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Antibacterial Assay of Secondary Metabolites Extracted from Endophytic Bacteria from *Plectranthus Amboinicus*

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Research Article

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ABSTRACT

Endophytic bacteria have been found in virtually every plant studied, where they colonize the internal tissues of their host plant. Literature survey revealed the occurrence 76 volatiles and 30 non-volatile compounds belonging to different classes of phytochemicals such as monoterpenoids, diterpenoids, triterpenoids, sesquiterpenoids, phenolics, flavonoids, esters, alcohols and aldehydes. Bacteria isolated from *Plectranthus amboinicus* have great potential pharmaceuticals properties. Preliminary phytochemical analysis endophytic bacteria isolated from *Plectranthus amboinicus* showed the presence of alkaloids, flavanoids, terpenoids, phenols, saponins, carbohydrates and protein. The antibacterial activity of Secondary metabolites extracted from Encophytic bacteria of P.amboinicus was assessed by well diffusion method with 20, 10, 05, 2.5 and 1.25 µg/ml were found to be having higher inhibition over the *E.coli*. *Pseudomonas* spp., And *Klebsiella* spp., *Staphylococci* spp., and *Streptococci* spp., selected for the study.

INTRODUCTION

Plants are constantly involved in interactions with a wide range of bacteria. The natural therapeutic compounds produced by endophytic bacteria do have several potential applications in pharmaceutical industry. Next to the clinically acknowledged antineoplastic agent, taxol, endophyte research has yielded potential drug, given compounds with antimicrobial, antioxidant, antiviral, antidiabetic, anti-alzheimer's disease and immunosuppressant [1]. These evidences arises a hope to combat incurable diseases, drug resistance, other challenges related to human health1. The compounds derived from medicinal plants form the ingredients of analgesics, antibiotics, heart drugs, laxatives, anti-cancer agents, ulcer treatments, contraceptives, diuretics Compounds from plants are

referred as plant secondary metabolites, phytochemicals, anti-nutritional factors, plant xenobiotics2. The potential of finding new drugs that may be effective candidates for treating newly developing diseases in humans is great. Herbal molecules are safe and would overcome the resistance produced by the pathogens as they exist in a combined form or in a pooled form of more than one molecules in the protoplasm of the plant cell. Even with the advent of modern or allopathic medicine it have noted that a number of important modern drugs have been derived from plants used by indigenous people [2].

MATERIALS AND METHODS

The present study includes determination of efficacy of secondary metabolites from endophytic bacteria of Plectranthus amboinicus, against *Staphylococci*, *Streptococci* and *Klebsiella* spp., *E.coli* and *Pseucomonas* spp.,

Isolation of endophytic bacteria from *Plectranthus amboinicus*

The fresh plant materials belong to the *Plectranthus amboinicus* were collected from the garden of the SDM college of Ayurveda. Symptomless disease free leaves of the selected *Plectranthus amboinicus* plant were used for the isolation of endophytes. Plant material was first cleaned by washing several times under running tap water. Surface sterilization was performed by sequentially rinsing the plant material with 70% ethanol for 30 seconds, then with 0.01% mercuric chloride for 5 minutes followed by 0.5% sodium hypochlorite for 2-3 minutes and finally with sterile distilled water for 2-3 times. Plant material was then dried in between the folds of sterile filter papers. After surface sterilization the plant materials were sectioned into pieces of about 2-3 inches using sterile scalpel. Sectioned plant material were placed at equidistance on petriplates with Mueller Hinton Agar medium. All the plates were incubated at 37°C for about 24 to 48 hours to promote the growth of endophytes. On observing the microbial growth, sub culturing was done.

Characterization of endophytic bacteria isolated

Characterization of isolated bacteria is very necessary to identify their characteristics and properties. The isolated bacteria were characterized on the basis of grams staining technique. Identification and morphological study of microorganisms were characterized based on their colony morphology (Table 1). Isolated 5 isolates were subjected to catalase test endosopre staining and motility [3].

Table 1. Colony Characters of Endophytic Bacteria Isolated from Plectrantus ambonicus.

Isolated organisms	Colony	Margin	Elevation	Texture	colour	Gram's staining
1	Circular	Entire	Flat	Smooth	Dull white	Gram –ve
2	Punctiform	Lobate	Umbonate	Rough	whitish	Gram-ve
3	Punctiform	Erose	Umbonate	Rough	whitish	Gram-ve
4	Punctiform	Erose	Umbonate	Rough	whitish	Gram-ve
5	Irregular	Undulate	Flat	Smooth	creamish	Gram-ve

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Extraction of metabolites

Each bacterial isolate was inoculated in an Erlenmeyer flask containing 5 L of Nutrient broth and incubated for 3 to 4 days. The fermentation flask was incubated at 110 rpm on a rotary shaker at room temperature for 7 days. After fermentation the culture broth was filtered and the filtrates extracted three times with ethyl acetate. Extracts were subjected to see the efficacy against pathogenic bacteria (Figure 1) [4].

Figure 1. Plant material and sectioned leaf showing.

Antimicrobial assay

Natural extracts were screened for their efficacy using the well diffusion method against potentially pathogenic bacteria *Staphylococci*, *Streptococci* and *Klebsiella* spp., *E.coli* and *Pseucomonas* spp., on Muller Hinton Agar plate swabbed with standard McFarland inoculums lid were replaced after 5 minutes. 8 equidistant wells were made on the plates with the help of sterile corn borer. 100 µl of standard Ampicillin (10 µg) and different concentration of secondary metabolites (20 µg /ml, 10 µg /ml, 5 µg/ml, 2.5 µg/ml and 1.25 µg/ml) were charged onto the labelled wells. All the plates were incubated at 37°C for 24 hours. After incubation period, the zone of inhibition were measured with a ruler millimetres (Figure 2) [5].

Figure 2. Sub culturing of endophytes and sensitivity test.

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RESULTS AND DISCUSSION

Endophytes are the chemical synthesizers within plants. Many of them are capable of synthesizing bioactive compounds that can be used by plants for defense against pathogens and some of these compounds have been proved for useful drug discovery. Up till date, most of the natural products from endophytes are antibiotics, anticancer agents, biological control agents' antivirals, antidiabetic agents and other bioactive compounds by their different functional roles6. In the present study Secondary metabolites were isolated from *Plectranthus amboinicus* and same has been subjected to see the antibacterial activity of metabolites extracted against pathogenic bacteria *Staphylococci*, *Streptococci* and *Klebsiella* spp., *E.coli* and *Pseucomonas* spp., revealed the presence of antibacterial property with Zone of inhibition of 18,20,22 and 24 mm with different concentrations of 20,10,5,2.5,1.25. Among five isolates screened for antibacterial activity against Streptococci and *E.coli* were showed significant zone of inhibition in all the concentrations. However some of the test isolates had fairly high activity as well. It is possible that there were novel compounds from the test isolates and this could be promising agents to replace drugs which resistance has developed. However further chemical analysis is required to determine this that is, if there are novel compounds. Consequently, the search for the secondary metabolites as lead compounds or templates in drug development is encouraged [6].

CONCLUSION

Isolated endophytic bacteria have the phytochemicals which act on the infectious bacteria. Microbial endophytes reside in the internal plant tissues were stimulate phytochemicals properties of plants with wide clinical application. Research findings could serve as a foundation for further research on this plant endophytic bacterial classification and in determining potential roles of its bacterial endophytes in producing novel therapeutic compounds. *P.amboinicus* is an important aromatic medicinal herb packed with many bioactive constituents and nutrients, which are important for maintaining good health. The plant has shown a wide range of biological properties and proved to be effective in curing respiratory, cardiovascular, oral, skin, digestive and urinary diseases.

Results could demonstrate the potential value of investigating metabolite production by endophytic bacteria from *P albicans*, since plant leaf are source of promising antimicrobial compounds against various human pathogenic bacteria. It would be of interest to find out which functional group is responsible for the bioactivity and also whether any of them is a novel compound with antibacterial activity which would make it a promising candidate for the production of new antimicrobials.

Further work on these metabolites will reveal this and will also aid in gaining insight of synergism among the different functional groups. Molecular characterization should be carried out to fully identify the 5 endophytic bacterial isolates responsible for the production of the active secondary metabolites.

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