Full Length Research Paper

Antimicrobial and antioxidative effects of Ugandan medicinal barks

Marta Kuglerova[1], Hana Tesarova[1], Jean T. Grade[2], Katerina Halamova[1], Olivia Wanyana-Maganyi[3], Patrick Van Damme[2] and Ladislav Kokoska[1]*

[1]Department of Crop Science and Agroforestry, Institute of Tropics and Subtropics, Czech University of Life Sciences Prague, Kamycka 129, 165 21 Prague 6-Suchdol, Czech Republic.
[2]Laboratory for Tropical and Subtropical Agriculture and Ethnobotany, Department of Plant Production, Faculty of Bio-Science Engineering, Ghent University, Coupure links 653, 9000 Ghent, Belgium.
[3]Department of Botany, Makerere University, P. O. Box 7062, Kampala, Uganda.

Despite a rich tradition of medicinal plants use by local communities in Uganda their direct antimicrobial effectives together with potential to protect human health against diseases induced by oxidative stress are still poorly documented. The aim of this study is to investigate the *in vitro* antimicrobial and total antioxidative activities of barks from five tree species selected using information on their traditional use by Karamojong healers in Uganda. The antimicrobial activity of crude ethanol extracts of *Fagaropsis angolensis* (Engl.) H. M. Gardener (Rutaceae), *Trichilia prieuriana* A. Juss. (Meliaceae), *Turraea floribunda* Hochst. (Meliaceae), *Warburgia salutaris* Sprague (Canellaeceae) and *Zanthoxylum chalybeum* Engl. (Rutaceae) were tested against four bacteria and one yeast species using the broth microdilution method. The total antioxidative activity was determined by 2,2-diphenyl-1-picrylhydrazil free radical scavenging assay. It was found that the extract of *F. angolensis* possess the strongest antimicrobial activity inhibiting growth of *Staphylococcus aureus* and *Candida albicans* with minimum inhibitory concentrations of 64 and 32 µg/ml, respectively. Among all plants tested, *W. salutaris* showed the most promising antioxidative properties (IC_{50} = 6.59 µg/ml). As a result of this study, *F. angolensis* and *W. salutaris* possessed significant antimicrobial and antioxidative effects which indicates prospective pharmacological properties of both species.

Key words: Antimicrobial activity, total antioxidative activity, crude extracts, medicinal plants, Uganda.

INTRODUCTION

At present, medicinal plants still represent largely un-explored resources for the development of new effective drugs. Herbal medicine continues to play an essential role to cover the basic health needs in many developing countries, including Uganda. However, despite a well-

documented and rich tradition of Ugandan medicinal plants use as anti-infective agents (Kamatenesi-Mugisha et al., 2008), the direct antimicrobial effectives of the species most frequently utilized by local communities in some areas for example, in Karamoja region are still poorly documented. Even though certain attempts to monitor antioxidative properties of Ugandan fruits have previously been made (Stangeland et al., 2007), the potential of medicinal plants to protect human health against diseases induced by oxidative stress still remains to be explored. Thus, we decided to investigate the antimicrobial and total antioxidative activities of five Ugandan tree species selected using information on their

*Corresponding author. E-mail: kokoska@its.czu.cz. Tel: +420 224382180. Fax: + 420 234381829.

Abbreviations: DMSO, Dimethyl sulfoxide; MICs, minimum inhibitory concentrations; DPPH, 2, 2-diphenyl-1-picrylhydrazyl.

Table 1. Ethnobotanical data on medicinal plants.

Species (family) and voucher specimen number	Common name	Ethnomedicinal uses[1]
Fagaropsis angolensis (Engl.) H.M.Gardener (Rutaceae) JTG-481	Ekakiret	Pneumonia, respiratory infection in humans, contagious bovine/caprine pleuropneumonia
Trichilia prieuriana A.Juss. (Meliaceae) JTG-355	Lomaran	Tetanus, malaria and chest pain in people, tetanus and pneumonia in livestock
Turraea floribunda Hochst. (Meliaceae) JTG-353	Doktor	Malaria
Warburgia salutaris Sprague (Canellaeceae) JTG-037	Abwach	Yellow fever in humans, anaplasmosis, theleriosis and babesiosis in livestock
Zanthoxylum chalybeum Engl. (Rutaceae) JTG-347	Eusugu	Headache, malaria, jaundice, yellow fever, mouth pain used as a toothbrush

[1] Ethnomedicinal indications obtained from direct interviews with Karamojong healers in northern Uganda.

Table 2. Antimicrobial and total antioxidative activities of ethanol extracts from stem barks of Ugandan medicinal plants.

Plant species or reference compound	Yield (%)	Microorganisms[1]/Minimum inhibitory concentrations(µg/ml)					DPPH test IC_{50} (µg/ml)
		Gram-positive bacteria		Gram-negative bacteria		Yeast	
		E. f.	*S. a.*	*E. c.*	*P. a.*	*C. a.*	
F. angolensis	3.20	128	64	-[2]	-	32	174.85 ± 4.86
T. prieuriana	5.47	512	256	-	-	128	377.02 ± 9.21
T. floribunda	7.93	512	-	-	-	256	-
W. salutaris	11.93	512	256	-	-	256	6.59 ± 2.84
Z. chalybeum	2.93	-	-	-	-	256	22.66 ± 7.88
C/N/T[3]		1	0.5	0.015	0.25	4	3.49 ± 0.19

[1]*C.a.*, *Candida albicans*; *E.c.*, *Escherichia coli*; *E.f.*, *Enterococcus faecalis*; *P.a.*, *Pseudomonas aeruginosa*; *S.a.*, *Staphylococcus aureus*; [2] not active (> 512 µg/ml); [3]C, Ciprofloxacin; N, Nystatin; and T, Trolox were used as positive controls for antibacterial, anticandidal and free radical scavenging tests, respectively.

traditional use by Karamojong healers as evidenced by ethnobotanical research.

MATERIALS AND METHODS

Plant material

Ethnomedicinal indications obtained from direct interviews with local healers together with botanical names, families, voucher specimen numbers and common names of tested plants are summarized in Table 1. Plant species were selected according to their use in folklore medicine to cure cattle and human diseases, based on reports from Karamojong healers. The stem bark was collected in northeast Uganda in Karamoja region, the districts of Moroto and Nakapiripirit by J. Grade and P. Van Damme in June 2006. Voucher specimens authenticated by O. Wanyana-Maganyi are deposited in Makerere University Herbarium in Kampala, Uganda. All plant materials together with ethnobotanical data were collected in cooperation with local non-governmental organization Karamoja Christian Ethnoveterinary Program (KACHEP) with respect to the rights of indigenous people and rules for biodiversity and traditional knowledge conservation. The details on methodology of the ethnobotanical part of this work are described in recent study on

ethnoveterinary knowledge in pastoral Karamoja published by Grade et al. (2009).

Preparation of extracts

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 to dryness using a rotary evaporator Rotavapor R-200 (Buchi, Switzerland) in vacuum at 40°C. For determination of antimicrobial activity, dried residue was dissolved in dimethyl sulfoxide (DMSO) to create a 51.2 mg/ml concentration of stock solution which was stored at +4°C until tested, whereas for antioxidative assay the mixture of dried extract at a concentration 2.048 mg/ml in methanol was prepared directly prior to testing. Dried residue yields are shown in Table 2. Ethanol (pharmacological grade), methanol (analytical grade) and DMSO (pure) were purchased from Lach-Ner (Neratovice, CZ).

Microorganisms and culture conditions

Antimicrobial effectiveness of extracts was evaluated against one yeast and five bacterial strains, selected as representatives of both

classes of Gram-positive and Gram-negative bacteria. The following American Type Culture Collection (ATCC) strains were used: *Enterococcus faecalis* ATCC 2912, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, 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). Ciprofloxacin and nystatin (Sigma-Aldrich, Prague, CZ) were used as positive controls (Table 2).

Antimicrobial assay

In vitro antimicrobial activity was determined by broth microdilution method (NCCLS, 2008) using microtiter 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). Ten two-fold serial dilutions 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 a bacterial suspension at a density of 10^7 CFU/ml. Microplates were incubated at 37°C for 24 h (or for 48 h in the 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 expressed as the lowest extract concentrations that resulted in 80% growth reduction compared to that of the extract-free growth control. Final concentrations of DMSO did not exceed 1% in any sample tested. All samples were tested as three independent experiments, each carried out in triplicate.

Antioxidative test

Total antioxidative activity of extracts was evaluated *in vitro* using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging method described previously by Brand-Williams et al. (1994) with slight modifications. In disposable microtiter plates (96 flat-bottomed wells), eleven two-fold serial dilutions of each extract were prepared in concentrations ranging from 512 to 0.5 μg/ml. Subsequently, 25 μl of freshly prepared 1 mM 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 absorbance of samples was read after 30 min 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 (Sigma-Aldrich, Prague, CZ) was tested as positive control (Table 2). Results are expressed as the average of three independent experiments where each sample was tested in triplicate.

RESULTS AND DISCUSSION

Antimicrobial screening showed that all plants tested in this study inhibited growth of at least one of the examined strains at concentrations ≤ 512 μg/ml (Table 2). In contrast to the relatively strong susceptibility of Gram-positive bacteria and yeast, both Gram-negative species were highly resistant to all plant extracts tested. Among the latter, the extract of *Fagaropsis angolensis* (Engl.) H.

M. Gardener exhibited the strongest antimicrobial effect, inhibiting growth of *S. aureus* and *C. albicans* with MICs of 64 and 32 μg/ml, respectively. Although certain alkaloids for example, canthin-6-one and 5-methoxycanthin-6-one isolated from root methanol extract of this tree have previously been shown to possess *in vitro* growth inhibitory effects against phytopathogenic fungi such as *Botrytis cinerea*, *Fusarium monoliforme*, *Ustilago maydis*, or *Streptomyces scabies* (Bettarini et al., 1993), antimicrobial effect of crude extract obtained from trunk bark against bacteria or yeast known to cause diseases in man and animals has not been previously described. In addition, since the extract assayed in our study exhibited stronger effects than that of previously tested compounds, which were found to be effective at a concentration of 200 μg/disk, we believe that some different substances for example, other types of alkaloids or limonoids that were earlier found in the stem bark (Waterman and Khalid, 1981) may be responsible for the marked antimicrobial activity of *F. angolensis* evidenced in our test.

Considering other species assayed in our study, the extracts of *Trichilia prieuriana* A. Juss., *Turraea floribunda* Hochst., *Warburgia salutaris* Sprague, and *Zanthoxylum chalybeum* Engl. showed weaker results (MICs ≥ 128 μg/ml) than those of *F. angolensis*, however, still indicating a certain degree of antimicrobial action especially against Gram-positive bacteria and yeast.

Results of the antioxidative assay summarized in Table 2 show that the best DPPH scavenging activity was produced by the extracts of *W. salutaris* (IC_{50} = 6.59 μg/ml), followed by *Z. chalybeum* (IC_{50} = 22.66 μg/ml), *F. angolensis* (IC_{50} = 174.85 μg/ml) and *T. prieuriana* (IC_{50} = 377.02 μg/ml). *T. floribunda* showed no antioxidative properties. Despite recorded antioxidant action of aqueous and methanol extracts from *W. salutaris* leaves (Frum et al., 2005) and several papers reporting various biological activities of crude extracts or compounds (mainly sesquiterpenes) derived from *W. salutaris* stem bark, such as antimycobacterial (Wube et al., 2005) or antiviral (Parker et al., 2007) effects, the marked antioxidative properties of stem bark ethanol extract of this species observed during our study have not been previously described. Previous phytochemical investigation of *W. salutaris* stem bark showed the main presence of drimane type sesquiterpenoids such as warburganal, isopolygodial, polygodial, mukaadial and ugandensidial (Kioy et al., 1990; Mashimbye et al., 1999), however, since two of these compounds, namely mukaadial and warburganal previously did not possess significant level of free radical scavenging effect in DPPH test (Frum et al., 2005), the general antioxidative potential of drimane sesquiterpenoids seems to be quite weak. Moreover, polygodial has been observed to accelerate production of reactive oxygen species in the cells of *Saccharomyces*

cerevisiae, which even indicates its pro-oxidative effect (Machida et al., 1999). Thus, we suggest that other types of chemical structures than drimane sesquiterpenoids seem to be responsible for significant free radical scavenging effect of *W. salutaris* bark extract observed in this study, whereas other substances previously identified in various parts of this species such as lactones (Mashimbye et al., 1999) or flavonols (Manguro et al., 2003) may be possible candidates competent for its antioxidative properties. In addition, the dichloromethane bark extract that exhibited anti-mycobacterial activity was found to be not toxic to cultured mammalian macrophage cells in experiments performed by Madikane et al. (2007), which indicate low toxicity of the biologically active compounds present in the tested part of the plant.

In this study, the barks of some Ugandan indigenous trees were found to be highly effective in tests focused on their antimicrobial and antioxidative properties, which suggest them as prospective materials for further traditional or modern medicinal uses. In connection with this, it should be noted that harvesting of bark from woody species may result in the death of individual species (Cunningham, 1991; Hamilton, 2004). Therefore, since all of them are collected from the wild (Grade et al., 2009), sustainable systems of cultivation and harvesting for example, through species specific strategies of bark removal or plant part substitution (Zschocke et al., 2000; Delvaux et al., 2009) must be warranted for their protection and availability in Uganda. Especially in case of *W. salutaris*, which is over exploited for traditional medicinal purposes in several African countries (Botha et al., 2004) the techniques for appropriate harvesting and cultivation practices must be developed for future conservation and sustainable utilization of this species.

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. salutaris* 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. salutaris* stem barks have not been identified yet, the 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.

REFERENCES

Bettarini F, Borgonovi GE, Fiorani T, Gagliardi I, Caprioli V, Massardo P, Ogoche JIJ, Hassanali A, Nyandat E, Chapya A (1993). Antiparasitic compounds from east African plants: Isolation and biological activity of anonaine, matricarianol, canthin-6-one, and caryophyllene oxide. Insect. Sci. Appl. 14: 93-99.

Botha J, Witkowski ETF, Shackleton CM (2004). The impact of commercial harvesting on *Warburgia salutaris* ('pepper-bark tree') in Mpumalanga, South Afr. Biodivers Conserv. 13: 1675-1698.

Brand-Williams W, Cuvelier ME, Berset C (1994). Use of a free radical method to evaluate antioxidant activity. Lebensm-Wiss u-Technol. 28: 25-30.

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

Cunningham AB (1991). Conservation of medicinal plants. In: Akerele O, Heywood V, Synge H eds. Development of a Conservation Policy on Commercially Exploited Medicinal Plants: a Case Study from Southern Africa. Cambridge: Cambridge University Press. pp. 337-358.

Delvaux C, Sinsin B, Darchambeau F, Van Damme P (2009). Recovery from bark harvesting of 12 medicinal tree species in Benin, West Afr. J. Appl. Ecol. 46: 703-712.

Frum Y, Viljoen AM, Drewes SE (2005). *In vitro* 5-lipoxygenase and anti-oxidant activities of *Warburgia salutaris* and drimane sesquiterpenoids. S. Afr. J. Bot. 71: 447-449.

Grade JT, Tabuti JRS, Van Damme P (2009). Ethnoveterinary knowledge in pastoral Karamoja, Uganda. J. Ethnopharmacol. 122: 273-293.

Hamilton AC (2004). Medicinal plants, conservation and livelihoods. Biodivers. Conserv. 13: 1477-1517.

Kamatenesi-Mugisha M, Oryem-Origa H, Odyek O, Makawiti DW (2008). Medicinal plants used in the treatment of fungal and bacterial infections in and around Queen Elizabeth Biosphere Reserve, western Uganda. Afr. J. Ecol. 46: 90-97.

Kioy D, Gray AI, Waterman PG (1990). A Comparative study of the stem bark drimane sesquiterpenes and leaf volatile oils of *Warburgia ugandensis* and *Warburgia stuhlmannii*. Phytochemistry, 29: 3535-3538.

Machida K, Tanaka T, Taniguchi M (1999). Depletion of glutathione as a cause of the promotive effects of polygodial, a sesquiterpene on the production of reactive oxygen species in *Saccharomyces cerevisiae*. J. Biosci. Bioeng. 88: 526-530.

Madikane VE, Bhakta S, Russell AJ, Campbell WE, Claridge TDW, Elisha BG, Davies SG, Smith P, Sim E (2007). Inhibition of mycobacterial arylamine N-acetyltransferase contributes to anti-mycobacterial activity of *Warburgia salutaris*. Bioorg. Med. Chem. 15: 3579-3586.

Manguro LOA, Ugi I, Lemmen P, Hermann R (2003). Flavonol glycosides of *Warburgia ugandensis* leaves. Phytochemistry, 64: 891-896.

Mashimbye MJ, Maumela MC, Drewes SE (1999). A drimane sesquiterpenoid lactone from *Warburgia salutaris*. Phytochemistry, 51: 435-438.

NCCLS (2008). Performance Standards for Antimicrobial Susceptibility Testing; Ninth Informational Supplement. NCCLS document M100-S9. Wayne, PA: National Committee for Clinical Laboratory Standards.

Parker ME, Chabot S, Ward BJ, Johns T (2007). Traditional dietary additives of the Maasai are antiviral against the measles virus. J. Ethnopharmacol. 114: 146-152.

Rios JL, Recio MC (2005). Medicinal plants and antimicrobial activity. J. Ethnopharmacol. 100: 80-84.

Stangeland T, Remberg SF, Lye KA (2007). Antioxidants in some

Ugandan fruits. Afr. J. Ecol. 45: 29-30.

Waterman PG, Khalid SA (1981). The biochemical systematics of *Fagaropsis angolensis* and its significance in the Rutales. Biochem. Syst. Ecol. 9: 45-51.

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

Zschocke S, Rabe T, Taylor JLS, Jager AK, van Staden J (2000). Plant part substitution-a way to conserve endangered medicinal plants? J. Ethnopharmacol. 71: 281-292.