African Ethnopharmacology and New Drug Discovery

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ABSTRACT

This review covers the general area of disease and problems such as malaria, bacterial and fungal infections, free radical damage and the decline in the immune system. After a brief history of ethnopharmacology, we discuss the scientific approaches that have been used in the screening of medicinal plants and identify some African medicinal plants that are used successfully in the treatment of these diseases. It is evident that African medicinal plants are continuously being screened for their pharmacological properties and many interesting results with crude extracts have occasionally been obtained through the isolation and identification of the active principles. However, as a source of new drugs, African medicinal plants are understudied, considering the high percentage of plants not yet screened for their biochemical composition or for their pharmacological properties.

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INTRODUCTION

Plants have formed the basis of traditional medicine (TM) systems which have been used for thousands of years. Traditional medicine refers to health practices, approaches, knowledge and beliefs incorporating plant, animal and mineral-based medicines, spiritual therapies, manual techniques and exercises, applied singularly or in combination to treat or to diagnose and prevent illnesses or maintain well-being (WHO 2003). The use of plant-based systems continues to play an essential role in health care. It has been estimated that approximately 80% of the population in developping countries depend on TM for their primary health care (Kirby 1996; Hostettman and Marston 2002). In China, traditional herbal preparations account for 30 to 50% of the medicines consumed. In Ghana, Mali, Nigeria and Zambia, the first line of treatment for 60% of the children with high fevers, resulting from malaria, is the use of herbal medicines at home (WHO 2003). In industrialized countries, adaptations of TM often termed complementary or alternative medicine or CAM, also play an important role in the health care system of 20% of the population (WHO 2003). In the United States, 158 million adults use CAM and according to the USA commission for alternative and complementary medicines, US$ 17 billion was spent on traditional remedies in 2000. In the United Kingdom, the annual expenditure on CAM is US$ 230 million (WHO 2003). In addition, despite the remarkable progress in synthetic organic chemistry of the twentieth century, over 25% of prescribed drugs in industrialized countries derive directly or indirectly from plants. This percentage can reach 50% when the over-the-counter market is taken into consideration (Newman *et al*. 2000).

In African societies, the tradition of collecting, processing and applying plants and plant-based medications have been handed down from generation to generation. Traditional medicine, with medicinal plants as their most important component are sold in marketplaces or prescribed

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by traditional healers in their homes (Von Maydell 1996). Because of this strong dependence on plants as medicines, ethnopharmacological studies have been conducted to determine their safety and their efficiency and on the other hand to find out new active principles from plants. Ethnopharmacology is the investigation of biologically active agents traditionally used or observed by humans (Brunht and Holmested 1981). In this definition we may consider as an agent, plant mixtures, whole plants, a portion of a plant, a special preparation or a molecule. Ethnopharmacology is a scientific approach, thus prayers or spiritual practices should not be considered as agents as it is usually observed in TM in some regions. Ethnopharmacology is by definition a multidisciplinary science, thus successful research in ethnopharmacology requires the interaction of ethnobotanists, natural products chemists, pharmacologists, taxonomists, traditional healers and/or user communities. If useful compounds that need development are isolated, collaboration with synthetic chemists, ethical and legal practitioners is required.

The frequent rationale behind plant use is the need for new active principles in the treatment of many diseases such as malaria, aids and cancers. The choice of plant species that should be screened to reduce the time and the cost of the studies is an important consideration for any ethnopharmacological investigation (dos Santos and Fleurentin 1991). The present review is focused on a brief history of ethnopharmacology and the role of ethnopharmacology in the development of new drugs against several illnesses with emphasis on African medicinal plants.

BRIEF HISTORY

The use of natural products as medicinal agents dates back to prehistory. Earliest humans used various but specific plants to treat illness. The first records, written on a hundred clay tablets in cuneiform are from Mesopotamia, and date from about 2600 B.C. Amongst the approximately 1000 plant-derived substances used, were oils of *Cedrus* species (cedar) and *Cypressus sempevirens* (cypress), *Glycyrrhizza glabra* (licorice), *Camphora* species (myrrh), and *Papaver somniferum* (poppy). All these plants are still used today for the treatment of ailments ranging from coughs and colds to parasitic infections and inflammations (Newman *et al.* 2000).

Egyptian medicine dates from about 2900 B.C., but their best known pharmaceutical record is "Ebers Papyrus" written in about 1500 B.C which includes over 700 drugs (mostly plants, animal organs together with some minerals) and formulae such as gargles, snuffs, poultices infusions, pills and ointments, with beers, milk, wine, and honey being commonly used as vehicles. The Chinese *Materia medica* has been extensively documented over the centuries with the first record dating from about 1100 BC, reporting the uses, medicinal and otherwise of over 600 plants. The philosopher and the natural scientist Theophrastus (370-285 B.C.) in the "History of Plants" began the scientific classification of plants and dealt with the medicinal qualities of herbs. Ibn Al Baita (1197-1248) listed over 1400 drugs and medicinal plants in *Corpus of Simples* (Sneden 2004).

In Europe, after the 10th century, much of the medicinal lore was based in the Church, particularly in monastic orders, but by the 1500's, after the invention of the printing press, herbals available to the public were popular, particularly in England (Sneden 2004). By the late 1700s, studies that described specific doses and gave administration instructions for herbal remedies begun to appear. In the United States (US), before the advent of specific pharmaceuticals, herbal remedies were relied upon to treat many illnesses. Development of drugs based on natural products has had a long history in the US, and in 1991, almost half of the best selling drugs were natural products or derivatives of natural products (Sneden 2004).

Natural products are chemical compounds derived from living plants or animals. Drugs derived from natural products are usually secondary metabolites and their derivatives.
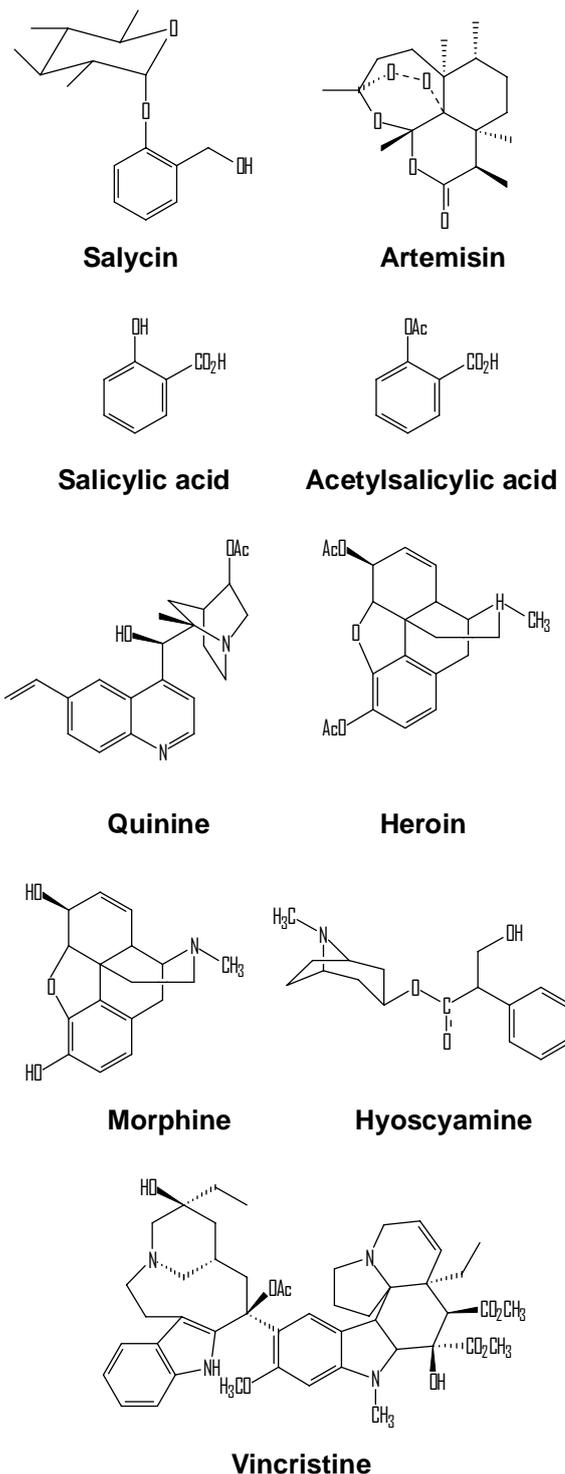

Fig. 1 Chemical structures of pharmacologically active compounds isolated from plants.

One of the earliest drugs developed from a natural product was aspirin (**Fig. 1**). The "Ebers Papyrus" indicates the use of willow leaves as an antipyretic treatment. Early English herbals recommended the use of the teas made from willow bark for this use. Following on these treatments, chemists and pharmacists began to isolate the compounds responsible for the remedy. Thus salycin (**Fig. 1**) was first isolated from the bark of white willow, *Salix alba* in 1825/26. The compound was then converted to salicylic acid (**Fig. 1**) via hydrolysis and oxidation. Salicylic acid was found to be a very good antipyretic but it showed a severe gastrointestinal toxicity. The problem was overcome after the compound

was converted into acetylsalicylic acid (ASA; **Fig. 1**) through acetylation. ASA, the first semi synthetic drug sold by Bayer Co. in 1899 under the trade name of aspirin (Newman *et al*. 2000) is today still the most widely used antipyretic and analgesic drug in the world.

Many of the earliest isolated compounds with biological activities were alkaloids because of their ease of isolation. An alkaloid is a plant-derived compound that is toxic or physiologically active, contains nitrogen in a heterocyclic ring, is basic, has a complex structure, and is of limited distribution in the plant kingdom. Less than 300 years ago, malaria was the scourge in Europe. When the Spanish and Portuguese began to colonize South America, they discovered the use of the bark of *Cinchona* trees by native Indians to treat malaria. Then the bark was introduced in Europe in 1633 and the first bark reached Rome 12 years later. Teas made from the bark cured people suffering from malaria and the bark became known as Jesuit's bark. Because of the philosophical differences between Protestants and Catholics, many Protestants refused to be treated with the bark. One of the most prominent Protestants, Oliver Cromwell, reportedly died of malaria because of this stubbornness. The principal antimalarial compound was isolated later from the bark of *Cinchona succiruba* and identified as quinine (**Fig. 1**; Sneden 2004). Quinine is one of 31 alkaloids occurring in the plant. Malaria is still a major public health problem throughout the world, so the search for new antimalarial agents from natural sources continues. One of the most promising new drugs is qinghaosu or artemisin (**Fig. 1**), a sesquiterpen isolated from *Artemisia annua*, a plant used in Chinese TM to treat malaria (Klayman *et al*. 1984; Teixeira da Silva 2004).

Amongst the famous alkaloids are tropane alkaloids isolated from *Solanaceae*. These plants have been used as poisons but many of their alkaloids were found to exert pharmacological activities. As examples, atropine, the racemic form of hyosciamine (**Fig. 1**), and scopolamine are central nervous system stimulants. They are used to dilate eye pupils and they can be used as topical anesthesics in ophthalmology.

Many other alkaloids were isolated from plants with a long history of traditional usage. Amongst them are morphine alkaloids (morphine and heroin, a semi synthetic compound) (**Fig. 1**) isolated from *Papaver somniferum*. They are powerful pain relievers and narcotics. Morphine was the first commercial natural product of E. Merck in 1826 (Newman *et al*. 2000). Vincristine (**Fig. 1**), one of the most potent antileukemic drugs in use today was isolated in a search for diabetes treatments from *Catharanthus roseus* in the 1950's along with vinblastine.

ETHNOPHARMACOLOGY AND THE DEVELOPMENT OF NEW DRUGS AGAINST SEVERAL ILLNESSES

Malaria

Malaria is a parasitic disease caused by a protozoan of *Plasmodium* genus. It is the most important infectious disease in the world (Breman 2001). Despite extensive control efforts the incidence of the disease is not decreasing because parasites are increasingly resistant to antimalarial drugs. Unfortunately, mortality from malaria appears to be increasing in the highest risk group, African children. Malaria control efforts include the attempt to develop an effective vaccine, eradicate mosquito-vectors and develop new drugs (Oask *et al*. 1991; Olliaro *et al*. 1996). However the development of a vaccine has proven very difficult and a highly effective vaccine will probably not be available in the near future. Efforts to control Anopheles mosquitoes have had limited success, although the use of insecticide-impregnated bed nets does appear to reduce malaria-related death rates (Alonso *et al*. 1997). The limitation of vaccine and vector control as well as the increasing resistance of

malaria parasites to existing drugs highlights the continued need for new antimalarial agents (Rosenthal 1998).

The critical consideration in antimalarial drug development is economic. Financial constraints are relevant in two key regards. First, to be widely useful, antimalarial drugs must be very inexpensive so that they are routinely available to the population in developing countries. Indeed, even a price of \$1 per treatment is probably unacceptable in many regions, considering severe poverty in most malarious regions. Second, since malaria markets are primarily in poor countries, marketing opportunities have been generally limited, so investment in antimalarial drug discovery and development has been small (Rosenthal 2003).

Considering these difficulties, plant-derived compounds offer an approach to chemotherapy. Importantly, this approach benefits from the knowledge of medicinal plants held by native people of malarious regions, since the use of plant products with specific clinical activity can be a useful starting point for medicinal chemistry. Some plants have been screened and many others are currently being screened in laboratories for antiplasmodial activity. Plant samples are often collected following leads supplied by local healers (Clarkson *et al*. 2004; Ména *et al*. 2005; Mbatchi *et al*. 2006). Chemical work includes preparation and purification of extracts, isolation of individual compounds and their identification using techniques such as chromatography, nuclear magnetic resonance and mass spectrometry. For biological activities, extracts or individual compounds are tested on parasite cultures in vitro to check if they affect the viability of parasites. Parasites are often fresh clinical isolates obtained from untreated malaria patients or reference chloroquine-sensible or chloroquine-resistant strains. Parasites are grown as described by Trager and Jensen (1976). The antiplasmodial activity can be quantified by light microscopy using giemsa-stained smears (Le Bras and Deloron 1983) or by flow cytometry with incorporation of [^{3}H] hypoxanthine (Desjardins *et al*. 1979; Schulze *et al*. 1997). Alternatively, a colorimetric method that includes 3-acetylpyridine as a substrate for malaria parasite lactate dehydrogenase has been used with the advantage that radio labelled substrates are not required (Makler *et al*. 1993).

Extracts of a large number of plant species that are used in TM have been evaluated for *in vitro* antiplasmodial activities (Benoit *et al*. 1996; El Tahir *et al*. 1999; Ancolio *et al*. 2002). In some cases, the constituents responsible for these activities have been isolated. Working on 14 South African plant species, Prozeky *et al*. (2001) found that two extracts had IC_{50} (drug concentration inhibiting 50% of the parasite growth) values below 2 µg/mL, seven extracts had IC_{50} values between 2 and 5 µg/mL, while chloroquine had a IC_{50} value of 0.043 µg/mL. Seven medicinal plants of Burkina Faso TM were screened for *in vitro* antiplasmodial activities against a *Plasmodium falciparum* chloroquine-resistant W2 strain. Significant activities were recorded with alkaloid extracts of *Pavetta crassipes* (IC_{50} < 4 µg/mL), *Acanthospermun hispidium*, *Crossopteryx febrifuga* and *Fadogia agrestis* ($4 < IC_{50} < 10$ µg/mL). *Terminalia macroptera* was the most active plant with an IC_{50} of 1 µg/mL (Sanon *et al*. 2003a, 2003b). Another study in which four plants were investigated revealed that *Sida acuta* had very good in vitro antiplasmodial activity (IC_{50} of 0.05 µg/mL). This may be related to its alkaloid content (Karou *et al*. 2003). Tona *et al*. (2004) screened seven medicinal plants of the Democratic Republic of Congo for their antiplasmodial activities. In this screening, the most active ethanolic extracts (IC_{50} < 3 µg/mL) were from *Cassia occidentalis* leaves, whole plants of *Euphorbia hirta*, the stem bark of *Garcinia kola*, and whole plants of *Phyllanthus niruri*. Their respective petroleum ether soluble fractions also exhibited good antiplasmodial activities. Petroleum ether fractions of *Vernonia amygdalina* leaves, *Tetracera poggei* leaves and *Morinda morindoides* leaves also displayed good activities, but their respective ethanolic extracts were less active. Working on Kenyan medicinal plants, Muregi *et al*. (2004), found synergic effects of

extracts of *Ekebengia capensis* and *Clerodentrum myricoides* in combination with chloroquine against a multi-drug resistant strain of *Plasmodium falciparum* V1/S. Synergistic effects were also observed between the total alkaloid extracts from the leaves of *Guiera senegalensis* and those of *Mitragyna inermis*, showing the rationale behind the traditional use of these two plants in combination in the treatment of malaria (Fiot *et al*. 2006).

Amongst medicinal plants with antiplasmodial activity, *Strychnos* species have been the most screened because of their great similarity with *Cinchona* genus (Philippe *et al*. 2005). Some individual compounds with antiplasmodial activities have been isolated from *Strychnos* species. Strychnopentamine and isostrychnopentamine (**Fig. 2**) isolated from these plants were active against chloroquine-sensitive and chloroquine-resistant strains (IC$_{50}$ ≈ 0.15 µM), while dihydrousambarensine (**Fig. 2**) a quasidimeric alkaloid isolated from the same *Strychnos* was more active against the chloroquine-resistant strain (IC$_{50}$ = 0.03 µM) than it was for the chloroquine-sensitive strain (Frederich *et al*. 1999). Another alkaloid, malagashanine (MG) (**Fig. 2**), the parent compound of a series of Nb,C(21)-secocuran alkaloids was isolated from Malagasy *Strychnos* species. MG co-occurs in the same plants with strychnobrazilline (SB) (**Fig. 2**), another Nb,C(21)-secocuran alkaloid, which is by far the major constituent (Rosanaivo *et al*. 1994). MG is devoid of intrinsic antiplasmodial activity, but when combined with chloroquine (CQ), MG was found to enhance *in vitro* and *in vivo* CQ action against CQ-resistant strains of *P. falciparum*. Its high lipophilicity may play a key role in its *in vivo* activity, since SB which is predominantly water-soluble at physiological pH, failed to enhance *in vivo* CQ activity (Rafatro *et al*. 2000).

More recently, bioguided fractionation of the petroleum ether extract of *Hyptis suaveoleus* led to the isolation of an abietane-type diterpenoid endoperoxide, 13α-epi-dioxabet-8 (14)-en-18-ol, displaying high antiplasmodial activity (Chukwujekwu *et al*. 2005). Previously, similar studies conducted on *Alchornea cordifolia* and *Sida acuta* extracts led to the identification of the active principles ellagic acid and cryptolepine (**Fig. 2**) (Banzouzi *et al*. 2002, 2004). Cryptolepine has been so far found to be the antimalarial compound of *Cryptolepis senguinolenta*, a plant traditionally used to treat malaria in Central and West Africa (Cimanga *et al*. 1996; Sharaf *et al*. 1996). The compound was first isolated from *Cryptolepis triangularis* (Clinquart 1929). Cryptolepine has good *in vitro* antimalarial activity, but it failed to cure malaria in mice by oral administration (Wright *et al*. 2005). By intraperitoneal administration, the compound showed toxic effects through DNA intercalating or topoisomerase II inhibition (Bonjean *et al*. 1998; Guittat *et al*. 2003). These properties suggest that the compound is not a good candidate for antimalarial drug development, but it may be a potent anticancer agent (Dassonneville *et al*. 2000).

Bacterial and fungal infections

Many pharmaceuticals dispended today in modern medicine have higher plant origins, but very few are intended for use as antimicrobials, since clinical microbiologists have relied on bacterial and fungal sources for these activities (Cowan 1999). Since the advent of antibiotics (ATB), the use of plant derivatives as antimicrobials has been virtually nonexistent, but clinical microbiologists have two reasons to be interested in the topic of antimicrobial plant extracts. Firstly it is likely that plant derived-antimicrobials may inhibit microorganisms through different mechanisms than conventional ATB do and therefore they can be of clinical value in the treatment of resistant microbial infections. Secondly, it is reported that on average, 2 or 3 ATB derived from microorganisms are launched each year, but the effective life span of any ATB is limited.

In developing countries, particularly in Africa, low levels of hygiene and sanitation exposed the people to a wider array of microbial pathogens which increases their susceptibility to bacterial and fungal infections (Fennell *et al*. 2004). It is reported that each year, 300,0000 children die of diarrhoeal diseases. The most common microorganisms responsible for intestinal diseases are *E. coli*, *Shigella* spp., *Salmonella* spp., and *Yersiania* spp. (Murray and Lopez 1997; Bonfiglio *et al*. 2002). In many regions, affected by these infections, local and indigenous plants are often the only available means of treating such infections.

Plants have an almost limitless ability to synthesize aromatic substances, which are phenols or their oxygen substituted derivatives. Some of them are secondary metabolites and are involved in plant defence against microorganisms. Cowan (1999) summarized useful antimicrobial phytochemicals in five groups including phenolics, terpenoids and essential oils, alkaloids, lectines and polypeptides,
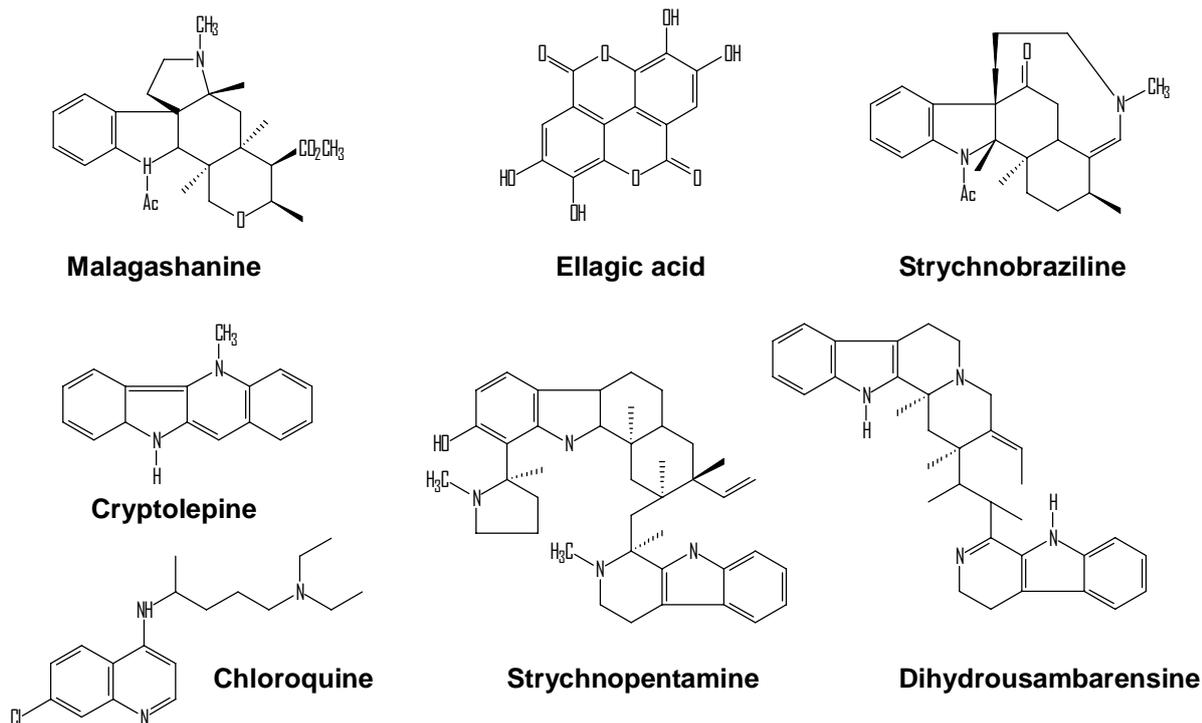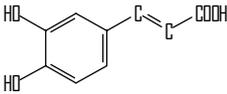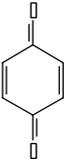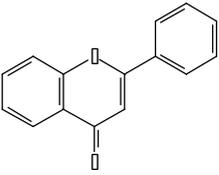

Malagashanine

Ellagic acid

Strychnobraziline

Cryptolepine

Chloroquine

Strychnopentamine

Dihydrousambarensine

Fig. 2 Chemical structures of plant-derived antimalarial compounds.

Table 1 Chemical structures of several plant phenolic compounds.

Simple phenols and phenolic acids	Quinones	Flavones and flavonoids	Coumarins
Caffeic acid	Quinone	Flavone	Coumarin
Catechol	Hypercin	Catechin	Warfarin
Euglenol		Chrysin	7-hydroxycoumarin
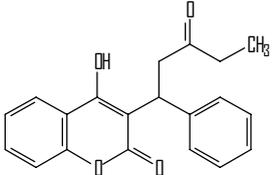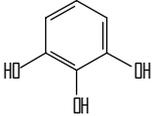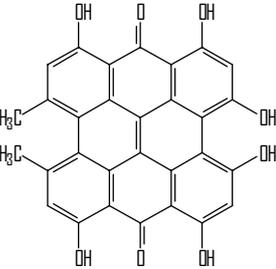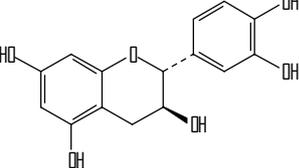
and polycetyles. Amongst these compounds, phenolic compounds are most cited and well documented as potential antimicrobial agents (Scalbert 1991; Cowan 1999). Phenolics are a group of highly hydroxylated compounds present in extractive fractions of several plant materials. Their most distinguishing characteristic is their reactivity with proteins and related polyamide polymers (Haslam 1996). Phenolic compounds in plants include several groups of compounds that are listed below and that are summarized in **Table 1**:

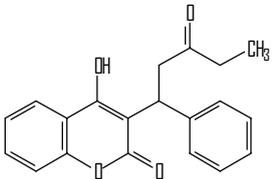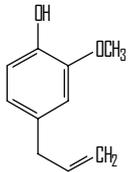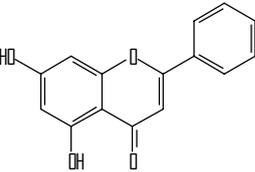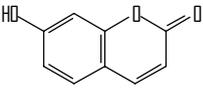1. Simple phenols and phenolic acids: single substituted phenolic ring as catechol or cafeic acid
2. Quinones: aromatic rings with two ketone substitutions
3. Flavones: phenolic structure containing one carbonyl group
4. Flavonols: flavones with a 3-hydroxyl group
5. Flavonoids: hydroxylated phenolics that occur as C_6–C_3 units linked to aromatic rings
6. Coumarins: phenolic substances made of fused benzene and α-pyrone rings.

Tannin is a general descriptive name of polymeric phenolic substances capable of tanning lather or precipitating gelatine from solution, a property known as astringency. Their molecular weights range from 500 to 3000 (Gollapudi *et al.* 1995). Tannins are either hydrolysable or condensed. Hydrolysable tannins are based on gallic acid; condensed tannins, often called proanthocyanidins are based on flavonoid monomers, flavone derivatives or quinone units.

The inhibition of microorganisms by phenolic compounds may be due to iron deprivation or hydrogen bounding with vital proteins such as microbial enzymes (Scalbert 1991). Phenolic compounds are vulnerable to polymerization in air through oxidization reactions. Therefore, an important factor governing their toxicity is their polymerization size. Oxidized condensation may result in the toxicification of microorganisms; on the other hand polymerization may result in detoxicification (Field and Lettinga 1992; Karou *et al.* 2005).

In laboratories, plant extracts are tested against micro-organisms using many assays. The most frequently used assay in antimicrobial plant extract screening is the agar disc diffusion assay. The method has been modified as the agar-well diffusion assay developed by Perez *et al.* (1990). The broth microdilution as recommend by the National Committee for Clinical Laboratory Standards is also used to quantify the antimicrobial activity of plant extracts resulting in minimal bactericidal concentration (MBC) and minimal inhibitory concentration (MIC) determination (NCCLS 1997, 2000). Bioautography combines thin layer chromatography with a bioassay *in situ*, therefore allowing the active compound within a sample to be localized (Hamburger and Cordell 1987).

Many plants have been screened in laboratories for antimicrobial activities. The plants are often selected according to their traditional uses. In these screenings, the gram-positive bacteria are often found to be more susceptible to plant extracts than the gram-negative ones (Kelmanson *et al.* 2000; Massika and Afolayane 2002; Fennell *et al.* 2004). Indeed, gram-positive bacteria have only an outer peptideglycane layer which is not an effective barrier (Scherrer and Gerhardt 1971). The gram-negative bacteria have an outer phospholipid membrane that makes the cell wall impermeable to lipophilic solutes, while the porines constitute a selective barrier to hydrophilic solutes with an exclusion limit of about 600Da (Nikaido and Vaara 1985).

The screening of extracts for antimicrobial activity *in vitro* often involves reference and clinical strains of microorganisms isolated from pathologic products or those that demonstrate resistance to several ATB or antifungals. Many plants screened for their antimicrobial activities showed interesting results with very low MIC and MBC values. For example, Longanga *et al.* (2006) found good antibacterial and anti fungal activities of alkaloids from *Epinetum villosum* (MIC ≈ 31 μg/mL). The alkaloids of *Sida acuta* were also found to exert good *in vitro* antibacterial activity against several pathogenic bacteria with MIC values ranging from 16 to 400 μg/mL (Karou *et al.* 2006). Among the antimicrobial extracts, essential oils received particular attention because of the ease of extrac-

tion. Thus essential oils of *Osmitopsis asteroides* (Viljoen *et al.* 2003), *Lippia multiflora* and *Lippia chevalieri* (Bassole *et al.* 2003) were found to be very active against pathogenic bacteria.

Some antimicrobial screening resulted in the isolation and the characterisation of the active principles. For example, the *in vitro* screening of *Warburgia salutaris, Vernonia colorata* and *Erythrina lysistemon* resulted in the isolation of muzigadial (**Fig. 3**; Rabe and Van Staden 2000), vernodalin (**Fig. 3**; Ried *et al.* 2001) and wighteone (**Fig. 3**; Pillay *et al.* 2001) respectively. More recently, new flavonoids: isogancaonin C, bolusanthin III and bolunsanthin IV (**Fig. 3**) with antibacterial and antioxidant activities have been isolated from *Bolusanthus speciosus* (Erasto *et al.* 2004). Two known sesquiterpene lactones: vernolide and vernodalol isolated from *Vernonia amygdalina* displayed good antimicrobial activity against 10 bacterial strains and 5 fungal species (Erasto *et al.* 2006). Cocsoline, a bisbenzylisoquinoloine alkaloid isolated from the root bark of *Epinetrum villosum* also showed good antimicrobial activety against several tested microorganisms (Longanga *et al.* 2006).
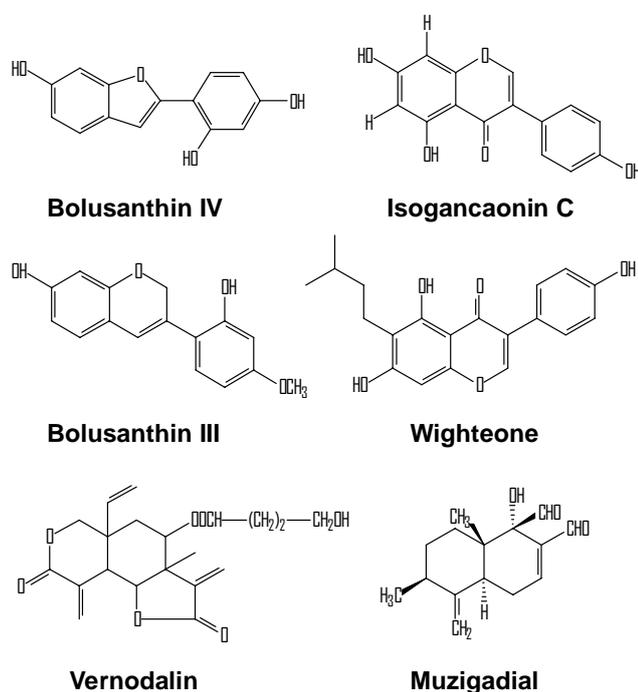

Fig. 3 Chemical structures of several compounds isolated from African medicinal plants.

Free radical damages

A free radical is a species capable of independent existence that contains one or more unpaired electrons. It is well known that oxygen plays an important role in many of the metabolic processes associated with aerobic existence. On the other hand, oxygen also leads to the formation of reactive oxygen species that have either unpaired electrons (e.g. $O2^{\bullet-}$, $OH^{\bullet}$), or the ability to attract electrons from molecules (e.g. H_2O_2) (Zhu *et al.* 2004). Free radicals contribute to the elimination of infected cells, but they can also react with cellular DNA or other macromolecules, either damaging them directly or setting in motion a chain reaction resulting in extensive damage of cellular structures. It is reported that many diseases such as brain dysfunction, cancer, heart diseases and immune decline could be the result of free radicals (Babbs 1992; Aruoma 1998). One of the factors contributing to oxidative stress is increasing O_2 concentration. In physiological conditions, the human body can compensate for a mild degree of oxidant stress and remove oxydatively damaged molecules by activating antioxidant enzymes like super oxide dismutase, catalase,

gluthation peroxidase etc. Oxidative stress is a state of imbalance between the level of antioxidant defence system and the production of oxygen-derivatives. Thus antioxidants have been of interest to pharmacologists, biochemists and other health professionals because they are supposed to reduce oxidative damages. Antioxidants are compounds that can delay or inhibit lipid oxidation or oxidation chain reaction propagation. Bors and Buettner (1997) summarized the free radical scavenging by ascorbic acid as below:

1. In physiological conditions ascorbic acid ($AsCH_2$) may be present as monodehydroascorbate ($AsCH^-$) and didehydroascorbate (ASC^{2-})
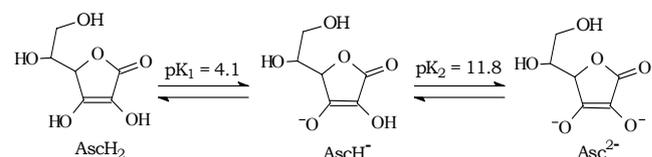
2. $AsCH^-$ donates a hydrogen atom ($H^{\bullet}$ or $H^+ + e^-$) to oxidizing radical ($R^{\bullet}$) to produce the resonance-stabilized didehydroascorbate free radical ($AsC^{\bullet-}$)
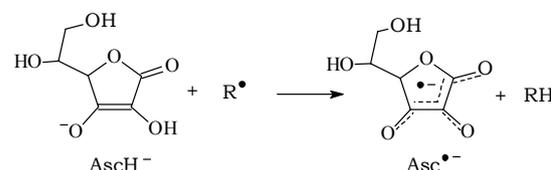
3. *In vitro*, $AsC^{\bullet-}$ is eliminated through dismutation reactions that involve two $AsC^{\bullet-}$.

$$2\ Asc^{\bullet-} + H^+ \rightleftharpoons AsCH^- + AsC^{2-}$$

However *in vivo*, ascobates are recycled by reducing enzymes (Hossain and Asada 1985).

Nowadays, there is a great interest in replacing synthetic antioxidants with natural ingredients because of the concern over the possible carcinogenic effects of synthetic antioxidants in foods (Zhu *et al.* 2004). Thus several plants are being screened for their antioxidant properties using many assays. The *in vitro* lipid peroxidation assay is frequently used to quantify the antioxidant activity of plant extract and interesting results (inhibition values greater than 70%) have been recorded. Extracts of *Amaranthus* sp., *Sisymbrium thellungii* and *Urtica dioica*, traditionally used to prepare "infimo", a South African diet, were found to exert good lipid peroxidation inhibition (Lindsey *et al.* 2002). Another assay used in the quantification of antioxidant activity is the *in vitro* ABTS (2,2'-azinobis-(3-éthylbenzothiazoline-6-sulfonic) acid) or DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging (Re *et al.* 1999) or the phosphomolybdenum reduction (Prieto *et al.* 1999). Using these methods, polyphenols extracts of *Khaya senegalensis, Pterocarpus erinaceus* and *Combretum micranthum* were found to be good free radical scavengers (Karou *et al.* 2005). Sorghum grains had good antioxidant activity related to their phenolic contents (Awika *et al.* 2003, Dicko *et al.* 2005). In the same way, Erasto *et al.* (2004) also found good free radical scavenging of *Botusanthus speciosus* flavonoids. From three South African *Salvia* species: *S. stenophylla, S. repens* and *S. runcinata*, screened for their antioxidant activity through DPPH-free radical scavenging, Kamatou *et al.* (2005) found that *S. runcinata* displayed the most important antioxidant activity ($IC_{50} = 6.09\ \mu g/mL$). It was also demonstrated that the hypotensive and the vasorelaxing activities of *Microdermis keayana* were due to its antioxidant properties (Zamble *et al.* 2006).

Immune system decline

The human immune response is a highly complex and extraordinarily sophisticated system involving both innate and adaptative mechanisms. Specific immune response uses adaptative mechanism. It is a result of interactions between T lymphocytes, B lymphocytes, and Antigen-Presenting Cell (APC). This coordination allows the system, to eliminate or neutralize potential harmful agents and to respond more rapidly and appropriately after a renewed antigen encounter. Small proteins, cytokines, mediate regulation and communication between cells. Cytokines are secreted by T lymphocytes, principally TCD4+ and TCD8+, and other cells such as Natural Killer cells, monocytes/macrophages, B cells, dendritic cells, neutrophilles and mast cell eosinophilles in a polarized fashion which permits their classification as type 1 and type 2 cytokines (Whiteside 1994; Lucey *et al.* 1996). A type 1 response is defined as a strong cellular immune response with normal or increased levels of IL-2, IFN-γ, TNF-β and/or IL-12, while a type 2 response is defined as an impaired cellular response with an increase in the levels of IL-4, IL-5, IL-6, IL-10 and/or IL-13. In health there is a delicate balance and a crossregulation maintained between the activity of the type 1 and type 2 cytokines. An imbalance can be observed under certain pathologies, chronic viral infections (HIV infection, measles, chronic active hepatitis B), bacterial diseases (leprosy, tuberculosis, intracellular bacterial infections), parasitic diseases (protozoan parasite infection, helminthic), fungal diseases (candidiasis, cryptococcosis), neoplastic diseases (Hodgkin's disease, Kaposi sarcoma), and inflammatory and autoimmune diseases (autoimmune thyroid diseases, systemic lupus erythematosus) (Lucey *et al.* 1996).

Studies focussing on the mode of action of plant substances on immune T-Cell response *in vitro* have traditionally relied on the use of radionucleides such as [^{3}H]-thymidine incorporation, for information on cell survival and/or proliferation, or the [^{51}Cr] release assay for cytotoxicity. These assays are based on the activities of the intracellular enzymes of the detected cells such as MTT (3-(4,5-dimethylthiazol-2-yl) 2,5-diphenyl tetrazolium bromide) assay (Mosmann, 1983) and data obtained reflects indirectly proliferation and cytotoxicity.

New assays of T-Cell specificity and functions have been introduced to evaluate immumomodulating activity. These assays are essentially based on immunofluorescence cytometry and cell sorting. Lymphocyte proliferation can be quantified by a flow cytometry assay (King 2000; Wang and Zheng 2002), bromodeoxyuridine (BrdU) incorporation (cell DNA synthesis) or carboxyfluoresceine-diacetate-succinimidyl ester (CFDA-SE) labelling. Cells reacting to plant extracts by DNA synthesis incorporate BrdU, which can be detected with a BrdU-specific fluorescent antibody (Thiel *et al.* 2004). Labelled with CFDA-SE, cells reacting to compounds by proliferation lose 50% of their florescent label after each cell division (Lyons 2000). Cytokine production can be detected at the level of a single cell by enzyme-linked immunosorbent assay and Spot-forming (ELISPOT) analysis, limiting dilution analysis, semi quantitative PCR and *in situ* hybridation (Baran *et al.* 2001). The multiparameter analysis in flow cytometry permits the simultaneous detection on one, two, or more cytokines which, when combined with the determination of the cell surface phenotype, allows Th1/Th2 subset detection (Maecker *et al.* 2000; Pala *et al.* 2000; Thiel *et al.* 2004). In this way, cell expansion is stimulated by the mitogen, phorbol myristate acetate-ionomycine (PMA) or phytohemagglutinin (PHA). The cytometric cytokine secretion assay (CCSA) allows analysis of cytokine secretion from cells (Thiel *et al.* 2004).

Plant natural products can have an immunomodulating potential. These include isoflavonoids, indoles, phytosterols, polysaccharides, sesquiterpenes, alkaloids, glucans and tannins (Bouic and Lamprecht 1999; Navarro e*t al.* 2000; Williams 2001; Suzuki *et al.* 2001; Hajto *et al.* 2005;

Chevrier *et al.* 2005; Vojdani and Erde 2006). Immuno-modulating activity refers to biological or pharmacological effects of compounds on humoral or cellular aspects of the immune system. Immunomodulation using medicinal plants can provide an alternative to conventional chemotherapy for a variety of diseases, especially when the host defence mechanism has to be activated under the conditions of impaired response or when a selective immunosuppression is desired in situations like autoimmune disorders.

Ethnopharmocological approaches have so far detected a number of African medicinal plants containing immuno-modulators. Ralamboranto *et al.* (1982) confirmed in their study immunomodulating effects of *Aloe vahombe* polysaccharides. Polysaccharides were also found to be responsible for the *in vitro* immunomodulating activity of *Cochlospermum tinctorium* (Nergard *et al.* 2005). An *in vivo* investigation of plant sterol/sterolin mixture effects on healthy individuals and patients infected with the Human Immunodeficiency Virus (HIV) in South Africa revealed the immunostimulating activity of this mixture (Breytenbach *et al.* 2001).

CONCLUSION

African medicinal plants are continuously screened for their pharmacological properties. Because of ethical reasons, the *in vitro* tests are the only approach used in these screenings. Plants are often collected following leads supplied by the local healers in geographical areas where they are found. Many interesting results are recorded with crude extracts showing the rationale behind plant usage in Africa, but it is not enough when we consider the large amount of plants which have not been screened for their biochemical composition or for their pharmacological properties. Bio-guided fractionation is commonly used in the isolation and identification of active principles. However few works have resulted in the isolation of single compounds and the isolated compounds are not used or modified for the development of new therapeutics. It is reported that cryptolepine, the main alkaloid of *Sida acuta* and *Cryptrolepis sanguinolenta* that is very active *in vitro* against *Plasmodium falciparum* failed to cure malaria *in vivo* and its synthetic analogues such as 2,7-dibromocryptolepine are under investigation (Wright *et al.* 2001; Onyeibor *et al.* 2005). Malagashanine another alkaloid isolated from *Strychnos* species has been tested for its *in vivo* chloroquine potentiating effects. Except for a few examples, the majority of studies always stop after the active compound is identified or the crude extract shown to be active. It may be concluded that much work has to be done in African ethnopharmacology and urgent attention has to be paid to as many African medicinal plants as possible in view of the rapid disappearance of tropical forests and associated species extinction. In addition, bio-guided fractionation must lead to the systematic biochemical screening of plant species. In the first approach, compounds present in the plant and that are not involved in the desired pharmacological activity may be omitted. The second approach allows the isolation and the identification of the majority of compounds in the plant and then each single compound can be tested for any pharmacological activity.

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