

Antimicrobial activity of some medicinal plants of the island Soqatra

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Abstract

Twenty-five selected plants belonging to 19 families were collected from different localities of the island Soqatra, dried and extracted with the solvents chloroform, methanol and hot water to yield 80 extracts. The extracts were tested for their antimicrobial activity against several Gram-positive and Gram-negative bacteria and against one yeast species using agar diffusion method. Antibacterial activity was demonstrated especially against Gram-positive bacteria including multiresistant *Staphylococcus* strains. The greatest activity was exhibited by the methanolic extracts of *Boswellia elongata*, *Boswellia ameero*, *Buxus hildebrandtii*, *Commiphora parvifolia*, *Jatropha unicostata*, *Kalanchoe farinacea*, *Pulicaria stephanocarpa*, *Punica protopunica*, *Withania adunensis* and *Withania riebeckii*. Only the methanolic extract of *Buxus hildebrandtii* displayed significant antifungal activity.

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1. Introduction

Herbal medicine represents one of the most important fields of traditional medicine in Yemen especially in rural areas. Thus, phytotherapy is practiced by a large proportion of Yemen population for the treatment of several physical, physiological, mental and social ailments. To promote the proper use of herbal medicine and to determine their potential as sources for new drugs it is essential to study medicinal plants, which have folklore reputation in a more intensified way (Schopen, 1983; El-Faky et al., 1995; Al-Dubai and Al-Khulaidi, 1996; Awadh Ali et al., 2001).

Within the recent years, infections have increased to a great extent and antibiotics resistance becomes an ever-increasing therapeutic problem (Austin et al., 1999). Natural products of higher plants may give a new source of antimicrobial agents with possibly novel mechanisms of

action (Hamil et al., 2003; Machado et al., 2003; Motsei et al., 2003; Barbour et al., 2004).

This paper reports the first attempt to study the antimicrobial activity of medicinal plants of the island Soqatra. We selected this part of Yemen because this island is undoubtedly a most precious natural asset not only for the Republic of Yemen but also for the whole world and human kind. 273 plants of about 850 plants are considered to be endemic. The selection of the plants for evaluation was based firstly on traditional use for the treatment of infectious and other diseases and secondly on the occurrence as endemic plants.

2. Material and methods

2.1. Plant materials

The plants were collected from different localities of the island Soqatra (Yemen) in the beginning of spring and in the winter 2002 and identified at the Botany Department, Faculty of Science, Sana'a University. Voucher specimens

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were deposited at the Pharmacognosy Department, Faculty of Pharmacy, Sana'a University.

2.2. Extraction of plant material

The air-dried and powdered plant materials (10 g of each) were extracted successively under shaking with chloroform (CHCl₃) for three to five times at room temperature, with 90% methanol (CH₃OH) in water-bath at 50 °C for three to five times and finely with water (H₂O) in a water-bath at 70 °C. The obtained extracts were filtered and evaporated by using vacuum evaporator or by freeze dryer to give the crude dried extract.

2.3. Antimicrobial assay

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 (*Staphylococcus epidermidis* 847, *Staphylococcus haemolyticus* 535 and *Staphylococcus aureus* North German reference strain) were also applied as test organisms. The strains 847 and 535 were isolated from hospitalized patients and stored at Institute of Hygiene of Mecklenburg-Vorpommern, Greifswald, Germany.

A modified agar diffusion method (Bauer et al., 1966) was used to determine the antimicrobial activity. Nutrient agar (OXOID LTD, Basingstoke, Hampshire, UK, 27 g/L water) was inoculated with microbial cell suspension (200 µl in 20 ml 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 extract solution (equivalent to 4 mg of the dried extract) and after evaporation placed on the surface of the inoculated agar plates. Ampicillin, gentamicin and amphotericin B were used as positive controls. Negative controls were done using paper discs loaded with 20 µl of the solvents. After preincubation for 2 h in a refrigerator the plates were incubated overnight (18 h) at 37 °C. In contrast, *Micrococcus flavus* was incubated at room temperature for 48 h and *Candida maltosa* was incubated at 28 °C for 48 h. At the end of the incubation period the antibacterial activity was evaluated by measuring the inhibition zones. An inhibition zone of 14 mm or greater (including diameter of the disc) was considered as high antibacterial activity.

3. Results and discussion

The paper describes the antimicrobial activity of a number of Yemeni plants from the island Soqatra against several Gram-positive and Gram-negative bacteria including

Table 1
List of plants screened for antimicrobial activity

Plant	Voucher specimen no.	Family	Part tested	Traditional uses ^a
<i>Boswellia ameero</i> Balf. f. ^b	SP-M106	Burseraceae	B	Common cold, bronchitis, asthma, rheumatism
<i>Boswellia elongata</i> Balf. f. ^b	SP-M102	Burseraceae	B	As <i>Boswellia ameero</i>
<i>Buxus hildebrandtii</i> Baill.	SP-M100	Buxaceae	L	Malaria
<i>Carphalea obovata</i> (Balf.f.) Verdcourt	SP-D213	Rubiaceae	L	Unknown
<i>Cassia socotrana</i> Serrato ^b	SP-A010	Caesalpiniaceae	L, T	Obstipation, skin diseases
<i>Cissus hamaderohensis</i> Radcliffe-Smith ^b	SP-N020	Vitaceae	L	Gastrointestinal troubles, skin diseases, burns
<i>Cissus subaphylla</i> (I.B.Balf.) Planch. ^b	SP-N025	Vitaceae	L	As <i>Cissus hamaderohensis</i>
<i>Cleome socotrana</i> Balf. f. ^b	SP-N030	Capparaceae	L, T	Migraine, otitis
<i>Commiphora parvifolia</i> Engl. ^b	SP-M108	Burseraceae	B	Antiseptic, diarrhea, dysentery, as uterine stimulant, emmenagogue
<i>Cystostemon socotranus</i> Balf. f. ^b	SP-M118	Boraginaceae	L, S	Unknown
<i>Dendrosicyos socotrana</i> Balf. f. ^b	SP-A015	Cucurbitaceae	L, S	Obstipation, however no uses
<i>Dorstenia socotrana</i> A.G.Miller ^b	SP-M122	Moraceae	S, L	Skin diseases
<i>Dracaena cinnabari</i> Balf. f. ^b	SP-D225	Agavaceae	L, F, R	Gingivitis, wounds, spasmolytic
<i>Euryops arabicus</i> Steud.ex Jaub.&Spach	SP-D203	Asteraceae	L, F	Unknown
<i>Exacum affine</i> Balf. f. ^b	SP-M112	Gentianaceae	L, F	Unknown
<i>Fagonia luntii</i> Baker	SP-C005	Zygophyllaceae	L, S	Headache, paresis, intestinal obstruction
<i>Gnidia socotrana</i> Gilg	SP-D207	Thymelaeaceae	L, T	Obstipation
<i>Jatropha unicostata</i> Balf. f. ^b	SP-N035	Euphorbiaceae	B, L, T	Wounds, eczema, scabies
<i>Kalanchoe farinacea</i> Balf. f. ^b	SP-D201	Crassulaceae	L, F	Tissue injuries, enlarged ganglia and peptic ulcer
<i>Pulicaria stephanocarpa</i> Balf. f. ^b	SP-C006	Asteraceae	L, F	Unknown
<i>Punica protopunica</i> Balf. f. ^b	SP-D223	Punicaceae	T, L	Anthelmintic, peptic ulcer, diarrhea, dysentery
<i>Trichocalyx obovatus</i> Balf. f. ^b	SP-D201	Acanthaceae	L, F	Unknown
<i>Withania adunensis</i> Vierh. ^b	SP-M110	Solanaceae	L, T	Inflammations, wounds, rheumatism
<i>Withania riebeckii</i> Schweinf.ex Balf.f. ^b	SP-M116	Solanaceae	L, T	As <i>Withania adunensis</i>
<i>Zygophyllum quatarense</i> M.N.Hadidi	SP-C007	Zygophyllaceae	L, S	Conjunctivitis, rheumatism, cough, as diuretic

^a The information about traditional uses has been taken from Al-Dubai and Al-Khulaidi, 1996, Schopen, 1983 and from consultation of native people.

^b Endemic plant, B: bark, F: flower, L: leaves, R: resin, S: stems, T: fruits.

Table 2 (Continued)

Plant species	Extract	Extract yield in % ^a	Inhibition zones (mm) ^b against								
			<i>S. a.</i>	<i>B. c.</i>	<i>M. f.</i>	<i>E. c.</i>	<i>P. a.</i>	<i>C. m.</i>	<i>S. e.</i> 847	<i>S. h.</i> 535	<i>S. a.</i> NGR
<i>Jatropha unicostata</i> (Leaves and fruits)	Chloroform	1.36	10	8	8	–	–	–	–	–	–
	Methanol	2.95	18	10	15	–	–	–	18	14	18
	Water	2.37	–	–	–	–	–	–	–	–	–
<i>Jatropha unicostata</i> (Bark)	Chloroform	0.24	11	10	11	–	–	–	10	8	10
	Methanol	5.26	10	10	12	–	–	–	12	–	12
	Water	3.01	–	–	–	–	–	–	–	–	–
<i>Kalanchoe farinacea</i>	Chloroform	4.52	–	–	–	–	–	–	–	–	–
	Methanol	10.28	15	10	17	–	–	–	16	12	16
	Water	5.37	–	–	–	–	–	–	–	–	–
<i>Pulicaria stephanocarpa</i>	Chloroform	1.40	–	–	–	–	–	–	–	–	–
	Methanol	2.98	19	14	17	–	–	–	18	14	20
	Water	2.20	–	–	–	–	–	–	–	–	–
<i>Punica protopunica</i>	Chloroform	2.60	10	8	10	–	–	–	–	–	–
	Methanol	8.43	18	14	25	–	–	–	22	18	22
	Water	12.22	18	17	18	–	–	–	24	20	22
<i>Trichocalyx obovatus</i>	Chloroform	1.12	–	–	–	–	–	–	–	–	–
	Methanol	7.64	–	–	–	–	–	–	10	–	10
	Water	3.65	–	–	–	–	–	–	–	–	–
<i>Withania adunensis</i>	Chloroform	1.47	17	17	25	–	–	–	16	16	16
	Methanol	7.54	16	16	21	–	–	–	16	14	16
	Water	4.12	10	10	14	–	–	–	10	–	–
<i>Withania riebeckii</i>	Chloroform	1.89	16	16	25	–	–	–	14	14	16
	Methanol	8.03	17	18	22	–	–	–	18	16	18
	Water	4.67	8	10	14	–	–	–	–	10	–
<i>Zygophyllum quatarense</i>	Chloroform	0.77	–	–	–	–	–	–	–	–	–
	Methanol	3.70	10	11	11	–	–	–	–	–	–
	Water	3.02	–	–	–	–	–	–	–	–	–
Ampicillin, 10 µg/disc			26	28	31	N.T.	N.T.	N.T.	–	–	–
Gentamicin, 10 µg/disc			N.T.	N.T.	N.T.	14	18	N.T.	N.T.	N.T.	N.T.
Amphotericin B, 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 6051; *M. f.*: *Micrococcus flavus* SBUG 16; *E. c.*: *Escherichia coli* ATCC 11229; *P. a.*: *Pseudomonas aeruginosa* ATCC 27853; *C. m.*: *Candida maltosa* SBUG; *S. e.* 847: multiresistant *Staphylococcus epidermidis*; *S. h.* 535: multiresistant *Staphylococcus haemolyticus*; *S. a.* NGR North German reference strain, multiresistant *Staphylococcus aureus*. –: no activity; N.T.: not tested. Negative controls did not show any activity.

^a Percentage extract yield (w/w) was estimated as dry extract weight/dry starting material weight × 100.

^b Inhibition zones including the diameter of the paper disc (6 mm).

multiresistant strains. A total of 80 extracts representing 25 plant species belonging to 19 families were submitted in the screening. Table 1 shows the botanical names, plant part used and the traditional uses of the plants in the island Soqatra. The results of the antimicrobial activity of the investigated extracts are shown in Table 2. The antimicrobial activity of the extracts was directed mainly against the Gram-positive bacteria. None of the extracts showed activity against Gram-negative bacteria. It was interesting to note that the multiresistant *Staphylococcus* strains showed more sensitivity to the investigated extracts than the antibiotic susceptible Gram-positive bacteria. Generally, among the investigated extracts the methanolic extracts exhibited the highest antibacterial effect followed by the chloroform extracts. The most pronounced activity with inhibition zones of more than 14 mm was shown by the methanolic extracts of *Boswellia*

ameero, *Boswellia elongata*, *Commiphora parvifolia*, *Buxus hildebrandtii*, *Jatropha unicostata*, *Kalanchoe farinacea*, *Pulicaria stephanocarpa*, *Punica protopunica*, *Withania adunensis* and *Withania riebeckii*. In addition, strong antibacterial activities were also shown by the chloroform extracts of *Cleome socotrana*, *Dracaena cinnabari* (resin), *Withania adunensis* and *Withania riebeckii*. The majority of the hot aqueous extracts of the investigated plants exhibited only low or no activity with the exception of the hot aqueous extracts of *Punica protopunica* and *Boswellia elongata*. *Cassia socotrana*, *Cissus subaphylla*, *Dendrosicyos socotrana*, *Dracaena cinnabari* (leaves), *Euryops arabicus*, *Exacum affine*, *Gnidia socotrana* and *Trichocalyx obovatus* did not display any antimicrobial activity. The methanolic extract of *Buxus hildebrandtii* was the only one that showed strong antifungal activity against *Candida maltosa*.

Although other species of the genera *Boswellia* (Adelakun et al., 2001), *Buxus* (Rahman et al., 1997), *Commiphora* (Asres et al., 1998), *Jatropha* (Aiyelaagbe et al., 2000), *Punica* (Machado et al., 2002), *Withania* (Budhiraja et al., 2000), *Kalanchoe* (Asiedu-Gyekye et al., 2002), *Cissus* (Beltrame et al., 2002) and *Cleome* (Samy et al., 1999) have been subjected to several studies and antimicrobial activities have been found, nothing is known about the species reported here. The results obtained in the course of the present study are in agreement to a certain degree with the traditional uses of the plants evaluated.

Punica protopunica, *Boswellia* species, *Commiphora parvifolia*, *Buxus hildebrandtii*, *Jatropha unicostata*, *Kalanchoe farinacea* and *Withania* species seem to be valuable sources for antibacterial drugs against Gram-positive bacteria, especially against multiresistant microorganisms. The bioassay-guided fractionation procedure to characterize and isolate the antibacterial active constituents is under way in our laboratory. In addition, these plants are currently being investigated for other pharmacological activities.

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