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Antimicrobial Activity of *Boswellia papyrifera* Essential Oil against Clinical Bacterial Pathogens

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Abstract

Background: *Bowsellia papyrifera*, also known as Sudanese frankincense, is a species of flowering plant and frankincense that is native to Ethiopia, Eritrea and Sudan. The tree is cultivated in Ethiopia because of its valuable resin. The incense smoke is characterized by a fresh lemon-pine scent, and is therefore highly esteemed.

Objective: To evaluate the antimicrobial activity of *Boswellia papyrifera* essential oil against clinical bacterial pathogens.

Materials and methods: The essential oil of *Boswellia papyrifera* (اللبان الحبشي الضكر) was obtained by hydro-distillation of Frankincense of *Boswellia papyrifera.* By disc diffusion method the minimum inhibition concentration (MIC) and the minimal bactericidal concentration (MBC) were determined against the clinical isolates: *Staphylococcus aureus (S. aureus), Escherichia coli (E. coli), Pseudomonas aeruginosa (P.aeruginosa), Klebsiella pneumoniae* (K. *pneumoniae)* and *Proteus vulgaris (P. vulgaris)* . Zones of inhibition were measured and compared with those of the standard antibiotics: tetracycline (30mcg), ciprofloxacin (5mcg), vancomycin (30 mcg) and amoxicillin (25mcg). Dimethyl sulfoxid (DMSO) was used as negative control.

Results: The essential oil of *Boswellia papyrifera* (300 µg) showed the highest *in vitro* anti-microbial activity against *S. aureus*, a moderate activity against *P. aeruginosa*, and a weak activity against *E.coli.* It was not effective against *K. pneumoniae* and *Proteus vulgaris.* Also it showed an antimicrobial activity stronger than that of tetracycline for *E.coli*, and it had a synergistic effect with amoxicillin against *S. aureus.*

Conclusion: The essential oil of *Boswellia papyrifera* had an antibacterial activity fair enough to inhibit growth of various clinical bacterial pathogens.

Keywords: *Boswellia papyrifera,* Essential oil, Antimicrobial activity, Bacterial pathogens.

Introduction

The genus *Boswellia* (family *Burseraceae*) consists of many, widespread species. It includes approximately 23 species of small trees that grow mainly in Arabia, on eastern coast of Africa and India. Olibanum is a natural oleo-gum-resin that exudes from tapping the bark of *Boswellia* trees. *Boswellia papyrifera* and *Boswellia scara* are most important multipurpose tree species in

Central and Eastern Africa. The *Boswellia papyrifera* was found to hold biofilms of oleo-gum resin activity against preformed 24 *S. epidermidis,* and *S. aureus* (ATCC 29213) when tested by MTT. At concentrations ranging from 217μml g^{-1} (25% v/v) to 6·8μml g^{-1} (0·75% v/v). The oil showed considerable activity. Inhibition percentages ranged from 99% to 71% or 95·3–59·1% against biofilms of *S. epidermidis* DSM 3269 and *S. aureus* ATCC 29213 respectively[1].
The main component of frankincense is oil (60%). It contains mono-(13%) and diterpenes (40%) as well as ethyl acetate (21.4%), octal acetate (13.4%) and methyl anisole (7.6%). The highest biological activity among terpenes is characteristic by 11-keto-ß-acetyl-beta-boswellic acid, acetyl-11-keto-ß-boswellic acid and acetyl-α-boswellic acid. Contemporary studies have shown that resin indeed has an analgesic, tranquillizing and anti-bacterial effects[2].
From the point of view of therapeutic properties, extracts from *Boswellia serrata* and *Boswellia carterii* are reported to be particularly useful. They support the inflammatory conditions of rheumatism by inhibiting leukocyte elastase and degrading glycosaminoglycans. *Boswellia* preparations can inhibit 5-lipoxygenase and prevent the release of leukotrien, thus having an anti-inflammatory effect in ulcerative colitis, irritable bowel syndrome, bronchitis, and sinusitis. Inhalation and consumption of *Boswellia* olibanum reduces the risk of asthma. In addition, boswellic acids have an anti proliferative effect on tumors. They inhibit proliferation of tumor cells of the leukemia and glioblastoma subset. They have an anti-tumor effect since they inhibit topoisomerase I and II-alpha and stimulate programmed cell death[3].

Materials and methods

This was an analytical study conducted at the Department of Medical Microbiology, Al Neelain University (Khartoum, Sudan) using standard microbiological techniques. Ethical approval was received from Al Neelain University (Khartoum) Ethical Board. Permission to collect the specimens was granted by the authorities of various medical laboratories in Khartoum State.
The plant sample of *Boswellia papyrifera* was collected form Khartoum North market by an aromatic plant specialist and taxonomically identified at the Medicinal and Aromatic Plants Research Khartoum, Sudan.
Volatile oil extraction: *Boswellia papyrifera* specimen (400g) was placed in 1000 ml rounded bottom capacity flask.1000 ml distilled water was added and the Clevenger receiver (lighter than water) and condenser attached to the top of the flask (Duran West Germany). System was heated at 100°C for four hours till the volume of oil above the water layer at the receiver constant. Oil was pipetted, dried over anhydrous sodium sulphate, and stored in dark container in a refrigerator till used[4]
Microbial strains: The essential oil was investigated for antimicrobial activity against standard strains: *Staphylococcus aureus* (ATCC29213), *Pseudomonas aeruginosa* (ATCC27853), *Escherichia coli* (ATCC25922), *Klebsiella pneumonia* (ATCC 13883) and *Proteus vulgaris* (ATCC13315). The essential oil was also investigated for antimicrobial activity of 10 species of *K. pneumonia,* 20 species of *E.coli,* 20 species of *S. aureus,* 20 species of *P. aeruginosa,* and 10 species of *Proteus vulgaris.*
Preparation of the Bacterial suspension: The turbidity of each bacterial suspension was prepared to match 0.5 McFarland standard ($1.5x10^8$ cfu/ml) and the turbidity was measured with the aid

of a spectrophotometer at an optical density 0.08-0.13 and turbid suspension at 625nm as per Bauer-Kirby method (1966)[5]

Antimicrobial testing: The agar disc diffusion method was employed for testing the antimicrobial activity of *Boswellia papyrifera* oil. The oil was dissolved in DMSO to a final concentration 30 mg/ml and sterilized by filtration through 0.45mm Millipore filters. The antimicrobial test was then carried out by the disc diffusion method using 100 μl of a suspension containing 10^8 cfu /ml of each bacterial species. The disc was impregnated with 10 μl essential oil (300 $\mu g/$ disk) and placed on an inoculated negative control agar.

After overnight incubation, the zones of inhibition of the test organisms were measured. Each antimicrobial sensitivity test was repeated twice according to Edris and Farrag (2003)[6]. The standard antibiotics: tetracycline (30 mcg), ciprofloxacin (5 mcg), vancomycin (30 mcg) and amoxicillin (25 mcg) were used as positive control (Bio Analysis) to determine the sensitivity of each bacterial species investigated. The inoculated agar plates were incubated at 37°C for 24hrs.

Minimum inhibitory concentration (MIC)**:** The MIC values were determined for the bacterial species that were sensitive to the essential oil using the disk diffusion method. The inoculated microorganism was prepared from 12 hr broth culture suspensions and adjusted to 0.5 McFarland standard turbidity. The essential oil of *Boswellia papyrifera* was serially diluted to give the concentrations: 500 µg/ml. 96 well-plates were then prepared by dispensing 95 µl nutrient broth and 5µl of the inoculum were dropped into each well. A set of 100 µl dilution of essential oil was prepared to give 500μg/ml concentration and dropped into the first well. Then 100μl from the serial dilutions was transferred to the six consecutive wells and the last well195$\mu l$of the nutrient broth without compound and 5μl of the inculums on each strip and used as negative control. The final volume of each well was 200μl. The plates were covered with sterile plate sealer, and the plate was shaken at 300 rpm for 20 sec and incubated at 37°C for 20 hrs. The microbial growth was determined by absorbance at 595 nm using a micro-plate reader. The MIC was recorded as lowest concentration of the extract to inhibit the growth of the microorganism. This was confirmed by placing 5μl of the sample collected from a clear well of nutrient agar.

Minimum bactericidal concentration (MBC): The MBC was determined by spreading 10μl of suspensions taken from wells showing no visible growth and inoculated on Mueller-Hinton agar plate for at 30°-37°C over night.

Results

The antibacterial activity of *Boswellia papyrifera* essential oil was determined by measuring the diameter of the inhibition zone. The essential oil at 300 $\mu g/$disk concentration gave the highest *in vitro* antimicrobial activity against most of the micro-organisms investigated. It gave a highly inhibition zone against *S. aureus*, a moderate zone against *P. aeruginosa*, a week zone against *E.coli*, and no inhibition zone against *K. pneumoniae* and *P. vulgaris.* Also, the essential oil showed an antimicrobial activity stronger than tetracycline for *E.coli* and showed a synergistic effect with amoxicillin against *S. aureus* (Table 1).

The MIC values were the lowest concentration of the essential oil that were no bacterial growth. MIC and MBC of essential oil of *B. papyrifera* against bacterial species are shown in Table (2). The essential oil MIC was found less than or around 0.25 mg//ml (250 µg/ml) against *S. aureus.*

Table (1): Inhibition zones (mm) of *Boswellia papyrifera* essential oil and antibiotics tested against the clinical bacterial isolates

Bacterial isolates	Essential oil	Amoxicillin	Vancomycin	Ciprofloxacin	Tetracycline
S. aureus	20	20	15	18	20
P. aeruginosa	15	19	10	15	10
E.coli	12	20	10	20	5
K. pneumoniae	0	0	15	0	15
P. vulgaris	0	8	10	0	12

Table (2): MIC and MBC (μg/ml) of *Boswellia papyrifera* essential oil against the clinical bacterial isolates

Bacterial isolates	MIC	MBC
S. aureus	250	250
P. aeruginosa	31.5	31.5
E.coli	7.8	7.8
K. pneumoniae	ND	ND
P. vulgaris	ND	ND

ND: Not determined

Discussion

The main chemical constituents of *B. papyrifera* essential oil are Limonene, Pinene, Borneol, Farnesol, Phellandrene, Myrcene, and other constituents. Limonene demonstrates antioxidant, anti-inflammatory, and anti-fungal properties. It is believed to reduce feelings of anxiety and to stimulate the immune system. Pinene is known to strengthen and invigorate the respiratory system and is reported to have diuretic properties based on empirical evidence. Borneol contributes tonic, anesthetic, sedative and anti-spasmodic properties to this oil. Farnesol is the component that allows this oil to reduce the user's signs of aging by smoothing the look of wrinkles and increasing skin's elasticity.

Used topically and cosmetically, its astringent and cytophylactic qualities help Frankincense oil to reduce the appearance of wrinkles and skin imperfections such as discoloration. It stimulates the growth of new cells, thus when used on cuts it promotes faster healing.

Used in aromatherapy, Frankincense works as an expectorant to clear the nasal passageway, promote the relief of congestion, and encourage easy breathing. It's sweet, woody aroma is sedative and enhances mood by diminishing feelings of stress and anxiety while improving concentration and memory.

Used medicinally, this anti-inflammatory oil is known to soothe inflamed skin by reducing the sensations of redness, swelling, and itching. It helps to disinfect and tighten the pores, thereby promoting the speedy healing of cuts, wounds, and scars. It is used to relieve flatulence,

stimulate the growth of new skin cells, and stimulate blood flow and circulation among other competencies.

Frankincense essential oil is reputed to have many therapeutic properties: .

* Cosmetic: astringent and cytophylactic.
* Odorous: carminative, expectorant, and sedative.
* Medicinal: antiseptic, anti-inflammatory, astringent, carminative, cicatrisant, cytophylactic, diuretic, emmenagogue, expectorant, and tonic.

Abdalla and his colleagues reported that the phytochemical investigation demonstrated the presence of phenolic compounds, alkaloids and saponins in the methanol extracts which is lacking in the petroleum ether and ethyl acetate extracts. The methanol extracts exhibited the highest antibacterial activity whereas the ethyl acetate extracts exhibited some degree of activity and the petroleum ether and water extracts exhibited no or least activity. The minimum inhibitory concentration (MIC) ranged between 31.25 and 250 µg/ml for oleo-gum resin methanol extract, and 62.5 to 500 µg/ml for *B. papyrifera* [6]. I
n this study the essential oil MIC was found less than or around 0.25 mg//ml (250 µg/ml) against *S. aureus.*

The findings of the present study confirmed the substantiation of previous studies which had reported that water is a good solvent for more consistent extraction of antimicrobial substances from medical plants compared to other solvents, such as methanol[7] .

The study data were analyzed by the Statistical Package for Social Sciences (SPSS) program. Data were presented in form of tables.

Conclusion: The essential oil of *Bowsellia papyrifera* had an antibacterial activity fair enough to inhibit growth of various clinical bacterial pathogens.

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