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Antibacterial activity of some endophytic fungi isolated from *Cyprus rotundus* L.

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Abstract
During present study attempt were made to screened antibacterial activity of endophytic fungi isolated from *Cyprus rotundus* L. Endophytic fungi *viz*., *Aspergillus niger, A. flavus, A. fumigates, Alternaria alternate, Fusarium oxysporum, Rhizoctonia oryzae, Cladosporium harbaroids, Curvularia clavata,* and *Penicillium citrinum* were isolated from different parts of *Cyprus rotundus. Aspergillus niger, Alternaria alternata,* Fusarium oxysporum and *Penicillium citrinum* were selected for present study. The efficiency of extract was tested against five human pathogenic bacteria *viz. Staphylococcus aureus, Salmonella typhimurium, Pseudomonas aeruginosa, Shigella flexneri* and *Escherichia coli*. The extract showed remarkable antibacterial activity against tested bacteria. This activity of endophytic fungi may due to presence of different secondary metabolites.

Keywords: Endophytic fungi, *Cyprus rotundus*, antibacterial activity.

Introduction
Medicinal plants are known to harbor endophytic fungi that are believed to be associated with the production of pharmaceutical products. Endophytes have a great potential to contribute to the discovery of new bioactive compounds, from the intensive studies it has been suggested that, endophytes have close biological association with their plant host which results in the production of a great number and diversity of bio-active molecules. However, the symbiotic relationship of endophytes indicates that, endophytic biologically active compounds are less toxic to the cell and these chemicals do not damage the eukaryotic host system. This phenomenon is particularly important in the medical point of view as potential drugs obtain from the endophytes may not adversely affect human cells (Monali Desale, 2016; Eyob and Raju, 2018) [3].

Recent investigations in the field of bioactive metabolites of endophytic fungi have been intensified by potentialities in the production of biologically active compounds such as alkaloids, flavanoids, steroids, terpenoids, phenols, phenolic acids, quinones, isocoumarin derivatives and peptides. (Padhi, *et al.*, 2013) [12]. Polyketide citrinin a chemical compound produced by *Penicillium janthinellum* from fruits of *Melia azedarach*, showed 100% antibacterial activity against *Leishmania* species (Kusari and Spiteller, 2011) [9]. Antibacterial compounds, including fumitremorgins B isolated from *Phomopsis* spp., and periconicins A and B from *Periconia* spp. (Liang, *et al.*, 2012) [10]. Endophytic fungi has potentialities in the production of pigments, bioactive metabolites, anticancer compounds and bio control agents (Indira Kalyansundaram, *et al.*, 2015) [6].

More than 20,000 bioactive metabolites are of microbial origin and among these; fungi are the most important groups that are well known for producing many novel secondary metabolites, which are directly used as drugs or as various bioactive products (Berdy, 2005; Kharwar, *et al*., 2011) [2]. Endophytic fungi are found to be potent sources of biologically active compounds which are of interest for specific health care applications; these fungal strains are also found to be promisingly useful in the production of bioactive metabolites, anticancer compounds, immune suppressants and bio-control agents (Gangadevi and Muthumary, 2007) [4]. The several drugs of fungal origin such as antibiotic penicillin derived from *Penicillium sp.*, griseofulvin antifungal agent from *Penicillium griseofulvum,* lovastatin as cholesterol biosynthesis inhibitor from *Aspergillus terreus*, and cyclosporine the immunosuppressant from *Tolypocladium inflatum* and *Cylindrocarpon lucidum*, and

β-lactam a antibiotics from various fungal taxa, these some important views has shifted the focus of drug discovery from plants towards endophytic fungi (Gunatilaka, 2006; Stadler and Keller, 2008) [5, 14].

Material and Methods

Isolation of Endophytic Fungi

Fungal endophytes were obtained from apparently healthy, living root, stem and leaf tissues of *Cyprus rotundus* L., collected from different localities of Aurangabad city. Above mention parts of selected species were randomly collected from each plant. For surface sterilization of samples, segments from each part were washed with running tap water followed by sequential immersion in 2% (v/v) sodium hypochlorite solution (bleach), 70% ethanol sterilized distilled water (Shekhawat and Shah, 2013) [15].

Surface sterilization of plant material was done to eliminate unwanted epiphytes and other superficial contaminants. A sterilized blade was used to cut plant material, three pieces of 5 mm long root, stem and leaves tissue were excised and transferred immediately to Petri plates containing Potato dextrose agar (PDA) medium. An antibiotic Streptomycin (1% solution) was added to the PDA, to eliminate bacterial endophytes contamination. Sterilization procedure followed in present studies has been widely used in endophytes research. The prepared Petri plates were kept at low temperature (4-8^{0}C) in laboratory and incubated for 2-3 weeks; emerging colonies were sub-cultured to obtain pure isolates (Pranita, *et al.*, 2016) [13].

Preparation of fungal extract

Endophytic fungi *viz*., *Aspergillus niger*, *Alternaria alternate*, *Fusarium oxysporum* and *Penicillium citrinum* isolated from *Cyprus rotundus* were cultured on glucose nitrate medium (GN) for one week, then cultured were filtered through Whatman filter paper and kept for drying at room temperature. The prepared fungal mat were crushed with the help of mortar-pestle, methanol was used as solvent and filtered through Whatman filter paper. The filtrate were dried on hot water bath and diluted by adding dimethyl sulfonate (DMSO) in it. Prepared extracts were used for antibacterial activity.

Antimicrobial Activity

Antibacterial Assay

Test organism

Authentic culture of human pathogenic bacteria *viz*. *Salmonella typhimurium* (NCIM-2501), *Pseudomonas aeruginosa*, (NCIM-5029), *Shigella flexneri* (NCIM-5265), *Escherichia. coli* (NCIM - 2931) and *Staphylococcus aureus* (NCIM-5021) were obtained from department of Microbiology, Vivekanand Arts, Sardar Dalipsingh Commerce and Science College, Aurangabad. In-vitro antibacterial assay of fungal extract was carried out by using 96- well plate method.

96-well plates method

About 100µl sterile Mueller-Hinton broths medium was loaded into each well along with 2µl serial diluted human pathogenic bacteria suspension, next 2, 4, 6, 8 and 10µl concentrations of methanol fungal extract was added to each well of 96-well plate. Control was prepared by nutrient broth and bacterial suspension without adding extract. The prepared experimental 96-well plate was sealed with parafilm and incubated in incubator at 37°C for 24 hours. Finally optical density (OD) at 540nm was measured on spectrophotometer of each sample (Ataee, *et al.*, 2012; Jadhao and Bhuktar, 2017) [1, 7].

Results and Discussion

Aspergillus niger extracts in various concentrations were tested against the different human pathogenic bacteria; it was cleared from the results that maximum inhibition of *S. typhi*, *S. flexneri and S. arueus* were found in 2 µl concentration, in case of *P. aurignosa* and *E. coli* it was 6µl. Regarding minimum inhibitory concentration (MIC) it was 2 µl for *S. typhi*, *S. flexneri and S. arueus* and 6µl for *P. aurignosa* and *E. coli* (table 1, graph 1).

Alternaria alternata extracts in various concentrations were tested against different human pathogenic bacteria; it was cleared from the results that maximum inhibition of *S. typhi* were found in 8µl concentration, for *S. flexneri* and *P. aurignosa* it was 6µl, for *S. arueus* 2 µl and for *E. coli* 10µl. Regarding minimum inhibitory concentration (MIC) it was 8µl for *S. typhi*, for *S. flexneri* and *P. aurignosa* 6 µl, 2µl for *S. arueus* and 10µl for *E. coli* (table 2, graph 2).

Fusarium oxysporum extracts in various concentrations were tested against different human pathogenic bacteria; it was cleared from the results that maximum inhibition of *S. typhi* were found in 8µl concentration, for *S. flexneri* 2,8 µl, for *P. aurignosa* it was 4µl, for *S. arueus* 6µl and for *E. coli* 10µl. Regarding minimum inhibitory concentration (MIC) it was 8µl for *S. typhi*, 2, 8µl for *S. flexneri*, 4µl for *P. aurignosa*, 6µl for *S. arueus* and 10µl for *E. coli* respectively (table 3, graph 3).

Penicillium citrinum extracts in various concentrations were tested against different human pathogenic bacteria; it was cleared from the results that maximum inhibition of *S. typhi* were found in 10µl concentration, for *S. flexneri* 8 µl, for *P. aurignosa* 4µl, for *S. arueus* 6µl and *E. coli* 10µl. Regarding minimum inhibitory concentration (MIC) it was 10µl for *S. typhi*, 8µl for *S. flexneri*, 4µl for *P. aurignosa* and 6µl for *S. arueus* and 10µl *E. coli* respectively (table 4, graph 4).

Table 1: Antibacterial activity of *Aspergillus niger* extract

Sr. no	*Aspergillus niger*	*S. typhi*	*S. flexneri*	*P. aurignosa*	*S. arueus*	*E. coli*
1	2 µl	0.19	0.24	0.23	0.22	0.26
2	4 µl	0.23	0.26	0.27	0.23	0.32
3	6 µl	0.29	0.29	0.19	0.27	0.24
4	8 µl	0.25	0.29	0.24	0.24	0.27
5	10 µl	0.27	0.31	0.25	0.23	0.29
MIC		2 µl	2 µl	6 µl	2 µl	6 µl
Control		0.02	0.01	0.01	0.00	0.00

Table 2: Antibacterial activity of *Alternaria alternata* extract

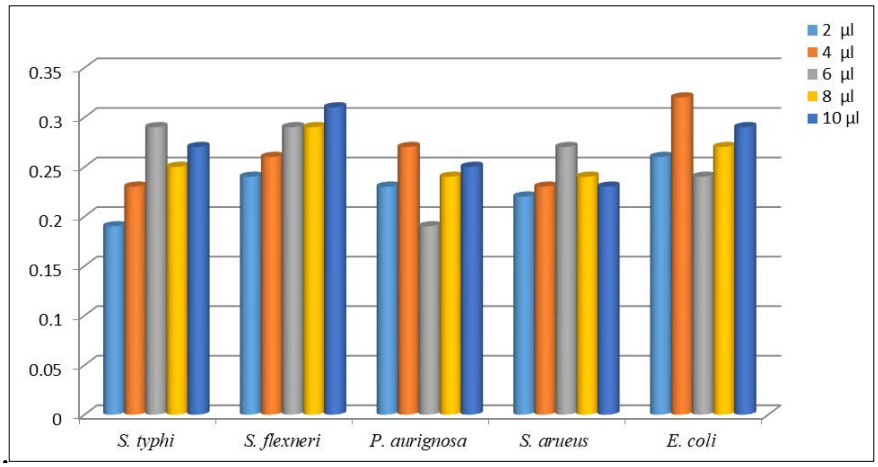
Sr. No	*Alternaria alternata*	*S. typhi*	*S. flexneri*	*P. aurignosa*	*S. arueus*	*E. coli*
1	2 µl	0.17	0.22	0.19	0.17	0.26
2	4 µl	0.21	0.25	0.27	0.21	0.30
3	6 µl	0.23	0.20	0.24	0.26	0.24
4	8 µl	0.15	0.29	0.24	0.24	0.25
5	10 µl	0.27	0.31	0.26	0.22	0.19
MIC		8µl	6µl	6 µl	2 µl	10µl
Control		0.01	0.01	0.02	0.00	0.01

Table 3: Antibacterial activity of *Fusarium oxysporum* extract

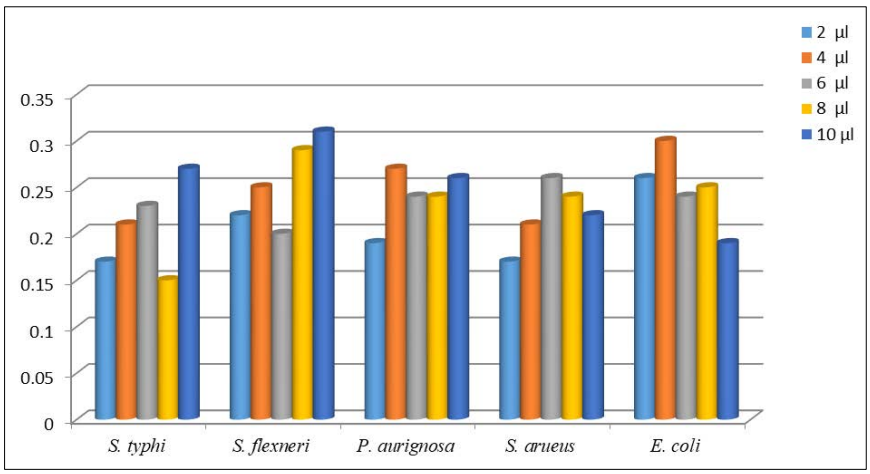
Sr. No	*Fusarium oxysporum*	*S. typhi*	*S. flexneri*	*P. aurignosa*	*S. arueus*	*E. coli*
1	2 µl	0.04	0.03	0.04	0.04	0.04
2	4 µl	0.08	0.06	0.02	0.05	0.05
3	6 µl	0.03	0.04	0.05	0.02	0.06
4	8 µl	0.02	0.03	0.03	0.07	0.05
5	10 µl	0.07	0.08	0.06	0.04	0.02
MIC		8 µl	2,8 µl	4 µl	6 µl	10 µl
Control		0.00	0.00	0.00	0.00	0.00

Table 4: Antibacterial activity of *Penicillium citrinum* extract

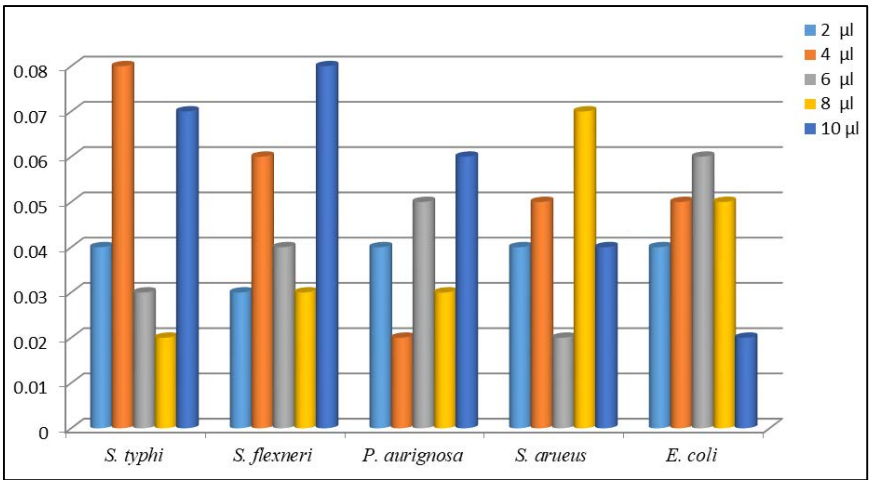
Sr. no	*Penicillium citrinum*	*S. typhi*	*S. flexneri*	*P. aurignosa*	*S. arueus*	*E. coli*
1	2 µl	0.12	0.16	0.09	0.04	0.04
2	4 µl	0.16	0.15	0.05	0.04	0.07
3	6 µl	0.16	0.14	0.10	0.05	0.05
4	8 µl	0.15	0.09	0.13	0.02	0.06
5	10 µl	0.09	0.12	0.08	0.06	0.03
MIC		10 µl	8 µl	4 µl	6 µl	10 µl
Control		0.00	0.00	0.00	0.00	0.00



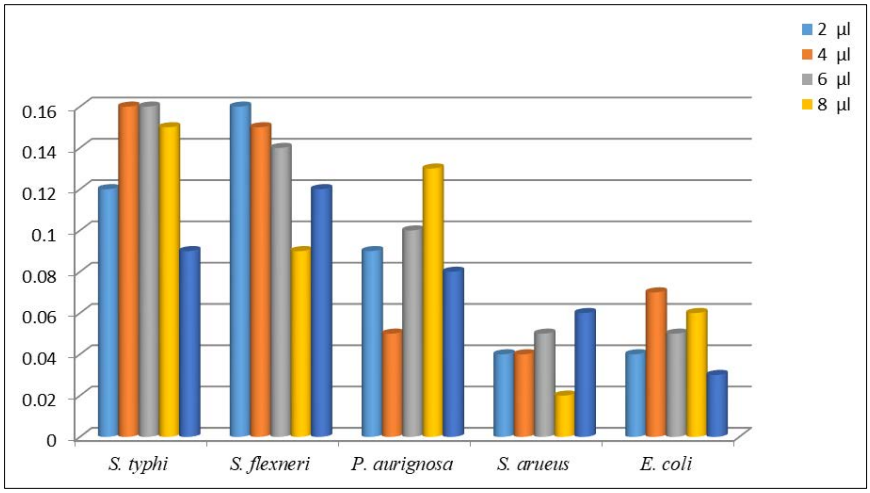
Graph 1: Antibacterial activity of *Aspergillus niger*



Graph 2: Antibacterial activity of *Alternaria alternata*



Graph 3: Antibacterial activity of *Fusarium oxysporum*



Graph 4: Antibacterial activity of *Penicillium citrinum*

Fig 1: *Cyprus rotundas* L.

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