CHAPTER 1

INTRODUCTION

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1.1 Medicinal plants

Throughout the ages, humans have relied on nature for their basic needs, for the production of food, shelter, clothing, transportation, fertilizers, flavours and fragrances, and medicines (Cragg and Newman, 2005). Plants have formed the basis of sophisticated traditional medicine systems that have been in existence for thousands of years and continue to provide mankind with new remedies. Although some of the therapeutic properties attributed to plants have proven to be erroneous, medicinal plant therapy is based on the empirical findings of hundreds and probably thousands of years of use. The first records, written on clay tablets in cuneiform, are from Mesopotamia and date from about 2 600 BC (Heinrich *et al*., 2004). Among the substances that were used are oils of *Cedrus* species (cedar) and *Cupressus sempervirens* (cypress), *Glycyrrhiza glabra* (licorice), *Commiphora* species (myrrh) and *Papaver somniferum* (poppy juice), all of which are still in use today for the treatment of ailments ranging from coughs and colds to parasitic infections and inflammation. In ancient Egypt, bishop's weed (*Ammi majus*) was reported to be used to treat vitiligo, a skin condition characterized by a loss of pigmentation (Staniszewska, *et al.,* 2003; Beissert and Schwarz, 2002). More recently, a drug (β-methoxypsoralen) has been produced from this plant to treat psoriasis and other skin disorders, as well as T-cell lymphoma (Beissert and Schwarz, 2002).

The interest in nature as a source of potential chemotherapeutic agents continues. Natural products and their derivatives represent more than 50% of all the drugs in clinical use in the world today. Higher plants contribute no less than 25% of the total (Farnsworth *et al*., 1985; Cragg and Newman, 2005). In the last 40 years, many potent drugs have been derived from flowering plants; including for example *Dioscorea* species (diosgenin), from which all anovulatory contraceptive agents have been derived; reserpine and other anti-hypertensive and tranquilizing alkaloids from *Rauwolfia* species; pilocarpine to treat glaucoma and 'dry mouth', derived from a group of South American trees

(*Pilocarpus* spp.) in the Citrus family; two powerful anti-cancer agents from the Rosy Periwinkle (*Catharanthus roseus*); laxative agents from *Cassia* sp. and a cardiotonic agent to treat heart failure from *Digitalis* species (Newman *et al.,* 2000).

Approximately half (125 000) of the world's flowering plant species are found in the tropical forests. Tropical rain forests continue to support a vast reservoir of potential drug species. They continue to provide natural product chemists with invaluable compounds as starting points for the development of new drugs. The potential for finding more compounds is enormous as to date only about 1% of tropical species have been studied for their pharmaceutical potential (Cragg and Newman, 2005). This proportion is even lower for species confined to the tropical rain forests. To date about 50 drugs have come from tropical plants. The probable undiscovered pharmaceuticals for modern medicine has often been cited as one of the most important reasons for protecting tropical forests. Therefore the high annual extinction rate is a matter for concern.

Although discovered through serendipitous laboratory observation, three of the major sources of anti-cancer drugs on the market or completing clinical trials are derived from North American plants used medicinally by native Americans: the papaw (*Asimina* spp); the western yew tree (*Taxus brevifolia*), effective against ovarian cancer and the mayapple (*Podophyllum peltatum*) used to combat leukaemia, lymphoma lung and testicular cancer (Gurib-Fakim, 2006).

1.2 Traditional medicine

Plants have been utilized as medicines for thousands of years (Samuelsson, 2004). These medicines initially took the form of crude drugs such as tinctures, teas, poultices, powders, and other herbal formulations (Balick and Cox, 1997; Samuelsson, 2004). The specific plants to be used and the methods of application for particular ailments were passed down through oral tradition. Eventually information regarding medicinal plants was recorded in herbal phamacopoeias (Balunas, 2005).

Modern allopathic medicine has its roots in ancient medicine, and it is likely that many important new remedies will be discovered and commercialized in the future, as it has been till now, by following the leads provided by traditional knowledge and experiences. While European traditions are particularly well known and have had a strong influence on modern western pharmacognosy, almost all societies have well-established herbal traditions, some of which have hardly been studied at all. The study of these traditions will not only provide an insight into how the field has developed but it is also a fascinating example of our ability to develop a diversity of cultural practices.

In some countries, the use of medicinal plants is often associated with witchcraft and superstition, because people do not have the scientific insight to explain and predict the curative action of plants. One example of such an irrational concept is the Doctrine of Signatures, elements of which are found in many of the healing cultures of the world (Boehme, 1982). It is based on the assumption that the appearance of plants may give clues to their medicinal properties—it is interpreted as God's signature on the plant. Red juice and sap, for example, is associated with blood and menstrual ailments; yellow flowers with bile and jaundice; the human shape of certain roots with the female form of fertility and so on. Sometimes this concept however, worked: *Chelidonium majus*, contains yellow flowers and a yellow alkaloid containing latex, and has been used successfully to treat jaundice (Gurib-Fakim, 2006).

1.2.1 African traditional medicine

African traditional medicine is ancient and perhaps the most diverse of all medicinal systems. Africa is considered to be the cradle of humankind, with a rich biological and cultural diversity and marked regional differences in healing practices. Unfortunately, even today the systems of medicines are poorly recorded. The documentation of medicinal uses of African plants is becoming increasingly urgent because of the rapid loss of the natural habitats of these plants due to human activities. The African continent is reported to have one of the highest rates of deforestation in the world. This loss is all the greater because the continent has a high rate of endemism, with Madagascar topping the list at 82% (Green and Sussman, 1990).

African traditional medicine in its varied forms is holistic, involving both the body and the mind. The healer typically diagnoses and treats the psychological basis of an illness before prescribing medicines to treat the symptoms. Well known African medicinal plants include *Acacia senegal* (gum arabic), *Agathosma betulina* (buchu), *Aloe ferox* (Cape aloes), *Aloe vera* (north African origin), *Artemisia afra* (African wormwood), *Aspalanthus linearis* (rooibos tea), *Boswellia sacra* (frankincense), *Catha edulis* (khat), *Commiphora myrrha* (myrrh), *Harpagophytum procumbens* (devil's claw), *Hibiscus sabdariffa* (hibiscus, roselle), *Hypoxis hemerocallidea* (African potato), *Prunus africana* (African cherry). Madagascar has contributed *Catharanthus roseus* (rosy periwinkle) and has the potential of contributing more in view of the diversity of the flora and fauna (Newman *et al., 2000;* Neuwinger, 2000).

1.2.2 American traditional medicine (North, Central and South)

1.2.2.1 North America

In the USA, just like in many other cultures, the indigenous healer or Shaman treated illnesses by addressing both the physical and spiritual dimension of diseases. These Shamanistic ceremonies involve chanting, dancing and other rituals aimed at expelling evil forces so that the patient or the community as a whole can be healed (Fabricant and Farnsworth, 2001). Early settlers learnt from native practices and they eventually adopted many of the herbal remedies, which later formed the basis of the early United States Pharmacopoeia. Among the well known medicinal plants of the United States are Echinacea (*Echinacea purpurea*) and Goldenseal (*Hydrastis canadensis*). During most of the 20th century, herbs or botanicals have been regarded with skepticism and the practice of herbal medicine went into decline. Plants were viewed mainly as a potential source of pure chemical compounds for the development of medicine. In recent years, herbs and botanicals have become very popular in the USA and Canada but they are still considered as nutritional supplements rather than medicines in their own rights (Pieroni *et al.,* 2000; Heinrich *et al.,* 2004; Gurib-Fakim, 2006).

1.2.2.2 Central and South America

Just like Africa, Central and South American countries have rich and diverse healing cultures, which are poorly known and have not been properly recorded. They will no doubt be a source of new herbal remedies in the years to come. South and Central America have made enormous contributions to agriculture and a large number of food crops such as maize, potatoes, tomatoes, pumpkins, cassava, peanuts and sweet potato originate from there. Traditional American Indian medicinal herbs are used extensively but the influence of Spanish, European, East Asian and African medical systems is obvious. Notable examples of medicinal plants are: *Cinchona pubescens* (peruvian bark), *Erythroxylum coca* (coca), *Ilex paraguariensis* (maté), *Myroxylon balsamum* (tolu balsam), *Paullinia cupana* (guarana), *Peumus boldus* (boldo), *Psidium guajava* (guava), *Spilanthes acmella* (Brazilian cress), *Tabebuia impetiginosa* (lapacho) and *Uncaria tomentosa* (cat's claw) (Fabricant and Farnsworth, 2001; Gurib-Fakim, 2006).

1.2.3 Australian and Southeast Asian medicine

This region has witnessed a resurgence of interest in traditional medicine and many countries now promote research into medicinal plants as a potential source of new remedies. The Aborigines had a complex healing system but much of the traditional knowledge in Australia was lost before it could be systematically recorded. In contrast, many healing practices such as those of Malaysia, Thailand, Vietnam, New Zealand, Borneo, and the Polynesian Islands remain intact and are being recorded and developed. A strong Chinese influence is being observed in most countries. Among the well-known medicinal products originating from this region are *Croton tiglium* (purging croton), *Duboisia hopwoodii* (pituri), *Eucalyptus globulus* (bluegum), *Melaleuca alternifolia* (tea tree), *Myristica fragrans* (nutmeg and Mace), *Piper methysticum* (kava kava), *Strychnos nux-vomica* (strychnine), *Styrax benzoin* (benzoin) and *Syzygium aromaticum* (cloves) (Maher, 1999; Kapoor, 1990; Newman, 2000; Gurib-Fakim, 2006).

1.2.4 Ayurvedic medicine (Indian traditional medicine)

Ayurveda is perhaps, the most ancient of all medicinal traditions. It is probably older than traditional Chinese medicine and is considered to be the origin of systemized medicine. It is actually a practical and holistic set of guidelines to maintain balance and harmony in the system. Dioscorides (who influenced Hippocrates) is thought to have taken many of his ideas from India. Ancient Hindu writings on medicine contain no references to foreign medicines whereas Greek and Middle Eastern texts refer to concepts and drugs of Indian origin (Magner, 1992; Chopra, 2000).

Ayurveda is derived from the Indian words '*Ayar*' (life) and '*veda*' (knowledge or science) and hence means the science of life. Following the system would help ensure a long life, which is considered to be the instrument for achieving righteousness (*dharma*), wealth (*artha*) and happiness (*sukha*).

In India, knowledge and wisdom have been passed on from one generation to the next through songs and poems, which scholars and physicians had to learn and recite by heart. The Veda is an ancient text in four parts (Rig Veda, Sama Veda, Yajur Veda and Atharva Veda), the earliest of which date back to 2 000 years BC. The principles of Ayurvedic medicine and the medicinal uses of plants are contained in thousands of poetic hymns in the Rig Veda. The first school to teach Ayurvedic medicine was at the University of Banaras in 500 BC where the great Samhita (or encyclopedia of medicine) was written. Another great enclyclopedia was written 700 years later, and these two together form the basis of the Ayurveda (Chopra, 2000).

Ayurveda is similar to Galenical medicine in that it is based on body humours (*dosas*) and the inner life force (*prana*) that is believed to maintain digestion and mental activity. The living and the non-living environment, including humans, are considered to be elements: earth (prithvi), water (jada), fire (tejac), air (vaju) and space (akasa). For an understanding of these traditions, the concept of impurity and cleansing is also essential. Illness is the consequence of imbalance between the various elements and it is the goal of the treatment to restore this balance (Magner, 1992).

Famous Ayurvedic medicinal plants include *Azadirachta indica* (neem), *Centella asiatica* (gotu kola), *Cinnamomum camphora* (camphor), *Elettaria cardamomum* (*ela* or cardamomum), *Rauwolfia serpentina* (Indian snake root), *Santalum album* (sandalwood), *Terminalia* species (myrobolan) and *Withania somnifera* (aswargandha) (Kapoor, 1990; Magner, 1992; Padua de, 1999; Gurib-Fakim, 2006).

1.2.5 Chinese traditional medicine

The civilizations of China and India were flourishing when only modestly sophisticated cultures were developing in Europe. Expectedly writings on medicinal plants and the aesthetics of vegetation were numerous. This ancient system of medicine, believed to be more than 5 000 years old, is based on two separate theories about the natural laws that govern good health and longevity, namely *yin* and *yang*, and the five elements (*wu xing*) (Kapoor, 1990; Patwardhan, 2005).

The legendary emperor Shen Nung discussed medicinal herbs in his works—which were probably written 2 500 years B.P. (Before Present) and not the traditional date of 3 500 B.P. The Traditional Chinese medicine was systematized and written between 100 and 200 BC (Before Christ). The most complete reference to Chinese herbal prescription is the Modern Day Encyclopedia of Chinese *materia medica* published in 1977. It lists nearly 6 000 medicines out of which 4 800 are of plant origin (Magner, 1992).

Treatment is based on symptoms and on a pattern of imbalances, often detected by taking the pulse or observing the patient's tongue. Warming or hot herbs, such as ginger and cinnamon, are used to treat ailments associated with cold symptoms such as cold hands, abdominal pains and indigestion (Kapoor, 1990; Padua de, 1999).

In common with Western and African traditional medicines, Chinese herbs are usually given in fixed mixtures or formulas of up to 20 herbs, carefully prepared according to traditional recipes. There are hundreds such recipes being used alongside with Western medicines. As in other healing cultures,

traditional recipes are used preferentially against chronic illnesses while acute or serious illnesses are cured by Western medicines.

The spread of traditional Chinese medicine to most continents has undoubtedly contributed to the current popularity of herbal medicines throughout the world. Examples of famous Chinese medicinal plants are *Angelica polymorpha* var. *sinensis* (dang gui), *Artemisia annua* (qing hao), *Ephedra sinica* (ma huang), *Paeonia lactiflora* (bai shao yao), *Panax ginseng* (ren shen) and *Rheum palmatum* (da huang) (Magner, 1992; Padua de, 1999; Gurib-Fakim, 2006).

1.2.6 European medicine

In the ancient Western world, the Greeks contributed significantly to the rational development of the use of herbal drugs. However, the European healing system is said to have originated with Hippocrates (460–377 BC) and Aristotle (384–322 BC), whose own ideas were rooted in ancient beliefs from India and Egypt. The philosopher and natural scientist, Theophratus (~300 BC), in his *History of Plants*, dealt with the medicinal qualities of herbs, and noted the ability to change their characteristics through cultivation. Dioscorides, a Greek physician (100 AD), during his travels with Roman armies, recorded the collection, storage and the use of medicinal herbs and Galen (130–200 AD) who practiced and taught pharmacy and medicine in Rome, published no less than 30 books on these subjects, and is well known for his complex prescriptions and formulas used in compounding drugs, sometimes containing dozens of ingredients (“galenicals”) (Weiher *et al.,* 1999).

Greek and Roman medicine was based on the belief that the world is composed of four elements—earth, wind, fire and water. Each of these has its corresponding humours, linked to the four vital fluids in the body. The four humours—blood, phlegm, black bile and yellow bile, influence both health and temperament (respectively sanguine, phlegmatic, melancholic and choleric). In order to restore balance, drastic measures such as blood letting (reducing excess blood) and purging (to remove excess black bile) was used. The four

humours were also associated with cold, heat, dampness and dryness and each of these had a corresponding range of cold, hot, damp or dry herbs that were supposedly able to restore imbalances. European tradition also had many regional influences that influenced local folk practices and traditions (Weiher *et al.,* 1999).

One of the most powerful influences was the famous book *De Materia Medica*, written by the Greek physician Dioscorides in the first century AD. It is generally accepted to be the first European herbal and was the standard reference in Europe for more than 1 000 years, providing the base for most of the later herbals. As early as AD 800, medicinal plants were cultivated according to a standardized layout in monasteries in Central Europe. One of the famous healers of this era was Hildegard of Bingen (1098–1179). In later years a Swiss alchemist known as Paracelsus (1493–1541) emphasized the importance of the correct dosage for medical treatments (Gurib-Fakim, 2006). Herbal medicine was part of everyday life in many countries in Europe and to this day has remained a popular method of treating ailments but is often considered to be supportive rather than curative. To date in several European countries, the use of herbal tea is still very popular. In addition to these, 'natural products' taken in their crude form (unprocessed) as teas or decoctions, more sophisticated phytomedicines (standardized and formulated extracts of plants, often subject to rigorous testing in humans) remain a popular alternative to medicinal products derived from pure synthetic chemicals (Vicker and Zollman, 1999).

A large number of traditional herbal remedies in Europe have become widely known as a result of commercialization and a number of active compounds have been isolated from medicinal plants and are used today as single chemical entities (Pieroni, 2000).

1.2.7 Classical Arabic and North African traditional medicine

The oldest written information in the Arabic traditions comes from the Sumerians and Akkadians of Mesopotamia, thus originating from the same areas as the archeological records of Shanidar IV (Heinrich *et al*., 2004). The earliest documented record, which presumably relates to medicinal plants, dates from 60 000 before the common era (BCE) found in the grave of the Neanderthal man from Shanidar IV, an archeological site in Iraq. Pollen of several species of plants, presumably used as medicines, was discovered among which are: *Centaurea solstitialis* (Asteraceae), *Ephedra altissima* (Ephedraceae), *Althea* sp. (Malvaceae) amongst others. Although this may not be a finding with direct bearing on the culture of Shanidar, these species or closely related ones from the same genus, are still important today in the phytotherapy of Iraq and also known from other cultural traditions. These species may well have been typical for the Neanderthal people and may also be part of a tradition for which Shaidar IV represents the first available record (Cragg and Newman, 2005).

The Middle East is known as the cradle of civilisation and many plants cultivated nowadays were domesticated in this region. The Babylonians, Assyrians and Sumerians recorded herbal remedies in cuneiform writing on numerous clay tablets. Of special interest is the Code of Hammurabi (ca. 1 700 BC), a comprehensive set of civil laws carved in stone and commissioned by the King of Babylon and which lists several medicinal herbs (Spiegel and Springer, 1997).

Similar documents have survived several millennia in Egypt. The Egyptians documented their knowledge (including medical and pharmaceutical) in wall paintings of tombs dating from the Old Kingdom and on papyrus which is made from *Cyperus aquaticus*. The most important of these writings is the Ebers Papyrus, which originates from around 1 500 BC and is reported to contain ancient medicinal knowledge from before 3 000 BC (Oubré *et al,* 1997). This famous 20 m papyrus scroll reputedly found in a tomb is inscribed in Egyptian hieroglyphics and named after Prof. Ebers Georges at Thebes in 1872. It was deposited at the University of Leipzig 1873 and two years later G.

Ebers published a facsimile edition (Ghalioungui, 1987). The Ebers Papyrus is a medical handbook covering all sorts of illnesses and includes empirical as well as symbolic forms of treatment. The diagnostic precision documented in this text is impressive. During the Dark and Middle Ages (5–12th Centuries, AD), the monasteries in countries such as England, Ireland, and Germany were responsible for preserving the remains of Western knowledge. But it was the Arabs who were responsible for the preservation of much of the Greco-Roman expertise, and for expanding it to include the use of their own resources, together with the Chinese and Indian herbs, till then unknown to the Greco-Roman world. The Arabs were the first to establish privately owned drug stores in the 8th century. The Persian pharmacist, physician, philosopher and poet, Avicenna, contributed much to the sciences of pharmacy and medicine throughout works such as *Canon medicinae*, regarded as the "final codification of all Greco-Roman medicine". *Canon medicinae* included elements of other cultures healing system and forms the basis for a distinct Islamic healing system known today as *Unani-Tibb* (Sheehan and Hussain, 2002).

Among the famous medicinal plants of the Middle East and Egypt are: *Allium cepa* (onion), *Astracantha gummifera* (tragacanth), *Carthamus tinctorius* (safflower), *Carum carvi* (caraway), *Ferula assafoetida* (asofoetida), *Lawsonia inermis* (henna), *Papaver somniferum* (opium poppy), *Peganum harmala* (syrian rue), *Prunus dulcis* (almond), *Punica granatum* (pomegranate), *Rosa* x *damascena* (damask rose), *Ricinus communis* (castor oil plant), *Salvadora persica* (toothbrush tree), *Senna alexandrina* (senna), *Sesamum indicum* (sesame), *Trachyspermum ammi* (ajowan), *Trigonella foenum-graecum* (fenugreek) and *Vitis vinifera* (grape) (Padua de, 1999; Neuwinger, 2000; Gurib-Fakim, 2006). A list of some botanical drugs used in traditional medicine, which have led to useful modern drugs are shown in Table 1.1. However, it should be noted that concern has been raised on the toxicity of Kava pyrones and that their anxiolytics have been positively reviewed (Schulze *et al.*, 2003).

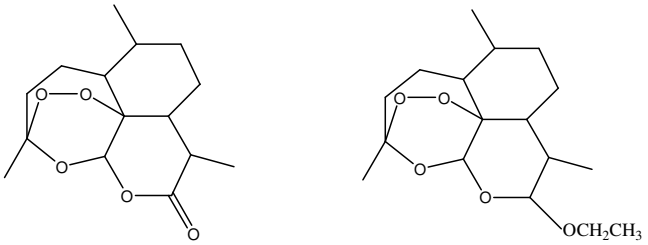
Table 1.1: Botanical drugs used in traditional medicine which led to useful modern drugs (Gurib-Fakim 2006).

Botanical names	English names	Indigenous use	Origin	Uses in biomedicine	Biologically active compounds
Adhatoda vasica	–	Antispasmodic, antiseptic, insecticide, fish poison	India, Sri Lanka	Antispasmodic, oxytocic, cough suppressant	Vasicin (lead molecule for Bromhexin and Ambroxol)
Catharanthus roseus	Periwinkle	Diabetes, fever	Madagascar	Cancer chemotherapy	Vincristine, Vinblastine
Condrodendron tomentosum	–	Arrow poison	Brazil, Peru	Muscular relaxation	D-Tubocurarine
Gingko biloba	Gingko	Asthma, anthelmintic (fruit)	Eastern China	Dementia, cerebral deficiencies	Ginkgolides
Harpagophytum procumbens	Devil's claw	Fever, inflammatory conditions	Southern Africa	Pain, rheumatism	Harpagoside, Caffeic acid
Piper methysticum	Kava	Ritual stimulant, tonic	Polynesia	Anxiolytic, mild stimulant	Kava pyrones
Podophyllum peltatum	May apple	Laxative, skin infections	North America	Cancer chemotherapy, warts	Podophyllotoxin and lignans
Prunus africana	African plum	Laxative, 'Old man's disease'	Tropical Africa	Prostate hyperplasia	Sitosterol

1.3 Drug discovery from medicinal plants

Drug discovery from medicinal plants has evolved to include numerous fields of inquiry and various methods of analysis. The process typically begins with a botanist, ethnobotanist, ethnopharmacologist, or plant ecologist who collects and identifies the plant(s) of interest. Collection may involve species with known biological activity for which active compound(s) have not been isolated (i.e traditionally used herbal remedies) or may involve taxa collected randomly for a large screening program. It is necessary to respect the intellectual property rights of a given country where plant(s) of interest are collected (Baker *et al*., 1995). Phytochemists (natural product chemists) prepare extracts from the plant material, subject these extracts to biological screening in pharmacologically relevant assays, and commence the process of isolation and characterization of the active compound(s) through bioassay-guided fractionation. Molecular biology has become essential to medicinal plant drug discovery through the determination and implementation of appropriate screening assays directed towards physiologically relevant molecular targets. Pharmacognosy encapsulates all of these fields into a distinct interdisciplinary science.

Numerous methods used to acquire compounds for drug discovery include: isolation from plants and other natural sources; synthetic chemistry; combinatorial chemistry, and molecular modeling (Ley and Baxendale, 2002; Geysen *et al*., 2003; Lombardino and Lowe, 2004). Despite the recent interest in molecular modelling, combinatorial chemistry, and other synthetic chemistry techniques by pharmaceutical companies and funding organizations, the natural products, and particularly that of medicinal plants, remain an important source of new drugs, drug leads, and chemical entities (Newman *et al*., 2000; Newman *et al*., 2003; Butler, 2004). In both 2001 and 2002, approximately one quarter of the best-selling drugs worldwide were natural products or were derived from natural products (Butler, 2004). An example is Arteether (Fig. 1.1), a potent antimalaria drug. It is derived from artemisinin, a sesquiterpene lactone isolated from *Artemisia annua* (Asteraceae), a plant used in traditional Chinese medicine (TCM) (van Agtmael *et al*., 1999; Graul, 2001).



Artemisinin Arteether

Figure 1.1: The structure of artemisinin and arteether.

Despite evident successes of drug discovery from medicinal plants, future endeavors face many challenges. Pharmacognosists, phytochemists, and other natural product scientists will need to continuously improve the quality and quantity of compounds that enter the drug development phase to keep pace with other drug discovery efforts (Butler, 2004). The process of drug discovery has been estimated to take an average of 10 years upwards (Reichert, 2003) and cost more than 800 million US dollars (Dickson and Gagnon, 2004). Much of this time and money is spent on the numerous leads that are discarded during the drug discovery process. It has been estimated that only one in 5000 lead compounds will successfully advance through clinical trials and be approved for use. Lead identification is only the first step in a lengthy drug development process (Fig. 1.2). There is also lead optimization (involving medicinal and combinatorial chemistry), development (including toxicology, pharmacology, pharmacokinetics, ADME [absorption, distribution, metabolism, and excretion], and drug delivery), and clinical trials which all take a considerable length of time.

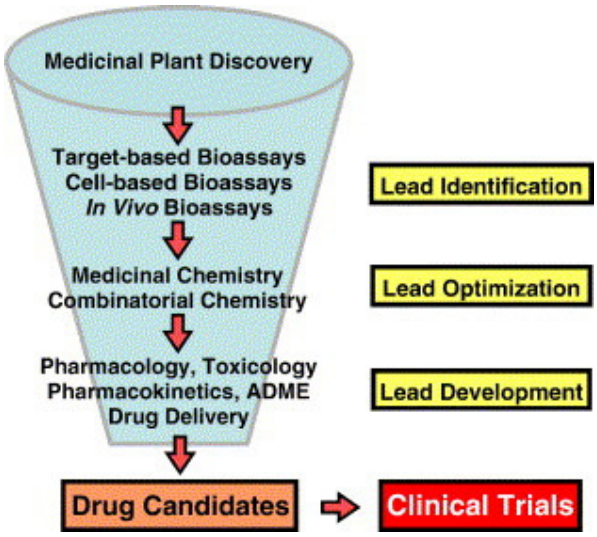


Figure 1.2: Schematic representation of a typical medicinal plant drug discovery process and development (Balunas and Kinghorn, 2005).

Drug discovery from medicinal plants has traditionally been lengthier and more complicated than other drug discovery methods. Therefore, many pharmaceutical companies have eliminated or scaled down their natural product research (Butler, 2004; Koehn and Carter, 2005).

Recently, there has been a rekindling of interest in 'rediscovering natural products'. As stated by one authority "We would not have the top-selling drug class today, the statins; the whole field of angiotensin antagonists and angiotensin-converting enzyme inhibitors; the whole area of immunosuppressives, nor most of the anticancer and antibacterial drugs. Imagine all of these drugs not being available to physicians or patients today". It is clear that nature has played and will continue to play, a vital role in the drug discovery process (Cragg and Newmann, 2005).

1.4 Synthesis and role of plant secondary metabolites

In plants, as a result of metabolic processes, many different kinds and types of organic compounds or metabolites are produced. These metabolites are grouped into primary and secondary metabolites. The primary metabolites like chlorophyll, amino acids, nucleotides, simple carbohydrates or membrane lipids, play recognised roles in photosynthesis, respiration, solute transport, translocation, nutrient assimilation and differentiation. The secondary metabolites also differ from primary metabolites in having a restricted distribution in the plant kingdom. That is, particular secondary metabolites are often found in only one plant species or a taxonomically related group of species, whereas the basic primary metabolites are found throughout the plant kingdom (Taiz and Zeiger, 2006). During the past few decades, experimental and circumstantial evidence has made it clear that many secondary metabolites do indeed have functions that are vital for the fitness of a plant producing them. The main roles are:

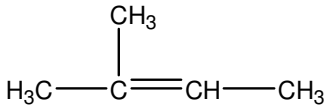
- Defence against herbivores (insects, vertebrates)
- Defence against fungi and bacteria
- Defence against viruses
- Defence against other plants competing for light, water and nutrients
- Signal compounds to attract pollinating and seed dispersing animals
- Signals for communication between plants and symbiotic micro-organisms (e.g. N-fixing Rhizobia or mycorrhizal fungi)
- Protection against UV-light or other physical stress (Wink, 1999)

They have also provided an invaluable resource that has been used to find new drug molecules (Gurib-Fakim, 2006).

Plant secondary metabolites can be grouped into three chemically distinct classes: terpenes, phenolics and nitrogen containing compounds. Figure 1.3 shows, in simplified form, the pathways involved in the biosynthesis of secondary metabolites and their interconnection with primary metabolites.

1.4.1 Terpenes

Terpenes, terpenoids or isoprenoids are dimmers, trimers or polymers of isoprene units, which are usually jointed in a head to tail fashion. In plants the



Isoprene unit

activated form of the isoprene unit (isopentenyl pyrophosphate) which is the building-block of each type of terpenoid is synthesised either by the mevalonic acid pathway (e.g. sesquiterpenoids) or the methylerythritolphosphate pathway (e.g. mono- and diterpenoids) (Taiz and Zeiger, 2006) (Fig 1.3). Isoprene units usually condense to form linear chain or ring compounds commonly containing carbon atom numbers of 10 (the monoterpenoids), 15 (the sesquiterpenoids, 20 (the diterpenoids), or 30 (the triterpenoids). Terpenoids with 25 carbons are rarely found.

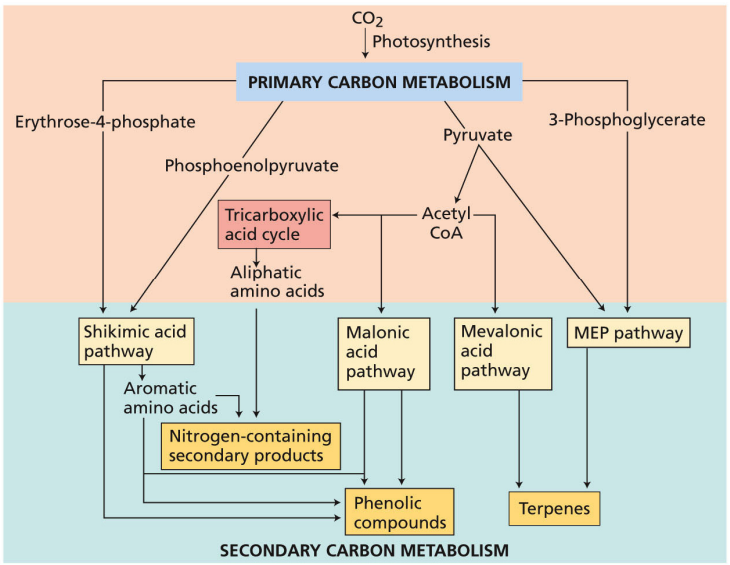
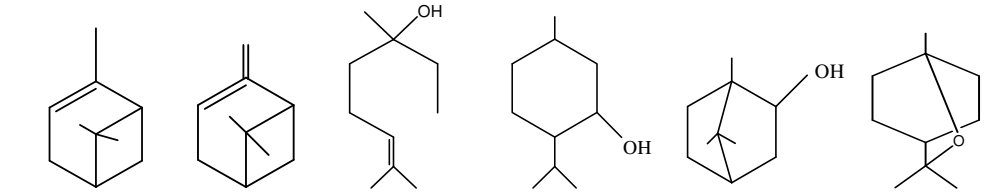


Figure 1.3: Main pathways leading to secondary metabolites (Taiz and Zeiger, 2006).

1.4.1.1 Monoterpenes

Monoterpenes are commonly found in essential oils. Iridoids and pyrethrins are included in this group. Examples of monoterpenes (Fig. 1.4) commonly found in essential oils are shown below (Taiz and Zeiger, 2006):



α- Pinene β-Pinene Linalool Menthol Borneol 1,8-cineol

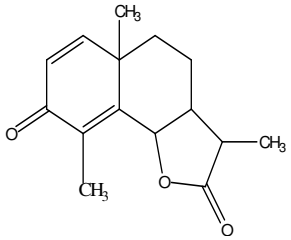
Figure 1.4: Monoterpenes commonly found in essential oils.

They are widely used as insecticides and their pharmacological properties range from analgesic to anti-inflammatory.

1.4.1.2 Sesquiterpenes

Sesquiterpenes are also constituents of essential oils of many plants, e.g. bisabolol, humulene and caryophyllene. Sesquiterpene lactones are well known as bitter principles and occur in families like the Asteraceae.

These compounds possess a broad range of activities due to the α-methylene-γ-lactone moiety and epoxides. Their pharmacological activities are antibacterial, antifungal, anthelmintic, antimalarial and molluscicidal. An example is santonin, which is used as anthelmintic and antimalarial (Gurib-Fakim, 2006).



α- Santonin

1.4.1.3 Diterpenes

Diterpenes are present in animals and plants and have some therapeutic applications, for example, the famous taxol and its derivatives are anticancer drugs. Other examples are forskolin, which has antihypertensive activity; zoapatanol is an abortifacient while stevoside is a sweetening agent (Gurib-Fakim, 2006).

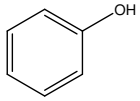
1.4.1.4 Triterpenes

Triterpenes are C_{30} compounds arising from the cyclization of squalene. They are comprised of a variety of structurally diverse compounds, which include steroids. Tetracyclic terpenes and steroids have similar structures but have different biosynthetic pathways (Taiz and Zeiger, 2006).

Steroids contain a ring system of three six-membered and one five-membered ring. Because of the profound biological activities encountered, many natural steroids together with a considerable number of synthetic and semi-synthetic steroidal compounds, are employed in medicine (e.g. steroidal saponins, cardioactive glycosides, corticosteroid hormones and mammalian sex hormones). The pharmaceutical applications of triterpenes and steroids are considerable (Gurib-Fakim, 2006).

1.4.2 Phenolic compounds

All phenolic compounds have an aromatic ring that contains various attached substituent groups such as hydroxyl, and methoxy (-O-CH_3) groups, and often other non-aromatic ring structures.



They range from simple structures with one aromatic ring to complex polymers such as tannins and lignins. Phenolics differ from lipids in being more soluble in water and less soluble in non-polar organic solvents. Some phenolics, however, are rather soluble in ether, especially when the pH is low enough to prevent ionization of any carboxyl and hydroxyl group present.

These properties greatly aid separation of phenolics from one another and from other compounds (Taiz and Zeiger, 2006). Other classes of phenolic compounds include coumarines, quinones and flavonoids.

Phenolic compounds are synthesised via the Shikimic acid or acetate pathway (Fig 1.5) and subsequent reactions. They have a wide range of pharmaceutical activities such as anti-inflammatory, analgesic, antitumour, anti-HIV, anti-infective (antidiarrhoeal, antifungal), antihepatotoxic, antilipolytic, antioxidant, vasodilatory, immunostimulant and antiulcerogenic. In plants they serve as effective defence against herbivores (Wink, 1999 and Gurib-Fakim, 2006).

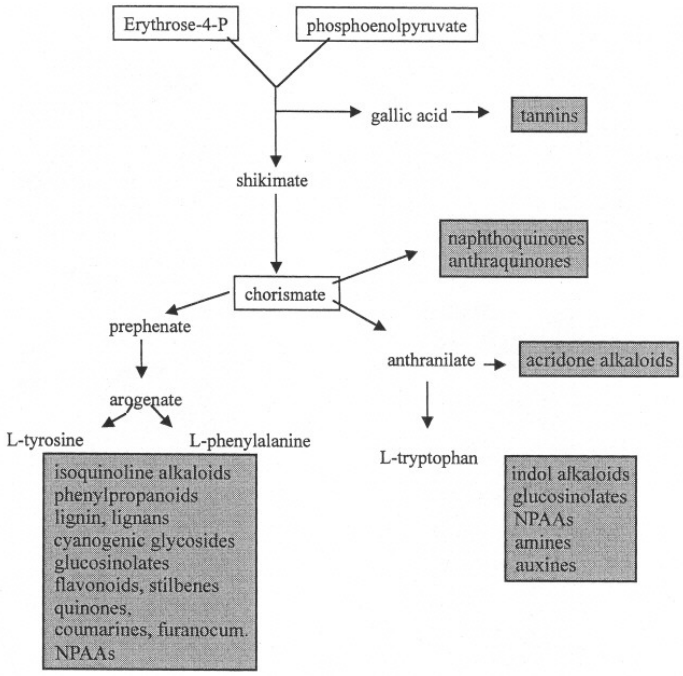


Figure 1.5: The pathways of secondary metabolites derived from precursors in the shikimate pathway (Wink, 1999).

1.4.2.1 Flavonoids

Flavonoids are 15-carbon compounds generally distributed throughout the plant kingdom (Fig. 1.6). More than 2000 have been identified from plants

(Taiz and Zeiger, 2006). They are responsible for the colour of flowers, fruits and sometimes leaves. Some may contribute to the colour by acting as a co-pigment. The name 'flavonoid' refers to the Latin word '*flavus*' meaning yellow.

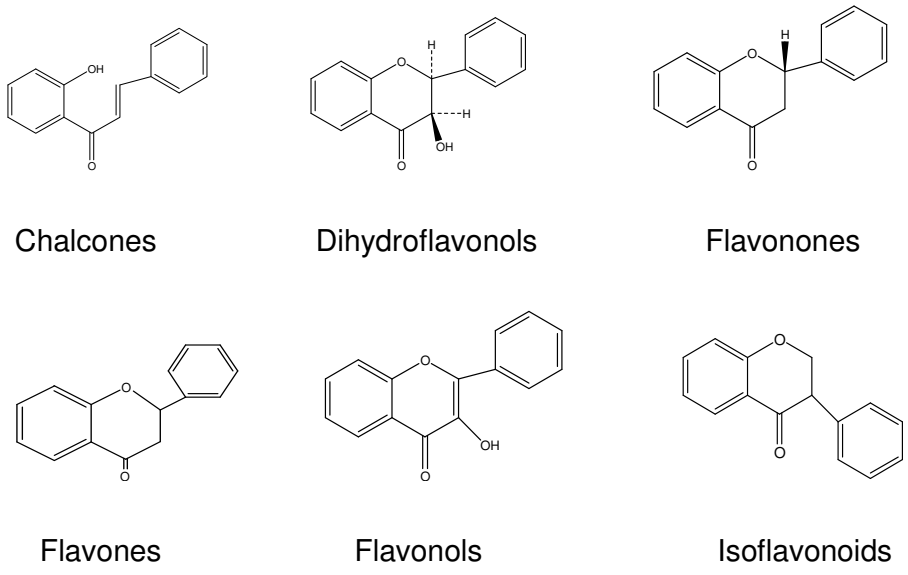


Figure 1.6: Basic structures of some flavonoids.

Flavonoids protect the plant from UV-damaging effects and play a role in pollination by attracting animals with their colours (Gurib-Fakim, 2006). The basic structure of flavonoids is 2-phenyl chromane or an Ar–C3–Ar skeleton. Biosynthetically they are derived from a combination of the Shikimic acid and the acetate pathways. Small differences in basic substitution patterns give rise to several sub-groups. In the plant, flavonoids can either occur as aglycones or as O- or C-glycosides (Gurib-Fakim, 2006). Recently, flavonoids have attracted interest due to the discovery of their pharmacological activities.

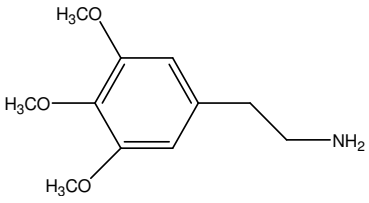
1.4.3 Nitrogen containing compounds

A large variety of plant secondary metabolites have nitrogen in their structures. Included in this category are such well-known antiherbivore compounds such as alkaloids and cyanogenic glycosides, which are of considerable interest because of their toxicity to humans and their medicinal

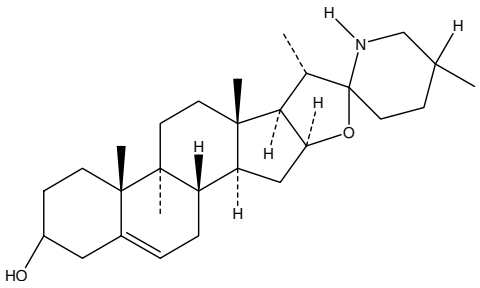
properties. Most nitrogenous secondary metabolites are biosynthesised from common amino acids (Taiz and Zeiger, 2006).

1.4.3.1 Alkaloids

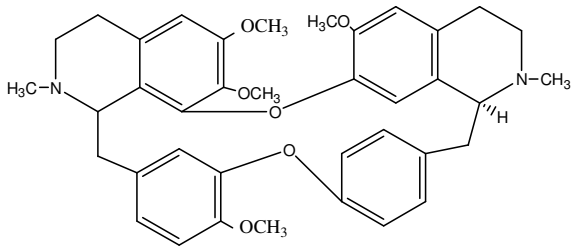
The term 'alkaloid' has been defined as a cyclic organic compound containing nitrogen in a negative oxidation state, which has limited distribution in living organisms (Taiz and Zeiger, 2006). Based on their structures, alkaloids are divided into several subgroups: non-heterocyclic alkaloids and heterocyclic alkaloids, which are again divided into 12 major groups according to their basic ring structure. Mescaline is an example of a non-heterocyclic or pseudo-alkaloid, tetrandrine is an example of a bisbenzylisoquinoline alkaloid while solasodine is a triterpene alkaloid (GuribFakim, 2006) (Fig. 1.7).



Mescaline



Solasodine



Tetrandrine

Figure 1.7: Structures of some alkaloids.

Free alkaloids are soluble in organic solvents and react with acids to form water-soluble salts. There are exceptions like berberine, which is a quartenary ammonium alkaloid. Most alkaloids are solids except for nicotine, which is a liquid.

Alkaloids, usually have a marked physiological action on humans or animals, and are sometimes believed to be waste products and a nitrogen source. They are thought to play an important role in plant protection, germination and plant growth stimulation.

Alkaloids are pharmaceutically significant, e.g. morphine as a narcotic analgesic, codeine in the treatment of coughs and pain, colchicines in the treatment of gout, quinine as an antimalarial, quinidine as an antiarrythmic and L-hyoscyamine (in the form of its racemic mixture known as atropine) as antispasmodic and for pupil dilation (Gurib-Fakim, 2006).

1.4.3.2 Cyanogenic glycosides

Perhaps the most obvious defence-related secondary metabolites are the cyanogenic glucosides (Bennett and Wallsgrove, 1994). They are not in themselves toxic but are readily broken down to give off volatile poisons when the plant is crushed. Cyanogenic glycosides release the well-known respiratory poisonous gas, hydrogen cyanide (Taiz and Zeiger, 2006).

1.5 Infectious diseases

Despite the tremendous progress in medicine, infectious diseases caused by bacteria, fungi, viruses and parasites continue to pose a threatening challenge to public health (Cos *et al.,* 2006). The burden of these diseases is felt the most in developing countries due to poverty, unavailability of medicines and the emergence of widespread resistance of pathogens to the available drugs (Okeke *et al.,* 2005). The World Health Organisation in 2002 has also reported that infectious and parasitic diseases account for 26.2% of the global cause of death, the vast majority of which occurred in the developing countries (WHO, 2003).

Every year, more than half of the deaths associated with infectious diseases continue to be attributed to three illnesses: HIV/AIDS, tuberculosis and malaria. These diseases are present in epidemic proportion, profoundly affecting and serving as major obstacles to the economic growth and development in many of the poorest countries in the world (Mandell *et al.,* 2005). Urgent solutions are required if the poorest regions in the world is to develop.

1.5.1 Malaria

Malaria is a protozoal disease caused by parasitic protozoa of the genus *Plasmodium*. It is transmitted to humans by the female *Anopheles* mosquito. There are over three hundred species of *Anopheles* mosquito, however, only about sixty are able to transmit the malaria parasite. Malaria commonly affects the populations of tropical and subtropical areas world wide, as well as increasing number of travellers to and from these areas. The following four species of *Plasmodium* cause the disease in its various forms: *P. falciparum, P. vivax, P. ovale* and *P. malariae*. *P. falciparum* is the most widespread and dangerous of the four as it can lead to the fatal cerebral malaria, which often results in death (Hyde, 2002). Today some 500 million people in Africa, India, South East Asia and South America are exposed to endemic malaria and it is estimated to cause 2.5 million deaths annually, one million of which are children. Although malaria is found in over 100 countries (Fig. 1.8 and 1.9), the major burden of the disease is carried by the nations of Africa, where over 90% of all falciparum malaria deaths are recorded, and where the high levels of morbidity and transmission place considerable strain on public health services and economic infrastructure (Hyde, 2002). In the absence of effective vaccines, management of the disease has depended largely upon chemotherapy and chemoprophylaxis. Of the various antimalaria drugs available, the aminoquinoline, chloroquine was for several decades the agent of choice, as it was safe, effective and cheap. Parasite resistance to this drug was first observed in Thailand in 1957 and then on the border of Colombia and Venezuela in 1959. By the late 1970s it had spread to East Africa and by the mid-1980s had become a major problem in several areas in Africa (Wernsdorfer and Payne, 1991).

Although the increasing prevalence of drug resistant *P. falciparum* has hindered the ability to control/treat the disease, it has at the same time intensified attempts to develop novel antimalaria drugs and agents to prolong

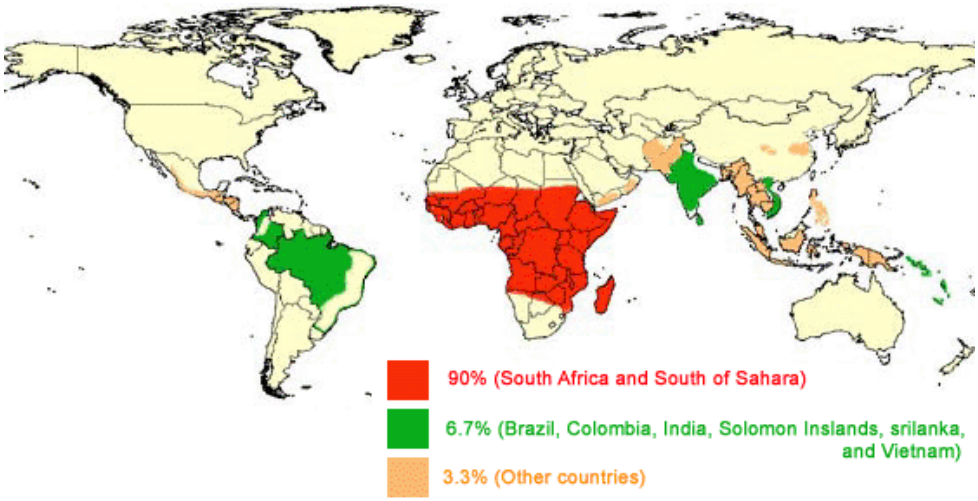


Figure 1.8: Global malaria distribution (WHO global atlas, 2005).

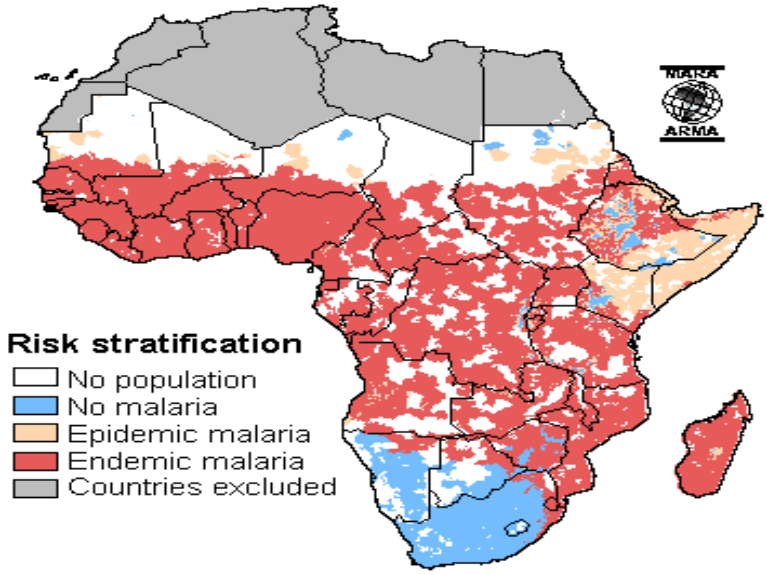


Figure 1.9: Distribution of malaria in Africa (WHO global atlas, 2005).

the clinical usefulness of the few currently available drugs (Singh and Puri, 2000). An increasing number of countries have been compelled to adopt a different class of drug, the antifolates, as the first line of alternatives to

chloroquine. The most widely used combination of this type consist of pyrimethamine (PYR) and sulfadoxine (SDX), known as fansider or SP, which is cheap and, until recently, was effective against the chloroquine-resistant parasites found in Africa. However, resistance to this formulation, long established in parts of south-east Asia and South America (Wernsdorfer, 1994), now threatens to leave Africa with no affordable treatment. Further combinations of antifolates with newer drugs such as the artemisinin derivatives, or the development of alternative combinations, may be the only way to limit the pace of the parasitic resistance to chemotherapy. For example, the antifolate prodrug, proguanil, has now been formulated together with a new type inhibitor, atovaquone, to yield malarone, recently licensed for clinical use (Hyde, 2002).

Developing countries, were malaria is epidemic, still depend on traditional medicine for the treatment of the disease. However, little scientific data are available to assess the efficacy of these herbal remedies. On the other hand, it is accepted that the recognition and validation of traditional medicinal practices could lead to new plant derived drugs, e.g. artemisinin from *Artemisia annua*, a Chinese traditional medicine plant (Ridley, 2002). Therefore it is important that medicinal plants which have a folklore reputation for antimalarial properties are investigated, in order to establish their efficacy and to determine their potential as a source of new antimalarial drugs (Tran *et.al.,* 2003). South Africa is an ideal place to search for a new drug because of its remarkable biodiversity and rich cultural traditions of plant uses.

1.5.2 Human immunodeficiency virus (HIV)

The Joint United Nations Programme on HIV/AIDS (UNAIDS) and the World Health Organisation (WHO), reported the number of people living with HIV at the end of 2006 to be 39.5 million globally. Of the people infected worldwide, 64% reside in sub-Saharan Africa, 77% of which are women. The severity of the epidemic has been associated with poverty, low status of women and other socio-economic factors (Nicoll and Gill, 1999; NSP, 2000).

A number of documents have described the seriousness of HIV/AIDS in the Southern Africa region with particular emphasis on Southern Africa being the most affected (UNAIDS, 2000). The prevalent rate for South Africa is estimated to be 12.5%, which is one of the highest national prevalent rates in the world (James *et al.,* 2006). Women are more hit by the epidemic of HIV/AIDS. Of the 5.54 million people living with HIV in South Africa in 2005, 18.8% are adults aged 15-49 years of which women account for approximately 55%. The infection is more pronounced in the age group 20-24 years and 25-29 where the HIV prevalence rates are 23.9% for women to 6% for men and 33.3% for women and 12.2% for men respectively (NSP, 2007). HIV was around 3% among children aged 2-14 year and nearly 4% for people in their sixties (Dinkelman *et al.,* 2007).

The United Nations General Assembly Session on HIV/AIDS (UNGASS) has identified young people of the age group 15-24 years as the target group for reducing new cases of HIV infection and set a global target of reducing incidence of HIV in this group by 20% by 2015. Young people represent the main focus for altering the course of this epidemic. UNAIDS data on the experience of several countries including South Africa, confirm that positive behavioural change is more likely in this group than in older ages (NSP, 2007).

The increase in pregnancy and HIV infection in young school leaving people has been linked with unemployment. The inability to secure a job plus pressure from family members to make financial contributions for the maintenance of their homes, predispose them to sex work as a way to make ends meet (Dinkelman *et al.,* 2007).

Children under the age of 18 comprise 40% of the population of South Africa. In 2004, it was reported that 13% of them have lost either mother or father, half of which was due to AIDS. Children from deeply impoverished households were worst affected by the impact of AIDS (UNAIDS, 2004). Children are exposed to HIV through sexual abuse, blood transfusion and

mother to child transmission (MTCT) which occurs mostly during birth and/or breastfeeding (Nicoll and Gill, 1999).

The immediate determinant of the spread of HIV relates to behaviours such as unprotected sexual intercourse, multiple sexual partnerships, and some biological factors such as sexually transmitted infections, the fundamental drivers of this epidemic in South Africa are deep rooted in the problem of poverty, underdevelopment, and low status of women, including gender-based violence in society (Dinkelman *et al.*, 2007).

Many countries in Africa and Asia have taken urgent steps to curb the epidemic with varying degrees of success. In South Africa, despite the effort invested, the HIV infection rate has increased significantly over the last 5 years (NSP, 2000). The country has the largest number of people enrolled on antiretroviral therapy in the world. There are still many more people in need of this and other related interventions to reduce the morbidity and mortality of HIV/AIDS. In particular, more eligible adults than children have accessed these services. There is need to develop more innovative strategies to improve access for children in schools (primary and secondary) (NSP, 2007).

At the moment there is no cure for HIV. Single drug therapy is no longer effective due the resistance developed by the virus. Combinational therapy is now the method of choice in treatments (Spencer, 2005). The adverse side effects of the available drugs or combination of drugs and non-compliance of patients on treatment did not help the situation (Spencer, 2005). There is a urgent need to develop drugs with less side effects for the treatment and a cure for the disease. The only effective way to combat HIV infection at the moment is in prevention-advocacy and the practice of abstinence, and safe sexual practice (Nicoll and Gill, 1999).

1.5.3 Tuberculosis

The bacterium (*Mycobacterium tuberculosis)* causing tuberculosis first emerged as a major disease threat more than 15 000 years ago. Today about 2 billion people are infected. However, not all these individuals will become ill or develop active tuberculosis (Mandell *et al.,* 2005). HIV infection is the

strongest risk factor for progression to active disease: 46% of people in the developing world with HIV are co-infected with tuberculosis (Nicoll and Gill, 1999; Anthony and Fauci, 2005). About 4 million people have active tuberculosis at any time resulting in about 2 million deaths each year. Most of the deaths occur in the developing countries predominantly in Africa and Asia (Anthony and Fauci, 2005). The fatalities of this disease are worsened by the development of resistance to the available antituberculosis drugs (Mandell *et al.,* 2005).

1.6 Antioxidant activity

Free radical decomposition results in a large number of human diseases, such as heart disease, cataracts, cognitive dysfunction, aging and cancer (Brahmachari and Gorai, 2006). These damages or diseases are caused by free radicals called reactive oxygen species (ROS). Examples of ROS include superoxide anions, singlet oxygens, hydroxyl radicals, lipid peroxyl radicals and peroxynitrite radicals (Erkoç *et al.,* 2003).

The human body has evolved with antioxidant systems to protect it against free radicals. These systems include some antioxidants produced in the body (endogenous), obtained from the diet (exogenous) and repair antioxidant (proteases, lipase, transferases, and DNA repair enzymes). The ones produced in the body are enzymatic defences, such as Se-glutathione peroxidase, catalase, and superoxide dismutase, which metabolize superoxide, hydrogen peroxide and lipid peroxides, thus preventing most of the formation of the toxic hydroxyl radicals. Exogenous antioxidants consist of non-enzymatic defenses, such as glutathione, histidine-peptides, the iron-binding proteins transferring and ferritin, dihydrolipoic acid etc (Erkoç *et al.,* 2003; Brahmachari and Gorai, 2006). Owing to the incomplete efficiency of our endogenous defence systems and the existence of some physiopathological situations (cigarette smoking, air pollutants, UV radiation, high polyunsaturated fatty acid diet, inflammation, ischemia/reperfusion, etc) in which ROS are produce in excess and at the wrong time and place, dietary antioxidants are needed for diminishing the cumulative effects of oxidative

damage over the life span. The antioxidants derived from diet are vitamins C, E and A, and carotenoids. Other antioxidants of value to health derived from plants include phenols, phenolic acids, flavonoids, tannins and lignans (Pietta, 2000).

Antioxidant activity of a drug candidate adds to its medicinal value. In this chapter the antioxidant activity of the compounds isolated has been investigated using both qualitative and quantitative assays.

1.7 *Croton steenkampianus*

Several species of the genus *Croton* (Euphorbiaceae) showed excellent results when crude extracts were tested for antiplasmodial activity previously. Of the species tested, the leaves of *C. steenkampianus* Gestner, had the best activity and was therefore selected for isolation of the active principles in this study (Prozesky, 2004). Before 2004, little or nothing was known regarding the chemical composition and medicinal use of *C. steenkampianus* other than that known for the family in general. However, flavonoids and terpenes that showed promising antiplasmodial activities had been isolated from its leaves (Prozesky, 2004). Therefore it was decided to attempt the isolation of more active principles from this species.

Generally, species in the family Euphorbiaceae have a variety of uses and commercial products include rubber *(Hevea*), tung oil (*Aleurites*), castor oil (*Ricinus*), and cassava (*Manhot*) and many are used as ornamentals (Leistner, 2000). Medicinally, despite reports that many species are poisonous, they are used for ailments such as malaria, hepatic and kidney disorders, obesity, hypertension, fever, dysentery, convulsions, snakebite, chest pains, gastrointestinal disturbances, sterility, eye and respiratory complaints (Pooley, 1993; Ngadjui *et al.,* 2002; Suarez *et al.,* 2006). Chemically, the genus contains very diverse compounds including alkaloids, flavonoids and triterpenes. Many structurally diverse diterpenes have also been isolated from the genus (Prozesky, 2004).

C. steenkampianus is a shrub to small tree (1.5-4 m) (Fig. 1.10), found on the margins of sand forests and thickets in the eastern parts of South Africa and

further north into Africa. The main stem is much branched from the base, with smoothish grey bark. The leaves are large, heart shaped, grey to olive-green above, white beneath, with a pointed tip (Pooley, 1993; Prozesky, 2004).

Figure 1.10 *Croton steenkampianus* leaves.

1.8 Objectives

The objectives of this study were:

- Isolation of biologically active compound(s) from *C. steenkampianus*
- *In vitro* testing of pure compound(s) for antibacterial activity, antiplasmodial activity, anti-HIV activity and antioxidant activity
- *In vitro* testing of the antiplasmodial activity of isolated compound(s) with and without chloroquine
- Cytotoxicity testing of isolated compound(s)

1.9 Scope of the thesis

The isolation and identification of three flavonoids, two new diterpenes (with a newly described skeleton) and one new indane is described in chapter two. The isolation was performed using bioassay-guided fractionation (antibacterial activity). The bio-activities (antiplasmodial, antibacterial and antioxidant, anti-HIV and cytotoxicity of the isolated compounds are described in chapters 3, 4,

5 and 6 respectively. Chapter 7 consists of a general discussion and conclusion.

1.10 Hypothesis

The determination of biological activities and isolation of compounds from *C. steenkampianus* was performed for the first time by Prozesky (2004). This species not being well studied plus the interesting results reported previously led to the conclusion that it contains more active principles and was reselected for further studies. The hypothesis of this study is therefore that *C. steenkampianus* contains compounds with valuable bioactivity.

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