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Antimicrobial and antioxidative activity of Ugandan medicinal plants

MSc thesis

Marta Kuglerová

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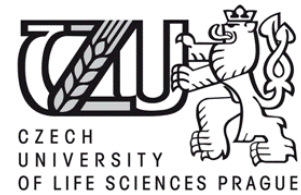

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Thesis Title: Antimicrobial and Antioxidative Activity of Ugandan medicinal Plants

Code for compiling this M.Sc. diploma thesis

1. Introduction
2. Background.

   (botany, chemistry, biological activities, ethnopharmacology and agronomy of selected species)
3. Material and Methods

   (plant material, preparation of extracts, antimicrobial assays)
4. Results and Discussion
5. Conclusions

Extent of the Thesis report:

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Jorgensen JH, Turnidge JD, Washington JA. Antibacterial susceptibility tests: Dilution and disk diffusion methods. In: Murray PR, ed., MANUAL OF CLINICAL MICROBIOLOGY. Washington, D.C., ASM Press, 1999, pp. 1526-1543.
Hamill FA, Apio S, Mubiru NK, Mosango M, Bukenya-Ziraba R, Maganyi OW, Soejarto DD (2000) Traditional herbal drugs of southern Uganda, I. JOURNAL OF ETHNOPHARMACOLOGY 70 (3): 281-300.
Hamill FA, Apio S, Mubiru NK, Bukenya-Ziraba R, Mosango M, Maganyi OW, Soejarto DD (2003) Traditional herbal drugs of Southern Uganda, II: literature analysis and antimicrobial assays JOURNAL OF ETHNOPHARMACOLOGY 84 (1): 57-78.
McFarland K, Mulholland DA, Fraser LA (2004) Limonoids from Turraea floribunda (Meliaceae) PHYTOCHEMISTRY 65 (14): 2031-2037.

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Certification

I, Marta Kuglerová, declare that this thesis, submitted in partial fulfilment of the requirements for the degree of MSc, in the Institute of Tropics and Subtropics of the Czech University of Life Sciences Prague, is wholly my own work unless otherwise referenced or acknowledged.

Prague, April 30 2008

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Signature

Abstract

The antimicrobial activity of crude ethanol extracts of seven barks traditionally used by Karamojong healers in Uganda, namely *Capparis tomentosa* (Capparaceae), *Dregea rubicunda* (Asclepiadaceae), *Fagaropsis angolensis* (Rutaceae), *Trichilia prieuriana* (Meliaceae), *Turraea floribunda* (Meliaceae), *Warburgia ugandensis* (Canellaeceae), and *Zanthoxylum chalybeum* (Rutaceae) was tested against five bacteria (*Enterococcus faecalis, Escherichia coli, Pseudomonas aeruginosa, Salmonella enteritidis* and *Staphylococcus aureus*) and one yeast species (*Candida albicans*) using the broth microdilution method. Results showed that all plant species possessed antimicrobial activity against at least one micro-organism tested in this study at concentrations of 512 µg/ml or below, whereas the extract of *F. angolensis* inhibited growth of *S. aureus* and *C. albicans* with minimum inhibitory concentrations (MICs) 64 and 32 µg/ml, respectively, representing the lowest MIC values achieved for all microbial strains tested in this study. Additionally, the same extracts were tested for Antioxidative activity using 2,2-diphenyl-1-picrylhydrazil free radical scavenging assay. Among the plants tested, *W. ugandensis* showed the most promising antioxidative properties (IC_{50}=7 µg/ml).

Key words: Antimicrobial activity, antioxidative activity, Ugandan medicinal plants, extracts

Abstrakt

Antimikrobiální aktivita sedmi kůr používaných tradičně v léčiteli kmene Karamoja v Ugandě, jmenovitě *Capparis tomentosa* (Capparaceae), *Dregea rubicunda*(Asclepiadaceae), *Fagaropsis angolensis* (Rutaceae), *Trichilia prieuriana* (Meliaceae), *Turraea floribunda* (Meliaceae), *Warburgia ugandensis* (Canellaeceae), a *Zanthoxylum chalybeum* (Rutaceae) byla testována proti pěti bakteriálním kmenům (*Enterococcus faecalis, Escherichia coli, Pseudomonas aeruginosa, Salmonella enteritidis* a *Staphylococcus aureus*) a jednomu druhu kvasinky (*Candida albicans*) za použití bujónové mikrodiluční metody. Výsledky prokázaly, že všechny rostlinné druhy vyvíjejí antimikrobiální aktivitu přinejmenším proti jednomu z testovaných patogenů v koncentracích 512 µg/ml a nižších. Zejména extrakt *F. angolensis* inhiboval růst *S. aureus* a *C. albicans* s minimální inhibiční koncentrací (MIC) 64 respektive 32 µg/ml, reprezentujíc tak nejnižší dosažené hodnoty MIC v této studii. Stejné extrakty byly podrobeny testům antioxidativní aktivity za použití 2,2-difenyl-1-picrylhydrazil vychytávání volných radikálů. Mezi testovanými rostlinami prokázala *W. ugandensis* nejslibnější antioxidativní vlastnosti (IC_{50}=7 µg/ml).

Klíčová slova: Antimikrobiální aktivita, antioxidativní aktivita, ugandské léčivé rostliny, rostlinné extrakty

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Contents

List of tables

List of figures

List of abbreviations

ATCC	American Type Culture Collection
CFU	Colony-forming unit
DMSO	Dimethyl sulfoxide
DPPH	2,2-diphenyl-1-picrylhydrazil
G+	Gram-positive (bacteria)
G-	Gram-negative (bacteria)
MIC	Minimum inhibitory concentration
TBS	Tris-buffer saline
MDR	Multi drug resistance
WHO	World Health Organisation

Foreword

Since the first human civilization, plants were used as a source of drugs and remedies. It is clear that the use of plants and their extracts has been an important part of human civilization (Rates, 2001; De Pasqual, 1984). At present, study of plants in order to find their medicinal properties is a big issue. The interest in studying plant based remedies is definitely increasing (Borris, 1996). The number of individuals using medicinal plants is estimated now to be increasing with increasing population in both developed and developing countries all around the world (Okello and Ssegava, 2007).

There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action because there has been an alarming increase in the incidence of new and re-emerging infectious diseases. Another big concern is the development of resistance to the antibiotics in current clinical use.
Substances derived from higher plants constitute more than 25 % of currently prescribed medicines (Hamburger and Hostettmann, 1991). Antimicrobial activity of plants can be detected by observing the growth response of various microorganisms to those plant tissues or extracts which are placed in contact with them (van den Berghe and Vlietinck, 1991). By the use of simple bioassays in conjunction with an approach involving bioactivity guided fractionation, it is possible to isolate novel compounds with interesting properties (Marston et al., 1993).

Drug discovery from plants involves a multidisciplinary approach combining botanical, ethnobotanical, phytochemical and biological techniques (Jachak and Saklani, 2007). Over the last few decades, a vigorous effort has been made to explore new, clinically useful, antibiotics. More than a thousand antibiotics have been prepared thanks to this big effort. However, only a few of those found their use in clinical practice. Still, certain diseases remain serious problems and many of the well known and often used antibiotics have notable disadvantages in terms of limited antimicrobial spectrum or serious side effects. For many years lower plants, with special emphasis on various fungi, have been screened for antimicrobial substances. Another potentially useful area for exploration is the higher plants (Mitscher et al., 1972). Traditional drugs still provide problems of a specific nature, in terms of the status of their technology, production and quality control (Labadie, 1986). In an attempt to conserve traditional medicine knowledge, it is necessary that inventories of plants with

therapeutic value are carried out, and the knowledge related to their use documented in systematic studies (Tabuti et al., 2003).

For many years, ethnobotanists have travelled around the world in the never-ending race for new remedies. The World Health Organization (WHO) estimates that more than 80% of health care needs in developing countries (especially Africa) are met through traditional health care practices (WHO, 2002). Many indigenous people still use plants as a source of medicine, using their own knowledge and abilities to cure diseases (Thring et al., 2007). In East Africa, 90% of the population relies on traditional medicines and traditional health practitioners as the primary source of health care (Miller, 1980). Medicinal plants in Uganda play a profound role in management and control diseases of poultry (Bukenya-Ziraba and Kamoga, 2007) and cattle (Gradé et al., 2008). The most common disease in Uganda is thought to be malaria and accounts for 25-40% of patient attendance in health facilities (Tabuti, 2008). A number of surveys have been carried out in Uganda to document the use of herbal medicines (Tabuti et al., 2003; Katuura et al., 2007; Okello and Ssegava, 2007; Kamatenesi-Mugisha et al., 2007). Thus we decided to test 7 medicinal plants used traditionally by local inhabitants of Karamoja region in Uganda for the treatment of various infections, and evaluated them for potential antimicrobial and antioxidative activity, in order to confirm their popular use by traditional health practitioners.

1. Introduction

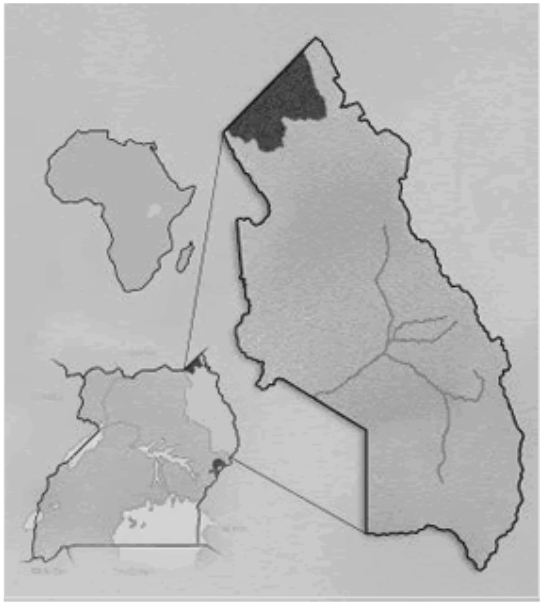
1.1. Study area

Karamoja is an isolated corner of land in Africa named after the people who have lived there for centuries: the Karimojong. With 27.319 km^2, this arid expanse of savannah and bush forms the northeast edge of Uganda where it borders Kenya and Sudan, with Ethiopia not far off. It covers about 10% of Uganda. A black and white map adopted from Anonymous, 2008a is illustrated in Fig.1. showing the location of the region.

Karamoja is naturally bordered with Kenya by the Rift Valley on the east. To the north lies the pristine basin of Kidepo National Park and also a large mountain range that leads into the Sudan. On the south there are the peaks of Mount Elgon National Park, which were formed by volcanic eruptions millions of years ago. Finally in the west, swamps enter into the Acholilands. The majority of Karamoja is more than 1000 metres above sea level, laid on plateau. It is surrounded by four main highest mountains- Mount Morungole in the north, Mount Moroto in the east, Mount Kadam in the south and Mount Napak in the west. According to the most recent Ugandan census figures, there live about 956,000 people in Karamoja, even though the actual numbers are estimated to be higher. Karamoja has five districts-Moroto, Kotido, Nakapiripirit, Abim and Kaabong .The capital of region Karamoja is Moroto town (Anonymous, 2008a).

Karamoja's climate is very tough. In many areas, rains do not exceed 800 millimetres per year (sometimes 500 millimetres). The rainy season is present from June to October, with rare precipitation. From November to March comes the hot and dry season. In recent years, drought has become more frequent. During the dry season, the men and youth herd livestock to migratory cattle areas, where they sleep in the open or in low grass huts (Gradé et al., 2007).

Fig. 1 Location of Karamoja region (enlarged) in Uganda



Source: Anonymous, 2008a

Beyond natural and national borders, inter-regional conflicts have kept Karamoja cut off from the rest of the world and its own country. The Karimojong, like other pastoral people, have beliefs in their traditional way of life (Anonymous, 2008a). External influences are minimal and 99% of the population relies exclusively on indigenous knowledge, medicines, and practices for themselves and their livestock (Gradé et al., 2007). Although Karamoja has limited sources of income for its people, it has natural resources that contribute to its development. Such resources include livestock and their products, minerals, gum arabic and other forest resources. At the moment, the local people are heavily dependent on livestock, sorghum production and small-scale trade (Egadu et al., 2007).

1.2. Ugandan folk medicine

Because African traditional medicine covers a broad range of practices, the term "healer" refers to practitioners specialized in various traditional approaches to health and illness, including spiritualists, priests, diviners, medium, faith healers, bone setters, herbalists, among others (Homsy et al., 1999).

Folk medicine is in Uganda often connected with ethnoveterinary medicine. It is obvious that inhabitants have observed self-healing abilities in animals and followed their example (Gradé

et al., 2008)Several studies were made on chimpanzee observation (Krief et al., 2005a; Krief et al., 2005b; Krief et al., 2006) and scientists followed feeding behaviour in cattle as well (Gradé et al., 2008). In Uganda, there is at least one traditional healer for nearly 290 people compared to one Western-trained medical practitioner for every 10,000 people in the urban areas and 50,000 people in the rural areas respectively. The majority of the population in Uganda has greater access to traditional than to western health care (IK Notes, 2003). Even studies on primary schoolchildren's knowledge of medicines and self-treatment have shown that children between 10and 18 years of age have a broad knowledge of herbal and biomedical remedies and use the frequently (Geissler et al., 2001).
In Uganda, important steps have been taken to recognize and promote traditional medicine and a draft bill for a law to recognize, coordinate and regulate the practice of traditional medicine in Uganda is under preparation (Ministry of Health, 2000). The majority of traditional healers is registered with Uganda ne dagala lye kinansi a traditional medicine healers association (Tabuti, 2004).

1.3. Biological activities of Ugandan medicinal plants extracts

As far as the biological activities of extracts from Ugandan medicinal plants are concerned, the most of all reports written are concentrated on their antimalarial effects. Few others have mentioned traditional use of plants in connection with other human or animal diseases, and not much laboratory testing has been done so far. Most of the studies are namely done by ethnobotanical method of questionnaires and are focused on traditional use of plants and their particular use in traditional medicine.

Screening of various Ugandan medicinal plants mentioned in literature is based on their traditional use in medicine as antimalarial anti-trypanosomal and often antiparasitic effects. Petroleum ether, dichloromethane, methanol and distilled water extracts of 9 plants species traditionally used to treat sleeping sickness in Uganda were prepared for *in vitro* screening of anti-trypanosomal activity. Evaluation of minimal inhibitory concentration and fluorescent dye method showed that of the plants tested eight of them at least one active anti-trypanosomal extract with *Alhizia gummifera* being the most active (Freiburghaus et al., 1996). A methanolic extract of *Albizia grandibracteata* leaves exhibited strong *in vitro* anthelmintic effect as well as inhibitory activity against certain tumoral cells (Krief et al., 2005). Specimens of 24 plant species included in the diet of Kibale chimpanzees were

collected and identified and their dried extracts tested against the following human parasites, presently used in biomedical research, *Leishmania donovani*, *Trypanosoma brucei brucei*, *Plasmodium falciparum* and a free-living worm *Rhabditis pseudoelongata*. Extracts were also tested against bacteria (*Staphylococcus aureus*, *Escherichia coli*), yeast and fungi (*Candida tropicalis*, *Penicillium crustosum*) and antitumour activities assayed. Seven of chosen species (*Albizia grandibracteata, Antiaris toxicaria, Blighia unijugata, Chaetacme aristata, Ficus exasperate, Phytolacca dodecandra* and *Trichilia rubescens*) presented good results especially as anti-tumoric and antimalarial (Krief et al., 2005b). Petroleum ether, chloroform and ethanol extracts of ten medicinal plants used to treat malaria (*Bothlioclines longpipes*, *Toddalia asiatica*, *Maesa lanceolata*, *Indigofera emerginella, Lantana trifolia, Vernonia lasiopus, Trimmeria bakeri, Rhus natalensis, Erythrophleum pyrifolia* and *Conyza* sp.) were subjected to *in vitro* antiplasmodial screening against *Plasmodium falciparum*. The highest antiplasmodial activity was produced by chloroform extract of *M. lanceolata* and *R. natalensis* followed by chloroform leaf extract of *B. longipes* and the petroleum ether root extract of *T. bakeri*. Despite those several reports there is still lack of information about medicinal plants used in our study.

1.4. Characteristics of studied plant species

Capparis tomentosa Lam.

Family: Capparaceae

Synonyms: *Capparis polymorpha* Guill. & Pers.; *Capparis alexandrae* Chiov.

Vernacular names: Woolly caper-bush, wollerige kapperbos, numero, sharube, khawa, gombor lik, mbada paka, andel

Origin and geographic distribution: The species grows from Natal, Swaziland, Transkei, Zululand, and eastern and northern Transvaal, westward across Botswana into northern Namibia, and northwards into tropical Africa. It occurs in coastal bush, forests, riverbanks, mountain slopes, evergreen forests, hot and dry thornveld, and in arid sandy plains. It grows most often as a spiny, scrambling bush or dense climber, hauling itself into the branches of trees and shrubs. It also grows on the tops of anthills, making a solid, tidy crown and occurs in the semi-arid and humid lowland and highland woodlands, forest edges and scrub. Plant may become a weed if not adequately controlled. Native is in Botswana, Eritrea, Ethiopia, Lesotho, Namibia, South Africa, Swaziland, exotic in Kenya (Anonymous, 2008b).

Description: *C. tomentosa* is often a spiny scrambler or a small tree that grows up to 10 m tall, with an upright trunk up to 13-15 cm in diameter and covered with scattered spines (Fig 3, Fig 4). Trees are well branched and branches are normally covered with thick yellow hairs; even the robust, recurved spines are often hairy. Leaves soft and velvety, light green to greyish-green, sometimes rusty coloured, alternate, 2.5-8 cm long, 1.3-3.8 cm wide, oval, oblong, or egg shaped, usually thickly velvet but sometimes smooth; tip usually rounded with a sharp, short point, sometimes notched or blunt; base rounded or narrowed; margin entire and rolled under. Spines grow in the axils of the leaves and are short, downward hooked and sharply pointed, broad based and vicious. Petiole is up to 10 mm long, densely velvety. Buds grow in clusters and open into large, fragrant flowers with pale yellowish-green petals, up to 3.5 cm in diameter, encircling a tuft of long, slender, white or pink stamens 3.5 cm long; conspicuous gynophore. The flowers develop into pendulous fruits from the size of a cherry to that of a golf ball, with a stout neck or stalk, globose, 3.5 cm in diameter, pink to bright orange when ripe, often hanging in great numbers, with a semi-transparent bluish-grey flesh surrounding and strongly adhering to the brown seeds. Seeds are oval and smooth. The

generic name is derived from the Arabic 'kapar', the name for *C. spinosa*. The specific name 'tomentosa' means 'densely hairy' in reference to the hairiness of the leaves and branches (Anonymous, 2008b).

Traditional uses: The Zulus of South Africa use it to cure madness, snakebite, headache, impotence and sterility (in women). It is also used to treat fever; mixed with dried hyena and antelope blood and ox fat, it is used in the ritual treatment of pneumonia. A decoction of the leaves is used for the treatment of asthma; a decoction prepared by scraping the bark and mixing it with goat soup is drunk for chest pains. Decoction of the root is a cough remedy, but it must be used with care, as it is highly poisonous when taken in large quantities. Other products: Roots are sometimes used as a love charm. A stick coated with powder from the roots is pointed to the sky as a safeguard against floods, and in Ethiopia, roots are mixed with roots of *Adatoda schimperi* to form a juice that is believed to ward off the evil eye (Anonymous, 2008b).

Biological activity: Aqueous and methanol extracts of *C. tomentosa* were tested for antibacterial activity against *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli* and *Pseudomonas aeruginosa* using a micro-well dilution method (Steenkamp et al., 2004).

Chemical composition: An oxindole alkaloid from the roots of *C. tomentosa* (Dekker et al., 1987) was previously isolated.

Dregea rubicunda K.Schum.

Family: Asclepiadaceae

Synonyms: *Marsdenia rubicunda* (K.Schum.) N.E.Br.

Origin, geographic distribution, and description: no data on origin and geographic distribution, climber/liana up to 4 m tall, leaves are mealy under, fruits 4-winged (Fig 5, Fig 6).

Traditional uses, chemistry and biological activity: According to our best knowledge there are no reports on folk uses, phytochemistry and biological properties of *D. rubicunda.*

Fagaropsis angolensis (Engl.) Dale.

Family: Rutaceae

Synonyms: *Clausena meliodes* Hiern., *Clausenopsis angolensis* (Engl.) Engl., *Fagaropsis oppositifolia* Mildbr., *Vepris angolensis* Engl.

Vernacular names: Mafu, Mfu, Mkunguni, Mtongoti (Tanzania), Muyinja (Kenya)

Origin and geographic distribution: East Africa found in rain and subtropical forests to elevations of about 1829 m.

Description: A large deciduous tree with opposite, imparipinnate leaves (Fig 7, Fig 8). Inflorescences are terminal and flowers unisexual (Waterman and Khalid, 1981).

Traditional uses: Plant is known to be traditionally used to treat malaria (Kirira et al., 2006).

Biological activity: Both methanol and aqueous extracts from stem bark of *F. angolensis* showed significant anti-plasmodial activity against chloroquine-sensitive and chloroquine-resistant strains of *Plasmodium falciparum*; however, methanol extract exhibited significant toxicity in a test with brine shrimp nauplii (Kirira et al., 2006). Chemicals derived from this plant showed fungicidal activity (Bettarini et al., 1993).

Chemical composition: From the stem bark of *F.angolensis* three alkaloids and two limonoids were previously isolated (Waterman and Khalid, 1981) namely new minor alkaloid 6-hydroxymethyldihydronitidine (Khalid and Waterman, 1985) as well as canthin-6-one and 5-methoxycanthin-6-one (Bettarini et al., 1993).

Trichilia prieuriana A.Juss.

Family: Meliaceae

Synonyms: *Trichilia senegalensis* C.DC., *Trichilia heudelotii* var.

Vernacular names: tchivi

Origin and geographic distribution: Often occurs gregariously in both middle and lower storeys and makes the forest appear heavily stocked; its relatively thin foliage permits the growth of shrubs and ground vegetation (Jones, 1955)

Description: Aforesttree, usually in the understorey and 3–14 m high, but sometimes up to 21.34 m high, bole conspicuously fluted, with grey-brown stringy flaking bark (Fig 15), flowers greenish white, fruits pink, seeds black with red arils, mainly in drier types of forest (Anonymous, 2008c).

Traditional uses: Traditionally is stem bark or twigs used against gonorrhoea, fevers and enema (Iwu, 1961; Ayensu, 1978).

Biological activity: According to our best knowledge there are no reports on biological activities of *T. prieuriana.*

Chemical composition: The chemical investigation of the leaves of *T. prieuriana* have resulted in the isolation of two protolimonoids, protolimonoid glucoside and tetracyclic triterpenoids (Olugbade, 1991; Olugbade and Adesanya, 2000).

Turraea floribunda Hochst.

Family: Meliaceae

Synonyms: *Turraea heterophylla* auct.

Vernacular names: wild honeysuckle tree

Origin and geographic distribution: Species is distributed throughout East Africa, being locally found along the east coast of South Africa stretching from the Transkei coast up to Mozambique (Mc Farland et al., 2004).

Description: Deciduous shrub or small tree up to 10 (13) m. tall, sometimes scrambling; first-year branchlets shortly pubescent, second-year purple-brown and glabrous (Fig 9, Fig 10). Leaf-lamina up to 14 × 7 cm, ovate to lanceolate, densely setose when young, more sparsely so later, except on nerves beneath, apex acuminate, base subtruncate, rounded or broadly cuneate; petiole up to 1.5 cm long. Inflorescence is a 2–7-flowered false raceme or flowers subfasciculate; peduncle 3–8 mm long; bracts 2 mm long, squamiform; pedicels 5–17 mm long. Calyx 2.5–5 mm long, densely puberulous to tomentellous. Petals (3.8) 4.5–5.2 × 0.3 cm., greenish-white, linear-spathulate, sparsely puberulous outside. Staminal tube (2.5) 3.6–4.5 cm long, pure white, cylindric, glabrous inside; appendages in pairs alternating with the anthers, 3 mm long, glabrous outside. Ovary 10-locular, puberulous; style (4) 4.8–5.5 cm

long; style-head globose. Capsule 1.3–2.4 × 2 cm, obovoid-cylindric, globose or depressed-globose, deeply ribbed and sulcate, woody, glabrous; aril covering about half of seed (Anonymous, 2008d).

Traditional uses: According to our knowledge gained from local healers in Karamoja region, plant is used to treat malaria symptoms.

Biological activity: Limonoids isolated from root bark of *T. floribunda* showed mosquito larvicidal activity (Ndung'u et al., 2004); leaf aqueous and ethanol extracts possessed *in vitro* anti-inflammatory activity in a cyclooxygenase assay (McGaw et al., 1997).

Chemical composition: Limonoids from the whole plant (Akinniyi et al., 1986), seeds (Fraser et al., 1994; McFarland et al., 2004), stem (Mulholland et al., 1998) and root barks (Torto et al., 1995; Torto et al., 1996) of *T. floribunda* were previously isolated.

Warburgia ugandensis Sprague.

Family: Canellaceae

Synonyms: *Warburgia breyeri* Pott

Vernacular names: Masuka, Mesuka, Sorget Balvigira, zogdom, East African green wood, East African greenheart, greenheart, Kenya greenheart, pepper-bark tree, mukuzanume, muwiya

Origin and geographic distribution: Lowland rain forest, upland dry evergreen forest and its relics in secondary bushland and grassland; termite-hills in swamnp forest; 800-1100-2400 m altitude (Eastern Africa)-Evergreen forest and Tecomaria-Buddleja-Dodonaea bracken thicket with scattered trees (Lebrun and Stork, 2003). Native in Democratic Republic of Congo, Ethiopia, Kenya, Malawi, South Africa, Swaziland, Tanzania, Uganda, exotic in India (Anonymous, 2008f).

Description: Tree up to 42 m tall but often quite small (about 5 m) in many Kenya localities, glabrous (Fig 11, Fig 12). Leaves oblong-lanceolate, elliptic or oblong-elliptic, often a little falcate with costa eccentric, very glossy and dark green above, paler beneath; venation sometimes a little more prominent beneath than in *W. stuhlmannii*, lamina acute at the apex, cuneate at the base and slightly involute, 3–15 cm long and 1.4–5.0 cm wide; petioles 3–5

mm long. Flowers solitary or in small cymes of 3–4, axillary. Bracts thick, 3 mm long and 3–3.5 mm wide, ciliate. Sepals 6–7 mm long and 4–4.5 mm wide; petals 5–7 mm long and 2.5–3 mm wide, obovate-spathulate, rarely with small lateral expansions at the middle. Staminal tube 4–5 mm long and 2–3 mm in diameter, thecae 1.5–2 mm long. Ovary 2.6–4 mm long; ovules 25–30. Style 0.5–1 mm long. Fruit at first greenish and ellipsoidal, later subspherical turning purplish, up to 5 cm in diameter. Seeds compressed, cordate, yellow-brown, 1–1.5 cm long (Verdcourt, B., 1997).

Traditional uses: Local people in the Sango bay forest reserve in southern Uganda swallow dry powdered bark against malaria. Alternatively, smoke from burnt dried powdered bark is inhaled in order to banish evil spirits from possessed victims (Ssegawa and Kasene, 2007).

Biological activity: The methanol extract and several compounds isolated from dichloromethane extract of *W. ugandensis* stem bark exhibited *in vitro* antiviral effect against the measles virus (Parker et al., 2007) and antimycobacterial activity against *Mycobacterium aurum*, *M. fortuitum, M. phlei* and *M. smegmatis* (Wube et al., 2005), respectively. Purification of methanol stem bark extract from *W. ugandensis* yielded muzigadial, a compound that has been found to be effective *in vitro* against the plant pathogenic fungi *Aspergillus niger* (MIC=5 µg/ml) and *Fusarium oxysporum* (MIC=50 µg/ml) using agar macrodilution method (Rugutt et al., 2006).

Chemical composition: Isolated were sesquiterpenes from stem bark (Kioy et al., 1990; Rajab and Ndegwa, 2000; Wube et al., 2005; Wube et al., 2006), flavonol glycosides from a methanolic leaf extract (Manguro et al., 2003).

Zanthoxylum chalybeum Engl.

Family: Rutaceae

Synonyms: *Fagara olitoria* (Engl.) Engl., *Fagara merkeri* Engl., *Fagara chalybea* Engl., *Zanthoxylum olitorium* Engl., *Fagara mpwapwensis* Engl.

Vernacular names: mndungu, mjafari, mtata, mpombo, mrungurungu, mnuwgu, mhombo, msele

Origin and geographic distribution: Range from Somaliland and Ethiopia to S. Rhodesia in dry woodland on termite mounds.

Description: Deciduous shrub or small tree up to 6 m tall; branches glabrous with terminal buds protected by dark scales, aculeate, aculei 2–10 mm long, ± recurved, reddish becoming greyish (Fig 13, Fig14). Leaves 6–20 cm long; petiole 1–5 cm long, somewhat flattened above at the base; rhachis subterete, slightly grooved above, usually aculeate below; leaflets papyraceous to subchartaceous, (1) 2–5-jugate, opposite or occasionally subopposite, sessile or the terminal one sometimes petiolulate with petiolule up to 15 mm long; lamina 2.5–7 × 1–2.5 cm, ovate-oblong to elliptic, acute or bluntly rounded at the apex, margin slightly crenulate or subentire, rounded or obtuse at the base, sparsely dotted with pellucid glands; lateral nerves 6–9 pairs. Inflorescence glabrous, of racemes or panicles up to 9 cm long, borne at the base of the new branches below the first leaves (rarely also in the axils); rhachis flexuous and ± pendulous in male and straight in female plants. Flowers are 4-merous. Male flowers: pedicellate, usually in clusters, with slender pedicels 1.5–2 mm long; sepals 4, 0.4 mm long, united at the base; petals 2.5 × 1 mm, imbricate, elliptic; stamens 4 with filaments as long as the petals; anthers basifixed, deeply 2-lobed at the base; gynophore very short; vestigial ovary 1.5 mm long, ellipsoid. Female flowers: subsessile, usually glomerate on pulvinoid nodes of the racemes; sepals 4; staminodes vestigial, reduced to the aborted anthers; ovary 1·5 mm. long, very oblique, 1-locular, 2-ovulate; ovules subapical, collateral, pendulous, 1 aborted; style short, incurved; stigma broadly discoid, peltate. Fruit is 6 mm in diameter, a subsessile or stipitate somewhat oblique subglobose follicle, glandular-foveolate, with stipe up to 1.5 mm long. Seed is black, shiny (Anonymous, 2008g).

Traditional uses: Local people in the Sango bay forest reserve in southern Uganda drink decoction against malaria (Ssegawa and Kasene, 2007). Traditionally is the infusion drunk against pyomyositis and sterility (Tabuti et al., 2003a).

Biological activity: Methanol extracts of *Z. chalybeum* stem bark exhibited a certain degree of antibacterial activity *in vitro* against *Micrococcus luteus* using agar disc diffusion assay at a concentration of 1 mg/ml and *in vitro* anti-inflammatory activity using the cyclooxygenase assay (Matu and van Staden, 2003). In another study, ethanol extracts of *Z. chalybeum* stem bark showed *in vitro* antimalarial effect against *Plasmodium falciparum* (Gessler et al., 1994).

Chemical composition: Alkaloids from roots of *Z. chalybeum* (Kato et al., 1996) were previously isolated.

2. Objectives

A. The main objective

The aim of this work is antimicrobial and antioxidative activity screening of seven medicinal Ugandan plants: *Capparis tomentosa*, *Dregea rubicunda*, *Fagaropsis angolensis*, *Trichilia prieuriana*, *Turraea floribunda*, *Warburgia ugandensis*, and *Zanthoxylum chalybeum*, which are used in traditional Ugandan folk medicine for treating conditions likely to be associated with pathogenic microorganisms or undesirable oxidative processes.

B. Specific objectives

1. Summarization of information on botany, origin and geographical distribution, ethnopharmacological uses, biological activities and chemical composition of these plants.
2. Determination of minimum inhibitory concentration (MIC) of ethanol extracts from different parts of selected plants using the broth microdilution method and evaluation of radical scavengering activity of aforementioned extracts.
3. Selection of prospective Ugandan medicinal plants as potential source of bioactive constituents for further study.

3. Materials and methods

3.1. Plant material

Plants were selected according to their use in folk medicine to cure cattle and human diseases likely to be associated with pathogenic microorganisms or undesirable oxidative processes by Karamojong healers in northern Uganda. Stem barks of the plant species were collected in northeast Uganda in region Karamoja, the districts of Moroto and Nakapiripirit by J. Grade and P. Van Damme in June 2006. Jean Grade and her team that helped with collecting samples consisted of local traditional livestock healers. Most of them she had a close relationship with as she was living there and helped them to treat their families and livestock. The main ones that were with her during those collections are: Joseph Lokure, Onyang Peter, Agan Joseph, Lopusko Peter, Sagal Joshua Mark, and Damac Felix.

The following samples were collected in the mountains (Kadam and Napak): *W. ugandensis* was collected (1345m 1*45 140N 34*39 846E) at Mt.Kadam, *T. floribunda* (1529m 1*45 305N 34*40 363E) at Mt Kadam, *T. prieuriana* at Mt Kadam while samples of *F. angolensis* were collected at Mt Napak. Samples of *C. Tomentosa* were collected at a mid-level elevation in the foothills of Mt. Kadem. And finally, samples of *D.Rubicunda* and *Z. Chalybeum* were collected in the plains. Voucher specimens authenticated by O. Wanyana-Maganyi are deposited in Makerere University Herbarium in Kampala, Uganda. Ethnomedicinal indications obtained from direct interviews with local healers together with botanical names, families, voucher specimen numbers and common names of tested plants are summarised in Table 1.

Rosmarinus officinalis L. for the antioxidative assay was collected in greenhouse of the Czech University of Life Sciences Prague (CZ).

Tab. 1 Ethnobotanical data of tested Ugandan medicinal plants

Species (family) and voucher specimen number	Common name	Part tested	Ethnomedicinal uses	Preparation
Capparis tomentosa Lam. (Capparaceae) JTG-305 [041]	Eworowogowete	Stem bark	Anaplasmosis in cattle, headaches and jaundice/liver disease in people*	pound root, mix with water, used orally
Dregea rubicunda K.Schum. (Asclepiadaceae) JTG-350 [86]	Lokakuon	Stem bark	sore mouth in children, for teething or gingivitis*	Crush and directly apply to tender area. Rinse and spit out
Fagaropsis angolensis (Engl.) H.M.Gardener (Rutaceae) JTG-481 163	Ekakiret	Stem bark	Snakebite*	Orally
Trichilia prieuriana (Meliaceae) JTG-355 [91]	Lomaran	Stem bark	tetanus, malaria and chest pain in people tetanus and pneumonia in livestock*	Pound bark, soak in water. Give orally
Turraea floribunda Hochst.(Meliaceae) JTG-353 [89]	Doktor	Stem bark	Malaria *	Pound bark, soak in water. Give orally
Warburgia ugandensis Sprague(Canellaeceae) JTG-352 [88]	Abwach	Stem bark	Malaria, yellow fever in people* anaplasmosis, theleriosis and babesiosis in livestock	Pound bark, soak in water. Give orally
Zanthoxylum chalybeum Engl.(Rutaceae) JTG-350 [83]	Euthugu	Stem bark	headache, malaria, jaundice, yellow fever, mouth pain used as a toothbrush*	Pound bark, soak in water. Give orally

* Ethnomedicinal indications obtained from direct interviews with Karamojong healers in northern Uganda

3.2. Extract preparation

Air-dried plant material (15 g of each species) was finely ground and macerated at room temperature in 80% ethanol (450 ml) for 5 days. The extract was subsequently filtered and concentrated using vacuum rotary evaporator (Rotavapor R-200, Büchi, Switzerland) at 40 °C. On analytical scales (Kern 770, Kern & Sohn GmbH, Balingen, Germany) 51.2 mg of each crude extract dried residue was weighed and dissolved in 1 ml of 100% DMSO to create a stock solution of concentration 51.2 mg/ml for determination of antimicrobial activity and stored in +4 °C, whereas for antioxidative assay the mixture of dried extract at a concentration 2.048 mg/ml in methanol was prepared directly prior to testing. The yields of dried residues are shown in Table 2. Ethanol (pharmacological grade), methanol (analytical grade) and DMSO (pure) were purchased from Lach-Ner (Neratovice, CZ).

Tab. 2 Yields of Ugandan plants extracts (in 450 ml of 80% ethanol) after evaporization

Species	Part tested	Yield (g)	Yield (%)
Capparis tomentosa	Stem bark	0.93	6.2
Dregea rubicunda	Stem bark	1.39	9.27
Fagaropsis angolensis	Stem bark	0.48	3.2
Trichilia prieuriana	Stem bark	0.82	5.47
Turraea floribunda	Stem bark	1.19	7.93
Warburgia ugandensis	Stem bark	1.79	11.93
Zanthoxylum chalybeum	Stem bark	0.44	2.93

3.3. Microorganisms

Microbial strains used in assays are known to cause infections indicated by the ethnopharmacological uses of the tested plants, and were selected to represent different groups of pathogenic bacteria and yeast and according to their physical and chemical characteristics and natural resistance pattern. Microorganisms, their taxonomy insertion and pathogenity description are summarized in Table 3. All bacteria used were obtained from the American Type Culture Collection (ATCC): *Enterococcus faecalis* ATCC 2912, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella enteritidis* ATCC 13076, and *Staphylococcus aureus* ATCC 25923. The yeast strain used in this study was

Candida albicans ATCC 10231. All microbial strains purchased from Oxoid (Basingstoke, UK) were grown in Mueller-Hinton broth (Oxoid, Basingstoke, UK).

Tab. 3 Microorganisms used in antimicrobial screening, their classification and pathogenity

Name	**Taxonomy group**	**Clinical Syndromes**
Enterococcus faecalis	G+ cocci	Abscesses, meningitis, wounds, nosocominal and urinary infections
Staphylococcus aureus	G+ cocci	Furunculosis, skin boils, pneumonia, osteomyelitis
Salmonella enteritidis	G- rods	intestinal infections, typhoid fever
Escherichia coli	G- rods	Diarrhoea, urinary infections, meningitis, pneumoniaions,
Pseudomonas aeruginosa	G- rods	Dermatitis, wound, GIT and respiratory infections
Candida albicans	Yeasts	Candidosis, nosocominal infections

Susceptibility test

The susceptibility of microorganisms to Ciprofloxacin was checked as antibiotic control. The antibiotic was prepared in concentrations from 4 to 0.125 μg/ml.

3.4. Chemicals and instruments

Laboratory instruments

Analytical scale KERN 770	Kern & Sohn GmbH,Balingen, GER
Densitometer Densi-La-Meter	Lachema, a.s., Neratovice, CZ
Multiscan Ascent Microplate Photometer	Thermo Fisher Scientific, Waltham, USA
Pipettes (single-, 12-channel, volume 0.1 – 1 ml)	Eppendorf AG, Hamburg, GER
Rotary evaporator	Rotavapor R-200, Büchi, CH
Sartorius filtration device	Sartorius AG, Goettingen, GER

Chemicals

Ascorbic acid (purity: 99%)	Sigma-Aldrich, Prague, CZ
Ethanol 96% pharm.	Lach-Ner, s.r.o., Neratovice, CZ
Dimethyl sulfoxid (DMSO) p.a.	Lach-Ner, s.r.o., Neratovice, CZ

2,2-diphenyl-1-picrylhydrazil (DPPH)	Sigma-Aldrich, Prague, CZ
Tris- buffer saline pH 7.6 (TBS)	Sigma-Aldrich, Prague, CZ
Trolox (purity: 98%)	Sigma-Aldrich, Prague, CZ
Antibiotics	
Ciprofloxacin	Sigma-Aldrich, Prague, CZ
Nystatin	Sigma-Aldrich, Prague, CZ
Cultivation media	
Mueller-Hinton broth (pH 7.4 ± 0.2)	Oxoid, Basingstoke, UK

Composition: Beef, dehydrated infusion 300.0 g/l
Casein hydrolysate 17.5 g/l
Starch 1.5 g/l

The required quantity of broth was weighed on analytical scales, dissolved in distilled water and heated up in case of needs. Later it was divided into penicillin bottles (volume 20 ml), sealed and sterilized by autoclaving. Finished media was stored in refrigerator.

Sterilization

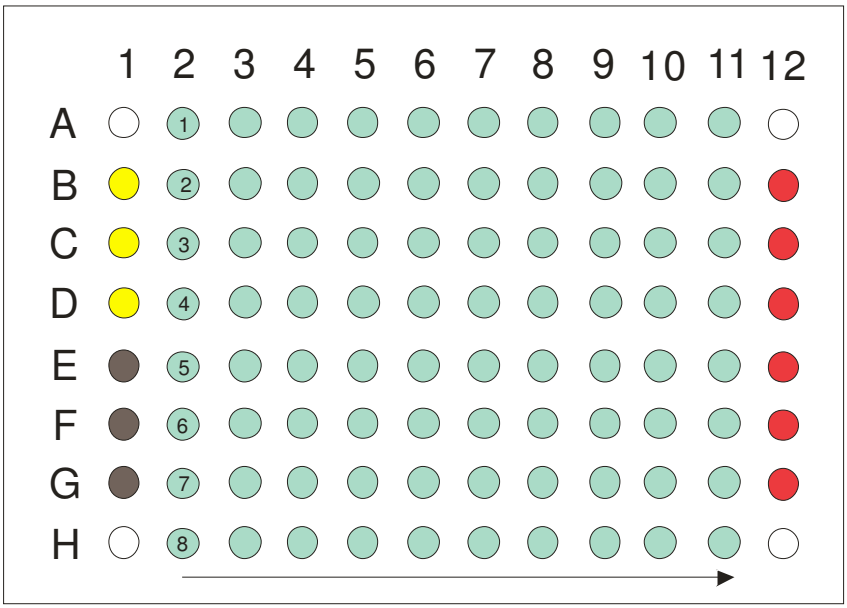
Single-use sterile needles, syringes, 96-well microtitration plates U-type and test tubes were used. Prepared media, TBS and all laboratory material were sterilized in the autoclave at 120°C and 1.2 MPa for 20 minutes.

3.5. Antimicrobial assay

In vitro antimicrobial activity was determined by the broth microdilution method (Jorgensen et al., 1999) using microtitre plates (96 U-shaped wells), modified according to the recommendations recently proposed for more effective assessment of anti-infective potential of natural products (Cos et al., 2006). Two-fold dilutions (ten) of each extract were prepared in Mueller-Hinton broth in concentrations ranging from 512 to 1 µg/ml. Each well was inoculated with 5 µl of bacterial suspension at a density of 10^7 CFU/ml. Finally, one well not containing an antimicrobial agent should be inoculated and used as a growth control and a second un-inoculated well should be used as a broth sterility control. The solution of DMSO (5% v/v) in TBS, for measuring the effect of solvent, was assayed as the negative control, simultaneously. The scheme for filling is shown in Fig.2. Microplates were incubated at 37 °C for 24 h (or for 48 h in case of yeast). Growth of microorganisms was observed as turbidity determined by Multiscan Ascent Microplate Photometer (Thermo Fisher Scientific, Waltham,

USA) at 630 nm. Minimum inhibitory concentrations (MICs) were calculated based on the density of the growth control and were the lowest extract concentrations that resulted in 80% reduction in growth compared with that of the extract-free growth control. Final concentrations of DMSO did not exceed 1% in any sample tested. All samples were tested in triplicate.

Fig. 2 Template for primary integrated in vitro screening



Template for 8 compounds with 10 serial dilutions
Corners of plate not used in data calculation
Non-infected medium control (0% growth of test organism)
Infected control (100% growth of test organism)
Reference drug control (concentrations: 4-0.125 µg/ml)
1 Samples 1- 8 (concentrations: 512-1 µg/ml)

3.6. Free radical scavenging activity assay

The antioxidative activity of all plant extracts was evaluated *in vitro* using 2,2-diphenyl-1-picrylhydrazil (DPPH) free radical scavenging method described previously by Blois (1958) and Brand-Williams (1994) with slight modifications. In disposable microtitre plates (96 flat-bottomed wells), two-fold dilutions (eleven) of each extract were prepared in concentrations ranging from 512 to 0.5 μg/ml. Subsequently, 25 µl of freshly prepared 1mM methanol solution of DPPH (Sigma-Aldrich, Prague, CZ) was mixed with the extract in each well (creating a final volume of 200 µl) to start the radical-antioxidant reaction. The mixture was

incubated in the dark at room temperature and the absorbance of samples was read after 30 minutes at 520 nm using Multiscan Ascent Microplate Photometer (Thermo Fisher Scientific, USA). IC_{50} values were calculated using Magellan V 6.3 software (Tecan Group, Austria). Trolox , ascorbic acid (Sigma-Aldrich, Prague, CZ) and leaf extract of *Rosmarinus officinalis* were tested as positive control. All samples were tested in triplicate.

4. Results

4.1. Antimicrobial activity

Antimicrobial screening showed that all plants tested in this study inhibited growth of at least one of the examined strains at concentration ≤ 512 µg/ml (Table 4). Moreover, five plants from total number of seven selected according to the ethnopharmacological data showed certain degree of antimicrobial properties, which confirms their popular use and justify the ethnobotanical approach in the search for novel biologically active compounds. In contrast to relatively strong susceptibility of Gram-positive bacteria and yeast, both Gram-negative species were highly resistant to all plant extracts tested.

Among them, the extract of *F. angolensis* exhibited the strongest antimicrobial effect, inhibiting growth of *S. aureus* and *C. albicans* with MICs of 64 and 32 µg/ml, respectively. By exhibiting moderate growth inhibitory activity against *E. faecalis* (128 µg/ml) this plant possessed high potential for further testing. The extract of *T. prieuriana* showed significant activity against Gram-positive bacteria and yeast with concentrations ranging from 128 to 512 µg/ml. The weakest results were detected for *T. floribunda*, *Z. chalybeum* and *W. ugandensis*, however, with MICs values (≥ 256 µg/ml) still indicating a certain degree of antimicrobial action. Both remaining species, *C. tomentosa* and *D. rubicunda* possessed no inhibitory properties against the microorganisms tested in this study.

The most susceptible microorganism was the yeast *C. albicans* which yielded to five of all tested plants namely *F. angolensis, T. prieuriana, T. floribunda, W. ugandensis* and *Z. chalybeum* with minimum inhibitory concentrations ranging between 32 µg/ml (*F. angolensis*) and 256 µg/ml (in case of *T. floribunda, W. ugandensis and Z. chalybeum*). Gram-positive bacteria *E. faecalis* was sensitive to *F. angolensis, T. prieuriana,* and *T. floribunda, W. ugandensis*. Other Gram-positive bacteria *S. aureus* was inhibited by three

(50%) of tested plants. None of the Gram-negative bacteria *S. enteritidis, E. coli, P. aeruginosa* were susceptible to plant samples used for this trial.

Tab. 4 Minimum inhibitory concentrations (µg/ml) of ethanol extracts of barks of seven medicinal plants form Uganda

Species, reference compound	Microorganisms					
	Gram-positive bacteria		Gram-negative bacteria			Yeast
	E.f.	S.a.	S.e.	E.c.	P.a.	C.a.
Capparis tomentosa Lam. (Capparaceae) JTG-305 [041]	NA	NA	NA	NA	NA	NA
Dregea rubicunda K.Schum. (Asclepiadaceae) JTG-350 [86]	NA	NA	NA	NA	NA	NA
Fagaropsis angolensis (Engl.) H.M.Gardener (Rutaceae) JTG-481 163	128	64	NA	NA	NA	32
Trichilia prieuriana (Meliaceae) JTG-355 [91]	512	256	NA	NA	NA	128
Turraea floribunda Hochst.(Meliaceae) JTG-353 [89]	512	NA	NA	NA	NA	256
Warburgia ugandensis Sprague(Canellaeceae) JTG-352 [88]	512	256	NA	NA	NA	256
Zanthoxylum chalybeum Engl.(Rutaceae) JTG-350 [83]	NA	NA	NA	NA	NA	256
C/N[a]	1	0.5	0.5	0.015	0.25	4

[a] C: Ciprofloxacin and N: Nystatin were used as positive controls for antibacterial and anticandidal tests. NA- not active (>512 µg/ml). *E.f., Enterococcus faecalis; E.c., Escherichia coli; P.a., Pseudomonas aeruginosa; S.a., Staphylococcus aureus; S.e., Salmonella enteritidis, C.a., Candida albicans*.

4.2. Antioxidative activity

Free radical scavenging activity of the extracts is concentration dependent and lower value of IC_{50} reflects better protective action. Only four extracts were able to reduce the stable free radical DPPH to yellow-coloured diphenylpicrylhydrazine. The results of antioxidative assay summarized in Table 5 show that the best DPPH scavenging activity was produced by the extract of *W. ugandensis* (IC_{50}=7 µg/ml), followed by *Z. chalybeum* (IC_{50}=23 µg/ml), *F. angolensis* (IC_{50}=174.82 µg/ml) and *T. prieuriana* (IC_{50}= 377.02 µg/ml). *C. tomentosa, D. rubicunda* and *T. floribunda* showed no antioxidative activity.

Even though the extract of *W. ugandensis* did not approach either of used positive chemical standards Trolox and ascorbic acid, which possess IC_{50} value of 2.56 mg/ml and 4.35 mg/ml, it has shown higher antioxidant effect than the extract of *Rosmarinus officinalis* L., which is widely considered as extract with high antioxidant properties (Wang et al., 2008; Perez et al., 2007).

Tab. 5 Antioxidative activity (µg/ml) of selected Ugandan plants

Sample	**IC 50**
Capparis tomentosa	> max
Dregea rubicunda	> max
Fagaropsis angolensis	174.82
Trichilia prieuriana	377.02
Turraea floribunda	> max
Warburgia ugandensis	7.0597
Zanthoxylum chalybeum	22.655
Rosmarinus officinalis[a]	7.84
Trolox[a]	3.487
Ascorbic acid[a]	2.3443

[a] *R. officinalis*, Trolox and ascorbic acid were used as positive controls for free radical scavengering test
> max (>512 µg/ml)

5. Discussion

The use of plants in Ugandan folk medicine against various health disorders has been already reported by several authors (Irvine, 1961; Ayensu, 1978; Ssegawa and Kasene, 2007). The most frequent use is for treatment of malaria and general body pains. The primary forms of usage are decoction, infusion and macerate; therefore in order to follow the ethnobotanical approach, 80% ethanol extracts were prepared.

For two plants, no scientific information about their antimicrobial or antioxidative properties was found in the literature. However, it must be said that the majority of plants showed significant responses against various tested microorganisms.

C. tomentosa was previously tested against *S. aureus*, *Streptococcus pyogenes*, *E. coli* and *P. aeruginosa*. The water extract showed activity at 4 mg/ml against *S. aureus* and at 1 mg/ml against *S. pyogenes*, whereas the methanol extract was active against *S. pyogenes* at 4 mg/ml (Steenkamp et al., 2004). Contrastingly, in tests carried out in our trial, *C. tomentosa* ethanol extract didn't show any properties against microorganisms tested. A possible cause for this disagreement in results could be the fact that our study followed the guide for *in vitro* screening of plants with potential antimicrobial properties (Cos et al., 2006). In our study we operated with much lower concentrations of our extracts starting from 512 µg/ml whereas in above mentioned study the samples were tested at higher concentrations (mg/ml). Methanol and water were used as solvents as opposed to ethanol in our study. No reports on antioxidative activity of this plant could be obtained from the literature and furthermore, results from our tests were rather inconclusive. The structure of oxonidole (namely 3-hydroxy-3-methyl-4-methoxyoxindole) was determined in *C. tomentosa* but no reports on activity of this solution are mentioned in the literature. The plant itself was studied for toxic effects of dried leaves and stems. Those ingested by Nubian goats developed signs of toxicosis with inappetence, locomotive disturbances, paresis and recumbency as features as well as anaemia developed and the results of kidney and liver function tests were correlated with clinical abnormalities and pathologic changes (Ahmed et al., 1993). It should be mentioned that, if further testing is to be done, care is needed, especially in vivo testing.

The extract of *D. rubicunda* exhibited no results in our testing. Moreover, in the literature, no mention is made of this plant in connection with antimicrobial or antioxidative properties and its toxicity was according to our knowledge neither proven nor denied.

Even though the extract of *F. angolensis* exhibited the strongest antimicrobial effect in our study, there are no reports on antimicrobial assays in literature. We gained minimum inhibitory concentration of 128, 64 and 32 µg/ml against *E. faecalis, S. aureus* and *C. albicans*, respectively, which shows a good potential for further examinations. Radical scavengering activity of *F. angolensis* extract showed rather promising values according to results achieved in this study whereas no antioxidative activity of this plant was previously described. Canthin-6-one and its dihydroderivative have previously been found to be antimicrobialy active (Bettarini et al., 1993). This compound may explain the strong antimicrobial effects of the extract of *F. angolensis*. However, further investigation on biological activity and phytochemistry of this species should be done in order to explore compounds responsible its for antimicrobial and antioxidative activities. Moreover, systematic toxicological investigations should be performed to confirm safety of this prospective plant-derived product.

T. prieuriana has been found to possess moderate activity against Gram-positive bacteria and yeast. In the literature there is mentioned extraction of significant compounds prieurianoside and prieurone from leaves of *T. prieuriana* (Olugbade, 1991; Olugbade and Adesanya, 2000) but no reports on activities of such compounds were found.

T. floribunda leaf aqueous and ethanol extracts possessed high *in vitro* anti-inflammatory activity in a cyclooxygenase assay (McGaw et al., 1997). Additionaly to this in our study we observed moderate antimicrobial activity of extract against *E. faecalis* and *C. albicans*. Different parts of *T. floribunda* have previously been extracted and showed to contain various limonoids (Akinniyi et al., 1986; Torto et al., 1995; Torto et al., 1996; Mulholland et al., 1998; Fraser et al., 1994; McFarland et al., 2004) where one of them presented mosquito larvicidal activity (Ndung'u et al., 2004). Many studies have been done on species from the same family all mentioning extraction of substances only.

Compounds isolated from stem bark of *W. ugandensis* were positively evaluated for inhibition of 12(S)-HETE which is implicated as a critical signalling molecule in tumour metastasis, atherosclerotic processes and the mediator in hyperproliferation of the skin (Wube et al.,

2006). The dichloromethane extract of the stem bark of *W. ugandensis* afforded sesquiterpene which was examined for its antimycobacterial activity against *Mycobacterium aurum*, *M. fortuitum*, *M. phlei* and *M. smegmatis* with minimum inhibitory concentration ranging from 4 to 128 µg/ml (Wube et al., 2005). Methanol extract of *W. ugandensis* had an extremely noticeable ability to neutralize measles virus during pre-incubation period (Parker et al., 2007) which supports our results, where the extract of *W. ugandensis* showed activity against both Gram-positive bacteria and yeast. Radical scavengering activity of *W.ugandensis* was very high and had even better IC_{50} value than commonly used extract of *Rosmarinus officinalis*.

Aqueous, hexane and methanol extracts of *Z. chalybeum* were screened for *in vitro* antibacterial and anti-inflammatory activities using the agar diffusion method and cyclooxygenase-1 assay, respectively. In this study the inhibitory activity was detected against Gram-positive bacteria (Matu and van Staden, 2003), which is in contrast to our results showing only *C. albicans* as a sensitive strain influenced by *Z. chalybeum* extract.

No Gram-negative bacteria were inhibited by any plant used in this study. In general, the literature describes higher susceptibility of Gram-positive bacteria to plant extracts and a greater resistance of Gram-negative bacteria (Shelef et al., 1980; Kudi et al., 1999; Palombo and Semple, 2001). A lack of activity against Gram-negative bacteria could be accounted for by the presence of a thick murein layer, which prevents the entry of inhibitors into the cell (Matu and van Staden, 2003; Murray and Moellering, 1981).

6. Conclusions

In summary, antimicrobial testing results show that *F. angolensis* stem bark ethanol extract possessed selective inhibitory activity against *S. aureus* and *C. albicans* at concentrations below 100 µg/ml, which according to criteria previously suggested by Rios and Recio (2005) would indicate its highly prospective antimicrobial properties. Additionally, the ethanol extract from *W. ugandensis* stem bark, even as a complex mixture, exhibited strong antioxidative action with IC_{50} value very close to the inhibitory effect achieved by reference compound Trolox (IC_{50}=3 µg/ml), suggesting its potent antioxidative properties. Since, according to our best knowledge, the main antimicrobial and antioxidative principles of *F. angolensis*, and *W. ugandensis* stem barks have not been identified yet, bioassay-guided fractionation of these two plants is currently underway in our laboratories with a goal to establish types of compounds responsible for their marked biological properties.

Antimicrobial and antioxidative study of seven Ugandan plants, all of them selected based on their relevant ethnomedical use, has provided various extracts with strong activity against several pathogenic microorganisms. The results of this study support the knowledge about plant activities which was gained from indigenous people. According to our best knowledge, this is the first report on antimicrobial activity of extracts from *D. rubicunda* and *T. prieuriana*.

The ethanol extracts of *F. angolensis* exhibited the most promising results suggesting its potential use in food or pharmaceutical industry for development of new antimicrobialy effective herbal-based nutraceuticals, functional foods, food additives and pharmaceutical or veterinary preparations.

Additionally the antioxidative activity of plant extracts was measured. The best radical scavengering activity was produced by sample of *W. ugandensis*. According to this result, *W. ugandensis* had significantly higher activity than well known and widely used rosemary essential oil, what makes it a promising source of new antioxidative agent.

However, it is still unknown, which compounds are responsible for significant biological activity of selected plants. Thus further bioassay-guided isolation and identification of the active principles of these plants are required. Furthermore extracts need to be tested for their potential toxicity.

7. References

Ahmed, S.A.R., Amin, A.E., Adam, S.E.I., Hapke, H.J., 1993. DTW. Deutsche tierärztliche Wochenschrift 100, 192 -194.

Akinniyi, J.A., Connolly, J.D., Mulholland, D.A., Rycroft, D.S., Taylor, D.A.H., 1986. Limonoid extractives from *Turraea floribunda* and *T. obtusifolia*. Phytochemistry 25, 2187–2189.

Anonymous, 2008a. Karamoja: The Region. Available [on-line] from http://www.karamoja.com/karamoja.html [Accessed 28 March2008].

Anonymous, 2008b. Agroforestry Tree database. Available [on-line] from http://www.worldagroforestry.org/Sea/Products/AFDbases/AF/asp/SpeciesInfo.asp?SpID=439 [Accessed 25 February 2008].

Anonymous, 2008c. Aluka-Species information. Available [on-line] from http://www.aluka.org/action/showCompilationPage?doi=10.5555/AL.AP.COMPILATION.PLANT-NAME-SPECIES.TRICHILIA.PRIEURIANA&cookieSet=1[Accessed 23 February 2008].

Anonymous, 2008d. Aluka-Species information. Available [on-line] from http://www.aluka.org/action/showMetadata?doi=10.5555/AL.AP.FLORA.FZ1428&pgs=[Accessed 23 February 2008].

Anonymous, 2008e. Aluka-Species information. Available [on-line] from http://ts-den.aluka.org/fsi/img/size2/alukaplant/pre/phase_01/pre0004/pre0272164-0.jp [Accessed 20 April 2008].

Anonymous, 2008f. Agroforestry Tree database. Available [on-line] from http://www.worldagroforestry.org/SEA/Products/AFDbases/AF/asp/SpeciesInfo.asp?SpID=1699 [Accessed 25 February 2008].

Anonymous, 2008g. Aluka-Species information. Available [on-line] from http://www.aluka.org/action/showCompilationPage?doi=10.5555/AL.AP.COMPILATION.PLANT-NAME-SPECIES.ZANTHOXYLUM.CHALYBEUM [Accessed 25 February 2008].

Ayensu, E.S., 1978. Medicinal plants of West Africa. Refernce Publications, Inc., Michigan, p.178.

Bettarini, F., Borgonovi, G.E., Fioranti, T., Gagliardi, I., Caprioli, V., Massardo, P., Ogoche, J.I.J., Hassanali, A., Nyandat, E., Chapya, A., 1993. Antiparasitic compounds from East-

African plants-isolation and biological activity-activity of anonaine, matricarianol, canthin-6-one and caryophyllene oxide. Insect Science and its Application 14, 93-99.

Borris, R.P., 1996. Natural products research: perspectives from a major pharmaceutical company. Journal of Ethnopharmacology 51, 29–38.

Blois, M.S., 1958. Antioxidant determinations by the use of a stable free radical. Nature 26, 1199–1200.

Brand-Williams, W., Cuvelier, M.E., Berset, C., 1994. Use of a free radical method to evaluate antioxidant activity. Lebensmittel Wissenschaft und Technologie 28, 25–30.

Bukenya-Ziraba, R., Kamoga, D., 2007. An inventory of medicinal plants used in treating poultry diseases in Jinja district, eastern Uganda. African Journal of Ecology 45, 31-38.

Cos, P., Vlietinck, A.J., Vanden Berghe, D., Maes, L., 2006. Anti-infective potential of natural products: How to develop a stronger *in vitro* 'proof-of-concept'. Journal of Ethnopharmacology 106, 290–302.

Dekker, T.G., Fourie, T.G., Matthee, E., Snyckers, F.O., 1987. An oxindole from the roots of *Capparis tomentosa*. Phytochemistry 26, 1845-1846.

De Pasquale, A., 1984. Pharmacognosy: the oldest modern science. Journal of Ethnopharmacology 11, 1–16.

Egadu, S.P., Mucunguzi, P., Obua, J., 2007. Uses of tree species producing gum arabic in Karamoja, Uganda. African Journal of Ecology 45, 17–21.

Fraser, L.A., Mulholland, D.A., Nair, J.J., 1994. Limonoids from the seed of *Turraea floribunda*. Phytochemistry 35, 455–458.

Freiburghaus, F., Ogwal, E.N., Nkunya, M.H.H., Kaminsky, R., Brun, R., 1996. *In vitro* antitrypanosomal activity of African plants used in traditional medicine in Uganda to treat sleeping sickness. Tropical Medicine and International Health 1, 765-771.

Geissler, P.W., Meinert, L., Prince, R., Nokes, C., Aagaard-Hansen, J., Jitta, J., Ouma. J.H., 2001. Self-treatment by Kenyan and Ugandan schoolchildren and the need for school-based education. Health Policy and Planning 16, 362-371.

Gradé, J.T., Tabuti, J.R.S., Van Damme, P., Arble, B.L., 2007. Deworming efficacy of *Albizia anthelmintica* in Uganda: preliminary findings. African Journal of Ecology 45, 18–20.

Gradé, J.T., Tabuti, J.R.S., Van Damme, P., 2008. Botany by instinct: Self medicating livestock of Karamoja, Uganda. Economic Botany (Submitted).

Gessler, M.C., Nkunya, M.H.H., Mwasumbi, L.B., Tanner, M., 1994. Screening Tanzanian medicinal plants for antimalarial activity. Acta tropica 56, 65-77.

Hamburger, M. and Hostettmann, K., 1991. Bioactivity in plants: the link between phytochemistry and medicine. Phytochemistry 30, 3864–3874.

Hamill, F.A., Apio, S., Mubiru, N.K., Mosango, M., Bukenya-Ziraba, R., Maganyi, O.W., Soejarto, D.D., 2000. Traditional herbal drugs of southern Uganda, I. Journal of Ethnopharmacology 70, 281-300.

Homsy, J., Katabira, E., Kabatesi, D., Mubiru, F., Kwamya, L., Tusaba, C., Kasolo, S., Mwebe, D., Ssentamu, L., Okello, M., King, R., 1999. Evaluating herbal medicine for the management of Herpes zoster in human immunodeficiency virus-infected patients in Kampala, Uganda. Journal of Alternative and Complementary Medicine 5, 553-565.

IK Notes, 2003. Traditional medicinal Practice in Contemporary Uganda. Available [on-line] from http://www.worldbank.org/afr/ik/default.htm. [Accessed 21April 2008].

Iwu, M.M., 1993. Handbook of African medicinal plants. CRC Press, Inc.Florida, p.46.

Jachak, S.M., Saklani, A., 2007. Challenges and opportunities in drug discovery from plants. Current science 92, 1251-1257.

Jones, E.W., 1955. Ecological Studies on the Rain Forest of Southern Nigeria: IV. The Plateau Forest of the Okomu Forest Reserve. The Journal of Ecology 43, 564-594.

Jorgensen, J.H., Turnidge, J.D., Washington, J.A.: Antibacterial susceptibility tests: Dilution and disk diffusion methods. In: Murray, P.R., Baron, E.J., Pfaller, M.A., Tenover, F.C., Yolken, R.H., editors. Manual of clinical microbiology. Washington, D.C., USA: ASM Press. 1999. p. 1526-1543.

Kamatenesi-Mugisha, M., Oryem-Origa, H., Olwa-Odyek, 2007. Medicinal plants used in some gynaecological morbidity ailments in western Uganda. African Journal of Ecology 45, 34–40.

Kato, A., Moriyasu, M., Ichimaru, M., Nishiyama, Y., 1996. Isolation of alkaloidal constituents of *Zanthoxylum usambarense* and *Zanthoxylum chalybeum* using ion-pair HPLC. Journal of Natural Products 59, 316-318.

Katuura, E., Waako, P., Ogwal-Okeng, J., Bukenya-Ziraba, R., 2007. Traditional treatment of malaria in Mbarara District, western Uganda. African Journal of Ecology 45, 48-51.

Khalid, S.A.,Waterman, P.G., 1985. 6-hydroxymethyldihydronitidine from *Fagaropsis angolensis*. Journal of Natural Products 48, 118-119.

Kioy, D., Gray, A.I., Waterman, P.G., 1990. A comparative study of the stem-bark drimane sesquiterpenes and leaf volatile oils of *Warburgia ugandensis* and *W. Stuhlmannii*. Phytochemistry 29, 3535-3538.

Kirira, P.G., Rukungab, G.M., Wanyonyi, A.W., Muregi, F.M., Gathirwa, J.W, Muthaura, C.N., Omarc, S.A., Tolob, F., Mungaid, G.M., Ndiege, I.O., 2006. Anti-plasmodial activity and toxicity of extracts of plants used in traditional malaria therapy in Meru and Kilifi Districts of Kenya. Journal of Ethnopharmacology 106, 403-407.

Krief, S., Thoison, O., Sevenet, T., Wrangham, R.W., Lavaud, C., 2005a. Triterpenoid saponin anthranilates from Albizia grandibracteata leaves ingested by primates in Uganda. Journal of Natural Products 68, 897-903.

Krief, S., Hladik, C.M., Haxaire, C., 2005b. Ethnomedicinal and bioactive properties of plants ingested by wild chimpanzees in Uganda. Journal of Ethnopharmacology 101, 1-15.

Krief, S., Huffman, M.A., Sevenet, T., Hladik, C.M., Grellier, P., Loiseau, P.M., Wrangham, R.W., 2006. Bioactive properties of plant species ingested by chimpanzees (Pan troglodytes schweinfurthii) in the Kibale National Park, Uganda. American Journal of Primatology 68, 51-71.

Kudi, A.C., Umoh, J.U., Eduvie, L.O., Gefu, J., 1999. Screening of some Nigerian medicinal plants for antibacterial activity Journal of Ethnopharmacology 67, 225-228.

Labadie, R.P., 1986. Problems and possibilities in the use of traditional drugs. Journal of Ethnopharmacology 15, 221–230.

Lebrun J.P., Stork A.L. Tropical African Flowering plants; Ecology and distribution; Volume 1: Annonaceae-Balanitaceae. Ville de Géneve: Département des affaires culturelles, editions des :conservatoire et jardin botaniques, 2003. ISBN 2-8277-0114-6.

Marston, A., Maillard, M., Hostettmann, K., 1993. Search for antifungal, molluscocidal and larvicidal compounds from African medicinal plants. Journal of Ethnopharmacology 38, 209-214.

Manguro, L.O.A., Ugi, I., Lemmen, P., Hermann, R., 2003. Flavonol glycosides of *Warburgia ugandensis* leaves. Phytochemistry 64, 891-896.

Matu, E.N., van Staden, J., 2003. Antibacterial and anti-inflammatory activities of some plants used for medicinal purposes in Kenya. Journal of Ethnopharmacology 87, 35-41.

Mc Farland, K., Mulholland, D.A., Fraser, L.A., 2004. Limonoids from *Turraea floribunda* (Meliaceae). Phytochemistry 65, 2031-2037.

McGaw, L.J., Jäger, A.K., van Staden, J., 1997. Prostaglandin synthesis inhibitory activity in Zulu, Xhosa and Sotho medicinal plants. Phytotherapy Research 11, 113-117.

Miller, N.N., 1980. Traditional medicine in East Africa: the search for a synthesis. American Universities Field Staff Reports. Africa Series 22, 1–15.

Ministry of Health. (2000) *Health Sector Strategic Plan 2000/01–2004/05*, Ministry of Health, Uganda. Available [on-line] from http://www.health.go.ug/docs/HSSPfinalEdition.pdf [Accessed 21 May 2008].

Mitscher, L.A., Leu, R.P., Bathala, M.S., Wu, W., Beal, J., White, R. (1972): Antimicrobial agents from higher plants. I. Introduction, rationale and methodology. Lloydia. 35(2):157-66.

Mulholland, D.A., Monkhe, T.V., Coombes, P.H., Rajab, M.S., 1998. Limonoids from Turraea holstii and Turraea floribunda. Phytochemistry 49, 2585-2590.

Murray, B.E., Moellering, R.C., 1981. Cephalosporins. Annual Review of Medicine 32, 559-581.

Ndung'u, M.W., Kaoneka, B., Hassanali, A., Lwande, W., Hooper, A.M., Tayman, F., Zerbe, O., Torto, B., 2004. New mosquito larvicidal tetranortriterpenoids from *Turraea wakefieldii* and *Turraea floribunda*. Journal of Agricultural and Food Chemistry 52, 5027-31.

Okello, J., Ssegawa, P., 2007. Medicinal plants used by communities of Ngai Subcounty, Apac District, northern Uganda. African Journal of Ecology 45, 76–83.

Olugbade, T.A., 1991. Tetracyclic triterpenoids from *Trichilia prieuriana* leaves. Phytochemistry 30, 698-700.

Olugbade, T.A., Adesanya, S.A., 2000. Prieurianoside, a protolimonoid glucoside from the leaves of *Trichilia prieuriana*. Phytochemistry 54, 867-870.

Oryem-Origa, H., Katende, A.B., Kakudidi, E.K.Z., 2001. Ethnobotanical studies of Mabira Forest area, Central Uganda. Discovery and Innovations (special edition), African Academy of Sciences, Nairobi, 169–181.

Palombo, E.A., Semple, S.J., 2001. Antibacterial activity of traditional Australian medicinal plants. Journal of Ethnopharmacology 77, 151-157.

Parker, M.E., Chabot, S., Ward, B.J., Johns, T., 2007. Traditional dietary additives of the Maasai are antiviral against the measles virus. Journal of Ethnopharmacology 114, 146-152.

Perez, M.B., Calderon, N.L., Croci, C.A., 2007. Radiation-induced enhancement of antioxidant activity in extracts of rosemary (Rosmarinus officinalis L.). Food Chemistry 104, 585-592.

Rajab, M.S., Ndegwa, J.M., 2000. 11 alpha-hydroxy muzigadiolide, a novel drimane sesquiterpene from the stem bark of *Warburgia ugandensis*. Bulletin of the Chemical Society of Ethiopia 14, 45-49.

Rates S.M.K., 2001. Plants as source of drugs. Toxicon 39, 603-613.

Rugutt, J.K., Ngigi, A.N., Rugutt, K.J., Neslut, P.K., 2006. Native Kenyan plants as possible alternatives to methyl bromide in soil fumigation. Phytomedicine 13, 576-583.

Shelef, L.A., Naglik, O.A., Bogen, D.W., 1980. Sensitivity of some common foodborne bacteria to the spices sage, rosemary and allspice. Journal of Food Science 45, 1042-1044.

Singh, G., Marimuthu, P., de Heluani, C.S., Catalan, C., 2005. Chemical constituents and antimicrobial and antioxidant potentials of essential oil and acetone extract of *Nigella sativa* seeds. Journal of the Science of Food and Agriculture 85, 2297-2306.

Ssegawa, P., Kasene, J.M., 2007. Medicinal plant diversity and uses in the Sango bay area southern Uganda. Journal of Ethnopharmacology 113, 521-540.

Steenkamp, V., Mathivha, E., Gouws, M.C., van Rensburg, C.E.J., 2004. Studies on antibacterial, antioxidant and fibroblast growth stimulation of wound healing remedies from South Africa. Journal of Ethnopharmacology 95, 353-357.

Tabuti, J.R.S., Lye, K.A., Dhillion, S.S., 2003a. Traditional herbal drugs of Bulamogi, Uganda: plants, use and administration. Journal of Ethnopharmacology 88, 19-44.

Tabuti, J.R.S., Dhillion, S.S., Lye, K.A., 2003b. Ethnoveterinary medicines for cattle (Bos indicus) in Bulamogi county, Uganda: plant species and mode of use. Journal of Ethnopharmacology 88, 279-286.

Tabuti, J.R.S., 2004. The traditional medicine practitioners (TMPs) and attitudes of the rural community of Bulamogi County (Uganda) towards traditional medicine: preliminary findings. African Journal of Ecology 42, 40-41.

Tabuti, J.R.S., 2008. Herbal medicines used in the treatment of malaria in Budiope county, Uganda. Journal of Ethnopharmacology 116, 33-42.

Thring, T.S.A., Springfield, E.P., Weitz, F.M., 2007. Antimicrobial activities of four plant species from the Southern Overberg region of South Africa. African Journal of Biotechnology 6, 1779-1784.

Torto, Bentley, M.D., Cole, B.J.W., Hassanali, A., Huang, F.-Y., Gelbaum, L., Vanderveer, D.G., 1995. Limonoids from *Turraea floribunda.* Phytochemistry 40, 239-243.

Torto, B., Hassanali, A., Nyandat, E., Bentley, M.D., 1996. A limonoid from *Turraea floribunda*. Phytochemistry 42, 1235-1237.

van den Berghe, D.A., Vlientick, A.J., 1991. Screening methods for antibacterial and antiviral agents from higher plants. In Methods in plant Biochemistry. Academic press. Vol.6 47-69.

Verdcourt, B.,1997. Flora of tropical east Africa. CRC Press. ISBN 9061913780

Wang, W., Wu, N., Zu, Y.G., Fu, Y.J., 2008. Antioxidative activity of Rosmarinus officinalis L. essential oil compared to its main components. Food Chemistry 108, 1019-1022 .

Waterman, P.G., Khalid, S.A., 1981. The biochemical systematics of *Fagaropsis angolensis* and its significance in the Rutales. Biochemical Systematics and Ecology 9, 45-51.

WHO, 2002 World Health Organisation, WHO Traditional Medicine Strategy 2002–2005, World Health Organisation, Geneva (2002) WHO/EDM/TRM/2002.1. Available [on-line] http://whqlibdoc.who.int/hq/2002/WHO_EDM_TRM_2002.1.pdf [Accessed 19 January 2008].

Wube, A.A., Bucar, F., Gibbons, S., Asres, K., 2005. Sesquiterpenes from *Warburgia ugandensis* and their antimycobacterial activity. Phytochemistry 66, 2309-2315.

Wube, A.A., Gibbons, S., Asres, K., Streit, B., Adams, M., Bauer, R., Bucar, F., 2006. *In vitro* 12(S)-HETE and leukotriene metabolism inhibitory activity of sesquiterpenes of *Warburgia ugandensis*. Planta Medica 72, 754-756.

8. Appendices

Appendix A: Photographic ilustrations of plant species

Fig 3 *Capparis tomentosa*

Original photo by J.Gradé

Fig 4 *Capparis tomentosa*-bark

Original photo by M.Kuglerová

Fig 5 *Dregea rubicunda*

Original photo by J.Gradé

Fig 6 *Dregea rubicunda*–bark

Original photo by M.Kuglerová

Fig 7 *Fagaropsis angolensis*

Source: Anonymous, 2008e

Fig 8. *Fagaropsis angolensis*-bark

Original photo by M.Kuglerová

Fig 9 *Turraea floribunda*

Original photo by J.Gradé

Fig 10 *Turraea floribunda*-bark

Original photo by M.Kuglerová

Fig 11 *Warburgia ugandensis*

Original photo by J.Gradé

Fig 12 *Warburgia ugandensis*-bark

Original photo by M.Kuglerová

Fig 13 *Zanthoxylum chalybeum*

Original photo by J.Gradé

Fig 14 *Zanthoxylum chalybeum*-bark

Original photo by M.Kuglerová

Fig 15 *Trichilia prieuriana*-bark

Original photo by M.Kuglerová

Appendix B. List of publications related to the thesis

Kuglerová, M., Halamová, K., Kokoška, L., Van Damme, P., Grade, J. Antimicrobial activity of Ugandan medicinal plants. 55th International Congress and Annual Meeting of the Society for Medicinal Plant Research, 02.09.-06.09 2007, Graz, Austria [published in: Planta Medica, 2007, 73 (9): 858-859; abstract P113].