

**STUDIES ON MOLLUSCICIDAL PROPERTIES OF SOME SOUTH AFRICAN MEDICINAL
PLANTS USED IN THE CONTROL OF SCHISTOSOMIASIS IN KWAZULU-NATAL**

By

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DECLARATION

I, WENDY CAROLINE TSEPE, HEREBY DECLARE THAT THIS DISSERTATION IS MY OWN ORIGINAL WORK AND HAS NOT BEEN PRESENTED FOR ANY DEGREE OF ANOTHER UNIVERSITY.

THE WORK REPORTED IN THIS DISSERTATION WAS PERFORMED IN THE DEPARTMENT OF PHARMACOLOGY OF THE UNIVERSITY OF DURBAN WESTVILLE, DURBAN 4000.

SIGNATURE _____

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ABSTRACT

Schistosomiasis is an important public health issue for rural communities located near, or around slow moving water bodies in the tropical and subtropical areas. Successful control of the disease involves multifaceted approaches, which include snail control, environmental sanitation, health education and chemotherapy. Although snail control might be an effective method of controlling schistosomiasis, there has been a general lack of control initiatives, largely due to the cost of available molluscicides. Plants offer a wide array of compounds which, on extraction, may show molluscicidal activity. If molluscicidal compounds that occur in indigenous plants can be extracted using local labour and simple technology, then there should be culturally acceptable and inexpensive molluscicides. The aim of this study was, therefore, to screen some Zulu medicinal plants for molluscicidal activity. We have also attempted to isolate the active chemical compounds from such plants.

Aqueous and methanolic crude extracts of ten (10) Zulu medicinal plants, used for different medicinal and domestic purposes, were screened for molluscicidal activity on *Biomphalaria pfeifferi* and *Bulinus africanus* snails reared in the laboratory during the time of bioassay. Bayluscide® (niclosamide) was used as a positive control for comparison, while de-chlorinated tap water was used as the negative control. Six of the plants were not active against the snails. Extracts from four of the plants demonstrated weak to moderate molluscicidal activities. These plants are: (i) *Sclerocarya birrea* stem-bark, (ii) *Psidium guajava* (hybrid) leaves, (iii) *Leonotis leonurus* aerial parts and (iv) *Ekerbegia capensis* stem-bark. The LC₅₀ values of the plant extracts were 78 ppm, 100

ppm, 398 ppm and 600 ppm respectively. Of the 4 plants that showed molluscicidal activity, *S. birrea* aqueous and methanol extracts were the most active against the snails, with LC50 values of 82 ppm and 78 ppm respectively. For the other plant extracts, only the methanolic extracts showed activity. Brine shrimp toxicity assay was performed with all the active extracts. *Psidium guajava* showed 10% survival of the shrimps at 1000 ppm, whereas no survival was observed for the other plant extracts at this concentration (1000 ppm). The results obtained in this study indicate that further studies have to be conducted, especially with *S. birrea* extracts, whose both aqueous and methanolic extracts showed significant activity against the snails.

CHAPTER ONE

1. INTRODUCTION

Schistosomiasis is a parasitic disease affecting more than 200 million people in 74 countries throughout South America, Africa and the Far East (El-Kheir & El-Tohami, 1997; WHO, 1998a). The disease currently ranks second to malaria in terms of socio-economic and public health importance in tropical and subtropical countries of the world (Dossaji & Oketch-Rabah, 1998). Many organizations have tried different methods to bring the disease under control (Ahmed & Ramsy, 1997; Obeng, 1976). However, successful control of the disease involves multifaceted approaches, which include environmental sanitation, health education and chemotherapy. One way of controlling this disease is by destroying the intermediate snail hosts that harbour the developing schistosomal larvae, and thus interrupting the parasite's life-cycle (Knudsen & Sloof, 1992). Snail control is the most efficient and commonly used method of controlling schistosomiasis. It is tried through different methods, the most important of which is the chemical control (Dossaji & Oketch-Rabah, 1998).

Although snail control might be an effective method of controlling schistosomiasis, there has been a general lack of control initiatives, largely due to the cost of available molluscicides (Dossaji & Oketch-Rabah, 1998). Furthermore, the insidious nature of schistosomiasis infection and its lack of drama, usually associated with other infections, has resulted in most developing countries' governments diverting their usually scarce manpower and financial resources to tackle other health issues where the "dollar benefit" is clearly visible. This, coupled with increasing emphasis on control of schistosomiasis-related morbidity through primary care approach, as

opposed to disease eradication, has given a new impetus to the study of plant secondary metabolites as potential molluscicides, as these would be easily accessible to the afflicted population.

1.1. A HOLISTIC APPROACH IN THE CONTROL OF SCHISTOSOMIASIS

There is, more than ever before, a need for safe and cheaper molluscicides. Schistosomiasis continues to be a menace in Africa, Asia and South America. Chemotherapy and the reduction of transmission are two main tools in the control of schistosomiasis. With the introduction of praziquantel to the pharmaceutical market, there has been a shift away from transmission control to the control of severe morbidity (Ndamba, 1993). However, despite the effectiveness of praziquantel, there is a high re-infectivity rate in endemic areas even after mass treatment. Furthermore, the cost of this drug, although reduced, remains prohibitive for many control programmes in schistosomiasis endemic areas. There is clearly a need for greater commitment to schistosomiasis control. Of necessity is a holistic approach, which should include not only reducing the disease burden in infected persons, but also interfering with the life-cycle of the parasite by eliminating the snail vector.

Together with chemotherapy, molluscicides are widely considered to be an important part of schistosomiasis control that can be used at selected sites to achieve immediate results. Measures such as improved sanitation and health education are likely to take much longer to affect the disease spread and prevalence. Mollusciciding should, therefore, be of importance in schistosomiasis control.

The lack of control initiatives is largely due to the cost of molluscicides. For example, bayluscide® (niclosamide), the most potent and safest molluscicide used in some irrigation schemes in many African countries over the years, is effective but to be able to achieve best results, its application has to be done at least twice a year. This is not affordable by the local communities in areas outside the irrigation schemes, where schistosomiasis is endemic and which act as reservoirs for the disease. Thus, while reasonable control has been realized in the irrigation schemes, the surrounding areas where there is high transmission remain a continuous source of miracidia for snails in other water bodies including irrigation dams (Knudsen & Sloof, 1992; Appleton, 1985).

1.2. PLANTS AS SOURCES OF MOLLUSCICIDAL DRUGS

The potential of plant secondary metabolites for schistosomiasis control is illustrated by the well-demonstrated activity of *Phytolacca dodecandra* fruits, so far the most promising plant molluscicide which have proved effective in clearing waterways of snails (Birrie *et al.*, 1998; Ndamba, 1993; Knudsen & Sloof, 1992; Baalawy, 1972;).

The use of natural products with therapeutic properties is as ancient as human civilization and, for a long time, mineral, plant and animal products were the main sources of drugs (De Pasquale, 1984). The Industrial Revolution and subsequent development of organic chemistry resulted in a preference for synthetic products for pharmacological treatment (De Pasquale, 1984). The reasons for this were that pure compounds were easily obtained, structural modifications to produce potentially more active and safer drugs could be easily performed, and economic power of the

pharmaceutical companies was increasing. Furthermore, throughout the development of human culture, the use of natural products has had magico-religious significance, and different points of view regarding the concepts of health and disease existed within each culture (Rates, 2001). Obviously, this approach was against the new *modus operandi* of the industrialized western societies, in which drugs from natural sources were considered either an option for poorly educated or low income people, or simply as a religious superstition of no pharmacological value.

However, even if we only consider the impact of the discovery of penicillin, obtained from micro-organisms, on the development of antimicrobial therapy, the importance of natural products is clearly enormous. About 65% of the drugs prescribed worldwide today have come from plants, one hundred and twenty one such active compounds being in current use. Of 252 drugs considered as basic and essential by the World Health Organization, 11% are exclusively of plant origin, and a significant number are synthetic drugs obtained from natural precursors (Rates, 2001). Examples of important drugs obtained from plants are digoxin from *digitalis* spp., quinine and quinidine from *Cinchona* spp., vincristine and vinblastine from *Catharanthus roseus*, atropine from *Atropa belladonna*, and morphine and codeine from *Papaver somniferum*. It is estimated that 60% of antitumour and anti-infective drugs already on the pharmaceutical market or under clinical trial, are of natural origin (Yue-Zhong, 1998). The vast majority of these drugs cannot yet be synthesized commercially, and are still obtained from wild or cultivated plants. In addition, compounds such as muscarine, physostigmine, cannabinoids, yohimbine, forskolin, colchicine and phorbol esters, all obtained from plants, are important tools used in pharmacology, physiology and biochemical studies (Williamson *et al.*, 1996).

1.2.1. SELECTING A PLANT

According to the Organizacion Panamericana de la Salud (OPS) (Arias, 1999), a medicinal plant is (i) any plant used in order to relieve, prevent or cure a disease, or to alter physiological and/or pathological processes, or (ii) any plant employed as a source of drugs or their precursors. A Phytopharmaceutical preparation or a herbal medicine is any manufactured medicine obtained exclusively from plants (aerial and non-aerial parts, juices, resins and oil), either in the crude state or as a pharmaceutical formulation. A medicine is a product prepared according to legal and technical procedures that is used for the diagnosis, prevention, suppression and treatment of a disease, and has been scientifically characterized in terms of efficacy, safety and quality (WHO, 1992). A drug is a pharmacologically active compound, which is a component of a medicine, irrespective of its natural, biotechnological or synthetic origin.

The approach for drug development from plant sources depends on what the drug is aimed for. Different strategies will result in a herbal medicine or in an isolated active compound. However, apart from this consideration, the selection of a suitable plant for a pharmacological study is a very important and decisive step. There are several ways in which this can be done, including traditional use, chemical content, toxicity, randomized selection or a combination of several criteria (Soejarto, 1996; Williamson *et al.*, 1996). The most common strategy is careful observation of the use of natural resources in folk medicine in different cultures. This is known as ethnobotany or ethnopharmacology. Information on how the plant is used by an ethnic group is extremely important. The preparation procedure may give an indication of the best extraction method. The formulation used will provide information about

pharmacological activity, oral versus non-oral intake, and the doses to be tested. However, certain considerations must be taken into account when the ethnopharmacological approach of a plant selection is chosen. For instance, each ethnic group has its own concepts of health or illness, as well as different healthcare systems (Elisabetsky and Posey, 1986). The signs and symptoms should be translated, interpreted and related to western biomedical concepts, thus allowing a focused study of a particular therapeutic property.

Selection based on chemical composition uses phylogenetic or chemotaxonomic information in the search, mainly in certain genera and families, for compounds from a defined chemical class with known pharmacological activity (Gottlieb and Boria, 1997; Souza, 1996).

Another method of selecting a plant is that the investigator decides on a well-defined pharmacological activity and performs a randomized search, resulting in active species to be considered for study. The search for anti-tumour drugs is a good example of the use of this strategy. Finally, it is possible, often desirable and inevitable, to use a combination of several criteria. Furthermore, apart from the chosen strategy, searching databanks and the scientific literature is crucial in finding active and/or toxic compounds that have already been identified, and can also be used as a criterion for choosing a medicinal plant (Rates, 2001).

Quantitative considerations regarding the average yield of active compounds and the amount of starting crude plant material required for the discovery, development and launch of a new drug on the market were presented by McChesney (1995). 50kg of raw material are necessary to provide 500 mg of pure compound for bioassays,

toxicology, and *in vivo* evaluation; full pre-clinical and clinical studies can require 2 kg of pure compounds obtained from 200 tons of raw material. Therefore, the choice of a biological material to be screened for active compounds (and the subsequent development of a drug) must take into account that the exploration of natural resources should meet global and regional needs for new, efficient and safe drugs, while preserving natural diversity and the environment.

The present situation of exploitation of the world's vegetation may endanger some plant species and lead to their extinction, which means not only the loss of their interesting chemical compounds as potential sources of drugs, but also the loss of genes, which could be of use in plant improvement or in the biosynthesis of new compounds. It is, therefore, crucial to protect and promote the rational exploitation of biodiversity as a source of chemical compounds that have direct biological activity, or can be used for the rational planning of new drugs. By following this principle, a new understanding of sustainable development emerges, involving preservation of the environment while developing new drugs, especially in developing countries, which, by coincidence, have the largest natural resources on the planet (Rates, 2001). Sensible use of these resources must be based on the amounts available, ease of access, the possibility of preservation and replanting, and establishment of priorities in relation to a desirable pharmacological activity (Sharapin, 1997).

1.2.2. TOXIC ACCIDENTS WITH HERBAL REMEDIES

Phytomedicines are freely marketed and, in underdeveloped or developing countries, the use of medicinal plants is widely accepted. This can result in toxic accidents from

the use of plants as food or for therapy, or from accidental ingestion by children or animals. Toxicity can result from over dosages, or from the state of conservation of plants and the form of use.

Accidents due to mistakes of botanical identification are one cause of toxic accidents with herbal remedies. The use of a wrongly identified plant is common, as is the substitution of different plants for the same indication. An example from Brazilian folk medicine is the use of a plant called “quebra-pedra” as a diuretic, and in the treatment of gallstone problems. The correct plant is *phyllanthus nirurrii*, which is commonly confused with the *Euphorbia* genus, which is potentially toxic. Popular remedies, made without legal authorization and sold by herbalists or even prescribed by religious leaders for use in rituals, have often resulted in toxic symptoms immediately after ingestion. Plants with a high content of cardiac glycosides, such as *Nerium oleander*, *Thevetia peruviana*, *Gomphocarpus fruticosos* and *Calotropis procera*, are used as decorative plants, and have caused a number of domestic accidents involving children and animals (Gilbet *et al.*, 1997).

1.3. PLANTS THAT INTERFERE WITH CONVENTIONAL PHARMACOLOGICAL THERAPY

- (a) *Plants containing coumarinic derivatives:* These compounds can lead to haemorrhagic accidents because of their chronic use or synergistic effects with oral anticoagulants, such as dicoumarol and the sodium coumarins. Among the coumarin-rich plants widely used in folk medicine as herbal medicines and to enhance flavour are *Mykania* spp., *Melilotus officinalis* and *Dypterix odorata*.

- (b) *Plants with a high tyramine content*: Tyramine is a phenylethylamine found in yeast products, such as cheese and wine, which can be responsible for hypertensive accidents in patients treated with monoamine oxidase inhibitors. Mushrooms and higher plants such as *Portulacca* spp., *Phoradendron* spp. and *Psittacanthus* spp., are also potentially dangerous (Rates, 2001).
- (c) *Plants containing oestrogenic compounds*: Ginseng (*Panax* spp.), used worldwide as a panacea, can have important oestrogenic effects and its use in combination with steroidal drugs is not recommended. This also applies to plants such as “inhome” (*Dioscorea* spp.).
- (d) *Plants that cause irritation and allergic problems*: Allergic reactions caused by contact with plants via pollen grains, secretions or volatile substances are not uncommon. The folk literature reports many plants that cause irritation; these include all species from families such as Urticaceae (*Urtica urens*), Euphorbiaceae (*Croton* spp., *Jatropha* spp., *Cnidocolus* spp.) and Leguminosae (*Mucuna pruriens*). Sesquiterpene lactones, found in Asteraceae, cause irritation. Furthermore, plants otherwise considered harmless such as camomille (*Maricharia recutita*) and *Arnica montana*, can cause dermatitis. Allergic reactions, caused by the roots of *Pfaffa* spp., are seen in workers in the herbal medicines industries, which use this plant as a substitute for *Panax* spp. (Subiza *et al.*, 1991).
- (e) *Plants containing photosensitive compounds*: Among the well-studied photosensitive compounds are the furocoumarins, present in plants used in

folk medicine as food. Furocoumarin derivatives are found in *Psoralea corylifolia*, *Conilla glauca* (Leguminosae), *Ficus carica*, *Brosimum gaudichandii* and in several species of *Citrus* (Rutaceae) (Rates, 2001).

In developing countries, the majority of people living in the rural areas almost exclusively use traditional medicines in treating all sorts of ailments including schistosomiasis. South Africa has a great environmental and biological (genomic) diversity compared with the rest of the world (Lin *et al.*, 2002). A range of medicinal plants with anti-schistosomiasis properties has been widely used by traditional healers of different tribes in South Africa. The effectiveness of many of these traditional medicines, however, has not been scientifically evaluated.

The aim of this study was, therefore, to screen some Zulu medicinal plants for molluscicidal activity. We have also attempted to isolate the active chemical compounds from such plants.

CHAPTER TWO

1. LITERATURE REVIEW

Schistosomiasis has a long history. As early as 50 BC, Egyptian pharaohs wrote of urinary disturbances. A schistosomal ovum was found in a cirrhotic liver from a mummy dated 1200 BC. A German pathologist named, Theodore Bilharz, found the causal parasite in 1851 at Kasr El-Eini Hospital in Cairo. In 1915, Lieper, an English Scientist, discovered the intermediate snail host. The disease was originally named after Bilharz, and subsequently became known as “Bilharziasis” (Cann, 1998).

Schistosomiasis is a parasitic disease that leads to chronic ill-health. It is endemic in seventy-four developing tropical countries. Six hundred million people are at risk, and it has infected more than two hundred million people. One hundred and twenty million people are symptomatic, while twenty million others suffer severe consequences of the disease. This disease has also caused twenty thousand deaths mainly from cirrhosis. Schistosomiasis is second only to malaria in human impact among tropical diseases, and is the most prevalent parasitic disease in the world (Shekhar, 2001).

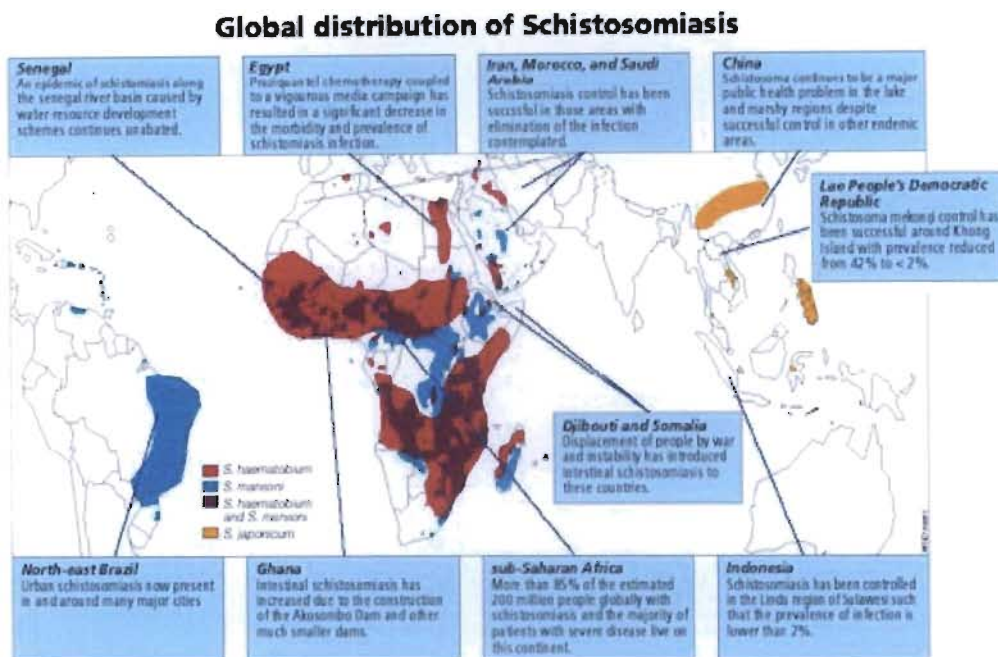
Schistosomiasis is caused by five species of flat worms, which live in fresh water in the tropics. The most common of all types is *Schistosoma mansoni*, which is customary in Africa and causes intestinal schistosomiasis. *Schistosoma japonicum* and *Schistosoma mekongi* also cause intestinal schistosomiasis but mainly in Asia and Pacific regions, Africa and the Eastern Mediterranean. *Schistosoma haematobium* causes urinary schistosomiasis (Kader, 2001; WHO, 1998a).

People are infected with schistosomiasis through contact with contaminated water. People could be infected while swimming or doing personal or domestic cleaning with water. It is also prominent in fishing practices and rice cultivation of developing countries. Due to lack of information and sanitation facilities, individuals contaminate their environment.

2.1. GEOGRAPHICAL DISTRIBUTION OF SCHISTOSOMIASIS

Schistosoma haematobium is found in 53 countries in the Middle East and Africa, including the islands of Madagascar and Mauritius. There is also an ill-defined focus of *S. haematobium* in India. With the recent introduction of *S. mansoni* to Mauritania, Senegal and Somalia, intestinal schistosomiasis is now found in 54 countries, including the Arabian peninsula, Egypt, Libya, Sudan, sub-Saharan Africa, Brazil, some Caribbean islands, Suriname and Venezuela. *S. intercalatum* has been reported from 10 countries within the rain forest belt of central Africa. *S. japonicum* is endemic in China, Indonesia and the Philippines and has been reported from Thailand. Another oriental schistosome is *S. mekongi* found in Cambodia and Laos, along the Mekong river (WHO, 1998a).

Figure 1. Geographical distribution of schistosomiasis (adapted from WHO, 1998a).



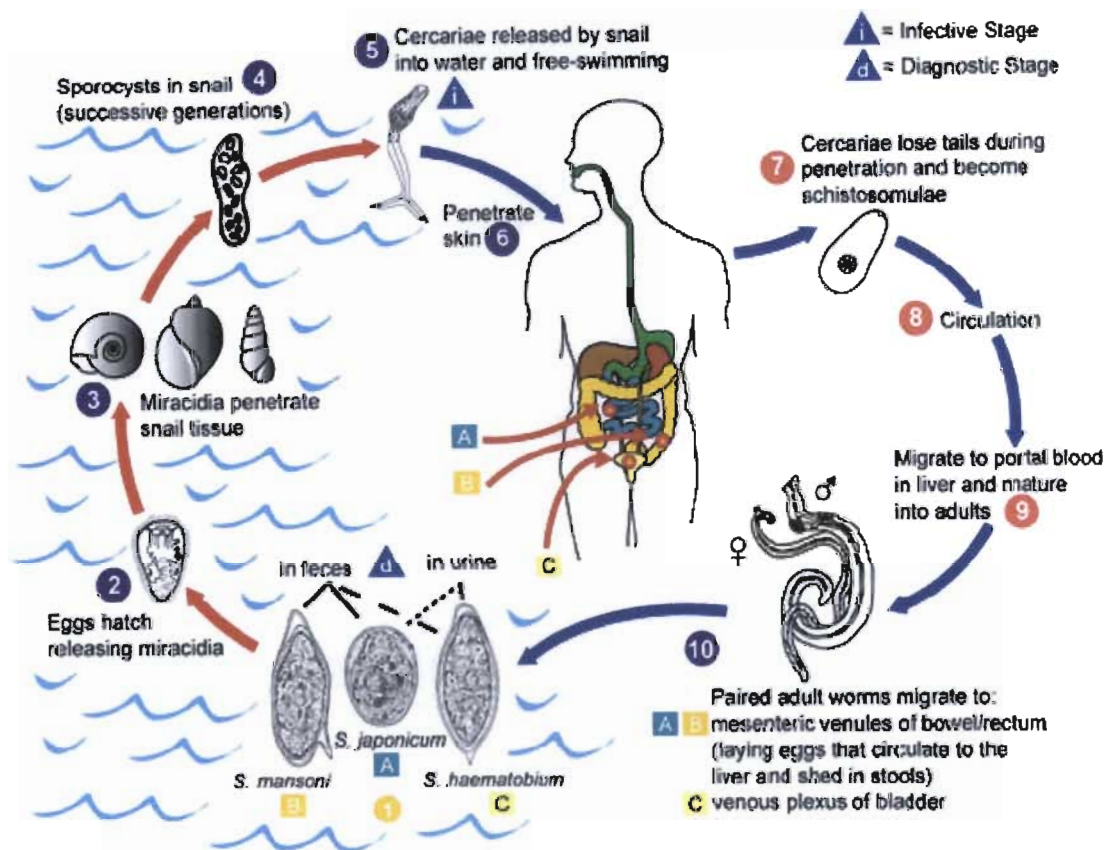
The global distribution of schistosomiasis has changed significantly in the past 50 years, with control successes achieved in Asia, the Americas, North Africa and Middle East. Schistosomiasis has been eradicated from Japan and some of the islands in the Lesser Antilles. Transmission has been stopped in Tunisia, and is very low in Morocco, the Philippines, Saudi Arabia, and Venezuela.

However, environmental changes linked to water resources development, and increasing population and population movements have led to the spread of the disease to previously low or non-endemic areas, particularly in sub-Saharan Africa (Chitsulo, 2000).

2.2. THE LIFE-CYCLE OF SCHISTOSOMIASIS

Schistosoma haematobium is mainly transmitted by *Bulinus* snails, *S. mansoni* by *Biomphalaria*, and *S. japonicum* by amphibious *Oncomelania* snails.

Figure 2. The life-cycle of schistosomes (adapted from WHO, 1998b).



The eggs hatch and release miracidia, which swim to find host snails in the fresh water. There are only a few species of snails that can act as a host, restricting this disease to tropical and subtropical areas. The stages in the snails include two generations of sporocysts, and the production of cercariae. Upon release from the snail, the infective cercariae enter the water. Here, they can survive for forty-eight hours before finding a new human host or die. Schistosomal parasites can penetrate the skin of a human host. Most of the eggs are excreted within a few weeks, but some

the skin of a human host. Most of the eggs are excreted within a few weeks, but some will stay and migrate through several tissues and stages to their residence in veins. Human contact with water is thus necessary for infections by schistosomes (WHO, 1998b).

Adult worms in humans reside in mesenteric venules in various locations, which at times seem to be specific for each species. For instance, *S. mansoni* occurs more often in superior mesenteric veins, and *S. japonicum* more frequently in the inferior mesenteric veins. However, both species can occupy either location, and they are capable of moving between locations, and as such, it is not possible to state unequivocally that one species occurs in one location. *S. haematobium* most often occurs in the venus plexus of the bladder, but it can also be found in rectal venules. The females (size 7 to 20 mm; males slightly smaller) deposit eggs in the small venules of the portal and perivesical systems. The eggs are moved progressively toward the lumen of the intestine (*S. mansoni* and *S. japonicum*) and of the bladder or ureters (*S. haematobium*), and are eliminated in faeces and urine respectively (WHO, 1998b).

2.3. SIGNS AND SYMPTOMS OF SCHISTOSOMIASIS

Within days after becoming infected, some people have a rash or an itchy skin. However, many infections are asymptomatic. Acute schistosomiasis, also known as 'Katayama fever', may occur weeks after the initial infection, especially by *S. mansoni* and *S. japonicum*. Manifestations include: fever, abdominal pains, diarrhoea, hepato-splenomegaly and eosinophilia (Cann, 1998). Symptoms are related to the

number and location of parasite eggs in the body. Occasionally, central nervous system lesions occur. Furthermore, continuous infection may cause granulomatous reactions and fibrosis in the affected organs. This may result in the symptoms that include: colonic polyposis with bloody diarrhoea (*Schistosoma mansoni* mostly); portal hypertension with haematemesis and splenomegaly, hepatic perinusoidal egg granulomas, Symmers' pipe stem periportal fibrosis, and occasional embolic egg granulomas in brain or spinal cord (*S. mansoni* and *S. japonicum*). Pathology of *S. haematobium* schistosomiasis includes: haematuria, scarring, calcification, squamous cell carcinoma, and occasional embolic egg granulomas in brain or spinal cord (WHO, 1998a).

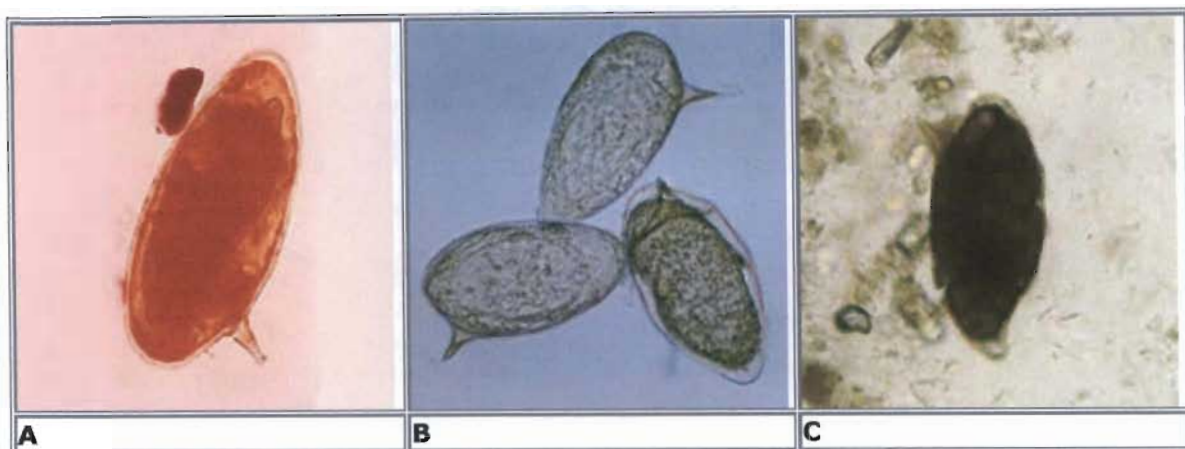
2.4. LABORATORY DIAGNOSIS

Microscopic identification of eggs in stool or urine is the most practical method for diagnosis. Stool examination should be performed when infection with *S. mansoni* and *S. japonicum* is suspected, whereas urine examination should be performed if *S. haematobium* is suspected. The examination can be performed on a simple smear. Since eggs can be passed in small amounts, their detection will be enhanced by repeated examinations. In addition, for investigational purposes, the egg output can be quantified by using the Kato-katz technique or the Ritchie technique (WHO, 1998a). Eggs can also be found in the urine in infections with *S. haematobium* and *japonicum*. Detection is enhanced by centrifugation and examination of sediment. Quantification is also possible by using filtration through a Nucleopore® membrane of a standard volume of urine, followed by egg counts on the membrane (WHO, 1998a).

2.4.1. MICROSCOPY

Schistosoma mansoni eggs are large (length 114 to 118 μm) and have a characteristic shape, with a prominent lateral spine near the posterior end. The anterior end is tapered and slightly curved. When the eggs are excreted, they contain a mature miracidium.

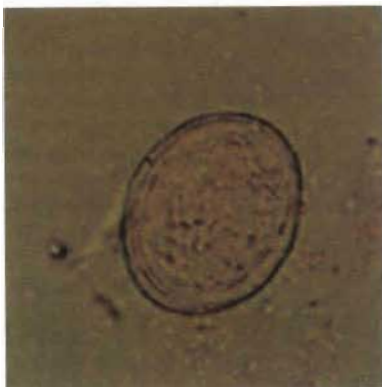
Figure 3. *Schistosoma mansoni* eggs.



- A:** *Schistosoma mansoni* egg (iodine stain).
B: *Schistosoma mansoni* eggs (wet preparation).
C: Non-viable *Schistosoma mansoni* egg.

Eggs of *Schistosoma japonicum* are typically oval or sub-spherical, and have a vestigial spine. *S. japonicum* eggs are smaller (68 to 100 μm by 45 to 80 μm) than those of the other species. Eggs of *Schistosoma haematobium* are large and have a prominent terminal spine at the posterior end. The eggs are 112 to 170 μm in length (WHO, 1998a).

Figure 4. *Schistosoma japonicum* egg.



A case history of a patient with symptoms and signs suggestive of endometriosis, who was found to have Schistosomiasis has been reported. The laparoscopic appearance was of gelatinous deposits throughout the pelvis, which were thought to be “non-pigmented” endometriosis. However, histological examination of the biopsy specimens revealed schistosomiasis (Jones *et al.*, 2003). This probably illustrates the importance of microscopy as a diagnostic tool in schistosomiasis.

2.4.2. ANTIBODY DETECTION

Antibody detection can be useful in indicating schistosomal infection in patients who have travelled to and stayed in schistosomiasis endemic areas, and in whom eggs cannot be demonstrated in faecal or urine specimens. The sensitivity and specificity vary among the many tests reported for serologic diagnosis of schistosomiasis, and are dependent on both the type of antigen preparations used (crude, purified, adult worm, egg, cercarial) and test procedure (Tsang and Williams, 1991).

2.5. PREVENTION AND CONTROL

Schistosomiasis can be prevented by avoiding swimming or wading in fresh water. Boiling the water before drinking should ensure safety of drinking water. Bath water should also be heated for at least 150°F. Most importantly, health education, good environmental sanitation and snail control through focal mollusciciding must be implemented to control and/or prevent this disease.

Collaborative studies have also identified some genetic factors contributing to the development of severe forms of malaria and schistosomiasis. In Thailand, the necrosis tumour factor (NTF)-alpha 5'-flanking region shows biallelic polymorphic sites at nucleotides- 238, -308, -857, -863, and -1031, and seven alleles have been identified in patients from Myanmar. It has been found that the TNF promoter-D allele is significantly associated with cerebral malaria in populations from Karen ($P < 0.0001$) (Bethony, 2002). In China, two major genes related to severity of liver fibrosis, one an HLA class II gene, and the other, the interleukin (IL)-13 gene, have been discovered. The frequency of the HLA- DRB5*0101 allele and that of the IL-13 promoter A/A genotype, were elevated in fibrotic patients, although the two genes are located on different chromosomes, chromosome 6p and 5q respectively. It was also found that the effects of the two susceptibility markers were synergistic rather than additive. This strongly suggests that the pathogenic Th2 response directly influences the prognosis of post-schistosomal liver fibrosis (Hirayama, 2002).

Immunity to schistosomiasis through vaccination, may be one of the strategies used to control the disease. A study was conducted to determine if the cell mediated immunity, induced by T-helper type-1 lymphocytes (Th1) response, during

Schistosomiasis mansoni has the potential to protect against infection, intensities of infections and re-infections. The egg count was followed up to 20 months among 119 individuals aged 5-22 years with different number of previous infections whose yearly levels and pattern of water contact were similar. They were classified into five groups. Delayed hypersensitivity skin tests (DHT) to adult schistosome excretory-secretory antigens (ESAg) and anti-schistosomula (ESAg) isotypes were measured on detecting re-infection. The group with a mean age of 8.6 ± 2.6 and infected less than five times showed only 6.5 percentage reduction of the egg count and low cellular and humoral responses. Th1-associated cellular (DHT) and antibody responses (IgG2, IgG3) to the five infections were significantly higher in the 13-year-olds than in 18-year age group. Th2-associated antibody responses (IgG1, IgG4, IgE) went on rising as patients allowed for age and number of infections increased over five, being significantly higher in the 19-year-olds than 14-year-olds (Abdel-Fattah *et al.*, 2001). These results imply a substantial protective role for cell mediated immunity in the pre-puberty stage and provide evidence that Th1-based vaccination strategy could work if augmented.

In recent years, cases of severe morbidity (fibrosis, ascites, haematemesis and hepatosplenomegaly) caused by *Schistosoma mansoni* infections have been increasing in Northern Senegal. The regulatory mechanisms that prevail in a minority of patients where infections lead to liver fibrosis, portal hypertension, porto-systemic collateral circulation, oesophageal varices and fatal bleeding are still unclear (Chatterjee *et al.*, 2003). In addition to distinct immunological factors that play a role in determination of morbidity, somatostatin has recently become a possible neuroimmune modulator (Weinstock & Elliott, 2000).

Somatostatin reduces fibrosis and morbidity in *schistosoma mansoni* infected animals (Mansy *et al.*, 1998), inhibits collagen formation by activated hepatic stellate cells, (which are responsible for hepatic fibrosis) in *in vitro* cultures (Reynaert *et al.*, 2001), and reduces variceal bleeding and portal hypertension in cirrhotic patients (Avgerinos *et al.*, 1997). Pathogenesis related to schistosomiasis may be regulated by inherent host-related factors (Chatterjee *et al.*, 2001), one of them being neuro-endocrine interactions. A study was conducted to delineate the role of somatostatin in *S. mansoni* caused pathogenesis, by studying host levels of somatostatin in the peripheral blood of uninfected and *S. mansoni* infected individuals. Subjects from the district dispensary at Richard Toll, in the Medical Region of Saint-Louis, Senegal, infected with *S. mansoni* and suffering from severe morbidity were selected. A separate group consisted of individuals resident in the same region but uninfected at the time of study. Significantly lower somatostatin levels were detected in severe morbidity patients, compared with the exposed but uninfected subjects. In patients with schistosomiasis, physiological levels of somatostatin may determine disposition of particular individuals towards severe morbidity, as opposed to others.

Whereas the antifibrotic and antimorbidity effects of somatostatin explain the inhibitory role of this neuropeptide in determining disease status, the reverse cannot be justified. Host pathology can thus be alleviated by the therapeutic ability to somatostatin to treat bleeding oesophageal varices, reduce portal pressure and prevent progression to severe fibrosis (Chatterjee *et al.*, 2003).

Somatostatin is a neuropeptide hormone for which there is emerging interest in schistosomiasis (Chatterjee *et al.*, 2001). The measurement of somatostatin levels in humans infected with *S. mansoni* may provide relevant information on how host-parasite interactions may be disrupted by circulating neuropeptide levels. Research

into the physiological somatostatin levels in such subjects could give insight into the possible pre-disposition of particular individuals towards severe morbidity as opposed to others, and could well explain the phenomenon why only a small percentage of *S. mansoni* infected individuals develop Symmers pipe-stem fibrosis (Chatterjee *et al.*, 2003).

2.6. TREATMENT OF SCHISTOSOMIASIS

2.6.1. SYNTHETIC DRUG THERAPY

Schistosomiasis, a grave and debilitating disease of socio-economic importance, is increasing in incidence despite efforts to control and contain the disease in all the endemic areas. While a multifaceted method of control using health education, sanitation and snail control has been used, chemotherapy and chemoprophylaxis play the most important role in preventing transmission of the disease (Shekhar, 2001).

Chemotherapy using praziquantel has been the cornerstone of schistosomiasis control for more than twenty years. Praziquantel is effective in the treatment of schistosome infections of all species (Katzung, 1998). The drug increases cell membrane permeability to calcium, resulting in marked contraction, followed by paralysis of worm musculature. Vacuolation and disintegration of the tegmen occur and parasite death follows.

Oxamniquine is a drug of choice for the treatment of *S. mansoni* infections. It is active against both mature and immature stages of *S. mansoni* but does not appear to be cercaricidal. Although its exact mechanism of action is not known, the drug may act by binding to DNA. Contraction and paralysis of the worms result in detachment

from terminal venules in the mesentery and shift to the liver, where they die. Surviving females return to mesenteric vessels but cease to lay eggs (Katzung, 1998).

Metrifonate is another safe, low-cost alternative drug for the treatment of *Schistosoma haematobium* infections. It is not active against *S. mansoni* and *S. japonicum*. The mode of action against both the mature and immature stages of *S. haematobium* is not established, but is thought to be related to cholinesterase inhibition. This inhibition temporarily paralyses the adult worms, resulting in the shift from the bladder venous plexus to small arterioles of the lungs, where they are trapped, encased and die (Katzung, 1998).

2.6.1.1. EFFECTS OF SCHISTOSOMAL INFECTION ON HEPATIC DRUG METABOLISING ENZYMES.

The metabolic fate of drugs is dependent, to a large extent, on the expression and activity of the microsomal drug metabolising enzymes (Jakoby & Ziegler, 1990). These enzymes include the microsomal cytochrome P-450 dependent monooxygenase system, and the uridine diphosphate glucuronosyl transferases as well as other cytosolic enzymes such as glutathione s-transferases. Several studies have shown that infection with *S. mansoni* results in altered activities of a number of drug metabolising enzymes (Hasler & Naik, 1998).

The available experimental evidence indicates that the altered drug metabolising enzyme activity is observed only in the presence of liver disease, which is observed consequent to granuloma formation. Mice harbouring a bisexual infection have decreased concentrations of cytochrome P-450 and NADPH cytochrome c-reductase levels, while animals having only male or female worms do not. Alterations are not

observed during parasite development, although they are observed after egg deposition by parasites, and the onset of liver disease. Furthermore, the alterations have been shown to be dependent on the degree of infection as judged by the number of eggs deposited or worm load (Hasler & Naik, 1998).

These alterations in enzyme activity caused by infection are reversible. Treatment with schistosomicides eliminates worms and also results in the gradual restoration of drug metabolising enzyme activity (Cha & Beuding, 1978). Interestingly, treatment of infected animals with classical inducers of drug metabolism, e.g. phenobarbital and 3 methylchloranthrene, is also able to restore the activities to normal *in vitro* and *in vivo* (Hasler & Naik, 1998).

While these studies suggest that infection with *S. mansoni* does indeed cause perturbations in hepatic drug metabolising enzyme activity, the actual causes of the alterations are not known. Preliminary evidence indicates, however, that alterations may be due to an oxidative stress. Such a stress would be caused by the egg granulomas which have been known to release reactive oxygen species and which are likely to cause membrane damage (Hasler & Naik, 1998). It is also possible that certain excretory products of worms released into the host circulation may affect the activity of drug metabolising enzymes in the liver (Lightwlers & Rickard, 1988).

Alterations in the metabolism of therapeutic agents could have potentially deleterous effects in infected humans. Delayed metabolism would cause an accumulation of drug, especially when prescribed in multiple doses, or for prolonged treatments.

2.6.2. HERBAL TREATMENT

While effective and safe drugs for mass chemotherapy are being developed, the problem of therapeutic failure and drug resistance is being reported from certain developing countries. Under these circumstances, alternative drugs must be resorted to.

Table 1: Some plants used in the treatment of schistosomiasis (Hutchings *et al.*, 1996)

Family, genus, species and (Zulu name)	Parts Used	Medicinal Uses	Chemical Constituents
Proteaceae <i>Faurea saligna</i> Harv. (isiqalaba)	Leaves, stem bark, roots	Schistosomiasis, menstrual pains, pneumonia	Tannins
Olacaceae <i>Ximenia Americana</i> L. var <i>Americana</i> (umkholotshwana)	Fruits, roots, stem bark	Schistosomiasis, headaches, diarrhoea, ulcers	Hydrocyanic acid, tannins
Olacaceae <i>Ximenia caffra</i> Sond. (amathunduluka)	Leaves, roots	Diarrhoea, fevers, leprosy, syphilis, bilharziasis	Hydrocyanic acid, tannins
Phytolaccaceae <i>Phytolacca dodecandra</i> L'Herit (ingubivumile)	Roots, leaves, fruits	Wounds, snake bite, inflammations, syphilis, schistosomiasis	Endod, oleanolic acid
Menispermaceae <i>Cissampelos mucronata</i> A. Rich (umbombo)	Roots, leaves	Malaria, rheumatic pains, schistosomiasis, syphilis, diarrhoea	Saponins, tannin pelosine
Fabaceae <i>Afzelia quanzensis</i> , Welw (umdlavusa)	Stem-bark, fruits, roots	Schistosomiasis, snake bite	Tannin
Papilionaceae <i>Abrus precatorius</i> L. subsp. <i>Africanus</i> verd. (umkhokha)	Roots, leaves, fruits	Asthma, malaria, contraception, schistosomiasis	Choline, trigoline, glucan
Fabaceae <i>Pterocarpus angolensis</i> DC. (umbilo)	Stem-bark, roots, leaves	Asthma, infertility, tuberculosis, schistosomiasis	Muningin, tannin
Balanitaceae <i>Balanites maughamii</i> Sprague (ipamu)	Stem-bark, roots	Schistosomiasis	Sapogenins, yamogen, balanits

Euphorbiaceae <i>Flueggea virosa</i> (oxb.ex wild) Voigt (umyaweyawe)	Roots, fruits, leaves	Malaria, diabetes, pneumonia, contraception, schistosomiasis	Norsecurine, nordenine
Euphorbiaceae <i>Antidesma venosum</i> E. Mey.ex Tull (isingowane)	Leaves, roots	Dysmenorrhoea, malaria, gnorrhoea, schistosomiasis	Saponin, tannic acid
Anacardiaceae <i>Sclerocarya birrea</i> (umganu)	Stem-bark, fruits, roots	Malaria, diarrhoea, schistosomiasis	Tannin, alkaloids, flavonoids, procyanidin
Anacardiaceae <i>Rhus queinzii</i> Sond (inhlokoshiyana)	Roots	Eye complaints, schistosomiasis	Malic acid, inositol, nanonic acid
Celastraceae <i>Maytenus senegalensis</i> (Lam) Excell (ubuhlangwe)	Roots, leaves	Snake bite, epilepsy, infertility, schistosomiasis	Tannin
Combretaceae <i>Terminalia sericea</i> Burch.ex DC. (amangwe)	Stem-bark, roots	Diabetes, schistosomiasis, tuberculosis	Tannins, triterpenoids
Ebenaceae <i>Euclea natalensis</i> A. DC (ichitamuzza)	Root, stem-bark	Venereal diseases, schistosomiasis	Lupeol, betulin
Periplocaceae <i>Mondia whitei</i> . Skeels (umondi)	Roots	Abdominal pain, constipation, schistosomiasis	Vanilla, glucosides, resin
Asteraceae <i>Berkheya speciosa</i> DC. Hoffm (ikhakhasanaomkhulu)	Roots	Abdominal disorders, schistosomiasis	Terpenoids, thiophene
Fabaceae <i>Tephrosia vogelli</i> Hook (ilozane)	Roots, fruits	Tuberculosis, schistosomiasis	Tephrosin, deguelin
Meliaceae <i>Trichisia emetcia</i> Vahl. (ixolo)	Bark, leaves	Leprosy, stomach complaints, malaria, schistosomiasis	Tannin, resin
Lecythidaceae <i>Barringtonia racemosa</i> (L.) Roxb. (iboqo)	Roots, bark, fruits	Malaria, stomach ache, skin diseases, othlamia	Saponins, tannin, barringtogenol, barringtonic acid
Euphorbiaceae <i>Jatropha curcas</i>	Fruits	Schistosomiasis, purgative	Phorbol esters

Despite the effectiveness of praziquantel, there is a high re-infectivity rate in endemic areas, even after mass treatment. Repeated treatment is thus necessary, although it has not been established what would be a suitable interval between such treatments (Bezerra, 2002; Rates, 2001). Control of vector snails is, therefore, relevant to the control of schistosomiasis. At present, only niclosamide (bayluscide®) is widely used in control programmes (Alam *et al.*, 2001; Diallo *et al.*, 2001; Perrett and Whitfield, 1996). On the other hand, molluscicidal activity has been observed in numerous plant families (Liu *et al.*, 1997) and attributed to several major classes of natural products including saponins, other terpenes and alkaloids (Mott, 1987; Marston & Hostettmann, 1985;). However, no plant molluscicide has so far gained wide application, and only a few plants have been extensively studied (Liu *et al.*, 1997; Singh, 1996; Kloos & McCullough, 1982;). In this study, the molluscicidal potential of some Zulu medicinal plants, which are also being used for other purposes in KwaZulu-Natal Province of South Africa, is investigated. The Plants include:

2.7. *SCLEROCARYA BIRREA* (FAMILY: ANACARDIACEAE)

2.7.1. DISTRIBUTION

Sclerocarya birrea ('marula tree') is a medium sized, deciduous tree of up to 15 meters in height. The tree is widely distributed throughout the African continent. In southern Africa, only the subspecies *caffra* is found. It is found in bushveld, woodland, on forest margins at low altitudes. It occurs from Natal south coast northwards to Transvaal, Mozambique, Swaziland and Tropical Africa (Moll, 1992).

2.7.2. BOTANICAL DESCRIPTION

The main stem is straight (up to 0.6 meters in diameter), branching high up, with spreading, rounded crown. The rough bark is flaky, with mottled appearance due to contrasting grey and pale brown patches. The leaves are divided into 10 or more pairs of leaflets, each about 60 mm long, dark green above, much paler below, with the tip abruptly narrowing to a sharp point. New leaves are coppery, turning shiny bright green. The leaves turn yellow before falling (Palgrave, 1977).

Figure 5. *Sclerocarya birrea* tree.



Sclerocarya birrea

The flowers are deep pink and white with dark reddish pink buds. Male and female flowers occur separately, usually but not always, on separate trees. Male flowers appear in long drooping sprays (50-80 mm long); female flowers singly or in small groups on 30 mm stalk, on bare tree or amongst new leaves in September to November. Large, rounded (up to 40 mm diameter), smooth, with thick, pale green

November. Large, rounded (up to 40 mm diameter), smooth, with thick, pale green skin, white flesh and a large, woody stone with 2-3 seeds are borne in profusion in late southern Africa summer to mid winter (Pooley, 1993). Fruits drop from the tree when still green, ripening pale-yellow on the ground. The smell of ripening and rotting fruit can be overpowering.

2.7.3. GENERAL USES

These trees are never cut down when clearing for fields because of the valuable food and shade they provide. The fruits are much sought after for their delicious pulp, high vitamin C content and edible nuts (Burgar *et al.*, 1987). The woody stones are laboriously cracked open to collect the nut-like kernels, which are small, very tasty (like walnuts) and highly nutritious. They are carefully stored, eaten raw, or cooked with maize meal. Archeological sites indicate that they have been used since earliest times (Pooley, 1993). In Botswana, a study was conducted to check the nutritive value of seeds of *S. birrea* among other plants. It was found that the seeds had adequate quantities of phosphorus, calcium, magnesium, potassium, iron and copper to meet requirements for beef, sheep and goat production. The content of sodium, manganese and zinc were, however, below recommended levels required for growth and productivity. The study suggests that these seeds serve as potential nutrient sources for grazing animals on the ranges of Botswana (Aganga & Mosase, 2001).

It has also become a commercial fruit crop in recent years, the fruit pulp being used to brew a refreshing and intoxicating drink, manufactured commercially in the Transvaal. A delicious jelly preserve can also be made from the fruit juice. The bark besides its popular medicinal uses, also provides a light brown dye used in basket ware. A number of butterflies and moths breed on this tree. Large caterpillars (larvae)

are collected, roasted and eaten, as are the cerambycid wood-boring beetle larvae (Pooley, 1993).

2.7.4. USE IN TRADITIONAL MEDICINE

In South Africa, diarrhoea, dysentery and unspecified stomach problems are treated with the bark (Galvez *et al.*, 1993; Galvez *et al.*, 1991), which is believed to be of value in combating fever and in the treatment of malaria. A study was conducted to investigate whether the ethnobotanical use of 'marula' against bacteria-related diseases by indigenous cultures in Africa, can be validated by laboratory studies. The acetone extracts of the stem-bark and leaves were used against *Pseudomonas aeruginosa*, *E. coli*, *Enterococcus faecalis*. All extracts were active with MIC values of 0.15 to 3 mg/ml. Based on the MIC values, the inner bark tends to be most potent followed by outer bark, then leaves. However, the differences were not statistically significant (Eloff, 2001).

It is also used as a general tonic. Chewing the fresh leaves and swallowing the astringent juice helps with indigestion. Elsewhere in Africa, the main use is in the treatment of diabetes. Decoctions of the bark or roots are taken orally or as enemas. Furthermore, leaf infusions or decoctions of the plant are drunk for diabetes (Van Wyk *et al.*, 1997).

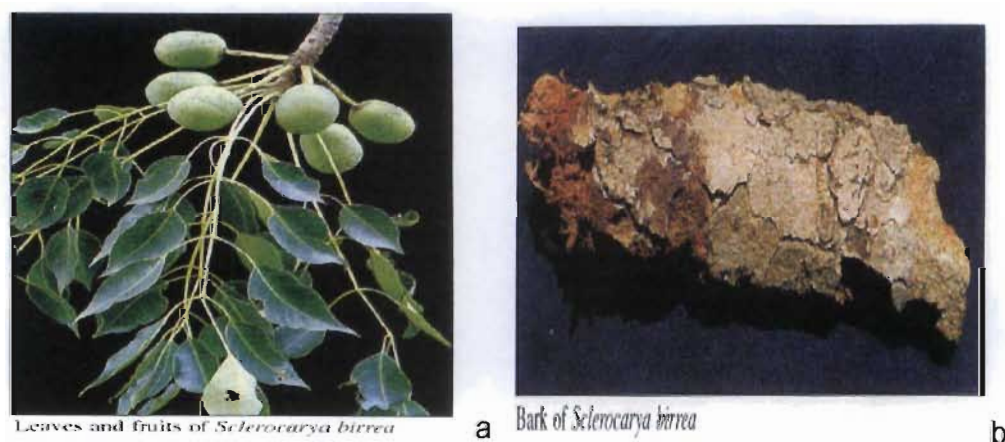
Sclerocarya birrea is a plant used widely in Africa to treat many ailments. The effects of its leaf extracts (crude decoction, aqueous, ethanolic and chloroformic) were investigated on calcium signaling in rat cultured skeletal muscle cells (Belemtougri,

2001). The results showed that different extracts of the leaf have significant antagonistic effect on caffeine-induced calcium release from sarcoplasmic reticulum. Crude decoction was the most active followed by ethanolic, aqueous, and chloroformic extracts in dose-dependent manner and can partly justify the use of the plant in traditional medicine.

2.7.5. PLANT PARTS USED

The leaves, stem-bark and roots are normally used for medicinal purposes.

Figure 6. *S. birrea* leaves and fruits (a) and the stem-bark (b).



2.7.6. CHEMICAL CONSTITUENTS AND THEIR BIOLOGICAL ACTIVITY

The bark contains procyanidins. The plant also contains gallotannins, flavonoids and catechins, but few details are available. In one study, (-)-Epicatechin-3-galloyl ester was isolated from the stem bark. The compound has secretagogue activity (Galvez *et al.*, 1992).

The bark has an astringent taste and antidiarrhoeal effects have been experimentally linked to procyanidins. There are also claims that the leaves have hypoglycaemic effects.

In another study, the physico-chemical composition and characterization of *Sclerocarya birrea* seed and seed oil was done and was found to contain 11.0% crude oil, 17.2% carbohydrate, 36.70% crude protein, 3.4% fibre and 0.9% crude saponins. The fatty acids distribution in the seed oil was obtained by fractionating the volatized fatty acid by GC-MS. The oil is made up of nine fatty acids, of which palmitic, stearic and arachidonic acids are the most dominant (Ogbobe, 1992).

2.8. *PSIDIUM GUAJAVA* (FAMILY: MYRTACEAE)

2.8.1. DISTRIBUTION

Guava occurs naturally in central America, but has become naturalized in many parts of the world, including Africa. In South Africa, it is found as a weed in the warm subtropical areas of KwaZulu-Natal, Mpumalanga and the Northern (Limpopo) Provinces.

2.8.2. BOTANICAL DESCRIPTION

Guava is a shrub or a small tree, usually not more than four meters in height. The bark peels off in flakes, revealing the characteristically smooth trunk. The large leaves

are formed opposite each other in pairs, with prominent veins, particularly on the lower side (Moll, 1992; Palgrove, 1977).

Small white flowers of about 25 mm in diameter, with numerous stamens, are produced in early summer, followed by rounded or pear-shaped yellow, many seeded fruit.

Figure 7. *Psidium guajava* tree.



Psidium guajava

2.8.3. GENERAL USES

The rounded or pear-shaped yellow, many-seeded guava fruits are an important commercial crop, due to their delicious taste and high vitamin C content.

2.8.4. USE IN TRADITIONAL MEDICINE

Guava leaves are commonly used in South Africa as a remedy for diarrhoea. The leaves are also used for other ailments, including diabetes, fever, cough, ulcers, boils and wounds (Jaiarj *et al.*, 1999; Tona *et al.*, 1999). The main ethnotherapeutic use of *P.guajava* in Africa is said to be for malaria. Leaf infusions are used in the Cape for diabetes (Roman-Romas *et al.*, 1995).

Crushed leaves are boiled in water and the infusion is either taken orally as tea or as an enema. For severe diarrhoea, an infusion of one crushed leaf in a litre of water is used (Van Wyk *et al.* 1997).

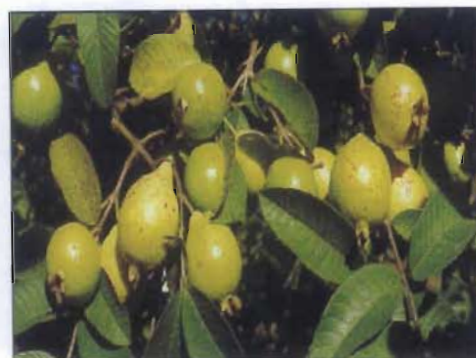
2.8.5. PLANT PARTS USED

The leaves are mainly used, but sometimes the unripe fruits, stem-bark or roots are also used.

Figure 8. *Psidium guajava* dried leaves (a) and fruits (b) .



Dried leaves of *Psidium guajava*



Fruits of *Psidium guajava*

a

b

2.8.6. CHEMICAL CONSTITUENTS AND THEIR BIOLOGICAL ACTIVITY

Numerous tannins and other phenolic compounds have been identified from *P. guajava*, of which amritoside is of particular importance. Amritoside is a glycoside (gentiobioside) of ellagic acid. Another biologically interesting compound in the plant is guijaverin, a glycoside (arabinopyroside) of quercetin. The leaves also contain

(gentiobioside) of ellagic acid. Another biologically interesting compound in the plant is guijaverin, a glycoside (arabinopyroside) of quercetin. The leaves also contain essential oils and triterpenoids such as oleanolic acid and ursolic acid. From the methanolic extract of the defatted leaves of *Psidium guajava*, a triterpene acid “psidiolic acid” has been isolated (Osman *et al.*, 1974). The psidiolic acid has been reported as a mixture of four acids, oleanolic acid, ursolic, maslinic acids together with guaijavolic acid.

Ellagic acid is a known intestinal astringent and haemostatic, which explains the therapeutic value of the plant against diarrhoea and dysentery. The tannins are generally of value because of their vasoconstricting effects and their ability to form a protective layer on the skin and mucosae. These effects, together with proven antibacterial and antifungal activity, result in effective treatment of both internal and external infections.

Quercetin (and its glycosides) undoubtedly also contributes to the efficacy of the plant, because quercetin is a known anti-oxidant with anticarcinogenic, anti-HIV and antibiotic effects. The traditional herbal remedy from *P. guajava* leaves has been medicinally proposed in Mexico as effective treatment for acute diarrhoea. A methanolic leaf extract was subjected to a bioassay-guided isolation of spasmolytic constituents. A trace of quercetin aglycone together with five glycosides was isolated from this active fraction. Biological activity of each compound was studied in the same bioassays. Results obtained suggest that the spasmolytic activity of *Psidium guajava* leaf remedy is largely due to the aglycone quercetin, present in the leaf and in the extract mainly in the form of five flavonoids, and whose effect is produced when these products are hydrolysed by gastrointestinal fluid (Loyoza *et al.*, 1994).

Although hypoglycemic effects have not been much documented, a study was conducted in Taiwan to determine the hypoglycemic effect of *Psidium guajava* in mice and human subjects. According to the folklore in Chinese Medicine, guava was useful in the treatment of diabetes mellitus (Cheng & Yang, 1983). In this study, acute intraperitoneal treatment with 1g/kg guava juice produced a marked hypoglycemic action in normal and alloxan-treated diabetic mice. Although effective duration of guava was more transient and it is less potent than chlorpropamide and metformin, blood glucose lowering effect of guava also can be obtained by oral administration in maturity-onset diabetics. Thus, it is suggested that guava may be employed to improve and/prevent diabetes mellitus (Cheng & Yang, 1983).

A study was also conducted to determine the effect of *Psidium guajava* leaves on some aspects of the central nervous system in mice. The leaves were extracted in hexane, ethyl acetate and methanol. The three extractives exhibited mostly dose-dependent antinociceptive effects in chemical and thermal tests of analgesia. The extracts also produced dose-dependent prolongation of pentobarbitone-induced sleeping time. However, they had variable and mostly non-significant effects on locomotor coordination, locomotor activity or exploration. In the pharmacological tests used, the ethyl acetate extract seemed to be the most active, followed by the hexane and the methanol extracts (Shaheen *et al.*, 2000).

Studies were also carried out on the suppression of both exploratory and spontaneous locomotor activities in the mouse by a non-polar fraction from methanol extract of the dried leaves of *P. guajava*. Shortly after intraperitoneal administration of this fraction, typical narcotic-like effects were observed, including catalepsy, analgesia, Straub tail,

shallow respiratory movements and exophthalmos. The duration of activity was dose-dependent and, for a dose of 13.2 mg/kg given i.p., it was found to be more than 6 hours. Qualitatively, similar results on exploratory activity were obtained when the extract was administered orally. A flavonoid compound was speculated to account for these results (Re *et al.*, 1999; Lutterodt & Maleque, 1988).

2.9. LEONOTIS LEONURUS (FAMILY: LAMIACEAE)

2.9.1. DISTRIBUTION

Leonotis leonurus has a wide natural distribution over large parts of South Africa, and has become a popular garden plant.

2.9.2. BOTANICAL DESCRIPTION

Leonotis leonurus is a shrub of two-to-five meters in height, with a thick, woody base and pale brown branches. All parts of the plant have a strong smell. The leaves are opposite each other on the stems, long and narrow, toothed in the upper half and distinctly hairy. Bright orange, tubular flowers are borne in characteristic rounded groups, which are neatly arranged along the branch ends. The hairy flowers resemble lion's ears, hence the name "leonurus" (which means lion's ears) (Van Wyk *et al.*, 1997).

Figure 9. *Leonotis leonurus* aerial parts.



2.9.3. GENERAL USES

Early reports claim that Nama people of South Africa smoked the leaves and used the powdered leaf to make small cakes, which were then chewed or eaten.

2.9.4. USE IN TRADITIONAL MEDICINE

Numerous traditional uses have been recorded (Hutchings *et al.*, 1996; Forbes, 1986; Smith, 1966). There is doubt about early reports of the plant being smoked as a substitute for dagga, because it is mildly narcotic (Watt, 1967). However, it has been smoked for relief of epilepsy. The leaves or roots are widely used as a remedy for snakebite and also to treat other bites and stings. Externally, decoctions have been applied to treat boils, eczema, skin diseases, itching and muscular cramps. Internally, decoctions are used for coughs, colds and influenza, as well as bronchitis, high blood

pressure and headaches. Leaf infusions have also been used for asthma and viral hepatitis.

2.9.5. PLANT PARTS USED

The leaves and stems are mainly used, but sometimes also the roots may be used.

Figure 10. *Leonotis leonurus* flowers(a) and dried aerial parts (b).



2.9.6. CHEMICAL CONSTITUENTS AND THEIR BIOLOGICAL ACTIVITY

Leonotis species contain several unusual diterpenoids (labdane type lactones). A typical example is marrubiin, which has been isolated from *L. leonurus*. There is evidence that premarrubiin actually occurs in the plant, and that marrubiin may be an artefact derivative from premarrubiin.

It is interesting to note that marrubiin is the main diterpenoid lactone in white horehound (*Marrubium vulgare*). The traditional European phytomedicine is used for the symptomatic treatment of coughs in acute bronchitis. The actual pharmacological effect is not known (Van Wyk *et al.*, 1997).

2.10. *EKEBERGIA CAPENSIS* (FAMILY: MELIACEAE)

2.10.1 DISTRIBUTION

This is a well-known and widely distributed species, which is never very plentiful. It occurs from Ethiopia and the Sudan in the north to as far south as South Africa. In this country, its distribution is much like that of all tropical plants, which is, through the northern and the north-eastern Transvaal to Natal, except that it occurs even further south, i.e. to the Cape Midlands and the southern Cape.

It is found throughout the Kruger National Park, and is not bound to a specific soil type. Almost invariably, however, it grows close to perennial water. In spite of its general distribution, prolific seed production and the effective means of dispersal offered by the waters, it is still a rare species. Because it grows so quickly and is one of the few big trees in South Africa, specimens have been planted on a large scale in all rest camps (Van Wyk, 1972).

2.10.2. BOTANICAL DESCRIPTION

It is a medium to large tree, growing up to about 18 meters in height with a spreading, dense crown of pendant branches. Stems may become about 90 cm in diameter, and are usually not very straight or tall, and in old trees are full of dents and grooves. The tree is usually evergreen, but away from water, most or all leaves turn yellow, dark-red or red-brown in autumn, and are shed progressively throughout the winter.

The ends of the twigs are rather thick, glabrous, green or pale brown and covered with distinct small, brown lenticels. Older branches are grey-brown with large and conspicuous leaf scars (Van Wyk, 1972).

Figure 11. *Ekebergia capensis* tree.



Ekebergia capensis

The particularly large leaves (up to 30 cm in length) are set spirally on the ends of old and new twigs and branches. The species shows great variations in certain morphological characteristics. The leaves are imparipinnate and usually composed of five pairs of lateral leaflets and single terminal one. Occasionally, there are seven pairs of leaflets and one tree was found on which not a single leaf had more than three pairs. All leaflets are more or less pendant, medium-thick, slightly brittle, moderately hard, glabrous, shiny and dark green above, dull and pale green underneath. The small, white, stellate florets are borne in long (up to 17 cm), sparse, branched racemes

at the bases of new twigs in the axils of the lowest pair of new leaves. The flowers appear just before or at the same time as the new leaves in October/November.

The fruits are borne in pedant clusters, on long, yellow-green stalks. They also are reminiscent of the exotic seringa. Most of the fruits are globose. Sometimes they may be drop-shaped or tapered at the bases and/or compressed at the apices so that they appear to be pear-shaped. They become up to 2 cm in diameter, are pale green in the juvenile stage and become attractively bright red when ripe, glabrous, smooth and glossy. A soft, thin exocarp encloses a white, slightly sticky, soft mush, which contains two or four seeds. Each seed is encased in a thin, firm, hard membrane. The seeds are bilobate, oblong, slightly curved so that they are almost bean-shaped, and are enclosed in a soft, thin, pale brown seed coat. Ripe fruits are found in February/March (Pooley, 1993).

2.10.3. GENERAL USES

The timber from *E. capensis* is suitable for the manufacture of all kinds of products, including furniture. Without treatment, however, it is not durable. The leaves are used for fodder in times of drought. Provided enough water is supplied, it is one of the fastest growing indigenous trees. For this reason, the species is particularly suitable for use as a decorative or shade tree (Van Wyk *et al.*, 1997).

2.10.4. USE IN TRADITIONAL MEDICINE

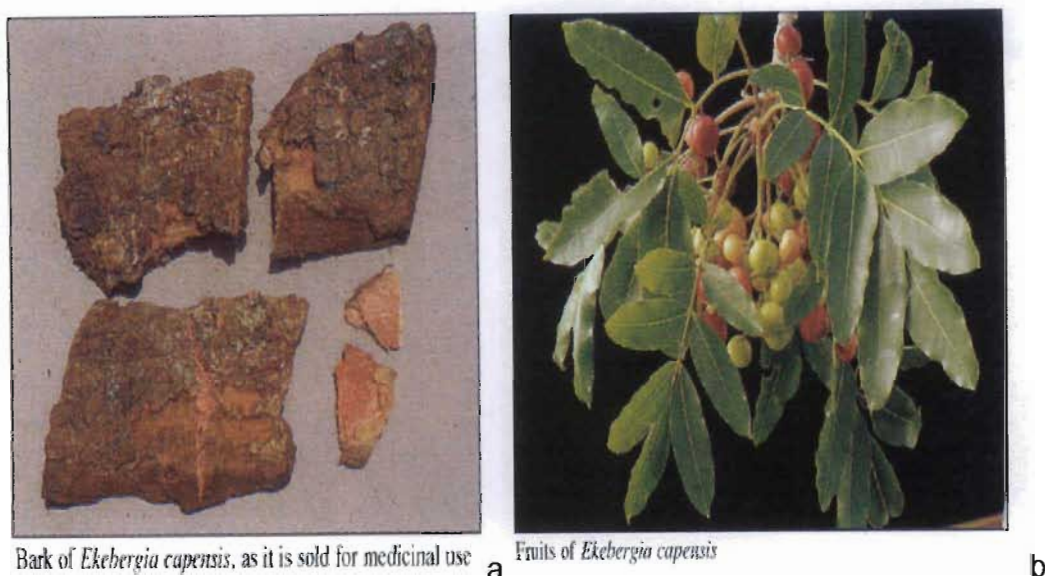
The bark is used as an emetic and as a remedy for dysentery and heartburn. An infusion of powdered bark is, sometimes mixed with flour, and is applied externally to abscesses, boils and acne. It is also used for tanning. The roots are also used for

chronic coughs, dysentery, acute gastritis, headaches, scabies and some skin diseases. A decoction of the leaves may be taken as a vermifuge (Van Wyk et al., 1997; Hutchings *et al.*, 1996).

2.10.5. PLANT PARTS USED

The stem-bark is mainly used, but sometimes the roots and the leaves are also used.

Figure 12. *Ekerbergia capensis* stem-bark (a) and fruits (b).



2.10.6. CHEMICAL CONSTITUENTS AND THEIR BIOLOGICAL ACTIVITY

The chemical compounds of *Ekerbergia* species are poorly known. Seed of *E. capensis* contain a limonoid-ekerbergin as the major constituent (Taylor, 1981). However, no limonoids were found in the bark or timber. The medicinal value is, therefore, unlikely to be due to these compounds. Limonoids are insect antifeedants and have been used to treat intestinal parasites.

2.11. *BARRINGTONIA RACEMOSA* (FAMILY: LECYTHIDACEAE)

2.11.1. DISTRIBUTION

There are about 39 species of *Barringtonia*, most of which occur in the Malaysian region, with outliers in Africa and northern Australia. *B. racemosa*, which is the most widely distributed species, occurs in the warmer areas bordering the Indian Ocean. It is very common in Natal, and a few trees are found in Port Elizabeth of South Africa (Van Wyk, 1972).

2.11.2. BOTANICAL DESCRIPTION

The tree is medium-sized, growing up to about 10 m in height, but usually smaller. The large leaves, which are produced in clusters at the end of the branches, are green or yellowish-red to bronze in colour, and have a pleasing appearance. Very conspicuous are the long, pendulous racemes, 50-75 cm long, arising from the wood or from the centre of the leaf crown at the end of the branch.

Figure 13. *Barringtonia racemosa* tree.



The flowers are large, and showy with numerous long pink and white filaments and a red style protruding from the centre. When the petals, together with the ring of filaments, have fallen off, the fleshy fruit develops to the size of a guava, and is green and/or red in colour. Flowering occurs twice a year, in June to September and again in January to April, and the flowers have a penetrating, somewhat nauseating smell in the morning (Van Wyk, 1972).

2.11.3. GENERAL USES

The bark and roots are used by Africans for tanning, and as fish poison. It is also recorded that young leaves are eaten as a salad. The wood is white and is of no value. The trees also make quite acceptable garden subjects in moist to wet, frost-free places.

In East Africa, the bark is used as tying material. The young leaf, after removal of the bitterness by soaking in limewater, is eaten as a vegetable (Van Wyk, 1972).

2.11.4. USE IN TRADITIONAL MEDICINE

Barringtonia species are reported to have insecticidal properties, which, although not comparable with those of nicotine, might be useful against thrip and aphid. The seed is used in Bengal as an insecticide. The root and the bark have been used for the relief of stomachache and in Netherlands, West Indies and India, for skin diseases. The fruit juice is applied to eczema in India. In Minahasa in the Netherlands Indies, the seed has been used, administered with homicidal intent, and coconut is eaten as an antidote. The seed has been used as an ophthalmic remedy, and in Madagascar, the seed is used as a vermifuge. It yields fixed oil and a saponin.

Ethnomedical survey has shown that the seeds of *Barringtonia racemosa* Roxb are traditionally used in certain villages of Kerala (India) to treat cancer-like diseases (Jose *et al.*, 2002). The seed extracts were tested for their antitumour activity and toxicity. Intraperitoneal (i.p.) daily administration of 50% methanol extract of this seed to mice challenged with 1 million Dalton's Lymphoma Ascitic (DLA) cells resulted in remarkable, dose-dependent anti-DLA activity in mice. The optimum dose was found to be 6 mg/kg. This dose protected all the animals challenged with the tumour cells. The efficacy of the drug was found to be better than that of the standard drug vincristine, in this tumour model. However, the oral administration showed only marginal activity compared to i.p. administration. The extract was found to be devoid of conspicuous acute and short-term toxicity to mice, when administered daily intraperitoneally for 14 days up to a dose of 12mg/kg. This was double the concentration of optimum therapeutic dose. The treated mice showed conspicuous

toxic symptoms only at 24mg/kg. The LD₅₀ in male mice for i.p. doses was found to be 36mg/kg. These results suggest that the seed extract is an attractive material for further studies leading to further drug development (Jose *et al.*, 2002).

2.11.5. PLANT PARTS USED

The stem-bark, and the fruits are mainly used.

Figure 14. *Barringtonia racemosa* fruits.



2.11.5. CHEMICAL CONSTITUENTS AND THEIR BIOLOGICAL ACTIVITY

Barringtonia racemosa contains a triterpenoid saponin which has yielded two neutral sapogenins, barringtogenin (C₂₂H₃₈O₄ or C₂₂H₃₈O₅) and barringoetin (C₂₀H₃₄O₄), and an acid sapogenin C has also been isolated from the plant. The ripe fruit yields large amounts of saponins, from which after hydrolysis, two triterpenoids sapogenins, *barringtogenol* and *barringtogenic acid* (Anantanaan & Pillai, 1956) have been

isolated. The yield of purified saponins from dried ripe fruit is approximately 13 percent, and of sapogenin 4.3 percent. The ultimate percentage yield of *barringtogenol* is 0.51 and *barringtogenic acid* 0.28 (Anantanaan & Pillai, 1956).

2.12. *JATROPHA CURCAS* (FAMILY: EUPHORBIACEAE)

2.12.1 DISTRIBUTION

The plant originates from tropical America, but has become naturalised in the northern parts of South Africa and in KwaZulu-Natal.

2.12.2. BOTANICAL DESCRIPTION

J. curcas is a small tree of up to six meters in height. The hairless leaves are heart shaped, usually with five large lobes (sometimes three-lobed or up to seven-lobed). Both the male and female flowers are small, greenish-yellow and hairy. The fruits are egg-shaped capsules, initially green but eventually turning dark brown or black.

Figure 15. *Jatropha curcas* flowers.



Flowers of *Jatropha curcas*

The fruits split into three parts at maturity, releasing the three large black seeds (nuts), each about 20 mm long and 10 mm in diameter (Van Wyk *et al.*, 1997).

2.12.3 GENERAL USES

In Mali, *J. curcas* has been traditionally grown as a hedge plant around gardens and fields. Also oil from the nuts is used both for the production of soap and, more recently, as a substitute for diesel oil (Liu *et al.*, 1997).

2.12.4 USE IN TRADITIONAL MEDICINE

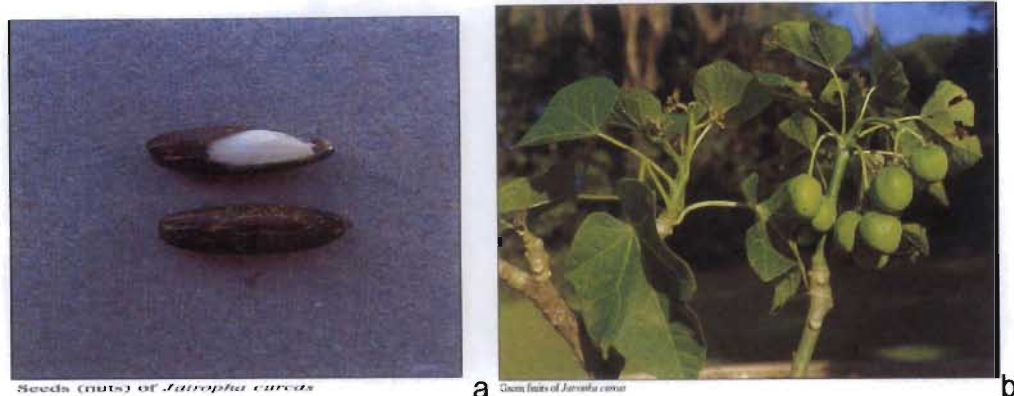
Nuts of *J. curcas* are taken in small quantity as a purgative, but leaves and bark have the same effect. Seeds are said to be strongly purgative, and larger numbers may cause severe diarrhoea, abdominal pain and vomiting.

As *J. curcas* is used for various purposes, Liu *et al.* (1997) investigated the molluscicidal activity of its seed extracts. It was tested against the schistosomal vector snails, *Oncomelania hupensis*, *Biomphalaria glabrata* and *Bulinus globosus*, which transmit *S. japonicum*, *S. mansoni* and *S. haematobium*, respectively. The seed extracts showed molluscicidal activity against both *B. glabrata* and *O. hupensis*, the latter being more sensitive (Rug & Ruppel, 2000).

2.12.5. PLANT PARTS USED

The seeds are mainly used.

Figure 16. Seeds (nuts) (a) and green fruits (b) of *J. curcas*.



2.12.6. CHEMICAL CONSTITUENTS AND THEIR BIOLOGICAL ACTIVITY

The seed oil contains irritant diterpenoids of the tiglian (phorbol) type, such as curcusion A and curcusion C. Curcusion C appears to be identical to jaherin, an active antimicrobial which was isolated from *J. zeyheri*. The activity of the seed oil is also partly ascribed to curcanoleic acid, which is similar to ricinoleic acid (from castor oil) and crotonoleic acid from (croton oil). The seeds also contain a toxic protein named curcin. The toxicity and gastro-intestinal irritation caused by the seed is ascribed to partially identified diterpenoid(s) esters, but the numerous diterpenoids, many with reported antimicrobial, antitumour, molluscicidal and even tumour-promoting activity, as well as toxalbumin curcin should also be considered (Dos santos & Kassamba, 1999; Van Wyk *et al.*, 1997). In a study conducted by Wiest *et al.* (1994), it was established that activation of protein kinase C by phorbol esters disrupts the tegument of *Schistosoma mansoni*.

2.13. RAUVOLFIA CAFFRA (FAMILY: APOCYNACEAE)

2.13.1. DISTRIBUTION

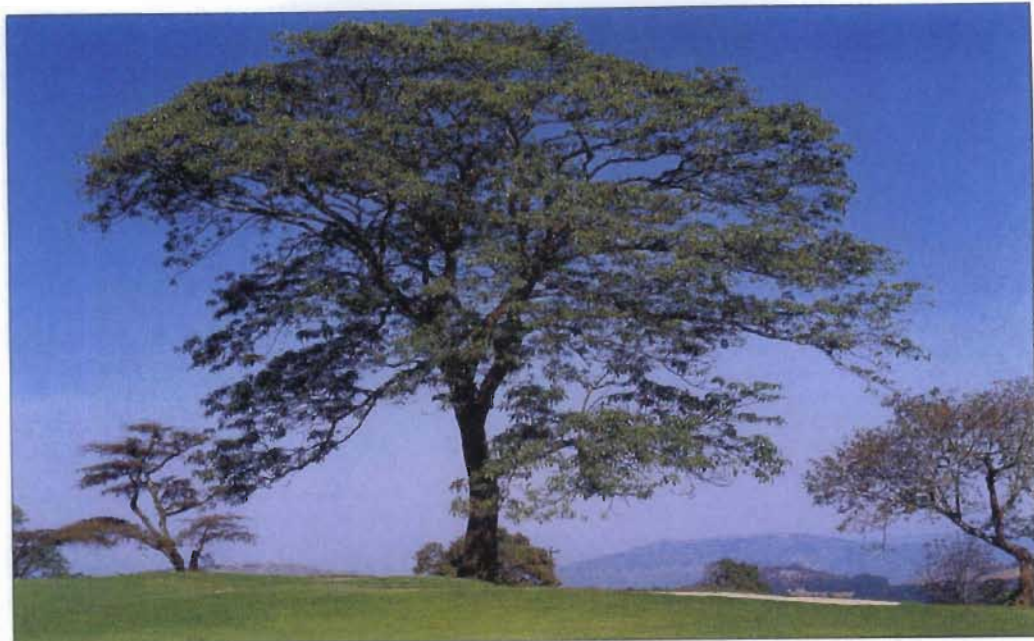
Rauvolfia caffra, also known as the 'quinine tree', varies in height from about 5 to 20 metres. It is found in forest, riverine forest, swamp forest and woodland at lower altitudes in Natal and Transkei. It also occurs in Eastern Cape, Transvaal, Swaziland and Mozambique to tropical Africa (Van Wyk *et al.*).

2.13.2. BOTANICAL DESCRIPTION

Mature plants have spreading crowns and pale-yellowish brown to grey bark, which is soft and corky, splitting into small rectangular blocks. The oblong leaves occur in groups of three to five on the branches and are oblong in shape, bright shiny green, hairless, with a prominent main vein. The stalk of the leaves is up to 20 mm long. Stipules form a rim between the leaves. The flowers are small, white, branched in terminal clusters, up to 200 mm in diameter on sturdy stalk. They have a strong scent. The flowers appear in May to October.

The fruits are rounded or egg-shaped berries. They are bright green, sometimes with conspicuous white spots, but become black and wrinkled when ripe. The fruits appear in October to March (Van Wyk *et al.*, 1997; Pooley, 1993).

Figure 17. *Rauvolfia caffra* tree.



Rauvolfia caffra

2.13.3. GENERAL USES

The fruits are eaten by birds and bush babies. Flowers, leaves and fruits are eaten by monkeys. The soft, light wood is used for drums. It is also a decorative tree.

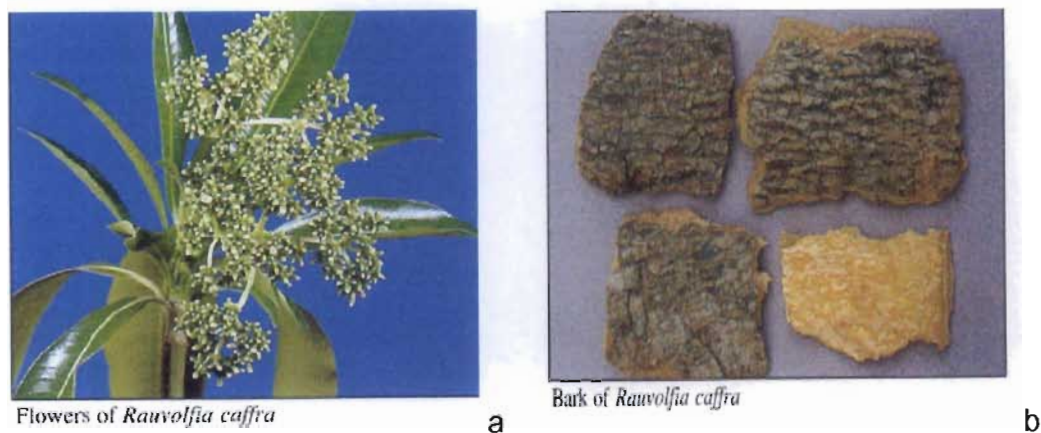
2.13.4. USE IN TRADITIONAL MEDICINE

R. caffra is used medicinally for a wide range of ailments. The main use of the stem-bark is to treat fevers and malaria, as well as insomnia and hysteria. The milky latex is applied to rashes as well as the rash caused by measles (Van Wyk *et al.*, 1997).

2.13.5. PLANT PARTS USED

The stem or root-bark is mainly used, rarely the leaves.

Figure 18. *R. caffra* flowers (a) and the stem-bark (b).



2.13.6. CHEMICAL CONSTITUENTS AND THEIR BIOLOGICAL ACTIVITY

A large number of indole alkaloids occur in *R. caffra*, of which reserpine and ajmalicine (sometimes also called raubasine) are of particular interest. Commercially, these alkaloids are obtained from *R. serpentina* (snake wood), *R. vomitaria* and *R. tetraphylla*. Reserpine is a well-known antihypertensive, widely used to reduce blood pressure, to reduce the heart rate and for its sedative effects. Reserpine has important side-effects, notably depression. Ajmalicine increases blood flow to the brain and forms an ingredient of products used to treat psychological and behavioural problems associated with senility, as well as cerebro-vascular and cranial traumas (Van Wyk *et al.*, 1997).

2.14. *SUTHERLANDIA FRUTESCENS* (FAMILY: FABACEAE)

2.14.1. DISTRIBUTION

The genus is restricted to southern Africa, and occurs in South Africa, Botswana and Namibia. *S. frutescens* is widely distributed and shows remarkable regional variation. Some species have become popular garden plants in many parts of the world (Van Wyk *et al.*, 1997).

2.14.2. BOTANICAL DESCRIPTION

The ‘cancer bush’ is an attractive small shrub of up to a meter in height. The leaves are slightly to densely hairy, often giving the plant a silvery appearance. Each leaf is divided into numerous small leaflets. The large red flowers are followed by characteristic bladder-like, papery pods (Van Wyk *et al.*, 1997).

Figure 19. The aerial parts of *S. frutescens*.



Typical form of *Sutherlandia frutescens*

2.14.3. USE IN TRADITIONAL MEDICINE

An overview of the recorded uses of the plant as well as some recent anecdotes, suggest that the *S. frutescens* is one of the most widely used but under-rated medicinal plants of southern Africa. It is an old Cape remedy for stomach problems and internal cancers. It is said to be a useful bitter tonic and a good general medicine. According to tradition, the virtues of the plant extend to include remedies for colds, influenza, chicken pox, diabetes, varicose veins, piles, inflammation, liver problems, backache and rheumatism. The medicinal use of the plant probably originated with the Khoi and Nama people, who used decoctions externally to wash wounds and internally for fevers and a variety of other ailments (Van Wyk *et al.*, 1997).

2.14.4. PLANT PARTS USED

The leaves are mainly used, but all the aerial parts are usually included.

Figure 20. Flowers and fruits of *Sutherlandia speciosa*.



Flowers and fruits of *Sutherlandia speciosa*

2.14.5. CHEMICAL CONSTITUENTS AND THEIR BIOLOGICAL ACTIVITY

Various chemical constituents have recently been found in the plant. These include, pinitol, amino acids, flavones and terpenoids (saponins). The seeds contain non-protein amino acid, canavanine. No alkaloids are found in the plant.

Canavanine has antitumourigenic properties, and it is possible that this or some other amino acids are responsible for reported benefits in treating cancer. It is speculated that the mechanism may be one which acts on the immune system (Van Wyk *et al.*, 1997).

2.15. *RICINUS COMMUNIS* (FAMILY: EUPHORBIACEAE)

2.15.1. DISTRIBUTION

The plant is an invasive alien found on disturbed soils and floodplains. It is believed to be indigenous to north-east Africa and India, but it is now widely distributed in the tropics. It occurs throughout South Africa as a weed and is also commonly cultivated (Van Wyk *et al.*, 1997).

2.15.2. BOTANICAL DESCRIPTION

It is a small plant of up to four metres in height, with very large, hand-shaped leaves on long, stout leaf stalks. The flower clusters appear near the tip of the branches. Female flowers occur above the male ones. The fruits are three lobed capsules, with spine-like projections on their surfaces. Each capsule has three seeds, which are about 10 mm long, conspicuously shiny, irregularly mottled with silver, brown and black. At the tip of the seed is a hard, white, fleshy aril (Van Wyk *et al.*, 1997).

10 mm long, conspicuously shiny, irregularly mottled with silver, brown and black. At the tip of the seed is a hard, white, fleshy aril (Van Wyk *et al.*, 1997).

Figure 21. *Ricinus communis* flowers and leaves.



RICINUS COMMUNIS

2.15.3. GENERAL USES

Castor oil is grown commercially on a large-scale for the oil, which is mainly an industrial product, used as a lubricant and as a starting material in the manufacture of polymers and various other products (Van Wyk *et al.*, 1997).

2.15.4. USE IN TRADITIONAL MEDICINE

Castor oil is a well-known purgative medicine, commonly referred to in South Africa as “blue bottle” medicine because of the characteristic blue bottle in which it was traditionally packed and sold. It is very effective but was much feared by children

because of its bitter taste. The seeds are not popular as purgatives in Sotho and Zulu traditional medicine, but the leaf infusions, administered orally or as enemas, are used for stomachache. Root and leaf poultices are widely applied to wounds, sores and boils (Van Wyk *et al.*, 1997).

2.15.5. PLANT PARTS USED

The seed oil is mostly used. Sometimes the leaves, seeds or fruits are also used.

Figure 22. Ripe fruit capsules of *R. communis*.



2.15.6. CHEMICAL CONSTITUENTS AND THEIR BIOLOGICAL ACTIVITY

Castor oil contains a fatty acid known as ricinoleic acid, which accounts for about 90% of the triglyceride fatty acids in the oil. The seeds also contain two highly toxic substances, which are not present in the oil- an alkaloid, ricinine; and a lectin-ricin. The latter is among the most toxic compounds known, and two seeds may cause fatal poisoning in humans. Like other anionic surfactants, ricinoleic acid, which is formed under the influence of lipase in the small intestine, reduces the net absorption of fluids and electrolytes and stimulates intestinal peristalsis (Van Wyk *et al.*, 1997).

CHAPTER THREE

3. MATERIALS AND METHODS

3.1. PLANT MATERIAL AND EXTRACTS

Plants analysed were selected on the basis of their ethnopharmacological information, indicating their medicinal uses in schistosomiasis endemic areas of KwaZulu-Natal Province of South Africa. The plant parts were collected in different areas around KwaZulu-Natal and identified by the Taxonomist/Curator of University of Durban-Westville's Botany Department (see Table 1). Voucher specimens were kept at the University of Durban-Westville's Herbarium. The plant materials were air-dried at ambient temperature in a shady area in order to stabilize the compounds. The dried plant materials were powdered and subjected to suitable extraction process. Since the aim of this study was to investigate the molluscicidal properties of the Zulu medicinal plants, methanol and water were used for extraction. The plant materials were soaked in methanol or water for 48 hours and then filtered. This was repeated for about three times to maximize the yield. The methanol and aqueous filtrates were concentrated *in vacuo* in a rotary evaporator at 55°C and 85 °C respectively. The solid, crude plant extracts obtained were removed and weighed.

3.2. TEST ORGANISMS

Snails (*Biomphalaria pfeifferi* and *Bulinus africanus*) were collected from a pond in Overport, Durban, and reared in the laboratory during the time of bioassay. The snails used were of a uniform size (8-10 mm). The snails were identified by the Zoologist of University of Durban-Westville's Zoology Department.

3.3. PREPARATION OF STOCK SOLUTIONS

A gram from each extract (methanolic and aqueous) was dissolved in 100 ml of pond or de-chlorinated tap water, to give a stock solution of 10 mg/ml. Other concentrations used for the tests were serially diluted from the stock solutions. For the methanolic extracts, the crude extracts were dissolved in 5 parts of methanol and then made up with 95 parts water to the desired concentrations. (This concentration of methanol had no adverse effects on the snails).

3.4. TESTING FOR MOLLUSCICIDAL ACTIVITY

For the screening tests, nine concentrations (1000 ppm, 800 ppm, 400 ppm, 200 ppm, 100 ppm, 80 ppm, 40 ppm, 20 ppm and 10 ppm) of the plant extracts were examined, and three replicates were used. Bayluscide® (niclosamide) was used as the positive control, while de-chlorinated tap water was used as the negative control. WHO, (1965) standards for preliminary screening of plants for molluscicidal activity were followed. *Biomphalaria* and *Bulinus* snails were used. Nine containers, each with 10 healthy snails containing 400 ml of the test solution, were set up for each concentration. In all tests, 24-hour exposure and 24-hour recovery periods were used. The LC_{50} and 95% confidence intervals were determined from the 24 hour counts of the dead snails by intersection.

Figure 23. Experimental setup during molluscicidal activity testing.



The snails were judged as being dead by not reacting to pricking their foot-soles with a sharp wooden object.

3.5. BIOACTIVITY-GUIDED FRACTIONATION

3.5.1. BASIC PRINCIPLES OF THIN LAYER CHROMATGRAPHY (TLC)

Chemical separation by TLC is effected by the application of the mixture or extract as a spot or thin line onto a solvent that has been applied to a backing plate. Analytical TLC plates, silica gel 60 F₂₅₄ purchased from Merck®, were used. Each plate was placed into a tank with sufficient, suitable solvent to just wet the lower edge of the plate/sorbent, but not enough to wet the part of the plate where the spots were applied (origin). The solvent front thereafter migrated up the plate through the solvent by capillary action, a process known as ‘development’. An important factor in quantifying migration of a compound on a particular sorbent and solvent is the R_f

value. This is defined as compound distance from the origin divided by solvent front distance from the origin.

As a consequence of development, compounds of a mixture will separate according to their relative polarities. Polarity is related to the type and number of functional groups present on a molecule, capable of hydrogen bonding.

3.5.2. MECHANISMS OF SEPARATION

There are three basic mechanisms of chromatography by which separation can occur, and more than one mechanism may be responsible during a given separation. These include, partition chromatography: this mechanism involves the relative solubility of the compound between the sorbent and the solvent. Compounds that are more soluble in the solvent will migrate faster. The other mechanism is size-inclusion/exclusion chromatography. Here, compounds may be separated by their sizes and by the inclusion (exclusion) into sorbent. Ion-exchange chromatography mechanism is limited to mixtures containing components that carry a charge.

3.5.3. COLUMN CHROMATOGRAPHY

To obtain active compounds, the plant extracts were first qualitatively analysed by thin layer chromatography (TLC) and/or column chromatography, and thereafter screened to determine their molluscicidal activity. For purification and isolation, the active plant extracts were sequentially fractionated on silica gel 60 (particle size 0.0063- 0.200 mm) saturated with hexane and ethyl-acetate (8:2) and the compounds were eluted with the same solvent system. All the solvents and silica gel were purchased from Merck. Fractions were pooled according to similar TLC profiles and the pooled fractions were evaporated to dryness in a rotary evaporator at 50°C. The residues obtained were removed with minimal amount of dichloromethane and put in

pre-weighed vials to air dry. The fractions obtained were then tested again on the snails.

3.5.4. STRUCTURE ELUCIDATION

Application of the newer spectroscopic techniques has tremendously eased the problem of structure elucidation of natural products which, in most cases, is now successfully achieved without resorting to the conventional chemical degradative procedures. Developments in Nuclear Magnetic Resonance (NMR) spectroscopy for structure elucidation are very remarkable (Mahato *et al.*, 1992). Although it was not possible to purify the compounds, due to technical reasons, some of the fractions obtained indicated the presence of oleanolic acid-like triterpenoids, and flavonoids in *Psidium guajava* and *Sclerocarya birrea* respectively after NMR analysis.

3.5.5. TOXICITY TESTS

Since most active principles are toxic at high doses, a possible approach to developing an effective general bioassay might be simply to screen for substances that are toxic to zoologic systems (Fatope *et al.*, 1993). Desiring a rapid, inexpensive, in-house, bioassay for screening and fractionation monitoring of our biologically-active plant extracts, we have used a tiny crustacean, brine shrimp, as the general toxicity assay.

The eggs of brine shrimp, *Artemia salina*, are readily available at low cost in pet shops as food for tropical fish, and they remain viable for years in dry state. Upon being placed in natural sea water, the eggs hatch within 48 hours and swim towards a light source, providing large numbers of larvae (nauplii) (Appleton, 1976; Meyer *et*

al., 1982). Compounds and extracts were tested at concentrations of 10, 100, 1000 ppm after being placed in vials containing 5 ml sea water and ten shrimp in each of the three replicates. Survivors were counted after 24 hours, and the percentage of the deaths at each dose was recorded. Since the extracts were dissolved in methanol, methanol solution (5 parts of methanol: 95 parts of water) was used as a negative control.

3.5.5.1 SAMPLE PREPARATION

Samples were prepared by dissolving 20 mg of extracts in 2 ml of methanol. Appropriate amounts of solution (5, 50, 500 μ l for 10, 100 and 1000 μ g/ml respectively) were transferred to discs of filter paper. The discs were dried in an oven for one hour.

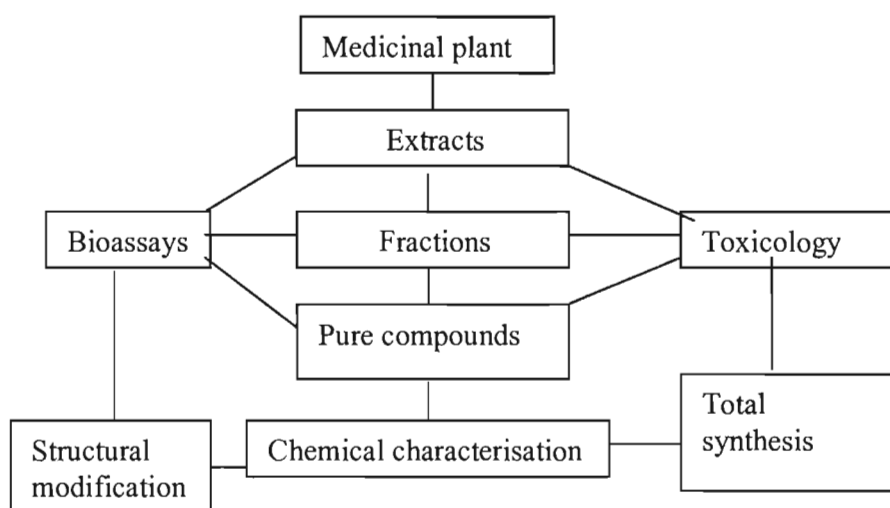
3.5.5.2. HATCHING THE SHRIMP

Brine shrimp eggs were hatched in a beaker filled with constantly oxygenated sea water. The eggs were sprinkled into the beaker, which was put in a dark room. After 48 hours, the phototropic nauplii were collected using a disposable pipette.

3.5.5.3. BIOASSAY

Ten shrimp were transferred to each sample vial using a disposable pipette, and sea water was added to make 5ml. The vials were maintained at 37°C. After 24 hours of exposure to the plant extracts, survivors were counted and percentage deaths at each dose and control were determined.

Figure 24. Flow Chart to show methods for obtaining active substances from plants



3.6. STATISTICAL ANALYSIS

The experimental results are expressed as means (\pm S.E.M.). Student's t-test was used to determine the statistical significance. Values of $P \leq 0.5$ were taken to imply statistical significance.

CHAPTER FOUR

4. RESULTS

Molluscicidal activity does not appear to be limited to any morphological part of the plants tested nor restricted to any family name. However, in some of the plants, some morphological parts seem to be more active than the others. This may be due to higher concentrations of active substances in the morphological part of that particular plant.

Table 2. Major classes of plant secondary metabolites with recognised molluscicidal activity (Spatafora & Tringali, 1996; Mott, 1987; Adewunmi & Sofowora, 1980).

Class of compound	Plant	Family
Alkaloids	<i>Culprinia aurea</i>	Fabaceae
Alkenyl phenols	<i>Anacardium occidentale</i>	Anacardiaceae
Anthraquinones	<i>Morinda lucida</i>	Rubiaceae
Chalcones	<i>Polygonum senegalensis</i>	Polygonaceae
Diterpenes	<i>Wedelia scaberrina</i> <i>Baccharis trimeria</i>	Compositae Compositae
Flavonoids	<i>Baccharis trimeria</i> <i>Polygonum senegalensis</i> <i>Polygonum nodosum</i>	Compositae Polygonaceae Polygonaceae
Furanocoumarins	<i>Ruta chalepensis</i>	Rutaceae
Iridoids	<i>Olea europaea</i>	Oleaceae
Isobutylamides	<i>Heliopsis longipes</i> <i>Fagara macrophylla</i>	Compositae Rutaceae
Monoterpenes	Genus <i>Lippia</i>	Verbanaceae
Naphthoquinones	<i>Diospyros usambarensis</i>	Ebenaceae
Sesquiterpenes	<i>Warbugia ugandensis</i> <i>Warbugia stuhlmannii</i> <i>Ambrosia maritime</i> <i>Podachaenium eminens</i>	Canellaceae Canellaceae Compositae Compositae
Spirostanol saponins	<i>Cornus florida</i> <i>Balanitis egyptiana</i> <i>Asparagus curillus</i>	Cornaceae Zygophyllaceae Liliaceae
Steroid glycoalkaloids	<i>Solanum mammosum</i>	Solanaceae
Tannins	<i>Acacia nilotica</i>	Fabaceae
Triterpenoid saponins	<i>Phytolacca dodecandra</i> <i>Hedera helix</i> <i>Lonicera nigra</i> <i>Swartzia madagascariensis</i>	Phytolaccaceae Araliaceae Caprifoliaceae Fabaceae

4.1. PLANT MATERIAL AND EXTRACTS

A total of 28 different extracts from 10 medicinal plants belonging to 8 plant families were screened for molluscicidal properties. Methanol and water were used. Table 3 shows the yield from methanolic and aqueous extracts of the different plants and plant parts used.

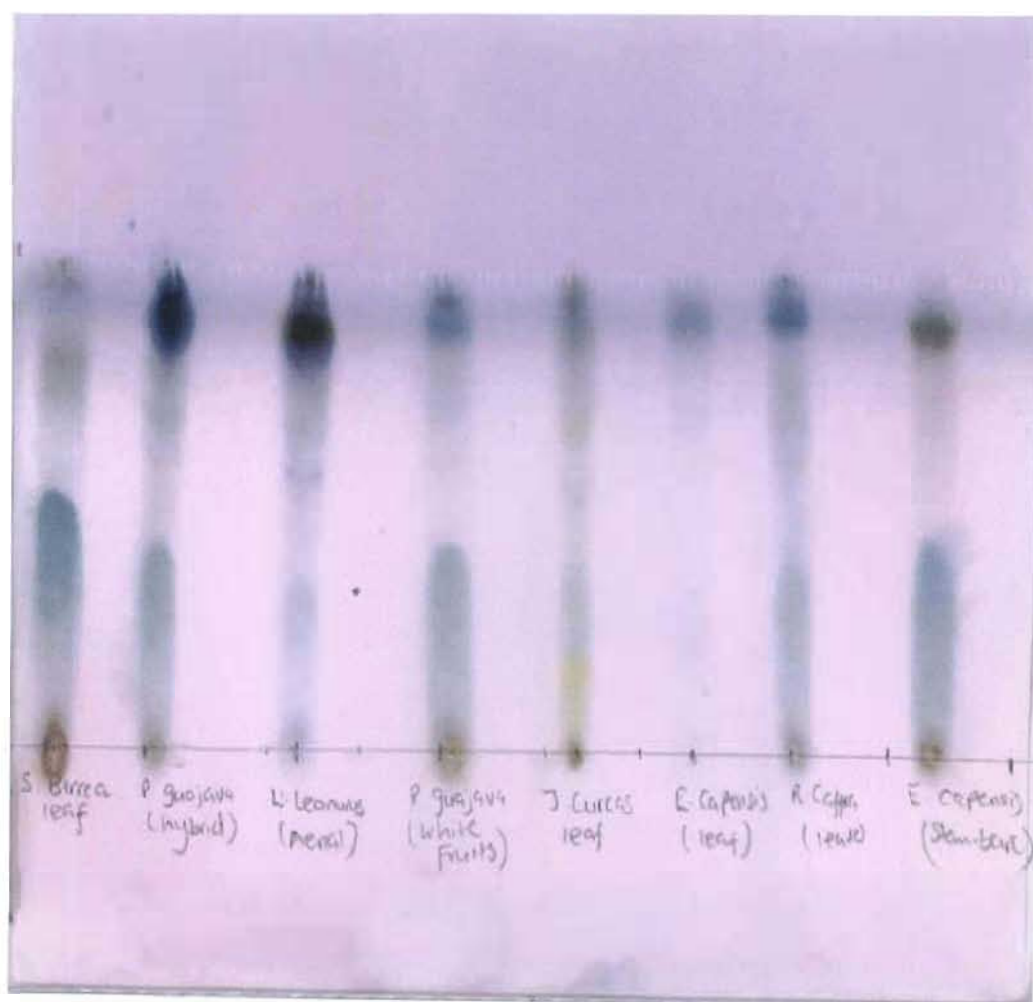
Table 3. Percentage yield of plant extracts

Plant name/part used/ code	Percentage yield (MEOH extract)	Percentage yield (aqueous extract)
<i>Sclerocarya birrea</i> stem-bark (WC/21D/E)	10.05	5.63
<i>Sclerocarya birrea</i> leaves (WC/15/D/E)	9.75	8.16
<i>B. racemosa</i> seeds (WC/5/D/E)	9.34	1.59
<i>B. racemosa</i> pericarp (WC/6/D/E)	6.14	2.25
<i>P. guajava</i> leaves (white fruits) (WC/23/D/E)	In viscous form	In viscous form
<i>P. guajava</i> leaves (hybrid) (WC/25/D/E)	In viscous form	In viscous form
<i>R. caffra</i> leaves (WC/24/D/E)	In viscous form	In viscous form
<i>J. curcas</i> leaves (WC/20/D/E)	3.33	In viscous form
<i>L. leonurus</i> aerial parts (WC/26/D/E)	In viscous form	In viscous form
<i>E. capensis</i> stem-bark (WC/219/D/E)	In viscous form	In viscous form
<i>E. capensis</i> leaves (WC/218/D/E)	In viscous form	In viscous form
<i>R. communis</i> seeds (WC/17/D/E)	0.1	In viscous form
<i>S. frutescens</i> aerial parts (WC/16/D/E)	-	0.05

4.1.2. THIN LAYER CHROMATOGRAPHY (TLC) OF EXTRACTS

TLC analysis of methanolic crude extracts of some plants tested for molluscicidal activity was performed, and each crude extract contained a mixture of compounds as the TLC plate (Fig. 25) illustrates.

Figure 25. TLC analysis of methanolic extracts of some plants screened for molluscicidal activity.



4.2. MOLLUSCICIDAL ACTIVITY

Extracts of 4 of the 10 plants (40%) were found to have molluscicidal activity on adult snails. These are: (i) *Sclerocarya birrea*, (ii) *Psidium guajava* (hybrid), (iii) *Leonotis leonurus* and (iv) *Ekebergia capensis*. Whereas methanol extracts were active in the 4 plants, only 1 (25%) of the 4 aqueous extracts was active at 100 ppm.

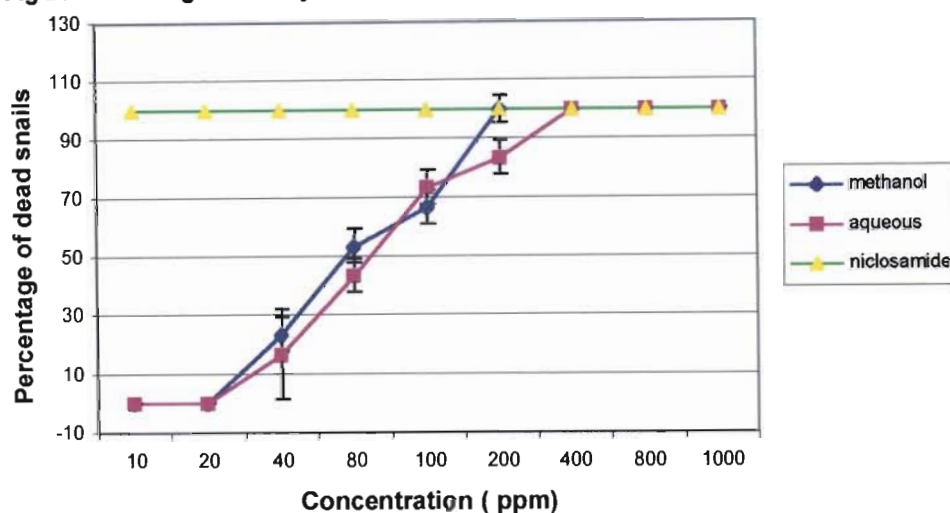
Both methanolic and aqueous *S. birrea* extracts showed molluscicidal activities. The stem-bark extracts of this plant were active whereas the leaf extracts were not. Three other methanolic plants extracts were molluscicidal. These are the leaves of *Psidium guajava* (hybrid), the aerial parts of *Leonotis leonurus* and the stem bark of *Ekebergia capensis*. The rest of the plant extractives, including the seeds of *Barringtonia racemosa* and *Riccinus communis*, and the leaves of *Jatropha curcas*, *Psidium guajava* (white fruits) leaves, *Rauvolfia caffra* and the aerial parts of *Sutherlandia frutescens* did not show molluscicidal activity with both methanolic and aqueous extracts. Niclosamide was used as a positive control. It produced 100% mortality of the snails at 10 ppm. In all, the methanolic extracts of the plants showed higher molluscicidal activities compared to the aqueous extracts. For the active extracts, it was observed that snails dropped to the bottom of the test solutions or became temporarily attached to the side of the beaker tanks, whereas for the inactive ones, the snails just swam in the test solutions.

Poisoning of the snails with the plant extracts caused adult snails either to retract into their shells or to become swollen and remain extended from the shell opening. The former behaviour was observed with the extracts of *Psidium guajava* (hybrid) and *Leonotis leonurus*. In addition to being swollen and remaining extended out of their

shells, as well as retracting into the shells, snails expelled haemolymph. This was observed mostly with *Sclerocarya birrea*.

The stem-bark and leaf extracts of *S. birrea* were tested for molluscicidal activity. However, only the stem-bark extracts of the plant were active.

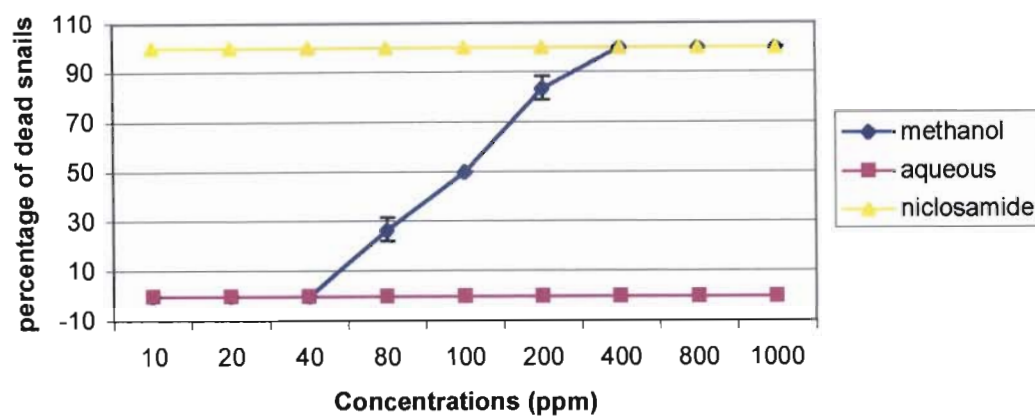
Fig 26. Percentage mortality of snails exposed to *S. birrea* extracts



The results presented in Figure 26 demonstrate that there was activity in both methanolic and aqueous stem-bark extracts of *S. birrea* against the snails. The methanolic extracts had a slightly higher activity compared to the aqueous extract. Median lethal concentration (LC_{50}) values of 78 ppm and 82 ppm were obtained for the methanol and aqueous extracts respectively. Niclosamide was used as a positive control in all cases. It produced 100% mortality at a concentration of 10 ppm.

The hybrid and white fruit sub-species of *P. guajava* leaf extracts were both tested for molluscicidal activity. Only the hybrid sub-species demonstrated activity against the snails.

Fig 27. Percentage mortality of snails exposed *P. guajava* extracts



However, as figure 27 illustrates, only the methanolic extract of the hybrid leaf extract was active, with LC_{50} value of 100 ppm. From the literature, no molluscicidal use of *P. guajava* has been reported. *P. guajava* grows abundantly in South Africa. Therefore, exploitation of this plant for mollusciciding may be a cheaper alternative.

The aerial parts of *Leonotis leonurus* were tested for molluscicidal activity. There is no record of use of this plant in the control of schistosomiasis, but because it is generally used by local people for the treatment of different ailments, our local herbalist advised that we should test it for molluscicidal activity.

Fig 28. Percentage mortality of snails exposed to *L. leonurus* extracts

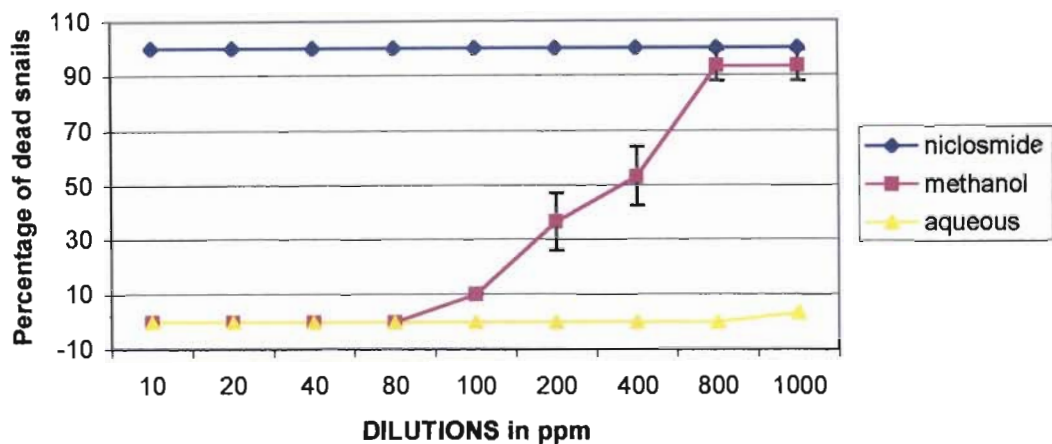
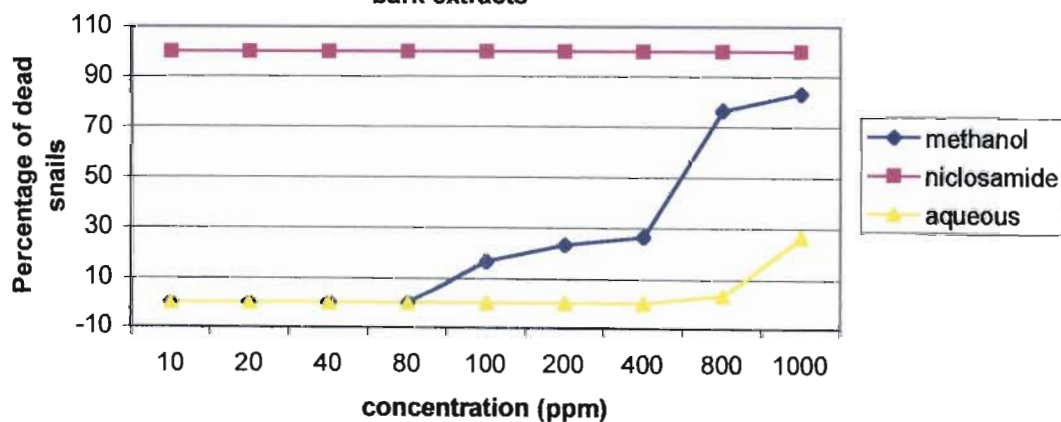


Figure 28 shows that only the methanolic extract of the plant was active with the LC_{50} value of 398 ppm. The aqueous extract was not active against the snails.

The chemical compounds of *Ekebergia* species are poorly known. Seeds of *E. capensis* contain a limonoid- ekebergin as the major constituent (Taylor, 1981).

Fig 29. Percentage mortality of snails exposed to *E. capensis* stem-bark extracts

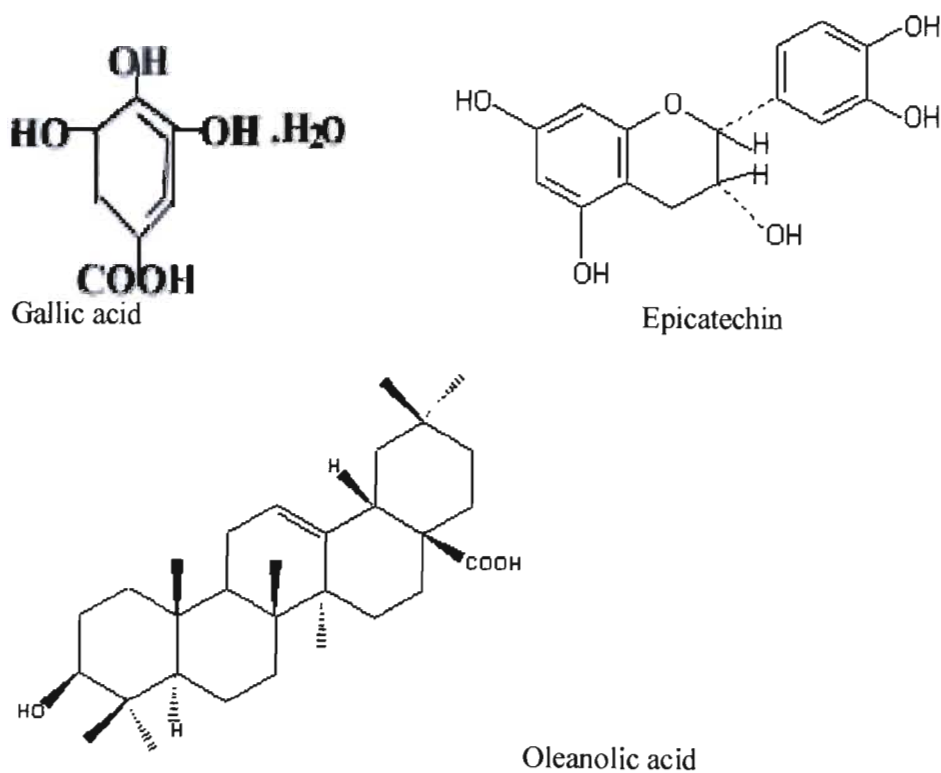


The stem-bark and leaf extracts of *E. capensis* were tested for molluscicidal activity. Only the stem-bark methanolic extract showed activity against the snails. The LC_{50}

was quite high, 600 ppm. This shows that only at high concentrations can the stem-bark be molluscicidal.

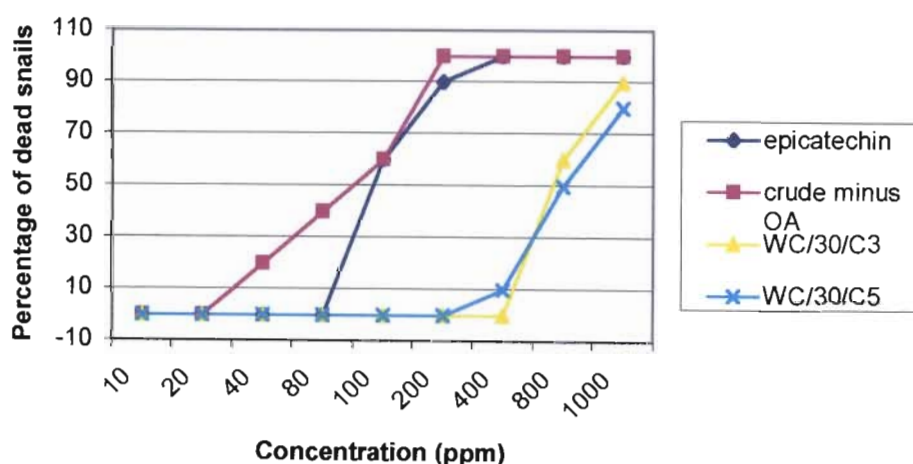
The methanolic extracts of the 4 plants that showed molluscicidal activities, namely: *S. birrea*, *P. guajava*, *L. leonurus* and *E. capensis* were purified using column chromatography with the aim of isolating active compounds in the extracts. Due to technical problems, however, pure compounds could not be isolated from the plant extracts. However, some of the fractions that were sufficient for bioactivity-guided assay were screened for molluscicidal activity. Below are the structures of some of the compounds tested against the snails.

Figure 30. Compounds tested for molluscicidal activity.



(-)-Epicatechi-3-galloyl ester was isolated from *Sclerocarya birrea* stem-bark methanol extract (Galvez *et al.*, 1992). Epicatechin and gallic acid (synthetic) were tested separately for molluscicidal activity. Also it has been established that *Psidium guajava* contains a mixture of oleanolic acid, ursolic and maslinic acid (Osman *et al.*, 1974). Research has also shown that saponins composed of oleanolic acid with a branched sugar side-chain possess molluscicidal activity. Therefore, oleanolic acid isolated from olive leaves was tested against the snails. The oleanolic-like compounds were also isolated from the crude extract of *P. guajava* and the crude extract without oleanolic acid was tested for molluscicidal activity. The extract without oleanolic-like compounds was further purified to yield two pooled fractions coded WC/30/C₃, and WC/30/C₅. The fractions were also tested against the snails.

Fig 31. Percentage mortality of snails exposed to various compounds



The results shown in Figure 31 demonstrate activity of epicatechin, crude extract without oleanolic acid, WC/30/C₃ and WC/30/C₅ against the snails. Their LC₅₀ values were 80 ppm, 85 ppm, 780 ppm and 820 ppm respectively.

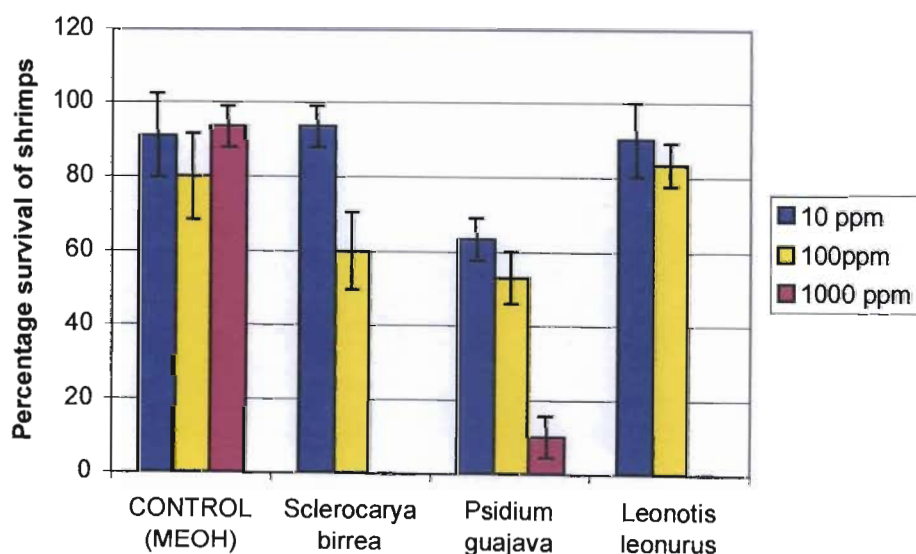
The results show that for *P. guajava*, the crude extract without the oleanolic acids demonstrate more activity than the crude with oleanolic acid (see fig. 27) (LC₅₀ values

85 ppm and 100 ppm respectively). Furthermore, the crude extract without oleanolic acid shows more activity as compared to the isolated fractions (with LC_{50} concentrations of 85 ppm and 800 ppm respectively).

TOXICITY TEST USING BRINE SHRIMP ASSAY

Since the most active plant extracts are toxic at high doses (Duncan, 1985), a simple toxicity bioassay using brine shrimp was carried out to identify the plant extracts that may be toxic to zoologic systems, and at what concentrations. Methanol was used as a negative control in this toxicity assay.

Fig 31. Percentage survival of brine shrimp exposed to extracts



Methanol did not kill the brine shrimps even at 1000 ppm concentration. This shows that the methanol used to dissolve the plant extracts is not responsible for killing the shrimps but the extracts themselves. During the counting of survived shrimps, it was also observed that some stuck onto the filter paper and died. *Sclerocarya birrea* and

Leonotis leonurus showed 0% survival at 1000 ppm, whereas *Psidium guajava* showed 10% survival at this concentration. Although *S. birrea* extracts have been reported to be cytotoxic, it showed 60% survival of brine shrimps at 100 ppm. *Psidium guajava* and *Leonotis leonurus* extracts respectively showed 56 % and 86% survival of the shrimps at 100 ppm. In general, the results show that the plant extracts tested may not be toxic to zoologic systems in water ponds. Furthermore, the results give a guideline as to what doses may be required to be used during mollusciciding.

CHAPTER FIVE

DISCUSSION

About 80% of South Africans still consult traditional healers, although most of them use modern medical services as well. This suggests a heavy dependence on medicinal plants, and from a conservation point of view, it may lead to total disappearance of the medicinal plant species. In this study, regenerating plant parts (fruits, leaves) and the stem-barks were used.

Methanolic and aqueous extracts of the various plants screened were used for testing for molluscicidal activity. This is because the use of plant molluscicides is more likely to be undertaken in rural areas where the use of special solvents and sophisticated technology may not be feasible. Moreover, some of these plants may be grown along the waterbeds and ponds so that their leaves and fruits can drop into the pond water and become active against the snails.

It is now generally agreed that control of snail intermediate host is one of the effective means of controlling schistosomiasis. The potential of plant's secondary metabolites for schistosomiasis control is illustrated by the well-demonstrated activity of *Phytolacca dodecandra* fruits, so far the most promising plant molluscicide, which have proved effective in clearing waterways of intermediate host snails. The present results have confirmed this possibility, based on the preliminary screening of potential plant molluscicides. Four out of the 10 plants screened showed a molluscicidal effect on *Biomphalaria* and *Bulinus* snail species.

From the results obtained in this study, methanolic extracts of the active plants examined showed higher molluscicidal activity compared to their aqueous extracts (with the exception of *Sclerocarya birrea* whose methanolic and aqueous extracts were both active against the snails). This probably indicates that the active constituents of the plants whose only methanolic extracts produced molluscicidal activity are more soluble in methanol than in water. However, the potency of some extracts as molluscicides may have been affected by the high temperature (85°C) used during evaporation and concentration of the aqueous extracts. This high temperature may have denatured the active compounds of the plant aqueous extracts.

Methanol is a polar solvent. Consequently, it extracts most chemical constituents in a plant including those that may also be soluble in water.

It has been reported that molluscicidal activity of plants is not restricted to any morphological part (Kela *et al.*, 1989). From the results obtained in this study, only the stem-bark of *Sclerocarya birrea* and *Ekebergia capensis* showed molluscicidal activity, whereas the leaf extracts of the same plants did not. This may suggest that the active molluscicidal compounds are more concentrated in the stem-bark of these plants. Furthermore, the leaves of *Jatropha curcas* did not show activity against the snails. However, Liu *et al.*, (1997) have reported that phorbol esters extracted from *J. curcas* seeds showed molluscicidal activity in schistosome vector snails. Ecological factors can also affect the concentration of some chemical constituents in a plant. Also sub-species of the same plant may contain different concentrations of chemical constituents as demonstrated by *Psidium guajava* where the hybrid demonstrated molluscicidal activity and the white fruits did not.

It has been suggested that molluscicides cause stress to the water balance system of snails by lowering the surface tension (Kela *et al.*, 1989). This could have accounted for the rapid submergence of snails with some of the plant extracts used, e.g., *Sclerocarya birrea* and *P. guajava* extracts, and to some extent, be the cause of snail mortality.

Sclerocarya birrea extracts showed the highest molluscicidal activity among all the plants screened. This may be due to its cytotoxic properties. A study conducted on the effect of *S. birrea*, aqueous bark extract on rat intestinal contractility (Katsoulis *et al.*, 2000), revealed that epithelial cells of the ileal tissue exposed to the plant extract had undergone necrosis. The cellular toxicity of the plant was confirmed using trypan blue exclusion assay, which showed the plant extract to significantly reduce cellular activity. Furthermore, *S. birrea* has been shown to contain flavonoids, and phenolic compounds are known to be cytotoxic. However, the activity of *S. birrea* on the snails cannot be attributed to its cytotoxic properties alone, since the results from the brine shrimp toxicity assay show 60% survival of the shrimps after exposure to the 'marula tree' extract.

Also isolated from *S. birrea* stem-bark methanol extract was (-)-epicatechin-3-galloyl ester (Galvaz *et al.*, 1992). This compound is said to have secretagogue activity. Using a tied-off rat colon technique (Galvez *et al.*, 1992), the fraction containing this compound resulted in net secretion of water, sodium, potassium and chloride. This compound, because of its secretagogue properties, would obviously affect the water and electrolyte balance of the snails, and consequently cause stress to the snails.

It has been reported that (-)-epigallocatechin-5,7-digallate and (-)-epigallocatechin-7-gallate showed molluscicidal activity against *Biomphalaria pfeifferi* and *Bulinus truncates* with 100% mortality at 75 ppm and 120 ppm respectively (Saad, 1984). In this study, epicatechin was found to have 50% mortality at 90 ppm, and this value does not differ much from the value obtained for *S. birrea* crude extract (82 ppm).

Triterpenoids are the most ubiquitous, secondary metabolites in terrestrial and marine flora and fauna. Their presence, even in non-photosynthetic bacteria, has created interest from both evolutionary and functional aspects. Although medicinal uses of this class of compounds are rather limited, considerable recent work in this regard strongly indicates their great potential as drugs (Mahato *et al.*, 1992). The wide occurrence and structural diversity of triterpenoids have always attracted attention for evaluation of their biological activity.

Oleanolic acid and its isomer, ursolic acid, are triterpenoid compounds that form part of chemical constituents found in *Psidium guajava*. Pharmacological studies on these two triterpenoid compounds have shown that the compounds are effective in protecting against chemically induced liver injuries in laboratory animals. Oleanolic acid has been marketed in China as an oral drug for human liver disorders. It also has been long recognized to have anti-inflammatory and antihyperlipidemic properties in laboratory animals as well as antitumour effects (Liu, 1995). Since oleanolic acid and ursolic acid are isomers, they have got the same molecular weight, so during TLC analysis the two compounds combined may appear as one compound.

Oleanolic acid-like compound was isolated from the methanolic crude extract of *P. guajava* using column chromatography. All the other fractions obtained (except the

fraction containing oleanolic acid) were combined together and tested for molluscicidal activity. The combined fractions showed activity against the snails with LC₅₀ value of 85 ppm. These results rule out the possibility that oleanolic acid may have been the compound responsible for molluscicidal activity. Because oleanolic acid obtained after purification of the crude extract was not sufficient for bioassay, oleanolic acid obtained from other commercial sources was tested for molluscicidal activity against the snails. The results obtained indicate that oleanolic acid has no molluscicidal properties. This finding probably confirms that oleanolic acid from the crude extract of *Psidium guajava* was not responsible for the molluscicidal activity of *P. guajava* extract.

The combined fractions from *P. guajava* methanol crude extract showed more molluscicidal activity compared to the individual fractions (WC/30/C₃) and WC/30/C₅) with LC₅₀ values of 85 ppm and 800 ppm respectively. This may be because the compounds might have a synergetic effect. Bioactivity-guided fractionation required when trying to isolate an active compound, may exclude compounds with relevant pharmacological activities. A good example of this is *Panax ginseng* in which the whole plant or its saponin fractions are more active than the isolated compounds (Hamburger and Hostettman, 1991). In addition, when only one activity is considered in pharmacological screens, it is not possible to detect other potentially useful activities. *Catharanthus roseus* was initially studied for its anti-diabetic activity described in folk medicine, but it also contains a powerful anti-tumour compound, currently in clinical use.

Low yield of material, the physico-chemical characteristics of the final compound and subsequent problems such as solubilization of extracts and fractions in solvents compatible with the animal system, are difficulties encountered during the pharmacological evaluation of natural products. In this study, *Leonotis leonurus* was one of the plants that showed molluscicidal activity. Purification of the methanolic crude extract of this plant was carried out using column chromatography. This plant extract yielded four fractions with major compounds in them (WC/29/C₄, WC/29/C₅, WC/29/C₆ and WC/29/C₈). However, none of the fractions could be assayed because they could not dissolve in water even after minimally dissolving them in methanol first. This is because in the crude extract, compounds co-solubilise each other, and therefore, solubility increases. Consequently, the crude extract was soluble in water. On the other hand, the individual fractions which contain purified, single compounds, are not soluble in water. In a crude extract, the compounds are bonded to each other by 'Van der Waals forces' such that the compounds with more -OH groups help the ones without -OH groups, so that overall, the number of -OH groups increases, and therefore, solubility also increases. These problems, in fact, may invalidate the entire pharmacological study because of false negative results, poor absorption through biological barriers and poor bioavailability of the products.

Furthermore, Hostettmann *et al.* (1982), reported on molluscicidal properties of various saponins. Their findings indicate that the sapogenins heterogenin and oleanolic acid as well as the dammarane glycosides showed no molluscicidal activity. It is noteworthy that structure-activity relationship plays an important role in pharmacology. According to the results obtained by Hostettmann *et al.*, (1982), the bidesmodic triterpenoids were not active, whereas the monodesmodic saponins

exhibited molluscicidal activity. Removal of sugar(s) bound to the -COOH group led to a high lack of activity of bidesmodic saponins. Also when carrying a sugar chain, heterogenin glycosides such as oleanolic acid are active as compared to oleanolic acid without the sugar moiety.

Furthermore, to emphasise the importance of structure-activity relationship, Saad, (1984), screened the molluscicidal activity of (-)-epicagallocatechin-5,7-digallate and (-)-epigallocatechin-7-gallate. He observed that (-)-epigallocatechin-5,7-digallate demonstrated more activity than epigallocatechin-7-gallate.

Some plant molluscicides have been studied for the chemical basis of their action. Flavonol glycosides have been reported to have molluscicidal activity (Mott, 1987; Adewunmi & Sofowora, 1980). Therefore, flavonoids present in *S. birrea* and *P. guajava* could have been responsible, at least in part, for the molluscicidal activities of the respective plant extracts. However, further studies on the chemical basis of the molluscicidal actions of these plants are certainly warranted.

PROPOSED MODE OF MOLLUSCICIDAL ACTIVITY

Research on the mode of molluscicidal activity of many compounds has followed two main paths. One is the study of physiology with the aim of explaining molluscicidal activity on molluscan metabolism in the hope that this could then be targeted in developing new molluscicides (WHO, 1992). This has not been successful yet, but with current molecular approaches, targeting specific enzymes in the snail metabolic pathway is promising. For example, the xenobiotic metabolizing enzymes, glutathione-S-transferase (GST) and esterases, recently demonstrated as the

antioxidant enzymes in freshwater snails, offer a potential target for designing new molluscicides.

The second approach has been bioassaying groups of structurally-related compounds for molluscicidal activity as a means of determining the structure-activity relationships. This has indicated some properties required for molluscicide molecules and led to the discovery of niclosamide, which is the most potent and safest synthetic molluscicide in use today (WHO, 1992). Some of the extracts screened in this study caused snails either to retract into their shells, expel haemolymph or become swollen, and remain extended from the shell opening. This last behaviour suggests loss of water-balance control, which is associated with certain carbamides.

Poisoning which caused the snails to remain extended from the shell opening could be due both to action on central nervous system and inhibition of the enzymatic activities of the snails (Kela *et al.*, 1989). Water balance is thought to be under neuro-secretory control, and compounds such as copper sulphate, which have molluscicidal activity, act in this way. Water flux in the snails falls in the presence of a number of molluscicides at concentrations around their LD₅₀ values. Molluscicides appear to cause stress on the water-balance system, which is lethal to snails. Reduction in water flow in the snail also precipitates other disturbances in metabolism or physiological functions, leading to death. Poisoning which caused the snails to expel haemolymph, could be due to destruction of the blood system. The cytotoxic flavonoids present in *Sclerocarya birrea* extract probably acted via this mechanism.

In a study conducted by Appleton (1985), it was indicated that the problem with bayluscide® is that it is psicidal at concentrations recommended for killing snails

(0.3-0.5 ppm). Large numbers of fish, mostly *Labeo* and *Cyprinus* species died within 10-20 minutes of spraying, whereas the bottom-living *Clarias* continued to float to the surface for up to 4 days afterwards. A great deal of time thus had to be spent collecting and burying dead fish. Furthermore, the presence of rotting fish at localities used for stock watering by farmers may result in a marked deterioration in the spray-teams public image. Therefore, toxicity assay against fresh water organisms in order to obtain a rough estimate of the ecological tolerance of plant extracts is important. It is strongly recommended that the toxic effects of extracts against fish and other animals in the water be investigated so as to determine the right concentrations, especially for use in fish ponds. However, it should also be taken into consideration that different species of fish differ in their susceptibility to molluscicides.

The results obtained in this study show that the extracts were toxic to brine shrimps at 1000 ppm. *Sclerocarya birrea* showed the highest molluscicidal activity and it has also been reported to have cytotoxic properties. The fact that it demonstrated toxicity to brine shrimps at 1000 ppm only, whereas relatively safe at lower concentrations, suggest that its cytotoxic properties may not be solely responsible for its molluscicidal activity. *Psidium guajava*, did not show 100 % mortality of the brine shrimp even at 1000 pm. This may mean that *P. guajava* extracts are relatively safe for use in fish ponds, even at high concentrations.

CHAPTER SIX

CONCLUSION

The choice of a biological material to be screened for active compounds and subsequent development of a drug must take into account that the exploration of natural resources should meet global and regional needs for new, efficient and safe drugs (Rates, 2001). The present situation of exploitation of the world's vegetation may lead to the extinction of some species. Therefore, sensible use of these resources must be based on amounts available, ease of access, the possibility of preservation and replanting, and the establishment of priorities in relation to a desirable pharmacological activity. By following this principle, a new understanding of sustainable development will emerge.

Pre-requisites for a viable candidate plant molluscicide are that, the crude extract from which the compounds are obtained should have an activity at concentrations lower than 100 ppm; the plant should grow abundantly in the endemic area; regenerating plant parts should be used and if possible not the roots, since this leads to destruction of the plant; extraction of the active constituents by water is an advantage; application procedures should be simple and safe to the operator; the plant extract or molluscicide should possess low toxicity to non-target organisms and cost should be low (Marston *et al.*, 1993).

Effective exploitation of a plant-derived compound depends on a sufficient supply of the plant material. In the case of *Sclerocarya birrea* and *Psidium guajava*, which grow

abundantly in tropical and subtropical regions, exploitation seems to be feasible. For *Psidium guajava* since only the leaves are needed for snail control, the plant would not need to be destroyed to obtain molluscicidal preparations.

The supply of *Scelerocarya birrea* and *Psidium guajava* could be ensured by multipurpose exploitation of the plants. The fruits of *P. guajava* are used commercially because of their rich vitamin C content. 'Marula tree' is also widely used commercially to brew a refreshing and intoxicating drink. It is also used in furniture making. Since all morphological parts of *Scelerocarya birrea* are used in traditional medicine, for example, for their antibacterial and antidiarrhoea properties; and the leaves of *Psidium guajava* are also used to treat diarrhoea, cultural acceptance to the use of the plants for controlling schistosomiasis is unlikely to be a problem.

The known plant molluscicides are a diversity of secondary metabolites representing a wide range of chemical structures. Of the many species of plants belonging to the families which show a notable level of molluscicidal activity, only a few can be short-listed as candidate agents (Dossaji *et al.*, 1998). Their mode of action is not understood. However, finding the relationship between molluscicidal activity and snail metabolism may provide the potential for discovering new agents, given the structural types of the plant molluscicides so far identified.

The present study revealed potent molluscicidal extracts from *Psidium guajava*, and yet it is not used as a molluscicide. These findings probably indicate the great potential of plants as sources of molluscicides. However, the issue today is whether to encourage further prospecting for molluscicides in plants or to pursue what is already

discovered and attempt to improve on their potency while at the same time reducing their toxicity. Attempts to elucidate the mode of action of known natural compounds may enable the development of more effective molluscicides with less toxicity to non-target organisms.

Toxicological investigations of the active extracts on fish and other organisms in the ecosystem are strongly recommended with a view to determining suitable mollusciciding concentrations, especially for plants that are established fish poisons. This, together with other toxicity tests such as cercariacidal, larvicidal and ovicidal effects may lead to selection of more potent, naturally-occurring plant molluscicides of acceptable efficacy for future integrated controls of snails and snail-borne diseases.

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Appendix 1

Table 1. Dates and location where plant materials were collected.

PLANT NAME & MATERIAL	LOCATION	DATE OF COLLECTION
<i>Sclerocarya birrea</i> leaves & stem-bark	Opposite Biomedical Resource Centre, UDW	April 2002
<i>B. racemosa</i> fruits	Opposite Biomedical Resource Centre, UDW	March 2002
<i>P. guajava</i> leaves (white fruits)	Road to Bio kinetic Building, UDW	May 2002
<i>P. guajava</i> leaves (hybrid)	Opposite T-junction to Sport centre, UDW	May 2002
<i>R. caffra</i> leaves	Opposite Biomedical Resource Centre, UDW	April 2002
<i>J. curcas</i> leaves	Durban Botanical gardens	May 2002
<i>L. leonurus</i> aerial parts	Collected by Pharmacy students	June 2002
<i>R. communis</i> seeds	Between Derby Downs building and UDW main gate	September 2002
<i>E. capensis</i> stem-bark & leaves	Along the road to Health Sciences Building (opposite Microbiology Building). UDW.	October 2002
<i>S. frutescens</i> aerial parts	Bought from Herbarium	October 2002



Table 2. Weights of the crude extracts (aqueous and methanolic) of the plants tested for molluscicidal activity

Plant name/part used/ code	Weight of methanol extracts (grams)	Weights of aqueous extracts (grams)
<i>Sclerocarya birrea</i> stem-bark (WC/21D/E)	107.07	41.63
<i>Sclerocarya birrea</i> leaves (WC/15/D/E)	43.95	21.7
<i>B. racemosa</i> seeds (WC/5/D/E)	55.89	22.52
<i>B. racemosa</i> pericarp (WC/6/D/E)	19.05	8.11
<i>P. guajava</i> leaves (white fruits) (WC/23/D/E)	30.67	11.5
<i>P. guajava</i> leaves (hybrid) (WC/25/D/E)	65.89	23.04
<i>R. caffra</i> leaves (WC/24/D/E)	36.95	35.35
<i>J. curcas</i> leaves (WC/20/D/E)	1.35	2,06
<i>L. leonurus</i> aerial parts (WC/26/D/E)	10.53	8.71
<i>E. capensis</i> stem-bark (WC/219/D/E)	25.263	21.495
<i>E. capensis</i> leaves (WC/218/D/E)	3.65	12.90
<i>R. communis</i> seeds (WC/17/D/E)	1.01	0.68
<i>S. frutescens</i> aerial parts (WC/16/D/E)	-	5.48

Appendix 2

Table1. Average percentage dead of snails* exposed to *Sclerocarya birrea* extracts

Average percentage of dead snails				
DILUTIONS (ppm)	STEM BARK		LEAVES	
	METHANOL	AQUEOUS	METHANOL	AQUEOUS
10	0	0	0	0
20	0	0	0	0
40	23.33±5.77	16.67±15.27	0	0
80	53.33±5.77	43.33±5.77	0	0
100	66.67±5.77	73.33±5.77	0	0
200	100±4.71	83.33±5.77	0	0
400	100	100	0	0
800	100	100	20	0
1000	100	100	20	0

Table2: Average percentage of dead snails* exposed to *Psidium guajava* leaf extracts

Average percentage of Dead snails				
DILUTIONS (ppm)	Hybrid		White fruits	
	METHANOL	AQUEOUS	METHANOL	AQUEOUS
10	0	0	0	0
20	0	0	0	0
40	0	0	0	0
80	26.67±4.71	0	0	0
100	50	0	0	0
200	83.33±4.71	0	0	0
400	100	0	0	0
800	100	0	0	0
1000	100	20	17	3.33

Table3. Average percentage of dead snails* exposed to *Leonotis Leonurus* extracts

Average percentage of Dead snails		
DILUTIONS (ppm)	METHANOL	AQUEOUS
10	0	0
20	0	0
40	0	0
80	0	0
100	10	0
200	36.67±20.54	0
400	53.33	0
800	93.33	0
1000	93.33	3.33

Table4: Average percentage of dead snails* exposed to *Ekebergia capensis* extracts

Average percentage of dead snails				
DILUTIONS (ppm)	STEM BARK		LEAVES	
	METHANOL	AQUEOUS	METHANOL	AQUEOUS
10	0	0	0	0
20	0	0	0	0
40	0	0	0	0
80	0	0	0	0
100	16.67±5.77	0	0	0
200	23.33±5.77	0	0	0
400	26.67±4.71	0	0	0
800	76.67±5.77	3.33	0	0
1000	83.33±5.77	26.67	3.33	0

Table 5: Average percentage of dead snails* exposed to *Rauvolfia caffra* leaf extracts

Average percentage of Dead snails		
DILUTIONS (ppm)	METHANOL	AQUEOUS
10	0	0
20	0	0
40	0	0
80	0	0
100	0	0
200	0	0
400	0	0
800	0	0
1000	0	0

Table6: Average percentage of dead snails* exposed to *Sutherlandia frutescens* leaf extracts

Average percentage of Dead snails		
DILUTIONS (ppm)	METHANOL	AQUEOUS
10	0	0
20	0	0
40	0	0
80	0	0
100	0	0
200	0	0
400	0	0
800	0	0
1000	0	0

Table7: Average percentage of dead snails* exposed to *Barringtonia racemosa* extracts

Average percentage of Dead snails				
DILUTIONS (ppm)	PERICARP		SEEDS	
	METHANOL	AQUEOUS	METHANOL	AQUEOUS
10	0	0		0
20	0	0	0	0
40	0	0	0	0
80	0	0	0	0
100	0	0	0	0
200	0.33	0	0	0
400	0.33	0	0	0
800	20	0	3.33	0
1000	43.33	0	6.67	0

Table 8. Average percentage of dead snails* exposed to *Ricinus communis* seeds extracts

Average percentage of snails (dead)		
DILUTIONS (ppm)	METHANOL	AQUEOUS
10	0	0
20	0	0
40	0	0
80	0	0
100	0	0
200	0	0
400	0	0
800	0	23.33
1000	3.33	23.33

Table 9: Average percentage of dead snails* exposed to *Jatropha curcas* extracts

Average percentage of snails (Dead)		
DILUTIONS (ppm)	METHANOL	AQUEOUS
10	0	0
20	0	0
40	0	0
80	0	0
100	0	0
200	0	0
400	0	0
800	0	0
1000	16.67	0

* *Biomphalaria* and *Bulinus* snails.