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Preliminary phytochemical screening and antibacterial activity of resins of *Boswellia serrata* Roxb.

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Abstract
In the present study the preliminary phytochemical screening and antibacterial activity of resins of *Boswellia serrata* Roxb. were studied. The Phytochemical screening of resins of *Boswellia serrata* reveals the presence of flavonoids, steroids, and tannins. Methanol extraxt, n-Hexane and Petroleum ether extract of resins of *Boswellia serrata* were prepared and antibacterial activity were studied by disc diffusion method against certain bacterial pathogens such as *Escherichia coli, Staphylococcus aureus, Klebsiella pneumonia, Enterobacter aerogenes, Pseudomonas aeruginosa, Salmonella typhimurium, Salmonella typhi, Staphylococcus epidermidis* and *Proteus vulgaris*. The Methanol extracts had wide range of antibacterial activity against enteric bacterial pathogens than the petroleum ether extract, where as n-Hexane extract were slightly higher antibacterial activity than petroleum ether extract. Antibacterial activity of various extract of resins of *Boswellia serrata* was carried in attempt to develop a new pharmaceutical drug from natural origin for prevention of bacterial infection. Further work is being carried out to isolate and identify the active constituents of the plants responsible for antibacterial activity.

Keywords: Phytochemical Screening, Antimicrobial activity, resins and *Boswellia serrata* Roxb

Introduction
Boswellia serrata (Family: Burseraceae) is a deciduous middle sized tree, which is mostly concentrated in tropical; parts of Asia and Africa. In India it occurs in dry hilly forests of Rajasthan, Madhya Pradesh, Gujarat, Bihar, Assam, Orrisa as well as central penisular regions of Andhra Pradesh, Assam etc. *B. serrata* gum resin has been reported to have analgesic, anti-inflammatory, antiarthritic and anti-pyretic activity. Boswellic acids are the major constituents of the gum derived from the plant *B. serrata.* The gum resin comprises of β-boswellic acids as the main triterpenic acid along with 11-keto-β-boswellic acids and their acetates. Raja *et al.* reported the antibacterial activity of acetyl-11-keto-β- boswellic acid and its inhibitory effect on biofilms generated by *S. aureus* and *Staphylococcus epidermidis* (Raja *et al.,* 2011) [7]. The extracts of Boswellia species have been reported to exert anticarcinogenic, antiproliferative, antitumor, apoptotic, and cytostatic activities (Singh *et al.,* 2007: Upaganlawar and Ghule 2009) [9, 11].

Resins are amorphous mixture of essential oil, oxygenated products of terpenes and carboxylic acids which are obtained as exudates from plants. They are considered as end products of metabolism. Terpenoids are secondary metabolic products which once were considered unimportant (Sharma *et al.,* 2010) [8]. Resins have many pharmaceutical applications and some of them are used as incense. Since ancient times, people have been exploring the nature for plants in the search of new drugs. This has resulted in the use of a large number of medicinal plants with curative properties that can treat various diseases. Nearly 80% of the world's population relies on traditional medicine for primary healthcare, most of which involve the use of plant extracts. In India, almost 90% of the prescriptions were plant based in the traditional systems like unani, ayurveda, homeopathy and siddha (Lee, 1999) [4]. A large number of phytochemicals belonging to several classes have been shown to have inhibitory effects on all types of microorganisms in vitro (Cowan, 1999) [2].

The gum resin obtained from its bark is called 'Indian Olibanum' or 'Salai guggul'and is credited for its astringent, stimulant, expectorant, diuretic, antipyretic and antiseptic properties; it has also reported to be useful in ulcers, goiter, piles, diarrohea etc. In recent years the gum resin has been used extensively in pharmaceutical formulations for relieving aches and pain, particularly associated with arthritis (Aman *et al.,* 2010) [1]. *Boswellia serrata* gum resin is used as an antiseptic, antifungal and antimicrobial, antiinflammatory, arthritis, anti-obesity, asthma, cardio-tonic, anti-convulsant (Sultana *et al.,* 2013) [10].

The qualitative phytochemical study of different extracts indicates presence of alkaloids, tannins, triterpenoids, steroids etc. in preliminary phytochemical screening (Pandey and Tripathi 2014) [6]. Many researchers had studied antimicrobial activity of other parts of plant like bark, leaves and fruits of *B. serrata* which are used to cure many infectious diseases in traditional system of medicine but still very, less work has been done on antimicrobial activities of oleo-gum resin of *B. serrata*. To prove the validity of traditional medicine the present work has been undertaken to evaluate the antimicrobial screening of resins of *Boswellia serrata* against the human bacterial pathogens.

Materials and Methods

Sample Collection: The resins were collected from the Local Ayurevdic store of the Kopargaon. The Resins of *Boswellia serrata* was identified and authenticated by Department of Botany, S.S.G.M College, Kopargaon (M.S), India.

Preparation of extracts: Solvent extraction method Thirty grams of dried resins of *Boswellia serrata* were extracted with methanol, n-Hexane and Petroleum ether using soxhlet apparatus for 48 hrs. The collected extracts were filtered with Whatman No.1 filter paper and used for estimation of phytochemicals and antibacterial activity.

Phytochemical screening: Preliminary qualitative phytochemical screening was carried out with the following methods (Khandelwal, 2001).

Test for Tannins: To 0.5 ml of extract solution, 1 ml of distilled water and 1 to 2 drops of ferric chloride solution was added, observed for blue or green black coloration.

Test for Saponins: Two ml of distilled water was added to 2 ml of the test solution shaken well and observed for frothing.

Test for Flavonoids: A volume of 1.5 ml of 50 % methanol was added to 4 ml of the extracts. The solution and magnesium metal was added and warmed. Then, 5 to 6 drops of concentrated hydrochloric acid was added to the solution and observed for red coloration.

Test for Steroids: (Salkwoski's test): Five drops of concentrated sulphuric acid (H2SO4) was added to 2 ml of each extract and observed for red coloration.

Test for Glycosides: To 4 ml of extract solution and add few drops of glacial acetic acid, few drops of ferric chloride and concentrated sulphuric acid and observed for a reddish brown coloration at the junction of 2 layers and bluish green colour in upper layer.

Test for Alkaloids: To 4 ml of extract filtrate, a drop of Mayer‟s reagent was added along the sides of test tube. Creamy yellow or white precipitate indicates that the test is positive.

Test for Anthraquinones: One gram of powdered plant material was taken and extracted with 10 ml of hot water for five minutes and filtered. Filtrate was extracted with 10 ml of CCl4 then CCl4 layer was taken off. Five ml water and 5 ml dilute ammonia solution was added. No free anthraquinones were revealed as absence of appearance of pink to cherry red colour. One gram of second sample of the same plant material was extracted with 10 ml of ferric chloride solution and 5 ml of hydrochloric acid then it was heated on water bath for 10 minutes and filtered. Filtrate was cooled and treated as mentioned above.

Test for phenolic compounds: Two ml of extract was diluted to 5 ml with distilled water. To this a few drops of neutral 5 % ferric chloride solution was added. A dark green colour indicates the presence of phenolic compounds

Bacterial cultures: The standard pathogenic bacterial cultures were procured from IMTECH, Chandigarh, India and used in the present study (Table 1). The bacterial cultures were rejuvenated in Mueller- Hinton broth (Hi-media laboratories, Mumbai, India) at 37^0C for 18h and then stocked at 4^0C in Mueller-Hinton Agar. The inoculum size of the bacterial culture was standardized according to the National committee for Clinical Laboratory Standards (NCCLS, 2002) [5] guideline. The pathogenic bacterial culture was inoculated into sterile Nutrient broth and incubated at 37^0C for 3h until the culture attained a turbidity of 0.5 McFarland units. The final inoculum size was standardized to 10^5 CFU/mL with the help of SPC and Nephlo-turbidometer.

Table 1: Bacterial cultures used in study (IMTECH, Chandigarh, India).

Bacterial Pathogens	MTCC Number
Proteus vulgaris	426
Staphylococcus epidermidis	435
Staphylococcus aureus	96
Escherichia coli	739
Pseudomonas aeruginosa	424
Klebsiella pneumoniae	109
Salmonella typhi	733
Enterobacter aerogenes	111
Salmonella typhimurium	98

Preparation of disc for antibacterial activities: The Methanol, n-Hexane and Petroleum ether extracts were prepared in their respective solvents and the sterile blotting paper disc (10 mm) were soaked in the diluted extract in such concentration that the amount of solution absorbed by each disc was 1mg, 2mg, 3mg, 4mg, 5mg of each extracts of resins of *Boswellia serrata*. The prepared disc were dried in controlled temperature to remove excess of solvent and used in study.

Antibacterial activity using disc diffusion method: The modified paper disc diffusion method was employed to determine the antibacterial activity of Methanol, n-Hexane and the Petroleum ether extracts of resins of *Boswellia serrata*. Turbidity of inoculums was matched with McFarland turbidity standard (NCCLS, 2002) [5]. Inoculums were spread over the Nutrient agar plate using a sterile cotton swab in order to get a uniform microbial growth. Then the prepared antibacterial disc were placed over the lawn and pressed slightly along with positive and negative controls. Ampicillin 10 mcg/disc (Hi-Media, Mumbai) were used as positive control while disc soaked in various organic solvents and dried were placed on lawns as negative control. The plates were incubated for 18h at 37^0C. The antibacterial activity was evaluated and diameters of inhibition zones were measured. Experiment was carried out in triplicate and the averages diameter of zone of inhibition was recorded. The antibacterial activity was classified as strong (>20mm), moderate (16-19mm) and mild (12-15mm) and less than 12mm was taken as inactive.

Results and Discussion

The present study was made in the resins (gum resin/oleo resin) of *Boswellia serrata,* The Table 2 represents the presence or absence of secondary metabolites in the resins of *Boswellia serrata,*. The results showed that the resins of *Boswellia serrata* possessed more abundant tannins, saponins and glycosides. The phenolic compounds were found to be normal in quantity and flavanoids were quite less. The alkaloids and anthroquinones were totally absent in all the three extracts. Methanol extract contains, Flavanoids, Glycosides, Saponins,, Tannins and Phenolic compounds, where as in n-Hexane extract only Steroids and Phenolics compounds were found. The presence of tannins and Steroid were present in Petroleum extract.

Table 2: Phytochemical analysis of resins of *Boswellia serrata*

S. No	Phytochemical Constitutes	Methanol extract	n-Hexane	Petroleum ether extract
1	Alkaloid	-	-	-
2	Flavonoids	+	-	-
3	Glycosides	+	-	-
4	Saponins	+	-	-
5	Steroids	-	-	+
6	Tannins	+	+	+
7	Anthroquinones	-	-	-
8	Phenolic compounds	+	+	-

Where: + = the presence of constitute, – = the absence of constitutes

Table 3: Antibacterial activity of resins of *Boswellia serrata* extracts against bacterial pathogens (Zone of inhibition of growth in mm, average of 3 readings)

Bacterial Pathogens	Methanol extract					n-Hexane extract					Petroleum ether extract					controls				Ampicillin (10mcg)
	5mg /disc	4mg /disc	3mg /disc	2mg /disc	1mg /disc	5mg /disc	4mg /disc	3mg /disc	2mg /disc	1mg /disc	5mg /disc	4mg /disc	3mg /disc	2mg /disc	1mg /disc	Pet ether	Chloroform	Ethyl acetate	Methanol	
P. vulgaris	15	13	12	-	-	14	-	-	-	-	15	13	-	-	-	-	-	-	-	16
S. epidermidis	23	20	17	15	13	20	17	15	14	12	20	18	17	14	12	-	-	-	-	25
S. aureus	22	21	18	17	16	24	22	20	18	16	20	19	17	15	13	-	-	-	-	24
E. coli	19	17	15	12	12	16	14	12	-	-	22	19	17	14	-	-	-	-	-	11
P. aeruginosa	16	15	14	13	12	15	13	-	-	-	21	17	15	14	12	-	-	-	-	16
S. typhi	21	20	17	14	12	14	12	-	-	-	18	17	16	15	-	-	-	-	-	18
E. aerogenes	17	15	15	13	12	18	16	15	13	11	20	19	17	14	13	-	-	-	-	14
S. typhimurium	18	17	15	13	11	15	12	-	-	-	20	17	17	15	-	-	-	-	-	19

According to antibacterial profile (Table 3), maximum inhibitory effect of the Methanol extract observed only on *Staphylococcus epidermidis, Staphylococcus aureus, Salmonella typhi* and moderate antibacterial against *Escherichia coli, Pseudomonas aeruginosa, Enterobacter aerogenes, Salmonella typhimurium* but mild inhibitory effect on *Proteus vulgaris.* N-Hexane extract showed strong antibacterial effect against *Staphylococcus epidermidis* and *Staphylococcus aureus* and moderate antibacterial against *Proteus vulgaris, Escherichia coli, Enterobacter aerogenes, Salmonella typhi* and *Salmonella typhimurium* but mild effect on *Pseudomonas aeruginosa.* Petroleum ether extract showed maximum inhibitory effect on *Staphylococcus aureus, Proteus vulgaris, Staphylococcus epidermidis, Pseudomonas aeruginosa, Salmonella typhi, Salmonella typhimurium,* but moderate inhibitory effect on *Escherichia coli, Enterobacter aerogenes.*

Conclusion

Resin is considered to be one of the important products of plants. Resins have commercial value, being used as incense (non-combustible, indirect-burning), have antioxidant, antimicrobial properties and many other medicinal uses. The result of the antibacterial assay show promising evidence for the antibacterial effect of resins of *Boswellia serrata*. Form the above evidence, it is clear that plant extracts have great potential as antibacterial compounds against bacterial pathogens and that they can be used in the treatment of bacterial infections. It is hoped that this study would lead to the establishment of some compounds that could be used to formulate new and more potent antimicrobial drugs of natural origin.

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