

# Studies on Antibacterial Activity of *Boswellia Ovalifoliolata* and *Syzygium Alternifolium* Against *Bacillus Subtilis* and *Pseudomonas Auriginosa*

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**Abstract:** - Medicinal plants have been used for centuries as remedies for human diseases because they contain components of therapeutic value. The two selected medicinal plants exhibited significant antibacterial activity on pathogenic bacterial strains. Methanol stem extract of *Boswellia ovalifoliolata* and *Syzygium alternifolium* showed potent antibacterial activity on *P. auriginosa* and *B. subtilis*.

**Keywords:** *Boswellia ovalifoliolata*, *Syzygium alternifolium*, *Pseudomonas auriginosa*, *Bacillus subtilis*.

## I. INTRODUCTION

Recently, the acceptance of traditional medicine as an alternative form of health care and the development of microbial resistance to the available antibiotics has led authors to investigate the antimicrobial activity of medicinal plants (Maoz and Neeman 1998; Hammer *et al.* 1999). During the last ten years the pace of development of new antimicrobial drugs has slowed down while the prevalence of resistance has increased astronomically (Hugo and Russell, 1984). In addition to this problem, antibiotics are sometimes associated with adverse effects on the host, including hypersensitivity, immunosuppression and allergic reactions (Ahmad *et al.* 1998). This situation forced scientists to search for new antimicrobial substances. Given the alarming incidence of antibiotic resistance in bacteria of medical importance (Monroe and Polk, 2000), there is a constant need for new and effective therapeutic agents (Bhavnani and Ballow, 2000). Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases from medicinal plants (Cordell, 2000).

Literature reports and ethnobotanical records suggest that plants are sleeping giants of pharmaceutical industry (Hostettmann and Hamburger, 1991). They may provide natural source of antimicrobial drugs that will provide novel compounds that may be employed in controlling some infection globally. According to World Health Organization medicinal plants would be the best source to obtain a variety of drugs. About 80% of individuals from developed countries

use traditional medicine, which has compounds derived from medicinal plants.

The use of plant extracts and phytochemicals, both with known antimicrobial properties, can be of great significance in therapeutic treatments. In the last few years, a number of studies have been conducted in different countries to prove such efficiency (Ikram and Inamul, 1984). The beneficial medicinal effects of plant materials typically result from the combinations of secondary products present in the plant. In plants, these compounds are mostly secondary metabolites such as alkaloids, steroids, tannins, and phenol compounds, which are synthesized and deposited in specific parts or in all parts of the plant. These compounds are more complex and specific and are found in certain taxa such as family, genus and species, but heterogeneity of secondary compounds is found in wild species (Balandrin *et al.* 1985). The medicinal actions of plants are unique to a particular plant species or group, consistent with the concept that the combination of secondary products in a particular plant is taxonomically distinct (Wink, 1999).

The plant's secondary products may exert their action by resembling endogenous metabolites, hormones, signal transduction molecules or neurotransmitters and thus have beneficial medicinal effects on humans due to similarities in their potential target sites. The presence of antimicrobial substances in the higher plants provide a source of inspiration for novel drug compounds as plants derived medicines have made significant contribution towards human health. The antimicrobial compounds from plants may inhibit microbial growth by different mechanism than those presently used antimicrobials and may have a significant clinical value in treatment of resistant microbial strains. Therefore, random screening of plants for active chemicals is as important as the screening of ethnobotanically targeted species (Principle, 1989).

## II MATERIALS AND METHODS

**Collection of medicinal plants:** Selected medicinal plants, *Boswellia ovalifoliolata* and *Syzgium alternifolium*, have been used in the present study and they were collected from Veligonda hills, Nellore district, Andhra Pradesh, India.

**Bacterial Cultures:** The bacterial cultures were procured from the Department of Microbiology, Sri Venkateswara Institute of medical Sciences (SVIMS), Tirupati. Pathogenic Gram positive bacteria such as *Bacillus subtilis*, and Gram negative bacteria *Pseudomonas aeruginosa*, were maintained on nutrient agar slants at 4°C until further used for experimental studies.

***Bacillus subtilis* :** *Bacillus subtilis* was a gram positive, rod shaped, endospore forming catalase-positive bacterium commonly found in soil (Madigan and Mortincko, 2005). *Bacillus subtilis* contaminate food and causes food poisoning (Ryan and Ray, 2004). It causes conjunctivitis followed by iridocheroiditis (Green wood *et al.*, 1997).

***Pseudomonas auriginosa* :** *Pseudomonas auriginosa* is a gram negative rod shaped, non-encapsulated, aerobic and non-spore forming bacterium with unipolar motility occurring as an opportunistic pathogen of plants and human, which typically infects the pulmonary tract, burns, wounds and causes blood infections. The most common cause of burn infections was *P.auriginosa* and post-operative infection in radical keratotomy surgery patients.

### 1. PREPARATION OF THE MEDIUM:

#### *Nutrient Agar media (pH 7.0)*

For the preparation of 1 litre of Nutrient Agar media ingredients like 1.5 g of beef extract, 1.5 g of yeast extract, 5 g of peptic digest of animal tissue and 5 g of sodium chloride were weighed and added in 500 ml of distilled water and heated with agitation to dissolve the constituents. Finally, the volumes were made up to 1 liter. Before the addition of agar (15 g) the pH of the medium was adjusted to 7.0 by adding few drops of 0.1 N NaOH or HCl using digital pH meter (Elico Pvt. Ltd., Hyderabad). These were then sterilized by autoclaving at 15 lbs pressure at 1200 C for 15 min; cooled to 400 C and approximately 20 ml of medium was poured to each 90mm sterilized petridish.

### 2. *Preparation of inoculum:*

**Bacteria:** 18 hrs old bacterial broth cultures were used as inocula after adjusting its population to 10<sup>6</sup> CFU/ ml (Colony Forming Units) using 0.9% (w/v) sterile saline by the method described by Forbes *et al.*, (1990).

### 3. *Preparation of plant extract:*

The methanolic extracts of the four selected medicinal plants were prepared by dissolving 10gm of fine powder of each medicinal plant separately in 100 ml of methanol solvent. The contents were kept in arbitrary shaker for 48 h. Then the extract was filtered and it is dried in hot air oven at 40°C. Then the extract was stored under refrigeration at 4°C for further studies.

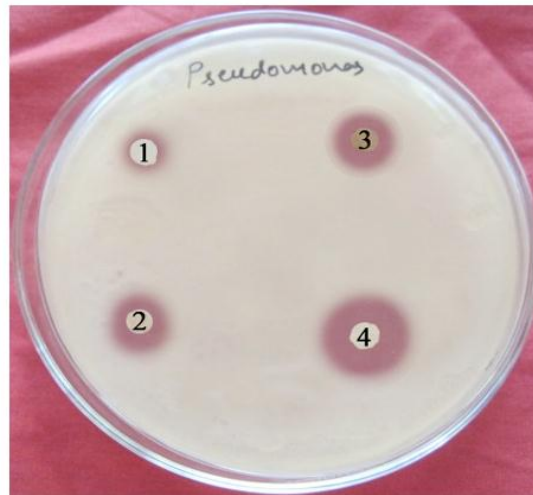
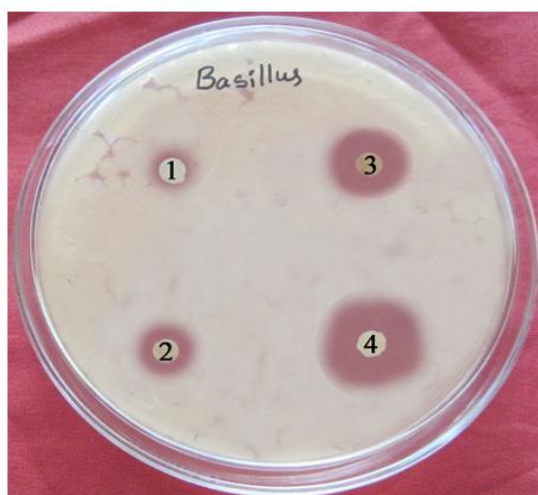
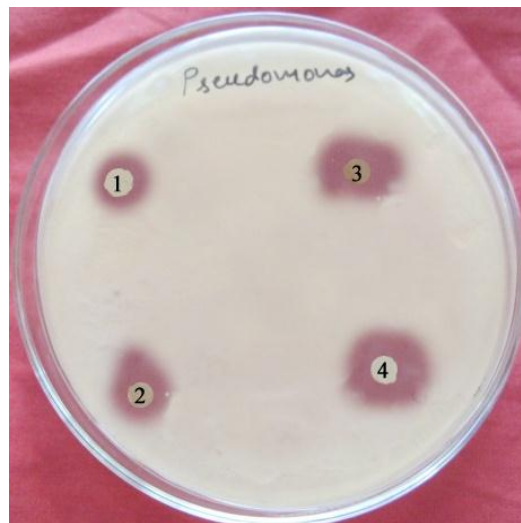
### 4. *Preparation of sterile disc:*

Whatman's No.3 filter paper was punched into 5 mm disc form and they sterilized, each sterile disc was incorporated individually with 20 – 60 mg/ml of extracts using micropipette. Precautions were taken to prevent the flow of the solvent extract from the discs to the outer surface. The condensed extracts were applied in small quantities on discs and they were allowed to dry in air. Then they were stored at 4°C.

### 5. *Assay of antimicrobial activity using Disc diffusion method:*

The antimicrobial activity was performed by employing the disc diffusion method adopted by Bauer *et al.*, (1966) and Cruickshank (1968). The 20 ml of sterilized Muller Hinton Agar was poured into sterile Petri-plates, after solidification, 100 µl of fresh culture of human pathogens were swabbed on the respective plates. Sterile discs of Whatman No. 1 filter paper of about 6 mm were prepared. The discs were kept over the agar plates using sterile forceps at various concentrations (20, 40, and 60 mg/ml). Streptomycin 10mg/ml containing disc used as standard control. The plates were incubated for 24 h at 37°C and the diameter of the inhibition zones was recorded. The assessment of antimicrobial activity was based on measurement of inhibition zones formed around discs. A zone of inhibition around the disc indicates that the compounds, which diffused into the agar from the disc, inhibited the growth of the organism.

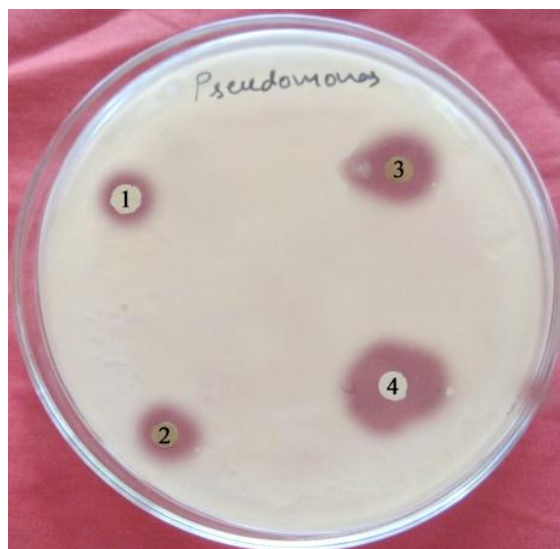
*Antibacterial activity of Boswellia ovalifoliolata:*



S. No	Name of the microorganism	20 mg/ml	40 mg/ml	60 mg/ml	Standard (Streptomycin 10 mg/ml)
1	<i>Bacillus subtilis</i>	8.14± 0.24mm	10.32 ±0.61mm	14.35 ± 0.34mm	23.45 ± 0.65mm
2	<i>Pseudomonas auriginosa</i>	7.54 ± 0.23 mm	10.3 ± 0.45mm	16.32 ± 0.29mm	26.25 ± 0.36 mm

*Antibacterial activity of Syzigium alternifolium:*





S. No	Name of the microorganism	20 mg/ml	40 mg/ml	60 mg/ml	Standard (Streptomycin 10 mg/ml)
1	<i>Bacillus subtilis</i>	7.21 ± 0.39mm	10.42 ± 0.56 mm	13.54 ± 0.49mm	23.45 ± 0.65mm
4	<i>Pseudomonas auriginosa</i>	7.5 ± 0.28mm	10.7 ± 0.88mm	15.5 ± 0.57mm	26.25 ± 0.36 mm

### RESULTS

Present investigation, anti bacterial activity of two selected medicinal plants, on pathogenic bacterial strains, all exhibited significant antibacterial activity. Methanol stem extract of *Boswellia ovalifoliolata* at the concentration 60 mg/ml showed potent antibacterial activity. *Pseudomonas auriginosa* (16mm) was more susceptible followed by *B. subtilis* (14mm). Methanol stem extract of *Syzigium alternifolium* at the concentration 60 mg/ml, *Pseudomonas auriginosa* (15.2mm) was more susceptible, followed by *B. subtilis* (13.5mm).

### III CONCLUSION

Among the two selected medicinal plants Methanol stem extract of *Boswellia ovalifoliolata* showed potent antibacterial activity, followed by *Syzigium alternifolium*. *Boswellia ovalifoliolata*, showed highest antibacterial activity and *Syzigium alternifolium* showed moderate antibacterial activity against the selected bacterial strains.

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