Antimicrobial, Antioxidant and Cytotoxic Activities and Phytochemical Screening of Some Yemeni Medicinal Plants

Ramzi A. A. Mothana¹, Salah A. A. Abdo², Sidgi Hasson², Faisal M. N. Althawab², Sama A. Z. Alaghbari² and Ulrike Lindequist³

¹Department of Pharmacognosy, Faculty of Pharmacy, Sana'a-University, PO Box 33039, ²Institute of Pharmacy, College of Medical science, University of Science and Technology, Sana'a, Yemen and ³Department of Pharmaceutical Biology, Institute of Pharmacy, Ernst-Moritz-Arndt-University, Greifswald, F-L-Jahn Str. 15a, D-17487 Greifswald, Germany

The traditional medicine still plays an important role in the primary health care in Yemen. The current study represents the investigation of 16 selected plants, which were collected from different localities of Yemen. The plants were dried and extracted with two different solvents (methanol and hot water) to yield 34 crude extracts. The obtained extracts were tested for their antimicrobial activity against three Gram-positive bacteria, two Gram-negative bacteria, one yeast species and three multiresistant Staphylococcus strains using agar diffusion method, for their antioxidant activity using scavenging activity of DPPH radical method and for their cytotoxic activity using the neutral red uptake assay. In addition, a phytochemical screening of the methanolic extracts was done. Antibacterial activity was shown only against Gram-positive bacteria, among them multiresistant bacteria. The highest antimicrobial activity was exhibited by the methanolic extracts of Acalypha fruticosa, Centaurea pseudosinaica, Dodonaea viscosa, Jatropha variegata, Lippia citriodora, Plectranthus hadiensis, Tragia pungens and Verbascum bottae. Six methanolic extracts especially those of A. fruticosa, Actiniopteris semiflabellata, D. viscosa, P. hadiensis, T. pungens and V. bottae showed high free radical scavenging activity. Moreover, remarkable cytotoxic activity against FL-cells was found for the methanolic extracts of A. fruticosa, Iris albicans, L. citriodora and T. pungens. The phytochemical screening demonstrated the presence of different types of compounds like flavonoids, terpenoids and others, which could be responsible for the obtained activities.

Keywords: antibacterial-cytotoxicity-medicinal plants-radical scavenging-Yemen

Introduction

Plant-derived drugs remain an important resource, especially in developing countries, to combat serious diseases. Approximately 60–80% of the world's population still relies on traditional medicines for the treatment of common illnesses (1,2). Traditional remedies have a long-standing history in many locations in Yemen and

continue to provide useful and applicable tools for treating ailments (3-5). Nevertheless, little scientific research was done to investigate the plants of Yemen used in herbal medicine. In the course of our investigations we found that several plants of the Yemeni ethnomedicine possess really interesting biological activities, which could be of interest for all parts of the world (6-11). The aim of this work was to continue these investigations and to determine the antimicrobial, antioxidant and cytotoxic activities of till now uninvestigated medicinal plants collected from different locations of Yemen. In this study, a total of 34 extracts prepared from

© 2008 The Author(s).

For reprints and all correspondence: Ramzi Mothana, Department of Pharmacognosy, Faculty of Pharmacy, Sana'a-University, PO Box 33039, Sana'a, Yemen. Tel: +9671-225097; Fax: +9671-374682; E-mail: r mothana@yahoo.com

This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (http://creativecommons.org/ licenses/by-nc/2.0/uk/) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

16 plants have been determined for their antibacterial and antifungal activity by means of the agar diffusion method, antioxidant activity using scavenging activity of DPPH radical method and cytotoxic activity using the neutral red uptake assay. Furthermore, a phytochemical screening of the methanolic extracts was performed.

The activities have been selected because of their great medicinal relevance. Within the recent years, infections have increased to a great extent and resistance against antibiotics becomes an ever-increasing therapeutic problem (12). Because natural products of higher plants may give a new source of antimicrobial agents, there are many research groups that are now engaged in medicinal plants research (13–15).

In the last years, interest in the antioxidant activity of plant extracts has become larger and very important (16–18) due to the fact that free radicals e.g. reactive oxygen species (ROS) can be responsible for various diseases, e.g. heart diseases, stroke, arteriosclerosis and cancer, as well as for aging process (19).

Methods

Plant Materials

The plants were collected from different localities of Yemen in July 2005 and identified at the Pharmacognosy Department, Faculty of Pharmacy, Sana'a University. Part of the identification of the investigated plants was done by Priv.-Doz. Dr Peter Koenig, at the botanical garden, Ernst-Moritz-Arndt-University, Greifswald, Germany. Voucher specimens were deposited at the Pharmacognosy Department, Faculty of Pharmacy, Sana'a University.

Extraction of Plant Material

The air-dried and powdered plant materials (10 g of each) were extracted with 400 ml methanol (CH₃OH) by using a Soxhlet apparatus for 8 h. The residue was dried over the night and then extracted with 250 ml water (H₂O) by using a shaking water-bath at 70°C for 2 h. The extraction with water was repeated thrice. The water-filtrates were mixed together. The obtained methanolic and water extracts were filtered and evaporated by using a rotary evaporator and freeze dryer, respectively to give the crude dried extract. The dried extracts were stored at -20° C until used.

Test Organisms

The following microorganisms were used as test organisms: *Staphylococcus aureus* (ATCC 6538), *Bacillus subtilis* (ATCC 6059), *Micrococus flavus* (SBUG 16), *Escherichia coli* (ATCC 11229), *Pseudomonas aeruginosa* (ATCC 27853) and *Candida maltosa* (SBUG). In addition, three multiresistant *Staphylococcus* strains namely, *S. epidermidis* 847, *S. haemolyticus* 535 and *S. aureus* North German Epidemic Strain (supply from the Institute of Hygiene of Mecklenburg-Vorpommern, Greifswald, Germany) were also applied as test organisms.

Antimicrobial Assay

The disc-diffusion assay (20) was used to determine the antimicrobial activity of the investigated extracts. Nutrient agar (OXOID LTD, Basingstoke, Hampshire, England) was prepared by dissolving of $27 \text{ g} \text{ l}^{-1}$ in water. The sterile nutrient agar was inoculated with microbial cells (200 µl of microbial cell suspension in 20 ml agar medium) and poured into sterile petri dishes. Sterile filter paper discs of 6mm diameter (Schleicher and Schuell, ref. No. 10321260, lot. DG0274-1) were impregnated with 20 µl of the extract solution (equivalent to 4 mg of the dried extract). The paper discs were allowed to evaporate and after that placed on the surface of the inoculated agar plates. Plates were kept for 2h in refrigerator to enable prediffusion of the extracts into the agar. Then, the plates were incubated overnight (18h) at 37°C. In contrast, M. flavus was incubated at room temperature for 48 h and C. maltosa was incubated at 28°C for 48 h. Ampicillin, gentamicin and amphotericin B were used as positive control. Negative controls were performed using paper discs loaded with 20 µl of organic solvents (chloroform and methanol). At the end of the incubation period the antibacterial activity was evaluated by measuring the inhibition zones (diameter of inhibition zone plus diameter of the disc). An inhibition zone of 14 mm or more was considered as high antibacterial activity.

Determination of Antioxidant Activity

Scavenging Activity of DPPH Radical

In order to measure antioxidant activity, DPPH free radical scavenging assay was used. This assay measures the free radical scavenging capacity of the investigated extracts. DPPH is a molecule containing a stable free radical. In the presence of an antioxidant which can donate an electron to DPPH, the purple color which is typical for free DPPH radical decays, and the change in absorbency at 517 nm is followed specrophotometrically. This test could provide information on the ability of a compound to donate a hydrogen atom, on the number of electrons a given molecule can donate, and on the mechanism of antioxidant action. The method was carried out as described by Brand et al. (21). The methanolic and aqueous extracts were redissolved in methanol and 5% ethanol, respectively and various concentrations (10, 50, 100, 500 and $1000 \,\mu g \,m l^{-1}$) of each extract were used. The assay mixture contained in total volume of 1 ml, 500 µl of the extract, 125 µl prepared DPPH (1 mM in methanol) and 375 µl solvent (methanol or 5%

Table 1. List of plants screened

Plant	Voucher specimen no.	Family	Part tested	Traditional uses ^a			
Acalypha fruticosa Forssk.	YH-05	Euphorbiaceae	L, S	Skin diseases, malaria and wounds (3, 4, a)			
Actiniopteris semiflabellata PicSer.	Mo-M08	Pteridophyta	L	Wounds and burns (a)			
Alkanna orientalis (L.) Boiss.	Mo-I03	Boraginaceae	R	Common cold, pharyngitis, rheumatism and toothache (4, a)			
Carthamus tinctorius L.	Mo-T10	Asteraceae	L, S	Skin diseases e.g. freckles, coloring agent and in food (4, a)			
Centaurea pseudosinaica Czerep.	Mo-S11	Asteraceae	L, T	Wounds, kidney diseases (4, a)			
Cleome schweinfurthii Gilg.	Mo-I01	Capparaceae	L, S	Otitis (a)			
Dodonaea viscosa (L.) Jacq.	Mo-T01	Sapindaceae	L, S	Malaria, wounds and burns (5, a)			
Forsskalea tinacissima L.	Mo-S08	Urticaceae	L	Diuretic and kidney diseases (a)			
Iris albicans Lange	Mo-I02	Iridaceae	R	Rheumatism and gout (4, a)			
Jatropha variegata Vahl	Mo-T05	Euphorbiaceae	L, T	Antiseptic, for wounds and hemostatic (3, 5)			
Lavandula pubescens Decne.	Mo-S10	Lamiaceae	L, F	Antiseptic, carminative and diuretic, (3, 4, 5, a)			
Lippia citriodora Kunth	Mo-S03	Verbenaceae	L	Spasmolytic, gastrointestinal troubles, common cold and sedative (4, a)			
Mentha longifolia (L.) Hudson	Mo-M01	Lamiaceae	L, S	Spasmolytic and digestive disorders (3, 4, a)			
Plectranthus hadiensis (Forssk.)	Mo-T04	Lamiaceae	L, R	Antiseptic and haemostatic (3, a)			
Schweinf. ex Sprenger							
Tragia pungens (Forssk.) MuellArg.	YT-20	Euphorbiaceae	L, S	Allergy and skin diseases (a)			
Verbascum bottae (Deflers) Huber-Mor.	Mo-I08	Scrophulariaceae	L, F	Cough, skin diseases and rheumatism (4, a)			

F: Flower, L: Leaves, R: Roots or rhizomes, S: Stems, T: Fruits.

^aInformation of traditional use has been taken from native people.

ethanol). After 30 min incubation at 25°C, the decrease in absorbance was measured at $\lambda = 517$ nm. The radical scavenging activity was calculated from the equation:

 $\begin{array}{l} Percentage \ of \ radical \ scavenging \ activity = \\ \left(Abs_{control} - Abs_{sample}\right) / Abs_{control} \times 100 \end{array}$

Cytotoxicity Assay

The cytotoxicity of the investigated extracts was measured by the neutral red uptake assay (22) using FL-cells, a human amniotic epithel cell line. Only living cells are able to manage the active uptake of neutral red. The effect of the plant extracts on the proliferation of the FL-cells was determined in 96-well tissue culture plates. Confluent monolayers were incubated with different concentrations (serial dilutions) in medium for 72 h. The 50% cellinhibitory concentration (IC₅₀) was determined.

Phytochemical Screening of the Methanolic Extracts

The screening of chemical constituents was carried out with the methanol extracts using chemical methods and thin-layer chromatography (TLC) according to the methodology given in (23).

Results

This article describes the antimicrobial, antioxidant and cytotoxic activities of a number of plants from different localities used in Yemeni traditional medicine. A total of 34 extracts representing 16 plant species belonging to 11 families were submitted in the screening. Table 1 shows the botanical names, plant part used and the traditional uses of the plants in the collected areas.

Characteristics of Plants

The results of the antimicrobial activity of the investigated extracts are shown in Table 2. The antimicrobial activity of the studied plant extracts was exhibited mainly against the Gram-positive bacteria. None of the extracts showed any activity against Gram-negative bacteria. It was interesting to note that the multiresistant Staphylococcus strains showed more sensitivity to the investigated extracts than the other antibiotic susceptible Gram-positive bacteria. Generally, among the investigated extracts the methanolic extracts exhibited the highest antibacterial effect. The most pronounced activity with inhibition zones more than 14 mm was shown by the methanolic extracts of Acalypha fruticosa, Dodonaea viscosa, Jatropha variegata, Lippia citriodora, Plectranthus hadiensis (roots) and Tragia pungens (Table 2). The majority of the hot aqueous extracts of the antibacterial active plants did not express any activity or exhibited only low activity. It is remarkable that no extract showed any antifungal activity against C. maltosa.

Antioxidant Activity

The methanol extracts of six plants namely, A. fruticosa, A. semiflabellata, D. viscosa, P. hadiensis, T. pungens and

Plant species	Extracts	Extract yield (%)	Microbial strains tested						Multiresistant strains tested		
			<i>S. a.</i>	В. с.	<i>M</i> . <i>f</i> .	Е. с.	<i>P. e.</i>	С. т.	S. e. 847	S. h. 535	S. a. North German epidemic strain
Acalypha fruticosa	Methanolic	8.8	14	14	21	-	-	_	12	_	10
	Hot aqueous	5.2	-	-	-	-	-	-	_	_	_
Actiniopteris semiflabellata	Methanolic	11.9	11	9	14	-	-	-	_	-	-
	Hot aqueous	8.1	-	-	_	-	-	-	_	-	20
Alkanna orientalis	Methanolic	17.5	10	10	14	-	-	-	18	20	16
	Hot aqueous	6.9	-	-	_	-	-	-	16	-	8
Carthamus tinctorius	Methanolic	10.2	12	9	12	-	_	_	12	-	_
	Hot aqueous	6.4	-	_	_	-	_	_	-	-	16
Centaurea pseudosinaica	Methanolic	13.1	13	13	14	_	_	_	14	12	_
	Hot aqueous	7.9	_	_	_	_	_	_	_	_	10
Cleome schweinfurthii	Methanolic	8.8	10	10	14	_	_	_	_	8	_
0	Hot aqueous	4.7	-	-	_	-	-	_	_	_	14
Dodonaea viscosa	Methanolic	10.0	15	11	21	-	-	_	12	10	12
	Hot aqueous	7.3	14	8	16	-	-	_	12	_	_
Forsskalea tinacissima	Methanolic	5.3	9	_	10	_	_	_	_	_	_
	Hot aqueous	4.2	_	_	_	_	_	_	_	_	8
Iris albicans	Methanolic	10.9	10	10	14	-	-	_	12	10	_
	Hot aqueous	9.4	-	-	_	-	-	_	_	_	12
Jatropha variegata	Methanolic	9.8	16	10	16	_	_	_	12	12	_
	Hot aqueous	6.8	-	-	_	-	-	_	_	_	12
Lavandula pubescens	Methanolic	8.0	14	11	12	_	_	_	12	12	18
	Hot aqueous	4.9	_	_	_	_	_	_	18	16	22
Lippia citriodora	Methanolic	8.4	17	13	17	_	_	_	20	20	_
	Hot aqueous	5.7	_	_	_	_	_	_	_	_	
Mentha longifolia	Methanolic	10.0	11	9	11	_	_	_	14	10	14
	Hot aqueous	6.8	_	_	_	_	_	_	22	18	20
Plectranthus hadiensis (Leaves)	Methanolic	9.8	12	11	20	_	_	_	12	10	12
	Hot aqueous	7.1	12	9	9	_	_	_	14	10	14
Plectranthus hadiensis (Roots)	Methanolic	7.9	16	14	20	_	_	_	24	16	22
	Hot aqueous	5.0	_	_	_	_	_	_	18	8	16
Tragia pungens	Methanolic	9.9	14	11	14	_	_	_	18	8	12
	Hot aqueous	5.9	8	_	_	_	_	_	14	_	12
Verbascum bottae	Methanolic	19.7	13	9	14	_	_	_	10	_	10
	Hot aqueous		_	_	_	_	_	_	_	_	_
Ampicillin 10 µg/disc	1		25	26	30	N.T.	N.T.	N.T.	_	_	_
Gentamicin 10 µg/disc						15	17	N.T.	N.T.	N.T.	N.T.
Amphotericin 10 µg/disc					N.T.				N.T.	N.T.	N.T.

 Table 2. Results of the antimicrobial activity of the investigated plants in agar diffusion method

S. a., Staphylococcus aureus ATCC 6538; B. c., Bacillus subtilis ATCC 6059; M. f., Micrococcus flavus SBUG 16; E. c., Escherichia coli ATCC 11229; P. e., Pseudomonas aeruginosa ATCC 27853; C. m., Candida maltosa SBUG; S. e. 847, multiresistant Staphylococcus epidermidis; S. h. 535, multiresistant Staphylococcus haemolyticus; S. a. North German species, multiresistant Staphylococcus aureus; –, no activity; N.T., not tested; Inhibition zones including the diameter of the paper disc (6 mm).

V. bottae showed a high effective free radical scavenging in the DPPH assay. These extracts exhibited a remarkable antioxidant effect at low concentrations. So the methanolic extracts of *A. fruticosa* and *T. pungens* exhibited at $10 \,\mu \text{g ml}^{-1}$ an extraordinary antioxidant effect (43% and 54% successively) whereas the ascorbic acid showed at this concentration an effect of 45% (Table 3). *A. semiflabellata*, *D. viscosa* and *P. hadiensis* started to exhibit a high effective free radical scavenging at $50 \,\mu g \,m l^{-1}$ (55, 50 and 54%, respectively). The water extracts of all investigated plants were only weak active. The free radical scavenging effect ranged between 10 and

Plant species	Extracts	$\frac{IC_{50}}{\mu gml^{-1}}$	Radical sc	avenging ac	Photochemical screening			
			$10\mu gm l^{-1}$	$50\mu gm l^{-1}$	$100\mu gml^{-1}$	$500\mu gml^{-1}$	$1000\mu gm l^{-1}$	
Acalypha fruticosa	Methanolic	70	43.37	44.07	52.75	81.92	92.26	Terpenoids, flavonoids, tannins
	Hot aqueous	>1000	0.0	0.0	8.57	15.87	22.94	
Actiniopteris semiflabellata	Methanolic	950	9.15	55.39	92.25	95.54	95.20	Isoflavonoids
	Hot aqueous	>1000	0.0	0.0	14.60	14.91	17.80	
Alkanna orientalis	Methanolic	700	6.02	17.34	15.63	98.10	94.98	Alkaloids, naphthoquinons
	Hot aqueous	>1000	0.0	0.0	0.0	0.0	6.29	
Carthamus tinctorius	Methanolic	100	0.0	0.0	10.78	89.73	88.70	Flavonoids, coloring substances
	Hot aqueous	>1000	0.0	0.0	0.0	10.34	16.21	
Centaurea pseudosinaica	Methanolic	540	0.0	0.0	18.53	74.40	99.98	Volatile oil, terpenoids, flavonoids
	Hot aqueous	>1000	0.0	0.0	0.0	9.44	37.90	
Cleome schweinfurthii	Methanolic	515	2.87	0.20	10.36	64.69	84.80	Glucosinolates, terpenoids, flavonoids
	Hot aqueous	>1000	0.0	0.0	0.0	0.0	2.65	
Dodonaea viscosa	Methanolic	650	29.98	50.72	80.84	94.29	92.45	Flavonoids, Steroids, terpenoids
	Hot aqueous	>1000	0.0	0.0	11.05	22.77	31.80	
Forsskalea tinacissima	Methanolic	100	22.37	19.08	21.42	41.56	63.60	Sterols
	Hot aqueous	>1000	0.0	0.0	0.0	0.0	4.02	
Iris albicans	Methanolic	15	13.78	17.13	33.51	40.10	58.69	Isoflavonoids, flavonoids
	Hot aqueous	>1000	0.0	0.0	0.0	12.64	17.32	
latropha variegata	Methanolic	100	0.0	5.62	51.0	99.0	99.62	Steroids, flavonoids
	Hot aqueous	>1000	0.0	0.0	0.0	0.0	13.96	
Lavandula pubescens	Methanolic	625	16.12	27.45	50.32	93.90	94.34	Terpenoids, Volatile oil and tannins
	Hot aqueous	>1000	0.0	0.0	0.0	0.0	23.83	
Lippia citriodora	Methanolic	30	3.22	7.95	14.04	60.52	99.41	Terpenoids, Volatile oil, tannin
	Hot aqueous	>1000	0.0	0.0	7.78	16.38	33.80	
Mentha longifolia	Methanolic	820	16.90	21.47	36.19	91.76	93.12	Flavonoids, volatil oil, terpenoids
	Hot aqueous	>1000	0.0	2.04	12.00	16.34	24.30	
Plectranthus hadiensis (Leaves)	Methanolic	150	0.0	0.0	12.84	80.10	83.82	Terpenoids, volatile oil, flavonoids
	Hot aqueous	>1000	0.0	0.0	2.05	8.10	8.73	
Plectranthus hadiensis (Roots)	Methanolic	>1000	26.06	54.85	92.07	93.36	95.25	Terpenoids, sterols
	Hot aqueous	>1000	4.30	3.51	10.24	39.90	38.80	
Tragia pungens	Methanolic	70	54.78	62.39	79.45	95.24	95.87	Terpenoids, tannins, flavonoids
	Hot aqueous	>1000	0.0	14.34	11.05	43.56	56.41	
Verbascum bottae	Methanolic	>1000	40.50	46.70	53.40	89.78	93.30	Iridoids, saponins
	Hot aqueous	>1000	0.0	4.02	13.00	23.51	29.00	
Ascorbic acid			45.28	96.81	96.51	97.60	96.37	

Table 3. Results of the free radical scavenging activity, cytotoxicity against FL-cells and phytochemical screening of the investigated plants

38% at the highest concentration namely $1000 \,\mu g \, m l^{-1}$ (Table 3).

I. albicans, *L. citriodora* and *T. pungens* exhibited noticeable activities with IC_{50} values below $100 \,\mu g \,ml^{-1}$ (Table 3).

Cytotoxic Activity

Among the 34 extracts tested for cytotoxicity against FL-cells only the methanolic extracts of *A. fruticosa*,

Phytochemical Screening

The results of the phytochemical screening of the investigated methanolic extracts showed the presence of

different types of active constituents like flavonoids, terpenoids, tannins, volatile oils, etc. (Table 3).

Discussion

The results of our screening assays confirmed the use of the investigated plants in Yemeni traditional medicine. It is the first report about antimicrobial, antioxidant and cytotoxic effects of *A. semiflabellata*, *F. tinacissima*, *I. albicans*, *J. variegata*, *P. hadiensis*, *T. pungens* and *V. bottae*. Whereas other plants like *A. fruticosa*, *A. orientalis*, *D. viscosa*, *L. pubescens*, *L. citriodora* and *M. longifolia* are partly well investigated. The existing knowledge about the other investigated plants is in many cases very limited.

The antibacterial effect of A. fruticosa was investigated among some Indian medicinal plants (24). It was found that A. fruticosa was one of the most active plants tested. Other species of Acalypha like A. siamensis and A. wilkesiana also showed antimicrobial activity (25,26). A search on A. guatemalensis demonstrated antioxidant and antimicrobial activity for this plant (27). The studies on A. wilkesiana and A. hispida as well as A. communis demonstrated the isolation of gallic acid, corilagin, geraniin and triterpenoids of cycloartane-type as compounds responsible for the observed antimicrobial activity (28,29). Flavonoids like quercetin- and kaempferol-derivatives were also identified. Thus, the estimated antimicrobial and antioxidant effects of the investigated A. fruticosa are in accordance with these data. Our phytochemical screening revealed the presence of terpenoids and flavonoids in the methanolic extract of A. fruticosa, which could be responsible for these noteworthy activities. In previous work (10), we reported about the anticancer potential effect of A. fruticosa against five cancer cell-lines. The methanolic extract showed a moderate cytotoxic effect against different cancer cell-lines $(IC_{50} > 50 \,\mu g \,m l^{-1})$ (10). The cytotoxicity of A. fruticosa against FL-cells with IC_{50} of $70 \,\mu g \,ml^{-1}$ was in accordance with that result.

It was reported that the methanolic extract of D. viscosa has an antimicrobial effect (30,31). Others described the isolation of diterpenoid- and flavonoid-derivatives from D. viscosa (32–34). Our phytochemical screening indicated the presence of these types of compounds, which are mainly responsible for the remarkable antioxidant and antimicrobial effect of this plant.

Whereas no reports about *J. variegata* were found, the extracts of other species of *Jatropha* namely *J. elliptica and J. gossypifolia*, exhibited antibacterial effect against gram-positive bacteria (35,36).

The high antibacterial effect of the investigated *L. citriodora* against both antibiotic susceptible and resistant Gram-positive microorganisms is due to the

high content of volatile oil. In earlier studies, it was found that the extracts and isolated volatile oil from L. citriodora and other Lippia species show a strong antimicrobial activity against Helicobacter pylori (37) and against different types of bacteria and fungi (38). Unlike this result, our investigated extract of L. citriodora exhibited activity only against Gram-positive and multiresistant bacteria. No effect was observed against C. maltosa. In addition, it was demonstrated that the infusion of L. citriodora has a potent superoxide radical scavenging activity and a moderate scavenging activity of hydroxyl radical (39). The scavenging activity of DPPH radical in our screening was shown only at the highest concentration $(1000 \,\mu g \,m l^{-1})$. This effect is due to the presence of several flavonoids and phenolic acids (39,40).

For P. hadiensis, no reports were found. However several Plectranthus species were investigated for their antimicrobial activity against different types of microorganisms. The methanolic extract of P. barbatus displayed a potent antibacterial activity against grampositive bacteria including S. aureus (41) and a remarkable antifungal effect against C. albicans (42). Many diterpenoids isolated from several types of Plectranthus like P. fruticosus and P. saccatus were responsible for the antimicrobial effect (43-45). In our screening, the roots of the investigated P. hadiensis exhibited more antimicrobial and antioxidant activity than the leaves. There are possibilities that similar or related compounds are present in the methanolic extract, which may be responsible for the strong antibacterial and antioxidant effect.

Besides the investigation of the leaves and roots of *Tragia involucrata* for their potential effect in wound healing, no reports about *T. pungens* or other species of this genus exist. *T. involucrata* displayed a high antibacterial effect against different bacterial strains especially *S. aureus* (46,47).

In comparison with the fact that no reports about V. bottae for any activity were found, V. macrurum and V. sinuatum showed antimicrobial activity (48,49). The presence of saponins and irridoids as major components could be the reason for the antimicrobial activity (50,51). Our phytochemical investigation showed the presence of saponins, iridoids and flavonoids, which may be responsible for the moderate antimicrobial and noteworthy antioxidant activities.

In conclusion, the results obtained in the present study are in agreement to a certain degree with the traditional uses of the plants estimated. The obtained results could form a good basis for selection of plant species for further investigation in the potential discovery of new natural bioactive compounds. A. fruticosa, C. pseudosinaica, D. viscosa, J. variegata, L. citriodora, P. hadiensis, T. pungens and V. bottae could be a source for antibacterial drugs against Gram-positive bacteria, especially against multiresistant microorganisms. In addition, these plants could represent striking antioxidant agents, which provide prophylaxis against various diseases like heart diseases, stroke, arteriosclerosis and cancers. The bioassay-guided fractionation procedure to characterize and isolate the antibacterial and antioxidant active constituents is needed.

Acknowledgments

The authors would like to thank Al-Saeed foundation for Science and culture, Taiz, Yemen and the University of Science and Technology, Sana'a, Yemen for the financial support to carry out this investigation.

References

- 1. World Health Organization. WHO Traditional Medicine Strategy 2002–2005. Geneva: World Health Organization, 2002.
- Zhang X. Traditional medicine: its importance and protection. In: Twarog S, Kapoor P (eds). Protecting and Promoting Traditional Knowledge: Systems, National Experiences and International Dimensions. Part 1. The Role of Traditional Knowledge in Healthcare and Agriculture. New York: United Nations; 2004, 3–6.
- Fleurentin J, Pelt J-M. Repertory of drugs and medicinal plants of Yemen. J Ethnopharmacol 1982;6:85–108.
- Schopen A. Traditionelle Heilmittel in Jemen. Berlin: Franz Steiner Verlag GmbH, 1983.
- Al-Dubai AS, Al-khulaidi AA. Medicinal and Aromatic Plants of Yemen (In Arabic), Sana'a, Yemen: Obadi Center for studies and Publishing, 1996.
- El-Fiky FK, Attif O, Aboul Ela M, Gaanem N. Antimicrobial evaluation of extracts from some Yemeni plants. *Alex J Pharm Sci* 1995;9:35–7.
- Awadh Ali NA, Juelich W-D, Kusnick C, Lindequist U. Screening of Yemeni medicinal plants for antibacterial and cytotoxic activities. *J Ethnopharmacol* 2001;74:173–9.
- Mothana RAA, Lindequist U. Antimicrobial activity of some medicinal plants of the island Soqotra. J Ethnopharmacol 2005;96:177–81.
- Mothana RAA, Mentel R, Reiss C, Lindequist U. Phytochemical screening and antiviral activity of some medicinal plants of the island soqotra. *Phytother Res* 2006;20:298–302.
- Mothana RAA, Gruenert R, Lindequist U, Bednarski PJ. Study of the anticancer potential of Yemeni plants used in folk medicine. *Pharmazie* 2007;62:305–7.
- Al-Fatimi M, Wuster M, Schroeder G, Lindequist U. Antioxidant, antimicrobial and cytotoxic activities of selected medicinal plants from Yemen. J Ethnopharmacol 2007;111:657–66.
- Austin DJ, Kristinsson KG, Anderson RM. The relationship between the volume of antimicrobial consumption in human communities and the frequency of resistance. *Proc Natl Acad Sci* U S A 1999;96:1152–6.
- Samy RP, Ignacimuthu S, Sen A. Screening of 34 Indian medicinal plants for antibacterial properties. *J Ethnopharmacol* 1998;62:173–81.
- Hamil FA, Apio S, Mubiru NK, Bukenya-Ziraba R, Mosango M, Maganyi OW, et al. Traditional herbal drugs of Southern Uganda, II: literature analysis and antimicrobial assays. *J Ethnopharmacol* 2003;84:57–78.
- Motsei ML, Lindsey KL, Van Staden J, Jaeger AK. Screening of traditionally used South African plants for antifungal activity against *Candida albicans. J Ethnopharmacol* 2003;86:235–41.
- Joyeux M, Moitier F, Fleurentin J. Screening of antiradical, antilipoperoxidant and hepatoprotective effects of nine plant extracts used in caribbean folk medicine. *Phytother Res* 1995;9:228–30.
- 17. Azaizeh H, Ljubuncic P, Portnaya I, Said O, Cogan U, Bomzon A. Fertilization-induced changes in growth parameters and antioxidant

activity of medicinal plants used in traditional Arab medicine. *Evid Based Complement Alternat Med* 2005;2:549-56.

- Alma MH, Mavi A, Yildirim A, Digrak M, Hirata T. Screening chemical composition and in vitro antioxidant and antimicrobial activities of the essential oils from Origanum syriacum growing in Turkey. *Biol Pharm Bull* 2003;26:1725–9.
- 19. Willcox JK, Ash SL, Catignani GL. Antioxidants and prevention of chronic disease. *Crit Rev Food Sci Nutr* 2004;44:275–95.
- Bauer AW, Kirby WMM, Sheriss JC, Turck M. Antibiotic susceptibility testing by standarised single method. Am J Clin Pathol 1966;45:493–6.
- Brand WW, Cuvelier HE, Berset C. Use of a free radical method to evaluate antioxidant activity. *Food Sci Technol* 1995;82:25–30.
- 22. Lindl T, Bauer J. Zell und Gewebekultur. Berlin: Gustav-fischer-Verlag Jena, 1989, 181.
- 23. Wagner H, Bladt S. Plants Drug Analysis: A Thin Layer Chromatography Atlas, 2nd edn. Berlin: Springer, 1996, 306-64.
- Duraipandiyan V, Ayyanar M, Ignacimuthu S. Antimicrobial activity of some ethnomedicinal plants used by Paliyar tribe from Tamil Nadu, India. BMC Complement Altern Med 2006;17:6-35.
- Wiart C, Hannah A, Yassim M, Hamimah H, Sulaiman M. Antimicrobial activity of *Acalypha siamensis* Oliv. ex Gage. *J Ethnopharmacol* 2004;95:285–6.
- 26. Akinyemi KO, Oladapo O, Okwara CE, Ibe CC, Fasure KA. Screening of crude extracts of six medicinal plants used in South-West Nigerian unorthodox medicine for anti-methicillin resistant *Staphylococcus aureus* activity. *BMC Complement Altern Med* 2005;11:5–6.
- 27. Navarro MC, Montilla MP, Cabo MM, Galisteo M, Cáceres A, Morales C, et al. Antibacterial, antiprotozoal and antioxidant activity of five plants used in Izabal for infectious diseases. *Phytother Res* 2003;17:325–9.
- Adesina SK, Idowu O, Ogundaini AO, Oladimeji H, Olugbade TA, Onawunmi GO, et al. Antimicrobial constituents of the leaves of *Acalypha wilkesiana* and *Aacalypha hispida*. *Phytother Res* 2000;14:371–4.
- Gutierrez-Lugo MT, Singh MP, Maiese WM, Timmermann BN. New antimicrobial cycloartane triterpenes from *Acalypha communis*. J Nat Prod 2002;65:872–5.
- Rojas A, Hernandez L, Pereda-Miranda R, Mata R. Screening for antimicrobial activity of crude drug extracts and pure natural products from Mexican medicinal plants. *J Ethnopharmacol* 1992;35:275–83.
- 31. Getie M, Gebre-Mariam T, Rietz R, Höhne C, Huschka C, Schmidtke M, et al. Evaluation of the anti-microbial and anti-inflammatory activities of the medicinal plants *Dodonaea* viscosa, Rumex nervosus and Rumex abyssinicus. Fitoterapia 2003;74:139–43.
- 32. Sachdev K, Kulshreshtha DK. Dodonic acid, a new diterpenoid from *Dodonaea viscosa*. *Planta Med* 1984;50:448–9.
- Abdel-Mogib M, Basaif SA, Asiri AM, Sobahi TR, Batterjee SM. New clerodane diterpenoid and flavonol-3-methyl ethers from *Dodonaea viscosa. Pharmazie* 2001;56:830–1.
- 34. Getie M, Gebre-Mariam T, Rietz R, Neubert RH. Evaluation of the release profiles of flavonoids from topical formulations of the crude extract of the leaves of *Dodonea viscosa* (Sapindaceae). *Pharmazie* 2002;57:320–2.
- 35. de Lima MR, de Souza Luna J, dos Santos AF, de Andrade MC, Sant'Ana AE, Genet JP, et al. Anti-bacterial activity of some Brazilian medicinal plants. *J Ethnopharmacol* 2006;105:137–47.
- Kumar VP, Chauhan NS, Padh H, Rajani M. Search for antibacterial and antifungal agents from selected Indian medicinal plants. J Ethnopharmacol 2006;107:182–8.
- Ohno T, Kita M, Yamaoka Y, Imamura S, Yamamoto T, Mitsufuji S, et al. Antimicrobial activity of essential oils against *Helicobacter pylori. Helicobacter* 2003;8:207–15.
- Oliveira DR, Leitão GG, Santos SS, Bizzo HR, Lopes D, Alviano CS, et al. Ethnopharmacological study of two *Lippia* species from Oriximiná, Brazil. *J Ethnopharmacol* 2006;108:103–8.
- 39. Valentão P, Fernandes E, Carvalho F, Andrade PB, Seabra RM, de Lourdes Basto M. Studies on the antioxidant activity of *Lippia citriodora* infusion: scavenging effect on superoxide radical, hydroxyl radical and hypochlorous acid. *Biol Pharm Bull* 2002;25:1324–7.

- Skaltsa H, Shammas G. Flavonoids from Lippia citriodora. Planta Med 1988;54:465.
- Matu EN, van Staden J. Antibacterial and anti-inflammatory activities of some plants used for medicinal purposes in Kenya. *J Ethnopharmacol* 2003;87:35–41.
- Runyoro DK, Matee MI, Ngassapa OD, Joseph CC, Mbwambo ZH. Screening of Tanzanian medicinal plants for anti-Candida activity. *BMC Complement Altern Med* 2006;30:6–11.
- Gaspar-Marques C, Simões MF, Duarte A, Rodríguez B. Labdane and kaurane diterpenoids from *Plectranthus fruticosus*. J Nat Prod 2003;66:491–6.
- Gaspar-Marques C, Simões MF, Rodríguez B. Further labdane and kaurane diterpenoids and other constituents from *Plectranthus fruticosus. J Nat Prod* 2004;67:614–21.
- Wellsow J, Grayer RJ, Veitch NC, Kokubun T, Lelli R, Kite GC, et al. Insect-antifeedant and antibacterial activity of diterpenoids from species of *Plectranthus. Phytochemistry* 2006;67:1818–25.

- Perumal Samy R, Gopalakrishnakone P, Sarumathi M, Ignacimuthu S. Wound healing potential of *Tragia involucrata* extract in rats. *Fitoterapia* 2006;77:300–2.
- Samy RP, Gopalakrishnakone P, Houghton P, Ignacimuthu S. Purification of antibacterial agents from *Tragia involucrata* a popular tribal medicine for wound healing. *J Ethnopharmacol* 2006;107:99–106.
- 48. Guarino C. Antimicrobial activity of *Verbascum macrurum* ten. (Scrophulariaceae). *Boll Chim Farm* 2002;141:238–42.
- Senatore F, Rigano D, Formisano C, Grassia A, Basile A, Sorbo S. Phytogrowth-inhibitory and antibacterial activity of *Verbascum sinuatum*. *Fitoterapia* 2007;78:244–7.
- Kalpoutzakis E, Aligiannis N, Mitakou S, Skaltsounis AL. Verbaspinoside, a new iridoid glycoside from *Verbascum spinosum*. J Nat Prod 1999;62:342–4.
- 51. Hartleb I, Seifert K. Triterpenoid saponins from Verbascum songaricum. Phytochemistry 1995;38:221-4.

Received August 24, 2007; accepted January 4, 2008



The Scientific **World Journal**



Gastroenterology Research and Practice





Journal of Diabetes Research



Disease Markers



Immunology Research





Submit your manuscripts at http://www.hindawi.com





BioMed **Research International**



Journal of Ophthalmology

Computational and Mathematical Methods in Medicine



Stem Cells International



CAM







Research and Treatment





Oxidative Medicine and Cellular Longevity





Behavioural Neurology