IN VITRO ANTIPLASMODIAL ACTIVITY AND PHYTOCHEMICALS SCREENING OF ETHNOMEDICINAL PLANTS USED TO TREAT MALARIA ASSOCIATED SYMPTOMS

A THESIS SUBMITTED IN FULFILLMENT

OF THE REQUIREMENT OF THE DEGREE OF

MASTER OF SCIENCE

OF

THE UNIVERSITY OF NAMIBIA

BY

Sylvia Ndeshihafela Nafuka

Student number: 200727826

April 2014

Main supervisor: Dr. D R. Mumbengegwi, (Multidisciplinary Research Center, University of Namibia)

Co-supervisor: Dr. R. Böck, (Department of Biological Science, University of Namibia)

Abstract

Malaria is a major health concern in Sub Saharan Africa and there are few effective treatment options. Namibia has diverse flora with potent antimalarial phytochemicals and high ethnomedicinal plants uses. This study investigated the phytochemical and *in vitro* antiplasmodial activity of ten ethnomedicinal plants namely: *Baikea plurijuga, Cyphostemma* spp*, Guibourtia coleosperma, Mundulea sericea, Neptunia oleracea, Diospyros mespiliformis, Acrotome inflata, Oxygonum dregeanum, Ziziphus mucronata* and *Vangueria infausta*. Crude methanol extracts from the ten ethno medicinal plants were screened for major classes of antiplasmodial phytochemical compounds i.e., terpenoids, alkaloids, flavonoids, anthraquinones and coumarins using thin layer chromatography (TLC). Total alkaloids and phenolics content of the ten crude methanol plant extracts were quantified spectrophotometrically. Based on the outcomes of phytochemical screen test, *M. sericea, D. mespiliformis* and *Cyphostemma spp* were selected for *in vitro* antiplasmodial microscopy assay of the crude methanol and aqueous extracts. The assay was done at 1% parasitaemia and 2% hematocrit in 96 wells plates for 24 and 48 hours, against *P. falciparum* 3D7A (chloroquine sensitive and sulfadoxine resistant strain). Coartem was used as a positive control while 0.5% DMSO in RPMI 1640 medium was used as negative control. Furthermore, *Cyphostemma* spp (whole plant) and *D. mespiliformis* (leaf and root) were fractionated through Sephadex LH-20 column eluted with methanol/DCM (1:1) and *n*- hexane/ethyl acetate (8:2) for the first and second round respectively. *In vitro* antiplasmodial assays and phytochemical screening of fractions were carried out at each round. TLC screening of the crude plant extracts revealed the presence of at least one of the phytochemicals screened

for. Moreover, all plant extracts were found to have *in vitro* antiplasmodial activity. The 50% inhibitory concentration (IC_{50}) values of the crude aqueous extracts were found to be 2.91 to 9.10 µg/ml for methanol extracts: 1.10 to 5.17 µg/ml. Also, the methanol plant extracts were more potent than their corresponding aqueous extracts. *M. sericea* (shoot) methanol extract had highest *in vitro* antiplasmodial activity. Fractionation of the crude methanol plant extracts yielded fractions that vary phytochemically as revealed by TLC screen. Additionally, *in vitro* antiplasmodial assay of the first round fractions indicated increased activity compared to the crude extracts at 24 and 48 hours. Lastly, anthraquinones, flavonoids and coumarins were identified as bioactive classes of antiplasmodial phytochemicals of *D. mespiliformis* (leaf & root) methanol extracts.

Keywords: *Cyphostemma* spp, *D. mespiliformis, M. sericea*, antiplasmodial activity, ethnomedicinal plants, malaria, and bioassay guided fractionation

Table of Contents

List of figures

List of tables

Acknowledgements

Most of all I would like to offer heartfelt gratitude to Almighty God for giving me this great privilege and wisdom to carry out and complete this work.

This work was funded by the Directorate of Science and Technology of the Ministry of Education, Republic of Namibia. Thank you very much for funding this work.

I take pride in acknowledging my main supervisor Dr. Davis R. Mumbengegwi, Deputy Director of the Multidisciplinary Research Centre at the University of Namibia for inspiring and motivating me to carry out this work successfully. His timely and relevant guidance and encouragements enabled me to carry out this research work successfully. Thank you very much.

My sincere gratitude goes to my co-supervisor Dr. Ronnie Bock, Department of Biological Science, University of Namibia for his valuable guidance and sacrificing his time for consultations.

I am very much grateful to Dr. Myrtle Morkel of the University of Namibia, School of Medicine, who tirelessly and in painful moments has availed herself to our bi-weekly call for phlebotomy. Thank you very much for making *P. falciparum* tissue culture progress smoothly.

I appreciate the very faithful blood donors to the Malaria Research Laboratory; Ms. Mildred Johnson, Mrs. Julia Hoveka, Ms. Justine Nakashole, Mr. Munyaradzi

Tambo, Ms. Florence Dushimemaria, Ms. Iwanette Du preez, Mr. Frankie Cloete, Ms. Julia Mesag, Ms. Mukosha Chinsenga, Mr. Wesly Pieters and Ms. Joyce Auala. Thank you very much for not hesitating to donate blood when we urgently needed it.

I will be in doubt if I do not appreciate Ms. Iwanette Du Preez (MRC), for training on *P. falciparum* culturing. Thank you very much for your insights, diligence and time that you invested in me during the training.

My sincere appreciation goes to Dr. Renate Hans and Dr. Stefan Louw of the Department of Biochemistry and Chemistry, University of Namibia for their guidance on fractionation. Thank you very much for affording me your valuable and limited time.

Lastly, I appreciate Ms. Trezia Shituula for her assistance with language editing.

Dedication

I dedicate this work to my sister, Selma Ndatoolewe Nafuka-Shaanika who is the source of my inspiration. This is for you.

To Researchers and Scholars who are tirelessly making all efforts at all cost to discover new chemical entities from natural resources with hope to alleviate infectious diseases. I am forever inspired

Declarations

I, Sylvia Ndeshihafela Nafuka, declare hereby that this study is a true reflection of my own research, and that this work, or part thereof has not been submitted for a degree in any other institution of higher education.

Signature…..…….....

Date…….....

Sylvia Ndeshihafela Nafuka

List of abbreviations

ACT- Artemisinin based Combination Therapy

BCG- Bromo Cresol Green

DCM- Dichloromethane

DMSO- Dimethyl sulfoxide

GPS- Global Position Satellites

HCL- Hydrochloride acid

IC_{50}- 50% inhibitory concentration

IRS- indoor residual spray

ITNS- insecticide treated nets

MoHSS- Ministry of Health and Social Services

MR4- The Malaria Research and Reference Reagent Resource Center

NBRI- Namibia Botanical Research Institute

PTFE- Polytetrafluoroethylene

RBC- Red Blood Cells

RPMI- Roswell Park Memorial Institute Medium

SPR- Slide Positivity Rate

TKMI- Trans Kunene Malaria Initiative

TLC- Thin Layer Chromatography

UV- Ultraviolet

WHO- World Health Organisation

List of publications

Posters and Conference presentations

Sylvia Nafuka, Davis R. Mumbengegwi & Ronnie Bock. (2012). Identification of chemical entities for antimalarial drug development from indigenous medicinal plants using bioassay guided fractionation, PowerPoint presentation at the annual IKS Symposium, at the University of Namibia, Windhoek, Namibia on the 12 October 2012

Sylvia Nafuka, Davis R. Mumbengegwi & Ronnie Bock, (2012). Identification of antiplasmodial chemical entities from Namibian indigenous ethnomedicinal plants using bioassay guided fractionation, Poster presented at the 61th American Society of Tropical Medicine and Hygiene (ASTMH) Annual Conference on the 12 November 2012, Atlanta, Georgia, USA

Sylvia Nafuka, Davis R. Mumbengegwi & Ronnie Bock, (2012). Bioassay guided fractionation of 3 Namibian ethnomedicinal plants for potential antiplasmodial new chemical entities, Oral presentation at the Multi-lateral Initiative on Malaria Conference at Durban, South Africa, 03-10 October 2013

Sylvia Nafuka, Davis R. Mumbengegwi & Ronnie Bock. (2012). Identification of antiplasmodial phytochemicals from ethno medicinal plants used to treat malaria associated symptoms, (2013) Oral presentation at the first Annual Faculty of Science Conference

Sylvia Nafuka, Davis R. Mumbengegwi & Ronnie Bock. (2012). Identification and quantification of classes of antiplasmodial classes of phytochemical compounds from medicinal plants, Poster presented at annual University of Namibia Research Day, September 2012

Sylvia Nafuka, Davis R. Mumbengegwi & Ronnie Bock. (2013). Identification of antiplasmodial chemical entities from Namibian indigenous ethnomedicinal plants using bio assay guided fractionation, Poster presented at the annual University of Namibia Research Day in October 2013

Peer reviewed journal article and book chapter

Sylvia Nafuka & Davis R. Mumbengegwi (2013). Phytochemical Analysis of selected ethnomedicinal plants used to treat malaria associated symptoms in Northern Namibia, *International Science and Technology Journal of Namibia*, 2(1), 78-93

Iwanette Du preez, **Sylvia Nafuka**, Davis Mumbengegwi & Ronnie Bock, (2012) Indigenous Knowledge of medicinal plants used as treatment for malaria & associated symptoms. *Book Chapter in press*

CHAPTER ONE: INTRODUCTION

Malaria is a parasitic disease that is transmitted to human beings by female *Anopheles* mosquitoes. There are four main malaria causing parasites namely: *Plasmodium vivax, Plasmodium ovale, Plasmodium malariae* and *Plasmodium falciparum*. Additionally, in some parts of Southern Asia there are reports of *P. knowlesi* (the simian *Plasmodium* species) infecting human beings, (White *et al*, 2013; Kantele & Jokiranta, 2011). The onset malaria symptoms are nonspecific. Usually, they are characterised by vague of general body weakness, reoccurring fever, nausea, vomiting, headache, fatigue, muscle aches, and abdominal discomfort. Generalised seizures are associated specifically with *falciparum* malaria and can be followed by coma.

Malaria is a worldwide public health concern. The World Health Organization (WHO) Malaria World Report, (2013) reports an estimate of 207 million cases and 627 000 deaths globally in 2012 alone. What is worse is that, over 70 countries are malaria endemic and globally an estimated 3.4 billion people are at risk of malaria infection. People living in Sub-Saharan Africa are at the highest risk of acquiring malaria. Particularly, 80% of cases and 90% of deaths due to malaria occur in the WHO African region, with children under the age of five and pregnant women mostly affected. In Namibia, malaria case incidences and mortality are declining due to the preventative and control measures that are implemented by MoHSS. According to the WHO World Malaria Report (2012, pp 54), Namibia has achieved reduction in malaria case incidences or malaria admission rates with more than 75 %. In 2011, the Ministry of Health and Social Services (MoHSS) reported 1860

confirmed malaria cases among 61 861 people tested, giving a Slide Positivity Rate (SPR) of 3% at national level, (MoHSS Malaria Strategic Plan 2010-2016). Based on low malaria case incidences, the continuations and the scaling up of interventions and control measures, it is propitious that Namibia is now moving toward pre-elimination phase in the malaria control continuum in near future, (Noor *et al*, 2012).

However, in some malaria endemic countries, malaria incidences, mortality and morbidity are escalating due to failures in the implementations of control interventions recommended by the WHO. Particularly, there are reports about *falciparum* malaria (the most lethal and wide spread form of malaria) treatment failures with ACTs (Artemisinin based Combination Therapies) which are the first line antimalarial drug for uncomplicated and severe malaria, (Beshir *et al*, 2013; Noedl *et al*, 2008; Rogers *et al*, 2009). Malaria treatment failures are due to *P. falciparum* developing reduced sensitivity to ACTs , (Whegang *et al*, 2010) and the parasites recrudescence after treatment with ACTs, (Dondorp *et al*, 2012). Reduction of ACT's sensitivity to *P. falciparum* is authenticated by reports at the Thailand-Myanmar and Thailand - Cambodia borders, (Dondorp *et al*, 2010: Lin *et al* 2010). It has been reported that ACT's reducing sensitivity is spreading to Africa as well, (Mita *et al*, 2009; Phyo *et al*, 2012).

Apart from the inherent weaknesses of antimalarial drugs, there is also a challenge of availability and high cost of antimalarial drugs to those that are mostly affected, especially in developing countries, (Matowe & Odeyi, 2010; Mutambingwa *et al*, 2005). Besides, ACTs are the sole antimalarial drugs that the *P. falciparum* parasite

did not develop resistance to, (Attaran *et al*, 2004; Davis *et al*, 2005; Mutabingwa, 2005). In the event of *P. falciparum* developing resistance to ACTs, there is no suitable replacement for ACTs. Therefore, lack of new antimalarial drugs in the pipeline and reduced sensitivity, highlight the urgent need to search for new antimalarial drugs to replace the currently used or to expand the antimalarial drug arsenal.

Nevertheless, when modern allopathic medicines are inaccessible to people living in developing countries, people tend to turn to natural resources such as plants for remedies, (Bodeker & Wilcox, 2004; Roessner & Dias, 2012). The use of plants for medicinal purposes has existed since antiquity and is still important for primary health care today, (Balunas & Kinghorn, 2005). According to the WHO, (2008) more than 80% of people living in developing countries still depend on herbal medicines to treat common diseases including malaria. Plants produce phytochemicals during metabolism. Although, primary roles of phytochemicals in plants are still unknown, it is known fact that many phytochemicals are pharmaceutically active against many human diseases, (Rates, 2001). Evidently, more than 62% of new small chemical drugs approved in the United States of America between 1981 and 2007 were isolated from medicinal plants or synthesized from compounds isolated form plants, (Ginsburg & Deharo, 2011). The preference for medicinal plants and their products may be because of the common belief that "what is natural is safe" and herbal medicines are readily available at low cost to people living in developing countries. Moreover, literatures have indicated that drugs

isolated from natural sources are usually more biologically active than synthetic drugs, (Ginsburg & Deharo, 2011).

Current used antimalarial drugs such as artemisinin and quinine based drug's parent molecules were indentified and isolated from medicinal plants. The above mentioned drugs were discovered with the help of the fact that their plants were they are isolated from are used in traditional setting to treat malaria associated symptoms, (Butler *et al,* 2010; Karunamoorthi *et al*, 2013). This supports the fact that ethnomedicinal plants are viable sources of novel antimalarial compounds or may provide chemical precursors as leads to new antimalarial compounds. This also presents an opportunity to explore ethnomedicinal plants for new antimalarials.

Namibia has an abundance of over 4300 plant species including 17% endemic plant species, (Botanical Society of Namibia, 2009). Most of these plant species can be found in the Northern region of the country where malaria is endemic and traditional medicines are used extensively, (Cheikhyoussef *et al,* 2010; Von Koenen, 2001; Hedimbi & Chinsembu, 2010). Some studies were conducted to document the use of plants to treat different ailments including malaria associated symptoms, (Cheikhyoussef *et al*, 2010; Koenen, 2001; Hedimbi & Chinsembu, 2010). Despite rich ethnomedicinal use of plant in Namibia, there is not enough data about the biological evaluation of plants to treat malaria in order to support their claimed use in traditional medicine. In addition, there is a lack of data on the presence and absence of major classes of antiplasmodial bioactive phytochemicals. Most vital, the bioactive phytochemicals responsible for antimalarial activity in traditional medicine are not yet screened for and isolated from ethno medicinal plants in Namibia.

With the advent of technologies to screen plants for their phytochemical content, it has been made easier and robust to explore ethnomedicinal plants for potential bioactive phytochemicals. Such techniques are: the use of preliminary test tube methods (Harbonius methods) and TLC analysis to screen plant extracts for the presence and absence of phytochemicals as well as chromatographic methods to partition multi constituent plant extracts into fractions and subsequently identify the bioactive phytochemicals. Bioactive phytochemicals from ethnomedicinal plants are usually isolated through bioassay guided fractionation. Bioassay guided fractionation regime entails the sequential separation of the active multi constituent plant extract into fractions. Each fraction is evaluated in a biological assay against a disease model and chemical constituents are identified at each step of fractionation, until new compound(s) is obtained and characterised. Simultaneously, the development of continuous *in vitro* culturing of *P. falciparum* by Trager and Jensen, (1976) and subsequent development of microscopy method to analyse *in vitro* bioassays, (Chodhury & Ghosh 1985: Warhurst, & Williams, 1996), also made it possible to screen plant extracts for antiplasmodial activity.

In Namibia, only one study has applied an ethnobotanical approach to evaluate plants for antiplasmodial activities, (Dupreez, 2012). But, this study is the first of its kind to attempt to isolate and identify phytochemicals compounds from ethnomedicinal plants using a bioassay guided fractionation regime. This study evaluated the phytochemical content of 10 ethnomedicinal plants used to treat

malaria associated symptoms in Ohangwena and Omusati region. The plants are as follows: *Baikea plurijuga, Cyphostemma* spp, *Guibourtia coleosperma, Mundulea sericea, Neptunia oleracea, Diospyros mespiliformis, Acrotome inflata, Oxygonum dregeanum, Ziziphus mucronata* and *Vangueria infausta*. Amongst the ten plants, the following three plants; *Mundulea sericea* (shoot and leaf), *Diospyros mespiliformis* (root and leaf) and *Cyphostemma* spp (whole plant) were selected for evaluation of *in vitro* antiplasmodial activity against *P. falciparum* 3D7A.

1.1. Statement of the problem

Many people living in Northern Namibia use ethnomedicinal plants to treat malaria associated symptoms. However, many plants are not evaluated for antiplasmodial activity in biological assays in order to confirm their efficacy. In addition, major classes of antiplasmodial phytochemical compounds are not yet screened for and quantified. Hence, there is a pressing need for the scientific evaluation of medicinal plants for antiplasmodial activity as well as the screening of bioactive phytochemicals. This can serve as basis for development of novel antimalarial drugs from plants in Namibia.

1.2. Research hypotheses

Namibia possesses unique, biological diverse and rich ethnomedicinal use of plants. Effectiveness of plants to treat malaria associated symptoms in traditional medicine is directly linked to plant's phytochemical content and the activity of the phytochemicals. Therefore, it is hypothesized that the selected ethnomedicinal plants contain potent antiplasmodial phytochemicals.

1.3. Objectives of the study

The main objectives for this study are:

* To screen crude plant extracts for the presence and absence of phytochemicals with known antiplasmodial activity.
* To screen crude plant extracts in an *in vitro* antiplasmodial bioassay in order to verify their ethnomedicinal malaria uses.
* To fractionate crude extracts in order to link antiplasmodial activities to a specific phytochemical compounds.

1.4. Research questions

* Do the aqueous and methanol extracts from the selected plants have antiplasmodial activity?
* What are the classes of antiplasmodial of phytochemicals of the selected ethnomedicinal plants?
* Can the antiplasmodial activity be linked to any identified classes of phytochemical compounds?

1.5. Significance of the study

Namibia possesses rich and diverse ecological system with unique plant species having unique properties. Plants contain various phytochemicals that are active to human pathogens. For this reason, plants are used effectively in traditional medicines and many allopathic drugs owe their origin from plants. This study provided scientific basis of the use of the selected medicinal plants to treat malaria

associated symptoms. The study, revealed the presence and absence of major antiplasmodial phytochemicals of the selected plants. The phytochemicals identified from the selected plants in this study can be the basis for pharmacological investigations and chemical modifications in order to develop new antiplasmodial drugs. The antiplasmodial efficacy of the selected crude plants extract revealed in this study also contributes to the evaluation of indigenous plants for possible antimalarial drug development. Additionally, this information can also partially assist in decision making by traditional healers and traditional medicine users about extracts preparations, dosage formulation and administration. This study can also assist the Traditional Medicine Council of MoHSS regarding the use of selected plants in traditional medicine as treatment options to people affected by malaria with limited access to modern allopathic medicines. Furthermore, this study is a basis for the development of herbal and complementary medicines. Finally, the results can be a rationale to sustainable utilization of these plants and the realization of their commercial values.

1.6. Limitations of the study

The main aim of this study was to evaluate the use of selected ethnomedicinal plants to treat malaria associated symptoms in traditional settings. In traditional settings, plant extracts preparation is a complex process that involves performance of rituals and the combination of different plant species and possibly other organisms to develop effective treatment concoctions. This study was limited to the therapeutic activity of single plant species and it disregards other aspects that are used in traditional settings to mix extracts. For this reasons, the conclusions drawn from this study about the efficacy of the selected plants is not a representation of the whole

traditional healing system. Secondly, crude extracts comprises a mixture of compounds. Some of these compounds could have the same R_f values or the spots could overlap making it difficult to detect colour changes. Also the detection limit for TLC is high thus making it difficult to detect compounds present in small amounts.

Thirdly, a laboratory *P. falciparum* 3D7A model was used to evaluate the *in vitro* antiplasmodial activity of the selected plants instead of a *P. falciparum* clinical model. Hence; the conclusions about the efficacy of the selected plants do not reflect their activity in clinical settings.

CHAPTER 2: LITERATURE REVIEW

2. Overview of the four malaria parasites

P. falciparum is the most lethal *Plasmodium* species. Hence, it causes the highest mortality. It accounts for 80% of all malaria infections and 90% of malaria related deaths in Namibia and the rest of the WHO Africa malaria regions. Infections with *P. falciparum* can lead to death within hours to few days post infection, (Trampuz *et al*, 2003). For this reasons, *P. falciparum* was used for *in vitro* antiplasmodial bioassay of crude plant extracts and fractions in this study. *P. malariae* causes benign malaria that can result in chronic infections that last for years (Mueller *et al*, 2007; Siala *et al*, 2005). *P. vivax* also causes benign malaria, but reoccurring and often less fatal. Due to the persistent liver stages, *P. vivax* can cause relapse up to 5 years post infection, (Pukrittayakamee *et al*, 2004). *P. ovale* is related to *P. falciparum* and *P.*

vivax, but relatively rare and less fatal. *P. ovale* also has a persistent liver stage which can result in relapse after several months.

The *Plasmodium* parasites are transmitted to human beings by a bite of a female anopheles mosquito that deposits its sporozoites under skin and then the sporozoites travel to the liver and invade the hepatocytes. 'Malaria can also be transmitted through perinatal transmission and through sharing of infected needles by drug users' (http://www.cdc.gov/malaria/about/faqs.html). According to the MoHSS Malaria strategic plan, 2010-2016 and the WHO World Malaria Report (2012), three *Plasmodium* vectors found in Namibia are; *Anopheles arabiensis, Anopheles gambiae* and *Anopheles funestus* of which *Anopheles gambiae* and *Anopheles funestus* are the most prevalent.

Typical onset malaria symptoms are vague and usually accompanied by fever, the hallmark symptom of malaria. The manifestations of other symptoms depend on the age of the patient. For example, typical symptoms in children and adults patients with uncomplicated malaria are usually fever and vomiting. Other symptoms are headache, a combination of vomiting and fever, chills and muscle ache (Banister & Mitchell, 2003). Additionally, severe malaria in children is manifested by symptoms such as: impaired consciousness, convulsion, respiratory distress, severe anaemia, hypoglycaemia, metabolic acidosis, and hyperlactataemia, (Crawely, 2010). On the other hand, pregnant women infected with malaria experience fever, anaemia and hyperglycaemia, (Warrel & Gilles, 2002). Other variable symptoms in pregnant women include paroxysms, enlargement of the spleen, a combination of diarrhoea

and vomiting and convulsion, (Bermen, 2001). The severity and course of clinical symptoms depend on factors such as the species and strain of the infecting *Plasmodium* parasite, age of the patient, genetic formation of the parasite, malaria-specific immunity, nutritional status of the patient and previous exposure to antimalarial drugs, (White *et al*, 2013).

2.1. Malaria situation in Namibia

In Namibia, malaria case incidences and admission rates have decreased with more than 75%, (WHO World Malaria Report, 2012), this encourage the feasibility of malaria elimination (see figure 2) in Namibia by 2020, (Alegana *et al*, 2013). However, there is a possibility of resurgent malaria transmission due to cross border transfer from neighbouring countries that are still in the control phase and malaria transmission is high, such as in Angola, (Noor *et al*, 2013). In Namibia malaria prevalence is highly seasonal and geographical. Malaria is endemic in the Northern regions, particularly Okavango East and West regions, Ohangwena and Omusati region, (see figure 1). The Central and Southern regions are characterised as malaria free zones. Generally, there are high malaria transmission and high case incidences during summer and spring.

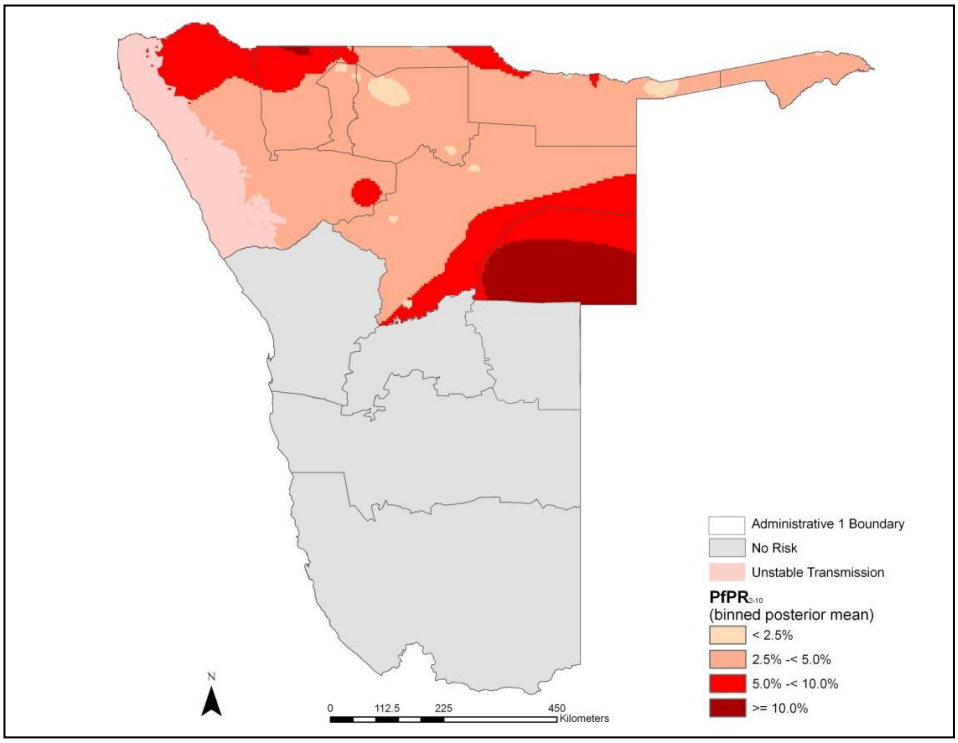


Figure 1: Current malaria transmission patterns in Namibia. Sources: (MoHSS Malaria Strategic Plan 2010-2016).

2.2. Malaria burden

The WHO has adopted a method to assess the burdens inflicted on human beings by diseases called disability adjusted life years (DALYs), (Bermen *et al*, 2004). DALY is defined as cumulative measure of premature mortality, morbidity, and disability, (Berman, 2004). This metric index can also be used for analyzing cost-effectiveness of the major interventions to combat diseases. According to Sumadhya *et al*, (2010), approximately, 35.4 million DALYs are lost in Sub Saharan African alone due to mortality and morbidity of malaria alone. Although, malaria transmission is gradually reducing in Namibia, many lives have been jeopardised by malaria. There is now evidence that uncomplicated and severe malaria have negative effects on the cognitive behaviour of children and therefore, impairs their school performance,

(Sumadhya *et al*, 2010). Additionally, pregnant women infected with malaria tend to give birth to infants with low birth weights and this can results in high infant mortality, (Huynh *et al*, 2011). (Sabot *et al*, 2010; Sachs & Malaney, 2002) indicated that, malaria control is one of the major barriers to socio-economic development in many countries. African governments use large portions of their gross domestic product for malaria control. For example, the WHO World Malaria Report (2012) stated that 90% of the finances used for malaria vectors control and treatment in Namibia in 2011 were channelled by the government and only 10% was donated. These finances could have been used for other purposes such as fighting poverty, improving educational services or creating more employment opportunities.

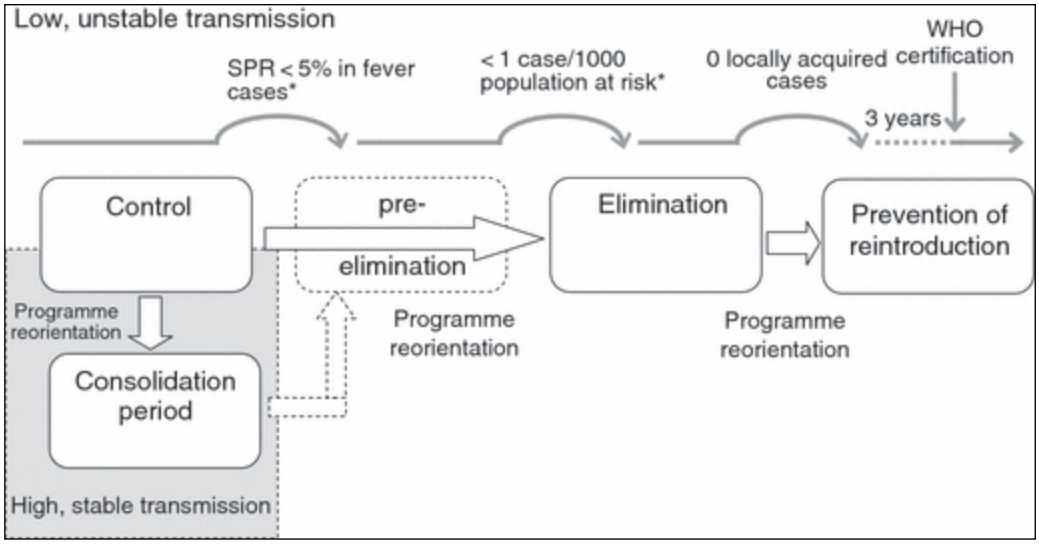


Figure 2: The malaria control to elimination continuum in high and low transmission settings. Sources: (Mendis *et al,* 2009).

2.3. Malaria prevention

Fortunately, malaria transmission can be prevented at household and government level. At household level, individuals that are living in malaria endemic regions must

ensure the use of external barriers against mosquito bites, such as by sleeping under ITNs every night, wearing long sleeved clothing and applying mosquito repellent. Furthermore, stagnant water in the proximity of homesteads should be avoided as this serves as a breeding site for mosquitoes.

The WHO has adopted various approaches that integrate preventative measures and chemotherapy for malaria endemic countries to adopt. These include:

- Vector control through the use of insecticide-treated nets (ITNs),
- Indoor residual spraying (IRS) and, in some specific settings, larval control Chemoprevention for the most vulnerable populations, particularly pregnant women and children under the age of five,
- Confirmation of malaria diagnosis through microscopy or rapid diagnostic tests (RDTs) for every suspected case
- Timely treatment with appropriate antimalarial medicines

The MoHSS Malaria Strategic Plan, 2010-2016 has stated that Namibia uses the following interventions to battle malaria: the use of ITNs and IRS, confirmation of malaria diagnosis through RDTs or microscopy and prompt treatment with appropriate medicine. Furthermore, a cross border programme between Angola and Namibia called Trans Kunene Malaria Initiative (TKMI) was established in 2009 to evaluate and monitor cross border malaria transfers between Angola and Namibia.

2.4. Malaria treatment

Plasmodium parasites can be killed by antimalarial drugs at both asexual and sexual stages. This is why strategies to eliminate malaria are spearheaded by antimalarial drugs. Historically, ethnomedicinal plants have always been sources of antimalarial drugs, (Wink, 2010). Two important antimalarial precursors' compounds were identified and isolated from ethnomedicinal plants. The first one is quinine where quinine based antimalarial drugs such as mefloquinine and chloroquine were modeled from. Quinine was isolated from a group of *Chinchona* species from Peru (Achan *et al*, 2011). The second is artemisinin. This is the parent compound were artemisinin based drug such as; artemether, artesenuate were modeled on, (White, 2008). The latter, was isolated from *Artemisia annua*, a Chinese ethno medicinal plants, (Miller & Su, 2011).

Classification of antimalarial drugs is based on their core functional chemical structures and therapeutic responses to the malaria parasites. Based on their therapeutic responses there are two groups namely; the blood schizonticides, these act on the asexual erythrocytes (blood stage) and partly on sexual stages of the parasites, (Saifi *et al*, 2013). The second group is the tissue schizonticides and these act on the hepatic stage (liver stage). Based on core chemical structures, there are three main groups; quinolines, sesquiterpenes and naphthaquinones.

2.4.1. Quinine based drugs

Quinine and its derivatives are the first pharmaceutical antimalarial drugs in the history of malaria treatment, (Duffy & Hobbs, 2010). Daily, (2006) described quinines as bitter tasting alkaloids that belong to the aryl alcohol group. Quinine derivatives are such as chloroquine, mefloquinie and amodiaquinine.

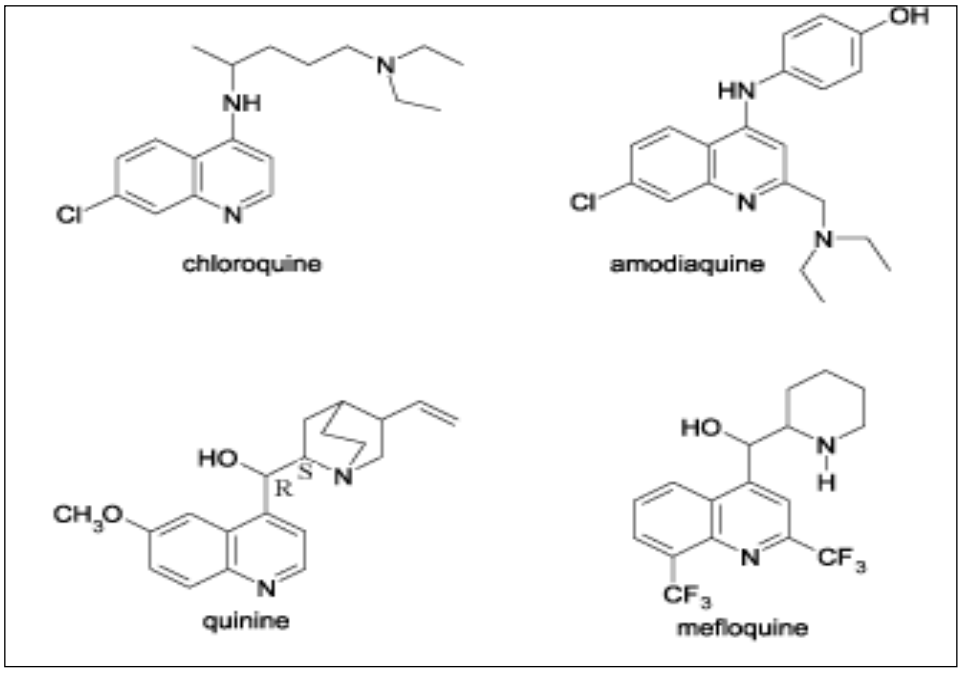


Figure 3: Chemical structure of quinine antimalarial drugs. Sources: (Delfino, *et al*, 2002)

Since its discovery until 2005, quinines and derivatives (see figure 3) were recommended to be used in combination with other antimalarial drugs as firstline treatment for uncomplicated and severe malaria, (Achan *et al*, 2009). The use of quinine as monotherapy had been banned by the WHO, (Bloland *et al*, 1993), although some African countries continue to use quinine as monotherapy for cost reasons, (Achan *et al*, 2009).

The quinolines act mostly during the blood stage of the parasite's life cycle, but some are believed to act at the liver stage as well, (Srinivasa *et al*, 2012). Nevertheless, *Plasmodium* parasites have developed resistance to quinine as from the 1950's in South America and Asia, (Wernsdorfer *et al*, 1980). Currently, it has spread throughout the world, (Baird, 2005).

2.4.2. Sesquiterpenes based drugs

The second group is artemisinin and its derivatives used in combination with other antimalaria drugs. These are now the first line treatment of uncomplicated treatment worldwide, (Sane de-Riddler *et al*, 2004). White, (2008) stated that artemisinins belong to sesquiterpenes lactones. Many derivatives such as; artesunate, dihydroartemisinin, artemotil (known as arteether) and artemether (see figure 4.) were later synthesized from the lead compound, (Krinshna *et al*, 2008). In 2006, the WHO recommended four ACTs namely; artesunate-sulfadoxinepyrimethamine, artesunate-amodiaquine, artesunate-mefloquine and artemether-lumefantrine for uncomplicated malaria treatment, (White, 2008; WHO, 2006). Parenteral artesunate is used for severe malaria treatment in adults, (Dondrop *et al*, 2010; WHO, 2010). Additionally, in 2010, the WHO added dihydroartemisinin-piperaquine to the existing list of four recommended ACTs, (Garner, 2013). Artemether-lumefantrine, commercially known as Coartem, is recommended for uncomplicated malaria treatment in Namibia, (MoHSS Malaria Strategic Plan 2010-2016). The efficacy and safety of ACTs depend largely on the partner drug, (Ngasala *et al*, 2009). "The artemisinins produce rapid clearance of parasitaemia and rapid resolution of symptoms, by reducing parasite numbers from 100- to 1000-fold per asexual cycle of the parasite (a factor of approximately 10 000 in each 48-hours asexual cycle)", (WHO, 2010).

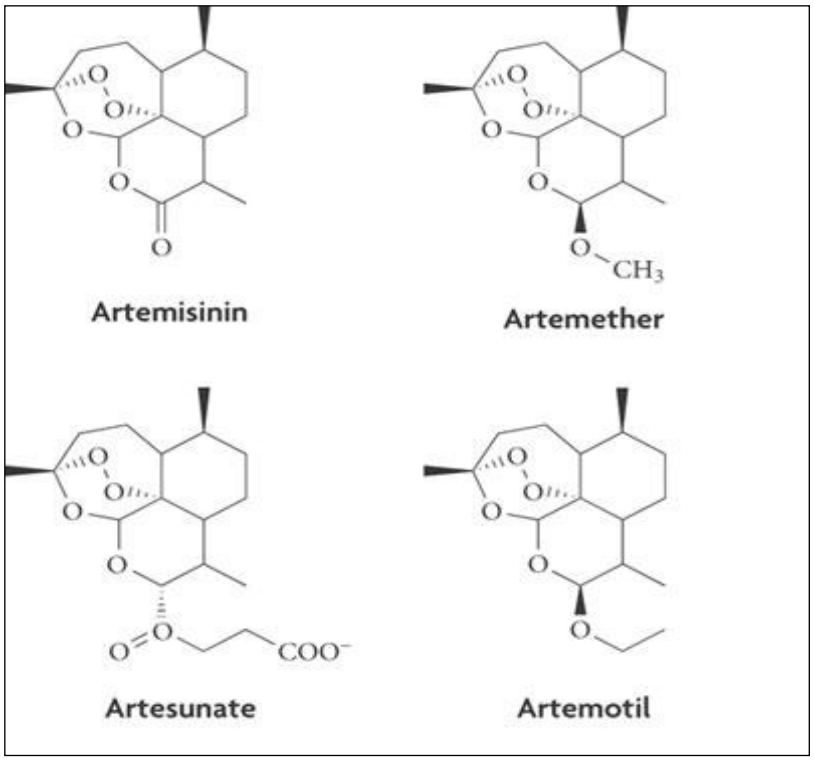


Figure 4: Chemical structure of artemisinins based drugs. Sources: (Dondrop *et al*, 2010)

According Sane de Ridder, (2008), Artemisinin derivatives kill intra erythrocytic forms of the parasites with a schizontocidal affect. The mechanism of action of artemisinin is by interacting with the haem to produce carbon centered free radicals that alkylates proteins and damage the parasites. ACTs do not have an effect on the hypnotic stage of the parasites, therefore a sequential drug; primaquine is administered to clear all hypnotic stages, (Dondrop *et al*, 2010).

2.4.3. Antifolates

Antifolates are not derived from plants but rather designed by combination of medicinal chemistry and cell biology. Antifolates are mainly used as chemoprophylaxis for travelers. According to Nzila (2006), antifolate agents used in

the treatment of malarial infection are subdivided into two classes: inhibitors of dihydropteroate synthase (DHPS), known as class I antifolates and class II anti-folates are inhibitors of dihydrofolate reductase (DHFR). The combination of DHFR and DHPS inhibitors is synergistic, hence their use in combination in the treatment of malaria. Their basic mode of action is the inhibition of the synthesis of folate co-factors that are required for nucleotide synthesis and amino acid metabolism, (Cunha-Rodrigues, *et al*, 2005).

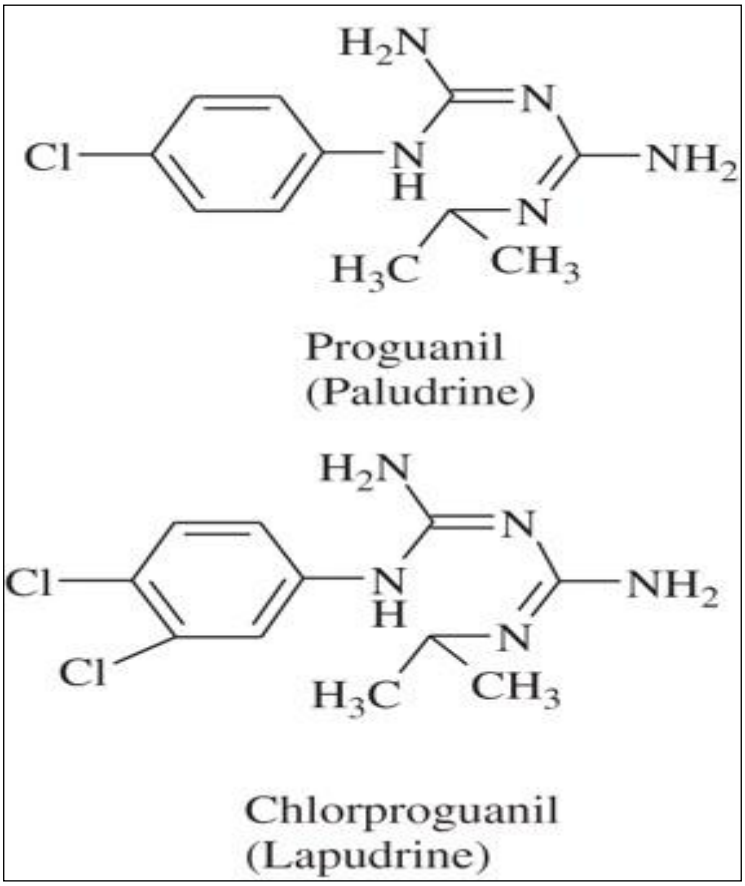


Figure 5 : Chemical structures of antifolates. Sources: (Nzila, 2006)

2.4.4. Atovaquone/proguanil combination

Atovaquone was discovered from high throughput screening of chemical libraries, most of which were hydroxynaphthoquinone. Atovaquone is used as a fixed-dose combination with proguanil (Malarone) for treating children and adults with uncomplicated malaria or as chemoprophylaxis for preventing malaria in travellers, (Nixon *et al*, 2013). Atovaquone is a hydroxynaphthoquinone that inhibit mitochondrial electron transport and collapse mitochondrion membrane potentials, (Shanks *et al*, 1998).

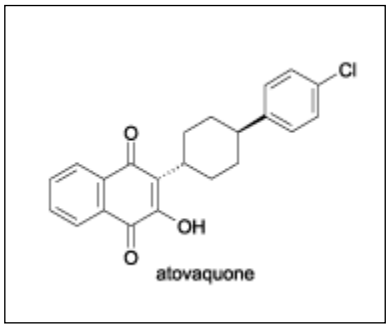


Figure 6 : Chemical structure of atovaquone antimalarial drugs. Sources: (Delfino, *et al*, 2002)

2.5. Ethnomedicinal plants uses - an overview

Medicinal plants have been used since antiquity to treat various ailments and diseases, (Kinghorn *et al*, 2011; Wells, 2011). Even when allopathic medicine is strongly emphasized, 80% of people in developing countries still depend on traditional medicine for primary health care needs, (WHO, 2008). People prefer to use medicinal plants over allopathic medicine for various reasons; relatively low cost, effectiveness, perceived safety and minimal side effects, (Fransworth & Fabricant, 2001).

Through metabolism, plants produce secondary metabolites that can be used as defence agents against predators, pathogenic microorganisms such as fungi, bacteria and virus, (Cox, 2001). Studies have indicated that some of the plant's secondary metabolites are potent to human diseases as well. Therefore, plants are widely used in traditional medicines and many natural medicinal products are derived from ethno medicinal plants.

Through trials and errors, people have built up a body of knowledge and experience about efficacy and safety of prepared plants and other natural product's extracts, (Michael *et al*, 1996). In some cultures, the use of medicinal plants is often associated with witchcraft, sorcery and superstition. This is perhaps due to the lack of scientific insights to explain and predict the curative actions of medicinal plants, (Michael *et al*, 1996).

Selection of ethnomedicinal plants for treatment of particular ailment in many traditional healing systems is mainly based on organoleptic properties of plants and Doctrine of Signature, (Leonti, 2011). Organoleptic properties of plants such as smell, taste, texture, appearance, gave lead to discovery of medicinal properties of some plants, (Leonti *et al*, 2002). For instance, through interactions with people in Omusati and Ohangwena region during the collection of plants for this project, the author learnt that the ability of some plants to treat specific aliments is correlated to smell and taste. For example, plants with strong odour and bitter taste are used for treating coughs and colds. In particular, the cold water root extract of *Pechuel-Loeschea leubnitziae* (Bitterbrush) is used for cough treatment due to its bitter taste by the Ovambo people in Namibia.

In some of the world's leading traditional healing systems, such as Ayurvedic, the Doctrine of Signatures concept is used to correlate ethnomedicinal plants to a particular ailment, (Leonti, 2011). The Doctrine of Signature is primarily based on the assumption that the physical appearance and inherent features of plants may point to the therapeutic values of ethnomedicinal plants, (Gurib-Fakim, 2006). For instance, red sap or resin exuding from plants represents effectiveness of the plant for treating blood and menstrual ailments. In addition, plant parts that are shaped in a particular way as human female body parts are correlated with the treatment of women fertility, (Parnham & Verbanac, 2011). This concept was used successfully in providing trails to the discovery of *Chelidonium majus*, an ethnomedicinal plant with yellow flowers and used in traditional settings to treat jaundice, (Gaman *et al*, 2010). A yellow coloured alkaloid, chelidonine was identified as bioactive phytochemicals responsible for jaundice treatment, (El Readi *et al*, 2012; Korien *et al*, 2013).

Masoko (2007) mentioned that the pharmacological screening of plants involves four different approaches. Firstly, the random screening approach whereby plants found in particular geographical regions are collected and screened for any disease model without relation to a specific disease. Anticancer drugs; paclitaxel, vinblastine and camptothecin were discovered using this approach, (Eisenberg *et al*, 2011). Secondly is the phytochemical targeting approach whereby plants that are a rich source of certain bioactive phytochemicals compounds are aimed. Thirdly, the taxonomic approach involves selecting plants based on taxonomic groups with

previous records of targeted pharmacological effects. Lastly, is the ethno directed approach, whereby plants are chosen based on traditional uses to treat specific diseases and related symptoms in traditional settings and subsequently screened for the similar diseases as in traditional settings. Quinine based drugs were discovered using this approach, (Allen, 2006). The last approach was used to select plants for this study because Namibia contains a diverse flora and rich ethnomedicinal plant uses whose potential is not yet explored and little research is conducted to identify bioactive. This maximise the probability of isolating novel bioactive phytochemicals from Namibian ethnomedicinal plants.

According to Fabricant & Fransworth, (2001), in an ethno botany directed approach, a typical pattern is followed to discover pharmacological potentials of plants. Firstly, traditional indigenous knowledge concerning possible therapeutic activity of plants accumulates, and then plants are used therapeutically by traditional healers. Through ethnobotanical studies, traditional healers communicate this knowledge to scientists. Plants are then collected; identified and crude extracts are screened in *vitro* bioassays for preliminary screening of desired pharmacological activities. Pure compounds are isolated by bioassay guided fractionation techniques to trace the source of the pharmacological activity in plant extracts, and the structures of the pure compounds are elucidated. *Artemisia annua* the sole source of artemisinin compounds was discovered using the ethno directed approach, (Tu, 2011).

2.6. The role of ethnomedicinal plants in diseases control in Namibia

Plants are major contributors to traditional medicine, (Greenwood *et al.,* 2008). In Namibia, fresh plants are harvested and eaten, prepared as fresh leaves teas, mashed for compression and poultices. Dried herbs can be soaked in liquid and drunk as cold or warm extracts. Plant materials can be crushed to powder and mixed with fat or calabash butter to make ointments. Herbs can also be prepared as steam bath, hot air and inhalation baths. Plants materials can also be prepared to be used as enema as a way of administration, (Von Koenen, 2001).

Plants that are used to treat various ailments have been document in ethnomedicinal studies, such as HIV/AIDS, in Zambezi region (Chinsembu & Hedimbi, 2009), various ailments in Oshikoto region, (Cheickyoussef *et al,* 2011) and among the Aakwanyama community, (Rodin, 1985). Some studies had also been conducted to evaluate the use of ethnomedicinal plants to treat bacterial diseases. (Hedimbi *et al,* 2012a; Hedimbi *et al,* 2012b) evaluated the *in vitro* antibacterial activity of *Sesamum alatum, Pechuel loceschea leubnitziae, Acrotome inflata* and *Acanthosicyos naudinianus* and against *S. flexneri*, *E. aerogenes*, *N. meningitidis*, *P. vulgaris*, and *P. aeruginosa*. Dushimemaria *et al,* (2012) evaluated the *in vitro* antimicrobial activity of *Sericea. tenuinervis* against *S. aureus* and *E. aerogenes* and its phytochemical content. Auala *et al,* (2012) evaluated the *in vitro* antibacterial activity of *Tarchonanthus camphoratus* plant extracts against *E. coli*, *S. typhii* and *S. aureus.* However, there is a gap in knowledge about evaluation of ethnomedicinal plants to treat malaria associated symptoms. Information about *in vitro* efficacy, safety, and their bioactive phytochemical constituents that may be responsible the

proposed activity as used in traditional setting is lacking. This expanded the need to venture into ethnomedicinal plant research to fill the gap in knowledge.

2.7. Phytochemicals

During biosynthesis, primary and secondary metabolites are produced. Plant's secondary metabolites are also known as phytochemicals. Phytochemicals are chemical compounds that occur naturally in plants but not important for plant's primary metabolic activities. Studies have indicated that, phytochemicals are mostly produced in response to environmental pressures such as adverse climatic conditions, immune response to infectious agents, (Lim & Bowles, 2012). Furthermore, phytochemicals serve many ecological functions such as; defence against microorganisms, insects, herbivores. Some are responsible for odour and flavours while others make the plants pigments, (Kayani *et al*, 2007). There are three main groups of phytochemicals namely; terpenoids and steroids, alkaloids, and phenolics, (Bourgard *et al*, 2001). Major classes of phytochemical with known antiplasmodial properties are; alkaloids, terpenoids, coumarins, flavonoids, chalcones, quinones and xanthones, (Dharani *et al*, 2010). In this study, emphasis was on selected major classes of antiplasmodial phytochemicals namely; alkaloids, flavonoids, coumarins, terpenoids and anthraquinones.

2.7.1. Flavonoids

Flavonoids are the most abundant polyphenols in our diets and medicinal phytochemicals, (Grotewold, 2008). The basic flavonoid structure is the flavan nucleus, containing 15 carbon atoms arranged in three rings (C6-C3-C6), (Cowan, 1999). (See figure 7) Flavonoids are sub classified into six groups namely, flavones,

flavonols, flavanols, flavanones, isoflavones, and anthocyanins, according to the oxidation state of the central C ring, (Cartea *et al*, 2011). Their structural variation in each subgroup is partly due to the structure of their side chain, (Rice- Evans & Parker, 2010). Some of the common flavonoids include quercetin, a flavonol abundant in onion and apple; the main flavanone in grapefruit; cyanidin-glycoside, an anthocyanin abundant in berry fruits and daidzein, genistein and glycitein, the main isoflavones in soybeans, (Dai & Russel, 2010).

Some hits flavonoids isolated from plants have previously shown in *vitro* antiplasmodial activity. For example, in a study done by Beldjoudi *et al*, (2003), four flavonoids namely: (R)-4-methoxydalbergione,obtusafuran, 7,4-dihydroxy-3-methoxyisoflavone and isoliquiritigenin isolated from the heartwood of *Dalbergia louvelii* indicated significant *in vitro* antiplasmodial activity against *P. falciparum* of IC_{50} values ranging from 5.8-8.7 µM. Furthermore, a novel isoprenylated flavone, artopeden was isolated from the barks of *Artocarpus champeden* and showed potent *in vitro* antiplasmodial activity with IC_{50} value of 0.045 µg/ml against *P. falciparum* 3D7, (Wahyuni *et al*, 2009).

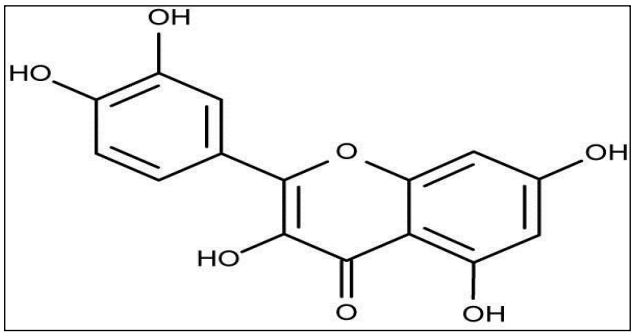


Figure 7 : A typical flavonoids, quercetin. Source; (Manach *et al*, 1995)

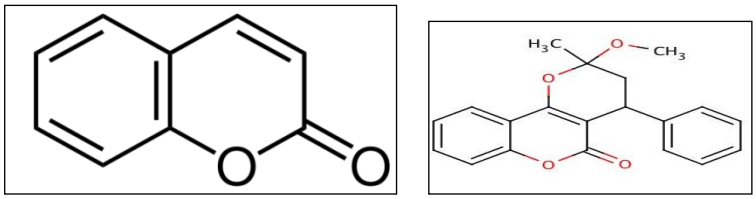
2.7.2. Coumarins

Coumarins are characterised by a benzopyrone ring (benzene ring fused a pyrone ring). Jain & Himanshu, (2012) mentioned that coumarins are classified into four sub groups namely; simple coumarins, furanocoumarins, pyranocoumarins and the pyrone-substituted coumarins. (See figure 8) Simple coumarins are hydroxylated, alkoxylated and alkylated derivatives of the parent compound, coumarin, along with their glycosides. While, furanocoumarins consist of a five-membered furan ring attached to the coumarin nucleus, divided into linear or angular types with substitution at one or both of the remaining benzoid positions. Pyranocoumarin members are analogous of furanocoumarins, but contain a six-membered ring.

Some examples of coumarins with known *in vitro* antiplasmodial activity are such as: two natural coumarins, (+)-4′-decanoyl-cis-khellactone and (+)-3′-decanoylcis-khellactone isolated from the methanolic extract of the rhizomes of *Angelica purpuraefolia*. Their *in vitro* antiplasmodial evaluation revealed growth inhibitory activity against *P. falciparum* D10 with IC_{50} values of 1.5 and 2.4 μM, respectively, (Chung *et al*, 2010). Furthermore, a novel coumarin, 5, 7-dimethoxy-8-(3′-hydroxy-

3′methyl-1′-butene)-coumarin was isolated from the root of *Toddalia asiatica* and showed *in vitro* antiplasmodial, (Oketch- Rabah *et al*, 2000).

A B



C

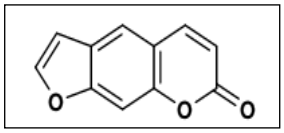


Figure 8: Core chemical structures of coumarins. A=simple coumarins, B= pyrano coumarins, C= furanocoumarins. Sources: (Jain & Himanshu, 2012)

2.7.3. Quinones

Quinones are similar to phenolic compound but they have a ketone group in the aromatic ring, (Dai & Russel, 2010). These compounds are naturally coloured. Two well-known quinones are naphthaquinones and anthraquinones (Cowan, 1999). Anthraquinones have three aromatic with a ketone group in the middle (see figure 9). Naphthaquinones have two aromatic rings and two ketone groups (see figure 9). Quinones are ubiquitous in nature highly reactive, and highly oxidized are responsible for the browning reaction in cut apple and onion, (Cowan, 1999). The antiplasmodial drug atovaquone is a naphthaquinones derivative as well, (Oliveira *et al*, 2009). Additionally, antiplasmodial properties of quinones compound, benzoquinones and its analogues isolated from the root extract of *Cordia globifera* (Boraginaceae) were reported, (Dettrakul *et al*, 2009). One naphthoquinoids isolated

from *Kigelia pinnata* root bark were assessed *in vitro* against chloroquine-sensitive (T9-96) and quinine resistant (K1) *Plasmodium falciparum* strains. 2-1-Hydroxyethyl naphtha 2, 3-b furan-4, 9-dione possessed good activity against both strains (IC_{50} values 627 nM (K1), and 718 nM (T9-96)), (Weiss *et al*, 2000).

A B

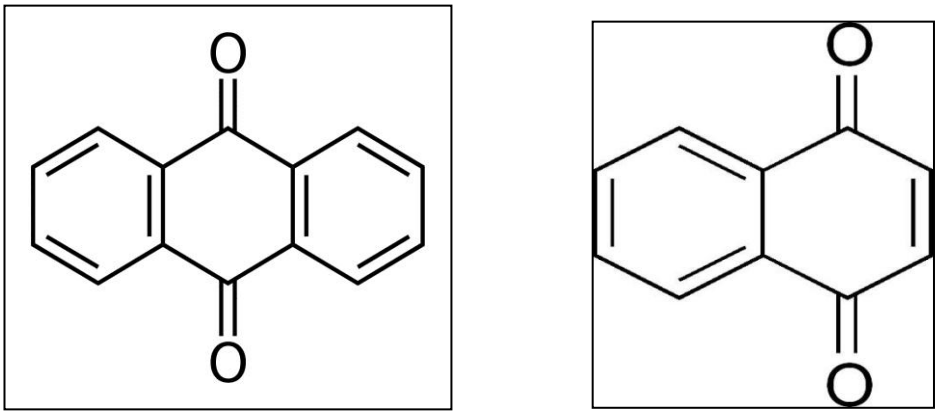


Figure 9: Core chemical anthraquinone (A) and naphthaquinones (B). Sources: (Cowan 1999)

2.7.4. Terpenoids

Terpenoids are modified terpenes whereby the methyl groups are absent and replaced by an oxygen moiety, (Zwenger & Chhandak, 2008). Terpenoids are classified according to the number of isoprene structures it contains, (see table 1). Terpenoids are found in more than 2000 plant species with representation of 60 families (Croteau *et al*, 2000). Terpenoids are the most wide spread phytochemicals. Mono and sesquiterpenoids are volatile tepernoids and chief components of essential oil. Examples of common terpenoids are menthol, camphor (monoterpenes), farnesol and artemisinin (sesquiterpenoids). Terpenoids play a particular role to malaria treatment

because, artemisinin and its derivative artemether, artesenuate etc, are currently used in combination as antimalarials.

Table 1: Classification scheme of terpenoids

Class of Terpenoids	Number of isoprene structures	Carbon structures
hemiterpenes	1	C5
monoterpenoids	2	C10
diterpenoids	4	C20
sesquiterpenoids	3	C30
triterpenoids	6	C60
tetraterpenoids	8	C40
Sesterterpenes	31/2	C25

n= number of isoprene structures

2.7.5. Alkaloids

Alkaloids are naturally occurring nitrogen containing compounds. Classification of alkaloids is based on its chemical structure, biological origin, other chemical features and biogenetic origin, (Bruce, 2008). 3 important classes of Alkaloids classes of alkaloids are such as true alkaloids, proto alkaloids and pseudo alkaloids. True alkaloids are derived from an amino acid precursor and the nitrogen atom, sourced from the amino acid precursor, forms part of the heterocyclic ring system. Proto alkaloids also derive from an amino acid precursor but the nitrogen is outside the ring system; proto alkaloids therefore do not contain a heterocyclic ring system. Pseudo alkaloids are not derived from an amino acid precursor, but the nitrogen atom forms part of a heterocyclic ring system, (Evans 2009). Alkaloids are most synthesized by plants however animal tissues e, g. Amphibian's skins also serve as sources of alkaloids. Alkaloids are one of the major classes and pioneers of natural products that exhibit antimalarial activity. In fact, quinine, the first antimalarial drug, belongs to this class, (Oliveira, *et al,* 2008).

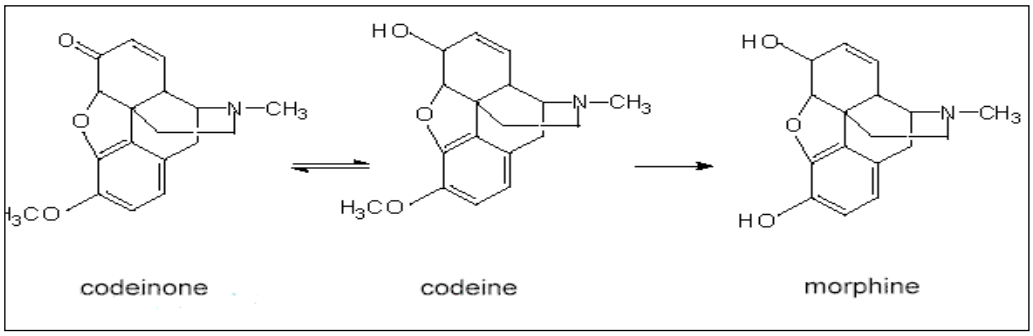


Figure 10: Alkaloids isolated from *Opium poppy*. Sources: (Francis *et al*, 2008)

CHAPTER 3: MATERIALS AND METHODS

3. Study design

The plants were collected from Omusati and Ohangwena region, Northern Namibia. The experiments were done at the Malaria Research Laboratory, at the University of Namibia, Windhoek Namibia. This study used both quantitative and qualitative approaches. The qualitative approach include information obtained in selecting the plants used in this study, the phytochemical screening for the presence and absence of major classes of antiplasmodial compounds in the crude plant extracts and fractions. The quantitative approach was used to determine total phenolics and alkaloids of the plant crude extracts, average percentage parasitaemia in the absence and presence of controls, crude plant extracts and fractions for 24 and 48 hours and to determine the IC_{50} of the parasites of the crude plant extracts.

3.1. Identification, collection and plant extracts preparation

3.1.1. Identification and collection of plants

A catalogue on medicinal, poisonous and edible plants in Namibia (Von Koenen, 2001) was used to identify and shortlist plants described to be used to treat malaria symptoms such as fever, headache, nausea, vomiting in traditional setting. The shortlisted plants were then checked against information obtained in the field through interactions with locals in the study areas.

A set of conditions used to select the plants were as follow:

* Plants species must actually be used to treat malaria associated symptoms in traditional settings in the study area.
* Plants species must be present abundantly in the study area for sustainable harvesting.
* Plants species should not be have been screened for antiplasmodial properties elsewhere with exception of *V. infausta*.
* Plant's species should belong to families that previously been shown to contain phytochemicals classes with known antiplasmodial activity.

Plants collection permit was obtained from the Ministry of Environment and Tourism in Namibia (see appendix five) and guidelines on sustainable harvesting of plants, provided by the National Botanical Research Institutes (NBRI), Windhoek, Namibia were followed. Plants materials were collected in March 2012 from Omunghete, Omusati region and Eehonge village, Ohangwena region. Roots, leaves, bark and in

some cases whole plant of selected plant species plant were collected. For taxonomical identification, collected plant's voucher specimens were prepared and submitted to the NBRI in Windhoek, Namibia. Lastly, GPS coordinates were captured and recorded at the exact locations where the plants were collected.

3.1.2. Preparation of plant extracts

The plant materials were dried at ambient temperature in a cool and dry room for 14 days. Pulverised plant's materials were prepared by grinding dry plant materials with a kitchen blender for light materials such as leaves and industrial blender for hard and coarse plant materials such as roots and bark. The very fine particles were removed by sifting and stored in labelled airtight plastic container at -20 ^{0}C.

The methanol and distilled water extracts were prepared by dissolving 10 g of the pulverised plant materials in 200 ml of the respective solvent. The methanol solvent mixtures were kept at room temperature for three days with occasionally swirling whilst the aqueous mixtures were kept in a conventional incubator at 37 ^{0}C for three days. After three days the mixtures were filtered using Whatmann no 1 filter papers. The filtrates were dried using rotary evaporator at 55 ^{0}C, 170 rotations per minutes (rpm) and 170 mbar. Remaining trace of moisture was removed by freeze dying. The dried plant extracts were weighed and stored in labelled airtight plastic conical tubes at -20 ^{0}C until further use.

3.2. Phytochemical screening of the crude methanol plant extracts

TLC was used to identify the phytochemical constituents of the methanol crude plant extracts. The methanol plant extracts were screened for presence or absence

terpenoids, alkaloids, anthraquinones, flavonoids and coumarins. The procedures used were adopted form Harbone (1998).

The TLC plates (20 x 20 cm, Merk, silica gel 60 F_{254}™), were sliced into halves. The origins were marked one centimetre above the edge of the TLC plates. The spots were marked 15 mm apart on the origin of the TLC plates. The rectangular glass tanks were presaturated with 100 ml of mobile phases as shown in table 2 for approximately 30 minutes.

The dry methanol plant extracts were reconstituted in methanol and 150 µl of the extracts were spotted on the TLC plates with capillary tubes. The spotted TLC plates were then air dried before inserting them into pre saturated glass rectangular tanks in such a way that the applied spot was above the surface of the mobile phase. The tank was then sealed.

The TLC plates were run for 20- 30 minutes in 100ml of each mobile phase for each phytochemical group as shown in table 2. After development, the solvent fronts were marked instantly with pencil and the TLC plates were air dried. The TLC plates were subsequently viewed under UV at 255 and 360 nm and later sprayed with suitable reagents to detect phytochemicals.

Table 2: TLC analysis protocol of methanolic plant extracts

Phytochemical Classes	Mobile phase	Staining reagent	UV colour before staining	Colour after staining
alkaloids	Methanol /concentrated nitric acid (200:3)	Dragendorrf reagent	Red and brown	Red and brown
coumarins	Hexane-ethyl acetate (4:1)	10% methanolic potassium hydroxide	Blue, violet, brown and yellow	Same
anthraquinones	Ethyl acetate-methanol -Water (100:17:13)	10% methanolic potassium hydroxide solution	Purple, brown and red	purple
flavonoids	Butanol: acetic acid: water (4:1:5)	1% methanolic aluminium chloride solution	Blue and yellow-brown	Blue and yellow
terpenoids	Hexane: ethyl acetate (17:3)	Liebermann-Burchard reagent	Purple	Purple

3.3. Quantification of major classes of phytochemicals

The following assays were used to quantify phenolics and alkaloids were to estimate the major classes of phytochemical content of the plants species.

3.3.1. Total alkaloids quantification

Total alkaloid content was determined by the (Shamsa *et al.*, 2008) methods. Quinine hydrochloride dehydrate, a quinine alkaloid was used as a standard. Standard solutions containing 5, 10, 15, 20 and 25 µg/ml of quinine hydrochloride dihydrate and 1mg/ml of methanol plant extracts were prepared. 1ml of each solution solutions were added to 1 ml of 2N HCl then filtered then one ml of the respective filtrates were transferred to a separatory funnel and washed with 10 ml

chloroform twice. The pH of this solution was adjusted to neutral with 0.1 N NaOH. Then 5 ml of BCG (bromocresol green) solution along with 5 ml of phosphate buffer were added to this solution. The mixtures were vigorously shaken and the complexes formed were extracted with 1, 2, 3, and 4 ml chloroform by vigorous shaking and venting. The extracts were combined while the rest of the aqueous phases were discarded. The absorbance of the complex in chloroform was measured with a GENESY spectrophotometer at 470 nm. The calibration curve of quinine hydrochloride dihydrate acid was generated as shown in appendix one.

3.3.2. Total phenolics quantification

The total phenolic content of plants was determined by using Folin Ciocalteu assay the methods were adapted from Mayank (2011). Gallic acid, a phenolic compound was used as standard. Standard solutions containing 5, 10, 15 and 20 µg/ml and methanol plant extracts were prepared. Standard solutions and plant extracts (1 ml) were added to separate 15ml conical tube. 1 ml of 8 X Folin Ciocalteu phenol reagents was added to the mixtures and homogenised. 2 m l of 1% (w/v) Na_2CO_3 was added. The mixtures were incubated for 90 minutes at room temperature. Lastly, the absorbencies of the standards and plant extracts against the blank were measured at 765 nm using a GENESY spectrophotometer. The Gallic acid calibration curve was generated as shown in appendix one.

3.4. Determination of *in vitro* antiplasmodial activity of the crude plant extracts

In order to determine *in vitro* antiplasmodial activity of the crude plant extracts, a *P. falciparum* culture is required. Therefore, *P. falciparum* was cultured. The following procedures describe how the culturing took place.

3.4.1. Cultivation of *P. falciparum* 3D7A

The *Plasmodium falciparum* tissue culturing was divided into two main steps: thawing *P. falciparum* and maintenance of *P. falciparum* culture. The *Plasmodium* culture is very sensitive to contamination therefore all reagents were sterile. The guidelines about decontaminations and safety precautions were followed. The entire culturing process was conducted in a validated biosafety level II cabinet. *P. falciparum* 3D7A, MRA-102, was obtained from the Malaria Research and Reference Reagent Resource Centre (MR4). The culturing process was performed according to the methods retrieved from (http://www.mr4.org/Publications/MethodsinMalariaResearch.aspx).

The procedures followed for thawing were as follow:

The cyrovial containing the parasites was thawed between two gloved hands before transferring the content into 15ml centrifuge tube in bio safety hood. 200µl of 12% NaCl was added drop wise using a sterile serological pipette with continuous agitation by taping the bottom of centrifuge tube vial with the index finger. The mixture was allowed to stand in the biosafety hood for five minutes. Furthermore, the NaCl- culture mixture was centrifuged at 250G for 5 minutes. The supernatants were aspirated following the addition of 10ml of 0.9%NaCl/0.2% D-glucose as in the

former step. The supernatants were aspirated again. The culture was then washed twice with 10 ml incomplete RPMI 16 medium (without serum). Following, 5ml of complete RPMI 16 (with 10% human) serum was added. The cell suspension was transferred to 75 ml culture flask. Finally, the cell culture was gassed with special gas i.e. N_2, O_2 and CO_2 mixture for 1 minute and swiftly tightly closed and incubated at 37 ^{0}C

3.4.2. Maintenance of *P. falciparum* tissue culture

The *P. falciparum* cell culture was maintained everyday by adding fresh human O^+ red blood cells and RPMI 16 medium supplemented with 10 % human serum, 10% glucose, 1M NaOH and 0.25 % gentamicin. The growth of the culture was monitored by determination of the culture's parasitaemia and hematocrit daily.

The detailed methods that were used as follow:

First of all the culture was transferred to a 15 ml conical tube. An aliquot of 100µl of the culture was removed and reserved for the preparation of thin smear. The rest of the culture was centrifuged at 400G for five minutes, the supernatants were aspirated. The pellet volume (volume of the residues) was determined in order to calculate the amount of red blood cells to add. If the culture volume was 5 ml, the amount of red blood cells added was 100µl-the pellet volume =RBCX2. If the culture volume was 10ml the red blood cells added was 200µl- pellet volume = RBCX2 (µl). The appropriate amount of the complete RPMI 16 medium and red blood cells is added. Finally, the culture is gassed with special gas mixture for 1 minute next, incubated at 37 ^{0}C.

Thin Giemsa stained smears were prepared to determine parasitaemia daily. The detailed procedures are as follows:

The 100µl of *P. falciparum* culture reserved in the Eppendorf tube was centrifuged for 30 seconds. The supernatants were aspirated (ensure a small amount is left to enable to mix the pellet). The pellet was mixed and a drop was placed onto clean, pre- labelled slide. A tilted slide was placed at an acute angle at one end of the slide and quickly slips through back and forth. The smear was then heat- fixed for 5-10 seconds. The smear was flooded with absolute methanol for 4-5 seconds. Next, smear was dried and slide was flooded with 10% Giemsa stain for 20 minutes. Finally, the Giemsa stain was washed off gently with water and the slide was air dried until ready for viewing with a microscope.

3.4.3. Preparation of extracts stock solutions

Stock solutions of the crude extracts were prepared at a concentration of 500µg/ml. Lyophilized aqueous and methanol extracts were dissolved in deionised water and Dimethyl sulfoxide (DMSO) respectively. Stock solutions of coartem (positive control) were similarly prepared in DMSO. The stock solutions were then diluted 1000 folds in the culture medium (RPMI 1640). All stock solutions were sterilized by filtration through 0.22 µm syringe microfilters.

M. sericea (root and shoot), *D. mespiliformis* (leaf and root) and *Cyphostemma*, (whole plant) were selected for *in vitro* antiplasmodial bioassay. The procedures used were according to Zongo, (2011). The antiplasmodial assay was done in three replicates in 96 well plates for 24 and 48 hours. Three concentrations of plant

extracts: 5, 10 and 50µg/ml were used. 0.5% DMSO in RPMI 16 medium was used as negative control, coartem was used as a positive control.

The suspension of *P. falciparum* 3D7A culture at 1% parasitaemia and 2% hematocrit was well mixed in the biosafety level II cabinet. 100µl of the cell culture was placed in pre- labelled 1ml centrifuge tube Following, was the addition of the pre mixed pooled volumes of desired concentrations of respective plant extracts from the stock solutions to the wells. For 50 µg/ml, 10µl was added, for 10 µg/ml, 2µl was added lastly, 5 µg/ml, 1µl was added. The 96 wells plates were placed in culture chamber gassed with special gas mixture for 5 minutes and incubated for 24 and 48 hours respectively at 37 ^{0}C.

3.4.4. Assessment of the antiplasmodial assay by microscopy method

Average percentages of parasitaemia after 24 and 48 hours of incubation with plants extracts and control treatments were analysed by microscopy according to the methods of Kamkumo *et al*, (2012). The smears were viewed at 100X magnification. An approximate of 100 non-infected red blood cells and the numbers of infected red blood cells were counted in 10 different microscopic fields of each slide. Parasitaemia was expressed as percentage of total infected red blood cells over the total non-infected red blood cell for each smear/replicate. The average percentage parasitaemia of the three replicates were calculated and recorded as shown in appendix two.

3.5. Bioassay guided fractionation of the selected crude extracts

In order to identify classes of phytochemical with known antiplasmodial activity of the selected plants extract, a bioassay guided fractionation regime was conducted. Below are the detailed procedures that were followed.

3.5.1. First and second round of fractionation

The choice of mobile phases for fractionation was based on the polarity of solvents. To ensures that a wide range of polar and non-polar phytochemicals are extracted. Methanol (polar) was mixed with dichloromethane (non-polar) in (1:1) ratio for first round of fractionation. For second round of fractionation, *n*-hexane a (non-polar) was combined with ethyl acetate (polar) in (8:2) ratio.

Column chromatography fractionation was performed according to the methods of Zafou *et al*, (2012) with modifications**.** Dry crude methanol extracts extract (300 mg) of *Cyphostemma* spp (whole plant), *Diospyros mespiliformis* (root and leaf) and *Mundulea sericea* (leaf and shoot) were dissolved in 10ml of methanol: DCM, (1:1), centrifuged at 2200g for 5 minutes and filtered with 0.45µm PTFE syringe filters. 500µl of the mixture was loaded on a Sephadex LH-20 column (1:10, w/v), eluted with methanol/dichloromethane (1:1). Averages of 15 ml of mobile phase were added in 5 ml portions with a Pasteur pipette. For the second round of fractions, the first round fractions were dried, reconstituted in (8:2) *n*-hexane/ethyl acetate and loaded onto the column eluted with *n*-hexane/ethyl acetate (8:2). Averages of 15 ml of mobile phase were added in 5 ml portions with a Pasteur pipette.

3.5.2. Phytochemical screening of first and second round fractions by TLC

TLC was used to identify the phytochemical constituents of the fractions. The fractions were screened for presence or absence of terpenoids, alkaloids, anthraquinones, flavonoids and coumarins. The procedures are the same as in section 3.2.

3.5.3. Antiplasmodial assay of the first and second round fractions

The fractions were dried by rotary evaporation at 20 ^{0}C, automatic pressure and 40 rpm. The dry extracts were then reconstituted in 1ml of DMSO, and then diluted 100 fold in incomplete RPMI 1640 medium. The antiplasmodial assay was done in three replicates at 1% parasitaemia and 2% hematocrit in 96 well plates for 24 and 48 hours. Coartem was used as a positive control, 0.1% DMSO in incomplete RPMI 1640 media was used as negative control. The average percentage parasitaemia of three replicates were calculated and recorded as shown in appendix three. The procedures used are the same as in section 3.5.

3.5.4. Determination of IC_{50} values of crude plant extracts

IC_{50} values of crude plant extracts were calculated from dose response curves of each plant extracts. Linear regression was used as model on Microsoft Office Excel 2007 software.

3.6. Data analysis

GeneStart Discovery Edition 4 software was used for data analysis. Total phenolics and alkaloids and average % percentage parasitaemia are expressed as mean ± SE (Standard error). The average % parasitaemia response data was tested for normality

using a Kolomogorov-Smirnov test. Level of significance was considered at 95 % confidence interval. One way-ANOVA was used to analyse significance difference across all concentrations at 24 and 48 hours for crude extracts and fractions. The data were found to be normally distributed hence; two ways-ANOVA was used to determine significance interaction between plant extracts and concentrations. The ANOVA tables are in appendix four.

CHAPTER FOUR: RESULTS

4. Selected plants and malaria associated symptoms ethnomedicinal uses

Six plants species, namely, *A. inflata, V. infausta, Z. mucronata, D. mespiliformis, N. oleracea* and *Cyphostemma spp* were collected form Omunghete village in Omusati region, while the remaining four: *G. coleosperma, M. sericea* and *B. plurijuga* were collected from Eehonge village in Ohangwena region. All plant's taxonomic identities were confirmed by NBRI in Windhoek, Namibia. Half of the plants species belong to the *Fabaceae* family while the rest belong to *Rhamnaceae, Ebeneceae, Lamiaceae, Rubiceae Polygonaceae, and Vitaceae* each. The pictures of the plants are shown in appendix 6.

Table 3: Summary of plants and their putative malarial related ethno medicinal uses in Northern Namibia. Information about traditional preparations and ethno medicinal uses were adopted from Von Koenen (2001).

Families	**Plant's names**	**Plant parts**	**GPS coordinates**	**Physical location of collection**	**Specimen numbers**	**Malaria related ethnomedicinal uses**	**Traditional preparation**
Fabaceae	*B. plurijuga,*	bark	$S17^0$ 35.234 $E017^0$ 15. 440	Eehonge, Ohangwena	SN 1	Fever and head ache	
		Leaves					
		Roots					
Vitaceae	*Cyphostemma spp*	Whole plant	$S17^0$ 60. 236 $E015^0$ 61.346	Omunghete, Omusati	SN 7	fever	A tea is administered orally
	G. coleosperma	Bark	S 17^0 37.549		SN 2	Headache, fever cough & malaria	Used in combination with *S. longependuculata*, *X. Tomentosa & P. angolensis* leaves and roots and administered as steam bath at regular intervals
		Leaves					
		Roots					
	M. sericea,	Shoots	S 17^0 37.549 $E017^0$ 23.493	Eehonge Ohangwena	SN 10	Headache, malaria and fever	A root extract enema is dried and root pulverised is strewn over glowing ember and used as smoke bath

		Leaves					
	N. oleracea	Whole plant	S 17^0 60.393 E015^0 61.201	Omunghete Omusati	SN 8	Malaria	
Ebeneceae	*D. mespiliformis*	Leaves	S 17^0 60.393 E015^0 61.201	Omunghete Omusati	SN 6	Fever	Leaf, twigs and bark are remedy of fever
		Roots					
Lamiaceae	*A. inflata*	Whole plant	S 17^0 60.303 E015^0 61.201	Omunghete Omusati	SN 3	Headache	
Polygonaceae	*O. dregeanum*	Whole plant	S17^0 36.679 E015^0 42.963	Eehonge Ohangwena	SN 9	Malaria and fever	Whole plant is used in combination with *Pergularia daemia* is made into a hot water extracts
Rhamnaceae	*Z. mucronata*,	Roots,	S 17^0 60.303 E015^0 61.201	Omunghete Omusati	SN 5	Fever, diarrhoea, and malaria	Teaspoon of cold water extract or decoction from crushed leaves is given to children with diarrhoea
		Leaves					
Rubiceae	*V. infausta*,	Shoots	S 17^0 37.549 E017^0 23.493	Omunghete, Omusati	SN 4	Fever, cold, cough and headache	A leaf and root tea is taken for cold
		Leaves					
		Roots					

4.1. Percentage yield of plant extracts

The root, leaf and bark extracts of *G. coleosperma* had the highest yield percentage per 10g of plant materials used for extraction, followed by *D. mespiliformis*, leaf extract. *Ziziphus mucronata* and *Vangueria infausta* had the least yield percentage per 10g of plant material used. The rest of the results are shown in figure 13.

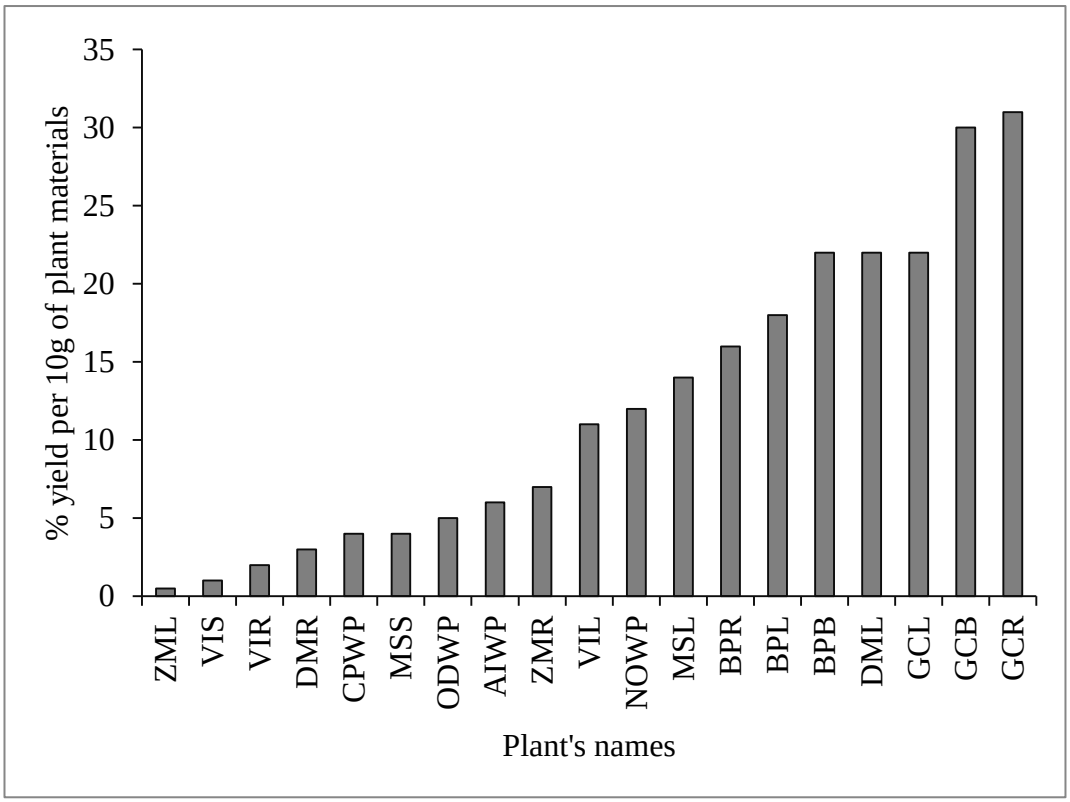


Figure 11: Yield precentages of methanol extracts. Keys: BPB: *Baikiaea plurijuga,* bark. BPL: *Baikiaea plurijuga,* leaf, BPR: *Baikiaea plurijuga,* root, CPWP: *Cyphostemma* spp whole plant, GCB: *Guibourtia coleosperma*, bark, GCL: *Guibourtia coleosperma* Leaf, GCR: *Guibourtia coleosperma,* root, MSL: *Mundulea sericea,* leaf, MSS: *Mundulea sericea,* shoot. NOWP: *Neptunia oleracea,* whole plant, DML: *Diospyros mespiliformis,* leaf, DMR: *Diospyros mespiliformis*, root AIWP: *Acrotome inflata,* whole Plant, ODWP: *Oxygonum dregeanum,* whole plant,

ZMR: *Ziziphus mucronata*, root, ZML: *Ziziphus mucronata*, leaf, VIS: *Vangueria infausta*, shoot, VIL: *Vangueria infausta* leaf. VIR; *Vangueria infausta*, root

4.2. Identification of antiplasmodial phytochemicals from plant extracts

Phytochemical screening of the crude plant extracts results are shown in table 4. Briefly, all plant extracts screened had at least one group of phytochemical screened for. The shoot of *M. sericea* contained all phytochemicals, while *A. inflata* whole plant extract showed the presence of terpenoids only. Terpenoids were detected the most (16/19), followed by anthraquinones (14/19), alkaloids (12/19). Coumarins and flavonoids (9/19) were the least detected. Barks extracts contain mostly terpenoids and alkaloid but no presence of flavonoids and coumarins were detected. The leaves, flavonoids and anthraquinones were mostly detected and the least were coumarins. Whole plant extracts showed mostly the presence of terpenoids and no alkaloid while the roots extracts exhibited mostly alkaloids and anthraquinones and no presence of flavonoids detected. Lastly the shoot extracts had mostly terpenoids, coumarins and alkaloids. The least detected group of phytochemicals were flavonoids and anthraquinones.

Table 4: Phytochemical screening of 10 medicinal plants for major classes of antiplasmodial compounds

Plants	Plant parts	flavonoids	alkaloids	anthraquinones	terpenoids	coumarins
A. inflata	Whole plant	-	-	-	+	-
B. plurijuga	Bark	-	+	+	+	-
	Leaf	+	-	+	-	-
B. plurijuga	Root	-	+	+	-	-
	Bark	-	+	-	+	-
Cyphostemma	Whole plant	+	-	+	+	+
D. mespiliformis	Leaf	-	+	+	+	-
	Root	-	+	+	+	+
G. coleosperma	Leaf	+	+	+	-	+
	Root	-	-	+	-	-
M. sericea	Leaf	+	-	-	+	-
	Shoot	+	+	+	+	+
N. oleracea	Whole plant	+	-	+	+	-
O. dregeanum	Whole plant	-	-	+	+	+
V. infausta	Shoot	-	+	-	+	+
	Leaf	+	-	-	+	+
	Root	-	+	+	+	+
Z. mucronata	Root	-	+	-	+	-
	Leaf	+	-	+	+	-

Keys: + = present and - = absent

4.3. Quantification of classes of antiplasmodial phytochemicals

Total phenolic and alkaloid content was determined by Folin Ciocalteu and Bromo Cresol Green method respectively and calculated from the calibration curves in appendix one. Total phenolics content range from 99.58 GAE/100µg of *D. mespiliformis* root extract to 343.55 GAE/100µg of *G. coleospema* bark extract. The richest source of total alkaloids was *O. dregeanum* whole plant extract, 24.45 µg/ 100mg of extracts and the poorest source of total alkaloids was *Z. mucronata*

leaf extracts 0.37 µg/100mg of plant extracts. The results are summarised in table 5.

Table 5: Total phenolic and alkaloids content of 10 medicinal plants used to treat malaria associated symptoms in Northern Namibia

Plants	Plant parts	Total phenolics content GAE/100µg	Total alkaloids content µg/100 mg extracts
A. inflata	Whole plant	118.43	4.99
B. plurijuga	Bark	199.33	17.44
	Leaf	275.38	0.84
	Root	113.40	4.88
Cyphostemma spp	Whole plant	160.55	21.11
D. mespiliformis	Leaf	99.58	6.67
	Root	226.38	18.41
G. coleosperma	Bark	343.55	9.97
	Leaf	123.98	18.77
	Root	272.09	17.60
M. sericea	Leaf	173.75	2.65
	Shoot	274.37	17.78
N. oleracea	Whole plant	246.90	18.73
O. dregeanum	Whole plant	247.91	24.45
V. infausta	Leaf	128.56	13.55
	Leaf	240.70	0.40
	Shoot	125.38	6.71
Z. mucronata	Leaf	207.09	2.97
	Root	278.89	0.37

4.4. *In vitro* antiplasmodial activity of *M. sericea, D. mespiliformis* and *Cyphostemma* spp's crude extracts

M. sericea (leaf and shoot), *D. mespiliformis* (leaf and root) and *Cyphostemma* spp (whole plant) were selected for *in vitro* antiplasmodial assay against *P. falciparum* 3D7A based on the wide range of phytochemicals as revealed by TLC (see table 4) and the high estimates of total phenolics and alkaloids quantified (see table 5).

The methanol and aqueous plant extracts were reconstituted in DMSO and distilled water respectively and further diluted to 0.5% DMSO in incomplete RPMI 1640 medium. Hence 0.5% DMSO was used as a negative control. Coartem at 50 µg/ml was used as a positive control. The non-treated control average percentages parasitaemia increased two fold forms 1% initial parasitaemia to 2.4% (0.07±SE) for 24 hours and three fold for 48 hours, 6.4% (0.06±SE). This indicate normal growth of the parasites, hence there was no natural death of the parasites. There was minimal carrier solvent effect (0.5% DMSO, 0.00% (0.35±SE) parasites inhibition for 24 hours and 26.6% (0.55±SE) for 48 hours. This confirms the ineffectiveness of the carrier solvent against *P. falciparum* 3D7A. Therefore, it can be said that the *in vitro* antiplasmodial activity observed for all crude plants extracts was solely due to phytochemicals constituents of the extracts.

Below are descriptive and comparative details of *in vitro* antiplasmodial activity of crude methanol and aqueous extracts from three plants at 24 and 48 hours at 5, 10 and 50 µg/ml.

4.4.1. Antiplasmodial activity of *M. sericea* leaf and shoot extracts

The results are summarised in figure 12. Briefly, there was parasitaemia reduction at all concentrations tested for both 24 and 48 hours in comparison with non-treated control as indicated in Figure 12. This confirms the efficacy of *M. sericea* leaf, crude methanol extract against *P. falciparum* 3D7A. In support of the above, at the lowest concentration (5µg/ml), a time dependent effect was observed. In other words, as the

time is increased, antiplasmodial activity is increasing. Conversely, at 10 and 50 µg/ml no time dependent effect was observed. Clearly, *M. sericea* leaf methanol extracts were mostly active during 24 hours with significant difference, (p value = 0.009) compared to 48 hours where no significant difference was observed, (p value =0.7).

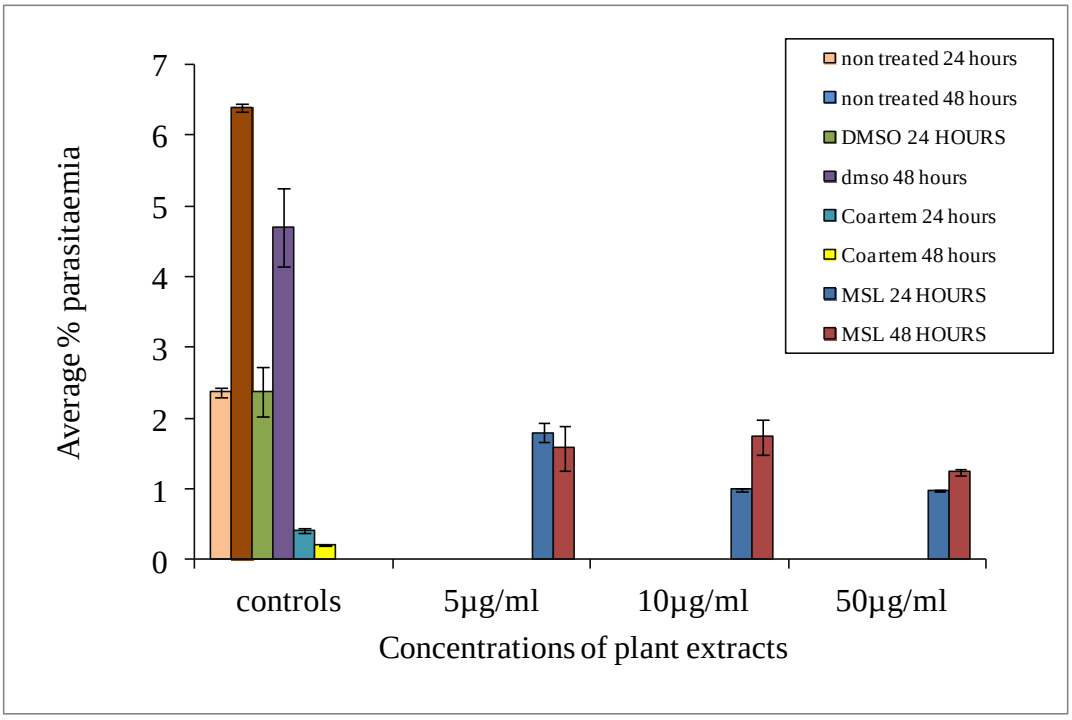


Figure 12: Antiplasmodial activity of *M. sericea* leaf, crude methanol extract at 24 and 48 hours.

The *in vitro* antiplasmodial activity of the *M. sericea*, leaf aqueous extract is shown in figure 13. Briefly, at 24 hours activity was directly proportional to increase in concentration of the leaf extracts and no significant difference, (p value =0.2) observed. Similarly, at 48 hours there was no significant reduction in parasitaemia (P value = 1.0). Average % parasitaemia reduction of *M. sericea* leaf aqueous extract at 10 µg/ml extracts for 24 hours 0, 74 (0.01±SE) and 48 hours 0.70

(0.07±SE) was almost equal. At 5 and 10 µg/ml, a time dependent effect was observed unlike at 50µg/ml, whereby no time dependent effect was observed. Clearly, the leaf extract was more effective at 24 hours' time of exposure to *P. falciparum* 3D7A.

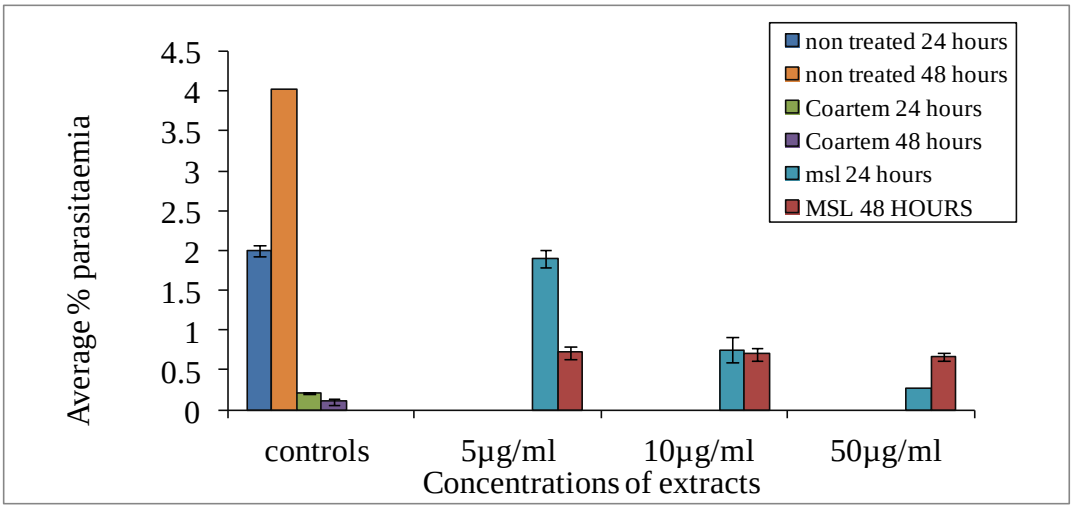


Figure 13: Graphical representation of antiplasmodial activity of *M. sericea,* leaf aqueous extract at 24 and 48 hours.

The *in vitro* antiplasmodial activity of *M. sericea* shoot extract is illustrated by figure 14. The results indicate reduction in average parasitaemia for 24 and 48 hours across all concentration in comparison to the non-treated control. This confers *in vitro* antiplasmodial activity of *M. sericea* shoot methanol extracts. The antiplasmodial activity at 24 and 48 hours was comparable at all concentration although, there was no concentration dependent effect observed. There was time dependent effect observed for only 5µg/ml. There was significant reduction in average % parasitaemia at 24 hours (p value =0.04) but not at 48 hours (p value = 0.8).

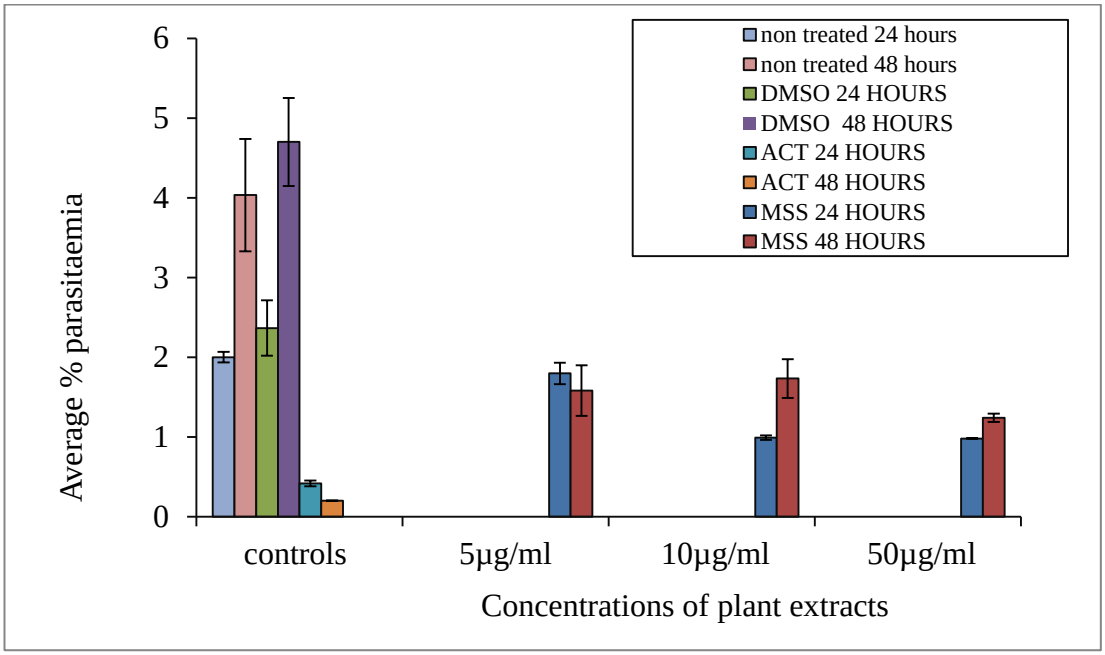


Figure 14: Antiplasmodial activity of *M. sericea* shoot methanol extract at 24 and 48 hours.

The antiplasmodial activity of *M. sericea* aqueous shoot extract is shown in figure 15. The results indicate reduction in average parasitaemia for 24 and 48 hours across all concentration in comparison to the non-treated control. Time dependent effect was observed for all concentrations as well as concentration dependent effect for all for 24 and 48 hours. Furthermore, the extract was mostly effective at 50µg/ml

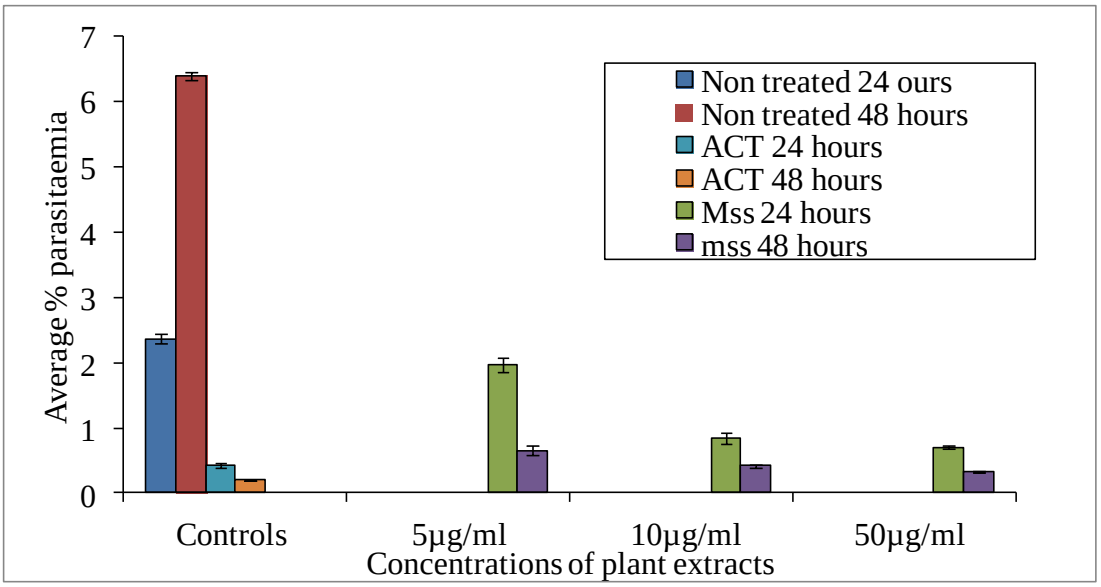


Figure 15: Antiplasmodial activity of *M. sericea* aqueous shoot extracts at 24 and 48 hours.

4.4.2. *M. sericea* leaf against shoot extracts at 48 hours

The shoot and leaf aqueous crude extracts were the most active against *P. falciparum* 3D7A strain at 48 hours. There was significant *in vitro* antiplasmodial activity between organic and aqueous of *M. sericea* shoot extracts. There was no significant difference in average % parasitaemia reduction between *M. sericea* leaf and shoot organic extracts (p value= 0.1) for 24 hours, similarly at 48 hours, (p value = 0.5). For aqueous leaf and shoot extracts of *M. sericea*, there was no significant difference in average % parasitaemia reduction, (p value = 0.2), but at 48 hours there was significant difference (p value =0.02). The rest of the results are summarised in figure 16.

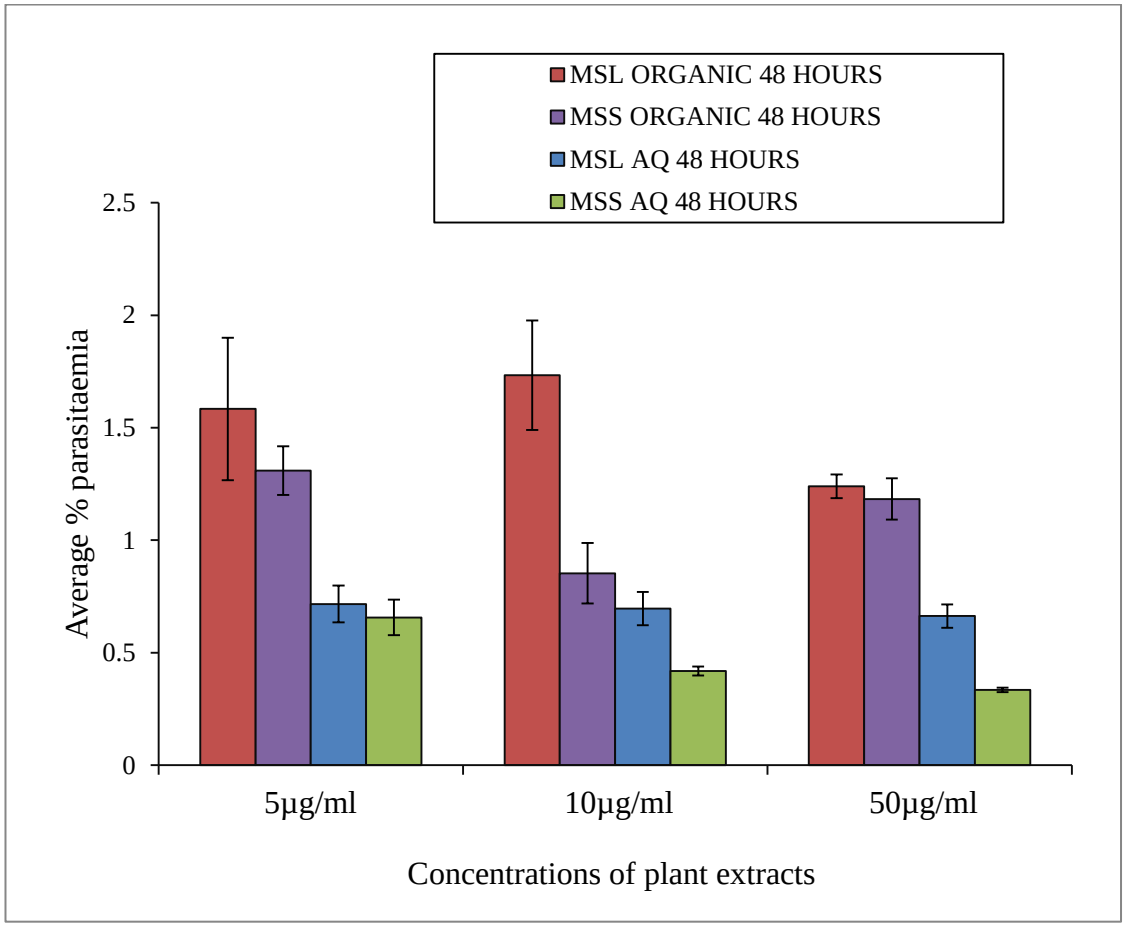


Figure 16: Antiplasmodial activity of *M. sericea*, leaf against shoot at 48 hours

4.4.3. Antiplasmodial activity of *D. mespiliformis*, leaf and root extracts

For *D. mespiliformis* methanol leaf extract, there was reduction in average % parasitaemia across all concentration after 24 and 48 hours exposure of *P. falciparum* 3D7A to *D. mespiliformis* crude methanol leaf extracts. This infers antiplasmodial activity as shown in figure 17. Conversely, the difference was not significant across concentrations for 24 hours (p values = 0.3) and for 48 hours as well, (p value = 0.5). Antiplasmodial activity was time dependent at 5µg/ml only. The extracts were mostly effective at 10 µg/ml for 24 hours.

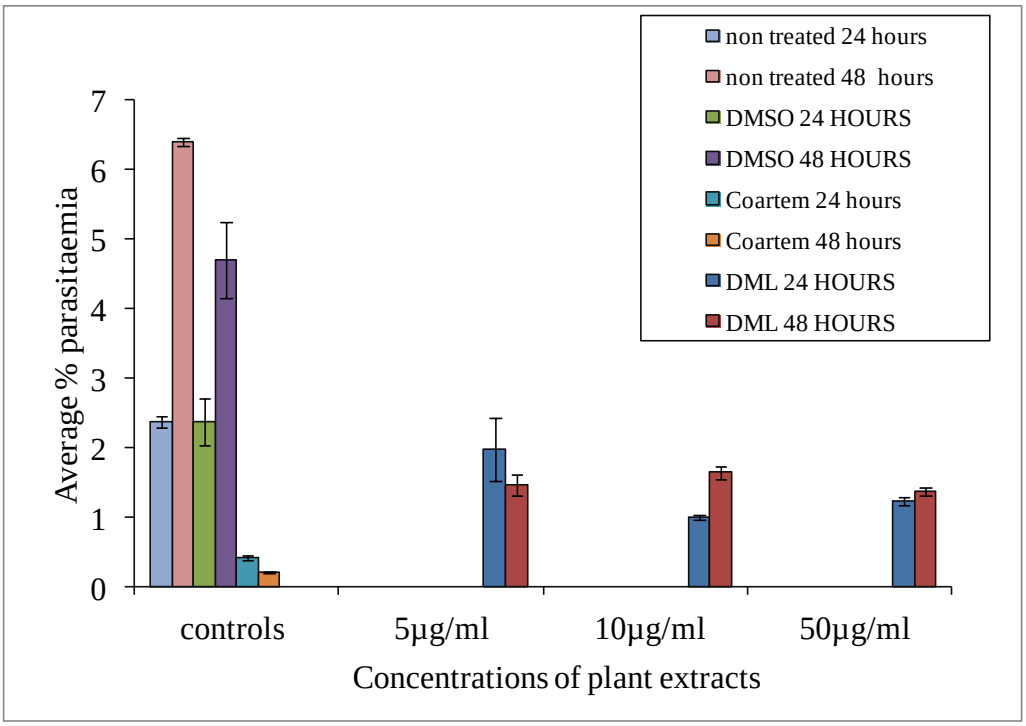


Figure 17: *In vitro* antiplasmodial activity of *D. mespiliformis* leaf crude methanol extracts at 24 and 48 hours.

Likewise, for the aqueous extract, there was no significant reduction in average percentage parasitaemia at 24 hours (p value =0.6) and 48 hours (p value =0.1). The leaf extract was most active against *P. falciparum* 3D7A at 24 hours than 48 hours. In other words, as the time of exposure increased, the parasites growth inhibition by the leaf aqueous decreased. There was no time dependent effect observed for all concentrations tested. The results are summarised in figure 18.

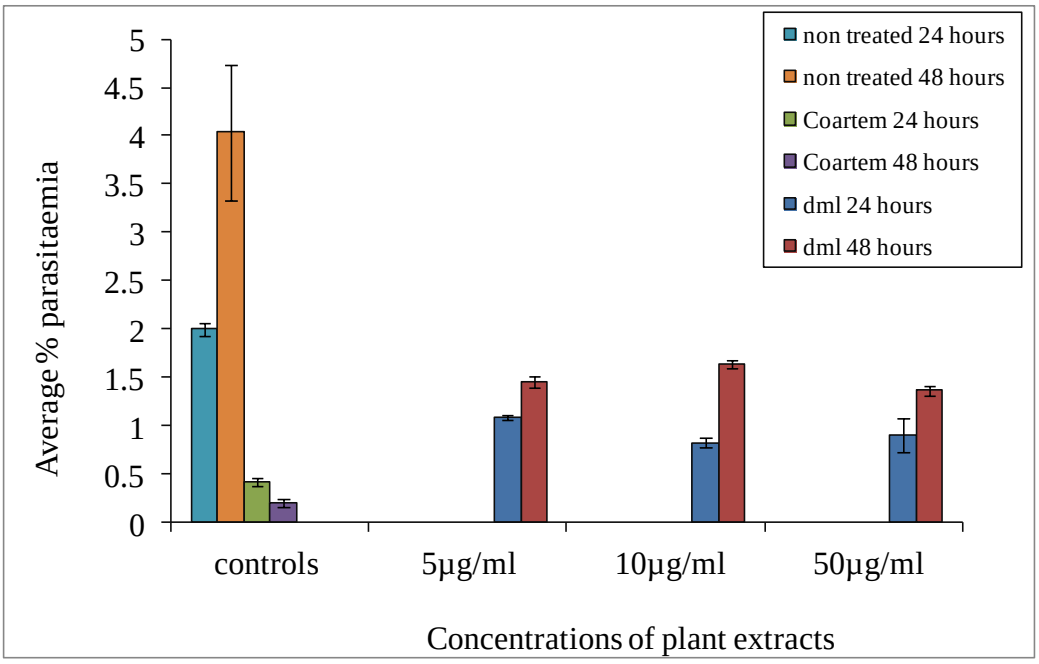


Figure 18: Antiplasmodial activity of *D. mespiliformis* aqueous leaf extracts at 24 and 48 hours.

D. mespiliformis root extract indicated similar pattern, (reduction in average % parasitaemia for all concentrations compared to the non-treated control). The root extract was effective with increased time exposure and concentration which infers that the methanol root extract was more effective at 48 hours. There was significant average parasitaemia reduction across all concentrations for 24 hours (p value = 0.09). On the other hand, at 48 hours, there was no significant difference (p value = 0.4). At 10 and 50µg/ml average reduction in parasitaemia was comparable. The results are summarised in figure 19.

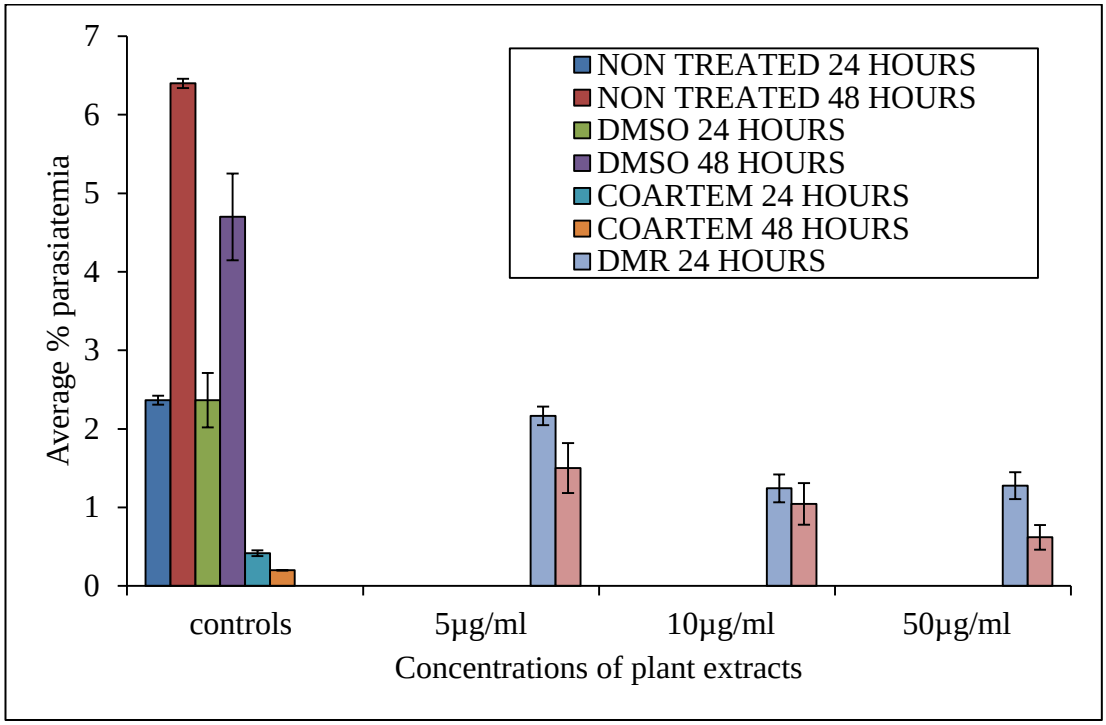


Figure 19: Antiplasmodial activity of *D. mespiliformis* methanol root extracts at 24 and 48 hours.

The *in vitro* antiplasmodial activity of *D. mespiliformis* aqueous root is shown in figure 20. There was reduction in parasitaemia for all treatments in relation to the non-treated control which proves that the crude aqueous root of *D. mespiliformis* has antiplasmodial activity. The extract was effective with increased time exposure and concentration which infers that the aqueous root extract was more effective at 48 hours. However, there was no significant average parasitaemia reduction across concentrations for 24 (p value = 0.9) and 48 hours (p value = 0.6).

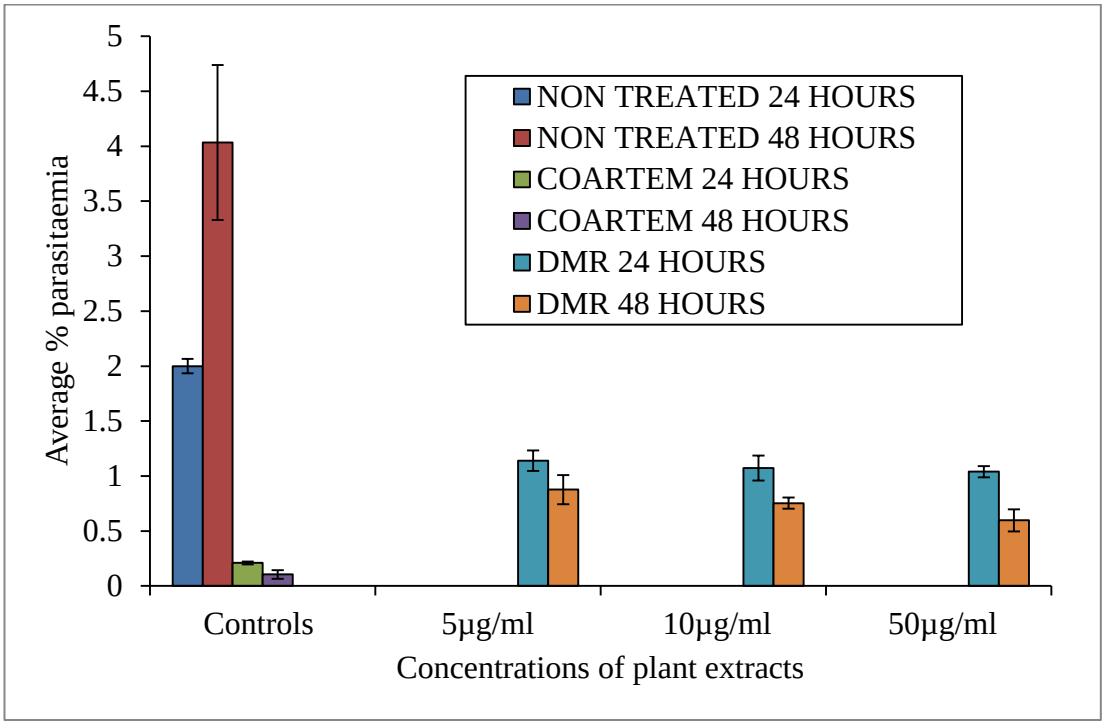


Figure 20: Antiplasmodial activity of *D. mespiliformis* aqueous roots extracts at 24 and 48 hours.

4.4.4. *D. mespiliformis* leaf against root extracts at 48 hours

There was no significant difference between *D. mespiliformis*, methanol leaf and root extracts across concentrations for 48 hours (p value =0.17). The methanol root extract was more potent at 48 hours than the leaf methanol extract with significant difference observed at 10 and 50 µg/ml. The aqueous leaf extract was more potent than the root methanol extract. Although, the difference was not significant across concentration at 48 hours (p value = 0.13). Overall, the aqueous extract had the least average % parasitaemia across concentration, while the leaf methanol extract had the highest average % parasitaemia. The summary of comparison is shown in figure 21.

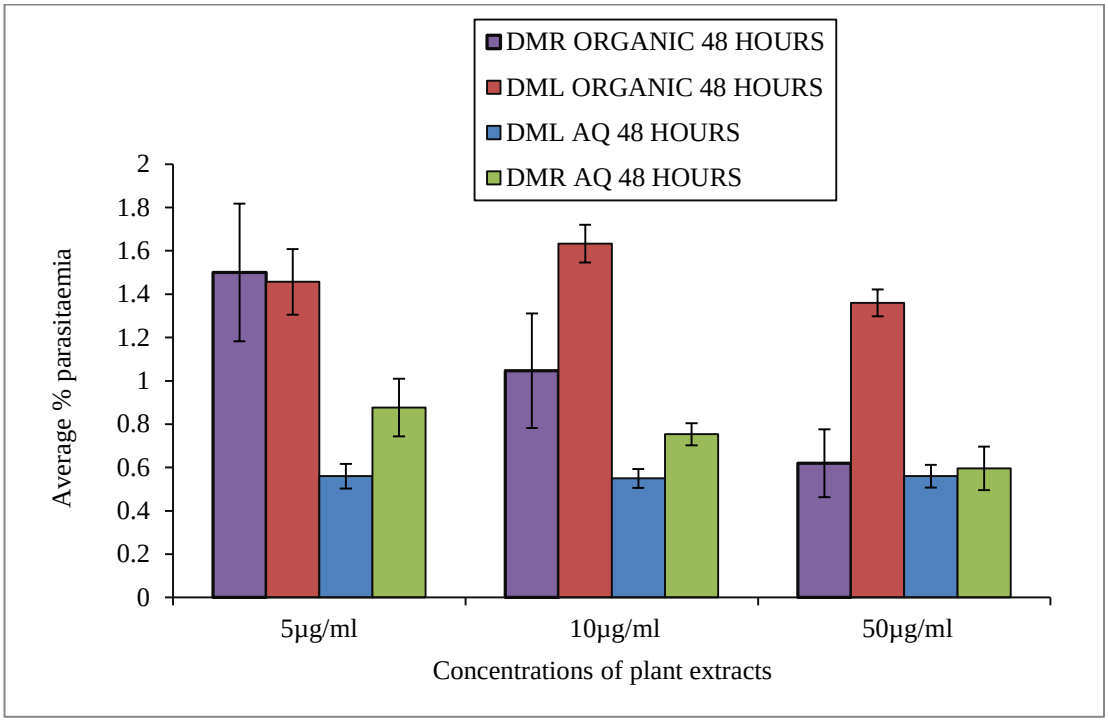


Figure 21: Antiplasmodial activity of *D. mespiliformis* aqueous extracts leaf against root at 48 hours

4.4.5. Antiplasmodial activity of *Cyphostemma spp* whole plant extract

The results are summarised in figure 22. There was a reduction in average % parasitaemia in comparison with the non-treated control for 24 and 48 hours. At 5 and 50 µg/ml; the methanol extract was most active at 24 hours than 48 hours therefore, a time dependent effect was observed at 5 and 50µg/ml. Conversely at 10µg/ml, the extract was most active at 48 hours. A concentration dependent effect and significant difference (p value =0.003) was observed at 24 hours with decreasing concentration. While at 48 hours, the antiplasmodial activity increased at 10 µg/ml and decreased at 50 µg/ml and no significant difference (p value = 0.1) was observed. Overall, the extract was more potent against *P. falciparum* 3D7A at 24 than 48 hours.

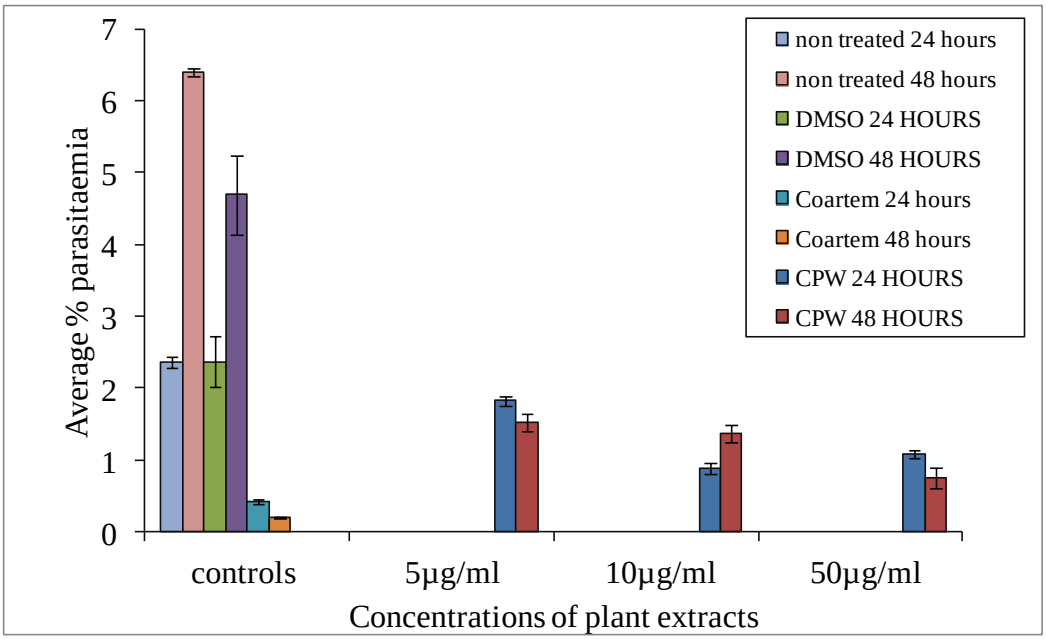


Figure 22: Antiplasmodial activity of *Cyphostemma spp* whole plant methanol extracts at 24 and 48 hours.

Similarly, for the *Cyphostemma* spp crude aqueous extract, there was reduction in average % parasitaemia across all concentrations at 24 and 48 hours compared to the non-treated control. At 24 hours, there was no significant difference (p value =0.8) across concentration, therefore no concentration dependent effect, observed. However, time dependent effect was observed at all concentrations. Hence, the crude aqueous extract was more potent at 48 hours than 24 hours. Even though, the difference across concentrate at 48 hours was not significant, (p value = 0.9). The results are summarised in figure 23.

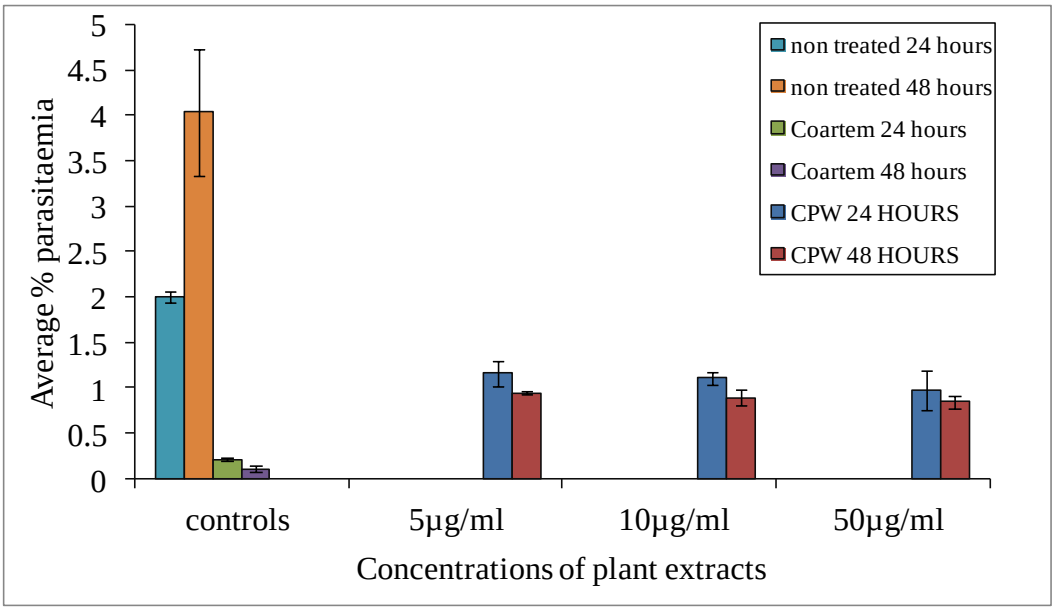


Figure 23: Antiplasmodial activity of *Cyphostemma spp* whole plant aqueous extracts post 24 and 48 hours.

4.4.6. *Cyphostemma* spp methanol against aqueous whole plant extracts at 48 hours

The methanol whole plant extract of *Cyphostemma* spp was most active at 5 and 10 µg/ml. The results are outlined in figure 24.

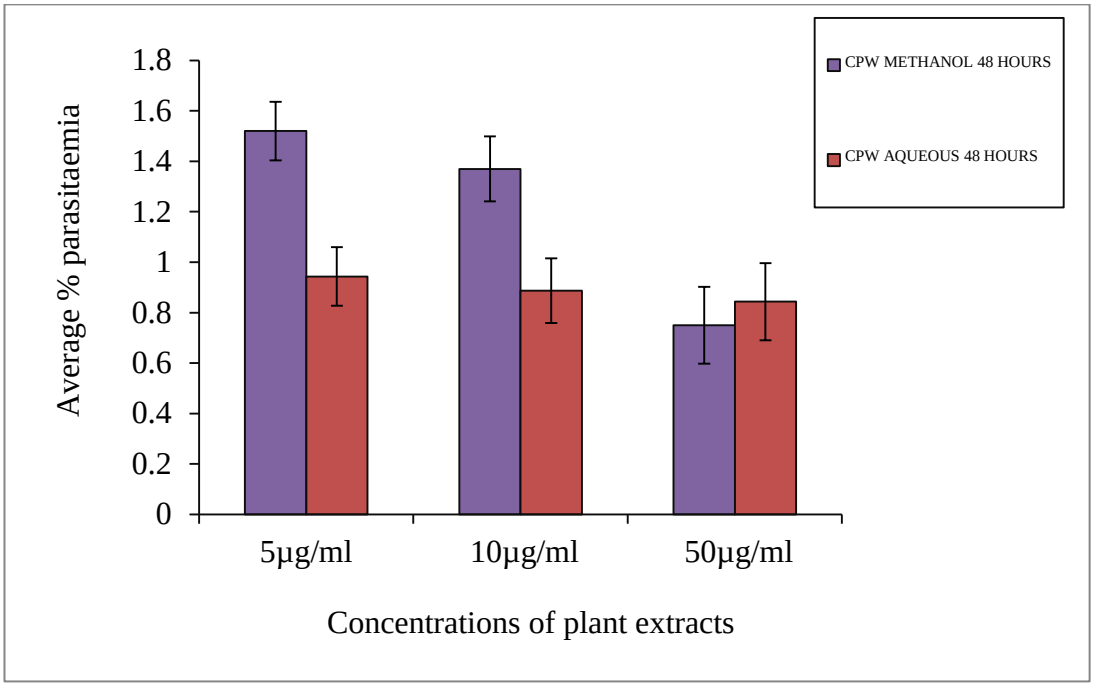


Figure 24: Antiplasmodial activity of *Cyphostemma spp* aqueous against methanol whole plant extracts at 48 hours.

4.4.7. IC_{50} values of the three plants crude extracts

According to the criteria set by Deharo (2001), plant extracts are considered highly active when their IC_{50} is less than 5µg/ml, moderately active when its IC_{50} value is between 5 and 10 µg/ml and inactive when it's IC_{50} is more than 10µg/ml. The IC_{50} shown by table 4, indicate that both aqueous and methanol extracts were active against *P. falciparum* 3D7A strain. The methanol extracts were more active than

their corresponding aqueous extracts of the same plant. The most active methanol extract was *M. sericea* shoot with IC_{50} value of 1.10 µg/ml while the least methanol extract was *Cyphostemma* spp whole plant with IC_{50} of 5. 17 µg/ml. For the aqueous extracts the most active was *D. mespiliformis* root, with IC_{50} of 2.91 µg/ml and least active was *M. sericea* shoot with IC_{50} of 9.90µg/ml. The overall best active extract was *M. sericea* methanol shoot with an IC_{50} value of 1.10 µg/ml and the least was the same aqueous extract with IC_{50} value of 9.10 µg/ml

Table 6: IC_{50} values of three ethnomedicinal plants used to treat malaria associated symptoms against *P. falciparum* 3D7A strain.

Plants	Plant parts	Methanol IC_{50} (µg/ml)	Aqueous IC_{50} (µg/ml)
M. sericea	Leaf	2.23	4.99
	Shoot	1.10	9.10
D. mespiliformis	Leaf	1.51	3.01
	root	2.12	2.91
Cyphostemma spp	Whole plant	5.17	5.03

4.5. Bioassay guided fractionation of the two medicinal plants

4.5.1. Phytochemical screening and antiplasmodial activity of *Cyphostemma spp* first round fractions

The phytochemical screening of different *Cyphostemma* fractions is shown in table 7, and *in vitro* antiplasmodial activity is depicted in figure 25. Briefly, there was no time dependent effect observed for all fractions however, significant reduction in

average % parasitaemia across fractions was observed at 48 hours, (p value = 0.02) and no significant difference at 24 hours (p value =0.2). Fraction 4 had the least antiplasmodial activity across time and single phytochemical entity detected by TLC. Fraction 1 and 2 showed the presence of terpenoids and coumarins and absence of anthraquinones in common and low average % parasitaemia at 24 hours and high average % parasitaemia at 48 hours. In addition antiplasmodial activity of fraction 1 and 2 has improved compared to the crude extract for 24 and 48 hours. Therefore, fraction 1, 2 and 3 were selected for sequential round of fractionation.

Table 7: Phytochemical screening of *Cyphostemma* spp first rounds fraction by TLC

Fraction ID	terpenoids	flavonoids	alkaloids	coumarins	anthraquinones
CPW	+	+	+	+	-
F1	+	-	-	+	-
F2	+	+	+	-	-
F3	+	+	+	+	-
F4	-	+	-	-	-

Keys: CPW= *Cyphostemma* whole plant crude extract and F1-F4= *Cyphostemma* whole plant fractions. + = present and - = absent

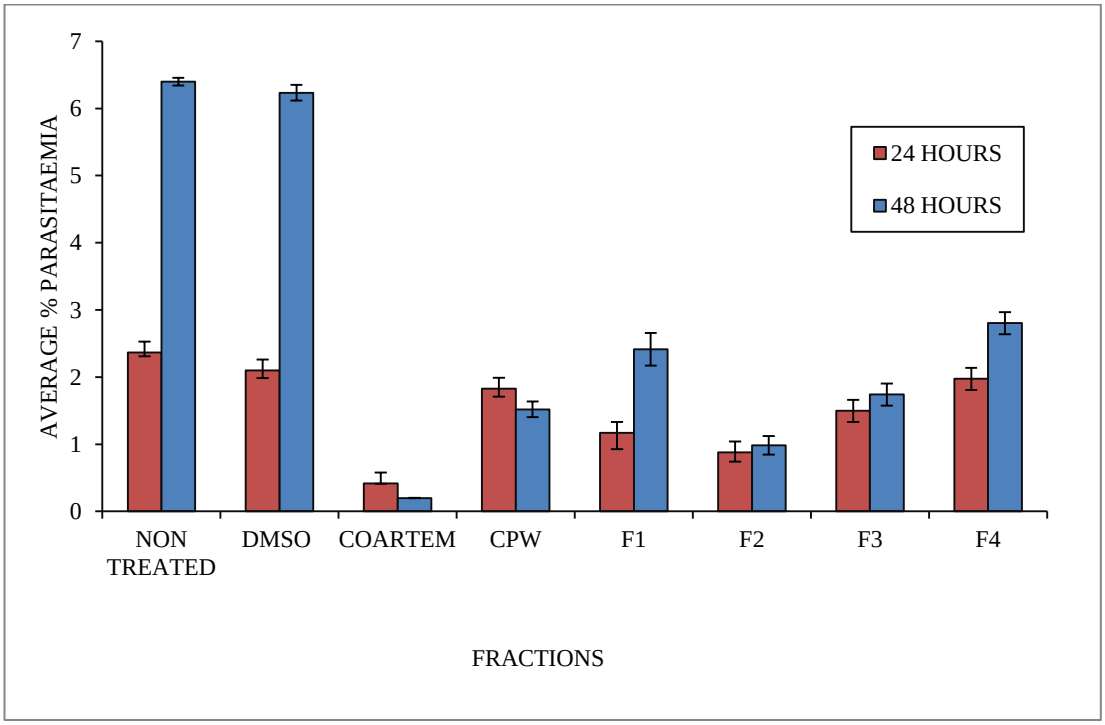


Figure 25: Antiplasmodial activity of *Cyphostemma*, whole plant first round fractions. Keys: CPW= crude *Cyphostemma* extract, coartem= positive control and DMSO = negative control.

4.5.2. Phytochemical screening and antiplasmodial activity of *D. mespiliformis* leaf first round fractions

The phytochemical screening of *D. mespiliformis* leaf fractions results are shown in table 8 and *in vitro* antiplasmodial activity is indicated in figure 26. In few words, a time dependent effect was observed for fraction 1 only and reduction in average % parasitaemia for all fractions at 48 hours. Antiplasmodial activity was highly significantly different across all fractions at 24 hours, (p value <0.001) and 48 hours (p value = 0.02). Fraction 1 and 2 were phytochemically distinct with only anthraquinones in common and almost equal average % parasitaemia reduction. On the contrary, fraction 3 and 4 were phytochemically similar with the presence of

terpenoids, coumarins and anthraquinones and absence of alkaloids and flavonoids and highest parasitaemia reduction at 24 and 48 hours. Furthermore, improved antiplasmodial activity was observed for fraction 3 and 4 compared to the crude extract, (DML). For this reason fraction 3 and 4 were selected for second round of fractionation.

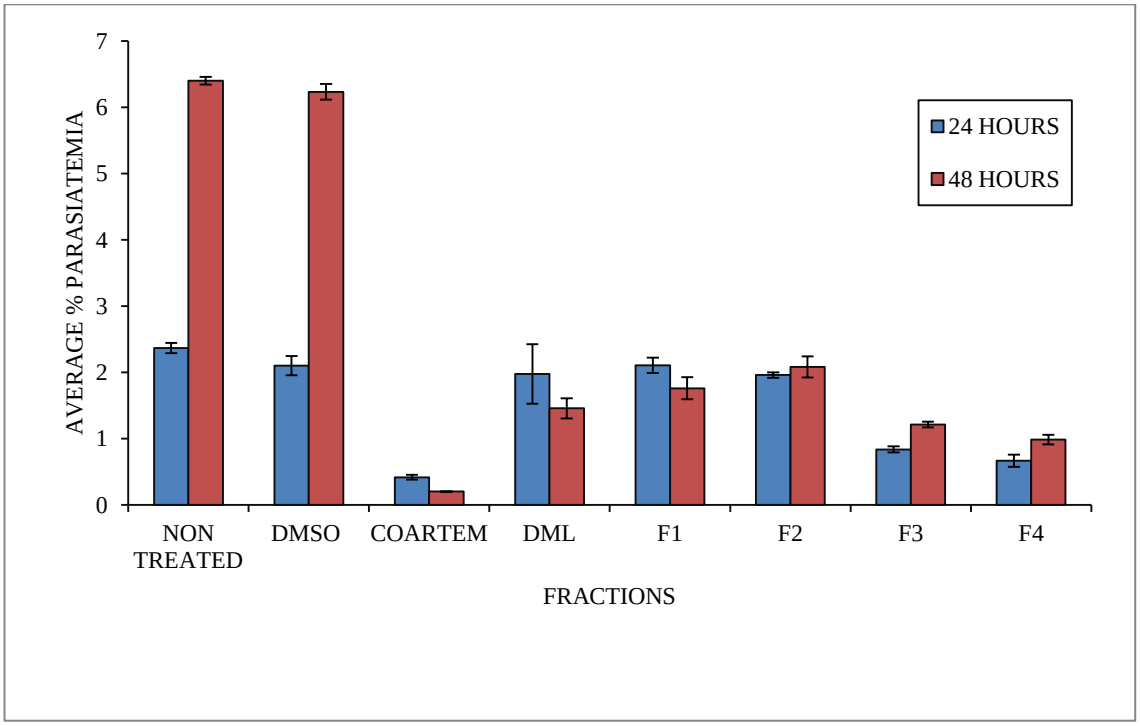


Figure 26: Antiplasmodial activity of *D. mespiliformis* leaf first round fractions. Keys: DML= crude *D. mespiliformis* leaf extract, coartem = positive control and DMSO= negative control

Table 8: Phytochemical screening of *D. mespiliformis,* leaf first round fractions by TLC

fraction ID	terpenoids	flavonoids	alkaloids	coumarins	anthraquinones
DML	+	+	+	+	+
F1	+	-	-	-	+
F2	-	-	-	+	+
F3	+	-	-	+	+
F4	+	-	-	+	+

Keys: DML= *D. mespiliformis* crude leaf extract F1-F4= *D. mespiliformis*, leaf fractions. + = present and - = absent

4.5.3. Phytochemical screening and antiplasmodial activity of *D. mespiliformis* root first round fractions

All root fractions had the presence of terpenoids and anthraquinones in common. Improved antiplasmodial activity of fraction with exception of fraction 6 in comparison with the crude extract (DMR) was indicated. However, average % parasitaemia reduction was not significantly different across all fractions for 24 hours, (p value = 0.2) and likewise at 48 hours (p value = 0.7). Fraction 1 and 2 had the presence of anthraquinones and terpenoids and absence of flavonoids in common (see table 9) and high average % parasitaemia reduction with time dependent effect shown for fraction 2. Hence, they were selected for sequential round of fractionation. Fraction 1 had the highest average % parasitaemia reduction while fraction 6 had the least average % parasitaemia reduction. The phytochemical screening of *D. mespiliformis* root fractions is summarised in table 9, and *in vitro* antiplasmodial activity is shown in figure 27.

Table 9: Phytochemical screening of *D. mespiliformis* root first round fractions by TLC

Fraction ID	terpenoids	flavonoids	alkaloids	coumarins	anthraquinones
DMR	+	+	+	+	+
F1	+	-	+	-	+
F2	+	-	-	-	+
F3	+	-	-	-	+
F4	+	+	-	-	+
F5	+	+	-	-	+
F6	+	+	-	+	+

Keys: DMR= *D. mespiliformis* crude root extract F1-F6= *D. mespiliformis* root fractions. + = present and - + absent

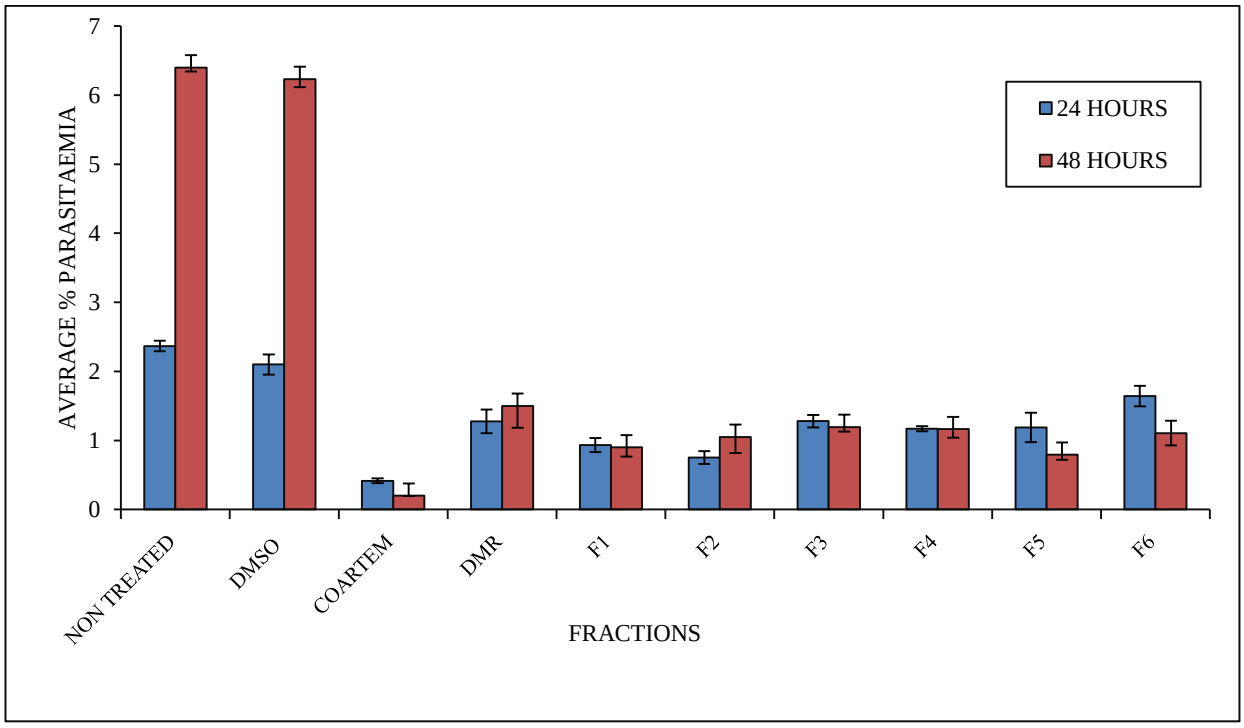


Figure 27: Antiplasmodial activity of *D. mespiliformis* root, first round fractions. Keys: DMR= crude *D. mespiliformis* root extract, coartem = positive control and DMSO = negative control.

4.6. Second round of fractionation

The selection of fractions from the first round of fractionation to proceed to the second round was based on the level of antiplasmodial activity and the number of classes of phytochemicals revealed by TLC screen. Only fractions that indicated highest of reduction average % parasitaemia and few classes of phytochemicals were selected for the second round of fractions. For *Cyphostemma spp* whole plant fraction 1, 2 and 3 were selected, *D. mespiliformis*, leaves fraction 3 and 4 were selected and *D. mespiliformis*, roots fraction 1 and 2 were selected. *Cyphostemma spp* fraction number 1 and 2 yielded three fractions and fraction number 3 of the same plant yielded 2 fractions. All roots and leaves of *D. mespiliformis* yielded two fractions.

Table 10: Second round fractions

first round fraction ID	second round fraction ID
CPW F1	A1F1
	A1F2
	A1F3
CPW F2	A2F1
	A2F2
	A2F3
CPW F3	A3F1
	A3F1
	A3F2
DML F3	B3F1
	B3F2
DML F4	B4F1
	B4F2
DMR F1	C1F1
	C1F2
DMR F2	C2F1
	C2F2

KEYS: The first letter represents the plant's names, e.g. A= *Cyphostemma*, B, = *D. mespiliformis* leaves, C= *D. mespiliformis* roots. The second number represents the fraction number from the first round of fractionation F# represents the second round fraction number in the decreasing elution order

4.6.1. Antiplasmodial activity and phytochemical screening of *Cyphostemma spp* second round fractions

Briefly, All *Cyphostemma* spp whole plant second round fractions remained multi constituent with the presence of more than 2 phytochemical compounds screened. All fractions had the presence of coumarins in common. There was significant difference in antiplasmodial activity across fractions at 24 hours (p value =0.02) and at 48 hours, the difference across fractions was not significant (p value =0.3). Fractions A3F1 and A3F2 indicated highest antiplasmodial activity at 24 and 48 hours respectively compared to the rest of the fractions. The phytochemical screening of *Cyphostemma* spp second fractions is summarised in table 11, and their antiplasmodial activity is shown in figure 28.

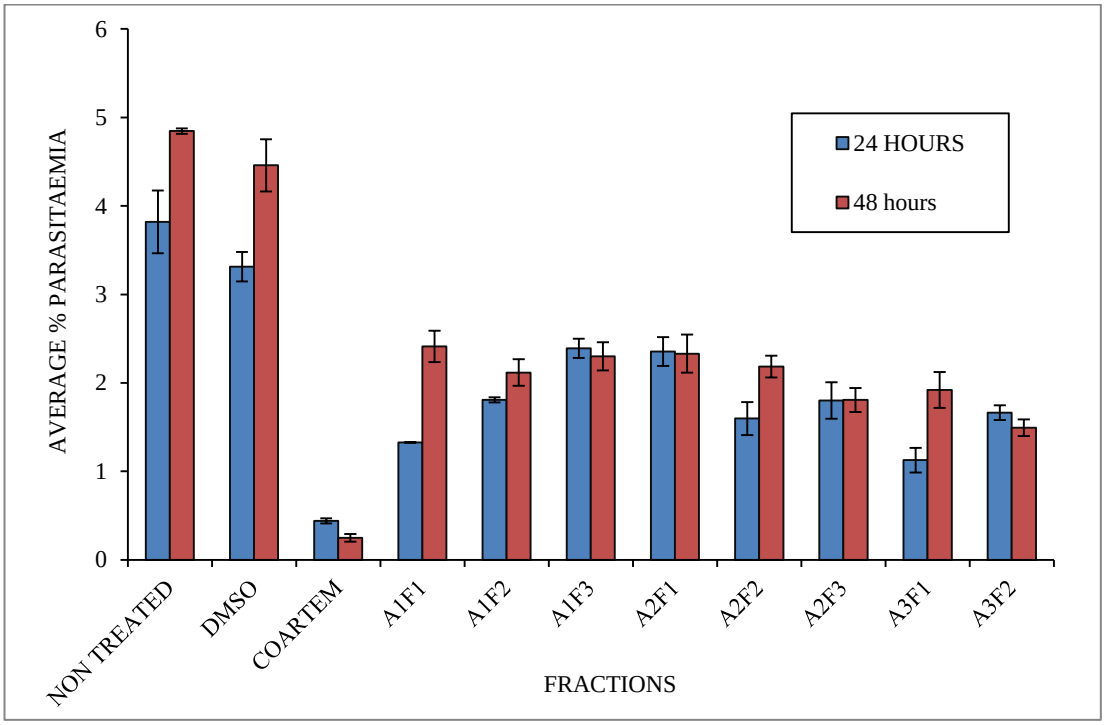


Figure 28: Antiplasmodial activity of *Cyphostemma* spp whole plant second round fractions.

KEYS: The first letter represents the plant's names, e.g. A= *Cyphostemma spp*. The second number represents the fraction number from the first round of fractionation. F (#) represents second round fraction number in decreasing elution order. DMSO = negative control

Table 11: Phytochemical screening of *Cyphostemma* spp whole plant second round fractions by TLC

Fraction ID	flavonoids	coumarins	terpenoids	anthraquinones	alkaloids
A1F1	+	+	+	+	+
A1F2	+	+	+	-	+
A1F3	+	+	+	-	-
A2F1	+	+	+	+	+
A2F2	-	+	-	+	+
A2F3	-	+	+	-	-
A3F1	+	+	+	-	+
A3F2	+	+	+	-	+

Keys: + = present and - = absent

4.6.2. Antiplasmodial activity and phytochemical screening of *D. mespiliformis,* leaf second round fractions

All *Diospyros mespiliformis*, leaf second round fractions were well fractionated with 1-2 anthraquinones and coumarins spots per fractions. The bioactive classes of antiplasmodial phytochemical of *D. mespiliformis* leaf are clearly identified as anthraquinones and coumarins (table 12). All fractions indicated high average % parasitaemia reduction in relation to non-treated control (see figure 29) after 24 and 48 hours. Fraction B3F2 had equal average % parasitaemia reduction at 24 and 48 hours as shown in figure 31. Additionally, there was no significant difference in average % parasitaemia reduction across for 24 hours (p value = 0.3) and likewise at 48 hours (p value = 0.7). Phytochemical screenings of fractions results are revealed in table 12 and their *in vitro* antiplasmodial activity is summarised in figure 29.

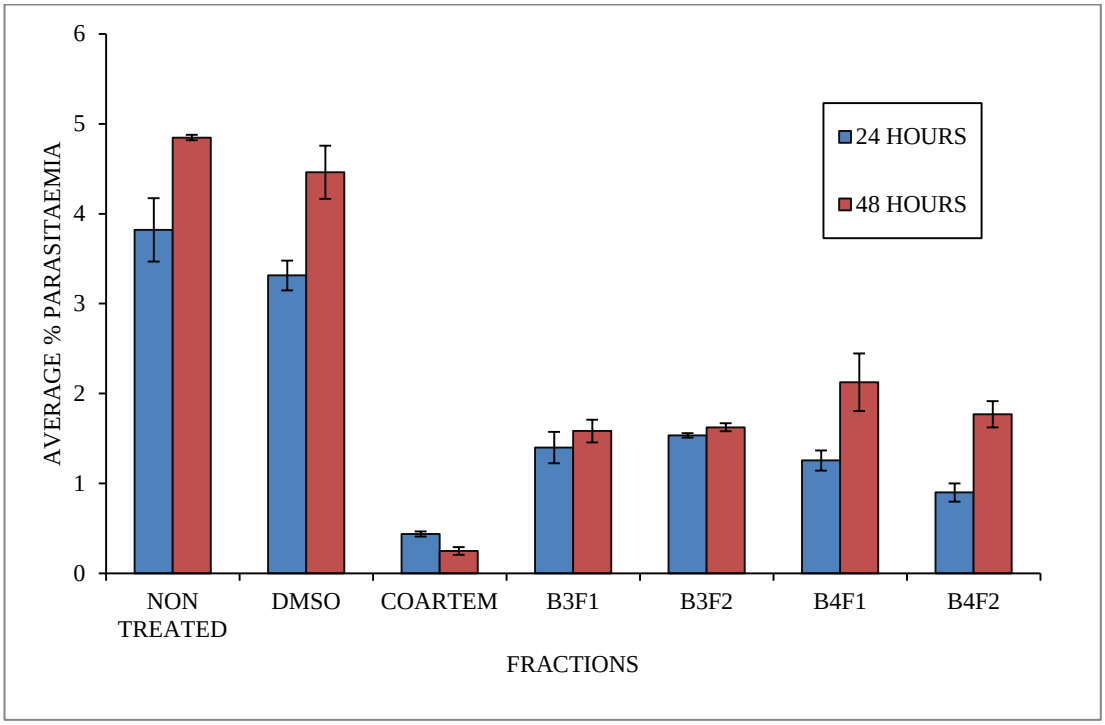


Figure 29: Antiplasmodial activity of *D. mespiliformis* leaf second round fractions.

KEYS: The first letter represents the plant's names, e.g. B, = *D. mespiliformis* leaf. The second number represents the fraction number from the first round of fractionation. F (#) represents second round fraction number in decreasing elution order. DMSO = negative control and coartem positive control

Table 12: Phytochemical screening of *D. mespiliformis* leaf second round fractions by TLC

Fraction ID	flavonoids	coumarins	terpenoids	anthraquinones	alkaloids
B3F1	-	-	-	+	-
B3F2	-	-	-	+	-
B4F1	-	-	-	+	-
B4F2	-	+	-	+	-

Keys: + = present and - = absent

4.6.3. Antiplasmodial activity and phytochemical screening of *D. mespiliformis* root second round fractions

Antiplasmodial activity of *D. mespiliformis* root fractions is shown in figure 30 and phytochemical screening of the fractions in table 13. Briefly, Most of *Diospyros mespiliformis*, root fractions did not show presence of any phytochemical screened for. Average % parasitaemia of all fractions was not significantly different for 24 hours (p value =0.5) and likewise at 48 hours, (p value = 0.7). However, in relation to the non-treated control, there was reduction in average parasitaemia at 24 and 48 hours. Therefore, this affirms the inhibitory activity of *D. mespiliformis* root fractions against *P. falciparum* 3D7A. Only one *Diospyros mespiliformis*, root fraction (C2F2) indicated the presence of flavonoids. For this, flavonoids were identified as a bioactive class of antiplasmodial phytochemical compounds of *D. mespiliformis* roots.

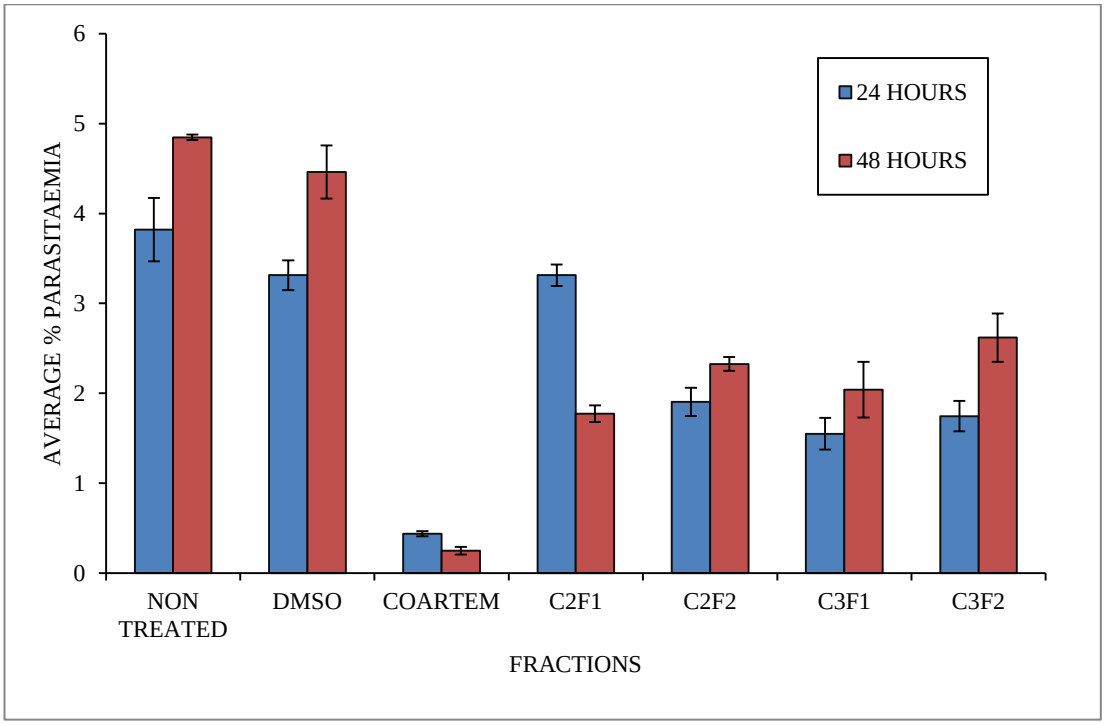


Figure 30: Antiplasmodial activity of *D. mespiliformis* root second round fraction.

KEYS: The first letter represents the plant's names, C= *D. mespiliformis* root. The second number represents the fraction number from the first round of fractionation. F (#) represents second round fraction number in the decreasing elution order. DMSO = negative control and coartem positive control.

Table 13: Phytochemical screening of *D. mespiliformis* root, second round fractions by TLC

Fraction ID	flavonoids	anthraquinones	coumarins	terpenoids	alkaloids
C2F1	-	-	-	-	-
C2F2	+	-	-	-	-
C3F1	-	-	-	-	-
C3F2	-	-	-	-	-

Keys: + = present and - = absent

CHAPTER 5: DISCUSSION

Plant species evaluated in this study where identified through an ethnobotanical approach whereby plants are selected based on their use in traditional settings to treat malaria associated symptoms. Other methods are used to identify plant species for biological evaluation such as random approach, chemotaxonomic approach and phytochemical approach, (Frabicant & Fransworth 2001). To avoid repetition of *in vitro* antiplasmodial evaluation of the same plant species in other countries, a background literature review was conducted for confirmation. Plant species were collected from Ohangwena and Omusati regions because these regions share borders with Angola where malaria transmission is high, (WHO Malaria Report, 2012, pp 95) and the use of ethno medicinal plants to treat common ailments is preferred as well, (Von Koenen, 2001).

The study focused mainly on the phytochemical screening of major classes of phytochemicals with known antiplasmodial activity namely; terpenoids, alkaloids, flavonoids, coumarins and anthraquinones. *M. sericea* shoot crude methanol extract indicated the presence all phytochemical compounds screened for and high total phenolics content (274.75 GA/E µg/ml) and total alkaloids content (17.78 µg/100 mg extracts). This can be linked to its role in treatment of malaria symptoms in traditional settings in Namibia, (Von Koenen, 2001). The presence of terpenoids in the methanol extract of *M. sericea* leaf is supported by the findings of Iyer *et al*., (2004). In Iyer's study, preliminary phytochemical analysis of the *M. sericea* leaf methanol crude extract revealed the presence of triterpenoids and saponins. It is noteworthy to mention that deguelin of the flavonoids group of phytochemicals and

an anticancer natural product, was first isolated from *M. sericea,* (Wang *et al*, 2012; Mehta *et al,* 2013). *M. sericea* has been studied phytochemically and several flavanones, flavanonols, rotenoids, chalcones, terpenoids and sesquiterpenoids have been isolated from this plant in Botswana, (Mazimba, 2012).

Acrotome inflata is one of the ubiquitous herbs found in Northern Namibia. It is known for its typical, strong and choking smell and its use as mosquito repellent. In this study, the crude methanol extract of this herb indicated the presence of terpenoids only, which is responsible for its typical odour. In the view of the fact that mono, di and sesquiterpenoids contribute to the odour of plants, (Baikal *et al*, 2008). Terpenoids are also significant to malaria treatment because artemisinin, the lead molecule of the artemisinin based derivatives belong to the sesquiterpenoids class of compounds (Abad *et al*, 2012; White, 2008). Furthermore, over 80% of plant extracts screened in this study indicated the presence of terpenoids as shown in table 3. These plants can be novel sources of biologically potent terpenoids that can be developed into potent antimalarias.

Alkaloids were detected in 10 of the 19 plant extracts screened. Typical alkaloids are derived from plant sources and are basic with one or more nitrogen atoms in a heterocyclic ring, (Evans, 2009). Alkaloids play a particular role to malaria treatment because quinine is the first chemically defined alkaloid that was indentified for malaria treatment, (Motley, 1998). In addition, some previous studies indicated that some alkaloids isolated from plants have antiplasmodial activities, (Astulla *et al,* 2008; Dolabelaa *et al*, 2008; Graziosea *et al*, 2011; Frederich, *et al*, 2008; Oliveira *et al*, 2009).

Anthraquinones were detected in 13 of the 19 plant extracts screened.

The presence of coumarins and flavonoids was detected in 8 of the 19 plant extracts screened. These compounds are part of a phenolic group which is distinguished by the presence of one or more hydroxyl group bonded directly to the benzopyrano ring, (Jain & Himanshu, 2012). Antiplasmodial activities of coumarins and flavonoids isolated from plants have been reported as well, (Bero & Joelle-, Quetin, 2010; Bero *et al*, 2009; Batista *et al*, 2009; Kaur, *et al*, 2009; Sturm *et al*, 2009; Voahangy *et al*, 2008). Most plant extracts indicate the presence of terpenoids (15) and the least detected were coumarins and flavonoids (8). This represents vast potentials of the selected plant to contain novel chemically diverse bioactive classes of antiplasmodial phytochemical compounds.

The presence of alkaloids, coumarins, anthraquinones and terpenoids in *Vangueria infaust*a, root and shoot extracts are in agreement with previous studies done in Botswana by Absol *et al*, (2006) and in Swaziland by Amusan *et al*, (2007). However, the presence of flavonoids in *Vangueria infausta* leaf extract determined in this study was contradicted by previous studies, (Amusan, 2007; Oluwole *et al*, 2007). Furthermore, flavonoids phytochemical compounds with antibacterial and antioxidant activity were isolated from the ethyl acetate leaf extract of *Vangueria infausta* in follow up studies by (Mbukwa *et al*, 2007; Temraz, 2011).

The presence of terpenoids and alkaloids in both root and leaf extracts of *D. mespiliformis* is consistent with the findings of Shagal & Kubmarawa, (2012) & Dangoggo *et al*, (2012). Nevertheless, the presence of flavonoids in both extracts

in current studies was contradicted by Shagal & Kubmarawa, (2012) & Dangoggo *et al,* (2012) and supported by Belemtougri *et al,* (2006) in Burkina Faso. Variations in phytochemical content of *D. mespiliformis* root and leaf extracts between the two studies can be a result of mode of preparation of the extracts and the method of phytochemical analysis used. In this study, methanol was used for extraction, while in Shagal & Kubmarawa's study, ethanol was used for extraction. Furthermore, TLC analysis was used for qualitative phytochemical analysis in the current study, while in the previous study, preliminary Harbonius methods were used. TLC methods are more accurate than preliminary Harbonius methods and the detection is more robust, (Harbone 1998). According to Belemtougri *et al,* (2006), phytochemical screening of *Diospyros mespiliformis*, flavonoids, tannins and saponins were found in aqueous leaf extract, flavonoids tannins and steroids/terpenoids existed in ethanolic leaf extract. Furthermore, triterpenoids phytochemical compounds were isolated from the petroleum ether extract of the stem bark of *D. mespiliformis* in Sudan, (Mohamed *et al,* 2009). The current study revealed the presence of terpenoids in both roots and leaves extract of *D. mespiliformis* as well.

Variations in the phytochemical content of plants may be influenced by external factors such as climatic, geographic, harvesting and storage conditions before and after extractions and analysis (Laher *et al,* 2013). These were not considered in this study. Extensive literature review published in major publications such as EBSCO Host, Springer Link Science Direct, HINARI, etc revealed that for the first time, TLC screening for alkaloids, terpenoids, anthraquinones, flavonoids and coumarins of *A. inflata*, *N. oleracea* and *O. dregeanum* is reported.

Total alkaloid content of the crude plants extracts ranges from 0.37 to 21.21 µg/100 mg extracts. Trace amount of alkaloids detected from the crude plant extracts in this study can be linked to two factors. Firstly; the method used for total alkaloid quantification might have contributed. The method used is based on reaction of alkaloids and BCG to form a yellow coloured complex, (Fazel *et al*, 2008). This is the best method to quantify total alkaloids in terms of simplicity, rapidity and sensitivity (Shamsa *et al*, 2008). However, the BCG method also has inherent limitations such as: BCG can only react with alkaloids that have nitrogen inside their structure; hence amine alkaloids do not react with BCG. Consequently, the data obtained may not provide the whole understanding of total quantity of alkaloid of plant extracts, (Ajanal *et al*, 2012). Secondary; alkaloids are naturally rare and confined to certain families of plants such as *Papaveraceae*, *Rubiceae* etc, (Gangwal, 2013). Additionally, alkaloids are difficult to extract, (Petruczynik, 2012).

Total phenolics content of the crude plant extracts range from 99. 58 to 336. 01 GA/E µg/ml. Similarly, the method used for total phenolic quantification contributed to the high amount of total phenolics obtained for various plant extracts in this study. The method used was the Folin Ciocalteu assay which depends on the transfer of electrons in alkaline medium from phenolic compounds to phosphomolybdic/phosphotungstic acid complexes to form blue complexes that are determined spectrophotometrically, (Dai & Russel, 2010). A study done by Everette *et al*, (2010) reported that the Folin Ciocalteu reagent can also react with other molecules in the extracts mixtures such as amino acid, vitamins, proteins and other

functional groups such as thiols. Consequently, frequently high amount of total phenolics are obtained.

Furthermore, phenolics compounds are abundant in many plant's species and ubiquitous in most plants parts, (Cartea *et al*, 2010; Crozier *et al*, 2010; D'Archivio *et al*, 2010).

Surprisingly, the root extract of *G coleosperma* did not indicate the presence of any phenolics compound analysed in this study but yet it indicated the highest total phenolics content of 343.55 GA/E µg/ml. This is due to other phenolic compounds such as tannins, saponins that were not analysed in this study. On that note, some flavonoids phytochemicals were also isolated from *G. coleosperma* bark, (Bekker *et al*, 2006).

The *in vitro* antiplasmodial assay was done at 1% parasitaemia and 2% hematocrit to mimic *in vivo* infection of *P. falciparum*. Since in 10 ml of *in vitro P. falciparum* culture, over 10000 are infected red blood cells, this is comparable to the percentage parasitaemia in the human blood stream 7 days after infection, (White *et al*, 2013). The IC_{50} values of plant extracts were solely calculated for 48 hour responses because the lifecycle of *P. falciparum* in the erythrocytes takes about 48 hours to complete, (White *et al*, 2013).

M. sericea shoot and leaf methanol crude extracts were not significantly different at 48 hours, (p value > 0.05). That means that the two plant extracts are equally potent and can be used for killing *P. falciparum* 3D7A. On the contrary, for *M. sericea* shoot and leaf aqueous crude extracts, were significantly different at 48 hours, (P values< 0.05). Based on the IC_{50} values, *M. sericea* shoot crude aqueous extract had

the lowest IC_{50} value of 4.99 µg/ml compared to the leaf extract (IC_{50} value =9.10). Therefore, in this case it is advisable to use the shoot extract.

Similarly, there was no significant difference between *D. mesipiliformis* crude methanol leaf and root extracts at 48 (p value >0.05) as well as the crude aqueous leaf and root extracts (p value >0.05). This implies that both aqueous and methanol extracts had similar effect on *P. falciparum* 3D7A.

The classifications of highly active, active and inactive antiplasmodial activity of crude extracts was based on criteria set by, (Deharo, 2001). In this study, extracts are considered highly active when the IC_{50} values of extracts are less than 5 µg/ml and active when the IC_{50} values are between 5 and 10 µg/ml and not active when is IC_{50} values are over 10 µg/ml. This is because inhibition of parasite growth at low concentration will indicate selective activity as opposed to high concentration where non-specific activity is usually observed, (Clarkson *et al*, 2004). Crude methanol plant extracts indicated lower IC_{50} values as opposed to crude aqueous plant extracts (see table 5). Water is the main and preferred solvent used in traditional settings. This is not surprising because some previous studies showed similar patterns of crude aqueous plant extracts less potent than their corresponding crude organic extracts, (Bero *et al*, 2009; Clarkson *et al*, 2004; Irungu *et al*, 2007). Possible reasons are: firstly, water is unable to extract lipophilic phytochemicals that are extracted by methanol (Clarkson *et al*, 2004). Secondary, aqueous extracts were not prepared the same way as in traditional settings. Actually, in traditional settings, different plant extracts are mixed into concoction which might enhance or inhibit their activity, (Von Koenen, 2001). Thirdly, perhaps the phytochemicals extracted by

water are more potent *in vivo* than *in vitro* model as presented here or active against another stage of parasite or other *Plasmodium* species.

In vitro antiplasmodial activity of the crude extracts observed can be attributed to the multi constituent phytochemicals of the crude extracts as revealed by phytochemical screening (table 3 and 4). The toxicity of DMSO to the parasites and red blood cells was reduced by diluting to less than 0.5% which is the recommended safe concentration, (Cos *et al*, 2006).

D. mespiliformis can be focus of antimalarial herbal development. One study recorded *in vivo* study in mouse whereby antiplasmodial activity of *D. mespiliformis* aqueous bark extracts exhibited significant ($p<0.05$) curative effects against *Plasmodium berghei berghei* and suppressive potency against early infection of the parasites (Adzu & Salawu 2009). Other *Diospyros* species namely; *Diospyros melanoxylon, Diospyros peregrina, Diospyros sylvatica, Diospyros tomentosa*, were evaluated for *in vitro* antiplasmodial activity, (Kantamreddi *et al*, 2008). Their IC_{50} values range from 16.5 to 4500 µg/ml. In another study, (Prachayasittiku *et al* 2010), a bioassay guided fractionation regime was applied to another *Diospyros* species namely; *Diospyros rubra* and led to the isolation of terpenoidal compounds with antiplasmodial activity such as; lupeol, lupenone, betulin, lupeol acetate, 28-*O*-acetylbetulin β-sitosteryl-3-*O*-β-D-glucopyranoside and a mixture of β-sitosterol and stigmasterol. This signifies the possibility of isolating novel antiplasmodial hits from *D. mespiliformis*.

Extensive literature review using search engines such as Google Scholar, Science Direct, Springer Link, Pub med etc., proved that there is no report of *in vitro* anti plasmodial activity of *M. sericea, D. mespiliformis* and *Cyphostemma spp*. Therefore for the first time *in vitro* antiplasmodial activity of *M. sericea, D. mespiliformis* and *Cyphostemma spp* is reported.

Cyphostemma spp whole plant extract remained phytochemically diverse throughout fractionation with presence of many phytochemicals per fractions, (see table 7 and 10). This can be due to the fact that the crude extract was concentrated with phytochemicals (Total phenolic content, 160.55 GAE/100µg and total alkaloids 21. 11 µg/100 mg extracts). First round fraction 4 indicated the presence of flavonoids only which can be linked to the *in vitro* antiplasmodial activity observed. Fraction 2 indicated the presence terpenoids and coumarins. The two phytochemicals can be working synergistically or additively to confer antiplasmodial activity of *Cyphostemma.*

Although, *Cyphostemma* first round fractions were phytochemically diverse, it appears that the bioactive compounds were working synergistically or additively to enhance antiplasmodial activity. As a matter of fact, increased antiplasmodial activity was observed for *Cyphostemma* spp multi constituents first round fractions for 24 hours compared to the crude extracts (see figure 25). Fraction 4 indicated the presence of single flavonoids and reduced antiplasmodial activity at 24 hours compared to the crude (see figure 25). In comparison with less constituent fractions, it appears that *Cyphostemma* fractions are mostly effective when multi constituent.

For the first time a bioassay guided fractionation regime was applied to *Cyphostemma spp* to identify antiplasmodial classes of phytochemical compounds. However, literature recorded attempts to isolate natural products with other biological properties from *Cyphostemma* spp. Bioassay guided fractionation of the ethanol bark extract obtained from *Cyphostemma greveana* led to the identification of one macrolide, one lasiodiplodin, three sesquiterpenoids: 12-hydroxy-15-oxoselina-4,11-diene, 1*β*,6*α*-dihydroxyeudesm-4(15)-ene, and (7*R*)-opposit-4(15)-ene-1*β*,7-diol. This included a new diterpenoid: 16, 18-dihydroxykolavenic acid lactone. The last two compounds indicated anticancer activity, (Cao *et al*, 2011). These highlight the fact that *Cyphostemma* spp are rich in phytochemicals and can be sources of novel antiplasmodial lead compounds.

Diospyros mespiliformis leaf first round fractions (F3 & F4) indicated the presence of terpenoids, coumarins and anthraquinones but when further fractionated with *n*-hexane/ethyl acetate (8:2) for the second round only anthraquinones were present. In this case the mobile phase was unable to elute terpenoids and coumarins from *D. mespiliformis* leaf fractions. Only F3 and F4 indicated increased antiplasmodial activity observed for only 24 hours with significant statistical difference in comparison with the crude leaf extract. However, at 48 hours all fractions indicated increased antiplasmodial activity with significant statistical difference. (See figure 28). This implies the dysergy effect of the crude extract bioactive phytochemicals.

Anthraquinones were identified as the bioactive class of phytochemicals responsible for antiplasmodial activity of *D. mespiliformis* leaf extract at the last round of

fractionation. Some anthraquinones isolated from plants in previous studies indicated antiplasmodial activities. For example, a bioassay guided fractionation regime was applied to the DCM root extract of *Rennellia elliptica* Korth and led to the isolation and characterization of one novel anthraquinone; 1,2-dimethoxy-6-methyl-9,10-anthraquinone (IC_{50} value of 1.10µM) and five known anthraquinones: nordamnacanthal (IC_{50} value of 72.46 µM), 2-formyl-3-hydroxy-9,10-anthraquinone (IC_{50} value of 0.63 µM), damnacanthal (IC_{50} values of 51.28 µM), lucidin-*ω*-methyl ether (IC_{50} value of 2.10 µM) and 3-hydroxy-2-methyl-9,10-anthraquinone (IC_{50} value of 0.35 µM), (Osaman *et al,* 2010).

D. mespiliformis root, only fraction 2 and 3 indicated increased *in vitro* antiplasmodial activity for 24 hours whilst at 48 hours all fractions indicated increased antiplasmodial activity in comparison with crude extract. (See figure 26). First round fractions (F3 & F4) were multi constituents with the presence of anthraquinones, terpenoids and flavonoids but when fractionated further with *n*-hexane/ethyl acetate (8:2) no presence of any phytochemical analysed was shown. However, *in vitro* antiplasmodial activity was indicated for all fractions. Although, other factors may be responsible for the observed change, it can also be due to the presence other phytochemicals not screened for in this study e.g. tannins, saponins etc. Only fraction C2F2 indicated the presence of flavonoids which can be linked to the *in vitro* antiplasmodial activity observed.

CHAPTER 6: CONCLUSION AND RECOMMENDATIONS

This study is a contribution to the evaluation of ethnomedicinal plants for search of new chemical entities with known antiplasmodial properties. Terpenoids, alkaloids, flavonoids, anthraquinones and were found to be present in the ten ethnomedicinal plants screened. In addition, the ten plants screened indicated total alkaloids and phenolics in substantial concentrations. *D. mespiliformis* (leaf & roots), *M. sericea* (leaf and shoot) and *Cyphostemma* spp (whole plant) crude aqueous and methanol extracts were evaluated in an *in vitro* antiplasmodial bioassay. All plants were found to be potent against *P. falciparum* 3D7A strain. Anthraquinones, coumarins and flavonoids were identified as the single class of antiplasmodial bioactive phytochemicals of *D. mespiliformis* (root and leaf) and *Cyphostemma* spp (whole plant) respectively.

The phytochemical screening of the crude plant extracts, *in vitro* antiplasmodial activity and the screening of the bioactive phytochemicals from the selected plants supported the use of these plants to treat malaria associated symptoms in traditional settings. The methanol plant extracts were more effective at 24 hours than 48 hours. Although, it is premature to say, it appears that the plant extracts are effective for a short period of time and should be used as such. The crude methanol extracts were found to be active than their corresponding crude aqueous extracts. The identification of the bioactive components of these plants serves as a basis for in-depth pharmacological evaluation and isolation of these bioactive phytochemicals for antimalarial drugs development. Additionally, this can also assist in herbal drug formulations of the selected plants. Further works will include actual identification

of single chemical entity responsible for antiplasmodial activity from *D. mespiliformis* (leaf and root) and *Cyphostemma* spp (whole plant). Additionally, *in vitro* cytotocixity studies to establish the selectivity index of the extracts crude plant extracts to human cells and to advice on safe dosage to the people who use traditional medicine is required. *In vivo* studies must also be conducted to show antiplasmodial activity of the selected plants in a closed system.

REFERENCE LIST

Abad Mara Jos´e, Bedoya Luis Miguel, Apaza Luis & Bermejo Paulina. (2012). The Artemisia L. Genus: A Review of Bioactive Essential Oils. *Molecules* 17(3), 2542–2566,

Abeer, T. (2011). Flavonoidal content of *Vangueria infausta* extract grown in Egypt: investigation of its antioxidant activity. *International Research Journal of Pharmacy*, *2*(3), 157-161

Abosl, A. O., Mbukwa, E., Majinda, R. R., Raserok, B. H., Yenesew, A., Midiwo, J. O., Waters, N. C *et al*. (2006). *Vangueria infausta* root bark*: in vivo* and *in vitro* antiplasmodial activity. *British Journal of Biomedical Science*, 63(3), 129-33

Achan, J., Talisuna, A. O., Erhart, A., Yeka, A., Tibenderana, J. K., Baliraine, F. N., D'Alessandro, U, *et al*. (2011). Quinine, an old antimalarial drug in a modern world: role in the treatment of malaria. *Malaria Journal*, *10*(144), 1475-2875.

Adzu, B., & Salawu, O. A. (2009). Screening *Diospyros mespiliformis* extract for antimalarial potency. *International Journal of Biological and Chemical Sciences*, *3*(2), 271-276

Ajanal, M., Gundkalle, M. B., & Nayak, S. U. (2012). Estimation of total alkaloid in *Chitrakadivati* by UV-spectrophotometer. *Ancient Science of Life*, *31*(4), 198.

Alegana Victor A. Peter M. Atkinson Jim A. Wright Richard Kamwi, Petrina Uusiku, Stark Katokele, Robert W. Snow Abdisalan M. Noor (2013).

Estimation of malaria incidence in Northern Namibia in 2009 using Bayesian conditional-autoregressive spatial-temporal models. *Spatial and Spatio-temporal Epidemiology* 7 (2013) 25–36

Allen, S. (2006). A History of quinine and artemisinin. *British Travel Health Association Journal*, *8*(19), 19-20.

Amusan, O. O., Sukati, N. A., Dlamini, P. S., & Sibandze, F. G. (2007). Some Swazi phytomedicines and their constituents. *African Journal of Biotechnology*, 6(3), 267-272.

Astulla, A., Zaima, K., Matsuno, Y., Hirasawa, Y., Ekasari, W., Widyawaruyanti, A., *et al*. (2008). Alkaloids from the seeds of *Peganum harmala* showing antiplasmodial and vasorelaxant activities. *Journal of Natural Medicines* 62(4), 470-472

Attaran, A., Barnes, K. I., Curtis, C., d'Alessandro, U., Fanello, C. I., Galinski, M. R., ... & Watkins, W. M. (2004). WHO, the Global Fund, and medical malpractice in malaria treatment. *The Lancet*, *363*(9404), 237-240.

Auala Joyce RN, Mumbengegwi Davis R, Du Preez Iwanette and Bock Ronnie A. (2012) Evaluation of *Tarchonanthus camphoratus* plant extracts for antimicrobial activity against food-borne pathogens. *Journal of Research in Microbes* 1(2):096-104

Baird J. Kevin, (2005). Effectiveness of antimalarial drugs. *New England Journal of Medicine*; 352(15):1565-77

Bakkali F, Averbeck S, Averbeck D & Idaomar M. (2008). Biological effect of essential oils- Review. *Food & Chemical Toxicology* 46(2), 446-475,

Balunas, M. J., & Kinghorn, A. D. (2005). Drug discovery from medicinal plants. *Life Sciences*, 78(5), 431-441.

Bekker, M., Bekker, R., & Brandt, V. E. (2006). Two flavonoid glycosides and a miscellaneous flavan from the bark of *Guibourtia coleosperma*. *Phytochemistry*, 67(8), 818-823.

Beldjoudi, N., Mambu, L., Labaïed, M., Grellier, P., Ramanitrahasimbola, D., Rasoanaivo, P., & Frappier, F. (2003). Flavonoids from *Dalbergia ouvelii* and their Antiplasmodial Activity. *Journal of Natural Products*, 66(11), 1447-1450.

Bero, J., Ganfon, H., Jonville, M. C., Frédérich, M., Gbaguidi, F., DeMol, P., Quetin-Leclercq, J, *et al*. (2009). *In vitro* antiplasmodial activity of plants used in Benin in traditional medicine to treat malaria. *Journal of Ethnopharmacology*, 122(3), 439-444.

Beshir, K. B., Sutherland, C. J., Sawa, P., Drakeley, C. J., Okell, L., Mweresa, C. K *et al* . (2013). Residual *Plasmodium falciparum* parasitaemia in Kenyan children after artemisinin-combination therapy is associated with increased transmission to mosquitoes and parasite recurrence. *Journal of Infectious Diseases*, 208(12), 2017-2024.

Bloland, P. B., Lackritz, E. M., Kazembe, P. N., Were, J. B., Steketee, R., & Campbell, C. C. (1993). Beyond chloroquine: implications of drug

resistance for evaluating malaria therapy efficacy and treatment policy in Africa. *Journal of Infectious Diseases*, 167(4), 932-937.

Bourgaud, F., Gravot, A., Milesi, S., & Gontier, E. (2001). Production of plant secondary metabolites: a historical perspective. *Plant Science*, 161(5), 839-851.

Breman Joel G., Martin S. Alilio, & Mills Anne, (2004). Conquering the intolerable burden of malaria: what's new, what's needed: A summary. *American Journal of Tropical Medicine and Hygiene* 71(Suppl 2), 2004; 1–15

Breman. G. Joel. (2003).The ears of the hippopotamus: manifestations, determinants, and Estimates of the malaria burden. *American Journal of Tropical Medicine and Hygiene*, 64(1, 2) S, 2001, pp. 1–11

Bruce, N. C. (2008). Alkaloids. *Biotechnology Set, Second Edition*, 327-361.

Brusotti, G., Cesari, I., Dentamaro, A., Caccialanza, G., & Massolini, G. (2014). Isolation and characterization of bioactive compounds from plant resources: The role of analysis in the ethnopharmacological approach. *Journal of Pharmaceutical and Biomedical Analysis*, *87*, 218-228.

Butler, A. R., Khan, S., & Ferguson, E. (2010). A brief history of malaria chemotherapy. *The Journal of the Royal College of Physicians of Edinburgh*, 40(2), 172.

Cao, S., Hou, Y., Brodie, P., Miller, J. S., Randrianaivo, R., Rakotobe, E, Kingston, D. G. *et al*. (2011). Antiproliferative compounds of *Cyphostemma greveana* from a Madagascar dry forest. *Chemistry & Biodiversity*, *8*(4), 643-650.

Cartea, M. E., Francisco, M., Soengas, P., & Velasco P. (2010). Phenolic compounds in *Brassica* vegetables. *Molecules*, 16(1), 251-280.

Cheikhyoussef, A., Shapi, M., Matengu, K., & Ashekele, H. M. (2011). Ethnobotanical study of indigenous knowledge on medicinal plant use by traditional healers in Oshikoto region, Namibia. *Journal of Ethnobiology and Ethnomedicine*, *7*(10), 1-11.

Chin, Y. W., Balunas, M. J., Chai, H. B., & Kinghorn, A. D. (2006). Drug discovery from natural sources. *The American Association of Pharmaceutical Scientists Journal*, *8*(2), 239-253.

Chinsembu, K. C., Hedimbi, M., & Mukaru, W. C. (2011). Putative medicinal properties of plants from the Okavango region, Namibia. *Journal of Medicinal Plants Research*, *5*(31), 6787-6797.

Choudhury, D. S., & Ghosh, S. K. (1985). Laboratory diagnosis of malaria. *The Indian Journal of Paediatrics*, *52*(3), 261-267.

Chung, I. M., Ghimire, B. K., Kang, E. Y., & Moon, H. I. (2010). Antiplasmodial and cytotoxic activity of khellactone derivatives from *Angelica purpuraefolia* Chung. *Phytotherapy Research*, *24*(3), 469-471.

Clarkson, C., Maharaj, V. J., Crouch, N. R., Grace, O. M., Pillay, P., Matsabisa, M. G., Folb, P. I, *et al*. (2004). *In vitro* antiplasmodial activity of medicinal plants native to or naturalised in South Africa. *Journal of Ethnopharmacology*, *92*(2), 177-191.

Cos Paul, Arnold J. Vlietinck, Dirk Vanden Berghe, Louis Maes (2006) Antiinfective potential of natural products: How to develop a stronger in vitro 'proof-of-concept' *Journal of Ethnopharmacology* 106, 290–302

Cowan, M. M. (1999). Plant products as antimicrobial agents. *Clinical Microbiology Reviews*, 12(4), 564-582.

Cox Paul Alan, (2001). Pharmacology, biodiversity and. *Encyclopaedia of Biodiversity, Volume 4*

Cox, P. A. (2008). Ethnopharmacology and the search for new drugs. *Bioactive Compounds from Plants*, *735*, 40.

Cox, R. J. (2000). 7Biosynthesis. *Annual Reports Section" B" Organic Chemistry)*, 96(1), 231-258

Crawley, J., Chu, C., Mtove, G., & Nosten, F. (2010). Malaria in children. *The Lancet*, 375(9724), 1468-1481.

Croteau, R., Kutchan, T. M., & Lewis, N. G. (2000). Natural products (secondary metabolites). *Biochemistry and Molecular Biology of Plants*, 1250-1318.

Crozier, A., Del Rio, D., & Clifford, M. N. (2010). Bioavailability of dietary flavonoids and phenolic compounds. *Molecular Aspects of Medicine* 31(6), 446-467.

Cunha-Rodrigues Margarida, Miguel Prudêncio, Maria M. Mota and Werner Haas., 2006. Antimalarial drugs – host targets (re)visited. *Biotechnology Journal*, 1, 321–332

Dai, J., & Mumper, R. J. (2010). Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. *Molecules*, 15(10), 7313-7352

Daily, J. P. (2006). Antimalarial drug therapy: the role of parasite biology and drug resistance. *The Journal of Clinical Pharmacology*, 46(12), 1487-1497.

Dangoggo, S. M., Hassan, L. G., Sadiq, I. S., & Manga, S. B. (2012). Phytochemical Analysis and Antibacterial Screening of Leaves of *Diospyros Mespiliformis* and *Ziziphus Spina-Christi*. *Journal of Chemical Engineering*, *1*(1) 31-37.

DArchivio, M., Filesi, C., Var, R., Scazzocchio, B., & Masella, R. (2010). Bioavailability of the polyphenols: status and controversies. *International Journal of Molecular Sciences* 11(4), 1321-1342

Das, K., Tiwari, R. K. S., & Shrivastava, D. K. (2010). Techniques for evaluation of medicinal plant products as antimicrobial agent: Current methods and future trends. *Journal of Medicinal Plants Research*, *4*(2), 104-111.

Davis, T. M., Karunajeewa, H. A., & Ilett, K. F. (2005). Artemisinin-based combination therapies for uncomplicated malaria. *Medical Journal of Australia*, *182*(4), 181-185.

Deharo, E., Bourdy, G., Quenevo, C., Muoz, V., Ruiz, G., & Sauvain, M. A. (2001). Search for natural bioactive compounds in Bolivia through a

multidisciplinary approach. Part V. Evaluation of the antimalarial activity of plants used by the Tacana Indians. *Journal of Ethnopharmacology* 77(1), 91-98. 90

Dettrakul, S., Surerum, S., Rajviroongit, S., & Kittakoop, P. (2009). Biomimetic transformation and biological activities of globiferin, a terpenoid benzoquinone from *Cordia globifera*. *Journal of Natural Products, 72*(5), 861-865.

Dharani, N., Rukunga, G., Yenesew, A., Mbora, A., Mwaura, L., Dawson, I., & Jamnadass, R. (2010). *Common antimalarial trees and shrubs of East Africa*. World Agroforestry Centre.

Dias, D. A., Urban, S., & Roessner, U. (2012). A historical overview of natural products in drug discovery. *Metabolites*, *2*(2), 303-336.

Dondorp, A. M., Nosten, F., Yi, P., Das, D., Phyo, A. P., Tarning, J., & White, N. J. (2009). Artemisinin resistance in *Plasmodium falciparum* malaria. *New England Journal of Medicine, 361*(5), 455-467.

Dondorp, A. M., Yeung, S., White, L., Nguon, C., Day, N. P., Socheat, D., & von Seidlein, L. (2010). Artemisinin resistance: current status and scenarios for containment. *Nature Reviews Microbiology*, *8*(4), 272-280.

Du Preez, I. (2012). *Evaluation of antimalarial properties of indigenous plants used by traditional healers in Namibia* (Master dissertation, University of Namibia).

Dushimemaria, F., Mumbengegwi, D., Du Preez, I., & Bock, R. (2012). Qualitative phytochemical screening and *in vitro* antimicrobial effects of plant extracts of *Searsia tenuinervis. Journal of Research in Microbes*, 1(2):088-095

Eisenberg, D. M., Harris, E. S., Littlefield, B. A., Cao, S., Craycroft, J. A., Scholten, R., *et al*. (2011). Developing a library of authenticated Traditional Chinese Medicinal (TCM) plants for systematic biological evaluation rationale, methods and preliminary results from Sino-American collaboration. *Fitoterapia*, *82*(1), 17-33.

El-Readi, M. Z., Eid, S., Ashour, M. L., Tahrani, A., & Wink, M. (2012). Modulation of multidrug resistance in cancer cells by chelidonine and *Chelidonium majus* alkaloids. *Phytomedicine* 20 (3-4), 282– 294.

Evans, W. C. (2009). *Trease and Evans pharmacognosy*. Elsevier Health Sciences.

Everette, J. D., Bryant, Q. M., Green, A. M., Abbey, Y. A., Wangila, G. W., & Walker, R. B. (2010). Thorough study of reactivity of various compound classes toward the Folin-Ciocalteu reagent. *Journal of Agricultural and Chemistry*, 58(14), 8139-8144.

Fabricant, D. S., & Farnsworth, N. R. (2001). The value of plants used in traditional medicine for drug discovery. *Environmental health perspectives*, *109*(Suppl 1), 69.

Francis, P. S., Adcock, J. L., Costin, J. W., Purcell, S. D., Pfeffer, F. M., & Barnett, N. W. (2008). Chemiluminescence detection of Opium poppy (*Papaver*

somniferum) alkaloids. *Journal of Pharmaceutical and Biomedical analysis*, *48*(3), 508-518.

Frederich, M., Tits, M., & Angenot, L. (2008). Potential antimalarial activity of indole alkaloids. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 102(1), 11–19.

Garner, P. (2013). Artemisinin Combination Therapy: A good antimalarial, but is the dose right? *PLoS Medicine*, *10*(12), e1001565.

Gilca, M., Gaman, L., Panait, E., Stoian, I., & Atanasiu, V. (2010). *Chelidonium majus* an integrative review: traditional knowledge versus modern findings.*Forschende Komplementärmedizin/Research in Complementary Medicine*, *17*(5), 241-248.

Ginsburg, H., & Deharo, E. (2011). A call for using natural compounds in the development of new antimalarial treatments-an introduction. *Malaria Journal*, 10(Suppl 1), S1.

Greenwood Brian M., Fidock David A., Kyle Dennis E., Kappe Stefan H.I., Alonso Pedro L., Frank H. Collins & Patrick E. Duffy, (2008). Malaria: progress, perils, and prospects for eradication. *Journal of Clinical Investigation* 118(4), 1266-1275.

Grotewold, E. (Ed.). (2007). The science of flavonoids. *Springer*.

Gurib-Fakim, A. (2006). Medicinal plants: traditions of yesterday and drugs of tomorrow. *Molecular Aspects of Medicine*, 27(1), 1-93.

Harbone .J.B., (1998). Phytochemical Methods: A guide to modern techniques of plant analysis, 3rd edition, *Chapman and Hall*, New York

Hedimbi Marius, Kaputjaza Diana K, Hans Renate H., Mumbengegwi Davis R., Böck Ronnie. (2012). Phytochemical analysis and antimicrobial screening of crude extracts *from Pechuel-Loeschea leubnitziae* (Kuntze) O. Hoffm (Asteraceae). *Journal of Research in Microbes* 1(1): 039-043

Hedimbi, M., Hamunyela, M. H., Hans, R. H., & Chinsembu, K. C. (2012a). Analysis of antimicrobial properties of *Acrotome inflata* and *Sesamum alatum*. *Journal of Research in Antimicrobials*, *1*(1), 043-048.

Hedimbi, M., Ndjoze-Siririka, V. N., & Hans, R. H. (2012). *In vitro* antimicrobial activity of *Acanthosicyos naudinianus* and *Pechuel-Loeschea leubnitziae* on five pathogenic microorganisms. *Journal of Research in Microbes.* 1(1): 033-038

Hobbs, C., & Duffy, P. (2011). Drugs for malaria: something old, something new, something borrowed. *F1000 Biology Reports*, *3*(24), 1-9

http://www.cdc.gov/malaria/about/faqs.html

http://www.mr4.org/Publications/MethodsinMalariaResearch.aspx

Irungu, B. N., Rukunga, G. M., Mungai, G. M., & Muthaura, C. N. (2007). *In vitro* antiplasmodial and cytotoxicity activities of 14 medicinal plants from Kenya. *South African Journal of Botany*, *73*(2), 204-207.

Iyer, M., Karode, R., Deshmukh, R., & Deshmukh, V. (2004). Antinociceptive activity of *Mundulea sericea* leaves. *Journal of Natural Remedies*, 4(2), 127-130.

Jain P. K. & Himanshu Joshi. (2012). Coumarin: Chemical and Pharmacological Profile. *Journal of Applied Pharmaceutical Science*, 2 (6); 236-240.

Joyeau, David Ramanitrahasimbola, Elisabeth Mouray *et al*. (2008) Antiplasmodial Phenolic compounds from *Piptadenia pervillei*. *Planta Medica*, , doi: 10.1055/s-2008-1034328

Kamkumo, R. G., Ngoutane, A. M., Tchokouaha, L. R., Fokou, P. V., Madiesse, E. A., Legac, J., Rosenthal, P. J. *et al*. (2012). Compounds from *Sorindeia juglandifolia* (Anacardiaceae) exhibit potent antiplasmodial activities *in vitro* and *in vivo*. *Malaria Journal*, *11*(1), 382.

Kantamreddi, V. S., & Wright, C. W. (2008). Investigation of Indian *Diospyros* species for antiplasmodial properties. *Evidence-based Complementary and Alternative Medicine*, *5*(2), 187-190.

Kantele, A., & Jokiranta, T. S. (2011). Review of cases with the emerging fifth human malaria parasite, *Plasmodium knowlesi*. *Clinical Infectious Diseases*, *52*(11), 1356-1362.

Karunamoorthi, K., Sabesan, S., Jegajeevanram, K., & Vijayalakshmi, J. (2013). Role of traditional antimalarial plants in the battle against the global malaria burden. *Vector-Borne and Zoonotic Diseases, doi:10.1089/vbz.2011.0946*

Kaur, K., Jain, M., Kaur, T., & Jain, R. (2009). Antimalarials from nature. *Bioorganic & Medicinal Chemistry* 17(9), 3229–3256.

Khaomek, P., Ichino, C., Ishiyama, A., Sekiguchi, H., Namatame, M., Ruangrungsi, N., & Yamada, H. (2008). *In vitro* antimalarial activity of prenylated flavonoids from *Erythrina fusca*. *Journal of Natural Medicines*, 62(2), 217-220.

Kokwaro, G. (2009). Ongoing challenges in the management of malaria. *Malaria Journal*, *8*(Suppl 1), S2.

Koriem, K. M., Arbid, M. S., & Asaad, G. F. (2013). *Chelidonium majus* leaves methanol extract and their chelidonine alkaloid ingredients reduce cadmium-induced nephrotoxicity in rats. *Journal of Natural Medicines*, *67*(1), 159-167.

Kubmarawa, D., Ajoku, G. A., Enwerem, N. M., & Okorie, D. A. (2007). Preliminary phytochemical and antimicrobial screening of 50 medicinal plants from Nigeria. *African Journal of Biotechnology*, *6*(14), 11-15.

Laher, F., Aremu, A. O., Van Staden, J., & Finnie, J. F. (2013). Evaluating the effect of storage on the biological activity and chemical composition of three South African medicinal plants. *South African Journal of Botany*, *88*, 414-418.

Lawrence Bannister & Graham Mitchell, (2003). The ins, outs and roundabouts of malaria. *Trends in Parasitology*, (19)5, 209-213

Leonti, M. (2011). The future is written: Impact of scripts on the cognition, selection, knowledge and transmission of medicinal plant use and its implications for ethno botany and ethno pharmacology. *Journal of Ethnopharmacology*, *134*(3), 542-555.

Lin, J. T., Juliano, J. J., & Wongsrichanalai, C. (2010). Drug-resistant malaria: the era of ACT. *Current Infectious Disease Reports*, 12(3), 165-173.

Manach, C., Morand, C., Texier, O., Favier, M. L., Agullo, G., Demigné, C., Rémésy, C *et al*. (1995). Quercetin metabolites in plasma of rats fed diets containing rutin or quercetin. *The Journal of Nutrition*, *125*(7), 1911-1922.

Masoko, P. (2007). *Characterization of antifungal compounds isolated from Combretum and Terminalia species (Combretaceae)* (Doctoral dissertation, University of Pretoria).

Matowe, L., & Adeyi, O. (2010). The quest for universal access to effective malaria treatment: how can the AMFm contribute? *Malaria Journal*, *9*(1), 274.

Maude, R. J., Woodrow, C. J., & White, L. J. (2010). Artemisinin antimalarials: preserving the "Magic Bullet". *Drug Development Research*, *71*(1), 12-19.

Mayank Gupta., Hayat M. Mukhtar and Sayeed Ahmad. 2011. *In vitro* evaluation of antioxidant activity and total phenolic content of *Tylophora indica* (burm f.) Merill. *International Journal of Pharmaceutical Science Research* 2(1), 121-126

Mazimba, O., Masesane, I. B., Majinda, R. R. T., & Muzila, A. (2012). GC-MS analysis and antimicrobial activities of the non-polar extracts of *Mundulea sericea*. *South African Journal of Chemistry*, *65*, 50-52.

Mbukwa, E., Chacha, M., & Majinda, R. R. (2007). Phytochemical constituents of *Vangueria infausta*: their radical scavenging and antimicrobial activities. *Archives for Organic Chemistry,* 9, 104-112.

Mehta, R., Katta, H., Alimirah, F., Patel, R., Murillo, G., Peng, X., & Mehta, R. G. (2013) Deguelin Action Involves c-Met and EGFR Signaling Pathways in Triple Negative Breast Cancer Cells, *PloS One* 8(6), e65113..

Mendis, K., Rietveld, A., Warsame, M., Bosman, A., Greenwood, B., & Wernsdorfer, W. H. (2009). From malaria control to eradication: The WHO perspective. *Tropical Medicine & International Health*, *14*(7), 802-809.

Michael, J. B., Elaine E., and Laid S. A. (1996). Medicinal resources of the tropical forest, biodiversity and its importance to human health. First edition, *Columbia University Press*.

Ministry of Health and Social Services. 2009. Malaria Strategic Plan (2010-2016). Windhoek, Namibia

Mita, T., Tanabe, K., & Kita, K. (2009). Spread and evolution of *Plasmodium falciparum* drug resistance. *Parasitology International*, *58*(3), 201-209.

Mohamed, I. E., El Nur, E. E., Choudhary, M. I., & Khan, S. N. (2009). Bioactive natural products from two Sudanese medicinal plants *Diospyros mespiliformis* and *Croton zambesicus*. *Records of Natural Products*, *3*(4), 198-203.

Motley, C. (1998). Cinchona and its product-quinine. *Ethnobotanical Leaflets, 1998(*1), 14.

Mueller, I., Zimmerman, P. A., & Reeder, J. C. (2007). *Plasmodium malariae* and *Plasmodium ovale* the 'bashful 'malaria parasites.*Trends in Parasitology*, 23(6), 278-283.

Mutabingwa, T. K. (2005). Artemisinin-based combination therapies (ACTs): Best hope for malaria treatment but inaccessible to the needy! *Acta tropica*, *95*(3), 305-315.

Ngasala, B. E., Malmberg, M., Carlsson, A. M., Ferreira, P. E., Petzold, M. G., Blessborn, D. *et al*. (2011). Efficacy and effectiveness of artemether-lumefantrine after initial and repeated treatment in children< 5 years of age with acute uncomplicated *Plasmodium falciparum* malaria in rural Tanzania: a randomized trial. *Clinical Infectious Diseases*, *52*(7), 873-882.

Noedl, H., Se, Y., Schaecher, K., Smith, B. L., Socheat, D., & Fukuda, M. M. (2008). Evidence of artemisinin-resistant malaria in western Cambodia. *New England Journal of Medicine*, *359*(24), 2619-2620.

Nogueira, C. R., & Lopes, L. M. (2011). Antiplasmodial natural products.*Molecules*, *16*(3), 2146-2190.

Noor A. M, Uusiku P, Kamwi R N. Katokele S, Ntomwa B, Alegana1 V A & Snow R W, (2013). The receptive versus current risks of *Plasmodium falciparum* transmission in Northern Namibia: implications for elimination. *BMC Infectious Diseases*, 13:1-10

Nzila, A. (2006). The past, present and future of antifolates in the treatment of *Plasmodium falciparum* infection. *Journal of Antimicrobial Chemotherapy*, *57*(6), 1043-1054.

Oketch-Rabah, H. A., Mwangi, J. W., Lisgarten, J., & Mberu, E. K. (2000). A new antiplasmodial coumarin from *Toddalia asiatica* roots. *Fitoterapia,71*(6), 636-640.

Oliveira A. B., Dolabela M. F., Fernão C. Braga, Rose I.R.P. Jácome.,Fernando P. Varotti and Marinete M. Póvoa. (2009). Plant-derived antimalarial agents: new leads and efficient phythomedicines. Part I. Alkaloids. *Anais da Academia Brasileira de Ciências* 81(4): 715-740

Oliveira, F. Q., Andrade-Neto, V., Krettli, A. U., & Brandão, M. G. L. (2004). New evidences of antimalarial activity of *Bidens pilosa* roots extract correlated with polyacetylene and flavonoids. *Journal of Ethno pharmacology*, *93*(1), 39-42.

Osman, C. P., Ismail, N. H., Ahmad, R., Ahmat, N., Awang, K., & Jaafar, F. M. (2010). Anthraquinones with antiplasmodial activity from the roots of *Rennellia elliptica* Korth. (Rubiaceae). *Molecules*, *15*(10), 7218-7226.

Parnham, M. J., & Verbanac, D. (2011). C9 Mild plant and dietary immunomodulators. *Principles of Immunopharmacology* (451-472). Birkhäuser Basel.

Payne, D. (1988). Use and limitations of light microscopy for diagnosing malaria at the primary health care level. *Bulletin of the World Health Organization*, *66*(5), 621.

Petruczynik, Anna, (2012). Analysis of alkaloids from different chemical groups by different liquid chromatography methods. *Central European Journal of Chemistry*. 10(3), 802-835

Phyo Aung Pyae, Standwell Nkhoma, Kasia Stepniewska, Elizabeth A Ashley, Shalini Nair, Rose McGready, Caritler Moo *et al*., (2012) Emergence of artemisinin-resistant malaria on the western border of Thailand: a longitudinal study. *The Lancet* 379(9830), 1960–1966.

Pindolia, D. K., Garcia, A. J., Wesolowski, A., Smith, D. L., Buckee, C. O., Noor, A. M., ... & Tatem, A. J. (2012). Human movement data for malaria control and elimination strategic planning. *Malaria Journal*, *11*, 205.

Prachayasittikul, S., Saraban, P., Cherdtrakulkiat, R., Ruchirawat, S., & Prachayasittikul, V. (2010). New bioactive triterpenoids and antimalarial activity of *Diospyros rubra* Lec. *Experimental and Clinical Science International Journal*, *9*, 1-10.

Pukrittayakamee, S., Imwong, M., Looareesuwan, S., White, N.J., (2004). Therapeutic responses to antimalarial and antibacterial drugs in *vivax* malaria. *Acta Tropica* 89, 351–356

Rates, S. M. K. (2001). Plants as source of drugs. *Toxicon*, *39*(5), 603-613.

Rice-Evans, C. A., & Packer, L. (Eds.). (2010). *Flavonoids in health and disease*. CRC Press.

Rieckmann, K. H., Campbell, G. H., Sax, L. J., & Ema, J. E. (1978). Drug sensitivity of *Plasmodium falciparum*: an *in vitro* micro technique. *The Lancet*, *311*(8054), 22-23.

Roberts, M. F., & Wink, M. (Eds.). (1998). *Alkaloids: biochemistry, ecology, and medicinal applications*. Springer.

Rodin, R. J. (1985). The ethnobotany of the Kwanyama Ovambos. *Monographs in Systematic Botany from the Missouri Botanical Garden*, *9*.

Rogers, W., Sem, R., Tero, T., Chim, P., Lim, P., Muth, S., ... & Wongsrichanalai, C. (2009). Failure of artesunate-mefloquine combination therapy for uncomplicated *Plasmodium falciparum* malaria in Southern Cambodia. *Malaria Journal*, *8*(1), 10.

Romanik, G., Gilgenast, E., Przyjazny, A., & Kamiński, M. (2007). Techniques of preparing plant material for chromatographic separation and analysis. *Journal of Biochemical and Biophysical Methods*, *70*(2), 253-261.

Sabot, O., Cohen, J. M., Hsiang, M. S., Kahn, J. G., Basu, S., Tang, L Feachem, R. G *et al*. (2010). Costs and financial feasibility of malaria elimination. *The Lancet*, *376*(9752), 1604-1615.

Sachs, J., & Malaney, P. (2002). The economic and social burden of malaria. *Nature Review*, 415(6872), 680-685.

Saifi, M. A., Beg, T., Harrath, A. H., Altayalan, F. S. H., & Al Quraishy, S. (2013). Antimalarial drugs: Mode of action and status of resistance. *African Journal of Pharmacy and Pharmacology, 7*(5), 148-156.

Sanne de Ridder, Frank van der Kooy, Robert Verpoorte, 2004. *Artemisia annua* as a self-reliant treatment for malaria in developing countries. *Journal of Ethnopharmacology* 120; 302-314

Shamsa, F., Monsef, H., Ghamooshi, R., & Verdian-rizi, M. (2008). Spectrophotometric determination of total alkaloids in some Iranian medicinal plants. *Thailand Journal of Pharmaceutical Science*, 32, 17-20.

Shanks G. Dennis, Daniel M. Gordon, Francis W. Klotz, Gladys M. Aleman, Aggrey J. Oloo, Deborah Sadie, and Trevor R. Scott. (1998). Efficacy and safety of Atovaquone/Proguanil as suppressive prophylaxis for *Plasmodium falciparum* malaria. *Clinical Infectious Diseases* 27(3):494–499

Siala, E., Khalfaoui, M., Bouratbine, A., Hamdi, S., Hili, K., & Aoun, K. (2005). Relapse of *Plasmodium malariae* malaria 20 years after living in an endemic area. *Presse Medicale (Paris, France: 1983)*, 34(5), 371.

Singleton, V. L., Orthofer, R., & Lamuela-Raventos, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. *Methods in Enzymology, 299*, 152-178

Srinivasa B. Reddy, T. Sujith, M. Sathish Kumar, A. Narendra Babu, N. Rama Rao, J. Manjunathan., (2012). Malarial drugs got resistance. *International Journal of Pharmaceutical and Biomedical Research 3*(4); 213-215

Sumadhya D Fernando1, Chaturaka Rodrigo, Senaka Rajapakse., (2010). The 'hidden' burden of malaria: cognitive impairment following infection. *Malaria Journal*, (9)366, 1-11

Trager, W., & Jensen, J. B. (1976). Human malaria parasites in continuous culture. *Science*, 193 (4254), 673-675.

Trampuz, A., Jereb, M., Muzlovic, I., & Prabhu, R. M. (2003). Clinical review: Severe malaria. *Critical care* 7(4), 315-323.

Tu, Y. (2011). The discovery of artemisinin (qinghaosu) and gifts from Chinese medicine. *Nature Medicine*, *17*(10), 1217-1220.

United Nations Industrial Development Organization, Handa, S. S., Khanuja, S. P. S., Longo, G., & Rakesh, D. D. (2008). *Extraction technologies for medicinal and aromatic plants*. Earth, Environmental and Marine Sciences and Technologies.

Voahangy Ramanandraibe, Philippe Grellier, Marie-Thrse Martin, Alexandre Deville, Roger Joyeau, David Ramanitrahasimbola, Elisabeth Mouray *et al*. (2008). Antiplasmodial phenolic compounds from *Piptadenia pervillei*. *Planta Medica*, doi: 10.1055/s-2008-1034328

Von Koenen, E. (1996). *Medicinal, poisonous and edible plants in Namibia* (Vol. 2). Klaus Hess Verlag.

Wahyuni, T. S., Ekasari, W., Widyawaruyanti, A., Hirasawa, Y., Morita, H., & Zaini, N. C. (2009). Artopeden A, a new antiplasmodial isoprenylated flavone from *Artocarpus champeden*. *Heterocycles*, *79*(1), 1121-1126.

Walker Naomi F, Behzad Nadjm Christopher & Whitty JM (2009), Malaria. *Medicine, Elsevier* 38(1); 41-46

Wang, Y., Ma, W., & Zheng, W. (2013). Deguelin, a novel antitumorigenic agent targeting apoptosis, cell cycle arrest and antiangiogenesis for cancer chemoprevention (Review). *Molecular and Clinical Oncology*, *1*(2), 215-219.

Warhurst, D. C., & Williams, J. E. (1996). ACP Broadsheet no 148. July 1996. Laboratory diagnosis of malaria. *Journal of Clinical Pathology*, *49*(7), 533-538.

Weiss, C. R., Moideen, S. V., Croft, S. L., & Houghton, P. J. (2000). Activity of extracts and isolated naphthoquinones from *Kigelia pinnata* against *Plasmodium falciparum*. *Journal of Natural Products*, *63*(9), 1306-1309.

Wells, T. N. (2011). Natural products as starting points for future antimalarial therapies: going back to our roots. *Malaria Journal*, *10*(Suppl 1), S3.

Wernsdorfer, W. H., & Kouznetsov, R. L. (1980). Drug-resistant malaria--occurrence, control, and surveillance. *Bulletin of the World Health Organization*, *58*(3), 341.

Whegang, S. Y., Tahar, R., Foumane, V. N., Soula, G., Gwét, H., Thalabard, J. C., & Basco, L. K. (2010). Efficacy of non-artemisinin and artemisinin-based

combination therapies for uncomplicated *falciparum* malaria in Cameroon. *Malaria Journal*, *9*, 56.

White Nicholas J, Pukrittayakamee , Sasithon, Hien Tran Tinh, Faiz, Olugbenga M Abul, Mokuolu A, Dondorp Arjen M. (2013). Malaria. doi.org/10.1016/S0140-6736 (13)60024-0

White, N. J. (2007). Cardiotoxicity of antimalarial drugs. *The Lancet Infectious Diseases*, *7*(8), 549-558.

White, N. J. (2008). Qinghaosu (artemisinin): the price of success. *Science*, 320(5874), 330-334

WHO. 2013. World malaria report. Geneva Switzerland

Willcox, M. L., Graz, B., Falquet, J., Diakite, C., Giani, S., & Diallo, D. (2011). A"reverse pharmacology" approach for developing an antimalarial phytomedicine. *Malaria Journal* 10(Suppl 1), S8.

Wink, M. (2012). Medicinal plants: a source of antiparasitic secondary metabolites. *Molecules*, *17*(11), 12771-12791.

World Health Organisation (2010). Guidelines for the treatment of malaria. Second edition, Geneva, Switzerland. World Health Organisation

World Health Organisation fact sheet no. 134: Traditional medicine. World Health Organization. 2008-12-01. Retrieved 2013-12-13.

World Health Organization (2006) Guidelines for the treatment of malaria, first edition. Geneva: World Health Organization.

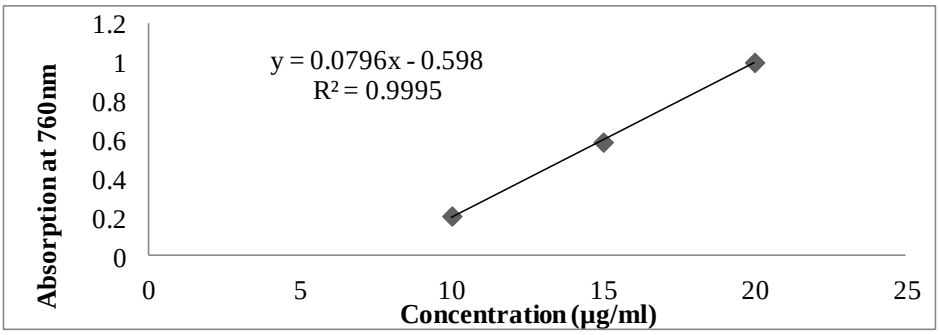
Zofou, D., Kowa, T. K., Wabo, H. K., Ngemenya, M. N., Tane, P., & Titanji, V. P. (2011). *Hypericum lanceolatum* (Hypericaceae) as a potential source of new antimalarial agents: a bioassay guided fractionation of the stem bark. *Malaria Journal*, 10, 167.

Zongo, C., Ouattara, L. P., Savadogo, A., Sanon, S., Barro, N., Koudou, J., *et al* (2011). *In vitro* antiplasmodial activity of some medicinal plants used in folk medicine in Burkina Faso against malaria. *Current Research Journal of Biological Sciences* 3(3): 216-222

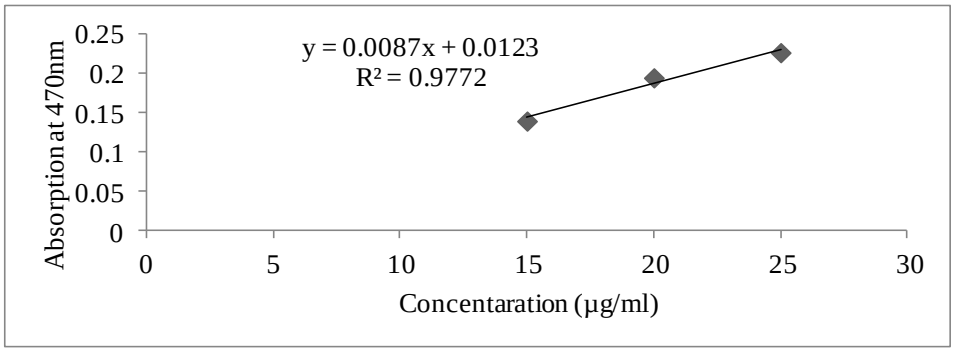
Zwenger, S., & Basu, C. (2008). Plant terpenoids: applications and future potentials. *Biotechnology Molecule Biology Review*, *3*(1), 1-7.

Appendix one: Calibration curves for total phenolics and alkaloids quantification

Standard callibration curve of of quercertin standard for total phenolic quantification



Standard callibration curve of of qunine hydroxide standard for total alkaloids quantification


y = 0.0087x + 0.0123
R² = 0.9772
Absorption at 470nm
0.25
0.2
0.15
0.1
0.05
0
0
5
10
15
20
25
30
Concentaration (µg/ml)

Appendix two: *In vitro* antiplasmodial activity of crude extracts raw data

Methanol extracts-48 hours

Concentrations		1	2	3	Average parasitaemia	Standard Deviation	standard error	Growth	% Growth
5 µg/ml	**NNT**	6.50	6.50	6.20	6.40	0.17	0.06	5.40	5.40
	DMSO	5.80	5.50	2.80	4.70	1.65	0.55	3.70	3.70
	COARTEM	0.21	0.19	0.20	0.20	0.01	0.00	-0.80	-0.80
	MSL	0.49	2.22	2.04	1.58	0.95	0.32	0.58	0.58
	MSS	1.66	1.25	1.02	1.31	0.32	0.11	0.31	0.31
	DML	1.00	1.91	1.46	1.46	0.46	0.15	0.46	0.46
	DMR	0.82	1.09	2.59	1.50	0.95	0.32	0.50	0.50
	CPW	1.41	1.91	1.24	1.52	0.35	0.12	0.52	0.52
10µg/ml	**MSL**	0.93	1.92	2.35	1.73	0.73	0.24	0.73	0.73
	MSS	1.31	0.70	0.55	0.85	0.40	0.13	-0.15	-0.15
	DML	1.34	1.72	1.84	1.63	0.26	0.09	0.63	0.63
	DMR	0.42	1.94	0.78	1.05	0.79	0.26	0.05	0.05
	CPW	1.38	0.98	1.75	1.37	0.39	0.13	0.37	0.37
50µg/ml	**MSL**	1.27	1.07	1.38	1.24	0.16	0.05	0.24	0.24
	MSS	1.49	1.10	0.96	1.18	0.27	0.09	0.18	0.18
	DML	1.37	1.54	1.17	1.36	0.19	0.06	0.36	0.36
	DMR	1.16	0.38	0.32	0.62	0.47	0.16	-0.38	-0.38
	CPW	0.41	1.27	0.57	0.75	0.46	0.15	-0.25	-0.25

Methanol extracts-24 hours

Concentrations		1	2	3	AVERAGE	STANDARD ERROR	ERROR BARS	INIHIBITION	% inhibition
	NNT	2.50	2.50	2.10	2.37	0.23	0.08	1.37	136.67
	DMSO	1.84	3.57	1.70	2.37	1.04	0.35	1.37	137.00
	COARTEM	0.54	0.38	0.33	0.42	0.11	0.04	-0.58	-58.33
5 µg/ml	**MSL**	2.19	1.82	1.38	1.80	0.41	0.14	0.80	79.67
	MSS	2.00	2.32	1.85	2.06	0.24	0.08	1.06	105.67
	DML	2.43	3.04	0.46	1.98	1.35	0.45	0.98	97.67
	DMR	2.15	2.53	1.82	2.17	0.36	0.12	1.17	116.67
	CPW	1.62	1.82	2.04	1.83	0.21	0.07	0.83	82.67
10 µg/ml	**MSL**	0.90	1.07	1.00	0.99	0.09	0.03	-0.01	-1.00
	MSS	1.86	0.94	0.93	1.24	0.53	0.18	0.24	24.33
	DML	0.90	1.07	1.00	0.99	0.09	0.03	-0.01	-1.00
	DMR	1.86	0.94	0.93	1.24	0.53	0.18	0.24	24.33
	CPW	0.79	1.14	0.71	0.88	0.23	0.08	-0.12	-12.00
50 µg/ml	**MSL**	1.00	0.97	0.97	0.98	0.02	0.01	-0.02	-2.00
	MSS	1.31	1.27	0.91	1.16	0.22	0.07	0.16	16.33
	DML	1.41	1.11	1.15	1.22	0.16	0.05	0.22	22.33
	DMR	1.15	1.84	0.84	1.28	0.51	0.17	0.28	27.67
	CPW	1.08	0.93	1.23	1.08	0.15	0.05	0.08	8.00

Aqueous extracts -24 hours

		1	2	3	Averages parasitaemia	Standard Deviation	Standard error
	NNT	1.80	2.00	2.20	2.00	0.20	0.07
5µg/ml	**COARTEM**	0.54	0.38	0.33	0.42	0.11	0.04
	MSL	1.90	1.60	2.20	1.90	0.30	0.10
	MSS	1.90	1.70	2.30	1.97	0.31	0.10
	DML	1.04	1.16	1.03	1.08	0.07	0.02
	DMR	1.22	0.83	1.37	1.14	0.28	0.09
	CPW	1.56	1.16	0.74	1.15	0.41	0.14
10µg/ml	**MSL**	0.54	1.10	0.62	0.75	0.30	0.10
	MSS	0.61	0.83	1.11	0.85	0.25	0.08
	DML	0.97	0.65	0.84	0.82	0.16	0.05
	DMR	1.40	0.72	1.10	1.07	0.34	0.11
	CPW	0.90	1.30	1.11	1.10	0.20	0.07
50µg/ml	**MSL**	0.00	0.80	0.00	0.27	0.46	0.15
	MSS	0.81	0.64	0.66	0.70	0.09	0.03
	DML	0.31	1.21	1.17	0.90	0.51	0.17
	DMR	1.02	0.90	1.20	1.04	0.15	0.05
	CPW	1.70	0.73	0.46	0.96	0.65	0.22

Aqueous extracts-48 hours

		1	2	3	parasitaemia AVERAGES	Standard deviation	standard ERROR
	NT	4.30	1.80	6.00	4.03	2.11	0.70
	COARTEM	0.21	0.00	0.20	0.14	0.12	0.04
5µg/ml	**MSL**	1.00	0.60	0.55	0.72	0.25	0.08
	MSS	0.93	0.53	0.51	0.66	0.24	0.08
	DML	0.58	0.72	0.38	0.56	0.17	0.06
	DMR	0.97	0.44	1.22	0.88	0.40	0.13
	CPW	0.96	0.90	0.97	0.94	0.04	0.01
10µg/ml	**MSL**	0.73	0.90	0.46	0.70	0.22	0.07
	MSS	0.45	0.46	0.35	0.42	0.06	0.02
	DML	0.43	0.53	0.69	0.55	0.13	0.04
	DMR	0.93	0.67	0.66	0.75	0.15	0.05
	CPW	0.65	1.15	0.86	0.89	0.25	0.08
50µg/ml	**MSL**	0.81	0.50	0.68	0.66	0.16	0.05
	MSS	0.35	0.30	0.35	0.33	0.03	0.01
	DML	0.39	0.70	0.59	0.56	0.16	0.05
	DMR	0.63	0.28	0.88	0.60	0.30	0.10
	CPW	1.03	0.90	0.60	0.84	0.22	0.07

Appendix three: *In vitro* antiplasmodial activity raw data of first round fractionation

Plants	Fractions	**1**	**2**	**3**	**Parasitaemia average**	**Standard deviation**
	DMSO	2.40	2.30	1.60	2.10	0.44
	NNT	2.50	2.50	2.10	2.37	0.23
CPW	**F1**	1.19	0.86	1.46	1.17	0.30
	F2	1.98	0.32	0.34	0.88	0.95
	F3	1.05	1.79	1.65	1.50	0.39
	F4	1.63	1.98	2.31	1.97	0.34
DML	**F1**	2.48	2.04	1.80	2.11	0.34
	F2	2.10	1.88	1.90	1.96	0.12
	F3	0.84	0.97	0.70	0.84	0.14
	F4	0.60	0.97	0.43	0.67	0.28
DMR	**F1**	1.07	0.58	1.15	0.93	0.31
	F2	0.56	0.63	1.07	0.75	0.28
	F3	1.04	1.57	1.23	1.28	0.27
	F4	1.27	1.18	1.06	1.17	0.11
	F5	0.75	1.92	0.89	1.19	0.64
	F6	1.21	2.10	1.62	1.64	0.45

Raw data of first round fraction antiplasmodial activity at 48 hours

Plants	Fractions	**1**	**2**	**3**	**average parasitaemia**	**Standard deviation**	**Standard error**
	NNT	6.50	6.50	6.20	6.40	0.17	0.06
	DMSO	5.90	6.60	6.20	6.23	0.35	0.12
CPW	**F1**	3.00	2.64	1.60	2.41	0.73	0.24
	F2	1.21	1.24	0.50	0.98	0.42	0.14
	F3	1.88	2.15	1.19	1.74	0.50	0.17
	F4	2.98	2.25	3.18	2.80	0.49	0.16
DML	**F1**	1.43	1.52	2.33	1.76	0.50	0.17
	F2	1.74	2.42		2.08	0.48	0.16
	F3	1.34	1.08	1.22	1.21	0.13	0.04
	F4	1.19	1.00	0.76	0.98	0.22	0.07
DMR	**F1**	1.30	0.50	0.90	0.90	0.40	0.13
	F2	0.50	0.81	1.84	1.05	0.70	0.23
	F3	1.32	1.29	0.97	1.19	0.19	0.06
	F4	1.36	1.40	0.73	1.16	0.38	0.13
	F5	0.76	1.03	0.59	0.79	0.22	0.07
	F6	1.69	0.64	0.99	1.11	0.53	0.18

Anti plasmodial activity of second round fractions at 24 hours

Plants	fractions	1	2	3	parasitaemia averages	Standard error	Standard error	Growth
	NNT	3.51	2.95	5.00	3.82	1.06	0.35	2.82
	DMSO	2.94	3.88	3.12	3.31	0.50	0.17	2.31
	ACT	0.36	0.53	0.43	0.44	0.09	0.03	-0.56
CPW	A1F1	1.32	1.34	1.32	1.33	0.01	0.00	0.33
	A1F2	1.90	1.79	1.73	1.81	0.09	0.03	0.81
	A1F3	2.03	2.48	2.66	2.39	0.32	0.11	1.39
	A2F1	1.80	2.54	2.72	2.35	0.49	0.16	1.35
	A2F2	2.05	0.97	1.77	1.60	0.56	0.19	0.60
	A2F3	1.09	2.12	2.19	1.80	0.62	0.21	0.80
	A3F1	1.60	0.81	0.97	1.13	0.42	0.14	0.13
DML	A3F2	1.91	1.41	1.67	1.66	0.25	0.08	0.66
	B3F1	1.53	1.85	0.82	1.40	0.53	0.18	0.40
	B3F2	1.48	1.59		1.54	0.08	0.03	0.54
DMR	B4F1	1.63	1.16	0.98	1.26	0.34	0.11	0.26
	B4F2	1.10	1.05	0.55	0.90	0.30	0.10	-0.10
	C2F1	0.86	1.43	1.53	3.31	0.50	0.17	2.31
	C2F2	1.57	2.24		1.91	0.47	0.16	0.91

Antiplasmodial activity of Second round fractionation at 48 hours

fractions	1	2	3	parasitaemia averages	Standard deviation	standard Error	%G	% inhibition
NNT	4.81	4.95	4.78	4.85	0.09	0.03	124.38	0.00
DMSO	5.48	3.91	3.99	4.46	0.88	0.29	106.48	0.39
COATRTEM	0.10	0.34	0.31	0.25	0.13	0.04	-88.43	4.60
A1F1	2.93	2.44	1.87	2.41	0.53	0.18	11.73	2.44
A1F2	1.94	2.63	1.78	2.12	0.45	0.15	-2.01	2.73
A1F3	1.81	2.33	2.76	2.30	0.48	0.16	6.48	2.55
A2F1	1.59	2.80	2.60	2.33	0.65	0.22	7.87	2.52
A2F2	2.24	1.79	2.52	2.18	0.37	0.12	1.08	2.67
A2F3	2.05	2.03	1.34	1.81	0.40	0.13	-16.36	3.04
A3F1	2.61	1.66	1.49	1.92	0.60	0.20	-11.11	2.93
A3F2	1.43	1.25	1.80	1.49	0.28	0.09	-30.86	3.36
B3F1	1.90	1.69	1.16	1.58	0.38	0.13	-26.70	3.27
B3F2	1.53	1.72	2..07	1.63	0.13	0.04	-24.77	3.23
B4F1	3.23	1.67	1.48	2.13	0.96	0.32	-1.54	2.72
B4F2	1.83	1.31	2.17	1.77	0.43	0.14	-18.06	3.08
C2F1	1.72	1.53	2.07	1.77	0.27	0.09	-17.90	3.08
C2F2	2.59	2.20	2.19	2.33	0.23	0.08	7.72	2.52
C3F1	1.20	3.04	1.88	2.04	0.93	0.31	-5.56	2.81
C3F2	3.47	1.87	2.52	2.62	0.80	0.27	21.30	2.23

Appendix four: ANOVA tables

Antiplasmodial activity of crude methanol extracts data analysis by one way-ANOVA

M. sericea shoots extract

Variate: average % parasitaemia 48 hours

Source of variation	Degree of freedom	Sum of squares	Mean of squares	variation ratio	P probability value
CONCENTRATIONS	2	1.466	0.733	5.62	0.042
Residual	6	0.7828	0.1305		
Total	8	2.2488			

D. mespiliformis root extracts

Variate: average % parasitaemia -24 hours

Source of variation	Degree of freedom	Sum of squares	Mean of squares	variation ratio	P probability value
units stratum					
Concentrations	2	1.6458	0.8229	3.67	0.091
Residual	6	1.347	0.2245		
Total	8	2.9928			

D. mespiliformis root extracts

Variate: average % parasitaemia - 48 hours

Source of variation	Degree of freedom	Sum of squares	Mean of squares	variation ratio	P probability value
Concentrations	2	1.162	0.581	0.99	0.425
Residual	6	3.5197	0.5866		
Total	8	4.6816			

D. mespiliformis leaf extracts

Variate: average % parasitaemia 24 hours

Source of variation	Degree of freedom	Sum of squares	Mean of squares	variation ratio	P probability value
CONCENTRATIONS	2	1.5955	0.7977	1.29	0.341
Residual	6	3.7041	0.6174		
Total	8	5.2996			

D. mespiliformis leaf extracts

Variate: average % parasitaemia -48 hours

Source of variation	Degree of freedom	Sum of squares	Mean of squares	variation ratio	P probability value
CONCENTRATIONS	2	0.1769	0.0884	0.82	0.483
Residual	6	0.6441	0.1074		
Total	8	0.821			

Cyphostemma whole plant extract

Variate: average % parasitaemia- 24 hours

Source of variation	Degree of freedom	Sum of squares	Mean of squares	variation ratio	P probability value
CONCENTRATIONS	2	1.49369	0.74684	18.84	0.003
Residual	6	0.23787	0.03964		
Total	8	1.73156			

Cyphostemma whole plant extract

Variate: average % parasitaemia - 48 hours

Source of variation	Degree of freedom	Sum of squares	Mean of squares	variation ratio	P probability value
CONCENTRATIONS	2	0.9998	0.4999	3.13	0.117
Residual	6	0.9576	0.1596		
Total	8	1.9574			

M. sericea leaf extracts

Variate: average % parasitaemia - 48 hours

Source of variation	Degree of freedom	Sum of squares	Mean of squares	variation ratio	P probability value
CONCENTRATIONS	2	0.3838	0.1919	0.39	0.69
Residual	6	2.9191	0.4865		
Total	8	3.3029			

M. sericea leaf extract analysis

Variate: average % parasitaemia -24 hours

Source of variation	Degree of freedom	Sum of squares	Mean of squares	variation ratio	P probability value
CONCENTRATIONS	2	1.31776	0.65888	11.49	0.009
Residual	6	0.34407	0.05734		
Total	8	1.66182			

M. sericea shoot extracts

Variate: average % parasitaemia- 48 hours

Source of variation	Degree of freedom	Sum of squares	Mean of squares	variation ratio	P probability value
CONCENTRATIONS	2	0.1095	0.0547	0.19	0.834
Residual	6	1.7531	0.2922		
Total	8	1.8626			

Antiplasmodial activity of crude aqueous extracts data analysis by one way-ANOVA

M. sericea leaf crude aqueous extract

Variate: Average Percentage Parasitaemia – 24 hours

Source of variation	Degree of freedom	Sum of squares	Mean of squares	variation ratio	P probability value
CONCENTRATIONS	2	0.4407	0.2203	1.81	0.243
Residual	6	0.7318	0.122		
Total	8	1.1725			

M. sericea, leaf crude aqueous extract

Variate: Average Percentage Parasitaemia -48 hours

Source of variation	Degree of freedom	Sum of squares	Mean of squares	variation ratio	P probability value
CONCENTRATIONS	2	0.00436	0.00218	0.05	0.953
Residual	6	0.2686	0.04477		
Total	8	0.27296			

M. sericea, shoot crude aqueous extract

Variate: Average Percentage Parasitaemia -24 hours

Source of variation	Degree of freedom	Sum of squares	Mean of squares	variation ratio	P probability value
CONCENTRATIONS	2	3.07069	1.53534	23.78	0.001
Residual	6	0.3874	0.06457		
Total	8	3.45809			

D. mespiliformis leaf crude aqueous extract

Variate: Average Percentage Parasitaemia -24 hours

Source of variation	Degree of freedom	Sum of squares	Mean of squares	variation ratio	P probability value
CONCENTRATIONS	2	0.18278	0.09139	4.97	0.053
Residual	6	0.11043	0.0184		
Total	8	0.29321			

D. mespiliformis leaf crude aqueous extract

Variate: Average Percentage Parasitaemia -48 hours

Source of variation	Degree of freedom	Sum of squares	Mean of squares	variation ratio	P probability value
CONCENTRATIONS	2	0.0002	0.0001	0	0.996
Residual	6	0.1422	0.0237		
Total	8	0.1424			

Cyphostemma spp whole plant crude aqueous extract

Variate: Average Percentage Parasitaemia -24 hours

Source of variation	Degree of freedom	Sum of squares	Mean of squares	variation ratio	P probability value
CONCENTRATIONS	2	0.0582	0.0291	0.14	0.874
Residual	6	1.2668	0.2111		
Total	8	1.325			

Cyphostemma whole plant crude aqueous extract

Variate: Average Percentage Parasitaemia -48 hours

Source of variation	Degree of freedom	Sum of squares	Mean of squares	variation ratio	P probability value
CONCENTRATIONS	2	0.01509	0.00754	0.2	0.824
Residual	6	0.2262	0.0377		
Total	8	0.24129			

D. mespiliformis leaf crude aqueous extract

Variate: Average Percentage Parasitaemia -24 hours

Source of variation	Degree of freedom	Sum of squares	Mean of squares	variation ratio	P probability value
Concentrations	2	0.01556	0.00778	0.11	0.9
Residual	6	0.43327	0.07221		
Total	8	0.44882			

D. mespiliformis leaf crude aqueous extract

Variate: Average Percentage Parasitaemia -48 hours

Source of variation	Degree of freedom	Sum of squares	Mean of squares	variation ratio	P probability value
Concentrations	2	0.11816	0.05908	0.65	0.555
Residual	6	0.5458	0.09097		
Total	8	0.66396			

First round fractionation antiplasmodial data analysis by One way ANOVA

Cyphostemma spp first round fraction analysis

Variate: average% parasitaemia - 48 hours

Source of variation	Degree of freedom	Sum of squares	Mean of squares	variation ratio	P probability value
Fractions	3	5.7495	1.9165	6.45	0.016
Residual	8	2.3774	0.2972		
Total	11	8.1269			

Cyphostemma spp first round fraction analysis

Variate: average % parasitaemia -24 hours

Source of variation	Degree of freedom	Sum of squares	Mean of squares	variation ratio	P probability value
Fractions	3	1.9793	0.6598	2.08	0.181
Residual	8	2.5361	0.317		
Total	11	4.5154			

D. mespiliformis leaf first round fraction analysis

Variate: average% parasitaemia -24

Source of variation	Degree of freedom	Sum of squares	Mean of squares.	variation ratio	P probability value.	Source of variation
fractions	3		5.1078	1.7026	26.43	<.001
Residual	7	-1	0.451	0.06443		
Total	10	-1	5.17905			

D. mespiliformis leaf first round fraction analysis

Variate: average% parasitaemia -48 hours

Source of variation	Degree of freedom	Sum of squares	Mean of squares.	variation ratio	P probability value.	Source of variation
fractions	3		2.2584	0.7528	6.2	0.022
Residual	7	-1	0.8493	0.1213		
Total	10	-1	2.7522			

Antiplasmodial activity of second round fractionation data analysis by one way-ANOVA

Cyphostemma spp whole plant first round fraction analysis

Variate: average % parasitaemia -24 hours

Source of variation	Degree of freedom	Sum of squares	Mean of squares	variation ratio	P probability value.
Fractions	3	1.9793	0.6598	2.08	0.181
Residual	8	2.5361	0.317		
Total	11	4.5154			

Cyphostemma spp whole plant first round fraction analysis

Variate: average % parasitaemia - 48 hours

Source of variation	Degree of freedom	Sum of squares	Mean of squares	variation ratio	P probability value.
Fractions	3	5.7495	1.9165	6.45	0.016
Residual	8	2.3774	0.2972		
Total	11	8.1269			

D. mespiliformis leaf first round fraction analysis

Variate: average % parasitaemia- 24 hours

Source of variation	Degree of freedom	Sum of squares	Mean of squares.	variatio n ratio	P probability value.	Source of variation
frations	3		5.1078	1.7026	26.43	<.001
Residual	7	-1	0.451	0.06443		
Total	10	-1	5.17905			

Comparison of antiplasmodial activity of crude extracts by plant parts. Two ways- ANOVA

M. sericea Leaf vs shoot crude methanol extract

Variate: Average % parasitaemia- 24 hours

Source of variation	Degree of freedom	Sum of squares	Mean of squares	variatio n ratio	P probability value.
CONCENTRATIONS	2	2.7783	1.38915	14.79	<.001
EXTRACTS	1	0.24267	0.24267	2.58	0.134
CONCENTRATIONS.EXTRACTS	2	0.00541	0.00271	0.03	0.972
Residual	12	1.12687	0.09391		
Total	17	4.15325			

M. sericea Leaf vs shoot crude methanol extract

Variate: Average % parasitaemia -48 hours

Source of variation	Degree of freedom	Sum of squares	Mean of squares	variation ratio	P probability value.
CONCENTRATIONS	2	0.4448	0.2224	0.57	0.579
EXTRACTS	1	0.186	0.186	0.48	0.503
CONCENTRATIONS.EXTRACTS	2	0.0484	0.0242	0.06	0.94
Residual	12	4.6723	0.3894		
Total	17	5.3516			

M. sericea Leaf vs shoot crude aqueous extract

Variate: Average % parasitaemia -24 hours

Source of variation	Degree of freedom	Sum of squares	Mean of squares	variation ratio	P probability value.
CONCENTRATIONS	2	6.95723	3.47862	37.28	<.001
EXTRCATS	1	0.18	0.18	1.93	0.19
CONCENTRATIONS.EXTRCATS	2	0.1267	0.06335	0.68	0.526
Residual	12	1.11967	0.09331		
Total	17	8.3836			

M. sericea Leaf vs shoot crude aqueous extract

Variate: Average % parasitaemia -48 hours

Source of variation	Degree of freedom	Sum of squares	Mean of squares	variation ratio	P probability value.
CONCENTRATIONS	2	0.11065	0.05533	1.7	0.223
EXTRCATS	1	0.22244	0.22244	6.85	0.023
CONCENTRATIONS.EXTRCATS	2	0.06119	0.0306	0.94	0.417
Residual	12	0.38969	0.03247		
Total	17	0.78398			

D. mespiliformis leaf vs root crude methanol extract

Variate: Average % parasitaemia – 24 hours

Source of variation	Degree of freedom	Sum of squares	Mean of squares	variation ratio	P probability value.
Concentrations	2	3.2099	1.6049	3.81	0.052
EXTRACTS	1	0.1233	0.1233	0.29	0.598
Concentrations. EXTRACTS	2	0.0313	0.0157	0.04	0.964
Residual	12	5.0511	0.4209		
Total	17	8.41			

D. mespiliformis leaf vs root crude methanol extract

Variate: Average % parasitaemia -48 hours

Source of variation	Degree of freedom	Sum of squares	Mean of squares.	variation ratio	P probability value.
EXTRACTS	1	0.7361	0.7361	2.12	0.171
Concentrations	2	0.8825	0.4412	1.27	0.316
EXTRACTS. Concentrations	2	0.4563	0.2282	0.66	0.536
Residual	12	4.1638	0.347		
Total	17	6.2387			

D. mespiliformis leaf vs root crude aqueous l extract

Variate: Average % parasitaemia -24 hours

Source of variation	Degree of freedom	Sum of squares	Mean of squares.	variation ratio	P probability value.
EXTRACTS	1	0.1058	0.1058	1.25	0.285
CONCENTRATIONS	2	0.09241	0.04621	0.55	0.592
EXTRACTS.CONCENTRATIONS	2	0.0273	0.01365	0.16	0.852
Residual	12	1.0126	0.08438		
Total	17	1.23811			

D. mespiliformis leaf vs root crude aqueous extract

Variate: Average % parasitaemia – 48 hours

Source of variation	Degree of freedom	Sum of squares	Mean of squares	variation ratio	P probability value.
CONCENTRATIONS	2	0.05884	0.02942	0.51	0.611
EXTRACTS	1	0.15494	0.15494	2.7	0.126
CONCENTRATIONS.EXTRACTS	2	0.05951	0.02976	0.52	0.608
Residual	12	0.688	0.05733		
Total	17	0.96129			

Appendix five: Plants collection permit and pictures of plants

MINISTRY OF ENVIRONMENT AND TOURISM

RESEARCH/COLLECTING PERMIT

Permit Number 1488/2010
Valid from 12 March 2010 to 28 February 2011

Permission is hereby granted in terms of the Nature Conservation Ordinance 1975 (Ord. 4 of 1975) to:

Name: **Dr RA Bock**
Address: **Private Bag 13301**
Windhoek
Namibia
Coworkers: **M. Hedimbi, C. du Preez and Dr D. Mumbengegwi**

To *study the evaluation of medicinal plants used by traditional healers for potential use as complementary medicine for treatment of Malaria throughout Namibia excluding protected areas,* subject to attached conditions.

IMPORTANT: This permit is not valid if altered in any way.

MINISTRY OF ENVIRONMENT AND TOURISM
REPUBLIC OF NAMIBIA
15 MAR 2010
WINDHOEK
PRIVATE BAG 13306, WINDHOEK
TEL: 2842111 FAX: 259101

Tuahengo
Authorising Officer

IMPORTANT
This permit is subject to the provisions of the Nature Conservation Ordinance, 1975 (Ordinance 4 of 1975) and the regulations promulgated thereunder, and the holder is subject to all such conditions and regulations.

Enquiries: Research Administrator, email tuahengo@met.na
Private Bag 13306, Windhoek, Namibia

Pictures of ethnomedicinal plants used to treat malaria associated symptoms in Northern Namibia

Diospyros mepiliformis, twigs and leaves

Vangueria infausta, aerial parts

Mundulea sericea, whole plant

Cyphostemma spp, whole plant

Acrotome inflata, whole plant

Neptunia oleracea, whole plant

Guibortia colesoperma, aerial parts

Baikea plurujuga, whole plant

Ziziphus mucronata, aerial parts

Oxygonum dregeanum, whole plant