A phytochemical study of selected medicinal herbs

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Declaration

I hereby declare that this dissertation, which I herewith submit for the research qualification

MASTERS DEGREE IN CHEMISTRY

to the North West University, Department of Chemistry, is, apart from the recognised assistance of my supervisors, my own work and has not previously been submitted by me to another institution to obtain a diploma or degree.

Signed.....

Kearabilwe Marua Kabasia

Signed.....

Dr Zimbili Mkhize

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- God, for being so good to me and providing me with the opportunity. This is a blessing.

Dedication

It is dedicated to my family and husband, without whose support I would have never been able to complete it. To my late grandmother, thank you for the love. I know you would have been very proud of this work. Wherever you are, you will always be a part of my life. I miss you dearly.

Abstract

Medicinal plants have played an important part in the establishment of human culture. They have played a crucial role in the development of some drugs currently in the market. This study illustrates the significance of traditional medicines in treating and managing human diseases such as malaria. Nine medicinal plants, *Lavandula x intermedia, Artemisia afra, Rosmarinus officinalis, Cymbopogan citratus*, *Verbascum thapsus*, *Pelargonium graveolens*, *Foeniculum vulgare*, *Lippia citriodora*, *Catharathus roseus* were studied and their petroleum ether, chloroform, ethyl acetate, ethanol and acetone extracts were used for the phytochemical screening to investigate the phytoconstituents in the medicinal herbs.

The results disclosed the occupancy of secondary metabolites mostly flavonoids, sterols and saponins. This was coupled with antimalarial tests to evaluate the therapeutic potential of the extracts of the nine selected plant species as potential antimalarial agents. The dichloromethane, ethanol and acetone extracts of the nine species were screened *in vitro* for antimalarial activity using NF_{58} sensitive strain using pLDH method. From the screening results *Artemisia afra* and *Cymbopogan citratus* warrantied further investigation and their IC_{50} values were 0.71 and 4.59 respectively. Based on the availability, *Artemisia afra* was investigated further resulting in the isolation of taurin (**4.24**) and marimatin (**4.25**). These two pure compounds were investigated for their antimalarial activity.

Keywords: Antimalarial activity, *Artemisia afra*, phytochemical study, structural elucidation.

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ACT	Artemisin-based Combination Therapy
^{13}C NMR	Carbon-13 Nuclear Magnetic Resonance Spectroscopy
COSY	Correlated Spectroscopy
d	doublet
DEPT	Distortionless Enhancement Polarization Transfer
FTIR	Fourier Transform Infrared Spectroscopy
HMBC	Heteronuclear Multiple Bond Correlation
HSQC	Heteronuclear Multiple Quantum Coherence
m	multiplet
MS	Mass Spectrometry
m/z	mass-to charge ratio
pLDH	Parasite Lactate Dehydrogenase Assay
ppm	parts per million
^{1}H NMR	Proton Nuclear Magnetic Resonance Spectroscopy
q	quartet
qd	quartet of doublet
s	singlet
SANBI	South African National Biodiversity Institute
TLC	Thin Layer Chromatography
WHO	World Health Organization

CHAPTER 1

Introduction

1.1. Drug discovery from medicinal plants

The plant kingdom is a source of potential drugs and the awareness about medicinal plants has increased over recent years.[1] Since time began, plant products have been inclusive of phytomedicines and they can be attained from different parts including leaves, roots and bulbs.[2] The understanding of plants chemical constituents is advantageous as the data are extremely useful for the synthesis of complex chemical substances.[2] Medicinal plants carry several phytochemicals which dispense precise change of activity on humans with bioactive substances.[3,4]

Phytochemicals are produced by primary or secondary metabolism in plants. Primary metabolites are the kind of metabolites which are openly involved in normal growth, development and reproduction. Secondary metabolites are chemically and taxonomically immensely multiple compounds with unforeseeable role, which serve as competitive weapons used against other bacteria, fungi, amoebae, plants, insect and metal transporting agents. Secondary metabolites are extensively used in human therapy, agriculture, veterinary, scientific studies and countless other areas. A greater number of secondary metabolites belonging to several chemical classes (tannins, alkaloids, carbohydrates, terpenoids, steroids, and flavonoids) have illustrated the restrictive effects on types of microorganisms *in vitro*.[5]

According to WHO report, in the African region and elsewhere, extracts of plants are still useful for treating ailments such as HIV/AIDS, tuberculosis and malaria.[6] In South Africa, though plant based traditional medicine occurs alongside conventional medicine, they remain popular in both rural and urban societies. An estimated 27 million (about 75% of the population) rely on plant based traditional medicine for health-care.[7] Apart from easy accessibility, affordability, safety and efficiency of plant based medicines the practice seems to be culturally relevant with some ailments identified as specifically requiring traditional medicine. It has been reported that South Africa possesses about 30 000 species which are mostly endemic and an estimated 3 000 of these are regularly used in traditional medicine.[8]

Agathosoma betulina, *Aloe ferox* and *Artermisa afra* are among the most popular and indigenous to South Africa. Their uses have been considered under several commercial applications and they are well assessed plants in the pharmaceutical, natural health and food industries.[8] *Agathosoma betulina* is the most known for its purgative action and as a tropical application to the skin, eyes and mucous membranes. Its gel has potent antiflammatory, antibacterial, antiseptic and antifungal properties enabling it to act as a natural antibiotic.[9-11] *Aloe ferox* is used widely for arthritis,

eczema, sinusitis, stress and hypertension.[12] *Artemisia afra* is used for the treatment of diabetes, bronchitis, diarrhoea and neuralgias.[13] Its herbal tea has been used as analgestic, antibacterial, antispasmodic and haemostatic agents in fork medicines.[14]

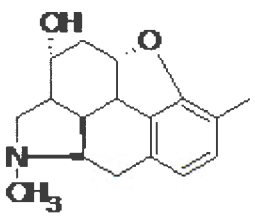
Agathosoma betulina[15]

Aloe ferox [16]

Artemesia afra[17]

Figure 1.1: Common South African indigenous medicinal plants.

Initially the medicinal use of plants was restricted to direct use as herbal mixtures, teas or functional foods.[6] The isolation of morphine (**1.1**) as a pure natural product in 1826 from the opium poppy *Papaver somniferum*, gave rise to the discovery in 1832 of codeine.[18] Historically, morphine has been used as an analgesic initiated drug development from plants, which generally remarks the value of indigenous knowledge as a starting point for the discovery and isolation of pure bioactive compounds. Since then a number of pure compounds have been isolated from plants and used as prescription drugs or analogues for the development of pharmaceutical drugs. At the start of the 21st century, an estimation of 11% of the 252 essential and basic drugs were of plant origin. Some examples of important derived pharmaceutical drugs in use today are shown in **Figure 1.2** which include morphine, codeine (**1.2**), atropine (**1.3**) and quinine (**1.4**).[19] Codeine has the ability to suppress chronic coughing and diarrhoea. Atropine was discovered in 1809 and isolated in 1831 from *Atropa belladonna*, which is used to treat some poisoning and muscle spasms of the gallbladder or urinary system. The discovery of quinine is considered the most unexpected medicinal discovery of the 17th century. In 1820, quinine was extracted from *Cinchona* bark that consists of medicinal properties of treating malaria and associated febrile states.



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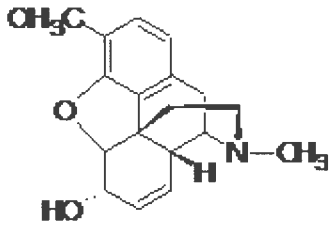

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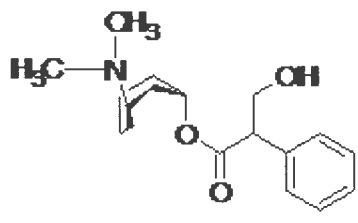

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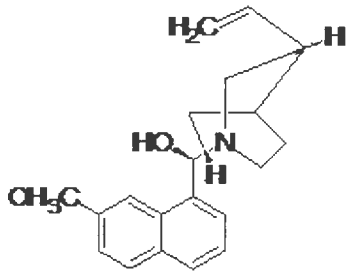


1.4

Figure 1.2: Some of early plant-derived malaria drugs.

Globally, traditional healers are using various medicinal plants for treating of malaria; however this practice is not really recognized by modern medicine. A wide variety of plants belonging to several families have been known through ethnobotanical and ethnopharmacological studies as antimalarial medicinal plants.[20] As a result of minimal scientific knowledge to endorse antimalarial properties of these medicinal plants, it is essential that their attested antimalarial properties are evaluated, fitting to initiate their efficiency and regulate their probity as sources of new antimalarial drugs.[8]

Malaria is a deadly blood disease, which is caused by parasites and is transmitted to humans by *Anopheles* mosquito. These parasites can multiply in the host's liver before infecting and destroying red blood cells once bitten by Anopheles mosquito. However, the disease can be controlled and treated if diagnosed early on. Sadly, this is not achievable in some areas of the world lacking medicinal facilities, where malaria outbreaks occur. The experience over the past decades have convincingly proved that conquering malaria is difficult. No one foresees a quick victory in fighting malaria even if new malaria drugs hit the market, or a vaccine substantiates highly promising. Instead, researchers and health planners expect their best chances to lie in many sides attack, drawing upon a variety of weapons suited to local environments. Skilfully combined several approaches, both old and new may at last make it possible to outmanoeuvre these persistent and deadly parasites.[21]

With the increasing levels of antimalarial drug resistance, the herbal knowledge of indigenous communities for malaria treatment can play an important role in the identification of any new antimalarial plant that is yet to be discovered.[21]

Drug research is older than a century as an interdisciplinary venture with an industrial base. The research profession in drug discovery began when chemistry had attained a level of full growth that permitted its problems and methods to be applied to complications outside chemistry itself and when pharmacology has become a well-defined scientific discipline in its own right.[22] There is an

estimation of 250 000 plant species worldwide and each plant producing hundreds or thousands of structurally diverse compounds. The potential of plants as sources of novel compounds are not possible to match. The advances in spectroscopic and chromatographic techniques used in the isolation of chemicals from plants further broaden their pharmaceutical potential.[23] Nine local medicinal plant species belonging to different families were selected for investigation. These plants can be a possible source for the development of new antimalarial drug.

1.2. Research aim

The main aim of this research was to perform phytochemical studies on selected medicinal herbs with potential antimalarial properties.

1.3. Research objectives

The main objectives of the study were:

- To perform preliminary phytochemical testing for determinations of the class of secondary metabolites present;
- To screen the medicinal plants for antimalarial activity using parasite lactate dehydrogenase assay;
- To prioritize a limited number of screens for further investigation based on the screening tests;
- To fractionate the active extract using column chromatography and isolate the active compounds;
- To determine the structures of the active compounds using different techniques: ^{1}H NMR), ^{13}C NMR, FTIR and MS.
- To screen the pure isolated compounds for antimalarial activity.

CHAPTER 2

Literature review

2.1. Malaria and its impact

Malaria, like febrile illnesses, was discovered since ancient times as fever that was periodic and associated with marshes and swamps. It was not until the 1880s and 1890s that Alphonse Laveran, Ronald Ross, Battista Grossi and others were able to identify the malaria parasite and link the transmission of malaria to mosquitos.[24] Malaria remains the dominant public health issue in many developing regions of the world. It is mainly transmitted in tropical regions such as Asia, Africa and South America, usually during periods of high temperature and rainfall while some transmission does occur outside the tropics. The worldwide distribution of malaria is illustrated in **Figure 2.1**.

Africa has the highest number of cases where over 90% of the deaths due to malaria occur. Malaria mostly accounts for childhood deaths. The disease also imparts much to anaemia among children, and is a vital cause of poor growth and development.[25,26]

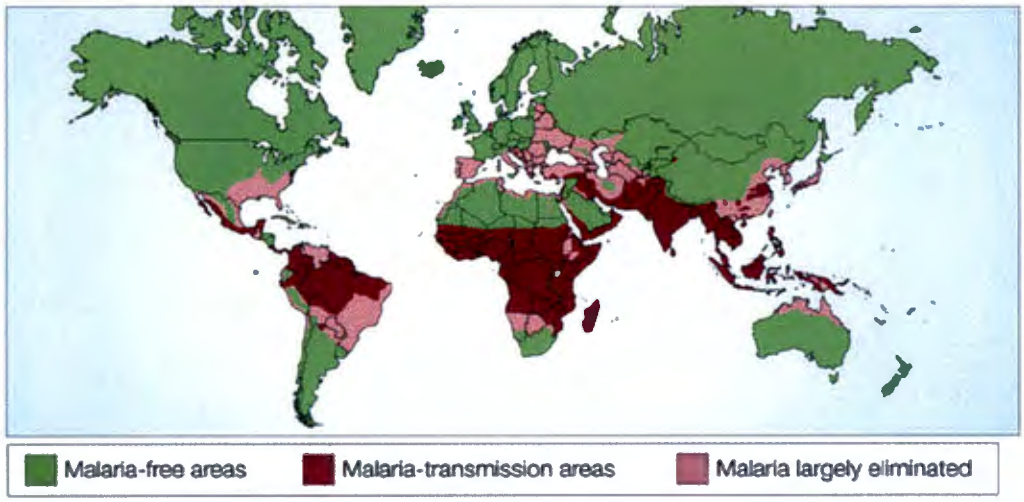


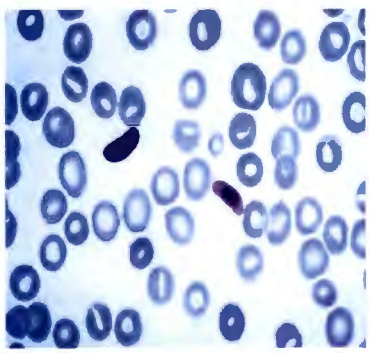
Figure 2.1: The distribution of malaria.[27]

Africa is facing serious economic impacts due to malaria as the costs of malaria are infinite when measured in economic terms and therefore obstruct overall economic development.[28] Traditional estimates have looked at some of the short-run costs of malaria without considering the long-term effects of malaria on economic growth and development. Malaria may hinder the flows of trade, foreign investment and commerce, by that affecting a country's entire population.[26]

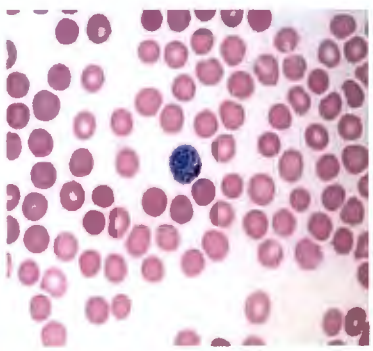
The infected protozoan parasites belonging to the genus *Plasmodium* by female *Anopheles* species mosquitoes cause malaria.[29] They fabricate malaria in animals and birds, as well as humans. Four types of *Plasmodium* species often vitiate human beings, namely, *Plasmodium falciparum*,

Plasmodium vivax, *Plasmodium malariae* and *Plasmodium ovale*. Each one has a particular appearance under the microscope and all produce different patterns of symptoms. Two or more species can live in the same stretch and infect a single person at the same time. The four species have been sequenced and they all have genomes of about 25 megabase organized into 14 chromosomes. The chromosomes vary in length from 500 kilobase to 3.5 megabase and it is presumed that this is the pattern throughout the genus.[30] The species are presented in **Figure 2.2**.

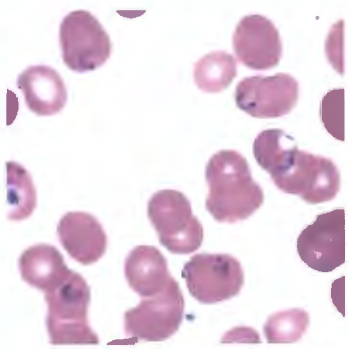
- *Plasmodium falciparum* is accountable for most fatalities, usually in the African region. The infection can develop suddenly, resulting in a production of several life-threatening complications such as kidney failure, fluid in the lungs and severe infection of the brain.[31]
- *Plasmodium vivax* is nearly all of spatially widespread species, which produce less frightful symptoms. Lapses can occur for up to three years.[32]
- *Plasmodium malariae* infections do not only fabricate predictable malaria symptoms but they can also endure in the blood for a lengthy spell without ever producing symptoms. A person with asymptomatic *Plasmodium malariae* can be infectious through blood donation or mosquito bites.[33]
- *Plasmodium ovale* is infrequent, it can establish clinical deterioration and normally exists in West Africa.[34]



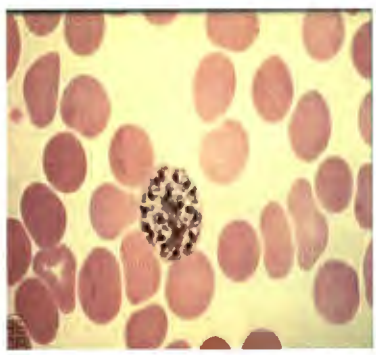
Plasmodium falciparum[35]



Plasmodium vivax[36]



Plasmodium malariae[37]



Plasmodium ovale [37]

Figure 2.2: *Plasmodium* species which often infect humans.

2.2. Malaria life cycle

The malaria parasite unveils a multiplex life cycle involving an insect and a human, which is demonstrated in **Figure 2.3**. The sporozoites are spread by a bite of infected female *Anopheles* mosquitoes to the vertebrate host. The sporozoites pierce hepatocytes just after inoculation into the blood circulation. This procedure has revealed that sporozoite invasion of hepatocytes entails surface proteins of the sporozoite during the 5-15 days depending on the *Plasmodium* species. *Plasmodium vivax, Plasmodium ovale and Plasmodium vivax* have an inoperative stage, named hypnozoite, that may remain in the liver for weeks to many years before the development of Preerythrocytic schizogony. This gives rise to the degeneration of malaria infection.[38]

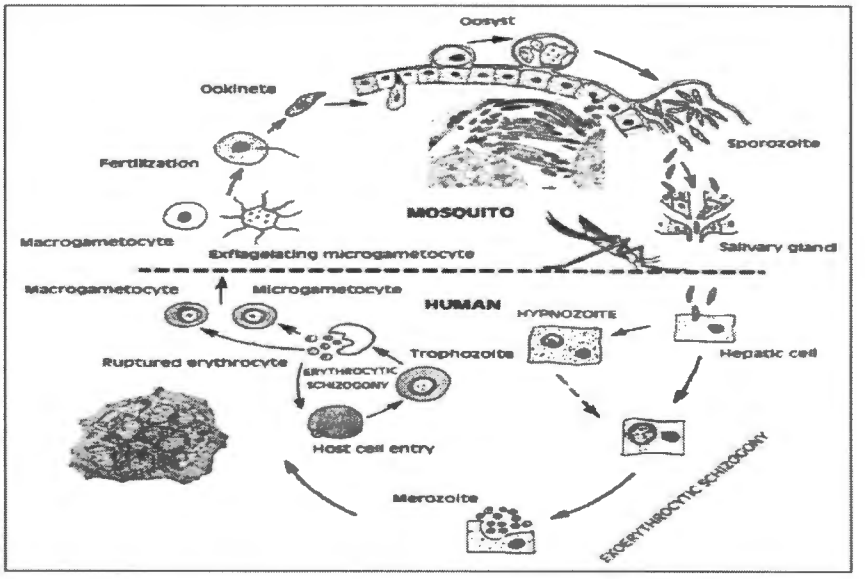


Figure 2.3: Illustrative diagram of the life cycle of malaria.[39]

The erythrocytic stages of malaria have various significant effects in clinical practice which is the only stage that causes the composition and diverse range of symptoms, as a result of characterized disease in human parasites recognized in the blood of a patient which allows the diagnosis of the infection and several species of the causing agent are differentiated. The necessitated time to complete the erythrocytic cycle is a define characteristic of the parasite species. *Plasmodium falciparum* and *Plasmodium vivax* have 48 hour development period, in *Plasmodium ovale* the cycle lasts up to 50 hours while *Plasmodium malariae* has a spun-out cycle of 72 hours.[40]

The zygote evolves into a motile ooikinetes that probes the midgut epithelial and matures into an oocyst. The oocyst undergoes through multiple rounds of asexual replication resulting in the production of sporozoites into the body cavity of the mosquito. The sporozoites roam to and invade the salivary gland, thus completing the life cycle. [41]

2.2.1. Symptoms of malaria

Malaria can be categorized into two categories: uncomplicated or complicated. Malaria is curable when it is promptly diagnosed and treated. The symptoms of malaria are linked with the erythrocycle stage because of the waste and toxins caused by the destruction of the red blood cell by *Plasmodium.* Malaria is normally diagnosed by the presence of *Plasmodium* in the patient's blood.[42]

Malaria is considered uncomplicated when symptoms are present but there are no clinical signs of severity. Malaria prodromal can arise as quickly as a week after infection or later after some months.[43] The attacks begin with prime side effects like shivering and fainting in children whilst their temperatures return to normal. This attack is persuaded by an asymptomatic period rearmost at 6-10 hours.[44] Tertian, together with quartan periodicities are associated with classical attacks. In tertian attacks, the symptomatic stages occur every second day. These attacks are caused by *Plasmodium vivax* and *Plasmodium ovale.* In the quartan attacks, the symptomatic stages occur every third day. *Plasmodium malariae* is the cause of periodicity.[42]

Complicated malaria is defined by the tangled infections as a result of serious organ relapses in the patient's metabolism and it is contemplated as a medical crisis in demand of an instant treatment.[44] Malaria is a threat to expectant women and infants/toddlers as they have a greater risk of evolving complicated malaria. *Plasmodium falciparum* infections are the cause of complicated malaria. Complicated malaria is normally complicated and has various crucial bacterium symptom like as drastic renal failure, serious respiratory distress syndrome, shock and high fever.[45]

2.3. The history of antimalarial drugs

Regardless of the fact that comprehending of mosquito cycle conducted new approaches in vector control in the early 20th century, malaria prophylaxis and therapy sustained to produce on earlier remedies. During the 19th and 20th centuries the western medicinal practice evolved due to pure chemical compounds, and later synthetic drugs gradually replaced herbal remedies. The treatment of malaria also experienced important scientific developments. Malaria is one of first diseases to be treated by pure chemical compound.[46,47]

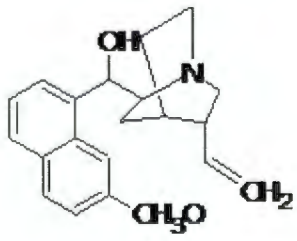
Cinchona officinalis bark became popular for its herbal medicine uses and by the fact that various *Cinchona* species were beginning to be no longer in existance.[48,49] Quinine (**2.1**) naturally in the bark of *Cinchona officinalis* trees, is an alkaloid that acts as a blood schizonticide and weak gametocide against *Plasmodium vivax* and *Plasmodium malariae*. It has a rapid schizonticidal action against intra-erythrocytic malaria parasite and it is also used in the treatment of acute cases for complicated *Plasmodium falciparum*.[47]

The most recent phytochemical agents were isolated from *Artemisia annua* and biologically characterized to show antimalarial potency, with artemisinin (**2.2**) having antipyretic properties.[50] Artemisinin reveals great potential in being able to treat resistant *falciparum* malaria and it can be used for uncomplicated malaria if the patient is able to take medication orally. It has been indicated as the fastest clearance of all antimalarial agents currently used and acts primarilly on the trophozite phase, hence averting further progression of the disease.[51] Certainly, what is interesting about malaria fevers is that two herbal treatments, cinchona bark and *Artemisia anuua*, presented in **Figure 2.4** were used to treat malaria adequately for century of years precedent to the understanding of the mosquito cycle.[24]

Cinchona bark[52]

Artemisia annua[53]

Figure 2.4: Early plant species for malaria treatment.



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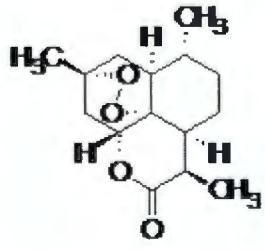


(2.2)

Figure 2.5: Prime important compounds used to control malaria.

Artemisinins are the most heady and fast antimalarial drugs accessible. They eliminate parasites resistant to other drugs. Artemisin therapy roots to a depletion in parasite load of larger than 90 percent within 24 hours.[54] WHO standards now endorse combination therapy of artemisinin with other antimalarial drugs with longer half-lives; known as the artemisin-based combination treatments (ACTs) for uncomplicated *Plasmodium falciparum* malaria.[55]

Artemisinin and its derivatives are broadly used in every part of the world. The process of action of these compounds appear to comprise the heme-mediated decomposition of the endoperoxide bridge to yield carbon-centred free radicals. The incorporation of heme explains why the drugs are

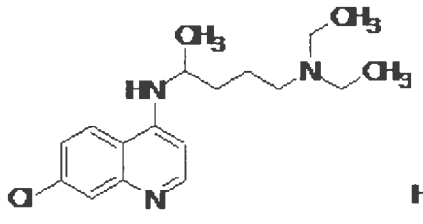
selectively toxic to malaria parasites.[56] The resulting carbon-centred free radicals are alkylate heme and proteins, one of which is the transnationally controlled tumour protein. Clinically, relevant artemisinin resistance has not been denoted but it is fit to occur since artemisinin resistance has been obtained in laboratory models.[57]

2.3.1. Treatment based on mechanism of action

Malaria is preventable, treatable and curative tools have been developed. Great progress has been made in the past years. Malaria death rates, which take into account population growth are estimated to have decreased by 60 percent globally across all age groups between 2000 and 2015. In the African region, malaria mortality rates decreased by 66% across all age groups and by 71% in children under 5 years of age. However, more of improved antimalarial drugs are needed to save lives.[58]

Many ancillary treatments have been suggested and tried in complicated malaria, but none have been shown unequivocally to affect outcome only antipyretics, anticonvulsants and exchange transfusion have been supported by sufficient evidence to warrant their use. Exchange transfusion should be performed if there are adequate facilities, the patient is seriously ill, and parasitemia exceeds 15 percent. [59]

Quinolone compounds are a family of synthetic broad spectrum antibiotic drugs which have been isolated from natural sources and can act as natural antimalarials.[60] In spite of the fact that most of the current research work points on identifying new drug targets, researchers have minimal mastery of the mechanism of action and the entire resistance mechanism to the quinolone compounds.[61] Artemisinin (**2.2**), chloroquine (**2.3**) and sulfadoxide/ pyrimethamine (**2.4**) drugs have been validated as primary medications against *Plasmodium*.[62] Nonetheless, the degree at which mutations conferring resistance occur is low in other medications like pyrimethamine and atovaquone (**2.5**). Halofontrine (**2.6**) was recognized in the 1940s and was not progressed until the 1980s. Very few new antimalarial drugs are undertaking clinical trials.[59]



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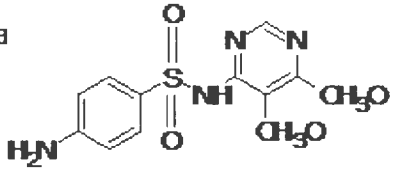

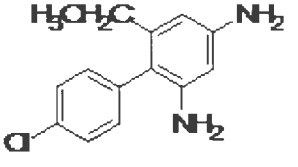

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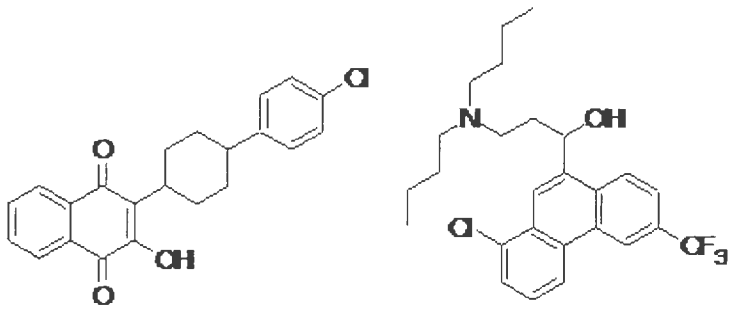


(2.5) (2.6)

Figure 2.6: Primary drugs against *Plasmodium*.

There has been much reduction by the ovulation and spread of chloroquine resistant malaria parasites due to clinical usefulness of chloroquine and, in some recent cases of quinine as well. The resistance mechanism include a reduced accumulation with possible explanations of energy-dependant efflux of preaccumulated drug via an unidentified transmembrane protein pump or an increase in vascular pH such that the protein gradient responsible for drug concentration is reduced.[63]

Combination therapy is considered the best for malaria management, which is the concurrent use of the two or more blood schizontocidal drugs with liberated modes of action and different biochemical targets in the parasites. The choice of the second drug will rely on resistance, cost, side effects profile and efficiency. The parasite's resistance to drugs such as artemisinin chloroquine combination therapies led to the new developments that are more effectual, with fewer side effects and affordable.[30] Thus it is crutial that the new drug targets are known and that the mode of action for documented antimalarial are clear so that compounds with improved effectiveness can be developed.[64]

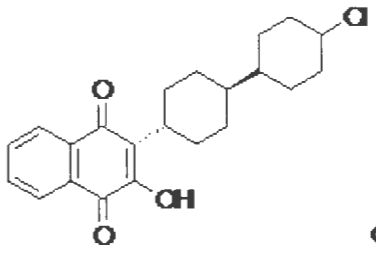
Malaria resistant parasite surface from some factors and elements inclusive of the exploitation of antimalarial chemoprophylaxis, poor adherence dosing regiments, a great extent of parasite and a huge parasite escalation rate. Poor manufacturing practices, counterfeiting or drug deterioration through poor storage or handling results in poor drug quality, which accelerates drug resistance. [65] Since many regularly used antimalarial drugs are chemically similar, the resistance growth to one has the ability to ease the evolvement of resistance to others.[66] Antimalarial expands since the resistant parasites have a greater endurance rate in the presence of the antimalarial than the sensitive parasites and sadly leads to the transmission of the resistant rather than the sensitive parasites. [67]

The purpose of WHO antimalarial treatment approach is to reduce the disease rate resulting in deaths via rapid and intact cure of infection, reduce the term of malaria infection during pregnancy

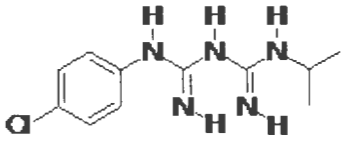
and limit or rather suppress the transmission of malaria. Malaria reign has two sectors composed of malaria prevention and case management. Malaria prevention comprises of mosquito vector control through removal of mosquito breeding sites, use of insecticides and human contact prevention by the use of screen and bed nets.[68]

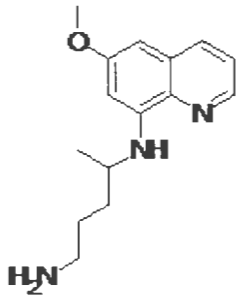
2.3.1.1. Casual prophylactics

Drug chemoprophylaxis has shown to be a potent preventive strategy in travellers to malaria endemic areas, both for *Plasmodium falciparum* and non-*falciparum* malaria; despite that it does not usually prevent the later relapses that can occur with *Plasmodium vivax* and *Plasmodium ovale*.[69] Drugs can act on different stages of the *Plasmodium* biological cycle, on the pre-erythrocytic liver forms and on the erythrocytic blood forms. Drugs in primary prophylaxis with causal activity include atovaquone/proguanil (**2.7**) or primaquine (**2.8**) that can be continued for a shorter time (seven days).[70]



(**2.7**)



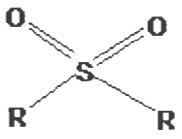


(**2.8**)

Figure 2.7: Casual prophylactics drugs.

2.3.1.2. Clinical curatives

Blood schizonticides are most important drugs in antimalarial chemotherapy and must be started as soon as the diagnosis is made or even suspected in severe disease. These drugs act on the blood forms of the parasite and thereby terminate clinical attacks of malaria.[71] These include chloroquine, quinine, halofantrine, pyrimethamine/sulfadoxine and sulfones (**2.9**).[72]



(**2.9**)

Figure 2.8: Clinical curatives drug.

2.3.1.3. Radical treatment

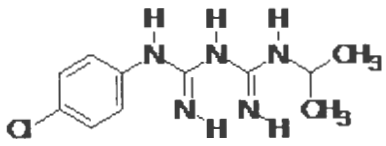
Primaquine is essentially used to avert the relapse of malaria due to *Plasmodium vivax* and *Plasmodium ovale*.[73] It eliminates hypnozoites that is dormant liver form of the parasite, after the *plasmodia* have been cleared from the bloodstream.[74] If primaquine is not administered to patients with proven *Plasmodium vivax* or *Plasmodium ovale* infection, a very high likelihood of relapse exists for weeks, months or sometimes years. The use in combination with quinine or chloroquine each of which is very effective at clearing *Plasmodium vivax* from blood, improves outcomes; they appear to also potentiate the action of primaquine.[75]

2.3.1.4. Gametocidal drug

Antimalarial drugs kill the asexual parasites responsible for causing the disease and some also kill the sexual transmission stages known as gametocytes. Gametocytocidal drugs destroy sexual forms in human, decreasing transmission.[76] Chloroquine and quinine have gametocytocidal activity against *Plasmodium vivax* and *Plasmodium malariae*, but not against *Plasmodium falciparum*. Primaquine has gametocytocidal activity against all plasmodia, including *Plasmodium falciparum*.[77]

2.3.1.5. Sporontocides

When given to a gametocyte carrier, they prevent the development of oocysts in mosquitoes feeding on that carrier. They therefore prevent the formation of sporozoits and thereby transmission of the disease.[78] Drugs with such action have also been called antisporogin drugs, which may or may not eliminate gametocytes from the bloodstream. Primaquine and proguanil (**2.10**) have this action, **Figure 2.9**.[79]



(2.10)

Figure 2.9: Sporontocides drug.

2.3.2. Treatment based on the mode of action

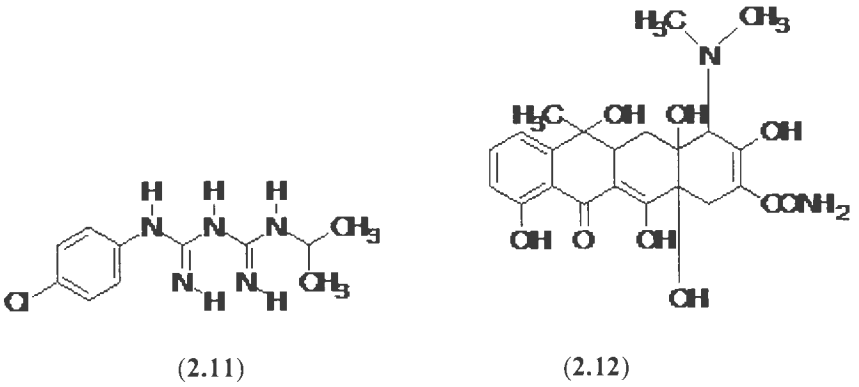
2.3.2.1. Antifolates

The antifolates set of antimalarial drugs was found in the 1940's. They possess the capability to prevent folate metabolism and thereby distorting the biosynthesis of amine acid and nucleotides. Speedily dividing cells, being cancer cells utilizing exogenous folates and rely on folates biosynthesis. Atovaquone is an effectual antimalarial drug though it is linked with recrudescence rates and reduced parasite susceptibility following treatment. Proguanil is dehydrofolate reductase

inhibitors with weak antimalarial activity, but it elaborates synergistic activity *in vitro* with atovaquone.[80]

2.3.2.2. Antibiotics

The term antibiotic is defined as any compound that has been used to treat bacterial infections and their echos developed if they were active against *Plasmodium falciparum*.[81] Lots of antibiotics like the tetracycline, lincosamides and macrolides category, have antimalarial activities. Antibiotics are used only in combination therapy with other antimalarial drugs because their parasitological response is slow and are not highly active against the malaria parasite. Doxycline (**2.11**) is a semi-synthetic derivative of tetracycline (**2.12**), presented in **Figure 2.10** was first developed in 1967.[82] Doxycline is especially useful as a prophylaxis in areas with chloroquine and multidrug-resistant *Plasmodium falciparum* malaria. It can be used by travellers to all malaria-endemic areas for malaria prophylaxis. When used in conjunction with other medication, doxycline can also be used to treat malaria.[83]

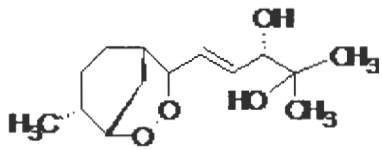


(**2.11**) (**2.12**)

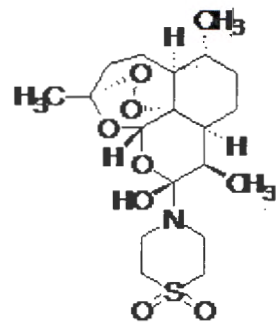
Figure 2.10: Antimalarial antibiotics.

2.3.2.3. Peroxides

The discovery of artemisinin has opened new approaches for combining malaria. Since early 80's hundreds of synthetic peroxides have been progressed and tested for their antimalarial activity.[84] Peroxides have remarkable antimalarial activities, illustrated in **Figure 2.11**. Yingzhaosu (**2.13**) is extracted from Chinese medicinal plant. This compound has become essential antimalarial drug with effective liveliness against the multidrug-resistant strains of *Plasmodium falciparum* malaria. Artemisone (**2.14**) is a new promising artemisinin derivative and health benefits of decosahexaenoic acid in terms of activity and safety.[54]



(2.13)



(2.14)

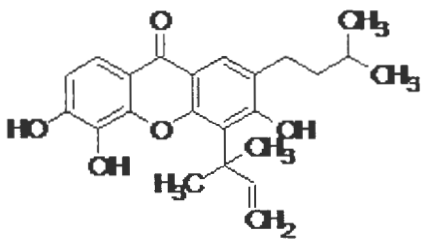
Figure 2.11: Antimalarial peroxides.

2.4 The promise of natural products

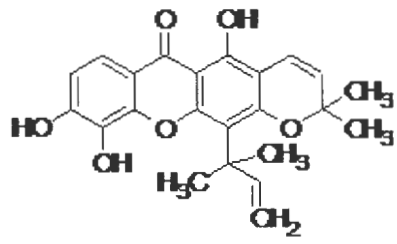
The African continent abounds in flowery biodiversity and its plant materials are enriched with natural products having fascinating chemical structures and promising biological activities. Based on this historically high success rate among natural products, the diversity of chemicals found in nature continues to be an important source of molecular templates in the search for new and novel antimalarial drugs. It is believed that the next generation of antimalarial of their composite may be established in plants currently used in African traditional medicine.[85] Numerous natural products with antimalarial activity have been discussed in the literature and their structures are elaborated.

2.4.1. Phenols

Phenols have a wide natural applications in different fields like medicines. Many phenolic compounds occur in nature and are used in the manufacturing of perfumes and flavours because of their pleasant odour.[86] Generally, phenolic compounds have strong antiseptic and antibacterial properties which act as nerve stimulants. Phenolic compounds shown in **Figure 2.12**, gerontoxanthone I (**2.15**) and macluraxanthone (**2.16**) were isolated from *Cassieae maingay* which were reported for the first time as metabolites. These compounds have the activity against malaria, with an IC_{50} of 0.20 and 0.22 μg/mL respectively.[87]



(2.15)



(2.16)

Figure 2.12: Antimalarial phenols.

2.4.2. Alkaloids

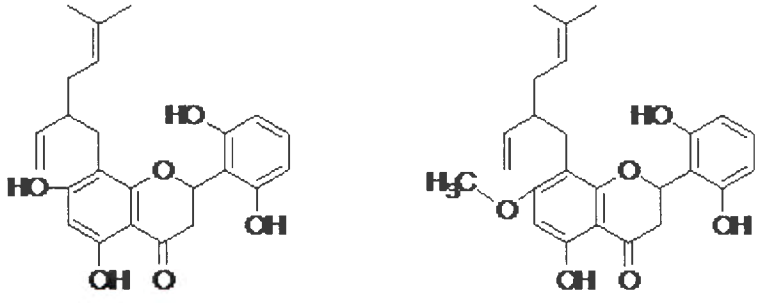
Alkaloids are one of the great classes of compounds having antimalarial activity. In fact, one of the oldest and most vital antimalarial drugs, quinine belongs to this class of compounds and is still relevant today.[3] The antimalarial activity of plant extracts was evaluated on the strains of *Plasmodium falciparum* maintained in continuous culture. The antimalarial activity of extracts was measured by the IC_{50}, representing the concentration of the drug that induced a 50% parasitaemia decreased compared with the control culture. The chloroform extract of *Guiera senegalensis* and methanol extract of *Feretia apodanthera* were fractionated. The antimalarial activities of revealed **Figure 2.13** harman (**2.17**) and tetrahydoharmine (**2.18**), are the highest activities with an IC_{50} lower than 4 µg/mL.[88]

(**2.17**) (**2.18**)

Figure 2.13: Antimalarial alkaloids.

2.4.3. Flavonoids

Flavonoids are any of a immense class of plant pigments having a structure based on or similar to that of flavone. The exact mechanism of antimalarial action of flavonoids is unclear but some flavonoids are shown to impede the influx into infected erythrocytes.[89] **Figure 2.14** illustrates Exiguaflavanone A (**2.19**) and Exiguaflavanone B (**2.20**), from *Artemisia indica* which revealed *in vitro* antiplasmodial activities (IC_{50}= 4.6 and 7.0µg/mL, respectively). Exiguaflavanones are drugs used in the treatment of malaria and antimalarials are usually classified on the basis of their action against *Plasmodia* at different stages in their life cycle in humans.[90]

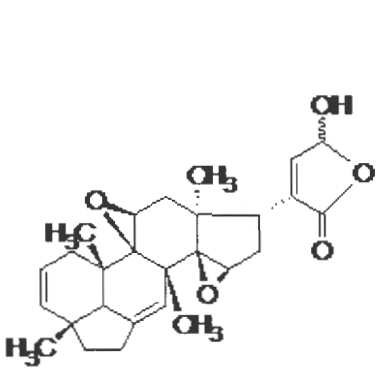


(**2.19**) (**2.20**)

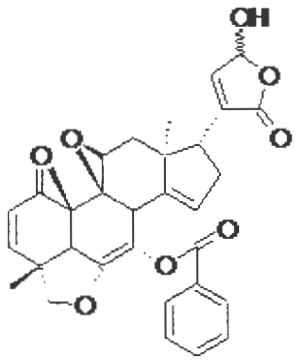
Figure 2.14: Antimalarial flavonoids.

2.4.4. Limonoids

Limonoids are phytochemicals, plentiful in citrus and other plants which are produced by species of Meliaceae. One known representative from this family is *Azarirachta indica*, commonly known as the Neem tree, which is widely used as an antiplasmodial plant in Asia.[91] Two limonoids, trichiburine A (**2.21**) and trichiburine B (**2.22**), illustrated in **Figure 2.15** have been isolated from *Thordisa rubescens* with significant antimalarial activity (IC_{50}= 0.3 and 0.2μg/mL, respectively).[92]



(**2.21**)

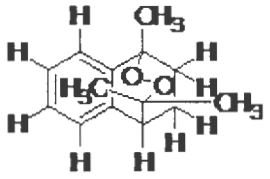


(**2.22**)

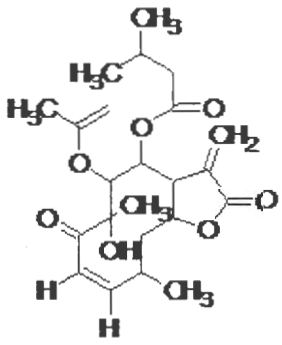
Figure 2.15: Antimalarial limonoids.

2.4.5. Sesquiterpenes

Artemisia annua prompted the analysis of some other naturally occuring peroxides for their schizonticidal activity. Artemisinin is a grade of antimalarials where the endoperoxide moiety plays a significant role. It is the 1,2,4 trioxane ring that is unique in nature and is important for the activity but the definite manner of action of this series of drugs is still not known. The endoperoxide sesquiterpene, 10,2-peroxycalamenene (**2.23**) exhibited strongest effect of *Plasmodia*. It was demonstrated in neurolenin B (**2.24**) that an unsaturated keto function is one of the structural requiremnets for high *in vitro* antiplasmodial activity.[93] **Figure 2.16** shows the antimalarial compounds with strong effect of *Plasmodia*.



(2.23)

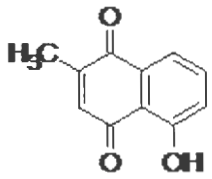


(2.24)

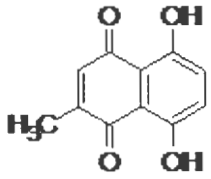
Figure 2.16: Antimalarial sesquiterpenes.

2.4.6. Quinones

Chemically, quinones are compounds with 1,4-diketo-cyclohexa-2,5-dienoid or a 1,2-diketocyclohexa-3,6-dienoid moiety. The structure of many naturally occurring quinones is grounded on the benzoquinone, naphthquinone or anthraquinone ring system. Naphthoquinones are fairly promising as blood schizonticides, since they are highly active against *Plasmodium falciparum in vitro*.[94] Roots of *Nepenthes thorellic* yielded plumbagin (**2.25**) and 2-methylnaphthazarin (**2.26**), displayed in **Figure 2.17** both of which were evaluated against *Plasmodium falciparum*, as a result they are active against *Plasmodium falciparum*.[95]



(2.25)



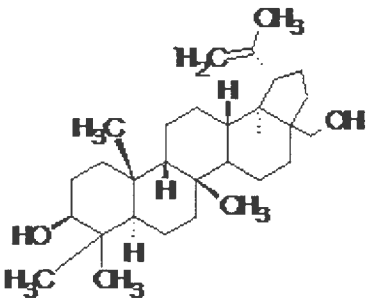
(2.26)

Figure 2.17: Antimalarial quinones.

2.4.7. Saponins

Saponins shown in **Figure 2.18** are a large family of amphiphilic glycosides of steroids and triterpenes found in plants and some marine organisms.[96] *Schefflera umbellifera* is a semi-decidous tree, widely distributed in Malawi. Its leaves have been used traditionally to treat rheumatism, colic, insanity and for malaria a bark is drunk.[97] The diclhoromethane/ methanol extract of leaves *Schefflera umbellifera* exhibited good antimalarial activity (IC_{50} =5μg/mL) when tested against the chloroquine-susceptible strain. From the compounds isolated, butelin (**2.27**) exhibited significact *in vitro* antimalarial occurancy (IC_{50} =3.2μg/mL) against *Plasmodium falciparum* chloroquine-suscepitible strain. Ent-kaur-16-en-19-ioc acid (**2.28**) was isolated fiom a Zataria multiflora plant

family has been tested against *Plasmodium falciparum* strains and showed moderate activity (12μg/mL).[98]



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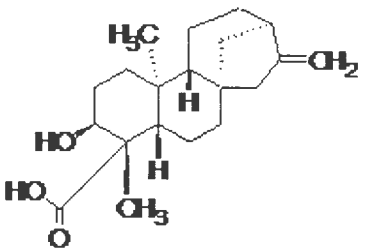
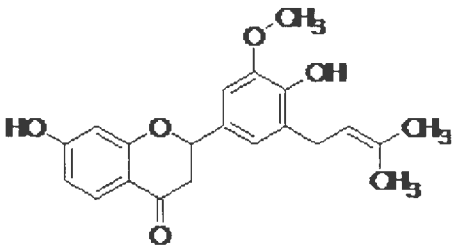


(2.28)

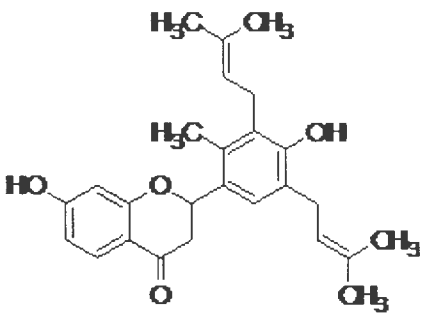
Figure 2.18: Antimalarial saponins.

2.4.8. Flavonones

The flavanones 5-deoxyabyssinin II (**2.29**), abyssinone IV (**2.30**) as shown in **Figure 2.19**, were isolated from the stem bark of *Erythrina abyssinica*.[99] The investigations by Yenesew *et al.* demonstrated that these compounds exhibited active antimalarial properties against the W2 and D6 strains of *P. falciparum* with IC_{50} values varying from 4.9 to 13.6 μM against the latter strain and from 5.9 to 13.3 μM against the former strain.[100]



(2.29)



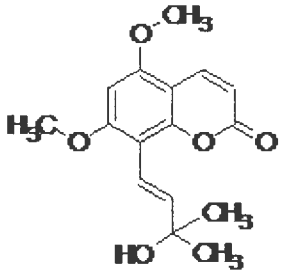
(2.30)

Figure 2.19: Antimalarial flavonones

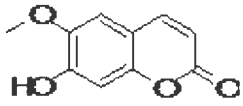
2.4.9. Coumarins

Oketch-Rabah *et al.* isolated a new antimalarial coumarin, 5,7-dimethoxy-8-(30-hydroxy-30-methyl- 10-butene) coumarin (**2.31**), from the roots of *Toddalia asiatica*[101]. This compound showed moderate activity against the chloroquine-sensitive K39 and chloroquine-resistant V1/S strains of *P. falciparum* strains, with IC_{50} values of 16.2 μg mL^{-1} and 8.8 μg mL^{-1}, respectively. The antimalarial coumarin 7-hydroxy6-methoxycoumarin or scopoletin (**2.32**) was isolated from

the dichloromethane leaf extract of *Schefflera umbellifera* (Araliaceae), harvested from Limpopo, South Africa.[102] This compound was evaluated *in vitro* against both the chloroquine-susceptible (D10) and chloroquine-resistant (K-1) strains of *P. falciparum* for anti-malarial activity, with an IC_{50} value of 28.2 μg mL^{-1}.[103] The structures (**2.31**) and (**2.32**) are illustrated in **Figure 2.20**.



(**2.31**)



(**2.32**)

Figure 2.20: Antimalarial coumarins

2.5. Conclusion

Plants species, with antimalarial activity, are wildly distributed in nature. Selected medicinal herbs were collected and their extracts were screened for phytochemicals. Also, the *in vitro* screening for antimalarial occupancy of nine plants used in traditional pharmacology was performed. The results of the screenings are presented and discussed in Chapter 3.

CHAPTER 3

Phytochemical screening and antimalarial activity of selected medicinal herbs

3.1. Introduction

Pharmacological plants have bioactive composites used for restoring different human diseases and play a vital role in healing. The phytochemical analysis of the plants has exceptional concern in medicament sectors for the assemble of new drugs for healing varied diseases, therefore it is important to use the phytochemical methods to screen and analyse bioactive components not only for the quality control of crude drugs but also for elucidation of the therapeutic mechanism.[104,105]

Malaria is a prime parasite illness in the world, especially in the African region.[106,107] With the rising problems of side effects and limited efficacy of antimalarial drugs, there is an urgent need for the development of alternative antimalarial substances and researchers are turning to natural products from plants as their main source of bioactive compounds with antimalarial properties to complement the existing synthetic antimalarials that are gradually becoming less potent against pathogenic parasites. Hence there is a need for the phytochemical screening and investigations into the antimalarial potential of plant extracts as it is claimed to have antimalarial activities.[108]

Plasmodium falciparum is the most widespread agent for human malaria which has become increasingly resistant to standard antimalarials such as peroxides and antifolates. Quinolines, peroxides, flavonoids, saponins and alkaloids have been found to possess antimalarial activity.[109] However, new drugs or drug unions are essentially entailed today for the reception of malaria. *In vitro* screens for activity constitute a key component for antimalarial drug screening. It is based on the ability to culture *Plasmodium falciparum* in human erythrocytes *in vitro*.[110] In this present work, nine plants were evaluated for the phytochemical screening and the *in vitro* antimalarial occupancy of their applications in traditional medicine against malaria. **Table 3.1** summarizes the selected plants and their traditional uses.

Table 3.1: Selected medicinal plants.

Plant name	Family	Uses
1. *Lavandula x intermedia*	Limiaceae	Treats burns, rashes, cold sores blisters and bruises.[111]
2. *Artemisia afra*	Asteraceae	Clears blocked nasal passages, used as body washes and lotions[112], also employed for treating coughs, loss of appetite, gout, asthma, malaria, kidney disorders and diabetes.[113]
3. *Rosmarinus officinals*	Limiaceae	Herb of memory[114], cures jaundice[115], used as an ointment to

		treat rheumatism sores.[116]
4. *Cymbopogan citratus*	Gramineae	Relief in digestion[117], muscle cramps[118], headaches and nausea.[118]
5. *Verbascum Thapsus*	Scrophulariceae	Used as domestic remedy for fever, allergies, migraine[119] and treats diarrhoea.[120]
6. *Pelargonium graveolens*	Geranaceae	Treats frequent diarrhoea[121], stops abdominal bleeding including that related to menstruation[122] and uterine problems.[123]
7. *Foeniculum valgare*	Umbelifererea	Relief from digestion, diarrhoea[124], respiratory disorders [125], menstrual disorders.[126]
8. *Lippia citrodora*	Lippi	Used for joint pain[127], trouble sleeping, asthma and chills.[128]
9. *Catharathus roseus*	Apocynaceae	Treats high pressure[129], diarrhoea, sore throat, toothache and intestinal pain.[130]

3.2. Experimental

3.2.1. Plant material

The present research included plant species which were *Artemisia afra, Rosmarinus officinalis, Foeniculum vulgare, Cymbopogan citratus, Verbascum thapsus, Lippia citriodora, Pelargonium graveolens, Cantharathus reseus and Lavundula x intermedia.*

3.2.2. Plant collection

Nine medicinal plants were collected locally from a medicinal herbs specialist in Unit 3 Mmabatho, Mr Sehlare. The plants are used medicinally for different ailments. These plants were identified botanically by Ms Botha Ndlovu from the South African National Biodiversity Institute (SANBI) (012 843 5000).

3.2.3. Plant extraction for phytochemical screening

After collection, all plants materials were put on top of newspapers to dry at room temperature in an open air laboratory, away from direct sunlight. The dry plants materials were then grounded to a fine powder using an electrical grinder. The powdered plants materials were stored in tightly closed glass bottles in the dark at room temperature.

The dry fine powdered plant materials were sequentially extracted with solvents of increasing polarity: petroleum ether, chloroform, ethyl acetate, ethanol and acetone. 2.5 grams of each dry sample were extracted sequentially with 75 mL of each of the five solvents. The mixtures were shaken gently and left at room temperature for 24 hours. Extraction solutions were filtered with Buchner funnel and the filtrates were placed inside the round bottom flask of the rotary evaporator in order to remove the excess solvent in each extract using distillation method. The temperature

range on the rotary evaporator was between 50-70 °C and the extracts left were placed inside labelled containers to remove organic solvents through evaporation under a steam of air at room temperature in the fume hood.[152] Dry extracts were weighed and extraction yields calculated as follows:

$$\text{Percentage yield} = \left(\frac{weight\ dry\ extract}{weight\ dry\ material}\right) * 100$$

After removal of solvents, percentage yields were estimated and plant extracts were stored in sample bottles in a refrigerator until needed for preliminary phytochemical screening and TLC analysis. The percentage yields of all samples are presented and discussed in the next section.

3.3. Preliminary phytochemical screening for different compounds

It involves testing of different extracts of samples for various phytochemicals by qualitative chemical tests to give general idea regarding the nature of constituents present in crude drugs.[153] The qualitative chemical tests for various phytoconstituents were carried out for all the extracts of all samples and explained as follows:

3.3.1. Test for tannins: ferric chloride test

5mL of the extracts for nine plants were placed inside test tubes and few drops of 0.1% ferric chloride were added. The presence of brownish green or blue black colour indicates the presence of tannins in the sample.[154]

3.3.2. Test for sterols and triterpenoids: salkowski test

In each of 5 mL of the extracts for nine plants that were placed inside test tubes, 2 mL of chloroform and 3 mL of concentrated sulphuric acid were added consecutively, well shaken and allowed to stand a few minutes. The red colour that appeared at the lower layer denotes the presence of sterols and the yellow coloured lower layer indicates the presence of triterpenoids.[155]

3.3.3. Test for flavonoids: alkaline reagent test

Small quantity drops of 1% liquor ammonia were taken in the test tube to which the sample was added. Yellow colouration of the solution confirmed the presence of flavonoids.[156]

3.3.4. Test for glycosides: bromine water test

Add 5 mL of bromine water to the test extract solutions; a yellow precipitate indicates the presence of glycosides.[157]

3.3.5. Test for saponins: foam test

To 10 mL of the sample, 3 mL of distilled water was added and shaken well, so as to obtain froth. To the froth formed, a few drops of olive oil were added. Formation of emulsion signifies the presence of saponins.[158]

3.3.5. Test for cardiac glycosides: kellar – kiliani test

To 5 mL of the sample, 2 mL of glacial acetic acid containing a drop of ferric chloride was added. This was followed by the addition of 1 mL of concentrated sulphuric acid. The brown ring obtained, yielded positive result for the test.[155]

3.4. Plants extraction for antimalarial screening

The dry fine powdered plant materials were sequentially extracted with solvents: acetone, dichloromethane and ethanol. 2.5 grams of each dry sample were extracted sequentially with 75 mL of each of the three solvents. The mixtures were shaken gently and left at room temperature for 24 hours. Extraction solutions were filtered with Buchner funnel and the filtrates were placed inside the round bottom flask of the rotary evaporator in order to remove the excess solvent in each extract using distillation method. The temperature range on the rotary evaporator was between 50-70 °C and the extracts left were placed inside labelled containers to remove organic solvents through evaporation under a steam of air at room temperature in the fume hood.[152] Dry extracts were weighed and stored in the refrigerator until their use in antimalarial assays.

3.5. *In vitro* antiplamsodial assays

All crude extracts were analysed for *in vitro* activity against *Plasmodium falciparum* chloroquine-sensitive strain (3D7) and the most outstanding extracts were evaluated against *Plasmodium falciparum* chloroquine-resistant strain (W2). For each crude extract, a series of 8 threefold dilutions (from 200 to 0.09 µg/mL) was prepared, placed in two row of a 96-well micro plate and tested in a triplicate. After 48 hours of incubation at 37 °C, the level of parasitaemia was determined by measuring lactate dehydrogenase activity. The results were conveyed as the mean IC_{50} (concentration of a drug that reduced the level of parasitaemia of up to 50%) for extracts that showed percentage death greater than 60%.[159]

3.6. Results and discussion

3.6.1. Percentage yields results

Figure 3.1 illustrates the percentage yields of the selected medicinal plants extracts with petroleum ether, chloroform, ethyl acetate and ethanol. The percentage yields (%) by petroleum ether, chloroform, ethyl acetate and ethanol ranged from 87.91 to 100.52, 88.44 to 156.47, 74.64 to 113.76. and 86.76 to 154.63 respectively.

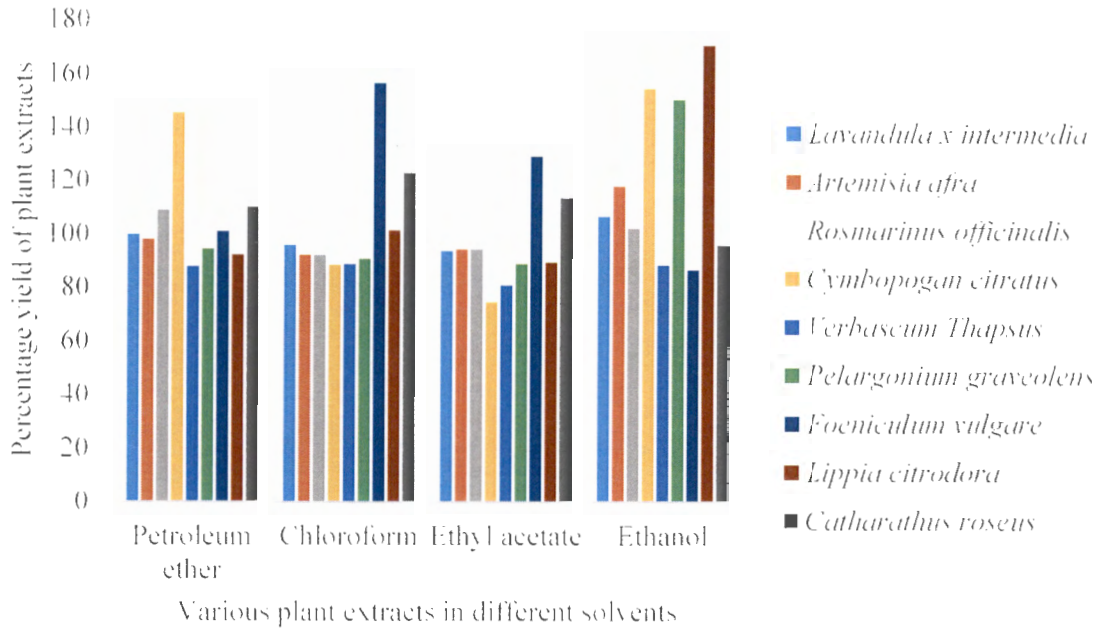


Figure 3.1: Percentage yield of crude extracts of nine selected plants in different solvents.

The calculation in this work suggests that the percentage yields below 100% was expected, because of the residual plant material that was disposed off after filtration and the percentage yields above 100% may be caused by the crudes that were not completely dry, extraneous solvents and impurities.

3.6.2. Phytochemical screening tests results

The plants were selected randomly, according to their availability. The plant extracts of *Lavandula x intermedia* (1), *Artemisia afra* (2), *Rosmarinus officinalis* (3), *Cymbopogan citratus* (4), *Verbascum thapsus* (5), *Pelargonium graveolens* (6), *Foeniculum vulgare* (7), *Lippia citriodora* (8), *Catharathus roseus* (9) were prepared using different solvents and were used for the phytochemical analysis to investigate the phytochemical constituents in all the selected medicinal plants as well as antimalarial screening.

For all the plants selected, the extracts of petroleum ether, chloroform, ethyl acetate, ethanol and acetone were prepared for different plant parts, as shown in **Table 3.2**.

Table 3.2: Plant parts used to prepare extracts with different solvents.

Plant	Plant part	Solvents
1	Leaves and stem	Petroleum ether Chloroform
2	Leaves and stem	
3	Flowers	
4	Leaves	

5	Leaves	Ethyl acetate
6	Flowers, leaves and stem	Ethanol
7	Flowers, leaves and stem	Acetone
8	Flowers, leaves and stem	
9	Leaves and stem	

Phytochemical screen was done on thirty nine extracts using preliminary standard methods. **Table 3.3** shows the results obtained.

Table 3.3: Phytochemical tests results of selected medicinal plants.

Plant	Solvents	Phytochemicals						
		Tannins and phenolics	Sterols	Triterpenoids	Flavonoids	Glycosides	Saponins	Cardiac glycosides
1	Petroleum ether	-	-	-	+	-	-	-
	Chloroform	-	+	-	+	+	+	+
	Ethyl acetate	+	+	-	-	-	+	-
	Ethanol	-	+	-	-	-	-	+
	Acetone	-	-	+	+	-	+	+
2	Petroleum ether	-	-	-	-	-	+	-
	Chloroform	+	-	+	+	+	+	+
	Ethyl acetate	-	+	-	+	-	+	+
	Ethanol	-	-	+	+	-	+	-
	Acetone	-	+	-	+	+	+	+
3	Petroleum ether	-	+	-	-	-	+	-
	Chloroform	-	+	-	+	+	-	-
	Ethyl acetate	-	+	-	+	-	+	-
	Ethanol	-	+	+	-	-	-	+
	Acetone	-	-	-	+	-	+	+
4	Chloroform	-	-	-	-	-	-	+

	Ethyl acetate	+	-	+	-	-	+	-
	Ethanol	-	-	-	+	-	+	-
	Acetone	-	+	-	+	-	+	+
5	Chloroform	-	-	-	-	+	-	-
	Ethyl acetate	-	-	-	-	-	-	-
	Ethanol	-	-	+	+	+	+	-
	Acetone	+	-	-	+	-	+	+
6	Petroleum ether	-	+	-	-	-	-	+
	Chloroform	-	+	-	+	-	+	-
	Ethyl acetate	-	-	-	+	-	+	-
	Ethanol	-	-	-	+	+	+	-
7	Petroleum ether	-	-	+	-	-	-	-
	Chloroform	-	-	-	-	+	+	-
	Ethyl acetate	+	+	-	-	+	+	-
	Ethanol	-		-	-	-	+	-
8	Petroleum ether	-	-	+	-	-	+	-
	Chloroform	-	+	-	+	+	+	-
	Ethyl acetate	+	-	-	+	+	-	+
	Ethanol	-	-	+	-	-	+	-
9	Petroleum ether	-	-	+	-	-	-	-
	Chloroform	+	+	-	+	+	+	+
	Ethyl acetate	-	+	-	-	-	-	+
	Ethanol	-	+	-	+	-	+	-

+ indicates present, - indicates not detected

The phytochemical screening of petroleum ether, chloroform, ethyl acetate, ethanol and acetone crude extracts exhibited the presence of secondary metabolites such as sterols, flavonoids and saponins. The study exhibited mostly the occupancy of phytochemicals regarded as outstanding pharmacological chemical constituents. For example, sterols derived from plants are known to have cardiotonic effect and have antibacterial and insecticidal properties. They are very often used in medicines due to their well-known biological activities.[131]

According to research, tannins are known to have antibacterial, antitumor, and antiviral activities. They work by making nutritional protein unavailable from them.[132,133] Saponins act as antimicrobial activity to cold blood animals. They are used in anticancer, inflammatory and weight loss. Plant phenols are the significant group of compounds acting as free radical scavenging, therefore it is justifiable to determine phenolic content in plant extracts.[134] The ability of flavonoids to act as potent antioxidants depends on their molecular structure, which are found in plants as their glycosides.[135] Other phytochemical such as cardiac glycosides have been used to treat congestive heart failure and cardiac arrhythmia,[136] and were found to be present in some plant extracts tested.

The leaves and stem of *Lavandula x intermedia* demonstrated the presence of tannins and phenolics, sterols, terpenoids, flavonoids, glycosides, saponins and cardiac glycosides. Previous study revealed that the leaf of *Lavandula x intermedia* has a high total amount of phenolic acids, flavonoids, procyanidis, tannnins, polyphenols as well as antimicrobial activity.[137]

Phytochemical screening of *Artemisia afra* leaves and stem also revealed the presence of tannins and phenolics, sterols, terpenoids, flavonoids, glycosides, saponins and cardiac glycosides. Degu Lere Keshebo observed similar results. Previous study has revealed the presence of antimicrobial, antioxidant, and antibacterial in *Artemisia afra*.[137]

Rosmarinus officinals flowers revealed the presence of sterols, terpenoids, flavonoids, glycosides, saponins and cardiac glycosides. Edrah Salem Mohamed observed similar results. The results signified the presence of tannins, saponins, flavonoids, alkanoids, phenols, terpenoids and phlobotanins as well as antimicrobial activity.[138]

The leaves of *Cymbopogan citratus* demonstrated the presence of tannins and phenolics, sterols, terpernoids, flavonoids, saponins and cardiac glycosides. The phytochemical analysis of the previous study showed that various plant secondary metabolites are present in the *Cymbopogan citratus* leaf extracts. All the phytochemicals tested for such as flavonoids, carbohydrates, steroids, tannins, alkaloids, steroids, glycosides, phenols and phytosteroids were found to be present. Previous study has also revealed antimicrobial and antibacterial activities of *Cymbopogan citratus*.[139]

Phytochemical screening of *Verbascum thapsus* leaves and stem revealed the presence of tannins and phenolics, sterols, terpenoids, flavonoids, glycosides, saponins and cardiac glycosides. Previous study revealed that in *Verbascum thapsus*, saponins, coumarins, steroids and phlobatanins were absent while alkaloids, terpernoids, cardiac glycosides and anthaquinones were present.[140] Previous study also revealed the presence of biological activities; that is antiviral, antigermination, anticancer and cytotoxic activities of *Verbascum thapsus*.[141]

Pelargonium graveolens flowers, leaves and stem revealed the presence of tannins and phenolics, sterols, terpenoids, flavonoids, glycosides, saponins and cardiac glycosides. Previous workdemonstrated the maximum occurrence of phytoconstituents such as flavonoids, phenols, tannins, saponins, reducing sugars, glycosides, terpenoids, anthraquinones and phlorotannins and absence of starch and steroids were observed.[142] Previous study has also revealed the presence of antioxidant and antimicrobial activities of *Pelargonium graveolens.*[143]

The flowers, leaves and stem of *Foeniculum valgare* demonstrated the presence of tannins and phenolics, sterols, terpernoids, flavonoids and saponins. Previous study revealed that phytochemical analysis were tested for various phytochemicals. Alkaloids, saponins, terpenoids, saponins, terpenoids and flavonoids were predominantly present in active extracts. Previous study signified the presence of antimicrobial and antioxidant activities of *Foeniculum valgare.*[144]

Phytochemical screening of *Lippia citrodora* flowers, leaves and stem revealed the presence of tannins and phenolics, sterols, terpenoids, flavonoids, glycosides, saponins and cardiac glycosides. The results of the previous study confirmed the presence of different types of active constituents like flavonoids, terpenoids and tannins. Previous study also showed a strong antimicrobial activity of *Lippia citrodora.*[145]

Catharathus roseus leaves and stem revealed the presence of tannins and phenolics, sterols, terpenoids, flavonoids, glycosides, saponins and cardiac glycosides. Previous study revealed the presence of alkaloids, terpenoids, saponins, proteins, phenols and tannins. In the previous study antioxidant, antimicrobial and anticancer activities of *Catharanthus roseus* were significant.[146]

The total content of the phytochemicals was not determined because the study was done to observe just their presence for further analysis.

3.6.3. Antimalarial screening results

In vitro screens for activity constitute a key component for antimalarial drug screening. It is based on the ability to culture *Plasmodium falciparum* in human erythrocytes *in vitro.*[110] The development of techniques for continuous cultivation of *Plasmodium falciparum* is a reliable source for screening of antimalarial drugs. *In vitro* methods are normally the methods of choice for large scale production by the pharmaceutical industry because of the ease of culture for production, compared with the use of animals and because of economic considerations.[147]

Scenery and specific plants are a probable origin of recent antimalarial drugs, as they possess a quantity of metabolites with a significant variety and medicinal activities.[148] The outcomes from Africa and other continents have been quite good, thus there has been a general request for the use of natural products as drugs, in order to possibly avoid problems related to drug resistance.[149]

One of the major objectives of this study was to assess the potential antimalarial properties of *Lavandula x intermedia* (1), *Artemisia afra* (2), *Rosmarinus officinalis* (3), *Cymbopogan citratus* (4), *Verbascum thapsus* (5), *Pelargonium graveolens* (6), *Foeniculum vulgare* (7), *Lippia citriodora* (8), *Catharathus roseus* (9) plants used in traditional medicine. Twenty-three extracts of acetone, dichloromethane and ethanol were screened for the potential antimalarial properties against chloroquine-sensitive *Plasmodium falciparum*. The antimalarial activity of extracts was defined according to the percentage parasite kill and IC_{50} values obtained for extracts with significant activity.

Initial tests were done on the ethanol extract. The assay was ran twice using the pLHD on the FCR-3 strain. **Table 3.4** below shows the summary of results. These results showed that the solvent used, ethanol was too polar to extract some active compounds from the plant. The IC_{50} values could not be determined since none of the extracts inhibited above 60% parasite growth.

Table 3.4: Antimalarial screening results of ethanol extracts.

Compound no.	Plant at 50mg/mL	% kill
1	*Lavandula x intermedia*	37.12
2	*Artermisia afra*	29.36
3	*Rosmarinus officinalis*	21.99
4	*Cymbopogan citratus*	6.72
5	*Verbascum Thapsus*	19.80
6	*Perlagonuim graveolens*	19.32
8	*Lippia citrodora*	0.10
9	*Catharanthus roseus*	35.76
CQ		99.99
DHA		93.47

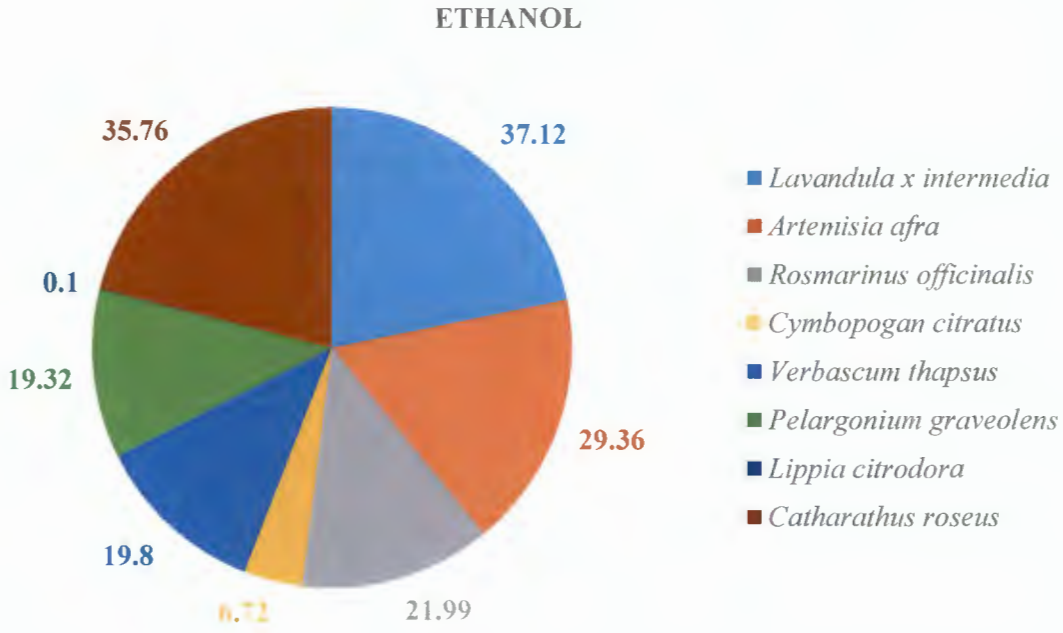


Figure 3.2: Percentage parasite growth inhibition of ethanol extracts.

The solvent was then changed to dichloromethane and acetone. Below are the results obtained for the *in vitro* screening (**Table 3.5**). As seen the *Artemisia afra* and *Cymbopogan citratus* extracts showed more than 60% parasite death. **Figure 3.3** shows the plant species that displayed significant inhibition of parasite growth.

Artemisia afra[150] *Cymbopogan citratus*[151]

Figure 3.3: Species with significant inhibitor of parasite growth.

The codes, AKM and DKM, were used for the acetone and dichloromethane extracts respectively. *In vitro* antiplasmodial study disclosed that acetone extract of *Cymbopogan citratus* (IC_{50} = 4.59 µg/mL and % parasite kill= 68.58%) and dichloromethane extract of *Artemesia afra* (IC_{50} = 0.71 µg/mL and % parasite kill= 65.09%) were the most active when compared to other extracts against chloroquine sensitive strain of *Plasmodium falciparum* displayed in **Figure 3.2**. From literature, an extract is viewed as highly active if IC_{50} < 10µg/mL, moderately active if IC_{50} is between 10µg/mL

and 50µg/mL and inactive if IC_{50} > 50µg/mL. Based on this classification, the two extracts were found to be highly active against *Plasmodium falciparum*.

Table 3.5: Antimalarial analysis results of dichloromethane and acetone extracts.

NF54 chloroquine sensitive strain of *P. falciparum*

Compound No	Compound ID	Plant name	% Parasite growth	% Parasite Kill	SD	IC50 (µg/mL)	SD
1	AKM1	*Lavandula x intermedia*	49,02	50,98	4,33		
2	AKM2	*Artemisia afra*	57,37	42,63	0,28		
3	AKM3	*Rosmarinus officinalis*	53,55	46,45	1,09		
4	AKM4	*Cymbopogan citratus*	31,44	68,56	0,81	4,59	0,90
5	AKM5	*Verbascum thapsus*	90,92	9,08	3,68		
6	AKM6	*Pelargonium graveolens*	52,87	47,13	1,25		
7	AKM8	*Lippia citrodora*	61,44	38,56	6,81		
8	AKM9	*Catharathus roseus*	65,89	34,11	8,79		
9	DKM1	*Lavandula x intermedia*	64,20	35,80	5,02		
10	DKM2	*Artemisia afra*	34,91	65,09	7,67	0,71	0,16
11	DKM3	*Rosmarinus officinalis*	52,93	47,07	5,96		
12	DKM4	*Cymbopogan citratus*	78,38	21,62	10,42		
13	DKM5	*Verbascum thapsus*	89,21	10,79	4,43		
14	DKM6	*Pelargonium graveolens*	54,14	45,86	2,03		
15	DKM9	*Catharathus roseus*	67,23	32,77	15,06		
16	CQ		0,10	99,90	3,64	0,0102	0,0021

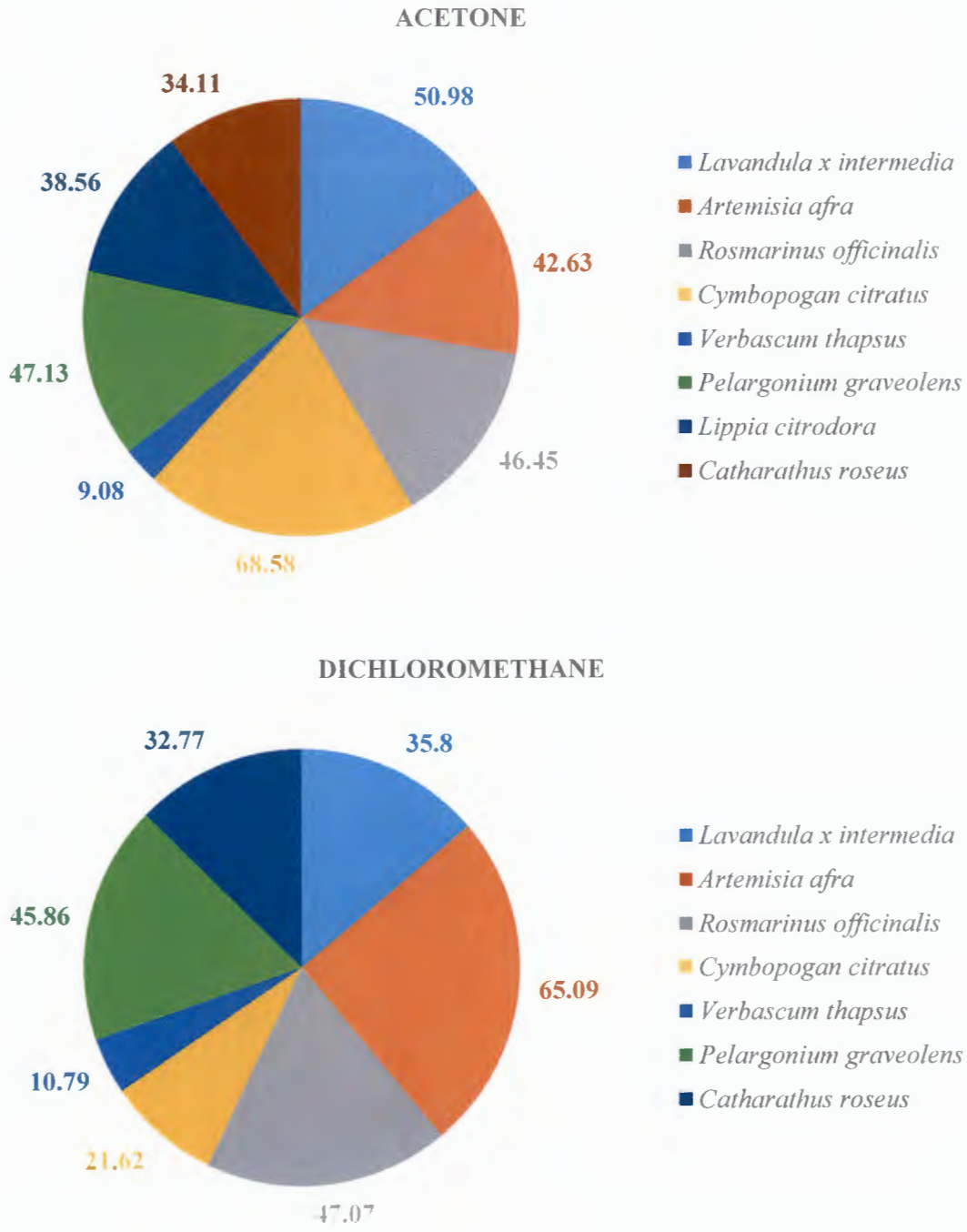


Figure 3.4: Percentage growth inhibition of acetone and dichloromethane extracts.

The antiplasmodial property of the plant extracts may be attributed to the presence of some phytochemicals that might have conversed some protective impact against oxidative stress induced in the host-parasitized red blood cells by malaria parasite.

3.7. Conclusion

The study first investigated the presence of the phytochemicals in the selected medicinal herbs and almost all the classes tested were found to be present. The study also investigated the antimalarial

activities of extracts in varying solvents of *Lavandula x intermedia, Artemisia afra. Rosmarinus officinalis, Cymbopogan citratus*, *Verbascum Thapsus, Pelargonium graveolens*, *Foeniculum vulgare*, *Lippia citriodora* and *Catharathus roseus*. The results revealed that *Artemisia afra* and *Cymbopogan citratus* are potential antimalarials but *Artemisia afra* exhibited more phytocontituents than *Cymbopogan citratus*. This is the reason why Chapter 4 focuses on the phytochemistry of *Artemisia afra*. Chapter 4 covers the botany, traditional uses and phytocontituents of *Artemisia afra* and its family members.

CHAPTER 4

Phytochemical studies of *Artemisia afra*

4.1. *Artemisia* genus

The large genus *Artemisia*, from the family Asteraceae and the largest tribe of Anthemideae comprises over 500 species which are widely spread in different parts of the world such as South West America, Europe, South Africa and mainly Africa.[160,161] The genus is composed of significant medicinal plants that are presently the discipline of phytochemical attention because of their essential oil production, biological and chemical diversity.[162] **Figure 4.1** illustrates common species in the genus with their common names, which include mugwort (*Artemisia vulgaris*), wormwood (*Artemisia afra*), segabrush (*Artemisia tridentata*) and sagewort (*Artemisia annua*).[163-166] Most species have intense aromas and bitter taste from terpenoids and sesquiterpene lactones, which exist as an adaptation to discourage herbivores.[167]

Artemisia vulgaris[168]

Artemisia afra[17]

Artemisia tridentata[169]

Artemisia annua[17]

Figure 4.1: Common species of *Artemisia.*

Most *Artemisia* species usually have silver-grey, at least beneath the leaves with flowers found in terminal clusters.[170] They either bloom at the end of summer or during autumn in disparity to other Anthemideae genera, which typically flower during spring and summer. The genus is mostly composed of perennial plants, shown in **Figure 4.2** (a-h) but some of them are known to act as

either annuals **Figure 4.2** (i-j) or biennials **Figure 4.2** (k).[171-174] Within the genus there exists a certain variability of biotypes, being pronominally considered as herbs (*Artemisia afra*, *Artemisia vulgaris*), subshrubs (*Artemisia changaica*, *Artemisia crithmifolia*) and shrubs which may come into being highly lignified stems (*Artemisia tridentate*). The capacity of this genus to inhabit many different ecosystems and environmental conditions is evident, ranging from deserts and semi-deserts, forests and deeply entropized meadows, to humid areas, from sea level to high mountains.[175]

a. *Artemisia desertorum*[176]

b. *Artemisia changaica*[177]

c. *Artemisia gmelinni*[178]

d. *Artemisia keiskeana*[179]

e. *Artemisia mendozana*[180]

f. *Artemisia messerschmidtiana*[181]

g. *Artemisia nova*[182]

h. *Artemisia selengensis*[183]

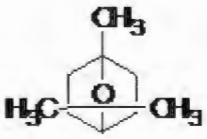
i. *Artemisia jacutica*[184]

j. *Artemisia palustris*[185]

k. *Artemisia biennis*[186]

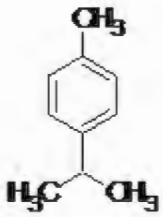
Figure 4.2: Species of genus *Artemisia.*

In recent times the active constituents responsible for medical actions of plants have been studied and observed. The active plant contituents are usaully classified by their chemical structure rather than action. This list is not conclusive but it provides a basic overview of the main constituents including alkoloids, flavonoids, minerals, saponins, vitamins, glycosides and volatile oils for better understanding of medicinal plants.



Cineole

(4.1)

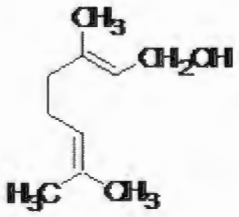


p-cymene

(4.2)

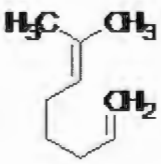
Caryophyllene

(4.3)



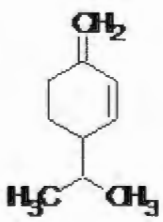
Geraniol

(4.4)



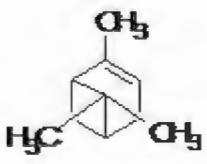
Myrcene

(4.5)



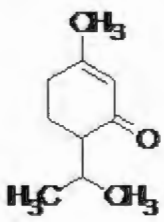
Phellandrene

(4.6)



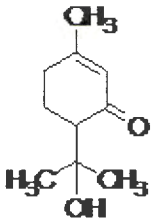
α-pinene

(4.7)



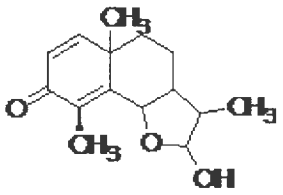
Piperitone

(4.8)



Terpineol

(4.9)



Santonin

(4.10)

Figure 4.3: Structures of the occurring major volatile components of *Artemisia* species.

The major chemical composition of essential oils from the *Artemisia* genus has been substantially researched in several species from around the world. Many studies have displayed that *Artemisia* species display vital intra specific variations in the terpene constituent of essential oils.[233]

Artemisia afra

4.1.1. Traditional uses of *Artemisia*

Medicinal herbs have a long histoy in improving human health and curing various diseases.[187] A wide interest has been made for researchers using herbal material in identification of the active components and verification of the efficiency[188]. Historically and up until now the *Artemisia* plants have been used for variety of digestive tract disorders, soothing the inflammation of intestinal tisssues, decrease stomach pain and cramping.[189-191] Herbal preparations of the plant are considered to be liver tonic as well.[192] They stimulate its cleaning by enhancing the draining of waste products with the help of the improved bile secretion. *Artemisia* is often used in jaundice and hepatisis treatment.[193]

Many of the other *Artemisia* species are aromatic perennials that are used medicinally.[3] *Artemisia afra* is included for its internal worm-expelling properties in the ancient Greek text of Dioscorides; Indians from New Mexico use similar varieties to treat bronchitis, and colds, and the Chinese still use wormwood rolled up in the nostril to stop nosebleeds.[194] Antibacterial properties of *Artemisia* are applied for treating conditions such as parastic and bladder infections, without damaging intestinal flora. *Artemisia* is used for the reproductive system disorders.[195] It decreases the bleeding in a prolonged menstrual cycle, warms the womb to enhance fertility and soothes menstrual pain.[196]

Many of the species have traditional medicinal uses such as *Artemisia afra* from which artemisinin is extracted to treat malaria, it kills organisms causing this condition and fights its symptoms. *Artemisia dracunculus* which is a culinary condiment used to prepare liquours and ornamental uses from *Artemisia arborescens*.[163] *Artemisia vulgaris* has long been considered a herbal ally for women with benefit in regulating the menstrual cycle and easing the transition to menopause.[197]

Artemisia campestris is used as an emmenagogue, a good stimulanttonic and has ome nervine principles.[198] *Artemisia abrotanum* is traditionally a remedy for hair growth stimulation, it is also used for coughs, brontilis, macus and congestion. *Artemisia tripartita* is used in the treatment of colds, sore throath, tonsillitis and headaches.[199] *Artemisia verlotorum* is used as a remedy for hypertension. *Artemisia parviflora* is used to treat skin diseases, burns, cuts and wounds.[170] *Artemisia roxburghiana* is used to treat chest colds, sore throat and toothache.[200]

4.1.2. Ethnopharmacology

The species of *Artemisia* are mostly perennial herbs dominating the vast steppe communities of Asia. *Artemisia* species are frequently utilized for the treatment of diseases such as malaria, hepatitis, cancer, inflammation and infections by fungi, bacteria and viruses.[17]

Artemisia absinthium has been used traditionally as antispasmodic, febrifuge, stomachic, cardiac stimulant, anthelmintic, for the restoration of declining mental function and inflammation of the liver and to improve memory.[201] *Artemisia annua* has been used as a cure of various fevers.[202,203] Traditionally, *Artemisia asiatica* has been used in the oriental medicine for the treatment of cancer, inflammation, infections and ulcerogenic diseases. *Artemisia dracunculus* has been used to treat antidiabetic and anticoagulant.[203] *Artemisia tripartile* has been used in the treatment of colds, sore throats, tonsillitis, headaches and wounds.[204] *Artemisia judaica* is an Egyptian medicinal plant that has been used to treat gastrointestinal disorders.[205] *Artemisia verlotorum* has been used as a remedy for hypertention.[205] *Artemisia vestita* is a typical traditional medicinal plant which has been widely used for treating various inflammatory diseases such as rheumatoid arthrities and contact dermatitis.[206] *Artemisia vulgaris* has been used as an analgesic, antiflammatory, antipasmodic and in liver diseases[207].

4.2. *Artemisia afra*

Artemisia afra shown in **Figure 4.4**, is a medical plant known as African wormwood and commonly found in most areas of Southern Africa, where it has a reputation for its claimed healing properties and use in specific aliments.[162] In fact, based on South African indigenous knowledge the plant is used traditionally for a wide range of specific illnesses inclusive of diabetes mellitus, fevers asthma, malaria, haemorrhoids and heartburns. [259-264] [265] A few laboratory studies have shown that *Artemisia afra* materials exhibit a wide span of biological and pharmacological activities that may substantiate the therapeutic use of this plant in traditional medicine.[113]

Artemisia afra is a perennial woody shrub, which grows up to 2 m tall with a leafy, hairy and ridged stem. **Figure 4.4** illustrates the leaves that are of soft texture, dark green on the adaxial surface and a lighter green on the biaxial surface, reaching a length of 8 cm and a width of 4 cm. It blossoms from January to June, producing yellow, butter coloured flowers with abundant bracts. The plant has a simply identifiable aromatic odour and smells pungent and sweet after bruising.

The fruit is approximately 1 mm in length, covered with a silvery-white coating and the shape is slightly 3-angled and curved as shown in **Figure 4.4**. In winter, the branches rapidly regenerate from the base directly after dying back.[113]

Artemisia afra plant[266]

Artemisia afra leaves[267]

Artemisia afra fruit[113]

Figure 4.4: *Artemisia afra* plant, leaves and fruit.

Artemisia afra is a common, widely distributed species in South Africa from Cedarburg Mountains in the cape, northwards to tropical east Africa and stretching as far north as Ethiopia, Kenya, Zimbabwe, Tanzania and Angola. Illustration in **Figure 4.5** is the South African geographic distribution of *Artemisia afra*. In the wild, it grows at altitudes between 20-2440 m on damp slopes, along streamsides and forest margins. It is the only indigenous species in this genus.[268]

Figure 4.5: Geographical distribution (orange) of *Artemisia afra* in South Africa.

4.2.1. Traditional uses

Artemisia afra is often used in common colds, cough, sore throat, influenza, asthma as it is to clear the respiratory and bronchail passages.[97,163,269,270] The leaves are heated and the vapours inhaled to alleviate symptoms of colds and flu.[97,271] It is also used to clear blocked nasal passages by inserting fresh leaves in the nostrils or by using as sniff to relieve fresh leaves in the throat in scarlet fever, either the hot infusion is used as a gargle or the throat is exposed to vapours.[97,165]

Artemisia afra is used in digestive complaints like indigestion, colic, constipation, flatulence, gastititis, dry dyspepsia and to get rid of intestinal worms.[163,272] It is devoured to overcome general delibity and as an appetizer. Watt and Breyer-Brandwijk report that the (i) extract is applied topically to ease pain and hasten bursting of boil carbuncles, large acne pimple; (ii) hot bath in the extraction is used to bring out the rash in measles, mumps, herpes, chicken pox; (iii) infusion is used to bathe hemorrhoids, herpes, vermicelli sores; (iv) poulice of the leaf is applied as a dressing to relieve neuralgia, to the swelling of mump and to other glandular or skin inflammations and (v) lotion is used to wash the body to rejuvenate the skin.[97] The use of *Artemisia afra* in combination with other medicinal plants is summarised [262] in **Table 4.1**.

Table 4.1: Traditional uses of *Artemisia afra* combined with other plant species for the treatment of respiratory complaints.

Plant combination	Uses
Artemisia afra and *Artemisia betulina*	Respiratory complaints
Artemisia afra, Eucalyptus globulus and *Leonotis microphylla*	Fever, chest infections and digestive disturbances
Artemisia afra and *Lippa javanica*	Fever, respiratory complaints, measles and as a prophylactic against lung inflammations
Artemisia afra, Tetradeni raparia and salt	Coughs
Artemisia afra and *Alepidea amatymbica*	Cold and flu
Artemisia afra, Warburgia salutaris and *Acorus calamus*	Chronic bronchitis and emphysema

4.2.2. Ethnopharmacology

Artemisia afra is a notable medicinal plant of South Africa, where it is known as "wilde als". The anthelmintic activity of the *Artemisia afra* is thought to be caused by lactones related to santonin, which is found in wormseed and other species of *Artemisia*. [273] The laboratory studies have found constituents in *Artemisia afra* to have antispasmodic activity, supporting its traditional use in stomach or intestinal cramping.[274] It is traditionally used as an antibacterial and antifungal agent.[261,275]

The roots, stems and leaves of *Artemisia afra* are used in many different ways and taken as enemas, poultices, infusions, body washes, lotions, smokes, snuffed or drunk as tea. Many Indian tribes induce visionary states during religious ceremonies, smoke *Artemisia afra* foliage. It is a strong narcotic, analgesic, antihistamine and an excellent smoke reputed for its hallucinogenic effects and psychoactive. In addition, a phytoconstituent of *Artemisia afra* called thujone can stun roundworms, which can then be expelled by normal intestinal peristalsis.[276,277]

4.2.3. Chemical constituents of *Artemisia afra*

Microchemical tests of *Artemisia afra* indicated the presence of tannins and saponins except of alkaloids or of cardiac, cyanogenic or anthraquinone glycosides. Other studies have identified the triterpenes α- and ß-amyrin and friedelin as well as the alkane's ceryl cerotinate and n-nonacosane in the leaves of South African collections of *Artemisia afra*. The analysis of leaf exudate flavonoids disclosed the presence of two luteolin methyl ethers. In an analysis of the sesquiterpene lactones of this species, 10 guaianolides and 5 glaucolides were detected in the overground parts of the plan. Some of the major essential oil components of *Aremisia afra* species are tran-anethole (**4.11**), artemisia ketone (**4.12**), β-bisabolol (**4.13**), borneol (**4.14**) and limonene (**4.21**).[278]

4.3. Isolation

4.3.1. General methods

The separation of the compounds was monitored by thin-layer chromatography (TLC) on aluminium sheets coated with Merck silica gel 60 F_{254}, cut into strips of various sizes, with visualization of the compound by inspection under UV (254 nm and 365 nm) and staining with a *p*-anisaldehyde solution. The *p*-anisaldehyde was prepared by 7.5 mL of acetic acid and 1.75 mL of *p*-anisaldehyde to 175 mL of ice cold ethanol. 25 mL of concentrated sulphuric acid was cautiously added drop wise to the mixture for 30 minutes.[279] The prepared solution was stored in the refrigerator. Column chromatography was performed on the glass column with Merck silica gel 60 (0.040-0.063 mm).

4.3.2. Isolation of *Artemisia afra* compounds

Silica gel was loaded gently on the glass column. The acetone extract of *Artemisia afra* (2.25 g) was loaded directly on dry silica gel in the column and small layer of silica gel was added to cover the extract. About 150 mL of hexane of non-polar hexane was added to allow the extract to stick to the stationary phase. Once the column was loaded, the tap was opened to allow the solvent level to drop in different colours, which were collected in individual containers. About150 mL of ethyl acetate, acetone were added into the column and 150 mL of ethanol was added to wash off the column.[280] The extract was fractionated using a step gradient hexane:ethyl acetate 9:1, 4:1, 3:2, 2:3 allowing the compounds to travel with the solvent. The fractions were further cleansed using

column chromatography to afford seven compounds. Of the seven compounds, two were characterized using different techniques to determine their structures.

4.4. Characterization of isolated compounds

4.4.1. Nuclear Magnetic Resonance Spectroscopy

NMR is a technique used to determine a compound's unique structure. It identifies the carbon-hydrogen framework of an organic compound. This type of spectroscopy determines the physical and chemical properties of atoms or the molecules in which they are contained.[281] It relies on the phenomenon of nuclear magnetic resonance and can provide detailed information about the structure, dynamics, reaction state, and chemical environment of molecules. The intramolecular magnetic field around an atom in a molecule changes the resonance frequency, thus giving access to details of the electronic structure of a molecule and its individual functional groups.[282] NMR samples were prepared by dissolving the isolated compounds in $CDCl_3$ and ran for analysis using Bruker 800 MHz.

4.4.2. Mass Spectrometry

MS is an important device for the identification and structural elucidation of natural products. It was primarily used to obtain molecular weights of the pure compounds.[283] Former ionization techniques, as electron ionization, limited this use to non-polar, volatiles and thermostable substances. The improvement of softest ionization techniques allowed gradually the analysis of polar and thermolabile compounds, having currently unlimited physical or chemical properties to be analyzed by MS.[284] Pure compounds from the extract of *Artemisia afra* were dissolved in methanol and ran for analysis using Agilent GC 200 MS.

4.4.3. Fourier-Transform Infrared Spectroscopy

FTIR has proven to be a valuable tool for the characterization and identification of compounds or functional groups (chemical bonds) present in an unknown mixture of plant extracts.[285] FTIR spectra of pure compounds are usually so unique that they are like a molecular "fingerprint". For most common plant compounds, the spectrum of an unknown compound can be identified by analysis comparison to a library of known compounds.[286] Pure compounds from the extract of *Artemisia afra* for FTIR were dissolved in hexane: ethyl acetate (6:4) solvent and ran for analysis using Bruker FTIR.

4.4.4. Ultraviolet–Visible Spectroscopy

The UV–Vis spectroscopy is a facile method to decipher herbals and herbal products for its characterization, identification, authentication, stability, adulteration, and purity.[287] The use of UV-Vis spectroscopy in the structure elucidation process is limited. Nevertheless, it plays an important role as probably the most often used tool for detection in separation.[288] Pure compounds

isolated from extract of *Artemisia afra* were dissolved in hexane: ethyl acetate (6:4) solvent and analysed with Cary 300 UV-Vis.

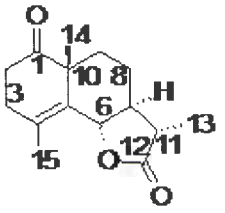
4.5. Results and discussion

The characterization of natural products and bioactive compounds in the past decades utilized spectroscopic techniques as well as chemical methods to determine the structures. The use of UV-vis, FTIR, NMR, and MS can yield complementary information that is used to determine the structures of the compounds. When NMR data and degradative studies are inconclusive, a total synthesis and spectroscopic comparison between the synthetic and natural product is the easiest way for stereochemical determination and confirmation. [289]

4.5.1. Structural elucidation of taurin (4.24)

Taurin (**4.24**) was obtained from the hexane: ethyl acetate (6:4) fraction. The NMR data of Plate 1 and 2 are summarised in Table 4.4. The ^{13}C NMR spectrum of the compound displayed fifteen signals. Carbon 4 and 5 signals were observed at δ_C 128.1 and δ_C 114.5, with no corresponding proton observed, indicating that the double bond is tetrasubstituted. This was also confirmed by their absence in the DEPT NMR. Three methyl signals at δ_C 14.2, δ_C 21.1 and δ_C 29.5 were observed. Two carbonyl peaks were observed at 171.3 and 211.1 possible corresponding to the ester and ketone respectively, four methylene signal at δ_C 21.1, 29.7, 317 and 38.0.

The ^1H NMR spectrum (Plate 1) exhibited signals of secondary methyl (δ_H 1.60, 3H) as a doublet and two tertiary methyl groups (δ_H 0.80 and 0.40), both appearing as singlets. Also observed was a doublet resonating at δ_H 5.3 (1H, d) corresponding to proton (H-6).



(**4.24**)

Plate 7, the HMBC spectrum of **4.24** showed the correlation between the methyl protons resonating at δ_H 1.30 and the carbonyl carbon δ_C 171.1, two methine carbons δ_C 53.0 and δ_C 46.1. The methyl proton resonating at δ_H 1.60 correlated with two double bonded carbons at δ_C 114.5 and δ_C 128.0. The DEPT spectrum confirmed the presence of three methine at δ_C 46.1, 53.0 and 90.3; four methylene at δ_C 21.1, 29.7, 31.7 and 38.0, as well as three methyl groups at δ_C 14.2, 22.0 and 29.6. The structure was confirmed to be taurin and the NMR data is illustrated in **Table 4.2**.

Table 4.2: ^{1}H and ^{13}C NMR data of **4.24** in $CDCl_3$.

Experimental values				Literature results[290]	
Position	δ_C	DEPT	δ_H	δ_C	δ_H
1	211.1	C		212.5	
2	31.7	CH_2	2.45 (m) 2.61 (m)	35.0	
3	29.7	CH_2	2.36 (m) 2.30 (m)	35.9	
4	114.5	C		130.2	
5	128.1	C		126.6	
6	90.3	CH	5.3 (1H ,d, J 6.0 Hz)	81.5	4.6 (1H, d, J 9.0 Hz)
7	46.1	CH	1.70 (m)	55.0	
8	21.1	CH_2	1.30 (m)	23.8	
9	38.0	CH_2	1.97 (m)	32.9	
10	54.0	C		48.8	
11	53.0	CH	2.20 (1H, m)	40.8	
12	171.1	C		177.8	
13	14.2	CH_3	1.60 (3H, d, J 7.3Hz)	12.3	1.23 (3H, d, J 7.0 Hz)
14	22.0	CH_3	0.40 (3H,s)	23.3	1.33 (3H,s)
15	29.6	CH_3	0.80 (3H,s)	19.7	1.98 (3H,s)

The FTIR spectrum of taurin, as show in **Figure 4.6** is in the range of 400-3500 cm^{-1}. The band at 2982 cm^{-1} is due to asymmetric stretching vibration of CH_2 stretching vibration. The peak at 1738 cm^{-1} is indicative of stretching vibration of C=O bands due to an ester. The peaks at 1685 cm^{-1} is due to the C=O stretching vibration of a ketone.

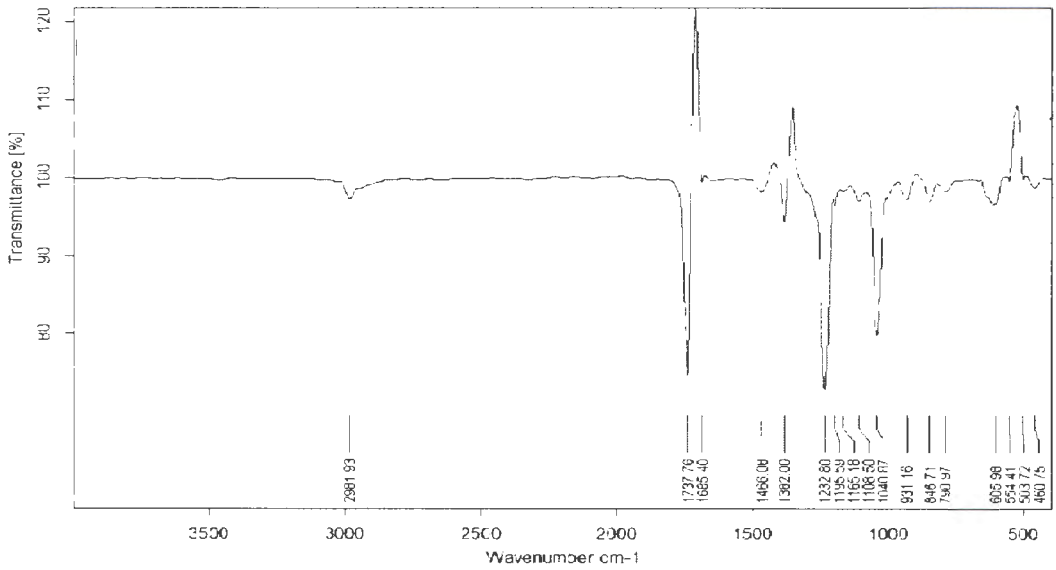


Figure 4.6: FTIR spectrum of **4.24**.

The qualitative UV-Vis spectra of *Artemisia afra* acetone sub fraction as shown in **Figure 4.7** was taken at the wavelength of 200 and 400 nm due to the sharpness of the peaks and proper baseline. The spectra showed the peaks at 238, 273 and 609 nm with the absorption at 0.16, 4.00 and 0.00 respectively. The peak at 273 indicated a ketone.

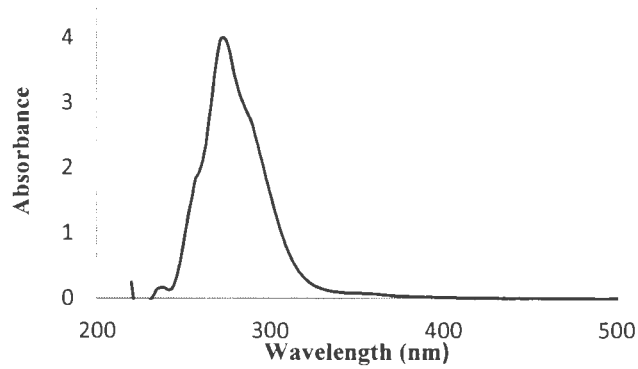


Figure 4.7: UV-Vis spectrum of **4.24**.

4.5.2. Structural elucidation of marimitin (4.25)

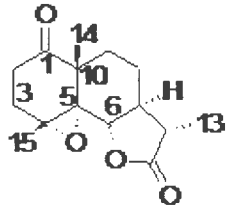
Maritimin (**4.25**) was also isolated from the acetone extract of *Artemisia afra* using the hexane and ethyl acetate mixture as eluent. The data obtained from NMR experiments are summarised in **Table 4.3**. Fifteen carbon signals could clearly be identified in the ^{13}C NMR (Plate 9). The fifteen carbon atoms signals included three methyl groups resonating at δ_C 14.1, δ_C 14.2 and δ_C 20.2; four methylene carbons resonating at δ_C 22.7, δ_C 26.6, δ_C 30.9 and δ_C 36.4; three methine carbons at δ_C 44.0, 45.0 and δ_C 77.2; one quaternary carbon at δ_C 48.9, two more quartenary carbons further

downfield at δ_C 60.4 and 70.1; and two carbonyl resonating at δ_C 172.5 and δ_C 211.0 possible for an ester and ketone respectively.

The ^1H NMR spectrum (Plate 8) showed a doublet at δ_H 3.59 (1H,d) which is characteristic of the lactone proton H-6. The presence of the two tertiary methyl groups δ_H 0.83 and 0.54 and a secondary methyl δ_H 2.49 were observed. In the HMBC experiment, the methyl group resonating at δ_C 2.49 showed correlation with the ketone carbon δ_C 211.1, a quaternary carbon resonating at δ_C 48.9, a methylene carbon resonating at δ_C 26.6, and the quaternary carbon resonating δ_C 70.1. In the DEPT spectrum, the presence of three methyl at 44.0, 45.0 and 77.2 ppm; four methylene at 22.7, 26.6, 30.9 and 36.4 ppm as well as three methyl groups at 14.1, 14.2 and 20.2 ppm were observed. This information enabled the construction of the maritimin indicated in **Figure 4.8**.

Table 4.3: ^1H and ^{13}C NMR data of **4.25** in $CDCl_3$.

Experimental values				Literature results[290]	
Position	δ_C	DEPT	δ_H	δ_C	δ_H
1	211.0	C		210.7	
2	30.7	CH_2	1.66 (m) 1.31 (m)	31.0	
3	36.4	CH_2	2.36 (m)	33.4	
4	60.4	C		66.0	
5	70.1	C		63.6	
6	77.2	CH	3.59 (1H, d, J 8.0 Hz)	76.6	4.34 (1H, d, J 9.0 Hz)
7	45.0	CH	2.00 (m)	48.5	
8	22.7	CH_2	1.04 (m)	22.9	
9	26.6	CH_2	2.11 (m)	27.9	
10	48.9	C		49.2	
11	44.0	CH	3.3 (m)	40.4	
12	171.1	C		178.0	
13	14.1	CH_3	2.49 (3H, d, J 7.2 Hz)	12.3	1.25 (3H, d, J 7.0 Hz)
14	14.2	CH_3	0.54 (3H,s)	20.7	1.27 (3H,s)
15	20.2	CH_3	0.83 (3H,s)	19.4	1.68 (3H,s)



(4.25)

Figure 4.8: Marimitin structure **(4.25).**

The FTIR analysis of acetone sub fraction of *Artemisia afra,* as illustrated in **Figure 4.9** provided significant information in the range of 400-3500 cm^{-1}. The band at 2981 cm^{-1} is due to assymetric stretching vibration of CH_2 stretching vibration of aliphatic acids. The peak at 1737 cm^{-1} is indicative of stretching vibration of C=O bands due to an ester. The peaks at 1685 cm^{-1} is due to the C=O stretching vibration of a ketone.

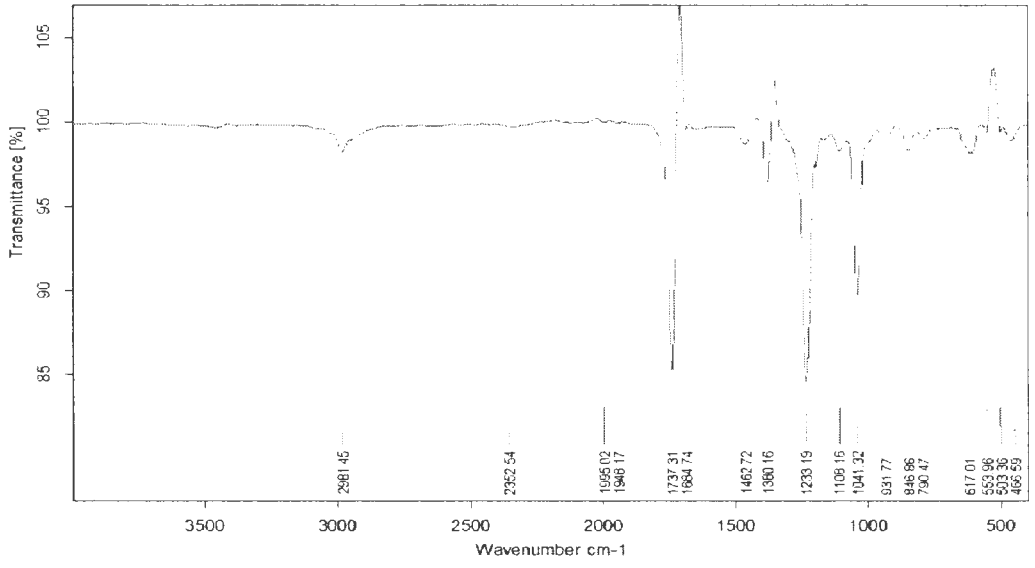


Figure 4.9: FTIR spectrum of **4.25**.

The Uv-Vis profile was recorded at 215 and 465 for the sub fraction of *Artemisia afra* acetone extract. The profile indicated the peaks at 249, 282 and 609 with the absorption 1.71, 6.66 0.05respectively. The peak at 282 showed the presence of a ketone. **Figure 4.10** represents the Uv-Vis spectrum of marimitin.

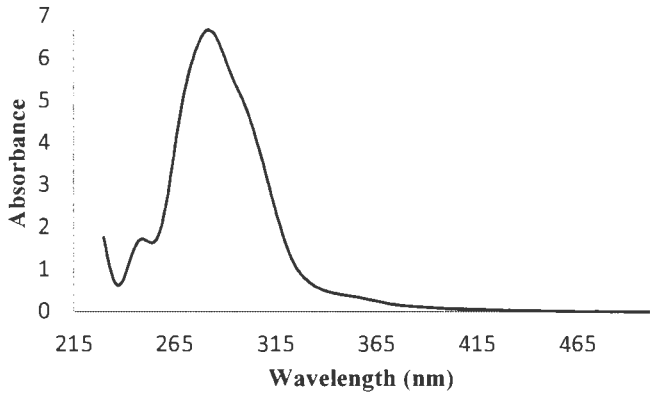


Figure 4.10: UV-Vis spectrum of **4.25**.

4.6. Isolation of compounds

4.6.1. Isolation of taurin

An acetone crude extract of *Artemisia afra* (2.25 g) was loaded directly on dry silica gel in the column and small layer of silica gel was added to cover the extract. A hexane: ethyl acetate (9.5:0.5) solvent mixture was used as an eluent and 10 mL fractions were collected in vials, 0.1372 g of the pure material of hexane: ethyl acetate (6:4), were obtained by spotting a full TLC plate with the hexane: ethyl fraction (6:4) and putting it into a TLC glass tank for elution to take place. The TLC plate was viewed under UV-Vis light and two long lines spottings appereared. The spottings were scrapped off, soaked into ethyl acetate, filtered off, washed with acetone and kept in the fume hood for the solvent to evaporate.

Appearance:	Green
Empirical formula:	$C_{15}H_{20}O_3$
Melting point:	110-112 °C
^{1}H NMR (CDC_3) δ_H:	0.40 (3H, s, H-14), 0.80 (3H, s, H-15), 1.30 (m, H-8), 1.60 (3H, d, J .3 Hz, H-13), 1.70 (m, H-7), 1.97 (m, H-9), 2.20 (1H, m, H-11), 2.25 (m, H-3), 2.30 (m, H-3), 3.00 (m, H-2), 3.46 (m, H-2), 5.3 (3H, d, J 7.3 Hz, H-6)
^{13}C NMR ($CDCl_3$) δ_C:	14.2 (d, C-13), 21.2 (m, C-8), 22.0 (s, C-14), 29.6 (s, C-15), 29.7 (m, C-3), 31.7 (m, C-2), 38.0 (m, C-9), 53.0 (m, C-11), 54.0 (C-10), 90.3 (d, C-6), 128.0 (C-5), 114.5 (C-4), 171.1 (C-12), 211.1 (C-1)
ESI-MS (positive mode) *m/z*:	273.1472

4.6.2. Isolation of marimitin

A filtered crude acetone extract of *Artemisia afra* (2.25 g) was loaded directly on dry silica gel in the column and small layer of silica gel was added to cover the extract. A hexane: ethyl acetate (9.5:0.5) solvent mixture was used as an eluent and 10 mL fractions were collected in vials, 0.2387 g of the pure material of hexane: ethyl acetate (6:4), were obtained by spotting a full TLC plate with the hexane: ethyl fraction (6:4) and putting it into a TLC glass tank for elution to take place. The TLC plate was viewed under UV-Vis light and two long line spottings appereared. The spottings were scrapped off, soaked into ethyl acetate, filtered off, washed with acetone and kept in the fume hood for the solvent to evaporate.

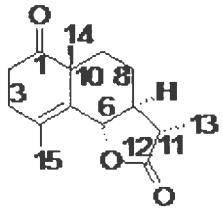
Appearance:	Gold
Empirical formula:	$C_{15}H_{20}O_4$
Melting point:	110-112 °C
^{1}H NMR ($CDCl_3$) δ_H:	0.54 (3H, s, H-14), 0.83 (3H, s, H-15), 1.04 (m, H-8), 1.31 (m, H-2), 1.66 (m, H-2), 2.00 (m, H-7), 2.11(m, H-9), 2.36 (m, H-3), 2.49 (3H, d, J 7.2Hz, H-13), 3.3 (m, H-10), 3.59 (1H, d, J 8.0 Hz, H-6)
^{13}C NMR ($CDCl_3$) δ_C:	14.1 (d, C-13), 14.2 (s, C-14), 20.2 (s, C-15), 22.7 (m, C-8), 26.6 (m, C-9), 30.9 (m, C-2), 36.4 (m, C-3), 44.0 (m, C-11), 45.0 (m, C-7), 48.9 (C-10), 60.4 (C-4), 70.1 (C-5), 77.2 (d, C-6), 172.5 (C-12), 211.0 (C-1)
ESI-MS (positive mode) *m/z*:	287.1259

Chapter 5

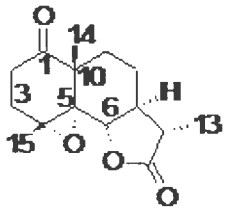
Conclusion and future work

There is an acute need for the development of the novel drugs to treat malaria. Many countries have an immeasurable experience in the use of medicinal plants and the required knowledge spans many centuries. It is key that efficacy and safety to traditional medicines be validated and their active constituents be identified so that reliable quality controls can be established. An independent screening method also needs to be done to determine the cytotoxicity of the plant extracts. This will help to determine the difference between antimalarial activity and the toxicity.

In vitro biological screening for antimalarial activity was conducted on the dichloromethane, ethanol and acetone extracts on NF_{54} chloroquine sensitive strain using the pLDH method. None of the dichloromethane and ethanol extracts showed more than 60% death of parasites thus the IC_{50} values were not determined. *Artemisia afra* and *Cymbopogan citratus* acetone extracts showed significant activity and *Artemisia afra* was selected for further investigaton. Phytochemical studies on *Artemisia afra* resulted in the isolation of two compounds taurin (**4.24**) and marimitin (**4.25**). The two compounds have been isolated before and our spectral data matched the literature. The two compounds have been sent for antimalarial screening. *In vivo* toxicity of the extracts and isolated compounds still need to be investigated.



(4.24)



(4.25)

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Appendix

NMR spectra of isolated compounds

Plate 1: 1H NMR spectrum of taurin (4.24)

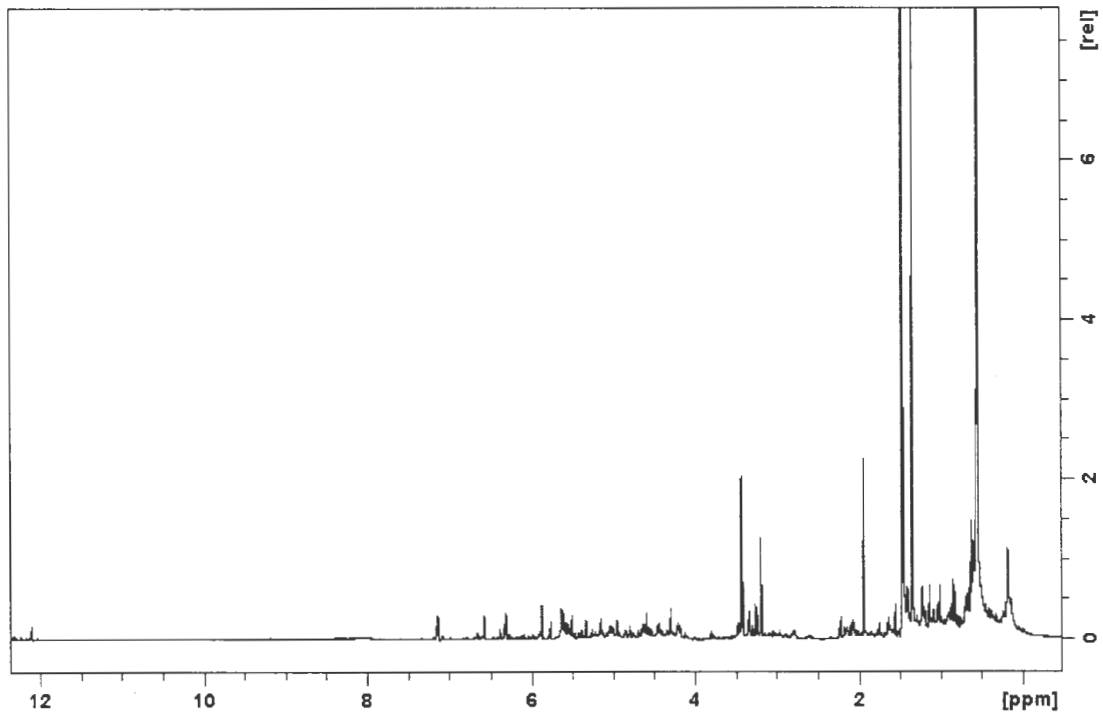

Plate 1a: ^{1}H-e NMR spectrum of taurin (4.24)

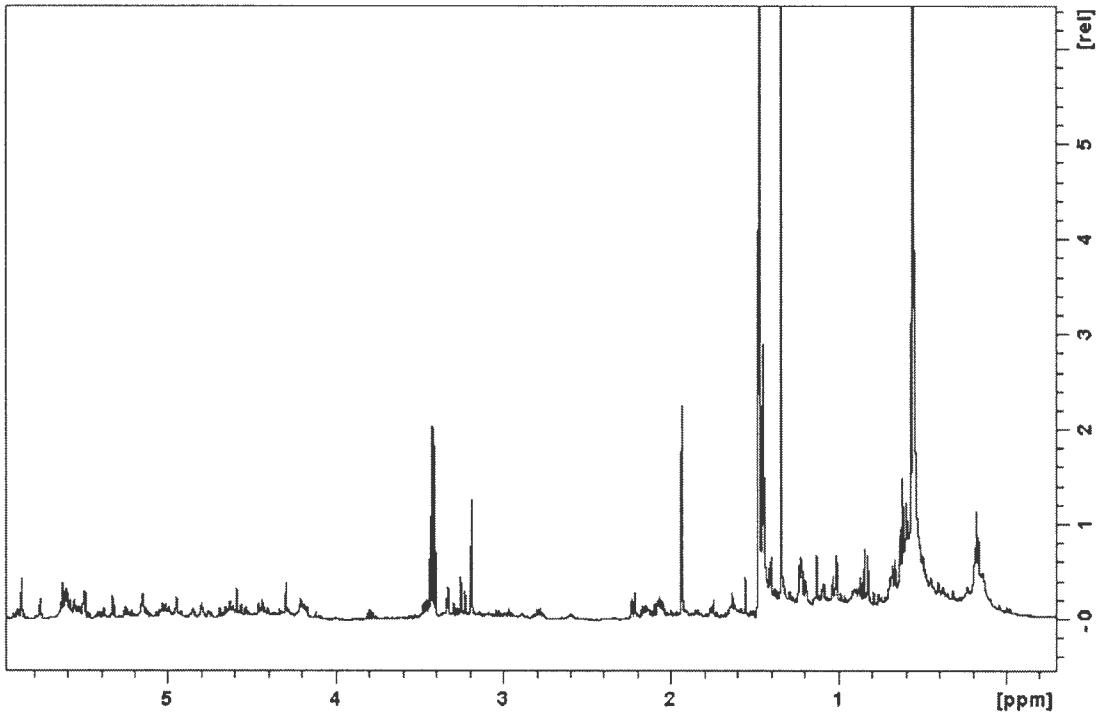

Plate 2: ^{13}C NMR spectrum of taurin (4.24)

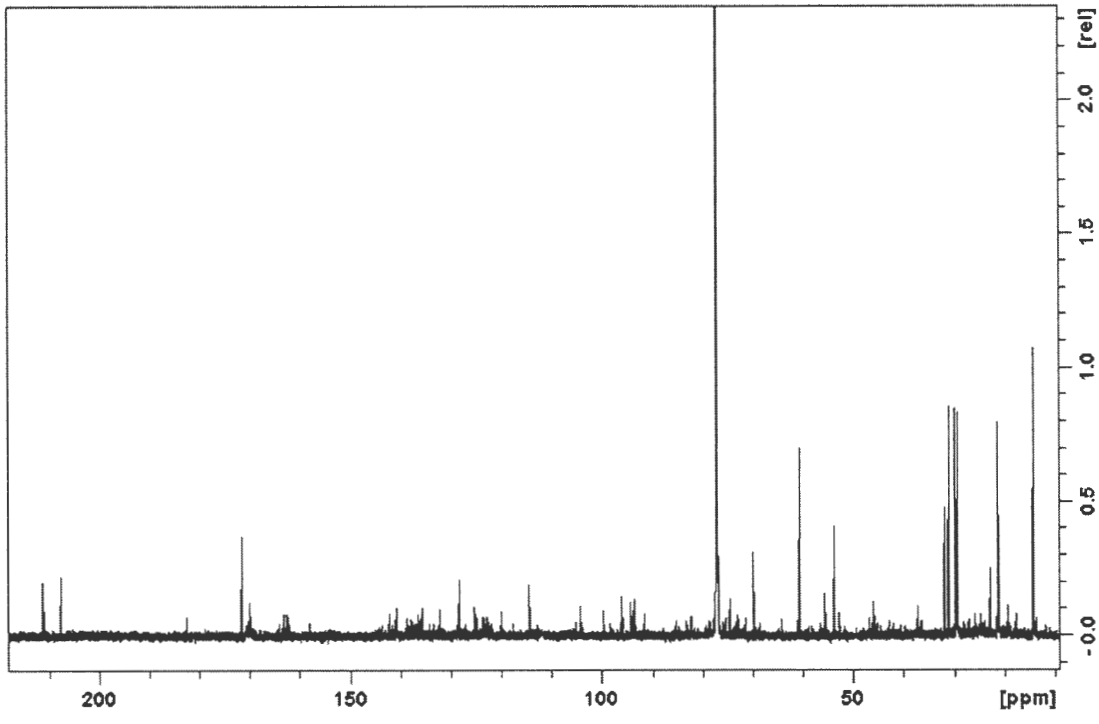

Plate 3: DEPT 135 spectrum of taurin (4.24)

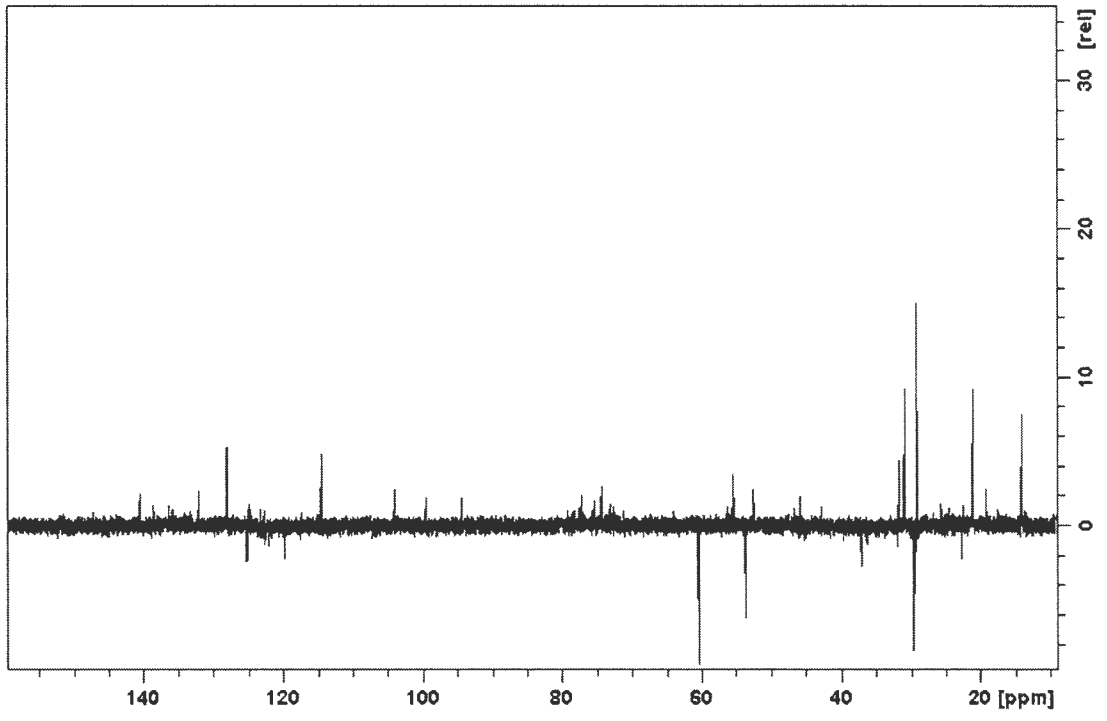

Plate 4: DEPT 90 spectrum of taurin (4.24)

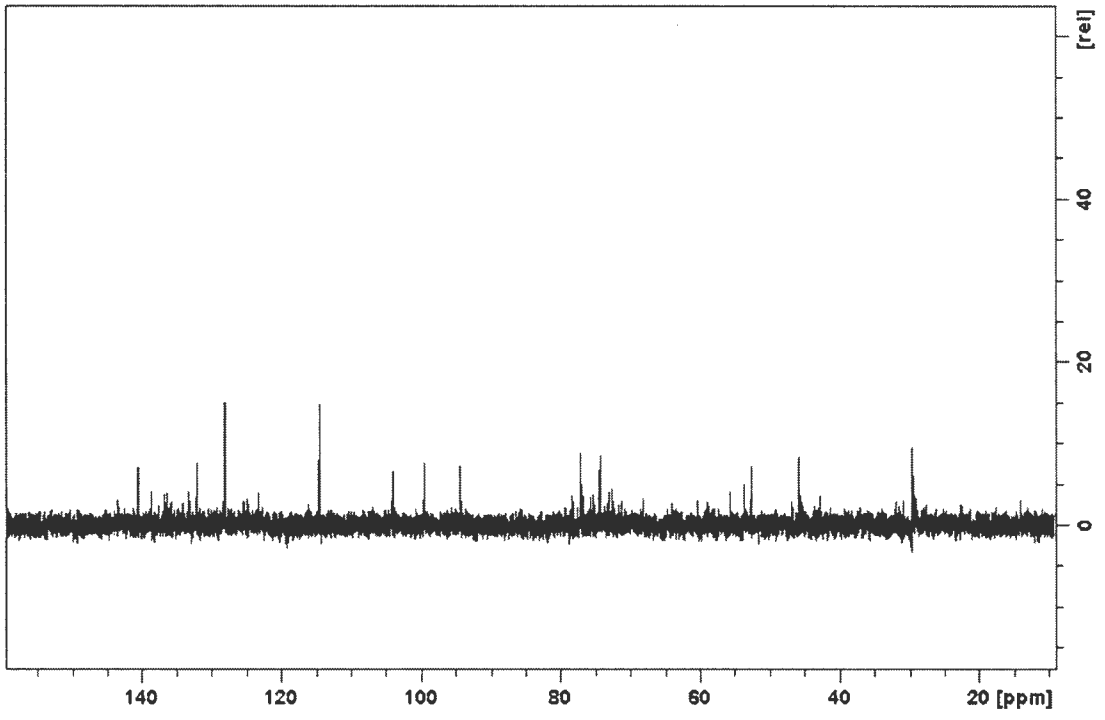

Plate 5: COSY spectrum of taurin (4.24)

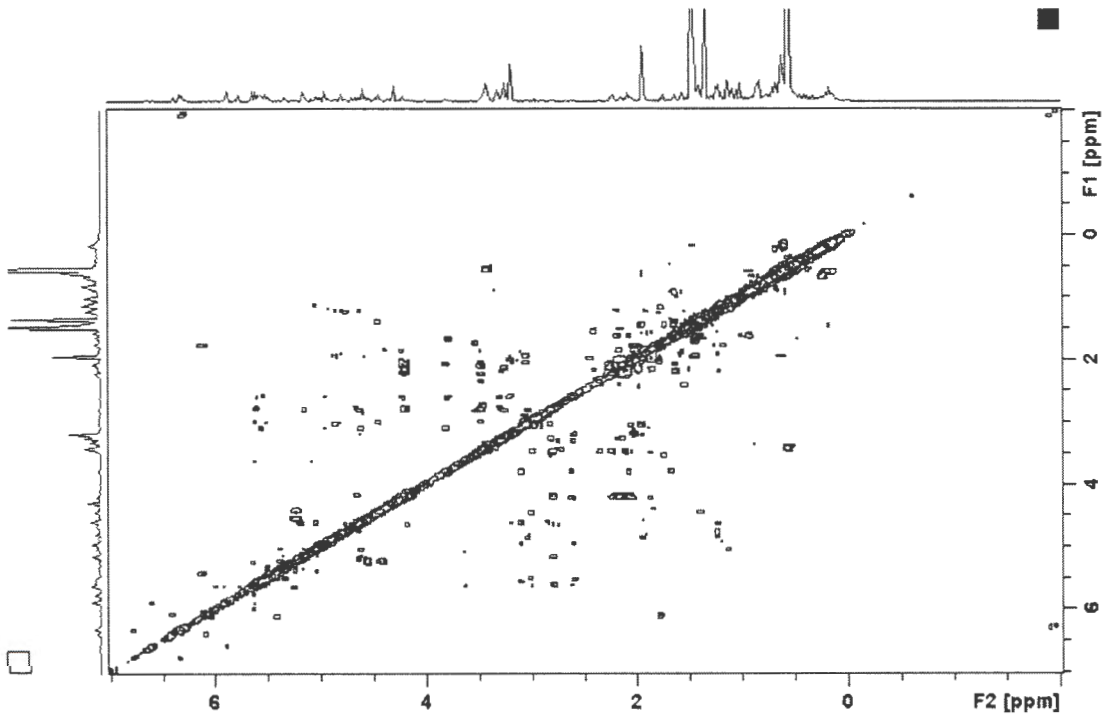

Plate 6: HSQC spectrum of taurin (4.24)

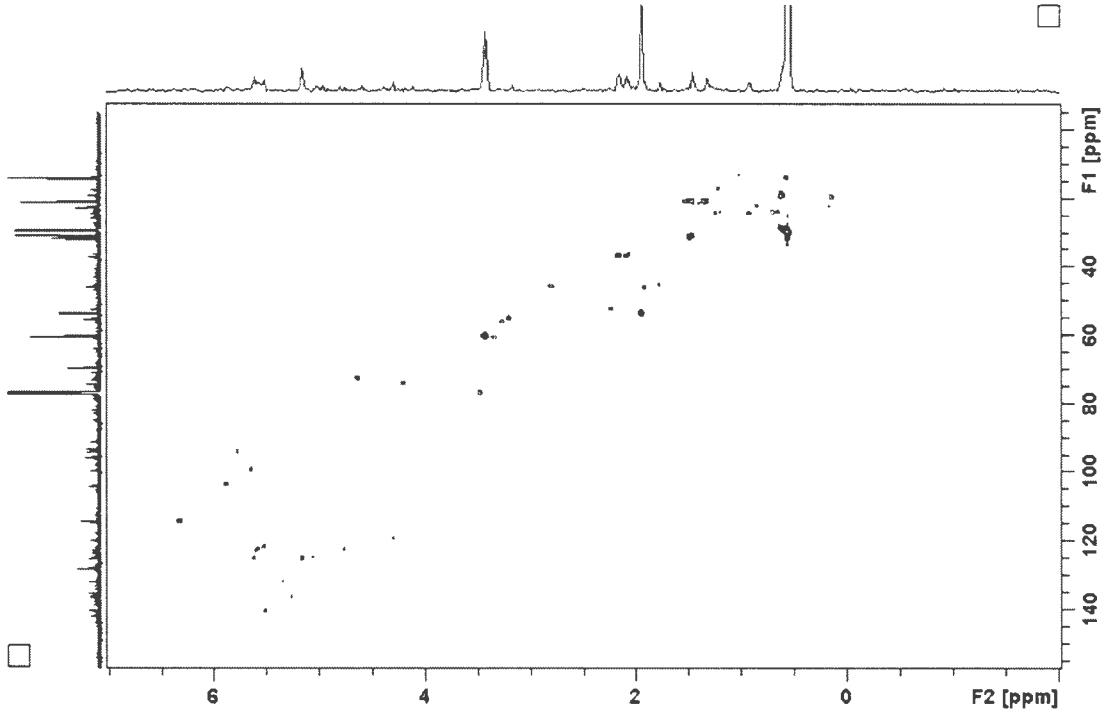

Plate 7: HMBC spectrum of taurin (4.24)

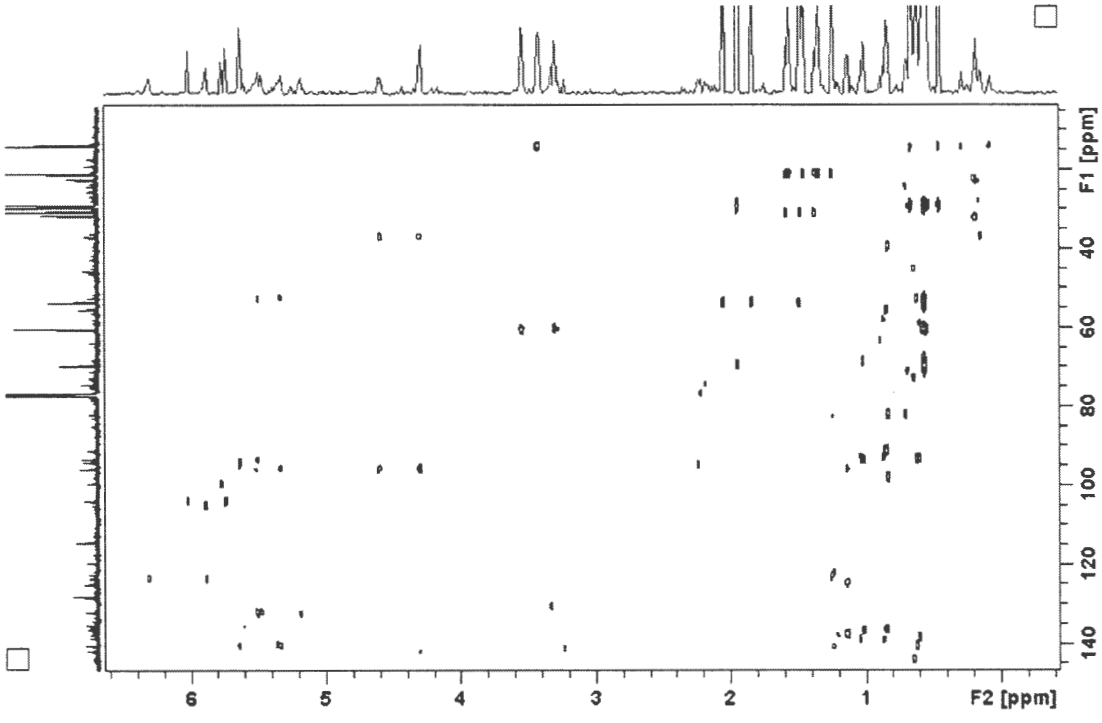

Plate 8: ^{1}H NMR spectrum of marimitin (4.25)

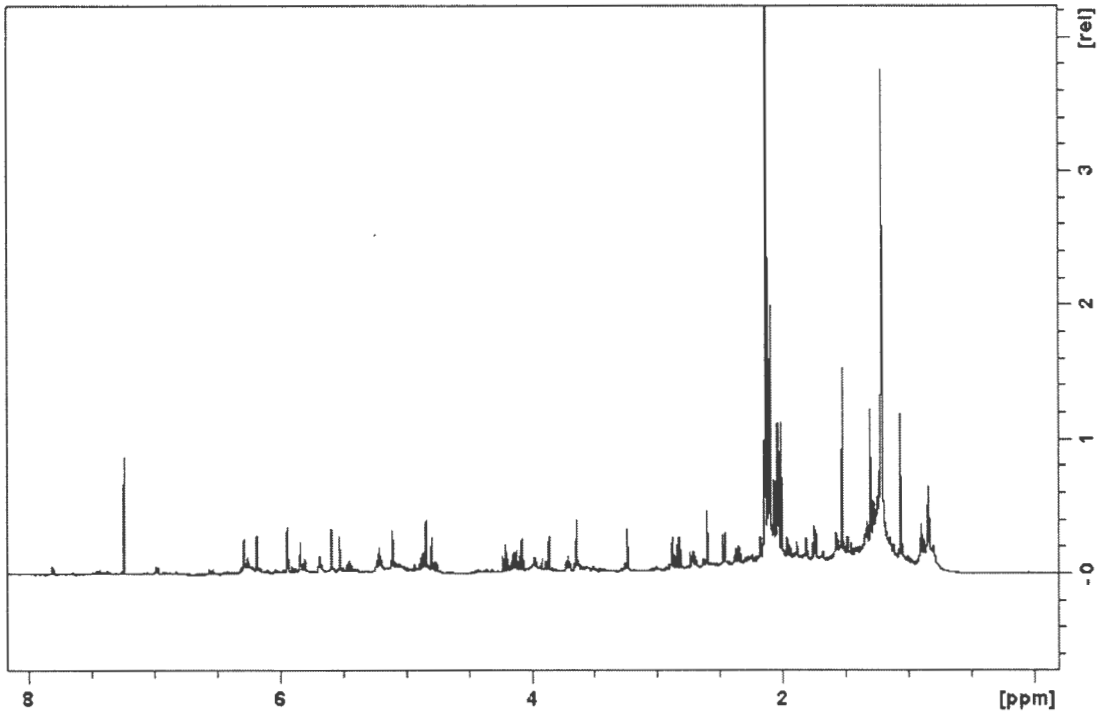

Plate 8a: ^{1}H NMR spectrum of marimitin (4.25)

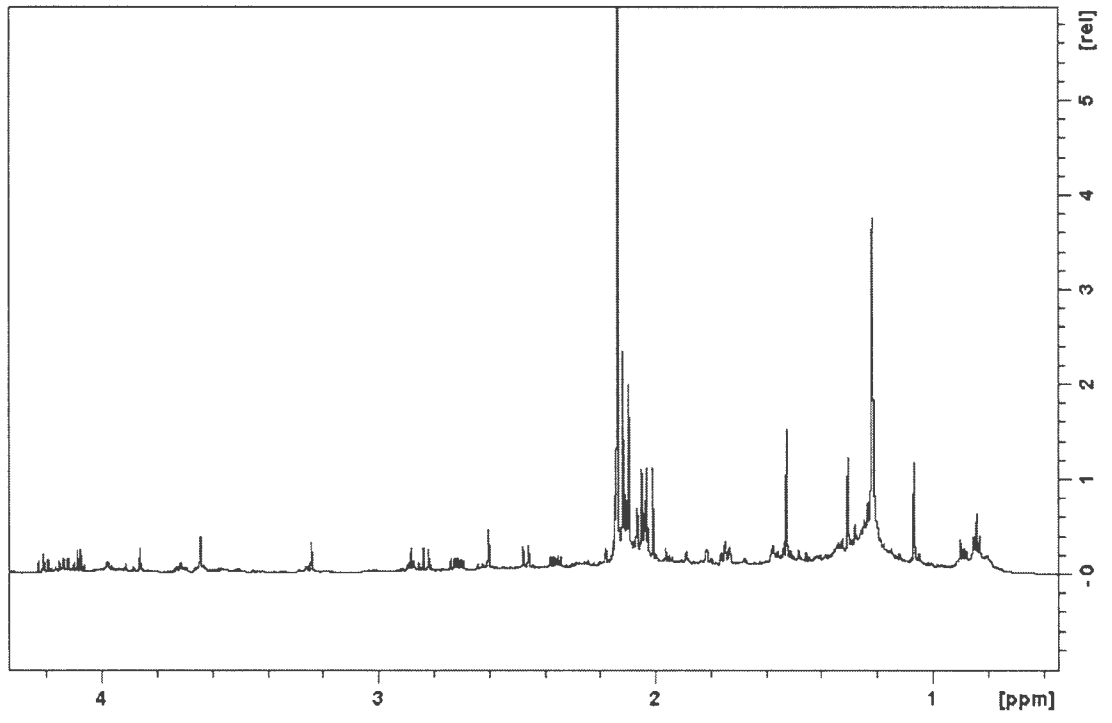

Plate 9: ^{13}CNMR spectrum of marimitin (4.25)

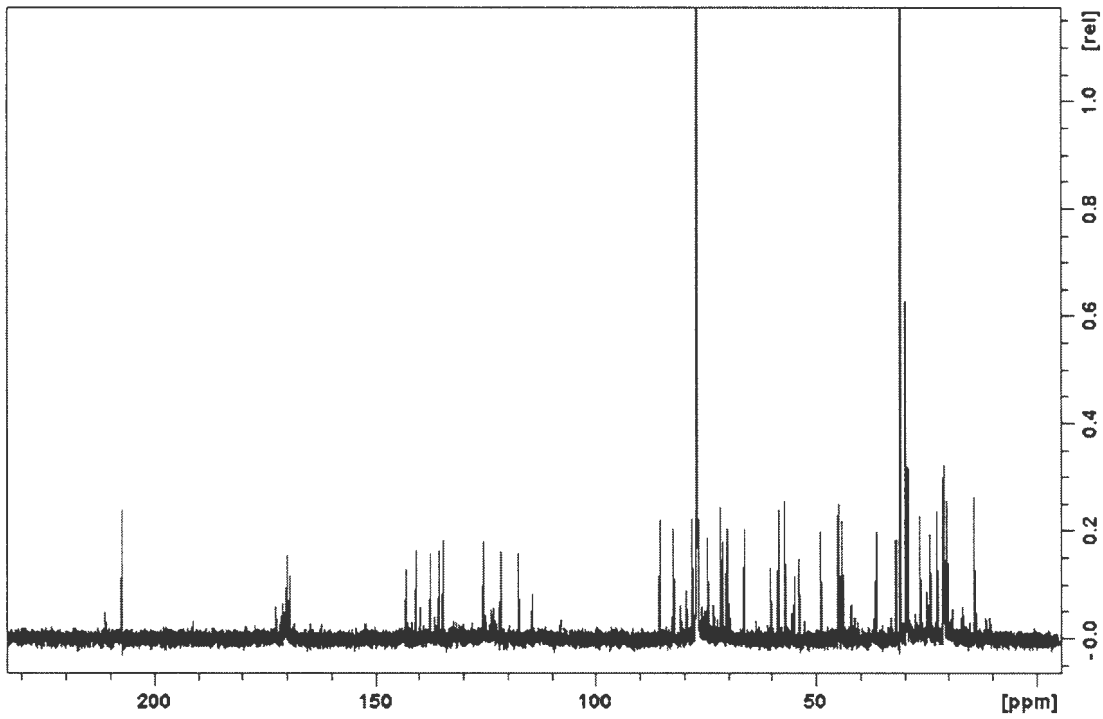

Plate 10: DEPT 135 spectrum of marimitin (4.25)

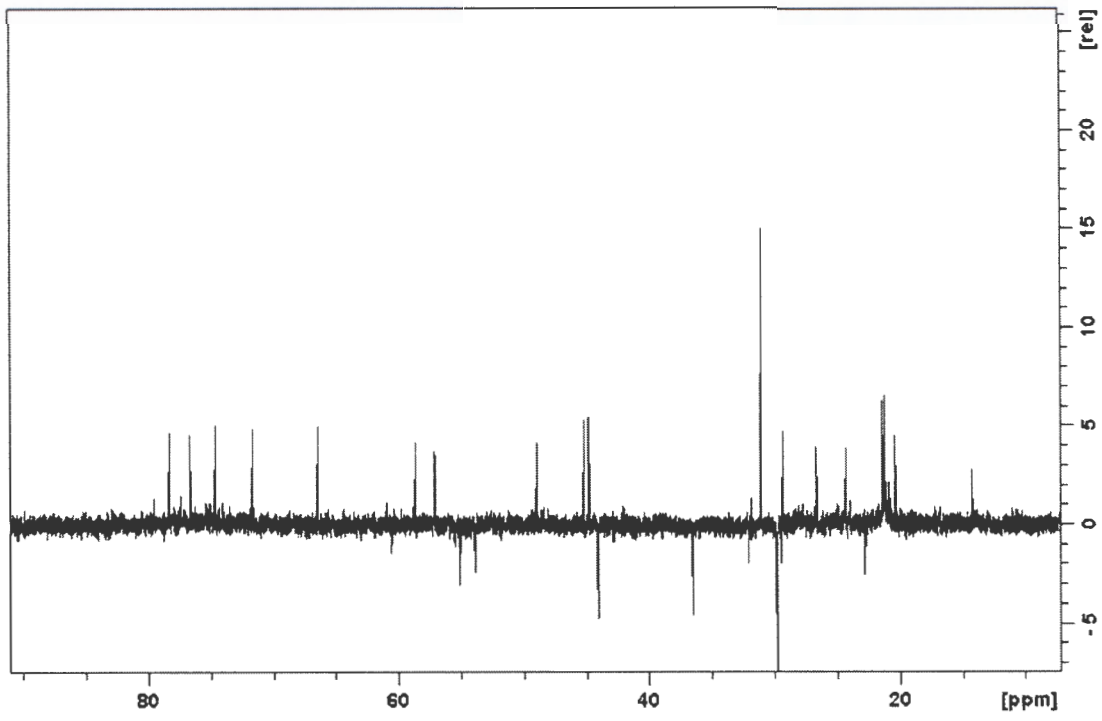

Plate 11: DEPT 90 spectrum of marimitin (4.25)

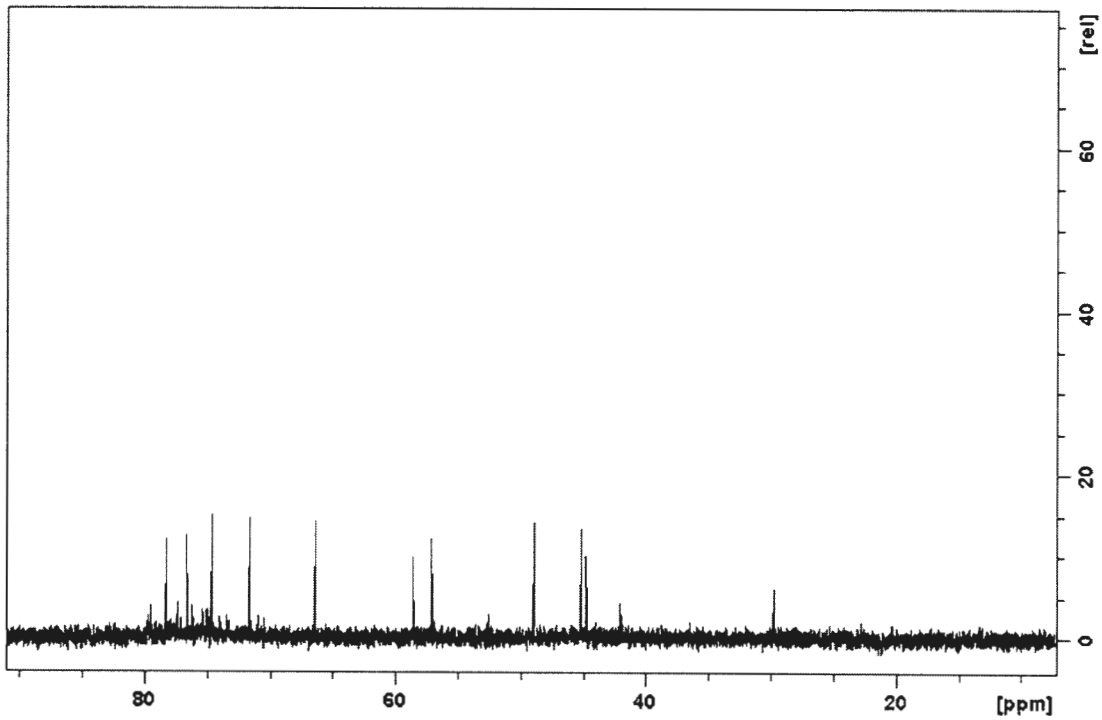

Plate 12: COSY spectrum of marimitin (4.25)

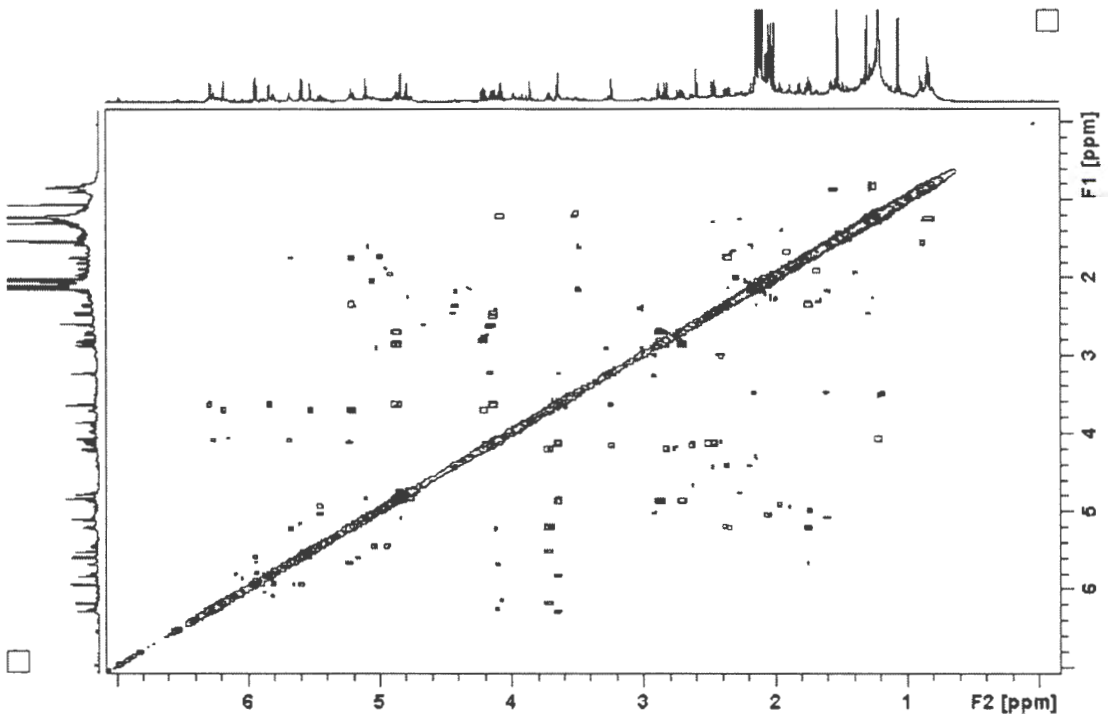

Plate 13: HSQC spectrum of marimitin (4.25)

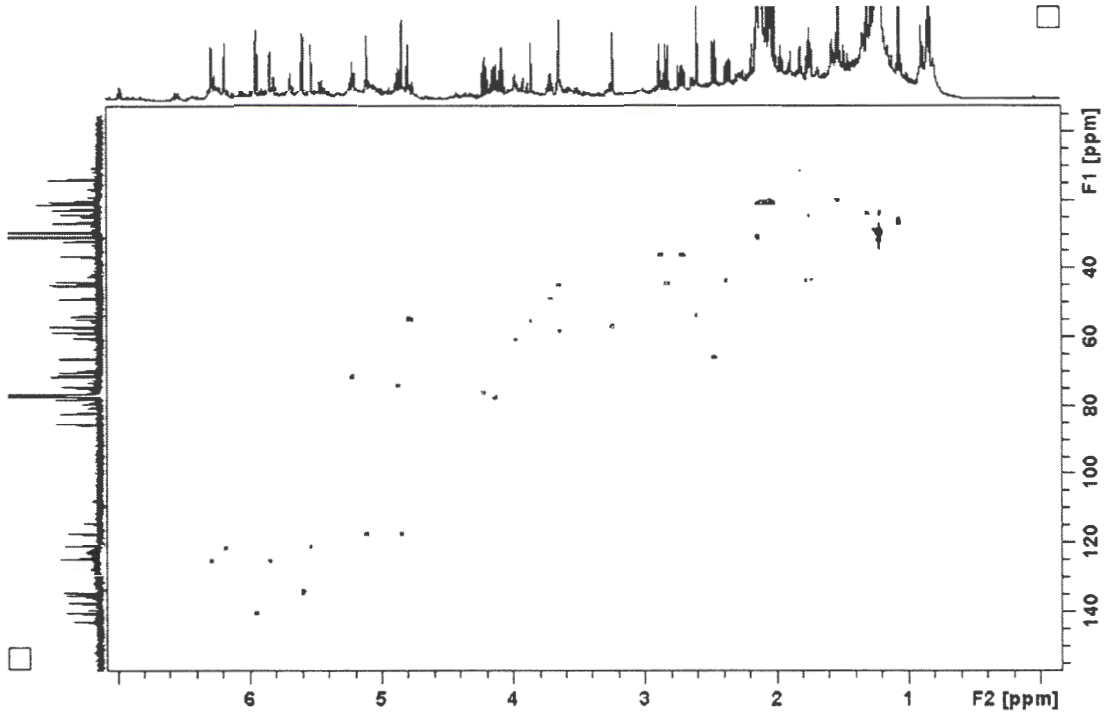

Plate 14: HMBC spectrum of marimitin (4.25)