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ANTIMICROBIAL ACTIVITY OF ENDOPHYTIC FUNGI ISOLATED FROM *FICUS RELIGIOSA* L.

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ABSTRACT

Endophytes have received attention of the scientific community due to their capacity to produce novel bioactive compounds. In the present study is to isolate endophytic fungi from ***Ficus religiosa*** (Peepal) and to assess their antimicrobial activity against water borne pathogens. In this study six endophytic fungi were isolated from bark of peepal tree. Out of six endophytic two isolates showed the inhibition against test microorganisms. The isolate ***A.niger*** and ***Fusarium*** exhibited a high antimicrobial activity against ***E.coil*** (25mm) by agar well diffusion method. The results of the study suggest that endophytic fungi associated with peepal are potential agents for antimicrobial activity.

KEY WORDS: Endophytic fungi, Antimicrobial activity, Peepal.

INTRODUCTION

Endophytic microorganisms are mutualistic symbionts of plants which colonise the healthy tissues of plants without causing any overt negative effects[1,2]. Many substances found in plants were extracted from their endophytes[3]. Therefore the focus on on the isolation and/or application of endophytes from medicinal plants has been increasing[4, 5]. These endophytes can produce several substances of biotechnological interest, including secondary metabolites with pharmaceutical application[6, 7], in the production of antimicrobials[8, 9]. Endophytes have proved to be the promising sources of biologically active products which are of interest for specific health care applications.[10,11] Endophytic fungal strains are also found to be potentially useful in the production of pigments, bioactive metabolites, immunosuppressants, anticancer compounds and bio-control agents[12,13]. The aim of this study is to isolate endophytic fungi from peepal (***Ficus religiosa***) and screen them for antimicrobial activities against water borne pathogens.

Ficus religiosa L. plant are considered as sacred plants in India. It is also called vishnoos tree. They show great potential for obtaining useful biologically active compounds. The genus ***Ficus religiosa*** belong to the family **moraceae** and are commonly found in tropical regions of South East Asia. It is not only a native economic tree, but also its bark can be used in traditional medicine for treatment of skin diseases, diabetes, ulcers, scabies, inflammation, fistula, gonorrhoea. It is also used to treat asthma, hiccup, indigestion, purgative and sterility[14].

MATERIALS AND METHODS

Collection of Plant Samples: The samples were collected during the month of May 2015 to June 2015 (between 10:30am and 4:30pm each day) in tribal area of chintapalli, Visakhapatnam district. The fresh-cut ends of plant bark samples were cut by alcohol sterile scissor, and placed in zip-lock plastic-bags and stored at 4^{o}c in the laboratory until the examination and isolation of endophytic fungi.

Isolation of Fungal Endophytes: Samples were cleaned under running tap water for 5 minutes and then air-dried. Before surface sterilization, the cleaned stems were cut into 5-cm long pieces were sterilized by immersion in 70% ethanol for 1 minute, 5% sodium hypochlorite solution for 5 minutes, and sterile distilled water for 1 minute twice[15]. The surface-sterilized leaves and stems were cut into small pieces about 0.5×0.5 cm2 using a sterile blade and placed on sterile half strength potato dextrose agar plates. The plates were incubated at room temperature for 24–72 hours. The hyphal tip of endophytic fungus growing out from the plant tissue was cut by a sterile pasture pipette and transferred to a sterile half strength potato dextrose agar plate. After incubation at room temperature for 7-14 days, colony morphology of each endophytic fungi was determined. Culture purity was obtained by several times sub culturing.

Test Organisms: The tested organisms were 4 bacterial (***Ecoli, Staphylococcus, Vibrio, Salmonella***) isolated from water samples collected from Well and spring waters (drinking water) from Chintapalli Mandal, Visakhapatnam district[16].

Antimicrobial assay: Antimicrobial activity of isolated Endophytic fungi was tested based on the protocol of Zhang et al., with slight modifications[17]. The petridishes containing respective media for the growth of bacteria and fungi were prepared and 100 µl of test organism was spread over the surface of the agar media using sterile cotton swab. Eight millimetre diameter of actively growing fungal culture from PDA plates were cut using a sterile cork borer and placed on the surface of the respective agar media seeded with test bacterial species. These plates were sealed with parafilm and incubate at 37^0c for 24 hours. After incubation the diameter of the inhibition zone was measured in millimetre by using scale. The experiment was carried out in triplicates.

RESULT AND DISCUSION

In the present study, a total of 6 isolates were obtained from the bark of ***Ficus religiosa.*** Among of them 2 fungal species were examined for antimicrobial and enzyme activity. The potential endophytic fungi have been identified based on macroscopic colony and spore or conidia characteristic as shown on Table no1.

Table No 1: Endophytic fungi which have interested Antimicrobial activity

Name of the Fungi	Macroscopic	Microscopic
Aspergillus niger	Black colonies	Single celled spores in chains
Fusarium sps	Woolly white colonies	Multicelled spores are in crescent shaped

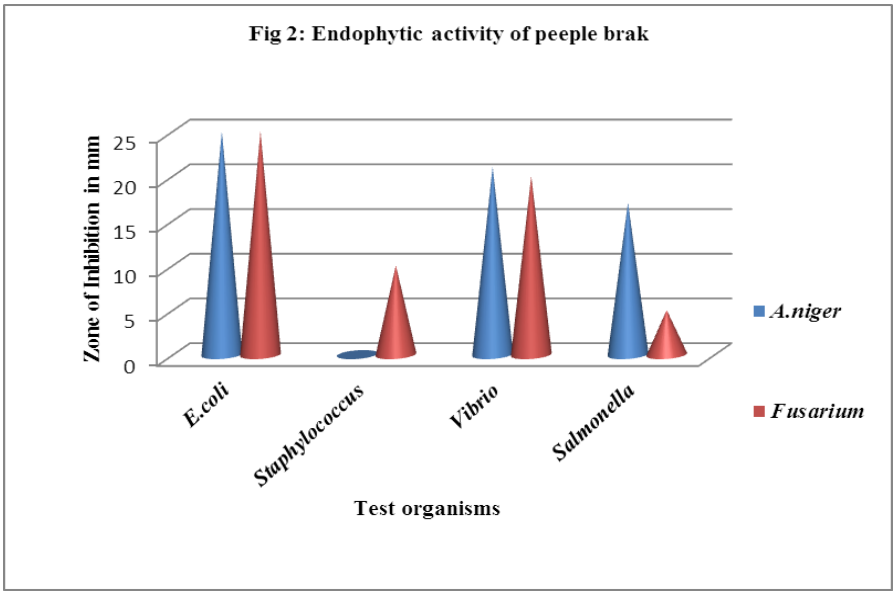
(a) **(b)**

Fig 1: Isolated Endophytic Fungi (a) *Aspergillus niger (b) Fusarium sps*

Table No 2: Endophytic Activity of Fungi Isolated from Peeple Bark.

S. No	Organism	Zone of Inhibition (mm)	
		A.niger	***Fusarium***
1	***E. coli***	25	25
2	***Staphylococcus***	Nil	10
3	***Vibrio***	21	20
4	***Salmonella***	17	5

Two endophytic fungi isolated from Peepal have significantly inhibited representative Gram positive, Gram negative bacteria. The zones of inhibition by these fungi are very much comparable to the standard antibiotics**.** ***A.niger*** showed maximum zone of inhibition towards ***E.coli*** (25mm) fallowed by ***Vibrio*** (21mm) and ***Salmonella*** (17mm). No zone of inhibition was observed in ***Staphylococcus*** against ***A.niger. Fusarium*** also showed maximum zone of inhibition towards ***E.coli*** (25mm) fallowed by ***Vibrio*** (20mm), ***Staphylococcus*** (10mm) and ***Salmonella*** (5mm).



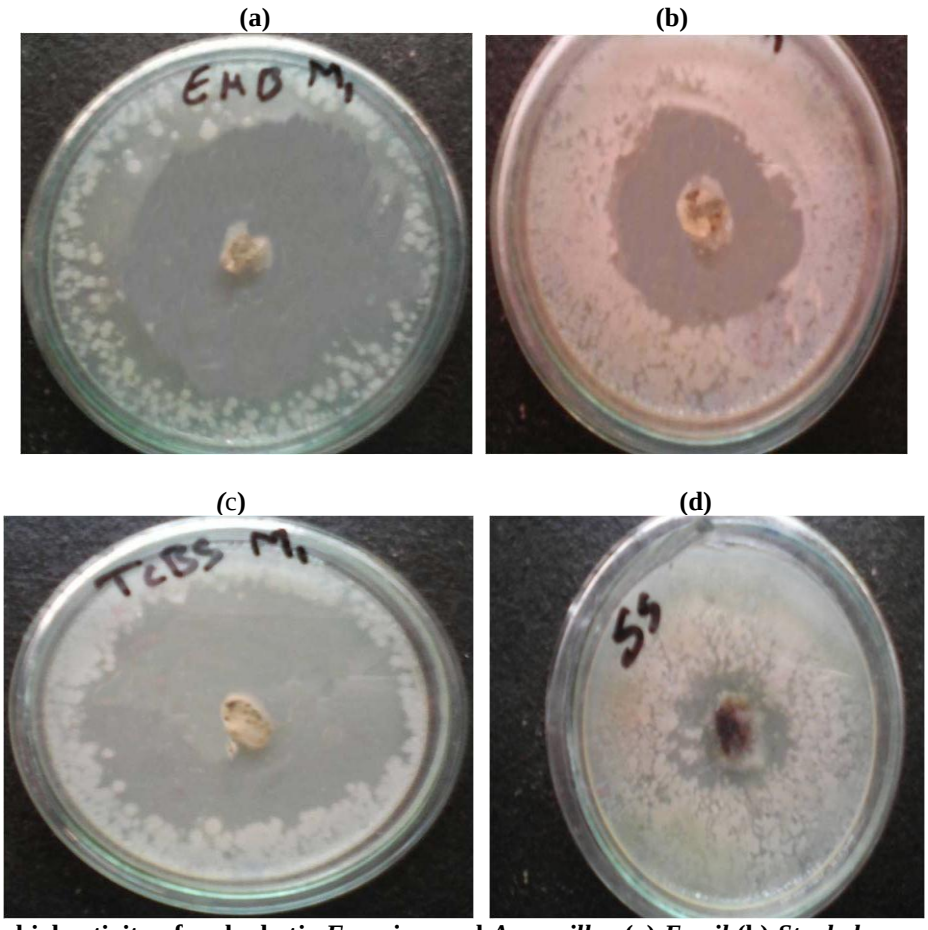


Fig 3: Antimicrobial activity of endophytic *Fusariem* and *Aspergillus* (a) *E.coil* (b) *Staphylococcus* (c) *Vibrio* (d) *Salmonella*

The differential susceptibility was attributed to the culture conditions, extraction procedure and the test strain used for antimicrobial analysis[18]. Our results indicate the broad antimicrobial spectrum and strong toxicity of the bioactive components agar well diffusion method. Conclusively, the antimicrobial activity of endophytic ***Aspergillus sp.*** is due to the intracellular bioactive components. Liu et al., 2010[19], the crude extracts of ***Fusarium oxysporum*** and ***Fusarium poae***, isolated from ***Ophiopogon japonicus***, were investigated for their prominent inhibition of several phytopathogens. Many studies have indicated that ***Fusarium sp***. is the most common species and a potent source of bioactive compounds among endophytes from medicinal plants.

Previous research has shown the abundance of secondary metabolites of ***Fusarium*** that have antimicrobial activity. The pentaketide (CR377:2-methylbutyraldehyde-substituted-α- pyrone) from a Fusarium sp. (in ***Selaginella pallescens***) showed positive activity against ***Candida albicans***[20]. ***Fusarium sp.*** from ***Tripterygium wilfordii*** produced antimicrobial compounds such as subglutinol A and B[21]. In addition, beauvericin from ***Fusarium oxysporum*** isolated from the bark of ***Cinnamomum kanehirae,*** suppressed growth of methicillin-resistant ***S. aureus*** and ***Bacillus subtilis***[22].

Souza et al. (2004) tested the antimicrobial activity of endophytes from Amazonian toxic plants ***Palicourea longiflora*** and ***Strychnos cogens***[23]. 19 fungal isolates inhibited at least one of the pathogenic microorganisms tested: ***Bacillus sp., B.subtilis, S.aureus, E. coli*** and ***Candida albicans***. Phongpaichit et al.[24] isolated fungal endophytes from Garcinia, a medicinal plant and verified that the metabolites produced by 70 isolates and extracted with ethyl acetate showed antimicrobial activity by agar diffusion test against pathogen microorganism tested: ***Staphylococcus aureus, C.albicans, Cryptococcus neoformans*** **and** ***Microsporum gypseu*****.** They identified the genera ***Aspergillus, Botryosphaeria, Eutypella, Fusarium, Guignardia, Penicillium and Phomopsis.***

CONCLUSION

Endophytes are gaining importance because of their enormous potential to produce novel bioactive compounds of agricultural and medicinal importance. The current study has presented the endophytic fungi from ***Ficus religiosa*** were abundant of an extremely diverse endophytic fungal flora and the extracts of endophytic fungi exhibited considerable antimicrobial activity. ***Fusarium sp.*** exhibited the most significant inhibitory activity against all the test human pathogens. Further studies on isolation of these antimicrobial compounds and identification of bioactive compounds can be a crucial approach to search of novel natural products.

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