Chapter 1

Introduction, hypotheses, aims and objectives

1.1 Introduction

1.1.1 Serious underestimation of plant-derived medicines

The importance of plant-derived medicines is seriously underestimated in modern medicine. Only approximately 15% of the Angiosperms (flowering plants) have been chemically investigated for their medical potential (Farnsworth, 1966; Farnsworth and Soejarto, 1991). Of the 300 plant species tested by Noristan Pty, Ltd. (Pretoria, RSA), 31% displayed high activity (activity being: analgesic, anti-inflammatory, anti-hypertensive, antimicrobial, antifungal, anti-ulcer, antagonism of acetylsalicylic acid induced gastric damage, narcotic analgesic, anti-convulsent, anti-depressant, anti-arrhythmic, diuretic and general toxicological and central nervous system effects), 48% were moderately active and 21% had no activity (Fourie *et al.*, 1992).

Plant secondary compounds are frequently associated with plant taxons. From data provided by Cunningham (1990), Eloff (1998) calculated that while the Combretaceae is a relatively small family the scale of use in KwaZulu-Natal is large, relative to most other plant families. This suggests that the Combretaceae plant family has the potential to offer novel or alternative phytomedicines. The intent of this research was therefore to investigate the assumed bioactivity potential of relatively unknown members in the Combretaceae namely; *Pteleopsis myrtifolia* and *Quisqualis littorea.*

1.1.2 Tendency towards unrefined or “natural’, assuming non-toxic

The majority of people from rural communities, also including some shack dwellers on the outskirts of cities, depend entirely on herbal medicines for their health. Due to increased

awareness of toxicities in refined products amongst modern city dwellers, individuals are progressively focusing their attention towards herbal medicine in an effort to find an alternative approach to living healthier. This is indicated by the steady increase in the number of retail outlets who solely merchandise herbal medicine and conventional pharmacies who stock herbal medicines. The United Nations Development Programme predicts (UNDP, 2002), that despite the severe impact of HIV/ AIDS, the African continent will still experience a slight growth in its human population (0,4% instead of 1,4%). The demand on natural resources will therefore not decline, but maintain the current pressure of intrusion into natural habitat areas of phytomedicines. A greater demand for land in terms of pastoral agriculture and commercial development will therefore reduce the available areas for conservation and subsequently impact negatively on areas for medicinal plants. A dynamic and clear cut plan for cultivating and conserving medicinal plants, as well as more knowledge about the Plant Kingdom's medicinal properties (many of which are undiscovered yet), urgently need to be implemented to ensure their future beneficial use.

In addition to identifying phytomedicines which can offer solutions to modern day diseases like AIDS and certain cancers, increased knowledge about phytomedicines can:

- serve as alternative solutions where orthodox medicines have limitations, for example antibiotics (in cases of antibacterial-drug-resistance), and anticancer drugs from plants, like tubulin polymerization inhibitors (which is less toxic than current anti-cancer drugs, such as Actinomycin D) and;
- provide man with necessary knowledge to avoid or minimize unwanted side effects from toxicities resulting from use of herbal medicines.

1.1.3 Ethno-medicinal uses represent leads for discovery of modern drugs

Folk or ethno-medicinal uses represent leads that may guide pharmaceutical researchers to

discover modern therapeutic drugs. Scepticism may still exist amongst some researchers (especially individuals who strongly advocate the traditional Western approach to medicine), regarding indigenous folk traditions that have been handed down from generation to generation. However, many important modern compounds for example, atropine, digitoxin, *d*-tubocurarine, ephedrine, morphine, reserpine and many other are of natural origin. Indeed, some have been discovered through following up leads derived from ethnographic research into folk use and information on integration of the indigenous people with their ecosystem. For example, the discovery of thiarubrine-A, a potent antibiotic, from the genus *Aspillia* (Compositae), took place by careful observation of chimpanzees' dietary habit (Rodriguez *et. al*, 1985). Winter (1955) investigated antimicrobial properties of two groups of plants. One group was randomly selected and the other obtained from Herbal Remedy sources, which was previously documented to have properties that are useful for the treatment of infections. Of the former randomly selected plants only 29,5% exhibited antimicrobial activity, while 65% of the latter selected group was active.

1.1.4 Expansion of human civilizations pose a threat to plant biomes

The rapid expansion of human civilization and its health care needs, poses a threat to certain plant biomes (such as rain forests) and human cultures living in and around such biomes. Their disappearance will be accompanied by the extinction of medicinal genetic resources as well as folk knowledge about how to use them for existence and survival. In each of the nine provinces of South Africa, savannas, grassland, forests, fynbos and karoo (to mention a few) are gradually being replaced by agriculture and urban development. These will deplete plant biomes from their resources and in future, they will virtually become extinct. Time is running short to investigate these biomes before they become extinct.

1.1.5 Plants' extraordinary ability to synthesize secondary metabolites

Plants' defence mechanisms are sophisticated which allow them to survive even though they have a sedentary existence. They have to be able to defend themselves against all the hazards to be able to survive. They do this with an enormous variety of secondary metabolites that they synthesize. Several tens of thousands of secondary metabolites have already been isolated and their structures elucidated (Wink, 1999).

The main roles of secondary metabolites have been identified to be:

- defence against herbivores (insects, vertebrates),
- defence against fungi and bacteria,
- defence against viruses,
- defence against other plants competing for light, water and nutrients,
- signal compounds to attract pollinating and seed dispersing animals,
- signals for communication between plants and symbiotic microorganisms (N-fixing Rhizobia or mycorrhizal fungi) and;
- protection against UV-light or physical stress (Wink, 1999).

1.1.6 Bioactive properties derived from plants

Large numbers of surveys have been conducted in which plant extracts have been evaluated for various biological activities. Only a small sample of species are listed in Table 1.1:

Table 1.1. Plants with medicinal uses indicating biological activity, drug name, type of extract or plant part, plant species and reference.

Biological activity	Drug/ extract/ plant part	Plant name	Reference
Antineoplastic	Combretastatin A-4 and B-1 (Subjects of patents)	*Combretum caffrum* *Combretum kraussi*	Pettit *et al*. (1987) Pettit *et al*. (1995)
Antibacterial	Juice	*Vaccinium* spp. (cranberry)	Ofek *et al*. (1996)
Antifungal	Grapefruit peel	*Citrus paradisa*	Stange *et al*. (1993)
Antiviral - AIDS	Glycyrrhizin (flavonoid)	*Glycyrrhiza rhiza*	Watanbe *et al*. (1996)
Antimalarial (plasmodium)	Solvent extract	*Mahonia aquifolia*	Omulokoli *et al*. (1997)
Insecticide	Phenantrenes	*Combretum apiculatum*	Malan and Swinny (1993)
Molluscicide Schistisomiasis	Mollic acid	*Combretum molle*	Rogers (1989)
Hypoglycemic	Leurosine sulphate (alkaloid)	*Catharanthus roseus*	Svoboda *et al*. (1964)
Cardiotonic activity	Extract	*Carissa sp.*	Thorpe and Watson (1953)
Andro- or Estrogenic	Extract	*Butea superba*	Schoeller *et al*. (1940)
CNS	Morphine	*Papaver somniferum*	Schmitz (1985)
Antihelminthic	Dried nuts	*Quisqualis indica* *Combretum molle*	Ladion (1985) Rogers and Verotta (1997)

1.1.7 Compounds from plants that regulate or participate in disease resistance

Plants have developed sophisticated active defence mechanisms against infectious agents (Barz *et al*., 1990). The main aim of these reactions appears to be inhibition of microorganisms with antibiotic compounds, hydrolytic enzymes, inactivation of microbial exoenzymes with specific inhibitors and isolation of lesions. These defence mechanisms operate at different stages of infection (Kuć, 1990a). The external plant surfaces are often covered with biopolymers (fatty acid esters) that are difficult to penetrate. In addition, external surfaces can be rich in compounds (phenolic compounds, alkaloids and steroid glycoalkaloids) that will inhibit

the development of fungi and bacteria (Reuveni *et al*., 1987). Once pathogens have passed the external barriers, they may encounter plant cells that contain sequestered glycosides (Kuć, 1990b). The glycosides may be antimicrobial *per se* or may be hydrolysed to yield antimicrobial phenols; these in turn may be oxidised to highly reactive quinones and free radicals (Noveroske *et al*., 1964). Damage to a few cells may rapidly create an extremely hostile environment for a developing pathogen. This rapid, but restricted disruption of a few cells after infection can also result in the biosynthesis and accumulation of low molecular weight antimicrobial, liphophylic compounds, called phytoalexins.

Phytoalexins differ in structure, with some structural similarities within plant families (Carr and Klessig, 1989). Some are synthesized by the malonate pathway, others by the mevalonate or shikimate pathways, whereas still others require participation of two or all three of the pathways (Kuć, 1990b). Phytoalexins can induce constitutive or other secondary metabolite pathways and link to various metabolic pathways (Barz *et al*., 1990). Since phytoalexins are not translocated, their protective effect is limited to the area of the infection, and their synthesis and regulation are accordingly restricted. Phytoalexins are degraded by some pathogens and by the plant; thus, they are transient constituents and their accumulation is a reflection of both synthesis and degradation.

Often associated with phytoalexin accumulation is the deposition around sites of injury or infection of biopolymers, which both mechanically and chemically restrict further development of pathogens (Hammerschmidt and Kuć, 1982). These biopolymers include: lignin, a polymer of oxidized phenolic compounds; callose, a polymer of β-1,3-linked glucopyranose; hydroxy-proline-rich glycoproteins, and suberin. The macromolecules produced after infection or some forms of physiological stress include enzymes, which can hydrolyse the walls of some pathogens (Carr and Klessig, 1989), including chitinases, β-1,3-glucanases and proteases.

Unlike the phytoalexins and structural biopolymers, the amounts of these enzymes increase systemically in infected plants even in response to localized infection. They are often found intercellular where they would contact fungi and bacteria. These enzymes are part of a group of stress or infection-related proteins commonly referred to as pathogenesis-related (PR) proteins. The function of many of these proteins is unknown. Some may be defence compounds; others may regulate the response to infection (Tuzun *et al.*, 1989).

Another group of systemically produced biopolymer defence compounds comprises the peroxidases and phenoloxidases (Hammerschmidt *et al.,* 1982). Both can oxidize phenols to generate protective barriers to infection, including lignin. Phenolic oxidation products can also cross-link to carbohydrates and proteins in the cell walls of plants and fungi to restrict further microbial development (Stermer and Hammerschmidt, 1987). Peroxidases also generate hydrogen peroxide, which is strongly antimicrobial. Associated with peroxidative reactions after infection is the transient localized accumulation of hydroxyl radicals and superoxide anion, both of which are highly reactive and toxic to cells. Both plant and microbial compounds regulate the expression of genes that encode products that contribute to disease resistance. The speed and degree of gene expression and the activity of the gene products (and not the presence or absence of genes for resistance mechanisms) determine disease resistance in plants (Kuć, 1990b).

The future will probably see the restriction of pesticide use and a greater reliance on resistant plants generated using immunization and other biological control technologies, genetic engineering and classical plant breeding. However, as with past and current technology, we may create unique problems. The survival of our planet may significantly depend upon anticipating these problems and meeting the challenge of their solution.

1.1.8 Antibacterial resistance – research to find alternative or natural antibiotics remains a matter of urgency

We are largely dependant on the pharmaceutical industry to continue to provide us with new antimicrobial agents to which bacteria have not yet developed resistance. Antibiotic resistance, which resulted from the frequent and unwise use of antibiotics, is a problem in hospital environments and can lead to the spread of resistant strains to communities. Resistance is determined by the bacterial genome, which may change rapidly (Berkowitz, 1995). A 'new' antibiotic may have a limited time in which bacteria have developed little or no resistance to it; thus the search for new antibiotics remains a continuous urgent priority.

The most common bacterial pathogens causing nosocomial infections are *Escherichia coli* (commonest pathogen in adult services), *Staphylococcus aureus* (commonest pathogen in paediatric and newborn services), *Enterococcus faecalis* (antibiotic resistant, some also against Vancomycin) and *Pseudomonas aeruginosa* (Sacho and Schoub, 1977). Despite the availability of a wide range of antibiotics (e. g. penicillin, cephalosporins, tetracycline, amino-glucosides, monobactams, carbapenems, macrolides, streptogranins and dihydrofolate reductase inhibitors), the percentage of people who die in hospitals is steadily increasing, because of resistant bacterial infections. Table 1.2 lists resistance mechanisms of pathogens to antimicrobial agents.

Table 1.2. Major resistance mechanisms of pathogens to antimicrobial agents (Jacoby and Archer, 1991).

Type of antimicrobial class	Specific resistance mechanism of pathogen
Quinolones	Altered DNA gyrase
Rifampicin	Altered RNA polymerase
Sulfonamides	New drug-insensitive dihydropteroate reductase
Tetracycline	Ribosomal protection
Trimetoprim	New drug-insensitive dihydrofolate reductase
Vancomycin	Altered cell wall stem peptide
Main action of antimicrobial and antibiotic that addresses it	**Specific resistance mechanism of pathogen**
Detoxifying enzyme	
Aminoglucosides (amikacin, gentamycin, kanamycin, netilmycin, tobramycin)	Acetyltransferase, nucleotidyltransferase, Phospho transferase
B-Lactam antibiotics (carbapenems, cephalosporins, monobactams, penicillin)	B-Lactamase
Chloramphenicol	Acetyltransferase
Decreased uptake	
Diminished permeability	
B-lactam antibiotics, chloramphenicol, quinolones, tetracycline, trimethoprim	Alterations in outer membrane proteins
Active efflux	
Erythromycin	New membrane transport system
Tetracycline	New membrane transport system

1.1.8.1 Useful antimicrobial phytochemicals

Useful antimicrobial phytochemicals can be divided into several categories, as shown in Table 1.3.

Table 1.3. Major classes of antimicrobial compounds from plants (Cowan *et al*., 1999).

Class	Subclass	Example(s)	Mechanism	References
Phenolics	Simple phenols	Catechol Epicatechin	Substrate deprivation Membrane disruption	Peres *et al*. (1997) Toda *et al*. (1992)
	Phenolic acids	Cinnamic acid		Fernandez *et al*. (1996)
	Quinones	Hypericin	Bind to adhesins, complex with cell wall, Inactivate enzymes	King and Tempesta (1994)
	Flavonoids	Chrysin	Bind to adhesins	Perrett *et al*. (1995)
	Flavones	Abyssinone	Complex with cell wall Inactivate enzymes Inhibit HIV reverse transcriptase	 Brinkworth *et al.* (1992) Ono *et al*. (1989)
	Flavonols	Totarol	?	Kubo *et al*. (1993)
	Tannins	Ellagitannin	Bind to proteins Bind to adhesins Enzyme inhibition Substrate deprivation, complex with cell wall, Membrane disruption, metal ion complexation	Stern *et al*. (1996) Scalbert (1991) Haslam (1996)
	Coumarins	Warfarin	Interaction with eucaryotic DNA (antiviral activity)	Bose (1958)
Terpenoids, essential oils		Capsaicin	Membrane disruption	Cichewicz and Thorpe (1995)
Alkaloids		Berberine Piperine	Intercalate into cell wall/ or DNA	Rahman and Choudhary (1995)
Lectins and polypeptides		Mannose-specific agglutinin Fabatin Polyacetylenes	Block viral fusion or adsoption 8S-Heptadeca-2(Z),9(Z)-diene-4,6-diyne-1,8-diol	Zhang and Lewis (1997) Estevez-Braun *et al*. (1994)

In recent studies, several antibacterial compounds were isolated in the Combretaceae plant family, some for the first time from this family. The flavanols: kaemferol, rhamnocitrin, rhamnazin and quercitin 5,3'-dimethylether and flavones apigenin, genkwanin and 5 hydroxy-7,4'-dimethoxyflavone were isolated from *Combretum erythrophyllum* (Martini, 2002), and the stilbene, 2',3,4-trihydroxyl,3,5,4'-trimethoxybibenzyl (combretastatin B5) from *C. woodii.* This is the first report of antimicrobial activity of combretastatin B5 (Famakin, 2002). Two flavanones: alpinentin, pinocembrin and one chalcone: flavokwavain were isolated from *C. apiculatum* subsp. *apiculatum* (Serage, 2003). Terminoic acid isolated from *Terminalia sericea*, was compared to commercial gentamycin cream for use as a topical antibacterial remedy on mice's skin by Kruger (2004).

1.1.9 Plant compounds' role in the treatment of cancers

Some confusion exists in the use of the terms 'cytotoxicity', 'antineoplastic' and 'antitumour'. The National Cancer Institute (NCI) has defined these terms: cytotoxicity refers to *in vitro* toxicity of tumour cells, while antineoplastic and antitumour should refer to *in vivo* activity in experimental systems (Ghisalberti, 1993). Between 1955 and 1982, NCI screened 35 000 plant species representing 1551 genera compromising 114 000 extracts for *in vitro* cytotoxicity and *in vivo* activity against various animal tumour systems (Hamburger *et al*., 1991). Estimates indicate that there are approximately 250 000 terrestrial species of higher plants. Since plants have four or five different plant parts, a comprehensive screening program would require a million or more samples per assay. Approaches that are more pragmatic are usually followed (for example: leads from ethno-medicines or chemotaxonomy). Existing *in vivo* test systems, for example, xenografts on immune deficient mice, are far too slow, complex, expensive and probably immoral to be used as a mass screen. Using cells derived from human cancers in an *in vitro* setting, on the other hand, is quite compatible with the desired goal (Lednicer and Narayanan, 1993). Plant constituents able to kill cancer cells, and hence described as being

"cytotoxic" exhibit a very large range of structural types. The presence of tannins or other polyphenolic materials should be taken into account when enzyme-based bioassays are being used, because false positive results are often observed (Cordell *et al*., 1993).

Conventional anti-cancer drugs are designed to arrest and kill rapidly dividing cancer cells. They are however, non-selective, chemotherapy and radiation will kill both normal and tumour cells therefore, drugs with selective pharmacodynamics are sought after. A number of secondary metabolites and their derivatives of plant origin, as well as natural products of marine and microbial origin are currently in preclinical and clinical trials as potential anticancer agents. One such is 'combretastatin' from the Combretaceae plant family. It was isolated from the bark of the South African *Combretum caffrum* by Dr Gordon Gragg (an ex-South African organic chemist) in the laboratories of Prof Pettit at Arizona State University (Pettit *et al*., 1982). Since the yield was very low – 26,4 mg was isolated from 77 kg dry stem bark – several forms were synthesized and tested. Experiments examining the effect of combretastatin A4 and combretastatin A4 phosphate on murine tumours demonstrated that combretastatin A4 phosphate caused selective extensive vascular shutdown of tumours (more detail in 1.1.9.2). The vascular shutdown was followed by large-scale cell death and necrosis within 24 h after administration (Chaplin *et al.*, 1999).

In Tanzania 47 plants were evaluated for cytotoxic activity by testing their methanolic extracts on three human cancer cell lines. Of the nine plants traditionally used to treat cancer, only two exhibited a cytotoxic effect. Of the 38 plants that are used to treat non-cancer diseases, 14 exhibited a cytotoxic effect. *Pteleopsis myrtifolia* was one of the plants not traditionally used to treat cancers that had cytotoxic effects: at 100 μg/ml *in vitro* 75-100% inhibition of growth was obtained for the HT29 (colon adenocarcinoma) and A431 (skin carcinoma), and 25-50% for HeLa (cervical carcinoma) cells (Kamuhabwa *et al.*, 2000).

Cancer itself creates oxidative stress and impairs antioxidant status in the organism as a whole. Chemotherapy can overwhelm the antioxidant defence systems in the cell, which will lead to an increase in lipid peroxidation, which in turn leads to a decrease in cellular proliferation and therefore to a decrease in the effectiveness of chemotherapeutic agents. Patients with an impaired antioxidant status may become relative resistant to chemotherapy. There is also evidence that antioxidants improve the antitumour response to antineoplastic agents (Drisko *et al.*, 2003).

1.1.9.1 Incidence of cancer

Cancer is the second leading cause of death amongst Americans. One out of every four deaths in the U.S. is due to cancer. Figures for the year 1990 showed that the rate of growth in cancer cases (2,1% per year) was superseding that of the overall population increase (1,7%/year) (Kinghorn *et al.*, 1999). In the United States in 1999, over 1500 people were expected to die of cancer each day. A United States Cancer Report was released (by the Centres for Disease Control and Prevention (CDC), National Cancer Institute (NCI) and North American Association of Central Cancer Registries (NAACCR)) in November 2003 (to date the most current available) with cancer incidence data up to the year 2000. In 1994, U.S. death rates (for all cancer sites combined) decreased up to 1998 and stabilized from 1998 through 2000. Increases in breast cancer amongst woman and prostate cancer amongst men are masked by statistics of a decrease in all cancer sites combined.

1.1.9.2 Combretastatin

When a study on cancer cell growth inhibitors of the African willow tree (*Combretum caffrum*) was carried out, several active phenanthrenes, stilbenes and bibenzyls were isolated. Two potent cell growth and tubulin polymerisation inhibitors, the bibenzyls combretastatin A-1 (Lin *et al*., 1989) and combretastatin A-4 (Pettit *et al*., 1989) were of particular importance. Combre-

tastatin was found to prevent astrocyte maturation (Baden *et al*., 1981) and to inhibit tubulin polymerisation (Boyd, 1993). Table 1.4 show results of an evaluation of the combretastins A-1 to A-6 in the US NCI screens (Boyd, 1993).

Table 1.4. Antitumour evaluation of combretastatins in the NCI *in vitro* panel of 60 human tumour cell lines.

Combretastatins	Mean panel GI_{50} (x 10^{-8}M)
A-1	1.62
A-2	3.16
A-4	0.32
A-5	165.00
A-6	>10000

Combretastatin A-4 has been studied intensively because of its potent cytotoxicity. The drug seems to induce apoptosis of cells, suggesting that it may activate at least one specific intracellular signalling pathway. *In vivo* studies support the suggestion that combretastatin A-4 causes a rapid vascular collapse by increasing tumour vessel permeability (Dark *et al*., 1997).

Although combretastatin A-4 exhibits potent biological activity, it has poor pharmacokinetic properties due to its high lipophilicity and low water solubility (Ohsumi *et al*., 1998). Synthesis of more water-soluble analogues in order to enhance and promote more desirable qualities such as chemical stability, bioavailability and decrease of side effects, are required. Several derivatives have been prepared and were evaluated as pro-drugs, but it was proven to be insoluble in water. The analogues with dipotassium and disodium phosphate (Figure 1.1) had good water solubility.

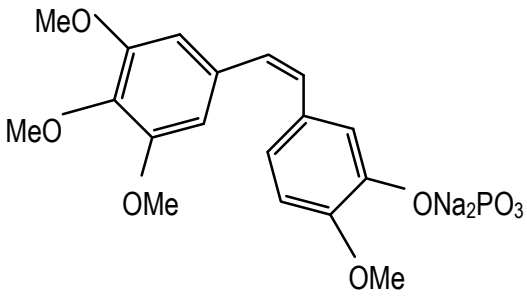


Figure 1.1. Structure of the combretastatin A-4 disodium phosphate analogue.

The combretastatin A-4 disodium phosphate analogue, known as CA4P, is undergoing phase I clinical trials (Dowlati *et al*., 2002) and has potential for cancer treatment in combination with other conventional antitumour drugs (Chaplin *et al*, 1999). CA4P is itself inactive but there is rapid phosphate hydrolysis *in vivo* to produce combretastatin A-4 (Chaplin *et al*, 1996). Histological studies carried out by the Pettit group (Chaplin *et al*., 1999), demonstrated that 90% of vessels were non-functional 6 h post-treatment with 100 mg/kg ip. Further, it showed that normal vessels were unaffected. This selectivity seems to come from a process of *in vivo* phosphate hydrolysis by endogenous non-specific phosphates, with greater rates in tumour vascular systems than in the normal vascular system (Griggs *et al*., 2001).

Since there was considerable interest in the possibility of separating the cytotoxic activity of CA4P from its ability to effect vascular shutdown, analogues have been prepared that do indeed display this type of selectivity (Hadimani *et al*., 2003). Future work in this area will be of interest to those studying the various retinopathies and other vascular diseases (Cirla and Mann, 2003).

1.1.10 Antioxidative properties of plants

Since reactive oxygen radicals play an important part in carcinogenesis, antioxidants present in consumable fruits, vegetables, neutraceuticals and beverages have received considerable attention as cancer chemopreventative agents (Muktar *et al.*, 1994). The balance between an

individual's intake of antioxidants and exposure to free radicals may literally be the balance between life and death (Holford, 1997). Several compounds from plants play cancer preventative roles. The antioxidant activity of several plant constituents, beyond the vitamins, in the form of crude extracts and isolated compounds, has been put into consideration (Gazzani *et al*., 1998). Many phenolic compounds, including flavonoids, have attracted considerable attention because the antioxidant activity thereof has been reported to be more powerful than vitamins, C, E and β-carotene (Vinson *et al*., 1998). Consumption of the flavonoids and their potential significance as antagonists of oxidative stress has been an interesting subject of many investigations. One of the best approaches for discovering new antioxidants, is the screening of plant extracts (Souri *et al*., 2004). Recently it has been found that proanthocyanidins from grape seeds inhibited the activation of mitogen-activated protein kinases (MAPK) and nuclear factor κB (NFκB) pathways in human prostate carcinoma cells, thus preventing cancer (Vayalil *et al*., 2004).

In more recent years, a variety of substances normally included in the diet have come under more critical investigation for the neutraceutical value thereof. The occurrence or lack of certain diseases in specific demographically defined areas of the world led to comparative analysis of the population's diets. Food items known for their antioxidant value have been investigated. Recently studies on tea (the most popularly consumed beverage aside from water and associated with decreased risk of various proliferative diseases such as cancer and arteriosclerosis in humans), provided evidence that green tea catechins, in addition to their antioxidative properties, also effect the molecular mechanisms involved in angiogenesis, extra cellular matrix degradation, regulation of cell death and multidrug resistance (Demeule *et al.*, 2002).

1.1.11 Phytochemistry of the Combretaceae plant family

Medicinal uses from the Combretaceae plant family by traditional healers in Africa have almost exclusively been of species from the genus *Combretum* and to a lesser extent, *Terminalia.* These species have been used for the treatment of a wide range of disorders, but only about 25% percent of the African species of *Combretum* have been subjected to scientific study. With the exception of a few species of *Terminalia, Annogeissus* and *Guiera*, very little have been reported on the phytochemistry of the remaining genera (Rogers and Verotta, 1997). In a preliminary investigation of the antibacterial activity of 27 members of the South African Combretaceae, Eloff (1999) found that other genera in the Combretaceae (like *Pteleopsis* and *Quisqualis*) displayed antibacterial activity similar to that of the *Combretum* genus.

Combretum genera secrete triterpenoid mixtures onto the surface of their leaves and fruit through epidermal trichomes. The anatomy of the trachoma's and the chemical composition of the triterpenoids are both species specific and are of taxonomic importance (Lawton *et al*., 1991). Treatment of leaves from South American and Indian species yielded mixtures of acidic triterpenoids similar to those found in South African species (Rogers, C. B., unpublished data). These results as suggest that their distribution must have been established when continents from Gondwanaland had separated by a significant amount approximately 120 million years ago. The genus *Pteleopsis* occurs in the sub-tribe Pteleopsidinae and comprises 10 spp. Moreover, the genus *Quisqualis* occurs in the sub-tribe Combretiae and comprises 16 spp.

For more information on metabolites isolated in Combretaceae so far, see 3.1.3 of Chapter 3.

1.1.12 Distribution of *Pteleopsis myrtifolia*

Pteleopsis *myrtifolia* (common names - Myrtle Bush willow, 'basterraasblaar' or 'stinkboswilg', Mnepa, Mgoji) of the family Combretaceae occurs in Botswana, Zimbabwe, Angola, Zambia,

Mozambique, Malawi, Tanzania, Kenya and South Africa. In South Africa, the occurrence thereof is north of the Soutpansberg in the vicinities of Messina and Sibasa, in the Punda Milia area in the Kruger National Park and in the North Eastern part of KwaZulu-Natal, from Ndumu and Kosi Bay reaching as far south as the Hluhluwe vicinity (Figure 1.2).

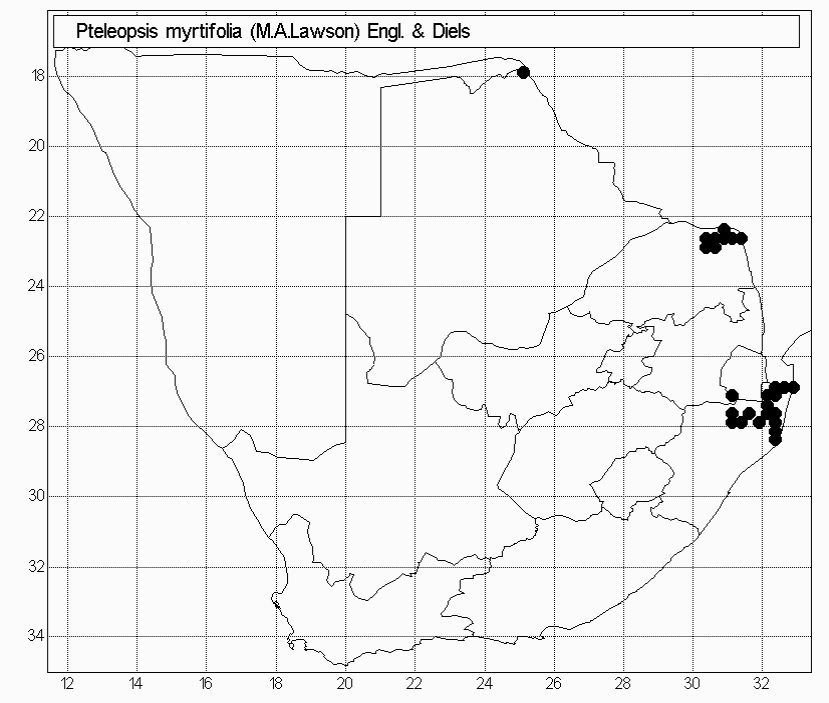


Figure 1.2. Distribution map of *Pteleopsis myrtifolia* (Precis data from SANBI, 2005).

This tree occurs in Brachystegia, Mopane and Baikieae woodland, also in *Acacia* or Combretaceae savannah, evergreen forest and riverine forest, from near sea level up to 1500m. It thrives in sand and under such conditions can be the dominant species. In sand, its growth form can be shrub in dense thickets, reaching 2 – 4 m. In *Acacia* or Combretaceae savannah, its growth form is trees that reach 10 – 12 m (30 m in Tanzania) (Carr, 1988; Van Wyk 1974). The growth form, flowers and fruit of *P. myrtifolia* are shown in Figure 1.3.

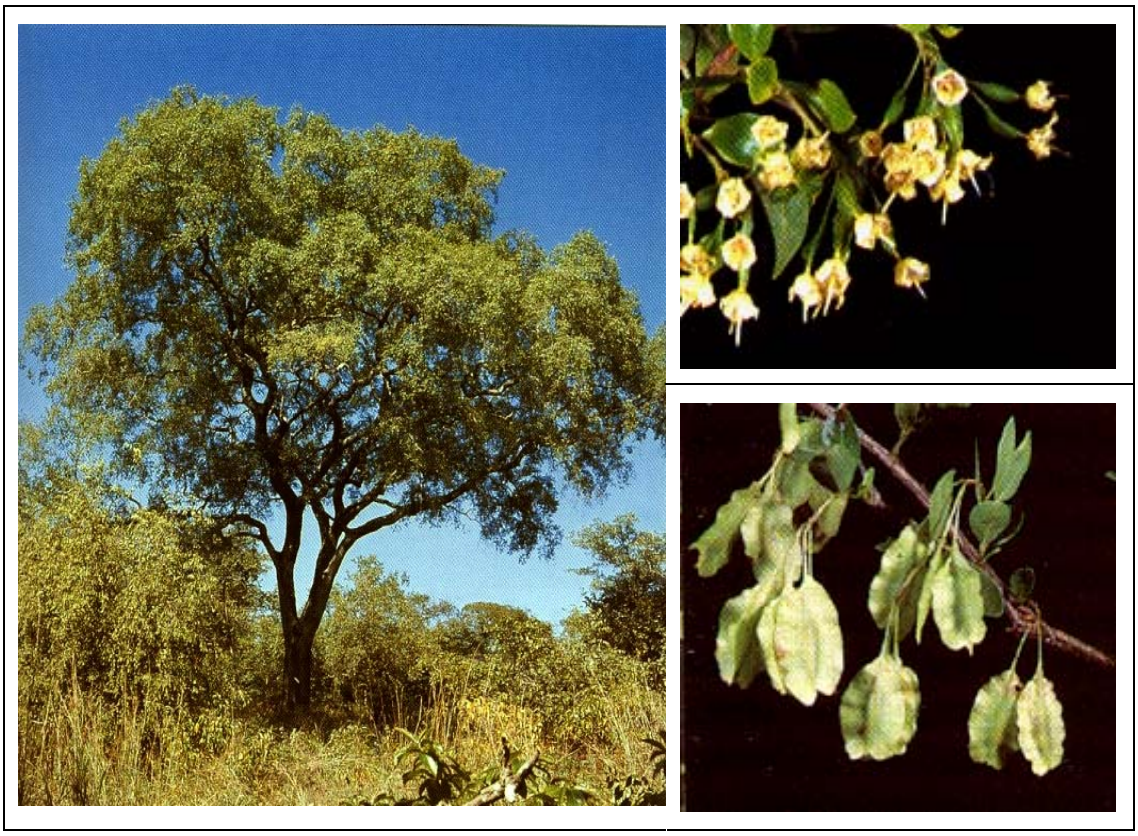


Figure 1.3. *Pteleopsis myrtifolia* tree (left), flowers (top right) and fruit (bottom right) (Van Wyk *et al*., 2000).

1.1.13 Distribution of *Quisqualis littorea*

The genus *Quisqualis* consists of 17 species of woody vines and climbing shrubs native to the Old World tropics. The name is from the Latin '*quis'*, who?, and '*qualis'*, what ? This name was given by the early botanist Rumphius as an expression of his surprise at the variability of the plant's growth and flower colour. *Q. littorea*'s growth form is a climber, it occurs mainly in forests in central Africa, with a few plants in the Laeveld National Botanical Garden (Jongkind, 1993). No distribution map could be found and the South African National Botanical Garden in Pretoria (SANBI) could not provide data for a map.

Blossoms and fruit of *Q. indica* are shown in Figure 1.4.

Figure 1.4. *Quisqualis indica* blossoms (left) and fruit (right) (A Short Story About *Quisqualis* Fruit, 2003).

1.1.14 Bioactivities of *Pteleopsis species*

Pteleopsis suberosa: The aqueous extract of bark has anti-ulcer activity against indomethacin-induced ulcers in rats (De Pasquale *et al.*, 1995). In Mali, it is used locally (10 ml/kg) for the treatment of gastric ulcers. The aqueous extract's mechanism of action appears to be similar to other known triterpenoids (derivatives of glycyrrhetinic acid, a triterpenoid saponin from liquorice roots), now used in treatment of gastric ulcers. The mechanism of action may be due to its coating property that has a protective effect on the gastric mucosa. Sodium carbenoxolone, a triterpenoid related compound, is effective as an anti-ulcer agent because it protects the mucosa from gastric effects by selectively inhibiting prostaglandin $PGF_{2\alpha}$ (Aguwa and Okunji, 1986). The methanolic extract of stem bark was effective (at MIC's of 31.25 – 250 μg/ml) against gastric ulcers associated with *Helicobacter pylori* infections in rat (Germano *et al.*, 1998).

Traditional use of this plant for the treatment of cough and other respiratory diseases was confirmed by the fact that a 1000 mg/ kg dose of a decoction, reduced citric acid induced cough in guinea pigs by 73.72 % (Occhiuto *et al.*, 1999).

Preliminary results of a previous investigation indicated that polar substances were responsible

for antimicrobial activity and that the tannins were also involved in antibacterial activity. The methanolic extracts had antimicrobial activity against some microorganisms that are responsible for skin infections (*Staphylococcus aureus*, *S. capitatis*, *S. epidermidis*, *S. saprophyticus*, *Bacillus subtilus*, *Pseudomonas aeruginosa*, *P. cepacia*, *Cochlospermum tinctorium* but not against *Escherichia coli*, *Proteus vulgaris* and *P. mirabilis*). The antibacterial activity established, possibly justify the traditional use of these plants in folk medicine for treatment of skin diseases (Bisignano *et al.*, 1996). Antifungal activity of shoot and stem-bark samples (MIC's 0.25 – 1 mg/ml) against *Candida albicans*, *Epidermophyton floccosum*, *Microsporum gypseum*, *Trichophyton mentagrophytes* and *Trichophyton rubrum* can be contributed to the tannins and saponins, which occurs richly in these plants (Baba-Moussa *et al.*, 1999).

Pteleopsis hylodendron is highly valued in folk medicine in Cameroon. The aqueous concoction of the stem bark is used in the treatment of sexually transmitted diseases, female sterility, liver and kidney disorders as well as dropsy. The ethyl acetate extract was found to be active against the bacteria *Bacillus cereus*, *Corynebacterium diptheriae*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Streptococcus pyogenes* (Ngounou *et. al*, 1999).

Pteleopsis myrtifolia: Decoctions of these trees' roots have been used by Zulus (Hutchings *et al.*, 1996) and by the traditional healers of Tanzania for venereal diseases (Kokwaro, 1976). Root decoctions and leaf sap have also been used for dysentery (Neuwinger, 2000). Methanolic extracts of roots (that contain many polar compounds) showed cytotoxic activity (100 μg/ml) *in vitro,* 75-100% inhibition of growth against HT29 (colon adenocarcinoma) and A431 (skin carcinoma) and 25-50% inhibition of growth against HeLa (cervical carcinoma) (Kamuhabwa *et al.*, 2000).

1.1.15 Bioactivities of *Quisqualis species*

Quisqualis littorea's synonyms are *Q. falcata, C. falcatum, C. mussaendiflorum, Q. mussaendiflora, C. sericogyne, C. pellegrinianum, Q. pellegriniana* and *Cacoucia littoria* (Jongkind, 1993).

Although Jongkind (1991) suggested that '*Quisqualis*' be incorporated into '*Combretum*', '*Quisqualis*' is still recognised as the official name in South Africa (Germishuizen and Meyer, 2003) and Central Africa (Lebrun and Stork, 1991).

Quisqualis indica is antiviral (at non-cytotoxic concentrations), minimally in the range of 30 – 80 μg plant material/ ml) against the double-stranded DNA murine cytomegalovirus (MCMV) and the single-strand RNA Sindbus virus (SV) (Yip *et al*., 1991). Fruit and roots were found to be antihelminthic (Monzon, 1995). Extracts (50% ethanol and ethanol) inhibited (> 30%) phosphodiesterase (Thein *et al*., 1995). Amoebicidal, antimalarial, antibacterial, and antispastic drugs appear active in phosphodiesterase inhibition tests (Weinryb *et al.* (1972). Further more, it was found to be antibacterial (Nyein and Zaw, 1995), and the acetone extracts of leaves displayed antifungal activity (inhibition of germ-tube elongation) (Ganesan, 1992). Leaf extracts in India were used as a vermifuge (Cirla and Mann, 2003).

Previous investigations of species from the Combretaceae at the University of Pretoria, have isolated and determined the structure and biological characteristics of compounds and extracts. Some extracts had such good activity that commercial applications in the protection of animal health are currently under way. There are a strong probability that similar activities and applications may be discovere*. myrtifolia* and *Q. littorea.*

P. myrtifolia and *Q. littorea* have previously only been included in preliminary investigations (Eloff, 1999; Kamuhabwa *et al*., 2000) and no report of a thorough investigation exists. The

absence of information on *P. myrtifolia* and *Q. littorea* motivated this study.

1.2 Hypothesis, aim and objectives of this study

1.2.1 Hypothesis: *Pteleopsis myrtifolia* and *Quisqualis littorea* are under-evaluated species of the Combretaceae and have antibacterial activity. *P. myrtifolia* has cytotoxic and antioxidant activity as well.

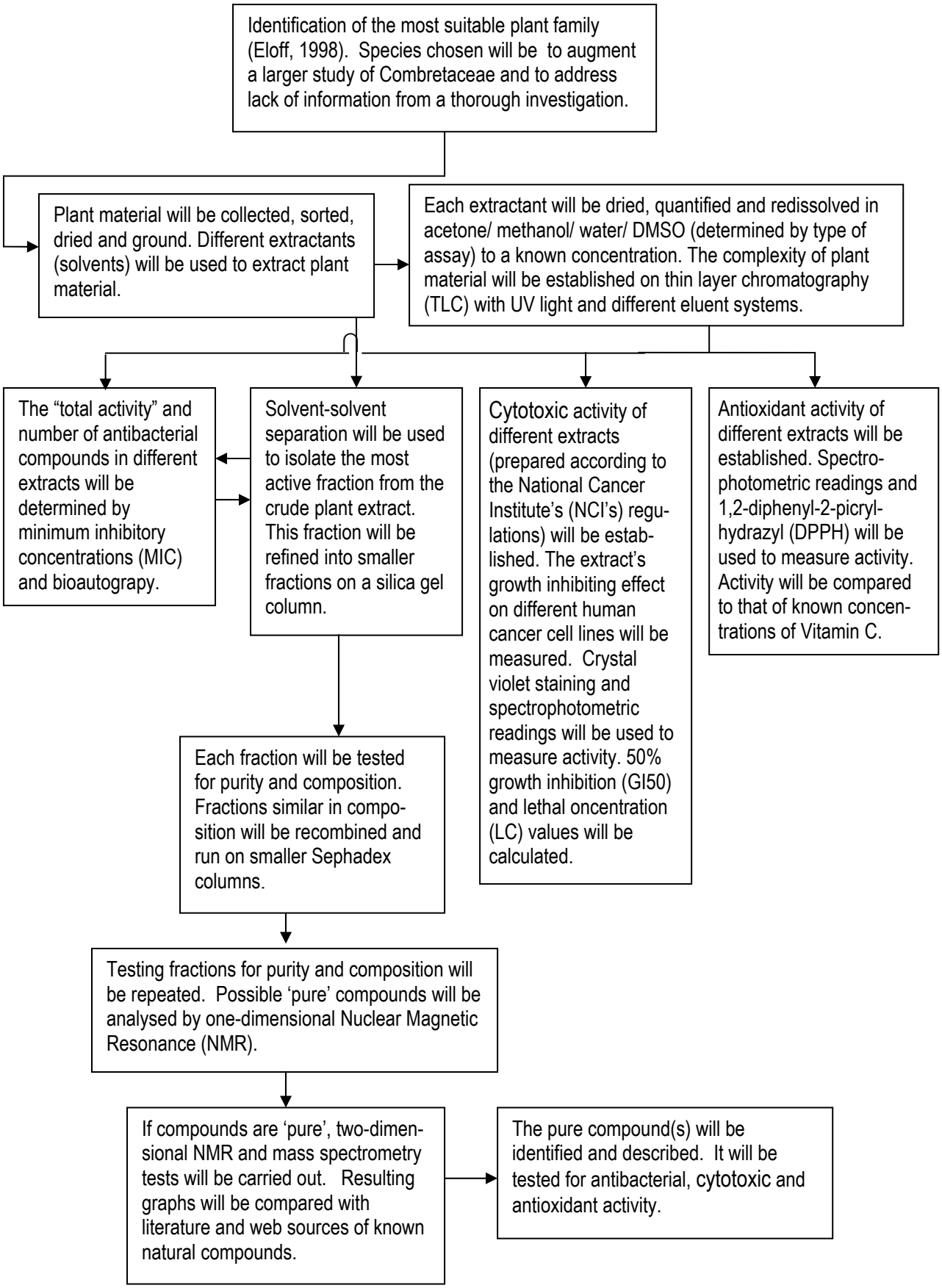
2.2 The aim of this study (as part of a comprehensive project to explore less known genera of the Combretaceae is):

- to investigate extracts of *P. myrtifolia* and *Q. littorea* for antibacterial activity, to investigate extracts of *P. myrtifolia* for cytotoxic and antioxidant activity, as well as to determine the chemical structure and activities (antibacterial, cytotoxic and antioxidant) of possible pure compound(s) isolated by bioassay- guided fractionation from *P. myrtifolia* leaves.

2.3 The objectives of this study are:

- to prepare extracts of *P. myrtifolia* and *Q. littorea* over a wide polarity range in order to identify range in order to identify extracts with antibacterial activity, using *Staphylococcus aureus, Enterococcus faecalis* (Gram-positive); *Pseudomonas aeruginosa* and *Escherichia coli* (Gram-negative) recommended by the National Committee for Clinical Laboratory Standards (NCCLS 1990).
- to investigate cytotoxic activity of different leaf extracts from *P. myrtifolia* on the following human cell lines: oesophagus (WHCO3), breast (MCF-7), lung (H157), cervix (HeLa) (transformed) and breast (MCF12) (non-transformed).
- to identify extracts from *P. myrtifolia* with oxidant scavenging activity.
- to isolate pure compounds from active extracts of *P. myrtifolia* and;
- to investigate antibacterial, cytotoxic and oxidant scavenging activity of pure compounds.

1.3 Schematic representation of the research methodology



1.4 Envisaged contributions of this study:

- Information about antibacterial activity of extracts from *P. myrtifolia* and *Q. littorea* will be determined.
- Cytotoxic activities of different *P. myrtifolia* leaf extracts will be established (Preliminary investigations indicated that *Q. littorea* material would not be sufficient for several assays).
- Antioxidant activities of different *P. myrtifolia* leaf extracts will be determined.
- Possible pure compound's structure from *P. myrtifolia* will be elucidated with NMR and MS. This may contribute to existing knowledge about Combretaceae's phytochemistry.
- Antibacterial, cytotoxic and antioxidant activities of possible pure compounds will be established.
- This knowledge may not only help in the discovery or development of new therapeutic agents, it will also contribute to the knowledge of where new sources of economic viable materials (such as tannins and gums, precursors for the synthesis of complex chemical substances) can be found.
- The results of this research will be submitted as articles (Chapters 2 and 3 combined, Chapter 5, Chapter 6, and Chapters 7 and 8 combined, with some modifications).

1.5 Statistical considerations:

Dr P. Becker from the Medical Research Council (MRC) in Pretoria was involved in the analyses and interpretation of the results. Growth inhibition, lethal concentration and 50% data (growth inhibition of 50% [GI_{50}]) were summarized and displayed graphically (taking into account extraction methods using descriptive statistics, mean and SD). Outcome data was analysed in appropriate analysis of variance for the functional designs of the different experiments. The main effects of the different experiments were cell lines, extraction methodology and concentration. Of great importance was the interpretation of interactions between the factors that were present. Testing was done at the 0.05 level of significance and the Strata Release

statistical software was employed.

1.6 Literature references

A Short Story About *Quisqualis* Fruit, Clearharmony (2003) http://www.zhengjian.org/zj/articles/2003/3/21/20901.html

Aguwa CN, Okunji CO (1986) Gastrointestinal studies of *Pyrenacantha staudtii* leaf extracts. Journal of Ethnopharmacology 15: 45-55

Baba-Moussa F, Akpagana K, Bouchet P (1999) Antifungal activities of seven West African Combretaceae used in traditional medicine. Journal of Ethnopharmacology 66: 335–338

Baden DG, Mende TJ, Lichter W, Wellham L (1981) Crystallisation and toxicology of T34: a major toxin from Florida's red tide organism, *Ptychodiscus brevis*. Toxicon 19: 455-462

Barz W, Bless W, Börger-Papendorf G, Gunia W, Mackenbrock U, Meier D, Otto, C, Süper E (1990) Phytoalexins as part of induced defence reactions in plants: their elicitation, function and metabolism. John Wiley & Sons, Chichester. pp. 140-156. ISBN 04719 26914

Berkowitz FE (1995) Antibiotic Resistance in Bacteria. Southern Medical Journal 88(8): 797–80

Bisignano G, Germano MP, Nostro A, Sanogo R (1996) Drugs used in Africa as Dyes: II. Antimicrobial activities. Phytotherapy Research 10: 161–163

Bose PK (1958) On some biochemical properties of natural coumarins. Journal of the Indian Chemical Society 58: 367-375

Boyd MR (1993) Section I. Introduction to cancer therapy. In: Niederhuber JE, (Ed.) Current Therapy in Oncology. BC Decker, Philadelphia. pp. 11- 22

Brinkworth RI, Stoermer MJ, Fairlie DP (1992) Flavones are inhibitors of HIV-1 proteinase. Biochemical and Biophysical Research Communications 188: 631-637

Cannell RJP (1998) How to approach the isolation of a Natural product. In: Cannell RJP (Ed.) Methods in Biotechnology, Volume 4: Natural Product Isolation pp. 1-87. Humana Press Inc., Totowa. ISBN 0896033627 (quote before contents)

Carr J D (1988) Combretaceae in Southern Africa. Tree Society of Southern Africa, Johannesburg. pp. 173-176.

Carr JP, Klessig DF (1989) The pathogenesis-related proteins from plants. In: Setlow JK (Ed.) Genetic engineering – principles and methods, vol 11. Plenum Press, New York & London, pp. 65-109

Carr JD, Rogers CB (1987) Chemosystematic studies of the genus *Combretum* (Combretaceae). 1. A convenient method of identifying species of this genus by a comparison of the polar constituents extracted from leaf material. South African Journal of Botany 53: 173–176

Chaplin DJ, Pettit GR, Hill SA (1999) Anti-Vascular approaches to Solid Tumour Therapy: Evaluation of Combretastatin A4 Phosphate. Anticancer Research 19: 189-196

Cichewicz RH, Thorpe PA (1995) Antimicrobial properties of chilli peppers (*Capsicum* sp) and their uses in Mayan medicine. Journal of Ethnopharmacology 52: 61-70

Cirla A, Mann J (2003) Combretastatins: from natural products to drug discovery. Natural Products Reports 20: 558-564.

Cordell GA, Kinghorn AD, Pezzutzo JM (1993) Separation, Structure elucidation, and Bioassay of Cytotoxic Natural Products. In: Colegate SM, Molyneux RJ (Eds.) Bioactive Natural Products. CRC Press, Inc., Boca Raton pp. 195-202. ISBN0849334720

Cowan MM (1999) Plant products as antimicrobial agents. Clinical Microbiology Reviews 12: 564-582

Cunningham AB (1990) People and medicines: the exploitation and conservation of traditional Zulu medicinal plants. Mitteilungen der Institute fur Algemeine Botanik, Hamburg 23b: 979–990

Dark GG, Hill SA, Prise VE, Tozer GM, Pettit GR, Chaplin DJ (1997) Combretastatin A-4, an agent that displays potent and selective toxicity toward tumour vasculature. Cancer Research 57: 1829-1834

De Pasquale R, Germano MP, Keita A, Sanogo R, Lauk L (1995) Anti-ulcer activity of *Pteleopsis suberosa*. Journal of Ethnopharmacology 47: 55–58

Demeule M, Michaud-Levesque J, Annabi B, Gingras D, Boivin D, Jodoin J, Lamy S, Bertrand Y, Béliveau R (2002) Green tea catechins as novel antitumour and antiangiogenic compounds. Current Medicinal Chemistry – Anti-Cancer Agents 2(4): 441-462

Dowlati A, Robertson K, Cooney M, Petros WP, Stratford M, Jesberger J, Rafie N, Overmoyer B, Makkar V, Stambler B, Taylor A, Waas J, Lewin JS, McCrae KR, Remick SC (2002) A Phase I Pharmacokinetic and Translational Study of the Novel Vascular Targeting Agent

Combretastatin A-4 Phosphate on a Single-Dose Intravenous Schedule in Patients with Advanced Cancer. Cancer Research 62: 3408-3416

Drisko JA, Chapman J, Hunter VJ (2003) The use of antioxidant therapies during chemotherapy. Gynecologic Oncology 88: 434-439.

Eloff JN (1998) Conservation of Medicinal Plants: Selecting Medicinal Plants for Research and Gene Banking. In: Adams RP, Adams JE (Eds.) Conservation of plant genes III: Conservation and utilization of African Plants. Missouri Botanical Garden Press, St. Louis, USA. pp. 209 – 222. ISBN 0915279568

Eloff JN (1999) The antibacterial activity of 27 southern African members of the Combretaceae. South African Journal of Science 95: 148-152

Estevez-Braun A, Estevez-Reyes R, Moujir LM, Ravelo AG, Gonzalez AG (1994) Antibiotic activity and absolute configuration of 8S-Heptadeca-2(Z),9(Z)-diene-4,6-diyne-1,8-diol from *Bupleurum salicifolium*. Journal of Natural Products 57: 1178-1182

Famakin JO (2002) Investigation of antibacterial compounds present in *Combretum woodii*. MSc thesis Pharmacology, University of Pretoria, Pretoria

Farnsworth NR (1966) Biological and Phytochemical screening of plants. Journal of Pharmaceutical Science 55: 225–276

Farnsworth NR, Soejarto DD (1991) Global Importance of Medicinal Plants. In Akerle O, Heywood V, Synge H (Eds.) Conservation of medicinal plants. Cambridge University Press, Cambridge, pp. 25-51. ISBN 0521392063

Fernandez MA, Garcia MD, Saenz MT (1996) Antibacterial activity of the phenolic acid fraction of *Scrophularia frutescens* and *Scrophularia sambucifolia.* Journal of Ethnopharmacology 53: 11-14

Fourie TG, Swart I, Snyckers FO (1992) Folk medicine: a viable starting point for pharmaceutical research. South African Journal of Science 88: 190–192

Ganesan T (1992) Antifungal properties of wild plants. Advances in Plant Sciences 7: 185 – 18

Germano MP, Sanogo R, Guglielmo M, De Pasquale R, Crisafi G, Bisignano G (1998) Effects of *Pteleopsis suberosa* extracts on experimental gastric ulcers and *Helicobacter pylori* growth. Journal of Ethnopharmacology 59: 167– 172

Germishuizen G, Meyer NL (Eds.) (2003) Plants of Southern Africa: An annotated Checklist. Strelitzia 14. National Botanical Institute, Pretoria. p. 317. ISBN 1919795995

Ghisalberti EL (1993) Detection and isolation of Bioactive Natural products. In: Colegate SM, Molyneux RJ (Eds.) Bioactive Natural Products. CRC Press, Inc., Boca Raton pp. 22-57. ISBN0849334720

Griggs J, Metcalfe JC, Hesketh R (2001) Targeting tumour vasculature: The development of combretastatin A4. Lancet Oncology 2: 82-87

Hadimani MB, Hua J, Jonklaas MD, Kessler RJ, Sheng Y, Olivares A, Tanpure RP, Weiser A, Zhang J, Edvardsen K, Kane RR, Pinney KG (2003) Synthesis, in vitro, and in vivo evaluation of phosphate ester derivatives of combretastatin A-4. Bioorganic and Medicinal Chemistry Letters 13: 1505-1508

Hamburger M, Marston A, Hostettmann K (1991) Search for new drugs of plant origin. Advances in Drug Research 20: 167–217

Hammerschmidt R, Kuć J (1982) Lignification as a mechanism for induced systemic resistance in cucumber. Physiological Plant Pathology 20: 61-71

Hammerschmidt R, Nuckles E, Kuć J (1982) Association of peroxidase activity with induced systemic resistance in cucumber for *Colletotrichum lachenarium*. Physiological Plant Pathology 20: 73-82

Haslam E (1996) Natural polyphenols (vegetable tannins) as drugs: possible modes of action. Journal of Natural Products 59: 205-215

Holford P (1997) The Optimum Nutrition Bible. Butler & Tanner Ltd., London. pp. 79-93. ISBN 0749918551

Hutchings A, Scott AH, Lewis G, Cunningham AB (1996) Zulu medicinal plants: an inventory. University of Natal Press; Pietermaritzburg, South Africa, pp. 213-217, 336, 347. ISBN 0869809237

Jacoby GA, Archer GL (1991) New mechanisms of bacterial resistance to antimicrobial agents. New England Journal of Medicine. 324: 601-612

Jongkind CCH, (1991) Novitates gabonensis 6[1]. Some critical observations on *Combretum* versus *Quisqualis* (Combretaceae) and description of two new species of *Combretum*. Bulletin du Museum national d'histoire. Paris 4[e] sér. 12, section B Adansonia 3-4: 275-280

Jongkind CCH, (1993) Novitates gabonensis 13. Three new species and one new combination

in *Combretum* (Combretaceae). Bulletin du Museum national d'histoire. Paris 4e sér. 14, section B Adansonia 2: 257-262

Kamuhabwa A, Nshimo C, De Witte P (2000) Cytotoxicity of some medicinal extracts used in Tanzanian traditional medicine. Journal of Ethnopharmacology 70: 143–149

King SR, Tempesta MS (1994) From shaman to human clinical trails: the role of industry in ethnobotany, conservation and community reciprocity. Ciba Foundation Symposium 185: 197-206

Kinghorn DA, Farnsworth NR, Soejarto DD, Cordell GA, Pezzuto JM, Udeani GO, Wani MC, Wall ME, Navarro HA, Krame RA, Menendez AT, Fairchild CR, Lane KE, Forenza S, Vyas DM, Lam KS, Shu Y-Z (1999) Novel strategies for the discovery of plant derived anticancer agents. Pure and Applied Chemistry 71: 1611–1618

Kruger JP (2004) Isolation, chemical characterization and clinical application of an antibacterial compound from *Terminalia sericea.* PhD thesis, University of Pretoria, Pretoria

Kubo I, Muroi H, Himejima M (1993) Combination effects of antifungal nagilactones against Candida albicans and two other fungi with phenyl propanoids. Journal of Natural Products 56: 220-226

Kuć J (1990a) Immunization for the control of plant disease. In: Hornby D, Cook RJ, Henis Y (eds.) Biological control of soil-borne plant pathogens. CAB International, Wallingford, pp. 255-374

Kuć J (1990b) Compounds from plants that regulate or participate In disease resistance. In: Ciba Foundation Symposium 154. Bioactive compounds from plants. John Wiley & Sons,

Chichester. pp. 213-224. ISBN 04719 26914

Ladion HG (1985) Healing wonders of herbs. Manila, Philippine publishing House. 158pp. ISBN 9711019019

Lawton JR, Govender H, Rogers CB (1991) Mollic acid Glucoside: A possible (Third World) Answer to the Control of Schistosomiasis in South and Central Africa. Planta Medica 57(S): A74

Lebrun J-P, Stork AL (1991) Énumération Des Plantes à Fleurs D'Afrique Tropicale. I Généralités et Annonaceae à Euphorbiaceae et Pandaceae. Ville de Genève, Genève. p.165. ISBN 2827701081

Lednicer D, Narayanan, VL (1993) Acquisition and Screening of Natural Products as Potential Anticancer and AIDS antiviral agents. In: Colegate SM, Molyneux RJ (Eds.) Bioactive Natural Products. CRC Press, Inc., Boca Raton pp. 159-162. ISBN0849334720

Lin CM, Ho HH, Pettit GR, Hamel, E (1989) Antimitotic natural products combretastatin A-4 and combretastatin A-2: studies on the mechanism of their inhibition of the binding of colchicine to tubulin. Biochemistry 28: 6984-6991

Malan E, Swinny E (1993) Substituted bibenzyles, phenanthrenes from the heartwood of *Combretum apiculatum.* Phytochemistry 34: 1139–1142

Martini ND (2002) The isolation and characterization of antibacterial compounds from *Combretum erythrophyllum* (Burch.) Sond. PhD thesis Pharmacology, University of Pretoria, Pretoria

Monzon RB (1995) Traditional medicine in the treatment of Parasitic diseases in the Philippines. Southeast Asian Journal of Tropical Medicine and Public Health 26: 421–427

Muktar H, Katiyar SK, Agarwal R (1994) Green tea and skin-anticarcinogenic effects. Journal of Dermatology 102: 3-7

Neuwinger HD (2000) African Traditional medicine. A Dictionary of Plant Use and Applications. Medpharm Scientific Publishes, Stuttgart. p. 426. ISBN 3887630866

Ngounou FN, Rahman A, Choudharry MI, Malik S, Zareen S, Ali R, Lontsi D, Sondengam BL (1999) Two saponins from *Pteleopsis hylodendron*. Phytochemistry 52: 917–921

Noveroske RL, Kuć J, Williams EB (1964) Oxidation of phloridzin and phloretin related to resistance of *Malus* to *Venturia inaequalis*. Phytopathology 54: 92-97

Nyein MM, Zaw M (1972) Effects of *Quisqalis indica* on some bacteria. Report of the Burma Medical Research Council. p. 18

Occhiuto F, Sanogo R, Germano MP, Keita A, D'Angelo V, De Pasquale R (1999) Effects of some Malian Medicinal plants on the respiratory Tract of guinea-pigs. Journal of Pharmacy and Pharmacology 51: 1299–1303

Ofek IJ, Goldhar J, Sharon, N (1996) Anti-*Escherichia coli* adhesin activity of cranberry and blueberry juices. Advances in Experimental Medical Biology 408: 179-183

Ohsumi K, Nakagawa R, Fukuda Y, Hatanaka T, Morinaga Y, Nihei K, Ohishi K, Suga Y, Akijama Y, Tsuji T (1998) Novel combretastatin analogues effective against murine solid tumours: design and structure-activity relationships. Journal of Medicinal Chemistry 41: 3022-

3032

Omulokoli EB, Khan B, Chabra CS (1997) Antiplasmodial activity of four Kenyan medicinal plants. Journal of Ethnopharmacology 56: 133–137

Ono K, Nakane H, Fukushima M, Chermann J-C, Barre-Sinoussi F (1989) Inhibition of reverse transcriptase activity by a flavonoid compound, 5,6,7-trihydroxixyflavone. Biochemical and Biophysical Research Communications 3: 982-967

Peres MTLP, Monache FD, Cruz AB, Pizzolatti MG, Yunes RA (1997) Chemical composition and antimycrobial activity of *Croton urucurana* Bailon (Euphorbiaceae). Journal of Ethnopharmacology 56: 233-226

Perrett S, Whitfield PJ, Sanderson I, Bartlett A (1995) The plant molluscicide *Melletia thonningii* (Leguminosae) as a topical antischistosomal agent. Journal of Ethnopharmacology 47: 49-54

Pettit GR, Cragg GM, Herald DL, Schmidt JM, Lohavanijaya P (1982) Isolation and structure of combretastatin. Canadian Journal of Chemistry 60*:* 1374-1376

Pettit GR, Singh SB, Niven ML, Schmidt JM (1987) Isolation, structure and synthesis of combretastatins A-1, B-1, potent new inhibitors of microtubule assembly, derived from *Combretum caffrum*. Journal of Natural Products 50: 119-131

Pettit GR, Singh SB, Hamel E, Lin CM, Alberts SD, Garcia-Kendall D (1989) Isolation and structure of the strong cell growth and tubulin inhibitor combretastatin A-4. Experientia 45: 209-211

Pettit GR, Singh SB, Boyd MR., Hamel E, Petit RK (1995) Antineoplastic agents. 291. Isolation

and synthesis of combretastatins A-4, A-5 and A-6. Journal of Medicinal Chemistry 38: 1666-1672

Rahman A-U, Choudhary MI (1995) Diterpenoid and steroidal alkaloids. Natural Products Report 12: 361-379

Reuveni M, Tuzun S, Cole JS, Siegel MR, Kuć J (1987) Removal of DVT from the surfaces of tobacco leaves increases their susceptibility to blue mould. Physiological and Molecular Plant Pathology 30: 441-451

Rodriguez E, Aregullin M, NishidaT, Uehara S, Wrangham R, Abramowski Z, Finlayson A, Towers GH (1985) Thiarubrin A, a bioactive constituent of *Aspillia* (Asteraceae) consumed by wild chimpanzees. Experientia 41: 419-420

Rogers CB (1989) Isolation of 1α-hydroxycycloartenoid mollic acid and α-L-arabinoside from *Combretum edwardsii* leaves. Phytochemistry 28: 279–281

Rogers CB, Verotta L (1997) Chemistry and biological properties of the African Combretaceae. Phytochemistry 46: 121–141

Sacho H, Schoub BD (1977) Current perspectives on nosocomial infections. GlaxoWellcome booklet, The Natal Witness Printing and Publishing Company (Pty) Ltd. pp. 1-100

Scalbert A (1991) Antimicrobial properties of tannins. Phytochemistry 30: 3875-3883

Schoeller W, Dohrn M, Hohlweg W (1940) An estrogenic substance from the tubers of the Siamese vine, *Butea superba.* Naturwissenschaften 28: 532

Schmitz R (1985) Friedrich Wilhelm Serturner and the discovery of morphine. Pharmaceutical Histology 27(2): 61-74

Serage A (2003) Isolation and characterization of antibacterial compounds present in *Combretum apiculatum* subsp *apiculatum*. MSc thesis, University of Pretoria, Pretoria

Stange RR (jr.), Midland SL, Eckert JW, Sims JJ (1993) An antifungal compound produced by grapefruit and Valencia orange after wounding of peel. Journal of Natural Products 56: 1627–1629

Stermer BA, Hammerschmidt R (1987) Association of heat shock induced resistance to disease with increased accumulation of insoluble extensin and ethylene synthesis. Physiological Plant Pathology 31: 453-461

Stern JL, Hagerman AE, Steinberg PD, Mason PK (1996) Phlorotannin-protein interactions. Journal of Chemical Ecology 22: 1887-1899

Svoboda GH, Gorman M, Root M (1964) Alkaloids in Vinca rosea (*Catharanthus roseus*) XXVIII. A preliminary report on hypoglycemic activity. Lloydia 27: 361-363

Thein K, Myint W, Myint MM, Aung SP, Than A, Bwin M (1995) Preliminary Screening of Medicinal Plants for Biological Activity based on Inhibition of Cyclic AMP Phosphodiesterase. International Journal of Pharmacology 33: 330–333

Thorpe RH, Watson TR (1953) A study of the occurrence of cardio-active constituents in plants growing wild in Australia. I. Families Apocynaceae and Asclepidaceae. Australian Journal of Experimental Biology and Medical Science 31: 529-532

Toda MS, Okubo H, Ikigai T, Suzuki Y, Hara Y, Shimamura T (1992) The protective activity of tea catechins against experimental infection by *Vibrio cholerae*. Microbiology and Immunology 36: 999-1001

Tuzun S, Rao N, Vogeli U, Schardl C, Kuć J (1989) Induced systematic resistance to blue mould; early induction of β-1, 3-glucanases, chitinases and other pathogenesis-related proteins in immunized tobacco. Phytopathology 79: 979-983

United Nations Development Programme Human Development Report (2002) Oxford University Press, New York.

Van Wyk NP (1974) Nasionale bome van die Krugerwildtuin Deel II. Perskor-uitgewery, Johannesburg, pp. 441– 442. ISBN 0628005784

Van Wyk B, Van Wyk P, Van Wyk B-E (2000) Photographic guide to trees of Southern Africa. Briza Publications, Pretoria. p. 257. ISBN 1875093249

Vayalil PK, Mittal A, Katayar SK (2004) Proanthocyanidins from grape seeds inhibit expression of matrix metalloproteinases in human prostate carcinoma cells, which is associated with the activation of MAPK and NFκB. Carcinogenesis 25(6): 987-995

Watanbe H, Miyaji C, Makino M, Abo T (1996) Therapeutic effects of glycyrrhizin in mice infected with LP-BM5 murine retrovirus and mechanisms involved in the prevention of disease progression. Biotherapy 9: 209–220

Weinryb J, Chasin MF, Harris DN, Goldenbery H, Paik MIM, Phillips M, Samnaniego F, Hess SM (1972) Effects of Therapeutic Agents on Cyclic AMP Metabolism *in vitro*. Journal of Pharmaceutical Science 61: 1556–1567

Wink M (1999) Introduction: biochemistry, role and biotechnology of secondary metabolites. In: Wink M (Ed.) Biochemistry of Plant Secondary metabolism. Annual Plant Reviews, Volume 2. Sheffield Academic Press, Sheffield. pp. 1-14

Winter AG (1955) Antibiotische therapie mit arzneipflanzen. Planta Medica 3: 1-16

Yip L, Pei S, Hudson JB and Towers GHN (1991) Screening of medicinal plants from the Yunnan Province in Southwest China for antiviral activity. Journal of Ethnopharmacology 34: 1–6

Zhang Y, Lewis K (1997) Fabatins: new antimicrobial plant peptides. FEMS Microbiology Letters 149: 59-64