b).** No { }

37).How common are malaria cases in this area of Msambweni District (**tick as necessary**).

a). Very common **b).** Common **c).** Not common

38). Do you associate malaria with mosquitoes? **a).** Yes **b).** No.

39). What other factors do you associate with malaria? **Please list them**.....

FACTORS RESPONSIBLE FOR CAUSING OR SPREAD OF MALARIA

40).In your opinion what factors are responsible for the development and spread of malaria.....

.....

.....

.....

.....

MALARIA CONTROL AND TREATMENT

41). In your opinion what methods do you use to protect yourself against malaria?.....

.....

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.....

.....

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42). Please tell me some of the traditional methods you use to control malaria?.....

.....

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……

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43). What do you do when you suffer from malaria?----------

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44). If self medication with herbal medicine, which plants do you use?.....

……

……

……

……

……

……

45). Do you prefer herbal medicine to drugs from hospitals? **a)**. Yes { } **b)**. No { }

46).Do you know how to treat malaria using traditional herbal remedies? **a).** Yes **b).** No

47). If yes, how do you treat a case of

malaria?.....

.....

.....

.....

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.....

HARVESTING, PROCESSING AND ADMINISTRATION OF HERBAL MEDICINES FROM MEDICINAL PLANTS.

48). Which medicinal plants do you use to treat malaria and how do you use them? Please fill in the table below.

Plant(Vernacular Name)	**Status e.g. Cv=cultivated; We=weed; Wi=Wild; SW=Semi wild, or both**	**Habitat e.g. Bm=boundary marker; Bu=bush; Cf=crop field; Cp=compound**	**Part used e.g. R=root; Bk=bark; L=leaves; T=tuber, F=fruit**	**How is it prepared**

DOSAGE, PREPARATION AND ADMINISTRATION

49).Among all the plants that you have just mentioned, which plants do you commonly use to prepare herbal remedies for the treatment of malaria in order of their efficacy. Please fill the table below.

50).Are the doses different for different groups of people. **a)**. Yes **b)**. No

Name of plant(Vernacular)	**Part used(Roots, Tubers, Bark, Leaves, fruits, Stem, Bulb, Other(specify)**	**Preparation (powder, boil single, mixture, Soaked in water-How much of plant part in how much water?**	**Administration(Oral,Rectal, inhalation, bathed, other(Please specify)**	**Dosage (How much is given, after how long and for how long?**	**Side effects reported/remedy**

51). If yes, how are they different?.....

.....

.....

52). How long does your medicine take before being spoiled?.....

TOXICITY

54).Can some plants cause problems if the dosage is exceeded? **a).** Yes **b).** No

55).Can you tell me the risks associated with too much dosage in the mixture you consider most risky. Please fill in the table below.

Names of most risky mixture(Name plants used in the mixture)	**Plants associated with the risk**	**Risk of overdose**	**Antidote**

56).Have you ever experienced a case of over dosage**? a**). Yes **b)**. No

57). If yes, what happened and how did you respond to the side effects?.....

.....

.....

.....

58). Do you use some plants to reduce toxicity (**if at all**) in the preparation of herbal medicine to treat a malaria case?

a). Yes { } **b)**. No { }

59). If yes, please fill the table below.

Name of plant used to reduce toxicity of antimalarial medicine	**Part used**	**Amount used in the mixture for malaria treatment e.g handful, half kg. etc…..**	**What is done to the plant part before mixing**

TOXIC PLANTS

60). Which plants do you consider to be toxic to both animals and humans. Please fill the table below.

Name of toxic plant (Vernacular Name)	Toxic effects/system affected