

Original Article

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

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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*, *Actinopterys 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

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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), *Micrococcus 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 g 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 6 mm 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 2 h in refrigerator to enable prediffusion of the extracts into the agar. Then, the plates were incubated overnight (18 h) 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 spectrophotometrically. 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 µg ml⁻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
<i>Acalypha fruticosa</i> Forssk.	YH-05	Euphorbiaceae	L, S	Skin diseases, malaria and wounds (3, 4, a)
<i>Actiniopteris semiflabellata</i> Pic.-Ser.	Mo-M08	Pteridophyta	L	Wounds and burns (a)
<i>Alkanna orientalis</i> (L.) Boiss.	Mo-I03	Boraginaceae	R	Common cold, pharyngitis, rheumatism and toothache (4, a)
<i>Carthamus tinctorius</i> L.	Mo-T10	Asteraceae	L, S	Skin diseases e.g. freckles, coloring agent and in food (4, a)
<i>Centaurea pseudosinaica</i> Czerep.	Mo-S11	Asteraceae	L, T	Wounds, kidney diseases (4, a)
<i>Cleome schweinfurthii</i> Gilg.	Mo-I01	Capparaceae	L, S	Otitis (a)
<i>Dodonaea viscosa</i> (L.) Jacq.	Mo-T01	Sapindaceae	L, S	Malaria, wounds and burns (5, a)
<i>Forsskalea tinacissima</i> L.	Mo-S08	Urticaceae	L	Diuretic and kidney diseases (a)
<i>Iris albicans</i> Lange	Mo-I02	Iridaceae	R	Rheumatism and gout (4, a)
<i>Jatropha variegata</i> Vahl	Mo-T05	Euphorbiaceae	L, T	Antiseptic, for wounds and hemostatic (3, 5)
<i>Lavandula pubescens</i> Decne.	Mo-S10	Lamiaceae	L, F	Antiseptic, carminative and diuretic, (3, 4, 5, a)
<i>Lippia citriodora</i> Kunth	Mo-S03	Verbenaceae	L	Spasmolytic, gastrointestinal troubles, common cold and sedative (4, a)
<i>Mentha longifolia</i> (L.) Hudson	Mo-M01	Lamiaceae	L, S	Spasmolytic and digestive disorders (3, 4, a)
<i>Plectranthus hadiensis</i> (Forssk.) Schweinf. ex Sprenger	Mo-T04	Lamiaceae	L, R	Antiseptic and haemostatic (3, a)
<i>Tragia pungens</i> (Forssk.) Muell.-Arg.	YT-20	Euphorbiaceae	L, S	Allergy and skin diseases (a)
<i>Verbascum bottae</i> (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:

$$\text{Percentage of radical scavenging activity} = \frac{(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}})}{\text{Abs}_{\text{control}}} \times 100$$

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% cell-inhibitory 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

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

Plant species	Extracts	Extract yield (%)	Microbial strains tested						Multiresistant strains tested		
			<i>S. a.</i>	<i>B. c.</i>	<i>M. f.</i>	<i>E. c.</i>	<i>P. e.</i>	<i>C. m.</i>	<i>S. e.</i> 847	<i>S. h.</i> 535	<i>S. a.</i> North German epidemic strain
<i>Acalypha fruticosa</i>	Methanolic	8.8	14	14	21	–	–	–	12	–	10
	Hot aqueous	5.2	–	–	–	–	–	–	–	–	–
<i>Actinopterys semiflabellata</i>	Methanolic	11.9	11	9	14	–	–	–	–	–	–
	Hot aqueous	8.1	–	–	–	–	–	–	–	–	20
<i>Alkanna orientalis</i>	Methanolic	17.5	10	10	14	–	–	–	18	20	16
	Hot aqueous	6.9	–	–	–	–	–	–	16	–	8
<i>Carthamus tinctorius</i>	Methanolic	10.2	12	9	12	–	–	–	12	–	–
	Hot aqueous	6.4	–	–	–	–	–	–	–	–	16
<i>Centaurea pseudosinaica</i>	Methanolic	13.1	13	13	14	–	–	–	14	12	–
	Hot aqueous	7.9	–	–	–	–	–	–	–	–	10
<i>Cleome schweinfurthii</i>	Methanolic	8.8	10	10	14	–	–	–	–	8	–
	Hot aqueous	4.7	–	–	–	–	–	–	–	–	14
<i>Dodonaea viscosa</i>	Methanolic	10.0	15	11	21	–	–	–	12	10	12
	Hot aqueous	7.3	14	8	16	–	–	–	12	–	–
<i>Forsskalea tinacissima</i>	Methanolic	5.3	9	–	10	–	–	–	–	–	–
	Hot aqueous	4.2	–	–	–	–	–	–	–	–	8
<i>Iris albicans</i>	Methanolic	10.9	10	10	14	–	–	–	12	10	–
	Hot aqueous	9.4	–	–	–	–	–	–	–	–	12
<i>Jatropha variegata</i>	Methanolic	9.8	16	10	16	–	–	–	12	12	–
	Hot aqueous	6.8	–	–	–	–	–	–	–	–	12
<i>Lavandula pubescens</i>	Methanolic	8.0	14	11	12	–	–	–	12	12	18
	Hot aqueous	4.9	–	–	–	–	–	–	18	16	22
<i>Lippia citriodora</i>	Methanolic	8.4	17	13	17	–	–	–	20	20	–
	Hot aqueous	5.7	–	–	–	–	–	–	–	–	–
<i>Mentha longifolia</i>	Methanolic	10.0	11	9	11	–	–	–	14	10	14
	Hot aqueous	6.8	–	–	–	–	–	–	22	18	20
<i>Plectranthus hadiensis</i> (Leaves)	Methanolic	9.8	12	11	20	–	–	–	12	10	12
	Hot aqueous	7.1	12	9	9	–	–	–	14	10	14
<i>Plectranthus hadiensis</i> (Roots)	Methanolic	7.9	16	14	20	–	–	–	24	16	22
	Hot aqueous	5.0	–	–	–	–	–	–	18	8	16
<i>Tragia pungens</i>	Methanolic	9.9	14	11	14	–	–	–	18	8	12
	Hot aqueous	5.9	8	–	–	–	–	–	14	–	12
<i>Verbascum bottae</i>	Methanolic	19.7	13	9	14	–	–	–	10	–	10
	Hot aqueous	14.8	–	–	–	–	–	–	–	–	–
Ampicillin 10 µg/disc			25	26	30	N.T.	N.T.	N.T.	–	–	–
Gentamicin 10 µg/disc			N.T.	N.T.	N.T.	15	17	N.T.	N.T.	N.T.	N.T.
Amphotericin 10 µg/disc			N.T.	N.T.	N.T.	N.T.	N.T.	10	N.T.	N.T.	N.T.

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 µg 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 µg ml⁻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

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

Plant species	Extracts	IC ₅₀ μg ml ⁻¹	Radical scavenging activity (%)					Photochemical screening
			10 μg ml ⁻¹	50 μg ml ⁻¹	100 μg ml ⁻¹	500 μg ml ⁻¹	1000 μg ml ⁻¹	
<i>Acalypha fruticosa</i>	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	
<i>Actinopterys semiflabellata</i>	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	
<i>Alkanna orientalis</i>	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	
<i>Carthamus tinctorius</i>	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	
<i>Centaurea pseudosinaica</i>	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	
<i>Cleome schweinfurthii</i>	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	
<i>Dodonaea viscosa</i>	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	
<i>Forsskalea tinacissima</i>	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	
<i>Iris albicans</i>	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	
<i>Jatropha variegata</i>	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	
<i>Lavandula pubescens</i>	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	
<i>Lippia citriodora</i>	Methanolic	30	3.22	7.95	14.04	60.52	99.41	Terpenoids, Volatile oil, tannins
	Hot aqueous	>1000	0.0	0.0	7.78	16.38	33.80	
<i>Mentha longifolia</i>	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	
<i>Plectranthus hadiensis</i> (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	
<i>Plectranthus hadiensis</i> (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	
<i>Tragia pungens</i>	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	
<i>Verbascum bottae</i>	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	

38% at the highest concentration namely 1000 μg ml⁻¹ (Table 3).

Cytotoxic Activity

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

I. albicans, *L. citriodora* and *T. pungens* exhibited noticeable activities with IC₅₀ values below 100 μg ml⁻¹ (Table 3).

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 ($IC_{50} > 50 \mu\text{g ml}^{-1}$) (10). The cytotoxicity of *A. fruticosa* against FL-cells with IC_{50} of $70 \mu\text{g ml}^{-1}$ 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 multi-resistant 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 ($1000 \mu\text{g ml}^{-1}$). 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 gram-positive 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, irridoids 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.

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