CHAPTER 1

Malaria and antimalarials from plants

1.1 History of Malaria

Malaria, a life-threatening disease that is transmitted by Anopheles mosquitoes, is probably one of the oldest diseases known to mankind. Mentions of this disease can be found in ancient Chinese, Indian and Egyptian manuscripts. In the 5th century BC Hippocrates, the Greek physician, was the first to describe the manifestations of the disease. In the 7th century AD, the Italians named the disease *mal'aria* meaning bad air, due to its association with ill-smelling vapours from the swamp near Rome.

The first recorded treatment of malaria dates back to 1600 when the bark of the Cinchona tree was first used by the native Peruvian Indians to treat the intermittent fevers associated with this illness.[1] It was not until 1889 that Alphonse Laveran discovered the protozoal (single celled parasite) cause of malaria and not until 1897 that Ronald Ross demonstrated that the Anopheles mosquito was the vector for the disease.[2] His pioneering work on establishing the main features of the parasitic life cycle earned Ross the Nobel Prize in Medicine in 1902.

Over the next century significant advances were made towards attempts to eradicate malaria particularly with respect to controlling mosquitoes, understanding the parasite and developing drugs to treat the disease.[3] The greatest challenge lies in the parasites ability to quickly adapt and overcome eradication efforts when these are fragmented and uncoordinated. Malaria quickly rebounded from the mass insecticide spraying campaigns in the 1950s and 1960s. Since the 1980s parasite resistance to chloroquine, the most commonly available antimalarial drug, has emerged as a major challenge.

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[1] M.R. Lee, *J. R. Coll. Physicians Edinb.*, 2002, **32**, 300.
[2] A. Robert, B. Françoise, O. Dechy-Cabaret and B. Meunier, *Pure Appl. Chem.*, 2001, **73**, 1173.
[3] London School of Hygiene and Tropical Medicine, 'Malaria : Waiting for the Vaccine', John Wiley & Sons, Chichester, 1991.

1.2 Malaria Today

Malaria is one of the biggest killers in the world. Current estimates place the clinical caseload at between 300 and 500 million people annually and nine out of ten of these cases occur in sub-Saharan Africa.[4] This is due to the majority of infections in Africa being caused by *Plasmodium falciparum*, the most dangerous of the human malaria parasites. It is also because the most effective and most difficult to control malaria vector - the mosquito *Anopheles gambiae* - is the most widespread in Africa. Climatic conditions over a large part of Africa favour malaria transmission and global warming together with changes in land use are extending the areas of transmission. Moreover, many countries in Africa lacked the infrastructures and resources necessary to mount sustainable campaigns against malaria and as a result few benefited from historical efforts to eradicate malaria. Also, methods aimed at eradicating the disease have been hindered by lack of governmental commitment, failure to use existing resources and poor health care facilities.

Today approximately forty percent of the world's population is at risk to malaria. Malaria is endemic in Africa, much of South and Southeast Asia, Central America, and northern South America (Figure1.1).[5]

In South Africa malaria is mainly transmitted in the low altitude areas of the northeastern parts of Southern Africa; this includes the lowveld region of Mpumalanga, Limpopo Province and the northeastern parts of KwaZulu-Natal. Malaria transmission is seasonal with the greatest number of cases occurring between October and May with a significant inter-annual variation in the number of malaria cases. In the year 2002 the annual number of reported malaria cases was approximately 15 582 while in 2003 it was 4392.[6] This variation is mainly attributed to favourable climatic conditions, population migration and the emergence of drug resistant parasites. The South African government has increased efforts to control malaria particularly due to its devastating impact on the economy.

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[4] World Health Organisation Fact Sheet No. 94, 2003, WHO information. http://www.int/inf~fs/en/fact094.html

[5] World Health Organisation, World Health Report, 2003. http://www.d2ol.com/malaria.html

[6] South African Department of Health, National Health Report, 2003. http://www.doh.gov.za/issues/malaria/year00_03.pdf

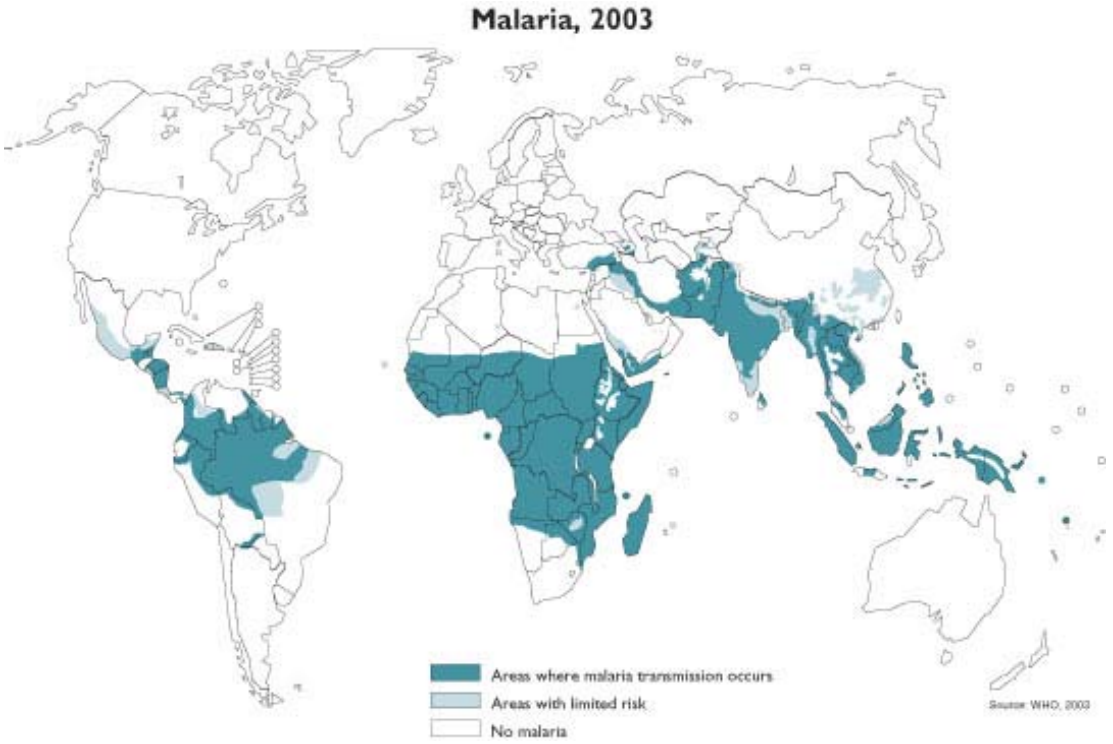


Figure 1.1 Malaria-endemic regions.

Annual economic growth in countries with high malaria transmission has historically been lower than in countries without malaria. The direct costs of malaria include a combination of personal and public expenditures on both prevention and treatment of the disease. The indirect costs of malaria include lost productivity or income associated with illness or death. Also, the prevalence of malaria in a country can lead to a decline in international trade and tourism and foreign investment, which are vital for economic growth.

1.3 The Malaria Parasite

The malaria parasite*, Plasmodium falciparum*, is a very small, single-cell blood organism, or 'protozoan'. There are three other parasite species (*P. malariae*, *P. vivax* and *P. ovale*) that also cause malaria but they are rare in South Africa. The parasite is transmitted to humans by a vector, namely the female *Anopheles* mosquito.

Knowledge of the life cycle of the malaria parasite is fundamental to understanding the methods of prevention, treatment and research pursuits. Interrupting the life

cycle will prevent malaria, but this has proven more difficult than anticipated. The *Plasmodium* parasite spends part of its life cycle in humans and partly in mosquitoes (Figure 1.2).[7,8]

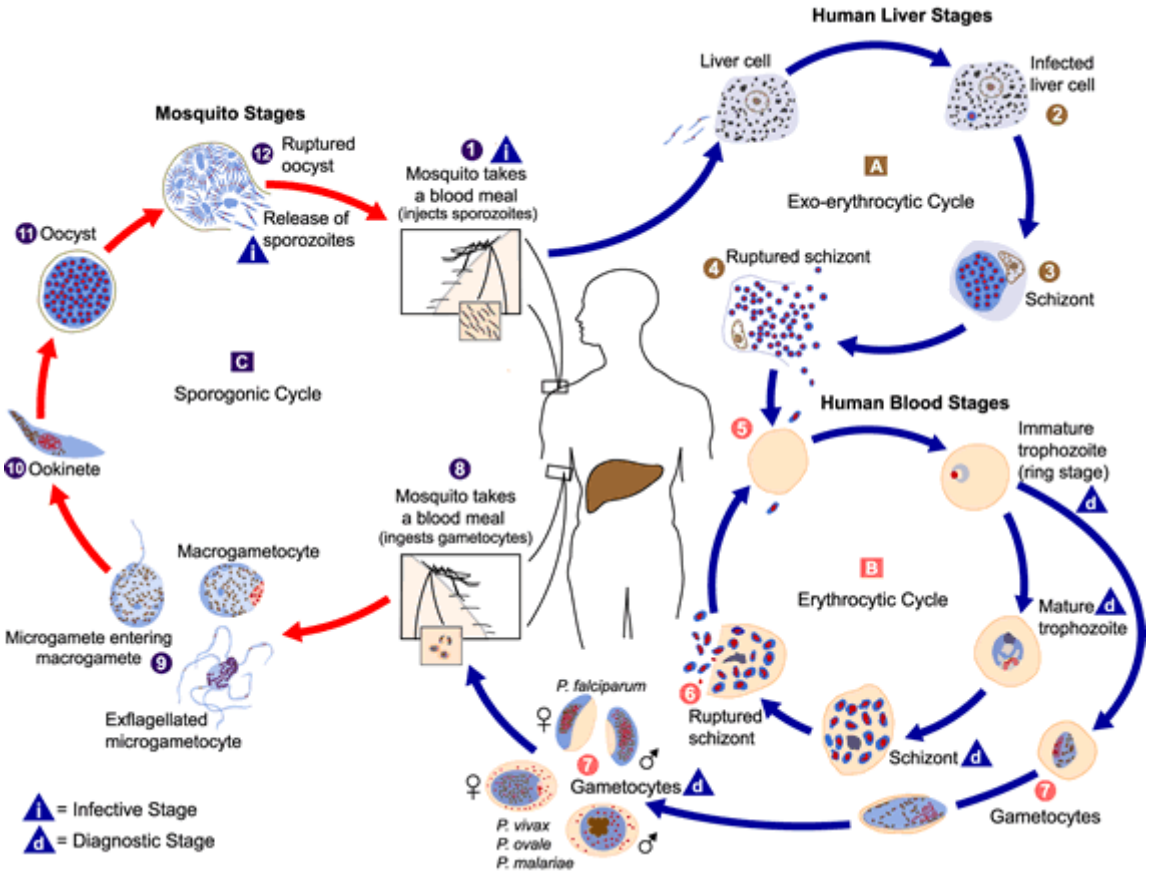


Figure 1.2 Life cycle of *Plasmodium* spp.[9]

While ingesting a blood meal, an infected mosquito injects sporozoites into a human host (1). Sporozoites move to the liver and infect liver cells (2) where they reproduce by mitosis and develop into schizonts (3), which rupture and release merozoites (4). Following this initial replication in the liver (exo-erythrocytic schizogony (A)), the merozoites are released into the bloodstream where they undergo asexual multiplication in the erythrocytes (erythrocytic schizogony (B)).

Merozoites infect red blood cells (5). The ring stage trophozoites mature into schizonts, which rupture releasing merozoites (6). Two out of every three red blood

[7] R. Caniato and L. Puricelli, *Crit. Rev. Plant Sci.*, 2003, **22**, 79.
[8] G. Taubes, *Science*, 2000, **290**, 435.
[9] http://www.netdoctor.co.uk/travel/disease/lifecycle of the malarial parasite.htm

cells soon become infected. The periodic fever and chills associated with malaria occur when the red blood cells rupture and release the merozoites. This is the blood stage of the disease. A fraction of the merozoites differentiate into gamete producing cells (gametocytes) (7).

The gametocytes, male (microgametocytes) and female (macrogametocytes), are ingested by an Anopheles mosquito during a blood meal (8). The parasites' multiplication in the mosquito is known as the sporogenic cycle (C). While in the mosquito's stomach, the male gametes fertilize the female gametes generating zygotes (9). The zygotes in turn become ookinetes (10) which invade the midgut wall of the mosquito where they develop into oocysts (11). The oocysts grow, rupture and release sporozoites (12), which make their way to the mosquito's salivary glands. Inoculation of the sporozoites into a new human host occurs and the cycle begins again (1).

1.4 Malaria Prevention and Control

There are a number of approaches towards the prevention and control of malaria and the choice of intervention in a country or region are usually most dependent on cost-effectiveness.[3]

The early diagnosis of malaria and prompt treatment with antimalarial drugs is essential in controlling the spread of the disease. By reducing the number of infected humans, the number of infected mosquitoes is effectively reduced. This type of control is especially important when outbreaks of malaria occur. When humans are treated the life cycle of the parasite is essentially interrupted.

Another approach is the use of personal protection. The first objective of this is to protect people from being bitten by an infected mosquito. Mosquito nets, screening doors and windows, wearing protective clothing, using insect repellants, coils and vapourizers are all ways of doing this. The other objective of personal control is the use of preventative or prophylactic drug treatment. For instance, travelers to regions where malaria is present often take prophylactics which help prevent the

development of the disease but not the initial infection. Cost and availability of drugs can be dictating factors in many countries.

A third approach is vector or mosquito control. Spraying of insecticides to kill the adult or larval mosquitoes can be quite effective. Managing the environment by reducing the mosquito breeding sites has helped to eliminate malaria in some areas. Using natural biological controls such as mosquito predators is also promising. In the last few years there has been growing interest in bioengineering insects that are unable to transmit the malaria parasite.[10] This is vector manipulation.

Ideally, a protective vaccine would be the most effective approach to controlling malaria. Attempts to develop a vaccine, however, have been hindered by the great genetic diversity of the parasite, its multistage life cycle, as well as the complex and inefficient human immune response.[11] It is anticipated that a vaccine will be available within the next ten years.[3]

1.5 Malaria Treatment

Drugs used for the treatment of malaria do not assist the natural healing processes of the body; instead they act chemically on the parasite as a controlled poison. In most cases antimalarial drugs target the asexual erythrocytic stage of the parasite. The parasite degrades haemoglobin in its acidic food vacuole, producing free haeme able to react with molecular oxygen and thus generating reactive oxygen species as toxic by-products. A major pathway of detoxification of haeme moieties is polymerization as malaria pigment. Most antimalarial drugs act by disturbing the polymerization of haeme, thus killing the parasite with its own metabolic waste. Antimalarial drugs fall into several chemical groups. The first and most commonly used are the quinoline based antimalarials, which include quinine **(1)** and its derivatives chloroquine **(2)**, amodiaquine **(3)** and mefloquine **(4)** (Figure 1.3).

[10] http://ecology.cwru.edu/malaria
[11] P. Newton and N. White, *Annu. Rev. Med.*, 1999, **50**, 179.

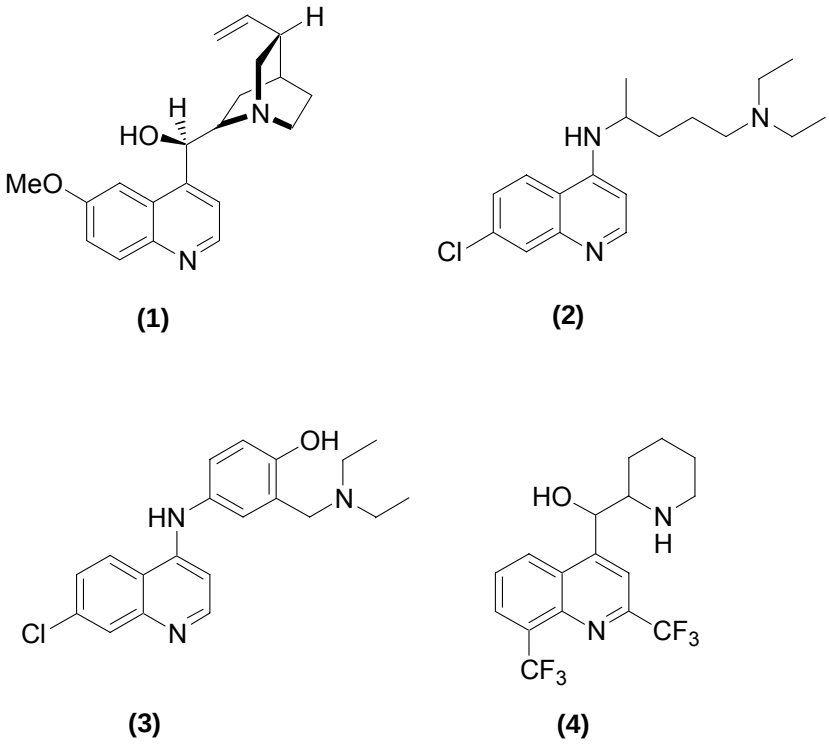


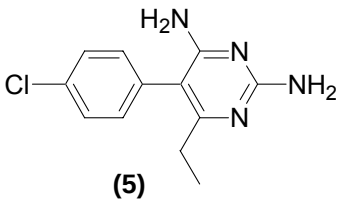
Figure 1.3 Quinoline based antimalarials.

Quinine **(1)** has been used for more than three centuries and until the 1930s was the only effective agent for the treatment of malaria. Of the 36 alkaloids found in the bark of the Cinchona tree, only four possess antimalarial properties, with quinine being the most effective.[1] It is able to bind strongly to blood proteins and forms complexes that are toxic to the malarial parasite. Due to its undesirable side effects it is now only used as an intravenous injection to treat severe malaria.

Chloroquine **(2)** was introduced in 1944 and soon became the mainstay of therapy and prevention, since this drug was cheap, non-toxic and effective against all strains of the parasite.[2] It is capable of blocking the polymerisation of haem to haemozoin (malaria pigment).[12] It is a chemically synthesized drug and remains the most widely used drug in the treatment of malaria, despite increasing parasite resistance. Chloroquine's reduced efficacy led to the development of the synthetic analogues amodiaquine **(3)** and mefloquine **(4)** that are used to treat cases of uncomplicated malaria in areas where chloroquine resistance is prevalent.

[12] J. Zhang, M. Krugliak and H. Ginsburg, *Mol. Biochem. Parasitol*., 1999, **99**, 129.

The second class of common antimalarials is the folate antagonists (Figure 1.4). These compounds inhibit the synthesis of parasitic pyrimidines and thus of parasitic DNA.[2] There are two types of antifolates, the dihydrofolate reductase (DHFR) inhibitors pyrimethamine **(5)** and proguanil **(6)**, and the dihydropteroate synthetase (DHPS) inhibitors, which include the sulphonamide drugs, sulphadoxine **(7)** and dapsone **(8)**. Due to a marked synergistic effect, a drug of the first group is usually used in combination with a drug of the second one. Pyrimethamine-sulphadoxine (SP), or Fansidar®, is the most widely used combination.[11]



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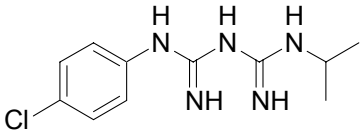

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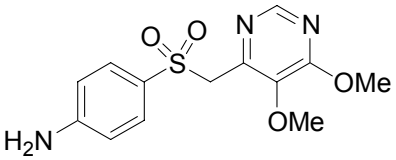

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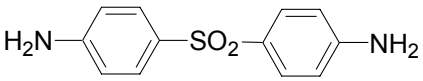

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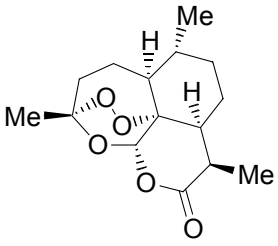
Figure 1.4 DHPS and DHFR inhibitors.

The third class of antimalarials is based on the natural endoperoxide artemisinin **(9)** which was first extracted from the Chinese traditional medicine, *Artemisia annua*, in 1972.[13] Artemisinin is not soluble in water or oil and because of this poor solubility the drug absorption and its bioavailability are also poor. However, since the peroxide bridge of the compound is stable under various chemical reactions, several oil and water-soluble derivatives of artemisinin have since been synthesized.[14] These include dihydroartemisinin (DHA) **(10)**, artemether **(11)**, arteether **(12)**, artesunate **(13)** and artelinic acid **(14)** (Figure 1.5).[2] The semi-

[13] J.A. Vroman, M. Alvim-Gaston and Mitchel A. Avery, *Curr. Pharm. Design*, 1999, **5**, 101.
[14] M.R. Lee, *J.R. Physicians Edinb.*, 2002, **32**, 300.

synthetic derivatives of artemisinin have improved pharmacokinetic properties and are also of current clinical use.



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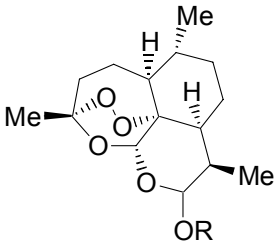

(10) R = H
(11) R = Me
(12) R = Et
(13) R = $CO(CH_2)_2COONa$
(14) R = $CH_2(p\text{-}C_6H_4)CO_2H$

Figure 1.5 Artemisinin and its semisynthetic derivatives.

Since an artemisinin derivative lacking the endoperoxide bridge (deoxyartemisinin) is devoid of antimalarial activity, the possible reactivity of this peroxide function within the parasite is the key factor of the pharmacological activity of these molecules.[15] This group of antimalarials is the most rapidly acting and is effective against multi-drug resistant strains of the parasite. Although the precise mode of action of artemisinin and its derivatives is not completely understood, it is proposed that the endoperoxide bridge is cleaved to generate free radicals. The free radicals are strong alkylating agents and form covalent bonds with various parasite proteins.

In addition to destroying the parasite, health care providers are attentive to treating the multiple symptoms of malaria. These symptoms include fever, chills, headaches, malaise, weakness, hepatomegaly (enlarged liver), splenomegaly (enlarged spleen) and dehydration. Malaria can also cause anemia, anorexia, nausea, vomiting, abdominal pain and diarrhoea. Deaths from malaria are normally caused by cerebral, renal or pulmonary fever, or a combination of the three.[16]

[15] D.L. Klayman, *Science*, 1985, **228**, 1049.
[16] G.T. Strickland and K.W. Hunter, 'The Pathophysiology of Human Malaria', Praeger, Westport, 1982.

1.6 Resistance to Antimalarial Drugs

Whilst the number of effective drugs to treat a malaria infection is limited, the rapid emergence of drug resistant strains of the parasite is outpacing the development of new antimalarials. The reasons for the development and spread of drug resistance involve the interaction of drug-use patterns, characteristics of the drug itself, human host factors, parasite characteristics, and vector and environmental factors.[17] However, only gene mutations confer resistance to the parasites in nature.

The rate of spread of resistance to a particular drug will depend on a number of factors including the starting frequency of resistant mutations to that drug, the intensity of malaria transmission, immunity of population, drug pressure and the pharmacokinetic/pharmacodynamic properties of the drug.

Chloroquine **(2)** is by far the most used antimalarial in conventional malaria therapy due to it being relatively inexpensive and readily available in many endemic areas. However, owing to widespread drug resistance, the drug is becoming increasingly ineffective in many parts of the world. The generally accepted explanation for chloroquine resistance is that resistant parasites accumulate less chloroquine than sensitive parasites. Thus lethal concentrations of the drug are prevented from reaching the parasitic food vacuole. The decrease in chloroquine accumulation can be attributed to a higher rate of chloroquine efflux, a lower rate of chloroquine uptake, or varying combinations of both these processes.[18]

There is also evidence of increasing antifolate resistance in malaria parasites. Antifolate resistance is generally due to a combination of mutations in the target enzymes and the use of an alternative pathway to recover folate. There is no solid data supporting the existence of resistance to artemisinin, although recurrence is associated with the monotherapy of artemisinin and its derivatives at a high rate.

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[17] R.G. Ridley, *Nature*, 2002, **415**, 686.
[18] K.J. Saliba, P.I. Folb and P.J. Smith, *Biochem. Pharmacol.*, 1998, **56**, 313.

In order to prevent this return artemisinins are used with longer-acting antimalarial medications in combined treatments.[19]

1.7 Need for New Antimalarials

Growing resistance to antimalarial drugs is perhaps the most important factor contributing to the current resurgence of malaria. The escalating mortality rate among African children is directly attributable to malaria and more specifically to the rapidly increasing resistance to antimalarial drugs. The number of effective drugs available to treat malaria is limited and the rate at which resistance is mounting is outpacing the development of new antimalarials. Nearly all the antimalarials that are in use today were developed almost thirty years ago and, in general, the pharmaceutical companies, particularly the multinationals, have little interest in developing a new cure despite the immense need.[20]

Most of the available drugs were developed through synthesis and screening – an approach which has proved inefficient and costly. With no vaccine on the immediate horizon, chemotherapy and chemoprophylaxis remain the major methods of controlling malaria. However, with the increase in cases of drug resistance there is an urgent need for new drugs with novel modes of action.

One of the biggest obstacles in the battle against malaria in areas where the disease is most prevalent is poverty. Thus there is a real need to find simple, affordable antimalarial medicines. One approach to this is the investigation of medicinal plants and natural products.

1.8 Traditional Medicine

Traditional medicine is the embodiment of the knowledge, skills and practices based on the theories, beliefs and experiences indigenous to different cultures that have been handed down from generation to generation. They are used in the maintenance of health as well as in the prevention, diagnosis, improvement or treatment of physical and mental illness.

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[19] P.B. Bloland, 'Drug resistance in malaria', Geneva: World Health Organisation, 2001.
[20] R. Ramachandran, *Frontline*, 2002, **19** http://www.frontlineonnet,com/fl1913/19130870.htm

In the last decade, there has been a global upsurge in the use of traditional medicine and complementary and alternative medicine in both developed and developing countries. Various reasons have been proposed for this increase, including affordability, but also changing needs and beliefs. The most widely used traditional medicine and complementary and alternative medicine therapies are herbal medicines and acupuncture.

Traditional medicine has been described by the World Health Organisation (WHO) as one of the surest means to achieve total health care coverage of the world's population. In spite of the marginalisation of traditional medicine practiced in the past, the attention currently given by governments to widespread health care application has stimulated research, investments and programme design in this field in several developing countries.[21] So far countries that have successfully integrated traditional medicine into their primary health care systems are China, North and South Korea and Vietnam.

In South Africa there are an estimated 200 000 practicing traditional healers. A traditional healer is the general term used to describe a practitioner of indigenous medicine. The popularity of traditional healers in South Africa is considered due to the strong cultural belief system and modern medical facilities often being inaccessible or unaffordable. A number of initiatives have been taken by the South African National Department of Health to investigate traditional medicines for efficacy, safety and quality with the aim to incorporate their use in the national health care delivery system.

1.9 Medicinal Plants

Plants of medicinal value have been used effectively for centuries in traditional medicine. Traditional health care systems using medicinal plants can be recognized and used as a starting point for the development of novel drug leads. Medicinal plants are considered a major source of biologically active natural products that may serve as commercially significant entities themselves or provide

[21] Conserve Africa Organisation, 'Overview on Medicinal Plants and Traditional Medicine in Africa', 2004 http://www.conserveafrica.org/medicinal_plants.rtf

lead structures for the development of modified derivatives possessing enhanced activity and/or reduced toxicity.

Traditional medicines include crude plant extracts, or combinations of several medicinal plants, which contain numerous components that are thought to contribute to the overall therapeutic effect. Because the chemical compounds in the different plant components are often quite different, usually only a specified plant part is used medicinally (*viz.* leaves, roots, bark or fruit). The method of preparation is crucial. Activities including the addition of appropriate volumes of solvents such as water or alcohol to a specified amount of fresh or dry plant material, boiling for a specified length of time or partial burning to achieve a desired colour are important and can serve to neutralize certain toxins. Dosage forms (*viz*. tinctures, extracts, ointments or enemas) as well as the method of administration (*viz*. orally, topically or nasally) are also critical and are conveyed by the healer.[22]

Plant-based traditional medicine systems continue to play an essential role in healthcare, and it has been estimated by the WHO that approximately 80% of the world's inhabitants use plants for their primary healthcare.[23] Because of the importance of medicinal plants the WHO encourages their use not only under an empirical basis, but also under a scientific approach. The profits gained from a scientific approach to traditional plant remedies in developing countries, where they have fundamental importance, are numerous.

Firstly it would allow natives to gain some independence from developed countries in the preparation of plant-derived medications, and it would promote the establishment of sustainable supply and extraction industries, which could prove to be a vital aspect for economic development. Proving the efficacy of traditional medicines, would also allow the local medium-large scale cultivation of medicinal plants with an obvious benefit for the national economy. Finally, from an environmental point of view, the proof of therapeutic value of selected medicinal

[22] B.E. van Wyk, B. van Oudtshoorn and N. Gericke, 'Medicinal Plants of South Africa', Briza Publications, Pretoria, 2000.
[23] World Health Organisation, 'Traditional Practitioners as Primary Health Care Workers', WHO/SHS/DHS/TRM/95.6, Geneva, 1995.

plants would help to conserve species that would otherwise be depleted by unsustainable harvesting activities.[7]

An estimated 70% of South Africans regularly use traditional medicines, most of which are derived from plant species indigenous to the region. South Africa represents only 0.04% of the land surface area of the world, yet nearly 10% of all known plant species occur here. There are over 24 000 plants indigenous to South Africa. Approximately 3000 species of plants are used as medicines, and some 350 of these are the most commonly used and traded medicinal plants. South African medicinal plants that are popular worldwide include Cape aloes (*Aloe ferox*), buchu (*Agathosma betulina*) and devil's claw (*Harpagophytum procumbens*).[22]

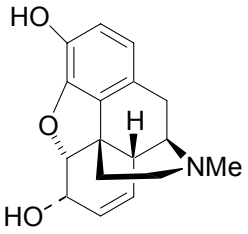
1.10 Drugs from Plants

There are two basic approaches to drug discovery: rational drug design and the traditional method of random screening. Rational design-engineering of new drug molecules from scratch with the aid of computers and molecular biology requires knowledge of the drug target such as a receptor or an enzyme. So far, it has had only limited payoffs, although it has promising potential. In random screening many synthetic chemicals or natural products are indiscriminately tested for biological activity. Because this method is both costly and time-consuming, there has been a great need for better efficacy in strategic research and development planning for pharmaceutical companies. The strategy of developing new drugs based on medicinal plants has an advantage over random screening, since it is guided by experience from a long history of clinical practice.

In 1819, the isolation of the analgesic morphine **(15)** from the opium poppy (*Papaver somniferum*) laid the foundation for the purification of pharmacologically active compounds from medicinal plants.[24] More than 50% of all drugs in clinical use today originated from plants or are derivatives of natural products. Well known examples include quinine **(1)**, extracted from the bark of the *Cinchona* species; the anticancer drug, taxol **(16)**, from the bark of *Taxus brevifolia*; and salicylic acid **(17)**

[24]M.S. Butler, *J. Nat. Prod*., 2004, **67**, 2141.

which served as a template for aspirin **(18)**, originally isolated from the bark of the *Salix* species. In addition, crude herbal preparations such as *Oenothera biennis* (Evening primrose), *Hypericum perforatum* (St John's wort), and *Panax ginseng* (Ginseng) are also popular. The isolation of an active compound, or the use of a herbal preparation with therapeutic efficacy is particularly relevant to diseases lacking effective chemotherapeutic agents.



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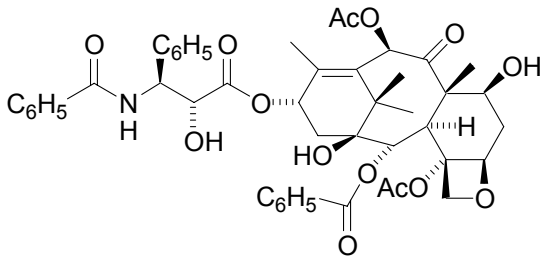

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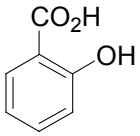

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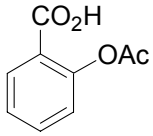

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Figure 1.6 Examples of plant-derived drugs.

The active ingredients in medicinal plants are chemical compounds that act directly or indirectly to prevent or treat a disease or ailment and maintain health. Plants investigated for pharmacologically active compounds are usually selected on the basis of ethnomedicinal information as there is a correlation between biological activity and the traditional use of the plant. In selecting plants that may contain biological agents, generally a targeted approach to plant collection is adopted based on the belief that plants that have acquired the status of a traditional herbal remedy have reasonable proof of safety and efficacy from their history of use and have a higher probability of yielding an active substance.

The chemical diversity and stereospecificity of complex natural products are the main attractions of working with plants as opposed to synthetic chemistry approaches. In addition plant constituents often occur as a group of structurally

related metabolites, making it possible to isolate analogues of the active compound and obtain information on structure-activity relations. Only a small percentage of the estimated 250 000 higher plant species have been investigated for pharmacological activity thus it is most likely that plants will continue to offer novel leads for drug development.

1.11 Antimalarials from Plants

Historically the majority of the antimalarial drugs have been derived from medicinal plants or from structures modeled on plant lead compounds. Quinine **(1)**, the first effective antimalarial drug is still in clinical use today and the more recently discovered artemisinin **(9)** has proved to be an incentive for further research into plants.

The investigation of a range of plants from various countries used in traditional medicine for the treatment of malaria has led to the discovery of a large number of antimalarial compounds with significant structural variety.[25] Table 1.1 lists some of these compounds which belong to different secondary metabolite classes and the traditional medicines from which they were isolated.[7] Some of these compounds are not particularly active, but are nevertheless interesting because they might strengthen chloroquine activity or restore chloroquine sensitivity in resistant strains of *P. falciparum*.

In researching plants which are frequently mentioned as antimalarials in literature it is often found that these do not necessarily show high activity in *in vitro* tests. This can partly be explained by the fact that many plants are used in the treatment of malaria, not for their antiparasitic effects but because of other therapeutic activities. These include reducing fever, calming convulsions and headache, and possibly even immuno-stimulatory effects. Another problem is that some plants are given in a mixture and are possibly only active in this combination due to synergistic effects. Also, an *in vitro* assay cannot precisely reproduce the *in vivo* situation. Certain plant extract components might only become active after specific metabolic processes *in vivo*.[26]

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[25] S. Schwikkard and F.R. van Heerden, *Nat. Prod. Rep.*, 2002, **19**, 675.
[26] M.C. Gessler, M.H.H. Nkunya, L.B. Mwasumbi, M.Heinrich and M.Tanner, *Acta Trop.*, 1994, **56**, 65.

Table 1.1 Examples of classes of compounds with antimalarial activity isolated from traditional medicines

Class of compound	Compound	Plant	Part of plant	Country
Quinones	1-hydroxybenzoiso-chromanquinone	*Psychotria camponutans*	Stem and roots	Panama
Triterpenes	Lupeol	*Vernonia brasiliana*	Leaves	Brazil
Sesquiterpenoids	16,17-Dihydrobrachy-calyxolide	*Vernonia brachycalyx*	Leaves	Kenya
Quassinoids	Bruceolide	*Brucea javanica*	Fruits	China
Liminoids	Fissinolide	*Khaya Senegalensis*	Bark	Sudan
Alkaloids	Ancistroheynine A	*Ancistocladus heyneanus*	Roots	India
Lignans	(+)-Nyasol	*Asparagus africanus*	Roots	Kenya
Coumarins	O-Methylexostemin	*Exostema mexicanum*	Stem bark	Latin America

Other problems commonly encountered when investigating medicinal plants as a source of antimalarial drugs is that crude extracts or compounds show *in vitro* activity but are extremely toxic or those which are active *in vitro* fail to display *in vivo* activity. If a compound destroys parasites, it is logical to screen for toxicity *in vitro* using human cells in culture. When a compound is found to destroy human cells at similar concentrations its potential as a useful drug is limited as the safety margins will be too slender. The difficulty arises from the fact that protozoa share many biochemical pathways with the human host thereby limiting the antimalarial drug's selectivity to kill the parasite without harming mammalian cells. The pharmacokinetic and pharmacodynamic properties of the extract or compound determine whether it will display *in vivo* activity. This includes absorption, distribution to the active site and whether the compound is metabolized too rapidly or to a less active form.[27] Out of the numerous potential antimalarial drugs discovered, only a limited number achieve drug candidate status.

[27] G.C Kirby, *Trans. R. Soc. Trop. Med. Hyg.*, 1996, **90**, 605.

1.12 Antimalarial Drug Discovery

In the drug discovery process, when a compound is identified in primary screens to have antiparasitic activity and lack of host toxicity, extensive biochemical studies are conducted to determine the mode of action. In terms of developing a drug from a plant-derived lead compound, attempts may be made to produce chemical analogues of the active principle with enhanced antiplasmodial activity and reduced host toxicity. Guides for conducting this work are often obtained from the original plant source since many plant compounds exist as groups of structurally related metabolites within a single species or within related species and genera of one or more families. Before a compound can be used as a drug it is essential to know its likely effects when used in humans. This is achieved after extensive laboratory testing followed by the application of clinical trials.

Biological testing for antimalarial activity in plants has progressed over the years. In the 1950s, the screening of crude plant extracts was based on avian malarias using *in vivo* tests against *P. gallinaceum* in chicks and against *P. cathemerium* and *P. lophurae* in ducklings. In the 1970s, *in vitro* procedures were developed utilizing *P. falciparum* cultures in human red blood cells, a technique that enabled the development of a microdilution assay. This technique, compared to previous ones active on human malarias, is useful to assess *in vitro* antimalarial activity of crude extracts prior to the isolation of active principles.[7]

One such *in vitro* assay is the parasite lactate dehydrogenase (pLDH) assay. pLDH is a terminal enzyme in the glycolytic pathway of *Plasmodium spp.* and plays an important role in the parasites anaerobic carbohydrate metabolism. As malaria parasites principally rely on anaerobic glycolysis, they require the regeneration of nicotinamide adenine dinucleotide (NAD) for the continuous flux of glucose through this pathway.[28] On the basis of the discovery that pLDH is distinguishable from host LDH using the 3-acetylpyridine dinucleotide analogue of NAD (APAD), Makler *et al*.[29] developed a drug-sensitivity assay that determines inhibition profiles by measuring the enzymatic activity of pLDH.

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[28] H. Noedl, C. Wongsrichanalai and W.H. Wernsdorfer, *Trends Parasitol.*, 2003, **19**, 175.
[29] M.T. Makler J.M. Ries, J.A. Williams, J.E. Bancroft, R.C. Piper, B.L. Gibbins and D.J. Hinrichs, *Am. J. Trop. Med. Hyg.*, 1993, **48**, 739.

An *in vivo* screening is possible in mice using a natural infection with *P. berghei*. These methods allow the development of strains resistant to chloroquine or to other antimalarials by a passage in the presence of increasing concentrations of the drug. *In vitro* tests are considered more practical, quicker and less expensive than *in vivo* cultures and not all antimalarial drugs are active in the *P. berghei* mouse model. In addition, the *in vivo* model requires significantly higher amounts of drugs (at least 1 g of extract) when compared to the *in vitro* assays which require a few mg of extract. The advantage of the *in vivo* model is that at the same time it gives a measure of toxicity.

Detailed evaluation of antimalarial drugs is done in the Aotus monkey (*Aotus trivirgatus*) using *P. falciparum* infection or in the Rhesus monkey (*Macaca mulata*) with *P. cynomolgi* B infection.[7] Of the numerous extracts and compounds studied in primary screens *in vitro*, very few reach this stage of investigation.

1.13 Scope of this Study

South Africa boasts remarkable biodiversity and a rich cultural heritage of medicinal plant use. A number of extracts from South African plants have been evaluated for *in vitro* antimalarial activity but little is known about their active constituents.[30] In light of this and the pressing need for new antimalarial drugs, the South African Department of Arts, Culture, Science and Technology (now the Department of Science and Technology) awarded an Innovation Fund to five South African institutions - the Medical Research Council, South African National Botanical Institute, Council for Scientific and Industrial Research, University of Cape Town and University of Pretoria - to scientifically validate South African medicinal plants for the treatment of malaria.[31]

Extracts of approximately 140 plant taxa, which were selected semi-quantitatively using weighted criteria, were tested *in vitro* against the D10 *P. falciparum* strain using the pLDH assay. Approximately 50% of these showed promising antiplasmodial activity ($IC_{50} \leq 10\mu g/ml$). Several South African plant species and

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[30] E.A. Prozesky, J.J.M. Meyer, and A.I. Louw, *J. Ethnopharmacol.*, 2001, **76**, 239.
[31] C. Clarkson V.J. Maharaj, N.R. Crouch, O.M. Grace, P. Pillay, M.G. Matsabisa, N. Bhagwandhin, P.J. Smith, P.I. Folb., *J. Ethnopharmacol.*, 2004, **92**, 177.

genera were shown for the first time to possess *in vitro* antiplasmodial activity. This study reports on two of the plants identified as potential sources of new antimalarial drugs and was aimed at:

1. Investigating the *in vitro* antiplasmodial activity of these two plant species (*Vernonia staehelinoides* and *Oncosiphon piluliferum*)
2. Identifying, isolating and characterising compounds with *in vitro* antiplasmodial activity from these two plants