IJMPR 2020, 4(6), 137-141

International Journal of Modern Pharmaceutical Research
www.ijmpronline.com

ISSN: 2319-5878
IJMPR
Research Article
SJIF Impact Factor: 5.273

ANTIMICROBIAL POTENTIAL OF ENDOPHYTIC FUNGI ISOLATED FROM LEAF SAMPLES OF THE MEDICINAL PLANTS, *ABUTILON INDICUM* L. AND *ACMELLA OLERACEA* L.

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Received on: 21/09/2020
Revised on: 11/10/2020
Accepted on: 30/11/2020

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ABSTRACT

During the study period, endophytic fungi were isolated from leaf samples of two medicinal plants, *Abutilon indicum* L. and *Acmella oleracea* L. collected from our college APJ Abdul Kalam Eco-garden. The isolated fungi were subjected to antimicrobial analysis by active agar plug method in order to find their potency against pathogenic MTCC bacterial and fungal strains procured from IMTECH, Chandigarh, India. There are three endophytic fungi viz., *Aspergillus niger*, *Cladosporium herbarum* and *Fusarium* sp. were isolated from the medicinal plant, *Abutilon indicum* and two fungi i.e., *Aspergillus japonicus* and *Trichoderma viride* from *Acmella oleracea* L. Pathogenic bacteria i.e., *Klebsiella pneumoniae* and *Bordetella pertussis* were found to be more susceptible towards five endophytic fungi followed by other bacteria and *Candida albicans*. *Escherichia coli* and *Candida albicans* were found to be least affected by the fungal extracts. *Pseudomonas aeruginosa* was documented as average susceptible pathogenic bacteria among all. Active agar plugs of endophytic fungus, *Aspergillus niger* was recorded as a good inhibitor of microbial growth of both bacteria and *Candida albicans* in vitro comparison to other endophytes.

KEYWORDS: Antimicrobial potential, Endophytic fungi, Medicinal plants, *Abutilon indicum* L. and *Acmella oleracea* L.

INTRODUCTION

In general, endophytic fungi are the endodermal inhabitants of the plants especially leaves, stems, roots without causing outward harm to host.[1] They used to dwell almost all classes of vascular plants and grasses examined till date [2]. In addition to fungi, various other groups of microbes i.e., bacteria, actinomycetes and mycoplasma are reported as endophytes of plants[3]). The research on endophytic fungi and their huge diversity among plants has a long history. Recently it has been reported that each plant harbors one or more endophytic fungi.[4,5] These endophytic fungi are an outstanding source of secondary metabolites which possess a variety of bioactive potency.[6,7] They have received considerable attention for last twenty years for their capacity to protect against deadly pathogens.[2,3] Besides this much interest has been taken by Botanists to carry out research into the plant endophyte relation.[1]

Endophytic fungi have been the foundation of several important complexes owning potential bioactivities. Moreover, many more compounds from endophytic fungi have been isolated, purified and characterized by various researchers. The present study deals with the isolation and identification of endophytic fungi from leaf samples of two medicinal plants, *Abutilon indicum* L. and *Acmella oleracea* L. collected from Dr. APJ Abdul Kalam Eco-garden followed by the antimicrobial analysis of these endophytic fungi by active agar plugs in order to find their potency against pathogenic microbes.

MATERIALS AND METHODS

Collection of samples

Fresh leaves of *Abutilon indicum* L. and *Acmella oleracea* L. were collected from Dr. APJ Abdul Kalam Eco Garden of our P.G. centre, KMGIPSR, Puducherry. Healthy and mature leaf samples were carefully collected and brought to the Microbiology laboratory, Department of Botany and kept in sterile conditions for the isolation and enumeration of endophytic fungi.

Surface sterilization of leaves

After the collection of healthy leaves, leaf samples were thoroughly washed in running tap water. Then the leaves were cut into small segments (about $1cm^2$) including midrib portion. The leaf samples were surface sterilized by 70 % ethyl alcohol for 60 seconds and then rinsed in sterile distilled water for 10 seconds (three times

Culture of leaf samples on agar plates

Five (5) leaf segments of a centimetre square, both sterile an unsterile were placed separately on the PDA media

plates equidistantly by the help of sterile forceps and pressed later on followed by incubation for 3 to 7 days.

Isolation of fungi

After sterilization, the excess water was blotted out by sterile filter paper from the leaf segments and kept separately. The surface sterilized leaf segments (each 5) were placed equidistantly in the PDA mediated petridishes containing with antibiotics by sterile forceps. All the plates were incubated at 25±3ºC for 7 to 8 days in the BOD incubator for fungal growth. Observation of the petriplate was done from second day onwards to check the growth for enumeration and identification of the fungal CFUs.

Identification of fungi

After two days of incubation, the fungal colonies were counted for individual CFUs and the fungi were identified. Microscopic slides stained with lacto phenol cotton blue were prepared from each colony of the fungus and observed microscopically under the light microscope to identify directly them up to species level. The colony which could not be identified directly from plates was sub cultured in SDA/PDA media again and identified later on. The laboratory experience and taxonomic literature were employed to identify the fungal taxa.[8,9,10,11,12,13] The presence and absence based on the occurrence of individual fungus in the phylloplane and endophytic were determined and plotted in the form of tables and figures.

Antibacterial activity of the active agar plugs of endophytic fungi

The active agar plugs of 6mm size were taken carefully from the three-day pure culture plates of the endophytic fungi and were studied for antimicrobial activity against pathogenic bacteria (clinical isolates) using agar plug assay method. The test organisms used were from MTCC culture i.e., *Pseudomonas aeruginosa, Klebsiella pneumoniae, Bordetella pertussis, Escherichia coli* and *Candida albicans.* The microbes were grown in nutrient broth for 12 h. Lawns of pathogenic bacteria and *Candida albicans* were prepared on nutrient agar plates using sterile cotton swabs. Active agar plugs were placed on nutrient agar plates and each plug was placed inside the wells prepared earlier by cork borer. The plates containing bacteria and active plugs of endophytic fungi discs were incubated at 37^{o}C for 24 to 48 hours in the BOD incubator. The plates were examined for the zone of inhibition after 24 hrs, which appeared as clear area around the wells. Inhibition zone diameter was measured in mm by the HI-Media scale.

RESULTS AND DISCUSSION

During the present study period, endophytic fungi were isolated from the leaf samples of two medicinal plants, *Abutilon indicum* L. and *Acmella oleracea* L. collected from our college Dr. APJ Abdul Kalam Eco-garden. The isolated fungi were subjected to antimicrobial analysis by active agar plug method in order to find their potency against pathogenic MTCC bacterial and fungal strains procured from IMTECH, Chandigarh, India. Altogether, five endophytic fungi viz., *Aspergillus niger, Cladosporium herbarum, Fusarium* sp., *Aspergillus japonicus* and *Trichoderma viride* were isolated from both the medicinal plants, *Abutilon indicum* L. and *Acmella oleracea* L. Among the bacterial strains, *Klebsiella pneumoniae* and *Bordetella pertussis* were found to be more susceptible towards five endophytic fungi followed by other bacteria and *Candida albicans*. Table 1 shows the antimicrobial potential of endophytic fungi of *Abutilon indicum* L. against pathogenic bacteria and fungus. Likewise, Table 2 shows about the endophytic fungi of *Acmella oleracea* L. *Escherichia coli* and *Candida albicans* were found to be least affected by the fungal extracts. *Pseudomonas aeruginosa* was documented as average susceptible pathogenic bacteria among all. Active agar plugs of endophytic fungus, *Aspergillus niger* was recorded as a good inhibitor of microbial growth of both bacteria and *Candida albicans* in vitro comparison to other endophytes (Table 1, Fig 1, 2 and 3). *Aspergillus japonicus* was found to be good against *Pseudomonas aeruginosa* and *Trichoderma viride* was good against *Bordetella pertussis* in order to control the growth of the pathogens (Table 2). Fig 1 established the antimicrobial effect of endophytic fungi isolated from *Abutilon indicum* against pathogenic microbes and Fig 2 confirmed the antimicrobial effect of endophytic fungi isolated from *Acmella oleracea*. Sandhu et al [14] worked on the isolation and identification of endophytic fungi from *Ricinus communis* L. and their antibacterial activity too. They isolated 10 fungal species as endophytic fungi from *Ricinus communis* including *Aspergillus fumigatus, Aspergillus japonicas, Aspergillus niger, Fusarium semitectum, Curvularia pallescens, Phoma hedericola, Alternaria tenuissima, Fusarium solani* and *Aspergillus repens*. The fungal extracts were assessed for antibacterial activity against six human pathogenic bacterial strains: *Bacillus subtilis, Enterococcous* sp., *Klebsiella pneumoniae, Escherichia coli, Salmonella typhimurium* and *Staphylococcus aureus.* Most of the extracts showed *in vitro* inhibition of bacterial growth. The phytochemical screening revealed the existence of a diverse group of secondary metabolites in the crude extracts of the endophytic fungi that resemble those in the host plant extracts.

Table 1: Antimicrobial potential of endophytic fungi of *Abutilon indicum* L. against pathogenic bacteria and fungus.

Sl. No.	Pathogens Bacteria/Fungus	Endophytic fungi	Zone of inhibition in mm
1	*Pseudomonas aeruginosa*	*Cladosporium herbarum*	15
		Aspergillus niger	19
		Fusarium sp.	14
2	*Klebsiella pneumoniae*	*Cladosporium herbarum*	18
		Aspergillus niger	22
		Fusarium sp.	17
3	*Bordetella pertussis*	*Cladosporium herbarum*	18
		Aspergillus niger	21
		Fusarium sp.	18
4	*Escherichia coli*	*Cladosporium herbarum*	10
		Aspergillus niger	12
		Fusarium sp.	12
5	*Candida albicans*	*Cladosporium herbarum*	10
		Aspergillus niger	12
		Fusarium sp.	13

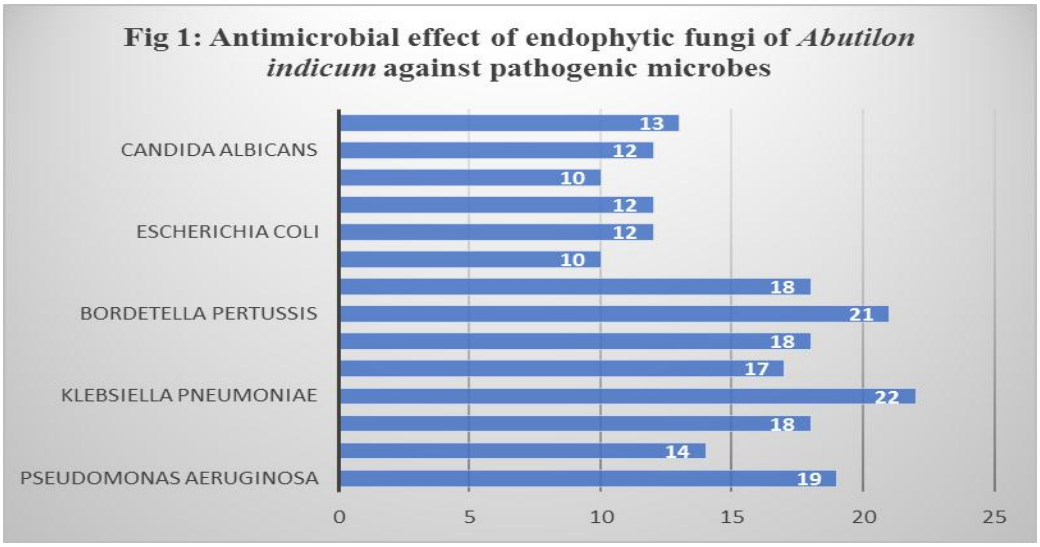


Fig 1: Antimicrobial effect of endophytic fungi of *Abutilon indicum* against pathogenic microbes

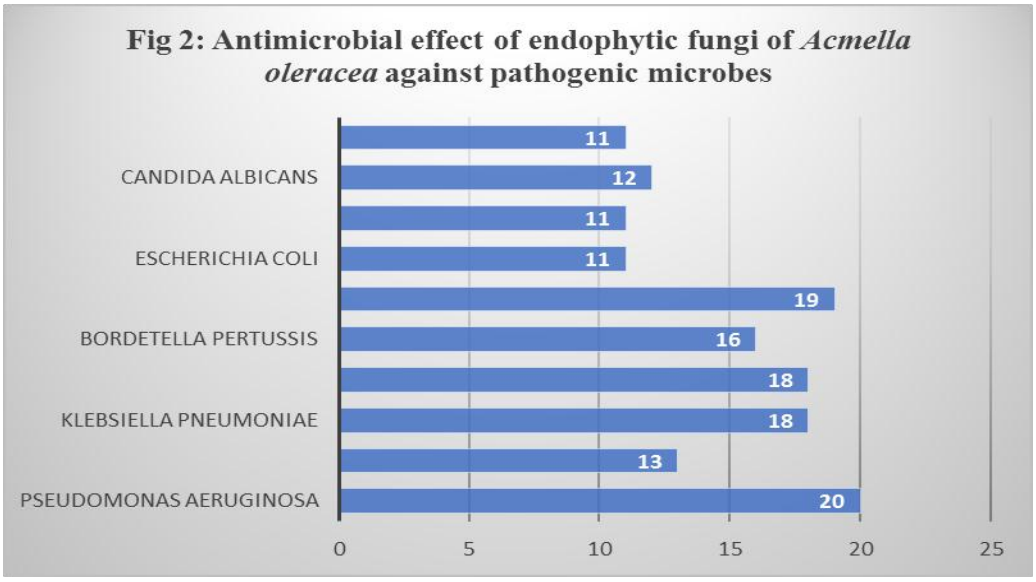
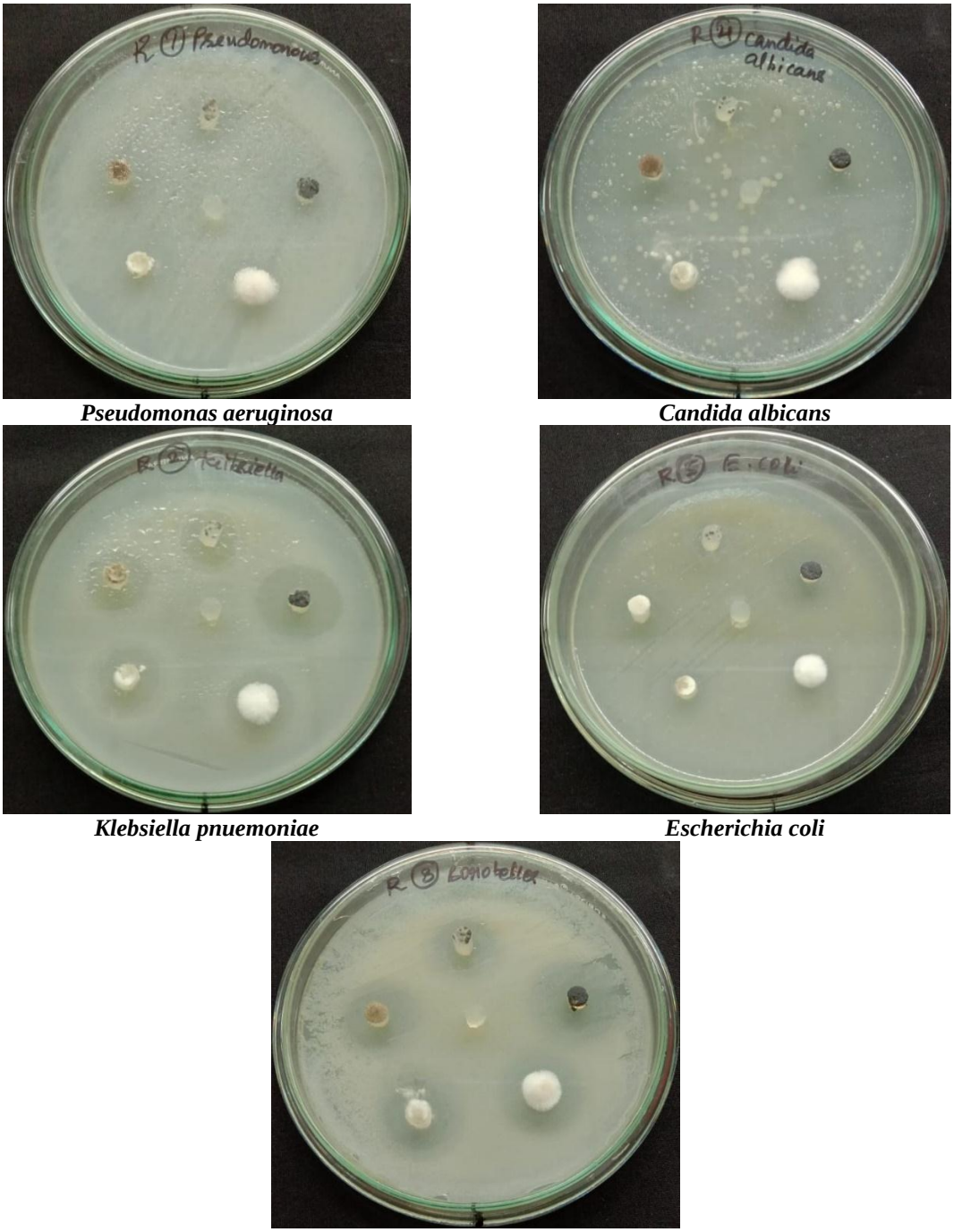


Fig 2: Antimicrobial effect of endophytic fungi of *Acmella oleracea* against pathogenic microbes



Pseudomonas aeruginosa ***Candida albicans***

Klebsiella pnuemoniae ***Escherichia coli***

Bordetella pertussis

Fig 3: Antimicrobial potential of active agar plugs of endophytic fungi against pathogens.

CONCLUSION

In the present study, endophytic fungi isolated from two medicinal plants i.e., *Abutilon indicum* L. and *Acmella oleracea* L. showed good antimicrobial activities against five pathogenic bacteria and *Candida albicans*. Endophytic fungus, *Aspergillus niger* was found to have more potential in order to prevent the growth of all microbes studied herewith. *Bordetella pertussis* and *Klebsiella pnuemoniae* were found to be more susceptible bacteria towards the endophytic fungi. *Escherichia coli* and *Candida albicans* were more resistant towards the endophytic fungi. It was documented that the endophytic fungi have good potency as antibiotics in our study.

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