ANTIBACTERIAL AND ANTIFUNGAL ACTIVITIES OF BOSWELLIA OVALIFOLIOLATA STEM BARK

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Abstract : This study was carried out with an objective to investigate the antibacterial and antifungal potentials of Stem bark of *Boswellia ovalifoliolata*. The aim of the study is to assess the antimicrobial activity and to determine the zone of inhibition of extracts on some bacterial and fungal strains. In the present study, the microbial activity of ethanolic extracts of stem bark of *Boswellia ovalifoliolata* was evaluated for potential antimicrobial activity against medically important bacterial and fungal strains. The antimicrobial activity was determined in the extracts using agar disc diffusion method. The antibacterial and antifungal activities of extracts (50, 100, 150 and 250 µg/ml) of *Boswellia ovalifoliolata* were tested against two Gram-positive— *Staphylococcus aureus, Bacillus subtilis* two Gram-negative— *Pseudomonas aerigunosa, E.coli*. bacteria. Two fungal strains— Aspergillus *Niger* and *Aspergillus oryzae*. Zone of inhibition of extracts were compared for antibacterial activity and nystatin and griseofulvin for antifungal activity. The results showed that the remarkable inhibition of the bacterial growth was shown against the tested organisms. The phytochemical analyses of the plants were carried out. The microbial activity of the *Boswellia ovalifoliolata* was due to the presence of various secondary metabolites. Hence, these plants can be used to discover bioactive natural products that may serve as leads in the development of new pharmaceuticals research activities**.**

Index Terms **- Boswellia ovalifoliolata, *in vitro* antibacterial activity, antifungal activity, secondary metabolites .**

I. INTRODUCTION

Antibiotics are important weapons in fighting bacterial infections and have vastly improved the quality of human life since their introduction. However, over the past few decades, these health benefits are under threat as many commonly used antibiotics have become less and less effective against certain illnesses, not only because many of them produce toxic element. Hence it is essential to investigate new drugs. Drugs derived from natural sources play a significant role in the prevention and treatment of human diseases. In many developing countries, traditional medicine is one of the primary healthcare systems.[1,2] Herbs are widely used in the traditional medicine and their curative potentials are well documented.[3] About 61% of new drugs developed between 1981 and 2002 were based on natural products and they have been very successful especially in the areas of infectious disease and controlling cancer.[4] Recent trends, however, show that the discovery rate of active novel chemical entities is declining.[5] Natural products of higher plants may give a new source of antimicrobial agents with possibly novel mechanisms of action.[6,7] The effects of plant extracts on bacteria have been studied by a very large number of researchers in different parts of the world.[8] Much work has been done on ethnomedicinal plants in India. [9] Plants are rich in a wide variety of secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids, glycosides, etc., which have been found *in vitro* to have antimicrobial properties. [10,11] Herbal medicines have been known to man for centuries. Therapeutic efficacy of many indigenous plants for several disorders has been described by practitioners of traditional medicine. [12] Antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world. The World Health Organization estimates that plant extracts or their active constituents are used as folklore endemic medicine in traditional therapies of 80% of the world's population. [13] The harmful microorganisms can be controlled with drugs and these results in the emergence of multiple drug-resistant bacteria and it has created alarming clinical situations in the treatment of infections. The pharmacological industries have produced a number of new antibiotics resistance to these drugs by microorganisms has increased. In general, bacteria have the genetic ability to transmit and acquire resistance to synthetic drugs which are utilized as therapeutic agents. [14] In an effort to expand the antibacterial agents from natural resources. In the present study *Boswellia ovalifoliolata* belonging to Burseraceae family has been selected. In the Indian literature, [15,16] reported to be useful against inflammations, joint pains, ulcers, arthritis, amoebic dysentery and diabetes has been suggested [17] and it is widely used by tribal people to treat various ailments including ringworm and other fungal skin infections.[18] *Boswellia ovalifoliolata* extract are known to be an important source of secondary metabolites, Indian people are using the leaves to treat inflammation *Boswellia ovalifoliolata* plant organs are notably phenolic compounds. [19] *Boswellia ovalifoliolata* exhibited significant antimicrobial activity and showed properties that was mentioned in folkloric as broad-spectrum antimicrobial agents. [20] Thus, *Boswellia ovalifoliolata* is well anchored in its traditional uses has now found wide-spread acceptance across the world. In the current investigation carried out, a screening of ethanolic extracts of

Boswellia ovalifoliolata stem bark against pathogenic bacteria and fungi is done in order to detect new sources of antimicrobial agents.

II. MATERIALS AND METHODS

2.1.Collection of plant materials

The fresh and healthy stem bark of the plant *Boswellia ovalifoliolata* were collected from Tirumala hills, near Thalakona chittoor district of Andhra Pradesh state India , and authentified by Dr. K. Madhava Chetty, Department of Botany S.V. University, Tirupati, and Andhra Pradesh, India. Voucher Specimen No. 1295 is kept for future reference at S.V. University, Andhra Pradesh, India.

2.2. Preparation of plant extract

Extraction

The extraction of the *Boswellia ovalifoliolata* stem bark was carried out using known standard procedures.[21] The plant materials were dried in shade and powdered in a mechanical grinder. The powdered plant materials were initially defatted with petroleum ether(60-80°C) followed by adding 900 ml of ethanol and extracting by Soxhlet extractor for 72 hours at a temperature not exceeding the boiling point of the solvent. The extracts were filtered using Whatman filter paper (No.1) while hot, concentrated in vacuum under reduced pressure using rotary flask evaporator, and dried in a desiccators. The yield was found to 5.750 g (23.0% w/w). More yields of extracts were collected by this method of extractions. The extracts stored in air tight container for further studies. The dry weight of the plant extracts was obtained by the solvent evaporation and used to determine concentration in mg/ml.

2.3. Preliminary phytochemical screening

The extracts were subjected to preliminary phytochemical testing to detect the presence of different chemical groups such as saponins, tannins, alkaloids, flavonoids, triterpenoids, steroids, glycosides, saponins, gum, mucilage, carbohydrates, reducing sugars, starch, protein and amino acids.

2.4. Test microorganisms and growth media

The following microorganisms *Staphylococcus aureus, Bacillus subtilis, Pseudomonas aerigunosa, Escherichia.coli*. and fungal strains *Aspergillus niger, Aspergillus oryzae, Candida albicans* were chosen based on their clinical and pharmacological importance. [23] The bacterial strains obtained from Asthagiri herbal research foundation Chennai, were used for evaluating antimicrobial activity. The bacterial and fungal stock cultures were incubated for 24 hours at 37 $\pm$ 00.5^{0}C for 24hrwhile the petridishes on nutrient agar and potato dextrose agar (PDA) medium (Microcare laboratory, Surat, India), respectively, following refrigeration storage at 4°C. The bacterial strains were grown in Mueller-Hinton agar (MHA) plates at 37°C (the bacteria were grown in the nutrient broth at 37°C and maintained on nutrient agar slants at 4°C), whereas the yeasts and molds were grown in Sabouraud dextrose agar and PDA media, respectively, at 28°C. The stock cultures were maintained at 4°C.

2.5. Antimicrobial Activity- Determination of zone of inhibition method

In vitro antibacterial and antifungal activities were examined for Ethanolic extracts. Antibacterial and antifungal activities of plant part extracts against four pathogenic bacteria (two Gram-positive and negative) and three pathogenic fungi were investigated by the well diffusion method. [24] Antimicrobial activity testing was carried out by using well diffusion method. Each purified extracts were dissolved in dimethyl sulfoxide, sterilized by filtration using sintered glass filter, and stored at 4°C. For the determination of zone of inhibition, pure Gram-positive, Gram-negative, and fungal strains were taken as a standard antibiotic for comparison of the results. The extracts were screened for their antibacterial and antifungal activities against the *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus pyogenes* and the fungi *Candida albicans, Aspergillus niger*, and *Aspergillus clavatus*. The sets of five dilutions (5, 25, 50, 100, and 250 µg/ml) of *Boswellia ovalifoliolata* extract and standard drugs were prepared in double-distilled water using nutrient agar tubes. Mueller-Hinton sterile agar plates were seeded with indicator bacterial strains (10^8 cfu) and allowed to stay at 37°C for 3 hours. Control experiments were carried out under similar condition by using ampicillin, chloramphenicol, ciprofloxacin, and norfloxacin for antibacterial activity and nystatin and griseofulvin for antifungal activity as standard drugs. The zones of growth inhibition around the disks were measured after 18 to 24 hours of in incubation at 37°C for bacteria and 48 to 96 hours for fungi at 28°C. The sensitivities of the microorganism species

to the plant extracts were determined by measuring the sizes of inhibitory zones (including the diameter of disk) on the agar surface around the disks, and values <8 mm were considered as not active against microorganisms.

III. RESULTS AND DISCUSSION

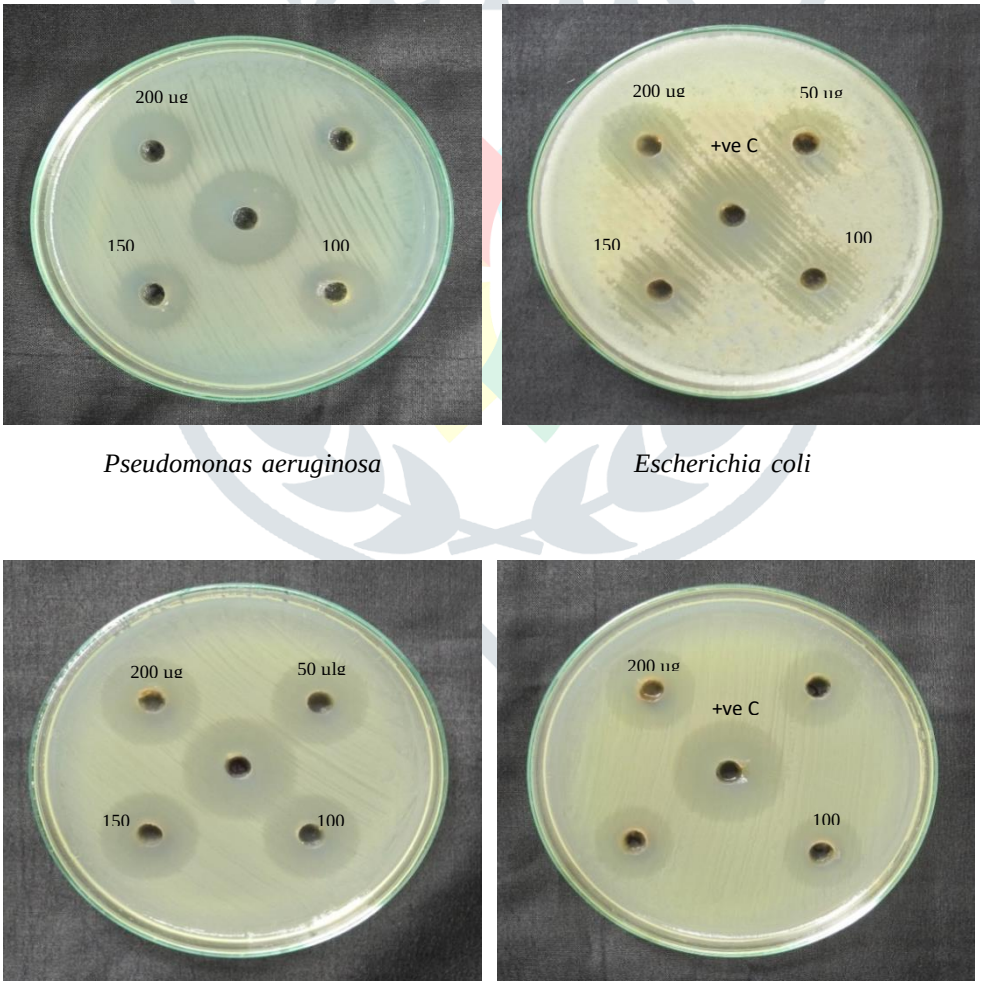
3.1. Preliminary phytochemical screening

It was found that ethanalic extracts of *Boswellia ovalifoliolata* stem bark contained tannins, flavonoids, saponins, triterpenoids, steroids, glycosides.

3.2. Microbial activity

The antimicrobial activity of the extracts of *Boswellia ovalifoliolata* were studied in different concentrations (50, 100, 150 and 250 µg/ml) against four pathogenic bacterial strains, two Gram-positive (*Staphylococcus aureus* , *Bacillus subtilis*) and two Gram-negative (*Escherichia coli* , *Pseudomonas aerigunosa*), and three fungal strains (*Aspergillus niger* and *Aspergillus oryzae*). These strains have been selected for the basis of its application purpose of further formulation study. Antibacterial and antifungal potential of extracts were assessed in terms of zone of inhibition of bacterial growth. The results of the antibacterial and antifungal activities are presented.

The results show that the ethanalic extracts of *Boswellia ovalifoliolata* were found to be more effective against all the microbes tested.



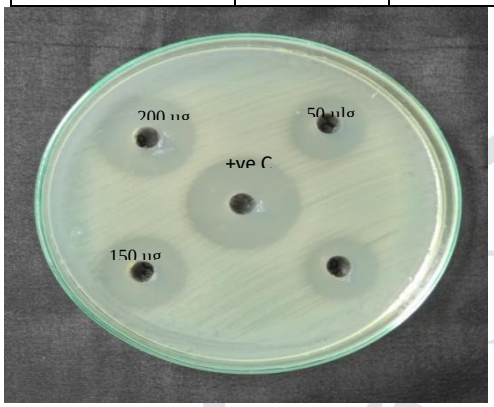
Pseudomonas aeruginosa *Escherichia coli*

Bacillus subtilis *Staphylococcus aureus*

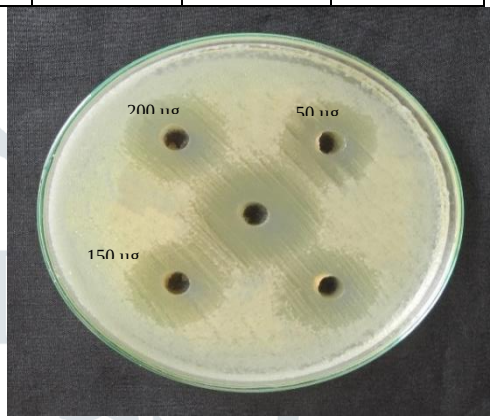
Figure 1. antimicrobial study of bo-1

Table 1. antimicrobial study of bo-1

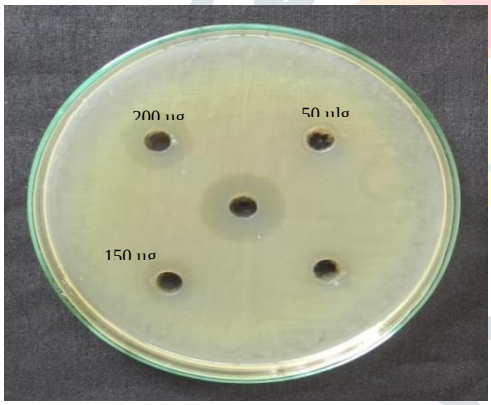
Organisms	Zone inhibition (mm in diameter)				
	50 µg/mL	100 µg/mL	150 µg/mL	200 µg/mL	20µg/mL Positive Control
P. aeruginosa	11	13	17	19	23
E. coli	18	20	21	23	26
S. aureus	15	17	18	21	23
B. subtilis	20	21	23	24	26



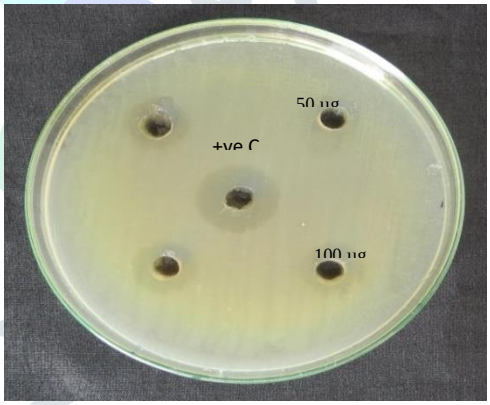
Pseudomonas aeruginosa



Escherichia coli



Bacillus subtilis



Staphylococcus aureus

Figure 2 antimicrobial study of bo-ii

Table 2. Antimicrobial study of BO-II

Organisms	Zone inhibition (mm in diameter)				
	50 µg/mL	100 µg/mL	150 µg/mL	200 µg/mL	20µg/mL Positive Control
P. aeruginosa	16	19	21	23	25
E. coli	20	21	22	25	27
S. aureus	08	10	11	15	19

B. subtilis	04	09	13	16	20

The ethanol extracts of *Boswellia ovalifoliolata* was subjected to antimicrobial activity in dose dependent manner against *B.subtili, E.Coli, Sacharomyces aureus* and *Pseuddomonas aeruginosa* of the species by well diffusion method for each isolated compounds of these plants and incubated for 24 hours for their growth.

After incubation, the plates were observed to identify the zone of inhibition. The zone of inhibition of each isolated compound and the standard were of different concentrations was recorded with the help of zone measuring scale (Hi-media). Zone of inhibition in different organisms were shown in the Table No.1.

The anti-microbial activity was observed is dose dependent manner when compound ethanalic extract positive and control. All these isolated compounds are having anti-microbial properties.

Antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world. The World Health Organization estimates that plant extract or their active constituents are used as folk medicine in traditional therapies of 80% of the World's population. In the present work, the extracts obtained from *Boswellia ovalifoliolata* show strong activity against most of the tested bacterial and fungal strains. The results were compared with standard antibiotic drugs. In this screening work, extracts of *Boswellia ovalifoliolata* were found to be active against any organism. Such as gram-positive, gram-negative, and fungal strains.

The above results show that the activity of ethanolic extracts of *Boswellia ovalifoliolata* shows significant antibacterial and antifungal activities. This study also shows the presence of different phytochemicals with biological activity. The result of phytochemicals in the present investigation showed that the plant contains more or less same components like saponin, triterpenoids, steroids, glycosides, flavonoids. It is also shown that the plant is rich in tannin and phenolic compounds and posses antimicrobial activities against a number of microorganisms. It gives a voluble importance in therapeutic index.

IV. CONCLUSION

In the current investigation, the ethanolic extract has been selected for the study of such a selected plant, Ethanolic extract gave higher yield of chemical constituents expected for this research work. The importance of this work is that very good results are obtained and it will be helpful to carry out other data with MIC and other formulation study. Ethanolic extract is more suitable for clinical study. The ethanolic extracts of *Boswellia ovalifoliolata* were found to be active on most of the clinically isolated microorganism and fungi, as compared with standard drugs. The present study justified the claimed uses of stem bark in the traditional system of medicine to treat various infectious disease caused by the microbes. However, further studies are needed to evaluate the potential effectiveness of the crude extracts as the antimicrobial agents. The present results will form the basis for selection of plant species for further investigation in the potential discovery of new natural bioactive compounds. Further studies which aimed at the isolation and structure elucidation of antibacterial active constituents from the plant have been initiated.

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