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ORIGINAL ARTICLE

Antimicrobial activity of traditional medicinal plants from Ankober District, North Shewa Zone, Amhara Region, Ethiopia

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Abstract

Context: Traditional medicinal plants have long been used in Ethiopia to treat human and livestock ailments. Despite a well-documented rich tradition of medicinal plant use in the country, their direct antimicrobial effects are still poorly known.

Objective: To investigate the antimicrobial activity of 19 medicinal plant species that were selected based on the ethnobotanical information on their traditional use to treat infectious diseases in Ankober District.

Methods: About 23 different ethanol extracts of plants obtained by maceration of various parts of 19 medicinal plant species were studied for potential antimicrobial activity using a broth microdilution method against *Bacillus cereus*, *Bacteroides fragilis*, *Candida albicans*, *Clostridium perfringens*, *Enterococcus faecalis*, *Escherichia coli*, *Listeria monocytogenes*, *Pseudomonas aeruginosa*, *Salmonella enteritidis*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Streptococcus pyogenes*.

Results: Plant extracts from *Embelia schimperi* Vatke (Myrsinaceae) showed the strongest antibacterial activity with a minimum inhibitory concentration (MIC) value of 64 µg/ml against *B. cereus*, *L. monocytogenes*, and *S. pyogenes*. Growth inhibitory activities were also observed for extracts of *Ocimum lamiifolium* Hochst. (Lamiaceae) against *S. pyogenes*, and those of *Rubus steudneri* Schweinf. (Rosaceae) against *S. epidermidis* at an MIC value of 128 µg/ml. Generally, 74% of ethanol extracts (17 extracts) showed antimicrobial activity against one or more of the microbial strains tested at an MIC value of 512 µg/ml or below.

Discussion and conclusions: Results confirm the antimicrobial role of traditional medicinal plants of Ankober and warrant further investigations on promising medicinal plant species so as to isolate and characterise chemicals responsible for the observed strong antimicrobial activities.

Keywords

Activity testing, ethnomedicine, plant extracts

History

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Introduction

Microbial diseases continued to be major threats to the world regardless of efforts and progress in developing modern medicine (Cos et al., 2006). The impact of microbial diseases is especially important in developing countries such as Ethiopia where there is limited access to modern drugs and prices are mostly unaffordable when the latter are available. Currently, the ever-increasing threat from drug-resistant bacteria calls for a global effort to search for novel solutions (Theuretzbacher, 2012) that can also be based on the natural products from plants that are selected on the basis of documented ethnomedicinal use (Verpoorte et al., 2005). A focused phytochemical screening, backed by ethnomedicinal

data, often leads to the discovery of new lead compounds that can play a role in the global efforts against pathogens (Savithramma et al., 2012).

According to WHO (2008), 80% of the population in developing countries depend on herbal medicine for primary health care. The situation is the same in Ethiopia where traditional medicine has been in use since time immemorial and found to be culturally entrenched in all communities (Kibebew, 2001). About 95% of traditional medicine preparations in the country are of plant origin (Demissew & Dagne, 2001) as evidenced by the wealth of indigenous knowledge on the utilisation of medicinal plants for treating human and livestock ailments.

This knowledge has been recorded in ancient medico-religious textbooks dating back to the Axumite Kingdom (7th–11th C) (Kibebew, 2001). Other documents on Ethiopian medicinal plants by foreign travelers including Cecchi (1886), Griaule (1928), Pearce (1831), Mérab (1912), Ganora (1929), and Strelcyn (1968, 1973) complemented the latter

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information. However, the efforts on antimicrobial screening of the country's medicinal flora remained minimal. Although there are a number of recent ethnobotanical documents on medicinal plants traditionally used in Ethiopia for the treatment of infectious diseases, such as those in Flatie et al. (2009), Giday et al. (2009, 2010), Lulekal et al. (2008), Mesfin et al. (2009), Sori et al. (2004), Teklehaymanot (2009), Teklehaymanot and Giday (2007), Wabe et al. (2011), Wondimu et al. (2007), and Yineger et al. (2007), only a few species have been tested up to now for their antibacterial or antifungal properties (Belay, 2011; Desta, 1993; Gebre-Mariam et al., 2006; Geyid et al., 2005; Tadeg et al., 2005; Tadesse et al., 2009; Taye et al., 2011; Yonathan et al., 2006) and this is found to be insignificant when compared to the wealth of Ethiopian ethnomedicinal plants and associated knowledge.

The present study reports the results of *in vitro* antimicrobial tests performed on 23 ethanol extracts of various parts of 19 different plant species, which are traditionally used for treating various ailments in the study area, against 12 various strains of microbes so as to show the antimicrobial efficacy of locally reported medicinal plants.

Materials and methods

Plant material

Plant specimens were collected from Ankober District (172 km northeast of the capital Addis Ababa), North Shewa Zone, Amhara Region Ethiopia, between 25 June and 26 September 2009, 9 January–20 February 2010, 22 May–27 August 2010 and 14 February–7 May 2011. Identification of specimens was performed both in the field and at the National Herbarium of Ethiopia (ETH) by Ermias Lulekal and Melaku Wondafrash, which was then confirmed by Prof. Ensermu Kelbessa, using taxonomic keys and floras (Edwards et al., 2000; Hedberg & Edwards, 1989; Hedberg et al., 2003) and by comparison with voucher reference herbarium specimens. Plant species were selected for screening based on local use reports from the study area and for which there are related ethnobotanical reports from other parts of the country (Table 1). The identified voucher specimens were deposited at the National Herbarium (ETH).

Preparation of extracts

About 15 g of air-dried plant material of each species was finely ground using a Grindomix apparatus (GM100 Retsch, Haan, Germany) and extracted at room temperature in 80% ethanol using a laboratory shaker for 24 h. Extracts from each specimen were subsequently filtered and concentrated to dryness using a rotary evaporator R-200 (Buchi, Switzerland) *in vacuo* at 40 °C. Dry residues were then dissolved in 100% dimethyl sulfoxide (DMSO) to create a concentration of 51.2 mg/ml stock solution of each extract that was stored at –20 °C until tested. Yield (%) of dry residues of each extract used is shown in Table 1.

Microorganisms

Antimicrobial activity was evaluated against one yeast and 11 bacterial strains obtained from the American Type Culture

Collection (ATCC) (Oxoid, Basingstoke, United Kingdom) and the German Resource Centre for Biological Material (DSM) (Braunschweig, Germany). Bacterial strains were selected as a representative of both classes of Gram-positive and Gram-negative bacteria. Microbial strains used were *Bacillus cereus* ATCC 11778, *Bacteroides fragilis* ATCC 25285, *Candida albicans* ATCC 10231, *Clostridium perfringens* DSM 11778, *Escherichia coli* ATCC 25922, *Enterococcus faecalis* ATCC 29212, *Listeria monocytogenes* ATCC 7644, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 29213, *Staphylococcus epidermidis* ATCC 12228, *Salmonella enteritidis* ATCC 13076, and *Streptococcus pyogenes* ATCC 19615.

Streptococcus pyogenes was grown in brain–heart infusion broth (Oxoid, Basingstoke, United Kingdom). *Bacteroides fragilis* and *C. perfringens* as representatives of anaerobic bacteria were grown in the Wilkins–Chalgren anaerobic broth (Oxoid) under anaerobic conditions using an anaerobic jar HP11 (Oxoid). Anaerobiosis was achieved in a Bugbox anaerobic chamber (BioTrace, Bridgend, United Kingdom). Other microorganisms were all grown Mueller–Hinton broth (Oxoid), which was enriched with glucose for *E. faecalis* cultivation. All cultivation media were obtained from Oxoid.

The sensitivity of tested aerobic bacteria and *B. fragilis* to a standard antibiotic ciprofloxacin (Sigma-Aldrich, Prague, Czech Republic) was checked. For *C. albicans* and *C. perfringens*, tioconazole and penicillin G (both obtained from Sigma-Aldrich) were used as positive controls, respectively.

Antimicrobial assay

In vitro antimicrobial activity was determined by the broth microdilution method (Jorgensen et al., 1999) using 96-well microtiter plates modified according to the recommendations recently proposed for the more effective assessment of anti-infective potential of natural products by Cos et al. (2006). Ten two-fold serial dilutions of each extract were prepared in the appropriate broth concentrations ranging from 512 to 4 µg/ml. Each well was then inoculated with 5 µl of bacterial suspension at a density of 10⁷ CFU/ml whereas microtiter plates were incubated at 37 °C for 24 h (or 48 h for *C. albicans*, *B. Fragilis*, and *C. perfringens*). All plates were then checked for minimum inhibitory concentrations (MICs).

Growth of microorganisms was observed as turbidity-determined spectrophotometrically using the Multiscan Ascent Microplate Reader (Thermo Fisher Scientific, Waltham, MA) at 405 nm. MICs were then calculated based on the density of the growth control and expressed as the lowest extract concentrations that resulted in ≥80% reduction in bacterial growth compared to that of the extract-free growth control. Tests were assayed in triplicate in three independent experiments.

Results

Among 23 plant extracts tested for antimicrobial activity, about 74% (17 extracts) showed activity at the concentration of 512 µg/ml or below against one or more of the 12 microbes (Table 2). Extracts of *E. schimperi*, *O. lamiifolium*, and *R. steudneri* showed the broadest spectrum of action as they

Table 1. Ethnobotanical data on medicinal plants selected for antimicrobial tests.

Scientific name	Family	Collection number (ErmiasL. x)	Local name (Amharic)	Part used	Extract yield (%)	Locally used in the study area to treat	Ethnomedicinal use reports from other regions in Ethiopia
<i>Bersama abyssinica</i> Fresen.	Meliastaceae	173	Azamid	Leaves and twigs	33.50	Diarrhoea, constipation	Ascariasis (Lulekal et al., 2008), febrile illness (Mesfin et al., 2009)
<i>Calpurnia aurea</i> (Lam.) Benth.	Fabaceae	76	Digit	Root	19.50	Diarrhoea, toothache, epiglottis and wound	Fungal infection (Wondimu et al., 2007), sore (Abebe, 1986), dysentery (Abebe, 1986; Teklehaymanot & Giday, 2007), amoebiasis (Giday et al., 2009), ophthalmia (Yineger et al., 2008)
<i>Carissa spinarum</i> L.	Apocynaceae	16	Agam	Root	14.50	Constipation, rheumatism, headache, evil spirit	Gingivitis (Abebe, 1986), bleeding (Giday et al., 2007), nasal infection (Yineger et al., 2008)
<i>Clematis hirsuta</i> Guill. & Perr	Ranunculaceae	18	Azo hareg	Leaves	30.00	Eczema, mumps, ringworm, diarrhoea	Wound (Giday et al., 2007), otorrhea (Yineger et al., 2008)
<i>Cluitia abyssinica</i> Jaub. & Spach	Euphorbiaceae	10	Fiyete fej	Root	9.50	Bloody diarrhoea, gastritis, constipation	Skin infection, diarrhoea (Lulekal et al., 2008)
<i>Croton macrostachyus</i> Hochst. ex Delile	Euphorbiaceae	17	Bisana	Leaves and twigs	21.00	Tinea versicolor, diarrhoea, eczema, allergic rashes	Athlete's foot (Abebe, 1986), eczema (Gedif & Hahn, 2003), diarrhoea, allergies (Yineger et al., 2008), gonorrhoea (Giday et al., 2007)
<i>Cyathula cylindrica</i> Moq.	Amaranthaceae	827	Yedem abinet	Roots	14.00	Epistaxis, minor bleeding	Rabies (Bussmann et al., 2011)
<i>Dodonaea angustifolia</i> L. f.	Sapindaceae	20	Kitkita	Leaves	28.50	Skin lesion, ringworm, diarrhoea, constipation	Haemorrhoids and sore (Lulekal et al., 2008), allergies (Yineger et al., 2008), malaria (Giday et al., 2007)
<i>Embelia schimperii</i> Vatke	Myrsinaceae	505	Inkoko	Seeds/fruit	25.50	Taeniasis, diarrhoea, ascariasis, constipation	Tape worm (Giday et al., 2007, Gedif & Hahn, 2003), leprosy (Mesfin et al., 2009)
<i>Jasminum abyssinicum</i> R.Br.	Oleaceae	577	Abita	Leaves and twigs	38.50	Bloody diarrhoea, gastritis and constipation	Tonsillitis (Getahun, 1976), skin infection (Goji et al., 2006)
<i>Maesa lanceolata</i> Forssk.	Myrsinaceae	42	Kelewa	Leaves and twigs	27.15	Diarrhoea and constipation	Skin infection (Lulekal et al., 2008), fever (Awas & Demissew, 2008)
<i>Ocimum lamifolium</i> Hochst.	Lamiaceae	828	Dama kessie	Leaves	11.5	Malaise, otorrhea and eye infection	Malaise (Giday et al., 2009; Gebre-Mariam et al., 2006), cough (Awas & Demissew, 2008; Tadesse et al., 2009), sore (Teklehaymanot & Giday, 2007), bloody diarrhoea (Gedif & Hahn, 2003)
<i>Olinia rochetiana</i> A. Juss.	Olimiaceae	102	Tife	Leaves	38.00	Eczema, diarrhoea	Stabbing pain (Abebe, 1986)
<i>Rubus steudneri</i> Schweinf.	Rosaceae	242	Injori	Roots	32.82	Gastritis, diarrhoea and constipation	Headache (Lulekal et al., 2008), rheumatism (Yineger et al., 2008)
<i>Rumex nepalensis</i> Spreng.	Polygonaceae	24	Lut	Leaves and twigs	23.00	Constipation, bloody diarrhoea	Diarrhoea (Lulekal et al., 2008; Giday et al., 2009), wound (Giday et al., 2007; Mesfin et al., 2009), tonsillitis (Abebe, 1986), amoebiasis (Gedif & Hahn, 2003)
<i>Thalictrum rhynchocarpum</i> Quart.-Dill. & A.Rich.	Ranunculaceae	535	Sire bizu	Leaves and twigs	8.11	Mumps, otorrhea, ascariasis	Stabbing pain (Lulekal et al., 2008), eczema (Yineger et al., 2008), tooth ache (Awas & Demissew, 2008)
<i>Verbascum sinaiticum</i> Benth.	Scrophulariaceae	829	Yeahya joro	Leaves	44.50	Diarrhoea and constipation	Sore (Teklehaymanot & Giday, 2007), diarrhoea (Abebe, 1986; Teklehaymanot & Giday, 2007)
<i>Vernonia amygdalina</i> Del.	Asteraceae	22	Girawa	Flowers	18.75	Taeniasis, ascariasis, constipation	Skin rash (Gedif & Hahn, 2003) malaise (Giday et al., 2009), purgative (Getahun, 1976), tonsillitis (Teklehaymanot & Giday, 2007), ascariasis (Giday et al., 2007)
<i>Zehneria scabra</i> Sond.	Cucurbitaceae	830	Hareg ireda	Leaves and twigs	17.5	Malaise, common cold, coughing	Malaise (Abebe, 1986; Teklehaymanot & Giday, 2007), malaria (Giday et al., 2007), febrile illness (Yineger et al., 2008), diarrhoea (Gedif & Hahn, 2003)

Table 2. MIC values of ethanol extracts of medicinal plant species with antimicrobial activities, Ankober District, Ethiopia.

Plant species/reference cpd. name	Part	Microorganisms/minimum inhibitory concentration ($\mu\text{g/ml}$)											
		Gram positive						Gram negative				Yeast	
		B.c.	C.p.	L.m.	S.ep.	E.f.	S.a.	S.p.	B.f.	E.c.	P.a.	S.en.	C.a.
<i>B. abyssinica</i>	R	–	–	512	512	–	–	–	–	–	–	–	512
<i>C. abyssinica</i>	R	–	–	–	–	–	–	256	–	–	–	–	–
<i>C. macrostachyus</i>	LT	–	–	–	–	–	–	256	–	–	–	–	–
<i>D. angustifolia</i>	L	–	–	–	–	–	–	512	–	–	–	–	–
<i>E. schimperi</i>	S/F	128	512	256	512	512	512	128	–	–	–	–	512
	LT	64	512	64	256	512	512	64	–	–	–	–	512
<i>J. abyssinicum</i>	L	512	–	512	512	–	–	256	–	–	–	–	–
<i>M. lanceolata</i>	LT	–	–	–	–	–	–	256	–	–	–	–	–
<i>O. lamifolium</i>	L	256	512	256	512	512	512	128	–	–	–	–	512
<i>O. rochetiana</i>	L	–	–	–	–	–	–	512	–	–	–	–	–
<i>R. steudneri</i>	R	256	–	512	128	512	256	512	–	–	256	512	–
	LT	512	–	512	256	512	512	512	–	–	512	–	512
<i>R. nepalensis</i>	R	512	–	–	256	512	–	–	–	–	–	–	–
	LT	–	–	–	–	–	512	512	–	–	–	–	512
<i>V. amygdalina</i>	F	–	–	–	512	512	512	–	–	–	–	–	–
	LT	512	–	–	–	512	–	256	–	–	–	–	–
<i>Z. scabra</i>	LT	–	–	–	–	–	–	–	–	–	–	–	512
C, T, P ($\mu\text{g/ml}$) ^a		0.125	0.125	1	0.125	0.5	0.25	0.5	8	0.015	0.625	0.031	0.125

R, root; LT, leaves and twigs; S, seed; F, fruit; L, leaves; B.c., *Bacillus cereus*; C.p., *Clostridium perfringens*; L.m. *Listeria monocytogenes*; S.ep., *Staphylococcus epidermidis*; E.f., *Enterococcus faecalis*; S.a., *Staphylococcus aureus*; S.p., *Streptococcus pyogenes*; B.f., *Bacteroides fragilis*; E.c., *Escherichia coli*; P.a., *Pseudomonas aeruginosa*; S.en., *Salmonella enteritidis*; C.a., *Candida albicans*; –, not active ($>512 \mu\text{g/ml}$).

^aC, ciprofloxacin; T, tioconazole; P, penicillin.

inhibited growth of *B. cereus*, *C. albicans*, *E. faecalis*, *L. monocytogenes*, *S. aureus*, *S. epidermidis*, and *S. pyogenes* strains with MICs ranging from 64 to 512 $\mu\text{g/ml}$. In addition, extracts of *E. schimperi* and *O. lamifolium* showed inhibitory activity against *C. perfringens* at an MIC value of 512 $\mu\text{g/ml}$, whereas those of *R. steudneri* were found effective against *P. aeruginosa* and *S. enteritidis* in an MIC range of 256–512 $\mu\text{g/ml}$. Moreover, extracts from *J. abyssinicum*, *B. abyssinica*, *R. nepalensis*, and *V. amygdalina* inhibited growth of three or more of the 12 microbes. No antimicrobial activity was observed with extracts of *C. aurea*, *C. spinarum*, *C. hirsuta*, *C. cylindrica*, *T. rhynchocarpum*, and *V. sinaiticum* against either one of the microbes tested.

The strongest antibacterial activity (MIC = 64 $\mu\text{g/ml}$) was shown by leaf and twig extracts of *E. schimperi* against *B. cereus*, *L. monocytogenes*, and *S. pyogenes*. Moreover, seed and fruit extracts of this species also showed strong activity (MIC = 128 $\mu\text{g/ml}$) against *B. cereus* and *S. pyogenes*. Similarly, strong growth inhibitory activities (MIC = 128 $\mu\text{g/ml}$) were observed for extracts of *O. lamifolium* against *S. pyogenes*, and those of *R. steudneri* against *S. epidermidis*.

Extracts of *R. steudneri* were the only extracts to show antimicrobial activity against Gram-negative bacteria *P. aeruginosa* and *S. enteritidis*. Two of the Gram-negative bacteria, i.e., *B. fragilis* and *E. coli*, were resistant to all extracts tested in this study. Among the Gram-positive bacteria, *C. perfringens* was found to be less sensitive as inhibited by the extracts of only two species, *E. schimperi* and *O. lamifolium*. *S. pyogenes* was shown to be the most sensitive bacterium which was inhibited by 76% of extracts (13 extracts) with MIC values ranging from 64 to 512 $\mu\text{g/ml}$. Gram-positive bacteria were generally found more susceptible to the extracts than the Gram-negative ones. The growth of yeast strain *C. albicans* was inhibited by extracts from *B.*

abyssinica, *E. schimperi*, *O. lamifolium*, *R. steudneri*, *R. nepalensis*, and *Z. scabra*. No growth inhibition was observed in the negative controls.

Discussion

The output of the present investigation substantiates the potential therapeutic role of traditionally used medicinal plants against some microbial diseases. About 17 (74%) of the 23 ethanol extracts obtained from 19 medicinal plant species have now been proven to show antimicrobial activity against one or more of the 12 microbial strains. Results also confirm the importance of considering ethnomedicinal background of medicinal plants to run antimicrobial activity tests for a high hit-rate (74% in this case). Similar investigations on antimicrobial activity tests based on ethnomedicinal background (Buwa & van Staden, 2006; Egualé et al., 2007; Gebre-Mariam et al., 2006; Geyid et al., 2005; Hussain et al., 2010; Kloucek et al., 2005; Tadesse et al., 2009; Tekwu et al., 2012) have also been reported for a high hit-rate in different countries.

The broad spectrum antimicrobial activity of extracts from *E. schimperi*, *O. lamifolium*, and *R. steudneri*, each inhibiting growth of 67% (eight) of the 12 microbes (Table 2) with a MIC range between 64 and 512 $\mu\text{g/ml}$ supports wide traditional use reports of these species in Ethiopia and neighbouring countries (Gedif & Hahn, 2003; Kokwaro, 1976; Mesfin et al., 2009; Pascaline et al., 2011; Teklehaymanot & Giday, 2007; Wondimu et al., 2007). In addition, Awino et al. (2008) have reported antimicrobial activity of extracts from *E. schimperi* against *S. aureus*, which complements results of the present work. A report from the same authors on antimicrobial activity of a pure compound, 2,5-dihydroxy-3-methyl-1,4-benzoquinone, from *E. schimperi*

against two of the Gram-negative bacteria i.e., *P. aeruginosa* and *E. coli*, which were shown not to be susceptible in the present work, suggests the presence of other antibacterial constituents in the extract tested in this study. Moreover, Bøgh et al. (1996) described anthelmintic usage of extracts from *E. schimperi* berries against *Taenia saginata*, which confirms diverse medicinal role of the species in the treatment of infective agents.

Observed strong antibacterial activity of *E. schimperi* against *B. cereus*, *L. monocytogenes*, and *S. pyogenes* correlates with its reported traditional use of treating diarrhoea which is one of the major bacterial diseases in the study area. Reports on chemical constituents of this broad spectrum species show that *E. schimperi* leaves possess embelin and rapanone (Midiwo & Manguro, 1993), schimperinone (Machocho et al., 2003), oleanane-type triterpenes (Manguro et al., 2006), and flavonol glycosides (Manguro et al., 2004). Embelin has been reported for its antibacterial properties against *S. aureus*, *S. pyogenes*, and *P. aeruginosa* (Chitra et al., 2003). A wide spectrum of biological and pharmacological property of embelin has also been described by Machocho et al. (2003). Hence, the broadest spectrum action (against 67% of the microbes in the present study) and the strong antimicrobial activity (MIC 64 µg/ml) shown by both leaf and twig extracts of *E. schimperi* against *B. cereus*, *L. monocytogenes* and *S. pyogenes* could be attributed to the existence of such active chemicals with pharmacological properties.

Runyoro et al. (2010) applied an agar-dilution technique and reported antimicrobial role of extracts from *O. lamiifolium* against *S. aureus* and *S. epidermidis*, which corresponds to the present results. In addition, the investigated strong growth inhibitory activity of extracts from *O. lamiifolium* and *R. steudneri* was also found to support recorded ethnobotanical uses of the species to treat common bacterial diseases, such as diarrhoea, in the District. Previous studies have described anti-inflammatory, anti-pyretic, and analgesic properties of aqueous and ethanol extracts of leaves from *O. lamiifolium* (Makonnen et al., 2003a,b; Mequanint et al., 2011). Growth inhibition of *S. aureus* and *P. aeruginosa* by methanolic extracts of *R. steudneri* has also been reported by Kamoga (2010), which also matches the current findings.

The presence of chemical compounds including bornyl acetate, *p*-cymene, camphene, α -pinene, and sabinene was also reported from the essential oil analysis of *O. lamiifolium* (Runyoro et al., 2010; Tchoumboungang et al., 2006). A literature search for relevant information on antimicrobial activities of chemicals from this plant shows the antimicrobial role of sabinene (Oji & Shafaghat, 2012; Tchoumboungang et al., 2006) and bornyl acetate (Runyoro et al., 2010). Therefore, the observed broad spectrum activity from extracts of *O. lamiifolium* could relate to these active components.

Although our results demonstrate significant antimicrobial effects of extracts from *R. steudneri*, inhibiting growth of 67% of microbes tested in this study, no report was found on its chemical constituents. Hence, this new observation calls for an in-depth investigation on *R. steudneri* to isolate and characterise its antimicrobial active components.

Antimicrobial action of extracts from *J. abyssinicum*, *B. abyssinica*, *R. nepalensis*, and *V. amygdalina* against three

or more strains also relates to their traditional use reports (Getahun, 1976; Goji et al., 2006; Lulekal et al., 2008; Mesfin et al., 2009). A report by Goji et al. (2006) described the antibacterial role of leaf extracts of *J. abyssinicum* against *S. aureus*, *P. aeruginosa*, and *S. pyogenes* through an agar well-diffusion method. The results of the present work are in agreement with the reported antimicrobial activity against *S. pyogenes* but not with that of *S. aureus* and *P. aeruginosa*. The discrepancy may be attributed to differences in methods followed for activity testing. A difference in geographical location, season, and developmental stages of samples collected could also be mentioned as factors for differences in outputs of activity tests (Runyoro et al., 2010). A report by Gallo et al. (2006) on chemical constituents of root bark of *J. abyssinicum* stated the presence of esters of a cyclopentanoid monoterpene but no information on their antimicrobial effect was available.

Geyid et al. (2005) used the agar-dilution method and reported antimicrobial role of root and stem bark extracts of *B. abyssinica* against *S. aureus* at concentrations between 500 and 2000 µg/ml, but in the present work, we found no activity for this species for the range we tested for. The antimicrobial effect of *R. nepalensis* on *S. aureus* reported by Hussain et al. (2010) was also found to be in agreement with the results of this work but their report against *E. coli* differs from our results. The observed discrepancy may be attributed to differences in solvents used for extraction and methods followed for activity testing besides aforementioned factors that could bring differences in output of activity tests.

Stem and leaf extracts of *V. amygdalina* have also been reported for antimicrobial role against *Klebsiella* spp. (Uzoigwe & Agwa, 2011). The same authors have also shown that *E. coli* is not susceptible to extracts of *V. amygdalina*, which matches our own finding. Ijeh & Ejike (2011) reviewed current perspectives on medicinal potentials of *V. amygdalina*. Chemical constituents of *V. amygdalina* include flavonoids, steroidal alcohols, and sesquiterpene lactones have also been reported as chemically active by Luo et al. (2010). The latter may be responsible for the relatively broad spectrum of actions of this species observed in the present work.

Our investigation has shown that Gram-positive bacteria are more susceptible to tested medicinal plant extracts than Gram-negative ones. This might relate to differences in cell wall morphology of the two groups of bacteria. Hodges (2002) explained that Gram-negative bacteria have an outer phospholipid membrane composed of lipo-polysaccharide constituents that make their cell wall impermeable to antimicrobial chemicals, whereas Gram-positive groups possess cell walls composed of a peptidoglycogen layer that is an inefficient permeability barrier. Although there are such barriers in Gram-negative bacteria, extracts of *R. steudneri* were found to be the only ones to be active and effective against two of the Gram-negative bacteria, i.e., *P. aeruginosa* and *S. enteritidis*. Moreover, among the Gram-negative groups, *B. fragilis* and *E. coli* were found susceptible to none of the extracts and this could also partly be attributed to cell wall impermeability. Multi-drug resistance of Gram-negative bacteria has also been reported by Sader et al. (2002). Earlier

reports also confirmed that Gram-negative bacteria are less susceptible to diverse medicinal plant extracts than Gram-positive groups (Kloucek et al., 2005; Tadege et al., 2005) hence supporting the present result.

Conclusions

In conclusion, herbal extracts from traditional medicinal plants have been attracting scientific interests due to their potential as sources of chemicals against microbes. We were able to show that 74% of the medicinal plant extracts tested exhibited antimicrobial effect against one or more of the 12 different microbial strains. We found *E. schimperi*, *O. lamiifolium*, and *R. steudneri* to be the most promising plants for a potential discovery of lead compounds against microbes. The antimicrobial activity of extracts from *J. abyssinicum*, *B. abyssinica*, *R. nepalensis*, and *V. amygdalina* are also promising and calls for further investigation. Among the most promising plants for antimicrobial properties, *E. schimperi* and *O. lamiifolium* were found with few reports on chemical constituents whereas no report was found for *R. steudneri*. Hence, further studies on isolation and characterisation of chemicals from these species that are responsible for the observed activities against microbes are highly recommended. More scientific studies on medicinal plants with traditional use reports will also bring more useful results that contribute significant role in the fight against pathogens.

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