EVALUATION OF INDIGENOUS NAMIBIAN MUSHROOMS AND PLANTS FOR ANTIMALARIAL PROPERTIES

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By

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ABSTRACT

Malaria is a parasitic disease caused by *Plasmodium* species and transmitted by Anopheles mosquitoes. The disease is currently ranked high among the most problematic infectious diseases around the world. Despite the significant progress that has been made toward reducing the global burden of malaria, it remains one of the most significant public health threats in sub-Saharan Africa and many other parts of the developing world. Current malaria control measures have adverse environmental and human effects. The synthetic repellents used for controlling vectors are causing irreversible damage to the ecosystem since the chemicals are non-degradable in nature. The current antimalarial drugs are also facing the specter of parasite resistance, hence the need to discover novel drugs from natural products. The overall objective of the study was to perform bioassay-guided fractionation and determine the antiplasmodial activities, phytochemical profiles, active compounds and cytotoxicities of mushroom and plant extracts. The study involved an ethnobotanical survey of putative antimalarial mushrooms and plants. Preparation of mushroom and plant organic extracts; evaluation of antiplasmodial activity of the extracts using the parasite lactate dehydrogenase (pLDH) assay; verification of phytochemical profiles (saponins, terpenoids, anthraquinones, coumarins, alkaloids, and flavonoids) using standard procedures; elucidation of active antiplasmodial compounds using Gas chromatography-Mass Spectrometry (GC-MS), Fourier Transform Infrared (FTIR) and Nuclear Magnetic Resonance spectroscopy (NMR) and determination of cytotoxicity using the (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) (MTT) method. Four mushroom and thirteen plant species used in this study were collected from north-central Namibian regions namely Kavango East, Kavango West, Ohangwena, and Oshikoto. Of

these, only two plants were active against 3D7 strains of the malaria parasite, *Plasmodium falciparum*. None of the evaluated mushrooms showed activity. The phytochemical screening of the plants revealed presence of anthraquinones, saponins, terpenoids, coumarins, alkaloids, and flavonoids. Two antiplasmodial compounds namely Npk1 F70 (IC_{50} = 2.63 ± 0.48 µg/mL) and NPk1 F78 (IC_{50} 2.64 ± 0.32 µg/mL) were isolated from Npk1 (*Pechuel-loeschea leubnitiziae*) dichloromethane (DCM) extracts. The compounds were screened for cytotoxicity on the Chinese hamster ovary (CHO) cell line using the MTT assay and were found to be toxic to the mammalian cells. GraphPad Prism was used to calculate the compounds' IC_{50} values of 2.7 µg/mL and 2.22 µg/mL for NPk1 F78, and Npk1 F70, respectively. The molecular weight of the two anti-3D7 compounds was determined by GC-MS to be 246 g/mol. When FTIR and NMR were performed, the structure of the active compound was identified as xerantholide. This is the first study to elucidate an active antiplasmodial compound from this indigenous Namibian plant *P. leubnitiziae* which is used by local people to manage malaria. This study, to the best of my knowledge is the first to report the antimalarial activity of xerantholide, a known guaianolide extracted from aerial parts of *P. leubnitiziae*. It is recommended that pharmacomodulation study be carried out on the compound identified in this study in order to decrease the compound's toxicity, while maintaining or improving its activity. Further studies also need to be done on xerantholide to determine the diastereomers (stereoisomers that are not mirror images of one another). Modification on xerantholide chemical structure also needs to be carried out so that the toxic compounds can be used for possible anti-cancer therapy..

Keywords: Active compounds, cytotoxicity**,** extracts, malaria, , mushrooms, plants , *Plasmodium falciparum*, *Pechuel-loeschea leubnitiziae*, Xerantholide

LIST OF PUBLICATIONS AND CONFERENCE PROCEEDINGS

One of the following articles has been published in peer reviewed journal as stated below, and the rest have been presented and published in conference proceedings. All the listed publications are part of the work carried out to complete this study.

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5. Kadhila, N.P., Sekhoacha, M., Tselanyane, M., and. Chinsembu, K. C. (2019). Antiplasmodial activity, phytochemical profile, active principles and cytotoxicity of *Pechuel-loeschea leubnitziae*O. Hoffm(Asteraceae): An endemic shrub used to manage malaria in Namibia

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LIST OF ABBREVIATIONS

ACT	Artemisinin-based combination therapy
AIDS	Acquired immunodeficiency syndrome
ALMAA	African Leaders Malaria Alliance
CDC	Centers for Disease Control
CHO	Chinese hamster ovary
CSIR	Council for Scientific and Industrial Research
DCM	Dichloromethane
DCs	Dendritic cells
DDT	dichlorodiphenyltrichloroethane
E8	Elimination 8 Initiative
FTIR	Fourier Transform Infrared
IPTp	Intermittent preventive treatment in pregnancy
IRS	Indoor residual spraying
ITN	Insecticide-treated mosquito net
KOH	Potassium hydroxide

LBr	Liebermann Burchard Reagent
LLIN	Long-lasting insecticidal net
MAP	Malaria Atlas Project
MRC	Multidisciplinary Research Centre
MTT	(3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide)
NCRST	National Commission on Research Science and Technology
NGSTP	Namibia Government Scholarship and Training Program
pLDH	Parasite lactate dehydrogenase
RPMI	Roswell Park Memorial Institute medium
SADC	Southern African Development Community
SMC	Seasonal malaria chemoprevention
SP	Sulphadoxine–pyrimethamine
WHO	World Health Organization
ZERI	Zero Emissions Research Initiatives

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DEDICATION

This dissertation is dedicated to my four boys Manasse, George, Cecil and Simon. This work must motivate you to always work hard and make your dreams come true, take your time and hit it hard when you know it is your time. Always remember our "moto" Romans 12:12-18. Mama will always love you.

This work is also dedicated to my parents, mama Emilia Jona Makili and papa Simon Kadhila Amoomo. I love you dearly and I thank God for keeping you to still give me the warm loving hugs. May the almighty keep you for more beautiful years that are full of overflowing blessings and profuse good health. I will forever remain your cheerful loving beautiful sheeli.

DECLARATION

I, Nailoke Pauline Kadhila, hereby declare 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 institute of higher education.

…… Date:

Nailoke Pauline Kadhila

CHAPTER 1

1. INTRODUCTION

1.1 General introduction

Malaria is a protozoan disease caused by parasites of the genus *Plasmodium* and transmitted by female *Anopheles* mosquitoes (Cox, 2010). It is an infectious disease caused by four *Plasmodium* species *Plasmodium falciparum*, *Plasmodium malariae*, *Plasmodium ovale,* and *Plasmodium vivax* (Nogueira & Lopes, 2011). According to the World Health Organization (2018), *P. falciparum* is the most prevalent malaria parasite on the African continent, making it also responsible for most malaria-related deaths globally, while *P. vivax* is the dominant malaria in most countries outside of sub-Saharan Africa.

The parasites are transmitted by Anopheles mosquitoes, with *Anopheles gambiae sensu stricto, Anopheles funestus,* and *Anopheles arabiensis* being the most prevalent in Africa (Nkumama, O'Meara, & Osier, 2017). The two, *An. gambiae* and *An. Funestus*, are strongly anthropophagic and consequently the most efficient malaria vectors in the world (Centers for Disease Control and Prevention, 2018). The disease is one of the leading causes of morbidity and mortality in the world, especially in children under the age of 5 years and pregnant women in developing countries. Malaria causes enormous medical and economic impacts (Alemu, et al., 2012). The disease remains the world's most devastating human parasitic infection, afflicting more than 500 million people and causing about 2.5 million deaths each year (Petritus & Burns, 2008). According to the World Malaria Report (2017), there were 216 million cases of malaria in 2016, up from 211 million cases in 2015. The estimated number of malaria deaths stood at 445 000 in

2016, a similar number to the previous year which was 446 000. The majority of the world's malaria cases and deaths occur in the African continent with over 75% of the estimated 2.5 million deaths each year occurring in children living in Sub-Saharan Africa (World Malaria Report 2011). World Malaria Report, (2017) stated that globally, more countries are moving towards elimination. In 2016, 44 countries reported fewer than10 000 malaria cases, up from 37 countries in 2010.

According to CDC and Malaria (2017), malaria caused an estimated 214 million cases and 438,000 deaths in 2015, mostly young children in Sub-Saharan Africa. In Africa alone, costs of illness, treatment, and premature death from malaria are at least $12 billion per year. In 2016, an estimated US$ 2.7 billion was invested in malaria control and elimination efforts globally (World malaria report 2017). Malaria's toll would be much higher without the efforts of CDC and other global partners. From 2000 through 2015, the massive scale-up of malaria prevention and treatment interventions saved approximately 6.2 million lives globally, and malaria death rates in Africa were cut by more than half. However, the disease remains a major public health problem, although it is both preventable and treatable. According to Roser & Ritchie, (2018) the world malaria deaths by region shows that most death occurred in Sub-Saharan Africa (**Fig. 1**).

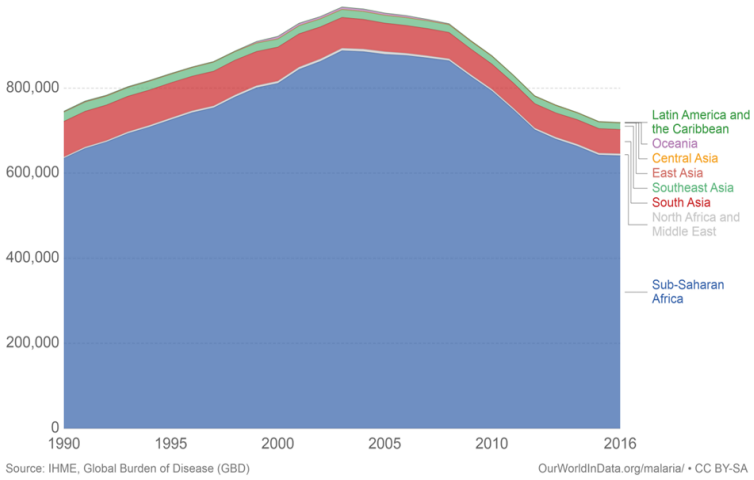


Figure 1. World malaria deaths by region. (Roser & Ritchie, 2018)

At the global level, the most vulnerable age group to malaria deaths is children under five years old. In the year 2016 they account for 72 percent of total deaths (Fig. 2). The number of deaths from malaria tends to decrease with age; with those over 70 years old accounting for less than four percent. When age categories are combined, children under 14 years old account for approximately 80 percent of mortality (Fig. 2). (Roser & Ritchie 2018).

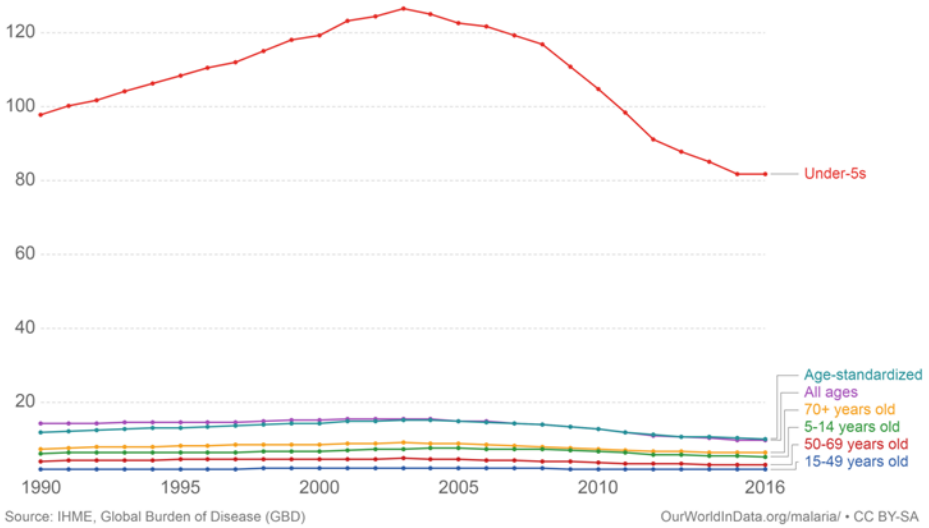


Figure 2. World malaria death by age group worldwide. Source: Our word in data

1.2. Malaria in Namibia

In Namibia, malaria is mainly found in the Northern and Eastern areas of the country, although it has also been reported in the Central region and occasionally in the South. Major *plasmodium* species found in Namibia are *P. falciparum, P. vivax* and the major anopheles species are *An. gambiae, An. arabiensis* and *An. funestus* (World Malaria Report, 2011).

Recent studies at vector sentinel sites confirm that the previous primary vectors *An. gambiae and An. funestus* have been eliminated (Aide Mémoire, 2011). According to 2011 WHO report, malaria deaths in Namibia reached 616 or 3.01% of total deaths and the country was ranked number 31 in the world. A total of 97% of malaria cases are due to infection with *P. falciparum*, while the remaining 3% are attributable to the remaining 3 species *P. vivax, P. ovale and P. malariae* respectively.

It is reported that malaria deaths in Namibia have declined over the past decade with a reduction rate of 90% and the incidence of malarial infections has also dropped significantly. In fact, Namibia is moving towards pre-elimination of the disease (i.e. <1 case/1000 people) within its borders (Du Preez, 2012). Despite the overall reduction of malaria, there remains low to moderate transmission in the northern regions bordering Angola (Gueye et al., 2014).

The historical review of malaria control in Southern African countries revealed that Indoor Residue Spray (IRS) mainly with Dichlorodiphenyltrichloroethane (DDT) is an effective control measure against malaria transmission, especially when supplemented with other interventions such as the use of insecticide-treated mosquito nets (ITNs) and new drug therapies (artermisinin-based combinations) (Kamwi et al., 2012).

Natural products contain a great variety of chemical structures and have been screened for antiplasmodial activity as potential sources of new antimalarial drugs (Nogueira & Lopes, 2011). In the search for new therapeutic substances, a great number of researchers have resorted to plant sources. This is due to the fact that many of these plants are used in African traditional medicine and drugs from natural products have been beckoned for use as origin of developing new antimalarials (Christy et al., 2016).

Recent developments in the field of biological control of malaria-transmitting mosquitoes have shown that certain fungi are virulent to adult *Anopheles* mosquitoes (Katsayal, et al., 2009). Mushrooms such as *Ganoderma lucidum* belonging to the Ganodermataceae family have been reported to have a number of pharmacological effects including immuno-modulating, anti-atherosclerotic, anti-inflammatory, analgesic,

chemopreventive, anti-tumour, radio-protective, sleep-promoting, anti-bacterial, anti-viral (including anti-HIV), hypo-lipidemic, anti-fibrotic, hepato-protective, anti-diabetic, antioxidative and radical-scavenging, anti-aging, hypoglycemic and anti-ulcer properties. *G. lucidum* has now become recognized as an alternative adjuvant in the treatment of leukemia, carcinoma, hepatitis, diabetes, and has anti-tumour, anti-inflammatory, antiplatelet aggregation, and hypocholesterolemic properties (Sanodiya, et al., 2009). This study postulates that indigenous Namibian *Ganoderma* and other medicinal mushrooms studied in Namibia may be useful in the treatment of malaria.

Medicinal plants play a major role in many communities over the world in the treatment and prevention of disease and the promotion of general health (Muthaura et al., 2015). The study of plants used traditionally as antimalarial is attractive due to the possibility of finding new drugs and because of the strong adhesion of local people for economic and cultural reasons (Ganfona, et al., 2013). Jan et al., 2014 stated that plant resources not only provide materials for survival, medicinal and forage values, but also possess and preserve cultural heritages, biological information and indigenous knowledge. They further stated that bioactive compounds currently extracted from plants are used as pharmaceuticals, agrochemicals, flavor and fragrance ingredients, food additives, and pesticides. Studies have suggested that the antimicrobial activity of plants is particularly related to the chemical composition of their essential oils. Vast majorities of prescription drugs used in the world today contain compounds that are directly or indirectly, via semi-synthesis, derived from plants.

Plant products continue to make an immense contribution to malaria chemotherapy, either directly as antimalarial agents, or as important lead compounds for the discovery of more potent anti-malarial drugs (Chinsembu 2015). As a result, the goal of this study is to screen some indigenous Namibia medicinal mushrooms and plants for their antiplasmodial properties.

1.3. Statement of the problem

This study was necessitated by the fact that current antimalarial drugs are becoming less effective against the parasites and the methods being used in the control of vectors are deemed harmful to the environment and humans. For example, mosquitoes are controlled using indoor residual spraying (IRS) primarily with dichlorodiphenyltrichloroethane (DDT), yet this control intervention has adverse environmental and human health effects. Although mushrooms and plants are putatively used to treat malaria in Namibia, there have been no empirical studies to investigate the antiplasmodial activities, toxicities, and active compounds of indigenous Namibian mushrooms and plants.

1.4. Aim of the study

The overall objective of the study was to perform bioassay-guided fractionation and determine the antiplasmodial activities, phytochemical profiles, active compounds and cytotoxicity for mushroom and plant extracts.

1.4.1. Specific objectives

1) To evaluate the *in vitro* activity of mushroom and plant extracts against the chloroquine-sensitive *Plasmodium falciparum* strain 3D7.
2) To determine the phytochemical profiles of antiplasmodial active mushroom and plant extracts.
3) To elucidate the structures of antiplasmodial active compounds.
4) To determine the cytotoxicity of antiplasmodial active mushroom and plant extracts.

1.5. Significance of the study

Although there is extensive research done on the medicinal properties of some mushroom and plant species on antiplasmodial activities, the indigenous Namibian *Ganoderma* and other medicinal mushrooms and some plants are still to be explored, thus making this research unique and necessary in Namibia. If the Namibian indigenous mushrooms and plants to be screened are found to contain bioactive compounds that have antiplasmodial activity, they can serve as candidates from which bio-control or drugs may be developed and be used in controlling the malaria parasite or in treating the disease itself. Community education and awareness on consumption and cultivation of mushrooms as well as conservation of plants that yield positive results will be conducted upon complete of this study.

CHAPTER 2

2. LITERATURE REVIEW

2.1. Malaria as a disease

Malaria is a parasitic disease caused by *Plasmodium* species and transmitted by Anopheles mosquitoes. The disease is currently ranked highly among the most important infectious diseases around the world (Jansen et al., 2010). Despite the significant progress that has been made toward reducing the global burden of malaria, this disease remains one of the most significant public health threats in sub-Saharan Africa and many other parts of the developing world (Merkord et al., 2017).

Tren & Bate, (2001) stated that the link between the plasmodium parasite, the anopheles mosquito and man was only made in 1898. They added that malaria has long been associated with swamps, marshes and wetlands without the knowledge that these were breeding grounds for mosquitoes. People thought that foul smelling air or miasmas were the cause of infection. Others believed that poisons seeped from the soil into drinking water, thereby infecting people. They elaborated that it was widely believed that malaria was caused not by disease-carrying mosquitoes, but somehow by the *Acacia xanthophloea* tree commonly known as the fever tree.

According to WHO (2018), the malaria cycle involves female Anopheles mosquitoes picking up the parasite from infected people when they bite to obtain blood needed to nurture their eggs. Gametocytes within a mosquito develop into sporozoites which are transmitted via the saliva of a feeding mosquito to the human bloodstream. From there

they enter liver parenchyma cells, where they divide and form merozoites which are then released into the bloodstream and infect red blood cells. Rapid division of the merozoites results in the destruction of the red blood cells, and the newly multiplied merozoites then infect new red blood cells. Some merozoites may develop into gametocytes, which can be ingested by a feeding mosquito, starting the life cycle over again. One to two weeks after a person is infected the first symptoms of malaria appear, usually fever, headache, chills and vomiting. If not treated promptly with effective medicines, malaria can kill by infecting and destroying red blood cells and by clogging the capillaries that carry blood to the brain or other vital organs. The life cycle of *P. falciparum* in both the human and a mosquito is shown in Fig. 3.

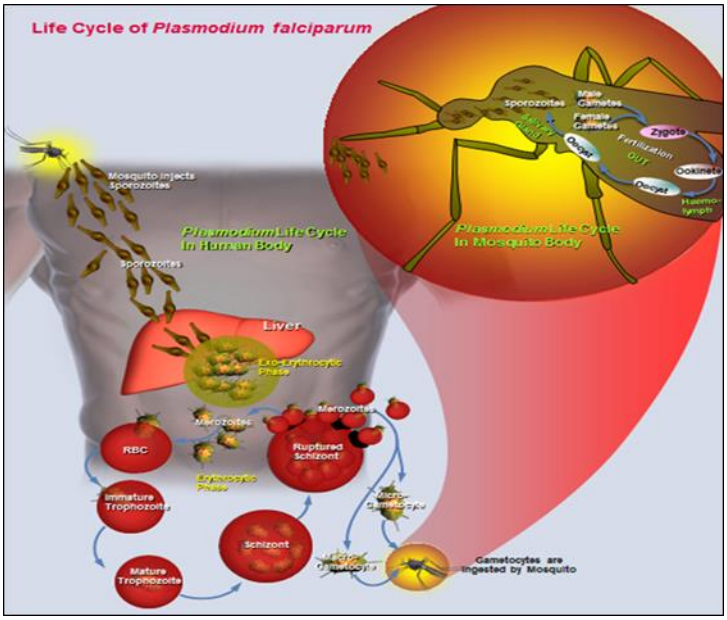


Figure 3. Life cycle of *Plasmodium falciparum*, in both human and mosquito.

(Pathway database, 2016)

Nkumama et al., (2017) stated that the patients present with nonspecific symptoms, including fever, rigors, and chills, and the majority will not require hospital admission. Severe malaria develops in a minority, and in children it may manifest as a fever, impaired consciousness, severe anemia, respiratory distress, convulsions, and hypoglycemia, among other symptoms.

P. falciparum is one of the five distinct species of malaria parasite and the most dangerous of the infective agents. It is found throughout the sub‑tropical and tropical regions of the world. Combinations of drugs containing an artemisinin derivative, artesunate, were introduced for the treatment of *P. falciparum* infections in endemic countries, however, recent reports show that drug resistance has developed to artemisinin (Ndjonka et al., 2012).

2.2. Parasite resistance

In many parts of the world, the parasites have developed resistance to a number of anti-malaria drugs such as chloroquine, sulphadoxine-pyrimethamine, and other conventional ones. Resistance to these drugs has been reported to be as high as 40 to 60% in some African and Asian countries. Some studies indicate that resistance to chloroquine, which was the most widely used for malaria, may be responsible for the increase in malaria-specific mortality in many malaria-endemic countries (Annan et al., 2012). In Africa, artemisinin resistance has not been reported to date and first-line ACTs remain efficacious in all malaria endemic settings (World Malaria Report, 2017).

2.3. Malaria in Namibia

In Namibia, malaria transmission occurs in the endemic northern regions which are considered to contribute almost the entire malaria burden in the country (Alegana et al., 2016). Malaria transmission occurs in nine regions (22 districts) of Namibia with 1.2 million people (65% of the population) live in malaria transmission areas. However, the intensity of malaria transmission is generally low except in Caprivi and Kavango regions. Endemicity is highest in the north-eastern part of the country along the borders with Angola and Zambia, and decreases towards the west, southwest and east **(Fig.4)** (Aide Mémoire, 2011). Chinsembu et al., (2015) reported that the highest incidence of malaria in Namibia is in Rundu, Kavango East region, with 56.3% and the two Kavango regions with 10,501 reported cases with 34,608 suspected and 1176 confirmed cases. They further stated that 71.1% of Rundu's inhabitants attend a public health facility for malaria diagnosis and treatment, while the remainder seek the use of traditional medicines.

According to Gueye et al., (2014), malaria diagnosis and treatment is available for free to both citizens and foreigners in all health facilities in Namibia. In 2005, the national guidelines called for clinical diagnosis with parasite confirmation using microscopy or a Rapid Diagnostic Test (RDT). They further stated that chloroquine was the first line treatment for *P. falciparum*, and sulfadoxine pyramethamine (SP), or oral quinine for pregnant women, was the second line treatment. However, increasing resistance to chloroquine led to a treatment policy change to use artemisinin combination therapy (ACTs) in 2005, which was then rolled out nationwide in 2006. Since then, all health facilities in Namibia offered malaria treatment with ACTs.

Although the country has made remarkable progress in reducing its malaria incidence since 2000, the challenge of eliminating the disease, particularly along the country's northern border, may be too high to overcome by 2023 (United Nations System in Namibia, 2018).

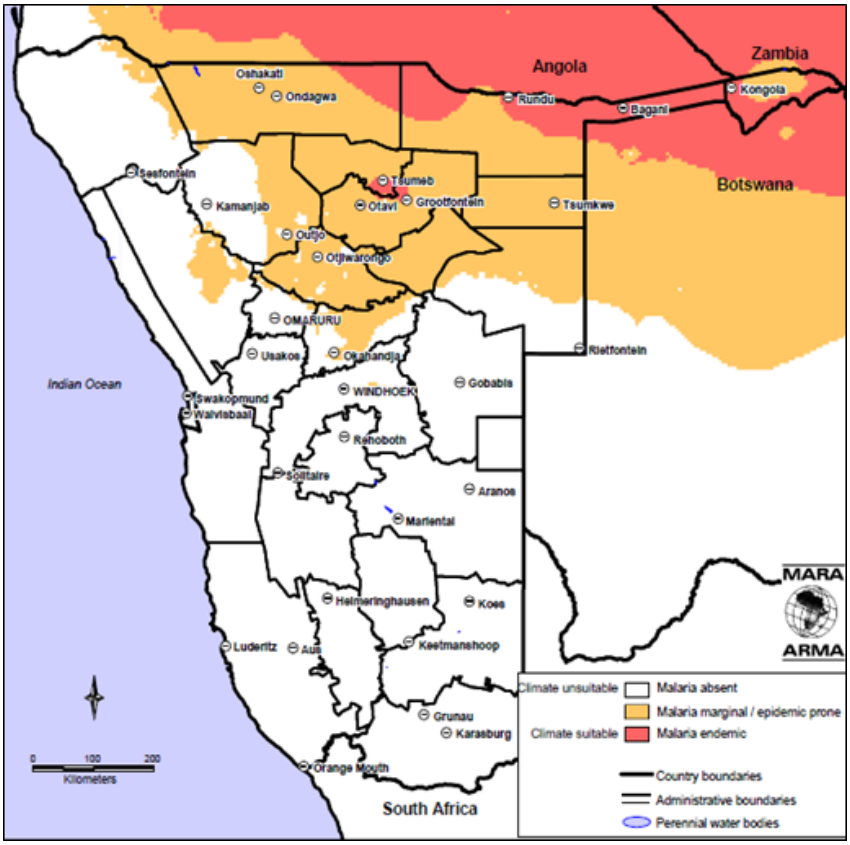


Figure 4. Distribution of endemic malaria in Namibia adopted from (MARA/ARMA 2001).

During the year 2017, Namibia had a malaria outreak that affected 4 600 in Kavango East and Kavango West, 582 in Zambezi and 1 180 in Ohangwena regions with 15 death recorded **Fig. 5**. (Shapwanale & Itamalo, 2017).

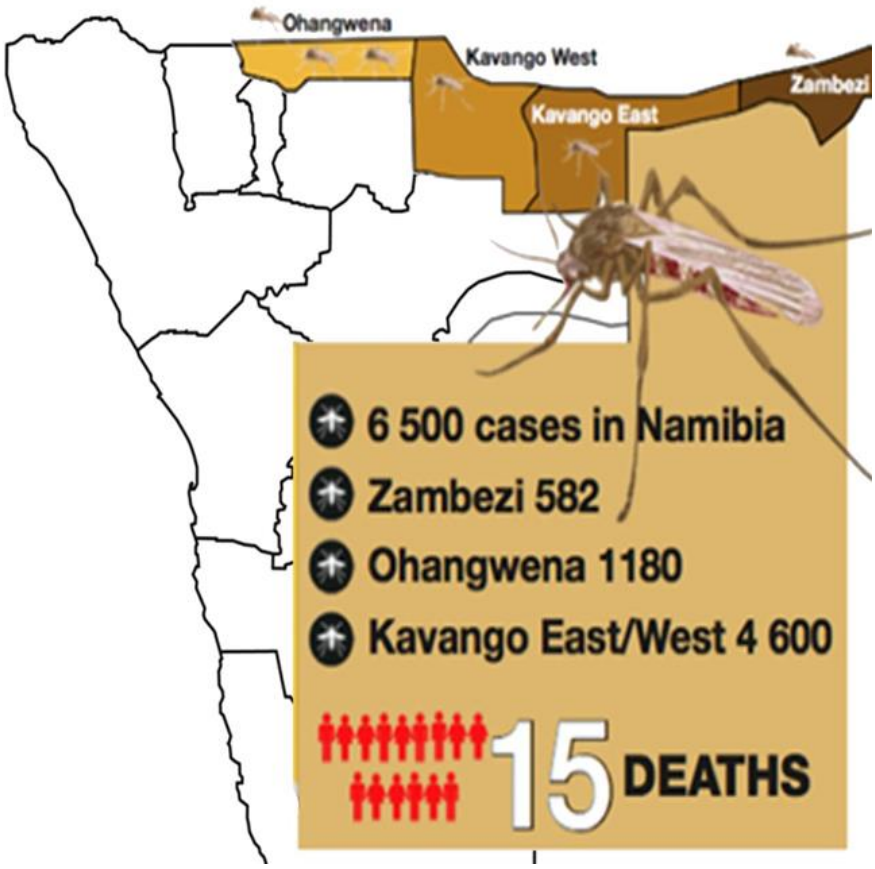


Figure 5. Reported malaria cases from the four endemic northern regions during the year 2017. (Shapwanale & Itamalo, 2017).

Namibia is one of the countries with elimination programs together with Botswana, Comoros, South Africa and Eswatini. The country is classified amongst countries with low transmission in southern Africa (World malaria report, 2017). The country is also a member of the Elimination 8 Initiative (E8) (**Fig. 6**) which was established by the Southern African Development Community (SADC) ministers of health to achieve malaria elimination by 2020 in the four southern-most countries viz. Botswana, Namibia, South Africa and Eswatini, and to reduce incidence and eventually eliminate malaria from their northern neighbors (Angola, Mozambique, Zambia and Zimbabwe).

The E8 complements national efforts by working towards the following objectives: Strengthen regional coordination in order to achieve elimination in each of the E8 member countries; elevate and maintain the regional elimination agenda at the highest political levels; promote policy harmonization, quality control and knowledge management to accelerate progress towards elimination; reduce cross-border malaria transmission through expanded access to early diagnosis and treatment in border districts; and secure resources to support the regional elimination plan and ensure long-term sustainable financing for the region's elimination ambitions (World Health Organization, 2016). However, Namibia and Eswatini are not on track due to the increase in cases in recent years. Namibia reported 556 cases in 2010, unfortunately the cases increased to 25 198 in 2016 which is a 45 times increase. Eswatini reported a 30% increase during the same period. Most populations within the E8 countries have access to LLIN or IRS Fig.7.

The increases in malaria cases in the two countries are due to many factors, including inadequate vector control, climatic factors such as El Niño in 2015–2016 and improved reporting. In Namibia, the outbreaks are linked to environmental and epidemiological changes in the country and in neighboring countries, and are particularly severe in areas with poor implementation of malaria interventions (Simasiku, 2017).

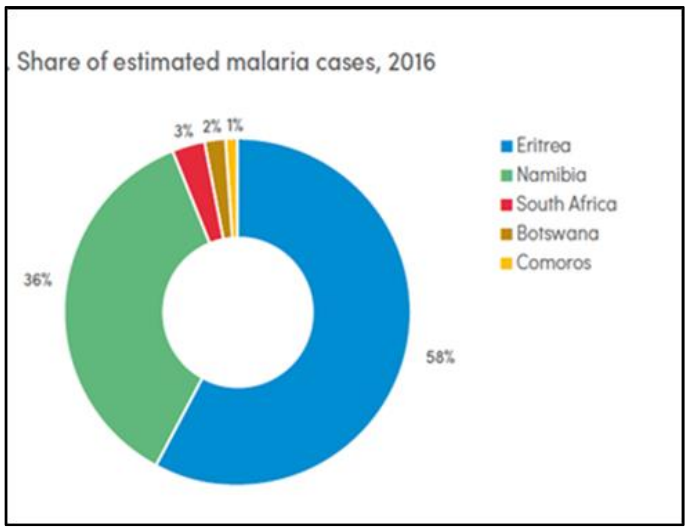


Figure 6. Elimination 8 Initiative share of eliminated cases during 2016 (World malaria report, 2017)

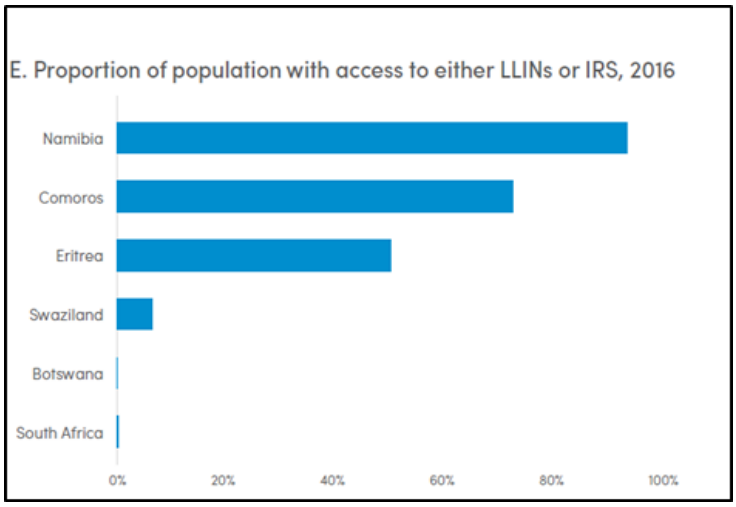


Figure 7. Proportion of population with access to LLIN or IRS in Elimination 8 Initiative during 2016 (World malaria report, 2017)

2.3. Mosquitoes as malaria vectors

Mosquitoes represent the major arthropod vectors of human disease worldwide transmitting malaria, lymphatic filariasis, and arboviruses such as dengue virus and Zika

virus. Unfortunately, there is no treatment in the form of vaccines or drugs available for most of these diseases and vector control is still the main form of prevention (Benelli, Jeffries, and Walker, 2016).

According to Naseem et al., (2016), mosquitoes are considered as very important group of insects, that act as vector for many viral, bacterial and protozoans' diseases. The exponential increase in population of mosquitos is a major problem for many countries because they spread different diseases such as filarial, Japanese encephalitis, Lyme disease, Yellow fever, encephalitis, malaria, chikungunya, dengue, and epidemic poly-arthritis. In tropical and subtropical countries mosquito borne diseases are main problem.

Anopheles mosquitoes are the only vectors of human malaria and they are the only vectors of *Plasmodium* species, the etiological agents of malaria to humans. Anopheles mosquitoes are found in tropical and neotropical regions, and up until 2016, 472 species had been identified (Rodrigures et al., 2017).

Primary malaria vectors in Africa are *Anopheles gambiae* and *Anopheles funestus* and they are strongly anthropophilic and consequently the most efficient malaria vectors in the world (Centers for disease control and prevention, 2017). Over its life span a female mosquito repeatedly takes a blood meal as protein source to complete egg development. By injecting the saliva which may contain pathogens into the host animal, the pathogens thus complete an obligatory life cycle phase and multiply in the mosquito's salivary glands. This thereby makes female mosquito an ideal transmitter of diverse blood borne pathogens and agents of devastating human diseases (Kalita, Bora, and Sharma, 2013).

Mosquito control and personal protection from mosquito bites are currently the most important measure to control this disease. Synthetic mosquito repellents used for control of vectors are causing irreversible damage to ecosystem since the chemicals are non-degradable in nature. To overcome the problem, there is need for the development of effective non N,N-diethyl-3-methyl toluamide (DEET) alternatives and prepare repellent by using biodegradable mosquito repellent (Chavare, et al., 2015). DEET is best synthetic mosquito repellent that can be available easily but it has harmful effects (Naseem et al., 2016).

Benelli, et al., 2016, stated that mosquitoes behavior play a key role in vector control programs and further knowledge regarding the chemical ecology of mate searching, swarming landmarks, and mate choice in swarming sites is required to improve control strategies. Vector control strategies have traditionally focused on killing mosquitoes using a variety of insecticides. Environmental management through reduction or removal of mosquito breeding sites has often been used alongside chemical or microbiological ovicides, larvicides, and pupicides in areas where endemic mosquito-borne diseases occur. They elaborated that the use of synthetic insecticides has to be regulated given that the development of insecticide resistance is widespread and that there is concern regarding the damage to the environment and effects on non-target organisms. They concluded by stating that use of insecticides for mosquito control, which include organophosphates, carbamates, and pyrethroids, can also have negative effects on human health.

Older methods of mosquito repellents include the rubbing of mana, vinegar and plant oils on the body. Ancient people also used to burn the bay, black cumin, oregano and galbanum to restrict the mosquitoes. Many research efforts revealed that essential oil compounds and their derivatives are alternative controlling measure for mosquitoes (Naseem et al., 2016).

Biocontrol strategies for mosquito-borne diseases are needed to help reduce the prolonged application of insecticides that are currently used as the primary method for mosquito control. Eco-friendly, safe, and sustainable methods should be developed that can target a range of different mosquito species (Benelli, et al., 2016).

2.4. Mushrooms

Mushrooms represent one of the world's greatest untapped resources of nutrition, palatable food and medicinal importance of the future (Wani, Bodha, & Wani, 2010). According to recent estimates, mushrooms constitute at least 12,000 species in the ecosystem and 2,000 species are reported as edible among the known. They are not only considered as nutritious protein-rich food but also serve as a potential source for producing pharmaceutical and nutraceutical compounds (Margret, Beulah, and Nelson, 2013). Mushrooms like truffles have gained popularity as they have a unique aroma profile which is highly desirable in various foods (Wang & Marcone, 2011). Several fungi with antiplasmodial properties have been proved as sources for novel antiplasmodial compounds (Kadhila-Muandingi et al., 2014).

After the discovery of the first wonder drug, penicillin, from filamentous fungi much more attention has been carried out in therapeutic usage of fungus especially from medicinal mushrooms (Margret et al,. 2013). Modern pharmacological research confirms large parts of indigenous knowledge regarding the medicinal effects of mushrooms due to their antifungal, antibacterial, antioxidant and antiviral properties, besides being used as functional foods (Wani et al., 2010).

Rahman & Choudhury (2012), stated that for medical purposes, mushrooms have been consumed to prevent cancer and cardiac diseases, to improve blood circulation, and to reduce cholesterol. Some of the mushrooms have also been used for the treatment of physical and emotional stress, osteoporosis, gastric ulcers, and chronic hepatitis; for the improvement of the quality of life of patients with diabetes; and especially for the stimulation of immunity (Fantuzzi et al., 2010).

According to Oluba et al., (2012), *G. lucidum* mushrooms are said to prevent the proliferation of malarial parasites in mice. Their study found that extracts of *G. lucidum* possessed significant antiplasmodial activity. In a different study, Katsayal et al., (2009) reported the antiplasmodial properties of the fungus *Chlorophyllum molybdites*. In Nigeria, *G. lucidum* aqueous extract is reported to have been used to establish changes in serum and liver lipoprotein cholesterols accompanying *Plasmodium berghei* malarial infection in mice (Oluba et al., 2012). Kadhila-Muandingi et al., (2014) reported the antiplasmodial activity of aqueous extracts for the indigenous *G. lucidum* and *Terfezia pfeilii* Namibian mushrooms against *P. falciparum* 3D7A. The two mushrooms showed antiplasmodial activity at concentrations ranging from 5-50 μg/mL, with *T. pfeilii*

extracts showing the highest activity with an IC_{50} of 0.022 µg/mL compared to *G. lucidum* at 3.66 µg/mL.

According to a study by Kadhila-Muandingi & Chimwamurombe (2012) on medicinal uses of some mushrooms in Namibia, *Ganoderma* mushrooms' smoke is inhaled for the relief of flu and body aches. They also stated that the boiled mixture of its water and that of other five species of mushrooms included in their study are used as nerve calming tonics and as treatment for skin infections including measles and many other health complications. The genus *Ganoderma* is a popular topic in the world for their medicinally important use as immuno-booster, being used as remedy for cancer, diabetes, high blood pressure, and in the improvement of the conditions of those living with HIV/AIDS. Though inedible due to its thick tough and cocky fruiting body, it is mostly taken as neutriceutical in the form of capsules, tonic, spice and tea (Khatun et al., 2011, & Sanodiya et al., 2009).

Stamets 2005 stated that there is promising evidence documenting the ability of the mushroom *Polyporus umbellatus* (Zhu ALing) to completely inhibit the parasite that causes malaria. He stated that *P. umbellatus* was 100% effective *in vitro* in inhibiting the malarial parasite *P. falciparum*. He also reported the effectiveness of *Pleurotus ostreatus* on *P. falciparum*.

De Silva et al., (2013) reported that the lanostanes from *G. lucidum* have shown moderate antiplasmodial activity in vitro with IC_{50} values of 6 to 20 µM. They further added that *Cordyceps nipponica* exhibited potent *in vitro* antimalarial activity with IC_{50}

values of 0.066 and 0.037 μg/mL, respectively. Other mushrooms such as *Cordyceps unilateralis, Cordyceps pseudomilitaris* and *Stereum ostrea* (false turkey-tail) have also been reported to be active against *P. falciparum* (Stamets, 2005 & Arko et al.,, 2017).

Lentinan, a (1-3)-beta glucan from *Lentinus edodes*, an effective immunostimulatory drug, is reported to induce protective Th1 immune responses to control the proliferation of malaria parasites during the blood stage of P.y17XL infection by stimulating maturation of dendritic cells (DCs) to inhibit negative regulation of the Th1 immune response by Tregs, so it can be established that lentinan has prophylactic potential for the treatment of malaria (Zhou et al., 2009). Numerous studies show that fungi can infect adult mosquitoes when applied to a range of substrates, suggesting potential for use as IRS or via novel delivery strategies such as impregnated eve curtains, resting targets or even impregnated bed nets (Blanford et al., 2012).

2.5. Plants as antimalarial agents

The discovery of the plant-based drug artemisinin for malaria treatment highlights the importance of screening plants and fungi as sources of metabolites for parasitological and mosquitocidal properties (Benelli et al., 2016). Plants and other natural products are starting points for future antimalarial therapies and novel antiplasmodial drugs. Many plant remedies are used to treat malaria in Africa, Asia and the Americas (Chinsembu, 2015). Many herbs used in folklore for the treatment of diseases in different parts of the world are being screened for antimicrobial activities and the results obtained from these scientific studies so far have rationalized the tradomedical use of many plants and plant parts (Theresa et al., 2011).

Plants have also been used for centuries in the form of crude fumigants where plants were burnt to drive away nuisance mosquitoes and later as oil formulations applied to the skin or clothes which was first recorded in writings by ancient Greek, Roman and Indian scholars. Most plants contain compounds that they use in preventing attack from phytophagous insects (Maia &Moore, 2011).

Recently, commercial repellent products containing plant-based ingredients have gained increasing popularity among consumers, as these are commonly perceived as "safe" in comparison to long-established synthetic repellents although this is sometimes a misconception. Although the primary functions of these compounds are defense against phytophagous insects, many are also effective against mosquitoes (Maia et al., 2011). Plant based repellents do not pose dangers of toxicity to domestic animals and humans and can be easily biodegraded (Naseem et al., 2016).

2.6. Malaria control and treatment

Current malaria control programs rely heavily on methods that target the mosquito vector using chemical insecticides, but recently alarming increases in insecticide resistance have been detected in malaria vectors throughout Africa. Insecticide resistance management initiatives require comprehensive quantification of resistance in field populations to the set of insecticides used in vector control (Hancock et al., 2018).

According to Hemingway et al., (2016), there are at least 25 projects in the global malaria vaccine pipeline, as well as 47 medicines and 13 vector control products. In addition, there are several next-generation diagnostic tools and reference methods

currently in development, with many expected to be introduced in the next decade. The development and adoption of these tools, bolstered by strategies that ensure rapid uptake in target populations, intensified mechanisms for information management, surveillance, response, and continued financial and political commitment are all essential to achieving global eradication.

There are three major interventions used globally which are insecticide treated nets (ITN), artemisinin-based combination therapy (ACT) and indoor residual spraying (IRS). Earlier work by Malaria Atlas Project (MAP) has generated detailed models of ITN, ACT and IRS coverage in parallel to spatiotemporal reconstructions of changing endemicity through time in Africa. MAP is working to extend the African ITN model to triangulate data from net manufacturers, national malaria control programmes and household surveys into coverage models. This will allow definitive evaluation of ITN coverage and enable future ITN distribution to be more targeted and risk driven. Similar models will be produced for ACTs, IRS, and intermittent preventive treatment in pregnancy (IPTp) and seasonal malaria chemoprevention (SMC) (Weiss at al., 2018).

Insecticide-treated bed nets have played a very important role in the reduction of *Plasmodium falciparum* infection prevalence in malaria endemic sub-Saharan Africa, which has seen the incidence of clinical disease fall by 40% between 2000 and 2015. However, bednets are only effective against mosquitoes that bite during the night and concern is growing that insecticide resistance, particularly due to the most commonly used class of pyrethroids, could reverse this trend and lead to rising incidence of malaria and increased fatalities (Benelli et al., 2016).

The world malaria report 2017 stated that the most commonly used methods to prevent mosquito bites are sleeping under an ITN and spraying the inside walls of a dwelling with an insecticide known as IRS. Use of ITNs has been shown to reduce malaria case incidence rates by 50% in a range of settings and to reduce malaria mortality rates by 55% in children under 5 years of age in sub-Saharan Africa. The report further stated that manufacturers have delivered a total of 582 million insecticide-treated mosquito nets (ITNs) globally between 2014 and 2016, of this amount, 505 million ITNs were delivered in sub-Saharan Africa. It is also reported that across sub-Saharan Africa, household ownership of at least one ITN increased from 50% in 2010 to 80% in 2016. However, the proportion of households with sufficient nets remains inadequate, at 43% in 2016.

The world malaria report (2017) reported that an estimated 409 million treatment courses of artemisinin-based combination therapy (ACT) were procured by countries in 2016 which is more than 311 million in 2015. Current strategies for malaria control center on the use of chemical insecticides against the adult mosquito vectors, unfortunately, the sustainability and effectiveness of these frontline technologies are being undermined by the exceptionally rapid spread of insecticide resistance in *Anopheles* populations. This growing resistance problem has led to calls for new control tools to help reduce the reliance on existing chemical insecticides (Blanford et al., 2011).

According to African Leaders Malaria Alliance Namibia (ALMA, (2012), report, the country has removed tariffs on anti-malarial medicines, malaria diagnostics, insecticides, and spray pumps for IRS. Namibia has also banned oral artemisinin-based

monotherapies and is introducing a policy on Community Case Management of malaria. Namibia has made significant progress in scaling-up malaria control interventions and has, in particular, sustained high levels of IRS whilst rolling out long-lasting insecticide treated nets (LLINs), and expanding to universal parasitological diagnosis and treatment. Namibia is one of the top 10 high-mortality countries with the sharpest increases in the annual rate of reduction in under-five mortality rate (ALMA, 2012). Disease prevention and elimination relies on operational control of Anopheles malaria vectors, which requires the deployment of effective insecticides (Coleman et al., 2017).

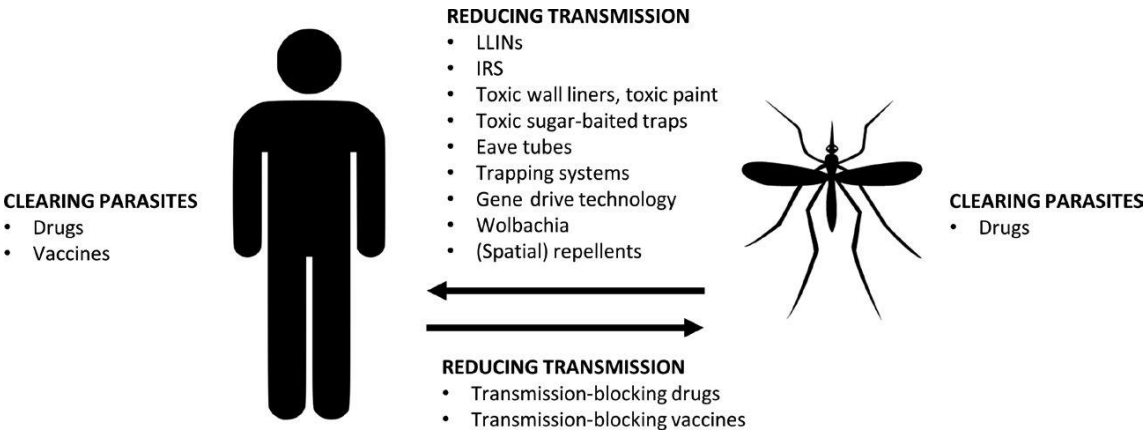


Figure 8. Current and novel interventions aiming at reducing malaria prevalence (Huijben & Paaijmans, 2018).

Current and novel interventions aiming at reducing malaria prevalence by clearing the parasites from humans and mosquitoes or by reducing the transmission probability from mosquito to human or vice versa (Fig. 8) (Huijben & Paaijmans, 2018).

2.6.1. Dichlorodiphenyltrichloroethane (DDT)

Dichlorodiphenyltrichloroethane is a colorless, crystalline, tasteless, and almost odorless organochlorine known for its insecticidal properties and environmental impacts. WHO recommends DDT only for indoor residual spraying, provided that several conditions are met. Concerns about the use of DDT are fueled by recent reports of high levels of human exposure associated with indoor spraying amidst accumulating evidence on chronic health effects. WHO recommends DDT for use in indoor residual spraying only because there is no alternative of both equivalent efficacy and operational feasibility, especially for areas with perennial and long seasonal malaria transmission (van den Berg, 2008).

Despite concerns regarding the health effects of exposure to DDT, it continues to be used for vector control in some developing countries. DDT and its primary degradation product and metabolite, DDE (dichlorodiphenyldichloroethylene), are lipophilic compounds that are persistent in the environment and have the ability to bioaccumulate (Whitworth et al., 2014). The problems with the use of DDT stems largely from its persistence, bioaccumulation, toxicity, and physicochemical capacity for long-range transport (Bouwmana et al., 2012).

There is evidence of adverse human health effects resulting from long-term DDT exposure. Effects include reproductive health effects at birth such as pre-term birth and low birth weight; earlier puberty in girls, adverse pregnancies and breast cancer in women and reduction in semen quality in men. There is also evidence of neurodevelopmental impairment in infants, neurotoxic effects in adults and development

of pancreatic cancers in men (Dalvie, 2011). Inhabitants of dwellings treated with DDT for indoor residual spraying show high DDT levels in blood and breast milk. This is of concern since mothers transfer lipid-soluble contaminants such as DDT via breast feeding to their children (Gyalpo et al., 2012).

Despite evidence of potential harmful human health effects of DDT exposure, there remains a gap in knowledge regarding determinants and strategies for reduction of exposure, particularly among non-occupationally exposed individuals (Whitworth et al., 2014). Efforts are needed to implement best practices to protect residents in treated households from exposures arising from IRS. Of particular concern would be women of childbearing age who live in DDT IRS-treated dwellings and transfer of DDT and DDE to the fetus in pregnancy and to the infant via lactation. (Bouwmana et al., 2012).

2.6.2 Parasite resistance

Antimalarial drug resistance is a threat to malaria control and has important implications for global public health. Resistance of malaria vectors to the four insecticide classes commonly used in ITNs or IRS threatens malaria prevention and control efforts. Of the 76 malaria endemic countries that reported standard monitoring data for 2010 to 2016, resistance was detected in 61 countries to at least one insecticide in one malaria vector from one collection site. In 50 countries there was resistance to two or more insecticide classes. Resistance to the four insecticide classes was detected in vectors present in all WHO regions except Europe (World Malaria Report, 2017).

According to (WHO, 2018), much of the success in controlling malaria is due to vector

control. Vector control is highly dependent on the use of pyrethroids, which are the only class of insecticides currently recommended for ITNs or LLINs. Pyrethroids also referred to as dalmatian chrysanthemum, is a perennial daisy loaded with chemicals called pyrethrins that are toxic to insects and nontoxic to humans. In recent years, mosquito resistance to pyrethroids has emerged in many countries. In some areas, resistance to all 4 classes of insecticides used for public health has been detected. Fortunately, this resistance has only rarely been associated with decreased efficacy of LLINs, which continue to provide a substantial level of protection in most settings. Rotational use of different classes of insecticides for IRS is recommended as one approach to manage insecticide resistance. However, malaria-endemic areas of sub-Saharan Africa and India are causing significant concern due to high levels of malaria transmission and widespread reports of insecticide resistance.

The use of two different insecticides in a mosquito net offers an opportunity to mitigate the risk of the development and spread of insecticide resistance; developing these new nets is a priority. Several promising products for both IRS and nets are in the pipeline. (WHO, 2018). The report further elaborated that, detection of insecticide resistance should be an essential component of all national malaria control efforts to ensure that the most effective vector control methods are being used. The choice of insecticide for IRS should always be informed by recent, local data on the susceptibility of target vectors.

Apart from IRS, three additional classes of insecticides are available (organochlorines, organophosphates and carbamates), the organochlorine DDT was predominantly used during the 50s and 60s as it was cheap and highly effective. More recently, pyrethroids

took over the IRS market with approximately 90% of President's Malaria Initiative (PMI)-funded countries using pyrethroids in their IRS campaigns around 2010, the dramatic increase in the prevalence and strength of insecticide resistance that is observed across Africa is likely due to applying single-class insecticides in public health. The use of a single insecticide in IRS campaigns (DDT in the 50s and 60s; pyrethroids up to very recent) or a single chemical class (only pyrethroids in/on ITNs/LLINs) resulted in selective pressure in the vector population (Huijben & Paaijmans, 2018).

Huijben & Paaijmans (2018), stated that resistance of *P. falciparum* parasites to chloroquine, the first widely available antimalarial, started to spread across the majority of malaria endemic areas in the 60s and 70s. The drug was replaced with sulphadoxine–pyrimethamine (SP) as the first-line treatment in several countries in the 90s. They further stated that, although falciparum parasites have developed resistance to nearly every available drug, ACTs still remain effective in most parts of the endemic world and have proven to be rather resilient against resistance evolution by not losing their efficacy as rapidly as chloroquine and SP did in the past. This success may be attributed to drug properties, as artemisinins act more rapidly and have a shorter half-life than all other antimalarials, resulting in a shorter window of selection and are protected by a partner drug with a longer half-life, so selective pressure is always for two drugs of different mode of action at the same time.

The main advantage of ACTs is that the artemisinin quickly reduces most of the malaria parasites and the partner drug clears the remaining ones. However, the efficacy of ACTs is threatened by the emergence of both artemisinin and partner drug resistance (Malaria

World Report, 2017). They concluded that an additional obstacle is that when it comes to evolutionary principles, there is a general disconnect between the academic world and policy makers, even though malaria programme managers and academics both acknowledge that the evolution of pathogen and vector is hampering progress of malaria control and eradication and that interventions may fail if evolutionary biology of the disease is disregarded (Huijben & Paaijmans, 2018).

CHAPTER 3

3. MATERIALS AND METHODS

3.1. Ethnobotanical survey

The Ministry of Environment and Tourism in Namibia issued the plants collection permit (Permit number 2124/2016) **appendix D**. The guidelines followed on sustainable harvesting of plants were provided by the National Botanical Research Institutes (NBRI), Windhoek, Namibia. The plants were collected after interviews with a well-known local herbalist Dr Simaata P Matengu who has been practicing as a traditional healer for 46 years and obtained his indigenous knowledge from his father and communicate all his needs during treatment with his ancestral spirits. Dr Simaata is a chairperson of the Traditional Healer Association in Namibia and he is registered with the Ministry of Health and Social Services (MoHSS).

Some community members in Kavango East and Ohangwena regions in North-Central Namibia gave information about the plants that are used for malaria treatment in their communities. Most of the plants were recommended by the herbalist and were collected with him in the forest at Ncuncuni village in Kavango East. One headwoman from Kaisosi in Rundu also provided information about the plants that were collected together with the herbalist. The collected plants were identified by the curator from the National Herbarium at the National Botanical Research Institute in Namibia (**appendix E**).

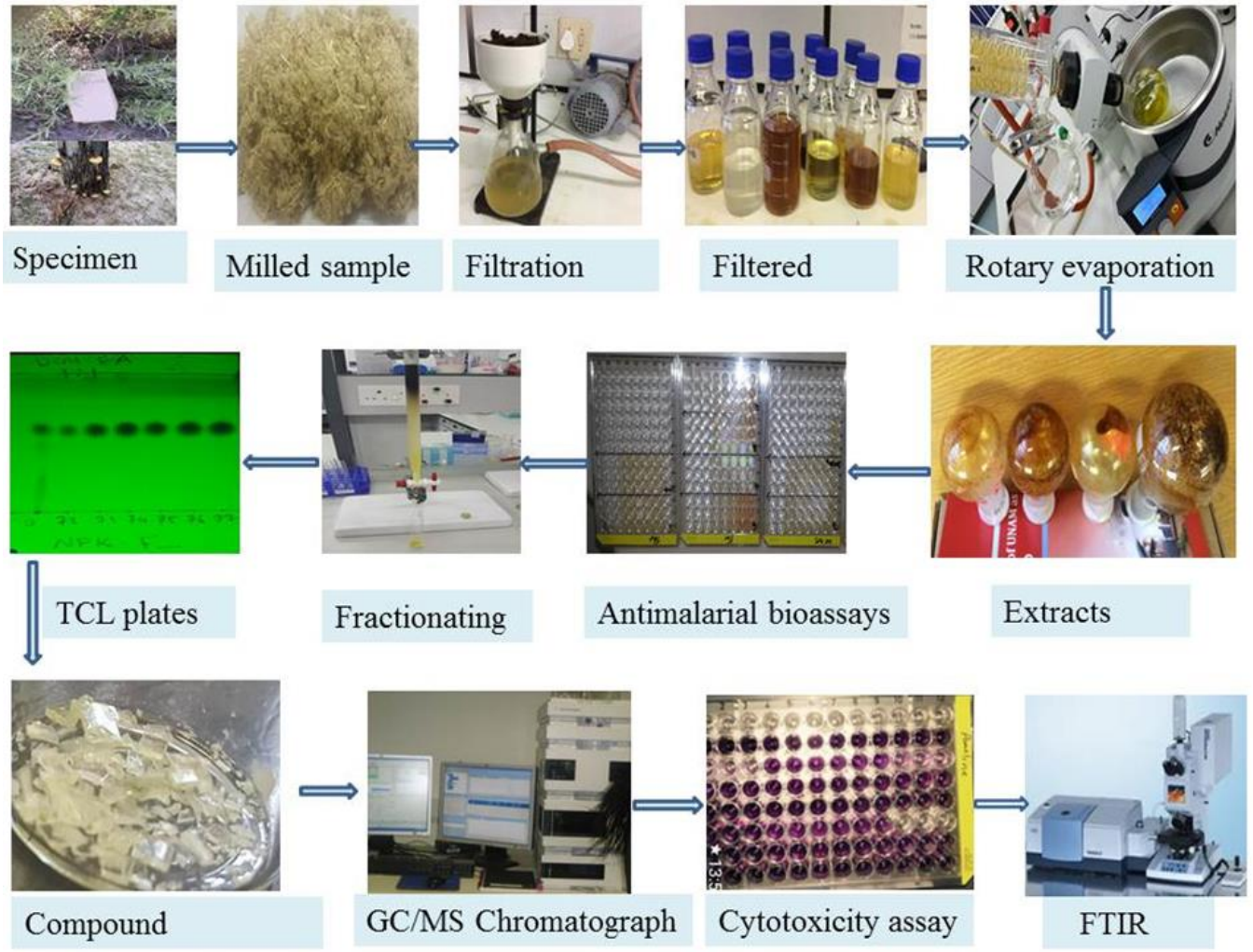


Figure 9. Schematic overview of methods used in this study

3.2. Mushroom collection and identification

Mushroom samples were collected from Kavango West, Ohangwena and Oshikoto regions. The regions were chosen due to the availability of tree stumps and cultivated fields where many mushrooms were found growing during the past studies. In the field, mushrooms were morphologically identified using a mushroom Field Guide book for Southern Africa by Van der Westhuizen & Eicker, (1994*)*.

Mushroom pictures were taken for further identification. The locality, soil type, vegetation type and the substrate on which the mushrooms were found growing were also noted to help in identification. The two species of mushrooms used in this study were chosen as a follow up study to that of Kadhila-Muandingi et al., (2014) with the aim of identifying and characterizing the bioactive molecules that are active against the malaria parasite observed in the past study.

3.3. Evaluation of mushroom and plant extracts for the *in vitro* activity against the 3D7 strain of the malaria parasite, *Plasmodium falciparum*

3.3.1 Extract preparation and *in vitro* screening for antiplasmodial activity

The selected collected mushrooms and plants were dried and ground to fine powder. Twenty (20 g) of each sample were added to 200 mL of solvent. The samples left on a shaker for 48 hours, filtered and extracted again with the same solvent for another 48 hrs. The extraction process was repeated sequentially with organic solvents in their increasing order of polarity. The solvents used are hexane, dichloromethane, ethyl acetate and methanol. The extracts were filtered through Whatman no.4 filter paper and

subsequently evaporated using a rotary evaporator (Heidolph GI, Germany) at 60^{o}C. The extracts were dried in the fume hood, weighed and placed in sterile sample bottles and stored in a refrigerator until required for use.

Stock solutions of 20 mg/ml were made in 100% dimethyl sulfoxide (DMSO) and the samples were screened at 100 µg/mL - 5.13 x 10^{-3} µg/mL for dose-response (3-fold dilution). The solvent control, DMSO, was also screened at corresponding concentrations of the test samples with the starting concentration of 1% DMSO at 100 µg/mL. The extracts were screened for *in vitro* antimalarial activity at full dose concentrations against the chloroquine-sensitive strain, 3D7 of *Plasmodium falciparum* (Nogueira & Lopes, 2011).

The 50% inhibitory activity (IC_{50} values) was determined using the parasite lactate dehydrogenase (pLDH) assay. Two-fold dilutions of the stock solution (2 mg/mL) to 200 µg/mL was carried out in complete RPMI 1640 culture medium, introduced to the parasite at trophozite stage, and incubated for 48 hrs at 37^{o}C in an atmosphere of 93% N_2, 4% CO_2, and 3% O_2. After incubation, parasite viability relative to the untreated controls were measured by pLDH assay, whereby the density of the parasite is indicated by production of quantifiable purple crystals of formazan salt. Dose-response curves were obtained by plotting percentage parasite survival against the logarithm of the concentration using the GraphPad Prism software package (GraphPad software, Inc, California, USA). The 50% inhibitory activities (IC_{50} values) were derived from dose-response curves.

3.3.2 Cultivation of malaria parasites

Plasmodium falciparum parasites were cultured as described by Trager and Jesen (1976), with minor modifications. The 3D7, and CQ-resistant strain K1 isolated in Kanchnaburi, Thailand and RSA11 isolated in Kwa-Zulu Natal were used. The parasites were maintained in Roswell Park Memorial Institute medium (RPMI) 1640 (BioWhittaker) culture medium supplemented with phenol red, albumax II (bovine serum albumin) (Gibco) (25 g/L), HEPES (*N*-[2-hydroxyethyl]-piperazine-*N*'-[2-ethansulphonic acid]) (6 g/L), 4.25%of sodium bicarbonate and gentamycin (50 mg/L). The reagents were purchased from Sigma-Aldrich, South Africa.

The parasites were cultured in sealed flat bottom flasks and maintained in washed O^+ human red blood cells (RBC) at 37^oC in an atmosphere of 93% N_2, 4% CO_2, and 3% O_2. The haematocrit and parasitaemia were kept between 2 - 4% by the addition of RBC. Parasites were synchronized at the ring stage regularly by treatment with 5% D-sorbitol. The parasitaemia was determined microscopically using a Giemsa stained thin blood smear of culture on the slide.

3.3.3. Fractionation of antiplasmodial active extracts by Column Chromatography

Extracts with desirable activity and selectivity index were fractionated using Column Chromatography to identify the fractions responsible for activity using Mass spectrophotometry and NMR analyses.

Silica (0.063 - 0.200 mm) was mixed with hexane to make a slurry, which was poured in to a glass column (60 x 4 cm) and left to settle. The active fraction (20 mg/mL) was

dissolved in 5 ml DCM:hexane (9:1) and added onto the column and washed with 50 ml hexane to wash out the DCM. The wash was collected in 20 ml of first 2 tubes. The washing was followed by a slow and constant drop by drop flow of 200 ml of mobile phase: DCM:Hex (60:40). The flow rate was an average of 65 drops per minute and 52 fractions of 10 mL each were collected. The polarity of the mobile phase was then increased to DCM:MeOH (9:1).

3.3.4 Thin Layer Chromatography

Thin Layer Chromatography (TLC) was performed on Whatman aluminium sheets coated with a 0.2 mm layer of silica (Si gel 60). TLC was used to determine the profiles of the eluted fractions generated from column chromatography. The fractions 1 to 15 were reconstituted in DCM:hexane (9:1) solvent system, while fractions 16 - 40 were reconstituted in 100% DCM. Fractions 41 - 52 were reconstituted in DCM:MeOH (9:1). Fractions were spotted on the TLC sheets using 10 µl capillary tubes. TLC plates were developed in a TLC tank in the mobile phase DCM:Hex:EtAc (6:3:1). The polarity of the mobile phase was increased to a DCM:Hex:MeOH (6:2:2) solvent system for the more non-polar fractions generated. The TLC plates were viewed under 254 nm and 365 nm UV light. Fractions with similar TLC profile were pooled together, which then yielded a total of 117 fractions including the wash, which was the last fraction.

3.3.5 Phytochemical profiling for the antiplasmodial active plant Npk1 (*Pechuel-loeschea leubnitiziae*)

For the detection of the compounds, universal reagents were used to react with many classes of natural products. The following classes of compounds were detected using freshly prepared universal reagents.

3.3.6 Detection of saponins and terpenoids

For the detection of terpenoids and saponins, freshly prepared Liebermann Burchard Reagent (LBr) reagent was used. The reagent was prepared by carefully adding 5 mL of concentrated H_2SO_4 to 50 mL of absolute ethanol on ice. The TLC plates were sprayed with 5 to10 mL of Liebermann Burchard Reagent (LBr), warmed at 100°C for 5 to 10 minutes and evaluated under visible or UV (365 nm) light. The presence of brown zones in UV365 nm, dark brown in visible light, grey zones in UV (365 nm) and weak grey in visible light are the presences of terpenoids and saponins.

3.3.7 Anthraquinones and coumarins detection

Freshly prepared KOH solution was used for the detection of anthraquinones, and coumarins. The TLC plates were sprayed with 10 mL of a 10% (w/v) of 96% ethanolic KOH solution, dried and then observed under UV (365 nm) or visible light without warming. The presence of yellow zones is an indication of anthraquinones and coumarins compounds.

3.3.8 Detection of flavonoids

Flavonoids were detected using aluminium chloride ($AlCl_3$). Each TLC plate was sprayed with 5 to 10 mL of a 1% (w/v) 95% ethanolic $AlCl_3$ solution and evaluated under UV (365 nm) light. The presence of blue, green and purple zones are an indication of flavonoids.

3.3.9 Detection of alkaloids

The Dragendorff reagent was prepared and used for the detection of alkaloids, which are heterocyclic nitrogen compounds. The reagent was prepared by dissolving 8.00 g of KI in 20 mL of distilled water. This solution was then added to a second solution containing 0.85 g of basic bismuth nitrate in 40 mL of distilled H_2O and 10 mL of glacial acetic acid. Each TLC plate was sprayed with 10 mL and observed under UV light at the wavelength of 365 nm. The presence of orange to red color and yellow zones were an indication of alkaloids, heterocyclic nitrogen compounds.

4. Structure elucidation of antiplasmodial active compounds

4.1 Fourier Transform Infrared (FTIR)

Fourier Transform Infrared Spectroscopy, also known as FTIR Analysis or FTIR Spectroscopy, is an analytical technique used to identify organic, polymeric, and, in some cases, inorganic materials. The FTIR analysis method uses infrared light to scan test samples and observe chemical properties. IR spectrum results from the absorption of energy by vibrating chemical bonds. However, for a vibration to give rise to absorption

of infrared radiation, it must cause a change in the dipole moment of the molecule. The intensity of absorption is proportional to the change in the dipole moment. There is a correlation between the wavenumber at which the molecule absorbs infrared radiation and the stiffness of the absorbing chemical bonds. Fourier Transform Infrared is most useful technique for identifying functional groups of compounds. The term Fourier Transform Infrared Spectroscopy (FTIR) refers to a development in the manner in which the data is collected and converted from an interference pattern to a spectrum. It is a powerful tool for identifying types of chemical bonds in a molecule by producing an infrared absorption spectrum that is like a molecular "fingerprint". It uses infrared light to scan test samples and elucidate the functional groups. The parameters used for FTIR are found in **Table 1**.

Table 1. Parameters used for Fourier Transform Infrared Spectroscopy

Advanced measurements	
Resolution	4 cm^{-1}
Sample scan time	32 Scans
Background scan time	32 Scans
Save data range	From 4000 to 400 cm^{-1}
Results spectrum	Transmittance
Optic measurements	
Source setting	MIR
Beam splitter	KBr
Optical Filter setting	Open
Aperture setting	6 mm
Measurement channel	Sample Compartment
Background meas. channel	Sample Compartment
Detector setting	RT-DLaTGS (Internal)
Scanner velocity	10 KHz
Sample signal gain	Automatic
Background signal gain	Automatic
Delay after device change	5
Delay before measurement	0
Optical bench ready	OFF
Preamp gain	A
Acquisition measurements	
Wanted high frequency limit	8000 cm^{-1}
Wanted low frequency limit	0 cm^{-1}
High Pass filter	Open
Low Pas filter	10 KHz
Acquisition mode	Double Sided, Forward-Backward
Correlation mode	OFF

5. Determination of cytotoxicity for antiplasmodial active plant compounds

5.1 Cytotoxicity assay with (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) (MTT)

MTT assay is based on the ability of viable cells to reduce the yellow water-soluble tetrazolium salt to a water-insoluble purple formazan product. Since this activity requires functional mitochondria, only viable cells can cleave the tetrazolium ring. The metabolic activity in the cells and the number of viable cells are directly proportional to the amount of formazan crystals formed.

Chinese hamster ovary (CHO) cells were cultivated in Dulbecco's Modified Eagle Medium (DMEM) and F-12 HAMS Medium (1:1), supplemented with 10% foetal bovine serum and 0.6% streptomycin. Cells were incubated with 10-fold serial dilutions (0.001-100 µg/mL) of test samples in a 5% CO_2 incubator for 48 hours. After the incubation, cell viability relative to untreated controls were determined using a colorimetric MTT (3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) assay and a IC_{50} (50% inhibition of cell viability) value derived from dose-response curves.

5.2. Gas chromatography-Mass Spectrometry (GC-MS)

Gas chromatography mass spectrometry (GC-MS) is an instrumental technique comprising a gas chromatograph (GC) coupled to a mass spectrometer (MS), by which complex mixtures of chemicals may be separated, identified and quantified. This makes it ideal for the analysis of the hundreds of relatively low molecular weight compounds found in environmental materials. The GC works on the principle that a mixture will

separate into individual substances when heated. The heated substances are carried in a column with an inert gas such as helium. GC-MS identifies different substances in a test sample. The operating conditions used for GC-MS spectrometry are found in **Table 2.** The sample was dissolved in dichloromethane. The Mass Spectra was Agilent 6890N network Gas chromatography and the system instrument was connected to Agilent 5973 network Mass Selectivity Detector using the HP5-MS, 30 m x 0.25 mm x 0.25 µm.

Table 2. Operating conditions used for GC and GC-MS spectrometry

GC OPERATING CONDITIONS	
Head Pressure (kPa)	60.5
Injector Temperature (^{o}C)	250
Carrier Gas	He
Split-Less Time (min)	1.5
Flow Rate (mL/min)	50
Start Temperature (^{o}C)	40
Hold Time (min)	2
End Temperature (^{o}C)	280
Ramp Rate (^{o}C)	10
Hold Time (min)	10
Total Run Time (min)	31
GC-MS OPERATING CONDITIONS	
Interface Temperature (^{o}C)	280
MS Source Temperature (^{o}C)	230
Ion Source Pressure (Torr)	4.4×10^{-5}
MS Quad Temperature	150
Electron Energy (eV)	70
Emission Current (µA)	34.6
Scan Range (amu)	40-450
Scan Time (s)	1.52

5.3. Nuclear Magnetic Resonance (NMR)

Nuclear Magnetic Resonance (NMR) spectroscopy is an analytical chemistry technique that determines the content, purity and unique molecular structure of a compound. It identifies the carbon-hydrogen framework of an organic compound. Here, the sample was dissolved in deuterated chloroform ($CDCl_3$) solvent. Varian 400 MHz spectrometer at 300 K was used and the coupling constant is given in Hertz (Hz). Chemical shift values are given as δ (ppm) relative to Tetramethylsilane (TMS) (^{1}H and ^{13}C) as internal standard.

6. Data Analysis

The parasitaemia was determined microscopically using the Giemsa stained thin blood smear of culture on the slide. The parasite viability was measured using the Plasmodium lactate dehydrogenase (pLDH) assay. The cytotoxicity was done using the (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) (MTT) assay. The IC50 value was derived from dose-response curves. The structure elucidation of the antiplasmodial active compound was done using standard procedures for gas chromatography-mass spectrometry (GC-MS), fourier transform infrared (FTIR) and nuclear magnetic resonance spectroscopy (NMR).

7. Research Ethics

Ethics approval for this study was obtained from the University of the Free State (**ECUFS 150/2015**) and the University of Namibia (**SEC/FOS/4/2014**). Collection permit was acquired from the Ministry of Environment and Tourism before the collection of mushrooms and plants (Permit number 2124/2016). The export permit was also granted by the Ministry of Environment and Tourism to send the samples to South Africa (106239) **Appendix D**.

CHAPTER 4

4. RESULTS

4.1. Study area

The two Kavango regions were chosen because the herbalist is mostly operating in those regions. The plants he uses are harvested in the two regions and the neigbouring country Angola.

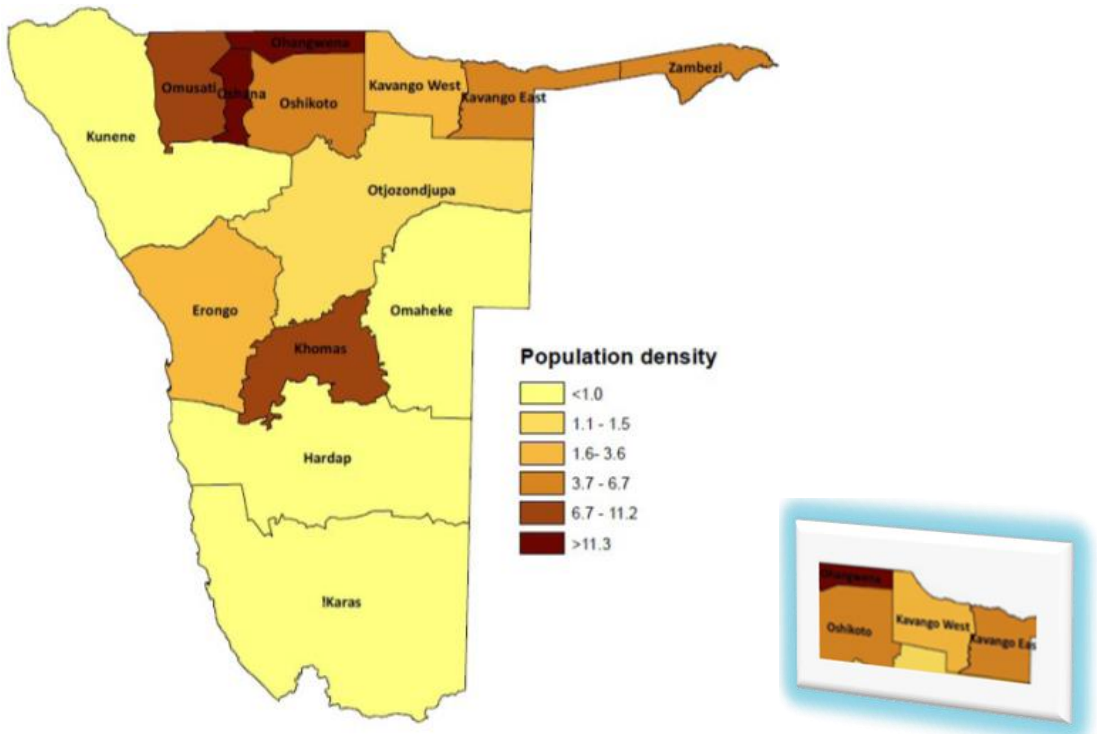


Figure 10. Map of Namibia and the extracted study area of Kavango West, Kavango East and Ohangwena Regions

4. 2. Mushrooms collected

The indigenous mushrooms included in this study are: Omapakululu (*Ganoderma* species), Omatumbula (*Kalaharituber pfeilii)*, well known as the Kalahari Desert Truffles consisting of samples from Oshikoto Region purchased at Omuthiya open market and Kavango West in Nkurenkuru and Owowa/Omajova (*Termitomyces schimperi*).

Mushrooms were collected based on the results from the previous study by Kadhila-Muandingi at al., (2014).

Ganoderma species *Kalaharituber pfeilii* *Termitomyces schimperi*

Figure 11. Medicinal mushrooms screened for antiplasmodial activities against the 3D7 strain of the malaria parasite, *Plasmodium falciparum*.

Table 3. **Mushrooms used in this study**

Voucher No	Scientific name	Family name	Local name
G	*Ganoderma lucidum (Curtis) P. Karst.*	Ganodermataceae	Omapakululu
To	*Terfezia pfeilii (Berber: Tirfas)* (Oshikoto)	Tuberaceae	Omatumbula
Tk	*Terfezia pfeilii (Berber: Tirfas)* (Kavango West)	Tuberaceae	Mafumbura
Tc	*Termitomyces schimperi* (Pat.) R. Heim	Lyophyllaceae	Owowa/Omajova

Table 4. Plants used for the treatment of malaria and its symptoms in Kavango East and Ohangwena regions in Namibia

Voucher No	Scientific name	Family name	Local name	Local uses	Mode preparation
npk 1	*Pechuel-loeschea leubnitiziae* (Kuntze) O.	Asteraceae	Oshizimba	Fever and chills	Boil whole plant and stream the sick person
npk 2	*Psidium guajava* L.	Myrtaceae	Guava	Fever, chills and headache	Leaves are ground and used in hot water to bath. Can be taken orally too
npk 3	*Acrotome inflata* Benth	Lamiaceae	Etse lya kuku	Mosquito repellent	Whole plant is crushed and spread in rooms to repel mosquitoes
npk 4	*Laggera decurrens* (Vahl) Merxm.	Asteraceae	Oshikuluzimba	Fever, chills and headache	Parts of the plant are boiled and the sick person is steamed
npk 5	*Acanthospermum hispidum* DC	Asteraceae	Okano ka kahandja	Fever and chills	Pounded leaves are sniffed to reduce pain and fever
npk 6	*Burkea africana* Hook	Leguminosae	Situnduwanga	Malaria, dizziness fever, cough, and headache	The crushed roots and leaves are put in water and the solution is taken orally
npk 7	*Hibiscus sabdariffa* L.	Malvaceae	Mutete	Malaria and appetiser	Young plants or leaves are crushed and the juice (raw or boiled) is drank

					as treatment
npk 8 fruits	*Colophospermum mopane* (Kirk ex Benth.) Kirk ex J.Léon.	Fabaceae	Omusati	Fever, chills and headache	The fruits and young leaves are crushed and inhaled to ease the pain. The patient can also bath with the solution
npk 9	*Steganotaenia araliacea* Hochst	Apiaceae	Mutukatabolo	Fertility, libido, malaria and pain in the stomach	The roots are crushed, put in water overnight and taken orally
npk 10	*Ipomoea hochstetteri* House	Convolvulaceae	Ndonga	Stomach pain and treats symptoms related to malaria	the roots are pounded and the solution is taken orally
npk 11 Roots	*Boscia albitrunca* (Burch.) Gilg & Gilg-Ben	Capparaceae	Omunkuzi	Roots are used to ferment milk,	
npk 12 Leaves	*Boscia albitrunca* (Burch.) Gilg & Gilg-Ben.	Capparaceae	Omunkuzi	most sickness including, colds, coughing and malaria	Leaves and twigs are crushed Leaves are crushed to use as sniffed for all kind of sickness
npk 13	*Dicoma tomentosa* Cass	Asteraceae	Okalupulupu	Fevers, vomiting and constipation	Whole plant is crushed and the concoction is drunk

Thirteen plants were collected **(Table 2)** and pictures **taken (Appendix F)** in this study. The most used method of prepration by the herbalist and the few community members was that of crushing the aerial parts of the plant (leaves and twigs) or roots, add water and then concoction is taken by the sick person for about 5 days before getting better.

4.1. Pictures of collected plants

Most of the plants were collected together with the herbalist, giving the plant parts that are used.

Figure 12. *Steganotaenia araliacea Hochst* (Mutakatadalo) collected at Ncuncuni village in Kavango east

This plant is reported to be used for malaria, stomach pains, libido and fertility in Kavango East region in Namibia.

Figure 13. *Ipomoea hochstetteri* House plant collected in the vicinity of Rundu town in Kavango East

This plant is used to treats malaria symptoms and stomach pain treats by drinking the infusion with water.

Figure 14. *Laggera decurrens* (Oshikuluzimba) collected from Kaisosi in Rundu Kavango East.

In both Ohangwena and Kavango East Regions, the leaves of this plant are crushed boiled and the person suffering from fever and headache is exposed to its steam.

Figure 15. *Acrotome inflata Benth* collected from Okamukwa village in Ohangwena Region

This plant is reported to be used in both regions of Ohangwena and Kavango East for repelling mosquitoes and for spiking children when suffering from fever and headache.

Figure 16. *Pechuel-loeschea leubnitiziae* collected from Kavango East near Rundu town

In both Kavango East and Ohangwena Regions, the leaves of this plant are crushed boiled and the person suffering from fever and headache is steamed.

Figure 17. *Acanthospermum hispidum* DC collected from Okamukwa village in Ohangwena Region

The plant grows as a weed in cultivated crop fields and its leaves are pounded and sniffed when locals are suffering from malaria in Ohangwena region.

Figure 18. *Colophospermum mopane* (Omusati) collected from Oilaati in Ohangwena Region

In Ohangwena Region of Namibia, *C. mopane* is regarded as a cure of everything, thus is also used to treat malaria related symptoms.

Figure 19. *Hibiscus sabdariffa L*. plant collected from Ncuncuni village in Kavango East

The roots of *H. sabdariffa L* are used for the treatment of stomach cramps, and it is believed that people who eat this plant as vegetable do not suffer from malaria. An infusion is prepared with the roots in cold water and left overnight and the concoction is taken orally for such treatment.

Figure 20. *Dicoma tomentosa Cass* (okalupulupu) collected from Oilaati in Ohangwena Region

The plant is found growing in cultivated field and disturbed areas around mahangu fields. The whole plant is crushed and used as medicine for most diseases including malaria. This plant seemed to be well researched when it comes to malaria elsewhere, but not in Namibia.

Figure 21. *Burkea africana Hook* collected from Ncuncuni village in Kavango East.

In Kavango East Region the locals use this plant for malaria and dizziness.

4.2. *In vitro* Antimalarial Activity for mushroom samples

Test samples that showed *in vitro* antimalarial activity of ≤ 10 µg/mL were considered active. Test compounds that show % parasite survival of 0 – 15% at a single concentration of 10 µg/mL were screened further for dose-response to determine the IC_{50}. The *in vitro* antimalarial activity of sixteen mushroom extracts was determined at single concentrations of 100 and 10µg/mL against the chloroquine-sensitive strain, 3D7 of *Plasmodium falciparum*.

Table 5. The *in vitro* antimalarial activity of tested 16 extracts at 100 and 10µg/mL on 3D7 strain

Test extracts	**Solvent**	**% Parasite Survival (100 µg/mL)**	**% Parasite Survival (10 µg/mL)**
Ganoderma species	Hexane	60.58	46.49
	Dichloromethane	23.06	68.16
	Ethyl acetate	75.93	65.15
	Methanol	80.28	69.49
Termitomyces schimperi	Hexane	67.55	68.25
	Dichloromethane	64.05	66.11
	Ethyl acetate	62.89	66.50
	Methanol	58.04	76.05
Truffles Kavango	Hexane	71.86	74.20
	Dichloromethane	66.13	67.86
	Ethyl acetate	60.01	66.39
	Methanol	61.98	68.86
Truffles Oshikoto	Hexane	79.76	77.26
	Dichloromethane	65.72	87.34
	Ethyl acetate	57.36	70.64
	Methanol	89.34	83.61

Sixteen extracts were screened for *in vitro* antimalarial activity against the chloroquine-sensitive strain, 3D7 of *P. falciparum* and none of the extracts showed activity. None of the mushroom extracts screened showed activity against the chloroquine-sensitive strain, 3D7 of *P. falciparum* (**Table 5**).

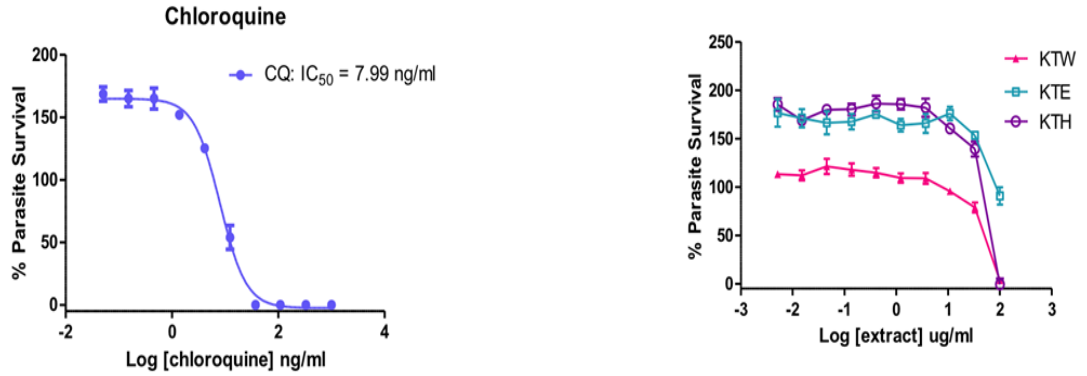


Figure 22. Log concentrations vs. % parasite survival data used to plot chloroquine and test samples dose-response curve for the tested mushrooms

Figure 23. Log concentrations vs. % parasite survival data used to plot test samples dose-response curve

The IC_{50} obtained for chloroquine (7.99 ng/mL) was in agreement with literature values (6 – 15 ng/mL). None of the extracts tested showed % parasite survival of zero at 100 µg/mL or less than 15% at 10 µg/ml. The reference standard, chloroquine showed 100% activity at 1 µg/mLi.e there was zero percentage survival of parasites. The Z' factor of 0.89 indicated that the assay worked very well (**Table 6**). The Z'-factor is used to quantify how well the assay works. A Z'-factor between 0.5 and 1.0 is an excellent assay while the one between 0 and 0.5 is a marginal assay. Therefore, a prerequisite for all experiments is to have a Z'-factor > 0.5.

Table 6. IC_{50} and Z'-factors of tested mushroom samples

Test sample	Z' factor	IC_{50} (ng/mL)
Chloroquine	0.57	7.99
OW	0.55	≥ 100
GE	0.52	≥ 100
GW	0.65	≥ 100
GMD	0.52	≥ 100
KTW	0.65	≥ 100
KTH	0.76	≥ 100
KTE	0.76	≥ 100
KTMD	0.78	≥ 100
KPMD	0.78	≥ 100

4.2. Mycochemical composition for the selected indigenous Namibian mushrooms

Analysis of mycochemical composition of the selected indigenous Namibian mushrooms revealed that *Terfezia pfeilii* contain sugars, triterpenes, steroids, alkaloids and flavonoids. *Termitomyces schimperi* contain reducing sugars, triterpenes, steroids, flavonoids and alkaloids and the *Ganoderma* species contain triterpenes, steroids (Saponins and bitter principles) and sterols (cholesterol and esters), essential oil, terpenes, phenols and sugars.

4.3. Plants

Thirty-six plant extracts were screened for *in vitro* antimalarial activity against the chloroquine-sensitive strain 3D7. Three samples namely **Npk-1D, MAM-D** and **MAM-H** showed *in vitro* antimalarial activity ranging from 4.21 µg/mL to 9.80 µg/mL.

Table 7. Percentage parasite Survival at 10 µg/mL against 3D7 strain of *P. falciparum*

Test Sample		% Parasite Survival ± SD at 10 µg/mL
Npk 1	Hex	19.55 ± 0.03
	DCM	16.11 ± 0.03
	MeOH	83.46 ± 6.07
	Ethyl Ac	94.57 ± 4.39
Npk 2	DCM	41.25 ± 2.00
	MeOH	88.84 ± 2.50
	Ethyl Ac	82.86 ± 4.21
Npk 3	Hex	44.44 ± 0.03
	DCM	75.26 ± 0.22
	MeOH	98.22 ± 4.20
	Ethyl Ac	73.03 ± 1.65
Npk 6	Hex	73.36 ± 0.06
	DCM	81.90 ± 4.26
	MeOH	91.06 ± 2.23

	Ethyl Ac	66.82 ± 0.02
Npk 7	DCM	82.16 ± 1.45
	MeOH	80.99 ± 0.50
Npk 8	Hex	11.66 ± 0.02
	DCM	28.51 ± 0.03
	MeOH	90.67 ± 0.83
	Ethyl Ac	71.62 ± 0.03
Npk 9	Hex	64.17 ± 3.93
	DCM	67.58 ± 0.01
	MeOH	89.78 ± 0.83
	Ethyl Ac	45.55 ± 0.02
Npk 10	Hex	80.99 ± 0.06
	DCM	94.84 ± 4.78
	MeOH	85.27 ± 1.00
	Ethyl Ac	89.95 ± 1.56
Npk 12	Hex	Not assayed
	DCM	65.91 ± 0.10
	MeOH	75.09 ± 1.39
	Ethyl Ac	83.27 ± 0.33

The Z'-factor for the single concentration experiments in **Table 6.** were 0.71 and 0.84. The chloroquine showed no parasite survival (i.e 0%) at 100 µg/mL.

Table 8. IC_{50} and *Z*'-factors of test samples against 3D7 strain of *P. falciparum*

	Test Sample	IC_{50} (µg/mL)	Z'-factor
1	Npk-1D	7.24	0.69
2	Npk-1H	73.87	0.65
3	Npk-8H	40.86	0.69
4	MAM-D	9.80	0.63
5	MAM-E	32.35	0.60
6	MAM-H	4.21	0.60
7	MAM-M	≥ 100	0.68

Three of the 7 extracts namely Npk-1D, Mam-D and MAM-H (Table 8) showed *in vitro* antimalarial activity against the 3D7 strain of *P. falciparum*. Npk-1D and MAM-D showed marginal activity (9.80 and 7.24 µg/mL, respectively) while MAM-H showed moderate activity (4.21 µg/mL). (**Figure 22**). Extracts Npk-1H and Npk-8H showed % parasite survival of 19.55 ± 0.03% and 11.66 ± 0.02%, respectively (**Figure 21).** The *Z*'-factors for these extracts ranged from 0.60 to 0.69 indicating that the assays worked well.

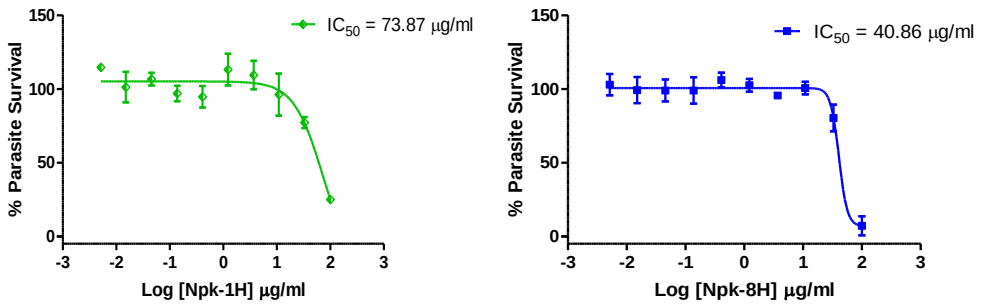


Figure 24. Log solvent percentage vs. % parasite survival data used to plot Npk-^{1}H and test samples Npk-8H dose-response curves

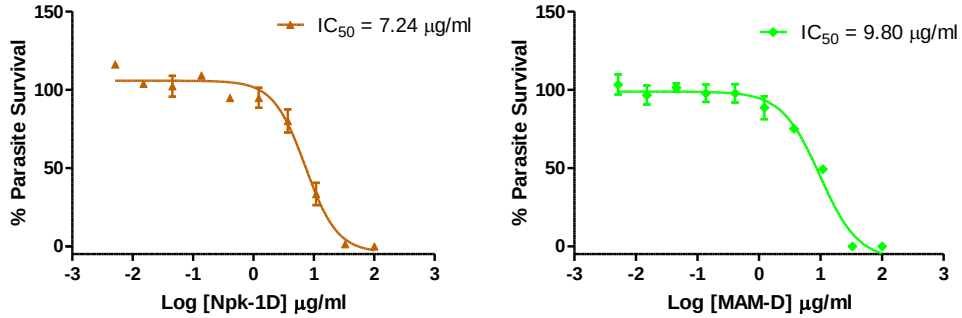


Figure 25. Log solvent percentage vs. % parasite survival data used to plot test samples Npk-1D (*Pechuel-loeschea leubnitiziae*) and MAM-D dose-response curves

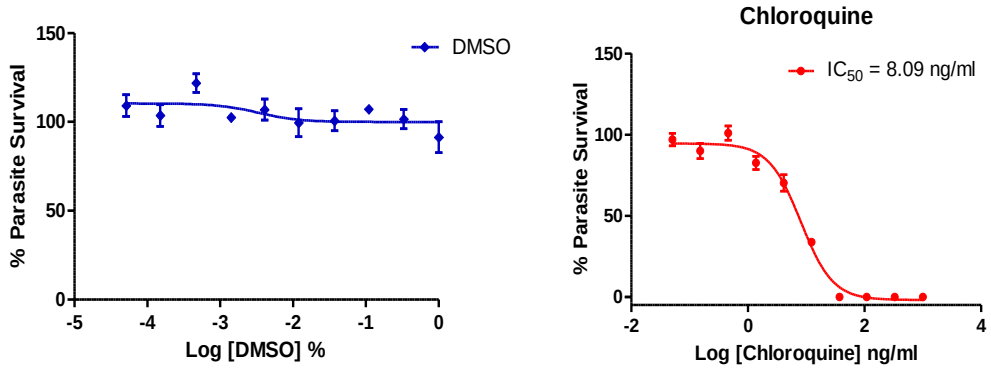


Figure 26. Log solvent percentage vs. % parasite survival data used to plot

test samples (DMSO and Chloroquine) dose- response curves.

The solvent control, DMSO was also screened at corresponding concentrations of the test samples with the starting concentration of 1% DMSO at 100 µg/mL. The concentration of DMSO from 1% to 5.08 x 10^{-5} % (100 µg/mL - 5.13 x 10^{-3} µg/mL) had no effect on the parasites (91.41% - 109.18% parasite survival) chloroquine showed IC_{50} of 8.09 ng/mL (**Figure 26**).

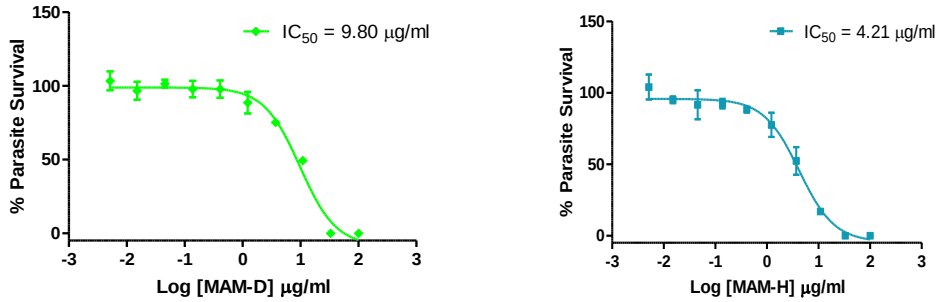


Figure 27. Log solvent percentage vs % parasite survival data used to plot test samples (MAM-D) and (MAM-H) dose-response curves.

4.4 Fractionation of the extracts and Column Chromatography

The active crude dichloromethane extract of Npk-1D, was subjected to purification in order to isolate compounds. Two compounds were isolated (**Figure 25**.), namely, Npk1 F70-77 and Npk1 F78-90 and showed increased activity *in vitro* antimalarial activity against 3D7 strain, Npk1 F70-77 (2.63 ± 0.48 µg/mL) and Npk1 F78-90 (2.64 ± 0.32 µg/mL), (**Table 9**).

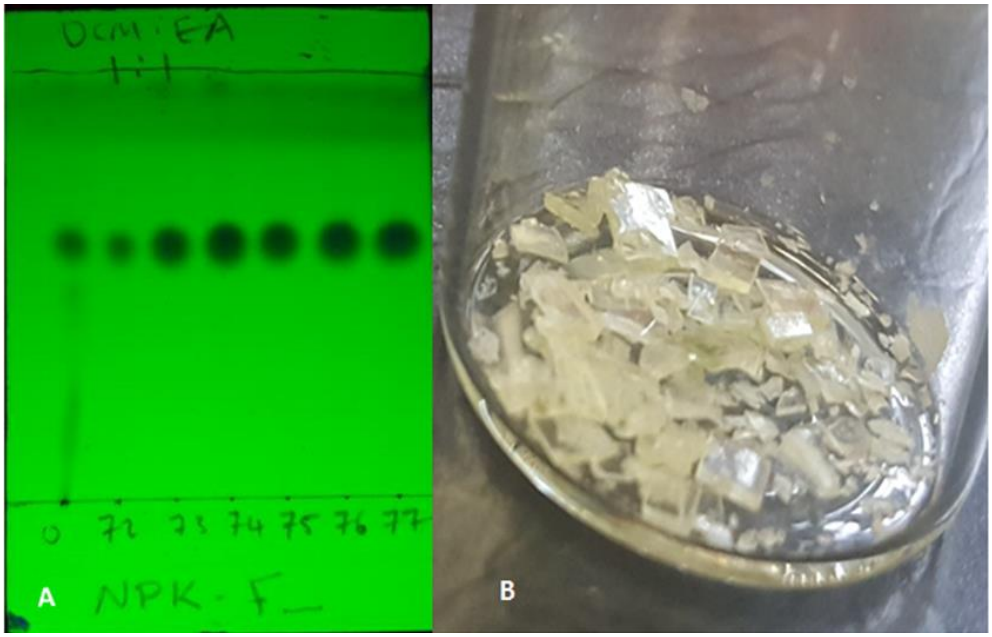


Figure 28. TLC profiles for the active fractions of Npk-1D on A and a purified square crystals compound of fraction F78-90 on B

Table 9. IC50 and Z'-factors of test Npk-1D fractionated samples and two compounds against 3D7 strain of *P. falciparum.*

Test sample	IC_{50} (µg/mL)	Activity	Z'-factor
Npk1 F21	1.58	High	0.64
Npk1 F25	2.09	High	0.64
Npk1 F29	1.42	High	0.78
Npk1 F32	1.33	High	0.78
Npk1 F39	4.06	Moderate	0.73
Npk1 F42	1.89	High	0.73
Npk1 F70-77	2.6 ± 0.5	High	0.84
Npk1 F78-90	2.64 ± 0.32	High	0.84

Table 9 above shows the results of the IC_{50} obtained for eight fractions and their Z'-factors. Six samples were fractions Npk1 F21-F42 while two were compounds Npk1 F70-77 and Npk1 F78-90, were from the Npk1 dichloromethane (DCM) extract. Seven samples were highly active (Npk1 F21 and Npk1 F25 in **Figure 29),** Npk1 F29 and Npk1 F32 in **Figure 30** , Npk1 F42 in **Figure 31**, Npk1 F70-77 and Npk1 F78-90) against the 3D7 strain of *P. falciparum*, with antimalarial activity ranging from 1.58 µg/mL to 2.64 µg/mL (**Figure 32**).

One sample, Npk1 F39, showed moderate activity with antimalarial activity of 4.06 µg/mL. The Z'-factors for the experiments ranged from 0.64 to 0.84 indicating that the

assay worked well. When tested, the concentration of DMSO from 1% to 5.08 x 10-5% (100 - 5.13 x 10^{-3} µg/mL) had no effect on the parasites (91.41% - 109.18% parasite survival) (**Table 9**). Chloroquine showed IC_{50} of 8.09 ng/mL.

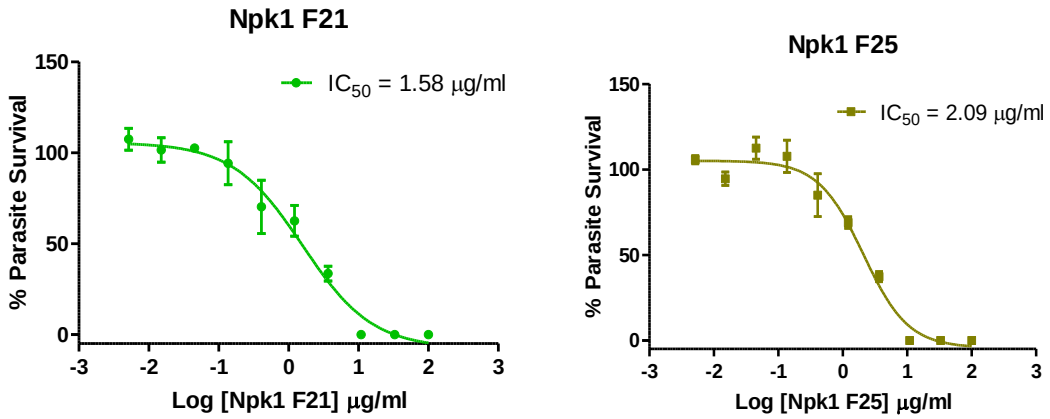


Figure 29. Log concentration vs % parasite survival data used to plot (Npk1 F21 and Npk1 F25) dose-response curves.

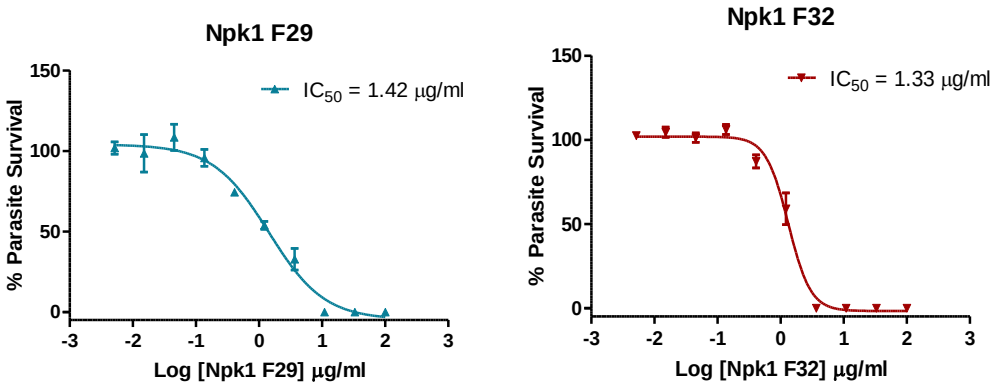


Figure 30. Log concentration vs % Parasite survival data used to plot (Npk1 F29 and Npk1 F32) dose-response curves

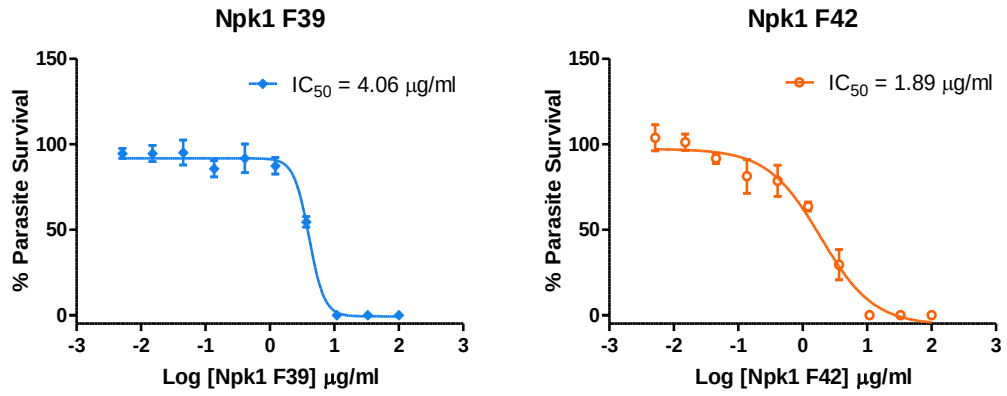


Figure 31. Log concentration vs. % Parasite survival data used to plot (Npk1 F39 and Npk1 F42) dose-response curves.

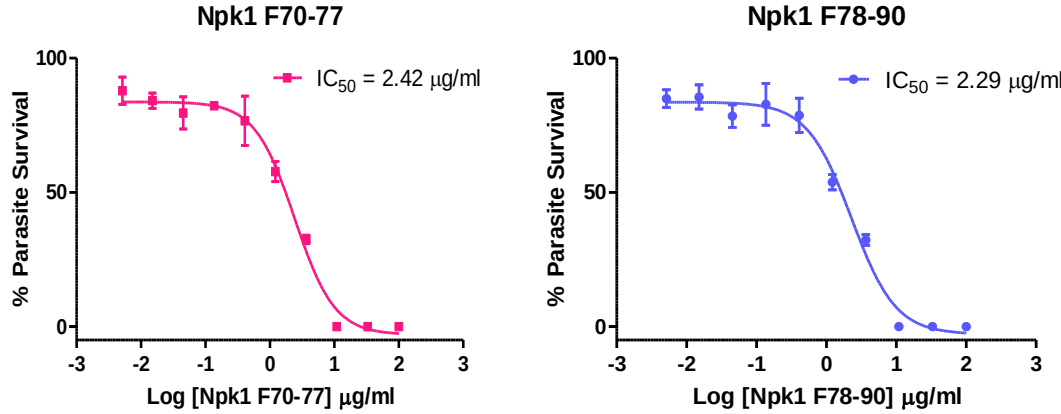


Figure 32. Log concentration vs % parasite survival data used to plot of Npk1 F70-77 and Npk1 F78-90 dose-response curves.

4.5. Elucidation for the structures of antiplasmodial active compounds Npk1 F70-77 and Npk1 F78-90

The chemical structure revealed that the two fractions contained the same compound which was identified as **Xerantholide** with a molecular weight of 246.3042 g/mol. Due to the chiral centers (molecules that possess two or more chirality centers) at position 1, 7, 8 and 10, it means the compound has diasteromers (stereoisomers that are not mirror images of one another) (**Figure 33**). There is however a mixture of similar compounds with different conformation or orientations of the groups attached.

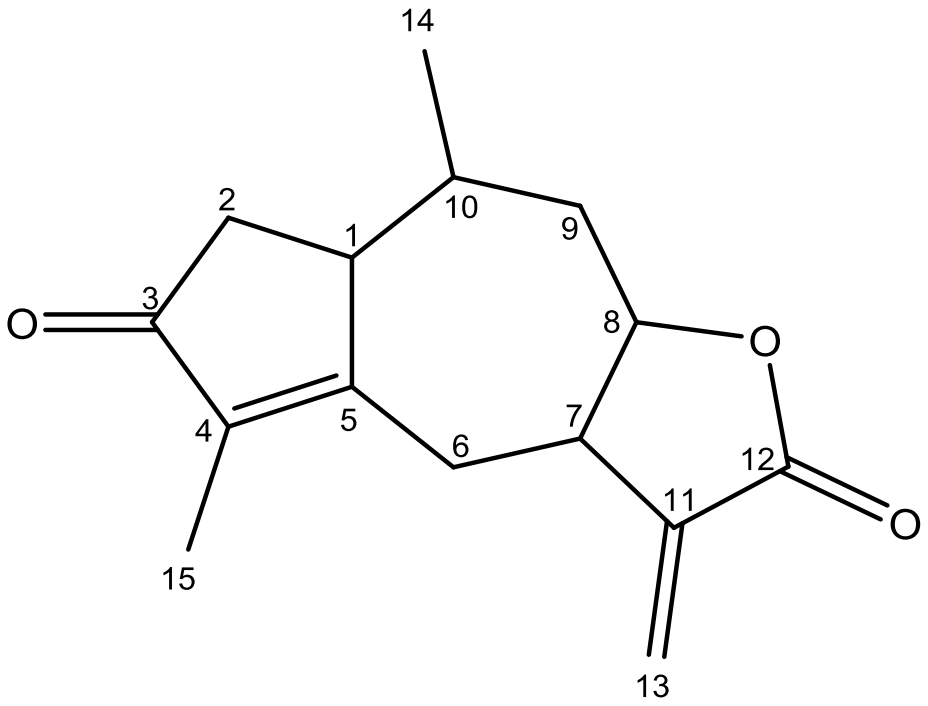


Figure 33. Chemical structure of the isolated active compound Xerantholide from NPK-1D F70-77.

The functional groups are defined as Vmax (KBr)/cm-^{1} 2967(C-15 and C-14 2 X CH_3) 2892(C-13 C=CH_2), 1755(C-12 O-C=O), 1688(C-3 C=O), 1632(C-5 C=C). A functional group is a specific group of atoms or bonds within a compound that is responsible for the characteristic chemical reactions of that compound. The same functional group will behave in a similar fashion, by undergoing similar reactions, regardless of the compound of which it is a part. Functional groups also play an important part in organic compound nomenclature; combining the names of the functional groups with the names of the parent alkanes provides a way to distinguish compounds.

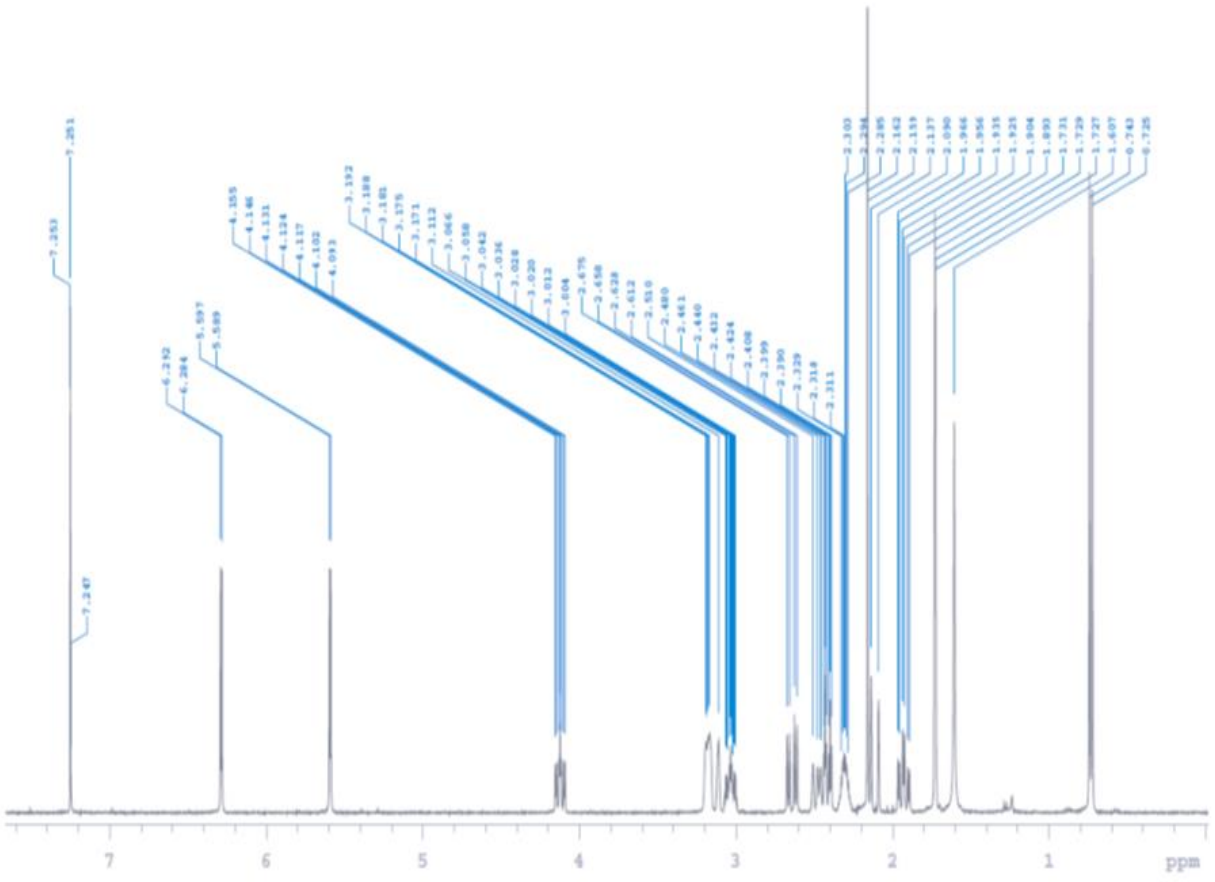


Figure 34. ^{1}H-NMR Spectrum of Npk F70-77 based on the data on **Table.11**.

The above figure shows the protons in the indentified compound.

Table 10. ^{1}H NMR and ^{13}C NMR Data of Xerantholide (400 MHz, $CDCl_3$)

H	δppm	Splitting
1	4.12	Dddd
2α	2.62	doublet of doublet (dd)
2β	2.46	broad doublet (brd)
6α	3.17	multiplet (m)
6β	2.51	Brd
7	3.01	Ddddd
8	2.30	multiplet (m)
9α	2.46	Ddd
9β	2.40	Ddd
10	2.31	M
11	-	
13	5.58	D
13’	6.28	D
14	0.73	doublet (d)
15	1.93	doublet doublet of doublet (ddd)

There is no proton observed at number 3, 4, 5, 11and 12

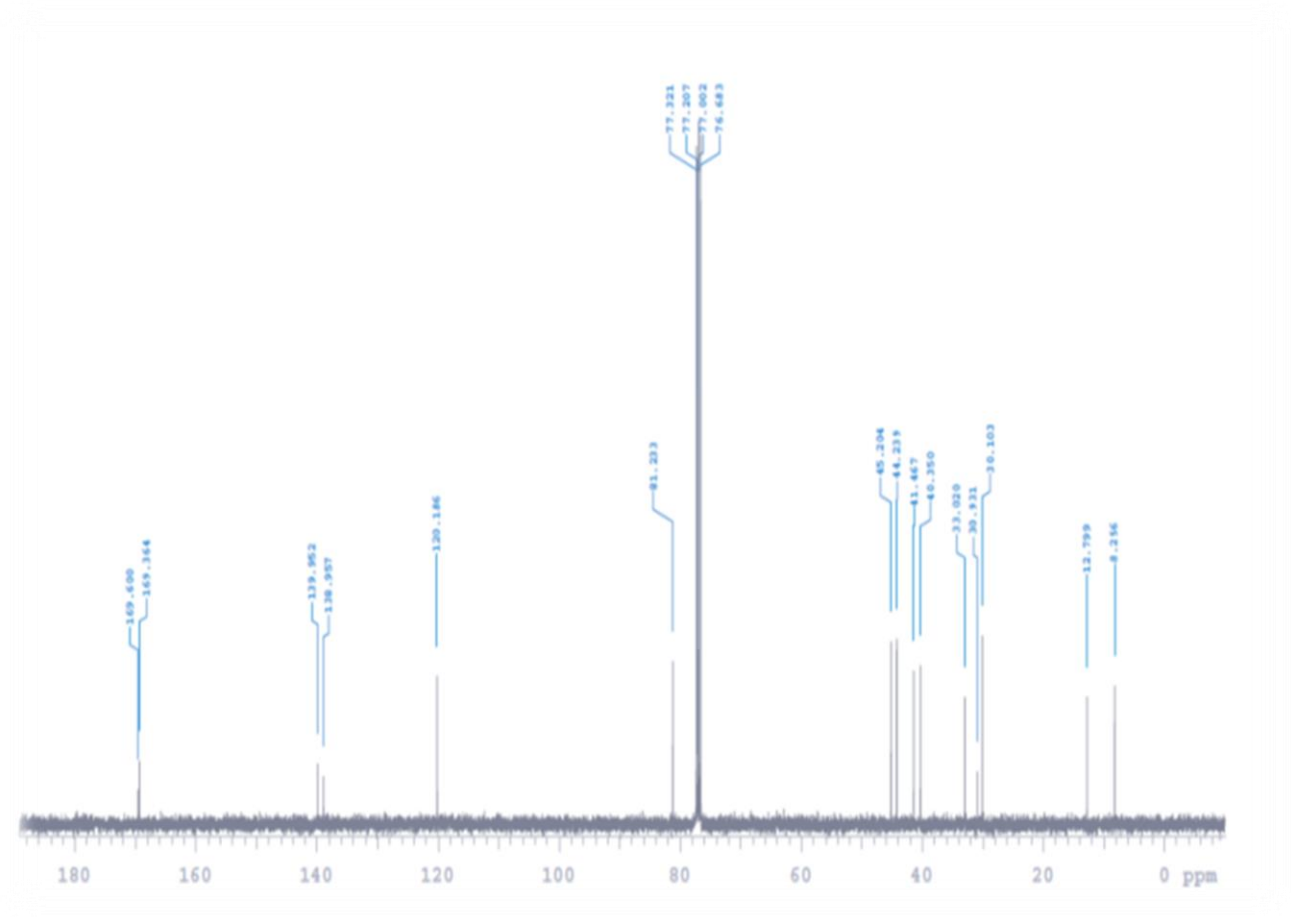


Figure 35. The ^{13}C-NMR Spectrum of Npk1 F70-77

Figure 35 shows the carbons that are present in the identified compound

Table 11. The ^{13}C NMR Data of Xerantholide (400 MHz, $CDCl_3$).

C	δppm
1	81.2
2α	41.5
2β	41.5
3	169.3
4	139.9
6α	45.2
6β	45.2
7	44.2
8	33.0
9α	40.4
9β	40.4
10	30.9
11	138.9
12	169.6
13α	120.1
13β	120.1
14	12.7
15	41.4

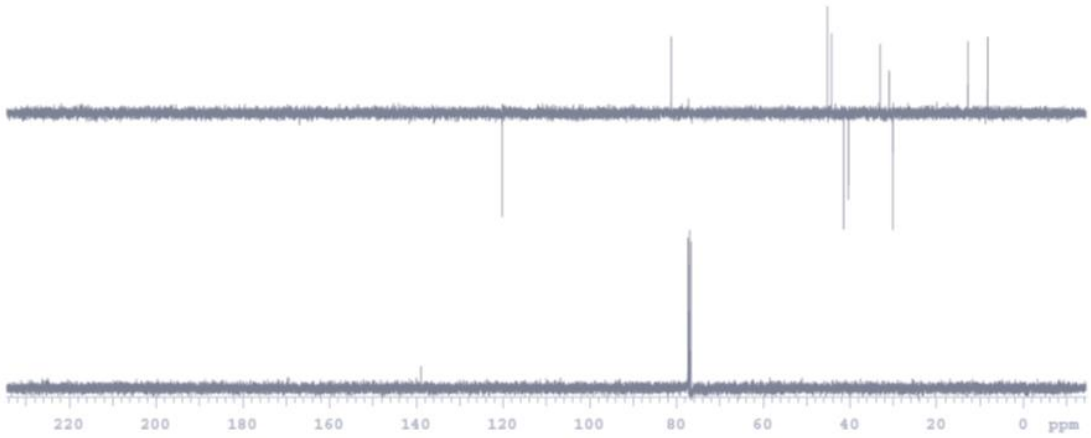


Figure 36. DEPT Spectrum of NPK1 F70-77

The above **Fig 36.** shows the CH_3 that are found in the compound. The upward shows the CH3, CH, and the C while the down ward are the CH_2.

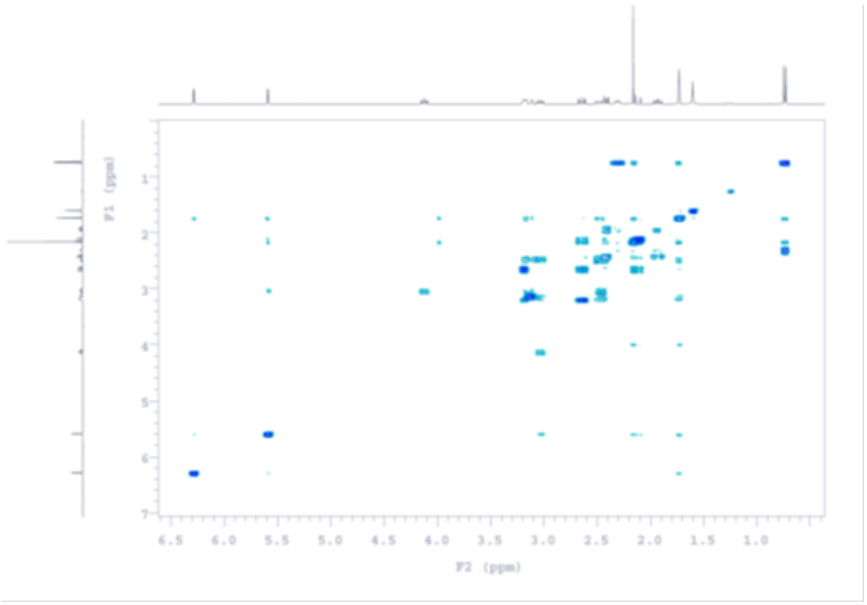


Figure 37. COSY Spectrum of NPK1 F70-77

The homonuclear correlation spectroscopy (**COSY**) is the first and most popular two-dimension NMR which is used to identify spins which are coupled to each other. It shows the interactions between hydrogens.

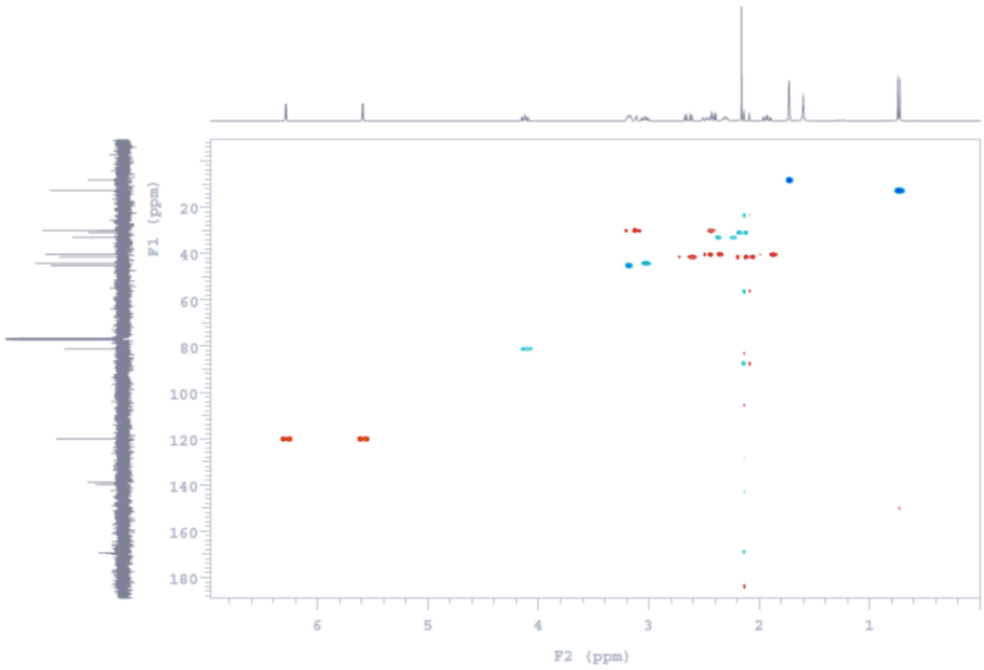
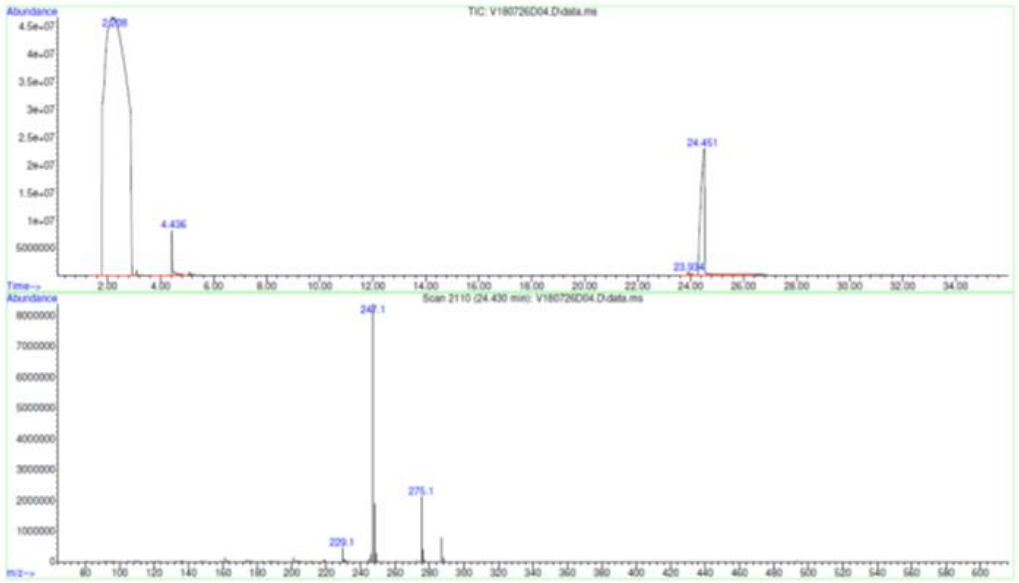


Figure 38. HSQC Spectrum of NPK1 F70-77

This is a heteronuclear single quantum coherence or correlation, it shows which proton or hydrogen is bonded to which carbon. It is a two-dimensional (2D) spectrum with one axis for proton (^{1}H) and the other for a heteronucleus, an atomic nucleus other than a proton which is usually ^{13}C. The above axes shows the hydrogens, while below is showing the carbons

GC-MS CI



GC-MS EI

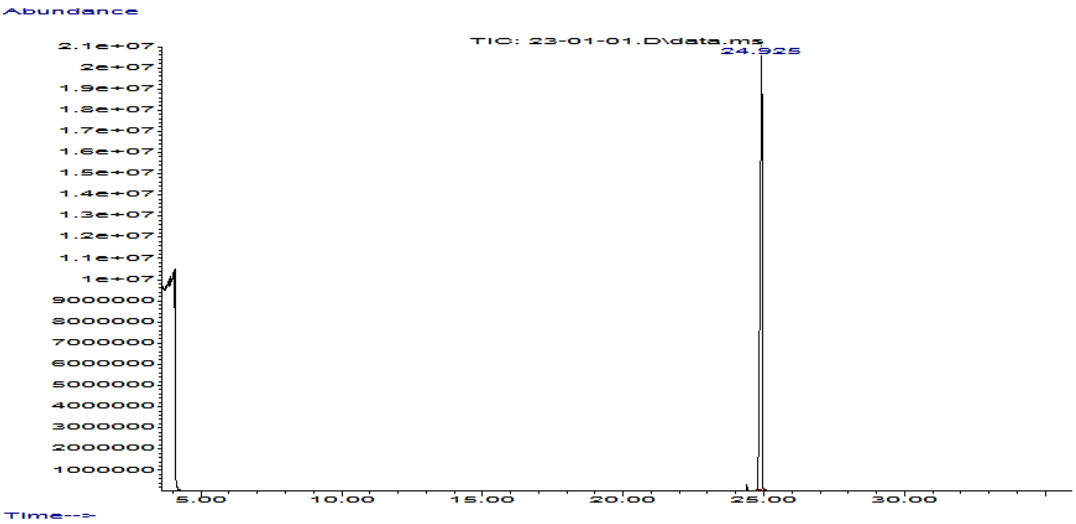


Figure 39. The GC chromatogram for NPK1 F70-77

Figure 39 above shows the retention the compound took to resonate. Retention time (RT) is a measure of the time taken for a solute to pass through a chromatography column. It is calculated as the time from injection to detection. The RT for a compound is not fixed as many factors can influence it even if the same GC and column are used.

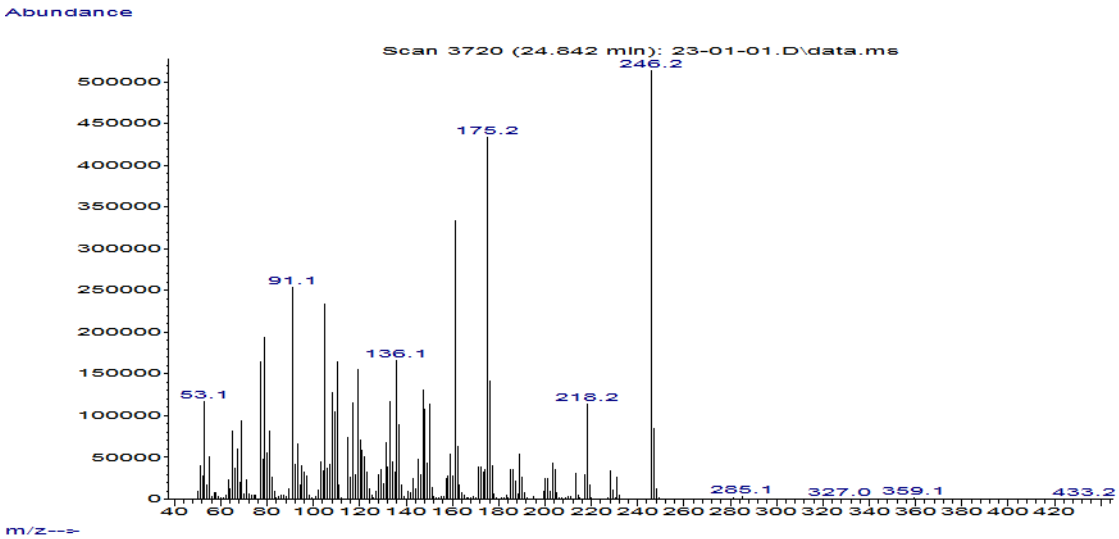


Figure 40. The MS CI and GC-MS EI for NPK1 F70-77

The above figure shows the fragmentation of the compound.

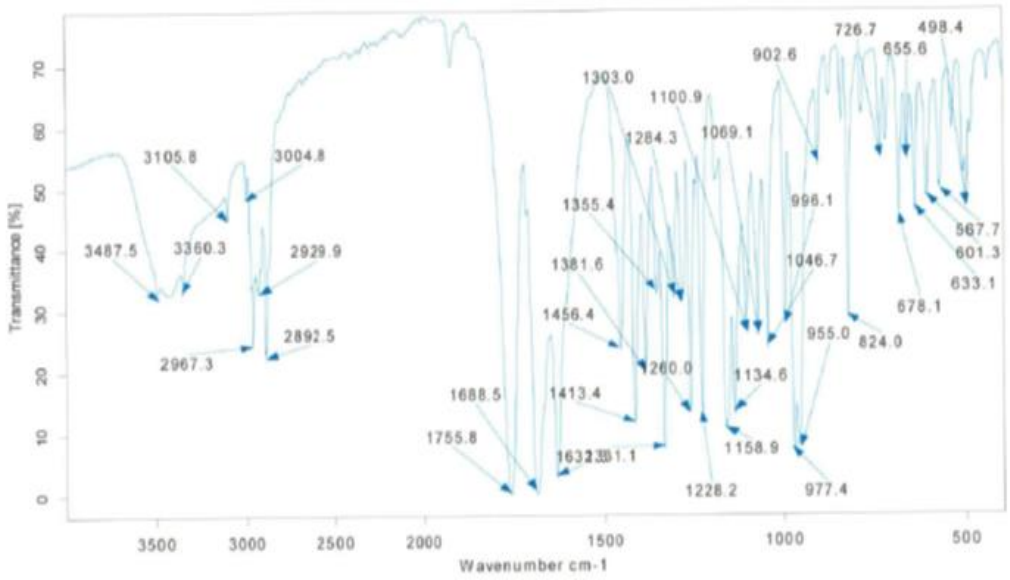


Figure 41. The FTIR spectra for NPK1 F70-77

The above figure 41 shows the functional groups that are found within the indentified structure.

The active crude extract for Npk-1 dichloromethane was tested for six classes of phytochemicals with known antimalarial properties namely anthraquinones, coumarins, flavonoids, terpenoids and saponins.

Table 12. Phytochemical profiling for the active plant Npk-1 (*P. leubnitiziae*)

Compound	Solvents			
	Hexane	DCM	Ethyl acetate	Methanol
Anthraquinones	**-**	**+**	**+**	**+**
Flavonoids	**+**	**-**	**-**	**+**
Saponins	**+**	**+**	**+**	**+**
Coumarins	+	+	+	+
Terpenoids	**+**	**+**	**+**	**+**
Alkaloids	**+**	**+**	**+**	**+**

Key:

+	Present
-	Absent

4.6 Cytotoxicity for antiplasmodial active of compounds

Cytotoxicity essay revealed that the isolated compounds are toxic with a IC_{50} values of 2.747 and 2.524 µg/mL for NPK-1D F78 and NPK-1D F70 respectively. These compounds are toxic to the mammalian cells (in this case CHO cells).

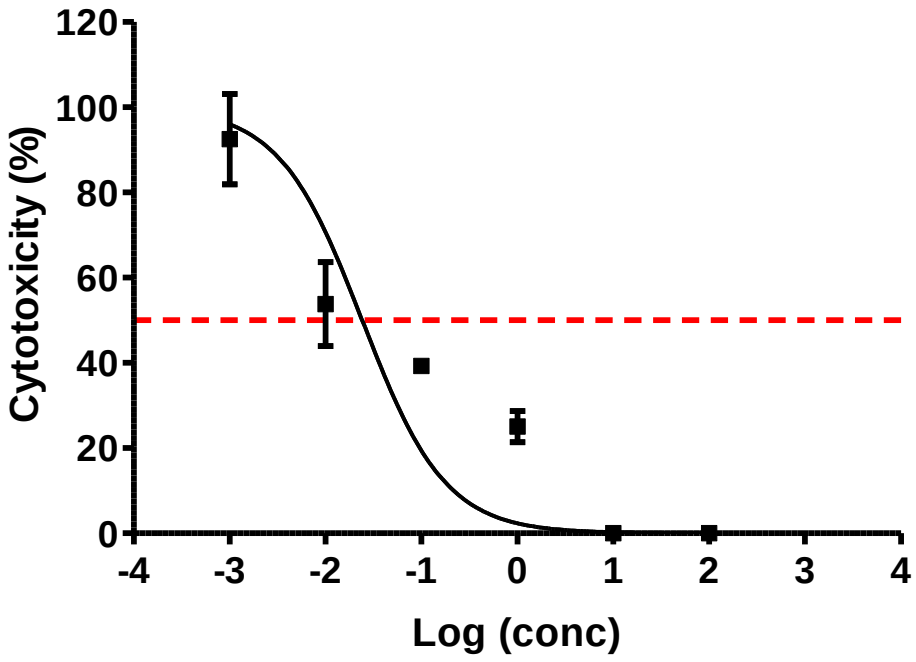


Figure 42. Percentage inhibition on CHO cells by the standard drug emetine with IC_{50} of 0.02411 µg/mL.

Emetine is the chloride salt of a white crystalline bitter alkaloid isolated from the root of the plant *Psychotria ipecacuanha*. It is one of the main active ingredients in ipecac syrup used as emetic, and has been used extensively in phytomedicine as an antiparasitic drug. It is able to inhibit both ribosomal and mitochondrial protein synthesis and interferes with the synthesis and activities of DNA and RNA. For this reason, it has been a vital

tool to pharmacologists and has demonstrated many biological properties, such as antiviral, anticancer, antiparasitic and contraceptive activities.

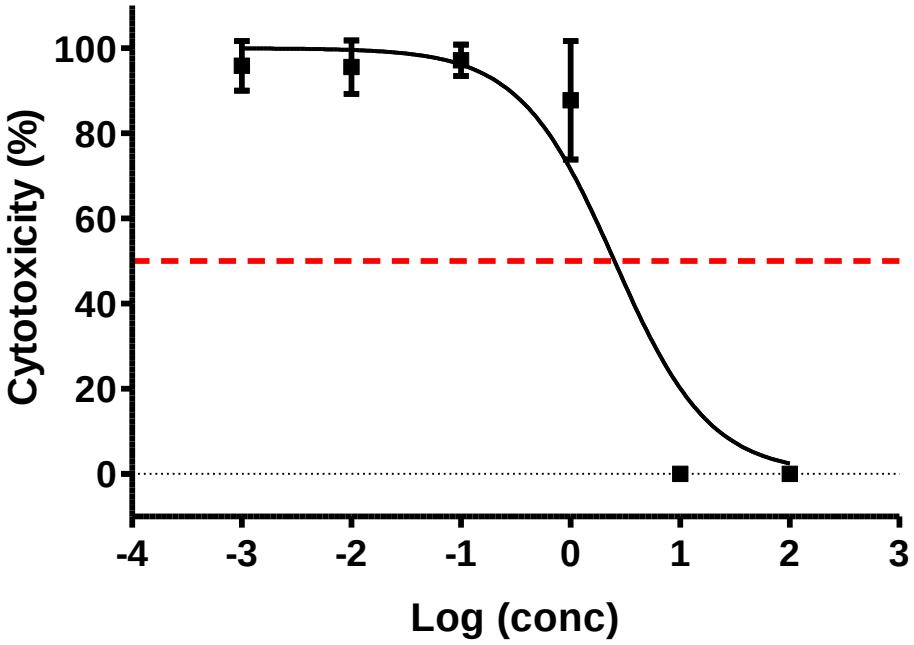


Figure 43. Percentage inhibition on CHO cells by the antiplasmodial active compound Npk1-D F70 with IC_{50} 2.524 µg/mL.

Npk1-D F70 is a semi purified compound that was fractionated from the the antiplasmodial active dichloromethane plant extracts of *P. leubnitiziae*. It can be observed from the graph that the compound was able to inhibit more than 50% of the CHO cells.

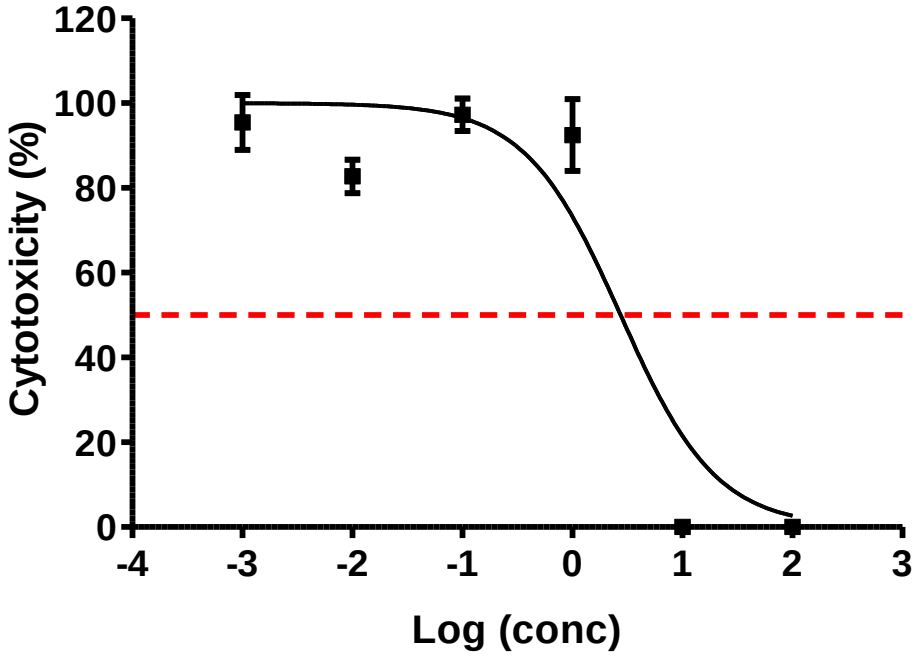


Figure 44. Percentage inhibition on CHO cell by the antiplasmodial active compounds Npk1-D F78 with IC_{50} 2.747 µg/mL

Npk1-D F78 is a semi purified compound that was fractionated from the the antiplasmodial active dichloromethane plant extracts of *P. leubnitiziae*. It can be observed from the graph that the compound was able to inhibit more than 50% of the CHO cells.

CHAPTER 5: DISCUSSION

5.1. Evaluated mushrooms

The idea to include the mushrooms in this study was a follow up on the study by Kadhila-Muandingi at al., (2014) in which it was reported that the antiplasmodial activity of aqueous extracts for the indigenous *G. lucidum* and *Terfezia pfeilii* Namibian mushrooms against *P. falciparum* 3D7A. Although they reported that the crude extracts for two mushrooms showed antiplasmodial activity at concentrations ranging from 5-50 µg/mL, with *T. pfeilii* extracts showing the highest activity with an IC_{50} of 0.022 µg/m: compared to *G. lucidum* at 3.66 µg/mL, the two mushrooms showed no antiplasmodial activities in the current study. It must however be noted that in this study, the two mushrooms were extracted using organic solvents instead of water. This alone might be the reason as to why there were no activities, regardless of the presence of mycochemical such as anthraquinones, flavonoids and steroids present in both mushrooms screened.

Kadhila-Muandingi at al. (2014) also used artemisinin-combination (ACT) therapy as their positive control whereas chloroquine was used in this study. The lifecycle stage of the parasites might have also played a role in these two studies when it comes to crude extracts antiplasmodial activities of mushrooms as well. Kadhila-Muandingi at al., (2014), used *P. falciparum* 3D7A parasites at the ring stage, while the same species was used in this study, but at trophozite stage due to the fact that there is more drug accumulation at that stage.

The antiplasmodial activities for the aqueous extract of *G. lucidum* have also been reported in Nigeria to have been used to establish changes in serum and liver lipoprotein cholesterols accompanying *Plasmodium berghei* malarial infection in mice by (Oluba et al. 2012,). De Silva et al., 2013), have also reported that the lanostanes from *G. lucidum* have shown moderate antiplasmodial activity raising more questions as to why the indigenous *Ganoderma* species included in this study did not yield positive result.

5.2. Evaluated plants

Youmsi et al., (2017), stated that plant-based repellents are generally target specific and relatively non-toxic, and are still extensively used traditionally as first intention and affordable tools in malaria endemic rural communities for protection against mosquito bites. In this study, the plant (Npk 3) *A. inflata* Benth from family Lamiaceae is reported to be used as a mosquito repellent in Ohangwena region where the whole plant is crushed and spread in rooms to repel mosquitoes. The crude extracts for this plant did not show antiplasmodial activity when tested, but it might be a good candidate as a malarial vector repellent. Nafuka, (2014), reported the ability of *A. inflata* to repel mosquitoes.

Chinsembu, (2015), listed more than 30 plants species that are used as traditional medicines for malaria in Namibia but the list does not include *Pechuel-loeschea leubnitiziae*, the plant that gave good antimalarial activities in this study. The plants species used as traditional medicines for malaria in Namibia in the report are also based mostly on ethnobotanical surveys without any studies having been conducted to elucidate antiplasmodial activities and active chemical compounds from the plants that

can be used for new drug development. In Namibia, *P. leubnitziae* have been studied for its *In vitro* antimicrobial activity and was found to possess compounds with antimicrobial activities against five pathogenic microorganism namely *E.* aerogenes, *Pseudomonas aeruginosa, Neisseria meningitides, Shigella flexneri* and *Proteus* vulgaris (Hedimbi et al., 2012, Ndongo 2017). Ndongo, (2017) also reported the antioxidant properties of *P. leubnitziae* and its effectiveness as mosquito repellent. Regardless of all the studies on *P. leubnitziae* in Namibia, none of the studies focused on antiplasimodial activities of the plant as in this study.

Crude extracts of *P. leubnitiziae* and *C. mopane* (Npk-1H and Npk-8H) showed % parasite survival of 19.55 % and 11.66 %, respectively. Npk-1H showed % parasite survival above 15%, but was screened for IC_{50} determination as the majority of compounds showed no activity at 10 µg/mL. When these two extracts were screened to determine their IC_{50}, they were inactive, thus they could not be considered for further analyses. Even though some of these extracts were screened again and still showed no activity, the screening was repeated at 10 µg/mL using a different batch of 3D7 strain but they were still inactive. Crude extracts of MAM-D showed marginal activity (9.80 and 7.24 µg/mL, respectively) while MAM-H showed moderate activity (4.21 µg/mL). This plant could not be fractionated to determine the active compounds due to the unavailability of the plant materials for further extraction. The plant was collected from the Eastern Cape in South Africa. The plant was also not identified and thus it is to be collected again for any further studies.

5.3. Plants included in the study that show no activities

Although most plants evaluated in this study were recommended by the traditional herbalist and despite strong associations with malaria and its treatment, they did not show any *in vitro* antiplasimodial activitiy. Clarkson et al., (2004), stated that possible explanation could be that the plants act as antipyretics or immune stimulants to relieve the symptoms of the disease, rather than having direct antiparasitic activity. He further stated that precursors of the active components may be present in the extracts but have to be modified, usually *in vivo*, before activity is exhibited. Harvesting and storage conditions could collectively influence the plant secondary metabolites prior to and following harvesting, which in turn would be reflected in the bioactivity. However, most of the plants have been reported to be antiplasmodial active in other studies in Africa.

Psidium guajava L. (Npk-2) is an important food crop and medicinal plant in tropical and subtropical countries widely used like food and in folk medicine around of the world. More recent ethnopharmacological studies show that *P. guajava* is used in many parts of the world for the treatment of a number of diseases, including anti-inflammatory, diabetes, hypertension, caries, wounds, pain relief and reducing fever (P´erez Guti´errez et al., 2008). *Psidium guajava* crude extract showed no antimalarial activities in this study, but its leaves are used for malaria in Nigeria as reported by Ayoola et al., (2008).

Laggera decurrens (Npk- 4) is harvested from the wild for local use as a medicine and insect repellent (Leffers, 2003). In both Ohangwena and Kavango East regions, the leaves of this plant are crushed, boiled and the person suffering from fever and headache

is exposed to its steam. It is reported for use on malaria related symptoms in Namibia (Chinsembu, 2015). *Laggera decurrens* crude extracts showed no antimalarial activities in this study, despite it being used traditionally for malaria symptoms.

Acanthospermum hispidum DC (Npk-5) (Okano ka Nahandja) is reported for use when locals are suffering from malaria symptoms in Ohangwena region. The leaves of this plant are crushed and sniffed for the cure of fever and chills. This plant's crude extracts showed no antimalarial activities during this study. It is reported that the ethanolic extract of leaves and flowering tops for *A. hispidum* have activities against a wide range of pathogenic bacteria. When crushed, it is used in the form of the paste to treat the skin aliments and leaf juice is used to relieve the fever (Chakraborty et al., 2012). The plant is reported for malaria use in Congo by Koukouikila-Koussounda et al., (2013), as well as in Benin (Chinsembu, 2015). Chakraborty et al., (2012), has also reported that the extract of *A. hispidum* was tested *in vitro* against two reference clones of *P. falciparum*, the W2 chloroquine-resistant and the D6 chloroquine-sensitive strains and the significant inhibitory activity was observed at (IC_{50} = 5.02 μg/mL), though it was not the case in this study.

The roots for *Burkea africana Hook* (Npk-6) are reported for use in wound healing and tuberculosis (Shomkegh et al., 2016; Chinsembu et al., 2015; Uzodimma, 2013). In Kavango East region the plant is used for malaria and dizziness, but the results from this study is that there are no activities from the extracts of this plant. Eboji et al., (2016), reported the use of *B. africana* in the traditional treatment of fever, inflammation, pneumonia and cancer in South Africa. *B. africana* Hook which is used in Kavango East

region for malaria and dizziness, did not show activities despite being reported in Mali that it is used against cerebral malaria (Malterud, 2017). This might be due to methods of extraction, solvents used or the geographical location (reference).

In Kavango East region it is believed that people who eat *Hibiscus sabdariffa L.* (Npk-7) as a vegetable rarely suffer from malaria. Traditionally it is used as a food, but in East Africa, the calyx infusion, called "Sudan tea", is taken to relieve coughs (Morton, 1987). In China, the plant is used to treat hypertension, pyrexia and liver damage. Recently, the sepal extract has been used as an effective treatment against leukemia due to its high content in polyphenols, particularly protocatechuic acid (Mohamed et al., 2012). The methanol extract of *H. sabdariffa* calyces contained effective antibacterial agents (Abdallah 2016). The plant is also reported to be used for chronic diarrhea (Chinsembu & Hedimbi, 2010). No antimalarial activities are reported for this plant and when tested in this study, there were no activities from its crude extracts.

The plant *Steganotaenia araliacea Hochst* (Npk-9) is used in traditional medicine especially in tropical Africa and savannah where it is mostly found. It is used to treat pneumonia, asthma, peptic ulcer, sore throat, fever, as a diuretic agent and other diseases of microbial origin (Ojerinde et al., 2013). The plant is said to be used for malaria, stomach pain, libido and fertility in Kavango East region in Namibia. Chinsembu et al., 2015) reported that the roots and leaves for *S. araliacea* are boiled in water and the concoction is taken orally. Root infusion is drunk as a remedy for fever and to ease breathing. When the root decoction is hot, it is also used as vapor treatment (Chinsembu,

2015). Despite all the above cited treatment of malaria symptoms, this plant did not yield good result as an antimalarial.

The *Ipomoea hochstetteri House* (Npk-10), genus *Ipomoea*, is used in different parts of the world for the treatment of several diseases such as diabetes, hypertension, dysentery, constipation, fatigue, arthritis, rheumatism, hydrocephaly, meningitis, kidney ailments and inflammations (reference). Some species have been reported to possess antimicrobial, analgesic, spasmolitic, spasmogenic, hypotensive, psychotomimetic and anticancer activities (Meira et al., 2012). This annual climbing herb is used in Zambezi and Kavango East regions for malaria related symptoms like fever and headache. When *I. hochstetteri* crude extract was tested, there were no antimalarial activities.

The plant *Boscia albitrunca* (Npk-11 & 12) roots and leaves are used in Ohangwena region for to cure malaria related symptoms, especially fever and headache. The leaves are crushed and inhaled. The roots are also used to ferment the milk for the production of omashikwa for human consumption among the Owambo and Herero tribes living in north and central Namibia (Bille, 2013). An infusion of the leaves is also used to treat eye infections in human and animals, the roots are reported to treat hemorrhoids and the fruit are used for the treatment of epilepsy (Leistner, 2000). No antimalarial activity was observed in this plant during this study.

Roots, fresh stems and young leaves of *B. albitrunca* are also used to treat syphilis in Kavango region in Namibia (Cheikhyoussef et al., 2011). The tree's mature leaves and

twigs are the preferred source forage/fodder for livestock because they have protein and are high in Vitamin A (Goitsemang, 2013).

The plant *Dicoma tomentosa Cass* (Npk-13) is a genus of flowering plants in the sunflower family, native to Africa and the Middle East. The whole plant is strongly bitter and is used as a febrifuge, particularly for children and women after childbirth. The plant is applied as a dressing to septic wounds, and is used in a fumigation to relieve itch skin. A roll of bruised leaves are inserted into the nostrils and left there for about an hour as a treatment for colds (Burkil, 1985).

Dicoma tomentosa is reported for anti-plasmodial activity in Burkina Faso (Jansen et al., 2012). In Ohangwena region, *D. tomentosa* is found growing in cultivated fields and disturbed areas around fields. The whole plant is crushed and used as medicine for most diseases including malaria. In a previous study dealing with the screening of several plants used in Burkina Faso in the traditional treatment of malaria, *D. tomentosa* was selected for its promising anti-plasmodial activity (Jansen et al., 2012).

Dichloromethane, diethylether, ethylacetate, and methanol extracts were found to be highly active with $IC_{50} \leq 5$ μg/mL. The above listed solvents DCM and ethylacetate used by Jansen et al., (2012) were used in this study, but no activities were observed. They further reported that hot water and hydroethanolic extracts for *D. tomentosa* showed a good activity ($IC_{50} \leq 15$ μg/mL), which confirmed the traditional use and the promising anti-malarial potential of the plant which was also confirmed *in vivo* and the identified main active compound is urospermal A-15-O-acetate. They further stated that

the active extracts exhibited cytotoxic activity, but no extract was found to display any haemolytic activity.

5.4. Antiplasmodial active compound Xerantholide

The chemical compound xerantholide has been identified from Npk1-D (*Pechuel-Loeschea leibnitziae*) in this study with a molecular formula of $C_{15}H_{18}O_3$ and a molecular weight of 246.306 g/mol. It is a known guaianolide that has been isolated from the aerial of *P. leibnitziae* (Bohlmann & Borthakur, 1982). The ^{1}H NMR spectral data of compound that was elucidated by Bohlmann & Borthakur (1982) is not much different from the result of this study (Table 8).

The compound Npk 1D F70-77 has a mixture of similar compounds with different conformation or orientations of the groups attached to it this might have to do with what is stated by Bohlmann & Borthakur (1982) when they referred to their compound as xerantholide and its probable precursor. They further stated that the compound could not be separated completely. This study found out that the isolated compound has diasteromers which are molecules that are not mirror images of each other, which might be the same scenario as observed by Bohlmann & Borthakur (1982). The compound xerantholide has also been isolated from the aerial parts of the *Xeranthemum cylindraceum* by Dekić et al., (2015).

The identified compound is listed among the 96 natural product fragments that have different chemotypes and are different from the known antimalarial aminoquinolines, quinolones, or diamidines. Xerantholide is listed as one of the sesquiterpene lactones,

which share the same backbone carbon structure as the current most effective antimalarial drug artemisinin which is a structural class known to kill *P. falciparum* parasites at submicromolar concentration (Vu et al., 2018). Although xerantholide is reported as a biologically active sesquiterpene lactones from the Asteraceae family (Ivanescu et al., 2015), its *in vitro* antimalarial activity is not reported as in the findings of this study.

The guaianolide Xerantholide which is the identified compound in this study is toxic to the CHO cells. Gasymova et al., (2018), stated that xerantholide exhibited cytostatic activity against HeLa and KB tumor cells during their study and this has also been reported by Dekić et al., (2015). This suggests that xerantholide is toxic to mammalian cells. Many sesquiterpene lactones isolated from asteraceae have already been described as anti-plasmodial and cytotoxic in the literature, but xerantholide has not been reported before. This is the first study to report on the *in vitro* antiplasmodial activity of xerantholide compound found in *P. leubnitiziae* plant from the family Asteraceae. In Namibia, *P. leubnitziae* is also used as a mosquito repellent. The use of *P. leubnitziae* to repel mosquitoes is warranted because Nawrot et al., (1986) also explained that Xerantholide isolated from *Xeranthemum cylindraceum* acts as a good deterrent and an antifeedant to beetles that infest stored grain. Sesquiterpene lactones including Xerantholide are known to have cytotoxic and anti-inflammatory activities (Ríos et al., 2014). This is consistent with our findings that Xerantholide is cytotoxic as well as the traditional applications of *P. leubnitziae* to treat inflammatory diseases in Namibia.

CHAPTER 6

6. CONCLUSION AND RECOMMENDATIONS

6.1. Conclusion

Traditional knowledge and the use of plant-based medicines remain important in the treatment of malaria. The discipline of ethno pharmacology, the study of biologically active agents traditionally employed or observed by man, has in recent years received increased attention, and there is presently a widespread interest in medicinal plants used by different cultures worldwide. Thirty six (36) plant extracts were screened for *in vitro* antimalarial activity against the chloroquine-sensitive strain, 3D7. Three of the plants extract *P. leubnitiziae* in dichloromethane (DCM) and MAM in DCM and hexane (Npk-1D, MAM-D and MAM-H) showed *in vitro* antimalarial activity ranging from 4.21 µg/mL to 9.80 µg/mL.

Pechuel-loeschea leubnitiziae showed *in vitro* promising antiplasmodial activity. Antiplasmodial activities were determined to be of IC_{50} 2.63 and µg/mL 2.64 µg/mL for NPk1 F70 and NPk1 F78, respectively. Phytochemical profiles were anthraquinones, saponins, terpenoids, coumarins, alkaloids, and flavonoids. This positive outcome supports the traditional use of this plant in Kavango East and Ohangwena regions. However, the plant's lack of selectivity would urge caution in its consumption. Thus further studies on its genotoxicity and chronic toxicity are needed to examine its toxicity.

One active compound was identified from *Pechuel-loeschea leubnitiziae* as **xerantholide**. The chemical compound is a known guaianolide that has been isolated from the aerial of *P. leibnitziae* before, but it's *in vitro* antimalarial activity has never been reported before. The cytotoxicity of xerantholide showed that it is toxic to the CHO mammalian cells. This makes it not to be a good antimalarial agent, unless if it is further purified, before it is developed as an antimalarial drug.

The four mushrooms used in this study showed no *in vitro* antimalarial activity against the chloroquine-sensitive strain, 3D7 strain of *P. falciparum*.

6.2 Recommendations

Many people living in developing countries do not have access to modern therapeutics such as ACT to treat malaria because of financial, socio-economical, geographical and/or cultural reasons. As result they use plants, often in combination, for the health care management of malaria (Jansen et al., 2012). People living in communities where the plant was collected must be informed of the outcome of this study in order to continue using it as per the indigenous knowledge. This is because better knowledge of plants from traditional pharmacopoeias and local valorization of traditional remedies in improved traditional medicine could lead to effective, standardized, available and affordable therapeutics for the management of malaria by local populations, when modern drugs are unavailable.

There is a need to do further studies on these mushrooms using aqueous extraction in order to isolate and identify the active compounds that were observed to possess

antiplasmodial activities by Kadhila-Muandingi at al., (2014). It is recommended that pharmacomodulation study should be carried out on the compound identified in this study in order to decrease its toxicity, while maintaining or improving activity. Further studies also need to be done on xerantholide to determine the diasteromers (stereoisomers that are not mirror images of one another). Modification on xerantholide chemical structure also need to be carried out so that such a toxic compound could be a possible anti-cancer therapy candidate.

CHAPTER 7

7. REFERENCES

Abdallah, E., M. (2016). Antibacterial efficiency of the Sudanese Roselle (*Hibiscus sabdariffa L.*), a famous beverage from Sudanese folk medicine. *Journal of Intercult Ethnopharmacol*, 5 (2): 186–190.

Action and Investment to defeat Malaria 2016-2030. For a Malaria-Free World. World Health Organization. ISBN 978 92 4 150897 1.

Aide Mémoire. (2011). Namibia Malaria Program Performance Review.

Alegana, V. A., Atkinson, P.M., Lourenço, C., Ruktanonchai, N.W., Bosco, C., zu Erbach-Schoenberg, E., Didier, B., Pindolia, D., Le Menach, A., Katokele, S., P., Uusiku and Tatem, A. J. (2016). Advances in mapping malaria for elimination: fine resolution modelling of Plasmodium falciparum incidence. *Sci. Rep.* 6, 29628; doi: 10.1038/srep29628.

Alemu, A., Muluye D., Mihret, M., Adugna, M., and Gebeyaw, M. (2012). Ten year trend analysis of malaria prevalence in Kola Diba, North Gondar, Northwest Ethiopia. *Parasites and Vectors*, 5:173

Annan, K., Sarpong, K., Asare, C., Dickson, R., Amponsah, K., Gyan, B., Ofori, M., … Gbedema, S., Y. (2012). In vitro anti-plasmodial activity of three herbal remedies for malaria in Ghana: *Adenia cissampeloides* (Planch.) Harms., *Terminalia ivorensis* A. Chev, and *Elaeis guineensis* Jacq. *Pharmacognosy Research*, 4 225-9.

Arko, P., F., Marzuki, B., M., and Kusmoro, J. (2017). The inventory of edible mushroom in Kamojang Nature Reserve and Nature Park, West Java, Indonesia. Biodiversitas: Volume 18, 530-540 DOI: 10.13057/biodiv/d180213.

article. Bangladesh J Med Biochem, 5, 24-32.

Ayoola G., A., Coker, H., A., B., Adesegun, S., A., Adepoju-Bello, A., A., Obaweya, K., Ezennia, E., C., Atangbayila T., O. (2008). Phytochemical screening and antioxidant activities of some selected medicinal plants used for malaria therapy in Southwestern Nigeria. *Tropical Journal of Pharmaceutical Research*, 7, 1019-1024.

Benelli, G., Jeffries, C. L., and Walker, T. (2016). Review: Biological control of mosquito vectors: past, Present, and Future. *Insects*, 7, 52; doi: 10.3390/insects7040052.

Betterton-Lewis, D. G. (2007). Malaria prize winners. *Medical Journal of Therapeutics Africa.* 1 (1).

Bhagwan, S., Sanodiyaa, Gulab, S., Thakura, Rakesh, K., Baghela, G. B. K. S. Prasad., and Bisena, P.S. (2009). *Ganoderma lucidum*: A potent pharmacological macrofungus. *Current Pharmaceutical Biotechnology*, 10, 717-742.

Bille, P., G. (2013). Effect of *Boscia albitrunca* (Omukunzi) Root on the Bacteriology and Viscosity of Omashikwa, traditional fermented buttermilk from Namibia. African *Journal of Food, Agriculture, Nutrition and Development*, 13, 4.

Blanford, S., Jenkins, N., E., Christian, R., Chan, B., H., K., Nardini, L., Osae, M., Koekemoer, L., Coetzee, M., Read, A., F., and Matthew B Thomas, M., B. (2012). Storage and persistence of a candidate fungal biopesticide for use against adult malaria vectors. *Malaria Journal*, 11:354.

Blanford, S., Shi, W., Christian, R., Marden, J., H., Koekemoer, L., L., Basil, D., Brooke, Coetzee, M., Andrew, F., Read, Matthew, B., T. (2011). Lethal and pre-lethal effects of

a fungal biopesticide contribute to substantial and rapid control of malaria vectors. *PLoS ONE,* 6(8): e23591. doi:10.1371/*journal.pone*.0023591.

Bohlmann, F., and Borthakur, N. (1982). Sesquiterpenes and norsesquiterpenes from Pechuel-Loeschea leibnitziae. Phytochemistry, 21(5), 116-162. https://doi.org/10.1016/S0031-9422(00)82441-3.

Bouwmana, H., Kylinb, H., Seredad, B., and Bornmane R. (2012). High levels of DDT in breast milk: Intake, risk, lactation duration, and involvement of gender. *Environmental Pollution,* 170, 63-70.

Brown, N., E., Cooke, T., and Skan, S., A. (1912). *Flora Capensis,* 5, 226.

Burkill, H., M. (1985). Entry for *Adenodolichos paniculatus* (Hua) Hutch. & Dalz. [family LEGUMINOSAE-PAPILIONOIDEAE]. In: The useful plants of west tropical Africa, Vol 3, Royal Botanic Gardens, Kew, UK.

CDC and Malaria. (2017). Center for Global Health, Division of Parasitic Diseases and Malaria. Retrieved 27/02/2017. Available at www.cdc.gov/malaria.

Centers for Disease Control and Prevention. (2018). Retrieved May 14th. Available at: https://www.cdc.gov/malaria/about/biology/index.html#tabs-2-5.

Centers for disease control and prevention. 2017. Accessed: 02/03/2017. Available at: https://www.cdc.gov/malaria/about/biology/mosquitoes/.

Chakraborty, A. K., Gaikwad, A. V., and Singh, K. B. (2012). Phytopharmacological review on Acanthospermum Hispidum. *Journal of Applied Pharmaceutical Science,* 2, 144-148.

Chavare, S. D., Karande, K. M., Aloorkar, N. H., Kulkarni, A. S., Majumdar, S. H. (2015). formulation of novel herbal mosquito repellent: a new approach in antimalarial management. *International Journal of MediPharm Research*, 1, 78-85.

Cheikhyoussef, A., Shapi, M., Matengu, K., Mu Ashekele, H. M. (2011). Ethnobotanical study of indigenous knowledge on medicinal plant use by traditional healers in Oshikoto

Chinsembu, K. C., (2015). Plants as antimalarial agents in Sub-Saharan Africa. *Acta Tropica* 152, 32–48.

Chinsembu, K.C., Hijarunguru, A., and Mbangu, A. (2015). Ethnomedicinal plants used by traditional healers in the management of HIV/AIDS opportunistic diseases in Rundu, Kavango East Region, Namibia. South African Journal of Botany 100, 33–42.

Christy, A. O., Mojisola, C. C., Taiwo, E. O., and Ola, O. O. (2016). The antimalaria effect of *Momordica charantia L*. and *Mirabilis jalapa* leaf extracts using animal model. *Journal of Medicinal Plants Research*, 10, 344-350.

Clarkson, C., Maharaj, V. J., Crouch, N. R., Olwen, M. G., Pillay, P., Matsabisa, M. G., Bhagwandin, N., Smith, P. J., and Folb, P. I. (2004). In vitro antiplasmodial activity of medicinal plants native to or naturalised in South Africa. *Journal of Ethnopharmacology* 92, 177–191.

Coleman, M., Hemingway J., Gleave, K. A., Wiebe A., Gething, P. W., and Moyes, C. L. (2017). Developing global maps of insecticide resistance risk to improve vector control *Malaria Journal*, 16:86.

Cox, F. E. G. (2010). History of the discovery of the malaria parasites and their vectors. *Parasites and Vectors.* 3:5.

Dalvie, M. A. (2011). DDT: Health Effects. Encyclopedia of Environmental Health, Pages 6-10.

De Silva, D. D., Rapior S., Sudarman E., Stadler M., Xu J., Alias S. A., and Hyde K. D. (2013). Bioactive metabolites from macrofungi: ethnopharmacology, biological activities and chemistry. *Fungal Diversity*, 62, 1-40.

Dekić, M. S., Radulović, N. S., Ranđelović, V., N., Stojanović-Radić, Z., Z., & Veljković, B. P. (2015). Essential Oils and Diethyl Ether Extracts of SerbianXeranthemum cylindraceumandX. annum: Chemical Composition, Antimicrobial Activity, and Chemotaxonomic Implications. *Chemistry & Biodiversity*, 12, 1378–1397. doi:10.1002/cbdv.201400316.

Demain, A. L. (1991). Production of beta-lactam antibiotics and its regulation. *Proc Natl Sci Counc Repub China B.* 15: 251-265. PMID 1815263.

Du preez, I., C. (2012). Evaluation of antimalarial properties of indigenous plants used by traditional healers in Namibia. MSc thesis, University of Namibia.

Eboji, O., Spies, L., Sowemimo, A., Koekemoer, T., Sofidiya, M., Van de Venter, M. (2016). Cytotoxic activity of the ethanolic extract of the bark of *Burkea africana hook*. (Caesalpinaceae). *Planta Med*, 82- PC73. DOI: 10.1055/s-0036-1578775.

Eliminating malaria, World Health Organization (2016). Retrieved on 16/11/2017. Available at: http://www.who.int.

Fantuzzi, E., Anastácio, L. R., Nicoli, J. R., de Paula, S.O., Arantes, R. M. E., Pinto da Matta, S. L. and Vanetti, M. C. D. (2010). Aqueous extract of culinary-medicinal royal sun mushroom, *Agaricus brasiliensis* S. Wasser et al. (Agaricomycetideae) Effects on Immunodepression in Mice. *International Journal of Medicinal Mushrooms*, 12, 227–234.

Ganfona, H., Beroa, J., Tchindae, A.T., Gbaguidid, F., Gbenoud, J., Moudachiroud, M., Frédérichb, M., Quetin-Leclercqa, J. (2013). Antiparasitic activities of two sesquiterpenic lactones isolated from *Acanthospermum hispidum* D.C. *Journal of Ethnopharmacology*, 141,411– 417.

Gasymova, S. A., Novruzov, E. N., & Mekhtieva, N. P. (2018). Chemical composition of fatty oil from *xeranthemum cylindraceum* seeds. *Chemistry of Natural Compounds*, 54, 760. https://doi.org/10.1007/s10600-018-2465-1.

Goitsemang, M. (2013). Motlopi: The tree that oozes life. Retrieved on 19 June 2018. Available at: http://www.dailynews.gov.bw/mobile/news-details.php?nid=7923&flag=.

Gueye, C. S., Gerigk, M., Newby, G., Lourenco, C., Uusiku, P., and Liu, J. (2014). Namibia's path toward malaria elimination: a case study of malaria strategies and costs along the northern border. *BMC Public Health*, 14:1190.

Gyalpo, T., Fritsche, L., Bouwman, H., Bornmanc, R., Scheringer, M., and Hungerbühler , K. (2012). Estimation of human body concentrations of DDT from indoor residual spraying for malaria control. *Environmental Pollution* 169, 235-241.

Han, C. (2010). Antinociceptive activity of agaricoglycerides extracted from mycelium of Ling Zhi or Reishi medicinal mushroom, *Ganoderma lucidum* (W. Curt.: Fr.) P. Karst. (Aphyllophoromycetideae).

Hancock, P. A., Wiebe A., Gleave KA., Bhatt S., Cameron E., Trett A., Weetman D., Smith D. L., Hemingway J., Coleman, M., Gething, P. W., and Moyes, C. L. (2018). Associated patterns of insecticide resistance in field populations of malaria vectors across Africa. *Proceedings of the National Academy of Sciences* of the United States of America, 115, 5938-5943

Hedimbi, M., Kaputjaza, D. K., Hans, R. H., Mumbengegwi, D. R., & Bock, R. (2012a). Phytochemical analysis and antimicrobial screening crude extracts from *Pechuel-Loeschea leubnitziae* (Kuntze) O. (Asteraceae). *Journal of Research in Microbes, 1*, 039-043.

Hedimbi, M., Ndjoze-Siririka, V. N., and Hans, R. H. (2012b). In vitro antimicrobial activity of

Acanthosicyos naudinianus and *Pechuel-Loeschea leubnitziae* on five pathogenic microorganisms. *Journal of Research in Microbes*, 1, 033-038.

Hemingway, J., Shretta R, Wells, T., N., C., Bell D., Djimdé, A., A., Achee, N., and Gao, Q. (2016). Tools and strategies for malaria control and elimination: what do we need to achieve a grand convergence in malaria? *PLoS Biol*, 14, e1002380. doi:10.1371/journal.pbio.1002380.

Huijben, S., Paaijmans, K., P. (2018). Putting evolution in elimination: Winning our ongoing battle with evolving malaria mosquitoes and parasites. *Evolutionary Applications*, 11, 415–430.

Ivanescu, B., Miron, A., Corciova, A. (2015). Sesquiterpene lactones from *Artemisia* genus: Biological activities and methods of analysis. Journal of Analytical Methods in Chemistry: 247685, 21 pages. http://dx.doi.org/10.1155/2015/247685.

Iyambo, N., K., Kibuule, D., and Ilonga, K., S. (2017). Antipseudomonal potential of *Colophospermum mopane* and *Acrotome inflata*, medicinal plants indigenous to Namibia. *African Journal of Pharmacy and Pharmacology*, 11, 78-86..

Jan, M., Singh, S., Kaloo, Z. A., and Maqbool, F. (2014). *International Journal of Advanced Research*, 1, 389-394.

Jansen O., Tits, M., Angenot L., Nicolas, J. P., De Mol, P., Nikiema, J. B., and Michel F. (2012). Anti-plasmodial activity of *Dicoma tomentosa* (Asteraceae) and identification of urospermal A-15-O-acetate as the main active compound. *Malaria Journal*, 11, 289.

Jansen, O., Angenot, L., Tits, M., Nicolas, J. P., De Mol, P., Nikiéma, J. B., Frédérich, M. (2010). Evaluation of 13 selected medicinal plants from Burkina Faso for theirantiplasmodial properties. *Journal of Ethnopharmacol*, 130,143–150.

Jansen, O., Tits, M., Angenot, L., Jean-Pierre, N., De Mol, P., Jean-Baptiste, N., and Frédérich, M. (2012). Anti-plasmodial activity of *Dicoma tomentosa* (Asteraceae) and identification of urospermal A-15-O-acetate as the main active compound. Available from: https://www.researchgate.net/publication/230713274. [accessed Oct 23 2018].

Kadhila-Muandingi, N. P., Nametso O, du Preez I, Mumbengegwi D. R. (2014). Antiplasmodial activity of indigenous *Ganoderma lucidum* and *Terfezia pfeilii* Namibian mushrooms, *Journal of Parasitology Photon,* 104, 186-193,

Kadhila-Muandingi, N., P., and Chimwamurombe, P., M. (2012). Uses of *Ganoderma* and other mushrooms as medicine in Oshana and Ohangwena regions of Northern Namibia. *Journal of Research in Agriculture,* 1, 146-151.

Kalita, B., Bora, S., and Sharma, A. K. (2013). Plant essential oils as mosquito repellent-a review. *International Journal of Research and Development in Pharmacy and Life Sciences,* 3, 741-747.

Kamwi, R., N., Mfune, J. K. E., Kaaya, G. P., and Jonazi, J. B. (2012). Seasonal variation in the prevalence of malaria and vector species in northern Namibia. *Journal of Entomology and Nematology.* 4 42-48.

Kangombe, F. N., Indongo, S. N., and Kwembeya, E. G. (2015). Ethnobotanical study of medicinal plants in Omusati Region. National Research Symposium Book of Abstracts, 23 - 25 September 2015 at Hilton hotel, Windhoek, Namibia.

Katsayal, U. A., Abdurahman, E. M., Abubakar, M. S., Musa, K. Y., Ambali, S. F., and Jahun, M. B. (2009). Fungi as potential source of antimalarial agents. *Nigerian Journal of Pharmaceutical Sciences,* 8, 135–142.

Kazhila, C. C. (2015). Plants as antimalarial agents in Sub-Saharan Africa. *Acta Tropica*, 152, 32–48.

Khatun, S., Islam, A., Cakilcioglu, U., and Chatterjee, N. C. (2011). Research on mushroom as a potential source of nutraceuticals: A review on indian perspective. University of Burdwan, Burdwan 713104, West Bengal, India. *American Journal of Experimental Agriculture,* 2, 47-73.

Koukouikila-Koussounda, F., Abena, A. A., Nzoungani, A., Jean-Vivien, M., Jean-Maurille, O., Jürgen, K., and Ntoumi, F. (2013). *African Journal of Traditional, Complementary and Alternative medicines,* 10, 270–276.

Leffers, A. (2003). Gemsbok Bean and Kalahari Truffle. Gamsberg MacMillan, Namibia ISBN 99916-0-491-X.

Leistner, O. A. (2000). Seed plants of southern Africa: families and genera. *Strelitzia* 10. National Botanical Institute, Pretoria.

Maia, M. F., and Moore, S. J. (2011). Plant-based insect repellents: a review of their efficacy, development and testing. *Malaria Journal,* 10, (Suppl 1):S11.

Malterud, K. E. (2017). Ethnopharmacology, chemistry and biological properties of four malian medicinal plants. Plants, 6, 11. Doi: 10.3390/plants6010011available at www.mdpi.com/journal/plants.

Margret A. A., Beulah, G. H., and Nelson, J. (2013). Marvelous medicinal mushrooms. International Journal of Pharmacy and Biological Sciences,3, 611-615.

Mathisen, E., Diallo, D., Andersen. Ø. M., and Malterud, K. E. (2002). Antioxidants from the bark of *Burkea africana*, an African *Medicinal Plant*. *Phytotherapy Research* 16, 148–153.

Meira, M., da Silva, E. P., David, J. M., and David, J. P. (2012). Review of the genus Ipomoea: traditional uses, chemistry and biological activities. *Brazilian Journal of Pharmacognosy,* 22, 682-713.

Merkord, C. L., Liu, Y., Mihretie, A., Gebrehiwot, T., Awoke, W., Bayabil, E., Henebry, G. M., Kassa, G. T., Lake, M., and Wimberly, M. C. (2017). Integrating malaria surveillance with climate data for outbreak detection and forecasting: the EPIDEMIA system. *Malaria Journal,* 16:89. DOI 10.1186/s12936-017-1735-x.

Mohamed, B. B., Sulaiman, A. A., and Dahab, A., A. (2012). Roselle (Hibiscus sabdariffa L.) in Sudan, cultivation and their uses. *Bulletin of Environment, Pharmacology and Life Sciences,* 1, 48-54.

MoHSS and ICF Macro. (2009). Namibia Health Facility Census Key Findings on Family Planning, Maternal and Child Health, and Malaria. Windhoek, Namibia.

Morton, J. (1987). Roselle. p. 281–286. In: Fruits of warm climates. Julia F. Morton, Miami, FL.

Muthaura, C. N., Keriko, J. M., Mutai, C., Yenesew A., Gathirwa, J. W., Irungu, B. N., Nyangacha, R., Mungai, G. M., and Derese, S. (2015). Antiplasmodial potential of traditional antimalarial phytotherapy remedies used by the Kwale community of the Kenyan Coast. *Journal of Ethnopharmacology*,170,148 –157.

Nafuka, S. (2014). In vitro antiplasmodial activity and phytochemicals screening of ethnomedicinal plants used to treat malaria-associated symptoms. MSc thesis, University of Namibia.

Namibia ALMA. (2012). Quarterly Report: Quarter Four. Scorecard for accountability and action.

Naseem, S., Faheem, M. M., and Munir T. (2016). Mosquito management: A review. *Journal of Entomology and Zoology Studies*, 4, 73-79.

Nawrot, J., Bloszyk, E., Harmatha, J. U. R. A., Novotny, L., Drozdz, B. (1986). Action of antifeedants of plant origin on beetles infesting stored products. *Acta Entomol. Bohemoslov*, 83, 327-335.

Ndjonka, D., Bergmann, B., Agyare, C., Zimbres, F. M., Lüersen, K., Hensel, A., Wrenger, C., and Liebau, E. (2012). In vitro activity of extracts and isolated polyphenols from West African medicinal plants against *Plasmodium falciparum*, Parasitology Research, 111, 827-834.

Ndongo, D. (2017). Antibacterial, antioxidant and phytochemical investigation of *Acacia arenaria*, *Aloe esculenta*, and *Pechuel-loeschea leubnitziae*. MSc thesis, University of Namibia.

Nkumama, I. N., O'Meara, W. P., Osier, F. H. A. (2017). Changes in Malaria Epidemiology in Africa and new challenges for elimination author links open overlay panel. *Trends in Parasitology*, 33, ttps://doi.org/10.1016/j.pt.2016.11.006.

Nogueira, C., R., & Lopes, L., M., X. (2011). Antiplasmodial Natural Products. *Molecules,* 16, 2146-2190; doi:10.3390/molecules16032146.

Ojerinde, S. O., Adepoju, B. A., Edache, J. J., Okundaye, E. M., and Alemika, T. E. (2013). Antioxidant and antibacterial constituents of *Steganotaenia araliacea* stem bark. *Journal of Pharmacy & Bioresources*,10, 1.

Oluba, O. M., Olusola, A. Nonyane, F., Masupa, T. (2016) *Burkea africana Hook*. Available online: http://www.plantzafrica.com/plantab/ burkeaafricana.htm (accessed on 14 November 2016).

Oluba, O. M., Olusola, A. O., Eidangbe, G. O., Babatola, L. J., and Onyeneke, E. C. (2012). Modulation of Lipoprotein Cholesterol Levels in *Plasmodium berghei* Malarial Infection by Crude Aqueous Extract of *Ganoderma lucidum*. *Cholesterol*, http://dx.doi.org/10.1155/2012/536396.

Orwa, C., Mutua, A., Kindt, R., Jamnadass, R., Anthony, S. (2009). Agroforestree Database: a tree reference and selection guide version 4.0. World Agroforestry Centre, Kenya.

P´erez Guti´errez, R. M., Mitchell, S., and Solis, R. V. (2008). Psidium guajava: A review of its traditional uses, phytochemistry and pharmacology. *Journal of Ethnopharmacology* 117, 1–27.

Petritus, P. M., and James M. Burns, J. M. (2008). Suppression of Lethal *Plasmodium yoelii* malaria following protective immunization requires antibody-, il-4-, and ifn-dependent responses induced by vaccination and/or challenge infection. *Journal of Immunology*, 180, 444-453.

Protein lounge. (2015). Life Cycle of *Plasmodium falciparum*. [Online] available at: http://www.proteinlounge.com/Pathway/LifeCycle of Plasmodium falciparum [Accessed 16/09/16].

Rahman, T., and. Choudhury, M. B. K. (2012). Shittake Mushroom: A Tool of Medicine. Review

Ribeiro, P. S. Josué, F., Wepf, A., Wehr, M. C., Rinner, O., Kelly, G., Tapon, N., Gstaiger, M. (2010). Combined Functional Genomic and Proteomic Approaches Identify a PP2A Complex as a Negative Regulator of Hippo Signaling. *Molecular Cell*, 39, 521-534.

Ríos, E., León, A., Chávez, M. I., Torres, Y., Ramírez-Apan, M. T., Toscano, R. A., Delgado, G. (2014). Sesquiterpene lactones from Mikania micrantha and Mikaniacordifolia and their cytotoxic and anti-inflammatory evaluation. *Fitoterapia*, 94, 155-163.

Rodin, R. J. (1985). The ethnobotany of the Kwanyama Ovambos. Monographs in Systematic Botany. Missouri Botanical Garden , https://doi.org/10.5962/bhl.title.149934.

Rodrigures, M. S., Batista, E. P., Silva, A. A., Costa, F. M., Neto, V. A. S., and Gil, L. H. S. (2017). Change in Anopheles richness and composition in response to artificial flooding during the creation of the Jirau hydroelectric dam in Porto Velho, Brazil. Malaria Journal, 16, 87. doi: 10.1186/s12936-017-1738-7.

Roser, M., and Ritchie, H. (2018). "Malaria". Published online at OurWorldInData.org. Available at: 'https://ourworldindata.org/malaria'. Retrieved on 14 June 2018.

Roux, J. P. (2003). Flora of South Africa. South African National Biodiversity Institute, Compton Herbarium, Cape Town (SAM).

Shai L., J., Chauke, M., A., Magano, S., R., Mogale A.,M., and Eloff, J., N. (2013). Antibacterial activity of sixteen plant species from Phalaborwa, Limpopo Province, South Africa. *Journal of Medicinal Plants Research*, 7, 1899-1906.

Shapwanale, N., and Itamalo, M. (2017) Malaria outbreak. Retrieved 18/11/17. Available at: https://www.namibian.com.na/162436/archive-read/Malaria-outbreak-kills-15-affects-6-000.

Shomkegh, S. A., Mbakwe, R., and Dagba, B. I. (2016). Utilization of wild plants for medicinal purposes in selected tiv communities of Benue State, Nigeria: An ethnobotanical approach. *European Journal of Medicinal Plants*, 14,1-14.

Shu-Ting, C., and Wasser, S. P. (2012). The Role of Culinary-Medicinal Mushrooms on Human Welfare with a Pyramid Model for Human Health. *International Journal of Medicinal Mushrooms*, 14, 95-134.

Simasiku, O. (2017). Malaria cases on increase in Oshikoto. [Online] Available at: https://neweralive.na/2017/01/30/malaria-cases-on-increase-in-oshikoto/. [Accessed

13/10/17].

Stamets, P. (2005). Mycelium running: how mushrooms can help save the world. Berkley/Toronto: Ten Speed Press, ISBN: 1580085792.

Tedder, M. J., Kirkman, K. P., Morris, C. D., Trollope, W. S. W., and Bonyongo, M. C. (2012). The influence of Pechuel-Loeschea leubnitziae (wild sage) on grass sward and soil seed bank composition. *African Journal of Range & Forage Science*, 29, 101–107.

Terán, M., J. (1998). Identific. Identificación de Especies Vegetales en Chuquisaca: *PLAFOR, Intercooperación, Fundación Ceibo*, 1–129.

Theresa I., Abass, E., and Olajire A. (2011). Two flavones from *Acanthospermum hispidum* DC and Their Antibacterial Activity. *International Journal of Organic Chemistry*, 1, 132-141.

Tren, R., and Bate, R. (2001). Malaria and the DDT Story. isbn 0 255 36499 7.

United Nations System in Namibia. (2018). United Nations Partnership Framework (UNPAF) 2019-2023. A Partnership for the eradication of poverty and inequality. [Online] available at: http://www.un.org.na/ . [Accessed 19/03/19].

Uzodimma. D., E. (2013). Medico-Ethnobotanical inventory of Ogii, Okigwe Imo State, South Eastern Nigeria. *Global Advanced Research Journal of Medicinal Plants* (GARJMP), 2, 030-044.

Van Damme, P., Van Den Eynden, V., and Vernemmen, P. (1922). Plant uses by the Topnaar of the Kuiseb valley Namib desert. *Afrika Focus*, 8, 223-252.

van den Berg, H. (2008).. Global status of DDT and its alternatives for use in vector control to prevent disease. Stockholm Convention on Persistent Organic Pollutants. UNEP/POPS/DDTBP.1/2.

Van Wyk, B-E., and Gericke, N. (2000). People's plants: A guide to useful plants of southern Africa. Briza Publications, Pretoria.

Vu, H., Pedro, L., Mak, T., McCormick, B., Rowley, J., Liu, M., Di Capua, A., Williams-Noonan, B., Pham, N. B., Pouwer, R., Nguyen, B., Andrews, K. T., Skinner-Adams, T., Kim, J., Hol, W., Hui, R., Crowther, G. J., Van Voorhis, W. C., and Quinn, R. J. (2018). Fragment-based screening of a natural product library against 62 potential malaria drug targets employing native mass spectrometry. *American Chemical Society infectious diseases,* 4, 431- 444.

Wang, S., and Marcone, M. F. (2011). The biochemistry and biological properties of the world's most expensive underground edible mushroom: truffles, *Food Research International*, 44, 2567–2581.

Wani, A. B., Bodha, R. H., and Wani, A. H. (2010). Nutritional and medicinal importance of mushrooms. *Journal of Medicinal Plants Research*, 4, 2598-2604.

Weiss, D., Lucas T., and Nguyen, M. (2018): MAP uses epidemiological data and geostatistical modeling approaches to produce maps and estimates of malaria burden. Retrieved on 21/09/2018, available at: https://map.ox.ac.uk/research-project/globa-malaria-epidemiology/Malaria Atlas Project.

Whitworth, K. W., Bornman, R. M., Archer, J. I., Kudumu, M. O., Travlos, G. S., Wilson, R. E., & Longnecker, M. P. (2014). Predictors of plasma DDT and DDE concentrations among women exposed to indoor residual spraying for malaria control in the South African study of women and babies (SOWB). *Environmental health perspectives,* 122, 545-552.

World Health Organization. (2018). Retrieved on 05/,06/2018, available at: http://www.who.int/news-room/fact-sheets/detail/malaria

World Malaria Report, World Health Organization, (2011). Retrieved on 20/11/2012, available at: http://www.who.int/malaria/world_malaria_report_2011/9789241564403_eng.pdf.

World Malaria Report, World Health Organization. (2017). Retrieved on 12/02/2018. Available at: http://apps.who.int/iris/bitstream/10665/259492/1/9789241565523-eng.pdf.

Youmsi, R. D. F., Fokou, P. V. T., Menkem, E. Z., Bakarnga-Via, I., Keumoe, R., Nana, V., & Boyom, F. F. (2017). Ethnobotanical survey of medicinal plants used as insects repellents in six malaria endemic localities of Cameroon. *Journal of Ethnobiology and Ethnomedicine,* 13.. doi:10.1186/s13002-017-0155-x.

Zhou L. D, Zhang Q. H., Zhang Y, Liu J and Cao Y. M. (2009). The shiitake mushroom derived immuno-stimulant lentinan protects against murine malaria blood stage infection by evoking adaptive immune-responses. *International Immunopharmacology*, 4, 455-462. doi: 0.1016/j.intimp.

APPENDICES

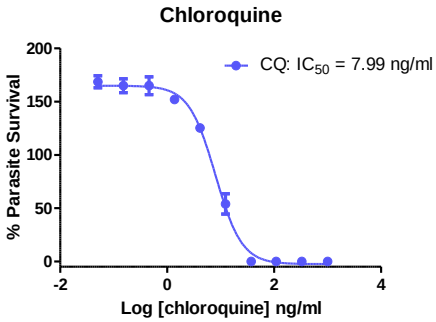
APPENDIX A: In vitro antimalarial assay for mushrooms crude extracts.

IC_{50} and Z'-factors of test samples

Test sample	Z' factor	IC_{50} (ng/ml)
Chloroquine	0.57	7.99
OW	0.55	≥ 100
GE	0.52	≥ 100
GW	0.65	≥ 100
GMD	0.52	≥ 100
KTW	0.65	≥ 100
KTH	0.76	≥ 100
KTE	0.76	≥ 100
KTMD	0.78	≥ 100
KPMD	0.78	≥ 100

Log concentration vs. % Parasite survival data used to plot chloroquine dose-response curve.

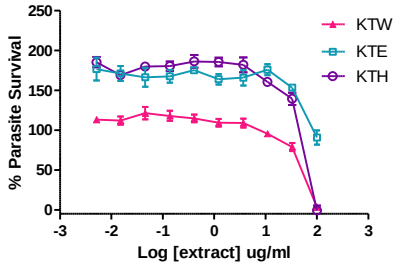
Log Conc (µg/ml)[a]	Chloroquine	
	%PS	SD
3.000	0	0
2.520	0	0
2.040	0	0
1.570	0	0
1.090	54	17
0.614	125	6
0.137	152	5
-0.337	165	15
-0.818	165	11
-1.294	169	10



[a]Data are expressed as means of duplicate values. SD = standard deviation; %PS = Percentage Parasite Survival.

Log concentration vs. % Parasite survival data used to plot KTW, KTE and KTH dose-response curves.

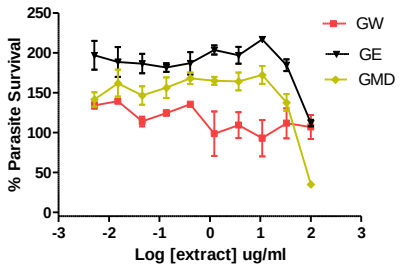
Log Conc (µg/ml)[a]	KTW		KTE		KTH	
	%PS	SD	%PS	SD	%PS	SD
2.00	4	3	91	15	4	6
1.52	79	9	153	6	139	13
1.04	96	4	176	12	161	8
0.57	109	10	166	17	182	16
0.09	109	8	164	12	186	9
-0.39	115	9	175	6	186	14
-0.86	118	11	168	14	180	10
-1.34	121	14	166	21	180	5
-1.82	112	9	171	16	169	8
-2.29	113	6	177	25	185	11



[a]Data are expressed as means of duplicate values. SD = standard deviation; %PS = Percentage Parasite Survival.

Log concentration vs. % Parasite survival data used to plot GW, GE and GMD dose-response curves.

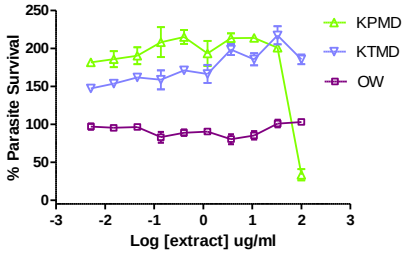
Log Conc (µg/ml)[a]	GW		GE		GMD	
	%PS	SD	%PS	SD	%PS	SD
2.00	107	26	112	7	35	3
1.52	112	33	185	13	138	18
1.04	93	39	217	4	172	20
0.57	109	28	197	18	164	20
0.09	99	49	204	10	165	9
-0.39	136	4	187	24	168	12
-0.86	124	6	182	10	156	22
-1.34	114	11	187	21	147	20
-1.82	139	6	189	32	162	29
-2.29	134	7	197	31	142	16



[a]Data are expressed as means of duplicate values. SD = standard deviation; %PS = Percentage Parasite Survival.

Log concentration vs. % Parasite survival data used to plot KPMD, KTMD and OW dose-response curves.

Log Conc (µg/ml)[a]	KPMD		KTMD		OW	
	%PS	SD	%PS	SD	%PS	SD
2.00	34	13	186	11	103	4
1.52	201	7	217	21	101	7
1.04	214	8	186	14	86	8
0.57	214	11	199	12	81	9
0.09	193	29	166	21	91	1
-0.39	215	16	171	8	89	6
-0.86	208	35	159	22	83	10
-1.34	190	19	162	8	97	5
-1.82	186	18	154	4	96	5
-2.29	182	7	147	5	97	6



[a]Data are expressed as means of duplicate values. SD = standard deviation; %PS = Percentage Parasite Survival.

APPENDIX B: In vitro antimalarial assay for Plants crude extracts

% Parasite Survival @ 10 µg/ml against 3D7 strain of *P. falciparum.*

Test Sample		**% Parasite Survival ± SD @ 10 µg/ml**
Npk 1	Hex	19.55 ± 0.03
	DCM	16.11 ± 0.03
	MeOH	83.46 ± 6.07
	Ethyl Ac	94.57 ± 4.39
Npk 2	DCM	41.25 ± 2.00
	MeOH	88.84 ± 2.50
	Ethyl Ac	82.86 ± 4.21
Npk 3	Hex	44.44 ± 0.03
	DCM	75.26 ± 0.22
	MeOH	98.22 ± 4.20
	Ethyl Ac	73.03 ± 1.65
Npk 6	Hex	73.36 ± 0.06
	DCM	81.90 ± 4.26
	MeOH	91.06 ± 2.23
	Ethyl Ac	66.82 ± 0.02

Npk 7	DCM	82.16 ± 1.45
	MeOH	80.99 ± 0.50
Npk 8	Hex	11.66 ± 0.02
	DCM	28.51 ± 0.03
	MeOH	90.67 ± 0.83
	Ethyl Ac	71.62 ± 0.03
Npk 9	Hex	64.17 ± 3.93
	DCM	67.58 ± 0.01
	MeOH	89.78 ± 0.83
	Ethyl Ac	45.55 ± 0.02
Npk 10	Hex	80.99 ± 0.06
	DCM	94.84 ± 4.78
	MeOH	85.27 ± 1.00
	Ethyl Ac	89.95 ± 1.56
Npk 12	Hex	Not assayed
	DCM	65.91 ± 0.10
	MeOH	75.09 ± 1.39
	Ethyl Ac	83.27 ± 0.33

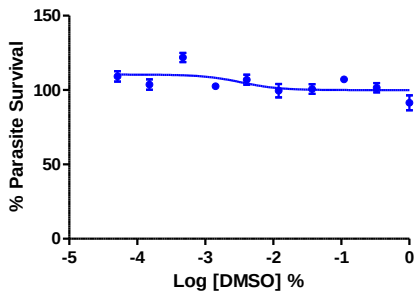
The Z'-factor for the single concentration experiments in table 1 were 0.71 and 0.84. The chloroquine showed 0% parasite survival at 100 ng/ml.

IC_{50} and Z'-factors of test samples against 3D7 strain of *P. falciparum.*

	Test Sample	**IC_{50} (µg/ml)**	**Z'-factor**
1	Npk-1D	7.24	0.69
2	Npk-1H	73.87	0.65
3	Npk-8H	40.86	0.69
4	MAM-D	9.80	0.63
5	MAM-E	32.35	0.60
6	MAM-H	4.21	0.60
7	MAM-M	≥ 100	0.68

Log solvent percentage vs. % Parasite survival data used to plot dose-response curves.

Log Solv.Perc (%)[a]	**DMSO**	
	%PS	**SD**
0.00	91.41	8.66

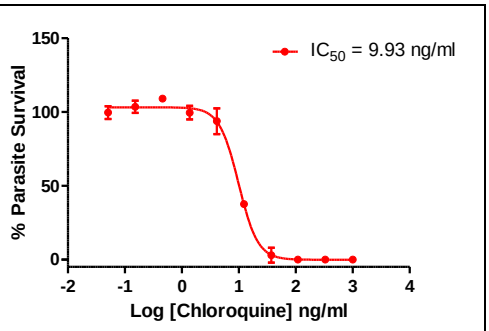


-0.48	101.62	5.42	
-0.96	107.17	1.17	
-1.43	100.72	5.58	
-1.92	99.56	7.82	
-2.39	106.94	5.85	
-2.85	102.51	1.52	
-3.33	121.92	5.28	
-3.82	103.65	6.05	
-4.29	109.18	6.16	

[a]Data are expressed as means of triplicate values. SD = standard deviation; %PS = Percentage Parasite Survival.

Log concentration vs. % Parasite survival data used to plot dose-response curves.

Log Conc (ng/ml)[a]	CQ	
	%PS	SD
3.000	0.00	0.00
2.520	0.00	0.00
2.040	0.00	0.00
1.560	2.94	5.09
1.090	37.56	1.49
0.614	93.69	8.74
0.137	99.48	4.64
-0.340	108.98	2.13
-0.818	103.43	4.07

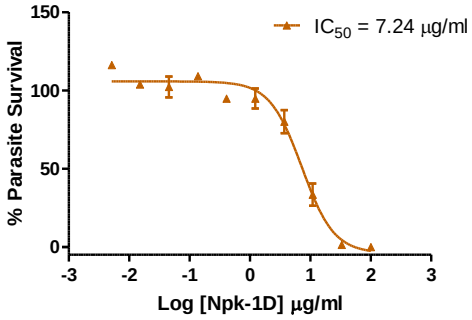


-1.294	99.54	4.33	

[a]Data are expressed as means of triplicate values. SD = standard deviation; %PS = Percentage Parasite Survival.

Log concentration vs. % Parasite survival data used to plot dose-response curves.

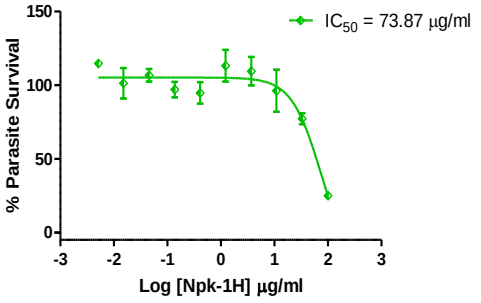
Log Conc (µg/ml)[a]	**Npk-1D**	
	%PS	**SD**
2.00	0.00	0.00
1.52	1.40	1.04
1.04	33.39	7.10
0.57	80.07	7.33
0.09	94.83	6.43
-0.39	94.78	0.74
-0.86	109.13	1.65
-1.34	102.24	6.65
-1.82	103.80	1.20
-2.29	116.20	2.22



[a]Data are expressed as means of triplicate values. SD = standard deviation; %PS = Percentage Parasite Survival.

Log concentration vs. % Parasite survival data used to plot dose-response curves.

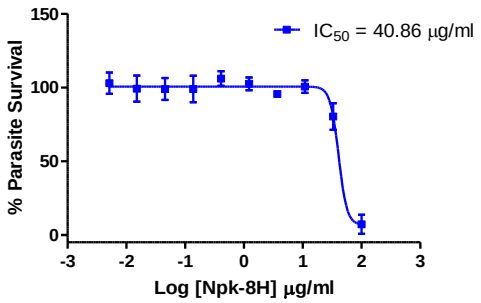
Log Conc (µg/ml)[a]	Npk-1H %PS	Npk-1H SD
2.00	25.09	2.00
1.52	77.18	3.76
1.04	88.10	3.89
0.57	109.42	9.65
0.09	113.11	10.74
-0.39	94.69	7.30
-0.86	96.95	5.20
-1.34	106.68	4.25
-1.82	101.26	10.31
-2.29	114.66	1.03



[a]Data are expressed as means of triplicate values. SD = standard deviation; %PS = Percentage Parasite Survival.

Log concentration vs. % Parasite survival data used to plot dose-response curves.

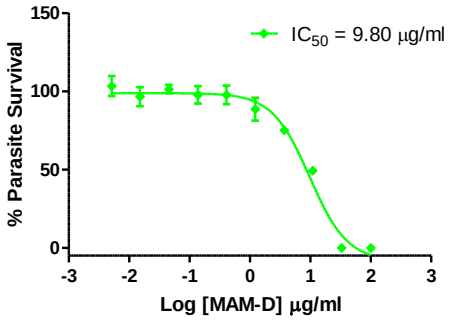
Log Conc (µg/ml)[a]	Npk-8H	
	%PS	SD
2.00	7.18	6.48
1.52	80.28	9.06
1.04	98.80	4.00
0.57	95.60	0.91
0.09	102.49	4.30
-0.39	106.08	4.95
-0.86	98.95	8.98
-1.34	98.94	7.44
-1.82	99.21	8.88
-2.29	102.87	7.22



[a]Data are expressed as means of triplicate values. SD = standard deviation; %PS = Percentage Parasite Survival.

Log concentration vs. % Parasite survival data used to plot dose-response curves.

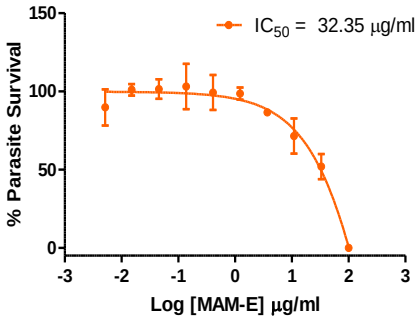
Log Conc (µg/ml)[a]	MAM-D	
	%PS	SD
2.00	0.00	0.00
1.52	0.00	0.00
1.04	50.16	1.22
0.57	75.15	0.82
0.09	88.52	7.31
-0.39	97.69	5.88
-0.86	97.76	5.56
-1.34	101.40	2.66
-1.82	96.58	6.09
-2.29	103.37	6.36



[a]Data are expressed as means of triplicate values. SD = standard deviation; %PS = Percentage Parasite Survival.

Log concentration vs. % Parasite survival data used to plot dose-response curves.

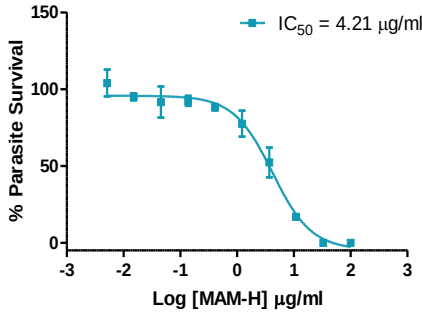
Log Conc (µg/ml)[a]	MAM-E	
	%PS	SD
2.00	0.00	0.00
1.52	51.84	8.01
1.04	65.22	10.57
0.57	86.45	1.31
0.09	98.51	3.91
-0.39	99.20	11.18
-0.86	103.01	14.45
-1.34	101.41	6.24
-1.82	100.93	3.70
-2.29	89.72	11.57



[a]Data are expressed as means of triplicate values. SD = standard deviation; %PS = Percentage Parasite Survival.

Log concentration vs. % Parasite survival data used to plot dose-response curves.

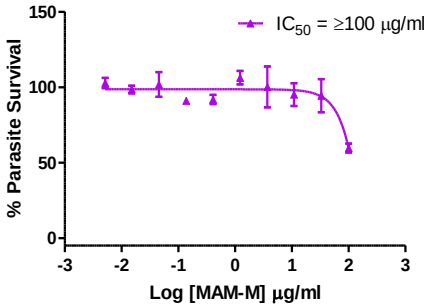
Log Conc (µg/ml)[a]	MAM-H	
	%PS	SD
2.00	0.00	0.00
1.52	0.00	0.00
1.04	16.94	0.25
0.57	52.29	9.69
0.09	77.51	8.46
-0.39	88.43	2.27
-0.86	92.38	3.25
-1.34	91.60	10.11
-1.82	95.07	2.33
-2.29	104.02	8.73



[a]Data are expressed as means of triplicate values. SD = standard deviation; %PS = Percentage Parasite Survival.

Log concentration vs. % Parasite survival data used to plot dose-response curves.

Log Conc (µg/ml)[a]	MAM-M	
	%PS	SD
2.00	59.67	2.95
1.52	94.38	10.93
1.04	91.89	10.40
0.57	100.18	13.59
0.09	106.34	4.42
-0.39	91.87	3.03
-0.86	91.03	1.15
-1.34	101.81	8.22
-1.82	98.46	2.47
-2.29	102.81	3.32

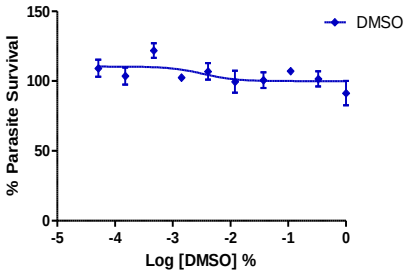


[a]Data are expressed as means of triplicate values. SD = standard deviation; %PS = Percentage Parasite Survival.

APPENDIX C: In vitro antimalarial assay for Npk-1D Fractions and compounds

Log solvent percentage vs. % Parasite survival data used to plot dose-response curves.

Log Solv.Perc (%)[a]	DMSO	
	%PS	SD
0.00	91.41	8.66
-0.48	101.62	5.42
-0.96	107.17	1.17
-1.43	100.72	5.58
-1.92	99.56	7.82
-2.39	106.94	5.85
-2.85	102.51	1.52
-3.33	121.92	5.28

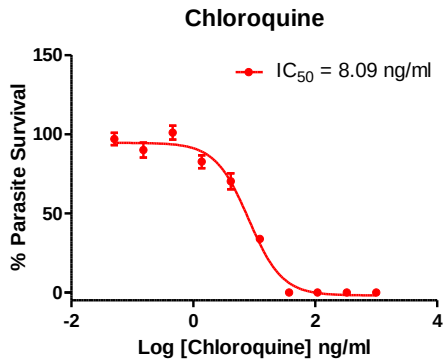


-3.82	103.65	6.05	
-4.29	109.18	6.16	

[a]Data are expressed as means of triplicate values. SD = standard deviation; %PS = Percentage Parasite Survival.

Log concentration vs. % Parasite survival data used to plot dose-response curves.

Log Conc (ng/ml)[a]	**Chloroquine**	
	%PS	**SD**
3.00	0.00	0.00
2.52	0.00	0.00
2.04	0.00	0.00
1.56	0.00	0.00
1.09	33.84	1.3

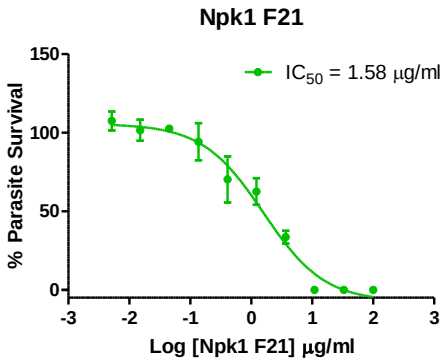


		6	
0.61	70.27	5.04	
0.14	82.59	4.03	
-0.34	100.95	4.39	
-0.82	89.95	4.68	
-1.29	96.95	3.90	

[a]Data are expressed as means of triplicate values. SD = standard deviation; %PS = Percentage Parasite Survival.

Log concentration vs. % Parasite survival data used to plot dose-response curves.

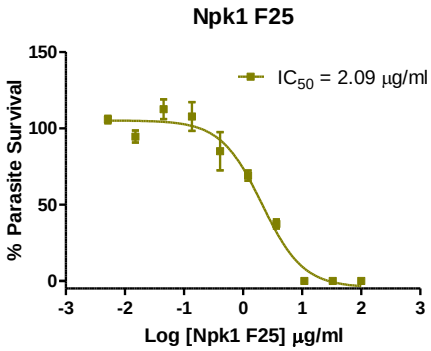
Log Conc (µg/ml)[a]	Npk1 F21	
	%PS	SD
2.00	0.00	0.00
1.52	0.00	0.00
1.04	0.00	0.00
0.57	33.49	4.01
0.09	62.55	8.47
-0.39	70.21	14.66
-0.86	94.16	11.83
-1.34	102.57	1.57
-1.82	101.53	6.71
-2.29	107.38	6.06



[a]Data are expressed as means of triplicate values. SD = standard deviation; %PS = Percentage Parasite Survival.

Log concentration vs. % Parasite survival data used to plot dose-response curves.

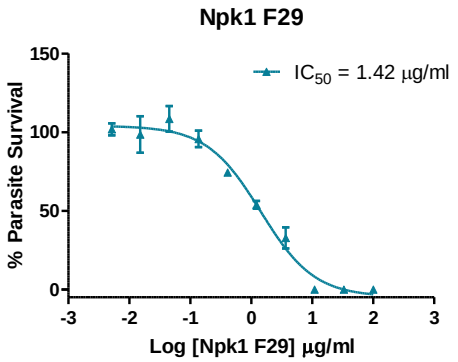
Log Conc (µg/ml)[a]	Npk1 F25	
	%PS	SD
2.00	0.00	0.00
1.52	0.00	0.00
1.04	0.00	0.00
0.57	37.30	2.95
0.09	68.95	3.32
-0.39	84.95	12.56
-0.86	107.70	9.41
-1.34	112.48	6.50
-1.82	94.57	3.96
-2.29	105.72	2.36



[a]Data are expressed as means of triplicate values. SD = standard deviation; %PS = Percentage Parasite Survival.

Log concentration vs. % Parasite survival data used to plot dose-response curves.

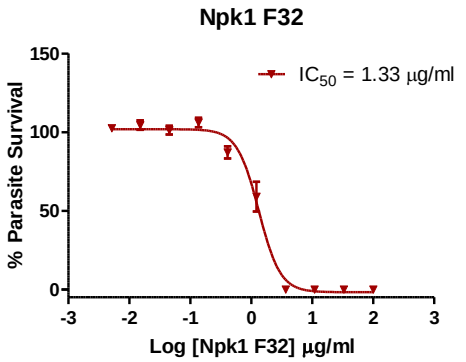
Log Conc (µg/ml)[a]	Npk1 F29	
	%PS	SD
2.00	0.00	0.00
1.52	0.00	0.00
1.04	0.00	0.00
0.57	32.81	6.70
0.09	53.78	2.46
-0.39	74.38	0.61
-0.86	95.74	5.25
-1.34	108.43	8.15
-1.82	98.53	11.68
-2.29	101.85	3.78



[a]Data are expressed as means of triplicate values. SD = standard deviation; %PS = Percentage Parasite Survival.

Log concentration vs. % Parasite survival data used to plot dose-response curves.

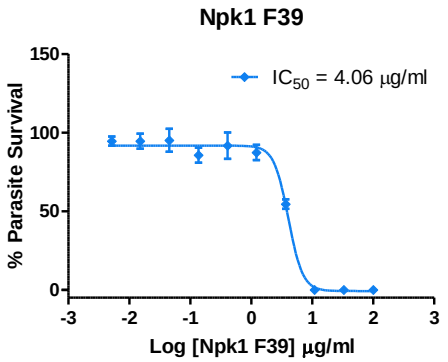
Log Conc (µg/ml)[a]	Npk1 F32	
	%PS	SD
2.00	0.00	0.00
1.52	0.00	0.00
1.04	0.00	0.00
0.57	0.00	0.00
0.09	58.99	9.39
-0.39	87.19	3.85
-0.86	106.15	2.91
-1.34	101.28	2.73
-1.82	104.52	2.99
-2.29	102.47	1.44



[a]Data are expressed as means of triplicate values. SD = standard deviation; %PS = Percentage Parasite Survival.

Log concentration vs. % Parasite survival data used to plot dose-response curves.

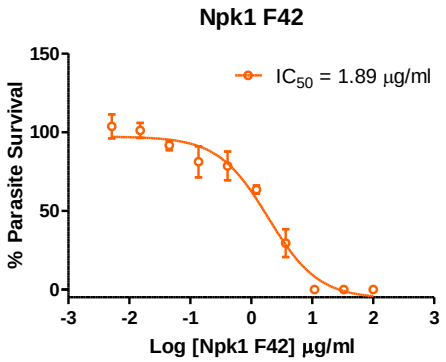
Log Conc (µg/ml)[a]	Npk1 F39 %PS	Npk1 F39 SD
2.00	0.00	0.00
1.52	0.00	0.00
1.04	0.00	0.00
0.57	54.51	3.05
0.09	87.33	4.88
-0.39	91.70	8.43
-0.86	85.62	4.82
-1.34	95.08	7.28
-1.82	94.54	4.81
-2.29	94.59	2.95



[a]Data are expressed as means of triplicate values. SD = standard deviation; %PS = Percentage Parasite Survival.

Log concentration vs. % Parasite survival data used to plot dose-response curves.

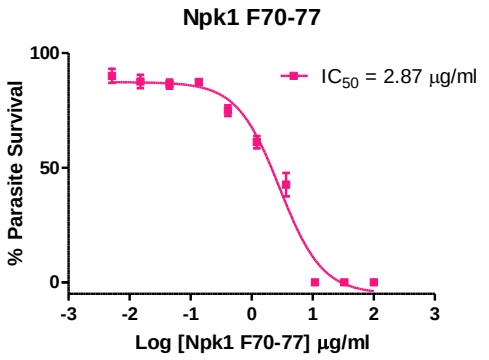
Log Conc (µg/ml)[a]	Npk1 F42	
	%PS	SD
2.00	0.00	0.00
1.52	0.00	0.00
1.04	0.00	0.00
0.57	29.47	8.85
0.09	63.54	2.59
-0.39	78.54	9.13
-0.86	81.21	9.89
-1.34	91.63	3.00
-1.82	101.12	4.73
-2.29	103.65	7.61



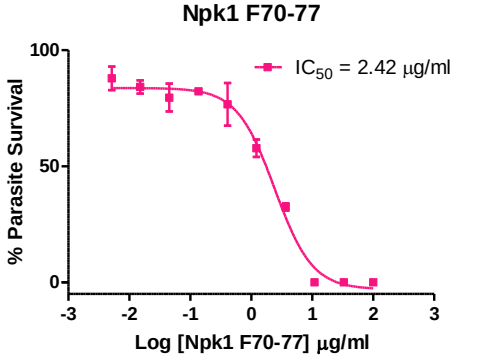
[a]Data are expressed as means of triplicate values. SD = standard deviation; %PS = Percentage Parasite Survival.

Log concentration vs. % Parasite survival data used to plot dose-response curves.

Log Conc (µg/ml)[a]	Npk1 F70-77	
	%PS	SD
2.00	0.00	0.00
1.52	0.00	0.00
1.04	0.00	0.00
0.57	42.66	5.08
0.09	61.15	2.66
-0.39	74.90	2.31
-0.86	87.41	0.23
-1.34	86.45	1.99
-1.82	87.60	2.89
-2.29	90.06	3.05



Log Conc (µg/ml)[a]	Npk1 F70-77	
	%PS	SD
2.00	0.00	0.00
1.52	0.00	0.00

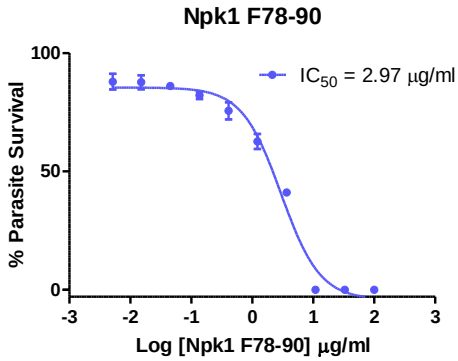


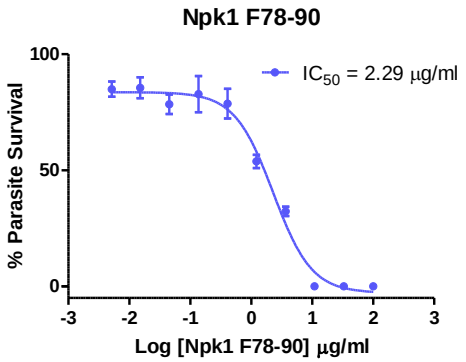
1.04	0.00	0.00	
0.57	32.50	1.59	
0.09	57.78	3.80	
-0.39	76.69	9.18	
-0.86	82.27	1.44	
-1.34	79.60	5.99	
-1.82	84.18	2.87	
-2.29	87.89	5.11	

[a]Data are expressed as means of triplicate values. SD = standard deviation; %PS = Percentage Parasite Survival.

Log concentration vs. % Parasite survival data used to plot dose-response curves.

Log Conc (µg/ml)[a]	**Npk1 F78-90**	
	%PS	**SD**
2.00	0.00	0.00
1.52	0.00	0.00
1.04	0.00	0.00
0.57	41.05	1.33
0.09	62.65	3.19
-0.39	75.59	3.62



-0.86	82.11	1.51	
-1.34	86.05	1.27	
-1.82	87.67	2.96	
-2.29	87.88	3.33	
Log Conc (µg/ml)[a]	**Npk1 F78-90**		
	%PS	**SD**	
2.00	0.00	0.00	
1.52	0.00	0.00	
1.04	0.00	0.00	
0.57	32.31	2.01	
0.09	53.81	2.84	
-0.39	78.68	6.42	
-0.86	82.81	7.78	
-1.34	78.41	4.22	
-1.82	85.56	4.53	
-2.29	84.94	3.26	

[a]Data are expressed as means of triplicate values. SD = standard deviation; %PS = Percentage Parasite Survival.

APPENDIX D: Research ethical clearance and permit

UNIVERSITY OF NAMIBIA

ETHICAL CLEARANCE CERTIFICATE

Ethical Clearance Reference Number: SEC/FOS/4/2014 **Date: 3March, 2014**

This Ethical Clearance Certificate is issued by the University of Namibia Research Ethics Committee (UREC) in accordance with the University of Namibia's **Research Ethics Policy and Guidelines. Ethical** approval is given in respect of undertakings contained in the Research Project outlined below. This Certificate is issued on the recommendations of the ethical evaluation done by the Faculty/Centre/Campus Research & Publications Committee sitting with the Postgraduate Studies Committee.

Title of Project: **EVALUATION OF INDIGENOUS NAMIBIAN MUSHROOMS FOR ANTIMALARIAL PROPERTIES**

Nature/Level of Project: Doctorate

Principal Researcher: Nailoke Pauline Kadhila-Muandingi

Host Department & Faculty: Biological Science, Faculty of Science.

Main Supervisor (s): Dr. Kazhila C. Chinsembu (Main), Dr. Mamello Sekhoacha (Co)

Take note of the following:

(a) Any significant changes in the conditions or undertakings outlined in the approved Proposal must be communicated to the UREC. An application to make amendments may be necessary.
(b) Any breaches of ethical undertakings or practices that have an impact on ethical conduct of the research must be reported to the UREC.
(c) The Principal Researcher must report issues of ethical compliance to the UREC (through the Chairperson of the Faculty/Centre/Campus Research & Publications Committee) at the end of the Project or as may be requested by UREC.
(d) The UREC retains the right to:
  (i). withdraw or amend this Ethical Clearance if any unethical practices (as outlined in the Research Ethics Policy) have been detected or suspected,
  (ii). request for an ethical compliance report at any point during the course of the research.

UREC wishes you the best in your research.

Prof. I. Mapaure
UNAM Research Coordinator
ON BEHALF OF UREC

UNIVERSITY OF THE FREE STATE
UNIVERSITEIT VAN DIE VRYSTAAT
YUNIVESITHI YA FREISTATA

UFS·UV
HEALTH SCIENCES
GESONDHEIDSWETENSKAPPE

IRB nr 00006240
REC Reference nr 230408-011
IORG0005187
FWA00012784

12 August 2015

MRS NP KADHILA-MUANDINGI
DEPARTMENT OF PHARMACOLOGY
FACULTY OF HEALTH SCIENCES
UFS

Dear Mrs NP Kadhila-Muandingi

ECUFS 150/2015 **DEPARTMENT OF PHARMACOLOGY**
PROJECT TITLE: SCREENING OF INDIGENOUS NAMIBIAN MUSHROOMS FOR THEIR ANTIMALARIAL ACTIVITIES AGAINST SENSITIVE AND RESISTANT STRAINS OF THE MALARIA PARASITES

1. You are hereby kindly informed that, at the meeting held on 11 August 2015, the Ethics Committee approved the above project.

2. Any amendment, extension or other modifications to the protocol must be submitted to the Ethics Committee for approval.

3. A progress report should be submitted within one year of approval of long term studies and a final report at completion of both short term and long term studies.

4. Kindly use the ECUFS NR as reference in correspondence to the Ethics Committee Secretariat.

5. The Ethics Committee functions in compliance with, but not limited to, the following documents and guidelines: The SA National Health Act. No. 61 of 2003; Ethics in Health Research: Principles, Structures and Processes (2015); SA GCP(2006); Declaration of Helsinki; The Belmont Report; The US Office of Human Research Protections 45 CFR 461 (for non-exempt research with human participants conducted or supported by the US Department of Health and Human Services- (HHS)), 21 CFR 50; 21 CFR 56; CIOMS; ICH-GCP-E6 Sections 1-4; The International Conference on Harmonization and Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH Tripartite), Guidelines of the SA Medicines Control Council as well as Laws and Regulations with regard to the Control of Medicines, Constitution of the Ethics Committee of the Faculty of Health Sciences.

Yours faithfully

DR SM LE GRANGE
CHAIR: ETHICS COMMITTEE

Cc: Dr MP Sekhoacha

Ethics Committee
Office of the Dean: Health Sciences
T: +27 (0)51 401 7795/7794 | F: +27 (0)51 444 4359 | E: ethicsfhs@ufs.ac.za
Block D, Dean's Division, Room D104 | P.O. Box/Posbus 339 (Internal Post Box G40) | Bloemfontein 9300 | South Africa
www.ufs.ac.za

MINISTRY OF ENVIRONMENT AND TOURISM

RESEARCH/COLLECTING PERMIT

Permit Number 2124/2016
Valid from 25 February 2016 to 31 January 2017

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

Name: **Mrs N.P. Kadhila-Muandingi**
Address: **P O Box 98848**
Pelican Square
Windhoek
Namibia
Coworkers: **Dr. K.C. Chinsembu, I.S.E. Uietile and Ms F.N. Mhanda**

To conduct a study on the evaluation of selected indigenous Namibian plants and mushrooms for antimalaria properties in Zambezi, Kavango West and Ohangwena Regions, subject to attached conditions.

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

MINISTRY OF ENVIRONMENT AND TOURISM
REPUBLIC OF NAMIBIA
02 MAR 2016
WINDHOEK
Private Bag 13306, Windhoek
Tel: 242111 • Fax: 258861

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: Conservation Scientist, email imatheus@met.na
Private Bag 13306, Windhoek, Namibia

RESEARCH/COLLECTING PERMIT CONDITIONS

****

1. You must report to the Regional Office of the Ministry of Environment and Tourism prior to arrival in fieldwork area, and must be shown your permit.
2. The permission of the land owner is required to work/collect on private lands.
3. The permission of the concession holder is required to work/collect in concession areas.
4. You are to seek permission from community authorities prior to entering a community for collection of the resource and or interviewing of members.
5. No further work, other than specified in the submitted proposal, is to be carried out without notification of and approval from the Ministry of Environment and Tourism and the respective communities.
6. You or Supervisor (s) are expected to provide the Ministry of Environment and Tourism a copy of the research work.
7. No commercial filming will be permitted without prior approval by the Ministry of Environment and Tourism.
8. Duplicates of publications and / or final report should be made available to MET Resource Centre and also the final report is to be submitted to the NBRI reference library.
9. No palaeontological and or archaeological samples may be taken without a permit from the National Heritage Council.
10. The specimens and their derivatives may be used for the purposes of this study only and may not be patented, commercialised, donated or sold to a third party without the written consent of the Ministry of Environment and Tourism.
11. All results (raw materials) or technology derived directly or indirectly from this research must be made available free of charge without reservations to the Ministry of Environment and Tourism.
12. Please submit a report on the work conducted under this permit to this office not later than one month after the expiry of this permit as well as to regional office where you have reported.
13. Applications for renewal of this permit must reach this office at least three months prior to the expiry of this permit.
14. Habitat destructive collecting methods must not to be used.
15. All field teams must be in possession of the permit and permit copy must accompany the transport of specimens.
16. It is your responsibility to make the necessary contacts and arrangements as specified above.

MINISTRY OF ENVIRONMENT AND TOURISM

Directorate Scientific Services
Private Bag 13306
Windhoek, Namibia
Enquiries: Chief Warden, Permit Office

Tel +264-61-284 2111
Fax +264-61-258 861

PERMIT No.:	106239
VALID FROM:	16 May 2016
VALID TO:	16 Nov. 2016
RECEIPT No.:	MET63110

GENERAL PERMIT

Name: P. N. Kadhila

Residential Address: 7 Marabouweg, Tauben Glen
Highland Park

Postal Address: P.O. Box 98848 Pelican Square

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

To export 6 (six) Bitter Herb to be Confirmed powder from Windhoek in Rep. of Namibia to Dr. Mamello, University of Free States Bloemfontein in Rep. of Namibia South Africa

Remarks - Conditions

- Subject to Veterinary and Import regulations
- From Research / Collecting permit no. 2124/2016

MINISTRY OF ENVIRONMENT AND TOURISM
REPUBLIC OF NAMIBIA
2016 -05- 16

Official MET date stamp

2016 -05- 1 ..

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

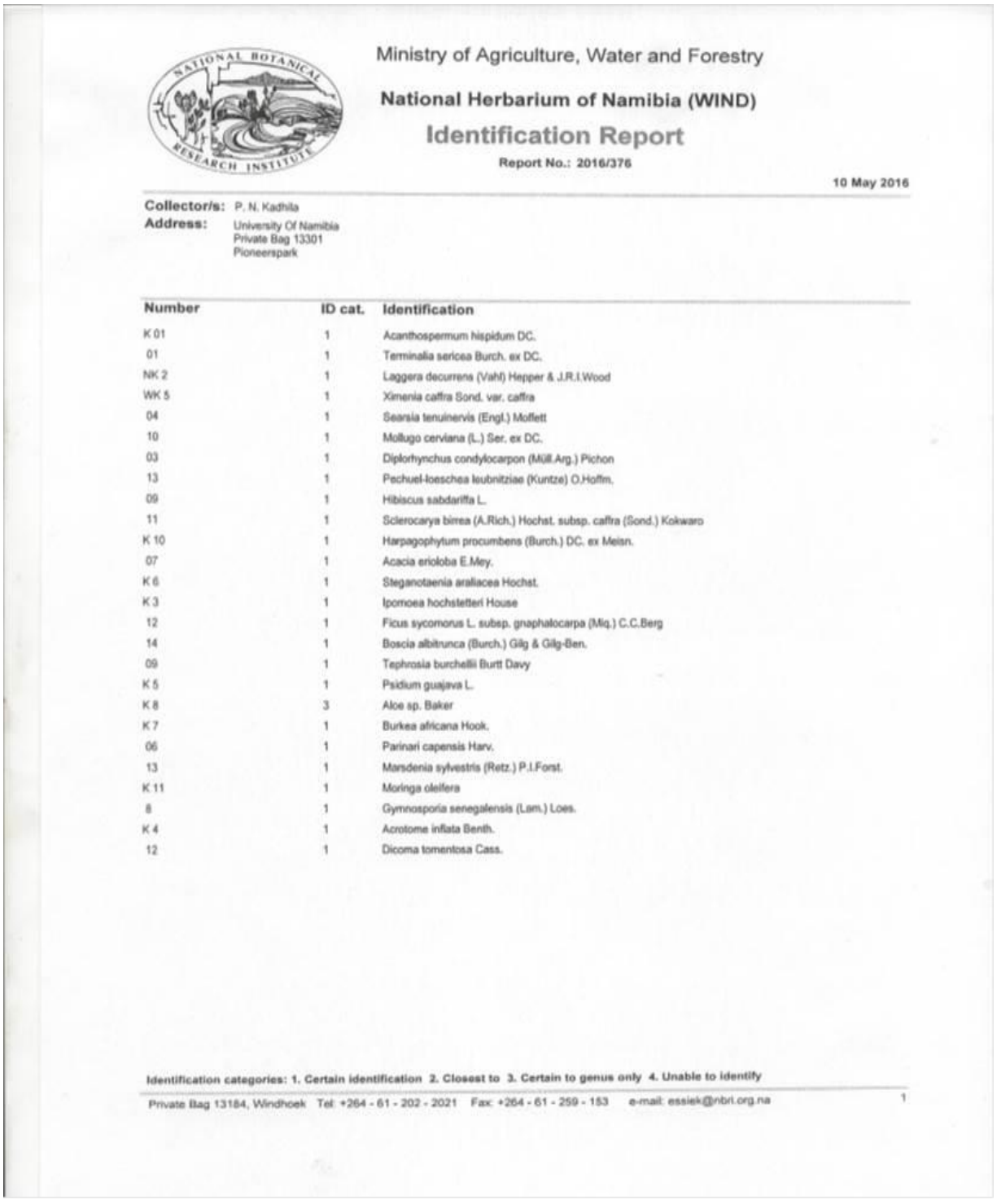
Authorizing Officer

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 prescribed conditions and regulations.

prime press 042010

APPENDIX E: Identification Report for the plants used



Ministry of Agriculture, Water and Forestry

National Herbarium of Namibia (WIND)

Identification Report

Report No.: 2016/376

10 May 2016

Collector/s: P. N. Kadhila

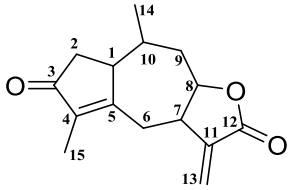
Address: University Of Namibia
Private Bag 13301
Pioneerspark

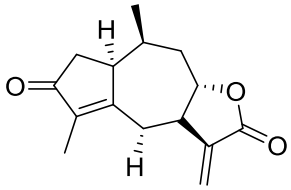
Number	ID cat.	Identification
K 01	1	Acanthospermum hispidum DC.
01	1	Terminalia sericea Burch. ex DC.
NK 2	1	Laggera decurrens (Vahl) Hepper & J.R.I.Wood
WK 5	1	Ximenia caffra Sond. var. caffra
04	1	Searsia tenuinervis (Engl.) Moffett
10	1	Mollugo cerviana (L.) Ser. ex DC.
03	1	Diplorhynchus condylocarpon (Müll.Arg.) Pichon
13	1	Pechuel-loeschea leubnitziae (Kuntze) O.Hoffm.
09	1	Hibiscus sabdariffa L.
11	1	Sclerocarya birrea (A.Rich.) Hochst. subsp. caffra (Sond.) Kokwaro
K 10	1	Harpagophytum procumbens (Burch.) DC. ex Meisn.
07	1	Acacia erioloba E.Mey.
K 6	1	Steganotaenia araliacea Hochst.
K 3	1	Ipomoea hochstetteri House
12	1	Ficus sycomorus L. subsp. gnaphalocarpa (Miq.) C.C.Berg
14	1	Boscia albitrunca (Burch.) Gilg & Gilg-Ben.
09	1	Tephrosia burchellii Burtt Davy
K 5	1	Psidium guajava L.
K 8	3	Aloe sp. Baker
K 7	1	Burkea africana Hook.
06	1	Parinari capensis Harv.
13	1	Marsdenia sylvestris (Retz.) P.I.Forst.
K 11	1	Moringa oleifera
8	1	Gymnosporia senegalensis (Lam.) Loes.
K 4	1	Acrotome inflata Benth.
12	1	Dicoma tomentosa Cass.

Identification categories: 1. Certain identification 2. Closest to 3. Certain to genus only 4. Unable to identify

Private Bag 13184, Windhoek Tel: +264 - 61 - 202 - 2021 Fax: +264 - 61 - 259 - 153 e-mail: essiek@nbri.org.na 1

APPENDIX F: Chemical structure for antiplasmodial active compounds





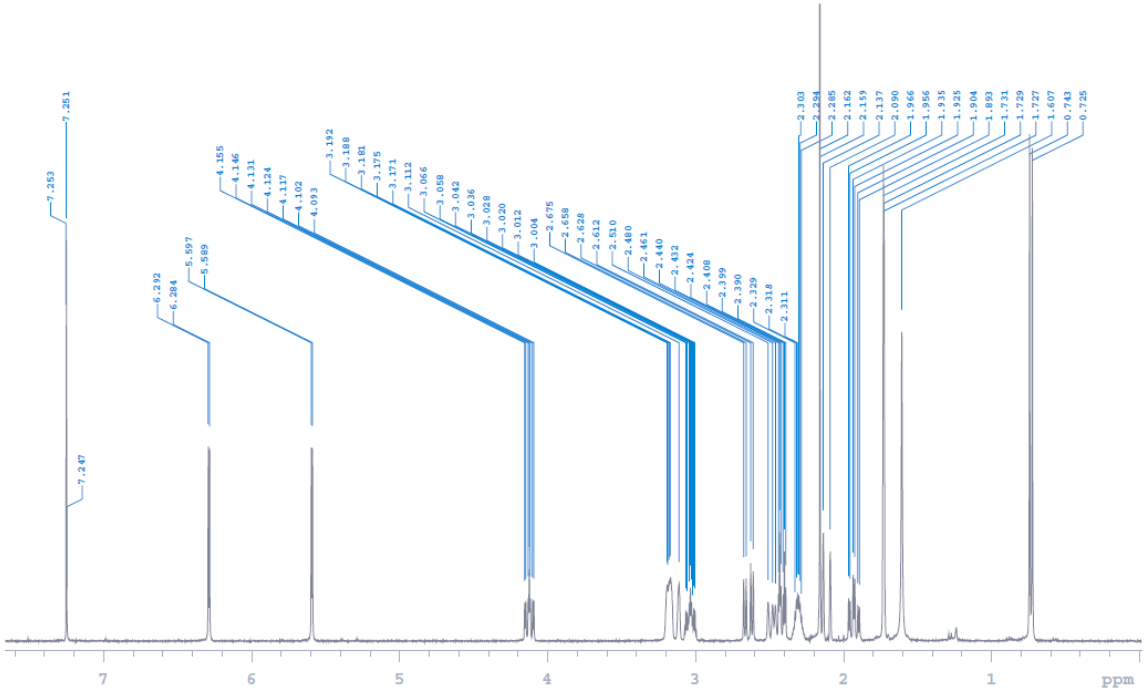


Figure 1. ^{1}H-NMR Spectrum of NPK F70-77

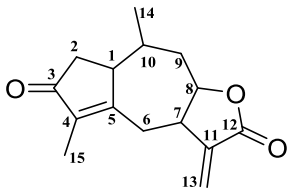
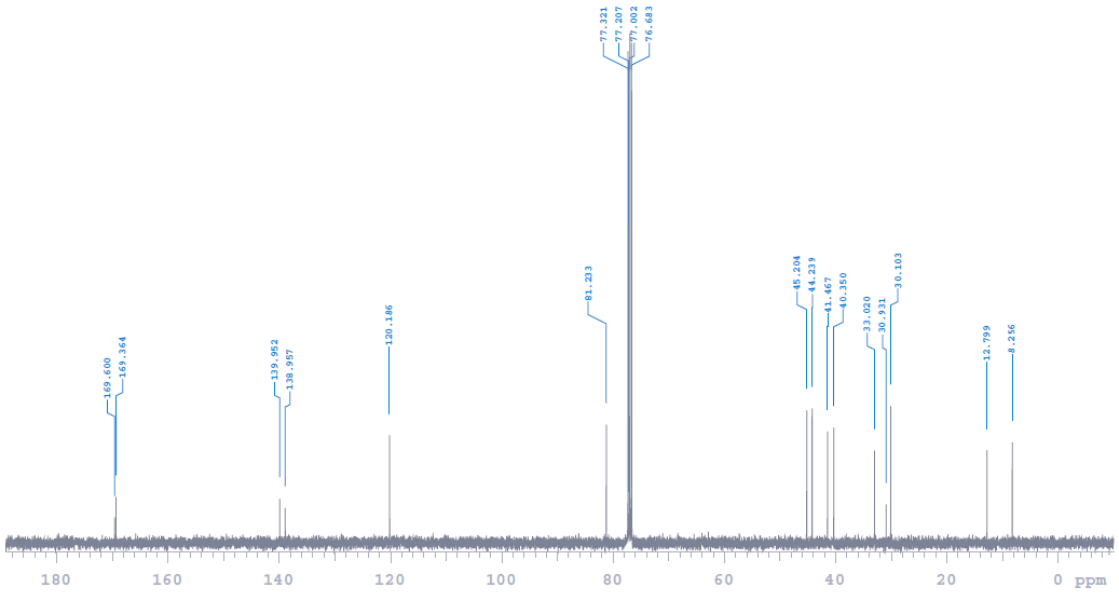


Table 1: 1H NMR and 13C NMR Data of Xerantholide (400 MHz, $CDCl_3$).

H	δppm	splitting
1	4.12	dddd
2α	2.62	doublet of doublet (dd)
2β	2.46	broad doublet (brd)
6α	3.17	multiplet (m)
6β	2.51	brd
7	3.01	ddddd
8	2.30	multiplet (m)
9α	2.46	ddd
9β	2.40	ddd
10	2.31	m
11	-	
13	5.58	d
13’	6.28	d
14	0.73	doublet (d)
15	1.93	doublet doublet of doublet (ddd)

No proton in number 11.



^{13}C-NMR Spectrum of NPK F70-77

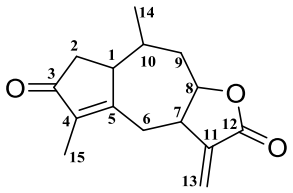
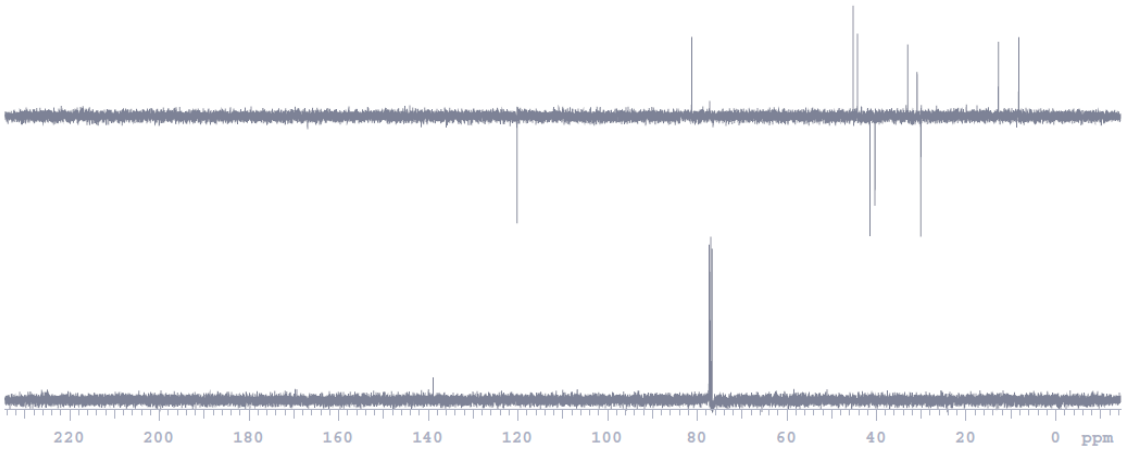


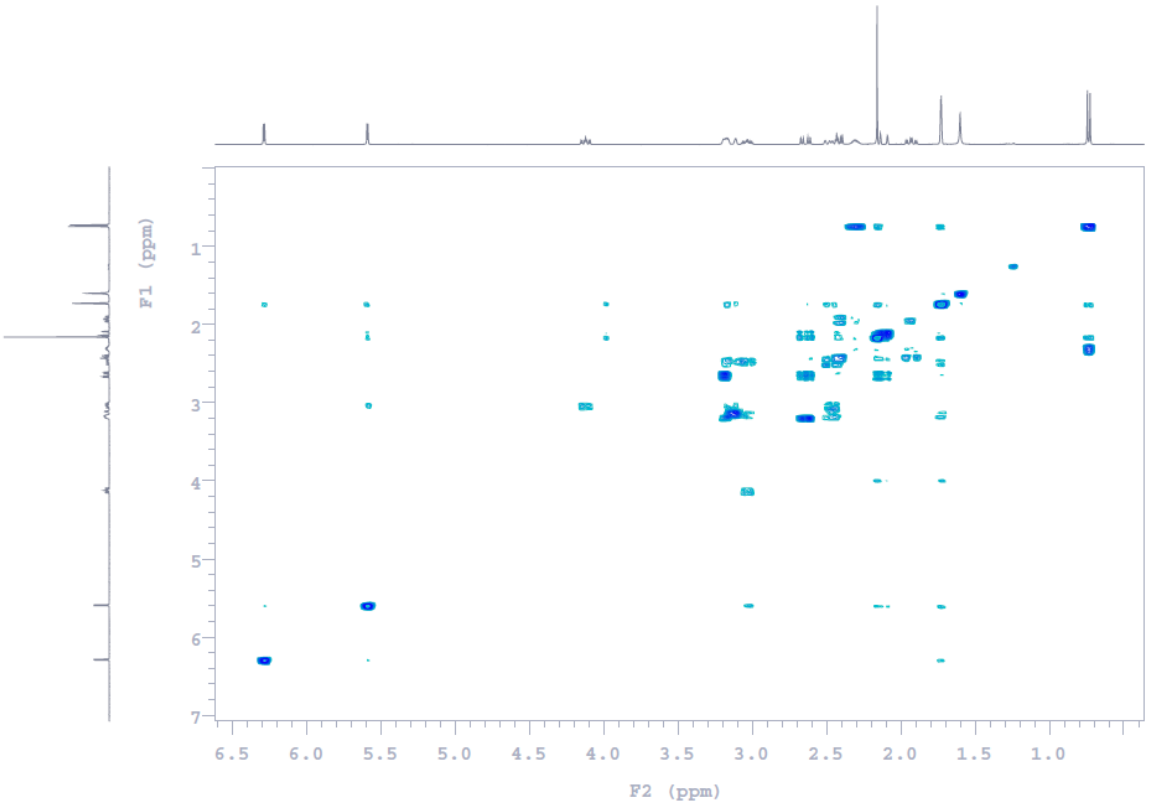
Table 1: 13C NMR Data of Xerantholide (400 MHz, $CDCl_3$).

C	δppm
1	81.2
2α	41.5
2β	41.5
3	169.3
4	139.9

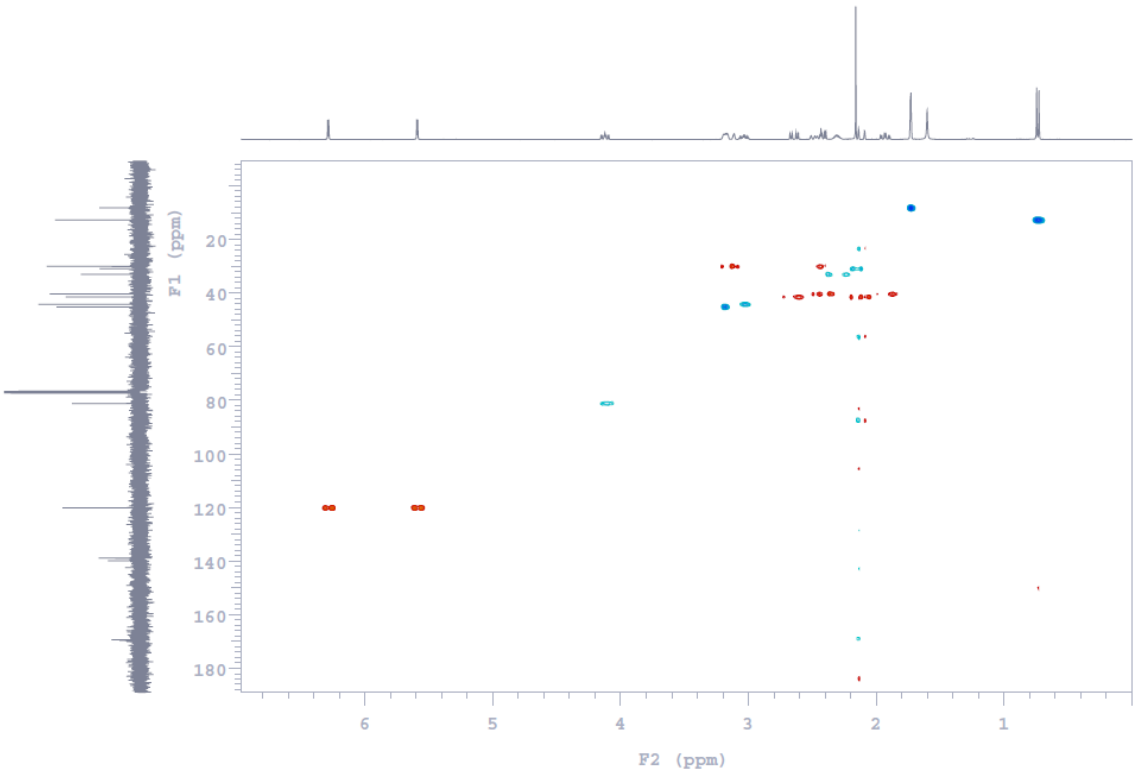
6α	45.2
6β	45.2
7	44.2
8	33.0
9α	40.4
9β	40.4
10	30.9
11	138.9
12	169.6
13α	120.1
13β	120.1
14	12.7
15	41.4



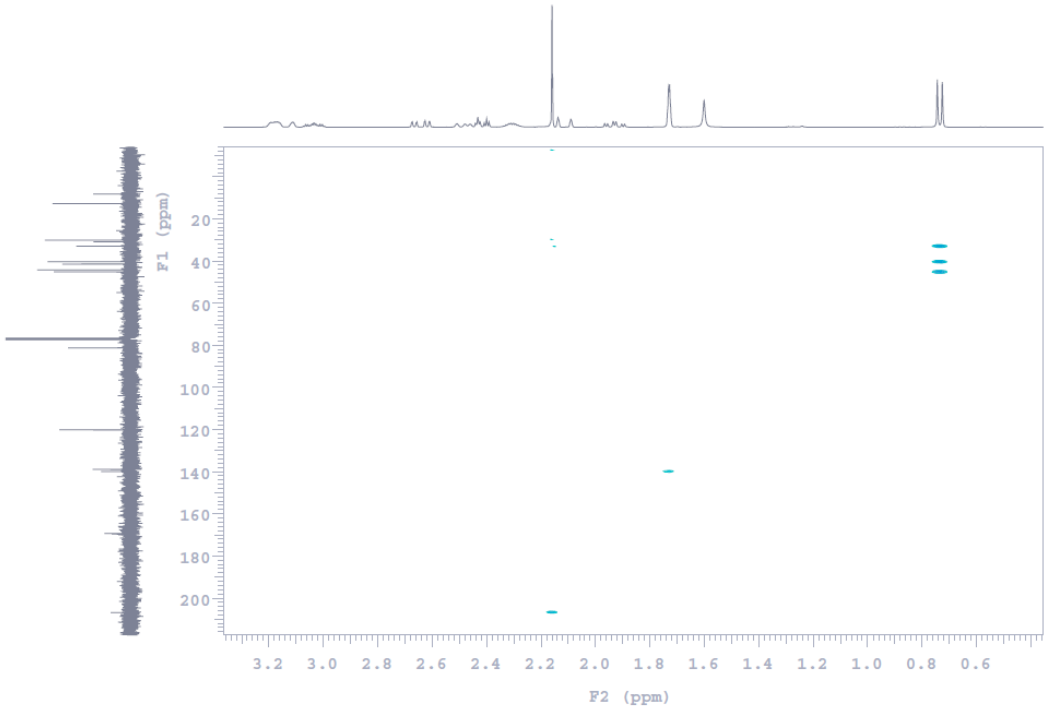
DEPT-NMR Spectrum of NPK F70-77



COSY-NMR Spectrum of NPK F70-77

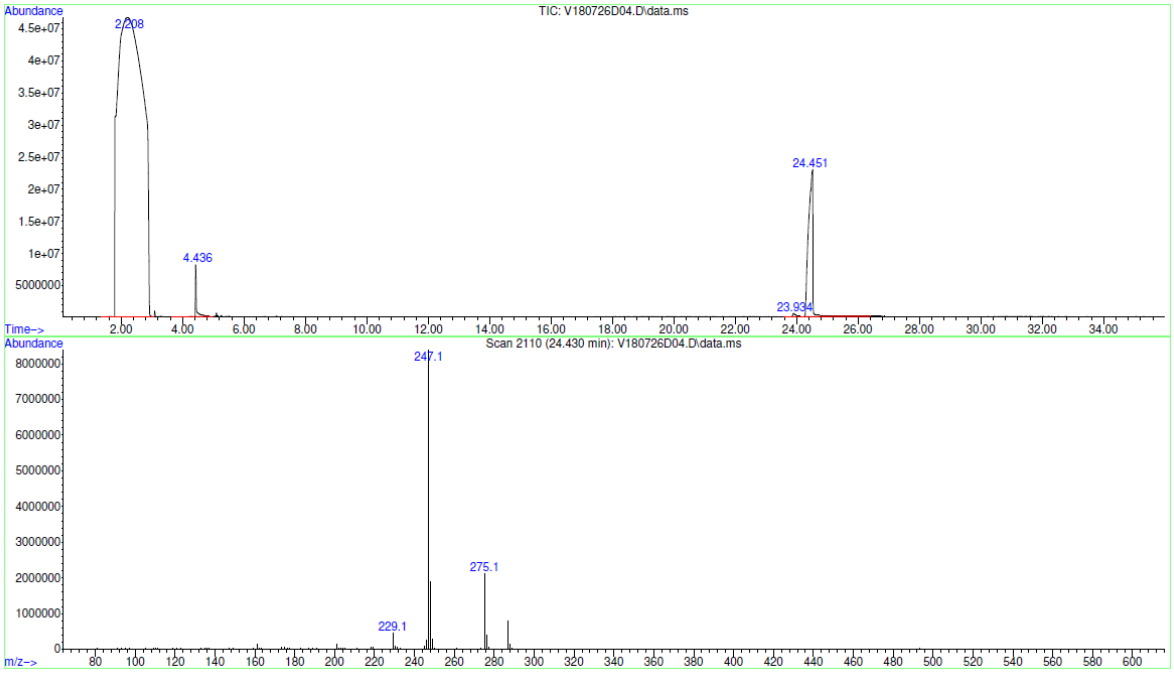


HSQC-NMR Spectrum of NPK F70-77

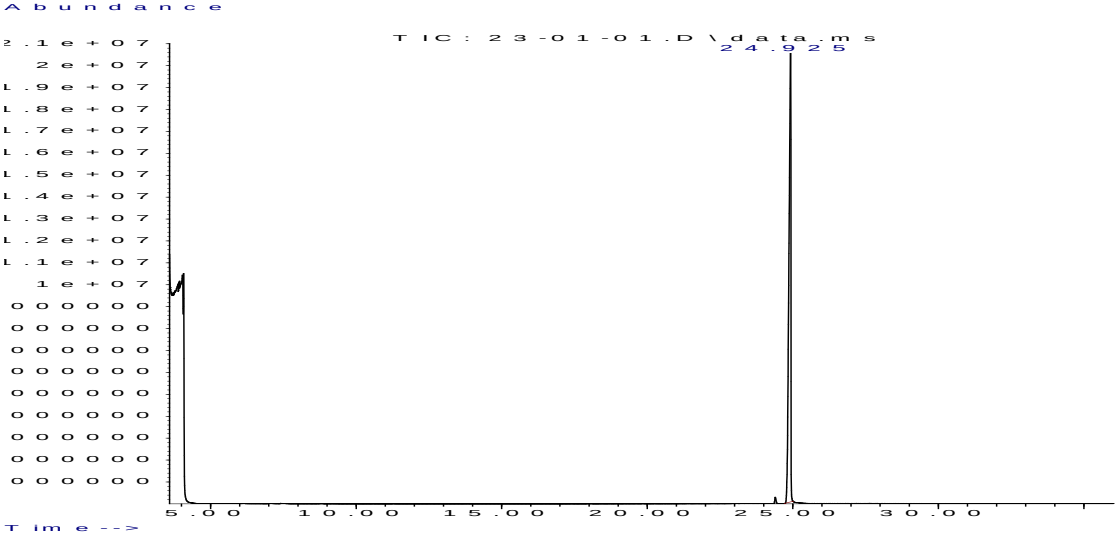


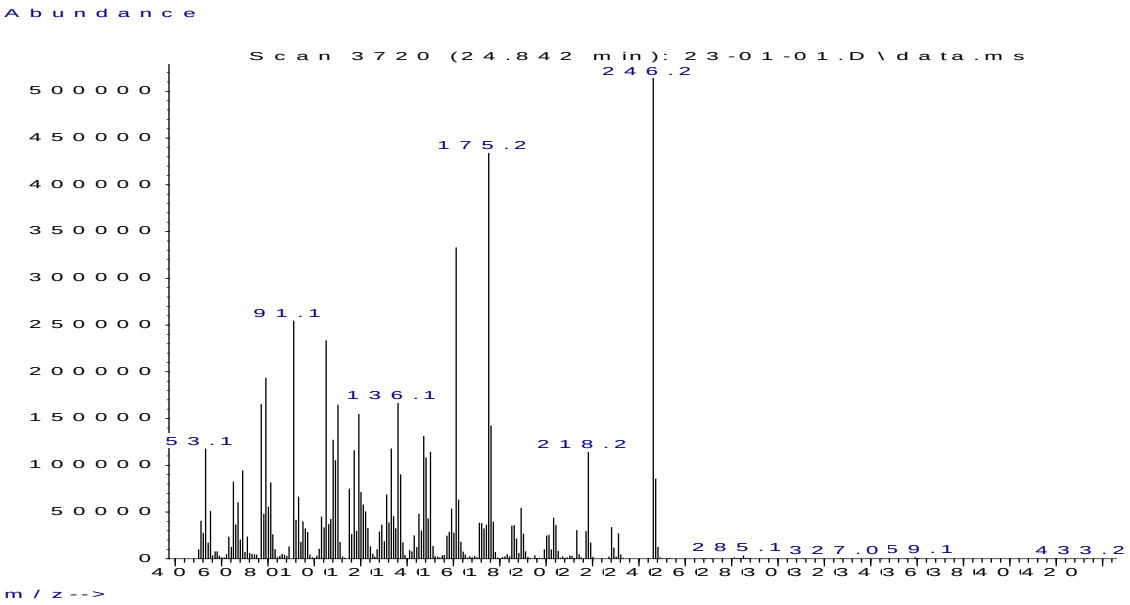
HMBC-NMR Spectrum of NPK F70-77

GC-MS CI

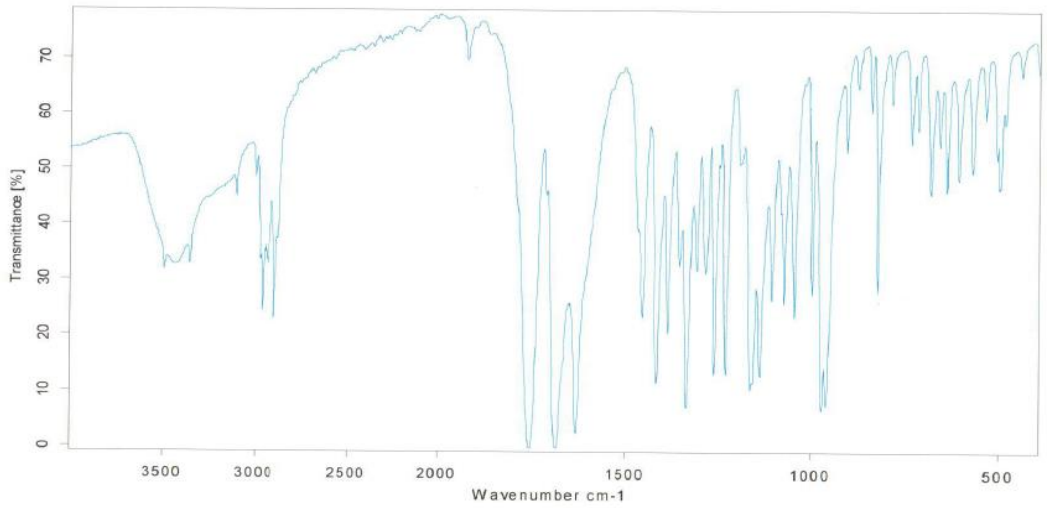


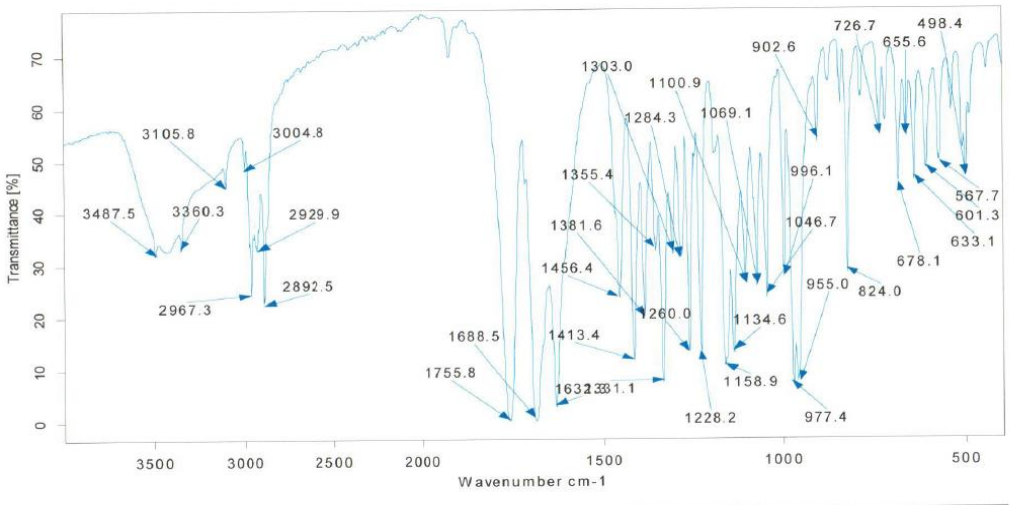
GC-MS EI



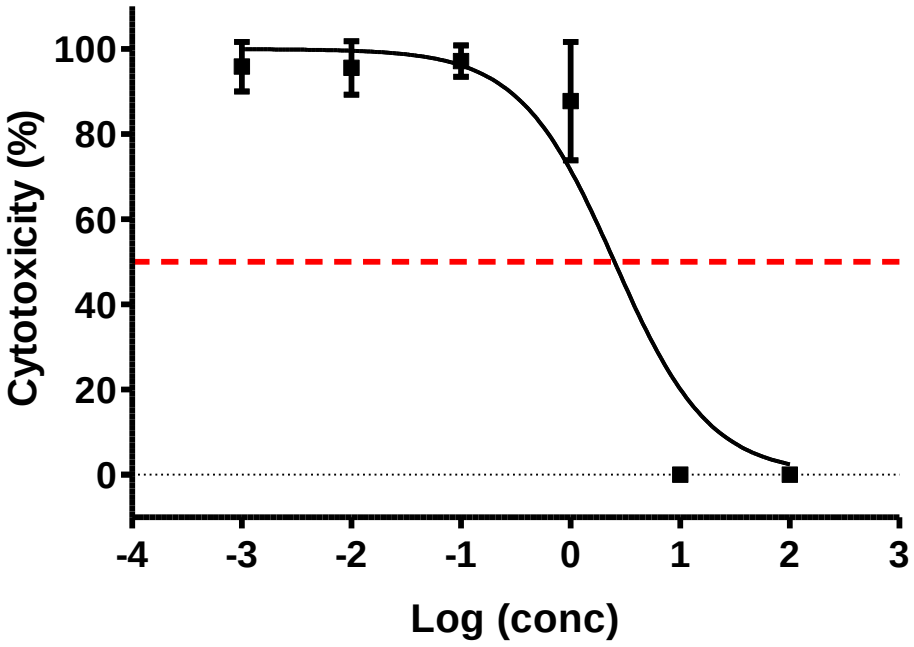


FTIR for NPK F70-77



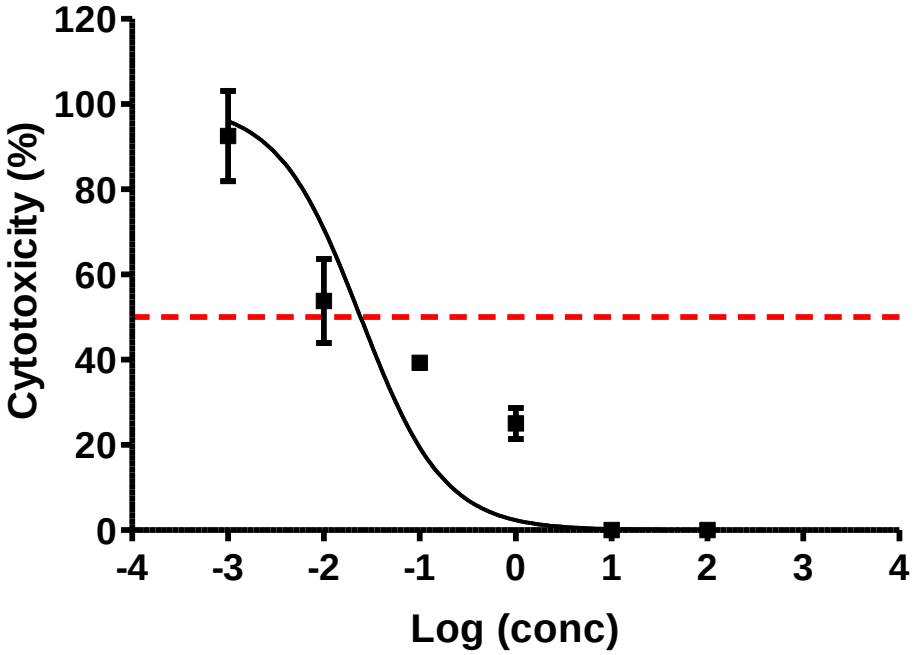

Transmittance [%]
Wavenumber cm-1
3487.5
3360.3
3105.8
3004.8
2967.3
2929.9
2892.5
1755.8
1688.5
1456.4
1413.4
1381.6
1355.4
1303.0
1284.3
1260.0
1228.2
1158.9
1134.6
1100.9
1069.1
1046.7
996.1
977.4
955.0
902.6
824.0
726.7
678.1
655.6
633.1
601.3
567.7
498.4

APPENDIX G: Cytotoxicity of antiplasmodial active compounds



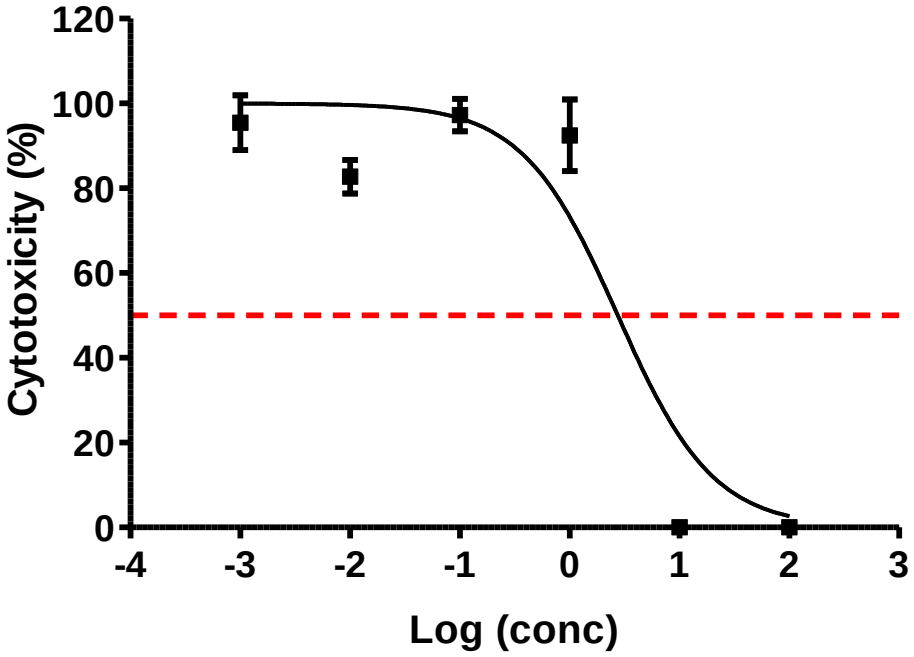
NPK-1 D F70

log(inhibitor) vs. normalized response	
Best-fit values	
LogIC50	0.4021
IC50	**2.524**
Std. Error	
LogIC50	0.1964
95% Confidence Intervals	
LogIC50	-0.1030 to 0.9071
IC50	0.7889 to 8.074
Goodness of Fit	
Degrees of Freedom	5
R square	0.9404
Absolute Sum of Squares	707.2
Sy.x	11.89
Number of points	
Analyzed	6



Emetine

log(inhibitor) vs. normalized response	
Best-fit values	
LogIC50	-1.618
IC50	**0.02411**
Std. Error	
LogIC50	0.2562
95% Confidence Intervals	
LogIC50	-2.276 to -0.9591
IC50	0.005291 to 0.1099
Goodness of Fit	
Degrees of Freedom	5
R square	0.8060
Absolute Sum of Squares	1208
Sy.x	15.54
Number of points	
Analyzed	6



NPK-1 D F78

log(inhibitor) vs. normalized response	
Best-fit values	
LogIC50	0.4388
IC50	**2.747**
Std. Error	
LogIC50	0.2508
95% Confidence Intervals	
LogIC50	-0.2060 to 1.084
IC50	0.6222 to 12.13
Goodness of Fit	
Degrees of Freedom	5
R square	0.8996
Absolute Sum of Squares	1146
Sy.x	15.14
Number of points	
Analyzed	6