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Original article

Antimicrobial activity of four medicinal plants widely used in Persian folk medicine

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Abstract

Background and objectives: *Commiphora habessinica* (O.Berg) Engl. (Burseraceae), *Boswellia sacra* Flueck (Burseraceae), *Phoenix dactylifera* L. (Arecaceae), and *Doronicum glaciale* (Wulfen) Nyman (Asteraceae) are of ethnomedicinal importance in Persian folk medicine and are widely used to treat infectious diseases**.** The aim of the present study was to investigate the antimicrobial properties of these herbal medicines to prevent misadministration. **Methods**: Antifungal and antibacterial (Gram-positive and Gram-negative) activities of the petroleum ether, dichloromethane and ethanol fractions obtained from oleo-gum-resin of *C. habessinica* and *B. sacra*, spathe of *P. dactylifera* and roots of *D. glaciale* were evaluated against standard species and clinical antibiotic resistant isolates using broth microdilution method. The fractions were tested at concentrations of 0.5 to 256 µg/mL. **Results**: The petroleum ether fraction of *C. habessinica* oleo-gum-resin exhibited the most anti-*Candida* activity with MIC_{50} of 0.5-16 µg/mL. The growth of *C. glabrata* and *C. tropicalis* was inhibited by the ethanol fraction of *C. habessinica* oleo-gum-resin with MIC_{50} of 1-16 µg/mL. *C. glabrata* was the most susceptible species. Among the tested fractions, only the petroleum ether fraction of *C. habessinica* oleo-gum-resin had an inhibitory effect on *Aspergillus* spp. with a MIC_{50} of 8-32 µg/mL. None of the fractions exhibited antimicrobial activity against the Gram-positive and Gram-negative bacteria at concentrations of 0.5 to 256 µg/mL. **Conclusions**: The sensitivity of fungi and bacteria to natural antimicrobials varies widely within species and it is essential to consider the sensitivity of the strains to prevent resistance.

Keywords: antimicrobial activity, *Boswellia sacra*, *Commiphora habessinica*, *Doronicum glaciale*, *Phoenix dactylifera*

Introduction

Antibiotic resistance is an increasing problem for health professionals as a result of misadministration and overuse of antibiotics [1-3]. Researchers and pharmaceutical companies are searching for new anti-microbial substances by synthesizing new derivatives or screening

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marine and herbal sources of traditional medicine [4,5]. It is a challenge to find sensitive bacteria or fungal strains for correct administration and prevention of misadministration and resistance [6]. Cost effectiveness is another challenge for antibiotic therapy, especially for developing countries. This makes it essential to find sensitive strains for different herbal medicines [7,8].

Four commonly-used medicinal plants with ethnomedicinal importance in traditional Persian medicine were selected for the present study (Table 1). The spathe of *Phoenix dactylifera* L. (Arecaceae) and its aromatic water called "tarooneh" in Persian folk medicine are used to treat pertussis, as a bladder and nerve tonic, sedative, tranquilizer and for rheumatoid arthritis. The composition of the volatile constituents and fractions of tarooneh has recently been evaluated. Triterpenes, flavonoids and oxygen containing monoterpenes (such as carvacrol, linalool, and thymol) have been found in this herb, which suggests a possible antimicrobial function [9].

Table 1. Selected plants, medicinal part and their herbarium code

Traditional name	Scientific name	Family	Part used	Code
Tarooneh	*Phoenix dactylifera* L.	Arecaceae	spathe	PM-172
Morre-makki	*Commiphora habessinica* (O.Berg) Engl.	Burseraceae	oleo-gum-resin	PM-155
Kondor	*Boswellia sacra* Flueck	Burseraceae	oleo-gum-resin	PM-177
Darunaj-e-agharabi	*Doronicum glaciale* (Wulfen) Nyman	Asteraceae	roots	PM-181

Commiphora habessinica (O.Berg) Engl. [Syn.: *C. abyssinica* (Engl.) Engl. and *C. assaortensis* Chiov.] is from the family Burseraceae. It is a small tree indigenous to northeast Africa and is found in southern Arabia and Iran. The oleo-gum-resin of this plant, which is called "morre-makki" has been used to treat and prevent infections (as antiseptic, in urinary disorders and skin diseases, for treatment of gonorrhea and for nasal catarrh and bronchitis) and to heal wounds. It is also used as aromatic for perfumes, in funerals, and as an insect repellent [10].

Boswellia sacra Flueck [Syn.: *B. carterii* Birdw., *B. bhaw-dajiana* Birdw. and *B. undulate crenata* (Engl.) Engl.] belongs to Burseraceae. The plant's oleo-gum-resin is called "kondor" in Persian and frankincense in English. It is routinely used in Persian folk medicine to heal wounds and treat infectious diseases (diarrhea, dysentery, urinary disorders, gonorrhea and bronchitis). It is burned with *Peganum harmala* to form smoke that acts as an air freshener and disinfectant. Reports have indicated that the oleo-gum-resin obtained from *B. sacra* (*B. carterii*) has neuroprotective, immunomodulatory [11], antioxidant [12] and anti-inflammatory [13] properties. Some studies have reported antimicrobial properties of the essential oils obtained from different species of *Boswellia genus*; there has been no study about the different fractions of this oleo-gum-resin [14,15].

Doronicum glaciale (Wulfen) Nyman [Syn.: *D. scorpioides* Willd., *D. latifolium* Bubani and *Aronicum scorpioides* (L.) D.C.] from Asteraceae is used in Persian folk and traditional medicine as a tonic for the liver, spleen, heart, nerves and also for treatment of some infectious diseases. It is said that the roots are an antidote for insect bites and venoms [10]. A preliminary phytochemical investigation on its roots has been reported by Hamedi *et al.* [16]. No report about the antimicrobial activity has been found, but another species of the genus *D. hookeri* has been reported to have antibacterial properties [17].

It is essential to find new antimicrobial agents and to apply antimicrobials correctly for susceptible microorganisms to prevent antimicrobial resistance. The present study was designed to determine the susceptibility of important pathogens to fractions of these four plants.

Experimental

Plant material

The dried medicinal parts of *B. sacra, C. habessinica, D. glacialeand and P. dactylifera* were purchased from Shiraz herbal market and authenticated by Sedigheh Khademyan, a taxonomist in the Department of Pharmacognosy

of Shiraz Faculty of Pharmacy. The voucher specimen of each medicinal part was preserved with the code as shown in table 1 and was kept in the herbal museum of the department for further reference. The plant material was powdered, passed through a no. 100 sieve number and kept in a dark closed container.

Plant powders (100 g) were extracted using petroleum ether in a Soxhlet apparatus for 6 h (petroleum ether fraction). The residuum was dried and macerated with dichloromethane for 2 days followed by ethanol for 2 days in a dark and closed glass container (solvents were purchased from Merck, Germany). Petroleum ether, dichloromethane and ethanol fractions were concentrated with a rotary evaporator and dried. The dried extracts were weighed and kept in Teflon caped tubes in -20 °C.

Determination of antimicrobial activity

Microorganisms

The antifungal activity of the extracts against fungi were determined by broth microdilution against the standard species *Aspergillus flavus* (ATCC 64025), *A. fumigates* (ATCC 14110), *A. clavatus* (CBS 514.65), *Candida albicans* (ATCC 10261), *C. tropicalis* (ATCC 750), *C. krusei* (ATCC 6258), *C. glabrata* (ATCC 90030), and *C. parapsilosis*.

The study also determined the *in vitro* activity of the extracts against standard species of *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Entrococcus faecalis* (ATCC11700), and the three clinical isolates of methicillin sensitive *S. aureus*, methicillin resistant *S. aureus*, third generation cephalosporin resistant *E. coli*, third generation cephalosporin sensitive *E. coli*.

Determination of minimum inhibitory concentrations

Minimum inhibitory concentrations (MICs) were determined using broth microdilution as recommended by Clinical and Laboratory Standards Institute (CLSI) with some modifications [18-20]. To determine the antifungal activity of the extracts against fungi, serial dilutions of the extracts (0.5-256 µg/mL) were prepared in 96-well microtiter plates using RPMI-1640 media (Sigma, St. Louis, USA) buffered with 3-(N-morpholino) propanesulfonic acid (MOPS) (Sigma, St. Louis, USA). Serial dilutions of the extracts (0.5-256.0 µg/mL) were prepared in Muller-Hinton Broth media (Merck, Germany) to determine their antibacterial activity.

The fungi and bacteria strains were suspended in media and the cell densities were adjusted to 0.5 McFarland standards at 530 nm wavelength using a spectrophotometer. This yielded stock suspensions of 1-5 × 10^6 cells/mL for yeast and 1-1.5 × 10^8 cells/mL for the bacteria. The working inoculums (0.1 mL) were added to the microtiter plates and incubated in a humid atmosphere at 30 °C for 24 to 48 h (fungi) or at 37 °C for 24 h (bacteria). The uninoculated medium (200 µL) was included as a sterility control (blank). Growth controls (medium with inoculums but without the extracts) were also included. The growth in each well was compared with the growth in the control well. MICs were visually determined and were defined as the lowest drug concentration at which a predominant decrease in turbidity (approximately 50% and 90% inhibition), compared with that of drug-free growth control well, was observed. Each experiment was performed in triplicate.

Media from wells with fungi showing no visible growth were further cultured on Sabouraud dextrose agar (Merck, Germany) and from wells with bacteria showing no visible growth on Muller-Hinton agar (Merck, Germany) to determine the minimum fungicidal concentration (MFC) and minimum bactericidal concentration (MBC). MBCs and MFCs were determined as the lowest concentration yielding no more than four colonies, which corresponds to a mortality of 99.9% of the microbes in the initial inoculums. Oxacillin disks were used (Mast Diagnostics, Merseyside, UK) for detection of methicillin resistant *S. aureus*, and ceftriaxone for third generation resistant *E. coli* [21].

Results and Discussion

Table 2 gives the yields of petroleum ether,

dichloromethane and ethanol fractions of the selected plants. As expected, the petroleum ether fraction of *B. sacra* which is rich in essential oils and resinous components had the highest yield.

Table 3 shows the antifungal activities of the plant extracts. The petroleum ether fraction of *C. habessinica* exhibited the most anti-*Candida* activity and inhibited the growth of *Candida* with MIC_{50} of 0.5-128 µg/mL. The growths of Candida species were inhibited by the ethanol fraction of *C. habessinica* with MIC_{50} 1-256 µg/mL, in which *C. glabrata* was the most susceptible species against this fraction. *C. glabrata* (*Torulopsis glabrata)* is a non-hyphae-forming yeast that creates infections most commonly observed in the urinary tracts of immunocompromised AIDS patients and in the elder people. *C. glabrata* has been reported to cause 20% of all urinary yeast infections. The most serious infections include fungaemias, meningitis and endocarditis [22].

Recently, attention has focused on fungal diseases caused by *C. albicans* over those by non-*albicans* species of *Candida*, such as *glabrata*, especially in ICU patients [23]. *C. glabrata* has the ability to produce sticky biofilms that adhere to living and non-living surfaces (such as catheters), making treatment more difficult. There have been several reports on the amphotericin B and fluconazole resistance of this yeast [24-27].

The petroleum ether and ethanol fractions of *C. habessinica* inhibited the growth of *C. tropicalis* with MICs of 0.5 and 16 µg/mL. *C. tropicalis* is taxonomically similar to *C. albicans*, one of the more common forms of *Candida* that causes disease in tropical countries at a rate of 3% to 66% of invasive candidaemia, depending on geography. This fungus is particularly virulent in neutropenic hosts with hematogenous seeding to peripheral organs [28-30]. Amphotericin B, an echinocandin, and triazoles are recommended for candidaemia and invasive candidiasis caused by *C. tropicalis*. Primary fluconazole resistance is uncommon but may be induced on exposure [31,32].

The essential oil extracted from *C. habessinica* oleo-gum-resin has been reported to contain β-elemene (32.1%), α-selinene (18.9%) and cadina-1,4-diene (7.5%) [33]. Extracts from different plants that contain these constituents are frequently reported to exhibit antimicrobial activity [34-36].

We could find no reports on the composition of the petroleum ether fraction of *C. habessinica*, but it is suspected to be rich in mono- and sesqui-terpenes such as curzerene, furanoeudesma-1,3-diene, β-elemene, α-selinene, cadina-1,4-diene and furanodiene [33, 37]. Two constituents of the hexane fraction obtained from resin of *C. molmol* were identified as uranodiene-6-one and methoxyfuranoguaia-9-ene-8-one.

They have shown potent antibacterial and antifungal activities against standard pathogenic strains of *E. coli*, *S. aureus*, *P. aeruginosa* and *C. albicans* with minimum inhibitory concentrations of 0.18 to 2.8 µg/mL [38].

2-Methyl-5-(4′(S)-hydroxy-1′(R),5′-dimethylhex-5′-enyl)-phenol which has been isolated from steam-distilled resin of *C. kua* has shown fungicidal activity against *Cladosporium cucumernum* in TLC assay [39].

Oleo-gum-resin obtained from another species of this genus, *C. sphaerocarpa* has been reported to contain furanosesquiterpenes such as (1*E*) 8,12-epoxygermacra-1,7,10,11-tetraen-6-one in its diethyl ether fraction.

None of the tested extracts showed inhibitory effects against *Aspergillus* spp. except for the

Table 2. Yields of different fractions obtained from selected plants (w/w %)

Plant / Fraction	*Phoenix. dactylifera*	*Commiphora habessinica*	*Boswellia sacra*	*Doronicum glaciale*
Petroleum ether	0.76	6.52	28.20	2.96
Dichloromethane	0.20	8.07	12.00	4.98
Ethanol	0.70	6.67	4.90	4.66

Table 3. Antifungal activities (MIC and MFC) of petroleum ether (P), dichloromethane (D) and ethanol (E) fractions of selected plants against yeasts and filamentous fungi

Plant	Fr	MI	Fungi								
			Aspergilus			*Candida*					
			flavus	*fumigatus*	*clavatus*	*albicans*	*parapsilosis*	*glabrata*	*tropicalis*	*krusei*	*dubliniensis*
Boswellia sacra	P	MIC_{50}	-	-	-	-	64	-	-	-	-
		MIC_{90}	-	-	-	-	-	-	-	-	-
		MFC	-	-	-	-	-	-	-	-	-
	D	MIC_{50}	-	-	-	-	-	-	-	-	-
		MIC_{90}	-	-	-	-	-	-	-	-	-
		MFC	-	-	-	-	-	-	-	-	-
	E	MIC_{50}	-	-	-	-	-	-	-	-	-
		MIC_{90}	-	-	-	-	-	-	-	-	-
		MFC	-	-	-	4	-	-	-	-	-
Commiphora habessinica	P	MIC_{50}	8	32	4	16	4	0.5	0.5	8	4
		MIC_{90}	32	128	16	128	16	1	1	16	16
		MFC	-	-	128	128	128	-	64	128	-
	D	MIC_{50}	-	-	-	-	-	-	-	-	-
		MIC_{90}	-	-	-	-	-	-	-	-	-
		MFC	-	-	-	-	-	-	-	-	-
	E	MIC_{50}	-	-	-	128	64	1	16	128	64
		MIC_{90}	-	-	-	256	128	4	64	256	128
		MFC	-	-	-	-	-	-	-	-	-
Doronicum glaciale	P	MIC_{50}	-	-	-	-	-	-	-	-	-
		MIC_{90}	-	-	-	-	-	-	-	-	-
		MFC	-	-	-	-	-	-	-	-	-
	D	MIC_{50}	-	-	-	-	-	-	-	-	-
		MIC_{90}	-	-	-	-	-	-	-	-	-
		MFC	-	-	-	-	-	-	-	-	-
	E	MIC_{50}	-	-	-	-	-	-	-	-	-
		MIC_{90}	-	-	-	-	-	-	-	-	-
		MFC	-	-	-	-	-	-	-	-	-
Phoenix dactylifera	P	MIC_{50}	-	-	-	-	32	-	-	-	-
		MIC_{90}	-	-	-	-	-	-	-	-	-
		MFC	-	-	-	-	-	-	-	-	-
	D	MIC_{50}	-	-	-	-	64	-	-	-	-
		MIC_{90}	-	-	-	-	-	-	-	-	-
		MFC	-	-	-	-	-	-	-	-	-
	E	MIC_{50}	-	-	-	-	4	-	-	-	4
		MIC_{90}	-	-	-	-	8	-	-	-	8
		MFC	-	-	-	-	-	-	-	-	-

Fr: fraction, MI: microbial inhibition

petroleum ether fraction of *C. habessinica*, which inhibited the growth of *A. fumigatus* and *A. clavatus* at a concentration of 32 µg/mL. *A. clavatus* is occasionally pathogenic but it can be allergenic and causes occupational hypersensitivity pneumonitis, also known as malt worker's lung [40]. The ethanol fraction of *P. dactylifera* could inhibit the growth of *C. parapsilosis* and *C. dubliniensis* with a MIC_{90} of 8 µg/mL. *C. parapsilosis* is an important cause of sepsis, wound and tissue infections in immunocompromised patients [41]. This fungus is not an obligate human pathogen and has been isolated from domestic animals, insects or soil. *C. dubliniensis* is also an opportunistic pathogen isolated from AIDS patients and immunocompetent individuals [42]. Infections of both species can be treated with amphotricine B and azole antifungals, but resistant isolates have recently been reported [43]. The ethanol

fraction of *P. dactylifera* has been previously reported to contain triterpenoids which could be responsible for this effect. Although essential oil components such as carvacrol, linalool and thymol have been reported for *P. dactylifera* but, in contrast to frequent reports of antimicrobial activity for these components [35], none of the tested microorganisms were susceptible to this fraction. None of the species exhibited the antimicrobial activity against the tested Gram-positive and Gram-negative bacteria at concentrations of 0.5 to 256 µg/mL.

No reports on antimicrobial activity of the different fractions of nonvolatile constituents of *B. sacra* oleo-gum-resin have been found. For frankincense essential oil obtained from different species of *Boswellia* genus [15,44,45], no significant growth inhibitory has been found for any of the tested strains except for *C. parapsilosis*. The results of the present study agree with those from Kumar *et al.* [17], who reported no significant antibacterial activity for oleo-gum-resin of *B. serrata* at concentrations of 500 and 1000 µg/mL except for *Bacillus pumilus* and, partially, for *Bacillus subtilis*. Frankincense is primarily dispersed or dissolved in vehicles or solvents such as fixed oils, water or other solvents with different pH and polarity values (rather than essential oil extraction using a Clevenger apparatus) to prepare oral or topical traditional Persian formulations. The antimicrobial properties reported in the literature for frankincense (primarily for its essential oil) should be investigated and revised for each formulation.

None of the fractions obtained from *D. glaciale* had acceptable MIC or MFC values against the tested fungi or bacteria. No published report was found on antimicrobial activity of this plant. The species *D. hookeri* [17] was evaluated for its antimicrobial activity at concentrations of 500 and 1000 µg/mL and found to cause complete inhibition of *S. faecalis*, *S. cerevisiae* and *C. albicans* only at concentrations of >500 µg/mL which is higher than the concentrations tested in this study (<256 µg/mL); in accordance with the results of the present study, it did not inhibit other bacteria.

In the present study, the petroleum ether and ethanol fractions of *C. habessinica* exhibited the most antifungal activities among the examined fractions. *C. glabrata* was the most susceptible species to the examined fractions. None of them exhibited antimicrobial activity against the tested Gram-positive and Gram-negative bacteria at concentrations of 0.5 to 256 µg/mL. Since the sensitivity of fungi and bacteria to natural antimicrobials varies widely within species; it is essential to consider the sensitivity of strains to prevent resistance.

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Declaration of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the content of the paper.

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