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**Traditional medicinal uses and biological activities of some
plant extracts of African *Combretum* Loeffl., *Terminalia* L.
and *Pteleopsis* Engl. species (Combretaceae)**

Pia Fyhrquist

Academic dissertation

To be presented with the permission of the Faculty of Biosciences of the University of Helsinki, for public criticism in Auditorium XV (4072) at University Main Building, Unioninkatu 34, on November 16th, 2007, at 12 noon

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LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications which are referred to in the text by their Roman numbers, and on unpublished results presented in the text:

- I **Fyhrquist, P., Mwasumbi, L., Hæggström, C.-A., Vuorela, H., Hiltunen, R., Vuorela, P.** (2002). Ethnobotanical and antimicrobial investigation on some species of *Terminalia* and *Combretum* (Combretaceae) growing in Tanzania. *Journal of Ethnopharmacology* 79: 169-177.
- II **Fyhrquist, P., Mwasumbi, L., Hæggström, C.-A., Vuorela, H., Hiltunen, R., Vuorela, P.** (2004). Antifungal Activity of Selected Species of *Terminalia*, *Pteleopsis* and *Combretum* (Combretaceae) Collected in Tanzania. *Pharmaceutical Biology* 42 (4-5): 308-317.
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LIST OF ABBREVIATIONS

A549	non-small lung cancer cell line
ACE	acetylcholine esterase
AIDS	acquired immunodeficiency syndrome
ATCC	American type culture collection
BBCE	bovine brain capillary endothelial cell line
BT-20	human breast carcinoma cell line
CA-1	combretastatin A-1
CA-2	combretastatin A-2
CA-3	combretastatin A-3
CA-4P	combretastatin A-4- disodium phosphate, the prodrug of CA-4
CA-4	combretastatin A-4
CH₂Cl₂	methylene chloride
CHO	Chinese hamster ovary cells
COS-7	green monkey fibroblasts
DMEM	Dulbecco`s modified Eagles medium
DMSO	dimethylsulfoxide
DNA	deoxyribonuclein acid
ED₅₀	effective dose leading to 50 % inhibition/mortality
FGF	fibroblast growth factor
HCT-15	colon cancer cell line
HeLa	cervical carcinoma cell line
HIV	human immunodeficiency virus
HL-60	human myeloid leukemial cell line
HOS-1	human osteosarcoma cell line
HPLC	high pressure liquid chromatography
HUVEC	human umbilical vein endothelial cells
IC₅₀	half maximal inhibitory concentration
J82	bladder cancer cell line
JNK-1	c-Jun N-terminal kinase
K562-DOX	human erythroleukemial cells
L1210	murine lymphocytic leukemia cell line
MCF 7	breast carcinoma cell line
MDR	multidrug-resistant
MREA	multidrug-resistant <i>Enterobacter aerogenes</i>
MeOH	methanol
MIC	minimum inhibitory concentration
MIC_{90%}	minimum inhibitory concentration killing 90 % of cells in population
MPLC	medium pressure liquid chromatography
MRSA	methicillin-resistant <i>Staphylococcus aureus</i>
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NAC	non-albicans <i>Candida</i> species
NCE	novel chemical entities
NDP	4-nitro- <i>o</i> -phenylene-diamine
NIH3T3	H- <i>ras</i> -transformed fibroblasts
P22	rat carcinosarcoma
P-388	murine lymphocytic leukemial cell line
PBS	phosphate buffer saline

PC-3	human prostate cancer cell line
PGF_{2α}	prostaglandin F 2 alpha
PRNG	penicillin-resistant <i>Neisseria gonorrhoeae</i>
PRSP	penicillin-resistant <i>Streptococcus pneumoniae</i>
RNA	ribonuclein acid
RPM	rounds per minute
RP-MPLC	reversed phase medium pressure liquid chromatography
RP-TLC	reversed phase thin layer chromatography
RP18	reverse phase
RP-18_{F254s}	reverse phase with fluorescence indicator
SAR	structure-activity relationship
SEM	standard error of mean
S155	mouse breast carcinoma
T138	murine breast tumor
T 24	bladder carcinoma cell line
TLC	thin layer chromatography
TMP	traditional medicinal practitioner
U-373	glioblastoma cancer cell line
UTI	urinary tract infections
VRE	vancomycin-resistant enterococci
VRSA	vancomycin-resistant <i>Staphylococcus aureus</i>
WHO	World Health Organization

BOTANICAL TERMS

Andromonoecious	Hermaphrodite and male flowers on same plant.
Axillary	Situated in or arising from an axil.
Calyx	The sepals of a flower collectively forming the outer floral envelope or layer of the perianth enclosing a developing bud; usually green.
Corolla	The petals of a flower collectively forming an inner floral envelope or layer of the perianth.
Disk of flower	The enlarged part of the flower to which sepals, petals and other flower organs are attached.
Filiform sepals	Threadlike sepals.
Glandular hair	A trichoma with enlarged unicellular or multicellular secretory hairs at the terminus.
Hermaphroditic flower	Flowers with both male and female reproductive organs.
Indumentum	Covering of fine hairs/scales on leaf.
Inflorescence	The flowering part of a plant or arrangement of flowers on a stalk.
Lamina	The blade of a leaf; the broad, expanded portion of a petal/sepal.
Monoecious	Unisexual reproductive units of both sexes appearing on the same plant.
Ovary	The organ (part of pistil) that bears the ovules (seeds) of a flower. Develops to a fruit in most flowering plants.
Panicle	A branched inflorescence.
Pedicle	A stalk which supports one flower/fruit.
Pericarp	The fruit wall.
Petals	Part of the perianth that is usually brightly colored (syn. flower petal).
Petiole	Stalk, petiolate leaf; having a stalk or petiole.
Pistil	The female ovule-bearing part of a flower composed of ovary and style and stigma (syn. gynoecium).
Raceme	Type of inflorescence that is unbranched and indeterminate and bears pedicellate flowers along the axis.
Receptacle	The apex of the flower stalk, from which the organs of the flower grow.
Scale	In subgenus <i>Combretum</i> : stalked multicellular scales on leaves. Can be used for species identification.
Sepals	One of the green parts that forms the calyx of the flower.
Sessile	Resting directly upon the main stem or branch, without a petiole or footstalk; a sessile leaf or blossom.
Short shoots	Shoots having very short internodia thus nodes situated very near each other. In <i>Terminalia</i> spp.
Spike	An indeterminate inflorescence bearing sessile flowers on an unbranched axis.
Stamen	The male reproductive organ of the flower.
Stigma	The apical end of the style where pollen is deposited.
Stipe	Supporting stalk or stemlike structure especially of a pistil.
Stipitate	Supported by a stipe.
Subulate sepals	Very narrow and tapering gradually to a fine point from a broadish base.
Zygomorphic flower	A bilateral flower consisting of two symmetrical halves.

Definitions from Webster`s dictionary and <http://www.wikipedia.org> .

ABSTRACT

In Africa various species of *Combretum*, *Terminalia* and *Pteleopsis* are used in traditional medicine. About 24 different species of *Combretum* are well known in traditional medicine and used for various ailments and diseases ranging from heart and worm remedies to wound dressings, treatment of the mentally ill, and scorpion stings. Many species of *Terminalia* are used for infections, diarrhea, venereal diseases and fever. Species of *Pteleopsis* are known to be effective for gastric ulcers, infertility, venereal diseases and dysentery. Some species of *Combretum*, *Terminalia* and *Pteleopsis* have already been documented for their antimicrobial as well as cytotoxic effects, and in some cases the active compounds have been isolated. Antimicrobial hydrolysable tannins, flavonoids, lignans, stilbenes, phenanthrenes and pentacyclic triterpenoids are known from some species of the three genera. Combretastatins, a group of stilbenes with cytotoxic, antiangiogenic and antivasular effects have been isolated from *Combretum caffrum* as well as from some other species of *Combretum*. Hydrolysable tannins from species of *Terminalia* have been shown to possess antimutagenic effects as well as being growth inhibitory against various cancer cell lines.

The aim of this work has been to document the ethnomedicinal uses of several species of *Combretum* and *Terminalia* in seven villages of the Mbeya region, south-western Tanzania, and to use this information for finding species with good antimicrobial and cytotoxic potential. The ethnomedical information I obtained from traditional healers was important, and facilitated the finding of *Combretum* and *Terminalia* species with excellent antimicrobial and cytotoxic effects.

I initiated this work with a five weeks expedition to Tanzania in spring 1999 (Study I). Sixteen different species of *Combretum* and *Terminalia*, as well as *Pteleopsis myrtifolia* were collected from various locations in the districts of Mbeya, Iringa and Dar-es-Salaam. During the expedition traditional healers in seven villages in the Mbeya region were interviewed in Swahili and Nyakyusa on the medicinal uses of the collected species of *Combretum* and *Terminalia* with the aid of a questionnaire. Interestingly, the results of the interviews correlated well between the different villages, the same species being used in similar ways in different villages. Of the ten species of *Combretum* and *Terminalia* I showed the traditional healers, six were frequently used as medicinal plants. *Combretum molle* was used in six villages out of the seven for treatment of bacterial infections, skin diseases, diarrhea, oedema and wounds. *Combretum zeyheri* was also a popular medicinal plant in the villages in Mbeya, as well as *C. fragrans*, *C. psidioides*, *Terminalia sericea* and *T. kaiserana*. Most of the species mentioned are used for the treatment of bacterial infections, such as gonorrhoea, syphilis and influenza, as well as for skin diseases, diarrhea and wounds. Roots and leaves were the most commonly used organs, although stem bark was reported to be used by one of the traditional healers I interviewed. Fruits and flowers from

Combretum and *Terminalia* were never used as medicine, and some of the healers reported that the seeds are poisonous. The most common way to prepare medicines from the plants was to make hot water decoctions from dried plant material. Other ways were to mix dried, powdered plant material with maize porridge, *Ugali* for oral use or with sheep fat for ointments. Infusions made from either dried or fresh plant material were also common. The extract of the leaves of *C. molle* was reported to be used for baths treating patients with bacterial infections. The process of preparation of the plants to medicine was never shown to us, since many traditional healers think that the phytomedicine loses its medicinal properties and power of healing in this way. In many cases different species of *Combretum* as well as *Terminalia* were mixed together, and generally it is thought that the medicinal properties of mixtures of plants are more effective than preparations made of one plant. I have screened twenty-one extracts of six *Combretum* species and four *Terminalia* species, collected from Tanzania, for their antibacterial effects against two gram-negative and five gram-positive bacteria, as well as the yeast, *Candida albicans*, using an agar diffusion method (Study I). Most of the screened plants showed substantial antimicrobial activity. The most potent extract was a methanolic root extract *Terminalia sambesiaca* which gave a MIC value of 0.9 mg/ml against *Enterobacter aerogenes*. Also root extracts of *Terminalia sericea* and *T. kaiserana* gave excellent antibacterial effects. Notably, a hot water decoction of *T. sericea*, the way in which this plant is prepared for traditional medicine in Mbeya, gave as good antibacterial effects as extracts of this species made in MeOH or EtOH. This indicates that some of the antimicrobial compounds in *T. sericea* might be polar. A leaf extract of *T. kaiserana* was the only extract used in this study to inhibit the growth of the gram-negative *E. coli*, and the effect was found to be bactericidal. *Combretum fragrans* and *C. padoides* gave the best antibacterial results of all the investigated *Combretum* species, and this is in accordance with the use of *C. fragrans* for diarrhea in Tanzanian traditional medicine.

In study II I have screened thirty-five crude extracts of five species of *Terminalia*, ten of *Combretum* and *Pteleopsis myrtifolia* for their antifungal effects against five species of yeast (*Candida* spp.) and *Cryptococcus neoformans*, using an agar-diffusion method. There were substantial differences between the species of *Combretum* and *Terminalia* in their antifungal effects, some being highly effective and others showing no effects. Interestingly, the most effective plant extracts gave antifungal effects comparable to the standard antibiotics itraconazole and amphotericin B, used in this investigation. The *Terminalia* species gave in general stronger antifungal effects than the species of *Combretum*, and the best effects were obtained with methanolic root extracts of *T. sambesiaca*, *T. sericea* and *T. kaiserana*, although also root extracts of *C. padoides*, *C. molle* and *C. fragrans* gave good antifungal results. *Candida glabrata*, *C. krusei* and *Cryptococcus neoformans* proved to be the most sensitive fungal species, whereas *C. alba*, *C. tropicalis* and *C. parapsilosis* were more resistant to the plant extracts. My investigation indicates that decoctions of *Terminalia sambesiaca*, *T. sericea* and *T. kaiserana* might be used for the treatment of fungal infections related to HIV.

Six antifungally and antibacterially active fractions were isolated from the roots of *Terminalia sambesiaca* with the aid of reversed-phase medium-pressure liquid chromatography (RP-MPLC) (Study **II**). The most polar fractions showed the strongest antifungal and antibacterial effects, although some less polar fractions also gave good growth inhibitory effects. This shows that *T. sambesiaca* roots contain antimicrobial compounds with a wide range of polarities.

In study **III** I have evaluated the cytotoxic effects of twenty-seven plant extracts of eight species of *Combretum*, five species of *Terminalia* and *Pteleopsis myrtifolia* collected in Tanzania against three human cancer cell lines (HeLa, cervical carcinoma; MCF 7, breast carcinoma; T 24 bladder carcinoma) and one endothelial cell line (BBCE, bovine brain capillary endothelial cells). The cytotoxic and antiproliferative effects were evaluated with the Alamar Blue and the Coulter count methods. The most outstanding effects were obtained with a leaf extract of *Combretum fragrans*, which nearly totally inhibited the proliferation of the T 24 and HeLa cells at a concentration of 25 µg/ml and inhibited 60 % of the growth of the HeLa cells at a concentration of 4.3 µg/ml. *C. fragrans* might induce apoptosis in HeLa cells since cells treated with this extract showed fragmented nuclei, a sign of apoptosis. The species of *Terminalia* were less cytotoxically potent than the *Combretum* species, although *T. sericea* and *T. sambesiaca* gave good cytotoxic effects (< 30 % proliferation). To the best of my knowledge I am the first to report that *Combretum fragrans* and *Terminalia sambesiaca* possess strong cytotoxic effects. *T. sambesiaca* is reported to be used for treatment of cancer in Tanzanian medicine, which is thus in accordance with the good cytotoxic results I have obtained for this species.

In summary the studies above indicate that many of the species of *Combretum*, *Terminalia* and *Pteleopsis*, used in Tanzanian traditional medicine, are powerful inhibitors of both microbial and cancer cell growth. In depth studies would now be needed to find the active compounds behind these biological activities.

1. INTRODUCTION

It has been estimated that less than 1 – 10 % of the large diversity of 250.000 – 500.000 plant species on the Earth have been studied chemically and pharmacologically for their medicinal properties (Farnsworth, 1991; Verpoorte, 2000). This is especially true for the tropical flora, as at date only 1 % of the species in these habitats have been studied for their pharmaceutical potential (Gurib-Fakim, 2006). Tropical forests and many other tropical ecosystems are rich sources of a diversity of plant derived chemical compounds, both because of the high species diversity but also because of the “eternal summer” which forces the plant species to the constant production of chemical defense compounds against herbivores and pathogens as well as against other plant species. Plants in a tropical rainforest also have to compete for space and light and this forces species to develop more efficient means of using energy and nutrients as well as to allocating resources for secondary compound production. For these reasons a greater portion of the tropical plant species contains secondary compounds, potentially useful as models for/as medicines (Wood-Sheldon et al., 1997). Plant derived compounds have been, and are still, important as such or as models (lead compounds) for medicines: 50 % of the prescription products in various countries in Europe and the US are either natural products or natural product derivatives (Cordell, 2002; Newman et al., 2003). To date about 50 drugs have come from tropical plants (Gurib-Fakim, 2006). Plants continue to be a potent source of lead compounds. Although combinatorial techniques have been used for the optimization of a number of recently approved agents, these methods have not been able to identify a *de novo* combinatorial compound (Newman et al., 2003). Examples of successful medicines derived from natural product leads include most antibiotics, the acetylcholine esterase (ACE) inhibitors, many anticancer agents, the immunosuppressants, cyclosporine and rapamycin and the antiparasitic avermectins (Harvey & Waterman, 1998).

People have used plants for millennia and vast information of the medicinal uses of plants has therefore accumulated especially in the tropical parts of the world. According to the World Health Organization (WHO), about 80 % of the people in developing countries rely primarily on medicinal plants for their primary health care (Wood-Sheldon et al., 1997). In many remote areas in African countries people consult the traditional healer of the village in case of illness. Western hospitals and medicines are often beyond the reach and Western medicines many times too expensive for the people to afford. Even in the big cities such as Dar-es-Salaam, with 2 500 000 inhabitants (http://en.wikipedia.org/wiki/Dar_es_Salaam), a large proportion of the inhabitants use traditional medicine for their health care (Swantz, 1974). In Africa the ethnopharmacological and –botanical knowledge on the uses of medicinal plants is often orally passed down from generation to generation. This abundance of information is in danger of disappearing since it is often kept secret until the last minutes of death of the traditional healer when they eventually call

on somebody to inherit the information (Kokwaro, 1976). Although traditional medicine has been recognized as a part of primary health care programmes in many African countries (WHO, 1978), there is a need to evaluate scientifically the crude extracts of plants for their medicinal and pharmacodynamic properties, clinical usefulness and toxicological potential (Kyerematen & Ogunlana, 1987).

Higher plants are still poorly explored as sources of new drugs (Hostettman & Terreaux, 2000). There are several ways in selecting plant materials when searching for new medicinal plants/active compounds. Ethnopharmacological information on medicinal plants is often of substantial importance for the finding of new potential medicinal plants/new ways of using an already known plant. It has been estimated that 74 % of the pharmacologically active, plant derived components were discovered after the ethnomedical uses of the plants started to be investigated (Farnsworth & Soejarto, 1991; Wood-Sheldon et al., 1997). Another important way of discovering new medicinal plants and lead compounds is the phylogenetic approach in which a number of closely related species of plants, assumed to contain related chemical compounds (chemotaxonomy), are screened for their biological effects (Cotton, 1996; Vuorela et al., 2004). Random sampling collecting plant samples from certain habitats with high species diversity (for example tropical rainforests) can be beneficial for finding novel chemical entities (NCEs), but is somewhat time-consuming and requires hard work (Vuorela et al., 2004). This kind of sampling is likely to be the industrial approach and most likely to be used for evaluating plants for bioactive compounds (Fabricant & Farnsworth, 2001).

Serious infections caused by bacteria that have become resistant to commonly used antibiotics have become a major global healthcare problem in the 21st century. In the developing countries bacterial infections are still the main cause of deaths (Iwu et al., 1999). The need for new antibacterial drugs is constant, because of the continuous development of antibiotic resistant strains of pathogenous bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA), penicillin-resistant *Streptococcus pneumoniae* (PRSP) and vancomycin-resistant enterococci (VRE). Recently, a simultaneous development of resistance to several antibiotic classes has created very dangerous multidrug-resistant (MDR) bacterial strains (Alanis et al., 2005). Over 75 % of the antibacterials in clinical use are of natural origin and most of them are obtained from fungal sources (Newman et al., 2003). A renewed interest in plant based antimicrobials has arisen during the last twenty years, but still plant based antimicrobials are poorly explored. Screening of plant extracts for antimicrobial activity has shown that higher plants represent a potential source of new anti-infective compounds (Press, 1996). Plant derived antimicrobial compounds might inhibit bacteria through different mechanisms than conventionally used antibiotics, and could therefore be of clinical value in the treatment of infections caused by resistant microbes (Eloff et al., 1998b). Only a small fraction of the known plant species of the whole world have been evaluated for the presence of antimicrobial compounds, and thus it is necessary to increase the

efforts in collecting and screening plants for the development of novel and environmentally safe antimicrobial agents (Stein et al. 2005).

Life-threatening fungal infections have increased dramatically in immunocompromised patients during the past decades (Rex et al., 1997). Although new antifungal agents are under evaluation, only a few have been licensed for the treatment of systemic fungal diseases. To date, only three classes of antifungal drugs are available for the treatment of systemic fungal infections: the polyenes, the azoles and flucytosine (Denning, 2003). Although many of these drugs have advanced the management of fungal infections, failure rates remain high. The prolonged use of antifungal drugs in the treatment of chronic infections have caused the emergence of amphotericin-B and azole resistant *Candida* species (Patel, 1998). There is a general consensus that new antifungal agents are needed (Selitrennikov, 2001). Recently two naturally derived antifungals, caspofungin acetate and micafungin sodium, have been approved for antifungal therapy. This is for the first time since the 1970s that two natural products are approved as antifungals. During the period 1981- 2002, most of the antifungals approved for clinical use have been synthetic (Newman et al., 2003). The Spanish Merck group have recently reviewed the importance of natural products in antifungal chemotherapy (Vicente et al., 2003), but the focus in this review is kept on micro-organisms as producers of new antifungals. Higher plants might contain valuable, not yet explored lead compounds for antifungal therapy.

Mortality that results from cancer is still unacceptably high and is becoming a growing public problem in the 21st century. The estimated worldwide new incidence of cancer is about 6 million cases per year. In the developed countries cancer is the second major cause of death after cardiovascular diseases (Srivastava et al. 2005). Natural products have been the mainstay in cancer chemotherapy for the past 30 years. Over 60 % of the clinically used anticancer drugs are of natural origin and most of them are derived from higher plants (Newman et al., 2003). Many of the chemotherapeutic medicines in current use have been discovered with the aid of traditional medicinal uses. This is the case with the powerful anti-leukemial drugs vinblastin and vincristin isolated from the native Madagascar plant, *Catharanthus roseus* and the podophyllins isolated from the roots of the mayapple, *Podophyllum peltatum*. The search for new anticancer lead compounds with enhanced biological properties and fewer adverse side effects is still needed. Natural products are likely to provide many of the lead structures also in the future (Mann, 2002).

2. REVIEW OF THE LITERATURE

2.1. *Traditional Medicine in Africa*

African traditional medicine is the oldest and perhaps the most diverse of all medicine systems. Africa is considered to be the cradle of mankind with a rich biological and cultural diversity, and there are marked differences between different regions of this continent when it comes to healing practices (Gurib-Fakim, 2006). Medicinal and poisonous plants, including a diverse array of woody plants, have always played an important role in African life. The traditions of collecting plants as well as processing herbal remedies and applying them have been handed down from generation to generation (von Maydell, 1996).

2.1.1. Documentation of African traditional medicine

Most of the African cultures have a verbal tradition, and therefore written information on cultural features in the past are not so readily available from Africa as from many other parts of the world (Hedberg & Staugård, 1989). There exists, however some documents on African traditional medicine, and the oldest of them is written by the famous Arab doctor and polyhistor, Avicenna, who lived 980-1037 A.D. With the colonization of Africa, European botanists started to explore the flora of various parts of the continent. The ethnobotanical information on the uses of the plants were sometimes documented on herbarium labels, and in this way ethnobotanical information on a number of plants began to accumulate (Hedberg & Staugård, 1989). Systematic accounts in written form dealing with medicinal plants in Africa are of fairly recent date, reports dealing with ethnopharmacological aspects being even more recent. An extensive review on African traditional medicine and on the use of plants for medicine is written by Maurice Iwu, a Nigerian pharmacognosist and ethnopharmacologist (Iwu, 1993). A number of traditional national pharmacopeias have appeared, starting with Madagascar in 1957, and research in the field of ethnobotany and ethnopharmacology has developed rapidly in many African countries (Hedberg & Staugård, 1989). The *African Pharmacopoeia*, covering traditional medicine of many African countries, has been published by the Scientific Technical Research Commission of the Organization of African Unity, starting with volume 1 in 1985 (*African Pharmacopoeia*, Vol. 1, 1985).

2.1.2. The importance of traditional medicine in different African countries

Traditional medicine is an important part of the health-care system in most of the African countries. About 80 – 90 % of the populations in African countries are dependent on traditional medicine for their primary health care (Hostettman et al., 2000). For example in Sudan, traditional medicine plays an important role for health care, since access to hospitals and other medicinal facilities is limited and a high percentage of the population are nomads (Elegami et al., 2002). In Tanzania, over 60 % of the health seeking population have a traditional healer as their first point of contact (Hedberg et al., 1982). In spite of an extensive programme to create health centers and to train Rural Medical Aids and Medical Assistants, the traditional healer is still the only medical practitioner available, within reasonable distance, to many Tanzanians living in the rural parts of the country (Hedberg et al., 1982). Traditional medicine is also important in the big cities of Tanzania, such as Dar-es-Salaam (Swantz, 1974). The number of registered traditional healers in Tanzania has been estimated to about 30.000 – 60.000, currently even 75.000 (Mhame, 2000), in comparison with about 600 Western-trained doctors (Weenen, 1990; <http://www.fao.org/docrep/X5327e/x5327e06.htm>). In South Africa it is estimated that about 27 million people depend on traditional herbal medicines for their primary health care (Meyer et al., 1996; Mander, 1998). In Nigeria traditional medicine is well acknowledged and established as a viable profession (Kafaru, 1994), and almost all plants seem to have some kind of application in traditional medicine (Babayi et al., 2004). Traditional medicine seems to have certain advances over imported systems of medicine because it is an integral part of the people's culture and is particularly effective in solving certain cultural health problems (von Maydell, 1996). The African Union and the O.A.U. Scientific Council for Africa work for the promotion of systematic research on traditional medicine in different African countries (Swantz, 1974).

The rising cost of Western medicine means that the people in African countries are increasingly turning to traditional medicine as an affordable alternative. The current policy in many African countries has been to incorporate traditional medicine into the formal health care sectors (Tsey, 1997). In some African countries it is thought that traditional medicine should be taught and practiced as part of formal health care sectors (Tamakloe, 1995). Gradually, traditional medicinal practitioners (TMP) are being officially accepted as part of African health services and their medical knowledge is finding its place in hospitals and clinics (Neuwinger, 2000). In South Africa, traditional medicinal practices have only recently been officially recognized as a legitimate form of health care and traditional medicine is now being integrated in the official health care system under the Reconstruction and Development Plan (RDP) (Pick, 1992). In many countries in Africa traditional methods are now being used for the treatment of HIV infection (Morris, 2002) and malaria (Njoroge & Bussman, 2006), including the use of medicinal plants

that help alleviate the symptoms of these diseases. These methods are sometimes claimed to give fewer side effects than conventional antiretroviral therapy (Morris, 2002).

2.1.3. The practice of traditional medicine in the African community

The holders of traditional medicinal knowledge differ in different indigenous groups. In some cases *all* members of the community may know how to treat a wide range of common diseases and only seek the advice of a traditional healer for the treatment of specific diseases when their own treatments have failed. For example in Tanzania, common plant treatments are known and used by the majority of rural people, as well as by many people in the cities, although these people are not recognized as “waganga” (medicine men) since they are not selling their services to others (Swantz, 1974). These day to day uses of medicinal plants are often included as a part of the diet. In many cases there are no clear indications of the extent to which medicinal plants grown in home gardens are used by households as opposed to being prescribed by traditional healers (<http://www.UNEP.org/CBD/WG8J/3/INF/3>). The TMP (traditional medicinal practitioners, medicine men) sell their services on a business basis (Swantz, 1974). In some indigenous groups TMP hold most of the medicinal knowledge, and in these cases the knowledge is often passed down through certain families/tribes from generation to generation under a system of apprenticeship (Swantz, 1974; Neuwinger, 2000). In Dar-es-Salaam, among the Zaramo, a considerable number of the traditional medicine men have learnt their “uganga” (medicinal practice) from other ethnic groups since their uganga is thought to be more powerful (Swantz, 1974). Traditional healers collect medicinal plants from the wild and/or cultivate some of them for their medicinal practice in their homes (Swantz, 1974; Hedberg & Staugård, 1989). Among the Zaramo in Dar-es-Salaam almost every tree, shrub or grass is believed to have medicinal value (Swantz, 1974). Traditional healers may sell some of their medicinal plants on local markets, as could be observed in Dar-es-Salaam (Fyhrquist, unpublished). In some African countries medicinal plants are sold in Amayeza stores, “medicine shops” (<http://www.UNEP.org/CBD/WG8J/3/INF/3>).

African traditional medicine involves both the body and the mind. Health in Africa is the condition of physical, psychological, social and economical well-being. The cornerstone of African traditional medicine is that illness is usually due to *outside influences* and the requirement is to know *who* or *what* could have caused it (Neuwinger, 2000). For example the Yoruba in Nigeria have three explanations for the cause of illness and misfortune; the natural (physical cause), the preternatural (witchcraft, sorcery as causes) and the supernatural (various spirits as causes) (Prince, 1964). These fore mentioned categories of causes for diseases are shared by many other people in Africa, such as the Zaramo in Dar-es-Salaam (Swantz, 1974). Thus, the natural physical cause of the disease is treated with herbs, the preternatural cause with

magical acts and the supernatural cause with ritual sacrifices, offerings or exorcism (Swantz, 1974). The healer typically diagnoses and treats the psychological basis of the illness before prescribing medicines to treat the symptoms. Most healers believe that the body requires treatment with *several different plants*, and it is the *combination* of these which produces a healing effect, either through complementary benefits or synergy and potentiation (Neuwinger, 2000). Many recipes consist of several plants, or *different parts* of the same plant, since different plant parts often cause different effects. The magical strength of a healing plant is often also introduced into the therapy. Often the healers use plants according to their analogy and morphological similarities to the ailment being treated, so that for example plants containing red juice are used for everything connected to blood, as menstruation problems and bleeding (Neuwinger, 2000).

2.1.4. Information on the medicinal uses of plants in Africa is in danger of disappearing

The abundance of information on the traditional medicinal uses of plants in Africa is in danger of disappearing since the knowledge of how to use medicinal plants is mostly passed down orally and even to date is poorly documented (Gurib-Fakim, 2006), although written information has been produced for some specific regions (see chapter 2.1.2). The large volume written by Iwu (1993) about medicinal plants and their uses in several African countries is extensive and covers much, but Africa is large and there is still large areas which have not been explored for their traditional medicine.

It is rare to find healers with written documents, apart from minor memory aids as to plant characteristics, which help to find medicinal plants similar in appearance but different in healing effects. Oral transfer of knowledge is vulnerable to disruption and interference and may result in the loss and distortion of valuable ethnomedical information. In indigenous groups where traditional healers hold most of the knowledge this is likely to be even more a problem. This is further made more problematical by the wish of keeping information secret and a refusal to reveal information - an issue of particular relevance to medicinal plants (Hedberg & Staugård, 1989). Transfer of knowledge between the generations is also a problem since the younger generation understand traditional medicine as being a profession mainly conducted by members of an older generation. Still, young people are using traditional medicine for their health problems, and believe in it (Ntemi & Bracebridge).

Sustainability of the use of medicinal plants is an actual and important issue. The demand for medicinal plants is increasing in Africa as the population grows and pressure on medicinal plant resources will become greater than ever. The interest in plant derived medicines has also

increased in the West, among the pharmaceutical companies, and thus extensive gathering of plants from tropical habitats are made in order to find NCE. Most medicinal plants are currently obtained from the wild, and there are already reports of some medicinal plant species becoming extremely rare, such as *Warburgia salutaris* (Canellaceae), *Cassine transvaalensis* (Celastraceae) and *Erythrophleum lasianthum* (Leguminosae) (Fennel et al, 2004). The major factor contributing to the depletion of natural resources is the loss of habitats due to anthropogenic activity (Iwu, 1995; Rukangira, 2001). Africa is estimated to have 216,634 ha of closed forest areas and with a calculated annual loss of about 1 % due to deforestation, one of the highest rates of deforestation in the world, many of the medicinal plants and other genetic materials become extinct before they are even documented. Habitat conversion threatens not only the loss of plant resources, but also traditional community life, cultural diversity, and the accompanying knowledge of the medicinal value of several endemic plant species (Iwu, 1995). A large proportion of the plants found in Africa are endemic to the continent, the republic of Madagascar having the highest rate of endemism (82 %) (Gurib-Fakim, 2006; <http://www.UNEP.org/CBD/WG8J/3/INF/3>). Different solutions for the sustainable use of medicinal plants have been presented: Medicinal plants can, for example be grown as crops through small-scale farming in their natural habitats (Iwu, 1995; Van Staden, 1999). On the other hand, there are traditional healers who believe that plants grown under agricultural conditions will not have the same medicinal properties as those harvested from the wild. Cultivated material is believed to lack the “power” of wild medicinal plants (Cunningham, 1993).

2.2. Description, brief systematics and geographical extension of the Combretaceae family

The widespread pantropical Combretaceae family comprises of about 600 species and 20 genera (Tan et al., 2002), of which 11 occur in tropical Africa (Heywood, 1978). The family includes forest trees, 50 m or more high, to dwarf shrubs with subterranean rhizomes and short aerial shoots. In forests the large trees and lianas predominate, while in grassland the shrublike growth form is more common. Plants belonging to Combretaceae have compartmented hairs, known as the “combretaceous hairs”, which apart from the Combretaceae occur only in Cistaceae and Myrtaceae (Wickens, 1973). The family has traditionally been placed in the order Myrtales since Robert Brown established it in 1810 (Dahlgren and Thorne, 1984), and this placement has recently been supported by molecular studies (Conti et al., 1996). Within the family, two subfamilies, Strephonematoideae and Combretoideae, have been recognized since Engler & Diels (1899). The subfamily Strephonematoideae contains a single genus comprising six species, distributed in West Africa. The subfamily Combretoideae contains 19 genera, including the mangrove species, and is characterized by a wholly inferior ovary and seeds with small, folded and spirally twisted cotyledons. Combretoideae is a taxonomically and phylogenetically complex

group. Species of this subfamily are not always easy to recognize on the basis of their morphological characters, because the variation is wide in flowers, fruits and vegetative shoot morphology (Stace, 1965).

The most known genera, as medicinal plants, are *Combretum* and *Terminalia*. Some species of *Pteleopsis* are also used for traditional medicinal purposes.

2.2.1. The genus *Terminalia* L.

The second largest genus of Combretaceae, *Terminalia*, consists of 200 species, distributed in the tropics and subtropics (Wickens, 1973; Tan et al., 2002). About 30 species of *Terminalia* are found in Africa (Wickens, 1973). Species of *Terminalia* vary greatly in morphology, anatomy and karyotype evidence (Excell 1954; Stace, 1965; Ohri, 1996). The species of *Terminalia* are small to large trees depending on the growth habitat. In rainforests they can reach heights of up to 50 m, and often grow as emergents, reaching above the upper tree stratum. In Africa *Terminalia* species growing in Miombo woodlands are smaller, and usually reach heights of 10-20 m.

Morphology

The African species of *Terminalia* are most often trees and rarely shrublike in growth form (Wickens, 1973). *Terminalia* trees show a very distinct, pagoda-like tree architecture, known as Aubréville's Model: the main stem produces whorls of horizontal lateral branches and each lateral branch is made up of a succession of branchlet units, each with the tip turned up and a cluster of leaves at its apex (van Wyk & van Wyk, 1997). Species of *Terminalia* lack scales or microscopic glands. In contrast to the Asian species of *Terminalia* with fleshy, hard, resinous, 5-winged (-ridged) fruits, the fruits of the African species are usually 2-winged, papery and quite thin and very variable in shape and size, usually 1-3 cm long and 0.5-1.8 cm wide. The leaves of *Terminalia* species are usually spirally arranged, often crowded at the ends of the branches, sometimes on short shoots, rarely opposite, petiolate or sessile. Two glands are often present at the base of the lamina or on the petiole. There are usually both hermaphroditic and male flowers in the same inflorescence. Usually the flowers are borne on axillary spikes with male flowers towards the apex and hermaphroditic flowers towards the base. Male flowers are stalked, the stalk resembling pedicels but corresponding to the lower receptacle with abortion of the ovary. Hermaphroditic flowers are sessile. The receptacle is divided into a lower part (lower receptacle) and an upper part, often scarcely developed, expanding into a shallow cup terminating into sepals. Petals are absent. Stamens are usually 10. The ovary is completely inferior; style free, not expanded at the apex. The flowers of *Terminalia* are remarkably uniform throughout the genus and scarcely ever provide any taxonomically useful characters and great reliance must therefore be placed on leaf, bark and fruit characters for species identification. (Wickens, 1973).

Most of the African species of *Terminalia* are growing in various kinds of woodlands such as coastal woodlands and Miombo woodlands including *Brachystegia* woodlands, as well as wooded grasslands, and some are typical components in riverine forests and rain forests (Wickens, 1973).



Figure 1. A large *Terminalia sambesiaca*- tree (15 m) growing in the Kitonga gorge, Tanzania.
Photo: Pia Fyhrquist



Figure 2. Fruits of *Terminalia spinosa*. Photo: Pia Fyhrquist.



Figure 3. Miombo woodland in Kitonga gorge, Tanzania. *Terminalia sambesiaca* along with other species of *Terminalia* and *Combretum* were collected from this forest. Photo: Pia Fyhrquist

2.2.2. The genus *Combretum* Loefl.

The largest genus of the family Combretaceae is *Combretum*, comprising of about 250 species and distributed throughout the tropics and subtropics. *Combretum* is absent from Australia and the Pacific Islands. Three subgenera are recognized by Excell and Stace; they are *Combretum*, *Cacoucia* (Aubl.) Excell & Stace and the monotypic Asian subgenus, *Apetalantum* Excell & Stace. On the worldwide scale the subgenera *Combretum* and *Cacoucia* are separable with certainty only on the character of the presence of either scales (subgen. *Combretum*) or microscopic stalked glandular hairs (subgen. *Cacoucia*) (Wickens, 1973).

Morphology

The species of *Combretum* are trees, shrubs, shrublets or woody climbers, very rarely subherbaceous. Scales (subgen. *Combretum*) or microscopic (sometimes macroscopic) stalked glands (subgen. *Cacoucia*) are present. The subgenus *Combretum* is sometimes, in addition, divided into eleven sections, based on the floral, scale and fruit anatomy (Wickens, 1973). In the genus *Combretum* the leaves are opposite, verticillate or rarely alternate, usually petiolate, almost always with entire margins. The petiole is sometimes persistent, and especially in climbers it forms a hooked woody spine when the leaf abscises. The flowers are hermaphroditic, regular or slightly zygomorphic, 4-5-merous and they are borne in elongated or subcapitate axillary or extra-axillary spikes or racemes or in terminal or terminal and axillary, often leafy panicles. The receptacle is usually clearly divided into a lower part (lower receptacle) surrounding and adnate to the ovary, and an upper receptacle which sometimes is differentiated into a lower part containing the disk and an often more expanded upper part. Sepals are 4-5 (rarely more), deltate to subulate or filiform, sometimes scarcely developed. Petals are 4-5, small and inconspicuous or showy (white, purple, red) and exceeding the sepals. Stamens are twice as many as the petals, inserted in 1 or 2 series inside the upper receptacle. The disk of the receptacle is glabrous or hairy, with or without a free margin, sometimes inconspicuous and absent. In East African species the style is free, the stigma is sometimes expanded and the ovary is completely inferior. The fruit is 4-5 winged and ridged or angled, sessile or stipitate, indehiscent or rarely dehiscent; the pericarp is usually thin and papery, sometimes leathery, more rarely fleshy. Even if the fruits are often used as a good species identification characters, species identification is not always easy at the fruiting stage (Wickens, 1973).

The shrublike growth forms are predominantly found in savanna-like habitats such as grasslands and wooded grasslands, whereas the treelike often grow in Miombo woodland. Climbers can be found in riverine forests and thickets as well as on ruderal habitats. Some species can be found in coastal and even swamp forests. Many species grow in lowland rainforests (Wickens, 1973).



Figure 4. Flowers of *Combretum constrictum*. Photo: Pia Fyhrquist.



Figure 5. Fruits of *Combretum hereoense*. Photo: Pia Fyhrquist.

2.2.3. The genus *Pteleopsis* Loefl.

There are probably about nine species of *Pteleopsis* in tropical Africa. The genus is intermediate in many characters between *Combretum* and *Terminalia*. The species of *Pteleopsis* are small to medium-sized trees or occasionally shrubs. They lack scales or stalked glands. The leaves are opposite to subopposite, petiolate, almost glabrous to hairy. The flowers are andromonoecious (hermaphrodite and male flowers in the same inflorescence) as in *Terminalia*, 4-(5)-merous, pedicellate, in terminal and/or axillary or extra-axillary subcapitate racemes. The upper receptacle is campanulate, joined to the lower part of the receptacle by a slender stalk-like region. The hermaphroditic flowers are usually at the apex of the flowers and they have deltate sepals and somewhat obovate white petals. Stamens are ten and 2-seriate in East African species of *Pteleopsis*. The style is not expanded at the apex. The male flowers are usually placed towards the base of the inflorescence, similar to the hermaphrodite flowers, but with the ovary not developing and with a slender stalk replacing the lower receptacle. The style is present or vestigial. The fruit is 2-5 winged, and the wings are often decurrent into the comparatively long slender stipe. (Wickens, 1973).

Species of *Pteleopsis* can be found in dry evergreen and riverine forest, deciduous woodland, coastal bushland and wooded grassland (Wickens, 1973).



Figure 6. *Pteleopsis myrtifolia*. Branches with fruits. Photo: Pia Fyhrquist.

2.3. Conventional antibiotics and the problem of microbial resistance

Major improvements in the early recognition and treatment of infectious diseases has been done in the last 60 years. This has resulted in a significant reduction in the morbidity and mortality associated with these diseases. Unfortunately, bacteria and fungi have developed resistance to all classes of different antibiotics discovered to date (Alanis, 2005). The use/misuse of antibiotics has led to an increasing prevalence of multiple-drug resistant (MDR) strains, and there is now an urgent need to develop new effective antibiotic agents (Cantrell et al., 2001). Serious systemic fungal and bacterial infections have become a major cause of morbidity and mortality among hospitalized patients around the world in the 21st century. The incidence of serious microbial infections is increasing also due to the increasing number of immunocompromised patients due to HIV/AIDS (Espinel-Ingroff et al., 1998). In tropical countries infectious diseases account for approximately *one-half* of all deaths (Iwu et al., 1999). Many bacterial diseases, which were thought to have been eradicated from Western countries might once again become a serious health problem. There is thus an urgent need for compounds that act on novel molecular targets that circumvent the established resistance mechanisms.

2.3.1. Conventional antibiotics and their targets in bacterial cells

At least 17 different classes of antibiotics, synthetic and of microbial origin, have been produced up to date (Alanis, 2005). These antibiotics act by a) *inhibiting the synthesis of the bacterial cell wall*, b) *inhibition of protein synthesis*, c) *inhibition of DNA synthesis*, d) *inhibition of RNA synthesis*, e) *competitive inhibition of folic acid biosynthesis*, e) *disorganizing membranes* and other mechanisms (Madigan et al., 2000).

Penicillins and the cephalosporins, derived from the fungal species *Penicillium chrysogenum* and *Cephalosporinum* sp. inhibit the synthesis of the bacterial cell wall by binding to the transpeptidase enzyme thus inhibiting the formation of peptidoglycan. Gram-negative bacteria are in general more resistant than gram-positive bacteria to the actions of antibiotics since they contain an outer membrane with a lipopolysackaride layer which render them impermeable to certain antibiotics and bactericidal compounds (Nikaido, 1996). Thus the naturally derived penicillin G does not have the ability to penetrate the gram-negative outer membrane. In synthetically modified penicillins (semi-synthetic penicillins) the N-acyl side chain is modified and enables these compounds to penetrate the outer membrane of gram-negative bacteria, and thus semi-synthetic penicillins have a much broader spectrum of activity than penicillin G. The β -lactam ring structure in the penicillines is susceptible to the actions β -lactamases, enzymes produced by penicillin-resistant bacteria. Some semi-synthetic penicillins, such as oxacillin and methicillin, are β -lactamase resistant. The cephalosporins are more resistant than the penicillins

to the β -lactamases and thus have a broader spectrum of antibacterial activity (Madigan et al., 2000).

The other three main classes of antibiotics, derived from bacterial sources, such as the aminoglycoside and macrolide antibiotics, as well as the tetracyclines inhibit protein synthesis by binding to either the 30S or the 50S subunits of the ribosome (Madigan et al., 2000).

Purely synthetic antibiotics act for example as growth analogues, such as the sulfa drugs, which block the synthesis of folic acid, a precursor to nucleic acids. A variety of analogues are known to different vitamins, amino acids, purines, pyrimidins and other compounds. The quinolones act with bacterial DNA gyrase and prevent the gyrase from supercoiling bacterial DNA, which is required for packing DNA in the bacterial cell (Madigan et al., 2000).

2.3.2. Antifungal agents and their targets in fungal cells

Invasive fungal infections have emerged as major causes of morbidity and mortality. During the last decades, the frequency of life-threatening infections has increased dramatically along with the number of potentially invasive species (Abi-Said et al., 1997; Berrouane et al., 1999; Boschman et al., 1998; Nguyen et al., 1996; Pfaller et al., 1998). For many years the treatment of fungal infections was essentially limited to amphotericin B. Therapeutic options did not emerge until the late 1980s, when fluconazole and itraconazole were introduced. The azole derivatives are the most widely used antifungals today, although resistance is emerging. During the 1990s there has been a major expansion in antifungal drug research (Groll et al., 1998). Current strategies for finding new antifungal drugs include screening of natural products, among them also higher plants. Although many new antifungal drugs have been developed, they belong to relatively few chemical classes. The antifungal drugs available can be classified into five major classes, based upon their molecular mechanism of action: 1) Inhibition of DNA or RNA synthesis (flucytosine), 2) Impairment of membrane function (for example amphotericin B), 3) Inhibition of the synthesis of ergosterol (azole derivatives, allylamines), 4) Inhibition of 1,3- β -D-glucan synthase (echinocandin derivatives), 5) Inhibition of fungal mitosis, probably by preventing sliding of microtubules (griseofulvin). Antifungal agents aim on different genera of fungi, being active against certain but excluding others. For example, griseofulvin is active against dermatophytes, but not against *Candida* or *Aspergillus* species. However, the major impact of antifungal resistance is not in the context of genus-level resistance but on the intra-species level, such as when earlier sensitive species suddenly become resistant against the antifungal therapy (Bossche et al., 1994).

2.4. Plant derived antimicrobials

The search for antimicrobial agents has mainly been concentrated on lower plants, fungi and bacteria as sources. Much less research has been conducted on antimicrobials from higher plants (Iwu et al., 1999). Since the advent of antibiotics, in the 1950s, the use of plant derivatives as antimicrobials has been virtually nonexistent. The interest in using plant extracts for treatment of microbial infections has increased in the late 1990s as conventional antibiotics become ineffective (Cowan, 1999). For example, none of the conventional antifungal drugs used to date seems to be ideal in efficacy, safety and antifungal spectrum (Di Domenico, 1998; Ablordeppey et al., 1999). In addition, many of the antimicrobial drugs in use have undesirable effects or are very toxic, produce recurrence, show drug-drug interactions or lead to the development of resistance (White et al., 1998). Although some new drugs have emerged for the treatment of obstinate fungal infections, such as allylamines and caspofungine (Vicente et al., 2003), and combination therapy is sometimes used to make the treatment more effective, there is a real need for a next generation of safer and more potent antifungal drugs (Bartoli et al., 1998). Also, it is increasingly difficult to deliver new antibacterial leads by modifying known antibacterial compounds. Therefore, the focus on much antibacterial research has moved to the identification of new chemical classes (Barker, 2005), and many smaller pharmaceutical companies have taken up this challenge (Boggs & Miller, 2004). Antimicrobial compounds from plants may inhibit bacteria/fungi through different mechanisms than conventional antibiotics, and could therefore be of clinical value in the treatment of resistant microbes (Eloff, 1998a). Phytomedicines derived from plants have shown great promise in the treatment of infectious diseases including opportunistic AIDS infections (Iwu et al., 1999). For example, plants containing protoberberines and related alkaloids, picralima-type indole alkaloids and *Garcinia* biflavanones used in African traditional medicine, have been found to be active against a wide variety of micro-organisms (Iwu et al., 1999). Investigations on plants used in traditional medicine for skin afflictions might provide new tropical antiseptics urgently needed in the Third World countries (Taylor et al., 2001). Rapid extinction of some habitats and plant species due to deforestation, especially in the tropical parts of the world, lead to a loss of valuable antimicrobial chemicals (Lewis & Elwin-Lewis, 1995). Thus, many pharmaceutical companies are now intensifying their screening programs on medicinal plants.

2.4.1. Defense chemicals produced by plants

Higher plants produce a great diversity of chemicals that have antimicrobial activity *in vitro* (Van Etten et al., 1994). Most of these defense molecules are secondary metabolites, of which at least 12 000 have been isolated (Schultes, 1978). There are two broad categories of plant produced

antimicrobials. 1) *Phytoalexins* are low molecular compounds which are produced in response to either microbial, herbivorous or environmental stimuli (Van Etten et al., 1994). These compounds are synthesized *de novo*, and thus require activation of certain genes and enzymes required for their synthesis. Phytoalexins are chemically diverse and include simple phenylpropanoid derivatives, flavonoids, isoflavonoids, terpenes and polyketides (Bailey & Mansfield, 1982; Dixon, 1986; Greayer & Harborne, 1994). 2) *Phytoanticipins* are low molecular compounds which are present in plants before the challenge by microorganisms or are produced from pre-existing constituents after infection (Van Etten et al., 1994). These phytoanticipin toxins, e.g. phenolic and iridoid glycosides, glucosinolates and saponins are normally stored as less toxic glycosides in the vacuoles of plant cells. If the integrity of the cell is broken when penetrated by the microbe, the glycoside comes into contact with hydrolyzing enzymes present in other compartments of the cell, releasing the toxic aglycone (Osbourn, 1996). There is no sharp boundary between phytoalexins and phytoanticipins, and in one plant species a certain chemical can function as a phytoalexin, whereas it has the function of a phytoanticipin in another species (McMurphy & Higgins, 1984; Higgins & Smith, 1972). The rich diversity of secondary metabolites in plants has partly arisen because of *selection for improved defense mechanisms* against a broad array of microbes, insects and other plants. Related plant families often make use of similar secondary compounds for defense purposes (isoflavonoids in Leguminosae; sesquiterpenes in Solanaceae). *Most antimicrobial secondary metabolites have relatively broad spectrum of activity.* The specificity is determined to whether the pathogen has the enzymes necessary to detoxify a particular host product (Van Etten et al., 1994). 2.4.2. Plant derived individual compounds with antimicrobial effects

2.4.1.1. Phenolic compounds

Simple phenolics

Simple phenolics, such as caffeic acid and cinnamic acid are known to possess antimicrobial effects (Brantner et al., 1996). Catechol and pyrogallol both are hydroxylated phenols shown to be toxic against microorganisms. Increased hydroxylation of the phenol group has been found to result in increased toxicity to microorganisms (Geissman, 1963). On the contrary, it has in some cases been found that *highly oxidized phenols* are more inhibitory (Scalbert, 1991). Phenolic compounds are thought to inhibit microbial enzymes possibly through reaction with sulfhydryl groups (the oxidized phenols) or through nonspecific interactions with the proteins (Mason & Wasserman, 1987).

Quinones

The potential range of quinone antimicrobial effects seems to be great. Probable targets for the quinones in the microbial cell are the surface –exposed adhesins, cell wall polypeptides and

enzymes bound to the membranes. Quinones are known to complex irreversibly with nucleophilic amino acids in proteins (Stern et al., 1996) thus leading to inactivation of the protein and loss of its function. It is also possible that quinones render substrates unavailable to the microorganism (Cowan, 1999). Anthraquinones, the largest group of quinones (Harborne et al., 1999), have been found to possess antibacterial effects by inhibiting nucleic acid synthesis, at least in *Bacillus subtilis* (Levin et al., 1988).

Stilbenoids

Stilbenoids are composed of two benzene rings separated with an ethane or ethene bridge, called bibenzyls and stilbenes, respectively. Phenanthrenes are biosynthetically derived from the bibenzyls and stilbenes. Stilbenes occur as aglycones or glycosides, and sometimes as polymers. Many higher plant families are known to produce stilbenes. Bibenzyls and their derivatives are rare in higher plants but occur in some families including *Orchidaceae*, *Combretaceae* and *Dioscoreaceae*, often alongside the corresponding phenanthrene or stilbene derivatives. Many stilbenoids are known for their antifungal and antibacterial properties (Bruneton, 1999). Eloff et al. (2005) have found that leaves of the South African *Combretum woodii* contain high concentrations of the antimicrobially active bibenzyl, combretastatin B5.

Flavonoids

Flavonoids are constitutive compounds but are also synthesized by plants in response to microbial infection (Dixon et al., 1983). Nearly half of the 200 phytoalexins characterized up to now belong to the flavonoids (Harborne, 1988). Flavonoids have been found to show *in vitro* antimicrobial activity against a wide range of microorganisms, some showing potent activity against MRSA (Iinuma et al., 1994). Their activity has been attributed to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls (Cowan, 1999). Lipophilic flavonoids may also disrupt microbial membranes (Tsuchiya et al., 1996). There are conflicting findings on the kind of molecular substitutions needed for a flavonoid in order to show antimicrobial activity. Some authors have found that flavonoids lacking hydroxyl groups on their β -rings are more active against microorganisms than flavonoids containing these groups and this finding supports the idea that their microbial target is the membrane (Chabot et al., 1992). Several authors have, however, also found the opposite effect; the more hydroxyl groups the greater antimicrobial activity (Sato et al., 1996). The low toxic potential of flavonoids makes them ideal as antimicrobial medicines (Cowan, 1999).

Tannins

Tannins are a large group of polyphenolic compounds which have received attention in recent years due to their claimed ability to cure a variety of diseases (Serafini et al., 1994). Tannins are subdivided into two groups: *hydrolysable tannins* and *proanthocyanidins (condensed tannins)*. Hydrolysable tannins are gallic acid and ellagic acid esters of core molecules that consist of

polyols such as sugars. Proanthocyanidins are polymers of flavan-3-ols (for example catechin) and flavan-3,4-diols linked through an interflavan bond that is not susceptible to hydrolysis (Haslam, 1989). A wide range of anti-infective actions have been assigned to tannins (Haslam, 1996). Tannins have the ability to complex with proteins through nonspecific forces such as hydrogen bonding and hydrophobic effects and also through covalent binding (Stern et al., 1996). The antimicrobial mode of action for tannins may thus be related to their ability to inactivate microbial adhesins, enzymes, cell envelope transport proteins, etc. (Cowan, 1999). There is also evidence that tannins *directly inactivate* microorganisms, because already low concentrations of tannin (0.063 mg/ml) modify the morphology of germ tubes of *Crinipellis pernicioso* (Brownlee et al., 1998). Tannins have also been found to induce changes in the morphology of several species of ruminal bacteria (Jones et al., 1994). Due to their ability to bind to proteins and metals, tannins also inhibit the growth of microorganisms through substrate and metal ion deprivation (Scalbert, 1991). Hydrolysable and condensed tannins have been found to possess similar antifungal (filamentous fungi) and antibacterial potency, but the hydrolysable tannins were found to be more effective against yeasts (Cowan, 1999). Latté and Kolodziej (2000) found that a panel of different hydrolysable tannins had low antibacterial effects, but that they possessed fairly high anticryptococcal effects.

Some research has been performed on the relationship between tannin structure and antimicrobial activity. The presence of a *hexahydroxydiphenoyl* moiety or its oxidatively modified entities was an important feature for the anticryptococcal activity of the ellagitannins corilagin, pelargoniin B and phyllanthusiin (Latté & Kolodziej, 2000). The pattern of B-ring hydroxylation of monomeric flavonols in condensed tannins has been shown to affect the level of growth inhibition of *Streptococcus sobrinus* and *Streptococcus mutans* (Sakanaka et al., 1989), *Clostridium botulinum* (Hara & Watanabe, 1989), *Proteus vulgaris* and *Staphylococcus* (Mori et al., 1987), and in all cases galliccatechins were more inhibitory than their catechin counterparts. The toxicity of tannins and lower molecular weight phenols has been discussed also in relation to their oxidation state; catechin was found to be devoid of any toxicity against methanogenic bacteria, whereas if oxidized it strongly reduced methane production (Field et al., 1989). The synthesis of red beet β -glucan synthase was found to be strongly inhibited by various oxidized phenols, but the effect of oxidation was less marked for tannic acid (a hydrolysable tannin) than for smaller phenols (Mason et al., 1987). It has also been proposed that tannin toxicity would be related to molecular size (M_r), since the larger the molecule the more effectively it binds to proteins. This has been observed in many cases; dimeric ellagitannins have been found to be more astringent than related monomers (McManus et al., 1985). On the other hand, in some cases the toxicity of tannins was found to be no higher than that of catechins (Siwaswamy et al., 1985), although catechins have very poor affinity to proteins. Kakiuchi et al. (1986) found that adding BSA to a glucosyl transferase solution before addition of gallotannins failed to remove the inhibition of the enzyme by the tannins and they concluded that inhibition of the enzyme is not necessarily due to the nonspecific binding of tannins to it. In their study of an array of different tannins and their

effects on ligand binding to various enzyme receptors, Zhu et al. (1997) found that some of the tannins inhibited ligand binding to specific receptors. Thus, this study shows that tannins *have specific activity at the receptor level*, and that these effects cannot solely be explained in terms of protein binding.

2.4.1.2. Terpenes

Terpenes are a large group of compounds responsible for the fragrance of plants and comprise the so called essential oil fraction. They are synthesized from isoprenoid units, and share origins with fatty acids. They differ from fatty acids in that they are branched and cyclized. Terpenes occur as monoterpenes, diterpenes, triterpenes, tetraterpenes, hemiterpenes and sesquiterpenes. When additional elements, such as oxygen, are added they are called *terpenoids* (Cowan, 1999). Terpenes and terpenoids have been found to possess antimicrobial activity (Amaral et al., 1998; Himejima et al., 1992; Mendoza et al., 1997). The mechanism of action of terpenes on microbes is not yet fully understood, but it is speculated to involve membrane disruption by the lipophilic compounds. Accordingly, Mendoza et al. (1997) found that addition of a methyl group on kaurene diterpenoids drastically reduced their antimicrobial activity. When different series of terpenoids were investigated for their antimicrobial effects it was found that the more lipophilic compounds were significantly more antibacterial than their more polar analogues (Cantrell et al., 2001).

2.4.2. Antimicrobial effects of plant extracts

In traditional and alternative medicine it is common to use medicinal plants as such, without isolating the active ingredients from them. Using crude extracts might be a more important way to use medicinal plants than has been realized in Western medicine, since plants contain numerous secondary metabolites, and pathogens in nature interact with many chemicals simultaneously (Izhaki, 2002). Traditional plant remedies or phytomedicines, include crude vegetable drugs (herbs) as well as galenical preparations (extracts, fluids, tinctures, infusions) prepared from them. Although a number of studies of the antimicrobial effects of plant *extracts* have been performed, many plants used in different traditional medicinal systems have never been evaluated for their antimicrobial effects. For example, in Africa, over 5000 plants are known to be used for medicinal purposes, but only a small percentage have been described or studied scientifically, and different combinations of plant species used in traditional medicines have been studied even to a lesser extent (Taylor et al., 2001). The major problem in investigations on the biological activities of plant extracts and phytomedicines lies in the fact that a variety of plants may be used in a single traditional medicine preparation, and in the possibility of synergistic

effects resulting from the interactions of the compounds in the extract. This can even result in a loss of activity as the extract is purified (Couzinier & Mamatas, 1986). Eloff & McGaw (2006) point out that biologically active extracts can be extremely useful in their entirety, taking into account synergistic and other effects, and according to them an approval of standardized and formulated plant extracts as drugs might be the starting point in developing countries for a successful pharmaceutical industry to be able to compete with Western pharmaceutical companies.

2.5. Antimicrobial effects and traditional medicinal uses of some species of *Terminalia*, *Combretum* and *Pteleopsis* reported in the literature

2.5.1. *Terminalia* species

A number of studies have been carried out on the ethnomedical uses of *Terminalia* species in Africa. Many *Terminalia* species have various applications in African traditional medicine, such as for the treatment of hypertension, diarrhea, bacterial and fungal infections and fever, just to mention some of them (Hedberg, 1982; Kokwaro, 1976; Chhabra, 1989; Fyhrquist et al. 2002) (Table 1). In Africa, all parts of the *Terminalia* species are used for medicinal purposes (Watt & Breyer-Brandwijk, 1962; Bouquet & Debray, 1974; Hedberg et al., 1982; Chhabra et al., 1989; Neuwinger, 2000), the fruits being reported to be used in only one case (Eldeen et al., 2005). In herbal remedies species of *Terminalia* are mostly used as hot water decoctions (woody plant parts) or infusions (leaves), but it is also common to mix dried, powdered plant parts with *Ugali*, maize porridge, and in some cases the fresh leaf sap is used. Sometimes decoctions of species of *Terminalia* are mixed with rice or maize porridge. Some of the species of *Terminalia* are used topically as well, mainly for the treatment of wounds, and are then applied as dressings or ointments. Sometimes the fumes of hot fomentations of the roots are inhaled for treatment of chest pains, presumably pneumonia or bronchitis.

According to Hartwell (1982) at least seven different species of *Terminalia* are used for the treatment of cancer in systems of Asian traditional medicine. Especially the fruits of the Asian species are frequently used for medical applications such as fever, cough, diarrhea, dysentery and skin diseases (Valsaraj et al., 1997) and even for food (Barthakur et al., 1991) (Table 2).

There are several investigations, most of them of quite recent date, on the antibacterial and antifungal effects of both African (Table 1), Asian (Table 2) and South American species of *Terminalia*. The antimicrobial effects have been evaluated with diverse methods, such as agar diffusion, agar dilution, direct bioautography and liquid serial dilution methods (microplates etc.) (Table 1 and 2). In most of the investigations crude extracts have been evaluated for their antimicrobial effects, but in some cases active fractions and compounds have been isolated and

even evaluated for their antimicrobial potential. Some of the results on the antimicrobial effects of *Terminalia* species support their use in traditional medicine for treatment of infectious diseases. The genus *Terminalia* seems to include species with potent antimicrobial effects and good effects have been obtained against both gram-positive and gram-negative bacteria as well as against yeasts (*Candida* spp.), the basidiomycet *Cryptococcus neoformans* and dermatophytes (*Trichophyton* spp., *Epidermophyton* spp. and *Microsporum* spp.). *Terminalia* species contain an array of different compounds with antimicrobial effects (Table 1 and 2) such as hydrolysable tannins (mostly ellagitannins) and their monomers gallic acid and ellagic acid, methylated ellagic acid derivatives, ethyl gallate, flavonoids, stilbenes, saponins, lignans, coumarins and triterpenoids. Antimicrobial compounds have been found from all plant parts of *Terminalia* species.

Many of the investigations on the *in vitro* antimicrobial effects of compounds isolated from *Terminalia* spp. lack studies on the toxicity of the compounds, and these studies would be essential to predict the safety of use. Some organs of some species of *Terminalia* are reported to be poisonous, such as the roots of *T. sericea* (Watt & Breyer-Brandwijk, 1962) and thus care should be taken to prescribe the correct dosages. The use of extracts, and especially of unstandardized extracts made of plants from the wild is not free of risks since the composition of the secondary compounds may show great intraspecific variation. Therefore standardized extracts should be used if possible.

2.5.1.1. African species of *Terminalia*

Some African *Terminalia* species reported to have uses in traditional medicine/antimicrobial effects are briefly presented (Table 1). Emphasis is put on East African species which are used in my doctoral thesis, but some other species are presented briefly as well.

Terminalia sericea

Terminalia sericea Burch ex DC., Silver cluster-leaf (vernacular name in Swahili: *Mhungweluwala*, *Mpululu*; Nyakusa: *Namatipo*). This species occurs in tropical and South East and South West Africa and reaches its northern limits in Zaire and Tanzania. It occurs in *Combretum-Terminalia*, *Brachystegia* and *Colophospermum* woodland (Miombo woodland) as well as on wooded grassland on sandy soils, where it is frequently dominant or co-dominant, and forms dense stands. *T. sericea* is a small-medium sized deciduous tree (3-16 m) (Wickens, 1973). The crown is rounded to flattish, characteristically layered as in most species of *Terminalia*. The flowers are small, whitish on spikes, unpleasantly scented, and appear on the trees from July-January (Drummond & Coates-Palgrave, 1973; van Wyk & van Wyk, 1997). The fruit is pinkish to purplish brown and surrounded by two wings. The leaves are clustered towards the tips of the

branches, narrowly obovate-elliptic and densely covered with silvery, silky hairs (van Wyk & van Wyk, 1997). The name *sericea* means silky (Drummond & Coates-Palgrave, 1973).

T. sericea has a wide range of uses in traditional medicine (Table 1) and has recently been selected as one of the fifty most important medicinal plants in Africa by the Association for African Medicinal Plant Standards (<http://www.aamps.net>). The roots are the most frequently used part of *Terminalia sericea* in African traditional medicine and are prepared for herbal remedy in various ways. Hot water decoctions of the roots are used to stop diarrhea and to relieve colic pains (Neuwinger, 1996; Tshikalange et al., 2005; Moshi & Mbwambo, 2005), as well as for treatment of certain eye diseases (Drummond & Coates-Palgrave, 1973). Decoctions of the roots of this plant are also used against bilharzia (Kokwaro, 1976) and gonorrhoea (Hedberg et al., 1982). Sometimes root decoctions of *T. sericea* are mixed together with *Carica papaya*, *Citrus limon* and bark of *Parinari excelsa*, and infusions of the roots of *T. sericea* with *Peltophorum africanum*, *Pterocarpus angolensis* and *Xymenia caffra* for the treatment of venereal diseases (Neuwinger, 2000). Menstrual cramps are treated with enemas of root decoctions of *T. sericea* (Neuwinger, 2000). Roots steeped in water for one hour are used for the treatment of *trachoma*, a disease in the eyes (Neuwinger, 2000). The roots are made into tea for treatment of skin diseases and cough (Neuwinger, 2000). Powdered root is swallowed together with food to treat headache and to treat general body weakness (Neuwinger, 2000). The outer layers of the roots are boiled in water to provide a liquid which is used in hot fomentation (Drummond & Coates-Palgrave, 1973) or chest is exposed to the vapours of this liquid (Neuwinger, 1994) to treat pneumonia. The powdered root bark is taken with corn meal against diabetes (Watt & Breyer-Brandwijk, 1962). The roots of *T. sericea* are also reported to be emetic. The root is considered poisonous by many traditional healers (Watt & Breyer-Brandwijk, 1962), why caution must be taken while using this plant organ for medicine. Other plant parts than the roots are more rarely used in traditional medicine, but there are some reports of the uses of leaves and stem bark and even the fruits of *T. sericea*. Leaf infusions are used to treat “chest complaints” (pneumonia?) and diarrhea (Neuwinger, 2000). Wounds are treated with dressings of stem bark or leaves of *T. sericea* (Neuwinger, 2000). The stem bark of *T. sericea* is exported to Europe and possibly to Germany from Mozambique according to some reports (Rukangira, 2000). The dried fruits are used for treatment of tuberculosis (Eldeen et al., 2005).

The uses of *T. sericea* for the treatment of infectious diseases in African traditional medicine are justified by several investigations which report that this species possesses great antimicrobial activity (Table 1). Aqueous (Tshikalange et al., 2005; Steenkamp et al. (2004) and methanol (Steenkamp et al., 2004) extracts of the roots of *T. sericea* have been found to inhibit the growth of gram-positive bacteria, but did *not affect the growth of gram-negative bacteria* up to concentrations of 4 mg/ml, whereas chloroform extracts were inactive against all the investigated bacterial species (Tshikalange et al., 2005). Moshi & Mbwambo (2005) found that in addition to being active against gram-positive bacteria, extracts of the roots *T. sericea* showed activity also against the gram-negative bacterium, *Escherichia coli*. Intermediate and polar extracts of the

roots of *T. sericea* have in general been found to possess the highest antimicrobial activity compared to more non-polar extracts (Moshi & Mbwambo, 2005; Tshikalange et al., 2005), and have also been found to be antifungal against *Candida albicans* and *Aspergillus niger*. In addition to the roots also the leaves of *T. sericea* have been found to be antimicrobial and activity was demonstrated both against gram-positive and gram-negative bacteria (Eloff, 1999). It is possible that the leaves contain different antimicrobial compounds as compared to the roots. Only one investigation has been done on the isolation of antimicrobially active compounds of *T. sericea*: Eldeen et al. (2005) have recently isolated Anolignan B from the roots of *T. sericea* and this compound showed activity both against gram-negative and gram-positive bacteria, and gave a MIC value of 3.8 µg/ml against *Bacillus subtilis*. In some previous investigations on the chemical constituents of *T. sericea* (Bombardelli et al., 1974 and 1986) the triterpenoids sericoside and arjunglucoside were isolated from the roots and stem bark of this plant, but no work on their biological effects was performed. On the other hand related terpenoids, such as arjungenin, sericic acid and arjunic acid, isolated from *T. macroptera*, have shown excellent antimicrobial effects (Conrad et al., 1998). Thus triterpenoids from *Terminalia* species seem to possess powerful antimicrobial effects.

Terminalia kaiserana

Terminalia kaiserana F. Hoffm. (vernacular name according to Makonde tribe: *Mnyenze*; Swahili: *Mpululu*; Nyakusa: *Bena*). This species of *Terminalia* grows to a small tree, up to 10 m, or is shrublike. The bark is fissured and the branchlets sericeous-tomentose to glabrous. The bark of the branches is purplish brown to purplish black, peeling off in strips to reveal a light brown, newly exposed surface. The leaves are spirally arranged and sparsely sericeous when young, but soon glabrescent. The inflorescences form axillary spikes, up to 11 cm long and flowers are creamy white. The fruit is purplish brown, broadly elliptic and surrounded by two wings. *T. kaiserana* occasionally forms hybrids with *T. sericea*. These hybrids are characterized by more persistent hairs on the leaves, and they are longer and less sericeous than those of *T. sericea*. *T. kaiserana* occurs in *Brachystegia* woodland (Miombo woodland) and wooded grassland. (Wickens, 1973).

Chhabra et al. (1989) report that the roots of *Terminalia kaiserana* are decocted and drunk for the treatment of headache and backache. According to Kokwaro (1976) and Haerdi (1964) the roots are used for gonorrhoea, bilharzia and as diuretics. Root decoctions of *T. kaiserana*, together with *Dalbergia (Dalbergiella?) nyassae* and *Bridelia cathartica* are massaged on the painful region to treat kidney pains. The leaf sap and root decoction are drunk for the treatment of schistosomiasis, scabies, cough and cardiac problems. Bark and root decoctions are taken together with rice or cooked in a meal gruel and eaten as an aphrodisiac (Haerdi, 1964). Although *T. kaiserana* is popular in African traditional medicine there are no reports on antimicrobial or other biological activity or the chemicals of this plant.

Terminalia sambesiaca

Terminalia sambesiaca (zambesiaca) Engl. & Diels., River cluster-leaf (vernacular name according to Pare tribe: *Mnyara-ndeghe*, *Mpuku*; according to Hehe-tribe: *Msame-dume*). *T. sambesiaca* grows into a tall tree, up to 39 m high. The bark is greyish, smooth to slightly rough and fissured. Young branchlets are tomentose, becoming glabrescent, with fibrous bark. The leaves are spirally arranged, petiolate; the lamina is elliptic to broadly elliptic or obovate-elliptic, the apex is rounded and distinctively acuminate, margin sometimes crenulate, base cuneate to obtuse or rounded, pubescent to pilose. The petiole is up to 4 cm long, tomentose. The inflorescences are axillary and occasionally terminal spikes, up to 15 cm long. The flowers are white, not sweetly scented. The fruits are reddish brown, elliptic and surrounded by two large wings. The apex of the fruits is subtruncate and sometimes emarginate; the base is narrowed into a stipe. *T. sambesiaca* has sometimes been confused with *T. kilimandcharica*, but can be readily distinguished from this species by the distinctively acuminate and sparsely haired leaves. In East Africa, *T. sambesiaca* extends up along river valleys into the range of *T. kilimandsharica*, and in some places it becomes difficult to differentiate from *T. kilimandsharica* without habit notes. (Wickens, 1973).

Powdered root bark of *T. sambesiaca* is mixed with porridge and eaten for the treatment of bloody diarrhea, whereas the stem bark and leaves are decocted and drunk to treat cancer, stomach ulcers and appendicitis (Chhabra, 1989). Extracts of the stem bark of *T. sambesiaca* showed antibacterial activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Shigella boydii* (Chhabra et al., 1989), which justifies the traditional uses of this plant for the treatment of bloody diarrhea, stomach ulcers and appendicitis. Leaf extracts of *T. sambesiaca* have been found to possess antifungal effects against *Candida albicans* and *Cryptococcus neoformans*, with average MIC values ranging from 0.232 – 0.64 mg/ml (Masoko et al., 2005; Masoko & Eloff, 2005) and the antifungal activity was concentrated to nonpolar hexane and dichloromethane fractions.

Terminalia spinosa

Terminalia spinosa Engl. (vernacular name according to Zaramo tribe: *Mtaba*) is a common species in East Africa. It grows to a tree, up to 20 m high. The bark is grey, longitudinally fissured and the branchlets are greyish or reddish brown. Long shoots zigzag with occasional spur shoots. 2 (-3) spines occur at the base of the spur shoots. The leaves are borne on the spur shoots, the lamina being broadly obovate. The inflorescences are up to 6 cm long spikes which are borne on the spur shoots. The flowers are white to purplish, shortly pedicellate and glabrous outside. The fruit is reddish purple, oblong-elliptic with no apical peg, 2-winged (Wickens, 1973). *T. spinosa* occurs in *Acacia*, *Commiphora* and coastal bushland, and less often in *Brachystegia* woodland and wooded grassland.

The fresh leaves of *T. spinosa* are pounded and the juice (fresh leaf sap) is drunk for the treatment of malaria. *T. spinosa* stem bark is chewed or added to tea to reduce fevers (Heine & Brenzinger,

1988). *T. spinosa* is believed to have magical properties: the wind makes a mournful wailing noise like a mad person when it blows through the thorns (Kokwaro, 1976).

Fabry et al. (1996) found good antibacterial activity of extracts of young branches of *T. spinosa* against *Staphylococcus aureus* and *Enterococci*, but no activity against *Mycobacterium tuberculosis*. *T. spinosa* has been found to contain coumarins and tannins (Okemo, 1996; Omulokoli, 1997) but these compounds have not been evaluated for their antimicrobial effects.

Terminalia avicenoides

Terminalia avicenoides Guill. & Perr. is a tree that is common in the savanna region in West Africa (Abdullahi et al., 2001). This plant is used in traditional medicine for treating wounds and ulcers, and decoctions of the roots are used for gastrointestinal disorders. Jukuns of Northern Nigeria use the roots of *T. avicenoides* for the treatment of syphilis (Irvine, 1961). The stem bark of *T. avicennioides* has been found to be fungicidal against the dermatophytic *Epidermophyton floccosum*, *Microsporum gypseum* and *Trichophyton mentagrophytes* and fungistatic against *Candida albicans* (Baba-Moussa et al., 1999), the first mentioned finding supporting the traditional use of this species for treatment of wounds and the second for gastrointestinal disorders. Extracts of the stem bark of *T. avicenoides* contain large quantities of saponins and tannins (Baba-Moussa et al., 1999), which might be responsible for the good antifungal effects. Aqueous root extracts of *T. avicenoides* possess antidiarrheal effects (Abdullahi et al., 2001), supporting the uses of root extracts of this plant for the treatment of gastrointestinal disorders.

Terminalia glaucescens

In the Central African Republic *Terminalia glaucescens* Planch. ex Benth is used for treatment of dysentery and other microbial infections and the leaves are reported to be useful in the last phase of AIDS (Koudou, 1995). The traditional use of this plant is in agreement with the good antimicrobial effects shown by this species against both bacteria and fungi. Leaf and root extracts of *T. glaucescens* have been found to be highly active against both *Candida* species (MIC 0.25 mg/ml) and dermatophytes such as *Trichophyton* spp. (Batawila et al., 2005). *T. glaucescens* is used as chewing sticks in some countries in West Africa, and extracts made from the sticks (the stem wood and bark) showed a wide spectrum of antibacterial activity against different bacterial species including methicillin-resistant *Staphylococcus aureus* (MRSA) and dentally relevant bacteria (Taiwo et al., 1999). A triterpenoid, Terminalin A, was isolated from the stem bark of *T. glaucescens* (Atta-ur-Rahman et al., 2002), but the biological effects of this compound have not been investigated.

Terminalia macroptera

Terminalia macroptera Guill & Perr. is a tree widely distributed in Africa. In Guinea-Bissau, West-Africa, it is used by traditional healers for the treatment of hepatitis and other infectious

diseases, including venereal diseases (Diniz et al., 1996). The traditional uses of this species agree well with the findings of several authors that *T. macroptera* possesses strong *in vitro* antibacterial and antifungal effects. Root extracts of *T. macroptera* have been found to give slight activity against *Candida albicans* as well as showing an interesting profile of activity against enteropathogenic microorganisms, including *Shigella dysenteriae*, ethanol and water extracts being especially active (Silva et al., 1996). The four major compounds in the root extract of *T. macroptera* were identified as ellagitannins (Silva et al., 2000) and thus some of these compounds might be responsible for the good antibacterial effects observed for *T. macroptera* roots. Also the leaves of *T. macroptera* possess antimicrobial potency, a diethyl ether fraction being especially active against *Neisseria gonorrhoeae* (Silva et al., 2002). The leaves were found to contain chlorogenic acid, quercetin, isoorientin, ellagitannins and their monomers gallic acid and ellagic acid (Silva et al., 2002). The stem bark of *T. macroptera* contains antifungal and antibacterial triterpenes active against *Bacillus subtilis* and *Pseudomonas fluorescens* at MIC values of 2.5 – 5 µg/ml (Conrad et al. (1998). The triterpenoids were less active against the fungal species, *Cladosporium cucumerinum*, except from sericic acid, which inhibited the growth of this fungus with a MIC value of 5 µg/ml. The hydrolysable tannins isoterchebulin and 4,6-*O*-isoterchebuloyl-D-glucose from the stem bark of *T. macroptera* showed bacteriostatic effects against *Bacillus subtilis* and none of the tannins were found to be active against *Pseudomonas fluorescens* even at the highest concentration used (1024 µg/ml) (Conrad et al. (2001).

Table 1. Antimicrobial activity of extracts and their active compounds as well as uses in traditional medicine of some African *Terminalia* species according to literature.

Species	Extract	Compounds	Screening method	Uses in traditional medicine
<i>T. avicennoides</i> Guill. & Perr.	Stem bark: dermatophytes (fungicidal), <i>Candida albicans</i> (fungistatic) (Baba-Moussa et al., 1999); roots: antidiarrheal (Abdullahi et al., 2001).	Saponins and tannins in stem bark * (Baba-Moussa et al., 1999)	Agar diffusion (Baba-Moussa et al., 1999)	Remedy for wounds and ulcers; decoctions of the roots for gastrointestinal disorders; Jukuns of Northern Nigeria use the roots for syphilis (Irvine, 1961).
<i>T. brachystemma</i> Welw. ex Hiern	Acetone and MeOH extr. of leaves: <i>Candida albicans</i> <i>Cryptococcus neoformans</i> (MIC 0.124- 0.163 mg/ml) (Masoko et al., 2005).	Unknown	Microwell plates, bioautography (Masoko et al., 2005)	For fungal infections in South African traditional medicine (Masoko et al., 2005).
<i>T. gazensis</i> Bak.	Acetone and MeOH extr. of leaves: <i>Candida albicans</i> <i>Cryptococcus neoformans</i> (MIC 0.124- 0.163 mg/ml) (Masoko et al., 2005).	Unknown	Microwell plates, bioautography (Masoko et al., 2005)	For fungal infections in South African traditional medicine (Masoko et al., 2005).
<i>T. glaucescens</i> Planch. ex Benth	Extracts of leaves, roots: <i>Candida</i> spp. (MIC 0.25 mg/ml), <i>Trichophyton</i> spp. (Batawila et al., 2005); extracts of stem wood and bark: MRSA, dental bacteria (Taiwo et al., 1999).	The triterpenoid Terminalin A from stem bark * (Atta-ur-Rahman et al., 2002)	Aqueous dilution (Batawila et., 2005) Agar diffusion (Taiwo et al., 1999)	In Central African Republic decoctions of leaves for dysentery and microbial infections related to AIDS (Koudou et al., 1995); as chewing sticks in West Africa (Taiwo et al., 1999).
<i>T. kaiserana</i> F. Hoffm.	No reports	Unknown	No reports	Roots in combination with <i>Dalbergia nyassae</i> and <i>Bridelia cathartica</i> to treat kidney pains; leaf sap and root decoction for schistosomiasis, scabies, cough and cardiac problems; bark and root decoctions with rice or cooked in a meal gruel as an aphrodisiac (Haerdi, 1964); roots for gonorrhoea, bilharzia and as diuretics (Kokwaro, 1976; Haerdi, 1964); roots for headache and backache (Chhabra et al., 1989).

Table 1. (continued)

<i>T. macroptera</i> Guill & Perr.	Extracts of roots: <i>Candida albicans</i> , <i>Shigella dysenteriae</i> (MIC 313 µg/ml), <i>Vibrio cholerae</i> (MIC ≤ 156 µg/ml) (Silva et al., 1996 and 1997); extracts of leaves: <i>Neisseria gonorrhoeae</i> (Silva et al., 2002).	Triterpenes from stem bark: <i>Bacillus subtilis</i> , <i>Pseudomonas fluorescens</i> (MIC 2.5 – 5 µg/ml) (Conrad et al., 1998); ellagitannins from stem bark: <i>Bacillus subtilis</i> (Conrad et al. 2001); ellagic acid, gallic acid, ellagitannins from roots and stem bark* (Silva et al., 2002); flavonoids, ellagitannins from leaves *(Silva et al., 2002).	Agar diffusion, agar dilution, microwell plates, bioautography (Silva et al., 1996, 1997 and 2002) Bioautography (Conrad et al., 1998) Microwell plates (Conrad et al., 2001)	In West-Africa for hepatitis and other infectious diseases, including venereal diseases (Diniz et al., 1996).
<i>T. prunioides</i> M. A. Lawson	Acetone and MeOH extr. of leaves: <i>Candida albicans</i> , <i>Cryptococcus neoformans</i> (MIC 0.124- 0.163 mg/ml) (Masoko et al., 2005).	Unknown	Microwell plates, bioautography (Masoko et al., 2005)	Used for fungal infections in South African traditional medicine (Masoko et al., 2005).
<i>T. sambesiaca</i> Engl. & Diels	Extracts of stem bark: <i>Staphylococcus aureus</i> , <i>Pseudomonas aeruginosa</i> , <i>Salmonella typhi</i> and <i>Shigella boydii</i> (Chhabra et al., 1989); extracts of leaves: <i>Candida albicans</i> , <i>Cryptococcus neoformans</i> MIC 0.232 – 0.64 mg/ml (Masoko et al., 2005; Masoko & Eloff, 2005).	Unknown	Agar diffusion (Chhabra et al., 1989) Microwell plates (Masoko et al., 2005 ; Masoko & Eloff, 2005)	The root bark for bloody diarrhea, and the stem bark and leaves for cancer, stomach ulcers and appendicitis (Chhabra, 1989).

Table 1. (continued)

<i>T. sericea</i> Burch ex DC.	Aqueous extracts of the roots: gram-positive bacteria; chlorophorm extracts inactive against the bacterial species used in study (Tshikalange et al., 2005); root extracts: <i>Aspergillus niger</i> , <i>Candida albicans</i> , <i>S. aureus</i> , <i>Escherichia coli</i> (Moshi & Mbwambo, 2005); MeOH and aqueous extracts of stem bark: <i>S. aureus</i> (MIC 1 mg/ml) and <i>Streptococcus pyogenes</i> (Steenkamp et al., 2004); ethylacetate extr. of roots: <i>Bacillus subtilis</i> , <i>Klebsiella pneumoniae</i> , MIC 0.3-1.5 mg/ml (Eldeen et al., 2005).	The triterpenoids sericoside and arjunglucoside from roots and stem bark * (Bombardelli, 1974 and 1986); the lignan Anolignan B from roots, MIC from 3.8-31 µg/ml against various bacterial species (Eldeen et al., 2005).	Agar dilution (Tshikalange et al., 2005) Agar diffusion (Moshi & Mbwambo, 2005) Microwell plates (Steenkamp et al, 2004)	Diarrhea, dysentery, colic, diabetes (Watt & Breyer-Brandwijk, 1962); colic, pneumonia, eyewash (Drummond & Coates-Palgrave, 1973); bilharzia (Kokwaro, 1976); gonorrhoea (Hedberg et al., 1982); stomach problems, wounds, inflammation and venereal diseases (Neuwinger, 1996; Tshikalange et al., 2005; Moshi & Mbwambo, 2005); skin diseases, cough, general body weakness, pneumonia, venereal diseases (Neuwinger, 2000); diarrhea, fever, hypertension, bacterial infections (Fyhrquist et al., 2002); dried fruits for tuberculosis (Eldeen et al., 2005).
<i>T. spinosa</i> Engl.	Young branches: <i>Enterococci</i> , <i>S. aureus</i> (MIC _{90%} 1 mg/ml), not active against <i>Mycobacterium tuberculosis</i> (Fabry et al., 1996)	Alkaloids?, coumarins and tannins * (Okemo, 1996)	Microwell plates (Fabry et al., 1996)	Leaf sap for malaria (Kokwaro, 1976); bark chewed or added to tea to reduce fevers (Heine & Brenzinger, 1988); young branches and leaves produce a juice which is used for wounds (Chhabra et al., 1989).

* no activity tests performed; MIC, minimum inhibitory concentration.

2.5.1.2. Asian species of *Terminalia*

Many Asian species of *Terminalia* have been investigated for their antimicrobial effects, both against bacterial and fungal species. Many of the species reported to have antimicrobial properties *in vitro* have applications in traditional medicine for the treatment of microbial infections, such as diarrhea and venereal diseases. Since the fruits of *Terminalia* spp. are frequently used in Asian traditional medicine (Barthakur et al., 1991), investigations of the antimicrobial effects of this organ predominate, although also other plant parts, such as leaves and bark have been screened for antimicrobial effects. Antimicrobial hydrolysable tannins, flavonoids, triterpenoids, saponins, anthraquinones and lignans have been isolated from Asian *Terminalia* spp. The antimicrobial effects of some Asian species of *Terminalia* are summarized in Table 2.

Table 2. Antimicrobial activity of extracts and active compounds as well as uses in traditional medicine of some Asian *Terminalia* species according to literature.

Species	Extract	Compounds	Screening method	Uses in traditional medicine
<i>Terminalia alata</i> D. Dietr.	MeOH extract of stem bark: <i>Staphylococcus aureus</i> , <i>Candida albicans</i> and <i>Sacharomyces cerevisiae</i> (Taylor et al., 1996).	Flavanone and triterpene saponin from roots active on <i>Candida albicans</i> and <i>Aspergillus niger</i> (Srivastava et al., 2001).	Agar diffusion (Taylor et al., 1996). Agar diffusion (Srivastava et al., 2001).	In Indian and Nepalesian traditional medicine for diarrhea, dysentery and ulcers and as a diuretic and cardiotoxic (Chopra, 1986).
<i>Terminalia arjuna</i> (Roxb.) Wight & Arn.	Leaves, fruits and bark active on <i>Bacillus subtilis</i> , MIC 2.89 mg/ml (Elegami et al., 2002).	Tannins, flavonoids, saponins and terpenes * (Elegami et al., 2002); gallic acid, ethyl gallate, ellagic acid, luteolin, arjunin (Pettit et al., 1989; Kandil & Nassar, 1997).	Agar diffusion and agar dilution (Elegami et al., 2002)	Treatment of dysentery and earache (Dwivedi & Udupa, 1988); bark as a tonic, astringent and febrifuge (Chopra et al., 1992; bark for abdominal pain, diarrhea and vomiting (D'Souza, 1993).
<i>T. bellerica</i> Roxb.,	EtOH extracts of fruits possess broad antimicrobial spectrum, hexane extr. inactive (Ahmad et al., 1998); 80 % EtOH extract of fruit active against gram-positive and gram-negative bacteria, <i>Candida albicans</i> and <i>Aspergillus niger</i> (Valsaraj et al., 1997).	Lignans and a flavan antifungal against <i>Penicillium expansum</i> (Valsaraj et al., 1997).	Agar diffusion (Ahmad et al., 1998) Direct bioautography (Valsaraj et al., 1997)	Component of the Ayurveda medicinal preparation called "Triphala" (three fruits), used for treatment of fever, cough, diarrhea, dysentery and skin diseases (Kirtikar & Basu, 1991; Pushpangadan et al., 1986).
<i>T. chebula</i> Retz.	Aqueous and EtOH fruit extracts and powder of dried fruits inhibit <i>Helicobacter pylori</i> , <i>Shigella dysenteriae</i> and <i>Shigella typhimurium</i> (Malekzadeh et al., 2001); EtOH extract of fruits active against methicillin resistant and sensitive <i>Staphylococcus aureus</i> (Sato et al., 1997); EtOH and aqueous extracts active, whereas hexane extract inactive (Ahmad et al., 1998); aqueous extract inhibits growth, adherence and aggregation of <i>Streptococcus mutans</i> (Jagtap et al., 1999); extracts of ripe seeds very active against <i>S. aureus</i> , MIC 0.62 mg/ml (Shahidi Bonjar, 2004).	Gallic acid, ethyl gallate, MIC 7- 62.5 µg/ml (Sato et al., 1997).	Agar diffusion (Malekzadeh et al., 2001). Agar diffusion, microwell plates (Sato et al., 1997). Agar diffusion (Shahidi Bonjar, 2004).	Remedy for human gastritis and peptic ulcers in Iranian traditional medicine (Malekzadeh et al., 2001); for prevention and treatment of caries (Chopra & Handa, 1958); fruits for chronic ulcers, wounds and as laxative (Iyenger, 1985); prevention of infectious diseases in Ayurvedic system (Barthakur & Arnold, 1991).

Table 2. (continued)

<i>T. citrina</i> Roxb.	Methanol extracts of fruits antimicrobial (Burapadaja & Bunchoo, 1995).	Five hydrolysable tannins with antibacterial and anti- <i>Candida</i> activity (Burapadaja & Bunchoo, 1995).	Microwell plates (Burapadaja & Bunchoo, 1995)	In Thai traditional medicine for treatment of diarrhea and infections of the skin (Burapadaja & Bunchoo, 1995).
<i>T. pallida</i> Brandis	MeOH extract of fruits gave broad spectrum of antibacterial activity, including gram-negative bacteria (<i>Shigella dysenteriae</i> and <i>Escherichia coli</i>) as well as activity against <i>Aspergillus niger</i> and <i>Candida albicans</i> (625 – 5000 µg/ml) (Gupta et al., 2002).	Tannins, steroids, triterpenoids, anthraquinones and saponins * (Gupta et al., 2002).	Agar diffusion (Gupta et al., 2002).	Fruits used for diarrhea, venereal diseases and ulcers by the tribal people of Thirupati hills, India (Gupta et al., 2002).

* no activity tests performed; MIC, minimum inhibitory concentration.

2.5.2. *Combretum* species

At least twenty four species of *Combretum* are well known in African traditional medicine, and used for the treatment of a variety of ailments and diseases, ranging from scorpion and snake bites, mental problems, heart and worm remedies to fever and microbial infections (Watt and Breyer-Brandwijk, 1962, Table 3). Of the African species of *Combretum*, none have been recorded for the treatment of cancer before our record on the use of the roots and leaves of *C. zeyheri* for the treatment of cancer in Mbeya, southern Tanzania (Fyhrquist et al., 2002). On the other hand it is questionable whether cancer (or other internal diseases with complicated and diverse symptoms) can be diagnosed by traditional healers. In Africa, all parts of the *Combretum*-species, in some cases even the fruits (Watt & Breyer-Brandwijk, 1962; Van Wyk & Van Wyk, 1997), are used for medicinal purposes. The fruits and seeds are, although, in general considered poisonous by traditional healers in various African countries and have been reported to give toxic effects on humans (Rogers & Verotta, 1996; Van Wyk & Van Wyk, 1997). In some cases different plant organs from the same species of *Combretum* are mixed as a herbal remedy. Species of *Combretum* are prepared for herbal remedies as hot water decoctions, cold water extracts or mixed with food, such as maize porridge. Sometimes fresh leaf sap is used as such. The remedies made from *Combretum* species are used both internally and externally. Sometimes the curing compounds of the plants are inhaled through fumes of steam baths of hot water extracts or from the smoke of burnt plant material. Dressings and ointments of different plant parts are used mainly for the treatment of wounds and infections on the skin. It is very common to mix different species of *Combretum* or to mix *Combretum* spp. with other medicinal plants for herbal remedies.

Since species of *Combretum* frequently are used for the treatment of microbial infections in traditional medicine, many authors have investigated the *in vitro* antimicrobial effects of various species of *Combretum*. Many species of *Combretum* have been found to possess powerful antibacterial and antifungal effects (Table 3). The large number of antimicrobial compounds found in species of *Combretum* might explain why they are so widely used in African traditional medicine (Martini & Eloff, 1998). In many cases the *in vitro* effects of decoctions and infusions have been investigated, since this is the way in which the traditional medicines from this plant group are prepared. In some cases these decoctions have proved to be as effective as alcoholic and acetone extracts, but in other cases water extracts/decoctions have not yielded any antimicrobial components (Martini & Eloff, 1998). Among antimicrobially active compounds isolated from *Combretum* spp. are combretastatins (bibenzyle compounds), acidic tetracyclic and pentacyclic triterpenes/triterpenoids, ellagitannins, phenanthrenes, flavonoids, saponins and cycloartane glycosides (Table 3). There exists a chemotaxonomic link between the genera *Terminalia* and *Combretum*, since pentacyclic triterpenes, based on the 23-hydroxylated aglycone

with antibacterial activities are found in both *Terminalia stuhlmannii* and *Combretum imberbe* (Katerere et al, 2003).

In the following some African species of *Combretum* which have been found to possess good antibacterial and antifungal effects, as well as having applications in traditional medicine are presented. Special emphasis is taken on species which are included in this doctoral thesis, such as *C. molle*, *C. apiculatum*, *C. padoides*, *C. zeyheri* and *C. fragrans*.

Combretum molle

Combretum molle R. Br. Ex G. Don., Soft-leaved combretum, Velvet bushwillow (Vernacular name in Swahili: *Mlama*; Nyakyusa: Mpula, Kapula) occurs throughout tropical Africa in areas where woodlands and wooded grasslands predominate, often forming pure stands on hillsides (Wickens, 1973; Drummond & Coates-Palgrave, 1973). It also occurs on the Arabian Peninsula. This species is one of the most common constituents in *Brachystegia-Julbernardia* (Miombo) woodland. It is a deciduous tree reaching heights of 5-7 m, sometimes even 10-17 m (Drummond & Palgrave, 1973; Wickens, 1973). The bark is dark grey to black, rough, reticulately fissured, resembling crocodile skin. The bark of the branchlets is peeling in grey, fibrous strips. The leaves are arranged oppositely (sometimes 3-verticillately). The lamina is narrowly elliptic or narrowly ovate-elliptic. The leaf apex is acute or obtuse. The lamina is typically pubescent above and densely grey tomentose beneath. Leaf scales are silvery to reddish. Flowers are borne in axillary spikes, 7- (11) cm long, the flowers being greenish-yellow and fragrant. The fruit is 4-winged, subcircular to elliptic in outline, pale straw coloured to yellow brown. The apical peg of the fruits is up to 1 mm long (Wickens, 1973). Wickens (1973) claims that *C. molle* is extremely variable in leaf shape, reticulation and size of the fruits. These characters appear in various combinations throughout the distribution range and further confusion is caused through the presence of intermediate forms.

Combretum molle is widely used in African traditional medicine for treatment of various ailments and diseases. Various organs such as the leaves, roots, and to a lesser extent the fruits and stem bark have applications in traditional medicine. The most commonly used part are the roots, although also the leaves of this species are used frequently for medicinal purposes. The inner part of the root is powdered and used as a wound dressing (Drummond & Coates-Palgrave, 1973; Watt & Breyer-Brandwijk, 1962). Decoctions of the roots of *C. molle* seem to have a variety of uses such as for hookworm, stomach pains, snake bite, leprosy, fever, dysentery, general body swellings, abortion (Kokwaro, 1976; Chhabra et al., 1989) as well as for swelling of the abdomen, sterility and constipation (Chhabra et al., 1989). Root extracts are used as galactogogues (Neuwinger, 2000). Extracts of the roots are used for cough remedy (Drummond & Coates-Palgrave, 1973). Very often *C. molle* is mixed with other species of *Combretum* as well as with other plant species since remedies made from mixtures of plant species are thought to be more powerful in their healing effects. The roots of *C. molle* are mixed with the roots of *Annona*

chrysophylla Boj. or *Annona senegalensis*, and this remedy is used as an expectorant. Decoctions of the roots of *C. molle* are mixed with *Securinega virosa* Pax et K. Hoffm. (Euphorbiaceae), *Psorospermum febrifugum* var. *ferrugineum* Keay & Milne-Redhead and *Premna senensis* Klotsch (Verbenaceae) and drunk for the treatment of syphilis (Hedberg et al., 1982). To treat snakebites a mixture of small chips of the roots of *C. molle* and roots of *Markhamia obtusifolia* Sprague (Bignoniaceae) and *Vangueria rotundata* Robyn (Rubiaceae) is applied to the bite (Hedberg et al., 1982) and wounds from poisoned arrows are treated in the same way (Haerdi, 1964). There are only two reports on the medicinal use of the stem bark of *C. molle*: an aqueous suspension of the stem bark is used for gargling and is drunk to treat angina (Kerharo, 1974) and decoctions of the inner bark is used for treatment of stomach problems (Watt & Breyer-Brandwijk, 1962). The leaves of *C. molle* are used in various ways for medicinal purposes. Decoctions of dried leaves are boiled with salt and water and sprinkled on wounds. Fresh leaves, or moistened dry leaves, are used as dressings for wounds (Drummond & Coates-Palgrave, 1973). The juice of the leaves of *C. molle* is mixed with a decoction of the roots to treat abortions and as an antidiarrhoic (Haerdi, 1964). Aqueous extracts of the leaves are used to treat chest complaints, as anthelmintics and as inhalants in steam baths. Leaves of *C. molle* are used externally together with the roots of *Senecio lyratipartitus* to heal wounds (Kokwaro, 1976). Leaf decoctions are used for the treatment of dropsy. The dried leaves are mixed with food for the same purpose (Kerharo, 1974). An infusion of the leaves is drunk as an aid in child birth and a hot application is applied to the vulva and abdomen (Watt & Breyer-Brandwijk, 1962). Even if the fruits of *Combretum* species in general are reported to be toxic, the fruits of this species are used as an aid in child birth in the same way as the leaves (Watt & Breyer-Brandwijk, 1962). In addition to the above mentioned uses *C. molle* is reported to be used for treatment of HIV/AIDS related infections (Bessong et al., 2005) and malaria, (Abebe & Ayehu, 1993).

C. molle has been found to possess both antibacterial (Eloff, 1998b; Eloff, 1999; Khan et al., 2000), antimycobacterial (Asres et al., 2001a) and antifungal effects (Pegel & Rogers, 1985), which is in accordance with the uses of this plant for treatment of infectious diseases and wounds in African traditional medicine (Table 3). Eloff (1999) found that a leaf extract of *C. molle* was especially active against *Enterococcus faecalis* (MIC < 0.2 mg/ml). Pegel & Rogers (1985) found that the compound responsible for the antifungal effects of the leaf extract was the triterpenoid mollic acid-3- β -D-glucoside. Also the stem bark of *C. molle* contains antibacterial compounds, and its acetone fractions have been found significantly to inhibit the growth of *Mycobacterium tuberculosis* typus humanus (ATCC 27294) (Asres et al., 2001a). A hydrolysable tannin, punicalagin, was found to be responsible for the antimycobacterial effect of the stem bark, whereas the isolated saponins were found to be inactive (Asres et al., 2001a). Peeled twigs of *C. molle* are used as chewing sticks in Tanzania, among other plant species, and the bark of these sticks was found to possess both antibacterial and antifungal effects, whereas extracts of the wood were inactive or showed slight activity (Khan et al., 2000). Thus Khan et al. (2000) recommended the use of *unpeeled* twigs of *C. molle* for chewing.

Combretum padoides

Combretum padoides Engl. & Diels is a tree or many-stemmed scandent shrub, up to 12 m tall. The leaves are oppositely to suboppositely arranged, elliptic to oblong-elliptic with an acuminate apex. The lamina is glabrous or very sparsely pubescent. The inflorescences occur solitary or rarely 2-3 in the axils of the upper leaves to form branched terminal panicles. The flowers are yellowish- white and scented. The fruit is subcircular- elliptic, 4-winged, golden, and glabrous with an apical peg. *C. padoides* occurs in riverine, coastal and swamp forests as well as deciduous tickets. (Wickens, 1973).

Combretum padoides was among the seven most efficient species in a survey over the antimicrobial effects of 27 species of members of the family Combretaceae (Eloff, 1999). Extracts of the fresh leaves were in general more active than extracts of dried leaves, and especially good MIC values were obtained against *E. coli* and *Enterobacter faecalis*, which both were inhibited at 0.8 mg/ml of the extract. Rogers (1989) isolated mono- and bi-desmosidic triterpenoids from the leaves of *C. padoides*, why it is possible that these compounds in part might be responsible for the antibacterial effects of this species. *Combretum padoides* is not used for the treatment of infectious diseases in African traditional medicine, but the leaves are used for snakebites and the roots for eliminating hookworms (Neuwinger, 2000).

Combretum apiculatum

Combretum apiculatum Sond., Red bushwillow, Rooibos (not to be confused with Rooibos tea, *Aspalanthus linearis*) (Vernacular name according to Zigua tribe: *Muhuluka*). This species can be found in East, South and Southwestern Africa. *C. apiculatum* grows into a small deciduous tree, up to 10 m high and is rarely a shrub. The bark is grey to grayish black, smooth or reticulated; leaf buds are black or dark brown. The leaves are opposite; lamina glutinous when young, broadly to narrowly obovate-elliptic or oblong-elliptic or ovate to subcircular, 3-14 cm long, 1.5-7.5 cm wide. Leaf apex is usually apiculate or mucronate and usually twisted. Mature leaves are usually glabrous in East-Africa. The inflorescences are axillary spikes, up to 3-7 cm long. The flowers are yellow with no fragrance recorded (Wickens, 1973). The fruit is four-winged, 20-30 × 15-25 mm, glutinous when young, yellowish green, often tinged with red (van Wyk & van Wyk, 1997). *C. apiculatum* occurs on granitic, rhyolitic and basaltic soils in *Brachystegia* woodland (Miombo), wooded grassland and in *Acacia-Commiphora* bushland, and is also common on rocky hillslopes, often on well-drained soils (Wickens, 1973; van Wyk & van Wyk, 1997).

The root decoction of *C. apiculatum* is used for the treatment of mental illness and a necklace of the roots is used for the same purpose (Chhabra et al., 1989). Roots are chewed and the sap is swallowed for the treatment of snakebite and leaf decoctions are drunk for treatment of scorpion sting (Kokwaro, 1976). Root decoctions of *C. apiculatum* are used for the treatment of leprosy and bloody diarrhea (Kokwaro, 1976). The leaves are used for abdominal disorders and the stem

bark for conjunctivitis (Watt & Breyer-Brandwijk, 1962). Von Koenen (1996) reports that stomach problems are treated by a vapor bath of *C. apiculatum*, combining this treatment with drinking the leaf decoction of this plant. Leaves of *C. apiculatum* are introduced vaginally for treatment of body exhaustion in women (Gelfland et al., 1985). The leaves are also used to disinfect the navel after birth (Van Wyk, 1997; Von Koenen, 1996; Kokwaro, 1976).

In accordance with the traditional uses of *C. apiculatum* for treatment of bacterial infections, this species has been found to possess substantial antibacterial activity. In a survey on the antibacterial effects of 27 South African species of Combretaceae, Eloff (1999) found that leaf extracts of *C. apiculatum* gave excellent effects against most of the bacterial species used in the investigation and, notably, showed that extracts of fresh leaves gave low MIC values against the gram negative bacteria *E. coli* (1.6 µg/ml). *C. apiculatum* leaf material was also found favourable to use, since the extraction yield was good; up to 16.2 % of the dry weight of the leaf material (Eloff, 1999). In a qualitative TLC survey of the patterns of components in different species of *Combretum*, Carr & Rogers (1987) found that *C. apiculatum* ssp. *leutweinii* leaves contains triterpenoids, but no assays on the biological activity of these compounds were performed. Substituted dihydrostilbenes, phenanthrenes and 9,10-dihydrophenanthrenes have been isolated from the heartwood of *C. apiculatum* (Letcher & Nhamo, 1971; Malan & Swinny (1993) and three of these compounds showed total inhibition against *Penicillium expansum* when 20 µg of the compounds were spotted on a TLC plate (Grayer & Harborne, 1994).

Combretum zeyheri

Combretum zeyheri Sond., Large-fruited combretum, Large-fruited bushwillow (vernacular name in Swahili: *Mlamamweupe*, *Mlama*; Nyakyusa: *Kakati*) occurs from Kongo and Tanzania southwards to South West Africa, Botswana, the Transvaal and Natal. It occurs in *Brachystegia* woodland, wooded grassland and *Acacia-Commiphora* bushland, usually on sandy soils, also on termite mounds. *C. zeyheri* is reported to be very tolerant to soils of high metal or serpentine content. In South Africa *C. zeyheri* is a common tree together with *Diplorhynchos condylocarpon* (Apocynaceae) (Drummond & Palgrave, 1973). *C. zeyheri* is a slender, deciduous tree reaching heights of 10-13 m, or rarely a shrub. The bark is brown or greyish-brown. The branchlets are light brown, pubescent. The leaves are oppositely arranged or 3-verticillate. The lamina is broadly to narrowly elliptic to obovate-elliptic, up to 14 (-16) cm long and 8 (-10) cm wide. The leaf apex is usually rounded to obtuse. The leaves are tomentose-pubescent to almost glabrous. Inflorescences are unbranched axillary spikes, up to 8 cm long. The flowers are greenish yellow. The scent, if any, is not recorded. The large fruit is subcircular to elliptic, 4-winged, usually 6.5 cm long and 5.5 -6 cm wide, straw coloured to light brown, usually glabrescent. The apical peg is very short or absent. The wings of the fruit are up to 4 cm wide.

The leaves, roots and stem bark of *C. zeyheri* are used medicinally. The leaves are used frequently and have a variety of uses in African traditional medicine. The smoke of burnt leaves is inhaled for treatment of coughs. Colic is cured with the bitter tasting water extracts of dried,

milled leaves. The Mankoya people in Zambia use the leaves and stem bark of *C. zeyheri* together with the roots of cassava (*Manihot esculenta*) to prepare a cure for smallpox. The treatment is divided into three parts; an enema, eye drops and an ointment. The enema is prepared by pounding the leaves of *C. zeyheri* and then warming these in water. The enema is then applied using a small calabash as a syringe. Eye drops are prepared by soaking the cassava roots in water for ten minutes and a few drops are put into the eye four times a day during a week. An ointment is prepared from the inner bark of *C. zeyheri* by pounding and stamping the bark, adding some water until a thick paste is formed. The pustules of smallpox are then pricked with a splinter of wood after which the ointment is smeared on them. The ointment is used only once. Throughout the treatment for smallpox the patient has to lie on a bed of earth made from a particular type of small termite nest (Drummond & Palgrave, 1973). Rheumatism and joint pain is treated with crushed leaves of *C. zeyheri*, which are mixed with melon seed oil and rubbed in on the hurting body parts (Kremnitz et al., 1988). Decoctions of finely ground leaves are reported to be very effective for treatment of eye inflammation and conjunctivitis (Kremnitz et al., 1988). Leaves are pounded with oil and rubbed in for the treatment of back pain (Von Koenen, 1996). The leaves are used externally to treat scorpion bites (Watt & Breyer-Brandwijk, 1962) or macerated together with roots for the same purpose (Von Koenen, 1996). The roots and stem bark of *C. zeyheri* are also used for medicinal purposes, although there are not such a variety of reports as for the leaves of this species. Hot water extracts and infusions of the roots and stem bark are added to porridge to treat diarrhea, dysentery, ankylostomiasis and vomiting (Kokwaro, 1976; Hedberg et al., 1982; Gelfland et al., 1985; Neuwinger, 2000). Roots are chewed for the treatment of schistosomiasis (Neuwinger, 2000). Powdered stem bark is put in the vagina to arrest menstrual flow (Watt & Breyer-Brandwijk, 1962). *Combretum zeyheri* is sometimes used together with other medicinal plants, such as the tree violet, *Securidaca longopedunculata* (Polygalaceae) and *Pterocarpus angolensis* (Fabaceae) for the treatment of nose bleeding. Malaria is treated with roots and leaves of *C. molle* together with *Ochna pulchra* (Ochnaceae), *Burkea africana* (Caesalpiniaceae) and *Diospyros chamaethamnus* (Ebenaceae) (Von Koenen, 1996).

Only a few authors have investigated the antimicrobial effects of *Combretum zeyheri*. Eloff (1999) reports that this species has some antibacterial potential, although it was not among the most promising of the 27 species of Combretaceae investigated. Three antimicrobial compounds have been isolated from the stem bark and leaves of *C. zeyheri*, but the identity of the compounds was not investigated in this study (Breytenbach & Malan, 1989).

Combretum fragrans

Combretum fragrans F. Hoffm., syn. *C. adenogonium*, Four-leaved bushwillow (vernacular name in Swahili: *Mlama*; Nyakusa: Hansebwe). This species is shrublike or a small tree, up to 10 (-12) m high. The bark is grey and reticulately fissured, the branches peeling to give dark reddish color. Leaves are opposite or 3-(4)-verticillate, the lamina is broadly ovate- elliptic. The leaves are

rarely tomentose in East Africa. The inflorescences are axillary, either single or simple spikes or clusters of such spikes, borne on very much reduced axillary shoots or single axillary spikes on elongated shoots. The shoots are leafless during flowering, thus giving the appearance of a branched inflorescence. The flowers are greenish yellow to white and fragrant, particularly at night. The fruit is subcircular to elliptic in outline, yellow-brown to brown. *C. fragrans* is common in deciduous woodland (Miombo) and wooded grassland associated with seasonally waterlogged clay soils and is sometimes also found growing on shallow, stony soils (Wickens, 1973).

Combretum fragrans has many uses in African traditional medicine. Root decoctions are drunk for the treatment of leprosy (Chhabra et al., 1989) cough and syphilis (Kokwaro, 1976) and root infusions as aphrodisiacs (Gelfland et al., 1985). The leaves are burnt to ashes, mixed with castor (*Ricinus communis*) oil and used topically for treatment of leprosy (Chhabra et al., 1989). Leaf decoctions are used to treat snakebites (Neuwinger, 2000) and for cleansing chronic wounds (Adjahoun et al., 1986). The roots of *C. fragrans* have been found to contain anthracene glycosides, coumarins, flavonoids, starch, steroids/triterpenoids and tannins. Chhabra et al. (1984) showed that the roots give antibacterial effects against *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Shigella boydii* and thus the traditional use of this plant for treatment of leprosy, syphilis and chronic wounds is in accordance with the *in vitro* antibacterial effects of this species.

Combretum collinum

Combretum collinum Fresen., Variable bushwillow (vernacular name according to the Mgunku tribe: *Mnama*; Swahili: *Mlama*) is widespread in tropical and subtropical Africa (Wickens, 1973). It grows to a small tree, up to 12 m high or to a coppicing shrub. *C. collinum* is considered to be a highly variable species and is thought to consist of several subspecies, differing from each other only in minor characters (Van Wyk & Van Wyk, 1997). At least 11 subspecies of *C. collinum* have been distinguished, of which six occur in East Africa (Rogers & Coombes, 1999), and some of them are distinguished from each other according to the pattern of triterpene glycosides in their leaves. Van Wyk & Van Wyk (1997) report that four subspecies can be distinguished in South Africa on the basis of the leaf morphology and occurrence of hairs on leaves. The bark is reddish brown to pale yellow. Leaves are opposite, alternate or verticillate; the lamina is very variable in shape, up to 22 cm long and 8 cm wide, the upper surface drying to reddish olive or yellowish brown, usually somewhat “metallic” in appearance. The inflorescences are simple spikes or panicles up to 10 cm long, axillary or supra-axillary from bracts or reduced leaves on the current year’s shoots. The flowers are yellow, cream or white and fragrant. The fruit is brown, reddish brown, greyish brown to dark purple, usually somewhat metallic in appearance, variable in shape, 2.5-5.9 cm.

Combretum collinum has various medicinal uses in African traditional medicine. Decoctions of the roots of *C. collinum* are used together with roots and bark of *Kigelia africana* Benth., the sausage tree (Bignoniaceae) for the treatment of excessive menstrual bleeding beyond the normal

time. *C. collinum* is hot water extracted together with other medicinal plants, such as *Combretum molle* (Mlama) and *Phyllanthus reticulatus* Poir. (Euphorbiaceae) for the treatment of diarrhea accompanied by mild anal bleeding. The powdered stem bark is mixed with porridge or put in tea and used against rectal prolapse (Hedberg et al., 1982). According to Haerdi (1964) decoctions of roots and leaves are drunk for the treatment of malaria. Kokwaro (1976) reports that the roots are decocted for the treatment of dysentery and chewed to treat snake bites. Root decoctions are used for the treatment of “Madi”, a blood disease and pains in the side (Watt & Breyer-Brandwijk, 1962). Vapours of leaf decoctions are used to treat malaria (Haerdi, 1964). Gastroenteritis is treated with three teaspoons of decoction of fresh roots (Adjanouhoun et al., 1993). The leaves of *C. collinum* are covered with trichomes which secrete triterpenoids (Rogers & Coombes, 1999), but no investigations have been performed on the antimicrobial effects of these compounds or crude extracts of *C. collinum*.

Combretum micranthum

The West-African species *Combretum micranthum* G. Don (name for the drug: kinkeliba) is widely used in traditional medicine and is even inscribed in the French Pharmacopoeia (Baba-Moussa et al., 1999). Leaf infusions of this species are drunk for the treatment of colds, fever, colic, vomiting and gastrointestinal problems (Prost, 1971). Root decoctions are used as anthelmintics as well as for washing wounds (Irvine, 1961). *C. micranthum* is also reported to be used for the treatment of malaria on the Ivory Coast in West Africa (Benoit et al., 1996). In accordance with the traditional uses, *C. micranthum* has been found to show antibacterial activities, but the compounds producing these activities are unknown. The leaves of *C. micranthum* were bactericidal against *Shigella dysenteriae*, *S. paratyphi* B and *Klebsiella ozenai*, and extracts made of fresh plant material were found to be more active than those made from dried leaves (Karou et al., 2005). Karou et al. (2005) demonstrated that the leaf extracts of *C. micranthum* are rich in polyphenols, and show good antioxidative activities, and thus they speculated that the polyphenols might be the antibacterially active compounds in this plant. The good antimicrobial effects *in vitro*, showed by extracts of *C. micranthum* are thus consistent with its uses in traditional medicine in West Africa.

Combretum woodii

Combretum woodii DuRoi is a bushveld species growing to a small tree found in northern Kwazulu-Natal, Swaziland and Mpumalanga in South Africa (van Wyk & van Wyk, 1997). Eloff et al. (2005) were the first to report that a stilbene, 2',3',4'-trihydroxy-3,5,4'-trimethoxybiphenyl (combretastatin B5), isolated from a chloroform fraction of *C. woodii* leaves gave significant antibacterial effects against *Staphylococcus aureus* (MIC = 16 µg/ml) as well as some activity against the gram negative *E. coli* and *P. aeruginosa* (MIC = 125 µg/ml), and was more potent than chloramphenicol and ampicillin. There are no reports on the uses of *C. woodii* in traditional medicine and the study of Eloff et al. (2005) now suggests that leaves of this species might be

used for the treatment of microbial infections in poor communities in South Africa. Combretastatin B5 has previously been isolated by Pelizzoni et al. (1992) from the seeds of *C. kraussii*, but they did not investigate its antimicrobial effects.

Combretum caffrum

Combretum caffrum Eckl. & Zeyh. grows on the banks of rivers in the Eastern Cape Province in Southern Africa. Local historians believe that for over 2000 years Arabians traded for the bark with the San people (Bushmen). The bark was probably used as a general tonic in those days, because, apart from its anti-cancer properties, the bark also produces a feeling of general well being. The Zulu use it as a poison for spears (Pettit et al., 1987), which illustrates that the extracts of this plant are poisonous at high doses (<http://www.chm.bris.ac.uk/motm/combretastatin/combh.htm>). Decoctions of the roots are added to bath water before bedtime to treat pains in the body (Bath & Jacobs, 1995).

Stilbenes, such as combretastatins A-1, A-2, A-3 and A-4 as well as combretastatins B-1, B-2, B-3 and B-4 have been isolated from the wood of the South African species of *Combretum caffrum*, and have mainly been noticed because of their outstanding antineoplastic activities (Pettit et al., 1987, 1988, 1989, 1995 and 1999). Masika & Afolayan (2002) showed that acetone, MeOH, water extracts and a water decoction of the stem bark of *C. caffrum* inhibited the growth of most of the gram-positive bacteria and some fungal pathogens used in their study. Interestingly, the hot water decoction, prepared in the traditional way, was more active than the water extract against the gram-positive bacteria. The MeOH extract showed good activity also against the gram-negative bacteria used in this study and was very active against most of the fungal species, including *Aspergillus* and *Penicillium* species. The compounds responsible for this excellent antibacterial and antifungal activities of extracts of *C. caffrum* might be combretastatins, since Eloff et al. (2005) found that combretastatin B5 gave good antibacterial activity.

Combretum pentagonum* and *C. hartmannianum

Elegami et al. (2002) have found in their screening of forty eight extracts of four members of Combretaceae that leaf, bark, fruit and stem bark extracts of both *Combretum pentagonum* Lawson and *C. hartmannianum* Schweinf. gave good effects against gram-positive and gram-negative bacteria, and most of the activity was found in water and methanol extracts, whereas chloroform extracts were devoid of activity. Extracts made from different parts of the plants did not differ much from each other in antimicrobial activity. The species of *Combretum* included in this study were found to be rich in flavonoids, saponins and terpenes as well as tannins, why the authors speculated that the good antibacterial effects shown by these species might be due to tannins and flavonoids. The antibacterial effects of *C. hartmannianum* are in accordance with the use of this plant in traditional medicine in Sudan, where it is used for treatment of fever, jaundice and bacterial infections (El Ghazali et al., 1994; Al Magboul et al., 1988).

Combretum erythrophyllum

Martini & Eloff (1998) have detected at least 14 antimicrobial compounds of a wide range of polarities from leaf extracts of *Combretum erythrophyllum* (Burch.) Sond. The chloroform fraction was found to contain the highest concentration of antimicrobial compounds. The most effective fraction (35 % MeOH) gave MIC values of 0.05 mg/ml (50 µg/ml) against *Staphylococcus aureus*, compared to 0.08 and 0.16 mg/ml for ampicillin and chloramphenicol. Good results were also obtained against *Enterococcus faecalis* and *E. coli*. A notable discovery in this investigation was also that water did not extract any antibacterial compounds with activity against *Staphylococcus aureus*, at least at the concentration used in this study. This means that all the antimicrobial compounds cannot be extracted when this plant is prepared as water decoctions (Arnold & Gulumian, 1984; Watt & Breyer-Brandwijk) in traditional medicine. Root and bark decoctions of *C. erythrophyllum* are used for treatment of cough and infertility as well as an aphrodisiac, whereas leaves are not known to be used in traditional medicine (Arnold & Gulimian, 1984; Watt & Breyer-Brandwijk, 1962). The excellent results on the antimicrobial activity of this species now indicate that it could be used for the treatment of bacterial infections in traditional medicine as well. Later on Martini (2001) and Martini et al. (2004) have isolated seven antimicrobial flavonoids from leaf extracts of *C. erythrophyllum*, and all compounds were found to give good activity against *Vibrio cholerae* and *Enterococcus faecalis*, with MIC values ranging from 25 – 50 µg/ml. Comparing these MIC values of the isolated compounds with the crude leaf extract suggested, however, either that the most effective antibacterial compounds had not been isolated or that the compounds in the extract act synergistically. Martini et al. (2004) thus suggested that leaf extracts of *C. erythrophyllum* might be used for the purification of water contaminated by *Vibrio cholerae*. This would be a cheap low-tech solution to cholera in rural areas in Africa. Schwikkard et al. (2000) have isolated combretastatin A-1 and (-)-combretastatin from the wood of *C. erythrophyllum*, but they did not investigate the antimicrobial effects of these compounds.

Combretum imberbe

Combretum imberbe Wawra grows up to a 33 m high tree or remains as a shrub (Wickens, 1973). It grows in dry, hot savanna areas with sandy soils, and is also found in savanna woodland. The local people in different African countries use both the leaves and roots for treatment of diarrhea and cough. Ashes of the wood is used for toothpaste (Neuwinger, 2000). Rogers & Subramony (1988) have isolated pentacyclic triterpenes from the leaves of *C. imberbe*. Also related glycosides have been isolated, all based on the aglycon imberbic acid (Rogers, 1988). Katerere et al. (2003) isolated two new glycosidic derivatives of hydroxyimberbic acid and they found that particularly imberbic acid had potent activity against *Mycobacterium fortuitum* and *Staphylococcus aureus*. They also found that one of the isolated pentacyclic triterpenes showed good activities against *Candida albicans*. The *in vitro* antibacterial effects of *C. imberbe* are thus

in accordance with the traditional use of this plant for the treatment of diarrhea and cough, symptoms which can be related to bacterial/fungal infection.

Combretum mkhuzense

Recently, antimicrobial lectin-like proteins have been isolated from the stem bark of *Combretum mkhuzense* (Gaidamashvili & van Staden, 2002). These proteins, which are non-enzymatic, bind with high specificity to carbohydrates, both oligosaccharides and monosaccharides, and thus are thought to bind to surface-exposed carbohydrates of microbes. In the plant these proteins might serve as a defense against microbial attacks. Gaidamashvili and van Staden (2002) were able to show that lectins isolated from *C. mkhuzense* elicited selective aggregation reactions within bacterial strains, and that the highest activities were achieved against *Staphylococcus aureus* whereas *Bacillus subtilis* was more resistant. This investigation is one of the few showing that plant derived lectins both elicit aggregation and possibly via this, inhibit the growth of microbial pathogens.

Table 3. Antimicrobial activity of extracts and active compounds and uses in traditional medicine of some African *Combretum* species according to literature.

Species	Extract	Compounds	Screening method	Uses in traditional medicine
<i>C. apiculatum</i> Sond.	Leaf extracts antimicrobial against gram-positive and gram-negative bacteria, <i>E. coli</i> (MIC 1.6 µg/ml) (Eloff, 1999).	Leaves contain triterpenoids * (Carr & Rogers, 1987); dihydrostilbenes, phenanthrenes (Letcher & Nhamo, 1971; Malan & Swinny, 1993) from heartwood active against <i>Penicillium expansum</i> (Malan & Swinny, 1993).	Bioautography (Malan & Swinny, 1993). Microwell plates (Eloff, 1999)	Stomach problems, scorpion stings, disinfect the navel after birth (Van Wyk, 1997; Von Koenen, 1996; Kokwaro, 1976)
<i>C. caffrum</i> Eckl. & Zeyh. (Kuntze)	Acetone, MeOH, water extracts and a water decoction of the stem bark antimicrobial against gram-positive bacteria and some fungal pathogens, MeOH extract active also against gram-negative bacteria (Masika & Afolayan, 2002).	Combretastatins A-1, A-2, A-3, A-4, B-1, B-2, B-3 and B-4 *from the wood (Pettit et al., 1987, 1988, 1989, 1995 and 1999).	Agar dilution (Masika & Afolayan, 2002).	The bark as a general tonic for production of general well being; the Zulu use it as a poison for spears (Pettit et al., 1987); for treatment of pains in the body (Bath & Jacobs, 1995).
<i>C. collinum</i> Fresen.	*	Triterpenoids * in leaves (Rogers & Coombes, 1999).	*	Decoctions of roots and leaves are drunk for malaria (Haerdi, 1964); Roots with <i>Kigelia africana</i> for excessive menstrual bleeding; roots with <i>Combretum molle</i> and <i>Phyllanthus reticulatus</i> for diarrhea; stem bark mixed with porridge or in tea for rectal prolapse. (Hedberg et al., 1982); roots for dysentery and chewed to treat snake bites (Kokwaro, 1976); decoctions for "Madi", a blood disease and pains in the side (Watt & Breyer-Brandwijk, 1962); leaf decoctions for malaria (Haerdi, 1964); fresh roots for gastroenteritis (Adjanouhoun et al., 1993).

Table 3. (continued)

<i>C. erythrophyllum</i> (Burch.) Sond.	14 antimicrobial compounds of different polarities detected from acetone extract of the leaves, most of them in chloroform fraction of this extract (MIC 0.08-0.16 mg/ml) (Martini & Eloff, 1998).	Flavones apigenin, genkwanin, and the flavonols kaempferol, rhamnocitrin, rhamnazin and quercetin-5,3-dimethylether, all active against <i>Vibrio cholerae</i> and <i>Enterococcus faecalis</i> (MIC 25-50 µg/ml) (Martini, 2001; Martini & Eloff, 2005); combretastatin A-1 and (-) combretastatin * from wood (Schwikkard et al., 2000).	Microwell plates, bioautography (Martini & Eloff, 1998)	Root and bark decoctions for cough and infertility and as an aphrodisiac (Arnold & Gulimian, 1984; Watt & Breyer-Brandwijk, 1962).
<i>C. fragrans</i> F. Hoffm.	Roots antibacterial against <i>Staphylococcus aureus</i> , <i>Klebsiella pneumoniae</i> and <i>Shigella boydii</i> (Chhabra et al., 1984).	Roots contain anthracene glycosides, coumarins, flavonoids, starch, steroids/triterpenoids and tannins (Chhabra et al., 1984).	Agar diffusion (Chhabra et al., 1984).	Root decoctions for leprosy (Chhabra et al., 1989) cough and syphilis (Kokwaro, 1976); root infusions as aphrodisiacs (Gelfland et al., 1985); leaves mixed with <i>Ricinus communis</i> oil for leprosy (Chhabra et al., 1989); leaf decoctions for snakebites (Neuwinger, 2000) and for cleansing chronic wounds (Adjanohoun et al., 1986).
<i>C. hartmannianum</i> Schweinf.	Water and MeOH extracts of leaves, fruits and stem bark active against gram-positive bacteria and <i>E. coli</i> , chloroform extract inactive (Elegami et al., 2002)	Flavonoids, saponins, terpenes, tannins * (Elegami et al., 2002).	Agar diffusion (Elegami et al., 2002).	Fever, jaundice, bacterial infections (El Ghazali et al. ; Al Magboul et al., 1988)
<i>C. imberbe</i> Wavra	Dichloromethane fraction of the leaves active against bacteria (Katerere et al., 2003).	Pentacyclic triterpenes (Rogers & Subramony, 1988); glycosides based on imberbic acid (Rogers, 1988); glycosidic derivatives of hydroxyimberbic acid, imberbic acid active against <i>Mycobacterium fortuitum</i> and <i>S. aureus</i> , no activity against <i>E. coli</i> (Katerere et al., 2003); five new pentacyclic triterpenoids active against <i>E. coli</i> and <i>Staphylococcus aureus</i> (Angeh et al., 2006).	Microwell plates (Katerere et al., 2003). Microwell plates, bioautography (Angeh et al., 2006).	Leaves and roots for diarrhea, coughs, ashes of wood for toothpaste (Neuwinger, 2000).

Table 3. (continued)

<i>C. micranthum</i> G. Don.	Aqueous acetone (70 % v/v) extracts of leaves bactericidal against <i>Shigella dysenteriae</i> , <i>S. paratyphi</i> B and <i>Klebsiella ozenai</i> , fresh plant material more active than dried (Karou et al., 2005).	Polyphenols * with antioxidative effects from leaves (Karou et al., 2005).	Agar diffusion (Karou et al., 2005)	Leaf infusions drunk for colds, fever, colic, vomiting and gastrointestinal problems (Prost, 1971); root decoctions as anthelmintics and for washing wounds (Irvine, 1961); for treatment of malaria on the Ivory Coast in West Africa (Benoit et al., 1996).
<i>C. mkhuzense</i>	Leaf extracts active against <i>Staphylococcus aureus</i> and <i>Bacillus subtilis</i> (Gaidamashvili & Van Staden, 2002).	Lectins from leaf extracts induce selective aggregation reactions in bacterial strains, highest activities against <i>Staphylococcus aureus</i> ; <i>Bacillus subtilis</i> more resistant (Gaidamashvili & Van Staden, 2002).	Dilution method and microscopy (Gaidamaswhili & Van Staden, 2002)	Not reported
<i>C. molle</i> R. Br. Ex G. Don	<p>Leaf extracts especially active against <i>Enterococcus faecalis</i>, MIC < 0.2 mg/ml (Pegel & Rogers, 1985; Eloff, 1998b; Eloff, 1999).</p> <p>Acetone fractions of stem bark inhibit the growth of <i>Mycobacterium tuberculosis</i> typus humanus (ATCC 27294) (Asres et al., 2001a).</p> <p>Bark of twigs antibacterial and anti-candidal, whereas extracts of stem wood inactive (Khan et al., 2000).</p> <p>Leaf extracts antifungal against <i>Microsporum gypseum</i> and <i>Trichophyton mentagrophytes</i> (Baba-Moussa et al., 1999).</p> <p>Root extract: large inhibition zones against <i>Candida albicans</i> (Runyoro et al., 2006).</p>	<p>Antifungal mollic acid-3-β-D-glucoside from leaf extracts effective against <i>Penicillium expansum</i> (Pegel & Rogers, 1985)</p> <p>The hydrolysable tannin, punicalagin, gives antimycobacterial effects (Asres et al., 2001a)</p>	<p>Bioautography (Pegel & Rogers, 1985)</p> <p>Microwell plates (Eloff, 1998b; Eloff, 1999)</p> <p>Broth dilution method with tubes (Asres et al., 2001a)</p> <p>Agar diffusion (Khan et al., 2000)</p> <p>Agar dilution, 24 well plates (Baba-Moussa et al., 1999).</p> <p>Agar diffusion (Runyoro et al., 2006).</p>	<p>Fresh leaves, moistened dry leaves and roots as wound dressings (Drummond & Palgrave, 1973); roots for cough, syphilis, snakebites (Hedberg et al., 1982); for treatment of poisonings from snakebites/arrows (Haerdi, 1964; Hedberg et al., 1982); roots for hookworm, stomach pains, snake bite, leprosy, fever, dysentery, general body swellings, abortifacient, swelling of the abdomen, abortion, constipation, sterility (Chhabra et al., 1989); stem bark for gargling and angina (Kerharo, 1974); malaria (Abebe & Ayehu, 1993) ; infections related to HIV/AIDS (Bessong et al., 2005).</p>

Table 3. (continued)

<i>C. padoides</i> Engl. & Diels	Acetone extracts of leaves antimicrobial (fresh leaves more effective than dried), MIC 0.8 mg/ml against <i>E. coli</i> and <i>Enterobacter faecalis</i> (Eloff, 1999).	Mono- and bi-desmosidic triterpenoids from leaves (Rogers, 1989)	Microwell plates (Eloff, 1999)	Leaves for snakebites and the roots for eliminating hookworms (Neuwinger, 2000).
<i>C. pentagonum</i> Lawson	Leaf, bark, fruit and stem bark extracts antimicrobial, most of the activity in water and methanol extracts whereas chloroform extracts were devoid of activity (Elegami et al., 2002).	Flavonoids, saponins, terpenes, tannins * (Elegami et al., 2002).	Agar diffusion (Elegami et al., 2002).	Roots for ankylostomiasis, wounds, oedema, gonorrhoea, loose tooth, gum bleeding; leaves for gonorrhoea, oedema (Haerdi, 1964)
<i>C. woodii</i> Dummer	Acetone extracts of leaves antimicrobially active (Eloff et al., 2005).	A stilbene, 2',3', 4-trihydroxyl-3,5,4'-trimethoxybenzyl, combretastatin B5, gave significant antibacterial effects against <i>Staphylococcus aureus</i> (MIC 16 µg/ml) and some activity against <i>E. coli</i> and <i>P. aeruginosa</i> (MIC 125 µg/ml) (Eloff et al., 2005)	Microwell plates (Eloff et al., 2005)	Not reported

Table 3. (continued)

<i>C. zeyheri</i> Sond.	Acetone extracts of leaves have some antibacterial potential (Eloff, 1999).	Three compounds with antimicrobial effects have been isolated from stem bark and leaves (Breytenbach & Malan, 1989).	Microwell plates (Eloff, 1999)	Leaves for coughs, colic, smallpox (Drummond & Palgrave, 1973), rheumatism, joint pain (Kremnitz et al., 1988), eye inflammation, conjunctivitis (Kremnitz et al., 1988), back pain (Von Koenen, 1996), scorpion bites (Watt & Breyer-Brandwijk, 1962; Von Koenen, 1996). Roots & stem bark for diarrhea and vomiting (Hedberg et al., 1982); root infusions for bloody diarrhea (Gelfland et al., 1985); root powder with porridge for dysentery, ankylostomiasis, schistosomiasis (Kokwaro, 1976 Neuwinger (2000)); powdered stem bark to arrest menstrual flow (Watt & Breyer-Brandwijk, 1962); in combination with <i>Securidaca longopedunculata</i> (Polygalaceae) and <i>Pterocarpus angolensis</i> (Fabaceae) for nose bleeding; roots and leaves of <i>C. molle</i> with <i>Ochna pulchra</i> (Ochnaceae), <i>Burkea africana</i> (Caesalpiaceae) and <i>Diospyros chamaethamnus</i> (Ebenaceae) for malaria (Von Koenen, 1996).
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* antimicrobial effect not investigated

2.5.3. *Pteleopsis* species

There are probably about nine species of *Pteleopsis* in tropical Africa. The genus is intermediate in many characters between *Combretum* and *Terminalia* (Wickens, 1973). Three species of *Pteleopsis* are used in African traditional medicine, such as the East-African *P. myrtifolia* and the West-African species, *P. hylodendron* and *P. suberosa* (Hedberg et al., 1982; Baba-Moussa et al., 1999; Ngounou et al. 1999). Two other East African species, *P. anisoptera* and *P. tetroptera*, do not apparently have any uses in traditional medicine.

Pteleopsis myrtifolia

Pteleopsis myrtifolia Engl. & Diels. (Vernacular name: *Mgonji*). This species occurs in tropical and south Africa (Hedberg et al., 1982). It is a tree and can grow up to 30 m high, but reaches more often heights of 8-20 m, and sometimes grows shrublike. The wood is red and very hard. Leaves are petiolate, opposite to subopposite, almost glabrous or hairy. The flowers are andromonoecious (male and hermaphrodite flowers on the same inflorescence), 4 (-5) merous, pedicellate, in terminal and/or axillary or extra-axillary subcapitate racemes, white to yellowish. The fruit is 2-5 winged, wings are often decurrent into the comparatively long, slender stipe. *Pteleopsis myrtifolia* can be found in dry evergreen and riverine forest, deciduous woodland, coastal bushland and wooded grassland (Wickens, 1973).

The roots of *P. myrtifolia* are boiled with chicken and this is eaten to treat sterility and infertility (Hedberg et al., 1982). Decoctions of the roots are used for the treatment of venereal diseases (Kokwaro, 1976). Juice from the leaves of *P. myrtifolia* is drunk against abortion; a mixture of the leaf juice of *P. myrtifolia* with leaf juice of *Royena amnicola* B. L. Burtt is drunk to treat dysentery (Haerdi, 1964). The leaf sap and root decoctions of *P. myrtifolia* are used for the treatment of dysentery together with *Diospyros zombensis* (Ebenaceae) (Haerdi, 1964; Hedberg et al., 1981; Kokwaro, 1976). These uses indicate that *P. myrtifolia* contains antimicrobial compounds. No reports of the antimicrobial effects of *P. myrtifolia* exist, however.

Pteleopsis suberosa

The West African species *Pteleopsis suberosa* Engl. & Diels is a popular medicinal plant in Senegal, Guinea, Ivory Coast, Ghana, Togo, Benin and Nigeria. In Mali folk medicine *Pteleopsis* is known by the common name “Terenifu” (De Pasquale et al., 1995). The bark decoction is used for the treatment amoebic dysentery; root and leafy twig decoctions for dysentery and the roots for jaundice (Adjanohoun et al., 1986). Decoctions of the stem bark have antitussive and antiviral properties (De Pasquale et al., 1995). The uses of *P. suberosa* in traditional medicine are in accordance with the finding of Baba-Moussa et al. (1999), who reported that extracts of the stem bark and the shoots of *P. suberosa* are effective inhibitors of the growth of dermatophytes (*Epidermophyton* and *Trichophyton* species) and *Candida albicans*. *P. suberosa* has also been

reported to exhibit antibacterial effects against different strains of *Helicobacter pylori* (Germanò et al., 1998), the causative agent of ulcers, and this justifies the use of *P. suberosa* for the treatment of gastric ulcers in Mali traditional medicine (De Pasquale et al., 1995). Germanò et al. (1998) found that especially the water decoctions of this species were active against the *Helicobacter* strains, and also, together with an earlier investigator (De Pasquale et al., 1995) that these decoctions contained a high content of tannins (27 %) as well as triterpenoid saponins, which are known to be both antibacterial and to protect the outermost layer of the gastric mucosa and render it less impermeable (tannins) as well as protecting the mucosa from acid by selectively inhibiting PGF_{2α} (triterpenoid saponins).

Pteleopsis hylodendron

Pteleopsis hylodendron Mildbr. grows into a big tree found in the forest regions of West and Central Africa (Irvine, 1961). *P. hylodendron* is highly valued in West African folk medicine. The aqueous decoction of the stem bark is used for treatment of sexually transmitted diseases, female sterility, liver and kidney disorders as well as dropsy. The indigenous use of *P. hylodendron* for the treatment of venereal diseases indicates that it contains antibacterial compounds. No investigations of the *in vitro* effects of this plant on bacteria has been performed, but Ngounou et al. (1999) have isolated saponins from the stem bark of this plant.

2.6. Anticancer drugs

Synthesis or modification of known anticancer drugs is an important aspect of research. However, a vast amount of synthetic work has contributed to relatively small improvements over the prototype drugs. There is a continued need for new templates to use in the design of potential chemotherapeutic agents. Natural products are important templates for chemotherapeutic agents. Studies on tumor inhibiting compounds originating from plants have yielded an impressive array of novel structures. Since 1961, nine plant-derived compounds have been approved for use as anticancer drugs in the United States; vinblastine (Velban), vincristine (Oncovin), navelbine (vinorelbine), etoposide (VP-16), teniposide (VM-26), taxol (paclitaxel), taxotere (docetaxel), topotecan (Hycamtin) and irinotecan (Camptosar) (Lee, 1999).

Apoptotic induction has been a target for innovative mechanism-based drug discovery. Resistance to programmed cell death (apoptosis) is an integral part of cancer cell development, and reestablishment of control of apoptosis is a known target mechanism for anticancer drugs (Gibb et al., 1997; Joshi et al., 2002). Certain products from plants are known to induce apoptosis in neoplastic cells, but not in normal cells, which would be the ideal characteristic of a successful anticancer drug (Hirano et al., 1995). Alkylating agents, such as cisplatin (Gibb et al., 1997), act directly on the DNA by cross-linking the guanine nucleobases and thus hindering the strands of

DNA to uncoil and separate and therefore making replication impossible. This in turn, triggers apoptosis of the cells. Plant derived compounds comprise a diverse group with different mechanisms of action, but ultimately seem to have the ability to induce apoptosis (Tharapadar et al., 2001). Understanding the modes of action of plant-derived anti-cancer compounds should provide useful information for their possible application in cancer prevention and perhaps also in cancer therapy (Tharapadar et al, 2001). It is thus important to screen apoptotic inducers from plants, either in the form of crude extracts or as components isolated from them (Tharapadar et al., 2001). Screening plants which are used as anticancer remedies in traditional medicine increases the chance of finding new active anti-cancer compounds.

Mitosis is a fundamental process in cellular development and depends on many factors including essential DNA enzymes and cellular microtubules which are composed of tubulin proteins. The correct topographic structure of DNA is maintained by the enzymes topoisomerase I and II during translation, transcription and mitosis. Polymerization/depolymerization of microtubules plays an essential role for cell division. All these systems are therefore important targets for new anticancer chemotherapeutic agents. (Lee, 1999).

One of the major problems in cancer chemotherapy is the development of multi drug resistance (MDR) against anti-cancer drugs. It has been discovered that drug resistance in cancer cells results from elevated expression of certain proteins, such as cell membrane ATP-binding cassette transporters (Choi, 2005), which can result in an increased efflux of the cytotoxic drugs from the cancer cells (Ambudkar et al., 1999; Thomas & Coley, 2003). The problems of acquired drug resistance may be circumvented by targeting endothelial cells associated to the tumor instead of the tumor cells themselves (Boehm et al., 1997). Endothelial cells are genetically more stable and thus are less likely to develop MDR compared to cancer cells. The process of angiogenesis, the development of new blood vessels, is an attractive target of cancer therapy since tumors are dependent on a functioning vascular system, and metastasis is dependent on the formation of new vessels around the cell foci (Griggs et al., 2001). Also already established tumor vasculature is a good target for anticancer therapy since it differs from normal vasculature in its permeability, the absence of vascular smooth muscle cells and lymphatic drainage (Matsumura & Maeda, 1986). There are a number of promising anti-angiogenic and antivascular agents, some of them originating from higher plants, and are undergoing clinical trials. Among them the stilbene combretastatin-A4, originally isolated from the South African tree *Combretum caffrum* (Pettit et al., 1987, 1988, 1989 and 1999).

2.6.1. Cytotoxic effects of species of *Combretum* reported in the literature

Some African species of *Combretum*, such as *C. caffrum* and to a lesser extent *C. erythrophyllum*, *C. woodii* and *C. kraussii* contain very interesting small molecular stilbene cytotoxic compounds, the combretastatins. Only two species of *Combretum*, *C. zeyheri* (Fyhrquist et al., 2002) and the Asian *C. latifolium* (Hartwell, 1982) have been reported to be used for the treatment of cancer in traditional medicine. Quite a few species of *Combretum* have been subjected to studies on their cytotoxic/antimutagenic effects, and even a smaller number of studies have contributed to the isolation of the compounds producing the cytotoxic/antimutagenic activity (Table 4). On the other hand the anticancer research on some *Combretum* species, such as *C. caffrum* has been extensive, and has led to the production of a new anticancer lead compound, combretastatin A4 (Pettit et al., 1989; Woods et al., 1995). *Combretum* species contain in addition to stilbenes also flavonoids (Martini, 2001; Martini et al., 2004; Elegami et al., 2002) and pentacyclic triterpenoids (Simon et al., 2003a, Pettit et al., 1987, 1988, 1989, 1995 and 1999; Schwikkard et al., 2000), some of them having antiproliferative and cytotoxic potential.

***Combretum caffrum* and the combretastatins**

Combretum caffrum Eckl. & Zeyh has a background of use in Zulu traditional medicine as a general tonic for improved well being, as a poison on Zulu spears and as a charm to harm the enemy (<http://www.chm.bris.ac.uk/motm/combretastatin/combh.htm>, Pettit et al., 1987). These uses in traditional medicine indicate that *C. caffrum* contains cytotoxic compounds. Pettit et al. (1987, 1988, 1989, 1995 and 1999) have isolated a large group of anti-tubulin stilbene compounds, the combretastatins, rare natural products which resemble colchicine in structure, primarily from the stem bark, but also from the twigs, leaves and the fruits of *C. caffrum* (Table 4). In total 15 different bibenzyls, stilbenes and dihydrophenanthrenes, designated the combretastatins, all with growth inhibitory properties against a murine lymphocytic leukemial cell line (P-388) were isolated (Pettit et al., 1987; Pettit et al., 1988). Combretastatins A-1, A-2, A-3, B-1 and B-2 were all found to be very effective inhibitors of the growth of the murine lymphocytic P-388 and L1210 leukemial cell lines, with IC₅₀ values ranging from 0.011 µg/ml to 0.6 µg/ml (Pettit et al., 1987 and 1988). All the combretastatins caused leukemial cells to accumulate in mitosis at cytotoxic drug concentrations. Many of the combretastatins have been found to inhibit microtubule assembly very effectively, and especially combretastatin A-1 and B-1 have been found to be very effective inhibitors, giving the same IC₅₀ values as podophyllotoxin (4 µg/ml) (Pettit et al., 1988).

In 1989 Pettit et al. isolated combretastatin A-4 from the stem wood and bark of *C. caffrum*, and they found that this compound was the most potent of all the combretastatins isolated in inhibiting the growth of murine lymphocytic leukemia and colon cancer cell lines as well as

inhibiting microtubule assembly, and that CA-4 was as potent as podophyllotoxin and more potent than colchicines and steganacin (Pettit et al., 1989). CA-4 shows potent cytotoxicity against a wide variety of human cancer cell lines, including multidrug-resistant cancer cell lines, and is thus an attractive lead molecule for the development of new anticancer drugs (El Zayat et al., 1993). Combretastatin A-4 has been found to competitively inhibit the binding of colchisin to tubulin, since it binds to the same site on β -tubulin, but with different characteristics that impart *selective toxicity to tumor vasculature* (Pettit et al., 1989; Woods et al., 1995).

A number of studies have reported on the relation between structure and activity (SAR) of combretastatins (Lin et al., 1989). Minimum requirements for activity are that the diaryl system should be separated through a double bond and the presence of a trimethoxy system in one of the rings (Srivastava et al., 2005). Also, it has been found that the *cis* (*Z*) isomer is preferred over the *trans* (*E*), as *cis* seems to be much more active than *trans* (Srivastava et al., 2005).

Both combretastatin (CA-4) and combretastatin A-4-phosphate (CA-4P), the more soluble phosphate salt prodrug of CA-4, have been found to show *in vitro* antiangiogenic effects (Kanthou et al., 2002; Kanthou et al. 2004). It has been shown that CA-4 and CA-4P induce apoptosis in human umbilical vein endothelial cells (HUVEC), impair HUVEC migration, and disrupt the endothelial cytoskeleton (Grosios et al., 1999; Iyer et al., 1998). Recent work shows that CA-4 can have dramatic effects on the three-dimensional shape and microtubule stability of newly formed endothelial cells, but less effects on quiescent cells (Dark et al., 1997; Galbraight et al., 2001; Kanthou et al., 2002). It is believed that newly formed endothelial cells are more sensitive than mature endothelial cells since the latter have a more highly developed actin cytoskeleton, which maintains cell shape despite depolymerization of the tubulin cytoskeleton (Chaplin & Dougherty, 1999).

A number of studies of the *in vivo* anti-vascular effects of CA-4 have been performed using rodent tumor models. Several studies in mice have shown that a single administration of combretastatin A4 (100 mg/kg) does not significantly affect the growth of the primary tumor (Grosios et al., 1999; Chaplin et al., 1999). Repeated administration of CA-4 at 12.5-25.0 mg/kg twice daily for periods of 10-20 days retarded the growth of Lewis lung carcinoma and T138 murine breast tumors about 50 %. In these tumors CA-4 induced haemorrhagic necrosis, consistent with the antivascular mode of action (Griggs et al., 2001). The antivascular effects of CA-4 seem to be rapid, since Dark et al. (1997) could measure a large reduction in red-blood-cell velocity of rat P22 carcinosarcoma in 10 minutes after treatment. CA-4P has also been shown to possess potent anti-metastatic activity in the Lewis lung carcinoma model and induced necrosis in metastases from an adenocarcinoma primary tumor (Grosios et al., 1999). The antivascular effects of CA-4P seem to be mediated through endothelial cell damage, but the acute vascular damaging effects seen both in rodents and humans suggest a mechanism distinct from apoptosis (El Zayat et al., 1993; Iyer et al., 1998; Griggs et al., 2001). Analysis with the aid of magnetic resonance imaging show that the antivascular effects of CA-4 are restricted to the core of the

primary tumor, leaving a small rim of viable cells remain at the periphery of the tumor, which can subsequently grow after withdrawal of drug (Beauregard et al., 1998).

The combretastatin A-4 prodrug, combretastatin A-4 disodium phosphate, began phase I clinical trials in October 1998 at the Ireland Cancer Center, as one of the most promising new antiangiogenic drugs (Pettit et al., 1999; Griggs et al., 2001). The clinical effects of combretastatin A-4-phosphate administered together with carboplatin in patients with advanced cancer have been investigated, and it seemed that the disease of some of the patients was kept stable during the treatment. However, this study showed that dose limiting thrombocytopenia occurs when CA-4P and carboplatin are administered together (Bilenker et al., 2005).

Combretum nigricans

Combretum nigricans Lepr. is a small tree widespread on the Sahelian savanna. It is used for the treatment of cataract, conjunctivitis, icterus and rheumatism, as well as for gastrointestinal disorders (Boullard, 2001; Kerharo, 1974). In a systematic search for cytotoxic compounds in Burkinabe plants, West Africa, Simon et al. (2003a) discovered that methanolic extracts of the leaves of *C. nigricans* gave cytotoxic effects against the glioblastoma (U-373), colon (HCT-15), non-small lung (A549) and bladder (J82) cancer cell lines (Table 4). They found that pentacyclic triterpenes, in part, were responsible for this activity, although the activity of these compounds was not very high. The pentacyclic triterpenoids arjungenin, arjunglucoside and combregenin, as well as the saponin combreglucoside have been isolated from this species as well (Jossang et al., 1995), but the cytotoxic activities of these particular compounds have not been investigated. Betulinic acid, a pentacyclic triterpenoid isolated from *Betula* species, has been found to selectively inhibit the growth of human melanoma tumors in nude mouse xenograft models and was shown to cause cytotoxicity by inducing apoptosis (Pisha et al., 1995). Various cycloartane-type triterpenoid glycosides from *Astragalus* species have been found to possess antileukemic effects (Calis & Sticher, 1996). It is possible that some of the triterpenoid compounds in *Combretum nigricans* might have good cytotoxic potential and that some of the triterpenoid compounds found from this species of *Combretum* would be able to induce apoptosis in certain cancer cell lines.

Combretum erythrophyllum

Schwikkard et al. (2000) found that methanol extracts of the stem wood of *C. erythrophyllum* (Burch.) Sond induced DNA damage in a yeast based assay. Bio-assay guided fractionation yielded combretastatin A-1 and (-) - combretastatin, as well as two new bioactive glucosides, combretastatin A-1,2'- β -D-glucoside and combretastatin B-1,2'- β -D-glucoside. Combretastatin A-1,2'- β -D-glucoside inhibited the growth of HeLa cervical and A549 lung carcinoma cells with IC₅₀ values of 1.5 and 7.0 μ g/ml respectively. For comparison the IC₅₀ values of AC-7739, a synthetic combretastatin analogue in clinical development, were 0.001 and 0.004 μ g/ml respectively, in these cell lines. All the isolated combretastatins were investigated for inhibitory

activity of topoisomerase I and where found inactive against this enzyme. The flavones apigenin, genkwanin, 5-hydroxy-7,4'-dimethoxyflavone and the flavonols kaempferol, rhamnocitrin, rhamnazin and quercetin-5,3-dimethylether have been isolated from the leaves of *C. erythrophyllum* (Martini, 2001; Martini et al., 2004), but the cytotoxic potentials of these compounds were not investigated. Many flavonoid compounds (e.g. luteolin, cryptocaryone) are known to possess cytotoxic effects (Middleton et al., 2000; Blank et al., 2004; Dumontet et al., 2001; Ko et al., 2002; Dumontet et al., 2001).

Combretum apiculatum, C. mossambicense and C. hereroense

In a survey of the biological effects of several African species of *Combretum*, McGaw et al. (2001) found that *Combretum apiculatum* Sond., *C. mossambicense* Klotzsch and *C. hereroense* Schinz. gave positive results in an assay measuring DNA damaging activity (Table 4). This suggests that these species have potential anti-cancer activity. No further investigations have however been performed on the *in vitro* cytotoxic effects of these species. Triterpenoids (Carr & Rogers, 1987), as well as dihydrostilbenes and phenanthrenes (Malan & Swinny, 1993) have been isolated from the leaves of *C. apiculatum*, and thus some of these compounds may be responsible for the DNA damaging effect of this species.

Table 4. Cytotoxic and antitumor effects as well as uses in traditional medicine of some *Combretum* species reported in the literature.

Species	Cytotoxic effects of extract	Compounds	Screening method	Uses in traditional medicine
<i>C. apiculatum</i> Sond.	MeOH, acetone and ethyl acetate extracts of leaves DNA damaging in a BIA assay (McGaw et al., 2001)	Leaves contain triterpenoids* Carr & Rogers (1987); substituted dihydrostilbenes and phenanthrenes in heartwood * (Malan & Swinny, 1993)	Biochemical Induction Assay (BIA) based on production of β -galactosidase by <i>E. coli</i> in response to DNA damage (McGaw et al., 2001)	Stomach problems, scorpion stings, disinfect the navel after birth (Kokwaro, 1976; Von Koenen, 1996; Van Wyk & Van Wyk, 1997).
<i>C. caffrum</i> Eckl. & Zeyh.	MeOH:CH ₂ Cl ₂ (1:1) extracts of stem wood and bark, twigs, fruits, leaves as well as chloroform and methylene chloride fractions of crude extract active against murine P-388 lymphocytic leukemia <i>in vitro</i> and <i>in vivo</i> as well as showing astrocyte reversal of 51-90 % (Pettit et al., 1987; Pettit et al., 1988).	<p>Combretastatins B1, B2, A-1, A-2, A-3 inhibitory against murine P-388 lymphocytic leukemia and L1210 leukemic cells causing cells to accumulate in mitosis (IC₅₀ 0.011 μg/ml to 0.6 μg/ml) (Pettit et al., 1987); CA-1, CA-2 and CA-3 inhibit tubulin polymerization and microtubule assembly with same IC₅₀ values (4 μg/ml) as podophyllotoxin (Pettit et al., 1988).</p> <p>Combretastatin A-4 powerful inhibitor of microtubule assembly, IC₅₀ 2-3 μM (Pettit et al., 1989); CA-4 shows potent cytotoxicity against a wide variety of human cancer cell lines (El Zayat et al., 1993).</p> <p>CA-4 antivasular and anti-angiogenic <i>in vitro</i>, alters cell shape and microtubule stability and causes apoptosis in HUVEC cells (Kanthou & Tozer, 2002; Kanthou et al., 2004; Grosios et al., 1999); CA 4 gave <i>in vivo</i> antivasular effects in rodent tumor models (Grosios et al., 1999; Chaplin et al., 1999; Griggs et al., 2001; Dark et al., 1997).</p> <p>Phase I clinical trials of CA-4P began in 1998 at Ireland Cancer Center (Stratford et al., 2000).</p>	<p>Astrocyte glioma cell reversal, murine P-388 cell growth and viability, <i>in vivo</i> effects using rodent tumor model (Pettit et al., 1987 and 1988).</p> <p>Tests for inhibitory capacity of tubulin polymerization and microtubule assembly (Pettit et al., 1987, 1988).</p> <p>HUVEC network formation assay, HUVEC migration assay, tubulin staining. Assessment of tumour growth by measuring tumor volume, haematoxylin and eosin staining, Hoechst 33342 staining (Grosios et al., 1999).</p> <p>Trypan blue exclusion cell viability assay, microtubule staining, Western blotting for β-tubulin, endothelial permeability assay, HUVEC cells (Kanthou & Tozer, 2002).</p>	Decoction of bark general tonic for improved well being and is reported to have anticancer effects; poison on Zulu spears, as a charm to harm the enemy (Pettit et al., 1987); decoctions of roots are used to treat body pain (Bath & Jacobs, 1995).

Table 4. Continued.

<i>C. erythrophyllum</i> (Burch.) Sond.	Methanol extracts of the wood induce DNA damage in yeast (Schwikkard et al., 2000)	Combretastatin A-1, (-)-combretastatin, combretastatin A-1,2'- β -D-glucoside cytotoxic against HeLa cervical cancer and A549 lung carcinoma cells (IC ₅₀ 1.5-7.0 μ g/ml), combretastatin B-1,2'- β -D-glucoside, all compounds inactive against topoisomerase I (Schwikkard et al., 2000); flavonoids* in leaf extracts (Martini & Eloff, 2004)	Yeast bioassay (<i>Saccharomyces cerevisiae</i>) for DNA damaging agents using 96-well microtiter plates (Schwikkard et al., 2000).	Root and bark for cough and infertility, as aphrodisiacs, leaves not known to be used in traditional medicine (Arnold & Gulimian, 1984; Watt & Breyer-Brandwijk).
<i>C. hereroense</i> Schinz.	MeOH, acetone and ethyl acetate extracts of leaves DNA damaging in a BIA assay (McGaw et al., 2001).	Phenanthrenes in heartwood* (Letcher et al., 1972; flavonoids in leaf extracts* (Carr & Rogers, 1987)	Biochemical Induction Assay (BIA) based on production of β -galactosidase by <i>E. coli</i> in response to DNA damage (McGaw et al., 2001)	Root decoctions for schistosomiasis (Kokwaro, 1976); leaves for chest problems, root decoctions for leprosy (Bally, 1937); powdered young shoots for toncillitis (Samuelsson et al., 1991).
<i>C. mossambicense</i> Klotzsch	MeOH, acetone and ethyl acetate extracts of leaves DNA damaging in a BIA assay (McGaw et al., 2001).	No reports	Biochemical Induction Assay (BIA) based on production of β -galactosidase by <i>E. coli</i> in response to DNA damage (McGaw et al., 2001)	Decoctions of roots and leaves for ailments of jaw and hip bones, facial swelling from tooth abscess, eye inflammation, nasal furuncle, mouthwash, eye bath (Von Koenen, 1996)
<i>C. nigricans</i> Lepr.	MeOH extracts of leaves cytotoxic against glioblastoma (U-373), colon (HCT-15), non small lung (A549), bladder cancer (J82) cell lines (Simon et al., 2003a).	Pentacyclic triterpenes in part responsible for the cytotoxicity (Simon et al., 2003a).	96 well microwell plates, MTT assay (Simon et al., 2003a).	Cataract, conjunctivitis, icterus, rheumatism (Kerharo & Adam, 1974 ; Boullard, 2001) ; expectorant, stomach problems (Kerharo & Adam, 1974).

* cytotoxic effects not investigated

2.6.2. Cytotoxic and antimutagenic effects of some species of *Terminalia* according to the literature

At least seven species of the genus *Terminalia* are used for treatment of cancer in traditional medicine (Hartwell, 1982). Studies on the cytotoxic and antimutagenic as well as anti-tumor effects, have mainly concentrated on Asian species of *Terminalia* (Pettit et al., 1996; Kandil & Nassar, 1997; Cheng et al., 2002; Kaur et al., 2002a), whereas African species of *Terminalia* have been investigated for their antimicrobial but not for their anti-cancer effects, although some of the African species of *Terminalia*, such as *T. sambesiaca*, are known to be used for treatment of cancer in traditional medicine (Chhabra et al., 1989). Asian *Terminalia* species have been found to possess antimutagenic (cancer preventive) as well as antitumor and cytotoxic effects (Table 5). The active compounds in these species have been found to be hydrolysable tannins, of which ellagitannins (Kandil & Nassar, 1997; Saleem et al., 2002) as well as their monomer, ellagic acid (Saleem et al., 2002) are especially well represented. Also gallic acid, ethyl gallate and the flavone luteolin, have been isolated from species of *Terminalia*, and all of them are reported to possess moderate anti-cancer effects (Pettit et al., 1996).

Terminalia arjuna

Terminalia arjuna (Roxb.) Wight & Arn. has been found to possess both antimutagenic (Kaur et al., 2000; Kaur et al., 2002a, Kaur et al., 2002b) as well as cancer cell growth inhibitory effects (Pettit et al., 1996; Kandil & Nassar, 1997) (Table 5). When the antimutagenic effects of a tannin rich fraction from this plant against the mutagen 4-nitro-*o*-phenylene-diamine (NDP) was compared to ellagic acid (also isolated from *T. arjuna*) using the Ames mutagenicity test, it was found that the tannin rich fraction was far more antimutagenically potent (Kaur et al., 2000). Thus, it seems that the antimutagenic potential of a hydrolysable tannin is related to its *degree of polymerization*, so that oligomeric forms are more active than monomeric (Kaur et al., 2002). Kaur et al. (2000) speculated that the hydrolysable tannins from *T. arjuna* might inactivate cytochrome P 448/P 450 dependent enzymes in rat liver microsome extract, used in the Ames test for investigating mutagenicity, and thus hinder these enzymes to activate chemical mutagens. This might be one of many possible anticancer, antitumor and anticarcinogenic mechanisms that hydrolysable tannins exert. Gallic acid, ethyl gallate and luteolin, isolated from the bark, stem and leaves of *T. arjuna* (Pettit et al., 1996) have been found to possess antiproliferative (Post & Varma, 1992; Chen et al., 1992) and anti-tumor effects (Ryu et al., 1994; Matsukawa et al., 1993). The hydrolysable tannin, arjunin, isolated from the leaves of *T. arjuna* showed moderate cytotoxic effects against BT-20 human breast carcinoma (Kandil & Nassar, 1997).

Terminalia chebula

Terminalia chebula Retz. (black myrobalan) is an important plant in both Indian as well as Korean and Chinese traditional medicine (Lee et al., 1995), where fruits of *T. chebula* are used for treatment of diarrhea, as an astringent, as an ingredient in *Triphala*, a mixture containing *T. chebula*, *T. bellerica* and *Embllica officinalis*, (Ram Chandra Reddy et al., 1990), as well as for their diuretic and cardiotoxic properties (Singh, 1990) (Table 5). Gallic acid, chebulinic acid, chebulagic acid and 1,2,3,4,6-penta-O-galloyl- β -D-glucopyranose, isolated from a methanolic fruit extract of *T. chebula* were found to show moderate cytotoxic effects against melanoma and ovarian cancer cell lines (Lee et al., 1995). Saleem et al., (2002) found that a 70 % methanol extract of the fruits of *T. chebula* decreased cell viability, inhibited cell proliferation and induced cell death in a dose dependent manner against human breast carcinoma (MCF 7), mouse breast carcinoma (S155), human osteosarcoma (HOS-1) and a prostate cancer cell line (PC-3). Chebulinic acid, tannic acid and ellagic acid were the most cancer cell growth inhibitory phenolics isolated from the fruits of *T. chebula* (Saleem et al., 2002) (Table 5). Ellagic acid may arise from the hydrolysis of ellagitannins in the human gut and it has been found to be ten times more antioxidative than tannic acid (Puech et al., 1999). Thus, the good in vitro anticancer effects Saleem et al. (2002) observed for this compound might be due to its antioxidative properties. Ellagic acid has also been found to induce cell cycle arrest and apoptosis (Narayanan et al., 1999), as well as inhibit tumor formation and growth in animals (Stoner & Morse, 1997; Khanduja et al., 1999). *Triphala* has been found to possess antimutagenic properties, and especially extracts made in acetone and chloroform were effective, whereas a water extract gave no activity (Kaur et al., 2002b).

Terminalia catappa

Terminalia catappa L. (Indian Almond) is a popular medicinal plant in Asia, and the leaves, trunk bark and fruits of this tree are used for treatment of dermatitis, hepatitis and fever as well as for its hemostatic effects in India, Philippines, Malaysia and Indonesia (Lin et al., 1997). Herbal tea of the leaves is used for treatment of liver cancer (hepatoma) and hepatitis in Taiwan (Lin, 1992; Chen et al., 2000). The leaves of *Terminalia catappa* have been found to contain many hydrolysable tannins, such as terflavin A and B, tergallicin, tercatin, punicalin, punicalagin, chebulagic acid, geraniin, granatin B and corilagin (Tanaka et al., 1986). Both the crude extract of the leaves of *T. catappa*, as well as its major tannin component, punicalagin, possess antioxidative (Lin et al., 2001) and anti-genotoxic effects (Liu et al., 1996; Chen et al., 2000) (Table 5). A leaf extract was found to significantly suppress the production of micronuclei in mitomycin-C treated CHO cells (Liu et al., 1996) and the major tannin component of this extract, punicalagin, effectively inhibited bleomycin induced mutagenesis in CHO cells (Chen et al. 2000). Mitomycin-C has been shown to damage DNA by generating reactive oxygen species (ROS) or by alkylating DNA to form adducts, and Chen et al. (2000) speculated that the water extract of *T. catappa* might exert its protective effect against DNA damage through inhibition of

ROS generation or reducing DNA adduct formation. Chen & Li (2005) studied the eventual mechanisms of action by which the extract of the leaves as well as punicalagin might act on cancer cells, and found that both the extract and punicalagin inhibited the proliferation and the anchorage-independent growth of *H-ras*-transformed NIH3T3 fibroblasts. The crude extract was found to be even more potent than punicalagin in inhibiting the proliferation of *H-ras*-transformed NIH3T3 cells, and thus it is possible that there are other effective inhibitory compounds in the crude extract in addition to punicalagin. Punicalagin inhibited the superoxide anion generation in *H-ras*-transformed NIH3T3 cells, and this modulation of the intracellular redox status might subsequently cause a reduction of JNK-1 kinase activation, thereby contributing to the suppression of proliferation. It has been found that approximately 30 % of human tumors have mutations in their *ras* oncogene, and thus punicalagin could be a novel compound for preventing *H-ras*-associated tumors (Chen & Li, 2005).

Table 5. Cytotoxic, antitumor and antimutagenic effects as well as uses in traditional medicine of some Asian *Terminalia* species reported in the literature.

Species	Cytotoxic effects of extract	Compounds	Screening method	Uses in traditional medicine
<i>T. arjuna</i> Roxb.	<p>1-butanol, methylenechloride and water fractions of a MeOH extract of stem, leaves and bark were active against ovarian (OVCAR-3), renal (A498), lung (NCI-H460), colon (KM20L2) and melanoma (MEL-5) human cancer cell lines (Pettit et al., 1996).</p> <p>Extract of leaves gave moderate activity against a BT-20 human breast carcinoma cell line (Kandil & Nassar, 1997).</p> <p>Acetone extract of bark antimutagenic against 4-nitro-o-phenylenediamine (Kaur et al., 2000); acetone and MeOH fractions of stembark extract antimutagenic (Kaur et al., 2002).</p>	<p>Gallic acid, ethyl gallate and luteolin (Pettit et al., 1996).</p> <p>Tannin rich fraction more antimutagenic than a fraction containing ellagic acid: antimutagenic capacity related to degree of polymerization. Bark reported to be rich in polyphenols (60-70 %), including flavones, phenylpropanoids and tannins (20-24 %) (Kaur et al., 2002); Hydrolysable tannins might inactivate P 448/P 450 dependent enzymes (Kaur et al., 2000).</p> <p>Arjunin, a tannin from leaves, active against BT-20 human breast carcinoma (Kandil & Nassar, 1997).</p>	<p>Growth inhibition of cancer cell lines, method not reported in paper, IC₅₀ values reported for pure compounds (Pettit et al., 1996).</p> <p>Ames <i>Salmonella</i> / mammalian microsome mutagenicity test (Kaur et al., 2000; Kaur et al., 2002).</p>	<p>Treatment of dysentery and earache (Dwivedi & Udupa, 1988); treatment of fractures, ulcers, blood diseases, anaemia, asthma, hepatic, congenital, venereal and viral diseases (Kumar & Prabhakar, 1987).</p>
<i>T. catappa</i> L.	<p>Water extracts of leaves suppress mitomycin-C induced micronuclei formation in CHO-K1 cells (Liu et al., 1996).</p> <p>Crude extracts of the leaves antioxidative (Lin et al., 2001) and water extracts active against bleomycin induced genotoxicity in CHO-K1 cells (Chen et al., 2000).</p> <p>Water extract of leaves inhibits selectively the proliferation of H-ras transformed NIH3T3 mouse fibroblasts, but to a lesser extent non-transformed cells (Chen & Li, 2005).</p>	<p>Punicalagin, a hydrolysable tannin, inhibits bleomycin induced genotoxicity in CHO-K1 cells (Chen et al., 2000).</p> <p>Punicalagin inhibits selectively the proliferation of H-ras transformed NIH3T3 mouse fibroblasts possibly partly through inhibition of superoxide anion formation which might decrease levels of phosphorylated JNK-1 and p38 leading to decreased cell proliferation (Chen & Li, 2005)</p>	<p>Micronucleus assay <i>in vitro</i> using CHO cells and mitomycin C as an inducer of mutagenesis (Liu et al., 1996).</p> <p>Antimutagenicity assay using Chinese hamster ovarian cells (CHO-K1) treated with the mutagen bleomycin (Chen et al., 2000).</p> <p>MTT assay for cell proliferation using 96 well microplates (Chen & Li, 2005).</p> <p>Cell cycle analysis using flow cytometer (Chen & Li, 2005).</p> <p>Western blot (Chen & Li, 2005).</p>	<p>The leaves, trunk bark and fruits are used for antidiarrheic, antipyretic and hemostatic purposes in Indian and Malesian traditional medicine (Lin, 1992); in Taiwan leaves are used as a herbal tea for hepatoma and hepatitis (Liu et al., 1996; Chen et al., 2000).</p>

Table 5. Continued.

<p><i>T. chebula</i> Retz.</p>	<p>70 % MeOH extracts of the fruits decreased cell viability, inhibited cell proliferation and induced cell death in a dose dependent manner against human breast carcinoma (MCF 7), mouse breast carcinoma (S155), human osteosarcoma (HOS-1) and a prostate cancer cell line (PC-3) (Saleem et al., 2002).</p> <p>In a mixture together with <i>T. bellerica</i> and <i>Emblica officinalis</i>, acetone and chloroform extracts showed inhibition of mutagenicity, whereas a water extract was found to be inactive (Kaur et al, 2002).</p>	<p>Chebulinic acid, tannic acid and ellagic acid the most growth inhibitory phenolics (Saleem et al., 2002).</p>	<p>Coulter count, [3H] thymidine incorporation, ATP levels in cells, Hoechst DNA staining, flow cytometry (Saleem et al., 2002).</p> <p>Salmonella histidine point mutation assay (Kaur et al., 2002).</p>	<p>Syn. black Myroblans, Harad (Hindi). Homeostatic, antitussive, laxative, diuretic, cardiotoxic, infectious diseases (Barthakur & Arnold, 1991; Singh, 1990); for human gastritis and peptic ulcers in Iranian traditional medicine (Malekzadeh et al., 2001); for prevention and treatment of caries (Chopra & Handa, 1958).</p>
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3. AIMS OF THE STUDY

The aims of this study have been to A) investigate the traditional medicinal uses of some selected species of the genera *Terminalia*, *Combretum* and *Pteleopsis* in some villages in Tanzania and B) to evaluate the biological activities of extracts of these plant species as well as C) to isolate the antimicrobially active fractions from the most promising species. The plant species were selected according to their uses in African (literature studies), especially Tanzanian traditional medicine (our own interviews with traditional healers) and on the basis of their taxonomic relations, all belonging to Combretaceae, as well as on the basis of a literature search on previous studies of the biological effects of related species.

The goals of this study have been achieved by the following investigations:

- 1) This work was initiated with a five weeks ethnobotanical expedition to Tanzania in spring 1999 during which a study on the traditional medicinal uses of selected species of *Combretum*, *Terminalia* and *Pteleopsis* collected in different field locations in the Iringa and Mbeya regions of Tanzania was conducted. The other important goal of this expedition was to collect plant material of Combretaceae for later investigations on the biological activities of these plants (**I**).
- 2) A suitable method (agar diffusion) was established for investigation of the antimicrobial effects of crude plant extracts (**I, II**).
- 3) A study on the antibacterial effects of twenty-one extracts of six species of *Combretum* and five of *Terminalia* was conducted. In this study the effects of the crude extracts of different parts of the plants were investigated against two gram-negative and five gram-positive bacteria, most of them common human pathogens. The antibacterial effects were compared with our results on the traditional medicinal uses of the plant species (**I**).
- 4) The antifungal effects of thirty-five crude extracts of five species of *Terminalia*, ten of *Combretum* and *Pteleopsis myrtifolia* were investigated on five pathogenous species of yeast, *Candida* spp. and the basidiomycet *Cryptococcus neoformans*, all causing serious systemic fungal infections in immunocompromised individuals. The results were again compared to the ethnopharmacological uses of the species. Antifungally active fractions of *Terminalia sambesiaca*, the most antifungal species in our screening, were isolated with RP18 column chromatography and thin layer chromatography (TLC) (**II**).

5) Twenty-seven extracts of eight species of *Combretum*, five species of *Terminalia* and *Pteleopsis myrtifolia* have been investigated for their *in vitro* antiproliferative and cytotoxic effects on three human cancer cell lines (HeLa cervical carcinoma, T 24 bladder carcinoma, MCF7 breast carcinoma) and an endothelial cell line (BBCE, bovine brain capillary cell line) **(III)**.

4. EXPERIMENTAL

4.1. Selection of study area, plant material and bioactivity assays

Tanzania was chosen as the area of study for this investigation since the flora is rich with an estimated number of 10.000 known plant species (<http://www.earthtrends.wri.org>), and the country has a long tradition of using medicinal plants as nearly 80 % of the population relies on traditional medicine for their primary health care (Hedberg et al., 1982). Since most of the existing information on the medicinal uses of the plants is oral, it is important to document it before it is forgotten. Young people move to the cities, and the practice of using plants for health care is diminishing. Although, still a quite large part of the population in the Tanzanian cities use traditional medicine for their primary health care, and medicinal plants can be seen on the marketplaces, for example in Dar-es-Salaam. The Mbeya region, which is situated in the southern parts of Tanzania was chosen, since this region is the home area of Mr. Leonard Mwasumbi (M. Sc., former super-intendent of the Botanical Museum, University of Dar-es-Salaam). Mr. Mwasumbi was also acting as the interpreter during the interviews with local traditional healers. The Mbeya region was also chosen since it seems to be relatively poorly known at least when it comes to traditional medicine, although some studies have been performed on religious and magical rites, some of them associated to traditional medicine of the Nyakyusa (Wilson, 1954; Swantz, 1966). Luoga et al. (2000) points out that the uses and ethnobotanical aspects of plants in Tanzania have not been documented adequately. There are still regions in Tanzania which are relatively unexplored for their medicinal plants.

In this study we have used both the ethnobotanical and the chemotaxonomical approach when selecting some species of *Combretum*, *Terminalia* and *Pteleopsis myrtifolia* (Combretaceae) for screening their antibacterial, antifungal and cytotoxic effects. This particular plant family and genera were chosen since Pettit et al. (1987) had isolated some powerful anti-tubulin stilbenes, the combretastatins, from the stem bark of *Combretum caffrum* and also since some species of *Terminalia* and *Combretum* were known to possess antibacterial and antifungal properties. It seemed thus worthwhile to study some other species of *Combretum* and *Terminalia*, some of which have not yet been investigated for their biological effects, since it is likely that closely related plants contain chemically similar compounds. Another criterion for choosing this particular group of plants was the frequent and successful use of *Combretum* and *Terminalia* species in African traditional medicine according to several authors (Watt & Breyer-Brandwijk, 1962; Haerdi, 1964; Bouquet & Debray, 1974; Kokwaro, 1976; Hedberg, 1982; Chhabra, 1989; Neuwinger, 2000). According to Farnsworth & Soejarto (1991), the possibility of finding plants

containing active compounds gets higher if the plants are chosen on the basis of their ethnopharmacological uses.

Bacterial infections are an important and increasing cause of mortality in Africa due to the AIDS epidemic (Iwu et al., 1999). The misuse and increasing use of antibiotics has also led to bacteria becoming more resistant to antimicrobial treatments. Because of these reasons we decided to study the antibacterial effects of the species of *Combretum*, *Terminalia* and *Pteleopsis* collected from Tanzania, some of them frequently used for the treatment of infectious diseases in Tanzanian traditional medicine. In this study we used some common pathogenous gram-positive and gram-negative bacteria. There is a constant need for new antibacterial molecules, and species of *Pteleopsis*, *Terminalia* and *Combretum* might be good sources for antibacterial compounds.

We studied the antifungal effects of some extracts and fractions of the selected species of plants against some of the most common species of *Candida* and *Cryptococcus neoformans*, causing serious infections in man. Some of the species of *Terminalia* and *Combretum* included in our study are used for the topical treatment of infections as well as diarrhea in Tanzanian traditional medicine, thus we assumed that these plants would give good antifungal effects. Fungal infections are often resistant and difficult to cure and there are only three classes of antifungal drugs available up to date. Because of the increasing number of immunocompromised patients, partly due to AIDS, the numbers of serious systemic fungal infections are increasing. Some of the species of *Candida* which cause serious systemic infections have developed resistance against the conventionally used drugs, so new molecules with new modes of action would be needed.

We investigated the antiproliferative effects of crude extracts of fourteen species of *Combretum*, *Terminalia* and *Pteleopsis* on cancer cell lines representing some common cancer forms with focus on some cancers common in Africa. Of the fourteen species we used in this investigation only *Combretum zeyheri* and *Terminalia sambesiaca* are used as anti-cancer remedies in Tanzania.

In this study a cervical (HeLa) and a breast cancer cell line (MCF 7) were included because they represent two common forms of cancer in women. Breast cancer accounts for approximately 30 % of all cancer diagnosed in women in the United States (Hilakivi-Clarke et al., 2004) and is the second leading cause of cancer death in women (Zafonte et al., 2000). In Western countries cervical cancer represents nearly 10 % of all cancers in women, and organized screening has contributed to a decline of this cancer form in the past 50 years. Recent trends show a resurgence of the disease in developed countries (Franco et al., 2001). Cervical cancer remains globally an important cause of female mortality (Critchlow & Kiviat, 2000). In Africa there seems to be an exceptionally high mortality among women with cervical cancer (Ferlay et al., 1998) and cervical cancer is the most common form of cancer in women in Sub-Saharan Africa (IDe Vuyst et al., 2003; http://www.nccc.online.org/malawi_project.php). Human papilloma virus (HPV) infections

have been found to be the main cause of cervical cancer (Lorincz et al., 1992; Muñoz et al., 1992; Schiffman et al., 1993) and in some populations in Sub-Saharan Africa the prevalence of HPV is high, 40 % of the women being HPV positive in some rural villages in Mozambique (Castellsague et al., 2001). HIV/AIDS is a serious problem on the African continent and is the cause of a multitude of diseases. A clear and significant relationship has been showed between HIV-infection, and the development of cervical intraperitoneal squamous lesions (SIL) (Leray et al., 1999).

The T-24 bladder cancer cell line was included in this study since bladder cancer is one of the most commonly occurring cancers in most African countries, and at least in Egypt it constitutes 30.3 % of all cancers (el-Mawla et al., 2001). In Africa bladder cancer arises in a background of schistosomiasis/bilharzias (Cooper et al., 1997; el-Mawla et al., 2001). The median age of diagnosis is rather young, being 46 years with a male preponderance (el-Mawla et al., 2001).

The *de novo* formation of blood vessels (angiogenesis), composed of endothelial cells, is crucial for both tumorigenesis and metastasis, the vessels supplying the tumor with oxygen and nutrients. Thus we also investigated whether some of the extracts of *Combretum* and *Terminalia* might have the ability to cause antiproliferative effects on BBCE endothelial cells. Since combretastatins with antiangiogenic and antivascular effects have been isolated from *Combretum caffrum*, we speculated that related compounds might be found in the species of *Combretum* we included in this study.

4.2. Ethnobotanical and -pharmacological survey in Tanzania

4.2.1. Description of the study area

The ethnobotanical and -pharmacological excursion was carried out in the Mbeya and Iringa regions of southern Tanzania. Iringa, the main city of the Iringa region, is situated 502 km from Dar-es-Salaam on the main Tanzania-Zambia road beyond the Mikumi National Park. Mbeya city is further west from Iringa and is situated 875 km from Dar-es-Salaam and only 114 km from the Zambian border. Both regions are located in the so called Southern highlands in the southern part of Tanzania. The Southern highlands of Tanzania form the largest blocks of highlands within East Africa. They mostly have a high rainfall and because of their high altitude they are cool. Temperatures average 22 °C (max) and 10 °C (min), and from May to July frosts are common on higher ground with temperatures dropping to - 7 °C on Kitulo Plateau (in Mbeya region). Like the rest of Southern Tanzania this region has, unlike to the northern parts of the country, one long wet season (October-May) and one long dry season (June-November). The southern highlands receive all its rainfall via convectional uplift from Lake Nyasa (syn. Lake Malawi) (<http://www.southernhighlandstz.org/southernhighlands.html>). As with most highland areas in Africa the southern highlands of Tanzania are associated with the Rift Valley System, and there

has been much volcanic activity in the area over the years. Due to rich soils and high rainfall this area is agriculturally productive in both food and crops and some coffee and tea which are the major cash crops of the area (Hodd, 1998). On the other hand there are also areas with very unfertile soil, and these are often covered by Miombo woodland vegetation.

The Southern highlands comprise mountain ranges and volcanoes capped by forest-grassland mosaic. The two highest peaks in southern Tanzania are included in the area: Mtorwi (2961 m) and Mt Rungwe (2960 m). Despite initial surveys revealing significant biodiversity this area has received very little conservation attention. Over 120 plant and animal taxa are endemic for the area and some 2 000 species of vascular plants occur, approaching a quarter of the total flora of East Africa. The main vegetation types of the area are: 1) woodland (Miombo woodland), 2) bushland, 3) wooded grassland, 4) bushland-grassland, 5) grassland , 6) afroalpine forest, 7) afroalpine grassland (Strömquist and Johansson, 1976). The forest-grassland mosaics of the Southern highlands form the WWF- designated ecoregion AT 1015, with its conservation status described as critical/endangered (<http://www.southernhighlandstz.org/southernhighlands.html>). As in the other parts of Tanzania the area of forests are declining due to the high demand for fuel wood. The forests of Tanzania are declining at 1.2 % a year, which is higher than the African average of 1 %.

4.2.1.1. Brief description of Miombo and *Combretum* woodlands

Most of the species of *Combretum*, *Terminalia* and *Pteleopsis* used in this study were found from Miombo and *Combretum* woodlands, and thus a brief description of these habitats is needed here. Miombo woodlands occur in the sub humid tropical vegetation zone and represent a biotope in the spectrum of different savannas. In Africa the Miombo forest covers an area of 2.7 million km² and extends over seven different countries: Angola, Democratic Republic of Congo, Malawi, Mozambique, Tanzania, Zambia and Zimbabwe. Miombo woodland in general occurs on old and nutrient poor soil. The canopy in the Miombo woodland is dominated by genera of leguminous plants, such as *Brachystegia*, *Julbernardia* and *Isoberlinia* (Caesalpiniaceae) (Haanpää, 1998), which vary in number of species in different areas (Chidumayo, 1994). Also *Combretum* and *Terminalia* as well as *Pteleopsis* species can be present in this kind of vegetation, but often to a lesser extent than the dominating leguminous trees. The composition of tree species is believed to depend on soil moisture, soil nutrients and on the frequency of fire. The dominance of the three leguminous tree genera might be related to their roots having ectomycorrhizae, facilitating the uptake of nutrients (Chidumayo, 1994). The species diversity is relatively high probably due to variation of nutrient retention and this diversity is important to maintain ecological stability and nutrient cycling (Chidumayo, 1994). The field layer is composed of grasses (*Digitaria* sp., *Sporobolus* sp., *Pennisetum* sp.) and to a lesser extent of herbs.

In some cases Miombo woodlands can be dominated by *Combretum* spp., and are then called *Combretum* woodland (Högberg, 1982). The soil in *Combretum* woodland is often shallow and therefore limit the growth of often more deep rooted *Brachystegia* and *Julbernardia* species (Savory, 1963). On deeper soil these woodlands can be composed of large Combretaceous trees, occasionally together with *Pterocarpus* spp. (Caesalpiniaceae) and then form almost closed canopies. In these forests very few shrubs are present, and the ground is covered by grasses (Strömquist and Johansson, 1976).

4.2.2. Methods for the fieldwork

4.2.2.1. Ethnobotanical fieldwork (I)

Seven different villages in the Mbeya region (Figure 7) were visited for the ethnopharmacological survey. These villages were Isalavani (Saadani division), Mayale, Itaka, Mlowo (Mbozi district), Ikumbilo (Ileje district) and Itumba (Chunya district). Seven traditional healers, one woman and six men, most of them belonging to the Nyakyusa and the others to the Bena, Mhehe and Mnyika ethnic groups, were interviewed on their traditional medicinal uses of species of *Combretum*, *Terminalia* and *Pteleopsis*. The structured interviews were performed with the aid of a questionnaire which consisted of general and more specific questions (Figure 8). Species of *Combretum*, *Terminalia* and *Pteleopsis* collected in the field were shown to the healers, and they were asked about their species preferences and uses of these plants. The interviews were usually started with general questions about the village after which more specific questions were asked about the medicinal plants shown to the healers. The questionnaire made it possible to get as comparable results as possible between the different villages.

Figure 7. Map of Tanzania (<http://www.maparchive.org>). Plant collections were carried out in the Mbeya and Iringa regions, as well as in the vicinity of Dar-es-Salaam. The interviews on the medicinal uses of the plants were performed in Mbeya region. The study area is indicated on the map.



Figure 8. Questionnaire used during field work in Tanzania in spring 1999.

Village and date:

Informant (Name, Age, Sex, Education, Ethnic group, Origin, Occupation):

A) General questions about village:

1. When was your village founded?
2. Could you tell me something about the history of the village?
3. Has it been isolated for a long time?
4. How many inhabitants do you have in your village?
5. How many women, men and children do you have?
6. How does the age-structure look like?
7. Is there some migration to or from the village?
8. How do people earn their living?
9. What kinds of plants do you cultivate? For domestic use or sale?
10. What kind of agricultural practice is the most usual?
11. Do you cultivate some medicinal plants?
12. Which are the most important cash crops?
13. Are some crops exported to cities nearby or far away?
14. Which are your main planted trees? For what purposes?
15. Do you sell timber?
16. From where do you collect firewood?
17. What kinds of domestic animals do you keep?
18. Could you tell me about the different kinds of work men and women do? Do women have the right for the ownership of land?

B) General questions about use of medicinal plants/Western medicines:

1. Do you prefer plant medicines or Western medicines (pills and capsules?)
2. How easily accessible are Western medicines?
3. Do you have a health station nearby, or do people rely on traditional medicine?
4. If you prefer plant medicines, why?
5. Do people in general prefer Western/plant medicines in your village?
6. From where do you collect the medicinal plants? Own garden/outside the village?
7. How do you collect medicinal plants?
8. Do you collect many species at the same time? How are the plants transported to the village?
9. Do you sell your remedies on the local marketplace? Do you then inform your customers how they should use the medicine?
10. Which are your favorite medicinal plants? How do you use them?
11. Do you store some medicinal plants (dried, milled?)

C) Specific question about specimens of *Combretum*, *Terminalia* or *Pteleopsis* shown to the traditional healer:

Specimen number:

1. Does this plant have any use/uses
2. Is it used as a medicinal plant?
3. Is it easy/difficult to find in nature?
4. Is it possible to confuse the medicinal plant with some other species with similar morphology?
5. How do you recognize this plant?
6. Is it widely known and used as a medicinal plant in your village?
7. Against what kinds of diseases/symptoms is this species used?
8. What parts of the plant are used?
9. How do you collect the plant?
10. Is there some special time of the day/season the plant should be collected?
11. Does the plant have to be in a particular state of its life when used as a medicine? (In bloom, bearing fruit)
12. Is a young tree better than an old or vice versa?
13. Is the medicinal plant used as such, or is it prepared in some way?
14. How is it prepared?
 - a) water extract
 - b) infusion
 - c) decoction
 - d) mixed with food
 - e) in some other way
15. Is the drug used orally or externally?
16. In what kind of dosages is the drug used?
17. Are there any myths, beliefs or rituals connected with the use of the plant as medicine?
18. Is the plant sold at the market-place? What parts of the plant are then for sale?
19. Who in the village has the authority to collect the plant? Is it for example collected only by traditional healers or do women/men/children of the village collect it?
20. Does the use of this plant as a medicine have a long tradition?
21. Has the use of this plant as a medicine decreased/increased if you compare to old times?
22. Is some part of the plant suitable as food? If it is, how is it prepared?
23. Are some parts of the plant known to be poisonous?
24. Is it possible to witness how you prepare this plant into medicine?
25. Does the plant have any economical value?

4.2.2.2. Botanical fieldwork (I)

The field work was carried out in the Mbeya and Iringa regions, as well as in the vicinity of Dar-es-Salaam. The plants were collected in the beginning of the rainy season (Dar-es-Salaam area) or in the middle of the rainy season (Mbeya region) (February-March) when most of the species of *Terminalia* and *Combretum* were in the fruiting stage in order to make the species identification more easy. During the expedition seventeen species, ten of *Combretum*, six of *Terminalia* and one species of *Pteleopsis*, *P. myrtifolia*, were collected (Table 6). Fresh plant material was collected in large sacks for grains and the specimen number was written, using a running number system. All different plant parts which were available were collected, such as roots, stem bark, leaves, fruits and in some cases even flowers. Because most of the species were abundant roots were also collected, and care was taken in the handling of the plants so that they were not destroyed. Voucher samples were collected for each specimen to deposit both in the Botanical Museum (H) of the Finnish Museum of Natural History, University of Helsinki and at the Herbarium of the University of Dar-es-Salaam, Tanzania. For each specimen the geographical location of growth, soil type, type of vegetation and associated plant species was noted (Table 6). Also, important characters of the plants were noted, such as height, growth habit (tree, shrub, climber), scent of flowers, characteristics of the bark, color of fruits etc. Pictures were taken with 200 ASA film of all the collected specimens. The voucher specimens were put in a plant press for tropical conditions with a large number of grey, absorbing papers between the plants. The rest of the plant material, to be used for assays on the biological activities, was dried as quickly as possible in the shade, after which the dried plant material was packed in small paper bags. 60 kg dried plant material with a phytosanitary certificate was transported to Finland through airline cargo transport. The plant material was then grained and stored dry and dark at room temperature until use. This dried material has been used for antimicrobial and cytotoxicity assays performed at both the Division of Pharmaceutical Biology, Faculty of Pharmacy, University of Helsinki and at the Institute for Preventive Nutrition, Medicine and Cancer, Folkhälsan Research center during the years 1999- 2006.

Figure 9. Fieldwork in Tanzania. Collections of *Terminalia kaiserana* in a Miombo forest. Photo: Pia Fyhrquist.



Table 6. *Combretum*-, *Terminalia* and *Pteleopsis*-species (Combretaceae) collected by the author and Mr. L. Mwasumbi for this thesis work in Tanzania in February –March 1999.

Genus and Species	Voucher specimen number	Place of collection	Vegetation type	Associated species
<i>Combretum</i> spp.				
<i>C. apiculatum</i> Sond.	1697025	Njelenje B, Mapogolo new settlement	Miombo woodland	<i>Pseudolachnostylis</i> sp., <i>Margaritaria</i> sp., <i>Parinari</i> , sp., <i>Annona senegalensis</i> , <i>Terminalia kaiserana</i>
	1697026	Njelenje B, Mapogolo new settlement	Miombo woodland	<i>Pseudolachnostylis</i> sp., <i>Margaritaria</i> sp., <i>Parinari</i> , sp., <i>Annona senegalensis</i> , <i>Terminalia kaiserana</i>
	112513	Njelenje B, Mapogolo new settlement	Miombo woodland	<i>Combretum</i> spp., <i>Terminalia</i> spp., <i>Parinari</i> sp.
<i>C. collinum</i> Fresen.	112481	Igoma village, Mufindi district	Miombo woodland	<i>Terminalia</i> sp., <i>Cussonia</i> sp., <i>Swartzia</i> sp., <i>Commiphora</i> sp., <i>Pterocarpus</i> sp., <i>Brachystegia</i> sp.
	1697031 1697033	Igoma village, near Madibira, Mafinga district	Miombo woodland	<i>Bachystegia</i> sp., <i>Julbernardia</i> sp., <i>Terminalia</i> sp.
<i>C. constrictum</i> Laws.	112476	Ruvu flood plain, 80 km from Dar-es-Salaam	Road embankment, ruderal vegetation, flooded during wet season	<i>Commicarpus</i> sp., <i>Solanum incanum</i> , <i>Panicum maximum</i> , <i>Pluchea discoides</i>
<i>C. fragrans</i> F. Hoffm.	112532	Mtipule village, near the roadside to Mkata ranch	<i>Brachystegia</i> woodland	<i>Maerua crassifolia</i> , <i>Commelina africana</i> , <i>Acacia robusta</i> , <i>Albizia</i> sp., <i>Acacia</i> sp., <i>Sclerocarya birrea</i>
	112501	Manyala village, Ileje district	Former farmland, meadow regenerating to secondary vegetation	<i>Piliostigma thonningii</i> , <i>Lonchocarpus capassa</i> , <i>Cassia singueana</i> , <i>Cassia auriculata</i> , <i>Thespesia daenis</i> , <i>Acacia nilotica</i>

Table 6. Continued.

<i>C. hereroense</i> Schinz.	112531	Mtipule village, near the road side to Mkata ranch	<i>Combretum-Acacia</i> woodland	<i>Acacia nilotica</i> , <i>Albizia</i> sp., <i>Lonchocarpus capassa</i> , <i>Pseudolachmostylis maprouneifolia</i>
<i>C. molle</i> G. Don.	112490	Luanda village, Mbozi district near Mbeya	Miombo woodland	<i>Brachystegia</i> spp., <i>Julbernardia</i> spp.
	112488	Itaka village, Mbozi district	Roadside vegetation, farmland	Poaceae
	112500	Manyala village, Ileje district	Former farmland, meadow regenerating to secondary vegetation	<i>Piliostigma thonningii</i> , <i>Lonchocarpus capassa</i> , <i>Cassia singueana</i> , <i>Cassia auriculata</i> , <i>Thespesia daenis</i> , <i>Acacia nilotica</i>
	112524	Inyala Mwakyenje, Mbeya district	Miombo woodland	<i>Brachystegia</i> spp., <i>Julbernardia</i> spp.
	112498	Manyala village, Ileje district	Former farmland, regenerating to secondary vegetation	<i>Piliostigma thonningii</i> , <i>Lonchocarpus capassa</i> , <i>Cassia singueana</i> , <i>Cassia auriculata</i> , <i>Thespesia daenis</i> , <i>Acacia nilotica</i>
	112478	Saadani village, Mufindi district	Degraded <i>Brachystegia</i> woodland	<i>Leptochloa panacea</i> , <i>Maytenus holstii</i> , <i>Maytenus heterophylla</i> , <i>Themeda triandra</i> , <i>Croton</i> sp., <i>Faurea</i> sp., <i>Hyparrhenia</i> sp., <i>Cyphostemma</i> sp., <i>Brachystegia</i> sp.
	112502	Manyala village, Ileje district	Former farmland, regenerating to secondary vegetation	<i>Piliostigma thonningii</i> , <i>Lonchocarpus capassa</i> , <i>Cassia singueana</i> , <i>Cassia auriculata</i> , <i>Thespesia daenis</i> , <i>Acacia nilotica</i>
<i>C. obovatum</i> F. Hoffm.	112509	Shalangwa village, Chunya district	In riverine thicket on termitaria	<i>Thespesia</i> sp., <i>Combretum molle</i> , <i>Entada abyssinica</i> , <i>Pappea capensis</i>

Table 6. Continued.

<i>C. padoides</i> Engl. & Diels	112528	Ruaha river valley, 6.6 km from the junction of the rivers Ruaha and Iyori	Riverine forest	<i>Tamarindus indica</i> , <i>Albizia glabryrhina</i> , <i>Ficus ingens</i> , <i>Pteleopsis myrtifolia</i>
<i>C. psidioides</i> Welw.	112497	Manyala village, Ileje district	Miombo woodland	<i>Piliostigma thonningii</i> , <i>Lonchocarpus capassa</i> , <i>Cassia singueana</i> , <i>Thespesia daenis</i> , <i>Acacia nilotica</i>
	112483	Igomaa village, Mufindi district	Miombo woodland	<i>Terminalia</i> sp., <i>Cussonia</i> sp., <i>Swartzia</i> sp., <i>Commiphora</i> sp., <i>Pterocarpus</i> sp., <i>Brachystegia</i> sp.
<i>C. zeyheri</i> Sond.	112512	Njelenje B village, Mapogolo new settlement, Mbeya district	Miombo woodland	<i>Julbernardia</i> sp., <i>Brachystegia</i> sp., <i>Terminalia</i> sp.
	112487	Chimala, Mbarari district. Near Chimala mission station.	Miombo woodland	<i>Brachystegia manga</i> , <i>Julbernardia</i> sp., <i>Friesodielsia</i> sp., <i>Cydolachnostylus maprouneifolia</i> , <i>Margaritaria discoidea</i>
	112533	Mtipule village near the roadside to Mkata ranch.	Miombo woodland	<i>Maerua crassifolia</i> , <i>Commelina africana</i> , <i>Acacia robusta</i> , <i>Albizia</i> sp., <i>Acacia</i> sp., <i>Sclerocarya birrea</i>
<i>Pteleopsis</i> spp.				
<i>P. myrtifolia</i> Engl. & Diels	112529	Ruaha river valley, 6.6 km from the junction of the rivers Ruaha and Iyori	Riverine forest	<i>Tamarindus indica</i> , <i>Albizia glabryrhina</i> , <i>Ficus ingens</i> , <i>Combretum padoides</i>
<i>Terminalia</i> spp.				
<i>T. glaucescens</i> Benth.	112480	Igomaa village, Mufindi district	Miombo woodland	<i>Sterculia quinqueloba</i> , <i>Lannea</i> sp., <i>Cussonia</i> sp., <i>Pterocarpus angolensis</i>

Table 6. Continued.

<i>T. kaiserana</i> F. Hoffm.	112482	Igomaa village, Mufindi district	Miombo woodland	<i>Terminalia</i> sp., <i>Cussonia</i> sp., <i>Swartzia</i> sp., <i>Commiphora</i> sp., <i>Pterocarpus</i> sp., <i>Brachystegia</i> sp.
	112484	Ilebula road junction, Mbozi district	Degraded Miombo woodland	<i>Brachystegia</i> sp., <i>Combretum molle</i>
	112491	Luanda village, Mbozi district	Miombo woodland	<i>Brachystegia</i> sp., <i>Julbernardia</i> sp.
	112492	Luanda village, Mbozi district	Miombo woodland	<i>Brachystegia</i> sp., <i>Julbernardia</i> sp.
	112493	Luanda village, Mbozi district	Miombo woodland	<i>Brachystegia</i> sp., <i>Julbernardia</i> sp.
	112525	Inyala Mwakyenje, Mbeya district	Miombo woodland	<i>Combretum molle</i>
<i>T. sambesiaca</i> Engl. & Diels	112526	Kitonga gorge	Roadside, near riverine course in primary Miombo woodland	<i>Brachystegia taxifolia</i> , <i>Pterocarpus angolensis</i> , <i>Dahlbergia nitidula</i> , <i>Ficus ingens</i> , <i>Ficus lutea</i> , <i>Combretum zeyheri</i> , <i>Dahlbergia melanoxylon</i> , <i>Diplorhynchus condylocarpon</i> , <i>Begonia</i> sp., <i>Ipomaea</i> sp., <i>Acalypha</i> sp., <i>Chlorophyton</i> sp., <i>Saint Paulia</i>
<i>T. sericea</i> Burch ex. DC.	112494	Ilebula road junction, Mbozi district	Degraded Miombo woodland	<i>Julbernardia</i> sp., <i>Brachystegia</i> sp.
	112477	Vigwasa, 80 km from Dar-es-Salaam	Miombo woodland	<i>Brachystegia</i> sp., <i>Pterocarpus</i> sp., <i>Hymenocardia</i> sp., <i>Acanthus walleria</i>

Table 6. Continued.

<i>T. spinosa</i> Engl.	112534	Tegeta village, 25 km north of Dar-es-Salaam.	Roadside with cashewnut trees	
<i>T. stenostachya</i> Engl. & Diels	112479	Igomaa village, Mufindi district	Hill slope in Miombo woodland	<i>Sterculia quinqueloba</i> , <i>Lannea</i> sp., <i>Cussonia</i> sp., <i>Pterocarpus angolensis</i>
	112514	Njelenje B, Mapogolo settlement, Mbeya district	Miombo woodland	<i>Julbernardia</i> sp., <i>Brachystegia</i> sp.

4.3. Extraction and fractionation of the plant material for antimicrobial and cytotoxicity assays

4.3.1. Crude extracts (I, II, III)

Extraction was performed on dried and milled plant material of the *Combretum*, *Terminalia* and *Pteleopsis* species collected from Tanzania (Table 6). The dried plant material had been stored in the dark prior to extraction. Several organs of the plants were used, such as roots, stem bark, leaves and fruits in order to compare their biological effects. Water, methanol, ethanol and acetone were used to compare the extraction capacity of the solvents.

For the methanol, ethanol and acetone extracts 4 g dried plant material was dissolved in 100 ml solvent and extracted for 2- 24 h of which 15 min in an ultrasonicator (Transsonic 460, Elma, Ultrasonics). The extracts were then filtered and evaporated under reduced pressure, after which they were freeze-dried and stored in -20 °C until use. 50 mg/ml of these extracts were dissolved in methanol for the antibacterial and antifungal assays (**I, II**) and in dimethylsulfoxide (DMSO) for cytotoxicity assays (**III**). The samples in DMSO for cytotoxicity screening were further diluted to 5 mg/ml with growth medium (DMEM or RPMI 1640) to reduce the percentage of DMSO.

The water decoctions were made in the same way as they are prepared in traditional medicine in Tanzania. The same amount of plant material to solvent was used as for the other extracts, but then the decoction was brought to the boil for 5 minutes. The decoction was allowed to cool down and the extraction was continued for two hours at room temperature. The decoctions were then centrifuged at 2000 RPM for 5 minutes and filtered through Whatman filterpaper No. 5. The filtered samples were evaporated under reduced pressure and the residual extract was then freeze-dried and stored at -20 °C until use. The freeze-dried extracts resulting from hot water decoctions were dissolved in MeOH to 50 mg/ml for the antimicrobial tests.

4.3.2. Fractionation with medium pressure liquid chromatography (MPLC) and thin layer chromatography (TLC) (II)

A glass column (460 mm x 26 mm, Büchi, AG, Flavil, Switzerland) filled with Lichrospher RP-18 (particle size 15-25 µm, Merck, Darmstadt, Germany) was used for the fractionation of the antimicrobially most potent extracts of *Terminalia* (**II**). The column was connected to an HPLC-pump (Waters M-6000A, Waters Inc., Milford, MA, USA).

In brief, twenty grams of root powder of *Terminalia sambesiaca* was extracted in 500 ml of MeOH (HPLC-quality, Rathburn) and freeze-dried for four days. 750 mg freeze-dried extract was dissolved in MeOH to a final concentration of 50 mg/ml. This extract was applied to the column and eluted with MeOH (HPLC-quality, Rathburn) and water (Milli Q purified), using a step gradient, flow rate 3.0 ml/min: 0-40 min 5 % MeOH, 40-125 min 25 % MeOH, 125-185 min 50 % MeOH, 185-235 min 80 % MeOH, 235-285 min 90 % MeOH. The fractions were collected with a fraction collector (LKB, Bromma, 2111 Multirac, Bromma, Sweden). The fractions obtained were investigated with reversed phase thin layer chromatography (RP-TLC) in order to be able to combine the fractions containing the same pattern of molecules. TLC was performed on RP-18_{F254s} (Merck, Darmstadt, Germany) TLC plates. The plates were developed with methanol: water 1:1 (v/v) containing 1 % formic acid (v/v). The plates were observed under UV-light at 254 and 366 nm. A Camaq video documentation system was used for documentation of the plates. Fractions were combined according to the pattern information obtained by RP-TLC and freeze-dried. Prior to use the fractions were dissolved in MeOH at a concentration of 20 mg/ml for the antifungal (II) and antibacterial investigations (unpublished results).

4.4. Biological assays

4.4.1. Bacterial and fungal strains (I, II)

Two gram-negative and five gram-positive bacterial strains, representing common human bacterial pathogens were used for the antibacterial screenings (I). The gram-negative bacteria were *Escherichia coli* ATCC 8739 and *Enterobacter aerogenes* FOMK (Division of Pharmacognosy, University of Helsinki, Finland). The gram-positive were *Staphylococcus aureus* FOMK, *Staphylococcus epidermidis* ATCC 12228, *Bacillus subtilis* FOMK, *Micrococcus luteus* YMBO (Division of General Microbiology, University of Helsinki, Finland) and *Sarcina* sp. FOMK.

The following five species of yeast, commonly causing systemic infections in immunocompromised patients were used (II): *Candida albicans* ATCC 90028, *C. krusei* ATCC 6258, *C. tropicalis* LT (T-185), *C. glabrata* NL 2238 and *C. parapsilosis* ATCC 22019. The basidiomycet, *Cryptococcus neoformans* LT (KT V2), was also chosen since it causes severe meningoencephalitis in immunocompromised patients.

4.4.2. Assay for antibacterial and antifungal activity (I, II)

An agar diffusion method (Barry & Thornsberry, 1991; Ojala et al., 2000; Rauha et al., 2000) was used to screen the plants for antibacterial and antifungal activities, as well as for estimating

minimum inhibitory concentrations (MIC) of the most promising extracts. The method is described in papers **I** and **II**. In brief, in the beginning of the experiments, the bacterial or fungal strains to be used were inoculated on Nutrient agar and Saboraud agar respectively, and grown for 24 h. A small amount of the culture was transferred to sterile isotonic sodium chloride, and the turbidity was measured spectrophotometrically at 625 nm. The suspensions were diluted in 0.9 % (w/v) NaCl to an absorbance of 0.1 at 625 nm. 240 μ l of diluted suspension of bacteria/fungi was inoculated on agar dishes containing Isosensitest and Saboraud agar for the bacteria and fungi respectively. Sterile Whatman filter paper discs ($\varnothing = 12.7$ mm) containing 200 μ l of extracts (50 mg/ml; 10 mg on filter paper disc) were applied to the dishes (**I**, **II**). Alternatively, steel cylinders ($\varnothing = 12.7$ mm) or holes ($\varnothing = 12.7$ mm) boared in the agar loaded with 200 μ l of extracts (10 mg extract/cylinder) were used (**I**). The petri dishes were kept in room temperature for one hour prior to incubation in the dark at + 35°C for 24 h. The diameter of the inhibition zones were measured after incubation and the results were expressed as the mean of 3-9 diameters (0.1 mm precision). Ampicillin and streptomycin (Sigma-Aldrich Chemicals, USA) were used as positive controls for the bacteria. For the fungal species amphotericin-B (Sigma-Aldrich Chemicals, USA) and itraconazol (Janssen-Cilag, Espoo, Finland) were used as positive controls.

4.4.2.1. Estimating minimum inhibitory concentrations, MIC (I, II)

The agar diffusion method was also used for determining minimum inhibitory concentrations (MIC) of the most promising plant extracts against the bacterial (**I**) and fungal species (**II**). For the bacteria five different concentrations; 50, 10, 2, 0.4 and 0.08 mg/ml were used for the first experiments. Then the next dilutions were made with a series of concentrations lying between the lowest concentration giving detectable inhibition (a clear zone of at least 1- 2 mm around the cylinder) and the next smallest concentration. A final series of 2.0, 1.75, 1.5, 1.2, 1.0, 0.95, 0.7 and 0.4 mg/ml were chosen for the antibacterially most efficient extracts. Ampicillin and streptomycin were used as references, and MeOH as the solvent blank. The MIC values against the fungal species (*Candida* spp. and *Cryptococcus neoformans*) were obtained essentially in the same way as for the bacteria, but for them concentrations of 50, 25, 12.5, 6.25, 3.125, 1.562 and 0.78 mg/ml of the plant extracts were used. All the tests were performed in triplicates.

4.4.3. Cell lines (III)

For the cytotoxicity and antiproliferative screening some cell lines representing common kinds of cancer were chosen. The following cancer cell lines were used: HeLa (cervical carcinoma,

human, ATCC CCL-2), MCF 7 (breast carcinoma, human, ATCC HTB-22) and T 24 (bladder carcinoma, human, ATCC HTB 4). Bovine brain capillary endothelial cells (BBCE) were used to study the eventual antiangiogenic effects of the extracts. The BBCE cells were grown in DMEM, low glucose variant (Gibco), containing 2 mM L- glutamine, non-essential amino acids (100 ×), penicillin-streptomycin (100 ×) and 10 % fetal calf serum (Gibco). 5 µl of fibroblast growth factor, FGF (Sigma) (0.5 µg/ml prepared in 0.2 % gelatin solution) was added to the BBCE cells once a week (in 10 ml cell suspension). The culturing of the cancer cell lines is described in paper **III**.

4.4.4. Assays for measuring cytotoxic activities of the extracts (III)

4.4.4.1. Alamar Blue method

In order to estimate the amount of viable cells after treating them with plant extracts, the Alamar Blue method was chosen. Alamar Blue (Promega, Biofellow, Finland) is a redox indicator which contains blue non-fluorescent resazurin which is reduced to pink fluorescent resorufin by the metabolic activity of living cells. In brief, 5×10^3 cells/ml (MCF 7, T 24) or 5×10^4 cells/ml (HeLa) were seeded on ELISA 96 well microtitre culture plates (Costar®, Corning Inc.) to a final volume of 199 µl and incubated for 24 h at + 37 °C. The following day 1 µl of extracts (5 mg/ml) were added to the wells so that the final concentration of extracts in the wells was 25 µg/ml. The cells with extracts were incubated for two days at 37 °C, after which the medium was replaced with fresh medium and the same amount of the extracts were added for the second time and incubated for two more days. After a completed time of incubation with the plant extracts the old medium with the extracts was aspirated and fresh DMSO medium without phenol red (Gibco) was added to the wells. The cells were allowed to grow in this medium for 4 h, after which 10 µl Alamar Blue reagent was added to each well and the cells were incubated for 24 h at 37 °C. The fluorescence of the wells was measured with a Victor (Wallac, Finland) spectrophotometer at 540 nm em./590 nm ex. The results were expressed as the percent cell growth of controls and were calculated as averages ± SEM of two-six experiments, each experiment with duplicate samples.

4.4.4.2. Coulter count

The antiproliferative effects of the plant extracts were also assayed by counting the cells after treating them with extracts, using Coulter count as a reference method to Alamar Blue. 5×10^3 cells/ml (MCF 7, T 24 and BBCE) or 5×10^4 cells/ml (HeLa) were seeded on 24- well cell culture plates to a final volume of 1000 µl/well (Costar®, Corning Inc.) and incubated for 24 h at + 37°C. The following day 5 µl of extracts (5 mg/ml, making final concentration to 25 µg/ml in

well) or serial dilutions of a leaf extract of *Combretum fragrans* (32.9 – 4.3 µg/ml) were added to the wells and the cells were incubated for an additional two days at + 37°C. The old medium was replaced with fresh medium and 5 µl of the extracts were added for a second time. The cells were incubated for an additional two days at + 37°C. The cell number was determined after five days of incubation when the untreated cells should be in their logarithmic phase of growth. For the cell count the medium was aspirated, the cells were washed with 500 µl PBS and detached from the wells with 500 µl trypsin-EDTA (Sigma). The cells in trypsin-EDTA were then added to 9.5 ml PBS and counted with a coulter counter apparatus (Beckman, USA). The experiments were done in duplicates and repeated four to six times. Cell proliferation was expressed as percent survival of control and the inhibition of proliferation as percent inhibition of control.

4.4.4.3. Hoechst staining

Hoechst staining was used to investigate if the antiproliferatively most effective extract caused fragmentation of the nucleus in the cancer cell lines. 5×10^3 or 5×10^4 cells/ml, depending on the cell line, were seeded on 24 well or 12 well tissue culture plates (Costar®, Corning Inc.) with aseptically inserted sterile glass coverslips. The cells were allowed to attach and grow for 24 h at 37 °C after which 5 µl of a series of concentrations (final concentrations 9.7 – 4.3 µg/ml in wells) of *Combretum fragrans* were added to the wells. The cells were incubated together with the extracts for two days at 37 °C after which the medium was changed and the extracts added for a second time. The cells with extracts were incubated for two more days. When incubation was completed the medium was aspirated and the cells were washed with PBS. The cells were fixed with 500 µl 3 % (w/v) paraformaldehyde (Sigma) for fifteen minutes. The fixed cells were treated with 500 µl 50 mM NH_4Cl_2 for 15 minutes and washed with PBS. 500 µl Hoechst 33258 (Bio-Rad) stain was added to the cells for 20 minutes at + 37 °C. The Hoechst stain was aspirated and the cells were washed with PBS and kept in this solution until mounted on microscope slides. The coverslips with the cells were put on a drop of gel/mount mounting medium (Biomed, USA) on microscope slides (O. Kindler, GmbH, Freiburg, Germany). The cells were observed under a Zeiss Axioplan II fluorescence microscope (Zeiss, Oberkochen, Germany).

5. Results and Discussion

5.1. Presentation of the traditional healers in the Mbeya region of Tanzania (I)

Traditional healers in seven different villages in the Mbeya region (Figure 7) were interviewed with the aid of a questionnaire on their uses of medicinal plants with focus on species of *Combretum*, *Terminalia* and *Pteleopsis*. The villages visited were Isalavano, Mayale, Itaka, Mlowo, Ikumbilo, Itumba and Segela and the number of inhabitants varied from 300-4000 people. Here I present the results of the general part of the questionnaire (Figure 8). This part of the questionnaire was used in order to obtain some general information of the villages and the people living in them, as well as getting familiar with the traditional healers to be interviewed. The median age of the traditional healers was 55 years, the youngest being 40 and the oldest 66 years. Six of the healers were men and one was a woman. Four of the seven healers belonged to the Nyakyusa tribe and the three others belonged to the Mhehe, Bena and Mnyika tribes respectively. Some of the healers could show us a certificate that they had been officially recognized by the Tanzanian health authorities as traditional medicinal practitioners (TMP). The current policy in many African countries, including Tanzania, has been to increasingly incorporate traditional medicine into the formal health care sectors, and thus, traditional healers have been officially recognized and accepted as a part of the health care system (Tsey, 1997).

All the traditional healers we interviewed were highly entrusted by the people in the villages. This is not only because of their skilful healing practices, but also due to the supernatural and magical capacities they are thought to hold, as well as participating in many aspects of life of the villagers. Traditional healers have been found to play an important role in promoting the traditional concepts and life of the ethnic groups in Tanzania (Swantz, 1974). The local people in the villages we visited seemed to prefer modern medicines if affordable and available, but have the traditional healer as their first point of contact (and in many cases only point of contact). Only in one village the people told us that they prefer traditional medicine because they think that they cannot get treated correctly with modern medicines. Most of the people in the villages we visited are not able to afford the expensive medicines or expensive treatments given in Western hospitals, and this seems to be the case in many African countries (Tsey, 1997). Most of the people in the villages we visited rely on traditional medicine for their primary health care and this is in agreement with Hedberg et al. (1982) according to whom 80 % of the population in African countries are dependent on traditional medicine for their primary health care.

In contrast to the rest of the people in the villages, the traditional healers naturally preferred traditional medicine to modern, but some of them were ready to use western medicines as well, either alone, or in *conjunction* with traditional remedies, if the disease they were treating was not cured by traditional remedies alone. Some of the healers had the possibility to get western

medicines from hospitals nearby. One of the healers had been treating patients since 1962, and wanted to point out that he has seen that traditionally prepared plant remedies effectively cure various diseases. Illustrative for the trust in traditional healers and traditional medicines is that people have come from the big cities, like Dar-es-Salaam, to be treated by traditional healers in Mbeya (over 900 km away), when Western health care fails (unpublished information from one of our interviews). Most of the traditional healers we interviewed, expressed their concerns on the declining interest in traditional medicine among the young people in the villages. Young people are moving to the cities (Dar-es-Salaam, Dodoma) in search for work, and in many cases they seem not to be interested in traditional medicine since it is a rather poor source of income.

All the traditional healers we interviewed do farming as their main income. Plant species generally grown in the villages are maize (*Zea mays*), beans (*Vicia faba*, *Phaseolus vulgaris*), cucumbers (*Cucumis sativus*), tobacco (*Nicotiana tabacum*), sunflowers (*Helianthus annuus*), groundnuts (*Arachis hypogaea*), coffee (*Coffea arabica*), millets (*Panicum miliaceum*), banana (*Musa paradisiaca*), tea (*Camellia sinensis*), mango (*Mangifera indica*) and papaya (*Carica papaya*), all of them mainly for domestic use. Groundnuts and maize are used in rotation for crops, the nitrogen-rich groundnuts fertilizing the soil. Tea and coffee are also cultivated as cash crops and sold to the marketplaces in Mbeya and Dar-es-Salaam. Two of the seven interviewed healers told us that they grow some of their medicinal plants in small gardens near their houses. Examples of medicinal plants grown in the gardens of the healers are species of *Dioscorea* (Mbinga), species of *Ozoroa* (Mwalukama), different species of Apocynaceae with milk sap (Mfua) and species belonging to Euphorbiaceae. The medicinal plants which were grown in the gardens were, however, not shown to us. During our stay in Tanzania we did not observe a single written document on how to use medicinal plants in the villages we visited. The traditional healers we interviewed sometimes used small memory notes, but most of the knowledge they keep in their minds. The information on how to use medicinal plants was mostly taught from father to son (daughter), and this is done during collection trips to the forests near the village as well as at home when preparing the plant remedies and treating the patients. There are quite big risks for making mistakes from this oral form of learning because the information taught may easily be wrongly remembered. There might also be some risks concerning the correct form of dosage and administration of the plant decoctions, even if we never heard of any poisonings during our expedition. Poisonings from traditional medicinal preparations are, however, reported to be a serious problem (Swantz, 1974).

5.2. Ethnopharmacological uses of species of *Combretum* and *Terminalia* by traditional healers in Mbeya, Tanzania (I)

By using a more specific part of the questionnaire (Figure 8), the traditional healers were interviewed on their medicinal uses of some species of *Combretum* and *Terminalia* shown to

them in the beginning of the interviews. The plants were collected from Miombo woodlands, riverine forests and ruderal vegetation (Table 6). In total ten different species, six of *Combretum* and four of *Terminalia*, were shown to the healers (Table 7).

Of the ten species of *Combretum* and *Terminalia* shown to the healers, six were commonly used as medicinal plants in the villages we visited (Table 7). These were *Combretum molle* (Swahili: *Mlama*; Nyakusa; *Mpula*), *Combretum fragrans* (Swahili: *Hansebwe*), *Combretum psidioides* (Swahili: *Mlama zambila*), *Combretum zeyheri* (Nyakyusa: *Kakati*; Swahili: *Mlama*), *Terminalia sericea* (Nyakyusa: *Namatipo*; Swahili: *Mplulu*) and *Terminalia kaiserana* (Swahili: *Mpululu*; Nyakusa: *Namatipo*). All the healers reported that they easily recognize these species from fruit and leaf characters. Among botanical professionals the fruits are considered to be the most species-specific organs of *Combretum* and *Terminalia* species and are the most used organs in species identifications (Wickens et al., 1973). In some cases also the characters of the stem bark, like the color and degree of smoothness/fissures are used, both among botanists and traditional healers. One of the healers reported that he also uses the flowers as characters to distinguish *Combretum* and *Terminalia* species from each other. Most of the healers admitted that it is easy to confuse species of *Combretum* and *Terminalia* with closely related ones, and that the only way to learn them is through experience. Most of the species of *Terminalia* and *Combretum* seem to be abundant and easy to find by the healers. The *roots* were the most common part of the plants to be used for medicine and were used by all the seven healers interviewed. The leaves of the species were used in four cases and the stem bark by two out of seven interviewed traditional healers.

In the following the species of *Combretum* and *Terminalia* which are used in traditional medicine in Mbeya are presented and the summary of these results can be read from Table 7 and I, Table 1.

5.2.1. Results from our interviews on the traditional uses of *Combretum* spp. in Mbeya region

Combretum molle (Soft-leaved combretum, velvet bushwillow, vernacular names in Swahili and Nyakyusa respectively: *Mlama*, *Mpula*) was the most popular species for medicinal purposes of all the *Combretum* species collected. It was used in six villages out of the seven visited, but our sample of interviews (n = 7) is not sufficiently large to draw any final conclusions of the popularity of this species among traditional healers in Tanzania. Most of the healers told us that *C. molle* is easy to find and very abundant, although it can be very variable in its leaf and fruit characters, and thus not very easy to identify. Wickens (1973) points out that *C. molle* can be very variable in leaf shape and size as well as in size of the fruits, and we also discovered this during the field collections of this species. The answers to the question of what time of the day

this species preferably should be collected were contradictory since one of the healers reported that he mainly collects it during daytime, and one of the healers that she collects it nighttime in order to obtain maximal healing properties. When the healers were asked whether they prefer young or more mature trees, most of them told us that they collect individuals of all ages, whereas some preferred young and others more mature individuals. It has been shown that several secondary components are most abundant at the juvenile phase of leaf and shoot development (Julkunen-Tiitto, 1989), at least in plants occurring on temperate latitudes, but this might not be the case with tropical plants which continuously must defend themselves against herbivores, insects, fungi and bacteria. This might be the reason why the species of *Combretum* seem to be equally good as medicinal plants independent of the time of the day or of the stage of development/age they are collected.

Combretum molle is used in a strikingly similar way among the healers in the different villages we interviewed. It is seldom used alone and often mixed together with other species of *Combretum* or with species of *Terminalia*. One of the healers reported that the roots of *C. molle* are mixed with *Terminalia kaiserana* for the treatment of diarrhea. The secondary compounds of these two species might act synergistically in these mixed decoctions, since both species are used for the treatment of diarrhea in single species decoctions. Most of the healers use dried samples of *C. molle*, but in one case also the use of fresh roots was reported. Gonorrhoea and syphilis are treated with mixtures of the roots, stem bark or leaves of this species and another plant called “Muwofi”, which both are grounded and one teaspoon of the powder is added to maize porridge, “Ugali”. Sometimes *C. molle* is used alone for the treatment of gonorrhoea and syphilis. Bacterial infections and diarrhea as well as venereal diseases are treated by allowing the patient to bath in an extract of the leaves, which are thought to be aseptic. Hot water decoctions of the dried roots and leaves or fresh roots cut into pieces, as well as mixtures of these plant parts, are put in maize porridge and used for the treatment of gonorrhoea, syphilis, influenza, severe cold and oedema. Both leaf and root decoctions of *Combretum molle* are used for the treatment of malnutrition in children. The dried, powdered roots of *C. molle* are also used topically for the treatment of wounds and diseases of the skin, and for this purpose the roots are mixed with sheep fat to form a paste that is smeared on the skin. Some of the healers in Mbeya informed us that they use the roots and leaves of *C. molle* for treatment of oedema. The uses of *C. molle* by the traditional healers in Mbeya are in agreement with the medicinal uses of this plant in many African countries reported by other authors (Table 7). Some additional uses which were not reported by the traditional healers in Mbeya are the use of *C. molle* for hookworms, snake bites, sterility, abortions and as an aid for child birth (Watt & Breyer-Brandwijk, 1962; Haerdi, 1964; Kokwaro, 1976).

Combretum zeyheri (vernacular names in Swahili and Nyakyusa respectively: *Mlama*, *Kakati*) seemed to be one of the most popular species of *Combretum* among the traditional healers in Mbeya. The Nyakyusa vernacular name, *Kakati*, means “always used”. This species is recognized

mainly from the leaf characters, and is abundant and easy to find from the forests according to the traditional healers interviewed. It is reported to be collected during any time of the day. *C. zeyheri* is used for treatment of diarrhea and tumors (Table 7; I, Table 1). It was the only *Combretum* species we included in this investigation to have uses for the treatment of tumors and cancer. Powdered, dried roots or leaves are made into hot water decoctions and one teaspoon of this decoction is mixed in a cup of hot water administered daily for the treatment of stomach tumours as well as diarrhea. Alternatively, the dried roots/leaves can be mixed into maize porridge (Ugali) for the same purposes as above. Sometimes *C. zeyheri* is mixed with other species of *Combretum* for the treatment of diarrhea.

Many other authors report traditional medicinal uses of *C. zeyheri* which agree with the ones we report: Hedberg et al. (1982) report that the roots and stem bark of *C. zeyheri* are made into hot water extracts and these are mixed into maize porridge for the treatment of diarrhea and vomiting; Gelfland et al. (1985) report that an infusion of the roots is used for the treatment of bloody diarrhea; Kokwaro (1976) reports that the root powder of *C. zeyheri* is mixed with porridge for the treatment of dysentery; the Mankoya people in Zambia use the leaves and bark of *C. zeyheri* to prepare a complex cure for treating smallpox (Drummond & Coates-Palgrave, 1973). The traditional healers in Mbeya seem to be the only ones who use *C. zeyheri* for the treatment of cancer and tumours, since to the best of our knowledge there exists no other reports on this kind of use of *C. zeyheri*.

Combretum psidioides (vernacular name in Swahili: *Mlama zambila*) is a large- and soft-leaved species, easy to identify and is fairly common on sandy soils in the Mbeya region. The leaves of this species are used as the main character for species identification by the healers in Mbeya. It is used either alone or in combination with other species of *Combretum*, such as *C. molle* and *C. zeyheri*, which are collectively called “Mlama” by the healers we interviewed. In combinations with the other species of *Combretum* it is used for the treatment of chest problems, pains in the spinal cord and oedema. All parts of *C. psidioides* are used for medicinal purposes, but especially the roots are popular for medicine. The roots are made into hot water decoctions for the treatment of diarrhea and muscle pain. The leaves, either fresh or dried, are pounded and extracted in hot water or mixed with porridge, Ugali, to treat oedema. One teaspoon leaf extract is mixed with hot water in a tea cup for the same purpose.

Just a few uses are listed for *C. psidioides* in the literature, and some of them are in accordance with the uses we report. For example, Haerdi (1964) reports that root decoctions of this plant are taken for treatment of rheumatic back pain and macerations of the stem bark for diarrhea. In addition this species is reported to have some uses we have not recorded during our stay in Mbeya. The stem bark is used as an aphrodisiac and the leaf sap and root decoction for the treatment of malaria (Haerdi, 1964).

Combretum fragrans (vernacular name in Swahili: Hansebwe) is fairly common in the Mbeya region and easy to identify from its fruit and leaf characters. The roots of this species are ground into a fine powder and mixed with hot water, brought to boil, filtered and drunk as a decoction for diarrhea (Table 7; I, Table 1). Sometimes the root powder is mixed into porridge or into tea for the same purpose. Many different medicinal uses of this species are reported by other authors, but none of them report of its uses against diarrhea. Apparently *C. fragrans* seems to contain antiseptic properties since it is used for the treatment of bacterial infections: Chhabra et al. (1989) report that both the roots and the leaves of *C. fragrans* are used for the treatment of leprosy; root decoctions are used for the treatment of coughs and syphilis (Kokwaro, 1976). Adjanohoun et al. (1986) report that leaves of this species are used for the cleaning of chronic wounds. In addition leaf extracts are used as an antidote against snake bites (Neuwinger, 2000) and according to Gelfand et al. (1985) root infusions are used as an aphrodisiac.

5.2.2. Results from our interviews on the traditional uses of *Terminalia* spp. in Mbeya

Two species of *Terminalia*, *T. sericea* and *T. kaiserana* were frequently used as medicinal plants among the traditional healers in Mbeya (Table 7; I, Table 1). Both species have a similar vernacular name in Swahili, *Mpululu* as well as two separate names in Nyakyusa (*Bena* for *T. kaiserana* and *Namatipo* for *T. sericea*) and it seems that the traditional healers often mix between these two. It is indeed known that hybrids between *T. sericea* and *T. kaiserana* occur, and they resemble *T. sericea* in terms of the degree of hairyness of the leaves (Wickens, 1973). It can be extremely difficult to distinguish between these two species if they are growing side by side.

Terminalia sericea is a popular medicinal plant in most of the villages we visited. This species is abundant and easy to find. It is willow like in its appearance, with small silvery, hairy leaves. The traditional healers told us that they distinguish it from other species of *Terminalia* from its leaf-, fruit- and flower-characters. All parts of *T. sericea* are used for medicinal purposes, except the flowers. In most cases dry plant material is used, and in this form it can be stored for long periods of time. Plants of all ages are used, and there seems not to be age preferences when collecting this plant for medicinal purposes. The leaves, roots or stem bark are powdered and made into hot water decoctions, mixed into maize porridge (Ugali) or made into tea for diarrhea, fever and hypertension (Table 7; I, Table 1). The used dose is usually one teaspoon of leaf powder in one cup of hot water for the tea. It is important to administer this plant in the correct dosage since it is claimed to be poisonous in large doses (Watt & Breyer-Brandwijk, 1962). Sometimes *T. sericea* can be mixed with another plant called “*Mololo*”.

Terminalia sericea is a well known plant in African traditional medicine. Our results of the uses of this species are in part in accordance with the uses reported by other authors. In most African countries the roots of *T. sericea* seem to be the most frequently used part, although the roots are considered to be poisonous by many traditional healers (Watt & Breyer-Brandwijk, 1962). In addition to the roots, also leaves and stem bark are used for medicinal purposes, although to a lesser extent than the roots. There is only one report on the uses of the fruits of *T. sericea* for medicinal purposes (Eldeen et al., 2005). The roots are considered antiseptic and are decocted or infused in water for oral treatment of diarrhea (Drummond & Coates-Palgrave, 1973), gonorrhoea (Hedberg et al., 1982) or used in mixtures with other medicinal plants such as *Carica papaya*, *Citrus limon*, *Parinari excelsa* for the treatment of venereal diseases (Neuwinger, 2000). Decoctions of the roots of *T. sericea* are also used topically for the treatment of certain eye-diseases, such as trachoma, as well as for the treatment of skin diseases (Neuwinger, 2000). The vapour of a hot water decoction of the outer layer of the roots is inhaled for the treatment of pneumonia (Neuwinger, 1996). Leaf infusions are used for the treatment of diarrhea as well as for the treatment of “chest complaints” (pneumonia?) (Neuwinger, 2000). Dressings of the leaves and stem bark of *T. sericea* are used for the treatment of wounds (Neuwinger, 2000).

Terminalia kaiserana is often used as a medicinal plant in the villages in Mbeya we visited. The use of it has, however declined, and a reason might be that it is extensively cut for fuel wood. Many of the traditional healers also told us that it is easily confused with other species of *Terminalia*, and they try to distinguish it from the other species with the aid of leaf and fruit characters. Both roots, leaves and stem bark of *T. kaiserana* are dried, grounded and made into hot water decoctions for the treatment of gonorrhoea, diarrhea and vomiting (Table 7; I, Table 1). Sometimes leaf powder can be added to tea or porridge for the same purposes. One teaspoon of dried plant material in a cup of hot water is the usual dose administered to the patients. The roots of *T. kaiserana* can also be used together with *C. molle* for the treatment of diabetes. The treatment of patients with *T. kaiserana* is sometimes connected with some spiritual prayers. Some of the medicinal uses of *Terminalia kaiserana* reported by previous authors are in accordance with those reported by us. According to Kokwaro (1976) and Haerdi (1964) the roots of *T. kaiserana* are used for treatment of gonorrhoea in the same way as the traditional healers in Mbeya, Tanzania use them. In addition the roots of *T. kaiserana* are used for treatment of bilharzia and as diuretics (Haerdi, 1964; Kokwaro, 1976). Sometimes root decoctions of *T. kaiserana* are mixed together with *Dalbergia nyassae* and *Bridelia cathartica* for the topical treatment of kidney pains. The fresh leaf sap and root decoctions are used for the treatment of schistosomiasis (bilharzia), scabies, cough and cardiac problems (Haerdi, 1964).

Table 7. Results from our investigation on uses of *Combretum*-, *Terminalia* and *Pteleopsis*-species (Combretaceae) in traditional medicine in some villages in the Mbeya region, Tanzania and medicinal uses reported by other authors.

Species	Vernacular name	Traditional use in Tanzania/our results	Preparation of plant for medicine/our results	Traditional use reported in literature
<i>Combretum</i> species				
<i>C. collinum</i> Fresen.	Mlama	No use reported in Mbeya region		Malaria (Haerdi, 1964); diarrhea, excessive menstrual bleeding, rectal prolapse (Hedberg et al., 1982); dysentery, snake bites (Kokwaro, 1976); "madi", a blood disease (Watt & Breyer-Brandwijk, 1962); gastroenteritis (Adjanouhoun et al., 1986).
<i>C. fragrans</i> F. Hoffm.	Hansebwe	For treatment of diarrhea	1) Decoctions: roots are ground into a powder and mixed with hot water, brought to boil, filtered and decoction is drunk 2) Mixed into porridge or made into tea: root powder	Coughs, syphilis (Kokwaro, 1976); aphrodisiac (Gelfland et al., 1985); chronic wounds (Adjanouhoun et al., 1986); leprosy (Chhabra et al., 1989); snakebites (Neuwinger, 2000);
<i>C. hereroense</i> Schinz.	Mlama	No use reported in Mbeya region		Cardiac problems, heartburn, venereal diseases, body pain, chest problems, coughs, stomach problems, headache, schistosomiasis (Watt & Breyer-Brandwijk, 1962); headache (Gelfland et al., 1985); schistosomiasis (Kokwaro, 1976).

Table 7. Continued.

<p><i>C. molle</i> G. Don.</p>	<p>Mlama, Mpula, Kapula</p>	<p>To treat gonorrhea, syphilis, influenza, oedema, skin diseases, diarrhea and wounds</p>	<p>In mixtures with <i>Combretum psidioides</i> and <i>Terminalia kaiserana</i>. Mostly dried for use, but use of fresh material was also reported.</p> <p>1) Added to maize porridge, Ugali: powder of dried roots, stem bark or leaves in mixture with plant called “Muwofi” (gonorrhea, syphilis).</p> <p>2) Baths in extracts: leaf extracts for bacterial infections, diarrhea, venereal diseases.</p> <p>3) Hot water decoctions of dried/fresh roots and leaves put in maize porridge: gonorrhea, syphilis, influenza, severe cold, oedema, malnutrition in children.</p> <p>4) Topically for treatment of wounds/diseases of the skin: roots mixed with sheep fat to form a paste smeared on the skin.</p>	<p>Wound dressing, stomach problems, snake bites, aid in child birth (Watt & Breyer-Brandwijk, 1962); abortions, antidiarrheic (Haerdi, 1964); wounds from poisoned arrows (Haerdi, 1964); wounds (Drummond & Coates-Palgrave, 1973); hookworm, stomach pains, snake bite, leprosy, fever, dysentery, general body swellings, chest complaints, abortifacient, wounds (Kokwaro, 1976); angina, dropsy (Kerharo, 1974); expectorant, syphilis, snakebites (Hedberg et al., 1982); swelling of abdomen, abortion, constipation, sterility (Chhabra et al., 1989); stomach problems, snake bites, wounds, dysentery, galactagogue (Neuwinger, 2000)</p>
<p><i>C. psidioides</i> Welw.</p>	<p>Mlama zambila</p>	<p>To treat diarrhea, muscle pain and oedema</p>	<p>Used either alone or in combination with <i>Combretum molle</i> and <i>C. zeyheri</i></p> <p>1) Hot water decoctions of roots for diarrhea and muscle pain and of leaves for oedema</p> <p>2) Extracts of leaves mixed with hot water in tea cup for oedema</p> <p>3) Mixed with porridge, Ugali: leaves, either fresh or dried to treat oedema.</p> <p>4) In combination with <i>C. molle</i> and <i>C. zeyheri</i> for chest problems, pains in the spinal cord and oedema.</p>	<p>Aphrodisiac, diarrhea, malaria, rheumatic back pain (Haerdi, 1964); galactagogue (Gilges, 1985).</p>

Table 7. Continued.

<i>C. zeyheri</i> Sond.	Mlama, Kakati (“always used”)	To treat diarrhea and cancer	<p>1) Hot water decoctions: powdered, dried roots/leaves decocted, one teaspoon of decoction mixed in a cup of hot water. Daily administration for stomach tumors as well as diarrhea.</p> <p>2) Mixed into maize porridge (Ugali): dried roots/leaves mixed in porridge for tumors and diarrhea.</p> <p>3) Sometimes mixed with other species of <i>Combretum</i> for the treatment of diarrhea.</p>	Scorpion bites (Watt & Breyer-Brandwijk, 1962); coughs, colic, smallpox (Drummond & Coates-Palgrave, 1973); schistosomiasis, dry wounds, rheumatism, back pain, bloody diarrhea, coughs (Kokwaro, 1976); diarrhea, vomiting (Hedberg et al., 1982); bloody diarrhea (Gelfland et al., 1985); rheumatism, joint pains, eye inflammation, conjunctivitis (Kremnitz et al., 1988); treatment of back pain, malaria (Van Koenen, 1996); schistosomiasis (Neuwinger, 2000)
<i>Terminalia</i> spp.				
<i>T. glaucescens</i> Benth.	Mpulu	No use reported in Mbeya region		Burns, headache, stomach problems (Bouquet & Debray, 1974); AIDS related fungal infections (Koudou et al., 1995); fungal infections of skin (Batawila et al., 2005); coughs, wounds, dental care (Neuwinger, 2002).
<i>T. kaiserana</i> F. Hoffm.	Mpululu, Bena	To treat gonorrhea, diarrhea, vomiting Together with <i>C. molle</i> for diabetes	<p>1) Hot water decoctions: dried roots, leaves and stem bark decocted for gonorrhea, diarrhea and vomiting</p> <p>2) Added to tea or porridge: leaf powder in tea or porridge for gonorrhea, diarrhea and vomiting. One teaspoon of dried plant material in a cup of hot water for the tea.</p> <p>- the roots of <i>T. kaiserana</i> can also be used together with <i>C. molle</i> for diabetes.</p>	Gonorrhea, bilharzia schistosomiasis, diuretics, aphrodisiac, headache, back and kidney pain, scabies, cardiac problems, cough (Haerdi, 1964; Chhabra et al., 1989)

Table 7. Continued.

<i>T. sambesiaca</i> Engl. & Diels.		No use reported		Bloody diarrhea, cancer, gastric ulcers, appendicitis (Chhabra et al., 1989).
<i>T. sericea</i> Burch ex. DC.	Namatipo, Mpululu	To treat diarrhea, fever, hypertension, bacterial infections	<p>1) Hot water decoctions: leaves, roots or stem bark powdered and decocted for diarrhea, fever and hypertension.</p> <p>2) Mixed into maize porridge (Ugali): leaves, roots or stem bark powdered and mixed with Ugali for diarrhea, fever and hypertension</p> <p>3) As a tea (infusion): one teaspoon dried powder of leaves, root or stem bark in cup of hot water for diarrhea, fever, hypertension</p> <p>- Sometimes mixed with another plant called <i>Mololo</i> for same purposes as above</p>	Diarrhea, dysentery, colic, diabetes (Watt & Breyer-Brandwijk, 1962); diarrhea, colic, pneumonia, eyewash for certain diseases in the eyes (Drummond & Coates-Palgrave, 1973); bilharzia (Kokwaro, 1976); gonorrhea (Hedberg et al., 1982); diarrhea, skin diseases, cough, general body weakness, pneumonia, venereal diseases (Neuwinger, 2000)

5.3. Antibacterial effects of extracts and fractions of *Combretum* and *Terminalia* spp. (I)

Since many of the species of *Combretum* and *Terminalia* included in this thesis work, are used for the treatment of infectious diseases in African traditional medicine the *in vitro* antibacterial effects of these species was studied.

A hole-plate agar diffusion method (Barry & Thornsberry, 1991; Ojala et al., 2002; Rauha et al. 2002) was applied to study the antibacterial effects of 21 extracts of six *Combretum* species and five species of *Terminalia*, collected from Tanzania. In this study seven bacteria, two gram-negative and five gram-positive species were used. All the species used were non-invasive, but as opportunists they are able to cause infections. Of the gram-positive bacteria used, *Staphylococcus aureus* is known to cause serious diseases such as pneumonia, meningitis, endocarditis and septicemia in immunocompromised hospital patients (http://www.en.wikipedia.org/wiki/Staphylococcus_aureus). The resistance of *S. aureus* to many commonly used antibiotics as well as to methicillin and vancomycin (VRSA) poses serious problems. Although serious infections caused by *S. aureus* are rare in otherwise healthy people, there have been some reports of strains of methicillin-resistant *S. aureus* (MRSA) isolated from children in the community (Chambers, 2001). *Staphylococcus aureus* is in addition a common cause of food-poisoning (Madigan et al., 2000). *Staphylococcus epidermidis* is responsible for a growing number of infections among hospital patients with weakened immune system. Infections with *S. epidermidis* often start at skin wounds caused by catheters. *Micrococcus luteus* is part of the normal flora of the skin, but seems to be an emerging nosocomial pathogen in immunocompromised patients. Among the gram-negative bacteria in our study, *Enterobacter aerogenes* is frequently isolated from nosocomial infections. In hospitalized intensive care unit patients *Enterobacter aerogenes* is known to cause bacteremia, lower respiratory tract infections, skin and soft tissue infections, urinary tract infections and endocarditis. *Enterobacter* species possess endotoxin which is known to play a major role in the pathophysiology of sepsis and its complications. Multidrug-resistant *Enterobacter aerogenes* (MREA) has gradually emerged as an important nosocomial pathogen in Belgium (Ronveux et al., 1999). Uropathogenic *Escherichia coli* causes 90 % of the urinary tract infections (UTI), and is also an important causative of travelers diarrhea as well as dysentery-like diarrhea (enteroinvasive *E. coli*, EIEC) (<http://www.textbookofbacteriology.net/e.coli.html>).

The results on the antibacterial effects of 21 extracts of six species of *Combretum* and four species of *Terminalia* showed that the species of *Terminalia* were in general more antimicrobial than the *Combretum* species (I, Table 2). Most of the species screened gave substantial antimicrobial effects (at 50 mg/ml), which is in accordance with the extensive use of various

species of *Combretum* and *Terminalia* in traditional medicine (Table 1, Table 2, Table 7, Table 9). The extracts of the roots of *Combretum* and *Terminalia* species generally gave the best antibacterial effects, although extracts of stem bark were also effective. Leaf extracts were less active than the other parts of the plants. Interestingly, none of the extracts, with the exception of a methanolic leaf extract of *T. kaiserana*, were active against *Escherichia coli* (I, Table 2), although Eloff (1999) reported good effects of many African species of *Terminalia* and *Combretum* against *E. coli*. Eloff (1999) used a more sensitive microplate dilution method, whereas our results were obtained with agar diffusion, which might explain this discrepancy. Eloff (1999) also used a different strain of *E. coli*, ATCC 27853, which might be more sensitive to extracts of Combretaceae than ATCC 8739 used in our investigation. All the species and extracts of *Terminalia* as well as those of *Combretum* were active against *Enterobacter aerogenes*, the other gram-negative bacterial species in our investigation. Taylor et al. (1996) did not find any antibacterial activity against *E. aerogenes*, when they screened an Indian species of *Terminalia*, *T. alata*, for its antibacterial potential. They, however, used ten times less extract than we, which might explain the result. Also, *T. alata* being an Asian species of *Terminalia*, might differ much in its chemical composition from the African species of *Terminalia*.

In the following the antibacterial effects of some of the most potent species of *Combretum*, *Terminalia* and *Pteleopsis*, collected from Tanzania will be summarized.

5.3.1. Results from the antibacterial screening of species of *Terminalia* collected from Tanzania

The results of the antibacterial potential of the most effective species of *Terminalia* in our investigation in relation to their uses in traditional medicine in Mbeya, Tanzania are summarized in Table 9.

Terminalia sambesiaca

Of the 21 crude extracts of *Combretum* and *Terminalia*, screened for their antibacterial effects, the most outstanding effects were obtained with a root extract of *Terminalia sambesiaca*, which gave the widest zones of inhibition of all the extracts investigated and showed excellent antibacterial activities both against *Enterobacter aerogenes* and all the gram-positive bacteria (I, Table 2). This plant gave, however, similarly to most of the other species of *Terminalia* and *Combretum* in our investigation, no effects against the gram-negative *Escherichia coli*. The methanolic root extract of *T. sambesiaca* showed bactericidal effects against the gram-negative *Enterobacter aerogenes* and gave a very low MIC value of 0.9 mg/ml against this bacterial species (I, Table 3). The root extract of *T. sambesiaca* was effective also against the gram-positive *Staphylococcus aureus* (MIC 1.8 mg/ml), *S. epidermidis* (MIC 1.5 mg/ml), *Micrococcus*

luteus (MIC 1.0 mg/ml) and *Sarcina* sp. (MIC 1.2 mg/ml), but the effects against these bacteria were bacteriostatic (I, Table 3). Six fractions of the root extract of *T. sambesiaca*, obtained by medium pressure liquid chromatography (MPLC) on reverse phase silica gel were also investigated for their antibacterial effects, and the crude extract was found to be less active than the polar fractions and as active as the less polar fractions. The polar fraction RF2 was most active of the fractions, although fractions RF1-RF4 were very similar to their antibacterial activities (unpublished results, Table 8). The more non-polar fractions (RF5-RF6) were not as active as RF1-RF4, although they also gave antibacterial effects. It thus seems that the root extract of *T. sambesiaca* contains several antimicrobial compounds of different polarity. This is in accordance with the finding of Martini & Eloff (1997) and Eloff (1999) that African species of *Combretum* and *Terminalia* contain a wide variety of different antibacterial compounds of different polarity. Similarly to us, also Tshikalange et al. (2005) and Moshi & Mbwambo (2005) found that the intermediate and polar extracts of the roots of *Terminalia* are antibacterially more active than less polar fractions (these authors used *T. sericea* for their investigations). Since hot water decoctions are one of the ways in which *T. sambesiaca* is prepared for medicine in Tanzania (Chhabra et al., 1989) this way of preparation would thus extract the highly antimicrobial polar compounds from *T. sambesiaca*. The antibacterial effects of the root extract of *T. sambesiaca* might be due both to polar compounds, such as hydrolysable ellagitannins which have been found in large quantities from many species of *Terminalia* (Burapadaja & Bunchoo, 1995; Baba-Moussa et al., 1999; Silva et al., 2000; Conrad et al., 2001; Silva et al., 2002) and saponins (Masoko et al., 2005), as well as more nonpolar compounds, such as flavonoids (Srivastava et al., 2001; Elegami et al., 2002; Silva et al., 2002), lignans (Valsaraj et al., 1997) and triterpenoids (Bombardelli, 1974 and 1976; Conrad et al., 1998; Srivastava et al., 2001; Atta-ur-Rahman, 2002) which are also known from many species of *Terminalia*. Also a methanolic extract of the stem bark of *T. sambesiaca* showed excellent antibacterial effects, although the zones of inhibition were smaller than those shown by the root extract (I, Table 2). The MIC value of the extract of the stem bark was also slightly higher than those shown by the extract of the roots against *Sarcina* sp. (I, Table 3). To the best of our knowledge there is only one earlier investigation on the antibacterial effects of *T. sambesiaca* (Chhabra et al., 1989) and according to this investigation an extract of the stem bark of *T. sambesiaca* gave antibacterial effects against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Shigella boydii*, but the active compounds were not identified. Masoko et al. (2005) and Masoko & Eloff (2005) found that leaf extracts of *T. sambesiaca* are antifungal, but they did not include bacterial species in their screening. In Tanzania powdered root bark of *T. sambesiaca* is mixed with porridge for the treatment of bloody diarrhea and the stem bark and leaves are decocted for the treatment of stomach ulcers and appendicitis (Chhabra et al., 1989). Our results on the antibacterial effects of *T. sambesiaca* are thus justified both by the uses of this plant in traditional medicine and by the good antibacterial results obtained by Chhabra et al. (1989). Our results show that it would be worth to investigate *T. sambesiaca* in depth for its antimicrobial potential

against a panel of bacterial species known to cause serious infections, and to isolate the active compounds responsible for the activity.

Table 8. Antibacterial effects of a crude extract (50 mg/ml) and fractions RF1-RF6 (20 mg/ml) isolated from a root extract of *Terminalia sambesiaca*. Average diameters of the zones of inhibition in mm (n = 4–6) ± S.E.M. Streptomycin and ampicillin (10 mg/ml) were used as positive controls.

Crude extract and fractions	<i>Enterobacter aerogenes</i>	<i>Micrococcus luteus</i>	<i>Staphylococcus aureus</i>
Extract	36.10 ± 0.53	39.30 ± 0.28	33.60 ± 1.26
RF 1	33.35 ± 0.30	35.68 ± 0.55	30.53 ± 0.06
RF 2	36.25 ± 0.60	37.53 ± 0.77	34.28 ± 1.36
RF 3	33.48 ± 0.19	29.13 ± 3.65	31.41 ± 0.18
RF 4	31.33 ± 0.09	32.23 ± 0.14	23.30 ± 0.41
RF 5	21.50 ± 1.46	18.45 ± 0.04	*
RF 6	18.30 ± 0.54	*	*
Ampicillin	51.00 ± 1.06	70.54 ± 1.78	60.80 ± 0.07
Streptomycin	44.10 ± 1.03	48.30 ± 0.45	41.25 ± 0.25

* not investigated

Terminalia sericea

Extracts of the roots of *Terminalia sericea* were nearly as effective as the root extract of *T. sambesiaca* against the bacteria used in our investigation (I, Table 2) and gave as low MIC values as the root extract of *T. sambesiaca* against *Micrococcus luteus* (1.0 mg/ml) and slightly higher values than the root extract of *T. sambesiaca* against *Enterobacter aerogenes* (1.5 mg/ml), *Staphylococcus aureus* (5.3 mg/ml) and *Staphylococcus epidermidis* (1.8 mg/ml) (I, Table 3). The MIC values we have found for the root extract of *T. sericea* correspond to the values given by Eloff (1999) for leaf extracts, although they report a lower MIC for *S. aureus*, 3.0 mg/ml, compared to our 5.3 mg/ml. The leaves and roots might differ to their chemical composition and quantity of compounds, and thus give totally different MIC values. We compared the antibacterial potential of a hot water decoction, a methanolic, acetone and ethanol extract of the roots to each other and found that they did not differ significantly from each other (I, Table 2). A chloroform extract obtained by liquid-liquid extraction of a methanol extract of *T. sericea*, which gave a yellowish, oily substance, was *completely devoid* of antibacterial activity in accordance with the findings of Tshikalange et al. (2005) who also found that this solvent did not extract any antibacterial compounds from the roots of *T. sericea*, at least against the bacteria they used in their investigation. The hot water decoction of the roots of *T. sericea* was as antibacterial as the methanol, ethanol and acetone extracts (I, Table 2), and this justifies the use of hot water decoctions of the roots of *T. sericea* in Mbeya, Tanzania and in many other African countries for the treatment of diarrhea, wounds, pneumonia, venereal, eye and skin diseases (Table 7; Table 9;

I, Table 1; **II**, Table 1; Drummond & Coates-Palgrave, 1973; Hedberg et al., 1982; Neuwinger, 2000), since the antibacterial compounds seem to be extracted in hot water as well. In accordance with our results also Tshikalange et al. (2005) found good antibacterial effects of hot water decoctions of the roots of *T. sericea* against gram-positive bacteria and also that this extract did not affect the growth of *E. coli*. On the contrary, Moshi & Mbwambo (2005) have found that root extracts of *T. sericea* are active against *E. coli*. The differences in these results might be due to the method of screening used, since we have used agar diffusion and Moshi & Mbwambo (2005) used a micro plate dilution method which is more sensitive in detecting antimicrobial activity. Similarly to our findings, both Tshikalange et al. (2005) and Moshi & Mbwambo (2005) found that intermediate and polar extracts of the roots of *T. sericea* are the antibacterially most active ones. The lignan, Anolignan B, isolated from the roots of *T. sericea* has been found to be antibacterial against both gram-negative and gram-positive bacteria and gave a MIC value of 3.8 µg/ml against *Bacillus subtilis* (Eldeen et al., 2005). Thus, part of the excellent antimicrobial activity we have seen for this extract might be due to this lignan. The triterpenoids sericoside and arjunglucoside have been isolated from the roots of *T. sericea* (Bombardelli, 1974 and 1986) but the antibacterial effects of these compounds have not been elucidated, to the best of our knowledge. The root is considered poisonous among traditional healers in Africa, and thus the plant is often administered in careful dosage (Watt & Breyer-Brandwijk, 1962). We found that methanolic extracts of the leaves of *T. sericea* were not as antimicrobial as those of the roots, although they also gave good antibacterial effects in accordance to the investigation of Eloff (1999), and especially good effects against *Enterobacter aerogenes* and *Micrococcus luteus* (**I**, Table 2). These results justify the use of leaf infusions for pneumonia and dressings of leaves for the treatment of wounds in African traditional medicine (Neuwinger, 2000) as well as leaves, roots and stem bark for diarrhea and bacterial infections in Tanzanian traditional medicine (**I**, Table 1; Table 7).

Terminalia kaiserana

We found that methanolic root extracts of *T. kaiserana* were as effective inhibitors of bacterial growth as the root extracts of *T. sambesiaca* and *T. sericea* (**I**, Table 2). This is, to our knowledge, the first time crude extracts of *T. kaiserana* are reported to possess excellent antibacterial activities. The leaf extract was less active than the extract of the roots, but was the only one of the plant extracts used in this investigation to inhibit the growth of *E. coli*, and the effect was found to be bactericidal. The excellent antibacterial activity of the root extract justifies the use of *T. kaiserana* in African traditional medicine for the treatment of diarrhea and gonorrhoea (**I**, Table 1; Table 7; Table 9; Haerdi, 1964; Kokwaro, 1976).

5.3.2. Antibacterial effects of the species of *Combretum* collected from Tanzania

The *Combretum* species showed in general less antibacterial activity than the species of *Terminalia*, although most of the species in our investigation were antibacterial to some extent. Three species of *Combretum*, *C. fragrans*, *C. padoides* and *C. molle* gave excellent antibacterial effects (I, Table 2), the results being summarized in Table 9 in relation to the uses of these plants in traditional medicine in Mbeya, Tanzania.

Combretum padoides

Methanolic extracts of the roots and the stem bark of *C. padoides* gave similar results against most of the bacterial species studied, and the best effects were obtained against *Staphylococcus aureus* and *Enterobacter aerogenes* (I, Table 2). This implies that the mechanism of action of the active compounds in these extracts are not very specific, but more general in nature, since it shows activity against both gram-positive and gram-negative bacteria. In accordance to us, Eloff (1999) found that leaf extracts of *C. padoides* are powerful inhibitors of the growth of *Enterobacter*, although they used another bacterial species, *E. faecalis* (MIC = 0.8 mg/ml). Eloff (1999) also found that a leaf extract of *C. padoides* inhibited the growth of *E. coli* (MIC = 0.8 mg/ml) whereas we found no effect of *C. padoides* on this bacterial species. This might be due to use of extracts from different parts of the plant, since we used roots and stem bark, whereas Eloff (1999) used leaves. The compounds responsible for the antibacterial activity of *C. padoides* are unknown. Mono- and bi-desmosidic triterpenoids (Rogers, 1989) and saponins (Rogers and Carr, 1987) have been isolated from the leaves of *C. padoides*, but as far as we know the antibacterial effects of these compounds have not been investigated.

Combretum fragrans

Extracts of the roots of *C. fragrans* gave excellent antibacterial activities against *Enterobacter aerogenes* and *Micrococcus luteus*. Leaf extracts were less active, but still gave very good effects against *Micrococcus luteus* (I, Table 2). To the best of our knowledge, this is the first time *C. fragrans* is reported to give good antibacterial effects. This species have been evaluated for antifungal effects by Batawila et al. (2005) and was found to possess excellent antifungal activity against different species of *Candida*, including *C. albicans*, and the leaf extract was found to contain antifungally active tannins, flavonoids and saponins. Rogers & Carr (1987) have isolated triterpenoids from the leaves of *C. fragrans*, and they might, in part, be responsible for the good antibacterial effects we observed. The good antibacterial effects we observed for *C. fragrans* are in agreement with the use of this species for treatment of bacterial infections, such as syphilis, leprosy and diarrhea, as well as for the treatment of incurable wounds in African traditional medicine (Kokwaro, 1976; Chhabra et al., 1989; Fyhrquist et al., 2002; Batawila et al., 2005).

The use of *C. fragrans* for the treatment of diarrhea in Tanzanian traditional medicine (**I**, Table 1; Table 7) is supported by the good activity this species showed against *S. aureus*, a common causative agent of food poisoning.

Combretum molle

A leaf extract of *Combretum molle* gave excellent antibacterial effects against *Micrococcus luteus* and *Enterobacter aerogenes* (**I**, Table 2). This is in accordance with the traditional use of leaf extracts and decoctions of this species for bacterial infections, diarrhea and venereal diseases in Mbeya, Tanzania (Table 7; **I** Table 1).

Combretum zeyheri, *C. psidioides* and *C. hereroense* gave also substantial activity against the investigated bacterial species. Of these species, *C. zeyheri* and *C. hereroense* have been screened for their antibacterial effects before by Eloff (1999). *C. psidioides* has to our knowledge not been screened for its antibacterial effects earlier. The compounds responsible for the antimicrobial effects of *C. hereroense* are unknown. Three compounds with activity against *S. aureus* have been isolated from the stem bark of *C. zeyheri* (Breytenbach & Malan, 1989).

Table 9. Summary of the antibacterial effects of the most effective extracts of *Combretum* and *Terminalia* in our screening and correlation to ethnomedical use in Mbeya. Extracts are placed in activity order. * not investigated.

Species and extract	Results in screening	MIC values	Traditional use in Mbeya
<i>Terminalia sambesiaca</i> , roots	The most antibacterial extract in this screening. Good activities against <i>Micrococcus luteus</i> , <i>Sarcina</i> sp., <i>S. aureus</i> and <i>E. aerogenes</i>	<i>Enterobacter aerogenes</i> , 0.9 mg/ml <i>Micrococcus luteus</i> , 1.0 mg/ml <i>Sarcina</i> sp., 1.2 mg/ml <i>Staphylococcus epidermidis</i> , 1.5 mg/ml <i>Staphylococcus aureus</i> , 1.8 mg/ml	No uses reported in Mbeya
<i>T. sambesiaca</i> , stem bark	Good activities against <i>Micrococcus luteus</i> , <i>Sarcina</i> sp., <i>S. aureus</i> and <i>E. aerogenes</i>	<i>Sarcina</i> sp., 4.3 mg/ml	No use reported in Mbeya
<i>T. sericea</i> , roots	Especially active against <i>Micrococcus luteus</i> , <i>Sarcina</i> sp., <i>Staphylococcus aureus</i> and <i>Enterobacter aerogenes</i>	<i>Micrococcus luteus</i> , 1.0 mg/ml <i>Enterobacter aerogenes</i> , 1.5 mg/ml <i>Staphylococcus epidermidis</i> , 1.8 mg/ml <i>Staphylococcus aureus</i> , 5.3 mg/ml	For diarrhea, hypertension, fever and bacterial infections
<i>T. kaiserana</i> , roots	Good activities against <i>S. aureus</i> , <i>Enterobacter aerogenes</i> , <i>Micrococcus luteus</i> and <i>Sarcina</i> sp. In general stronger antimicrobial effects than leaf extract.	<i>Sarcina</i> sp., 4.3 mg/ml <i>Staphylococcus epidermidis</i> , 4.7 mg/ml	For gonorrhoea, diarrhea and vomiting
<i>T. kaiserana</i> , leaves	The only extract inhibiting growth of <i>E. coli</i> . Good activities also against <i>Staphylococcus aureus</i> , <i>Micrococcus luteus</i> and <i>Sarcina</i> sp.	*	For gonorrhoea, diarrhea and vomiting
<i>T. sericea</i> , leaves	Good antimicrobial effects against <i>Micrococcus luteus</i> and <i>Enterobacter aerogenes</i>	*	For diarrhea, hypertension, fever and bacterial infections
<i>Combretum fragrans</i> , roots	Excellent antimicrobial activity against <i>Micrococcus luteus</i> and <i>Enterobacter aerogenes</i> .	<i>Sarcina</i> sp., 7.3 mg/ml	For diarrhea
<i>C. fragrans</i> , leaves	Excellent activity against <i>Micrococcus luteus</i>	*	No uses reported in Mbeya
<i>C. padoides</i> , roots	Good activities against <i>Staphylococcus aureus</i> and <i>Enterobacter aerogenes</i> .	*	No uses reported in Mbeya
<i>C. padoides</i> , stem bark	Good activities against <i>Staphylococcus aureus</i> and <i>Enterobacter aerogenes</i> .	*	No uses reported in Mbeya
<i>C. molle</i> , leaves	Excellent effects against <i>Micrococcus luteus</i> and <i>Enterobacter aerogenes</i>	*	To treat gonorrhoea, syphilis, influenza, oedema, skin diseases, diarrhea and wounds

5.4. Antifungal effects of extracts and fractions of *Combretum*, *Terminalia* and *Pteleopsis* spp. (II)

The incidence of opportunistic fungal infections in patients treated with immunosuppressive drugs, intensive chemotherapy, suffering from AIDS and neonates is increasing at an alarming rate (Denning et al., 1997). Mycotic infections are very difficult to eradicate and they constitute an enormous challenge for health care providers. The fungi, like their hosts, are eukaryotic organisms, making it difficult to choose intracellular targets whose inhibition would not also be deleterious to the host cells (Di Domenico, 1999). Although an array of different treatments are used for fungal infections, none of them are ideal in terms of efficacy, safety or antifungal spectrum (Di Domenico, 1998; Ablordeppey et al., 1999). Many of the current drugs are very toxic (Amphotericin B), lead to the development of resistance (fluconazole and 5-flucytosine) or exhibit drug-drug interactions (azoles) (White et al., 1998). Potent antifungal drugs might be found from the plant kingdom. Some research projects have been going on to find plant derived antifungal compounds: In a screening of African and Panamian plants, Hostettman & Marston (1994) found antifungal activity in 15 % of the investigated plants of which the most promising species were used in traditional medicine. In the search of the underlying active principles of *Pelargonium* species, used for treatment of respiratory infections, antifungal hydrolysable tannins were identified (Kayser & Kolodziej, 1997; Latté & Kolodziej, 2000). Several methods have been used for the screenings of antifungal compounds in plants, the most common ones being agar diffusion, broth microdilution techniques and bioautography. Bioautography allows the combination of a bioassay and localization of the active compounds on the TLC plate employed for the assay (Hostettman & Marston, 1994).

We have investigated the antifungal effects of thirty-five extracts of five species of *Terminalia*, ten species of *Combretum* and *Pteleopsis myrtifolia* (II, Table 2) with the aid of a filter paper agar diffusion method, and the results in relation to the traditional medicinal uses of the plants in Mbeya are summarized in Table 10. Some of the plant species included in our investigation are used for the treatment of topical fungal infections and diarrhea as well as for other infectious diseases in African traditional medicine (II, Table 1; Table 7). Some of the plants in this screening have to our knowledge not been investigated for their antifungal effects before.

Five species of yeasts (*Candida*) and the yeastlike basidiomycet, *Cryptococcus neoformans*, most of them common opportunistic pathogens, were used as test organisms (II, Table 2). All the fungal species used in our screenings are usually harmless to healthy persons, but have the ability to cause opportunistic systemic infections in immunocompromized hosts, although also healthy persons can get topical infections on the skin and mucosal surfaces. *Candida* species are also known to cause subcutaneous infections in tropical parts of the world, usually introduced through wounds. *Candida albicans*, also part of the normal flora in humans (in 30- 50 %), is the most frequently encountered human fungal pathogen causing systemic fungaemias, although

physicians report the increasing occurrence of non-albicans (NAC) *Candida* species, such as *Candida parapsilosis*, *C. tropicalis*, *C. krusei* and *C. glabrata*, during the last two decades, some of them exhibiting natural resistance to antifungal drugs (Sobel, 1998; Viscoli et al., 1999; Krcmery & Barnes, 2002). Reports within the last years have indicated the rapid development of fluconazole and amphotericin B resistance in *Candida tropicalis* (Krcmery & Barnes, 2002). *Cryptococcus neoformans* is the only significant human pathogen amongst the basidiomycetous fungi and causes life-threatening meningoencephalitis in immunosuppressed patients, especially in those with HIV infection (Harrison, 2000). In Africa and South-East Asia, in particular, AIDS related cryptococcosis appears to be more common than it was in Europe and North America prior to combination antiretroviral therapy (Kayembe et al., 1994). Despite conventional treatment, infections with *Cryptococcus neoformans* var. *gattii* strain are associated with significant mortality (Seaton et al., 1996), and not infrequently leads to blindness in survivors (Harrison, 2000).

5.4.1. Antifungal effects of the species of *Terminalia* collected from Tanzania

We found that the species of *Terminalia* in general gave stronger antifungal effects than the *Combretum* species and *Pteleopsis myrtifolia* used in this study, although also some of the species of *Combretum* gave good antifungal effects (II, Table 2). There was a large variation in activity between different species of *Terminalia* and *Combretum*, as well as between different extracts made from different plant parts (roots, leaves, stem bark and fruits). Some species/extracts did not show any antifungal activities compared to others with strong activity. The strongest antifungal effects of all the species used in our screenings were shown by *T. sambesiaca*, *T. sericea* and *T. kaiserana* (II, Table 2; Table 10) which also gave the best antibacterial effects (See previous chapter, Table 9; I, Table 2). In the following the species with the strongest antifungal activities are presented.

Terminalia sambesiaca

A root extract of *T. sambesiaca* (50 mg/ml) gave the strongest antifungal effects of all the investigated extracts and species of *Terminalia* and was especially active against *Candida glabrata* (MIC = 3.13 mg/ml) and *Cryptococcus neoformans* (MIC = 1.56 mg/ml) (II, Table 3). The root extract gave an antifungal activity comparable to the positive controls, itraconazol and amphotericin B used in our screening. Another research group has recently screened *T. sambesiaca* for antifungal effects (Masoko et al., 2005; Masoko & Eloff, 2005), and in agreement with our results they found that this species possesses potent antifungal activity against *Candida albicans* and *Cryptococcus neoformans*, with average MIC values ranging from 0.232 – 0.64 mg/ml, although they used leaf material and not roots and stem bark. We did not detect any

antifungal activity of a leaf extract of *T. sambesiaca* against *Cryptococcus neoformans* (II, Table 2) but this might be due to the use of different methods, Masoko et al. (2005) using a microdilution assay. Masoko & Eloff (2005) found that the antifungal activity of the leaves of *T. sambesiaca* was concentrated to nonpolar fractions, such as hexane and dichloromethane, whereas we found that the most polar fractions (RF 1 and RF 2) isolated from a methanolic root extract of *T. sambesiaca* with column chromatography were the antifungally most active ones (II, Table 2). It seems that the roots and leaves of *T. sambesiaca* contain different kinds of antifungal compounds, and that this species contains a large spectrum of antifungal compounds differing from each other in their polarities. Carpano et al. (2003) studied the antifungal effects of the aerial parts of the South-American species *Terminalia australis* and found, similarly to us, that the methanol and aqueous extracts gave the strongest antifungal activities against *Candida albicans* and *C. krusei*, whereas a nonpolar dichloromethane extract was inactive. Hydrolysable tannins might be present in higher concentrations in more polar extracts. Hydrolysable tannins have been isolated from both Asian and African species of *Terminalia* (Burapadaja & Bunchoo, 1995; Silva et al., 1997; Silva et al., 2000; Lin et al., 2001), and have been found to be especially potent against *Cryptococcus neoformans*, showing MIC values between 0.02 and 0.1 μM , corresponding to 16-125 $\mu\text{g/ml}$ (Latté & Kolodziej, 2000). Corilagin, also known from some *Terminalia* species (Burapadaja & Bunchoo, 1995), was found to be more active than the antibiotics sertaconazol and amphotericin B against *C. glabrata*, and some hydrolysable tannins were more potent against non-albicans species of *Candida* than against *C. albicans* (Latté & Kolodziej, 2000).

The strong antifungal activity shown by *Terminalia sambesiaca* in our investigation shows that this plant has antimicrobial potential and this is in agreement with the traditional use of this plant in Africa where the root bark is mixed with porridge for the treatment of bloody diarrhea (Chhabra et al., 1989). The way of preparing this plant into water decoctions for traditional medicine (Chhabra et al., 1989) might lead to the extraction of antifungally active water soluble compounds, since we detected strong antifungal activity for polar fractions obtained from a methanolic extract of the roots of *T. sambesiaca*. Our results indicate that this species can be used for antifungal therapy in remotely situated villages in Africa, where conventional antibiotics are not available. The strong activity of this plant against *Cryptococcus neoformans* makes it a valuable medicinal plant in Africa, where a high proportion of AIDS-related cryptococcal infections occur (Kayembe et al., 1994).

Terminalia kaiserana

A root extract of *Terminalia kaiserana* gave the second best antifungal effects of the four species of *Terminalia* used in our screening (II, Table 2), and was the most effective extract to inhibit the growth of *Candida krusei* and was also effective against *C. glabrata* (MIC = 1.56 mg/ml) (II, Table 3). In addition the root extract of *T. kaiserana* was as effective as the root extract of *T. sambesiaca* against *Cryptococcus neoformans* (MIC = 1.56 mg/ml) (II, Table 3 and Table 2). A

leaf extract of *T. kaiserana* did, interestingly, not show any antifungal activity, at least against the species of yeast we used in this screening, although in a previous screening (I, Table 2) we found some activity against *C. albicans*. This might be explained by the fact that two different strains of *C. albicans* were used which might differ from each other in their sensitivity. Differences between different plant parts in antifungal and biological activity in general is well known and for example Julkunen-Tiitto (1989), who studied the differences in phenolic composition between plant organs of Finnish *Salix* species, found that different plant organs can differ significantly from each other in terms of phenolic compound composition and quantity. The same trend of the leaf extracts being less active than the extracts of the roots could be seen in *Terminalia sambesiaca* and *T. sericea* as well, but not in *T. stenostachya* in which the leaf extract gave good effects against most of the yeasts and was more effective than an extract of the stem bark (II, Table 2). The chemical composition of *T. kaiserana* is unknown, but probably also this species of *Terminalia* contains hydrolysable tannins, as well as flavonoids and saponins, which have been found from many species of *Terminalia* (Baba- Moussa et al., 1999; Silva et al., 2002). The antifungal potential of root extracts of *T. kaiserana* is so great that it would be worth to elucidate which compounds are responsible for these effects.

T. kaiserana is a well known plant among traditional healers in Africa (I, Table 1; II, Table 1; Chhabra et al., 1989; Kokwaro, 1976; Haerdi, 1964). The roots are the most used part of this species, and this is in accordance with our findings that root extracts are more effective than the leaves in inhibiting fungal growth (Table 10). Kokwaro (1976) and Haerdi (1964) report that the roots of *T. kaiserana* are used for treatment of gonorrhoea and bilharzia and the leaf sap for cough, and traditional healers in Mbeya use both leaves, roots and stem bark as hot water decoctions or mixed in porridge to treat diarrhoea, gonorrhoea and vomiting (I, Table 1, Table 7). These uses in traditional medicine agree with our results on the antifungal potential of *T. kaiserana* since diarrhoea can be a symptom of *Candida* infection. The excellent antifungal activity shown by *T. kaiserana* indicates that this species could have an additional use specifically for the treatment of fungal infections in African traditional medicine. To our knowledge we are the first to report on the excellent antifungal activity of this species of *Terminalia* and so far no other research group have included this species in biological screenings, although Eloff (1999) have screened the antibacterial effects of 27 species of South African members of Combretaceae.

Terminalia sericea

Terminalia sericea was the third species of *Terminalia* in our investigation to give excellent antifungal effects (II, Table 2). Methanolic root extracts of this species were especially active against *Candida glabrata* and gave the lowest MIC value of all the extracts used in this investigation against this species of yeast (0.78 mg/ml) (II, Table 3). When different kinds of extracts of the roots were compared for their antifungal effects we found, interestingly, that a hot water decoction gave the best results against *C. albicans*, the acetone extract being second most effective and ethanolic and methanolic extracts being less effective. The roots of *T. sericea* are

generally reported to be prepared into hot water decoctions for the treatment of stomach problems in East Africa (Drummond & Coates-Palgrave, 1973; Fyhrquist et al., 2002), and thus we have shown that this way of preparing the plant for medicine seems to extract many antifungal compounds. Acetone seems also to be a good solvent for extracting antifungal compounds from *T. sericea*, since this extract gave good effects against *C. albicans*. Also Eloff (1999) and Martini and Eloff (1997) have reported that acetone is a good extractant for antimicrobial compounds in *Terminalia* spp. and *Combretum* spp. The differences we found in the antifungal activity of methanol, ethanol and acetone extracts were, however, very small and the extracts gave nearly equal antifungal effects against *Candida glabrata* and *Cryptococcus neoformans*. Only a few other investigations have been performed on the antifungal effects of *T. sericea*; In their screening of the antifungal effects of six species of *Terminalia*, Masoko et al. (2005) and Masoko & Eloff (2005) found that *T. sericea* was the antifungally most effective species, and that the best effects were obtained with dichloromethane and hexane extracts of the leaves, although good effects were also obtained with acetone and methanol extracts against *Candida albicans* and *Cryptococcus neoformans*. When the MIC values of the six *Terminalia* species were compared, they were nearly similar, but *T. sericea* contained more extractable antifungal compounds/g plant material. It would, as Masoko et al. (2005) also point out, be interesting to know if it is the same compound which is active against different fungal species, and also to know whether the same antifungal compound is present in different species of *Terminalia*. Moshi & Mbwambo (2005) detected good antifungal activities in both methanol and butanol extracts of the roots of *T. sericea* but found less activity of an aqueous root extract against *Candida albicans*. Since both Masoko & Eloff (2005) and Moshi & Mbwambo (2005) have found that relatively non-polar extracts of *T. sericea* give good antifungal effects and we have found good activity of hot water decoctions as well as acetone extracts, the antifungal activity of this species might due to both tannins as well as less polar compounds. Resveratrol-3-O- β -D-rutinoside (Bombardelli et al., 1975) and the triterpenoids sericoside, arjunglucoside as well as the aglycone of sericoside have been isolated from the roots and stem of *T. sericea* (Bombardelli et al., 1974; Bombardelli et al., 1986; Rode et al., 2003). Resveratrol (Chan, 2002; Leiro et al., 2004) as well as triterpenoids (Abad et al., 2007) are known to possess antifungal potential, and thus some of the compounds mentioned above might be responsible for the excellent antifungal effects we have observed for *T. sericea*.

Our results on the antifungal effects of *Terminalia sericea* agree with the use of hot water decoctions of this plant as well as in tea and porridge for the treatment of diarrhea and stomach problems in African traditional medicine since these ailments might be due to fungal infections (Watt & Breyer-Brandwijk, 1962; II, Table 1; Table 7; Table 10).

5.4.2. Antifungal effects of species of *Combretum* collected from Tanzania

Ten species of *Combretum* were screened for their antifungal effects (II, Table 2). Some of the species, such as *C. constrictum* and *C. psidiodes*, have not been investigated for their antifungal effects before. Since some of the species of *Combretum* we screened are known to be used for the treatment of diseases and ailments related to fungal (microbial) infections in African traditional medicine (II, Table 1; Table 7) we expected to find at least some antifungally active species among the ten screened ones. Root extracts of *Combretum padoides* and of *C. molle* gave the most outstanding antifungal effects of all the investigated extracts, whereas some other extracts, such as a leaf extracts of *C. fragrans* and *C. molle* showed only slight or not any activity against the fungal species used in our investigation. The antifungal activity was not related to/restricted to any particular plant organ in *Combretum* spp. Rather, it seemed to be *species specific* whether to which organ would show the highest activity. In some cases extracts showed species specificity in their antifungal effects, such as a leaf extract of *C. apiculatum* which gave slight antifungal effects against *Cryptococcus neoformans* but no activity against the *Candida* species. This arouses a question to whether this extract acts with a more specific inhibitory mechanism against *C. neoformans*. The extracts of the fruits were generally relatively inactive compared to the other plant parts of the *Combretum* species, with an exception of a fruit extract of *C. psidiodes* which inhibited the growth of *Candida krusei*, but none of the other species of *Candida*. *Candida glabrata* and *C. krusei* were the most sensitive fungal species whereas *Cryptococcus neoformans* was more resistant to the extracts of *Combretum*. Although some of the extracts gave high inhibitory values against *C. glabrata*, there were altogether more extracts showing activity against *C. krusei*, but giving smaller inhibition zones against this fungal species. *Candida albicans* seemed to be the most resistant species of all the fungal species used in our study.

The *Combretum* species showing the most potential antifungal effects in our screenings are presented below.

Combretum padoides

The most outstanding antifungal effects were observed with a root extract of *C. padoides* which gave fungicidal inhibition zones against *Candida glabrata* (MIC 6.25 mg/ml) and excellent inhibitory effects also against *C. parapsilosis* and *Cryptococcus neoformans* (MIC 6.25 mg/ml) (II, Table 2 and Table 3). This species of *Combretum* is used for treatment of snakebites and hookworms (Neuwinger, 2000), but not for ailments caused by microbial infections, and thus it was surprising that it gave the best antifungal results of all the investigated species of *Combretum*. On the other hand, both saponins (Carr & Rogers, 1987) as well as five triterpenoid desmosides (Rogers, 1989) have been isolated from leaf extracts of *C. padoides*, and both groups of compounds are known to possess antifungal potency. The chemical composition of the root

extract of *C. padoides* is however unknown, and the compounds responsible for the antifungal effects of the roots of this plant might be very different from them found in the leaves. Our results on the excellent antifungal effects of *C. padoides* root extracts indicate that this species might be used in a new way in African traditional medicine for treatment of fungal infections.

Combretum molle

C. molle root extracts gave excellent antifungal effects against all the species of *Candida* and *Cryptococcus neoformans*, and gave outstanding inhibitory effects against *Candida glabrata* (MIC 6.25 mg/ml) (II, Table 2 and Table 3). Extracts of the leaves were not as antifungal as the root extracts and were inhibitory only against the growth of *Candida krusei*, whereas none of the other fungal species were affected by this extract. This shows clearly that the leaves and roots contain active compounds in different amounts and might even contain different kinds of compounds. Some other authors have investigated the antifungal effects of *C. molle*: Khan et al., (2000) found that the stem bark of *C. molle* inhibited the growth of *C. albicans* with a MIC value of 5 mg/ml and Runyoro et al., (2006) observed that a root extract of *C. molle* gave fairly large inhibition zones against *C. albicans*. Baba-Moussa et al. (1999) found that a leaf extract of *C. molle* gave excellent antifungal effects against some dermatophytes, such as *Microsporum gypseum* (MIC = 0.25 mg/ml) and *Trichophyton mentagrophytes* (0.25 mg/ml). In many African countries, peeled twigs of *C. molle*, among many other plant species, are used as chewing sticks, “Miswaki” (Runyoro et al., 2006) and this kind of use of *C. molle* might be beneficial in treating *Candida* infections in the mouth often occurring in immuno-compromised patients such as those suffering from AIDS. *C. molle* is also used both internally as water decoctions for the treatment of diarrhea and stomach problems as well as topically, as an ointment for fungal infections on the skin (I, Table 1; Table 2, Table 7), and all these traditional uses are now justified by the results of us and other authors on the good antifungal potential of this species of *Combretum*. The excellent antifungal effects of *C. molle* might be due to hydrolysable tannins, since punicalagin and another tannin designated CM-A, have been isolated from the stem bark of *C. molle* (Asres et al., 2001a, Asres et al., 2001b). Punicalagin is an ellagitannin containing a galloyl moiety. It was originally isolated from *Punica granatum*, and is reported to possess antimicrobial activity against *Candida albicans* (Burapadaja & Bunchoo, 1995). As mentioned before, many hydrolysable tannins are known to possess good anti-*Candidal* and anti-*Cryptococcal* effects and the pyrogallol element in the tannin molecule seems to be crucial for the antifungal response (Latté & Kolodziej, 2000). Interestingly, when Asres et al. (2001b) investigated the antimalarial effects of punicalagin and CM-A they found that they were less effective than the crude extract of *C. molle* and speculated that the different tannins in the extract act synergistically with each other or with other compounds in the extract, or that some very active compounds were lost during the purification of the extract. In addition to tannins, *C. molle* has been found to contain many other compounds with potential antifungal activity: Letcher et al. (1972) have isolated a bibenzyl from the heartwood of *C. molle*; acid saponins like mollic acid-3- β -D-glucoside have been isolated from

the leaves of *C. molle*, and was found to give antifungal effects against *Penicillium* sp. (Pegel & Rogers, 1985). Thus, our results and the results of other authors indicate that *C. molle* is a rich source of antifungal compounds and this explains why the plant frequently is used for the treatment of fungal infections in African traditional medicine.

Combretum psidioides* and *Combretum fragrans

An extract of the stem bark of *Combretum psidioides* and a root extract of *C. fragrans* both gave excellent antifungal effects against *Candida glabrata* (II, Table 2). To our knowledge there are no previous reports on the antifungal effects of *C. psidioides*. Some phenanthrene compounds have been isolated from *C. psidioides* but the antimicrobial effects of these compounds were not investigated (Pettit et al. 1987). This species shows good antifungal potential also against *Cryptococcus neoformans* and *Candida parapsilosis*. Interestingly, extracts made from the fruits, leaves and stem bark of *C. psidioides* all showed activity against *C. krusei*. The use of *C. psidioides* for the treatment of diarrhea in African traditional medicine (Haerdi, 1964; I, Table 1; II, Table 1; Table 7) is now justified by the promising antifungal profile shown by this species (Table 10). A root extract of *C. fragrans* gave good antifungal activities against all the *Candida* species except *C. tropicalis* and was inactive against *Cryptococcus neoformans*. In addition to our investigation *C. fragrans* has been studied by Batawila et al. (2005) who found that a leaf extract of this species possessed good antifungal effects against *Candida zeylanoides*, *C. tropicalis*, *C. albicans* and *C. glabrata* and *C. guilliermondii* with MIC values ranging between 0.25 – 2 mg/ml. We did, however, not detect any antifungal effects of the leaf extract of this species. The discrepancies in the results of the antifungal effects of leaf extracts of this species between Batawila et al. (2005) and our group might be due to the use of different screening methods, Batawila et al. (2005) using an aqueous medium dilution method, whereas we used an agar filter paper diffusion method. Batawila et al. (2005) found that the leaves of *C. fragrans* contain large amounts of tannins, flavonoids and a few saponins, which indicates that these compounds might be responsible for the good antifungal activities shown by this species.

5.4.3. Antifungal effects of *Pteleopsis myrtifolia*

Pteleopsis myrtifolia was the only representant of the genus *Pteleopsis* included in our investigation. Since it is used for the treatment of wounds in African traditional medicine (Hedberg et al., 1982) it was not surprising that a root extract of *P. myrtifolia* gave good antifungal effects against *Candida glabrata* and *C. krusei* and some antifungal activity against the rest of the *Candida* species as well as against *Cryptococcus neoformans* (II, Table 2). As far as we know we are the first to report good antifungal activity for this species. Another species of *Pteleopsis*, *P. suberosa*, has been found to possess powerful antifungal effects against both dermatophytes and *Candida albicans* (Baba-Moussa et al., 1999) and this species was found to be

more active than the species of *Combretum* and *Terminalia* used in the investigation. Saponins and tannins were found in *P. suberosa*, why the same kind of compounds might be found from *P. myrtifolia* as well and be responsible for the good antifungal profile of this species.

Table 10. Summary of the antifungal effects of the most effective extracts of *Terminalia*, *Combretum* and *Pteleopsis* in our screening and correlation to ethnomedical use in Mbeya. * not investigated.

Species and extract	Results in screening	MIC values	Traditional use in Mbeya
<i>Terminalia sambesiaca</i> , roots	Antifungal against all <i>Candida</i> species and <i>Cryptococcus neoformans</i> . Produced the largest zones of inhibition of all the extracts.	<i>Cryptococcus neoformans</i> , 1.56 mg/ml <i>Candida glabrata</i> , 3.13 mg/ml	No use reported
<i>T. kaiserana</i> , roots	The second best extract to inhibit fungal growth. Most effective of all the extracts to inhibit the growth of <i>C. krusei</i> .	<i>Candida glabrata</i> , 1.56 mg/ml <i>Cryptococcus neoformans</i> , 1.56 mg/ml	To treat gonorrhea, diarrhea and vomiting; together with <i>C. molle</i> for diabetes
<i>T. sericea</i> , roots	Hot water decoction stronger inhibitor than a MeOH extract of the growth of <i>C. albicans</i> . Acetone, EtOH and MeOH extracts gave good antifungal profiles against all <i>Candida</i> species.	<i>Candida glabrata</i> , 0.78 mg/ml <i>Cryptococcus neoformans</i> , 6.25 mg/ml	To treat diarrhea, fever, hypertension and bacterial infections
<i>T. stenostachya</i> , leaves	Good antifungal profile against all <i>Candida</i> species except <i>C. tropicalis</i> . Active against <i>Cryptococcus neoformans</i> .	*	*
<i>Combretum padoides</i> , roots	Most antifungal of the <i>Combretum</i> species screened. Good antifungal profile against all <i>Candida</i> species and <i>C. neoformans</i> . Fungicidal against <i>C. glabrata</i> .	<i>Candida glabrata</i> , 6.25 mg/ml <i>Cryptococcus neoformans</i> , 6.25 mg/ml	*
<i>C. molle</i> , roots	Good antifungal profile against all <i>Candida</i> species and <i>C. neoformans</i>	<i>Candida glabrata</i> , 6.25 mg/ml <i>Cryptococcus neoformans</i> , 12.50 mg/ml	To treat gonorrhea, syphilis, influenza, oedema, skin diseases and wounds
<i>C. fragrans</i> , roots	Good antifungal activity against all species of <i>Candida</i> except <i>C. tropicalis</i> . Not active against <i>C. neoformans</i> .	*	To treat diarrhea
<i>C. psidioides</i> , stem bark	Antifungal against <i>C. krusei</i> , <i>C. glabrata</i> , <i>C. parapsilosis</i> and <i>Cryptococcus neoformans</i> .	*	To treat diarrhea, muscle pain and oedema
<i>Pteleopsis myrtifolia</i> , roots	Antifungal against all <i>Candida</i> species, but especially active against <i>C. glabrata</i> and <i>C. krusei</i> , as well as <i>C. neoformans</i>	*	*

5.5. Antiproliferative and cytotoxic effects of extracts of *Combretum*, *Terminalia* and *Pteleopsis* against some human cancer cell lines and BBCE endothelial cells (III)

Seven species of *Terminalia* have a long history in African and Asian traditional medicine for the treatment of cancer (Hartwell, 1982). Of the species of *Combretum*, only *C. latifolium* appears to have been recorded as a folk medical treatment of cancer (Hartwell, 1982). Based on my results the roots and leaves of *C. zeyheri* are used for the treatment of cancer (tumors) in some villages in Mbeya region (I, Table 1; Table 7). The genus *Pteleopsis* does not include species used for treatment of cancer in African traditional medicine, but was included in this study because of its relationship to *Combretum* and *Terminalia*.

The genus *Combretum* seems to be particularly interesting in terms of anticancer compounds, and the South-African species *C. caffrum*, along with some other species of *Combretum*, are known to contain a series of rare small molecular bibenzyles, the combretastatins, which have a high affinity for the colchisin binding site of tubulin (situated between α - and β -tubulin in the tubulin-heterodimer), inhibiting tubulin polymerization and microtubule assembly (Pettit et al., 1988; Pettit et al., 1989; Zhou & Giannakou, 2005). Combretastatin A-4 has been found to inhibit the proliferation of a variety of cancer cell lines, including multidrug resistant (MDR) cancer cell lines (McGown & Fox, 1990; El Zayat et al., 1993) and to trigger apoptosis (Cirla & Mann, 2003). In addition combretastatins have been shown to exhibit both antivasular and anti-angiogenic effects (Dark et al., 1997).

Because of this background of traditional use as well as some species of *Combretum* containing powerful anticancer compounds, we decided to elucidate the *in vitro* anticancer effects of selected species of *Combretum* and *Terminalia* as well as *Pteleopsis myrtifolia*, some of them not studied before for their anticancer effects. A summary of our results on the cytotoxic effects of the species of *Combretum*, *Terminalia* and *Pteleopsis* is presented in Table 12 and III, Table 2.

5.5.1. Antiproliferative effects of *Combretum* spp.

At a concentration of 25 $\mu\text{g/ml}$ six extracts of *Combretum* showed pronounced antiproliferative effects (> 70 % inhibition of proliferation) against the T 24 bladder cancer cells, three against the HeLa cervical cancer cells and three against the MCF 7 breast cancer cells (III, Table 2; Table 11) and one extract, a leaf extract of *C. fragrans* gave excellent antiproliferative activity against an endothelial cell line (Table 11). The cytotoxically most active *Combretum* species were in general more active than the *Terminalia* species showing the best activities. At 250 $\mu\text{g/ml}$ all the

extracts of *Combretum* spp. were lethal to the cancer cell lines, and resulted in empty wells compared to control cells.

Below those species of *Combretum* giving the most outstanding results in the screenings are presented.

Combretum fragrans

Of all the investigated fourteen species of *Combretum*, *Terminalia* and *Pteleopsis myrtifolia*, a methanolic leaf extract of *Combretum fragrans* gave the most outstanding antiproliferative effects against the human cancer cell lines (III, Table 2) as well as the BBCE endothelial cells (Table 11). At 25 µg/ml this extract reduced the growth of all the cancer cell lines as well as the BBCE endothelial cells (Table 11; III, Table 2) to 6.8 – 13.3 % growth of control, and the growth inhibitory effect was dose and cell line dependent (III, Figure 1), the MCF 7 breast cancer cells being more resistant than the bladder and cervical cancer cell lines. Still at 4.3 µg/ml this extract inhibited the proliferation of 60 % of the HeLa cervical cancer cells compared to the control cells (III, Figure 1). All the cancer cell lines exposed to this extract appeared to round up and failed to thrive, while the non-treated cancer cells were adherent. Sublethal concentrations of the leaf extract of *Combretum fragrans* (4.3 µg/ml) might induce apoptosis at least in some cancer cell lines, since some fragmentation of the nucleus as well as formation of apoptotic bodies could be observed in HeLa cervical cancer cells stained with Hoechst. Further investigations on the effects of this extract on expressions of marker enzymes for apoptosis (p53, caspases etc.) are needed to verify this observation. Notably, the leaf extract of *C. fragrans* might act by *specific cytotoxicity* against cancer cell lines, not attacking normal cells, since we found that it only to a minor extent affected the division of COS-7 green monkey fibroblasts.

The good antiproliferative effects of the leaf extract of *C. fragrans* on BBCE endothelial cells we have observed is interesting (Table 11), since tumorigenesis is dependent on the formation of new blood vessels (angiogenesis). Indeed, the cis-stilbene combretastatin A-4, originally isolated from the stem bark of *C. caffrum* (Pettit et al., 1989), has been found to possess both *in vivo* and *in vitro* anti-angiogenic and antivasculature effects (Dark et al., 1997), and it is possible that this kind of compounds would be responsible for the good antiproliferative effects on BBCE cells we observed by the leaf extract of *C. fragrans*.

Compared to the leaf extract the root extract of *Combretum fragrans* showed only slight or no cytotoxic effects on the cell lines investigated (III, Table 2). This is particularly interesting and is an example of that screenings of medicinal plants should always include different parts of the plants, since the variation in secondary compounds between different plant organs can be enormous.

To our knowledge we are the first to report on the excellent cytotoxic effects of *C. fragrans*. Batawila et al. (2005) have isolated tannins and flavonoids from a leaf extract of this species and found that it possesses good antifungal effects, but they did not study its cytotoxic effects. The excellent cytotoxic effects of the leaf extract of *C. fragrans* might thus be due to tannins and flavonoids, since these classes of compounds have been reported to possess anticancer effects (Ko et al., 2000; Saleem et al., 2002). The leaves of several species of *Combretum* have been found to contain powerful cytotoxic compounds: Pentacyclic triterpenes have at least partly been found to be responsible for the cytotoxic effects of a leaf extract of *C. nigricans*, and gave good activities against glioblastoma (U-373 MG), colon (HCT-15), non-small lung (A549) and bladder cancer (J82) cell lines (Simon et al., 2003a). *Combretum woodii* (Eloff et al., 2005) and *C. erythrophyllum* (Schwikkard et al., 2000) have been found to contain combretastatins in their leaves, and thus it may be possible that leaf extracts of *C. fragrans* also contain these kinds of compounds, which might be responsible for the remarkable antiproliferative effects we have observed for this extract against all the investigated cancer cell lines and the BBCE endothelial cells.

Since *C. fragrans* is frequently used in African traditional medicine, although not for treatment of cancer, the excellent cytotoxic effects we observed for this species could add an important new aspect of the use of this plant for the treatment of cancer in rural parts of Africa. On the other hand, careful studies on the toxicity of leaf extracts of this species must be performed to assess the proper and safe way to use it. The fact that mainly the leaves of this plant seem to contain cytotoxic compounds is beneficial since leaves are the easiest part to collect, and also makes non-destructive collection possible (compared to collections of roots).

Combretum zeyheri

A methanolic fruit extract of *C. zeyheri* gave antiproliferative effects comparable to those of the leaf extract of *C. fragrans*, showing excellent effects against all the human cancer cell lines (III, Table 2). Fruits of *Combretum* species are, however, considered poisonous in African traditional medicine (Rogers & Verotta, 1996). An extract of the roots of *C. zeyheri* was less cytotoxic than the fruit extract, and exhibited only moderate cytotoxicity against the T 24 bladder cancer cells and nearly no toxicity against the HeLa cervical cancer and MCF 7 breast cancer cells (III, Table 2). An extract of the stem bark of *C. zeyheri* gave moderate antiproliferative effects against the BBCE endothelial cell line (Table 11), showing that this species of *Combretum* might contain antiangiogenic and -vascular compounds. The stem bark extract gave only moderate antiproliferative effects against the T 24 bladder cancer cell line, weak effects against the MCF 7 breast cancer cell line, and was growth stimulatory of the HeLa cervical cancer cells. Previously, triterpenoids and saponins have been isolated from the leaves of *C. zeyheri* (Carr & Rogers, 1987) and these compounds might also be responsible for the cytotoxic effects of the fruit extract we observed.

In some villages of Mbeya region hot water decoctions of the stem bark and roots of *C. zeyheri* are used for the treatment of cancer (I, Table 1; Table 7). However, we have now found that extracts of the roots and stem bark of this species only possess minor antiproliferative effects compared to the fruits against the cancer cell lines in our study. On the other hand, the extract of the stem bark gave antiproliferative effects against the BBCE endothelial cell line, and this might justify the use of extracts of stem bark of *C. zeyheri* for the treatment of tumors in Mbeya region.

Combretum padoides

Good antiproliferative activities were also shown by an extract of the stem bark of *C. padoides*, which gave strong cytotoxic effects against HeLa cervical cancer cells (17.4 % growth of control) and good effects against T 24 bladder cancer cells (31.4 % growth of control) (III, Table 2). An extract of the roots was not as cytotoxic as the stem bark. No reports exist on the secondary compounds in the stem bark of *C. padoides*, but the leaves are known to contain mono- and bi-desmosidic triterpenoids (Rogers, 1989).

Combretum molle

Methanolic extracts of the roots and leaves of *C. molle* gave almost similar results against the T 24 bladder cancer cells, and both extracts were good inhibitors of the growth of this cell line reducing the growth to 26.3 – 27.7 % of the control (III, Table 2). Since this species is so frequently used in Tanzanian traditional medicine (Table 7; I, table 1), and showed mildly cytotoxic effects, we speculate the possibility of using it for the long term treatment of cancer in rural regions of Africa. Substituted phenanthrenes and a substituted bibenzyl have been isolated from the heartwood of *Combretum molle* (Letcher et al., 1972). *C. molle* has been found to be cytotoxic against a murine P-388 lymphocytic leukemial cell line, but the compound/-s responsible for this action was/were not identified (Pettit et al., 1987). The triterpenoid mollic acid 3 β -D-glucoside, a well known anti-molluscicide, has been isolated from acetone extracts of the leaves of *C. molle* (Pegel & Rogers, 1985), but no investigations on the cytotoxic effects of this compound have been performed.

5.5.2. Antiproliferative effects of *Terminalia* spp.

The most cytotoxic species of *Terminalia* were not at all as antiproliferative as the most potent species of *Combretum*, although some of the species gave good antiproliferative effects (> 70 % inhibition of proliferation) at 25 μ g/ml against both the cancer cell lines (III, Table 2) and the BBCE endothelial cell line (Table 11). Similarly to the species of *Combretum*, most of the species of *Terminalia* gave strong cytotoxic effects at 250 μ g/ml killing nearly all the cells in the wells when observed in light microscope. Methanolic extracts of the roots of *T. sambesiaca* and

T. sericea gave the strongest cytotoxic effects of all the *Terminalia* species studied. Root extracts of *T. sambesiaca* and *T. kaiserana* were potent inhibitors of the growth of the BBCE endothelial cell line (Table 11).

Terminalia sambesiaca

A root extract of *T. sambesiaca* gave the strongest antiproliferative effects of all the *Terminalia* species against the HeLa cervical cancer cells (18 % growth of control), and gave also good effects against the T 24 bladder cancer cells (29.5 % growth of control) (III, Table 2). To the best of our knowledge we are the first to report that this species possesses excellent-good cytotoxic activity. The extract of the stem bark of *T. sambesiaca* was not at all as active as the root extract, and interestingly showed only weak antiproliferative effects against the HeLa cervical cancer cells (81 % growth of control), but exhibited modest activity against the T 24 bladder cancer cells (58.5 % growth of control) (III, Table 2). The ethnomedical use of *T. sambesiaca* in Tanzania for treatment of cancer (Chhabra et al., 1989), correlates well with the good antiproliferative effects we found for this species, although in Tanzania extracts of leaves and stem bark are used for this purpose, but not the roots. We did not include leaves in our investigation due to lack of plant material, but it would be interesting to explore also the cytotoxic potential of this organ of *T. sambesiaca* in comparison to roots and stem bark.

Interestingly, root extracts of *T. sambesiaca* gave very good antiproliferative effects against the BBCE endothelial cell line (Table 11), and thus this species seems also to contain anti-angiogenic compounds. Flavonoids, such as luteolin and genistein, of which luteolin has been isolated from the leaves, bark and stem of *Terminalia arjuna* (Pettit et al., 1996), have been found to possess anti-angiogenic effects against HUVEC umbilical vein endothelial cells (Bagli et al., 2004). It is therefore possible that the root extract of *T. sambesiaca* might contain flavonoids responsible for its good antiproliferative effects against the BBCE endothelial cells.

A part of the good cytotoxic effects shown by *T. sambesiaca* might be due to hydrolysable tannins and their monomers, since several authors have found this group of molecules from *Terminalia* spp. (Baba-Moussa et al., 1999; Chen et al., 2000; Saleem et al., 2002) and have found that some of these compounds, such as chebulinic acid, tannic acid and ellagic acid possess antiproliferative effects (Saleem et al., 2002). To the best of our knowledge the tannin constituents of *T. sambesiaca* are unknown. Our investigation indicates that it would be worth to perform an activity guided isolation of the compounds responsible for the good cytotoxic activity of the root extract of *T. sambesiaca*. Perhaps, the scarce occurrence of *T. sambesiaca* in nature and thus the difficulty of getting plant material for performing biological assays might be one reason why this species has been studied for its biological (antimicrobial) effects only for a few times, both investigators being Africans (Chhabra et al., 1989; Masoko et al., 2005).

Terminalia sericea

A root extract of *Terminalia sericea* gave as good cytotoxic effects as the root extract of *T. sambesiaca* against the T 24 bladder cancer cells and was slightly less effective than *T. sambesiaca* against the HeLa cervical cancer cell line, but showed better effects against the MCF 7 breast cancer cell line (III, Table 2). The root extract of *T. sericea* did, however, not give so good effects against the BBCE endothelial cell line (Table 11). An extract of the leaves was not as antiproliferatively potent as the root extract. The good antiproliferative effects of *T. sericea* on the cancer cell lines indicates that hot water decoctions of the roots might be used for the treatment of cancer in African traditional medicine. *T. sericea* is a popular medicinal plant in many African countries, and is used for the treatment of various ailments and diseases but not for cancer (Hedberg et al., 1982; Arnold & Gulimian, 1984). Ethyl acetate extracts of *T. sericea* have been found to inhibit topoisomerase I, an enzyme responsible for the uncoiling of DNA and thus these extracts seem to inhibit replication of DNA (Wall et al., 1996). The compound responsible for this action is, however, unknown. Anolignan B, which has been isolated from the roots of *T. sericea* and *Anogeissus acuminata* has been found to possess cytotoxic effects against a fibrosarcoma cell line (Rimando et al., 1994). Some triterpenoids such as sericoside, arjunglucoside and an aglycon of sericoside have been isolated from the roots and stem bark of *T. sericea* (Bombardelli et al., 1974; Bombardelli et al., 1986) but none of them have been explored for their cytotoxic effects. Triterpenoids are known to exert cytotoxic effects (Simon et al., 2003a), and thus the triterpenoids isolated from *T. sericea* may in part be responsible for the good cytotoxic effects shown by the root extract of this species.

5.5.3. Antiproliferative effects of *Pteleopsis myrtifolia*

A root extract of the only species of *Pteleopsis* in this investigation, *P. myrtifolia* gave strong antiproliferative effects against the T24 bladder cancer cells, but was less effective against the HeLa cervical cancer and MCF7 breast cancer cell lines (III, Table 2). We are the first to report about the cytotoxic potential of this species. Saponins and triterpenoids have been isolated from the stem bark of *Pteleopsis hylodendron* (Ngounou et al., 1999) and *P. suberosa* contains saponins and tannins (Baba-Moussa et al., 1999), and similar compounds might therefore be present also in *P. myrtifolia*. *P. myrtifolia* is frequently used in African traditional medicine (Chhabra et al., 1989) but there are no reports that it would be used for the treatment of cancer. Thus our results indicate that this species might be used for the treatment of schistosomiasis induced bladder cancer, which is a common form of cancer among the male population in African countries (el-Mawla et al., 2001).

Table 11. Antiproliferative effects of extracts of *Combretum* and *Terminalia* against BBCE endothelial cells. Results are shown as the % of growth of control and obtained with Coulter count. The most antiproliferative extracts indicated in bold. Results are presented as means \pm S.E.M % of triplicates in two experiments.

Cell line Extracts, 25 μ g/ml	BBCE endothelial cell lines % growth
<i>Combretum</i> spp.	
<i>C. fragrans</i> ^L	14.08 \pm 3.02
<i>C. fragrans</i> ^R	62.23 \pm 2.97
<i>C. molle</i> ^L	74.76 \pm 1.62
<i>C. zeyheri</i> ^S	56.48 \pm 0.15
<i>Terminalia</i> spp.	
<i>T. kaiserana</i> ^R	20.94 \pm 1.49
<i>T. sambesiaca</i> ^R	17.54 \pm 1.18
<i>T. sambesiaca</i> ^S	34.34 \pm 2.87
<i>T. sericea</i> ^R	68.97 \pm 4.67
<i>T. stenostachya</i> ^S	40.46 \pm 1.03

L, leaves; R, roots; S, stem bark.

Table 12. Summary of the antiproliferative effects of the most effective extracts of *Combretum*, *Terminalia* and *Pteleopsis myrtifolia* in our screening and correlation to ethnomedical use in Mbeya. * not investigated.

Species	Cytotoxicity	Ethnomedical use in Mbeya	Secondary compounds
<i>Combretum</i> spp.	Our results/results from literature		
<i>C. fragrans</i>	<p>Extracts of the leaves gave the strongest inhibitory effects of all the extracts used in this study. At 4.3 µg/ml this extract inhibited the proliferation of 60 % of the HeLa cervical cancer cells. Root extracts are only slightly cytotoxic.</p> <p>Leaf extract not toxic against COS-7 green monkey fibroblasts.</p> <p>Leaf extract may cause apoptosis in the HeLa cell line at concentrations of 4.3 µg/ml.</p> <p>No other author has investigated this plant for cytotoxic effects.</p>	Roots for treatment of diarrhea.	Leaf extracts contain tannins, flavonoids and saponins (Batawila et al., 2005).
<i>C. molle</i>	<p>Extracts of roots and leaves good inhibitors of the proliferation of T 24 bladder cancer cells.</p> <p>Cytotoxic against a murine P-388 lymphocytic leukemia cell line (Pettit et al., 1987).</p>	Leaves, roots and stem bark to treat gonorrhea, syphilis, oedema, skin diseases and wounds.	Mollic acid β-3-D-glucoside from the leaves (Pegel & Rogers, 1985); Substituted phenanthrenes and a substituted bibenzyl from the heartwood (Letcher et al., 1972).
<i>C. padoides</i>	<p>An extract of the stem bark showed good cytotoxic activity against HeLa cervical cancer cells and T 24 bladder cancer cells.</p> <p>No previous reports on cytotoxic activity of this plant.</p>	*	Mono- and bidesmosidic triterpenoids from leaves (Rogers, 1989); Saponins in leaves (Carr & Rogers, 1987).
<i>C. zeyheri</i>	<p>A fruit extract as cytotoxic as the leaf extract of <i>C. fragrans</i>, effective against all the cancer cell lines. An extract of the roots only mildly cytotoxic and stem bark had growth stimulatory effects on HeLa cervical cancer cells.</p> <p>No previous reports on cytotoxic activity of this plant.</p>	Roots and leaves to treat diarrhea and cancer.	Triterpenoids and saponins from leaves (Carr & Rogers, 1987).

Table 12. Continued.

<i>Pteleopsis</i> spp.			
<i>P. myrtifolia</i>	A root extract gave strong antiproliferative effects against T 24 bladder cancer cells; less effective against HeLa cervical cancer and MCF 7 breast cancer cells. No previous reports on cytotoxic activity of this plant.	*	A related species, <i>P. suberosa</i> is known to contain saponins and tannins (Baba-Moussa et al., 1999); <i>P. hyladendron</i> contains triterpenoids and saponins in stem bark (Ngounon et al., 1999).
<i>Terminalia</i> spp.			
<i>T. sambesiaca</i>	A root extract was strongly active against HeLa cervical cancer cells. No previous reports on cytotoxic activity of this plant.	No use reported.	May contain saponin-like compounds in leaves (Masoko et al., 2005).
<i>T. sericea</i>	As effective as <i>T. sambesiaca</i> against T 24 cells and slightly less effective against HeLa cells. Good cytotoxic effects against the MCF 7 breast cancer cell line. Anolignan B from roots of <i>T. sericea</i> cytotoxic against a fibrosarcoma cell line (Rimando et al., 1994); Ethyl acetate extract inhibits topoisomerase I (Wall et al., 1996).	Leaves, roots and stem bark to treat diarrhea, fever, hypertension and bacterial infections.	The triterpenoids sericoside, arjunglucoside and an aglycon of sericoside have been isolated from roots and stem bark (Bombardelli et al., 1974; Bombardelli, 1986; Rode et al., 2003); the lignan Anolignan B from the roots (Rimando et al., 1994).

6. Conclusions and Summary

The results indicate that finding medicinal plants with good biological activities is enhanced when plants are chosen on the basis of ethnomedical use and when a group of related plants are screened. The chemotaxonomic approach on choosing a group of taxonomically related species, in our case species of Combretaceae, seems to be successful when trying to find plant species with new/improved biological activities. Although, some of the species of *Combretum*, *Terminalia* and *Pteleopsis*, included in this study, have been investigated for their traditional medicinal uses before by various authors, we have, in some cases reported on different or additional ethnomedical uses of certain species. This work has also elucidated the ways in which *Combretum* and *Terminalia* species are prepared for traditional medicines in Mbeya region, Tanzania, different countries and regions of Africa differing from each other in terms of methods for preparing herbal medicines from this plant group. Since ethnopharmacological knowledge is often handed down orally from generation to generation and usually no written documents are available, it is important to documentate this valuable information. Also, in many countries in Africa over 80 % of the population is entirely dependent on traditional medicine for their health care, and thus written information might aid and improve the health care of the local people, as well as help to preserve the information.

Ethnomedical uses of Combretaceae in Mbeya, Tanzania. Of ten species of *Combretum* and *Terminalia* six were commonly used as medicinal plants by the traditional healers in the villages we visited. These were *Combretum molle*, *C. fragrans*, *C. psidioides*, *C. zeyheri*, *Terminalia sericea* and *T. kaiserana*. The ethnomedical uses reported by the healers in Mbeya region correlated well with traditional uses of this plant group practiced by healers in other African countries as well as in Tanzania, reported by other authors. Common medicinal uses of *Combretum* and *Terminalia* species in Mbeya region was for the treatment of infectious diseases (gonorrhoea, syphilis, “stomach problems”, diarrhoea, influenza), fever, wounds, pains in the muscles (general body pain), oedema and hypertension. One species of *Combretum*, *C. zeyheri* was also reported to be used for the treatment of stomach tumors. In Mbeya region all plant parts of *Combretum* and *Terminalia* species were reported to have medicinal uses except from the fruits and flowers, the fruits of *Combretum* spp. being considered poisonous by many of the healers we interviewed. The traditional healers in Mbeya reported that most of the species of *Combretum* and *Terminalia* we showed them are abundant and easy to find. The most common way of preparing these plants for medicine is to use dried plant material and make hot water decoctions of it, although it was also common to mix either hot water extracts or the dried plant material itself in maize porridge. Leaves are sometimes prepared as teas (infusions). Fresh plant material was also used sometimes, although it was more common to use dried material. In some

cases the plants are used topically, mainly to treat wounds and infections on the skin, and for these purposes the dried plant material is mixed with sheep fat for ointments.

Correlation between ethnomedical use and biological effects. In many cases we found that the ethnomedical use of *Combretum* and *Terminalia* species correlated well with the antimicrobial effects of extracts made of them, and in some cases even with the cytotoxic properties of the plants. For example, species which gave the most outstanding/strong antibacterial and antifungal effects, such as *T. kaiserana*, *T. sambesiaca* and *T. sericea*, are used for the treatment of infectious diseases and diarrhea in Tanzania. Interestingly, two of the *Combretum* species, *C. fragrans* and *C. molle*, which both are used for diarrhea, “stomach problems” and wounds, gave excellent antibacterial and antifungal effects. The cytotoxic activity of the extracts of Combretaceae was in some cases related to their uses in traditional medicine: a fruit extract of *C. zeyheri*, the only species of *Combretum* used for treatment of tumors in Tanzania, Mbeya, showed excellent antiproliferative effects against the human T 24 bladder cancer, HeLa cervical cancer and MCF 7 breast cancer adenocarcinoma cell lines, and a root extract of *T. sambesiaca*, also used for cancer in Tanzanian traditional medicine, effectively inhibited the growth of the HeLa cells. Very good cytotoxic effects were however also observed for a leaf extract of *C. fragrans*, which is not reported to be used for cancer in traditional medicine, either in Mbeya, Tanzania or in other countries in Africa.

Antibacterial effects. The species of *Terminalia* were in general more antibacterial than the species of *Combretum*, although good antibacterial effects were also obtained with some of the *Combretum* species. Of the *Terminalia* species screened, *T. sambesiaca*, *T. sericea* and *T. kaiserana* gave the best effects, and for the first two the root extracts were more effective than extracts of the leaves or stem bark. A leaf extract of *T. kaiserana* was the only extract to inhibit the growth of the gram-negative *E. coli*. In a root extract of *T. sambesiaca* the antibacterial effects were highest in the more polar fractions. A hot water decoction of the roots of *T. sericea* was as effective as EtOH, acetone and MeOH extracts against all the bacterial species. Thus, it seems, that antibacterial compounds are extracted in hot water decoctions, the traditional way of preparing *T. sericea* for medicine. Our results on the good antibacterial effects of *Terminalia* species correlate well with the results published by Eloff (1999). Similarly to us, many authors report that especially polar extracts of *Terminalia* show a broad activity spectrum against both gram-positive and gram-negative bacteria (Ahmad et al., 1998; Malekzadeh et al., 2001; Gupta et al., 2002; Steenkamp et al., 2004; Moshi & Mbwambo, 2005). Antimicrobial ellagitannins (Burapadaja & Bunchoo, 1995; Conrad et al., 2001; Lin et al, 2001), lignans (Valsaraj et al., 1997; Eldeen et al., 2005), flavonoids (Valsaraj et al., 1997; Srivastava et al., 2001) and triterpenoids (Conrad et al., 1998; Atta-ur-Rahman et al., 2002) have been found from different species of *Terminalia*, which shows that this genus contains a broad range of different antimicrobial compounds.

Three species of *Combretum*, *C. fragrans*, *C. padoides* and *C. molle* gave excellent antibacterial effects. In summary, many *Combretum* species are known to possess powerful antibacterial effects against both gram-negative and gram-positive bacteria (Martini & Eloff, 1998; Eloff, 1999; Elegami et al., 2002; Katerere et al., 2003; Eloff et al., 2005; Eloff & Katerere, 2005; Karou et al., 2005). Tannins, flavonoids, saponins, coumarins, phenanthrene derivatives, anthracene glycosides and triterpenoids, some of them with antibacterial effects, have been isolated from species of *Combretum* (Bombardelli et al., 1974; Carr & Rogers, 1987; Malan & Swinny, 1993; Jossang et al., 1994; Udem et al., 1996; Martini & Eloff, 1998; Martini, 2001; Martini & Eloff, 2004).

Antifungal effects. We found that the species of *Terminalia* in general gave stronger antifungal effects against the species of yeast and *Cryptococcus neoformans* than the species of *Combretum*. Three species of *Terminalia*, *T. sambesiaca*, *T. sericea* and *T. kaiserana* gave excellent anticandidal and anticryptococcal activities, and the effects were in some cases comparable to the standard antibiotics, itraconazol and amphotericin used in our investigation. A hot water decoction of the roots of *T. sericea* was more effective than MeOH, EtOH and acetone extracts in inhibiting the growth of *C. albicans*. This indicates that many antifungal compounds are extracted especially in hot water extracts of this species and justifies this kind of preparation of medicine from *T. sericea* in African traditional medicine. Amphotericin B gave very poor inhibition against *Candida glabrata* compared to the extracts of the most effective species of *Terminalia*. *Candida albicans*, *C. tropicalis* and *C. parapsilosis* seemed to be the most resistant species, while *C. krusei*, *C. glabrata* and *Cryptococcus neoformans* were more sensitive to the extracts of *Terminalia* spp. Extracts of the roots seemed to be much more antifungal than extracts of the leaves, and in some cases extracts of the leaves were inactive against all the species of yeasts, although a leaf extract of *T. stenostachya* showed good antifungal activity. Thus, antifungal compounds, at least in the African species of *Terminalia* we have screened, seem mostly to be concentrated to the roots. Interestingly, we found that all fractions of a root extract of *T. sambesiaca* gave antifungal effects, although the more polar ones were more active. This result and studies of other authors on *Terminalia* species indicate that they contain a diverse array of antimicrobial compounds of different polarity. Hydrolysable tannins are known from many species of *Terminalia* and might be an attractive group of antifungals due to their high anti-cryptococcal and anti-*Candida* (non-*albicans*) inhibitory activity, as well as most of them showing low toxicity in the mammalian body and being water soluble, thus easily extracted from plant material into water decoctions (such as in traditional medicine). The excellent antifungal effects we found for some extracts of *Terminalia* indicate that this genus in general might be used for treatment of fungal infections in remote areas in Africa where conventional antifungals are not available.

The species of *Combretum* gave in general less prominent antifungal effects than those of *Terminalia*, although some species and extracts, such as the root extracts of *C. padoides* and *C.*

molle gave excellent antifungal effects and were active against all the fungal species investigated. In contrast to the genus *Terminalia*, relatively few African species of *Combretum* have been subjected to antifungal studies (Batawila et al., 2005; Katerere et al., 2003; Masika & Afolayan, 2002; Pegel & Rogers, 1985; Malan & Swinny, 1993; Baba-Moussa et al, 1999).

Cytotoxic effects. The antiproliferative effects of the *Combretum* species and extracts made from different plant organs of the same species, differed markedly from each other. There was no clear trend to which plant organ would be the cytotoxically most active among the species of *Combretum*, since the species differed from each other in terms of which organ was the most cytotoxic. To my knowledge we are the first to report on the outstanding cytotoxic effects of a leaf extract of *C. fragrans*. This extract effectively inhibited the proliferation of all the cancer cell lines as well as a BBCE endothelial cell line. This extract might induce apoptosis in HeLa cervical cancer cells at a concentration of 4.3 µg/ml since some fragmentation of the nucleus of the cells could be observed. Cytotoxic compounds identified from some African species of *Combretum* include the combretastatins (Pettit et al., 1987, 1988, 1989, 1995), pentacyclic triterpenoids (Simmons et al., 2003), flavonoids (Martini et al., 2004), an ellagic acid derivative (Asami et al., 2001) and hydrolysable ellagitannins (Jossang et al., 1994). Hydrolysable tannins have been found to show potent host-mediated anti-tumor effects in mice (Okuda, 2005). Punicalagin has been found to inhibit cell growth and anchorage-independent growth in H-ras transformed NIH3T3 fibroblasts, and possibly the effect is mediated through modulation of the redox status of the cells (Chen et al., 2005). Thus, tannins might in part be responsible for the good cytotoxic effects we have found in some of our extracts of *Combretum* since these kinds of molecules are found from species of *Combretum*.

Of the five investigated species of *Terminalia*, *T. sambesiaca* and *T. sericea* showed the most promising cytotoxic potential and the root extracts of both species gave strong effects against the HeLa cervical cancer and T24 bladder cancer cell lines. *T. sambesiaca* has not been investigated for its *in vitro* antiproliferative effects before. Our results are in agreement with those of other authors who have found several, mainly Asian species of *Terminalia* with good cytotoxic potential. The Asian species *T. catappa*, *T. chebula*, *T. bellerica* and *T. arjuna*, to mention a few, have been reported to possess cytotoxic (Lee et al., 1995; Saleem et al., 2002) chemopreventive (Chen et al., 2005) and antimutagenic (Liu et al., 1996; Chen et al., 2000; Kaur et al., 2000) properties. African species of *Terminalia* have mainly been studied for their antimicrobial effects, while studies on their cytotoxic effects on cancer cell lines are rare, an exception being the study on *T. sericea* crude extracts which were found to inhibit topoisomerase I activity (Wall et al., 1996). Hydrolysable ellagi- and gallotannins are thought to be responsible for much of the chemopreventive (Chen et al., 2000) and cytotoxic activity shown by species of *Terminalia* (Saleem et al., 2002; Pettit et al., 1989; Lee et al., 1995), which may explain the good cytotoxic effects for *T. sambesiaca* and *T. sericea* in my study.

My study demonstrated for the first time that root extracts of *Pteleopsis myrtifolia* possess excellent antiproliferative effects against T 24 bladder cancer cells. Decoctions of this species might thus be used for the treatment of schistosomiasis caused bladder cancer which is very common in African countries.

The Combretaceae plant family seems to include many medicinally interesting genera and species and there is still much to elucidate of biological activity of crude extracts and active compounds from these plants. New techniques for bioactivity guided isolation of active compounds, such as microfractionation, may enhance the finding of new compounds/known compounds with new biological activities from this plant group. The investigation of the biological effects of Combretaceae seems to be just in its beginning, but has expanded during the beginning of the new millennium with many new investigations.

Table 13. Summary of our results on the antibacterial, antifungal and cytotoxic effects of *Combretum*, *Terminalia* and *Pteleopsis*, ethnomedical use as well as compounds isolated from the species. * not investigated.

Species	Antibacterial effects (I)	Antifungal effects (II)	Cytotoxic effects (III)	Ethnomedical use; other authors/our results (I)	Major compounds
<i>Combretum</i> species					
1. <i>C. apiculatum</i> Sond.	*	Leaf extract inactive against all <i>Candida</i> spp., slightly active against <i>Cryptococcus neoformans</i> . Root extract showed slight activity against <i>Candida krusei</i> and <i>C. tropicalis</i> .	Cytotoxic against MCF 7 breast cancer cells (ca. 40 % growth of control).	Abdominal disorders, conjunctivitis (Watt & Breyer-Brandwijk, 1962); leprosy, bloody diarrhea, snake bite, scorpion sting (Kokwaro, 1976); body exhaustion in women (Gelfland et al., 1985); mental illness (Chhabra et al., 1989). Not investigated in Mbeya (Fyhrquist et al., 2002).	phenanthrenes, dihydrophenanthrenes, bibenzyls (Letcher & Nhamo, 1971).
2. <i>C. collinum</i> Fresen.	*	Root extract inactive against all fungal species in our screening. Leaf extract inactive against all other fungi except <i>Candida krusei</i> .	A leaf extract very active against MCF 7 breast cancer cells.	Malaria (Haerdi, 1964); Diarrhea, excessive menstrual bleeding, rectal prolapse (Hedberg et al., 1982); dysentery, snake bites (Kokwaro, 1976); "madi", a blood disease (Watt & Breyer-Brandwijk, 1962); gastroenteritis (Adjanouhoun et al., 1993). No use reported in Mbeya (Fyhrquist et al., 2002)	triterpenoids in leaf trichomes (Rogers & Coombes, 1999).
3. <i>C. constrictum</i> Laws.	*	Leaf extract slightly active against <i>Candida krusei</i> and extract of stem bark slightly active against <i>Cryptococcus neoformans</i> .	*	Snakebites, aphrodisiac (Kokwaro, 1976). Not investigated in Mbeya. (Fyhrquist et al., 2002)	*
4. <i>C. fragrans</i> F. Hoffm.	Root extracts gave excellent activity against <i>Micrococcus luteus</i> and <i>Enterobacter aerogenes</i> , stem bark extract against <i>M. luteus</i> . MIC against <i>Sarcina</i> sp.: 7.3 mg/ml	Root extract gave good antifungal activity against all species of <i>Candida</i> except <i>C. tropicalis</i> . No activity against <i>C. neoformans</i> . Leaf extract inactive against all the fungal species in our screening.	A leaf extract gave the most outstanding cytotoxic effects of all the extracts used in our screening. At 4.3 µg/ml it inhibited 60 % of the proliferation of HeLa cervical cancer cells. Also very good antiproliferative activity against BBCE endothelial cells. Root extract inactive or just slightly active against the cancer cell lines.	Coughs, syphilis (Kokwaro, 1976); aphrodisiac (Gelfland et al., 1985); chronic wounds (Adjanouhoun et al., 1986); leprosy (Chhabra et al., 1989); snakebites (Neuwinger, 2000) Diarrhea (Fyhrquist et al., 2002)	leaf extracts contain tannins, flavonoids and a few saponins (Batawila et al., 2005).

Table 13. continued.

5. <i>C. hereroense</i> Schinz.	Extracts of stem bark gave good activity against most of the bacterial species, especially <i>Enterobacter aerogenes</i> and <i>Sarcina</i> sp.	Extracts of stem bark gave moderate antifungal activity against most of the <i>Candida</i> species, but was inactive against <i>C. neoformans</i> .	Stem bark: some cytotoxicity against T 24 cells.	Cardiac problems, heartburn, venereal diseases, body pain, chest problems, coughs, stomach problems, headache, schistosomiasis (Watt & Breyer-Brandwijk, 1962; Gelfland et al., 1985); Schistosomiasis (Kokwaro, 1976). Not investigated in Mbeya (Fyhrquist et al., 2002).	Phenanthrene derivates in heartwood (Letcher & Nhamo, 1973); flavonoids in leaf extracts (Carr & Rogers, 1987).
6. <i>C. molle</i> G. Don.	Good antibacterial activity of leaf extract against <i>Enterobacter aerogenes</i> and <i>M. luteus</i> .	Good antifungal profile of root extract against most of the <i>Candida</i> species and <i>C. neoformans</i> MIC 12.5 mg/ml, <i>C. glabrata</i> MIC 6.25 mg/ml.	Extracts of roots and stem bark showed good activity against T 24 bladder cancer cells.	Wound dressing, stomach problems, snake bites, aid in child birth (Watt & Breyer-Brandwijk, 1962); abortions, antidiarrheic (Haerdi, 1964); wounds from poisoned arrows (Haerdi, 1964); wounds (Drummond & Coates-Palgrave, 1973); hookworms, stomach pains, snake bite, leprosy, fever, dysentery, general body swellings, chest complaints, abortifacient, wounds (Kokwaro, 1976); angina, dropsy (Kerharo, 1974); expectorant, syphilis, snakebites (Hedberg et al., 1982); swelling of abdomen, abortion, constipation, sterility (Chhabra et al., 1989); stomach problems, snake bites, wounds, dysentery, galactagogue (Neuwinger, 2000). Gonorrhoea, syphilis, influenza, oedema, skin diseases, wounds (Fyhrquist et al., 2002).	Substituted phenanthrenes and a substituted bibenzyl from the heartwood of <i>Combretum molle</i> (Letcher et al., 1972); Mollic acid 3 β -D-glucoside from an acetone extract of the leaves (Pegel & Rogers, 1976; Valsaraj et al., 1997).
7. <i>C. obovatum</i> F. Hoffm.	*	Stem bark extract did not show any antifungal activity against <i>C. albicans</i> and <i>C. krusei</i> . Not investigated against the rest of the fungal species.	*	No reports in literature. Not investigated in Mbeya (Fyhrquist et al., 2002).	*
8. <i>C. padoides</i> Engl. & Diels	Both stem bark and root extracts gave good antibacterial activity against <i>S. aureus</i> and <i>E. aerogenes</i> .	Most antifungal of all the species of <i>Combretum</i> in our screening. A root extract gave good activity against all the fungal species in our screening. Fungicidal against <i>C. glabrata</i> , MIC 6.25 mg/ml, <i>C. neoformans</i> , MIC 6.25 mg/ml.	An extract of the stem bark gave good activity against the HeLa cervical cancer cell line (17.4 % growth of control)	Snake bites and hookworms (Kokwaro, 1976). Not investigated in Mbeya (Fyhrquist et al., 2002).	Mono- and bidesmosidic triterpenoids from the leaves (Rogers, 1989); saponins in leaves (Carr & Rogers, 1987).

Table 13. continued.

9. <i>C. psidioides</i> Welw.	Leaf extract good antibacterial effects against <i>E. aerogenes</i> and <i>M. luteus</i>	Extracts of stem bark antifungal against <i>C. krusei</i> , <i>C. glabrata</i> and <i>C. neoformans</i> .	Not cytotoxic.	Aphrodisiac, diarrhea, malaria, rheumatic back pain (Haerdi, 1964); galactagogue (Gilges, 1985). Diarrhea, muscle pain, oedema (Fyhrquist et al., 2002).	Phenanthrenes (Pettit et al., 1987).
10. <i>C. zeyheri</i> Sond.	Extracts of fruits, stem bark and roots gave good antibacterial effects.	Slight or no antifungal effects of stem bark, fruits and roots.	The fruits are very cytotoxic against all the investigated cancer cell lines. Extracts of roots and stem bark just slightly cytotoxic or not active at all.	Scorpion bites (Watt & Breyer-Brandwijk, 1962); coughs, colic, smallpox (Drummond & Coates-Palgrave, 1973); Schistosomiasis, dry wounds, rheumatism, back pain, bloody diarrhea, coughs (Kokwaro, 1976); diarrhea, vomiting (Hedberg et al., 1982); bloody diarrhea (Gelfand et al., 1985); rheumatism, joint pains, eye inflammation, conjunctivitis (Kremnitz et al., 1988); treatment of back pain, malaria (Van Koenen, 1996); schistosomiasis (Neuwinger, 2000); Diarrhea, cancer (tumours) (Fyhrquist et al. 2002).	Triterpenoids and saponins from leaves (Carr & Rogers, 1987).
<i>Pteleopsis</i> spp.					
1. <i>P. myrtifolia</i> Engl. & Diels	*	A root extract antifungal against all the species of <i>Candida</i> and <i>C. neoformans</i>	Cytotoxic against T 24 bladder cancer cells.	Dysentery (Haerdi, 1964); venereal diseases (Kokwaro, 1976); dysentery, menorrhagia, swellings of the stomach, wounds, sterility, infertility (Hedberg et al., 1982). A related species of <i>Pteleopsis</i> , <i>P. suberosa</i> is used for treatment of gastric ulcers (Baba-Moussa et al., 1999) Not investigated in Mbeya (Fyhrquist et al. 2002).	No reports for <i>P. myrtifolia</i> , but related species such as <i>P. suberosa</i> contains saponins and tannins (Baba-Moussa et al., 1999) and <i>Pteleopsis hylodendron</i> triterpenoids and saponins in stem bark (Ngounou et al., 1999)
<i>Terminalia</i> spp.					
1. <i>T. glaucescens</i> Benth.	*	*	*	Burns, headache, stomach problems (Bouquet & Debray, 1974); AIDS related fungal infections (Koudou et al., 1995); fungal infections of skin (Batawila et al., 2005); coughs, wounds, dental care (Neuwinger, 2002). No use reported in Mbeya (Fyhrquist et al., 2002)	*

Table 13. continued.

2. <i>T. kaiserana</i> F. Hoffm.	A leaf extract the only extract in our screening which showed activity against <i>E. coli</i> . Both leaf and root extracts showed good antibacterial activity. <i>S. aureus</i> , MIC 4.7 mg/ml, <i>Sarcina</i> sp., MIC 4.3 mg/ml.	Powerful inhibitor of fungal growth. Most effective of all extracts on inhibiting <i>C. krusei</i> . <i>Candida glabrata</i> , MIC 1.56 mg/ml, <i>C. neoformans</i> , MIC 1.56 mg/ml.	Root extract cytotoxic against HeLa cervical cancer cells and BBCE endothelial cells.	Gonorrhea, bilharzia schistosomiasis, diuretics, aphrodisiac, headache, back and kidney pain, scabies, cardiac problems, cough (Haerdi, 1964; Chhabra et al., 1989) Gonorrhea, diarrhea (Fyhrquist et al., 2002).	*
3. <i>T. sambesiaca</i> Engl. & Diels	A root extract gave the best antibacterial effects of all extracts of <i>Combretum</i> and <i>Terminalia</i> . <i>Enterobacter aerogenes</i> MIC 0.9 mg/ml, <i>M. luteus</i> MIC 1.0 mg/ml, <i>Sarcina</i> sp. 1.2 mg/ml, <i>S. epidermidis</i> 1.5 mg/ml, <i>S. aureus</i> , 1.8 mg/ml. Also stem bark extracts antibacterial.	Together with <i>T. sericea</i> and <i>T. kaiserana</i> the most powerful antifungal extract of all screened species of <i>Terminalia</i> and <i>Combretum</i> . <i>Cryptococcus neoformans</i> MIC 1.56 mg/ml, <i>Candida glabrata</i> MIC 3.13 mg/ml. Fractionated extract of the root: antifungal activity was found especially in polar fractions . Active compounds might be tannins?	A root extract the most effective inhibitor of the growth of HeLa cervical cancer cells (18 % growth of control) of all the species/extracts of <i>Terminalia</i> we investigated. Root extract very active against BBCE endothelial cells. Extract of stem bark only slightly cytotoxic.	Bloody diarrhea, cancer, gastric ulcers, appendicitis (Chhabra et al., 1989). No uses of this plant were reported in Mbeya (Fyhrquist et al., 2002)	May contain saponin-like compounds in the leaves (Masoko et al., 2005).
4. <i>T. sericea</i> Burch ex. DC.	Extracts of roots especially active against <i>Micrococcus luteus</i> , <i>Sarcina</i> sp., <i>S. aureus</i> and <i>E. aerogenes</i> . When EtOH, MeOH, acetone extracts and a hot water decoction were compared, they were found to be equally active against all the bacterial species. Extracts of leaves active against <i>M. luteus</i> and <i>E. aerogenes</i> , although not at all as potent as the extracts of the roots.	Acetone-, EtOH- and MeOH-extracts of roots gave excellent antifungal effects against all <i>Candida</i> species and <i>Cryptococcus neoformans</i> . A hot water decoction gave the best effects against <i>C. albicans</i> compared to a MeOH extract.	Root extract gave strong antiproliferative effects against T 24 bladder cancer cells.	Diarrhea, dysentery, colic, diabetes (Watt & Breyer-Brandwijk, 1962); diarrhea, colic, pneumonia, eyewash for certain diseases in the eyes (Drummond & Coates-Palgrave, 1973); bilharzia (Kokwaro, 1976); gonorrhea (Hedberg et al., 1982); diarrhea, skin diseases, cough, general body weakness, pneumonia, venereal diseases (Neuwinger, 2000) Diarrhea, fever, hypertension, bacterial infections (Fyhrquist et al., 2002).	The triterpenoids sericoside, arjunglucoside, aglycone of sericoside in roots and stem (Bombardelli et al., 1974; Bombardelli et al., 1986; Rode et al., 2003). Resveratrol-3-O- β -D-rutinoside (Bombardelli et al., 1975).

Table 13. continued.

5. <i>T. spinosa</i> Engl.	*	A leaf extract was slightly antifungal against <i>Candida krusei</i> and <i>C. tropicalis</i>	A leaf extract was cytotoxic against T 24 bladder cancer cells.	Fresh leaves for treatment of malaria (Chhabra et al., 1989) Not investigated in Mbeya (Fyhrquist et al., 2002).	Methanolic extracts contain coumarins and tannins (Okemo, 1996).
6. <i>T. stenostachya</i> Engl. & Diels	Extracts of stem bark very active against <i>S. aureus</i> and <i>E. aerogenes</i> .	Leaf extract gave good antifungal profile against all the <i>Candida</i> species in our investigation, except <i>C. tropicalis</i> . Active against <i>C. neoformans</i> . Stem bark also active against some of the species of yeast.	Extract of stem bark active against T 24 bladder cancer cells and BBCE endothelial cells.	Treatment of epilepsy and poisoning (Neuwinger, 2000) Not investigated in Mbeya (Fyhrquist et al., 2002).	*

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