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ANTI BACTERIAL ACTIVITY OF STEM BARK OF ETHANOL EXTRACT OF *BOSWELLIA OVALIFOLIOLATA*

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ABSTRACT

Plants produce a wide variety of phyto chemical constituents, which are secondary metabolites and are used either directly or indirectly in pharmaceutical industry. Medicinal plants play an important role in health care. There are number of plants used for treating diseases which are not comprehensively documented due to a lack of communication and low frequency of their use. Such a group of medicinal plants are some endemic plants of Tirumala -Tirupati. These plants have medicinal properties and are used in herbal therapy to cure a wide range of health problems. There is a need to bridge the gap for data access on these plants to the scientific community. Very small amount of work has been down on these plants and a lot is to be done. We select endemic medicinal plants of Tirupati flora *boswellia ovalifoliolata*, member of a burseraceae family and grow in subtropical temperature. This study was intended to evaluate the anti-bacterial activity ethonol extracts of *boswellia ovalifoliolata* stem bark in gram positive and gram negative bacteria. ethnol extracts exhibited significant antibacterial activity, which supports the traditional medicinal utilization of the plant. This study established anti-bacterial activity of Stem bark of *Boswellia ovalifoliolata*.

INTRODUCTION:

Boswellia ovalifoliolata is a narrow endemic endangered and threatened medicinal tree species. It is deciduous medium sized tree belongs to the family Burseraceae. This tree harbours on Tirumala hills of seshalam hill range of Eastern Ghats of India. [1,2,3]. The plant used by tribal's like Nakkla, sugali and chanchu and indigenous community to treat number of aliments The plant is over exploited for its medicinal uses. Especially the stem bark are used to reduce pain inflammation. The leaf decoction is used as antibacterial, antiulcer, and anti-rheumatoid.[4]

MATERIALS AND METHOD:

Plant materials:

The fully mature B*oswellia ovalifoliolata* (BO) Stm bark were collected in June-July 2014 fromfoot slops of Tirumala hills forest chittoor district Andhra Pradesh state India. Stem bark were identified and authenticated by Botany department, SV University, Tirupathi. Ethnol was procured from SD fine chemicals, Bombay, anti biotic chlorampenicol procured from Casven Pharma Limited, Hyderabad.

Preparation of extracts

The B*oswellia ovalifoliolata* Stem bark were first washed several times with distilled water. The Stem bark were dried at room temperature and coarsely powdered by using mechanical grinder. The powder was successively extracted with Ethonol using cold Soxhlet apparatus method.[5,6,7] The percentage yields were 2.3% in ethanol . The extract was stored in air tight container for further studies.

Phyto-chemical Studies

The following qualitative phytochemical screening was carried out for stem bark extract of BO ie. Triterpeniods, saponins and tannins, steroids and alkaloids. Phyto-chemical Studies were carried out for qualitative procedures as described by Trease and Evans [8].

a) Test for alkaloids

1 ml of 1% HCL was added to the 3ml of extract in a test tube. Then it was treated with a few drops of Meyer`s reagent .A creamy white precipitate indicated the presence of alkaloids.

b) Test for flavonoids

A few drops of 1%NH3 solution was added to the 2ml of extract in a test tube. A yellow coloring was observed for the presence of flavonoids.

c) Test for terpenoids

5ml of extract was mixed with 2ml of CHCl3 in a test tube .3ml of concentrated H2SO4 was carefully added along with the wall of the test tube to form a layer. An interface with a reddish brown coloration indicated presence of terpinoids.

d) Test for Steroids

0.5ml of extract was treated with 0.5 ml of acetic anhydride and 0.5 ml of chloroform. Then concentrated H2SO4 was added slowly. Bluish green color was observed for steroids.

e) Test for Tannins/phenols

Gelatin test: The test solution was evaporated to dryness and the resulted residue was dissolved in 1% liquefied gelatin. To this 10%Nacl solution was added. A white precipitate was obtained indicated presence of Tannins. [11,12]

Microbiological Evaluation

Antimicrobial agents play an important role in treatment of many infectious diseases. However repeated use of some antibiotics results in resistance. Drug resistance happens when microbes developed way to survive against drugs meant to kill or weaken them. If a microbe is resistant to many drugs, then treating the infection can become more difficult or impossible.[13] The development of resistant to an antimicrobial is complex. Susceptible bacteria can become resistant by acquiring resistant gene from other bacteria or through mutations in their own genetic material (DNA).[14] Once acquired, the resistance characteristic is passed on to future generations and sometimes to other bacterial species.

Environmental factors

In order to ensure the therapeutic efficacy of a substance, one should determine the susceptibility of the microorganisms to that substance.[15] In vitro tests like cup-plate method or the paper disc plate method or tube dilution technique used for this purpose. The commonly used technique for determining the susceptibility of microorganisms is the cup plate method or disc plate technique, in which zone of inhibition surrounding each cup/disc, placed with the substance on an inoculated surface of an agar medium measured. By cup plate method, either single or multiple cups containing varying concentrations of sample are used. In disc plate method paper discs impregnated with different concentrations of samples are placed on an inoculated surface of agar medium. Method of interpretation is based on the measurement of inhibition zone diameter produced around cup/disc. This method is satisfactory for most common pathogenic bacteria and fungi. By tube dilution method one can determine the smallest amount of antimicrobial agent required to inhibit the growth of organism referred to as minimum inhibitory concentration (MIC).

Procedure

a. Cup-plate method

Cup-plate method was used for the evaluation of microbial sensitivity of the sample. Circular cups were filled with specific amount of test compound in suitable agar medium, which was inoculated with the test organism. After incubation the Petri dishes were observed for growth inhibition zone around the cups. The diameter of zone of inhibition is directly proportional to antimicrobial activity of the compound. The diameter of zone of inhibition was compared with that of standard antibiotic Tetracycline.

b. Minimum Inhibitory Concentration (MIC)

Minimum Inhibitory Concentration of the synthesized compound was determined by tube dilution techniques. Serial dilution of the substance under examination was placed into culture tubes containing suitable medium and inoculated with the test organism. After incubation, the minimum concentration of test compound that inhibited the growth of the organism was observed. The following microorganisms were used to study the antimicrobial activity. Test organisms (bacteria) Type:

Staphylococcus aureus - strain number: 2602
Streptococcus griseus - strain number: 2183
Bacillus subtilis - strain number: 2480s
Escherichia coli - strain number: 2981

Antibacterial Activity Studies

Cup Plate Method

Preparation of standard and test solutions

10 mg of Chloramphenicol is dissolved in water and made up to 100 ml. 100µl of above stock solution was used that contains 10µg of standard (chloramphenicol) 5 mg of extracts and isolated compound were separately dissolved in 5 ml of dimethyl formamide DMF (stock solution). From the stock solution, 1.5 ml was taken aseptically and made up to 10 ml which give a concentration of 150 µg/ml. 1 ml of the solution containing 150µg of the sample was transferred into the cups in the petri plate. Solution of the aqueous extract also prepared in similar way in distilled water. Ethonol extract were also tested for antimicrobial activity.

Preparation of Muller-Hinton agar

The composition of Muller-Hinton agar medium is: Beef infusion 300 ml; Casein hydrolase 17.5 g; Starch 1.5 g; Agar 17 g; Distilled water 1000 ml; Composition of Muller-Hinton agar medium (pH - 5.6 ± 0.2). The medium was prepared by dissolving the specified quantities of the dehydrated medium (Hi-media) in purified water by heating on water bath and dispensed in 20 ml test tubes. The test tubes were closed with cotton plugs and were sterilized by autoclaving at 121° C (15 lb psig) for 15 minutes. The contents of the test tubes were poured into sterile Petri dishes under aseptic conditions and allowed to solidify.

Procedure:

Each Petri dish was inoculated with one of the bacterial cultures suitably diluted to contain above 106cells/ml by spreading 0.1ml suspension of the organism with a sterile cotton swab. In each plate cups of 6 mm diameter were made at equal distances using sterile cork borer. One cup was filled with 0.1 ml of standard drug i.e. tetracycline solution, one was filled with 0.1 ml of DMF; others were filled with 0.1 ml of samples in sterile DMF. All the plates were kept in the refrigerator for 30 minutes to allow the diffusion of sample in to the surrounding agar medium. The Petri dishes were incubated at 37° C for 24 hours. Diameter of the zone of inhibition was measured and the average diameter for each sample was calculated.[16] The diameter obtained for the test samples were compared with that of the diameter produced by standard chloramphenicol . The diameter of zone of inhibition is proportional to the antibacterial activity of the substance.

Determination of MIC by tube dilution technique

Preparation of nutrient broth

The composition of the media is: Ingredients Quantity Beef extract 10 gm, Peptone 10 gm, NaCl 05 gm, Purified water 1000 ml. *Composition of nutrient broth* (pH 7.2 ± 0.2) The media was prepared by dissolving the specified quantities of dehydrated medium (Hi-media) in purified water. The medium was distributed in 4 ml quantities in test tubes. The test tubes were closed with cotton plugs and sterilized by autoclaving at 121ºC (15 lbs psig) for 15 minutes.

Procedure

5 mg of the extracts were dissolved in 5 ml of DMF that gave 1000 µg/ml concentration. From the above solution 1 ml was transferred to a test tube containing 1 ml of nutrient broth and the resultant concentration was half of the previous one. From the above test tube 1 ml was taken and transferred to another test tube containing 1 ml of nutrient broth. This was repeated up to six dilutions. 1 ml was discarded from the last test tube. The tubes were mixed well after each addition. The test tubes were closed with cotton plugs. Aseptic conditions were maintained throughout the process of sample transfer to the test tubes.

Table 1: Results of Preliminary Qualitative Phyto-chemical Studies of *boswellia ovalifoliolata* Leaves

S.no	Test	Ethnolic extract of BO
1	Alkaloids	+
2	Steroids	+
3	Triterpenoids	+
4	Flavonoids	+
5	Tannins/phenols	+

Table.2: Antimicrobial activity of *Boswellia ovalifoliolata* Stem bark extracts on selected bacteria

Test Organism	Strain Number	Zone Of Inhibition	
		Antibiotic	Extract
Streptococcus Griseus	2183	16.4mm	11.2mm
Staphylococcus	2602	16.6mm	10.1mm
Bacillus subtilis	2480	19.2mm	09.1mm
E-coli	2981	17.8mm	14mm

A positive control and negative control were also prepared to confirm the nutritive and sterility properties of the prepared medium respectively. All the tubes were incubated at 37ºC for 24 hours. The presence or absence of growth of organisms was observed and minimum inhibitory concentration of the isolated compounds against each compound.

RESULT AND DISCUSSION

The crude stem bark of *boswellia ovalifoliolata* Ethonolic extracts, were studied for antibacterial activity by cup plate method. The fractions and pure compounds exhibited potential inhibitory activity against gram positive and gram negative bacteria; This may be due to the presence of tannins phenols and triterpenoids in the extracts of *boswellia ovalifoliolata* stem bark

CONCLUSION

It was concluded that the results of the present study support to the traditional use of *boswellia ovalifoliolata* in antibacterial activity. *Boswelliaovalifoliolata*, possessing significant antibacterial activity. This may be due to the presence of tannins, triterpenoidss, which deserves further studies to establish its therapeutic value as well as its mechanism of action.

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