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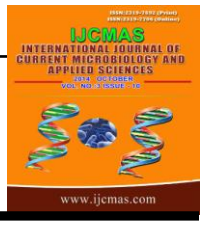

Original Research Article

Antimicrobial activity of frankincense of *Boswellia serrata*

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A B S T R A C T

This work describes the *in-vitro* screening of *frankincense* anti microbial activity of *Boswellia serrata*. Different concentrations (25, 50, 75 and 100 mg /ml) was evaluated for the investigation of antimicrobial efficacy using Gram positive (*Streptococcus pneumonia*, *Staphylococcus aureus* and *Bacillus subtilis*) and Gram negative (*E. coli*, *Proteus vulgaris*, *Klebsiella pneumonia*, *Enterobacter aerogenes* and *Pseudomonas aeruginosa*) microbes. Inhibition halos were evaluated and by the use of the antibiotic Ciprofloxacin (5µg/ml) as positive control. DMSO was used as a negative control. Results demonstrated significant antimicrobial activity. In this assay, extracts of *frankincense* showed antimicrobial activity comparable with standard and can be used in combating the bacterial infested diseases caused by the studied bacterial strains.

Keywords

Boswellia serrata, *Frankincense*, Antimicrobial Activity, Ciprofloxacin

Introduction

Plant antimicrobials tender prospective agent to deal with the hazard of biological warfare (Gibbons, 2008). Consideration to the sighting of novel plant antimicrobials must be paid in this new era of chemotherapeutic healing of infection by using plant-derived principles and on discovery of new antiinfective agents (Cowan, 1999). Herbal preparations can supplement other systems of medicine for the treatment of diseases caused by bacteria (Archana and Abraham, 2011). In fact, efforts are going on to identify and isolate secondary metabolites from plants as prospective modulators of bacterial resistance (Stavri *et al*., 2007).

Taxonomic status of *Boswellia serrata*

Boswellia serrata Roxb. ex Colebr. (Burseraceae) is a deciduous moderate to large sized branching tree that grows abundantly in the seshachalam hill valleys.The morphological characters have crown spreading and flat, bark greenish, ashy grey, ex-foliating in thin flakes. Leaves apically clustered, imparipinnate. Leaflets 14-26, thin-coriaceous, oblong-lanceolate, entire or crenate, obtuse or subacute, secondary nerves more than 16 pairs. Flowers pinkish white, in little branched panicles. Sepals and petals 5-7 each. Stamens 10-16, inserted below disc; disc

annular. Ovary 3-locular; ovules 2 per locule, pendulous. Fruits trigonous, brown, pyrenes 3, heart shaped, each one seeded.

B. glabra Roxb.; *B. serrata* Roxb. var. glabra (Roxb.) Benn. are the synonyms. *B. serrata* vernacularly (Telugu) known as Guggilum, Anduga, Dhupamu, Guggiladhpuam. Commonly known as Salai, White dammar, 'Indian olibanum', 'Indian frankincense', '*dhup*' and '*salai*' or '*salai guggul*' (Siddiqui, 2011).

Distributed commonly in lower hill slopes of Tirumala and Talakona of Seshachalam hill ranges. Flowering and Fruiting occurs in the season of March-August. Traditional usage of Gum is diuretic, diarrhoea, dysentery, stomachic, cardiac diseases, cough, haemorrhage, dyspnoea, polyuria, leucorrhoea, oligospermia, urinary troubles, piles, ulcers, burns (Madhava chetty *et al*., 2013).

When incisions are made in the trunks of the *Boswellia serrata* trees to produce exuded gum (Oleo gum-resin), which appears as milk like resin. The resin hardens (solidification) into orange-brown gum resin known as frankincense or olibanum. There are numerous species and varieties of frankincense trees, including *Boswellia serrata* in India.

The resins of *Boswellia serrata* have been used for the treatment of rheumatoid arthritis and other inflammatory diseases (Banno, 2006) such as Crohn's disease (Langmead, 2006) in traditional medicine of many countries. The anti-inflammatory activity has been attributing to the resin's ability in regulating immune cytokines production (Chevrier, 2005) and leukocyte infiltration (Sharma *et al.*, 1988; Singh and Atal, 1986). *Boswellia serrata* extract also exhibits anti-bacterial and anti-fungal activities (Weckesser, 2007).

Fig. 1 . *A* .Flowering and Fruiting of *Boswellia serrata* B. Tree Habitat C. Crude Frankincense resin *of Boswellia serrata*

Additionally, extracts from *Boswellia* species gum resins might possess anti-cancer activities, based on their anti-proliferative and pro-apoptotic activities in rat astrocytoma cell lines and Clinically, extract from the resin reduces the peritumoral edema in glioblastoma patients (Winking, 2000) and in human leukemia cell lines (Hostanska, 2002), as well as their anti-carcinogenic activity in chemically induced mouse skin cancer models (Huang, 2000).

The pharmacological characteristics and clinical efficacy of *Boswellia serrata* have been studied, with research published and systematically reviewed in the medical literature (Ernst, 2008). These results suggest that frankincense resin contains active ingredients that modulate important biological activities. *B. serrata* flowers and leafs showed significant antibacterial activity (Mohammed Aman *et al*., 2010). In addition *B. serrata* has versatile pharmacological activities (Arshiya Sultana *et al*., 2013).

However, there are no enough scientific reports to support these supposed antimicrobial activity. The present investigation was undertaken which deals with the evaluation of antimicrobial activity of aqueous crude extraction of frankincense resin of *Boswellia serrata*.

Materials and Methods

The crude gum is collected and processed. The collected material is dried under shade and made into powder and subjected to hot percolation by using soxhlet apparatus with water. The extract was filtered using Whatsman-No. 1 filter paper and the extraction procedure were repeated three times. The filtrate was used for the biological assay.

Microorganisms

Clinical isolates of Gram positive (*Streptococcus pneumonia*, *Staphylococcus aureus* and *Bacillus subtilis*) and Gram negative (*E. coli*, *Proteus vulgaris*, *Klebsiella pneumonia*, *Enterobacter aerogenes* and *Pseudomonas aeruginosa*) authentic bacterial strains were used in the study which are procured from Institute of Microbial Technology (IMTECH), Chandigarh. The stock culture maintained at Division of Animal Biotechnology, Sri Venkateswara University. All the microorganisms were maintained at 4°C on nutrient agar slants.

Preparation of the Bacterial Suspension

The turbidity of each of the bacterial suspension was prepared to match to a 0.5 McFarland standard ($1.5x10^8$ CFU/ml). Measure the turbidity with the aid of a spectrophotometer at an optical density 0.08-0.13 and turbid suspension at 625 nm as per Bauer-Kirby Method (1966).

Determination of antimicrobial activity

Culture of the bacterial organism was aseptically introduced and evenly spread using sterile 'L' rod on the surface of sterile Mueller Hinton agar (M173/M1084, HiMedia) plates. The agar disc diffusion test is the most convenient and widely used method for routine antimicrobial susceptibility testing according to CLSI (Clinical Laboratory Standards Institute; formerly NCCLS). 25 µL of different concentrations (25, 50,75,100 mg/ml) of the resin coarse powder extract were added on whatman No. 1 filter paper disc (6mm) and was inoculated with a loopful of the test organism previously diluted to 0.5 McFarland turbidity standards seeded on the medium. The experiment was run in

triplicate for each extract and each organism tested. The plates were incubated at 37°C for 24 hours and the zones of inhibition were measured in millimeters using a calibrated instrument like zone scale (HiMedia). DMSO was used as a negative control. These inoculated tubes were then incubated Control experiments comprising inoculums without the extract were set up.

Determination of MIC

The determination of the MIC was done with different dose levels of (25, 50, 75 and100 mg/ml) of extract. The procedure was repeated on all the test organisms using the standard antibiotic Ciprofloxacin (5 µg/mL Disc, Himedia, Mumbai, India).
The statistical analysis was undertaken using *t-test* in SPSS statistics software (Version 20, IBM Corporation, New York, USA), considered significant when $p \leq 0.05$.

Results and Discussion

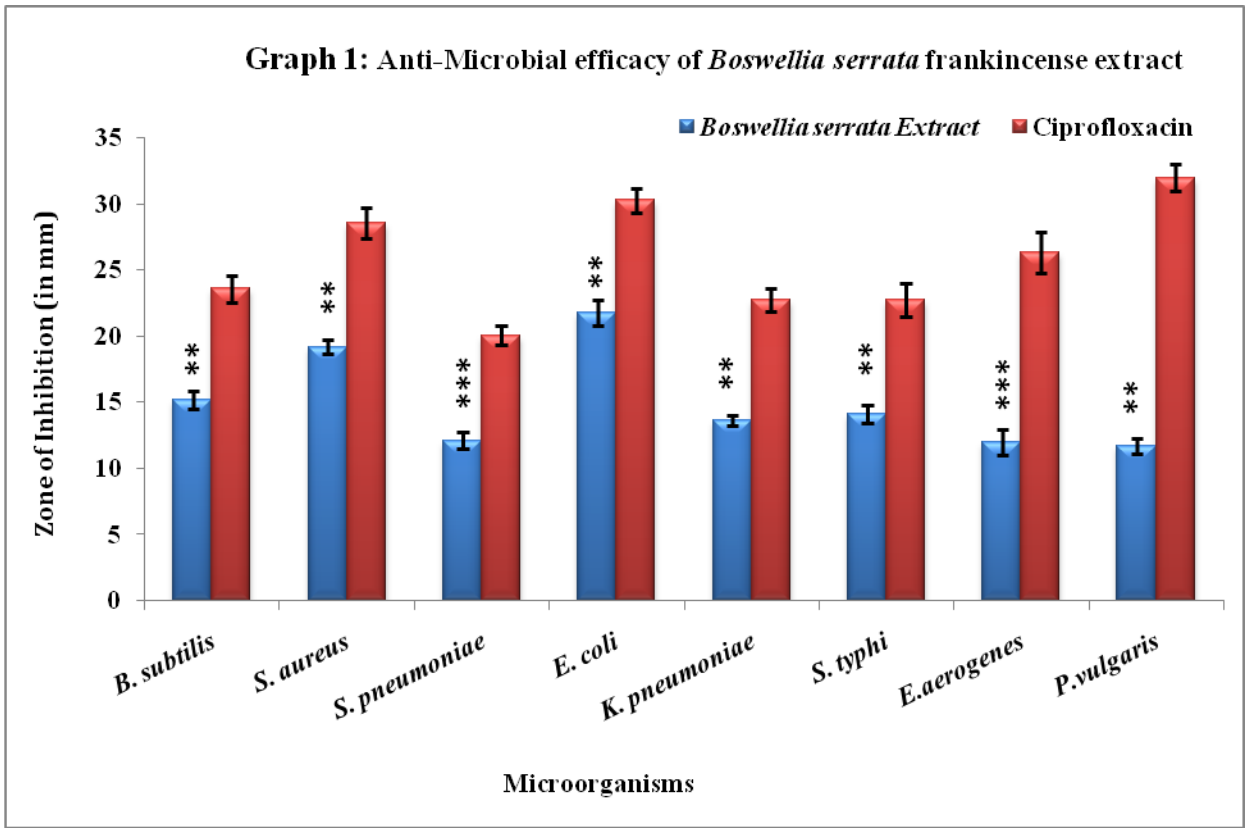
In this study the aqueous extract of commercially available *Boswellia serrata* powder was evaluated for their antimicrobial properties. Preliminary antimicrobial screening assay of commercially available *Boswellia serrata* gave relatively wide inhibition zone against the test strains. The MIC values of both revealed similar results. Showed different activities *in vitro* against the nine tested bacteria at the concentrations 10, 25, 50, 100 mg/ml. To determine the antibacterial sensitivity of B.S Frankincense resin aqueous extracts. All the bacterial strains were compared with Ciprofloxacin under the same experimental conditions. In this study both the extract and commercial product have shown greater antimicrobial activity which may explain anonymous claim on the topical use of *Boswellia serrata* for microbial infections (Table-1). *Boswellia serrata* has much known ethnobotanical value and has shown potent activity against the tested pathogens. Investigation in the potential discovery of new natural bioactive compounds (Graph-1).

The highest antimicrobial activity was observed on *E. coli* with zone of inhibition as 21.87±0.98 and the lowest effect was on *E. aerogenes with* a zone of inhibition of 11.67±1.00 mm. Whereas, Standard showed highest activity against *E.aerogenes* (30.27±1) and lowest activity against *S. pneumoniae* (20.07±0.74). The order of inhibitory activity of extract against different organisms is: *E. coli*>*S. aureus*>*B. subtilis*>*S. typhi*>*K. pneumoniae*>*S. pneumoniae*> *E. aerogenes* > *P. vulgaris*. The order of inhibitory activity of standard against different organisms are summarized as *P. vulgaris* > *E.coli* >*S. aureus*> *E. aerogenes*> *P. aureginosa*> *B. subtilis*> *K. pneumonia.* This shows that the studied *Boswellia Serrata* frankincense extract mostly exhibited bacteriostatic effects.

Extract inhibitory activity was statistically compared to the inhibitory activity of standard and observed that extract inhibitory activity on the microbes has significantly lower activity than the standard ($P \leq 0.01$, $P \leq 0.001$).

Minimum inhibitory concentration (MIC) is defined as the lowest concentration that will inhibit the growth of a test organism over a defined interval related to the organism's growth rate, most commonly 18-24 h (Turnidge *et al.,* 2003). The zone size is inversely proportional to the minimum inhibitory concentration (MIC). Disc diffusion test is a qualitative test method. The recommended medium for disc diffusion testing is Mueller-Hinton agar (MH; Himedia, Mumbai, India) (CLSI, 2012).

Graph.1 Antimicrobial efficacy of *Boswellia Serrata* frankincense resin extracts against some bacterial pathogens *vs* Standard (Ciprofloxacin -5 µg/ml)



Values are the Mean ± SD; Astringent indicate level of significance (** = p≤0.01, *** = p≤0.001)

Table.1 Minimum Inhibitory Concentrations (MIC)

S. No	Test Organism	G^+ / G^-	I	II	III	IV
1.	*B. subtilis*	G^+	++	+	+	*
2.	*S. aureus*	G^+	+	+	*	-
3.	*S. pneumoniae*	G^+	+	+	+	*
4.	*E. coli*	G^-	+	*	-	-
5.	*K. pneumoniae*	G^-	++	+	+	*
6.	*P. aeruginosa*	G^-	++	++	+	+
7.	*E. aerogenes*	G^-	++	++	+	*
8.	*P. vulgaris*	G^-	++	++	++	+

I = 25mg/ml; II =50 mg/ml; III =75 mg/ml; IV =100 mg /ml,*=MIC, - = No growth,
+ = Moderate growth, ++ =Dense growth

This medium demonstrates good batch-to-batch reproducibility, and supports the growth of most non fastidious bacterial pathogens (Jorgensen and Turnidge, 2003). Well-variant of the diffusion method was more sensitive and best conditions for the determination of minimal inhibitory concentration (Valgas *et al*., 2007). Dimethyl sulfoxide (DMSO) used as solvent for natural as well as synthetic antibacterial compounds (Wadhwani *et al.*, 2009; Houghton and Raman, 1998).

However, exclusive focus on individual biochemical targets neglects the fact that strong synergy of multiple constituents in a crude drug may prove more potent and effective than any single purified compound, or that interactions of co-occurring phytochemicals may help nullify the toxic effects of individual constituents. So while it is important to understand the active agents within medicinal plants, it should also be with caution that we extract and use constituents in isolation.

In Conclusion, the screening of antimicrobial activity performed on aqueous fractions of 25, 50, 75, 100 mg/ml of *Boswellia serrata*, traditionally important medicinal plant proved to be a bacteriostatic agent. The current work will provide new reference data for the drug development and possesses the ability to inhibit pathogenic bacteria. Further studies should be done on fractionation and identification of bioactive constituents which are responsible for antibacterial activity.

References

Archana S. and Abraham J. (2011). Journal of Applied Pharmaceutical Science. 1(8): 149-152

Arshiya Sultana, Khaleeq Ur Rahman, A.R. Padmaja and Shafeeq Ur Rahman (2013). *Boswellia serrata* Roxb. A Traditional Herb with Versatile Pharmacological Activity: A Review. *IJPSR* 4(6): 2106-2117.

Banno N, Akihisa T, Yasukawa K, Tokuda H, Tabata K, Nakamura Y, Nishimura R, Kimura Y, Suzuki T (2006): Anti-inflammatory activities of the triterpene acids from the resin of *Boswellia carteri*. *J Ethnopharmacol*. 107:249-253.

Bauer, Kirby, Sherris and Turck, (1966), Antibiotic susceptibility testing by standard single disc diffusion method Am. J. Clin. Path., 45: 493.

Chevrier MR, Ryan AE, Lee DY, Zhongze M, Wu-Yan Z, Via CS (2005). *Boswellia carterii* extract inhibits TH1 cytokines and promotes TH2 cytokines *in vitro*. *Clin Diag Lab Immunol*, 12:575-580.

CLSI (Clinical and Laboratory Standards Institute). (2012). Performance standards for antimicrobial susceptibility testing. National Committee for Clinical Laboratory Standards, Wayne, Pa.

Cowan M. (1999). Plant products as antimicrobial agents. Clinical Microbiology Reviews, 12(4):564-582.

Ernst E. (2008). Frankincense: systematic review. *BMJ*. ;337:a2813.

Gibbons S. (2008). Phytochemicals for bacterial resistance - Strengths, weaknesses and opportunities. *Planta Medica 74, 594–602.*

Hostanska K, Daum G, Saller R (2002). Cytostatic and apoptosis-inducing activity of boswellic acids toward malignant cell lines *in vitro*. *Anticancer Res* 22:2853-2862.

Houghton P and Raman A. (1998). Laboratory handbook for the fractionation of natural extracts. Chapman and Hall. 1-199.

Huang MT, Badmaev V, Ding Y, Liu Y, Xie JG, Ho CT (2000). Anti-tumor and anti-carcinogenic activities of triterpenoid, β-boswellic acid. *BioFactors* 13:225-230.

Jorgensen J and Turnidge J. (2003). Susceptibility test methods: Dilution and disk diffusion methods. Manual Clinical Microbiology 1:1108-1127.

Langmead L and Rampton DS (2006). Complementary and alternative therapies for inflammatory bowel disease. *Aliment Pharmacol Ther*, 23:341-349.

Madhava Chetty, Sivaji K and Tualsi Rao K. (2013). Flowering plants of Chittoor district. Student offset Printers, Tirupati. 4:61.

Mohammed Aman, Ravishankar Rai V., Pradeepa V. Samaga (India) Antimicrobial and Phytochemical Screening of *Boswellia serrata* Roxb., *Rhus mysorensis* Heyne, *Strychnos potatorum* Linn. F. and *Schefflera stellata* Gaertn. *Medicinal and Aromatic Plant Science and Biotechnology* 4(1): 69-72.

Sharma ML, Khajuria A, Kaul A, Singh S, Singh GB, Atal CK (1988). Effect of salai guggal ex-*Boswellia serrata* on cellular and humoral immune responses and leucocyte migration. *Agents Actions* 24:161-164.

Siddiqui M. Z. (2011). *Boswellia serrata:* A Potential Anti-inflammatory Agent: An Overview Indian J Pharm Sci. 73(3): 255–261.

Singh GB and Atal CK (1986). Pharmacology of an extract of salai guggal ex-Boswellia serrata, a new non-steroidal anti-inflammatory agent. *Agents Actions* 18:407-412.

Stavri M., Piddock, LJV., and Gibbon S., (2007). Bacterial efflux pump inhibitors from natural sources. J. Antimicrob. Chemother. 59: 1247–1260.

Turnidge J, Ferraro M and Jorgensen J. (2003). Susceptibility test methods: General considerations. In: Manual Clinical Microbiology.1:1102-1107.

Valgas C, de Souza S M, Smânia E F A, Smânia Jr A. (2007). Brazilian Journal of Microbiology 38:369-380.

Wadhwani T, Desai K, Patel D, Lawani D, Bahaley P, Joshi P, Kothari V. (2009). Effect of various solvents on bacterial growth in context of determining MIC of various antimicrobials. The Internet Journal of Microbiology, 7(1).

Weckesser S, Engel K, Simon-Haarhaus B, Wittmer A, Pelz K, Schempp CM (2007). Screening of plant extracts for antimicrobial activity against bacteria and yeasts with dermatological relevance. *Phytomedicine* 14:508-516.

Winking M, Sarikaya S, Rahmanian A, Jodicke A, Boker DK (2000). Boswellic acids inhibit glioma growth: a new treatment option? *J Neurooncol* 46:97-103.