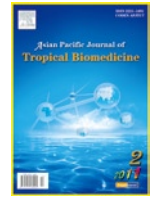




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Document heading

## *In vitro* antibacterial activity of three medicinal plants—*Boswellia* (Luban) species

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## ABSTRACT

**Objective:** To study *in vitro* antibacterial and antifungal activity of hot water and methanolic extracts of the three medicinal plants—*Boswellia* (Luban) species. **Methods:** Three selected plants were collected from different localities of Soqatra (Republic of Yemen), Dhofar (Sultanate of Oman) and Republic of Somalia. The plants were dried and extracted with two different solvents (methanol and hot water) to yield six crude extracts. The obtained extracts were tested for their antibacterial activity against eleven different bacterial strains and two fungi using the standard well–diffusion and micro–dilution methods. The following microorganisms were used: methicillin–resistant *Staphylococcus aureus* (ATCC 6538), multi–drug resistant *Pseudomonas aeruginosa* (ATCC 27853), enterohemorrhagic *Escherichia coli* (0157 EHEC), *Salmonella typhi*, *Proteus vulgaris*, *Klebsiella pneumoniae*, *Bacillus subtilis* (ATCC 6059, reference strain), *Streptococcus pneumoniae*, *Klebsiella pneumoniae*, MRSA, *Corynebacterium*, *Corynebacterium diphtheriae* and two fungus: *Candida maltosa* and *Candida albicans*. **Results:** The different extracts possessed different inhibitory activity against different types of bacterial species. The patterns of inhibition varied with the plant extract, the solvent used for extraction and the organisms tested. The antimicrobial activity exhibited by the methanolic extracts of *Boswellia sacra* from the Soqatra and Dhofar regions was greater than that of *Boswellia frereana* collected from Somalia. The methanolic extract of the oleo–gum–resin showed higher efficacy to inhibit all the tested bacterial strains than the methanolic extract of frankincense–resin. The *Boswellia frereana* collected from Somalia showed lower activity compared with the two other *Boswellia* species. The plant extracts showed bacteriostatic activity at lower concentrations and bactericidal activity at higher concentrations. Neither water nor methanolic extracts showed any activity against the fungi *Candida maltosa* and *Candida albicans*. **Conclusions:** It can be concluded that the methanolic extracts of *Boswellia* (Luban) possess the highest antibacterial activity. Neither water nor methanolic extracts show any activity against *Candida maltosa* and *Candida albicans*.

### 1. Introduction

Plant–derived drugs remain an important resource, especially in developing countries, to combat serious diseases[1]. Approximately 60%–80% of the world's population still relies on traditional medicine for the treatment of common illnesses[2–4]. And about 60%–90% of patients with arthritis who have used complementary and alternative medicine, most used Traditional Chinese

medicine[5].

For at least 3000 years, olibanum which is also known as frankincense had been an important trade material for the civilizations located in the Arabian Peninsula as well as in North Africa such as Somalia. It has been a precious commercial material because of the interest in its incense material of the old kings and queens such as the Queen of Sheba 700 B.C in Yemen[6].

Olibanum is a natural oleo–gum–resin which is obtained through incisions made in the trunks of trees of the genus *Boswellia*. The genus *Boswellia* belonging to the Burseraceae family includes several species growing in a range of countries and is approximately represented by 43 different trees and shrubs distributed mostly in the Arabian Peninsula, India and East Africa[7]. There are numerous

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species and varieties of frankincense trees, including *Boswellia serrata* (*B. serrata*) in India, *Boswellia carteri* (*B. carteri*) in East Africa and China, *Boswellia frereana* (*B. frereana*) in Somalia, and *Boswellia sacra* (*B. sacra*) in Arabia (Yemen and Oman), each producing a slightly different type of resin. Differences in soil and climate create more diversity in the resins, even within the same species. On the island of Soqatra which is a Yemeni island in the Indian Ocean a unique and endemic flora occurs as a result of its long geological isolation and the present hot and dry climate conditions. Within this flora the genus *Boswellia* is represented by eight endemic species<sup>[8,9]</sup>.

In the last two decades, olibanum has gained increasing attention from scientists and pharmaceutical companies to better define its medical effects and identify the constituents responsible for these effects. Consequently, several studies have been reported on the anticancer, anti-inflammatory, immunomodulatory, antimicrobial and antiviral activities of several *Boswellia* species<sup>[10–16]</sup>. In addition, olibanum has been reported to be a rich source of non-volatile triterpenoid constituents such as ursane, oleanane and lupine, which are in many cases responsible for the observed activity<sup>[17–22]</sup>.

The present study aims to examine three *Boswellia* species from three endemic regions including *B. sacra* from Soqatra Republic of Yemen, *B. sacra* from Dohfar (The Sultanate of Oman) and *B. sacra* from the Republic of Somalia for their antibacterial and antifungal activity.

## 2. Materials and methods

### 2.1. Plant materials

The plants were collected from different localities of Yemen and Oman in June and August 2010 and identified at the Pharmacognosy Department, Faculty of Pharmacy, Sana'a University. Part of the identification of the investigated plants was done by Dr. Wadieh A, Department of Botany, Naser College, Lahj Governorate, University of Aden, Republic of Yemen. Voucher specimens were deposited at the Pharmacognosy Department, Faculty of Medicine, University of Science and Technology, Sana'a, Republic of Yemen.

### 2.2. Extraction of plant material

#### 2.2.1. Methanol extract

Ten grams of air-dried and powdered plant materials were extracted with 400 mL methanol (CH<sub>3</sub>OH) by using a Soxhlet apparatus for 8 h. The residue was dried over night and then extracted with 250 mL water 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 room temperature until tested.

#### 2.2.2. Water extract

As previously reported by Bauer *et al*<sup>[23]</sup>, 25 grams of dried plant materials were added to 100 mL of distilled water. The mixture was homogenized in an electric blender for approximately 5 min and incubated for one day at 30 °C to release the active agent from the glycoside and the extract was then diluted to 100 mL in distilled water prior to filtration through Whatman filter paper No. 3. The filtrate was then sterilized using a 0.45 μm Millipore filter and used immediately for antimicrobial testing.

### 2.3. Test organisms

The test organisms used in the screening included: methicillin-resistant *Staphylococcus aureus* (ATCC 6538), multi-drug resistant *Pseudomonas aeruginosa* (ATCC 27853), enterohemorrhagic *Escherichia coli* (0157 EHEC), *Salmonella typhi*, *Proteus vulgaris*, *Klebsiella pneumoniae*, *Bacillus subtilis* (ATCC 6059), *Streptococcus pneumoniae*, *Klebsella pneumonia*, MRSA, *Corynebacterium*, *Corynebacterium diphtheriae*, *Candida maltosa* (SBUG) and *Candida albicans* (BNI 33).

### 2.4. Antimicrobial assay

The disc-diffusion assay<sup>[24]</sup> 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 in water. The sterile nutrient agar was inoculated with microbial cells (200 mL of microbial cell suspension in 20 mL agar medium) and poured into sterile petri dishes. Sterile filter paper discs of 6 mm in diameter (Schleicher and Schuell, Ref. No. 10321260, lot. DG0274–1) were impregnated with 20 mL of the extract solution (equivalent to 4 mg of the dried extract). The paper discs were allowed to dry and then 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 for 18 h at 37 °C. In contrast, both *Candida albicans* and *Candida maltosa* were incubated at 28 °C for 48 h. Methicillin and oxacillin were used as positive controls. Negative controls were performed using paper discs loaded with 20 mL of organic solvents (chloroform and methanol). At the end of the incubation time the antibacterial activity was evaluated by measuring the inhibition zones (diameter of inhibition zone plus diameter of the disc). An inhibition zone of 12 mm or more was considered as high antibacterial activity.

### 2.5. Determination of minimum inhibitory concentration and minimum bactericidal concentration

Minimum inhibitory concentration (MIC) was determined by micro-dilution method using 2-folds serial dilution of plant extracts according to the National Committee for Clinical Laboratory Standards (NCCLS) (National Committee for Clinical Laboratory Standards, 2000). MIC of the extracts was determined by dilution of the plant extracts of various concentrations of 0.0–45.0, 0.0–55.0, 0.0–42.0, 0.0–55.0, 0.0–36.0, 0.0–45.0 mcg/mL. Equal volume of each extract

and nutrient broth were mixed in a test tube. Specifically 0.1 mL of standardized inoculum ( $1-2 \times 10^7$  cfu/mL) was added to each tube. The tubes were incubated aerobically at 37 °C for 18–24 h. Two control tubes were maintained for each test batch. These included antibiotic control (tube containing extract and growth media without inoculum) and organism control (tube containing the growth medium, saline and the inoculum). The lowest concentration (the highest dilution) of the extract that produced no visible bacterial growth (no turbidity) when compared with the control tubes was regarded as MIC. However, the minimum bactericidal concentration (MBC) was determined by sub-culturing the test dilution onto a fresh drug free solid medium and

incubated further for 18–24 h. The highest dilution that yielded no signal bacterial colony on the solid medium was taken as MBC.

### 3. Results

Table 1 showed the properties and the local names of the plant extracts.

The results of the antimicrobial activity of the investigated extracts of *B. frereana* Luban (oleo–gum–resin) from Yemen, Soqotra Luban Oman, Dohfar Luban, Somalia Laban–Laess, were shown in Table 2. Methanolic extracts exhibited the

**Table 1**

List of plants screened.

Botanical name	Origin	Genus	Family	Local name	Part of the plant
<i>B. sacra</i>	Yemen, Soqotra	<i>Boswellia</i>	Burseraceae	Luban (Yl)	Oleo–gum–resin
				Luban–Dakar (Yb)	Frankincense–resin
<i>B. sacra</i>	Oman, Dohfar	<i>Boswellia</i>	Burseraceae	Luban (Ol)	Oleo–gum–resin
				Luban–Bakhur (Ob)	Frankincense–resin
<i>B. frereana</i>	Somalia	<i>Boswellia</i>	Burseraceae	Laban–Laess (Sl)	Oleo–gum–resin
				Luban (Sb)	Frankincense–resin

**Table 2**

Antibacterial activity of the crude plant extracts.

Plants	Zone of inhibition (mm)																			
	Buffered methanol extract*										Water extract*									
	Sa	Pa	Ec	St	Pv	Kp	Bs	Sp	Mr	Cd	Sa	Pa	Ec	St	Pv	Kp	Bs	Sp	Mr	Cd
Yl	40	48	42	47	45	55	34	47	42	44	26	32	34	25	20	11	29	32	27	30
Yb	34	38	37	19	25	31	39	36	29	31	35	30	36	27	37	23	30	24	30	26
Ol	44	39	47	44	33	29	29	44	39	55	31	37	22	24	18	32	39	31	26	22
Ob	36	23	55	33	44	35	21	37	29	23	20	30	31	35	20	31	31	27	33	18
Sl	29	30	39	30	32	29	32	28	31	25	29	21	30	19	16	19	43	23	12	26
Sb	30	28	29	28	21	19	31	23	20	24	35	25	33	29	28	30	28	30	26	31
M	14	17	13	20	nd	18	21	18	19	17										
O	23	13	26	17	11	21	27	20	26	14										

nd=not detected; M=Methicillin; O=Oxacillin; Sa=*Staphylococcus aureus*; Pa=*Pseudomonas aeruginosa*; Ec=*enterohemorrhagic Escherichia coli*; St=*Salmonella typhi*; Pv=*Proteus vulgaris*; Kp=*Klebsiella pneumoniae*; Bs=*Bacillus subtilis*; Sp=*Streptococcus pneumoniae*; Mr=MRSA; Cd=*Corynebacterium diphtheriae*.

\*: No inhibition was observed against *Candida maltosa* and *Candida albicans*.

**Table 3**

Antibacterial activity of methanolic and water plant extracts on the different bacterial strains.

Extracts	Plants	Sa	Pa	Ec	St	Pv	Kp	Bs	Sp	Mr	Cd										
		MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC								
Methanol	Yl	44.2	48.2	40.7	52.1	45.2	41.9	43.2	49.8	40.2	39.1	42.0	45.4	40.5	32.9	36.8	29.9	42.1	37.9	32.1	32.1
	Yb	32.5	41.7	35.2	29.9	39.3	39.5	32.5	33.5	31.2	27.3	30.4	29.3	32.1	30.7	30.7	31.9	33.8	38.4	37.3	29.5
	Ol	38.9	32.2	37.3	39.8	45.2	45.4	55.3	39.0	39.3	30.4	35.6	18.4	35.5	28.4	39.9	33.7	39.5	22.8	33.6	38.3
	Ob	29.8	31.9	35.2	29.4	40.5	35.1	39.4	33.8	31.4	18.9	20.3	29.5	25.2	27.3	22.2	34.4	29.8	31.7	21.5	29.2
	Sl	26.7	30.8	31.3	31.5	29.9	24.8	27.2	32.4	28.8	22.3	34.5	18.5	29.4	25.3	30.1	22.7	34.7	29.2	32.3	26.8
	Sb	23.2	29.9	35.8	23.2	22.2	25.1	21.4	24.9	27.3	19.0	26.2	21.3	22.5	23.4	25.6	19.8	22.8	26.1	23.2	20.1
Water	Yl	52.2	44.2	40.7	52.1	35.2	37.9	40.2	49.8	25.4	32.5	32.9	40.0	40.1	42.0	39.7	39.2	44.1	52.3	44.4	43.1
	Yb	32.5	41.7	35.2	39.9	35.3	39.5	32.5	33.5	29.3	40.1	30.7	31.2	27.3	30.4	35.1	37.3	41.7	33.7	40.1	38.3
	Ol	28.9	32.2	27.3	29.8	41.2	45.4	49.3	55.0	18.4	25.5	28.4	30.3	20.4	25.6	28.1	27.2	29.8	30.4	31.1	33.9
	Ob	29.8	31.9	29.2	35.4	29.5	40.0	34.4	39.8	29.5	35.2	27.3	31.4	18.9	20.3	31.4	29.6	35.6	38.1	32.1	30.1
	Sl	22.7	27.8	21.3	22.5	22.9	24.8	28.2	32.4	18.5	24.4	25.3	27.8	19.3	24.5	29.9	30.4	33.6	21.9	1835	22.1
	Sb	30.2	18.9	20.8	35.2	19.2	25.1	25.4	28.9	21.3	29.5	23.4	27.3	20.0	26.2	34.7	28.2	17.3	24.4	21.9	19.5

Minimum inhibitory concentration values are given as mg/mL for essential oils and  $\mu$ g/mL for standard antibiotics.

Sa=*Staphylococcus aureus*; Pa=*Pseudomonas aeruginosa*; Ec=*enterohemorrhagic Escherichia coli*; St=*Salmonella typhi*;

Pv=*Proteus vulgaris*; Kp=*Klebsiella pneumoniae*; Bs=*Bacillus subtilis*; Sp=*Streptococcus pneumoniae*; Mr=MRSA;

Cd=*Corynebacterium diphtheriae*.

highest antibacterial effect with inhibition zones of more than 14 mm (Table 2). Neither water nor methanolic extracts showed any activity against the fungi *Candida maltosa* and *Candida albicans* as judged by zones of inhibitions (Table 2).

The different extracts possessed different inhibitory activity against the different types of bacterial species. The patterns of inhibition varied with the plant extract, the solvent used for extraction and the organisms tested. Methanolic extracts of *B. sacra* from the Suqotra and Dhofar regions showed higher antibacterial activity than that of the *B. frereana* collected from Somalia. The methanolic extract of the oleo–gum–resin showed higher efficacy to inhibit all the tested bacterial strains than the methanolic extract of the frankincense–resin.

The MIC and MBC values obtained for extracts against the bacterial strains varied among the 3 plant extracts. The MIC values corresponded to the MBC values (Table 3). However, the *B. frereana* collected from Somalia showed lower activity compared with the two other *Boswellia* species. These plant extracts showed bacteriostatic activity at lower concentrations and bactericidal activity at higher concentrations as indicated by MIC and MBC (Table 3).

#### 4. Discussion

Traditionally, the oleogum resins of some *Boswellia* species like *B. serrata* and *B. carteri* have been used in many countries for the treatment of rheumatic and other inflammatory diseases, such as Crohn's disease and ulcerative colitis[13,24–28]. Frank *et al* reported that the frankincense oil derived from *B. carteri* induces tumor cell specific cytotoxicity. Frankincense resin is reported to contain about 5%–9% essential oil, 65%–85% alcohol-soluble resin and the remaining water-soluble gum (polysaccharidic fraction)[29–32]. Furthermore, the extracts and essential oils of frankincense have been used as antiseptic agents as a mouthwash as well as in the treatment of cough and asthma[33].

The antibacterial activity of the studied plants' extracts was exhibited mainly against the gram-positive bacteria. It was interesting to note that the multi-resistant *Staphylococcus* strains showed greater sensitivity to the investigated extracts than the other antibiotic susceptible gram-positive bacteria. Both water and methanolic extracts of these plants were effective on bacterial strains. However, neither water nor methanolic extracts showed any activity against the fungi *Candida maltosa* and *Candida albicans*, although early study by Camarda *et al* demonstrated that the essential oils of four *Boswellia* species exhibited a significant antifungal activity against both *Candida albicans* and *Candida tropicalis*[7]. Furthermore, Shao *et al* reported that limonene present in the essential oils is found to be the main component responsible for the antifungal activity[34]. Methanolic extracts exhibited the highest antibacterial effect with inhibition zones of more than 14 mm. The majority of the hot aqueous extracts of the antibacterial active plants exhibited only low activity in comparison to the methanolic extract.

The MIC and MBC values obtained for extracts against the bacterial strains varied among the 3 plant extracts. The MIC values corresponded well to the MBC values except for *Proteus vulgaris* which showed a significant lower value as compared with the rest. However, the *B. frereana* collected from Somalia showed lower antibacterial activity as compared with the other two *B. sacra* species, from Oman and Yemen. Moreover, these plant extracts demonstrated bacteriostatic activity at lower concentrations and bactericidal activity at higher concentrations as indicated by MIC and MBC.

These observations may be attributed to two reasons. Firstly, the nature of photochemical active components (alkaloids, anthraquinone, saponins and tannins) could be enhanced in presence of methanol. It has been well documented in early study by Tshesche that these components are well known as having anti-microbial activity[35]. Secondly, the stronger extraction capacity of methanol may have produced greater active constituents responsible for antibacterial activity.

Traditionally, in Yemen and Oman people allowed these plants to be soaked in water for days in large quantities before administrating to patients. Using such large quantities may compensate for the low activity of the water extracts as compared to the methanolic extracts.

The present results provide a scientific bases for the traditional use of *B. frereana* Luban (oleo–gum–resin) from Yemen, Suqutra (Luban), Oman, Dhofar (Luban), Somalia (Luban–Laess) as an antibacterial agent of these medicinal plants. Finally the results obtained from this study represent a preliminary report on the antibacterial activity of these medicinal plants regardless of its growth regions. The bioassay-guided fractionation procedure is recommended to characterize and isolate antibacterial active constituents.

#### Conflict of interest statement

We declare that we have no conflict of interest.

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