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ANTIMICROBIAL POTENTIAL OF *BOSWELLIA SERRATA* ROXB. EX. COLEBR.EXTRACTS AGAINST HUMAN PATHOGENS.

Dr. Aarti R. Deshpande*

Department of Microbiology, Shankarlal Khandelwal College, Akola (Maharashtra) (India).

***Corresponding Author: Dr. Aarti R. Deshpande**
Department of Microbiology, Shankarlal Khandelwal College, Akola (Maharashtra) (India).

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ABSTRACT

In the context of the growing problem of multidrug resistant strains of microorganisms, discovery and introduction of novel alternative antimicrobial compounds is a major challenge. Medicinal plants that have been traditionally used for the treatment of diseases with microbial etiology, could offer potent antimicrobial drugs. Considering the medicinal importance of *Boswellia serrata* an attempt was made in present study to evaluate the antimicrobial potential of this plant against human pathogenic bacteria and fungi. The sequential extracts of leaves and bark of this plant were separately prepared in petroleum ether, acetone, 90% methanol and water. Antibacterial activity of these extracts were tested against standard cultures of *Staphylococcus aureus, Bacillus cereus, Escherichia coli, Proteus vulgaris, Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Salmonella typhimurium* using well plate method. Significant antimicrobial activity was exhibited by acetone and methanol extracts. The relative minimum inhibitory concentrations of these extracts were determined for four bacteria and were found to be in the range of 3.12 mg/ml to 12.5 mg/ml for different bacteria. Antifungal activities of the organic extracts were tested against *Microsporum gypseum, Candida albicans,* and *Cryptococcus neoformas.* Appreciable antifungal activity was recorded for *B. serrata* leaves extract.

KEYWORDS: Antimicrobial activity, Antifungal activity, Antibacterial activity, *Boswellia serrata*.

INTRODUCTION

Rapidly increasing multidrug resistant strains of pathogenic microbes is of major concern considering the problems they create for treatment of common infectious diseases. Insensitivity of such strains to currently available antimicrobial drugs is a factor contributing to spread of the diseases and increasing the frequency of hospital acquired infections. This problem is posing a great threat to immuno-compromised and AIDS patients.[1] Indiscriminate, inappropriate and extensive use of antibiotics, a common practice in past few decades, has created this alarming and dreadful situation. This has resulted in augmented efforts world- wide for the discovery of novel antimicrobial agents. Combination therapy is considered one of the strategies for effective treatment and for restricting the growing antimicrobial resistance in microorganisms.[2]

Enormous knowledge of traditional medicine such as Ayurveda holds immence potential for modern drug development.[3,4] Many plant based antimicrobial compounds have been isolated and found effective against microorganisms.[5] Such plants derived compounds could be utilized alone or in combination with other modern drugs to increase their potency or to remediate the problem of antimicrobial resistance.

Boswellia serrata Roxb. ex. Colebr, is a plant belonging to the family Buseraceae. In ayurvedic literature this plant is known by the *Sanskrit* name *Sallaki* and has been used since ages for treating arthritis. Its gum exudate is known as frankincense which has anti-inflammatory properties. According to *Bhavaprakash* (Ancient Ayurvedic literature) its bark is mentioned as "*Jantughna*" i. e. which kills the germs. It is useful in infectious diseases such as skin diseases, dysentery, diarrhea, boils, mouth sores and bronchitis.[6] Boswellic acids extracted from frankincense (*Salai Guggul*) are the pharmacological active compounds responsible for anti-inflammatory action,[7] Regulation of immune cytokine production has been considered important as mechanism responsible for anti-inflammatory action[8] along with infiltration of leucocytes.[9]

Extensive studies have been undertaken by researchers regarding the pharmacological properties of gum exudates of this plant[10] such as anti-arthritic,[11] anti-inflammatory and analgestic,[12] anti-proliferative[13] and antioxidant.[14] Few reports also indicate the

antimicrobial properties of resins and essential oils derived from this plant.[15-18] However more investigations are needed to explore the application potential of the antibacterial and antifungal components of this plant for treatment of human infections. The present study was initiated to test the antimicrobial activity of *Boswellia serrata* leaves and bark extracts against common bacterial and fungal pathogens implicated in human diseases.

MATERIALS AND METHODS

Preparation of extracts

Powders of leaves and bark were successively extracted with petroleum ether, acetone and 90% methanol at room temperature by mixing solvents with the powder in stoppered reagent bottles and shaking for 16 to 18 hrs. at room temperature. The bottles were allowed to stand for few hours and then the extract was filtered through triple layered ordinary filter paper. The solvents were concentrated by evaporating at room temperature. Aqueous extract was prepared separately by same procedure.

Screening for Antibacterial Activity

Bauer –Kirby disc diffusion method was used.[19] for which 6mm discs of Whatman paper number 42 were soaked with plant extracts, allowed to dry and placed on plates of sensitivity test medium (Hi media M-296) that was surface inoculated with test culture of required turbidity .The plates were kept in refrigerator for 30 minutes for pre-diffusion and then incubated at 37°C for 18 to 24 hours. Bacterial cultures used were *Bacillus cereus* (NCIM 2322), *Staphylococcus aureus* (ATCC 6538), *Escherichia coli* (NCIM 2931), *Proteus vulgaris* (MTCC 742) *Pseudomonas aeruginosa* (NCIM 2200), *Salmonella typhimurium* (NCIM 2501), *Klebsiella pneumoniae* (MTCC 432).

Antibacterial Assays For Relative MIC

Agar well diffusion method.[20] was used in which the wells were made in sensitivity test agar seeded with test cultures. Different concentrations of plant extracts were added (50ul) to wells cut on the agar plates. The plates were kept in refrigerator for 30 minutes for pre-diffusion and then incubated at 370c for 18 to 24 hours.

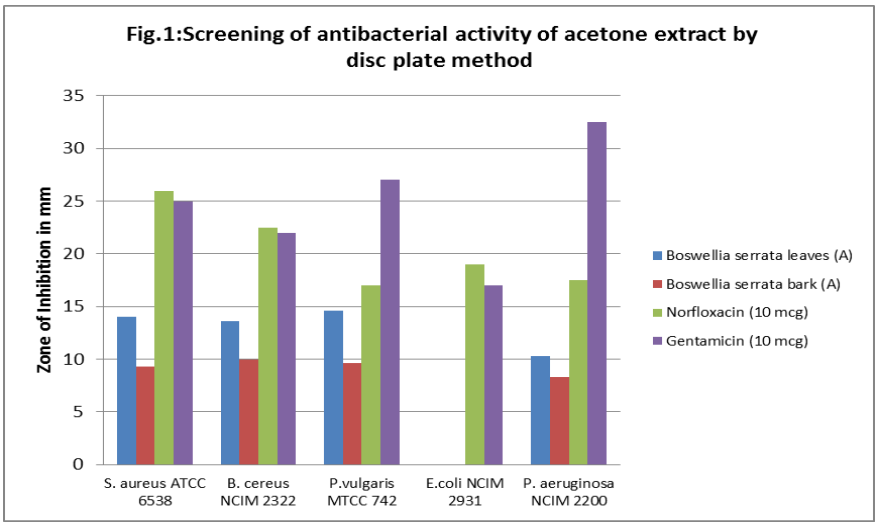
Relative MICs of extracts were determined as per Vahidi *et al.* [21]. Stock solutions of extracts were prepared (100mg/ml) in di methyl sulfoxide (DMSO) and twofold dilutions were prepared to get linear relationship between log concentrations and diameter of zones of inhibition .Lowest concentration giving visible zone of inhibition was recoded as MIC for each extract.

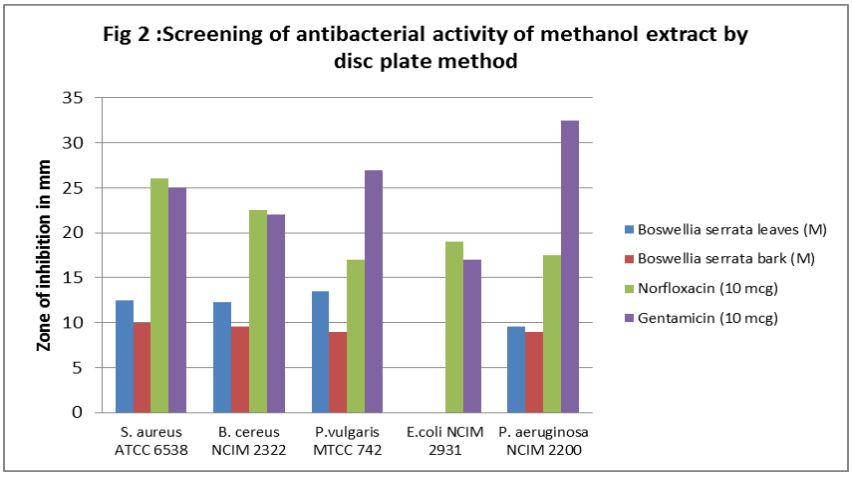
Screening of Antifungal Activity

Antifungal activities were tested by disc plate method against three fungal cultures on potato dextrose agar containing 0.5mg/ml chloramphenicol.[22] Inoculum was prepared by suspending two loopfuls of fresh culture in 5ml of sterile distilled water. The plates were seeded by swabbing so as to get uniform distribution of the culture on the surface of the agar. The disc impregnated with the extracts were placed on the surface of the agar. The plates were kept in refrigerator for 30 minutes for pre-diffusion and then incubated at 30^0c for 18 to 24 hours.

RESULTS AND DISCUSSION

Preliminary screening of the extracts was done by disc plate method against the standard cultures of five bacteria The zones of inhibition of extracts were compared with those of standard antibacterial agents viz. Norfloxacin (10mcg) and Gentamicin discs (10 mcg) (fig 1,2). Acetone and methanol extracts of both leaves and bark demonstrated antibacterial activity against four of the bacterial cultures excluding *E. coli*. The zones of inhibition were mostly in the range of 9 mm to 14.6 mm. The acetone and methanol extracts of leaves were more potent as compared to the bark extracts.. The inhibitory acetone of extracts were not as strong as the standard antibacterial compounds. Nevertheless their antibacterial potential appeared to be significant considering their crude nature.





Crude and un-concentrated aqueous and petroleum ether extracts did not reveal any antibacterial activity by disc plate method. Hence more detailed studies were performed by employing quantitative comparison of the antibacterial action of the dried extracts (Sticky/ Powders) by well plate method against seven standard bacterial cultures using di methyl sulfoxide (DMSO) as solvent (Table- 1). All the extracts demonstrated greater zones of inhibition against all the bacterial culture when tested at 0.1 g/ml concentration. Aqueous extracts of leaves (0.5g/ml concentration) displayed antibacterial action against most of the cultures except *E.coli* and *K. pneumoniae*. However the zones of inhibition were smaller as compared to the action and methanol extracts. None of the petroleum ether extracts of the leaves as well as bark showed antibacterial activity.

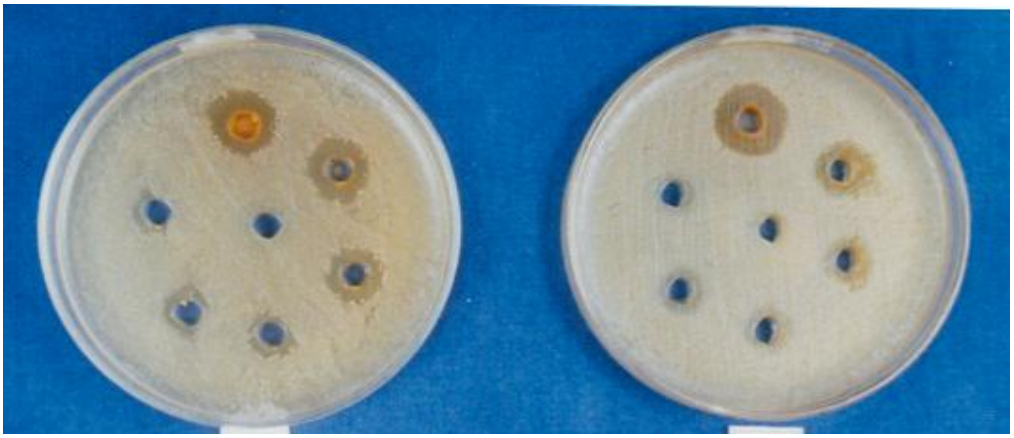
Table 1: Antibacterial activity of *Boswellia serrata* extracts by well plate method.

Bacteria	***Zone of inhibition in mm***							
	P. ether (0.1 gm/ml)		**Acetone (0.1 gm/ml)**		**Methanol (0.1 gm/ml)**		**Aqueous (0.5 gm/ml)**	
	Leaves	**Bark**	**Leaves**	**Bark**	**Leaves**	**Bark**	**Leaves**	**Bark**
S. aureus (ATCC 6538)	00	00	18	12	17.5	13	9	10
B. cereus (NCIM 2322)	00	00	16	14.5	15	12.5	9	10
P. vulgaris (MTCC 742)	00	00	18.6	15	15	15	10.5	11
P.aeruginosa (NCIM 2200)	00	00	18	16	17	18	10	8
E. coli (NCIM 2931)	00	00	8 (zs)	8 (zs)	8 (zs)	8 (zs)	00	00
S.typhimurium (NCIM 2501)	00	00	9 (zs)	8	8 (zs)	8.3	12	7.5
K. pneumoniae (MTCC 432)	00	00	13	13(zs)	17	12(zs)	00	8 (zs)

(zs = zone of suppression)

Patel had studied the antibacterial activity of the extracts of gum exudates of this plant against UTI pathogens *P. aeruginosa, K. pneumonia, E. coli* and *P. vulgaris* and have reported significant activity against all the test bacteria in two or more extracts including *E. coli*.[23] In his studies the acetone and petroleum ether extracts were found to be active against *K. pneumonia*. In present investigations, zone of inhibition was not detected against *E. coli* NCIM (2931) and *K. pneumoniae* MTCC (432) by acetone extracts and no antibacterial action was detected with petroleum ether extract. These differences in the two reports could be most likely due to different plant materials used in the studies. Similar differences were also noted with the reports of Shaikh Mannur *et al.*, regarding aqueous extracts.[15] Their reports indicate strong antibacterial activity in *B. serrata* aqueous extracts against test cultures of *E. coli* and *K. pneumoniae*. The differences might be due to their extraction procedure (Soxhlet Extraction) and the material used in their work (Frankinsence). These findings strongly suggest that frankincense, the dried and concentrated form of essential oils of the plant, appear to be far more enriched with the antibacterial compounds as compared to bark and leaves.

Relative MISCs of *Boswellia serrata* leaves and bark extracts were evaluated against four test bacteria to compare the degree of their antibacterial action (Table-2). MICs of methanol extract of leaves and bark and acetone extracts of leaves were lower for the two Gram positive test bacteria as compared to the Gram negative test bacteria. Higher MIC values were recorded for *Proteus vulgaris* and *Pseudomonas aeruginosa* suggesting their lesser sensitivity to the extracts.



Bark extract **Leaves extract**

Fig. 3: Antimicrobial assays of *Boswellia serrata* extract against *Basillus cereus.*

Table 2: Relative MICs of *Boswellia serrata* extracts (mg/ml).

Bacteria	**Acetone extract**		**Methanol extract**	
	Leaves	**Bark**	**Leaves**	**Bark**
S.aureus	3.12	6.25	3.12	12.5
B.cereus	6.25	3.12	6.25	6.25
P.vulgaris	12.5	6.25	12.5	12.5
P.aeruginosa	12.5	6.25	12.5	12.5

Acetone extract of *Boswellia serrata* leaves showed strongest inhibitory action against *Microsporum gypseum* (Table-3, Fig-4). Significant antifungal activity was also present in its methanol extract of leaves and acetone extract of bark against the same organism. Acetone and methanol extracts of leaves were also active against *C. neoformans*. However no antifungal activity against *Candida albicans* was detected in any of the extracts of this plant. Petroleum ether extract were inactive against all the three test fungi.

Leaves extract appeared to possess more antifungal potential against two of the three test fungi as compared to the bark extracts. Test culture of *M. gypseum* was more sensitive to these extracts than the test culture of *Cryptococcus neoformans*. Test culture of *Candida albicans* was insensitive to the inhibitoty action of the extracts. *M. gypseum* had more sensitivity also to standard antifungal drugs Amphotericin B, Clotramizole and Nystatin as compared to the other two test fungi (Table 3 and 4).

Table 3: Antifungal activity of *Boswellia serrata* extracts by disc plate method.

Fungi	**Zone of inhibition in mm**					
	P. ether (0.1 gm/ml)		**Acetone (0.1 gm/ml)**		**Methanol (0.1 gm/ml)**	
	Leaves	**Bark**	**Leaves**	**Bark**	**Leaves**	**Bark**
M.gypseum (MTCC 2829)	00	00	19	14	13.5	00
C.albicans (NCIM 3100)	00	00	00	00	00	00
C.neoformans (Clinical isolate)	08	00	10	00	13	10 (zs)

(zs = zone of supression)

Table 4: Sensitivity of test fungi to standard antifungal drugs.

Antifungal Drugs	**Zone of inhibition in mm**		
	***C. albicans* NCIM 3100**	***C. neoformans* (clinical isolate)**	***M. gypseum* MTCC 2829**
Amphortecin B (100 U/disc)	13.75	16.5	24.0
Clotramizole (10 mcg/disc)	12.5	13.0	21.5
Nystatin (100 U/disc)	18.0	21.5	30.0

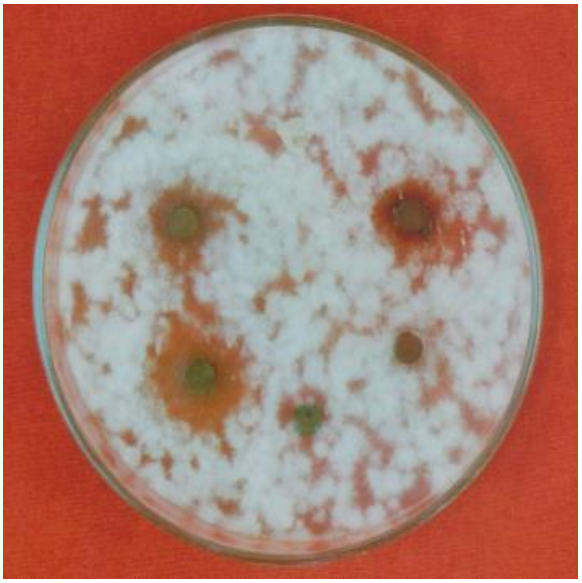


Fig. 4: Antifungal activity of *Boswellia serrata* extracts against *Microsporum gypseum*.

Saddhasivam *et al*., had reported antimycotic activity of essential oil of *B. serrata* against *Trichophyton* and *Candida albicans* and had found more sensitivity of the former than the latter.[24] Result of the present investigation also indicate the antimycotic potential of aceton and methanol extract of *B. Serrata* leaves and acetone extract of bark against *M. gypseum*, a dermatophyte. However these extracts were ineffective against the test strain of *C. albicans*. Mohammadi Rasoul *et al.* had reported inhibitory action of essential oil of the plant on *C. albicans* isolates.[25] Our findings suggested that the acetone and methanol extracts of *B. serrata* did not appear to possess the components inhibitory to *Candida albicans*. Present findings are in agreement with finding of Hassan *et al*., regarding the absence of antimycotic activity in methanol extract of the plant against *C. albicans.*[26]

CONCLUSIONS

Acetone, methanol and aqueous extracts of *B. serrata* have shown antibacterial and antifungal activities against one or more test cultures. The leaves extracts appeared to possess more antimicrobial potential than the bark extract. Growth of *Microsporum gypseum* MTCC 2829 was inhibited by the extracts. This supports the ancient knowledge of Ayurveda which states the use of this plant for skin diseases. Intensive future studies may lead to antimicrobial products for topical application and use of *B.serrata* derived antimicrobial compounds for treatment of infectious diseases.

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