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Research Article

CYTOTOXIC POTENTIAL AND PHYTOCHEMICAL ANALYSIS OF *JUSTICIA BEDDOMEI* AND ITS ENDOPHYTIC *ASPERGILLUS SP*

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ABSTRACT

Introduction; Endophytic fungi are mutualistic symbionts of plants producing bioactive secondary metabolites.
Objective; The study was carried out to screen for phytochemicals and demonstrate the cyotoxicity of the ethyl acetate extracts of the plant *Justicia beddomei* and its endophytic fungi.
Methods; Preliminary phytochemical screening was carried by standard procedures. The MTT assay of the extracts was carried out on A549 lung adenocarcinoma cells. Results; The endophytic Aspergillus had an IC 50 of 22.73µg/ml, while the plant extract had an IC 50 of 6.25µg/ml. The phytochemical screening of the extracts showed the presence of phenols, flavonoids, alkaloids, tannins and glycosides.
Conclusion; The present study indicates the presence of anticancer compounds in the plant and its endophytic *Aspergillus sp*. The endophyte was shown to possess bioactivity three times than that of the host plant.

Keywords: endophytic fungi, *Justicia beddomei, Centella asiatica.*

INTRODUCTION

Endophytes are microorganisms which live within the plant tissues without causing any noticeable symptoms of disease [1]. An estimate of about a million undescribed species of endophytic fungi occur within the plant aerial tissues [2]. Endophytic fungi have a mutualistic relationship with the host, protecting the host against pathogen and in some cases may be an opportunistic pathogen [3].During the long co evolution endophytes biosynthesize phytochemicals that are originally associated with the host plant[4]. A large number of secondary metabolites have been extracted, isolated and characterized from endophytic microbes [5]. Endophytes comprise a rich and reliable source of genetic diversity and biological novelty with application in pharmacology and agriculture [6].

In India, medicinal plants have long been used for the treatment of various diseases. Medicinal plants provide a special environment for endophytes. Many previous reported endophytic fungi with novel and bioactive natural products are obtained from medicinal plants [7]. In view of these earlier observations, the present study was carried out to investigate the bioactivity of the endophytic fungi of *Justicia beddomei* and compare it with plant itself. . *Justicia beddomei* is a well known Indian traditional medicine used for antimicrobial, diuretic, antidiabetic, antispasmodic properties. The leaves are used for irritable cough and bleeding in the diarrhea [8].

Very few reports are available on the bioactive compounds from *Justicia beddomei* and its endophytes. In the present study, preliminary phytochemical analysis was carried out on the ethyl acetate extracts of *Justicia beddomei leaves and* the isolated endophytic fungi. We also report the cytotoxic potential of the extracts on A549 lung adenocarcinoma cells.

MATERIALS AND METHODS

Isolation of endophytes and extraction of bioactive compounds

The fresh healthy leaves of *Justicia beddomei* were obtained from Siddha Institute, Chennai. The endophytes were isolated by standard procedures [9]. The endophytic fungi grown on plates were transferred to sterile PDA slants to maintain culture purity. The fungi were identified by colony morphology and LPCB mount.

The fungal endophytes were recultivated on potato dextrose broth by placing agar block of actively growing pure culture in 250ml Erlenmeyer flask containing 100ml of the medium. The flasks were incubated at 27°c for 7 days with periodical shaking at 150 rpm. After the incubation period, the cultures were taken out and filtered through sterile mesh cloth. Fungal metabolite in the mycelial mat was extracted by solvent extraction procedure with ethyl acetate as organic solvent [10]. The mycelia mat was soaked in ethyl acetate for 2hrs and ground in a mortar pestle. The solvent was evaporated in a Soxhalate Apparattus. The resultant crude extract was dissolved in DMSO for further analysis

Preparation of plant extract

The collected leaves were shade dried, coarsely powdered subjected to solvent extraction by ethyl acetate in a Soxhlet Extractor. The extract was concentrated in a vacuum under reduced pressure using rotary flash evaporator to yield a dry powder[11].

Phytochemical analysis of fungal and plant extract

The prepared plant extracts and the fungal extracts are subjected to phytochemical tests for presence tannins, saponins ,terepenoids, glycosides, cardiac glycosides, steroids and flavonoids using standard methodology[12] .

Cytotoxic activity

The A549 cells lung adenocarcinoma cells were procured from National Centre for Cell Science (NCCS), Pune with passage number 11. The cells were cultured in DMEM (Dulbecco's minimum essential medium) in the presence of 10% fetal bovine serum, with 100units/ml penicillin and 100µg/ml streptomycin. Cells were cultured in a humidified atmosphere with 5% CO_2 at 37°C.

Proliferation of A549 cells was assessed by MTT assay [13]. Cells were plated in 96-well plate at a concentration of 5×10^4 cells/well 24 h after plating. After 24h of cells incubation, the medium was replaced with 100µl medium containing test extract at different concentrations and incubated for 24 h. At the end of treatment, media from control and extract -treated cells was discarded and 20µl of MTT (0.5 mg/ml Phosphate Buffered Saline) was added to each well. Cells were then incubated for 4 h at 37°C in CO2 incubator. MTT was then discarded and the colored crystals of produced formazan were dissolved by adding 200µl of DMSO. Spectrophotometrical absorbance of the purple blue formazan dye was measured using an ELISA reader (BIORAD) at 570 nm. Optical density of each sample was compared with control optical density and graphs were plotted

RESULTS AND DISCUSSION

The fungi isolated from the plant *Justicia beddomei* was maintained as a pure culture. LPCB mount was prepared and the micrograph observed is given in Figure 1.

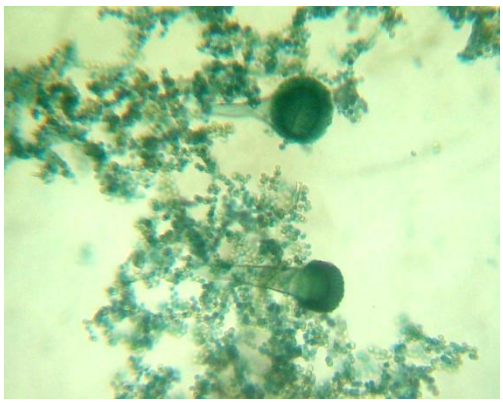


Fig.1: LPCB mount of the endophytic fungi isolated from *Justicia beddomei*

Fungal identification based on conventional methods is laborious and time consuming. Genetic methods present high sensitivity and specificity, is used for classifying microbial strains in diverse hierarchical taxonomic levels [14]. So the DNA was isolated from the endophtyic fungi and the PCR reactions were performed using primers ;

Forward primer: 5'- GTAGTCATATGCTTGTCTC-3'

Reverse primer: 5'- GAAACCTTGTTACGACTT-3'

The obtained sequence was submitted for BLAST query in NCBI (National Center for Biotechnology Information). Based on the colony morphology, LPCB mount and BLAST results, the isolated species has been identified to have a similarity with *Aspergillus fumigatus* .

The results of the phytochemical screening of the ethyl acetate extract of the endophytic fungi and the plant is given in the Table 1.

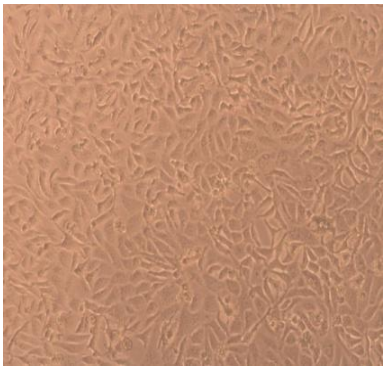
Table 1: Table showing the phytochemical analysis of the ethyl acetate extracts of the endophytic fungi and *Justicia beddomei*

Compound	*Justicia beddomei*	*Aspergillus sp*
Phenol	++	++
Triterpenoids	-	-
Flavones	++	+++
Alkaloids	++	-
Reducing sugars	+	-
Glycosides	+	+
Saponins	+	+
Quinones	-	-
Proteins	++	++
Tannins	+++	+++
Anthraquinones	+	-
Steroids	-	-

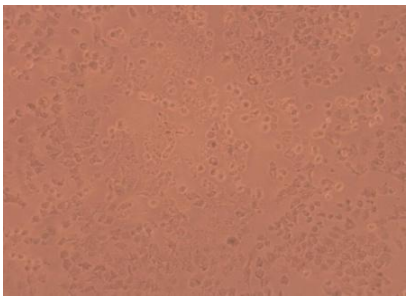
The phytochemical analysis showed the presence of different phytochemicals, phenolics, steroids, glycosides, tannins, alkaloids and flavonoids. Similar results were observed with Penicillum sp isolated from *Centella asiatica* [15]. It was observed that both the extracts contained phenols and tannins .Comparatively the flavanoid content seems to be more in the fungal extract than the plant extract. In the present study, the presence of alkaloids was not observed in the fungal extract. The bioactivity of the plant extract could be attributed to the alkaloid. Earlier studies showed the major alkaloids vasicine and vasicinone of *Justicia adhathoda,* to be biologically active and are the area under discussion of many chemical compounds and pharmacological studies [16].

Chemoprevention by phenolic phytochemicals is an inexpensive, readily applicable, acceptable, and accessible approach to cancer control and management .The major natural products of secondary metabolism in plants and fungi are phenolic compounds. Phenolic compounds possess a diverse range of beneficial biological activities, which contribute to their potent effects on inhibiting carcinogenesis. Many flavonoids possess diverse bioactivities. Flavonoids are known to be anti cancer agents because they have powerful antioxidant activities in vitro, being able to scavenge a wide range of reactive species. Tannins also exhibit strong antibacterial, antiulcer, anti-inflammatory,antileishmanial, antimutagenic, enzyme regulating, signal transduction pathways blocking, and apoptotic activities; thus, they have attracted wide attention for cancer treatment. [17].

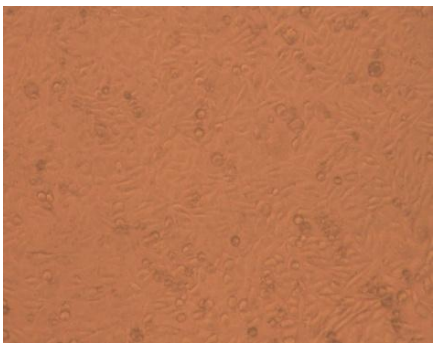
Some endophytes produce phytochemicals that were originally of the host plant. The use of endophytes for production of bioactive compounds would reduce the need to harvest the slow growing and possible rare plants. Further a microbial source for metabolite production is economical [18].The cytotoxic potential of the extracts on the A549 lung adenocarcinoma cell lines are given in the figure 2, 3and 4. The IC 50 values were observed to be 6.25µg/ml for the fungal extract and 22.73 µg/ml for the plant extract. The presence of the phenolics, flavonoids, tannins in the extracts could be attributed to the observed cytotoxic potential. As has been previously reported, the endophytic fungi exhibit greater bioactivity than the original host plant [19].



(a)



(b)



(c)

Fig.2: Figure showing the cytotoxic activity. (a) Control cells (b) fungal extract (c) plant extract

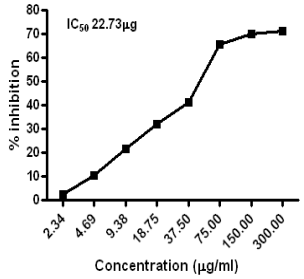


Fig.3: Cytotoxicity Potential of *Aspergillus fumigatus* against A549 lung adenocarcinoma cell line

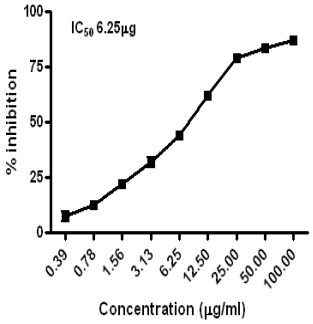


Fig.4: Cytotoxicity Potential of *Justicia beddomei* against A549 lung adenocarcinoma cell line

The anticancer properties of several secondary metabolites from endophytes have been investigated recently. The ethyl acetate extracts of the endophytic fungi isolated from Srikaya plants have shown to exhibit anticancer activity against breast cancer cell lines [20]. The in vivo anti cancer activity of taxol from dichloromethane extract of endophytic *Pestalotiopsis sp* has been demonstrated by Vennila etal [21].

In our present study, we have demonstrated the cytotoxic potential of the plant and its endophytic fungi. A preliminary phytochemical screening has indicated the nature of the bioactive compounds. The endophytic fungi thus could be an alternative source of anticancer compounds. Further studies on the quantification of these bioactive compounds and the correlation between the compounds of plant and the endophyte are necessitated.

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