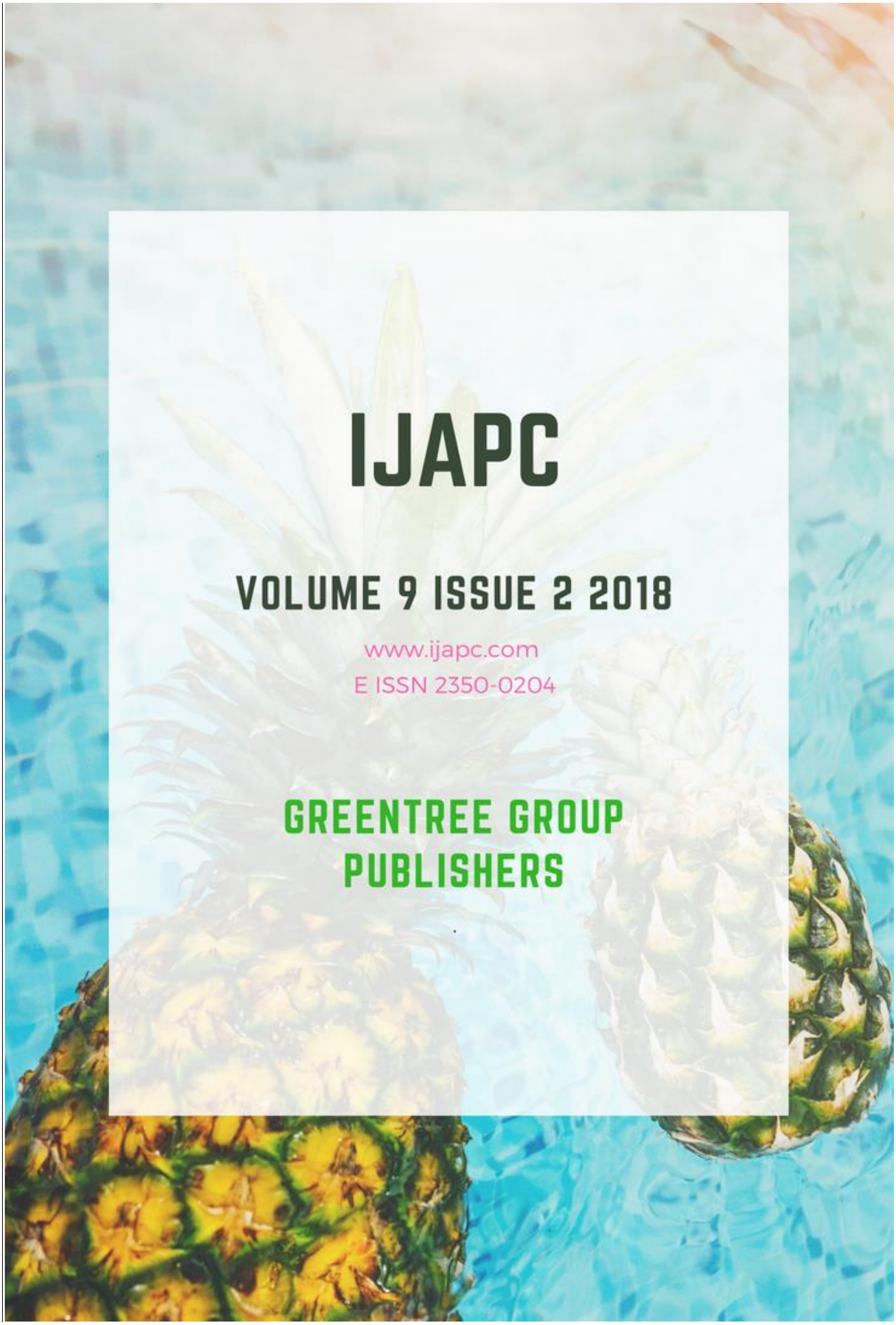

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Isolation, Characterization and Phytochemical Analysis of Endophytic bacteria Isolated from *Plectranthus amboinicus*

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ABSTRACT

Symbiotic association of bacteria, fungi and actinomycetes inside the tissues of plant were known as endophytes. Medicinal plants have diverse and capable symbiotic microbial flora. In this present study endophytic bacteria were isolated from *Plectrantus amboinicus* a medicinal plant by surface sterilization method. Sterile plant sections were placed on sterile nutrient agar plates and incubated for 24-48 hours in incubator. Plates were observed for the growth revealed the presence of bacterial isolates surrounding the plant sections. Five bacterial isolates were Isolated, endophytic bacteria isolated were subjected to characterization by morphological, microscopically and serologically to study the isolates. These endophytic bacteria were subjected to extract secondary metabolites, extracted secondary metabolites were processed to check the presence of preliminary phytochemicals.

KEYWORDS

Endophytic, Plectranthus amboinicus, Secondary metabolites

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INTRODUCTION

Endophytes are endosymbiont, these may bacteria are fungi. Symbiotic association of these endophytic bacteria may varies from plants to plants these may include fungi actinomycetes and bacteria. Endophytic fungi are present in the medicinal plant tissues boosting up the survival of the host. This enhanced survival of the host is due to because of the production of secondary metabolites from the endophytic fungi present in the host. Not only helps in the survival and also increases the uptake of nutrients and protection form the herbivory environment[1].

Endophytic bacteria behave as biotrophic symbionts either obligate or facultative. The secondary metabolites secreted from endophytic microorganism are having therapeutic and pharmaceutical properties like antioxidant, antiviral, antidiabetic, anti-alzheimer's disease anti-microbial and immunosuppressant[2].

METHODOLOGY

1. **Endophytic bacteria isolation from *P. amboinicus***

The fresh plant materials belong to the *Plectranthus amboinicus* were collected from the garden of the SDM college of Ayurveda. (Fig 1). selected *Plectranthus amboinicus* plant leaf were used for the isolation of endophytes. Leaf were washed under tap water to clean the soil adherence, later cleaned leaf were subjected to surface sterilization with 70% ethanol for about 30S, 5minutes with 0.01% mercuric chloride next with 3 minutes in 0.5% sodium hypochlorite and lastly wash leaf material with distilled water.

Fig 1 Plant material

Fig 2 Sectioned leaf showing endophytic bacteria

Symptomless disease free leaves of the Leaf were cleaned in blot dried with clean bolter paper. 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 petri-plates with Mueller Hinton Agar medium (Fig 2) and incubated in incubator for 24-48 hours at 37 º C. Incubation temperature promote the growth of endophytes (Fig 2). On observing the microbial growth, sub culturing were done (Fig 3).

2. Characterization of isolated endophytic bacteria:

Characterization of isolated bacteria is very necessary to identify their characteristics and properties. Characterization of endophytic bacteria isolated were did on the basis of grams staining technique (Table 1).

Fig 3 Sub-culturing of endophytic bacteria

Table 2 Isolated endophytic bacterial colony characteristic

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

Identification and morphological study of microorganisms were differentiated on the basis of their colony morphology (Table 2). Characterisation and identification of bacteria were did and isolates were processed to see the production of enzymes catalase by catalase test, identification of endospores by staining method and motility check by hanging drop method.(Table 3).

Table 1 Gram's staining results

Isolate	1	2	3	4	5
Catalase	Positive	Positive	Positive	Positive	Positive
Endospore	Negative	Negative	Positive	Positive	Positive
Motility	Negative	Positive	Positive	Positive	Negative

3. **Extraction of metabolites**

Each endophytic bacteria 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 at room temperature for 7 days in rotary. After fermentation the culture broth was filtered and the filtrates were processed to check the presence of phytochemicals.

Table 3 Results of catalase, Endospore staining and motility tes

Isolate	1	2	3	4	5
Result	Gr-ve bacilli	Gr-ve bacilli	Gr-ve bacilli	Gr-ve bacilli	Gr-ve bacilli

t

Phytochemical Analysis of Secondary Metabolites Extracted From Endophytic Bacteria (Table 1)

Qualitative Phytochemical Study

❖ **Test for Alkaloids**

❖ **Wagner' s test**

❖ Take 1ml of extract, by the sides of the test tube add 3-4 drops of Wagner's reagent.. A reddish brown precipitate confirmed the test as positive[3].

❖ **Test for Flavonoids**

- **Alkaline reagent test:** 4-5 drops of 10 % NaOH solution were mixed with1ml of drug extract solution to observe the dark yellow colour confirms the presence of Flavonoids[4].

❖ **NH_4OH TEST: 2-3ml extract were mixed with 5 drops of 10% NH_4OH** solution. Yellow fluorescence colour indicates positive test result.

❖ **Test for gums and mucilage**

Under constant stirring add absolute alcohol of 25ml and 10ml of the extract. formation of precipitation indicates the presence of gum and mucilage[5].

❖ **Test for fixed oils and fats.**

- Take filter paper drug extract were placed in the middle of paper pressed firmly. Presence of oils and gums indicated by the formation of oil stain on the filter paper[5].

❖ **Test for Saponins**

- **Frothing test:** 2mg of extract was treated with 5ml of water and shaken well. Froth formation was observed for the positive results. Which was found to be stable for 15 minutes[6].

❖ **Detection of pholabatanins**

Few drops of 1% diluted Hydrochloric acid was added to extract in boiling tube and then observed for the development of red precipitate[6].

❖ **Test for tannins:**

- **Ferric Chloride Test:** About 0.5 g extract was taken in a boiling tube and boiled to distilled water then filtered. Add 3-4 drops of 0.1% $FeCl_3$ were gently shaken and allowed to stand for 5 minutes. Brownish green or blue black color formation indicated the presence of tannins[6].

- 0.5ml of extract were taken in test tube 1ml of water and 1- 2 drops of ferric chloride solution were added. Blue color indicates positive for gallic tannins and green black color indicates positive for catecholic [7].

❖ **Detection of Phenolic compound:**

- **Ferric Chloride test**

3-4 drops of neutral ferric chloride were mixed with the 1ml of extract solution,

presence of phenolic were indicated by the formation of dark green color[3].

❖ **Detection of glycosides:**

- **Legal's test:** 10% 3-4 drops of NaOH were mixed with the 1ml of extract, add sodium nitroprusside solution to get the green coloration indicates the positive result[3].
- Take 3-4 drops of ferric chloride and concentrated sulphuric acid in clean test-tube, add 0.5ml of glacial acetic acid and 1ml of extract mixed well and observe for the reddish brown coloration at the junction of the two layer and bluish green color in the upper layer indicates the positive result[3]

Table4 Inference of Phytochemical Tests

Test	1	2	3	4	5	6
Alkaloids	-	+	+	+	+	+
Amino acids	+	-	-	-	-	+
Carbohydrates	+	-	-	-	-	+
Fixed oils and fats	-	+	+	+	+	-
Test for glycosides	-	-	-	-	-	-
Phenolic compounds and tannins	+	-	+	-	+	+
Proteins	-	+	+	+	+	+
Gum and mucilages	_	+	+	+	_	+
Phytosterols	+	+	+	+	+	+
Saponins	_	+	+	+	_	+

RESULTS

Present work is concentrated on isolation of symbiotic endophytic bacteria from *P amboinicus* leaf, isolated all five bacteria were Gram-negative bacilli. Out of five, three strains were positive for endospore staining, all five were catalase positive, and three isolates were showing motility. Isolated bacteria were observed for colony characterization. Bacteria showed the circular colony with entire margin, flat elevation, and smooth in texture and dull white in color. The isolated second organism showed the punctiform colony with lobate margin, umbonate elevation, rough in texture and whitish color. The third isolate also showed punctiform colony with erose margin, umbonate elevation, rough in texture and whitish in color. The fourth isolate also showed punctiform colony with erose margin, umbonate elevation, and rough in texture and whitish in color. The fifth isolated organism showed irregular colony with undulate margin, flat elevation, smooth in texture and creamish in color (Table 1). Endophytic bacteria isolated were subjected to secondary metabolite extraction by fermentation method, secondary metabolites were screened for presence of phytochemical analysis. Secondary metabolites subjected to phytochemical

analysis showing positive for alkaloids, flavanoids, tannins, and phenolic compounds (Table 4).

DISCUSSION

Endophytic bacteria were isolated from *P. amboinicus* is a genuine study as till date no work had been carried and no reports regarding the study was found. *Plectranthus amboinicus* leafs were subjected to surface sterilization and endophytic bacteria were isolated by taking sections of plant leaf and placing it on a muller hinton agar by incubating it. After isolation, endophytic bacteria were processed to extract the secondary metabolites, extracted secondary metabolites were subjected to see the presence of phytochemical. Presence of alkaloids, tannins , carbohydrates, phenols, amino acids, phytosterols, proteins, saponins, gum and mucilage analysed by preliminary phytochemical assay[8.9]. Microbial endophytes are now a days gaining attention from scientific community worldwide. Five endophytic bacteria were isolated in this present study, Which were all confirmed gram negative by bacteria gram's staining technique and catalase positive. Then out of five, three of the isolates were endospore forming and actively motile. Microbial endophytes reside in the internal plant tissues; these endophytic bacteria synthesize secondary metabolites with huge medicinal properties which promotes plants to show wide clinical application. Chewing of these *P. amboinicus* leaf is a remedy for dry cough and fever due to presence of alkaloids, tannin and phenols. Present study reveals that *P. ambonicus* leaf gives habitat to endophytic bacteria these endophytic bacteria will be containing secondary metabolites.

CONCLUSION

Isolated endophytic bacteria subjected to extract secondary metabolites which act on the infectious bacteria. Entophytes reside in *P. amboinicus* internal plant tissues which stimulate phytochemical properties of plants with wide clinical application. Research results may give strong evidence for future research on these plant endophytic bacterial classifications and in determining potential roles of its bacterial endophytes in producing novel therapeutic compounds.

P.amboinicus is an important aromatic medicinal herb packed with symbiotic endophytic microorganisms these synthesize many bioactive constituents, which are important for maintaining proper growth and metabolization of plant. *P.amboinicus* showed various level of biological properties and proved to be effective in curing respiratory,

cardiovascular, oral, skin, digestive and urinary diseases, indirectly endophytic microorganisms present in this plant tissue may responsible for it.

To conclude, the study reveals that *P. amboinicus* contains enormous endophytic bacteria and its secondary metabolites can be used as novel therapeutic compounds.

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