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## RESEARCH ARTICLE

### *In-Vitro* Antimicrobial Screening of *Boswellia serrata*

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#### Abstract

The present study shows *in vitro* antimicrobial screening of leaves, bark and fruits of *Boswellia serrata* Roxb. ex Cocls. which is collected from southern Aravalli hills of Rajasthan. To prepare crude drugs extraction was done successively and separately in four different solvents i.e. petroleum ether, chloroform, ethyl acetate and methanol. The antibacterial and antifungal activity of all the extracts tested by agar-well diffusion assay. All extracts displayed varied level of antimicrobial activity. Methanolic extract of leaves was found to be most active against *Streptococcus pneumoniae* ( $18 \pm 0.00$ ) and *Escherichia coli* ( $16.33 \pm 0.57$ ) while in bark, ethyl acetate extract showed more antimicrobial activity against *Klebsilla pneumoniae* ( $15.66 \pm 0.57$ ) and *Staphylococcus aureus* ( $15 \pm 0.0$ ) and among the four extracts of fruit ethyl acetate extract was found to be more active against all tested organisms, specifically *Escherichia coli* ( $16 \pm 0.0$ ) and *Streptococcus pneumoniae* ( $15.33 \pm 0.57$ ). Methanolic and ethyl acetate extract of all parts also shows good antifungal activity against *A. niger* with a zone of inhibition 20 to 22 mm. In *C. albicans* ethyl acetate extract of leaves found to be most active with a zone of inhibition  $11 \pm 0.0$  mm. Phytochemical screening showed the presence of phytochemicals such as tannins, phenols, steroids, flavonoids, and saponins. The results obtained in this study confirm the antimicrobial potential of this plant and its usefulness in the treatment of diseases that may be caused microorganisms.

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#### Introduction

*Boswellia serrata* belonging to family Burseraceae, is commonly known as 'Salai' or 'Indian frankincense'. It is also known by different names such as 'Shallaki' in 'Sanskrit', 'Kundur' in 'Unani', 'Olibanum indicum', in 'latin etc. It is found in West Asia, Oman, Yemen, South Africa, Southern Arabia and many parts of India and then extends into Pakistan through Punjab region. *B. serrata* is medium to large-sized, a balsimorous and deciduous tree. The tree, on injury, exudates an oleo-gum resin known as *salai guggul*.

*B. serrata* is an important part of Indian Ayurveda and Unani traditional systems of medicine. Every part of the tree **bark, Leaves, Gum, Resin, Oleo resin**, young drupe, fruits possesses medicinal potential and indigenous communities use it for treatment of various human and animal ailments like joint pain, muscular pain and rheumatism (Pawar and Patil, 2006; Jain et al., 2011), cough and stomachache (Jagtap et al., 2008), General weakness- (Reddy et al., 2010), relief from sciatica, menorrhagia (Bhogaonkar and Kadam, 2006), conjunctivitis (Patil and Bhaskar, 2006), eye infection (Jain et al., 2010), skin to cure burns (Jagtap et al., 2008), tuberculosis - (Patil and Bhaskar, 2006), towards off germs & mosquito repellent (Jagtap et al., 2008; Snajeev and Sasidharan, 1997), scorpion sting, antiseptic, heal wound (Reddy et al., 2010; Meena and Yadav, 2011), arthritis (Bhogaonkar et al., 2010), etc. Bark is reported to cure animals ailments like arthritis, indigestion, windiness and flatulence (Galav et al., 2013). *Boswellic acid* isolated from gum & resin possesses anti-inflammatory, immunomodulator activity (Singh, 2005). Its anti-inflammatory, anti-arthritis, analgesic and anti-oxidant activities have also been reported (Ammon et al., 1993; Kimmattkar et al., 2003; Sharma et al., 2010; Singh et al., 2012). This plant contains flavonoids, alkaloids, tannin, steroids saponins, glycosides and terpenoids (Aman et al., 2010; Zeeyauddin et al., 2011).

Many of its folk claims had also been scientifically validated in various parts of world (Sultana et al., 2013). Antibacterial and antifungal screening of oleogum -resin of *Boswellia serrata* was studied by many workers (Kumar et al., 2006; Camarda et al., 2007; Rajendra et al., 2013) while Aman et al. (2010), studied antibacterial activity of leaves and flower. Kudle et al. (2013), synthesized silver nanoparticles from the flower extract of *B. serrata* and done its antimicrobial screening. Many researchers had studied antimicrobial activity of oleo-gum resin of *B. serrata* but still very, less work has been done on the antimicrobial activities of other parts of plant like bark, leaves and fruits, which are used to cure many infectious diseases in traditional system of medicine. To prove the validity of traditional medicine the present work has been undertaken to evaluate the antimicrobial screening of leaves, bark and fruits of *Boswellia serrata* against the array of human pathogen.

## Materials and Methods

### Collection of Plant material

The plant materials were collected from the Southern Aravalli hills of Rajasthan. The plant was identified from its morphological features as mentioned in different standard text and flora (Hooker, 1872-1897; Singh et al., 1991). The voucher specimen has been deposited at VBRI, Udaipur for further reference.

### Preparation of Extracts

All parts of the plants were washed, shade dried, powdered by using a pulverizor. The coarse powder (100gm of each) was subjected to successive extraction with organic solvents such as petroleum ether, chloroform, ethyl acetate and methanol by Soxhlet method for 12 hrs. The extract were filtered and filtrate was concentrated to dryness under reduced pressure in rotary vacuum evaporator and stored at 4°C.

To make stock solution of 100mg/ml of each extract (crude drug) the appropriate amount is weighed and dissolved in DMSO. The stock solution was passed through 0.2µm pyrogesic filter to sterilize the solution and further concentrations of 50 mg/ml, 25 mg/ml and 12.5 mg/ml was made by diluting with DMSO.

### Test Microorganism

The pathological strains of test organism *i.e.* *Escherichia coli* (MTCC 118), *Staphylococcus aureus* (MTCC 96), *Klebsiella pneumoniae* (MTCC 39) *Pseudomonas aeruginosa* (MTCC 424) and *Streptococcus pneumoniae* (MTCC \*655), *Aspergillus niger* (MTCC 281) and *Candida albicans* (MTCC 183) were obtained from MTCC, Chandigarh, India and again identified by standard methods of identification (Collee et al., 1996).

## Antimicrobial Susceptibility Testing

### Well Diffusion Method

The *in vitro* antimicrobial activity was determined by the agar well diffusion method (Guyen et al., 2006). Cell suspensions containing 10<sup>6</sup> CFU/ml cells for bacteria and yeasts and 10<sup>5</sup> spore/ml of fungi were prepared and 100µl was evenly spread on the surface of the nutrient agar for bacteria and Sabouraud dextrose agar medium for yeasts and fungi using glass spreader. The wells of 6 mm diameter were made at equidistant. 100µl volumes of crude extract of each concentration were dispensed into wells, the plate were incubated at 37° C for 24 hrs for bacterial

strains, 48 hrs for yeasts and 72 hrs for fungi at 28° C. The zone of inhibition was measured. As reference antibiotic Meropenam (5µg/ml) was used against all the tested bacteria and Amphotericin-B (30 µg/ml) for yeast and fungi.

#### **Minimum Inhibitory Concentration (MIC)**

The estimation of MIC of the crude extracts was carried out using the method of agar well diffusion (Mohana et al., 2008; Bais et al., 2013) with some modification. Approximate amount of extract was taken from the solution of the crude drug sample (12.5mg/ml) with DMSO and diluted it serially (1:1) with DMSO to the concentration of 0.012mg/ml. As a result, a series of the sample solution in decreasing concentration was obtained by a ratio of 0.5 (final concentration: from 6.25mg/ml to 0.012mg/ml). In this method the least concentration of each extract showing a clear zone of inhibition was taken as the MIC. The MIC value was defined as the lowest concentration to inhibit visible growth of microbes.

#### **Preliminary Phytochemical Screening**

All the extracts of leaves, bark and fruits of *Boswellia serrata* were screened for various phytochemicals such as tannins, alkaloids, phenols, steroids, flavonoids, and saponins using standard methodology (Panday and Tripathi, 2014).

Table 1. Showing zone of inhibition of different extracts of *Boswellia serrata* against bacteria

Name of Organism	Cons. Mg/ml	<i>B. serrata</i>											
		Leaf Extracts				Bark Extracts				Fruit Extracts			
		PE	Chlo	EA	Meoh	PE	Chlo	EA	Meoh	PE	Chlo	EA	Meoh
<i>E. coli</i>	100	-	-	13 ± 0.0	16.33±0.57	-	-	13 ± 0.0	12 ± 0.0	12 ± 0.0	14.66±0.57	16 ± 0.0	13 ± 0.0
	50	-	-	12.33±0.57	14 ± 0.0	-	-	12 ± 0.0	11.33±0.57	11 ± 0.0	14 ± 0.0	14 ± 0.0	12 ± 0.0
	25	-	-	11.33±0.57	12 ± 0.0	-	-	11 ± 0.0	10.33±0.57	-	13 ± 0.0	11.33±0.57	11.66±0.57
	12.5	-	-	10.66±0.57	11 ± 0.0	-	-	10.33±0.57	10 ± 0.0	-	10.33±0.57	10.66±0.57	10.33±0.57
	6.25			10.33±0.57				10 ± 0.0				10 ± 0.0	
	3.125			10 ± 0.0				-				-	
	1.562			-				-				-	
	0.781			-				-				-	
<i>Kp</i>	100	12 ± 0.0	11 ± 0.0	13 ± 0.0	12.66±0.57	13.66±0.57	12.33±0.57	15.66±0.57	12.33±0.57	11.33±0.57	10.33±0.57	11 ± 0.0	13 ± 0.0
	50	11.66±0.57	10 ± 0.0	12 ± 0.0	12 ± 0.0	13 ± 0.0	12 ± 0.0	14.33±0.57	11.66±0.57	11 ± 0.0	10 ± 0.0	10.33±0.57	12.33±0.57
	25	10.66±0.57	-	11.33±0.57	11 ± 0.0	12 ± 0.0	11 ± 0.0	13 ± 0.0	11 ± 0.0	10 ± 0.0	-	10 ± 0.0	12 ± 0.0
	12.5	10 ± 0.0	-	11 ± 0.0	10 ± 0.0	11 ± 0.0	10 ± 0.0	12 ± 0.0	10 ± 0.0	-	-	-	11 ± 0.0
	6.25			10.33±0.57				11.66±0.57				-	
	3.125			10 ± 0.0				11 ± 0.0				-	
	1.562			-				10 ± 0.0				-	
	0.781			-				-				-	
<i>Sa</i>	100	10.66±0.57	-	15 ± 0.0	12 ± 0.0	12.33±0.57	11 ± 0.0	15 ± 0.0	13 ± 0.0	11.66±0.57	11.66±0.57	13.66±0.57	13 ± 0.0
	50	10 ± 0.0	-	13.33±0.57	11 ± 0.0	11 ± 0.0	10 ± 0.0	13.33±0.57	11.66±0.57	11 ± 0.0	11 ± 0.0	12.33±0.57	11.33±0.57
	25	-	-	12 ± 0.0	10 ± 0.0	10.33±0.57	-	12.66±0.57	11 ± 0.0	10.33±0.57	10 ± 0.0	12 ± 0.0	10.33±0.57
	12.5	-	-	11.33±0.57	-	10 ± 0.0	-	12 ± 0.0	10 ± 0.0	10 ± 0.0	-	11 ± 0.0	10 ± 0.0
	6.25			11 ± 0.0				11 ± 0.0				10 ± 0.0	
	3.125			10.33±0.57				10 ± 0.0				-	
	1.562			10 ± 0.0				-				-	
	0.781			-				-				-	
<i>Pa</i>	100	-	-	14 ± 0.0	12 ± 0.0	-	12±0.0	13 ± 0.0	12±0.0	-	-	13.66±0.57	12 ± 0.0
	50	-	-	12.66±0.57	11 ± 0.0	-	11 ± 0.0	12±0.0	11 ± 0.0	-	-	13.33±0.57	10.66±0.57
	25	-	-	11 ± 0.0	10.33±0.57	-	10.66±0.57	11.66±0.57	10.33±0.57	-	-	12 ± 0.0	10 ± 0.0
	12.5	-	-	10.66±0.57	10 ± 0.0	-	10 ± 0.0	11.33±0.57	10 ± 0.0	-	-	11 ± 0.0	-
	6.25			10 ± 0.0				11 ± 0.0				-	

	3.125			-				10.33±0.57				-	
	1.562			-				10 ± 0.0				-	
	0.781			-				-				-	
Sp	100	-	13 ± 0.0	15.33±0.57	18 ± 0.0	-	12±0.0	14 ± 0.0	13 ± 0.0	12.33±0.57	13.33±0.57	15.33±0.57	12.66±0.57
	50	-	12 ± 0.0	14 ± 0.0	16.33±0.57	-	11.66±0.57	13.33±0.57	12.33±0.57	12 ± 0.0	12.33±0.57	14 ± 0.0	11 ± 0.0
	25	-	10.66±0.57	12.66±0.57	14.3 ± 0.57	-	10 ± 0.0	12 ± 0.0	11 ± 0.0	11 ± 0.0	11 ± 0.0	12 ± 0.0	10.66±0.57
	12.5	-	10 ± 0.0	12 ± 0.0	13 ± 0.0	-	-	11 ± 0.0	10 ± 0.0	10 ± 0.0	10 ± 0.0	11 ± 0.0	10 ± 0.0
	6.25			10 ± 0.0				10 ± 0.0				10 ± 0.0	
	3.125			-				-				-	
	1.562			-				-				-	
	0.781			-				-				-	

PE- Petroleum ether, Chlo- Chloroform, EA- Ethyl acetate, MeOH- Methanol, *E. coli*- *Escherichia coli*, *Kp*- *Klebsiella pneumoniae*,

*Sa*-*Staphylococcus aureus*, *Pa*- *Pseudomonas aeruginosa*, *Sp*- *Streptococcus pneumoniae*.

Table 2. Showing zone of inhibition of different extracts of *Boswellia serrata* against fungi

Name of Organism	Cons. mg/ml	<i>B. serrata</i>											
		Leaf Extracts				Bark Extracts				Fruit Extracts			
<i>Aspergillus niger</i>		PE	Chlo	E A	Meoh	PE	Chlo	E A	Meoh	PE	Chlo	E A	Meoh
	100	12 ± 0.0	17 ± 0.0	22 ± 0.0	22 ± 0.0	13 ± 0.0	20 ± 0.0	21 ± 0.0	16 ± 0.0	-	14 ± 0.0	21 ± 0.0	22 ± 0.0
	50	11 ± 0.0	15.66±0.57	21 ± 0.0	21 ± 0.0	11.33±0.57	18.33±0.57	19 ± 0.0	14 ± 0.0	-	12.66±0.57	19.33±0.57	20 ± 0.0
	25	-	13 ± 0.0	18.66±0.57	20.33±0.57	10 ± 0.0	17 ± 0.0	16.66±0.57	12 ± 0.0	-	11 ± 0.0	17 ± 0.0	17.66±0.57
	12.5	-	11.66±0.57	18 ± 0.0	18 ± 0.0	-	16 ± 0.0	16 ± 0.0	10.66±0.57	-	-	14 ± 0.0	14 ± 0.0
	6.25			17 ± 0.0				14 ± 0.0				-	
	3.125			15 ± 0.0				13 ± 0.0				-	
	1.562			13 ± 0.0				11 ± 0.0				-	
	0.781			-				-				-	
<i>Candida albicans</i>	100	-	10.33±0.57	11 ± 0.0	-	10.33±0.57	-	10 ± 0.0	-	10 ± 0.0	-	-	-
	50	-	10 ± 0.0	10 ± 0.0	-	10 ± 0.0	-	-	-	-	-	-	-
	25	-	-	-	-	-	-	-	-	-	-	-	-
	12.5	-	-	-	-	-	-	-	-	-	-	-	-
	6.25			-				-				-	
	3.125			-				-				-	
	1.562			-				-				-	
	0.781			-				-				-	

PE- Petroleum ether, Chlo- Chloroform, EA- Ethyl acetate, Meoh- Methanol

**Table 3:-** Antimicrobial activity of ethyl acetate extract of leaves, bark and fruit of *Boswellia serrata* in term of MIC

Name of organism	EA extract of <i>B. serrata</i>		
	Leaves MIC (mg/ml)	Bark MIC (mg/ml)	Fruit MIC(mg/ml)
<i>Escherichia coli</i>	3.125	6.25	6.25
<i>Klebsiella pneumoniae</i>	3.125	1.562	-
<i>Staphylococcus aureus</i>	1.562	3.125	6.25
<i>Pseudomonas aeruginosa</i>	6.25	1.562	12.5
<i>Streptococcus pneumoniae</i>	6.25	6.25	6.25
<i>Candida albicans</i>	-	-	-
<i>Aspergillus niger</i>	1.562	1.562	12.5

**Table 4:-** Qualitative examination of secondary metabolites of extracts of *Boswellia serrata*

S. No.	Phytochemicals	Petroleum ether extract	Chloroform Extract	Ethyl acetate	Methanol extract
1.	Alkaloids	-	-	-	-
2.	Flavonoids	-	-	+	+
3.	Steroids	+	-	+	+
4.	Phenols	-	+	+	+
5.	Tannins	-	-	+	+
6.	Saponins	-	-	+	+

## Result and Discussion

The antimicrobial activity of leaves, bark and fruits of *B. serrata* was determined against the tested strains are shown in Table 1 and 2. The antimicrobial activity was observed to be in dose dependent manner i.e. 100 mg/ml shown more level of activity than other concentration against the entire tested microorganism. As shown in Table-1, the extract from the *B. serrata* leaves, bark and fruits displayed antimicrobial activity against the tested microorganism, with the diameter of zone of inhibition ranging between 10mm to 18mm. Among the four extracts of leaves methanolic extract was found to be most active against *Streptococcus pneumoniae* ( $18 \pm 0.00$ ) and *Escherichia coli* ( $16.33 \pm 0.57$ ) while in bark extracts, ethyl acetate extract showed higher antibacterial activity against *K. pneumoniae* ( $15.66 \pm 0.57$ ) and *S. aureus* ( $15 \pm 0.0$ ) and among the four extracts of fruit ethyl acetate extract was found to be more active against all tested organisms, specifically *Escherichia coli* ( $16 \pm 0.0$ ) and *Streptococcus pneumoniae* ( $15.33 \pm 0.57$ ).

Furthermore, among the fungi studied (Table-2), both ethyl acetate and methanolic extracts of leaves showed higher antifungal activity against *A. niger* with a zone of inhibition  $22 \pm 0.0$  mm. In *C. albicans* ethyl acetate extract of leaves found to most active with a zone of inhibition  $11 \pm 0.0$  mm. In bark extracts, ethyl acetate and chloroform extracts showed higher antimicrobial activity against *A. niger* with a zone of inhibition  $21 \pm 0.0$  mm and  $20 \pm 0.0$  mm respectively while in fruit methanol and ethyl acetate extract showed moderate antifungal activity against *A. niger* with a zone of inhibition  $22 \pm 0.0$  and  $21 \pm 0.0$  mm respectively.

The MIC of ethyl acetate extract against the entire tested microorganism was observed to be a range of 1.562 to 12.5 mg/ml (Table-3).

A preliminary screening was carried out to check the presence of various phytoconstituents in the extracts (Table-4). On phytochemical screening of the different solvent extracts of *B. serrata*, it was found that petroleum ether extract shows presence of steroids, chloroform extract shows presence of phenols while ethyl acetate and methanol extract shows presence of phenols, flavonoids, steroids, tannins and saponins.

Successive isolation of botanical compounds from plant material is largely dependent on the type of the solvent use in the extraction procedure. The present study showed that plant extracts prepare with ethyl acetate and methanol

provided a good inhibition index compare than other two extracts, were found to be less active against the test organism (Table 1 and 2).

The present investigation therefore clearly establishes the antimicrobial potential of the plant and suggests the need to further exploit in the management of microbial diseases caused by these bacteria in humans. From the result obtained it supports the folkloric usages of *B. serrata* as a therapeutic agent. Further phytochemical investigation suggests that all the extract contain certain constituents with antimicrobial properties that can be used as antimicrobial agents in new drug for the therapy of infectious diseases caused by pathogen.

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