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INVITRO ANTIBACTERIAL ACTIVITY OF ENDOPHYTIC FUNGAL EXTRACTS ISOLATED FROM A PHARMACEUTICALLY IMPORTANT PLANT *FICUS RELIGIOSA L.*

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ABSTRACT

Ficus religiosa L is one of the important medicinal plant widely used in pharmaceutical research. It is widely distributed in India and a religiously important host. In the present study, antibacterial activity of endophytic fungi isolated from *Ficus religiosa* was carried out. Organic extract of five dominant endophytic fungi viz *Botryodiplodia theobromae, Colletotrichum gloeosporioides, Phomopsis* sp*, Curvuleria lunata, Drechslera hawaiiensis* were tested against important gram positive and gram negative bacteria. The *C.lunata* extract showed maximum activity against tested organisms followed by *Colletotrichum gloeosporioides.* However, *Botryodiplodia theobromae* does not inhibit any of the test organisms.

KEY WORDS: ***Ficus religiosa*, Endophytic fungi, Anti-bacterial activity, *Curvularia lunata,* Gram positive bacteria and Gram negative bacteria.**

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INTRODUCTION

Ficus religiosa L a Pharmaceutically important plant belonging to the family Moraceae, commonly called as fig family. It is a large dry deciduous or semi- evergreen tree with plenty of medicinal value in its bark and leaves. It is religiously very important tree in India. The aim of the present study was to investigate the antimicrobial potential of endophytic fungi isolated from the leaves of *F. religiosa*. Medicinal plants were reported to harbor endophytes[1], which in turn provide protection to their host from infectious agents and they are unexpected producers of metabolites useful to pharmaceutical and agricultural industries[2]. Many important anticancer, antifungal and antibacterial chemotherapeutics are either microbial metabolites or their semi synthetic derivatives. A single endophytic strain may produce multiple bioactive principles. Many of these compounds are alkaloids, steroids, terpenoids and peptides etc[3]. Some of the more interesting compounds produced by endophytic fungi are taxol, cryptocin, cryptocandin, jesterone, oocydin, isopestacin, pseudomycins and ambuic acid[4,5] Many endophytic fungi are known to produce antimicrobial substances[6]. Antimicrobial metabolites can be defined as low molecular weight organic natural substances produced by microorganisms particularly by endophytic fungi that are active at low concentrations against other micro organisms[7]. The crude extracts from the culture filterate of endophytic fungi have shown antimicrobial activity against pathogenic fungi, bacteria and yeasts. Some endophytic fungi metabolite shows cytotoxic activity on human cell line, anti-Herpes simplex virus type 1 activity (anti-HSV) and antimalarial activity against protozoan *Plasmodium falciparum*[8]. There are numerous studies on antimicrobial activity of endophytic fungi isolated from different geographical locations [9-18]. Thus, in the continuous search for novel drug sources, endophytic fungi have proven to be a promising reservoir of natural products,with great chemical diversitywhich is largely untapped. Thus, endophytes are the chemical synyhesizers inside the plant. Many of these fungal endophytes are capable of synthezing bioactive compounds which are similar to the host metabolite that can be used as potential sources of many pharmaceutical leads. Though, the feasibility of industrial production of bioactive compounds by endophytic fungal sources has still to be proven.

MATERIALS AND METHODS

Extraction of Metabolites

Endophytic fungi were grown in Petriplate, for 5 – 7 days. Fermentation was carried out in a 500 ml Erlenmeyer flasks containing czapek's broth and incubated for 21 days in 25 ^{o}C at 120 rpm. The fermentation broth of each endophytic fungi was sonicated and filtered using Whatman no.1 filter paper. The filtrate was extracted thrice with equal volume of ethyl acetate. The extracts from different endophytic fungus were concentrated in a rotary evaporator at 45 ^{o}C. the semisolid residue was redisolved in ethyl acetate for further study[19,20]

Invitro antibacterial assay

Dominant endophyte like *Botryodiplodia theobromae, Colletotrichum gloeosporioides, Curvularia lunata, Drechslera hawaiiensis* and *Phomopsis* sp crude extracts were tested against both gram positive and gram negative bacteria using disc diffusion method[21,22]. About 25µl of crude extracts from each endophytic fungus were added on to a sterile disc with a size of 5 mm and allowed to dry for 10minutes. The disc contains extracts were placed on the medium. The test was conducted in triplicates. A standard antibiotic chloremphenicol was used as a control. Chloramphenicol (1NN) is an antibiotic used for the treatment of a various of bacterial infections. It is considered as a broad-spectrum antibiotic and it is effective against a wide variety of gram (+) and (-) bacteria. The plates were incubated in culture rack at 25^{o}C to 28 ^{O}C ± 1 for 48 hours. The diameter zone of inhibition around the disc was measured using ruler[22,23].

Table 1
Inhibition zones resulting from crude extracts of endophytic fungi against the bacterial pathogens

Endophytes	*S. aureus* (+)	*S. epidermidis* (+)	*M. luteus* (+)	*E. aerogenes* (-)	*S. typhi* (-)	*K. pneumonia* (-)
Botryodiplodia theobromae	8.7±0.42	9.06±0.09	8.16±0.12	9.10±0.14	10.1±0.15	14.16±0.09
Colletotrichum gloeosporioides	14.13±0.12	18.3±0.21	19.23±0.2	13.8±0.50	8.3±0.21	18.66±0.40
Phomopsis sp	17.1±0.08	17.66±0.40	18.06±0.0	17.3±0.21	16.0±0.16	16.13±0.09
Curvularia lunata	18.03±0.04	21.10±0.08	9.76±0.47	19.10±0.08	19.23±0.1	17.73±0.44
Drechslera hawaiiensis	-	11.23±0.16	8.23±0.20	18.23±0.16	13.13±0.1	8.77±0.20
Control	17.13±0.04	16.1±0.9	17.18±0.3	18.11±0.1	17.0±0.4	18.0±0.3

Values are means of three replicates ± Standard Deviation

RESULTS AND DISCUSSION

Endophytic fungi were isolated from a wide variety of tropical trees, medicinal plants and herbs[24-29]. Presently there is a need to look for new bioactive compounds particularly microbial origin. The present antibotics are chemically synthesized and cause side effects. But infectious diseases are continue to be a universal problem because of the rapid development and spread of drug resistance among pathogens[30,31]. Culture extracts of five dominant endophytic fungi isolated from *Ficus religiosa* exhibited a wide variety of antibacterial activity (Table 1). The organic extract of *Botryodiplodia theobromae* does not have any activity against both gram positive and gram negative bacteria tested in the study (Table-1). *Colletotrichum gloeosporioides* extract showed poor activity against *Salmonella typhi* and moderate activity against *Enterobacter aerogenes* and *Staphylococcus aureus*. However, *Colletotrichum gloeosporioides* extract exhibited significant activity against *Staphylococcus epidermidis, Micrococcs luteus and Klebsiella pneumonia*. *Phomopsis* sp showed higher activity against. *Curvularia lunata* showed maximum activity against all tested organism except *Micrococcs luteus* where it showed least activity (Table 1). *Drechslera hawaiiensis* showed moderate activity against *Enterobacter aerogenes* and least activity against other tested organisms. There have been similar reports of antibacterial activity of endophytes from the tree as well as herbs[32-34]. Endophyte, *Quambularia pitereka,* extract was active against *Escherichia coli, Staphylococcus aureus, Salmonella typhimurium, Pseudomonas aeruginosa, Xanthomonas citri, Mycosphaerella fijiensis, Saccharomyces cerevisae* and *Bacillus subtilis*[35]. Ethyl acetate extract of the endophytes like *Chaetomium* sp. and *Colletotrichum gloeosporioides* isolated from *Acalypha indica*, showed more antibacterial activity against three bacteria viz., *B. subtilis*, *K. pneumoniae* and *S. aureus*[36]. A similar trend observed in the present study also. Antibacterial resistance especially among gram negative bacteria is an important issue that has created a number of problems in treatment of infectious diseases[37]. It is reported that the structural differences in the cell walls of different types of bacteria and fungi are likely to affect the performance of the crude extract but purification of compounds may further explain why the performance of the extract was poorer in gram-negative bacterium and pathogenic fungi. In the present investigation *C.lunata* and *Phomopsis* sp extracts were inhibited gram negative bacteria more significantly (Table 1). *Colletotrichum gloeosporioides* showed maximum activity against *Micrococcs luteus* (19.23 mm), *Phomopsis* sp showed maximum activity against *Micrococcs luteus* (18.06 mm) and least activity against *Salmonella typhi* (16.0 mm). Similarly *C. lunata* showed maximum activity against *S. epidermidis* (21.10 mm) and least activity against *Micrococcs luteus* (9.76 mm) (Table 1). *C. lunata* and *Phomopsis* sp showed good activity against both gram (+) and gram (-) bacteria. It was found that there were large differences among isolates from the same species with respect to their ability to produce metabolites with antimicrobial activity[38]. In the present study also, the antimicrobial spectrum of endophyte exhibited certain differences between different genera. The endophytic fungi, *Curvularia lunata, Phomopsis* sp and *Colletotrichum gloeosporioides* showed good antagonistic

activity towards the tested pathogens when compared to the *Drechslera hawaiiensis* and *Botryodiplodia theobromae.* The results of present and previous studies strongly suggest that metabolites of plant endophytic fungi could be good potential sources for screening programs of bioactive natural products. Moreover the effect of endophytic fungal strain in this study is quite prominent towards gram negative bacteria which are a causative agent for many infectious diseases. Hence screening other pharmaceutically important host species will broaden our knowledge of diversity of endophytic fungi and metabolite. However use of different solvent system, media and culture conditions must be standardized as purification and characterization of individual compound is essential for drug development.

CONCLUSION

To conclude documentation of endophytic fungi from other medicinal plants particularly pharmaceutically important plants may provide an endophytic fungi in large number that could be used to produce bioactive substances. Bioactive compounds which show maximum activity could be made to produce large quantities in commercial scale because the culture and maintance of endophytic fungi in laboratory and fermentor are comparatively easy. Further this prevents harvesting plants in large scale for extraction of bioactive compounds and affecting the environmental biodiversity.

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