ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Diversity of Endophytic Fungi from A Medicinal Plant, *Ormocarpum Cochinchinense* And Its Antibacterial Activity.

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ABSTRACT

The medicinal plant, Ormocarpum cochinchinense belonging to family Papilionaceae (Fabaceae) is widely used for mending bone fracture. This is popularly called as "Elumbotti" in vernacular Tamil which means mending the bone. The plant is widely distributed in scrub jungles of South India. Fungi in general are omnipresent, among them, Endophytic fungi are the ones associated with the plant as asymptomatic pathogens. The benefit of association between the fungus and the plant is to serve as a defence mechanism against other microbes. These fungi in general are found to possess medicinal values. Thus, the diversity of endophytic fungi present in the plant, Ormocarpum cochinchinensewas studied using Potato Dextrose Agar (PDA) amended with Streptomycin antibiotic to control bacterial growth. The tissues of leaf, stem and root of the plant were examined for the presence of endophytes. A total of 150 segments inoculated onto the plates containing PDA yielded the presence of 57 isolates. The species of Acremonium, Alternaria, Aspergillus, Aureobasidium, Chaetomium globosum, Fusarium, Humicola, Oidium, Trichocladiumand Ulocladiumwere recorded. Among them the species of Acremonium, Phomaand Trichocladiumare found to be dominant. Thus, the culture filtrate of dominant fungi were studied for their antibacterial activity. The methanol chloroform extract of culture filtrate were examined for their antibacterial activity against, Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus and Streptococcus mutans were studied using disc diffusion method.

Keywords: Ormocarpum cochinchinense, Endophytic fungi, Acremonium,Phoma,Myrothecium sp,Antibacterial activity

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ISSN: 0975-8585

INTRODUCTION

Ormocarpum cochinchinense (Lour.) Merr. Belonging to papillionaceae (Fabaceae) is a shrub,found in dry evergreen to dry deciduous forests, moist deciduous and semi – evergreen forests. The plant is widely distributed in South and South East Asia. The roots of the plant are considered to be tonic and stimulant which is used in the treatment of lumbago and paralysis. Ormocarpum cochinchinenseis a shrub which is extremely efficacious in mending bone fractures, but at present its use is known only to handful of villagers in the tropical dry evergreen forest areas of Tamil Nadu for healing fractures (Maria john et al., 2011[1]. Dinesh Kumar et al., 2013) [2].

Endophytes are symbiotic microbial organisms that inhabit the interior of plants without causing an apparent harm to the host (Hirsch &Baraun, 1992) [3]. Endophytes belong to diverse groups of bacteria and fungi (Bandara et al., 2006) [4]. Plant endophytic fungi have the ability to produce the same or similar compounds to those originating from their plants (Erbert at al 2012) [5]. As well as a great number of diverse bioactive compounds (Devarju and Satish, 2010) [6]. Which have been implicated in the protection of its host against pathogens and herbivores (Wicklow et al., 2005) [7]. Endophytic fungi are the ones associated with the plant as symptomatic pathogens. The benefit of association between the fungus and the plant is to serve as a defence mechanism against other microbes. These fungi in general are found to possess medicinal values and antimicrobial activity. In this study, the diversity of endophytic fungi present in the medicinal plant, Ormocarpum cochinchinensewas studied. The methanol:chloroform extracts of fungal exudates of few endophytes isolated were studied for their antibacterial activity.

MATERIAL AND METHODS

Samples collection

The plant samples of Ormocarpum cochinchinenseare collected from Magamai Thirumani villages belonging to the District of Thiruvannamalai, Tamil Nadu State in India. The plant was authenticated and identified by a plant taxonomist Dr.Jayaraman of Plant Anatomy Research Centre, Chennai. The plant herbarium is maintained in the Herbarium collection of Presidency College, Chennai

ISOLATION OF ENDOPHYTES

Tissue samples of healthy leaves, stem and root of the plant collected were washed thoroughly in running tap water followed by double distilled water before processing. The samples were cut into small pieces of the size of 1.0 × 1.0 cm pieces; leaves were cut into small discs using sterile cork borer. The samples were immersed in 70% ethanol 1 – 3 min, and then mercury chloride for a period of 30 sec. Each sample was then dried under aseptic conditions. Segments of samples were placed on potato dextrose agar (PDA). The parafilm sealed Petri dishes were then incubated at 28^{o}C ± 2^{o}C and the plates were examined on alternate days and hyphal tips of actively growing fungi were subcultured. The sporulatingcolonies were identified using standard manuals. (Guba, 1961; Ellis, 1971[8]. Sutton 1980 [9]. And Nagraj, 1993) [10].The non-sporulating isolates were classified under morphospecies.

STATISTICAL ANALYSIS

$$\text{Endophytic Infection Rate (EIR \%): EIR} = \frac{\text{Total no. of endophytic fungi recorded}}{\text{Total no. of segments}}$$

$$\text{Relative Density of Colonization: rD} = \frac{\text{Total no. of individual cfu recorded}}{\text{Total no. cfu recorded}}$$

Preparation of fungal extract

Among the species of endophytes isolated, Acremoniumstrictum, Phomasp. and Trichocladiumsp. are found to be dominant. Thus, dominant fungal species were cultured, and the methanol:chloroform(2:1)

extracts of the culture filtrate was obtained. The fungal species were sub-cultured in 500ml Ehrlenmeyer's flask possessing 300ml of PD broth. Ten days fungal culture filtrate was mixed with equal amount of methanol chloroform solvent and the extract was allowed to dry. These extract was used for studying antibacterial efficacy.

Antibacterial activity

Source of Microorganisms:

Microbial cultures of Bacillus subtilis(MTCC121), Escherichia coli (MTCC443),Klebsiella pneumoniae (MTCC1320), Staphylococcus aureus (MTCC96) and Streptococcus mutans (MTCC 890) were obtained from Microbial Type CultureCollection and Gene Bank, Chandigarh, India.

Kirby-Bauer's Disc diffusion method

Mueller Hinton agar plates were spreadwith 100 µl of actively growing broth cultures ofthe respective bacteria and are allowed to dry for10 minutes. The sterile readymade discs loadedwith each extract individually (15 µl/disc, 20µl/disc and 25 µl/disc) were imposed on theinoculated plates. The plates were thenincubated at 37^{o}C for 36 hours. Thedevelopment of the inhibition zone around theextract loaded disc was recorded. Sterile discwith respective solvent of 25 µl was used asnegative control and Streptomycin at 10mg/disc was used as positive control.

RESULT AND DISCUSSION

The study on the endophytic diversity leaf, stem and root of the plant, Ormocarpum cochinchinense resulted in isolation of 57 isolates classified under 12 different species. All the species belongs to Mitosporic fungi except that of Chaetomium globosum which belongs to Ascomycetes. Among Mitosporic fungi, 2 belongs to Coelomycetes and all other species to Deuteromycetes[10]. Few non sporulating fungi were also recorded. These non sporulating species were classified under morpho species in this study. The list of fungi isolated, their infection rate in the tissues of leaf, stem and root are presented in Table 1.

The non sporulating colonies or morphospecies are found to be dominant as leaf endophyte followed by species of Oidium, Acremonium strictumand Fusarium oxysporum. The dominance of Humicolagriseawas seen in stem as well as root of the plant Ormocarpum cochinchinense. The second dominance was found to be Aureobasidium pullulansin caseof stem and it was Myrothecium sp case of root. All other species like Aspergillus flavus, Chaetomium globosum, Myroecium sp, Fusarium oxysporum, Phomasp. and Trichocladium sp. were found to occur equally in stem. The species, i.e. Acremonium strictum, Alternaria alternate, Trichocladium sp. and Uloclasdium botrytis were contributed equally in root of the plant. The relative density of colonization recorded for the present study is presented in Table II.

The antibacterial activity evaluated for the dominant fungal species, i.e. Acremonium strictum, Phomasp. And Myrothecium sp. showed different antibacterial potency of individual fungus. All the three fungi studies showed their inhibition against the bacteria, Bacillus subtilis, Staphylococcus aureus and Streptococcus mutans. However, their efficacy was found to differ . It was Acremoniu strictumwhich showed maximum efficacy against the three bacteria. The antibacterial was recorded only by Acremonium strictumagainst the bacteria, Escherichia coli. Similarly, Phomasp. recorded a slight zone of inhibition against Klebsiella pneumoniae. The antibacterial activity recorded against different bacterial pathogens is presented in Table III.
The study revealed the presence of endophytes of different forms, i.e. Ascomycetes, Coelomycetes and Deuteromycetous fungi. The presence of Basidiomycetous fungi might have been hidden as a non-sporulating fungi in this case as most of the basidiomycetes are non-sporulating or non-fruit body formers in laboratory cultures. The endophytic diversity recorded from the plant Ormocarpum cochinchinense includes few pathogenic forms like Alternaria alternata, Fusarium oxysporumand Oidiumsp. These fungi are characterized as a pathogenic form without forming a symptoms or asymptomatic fungal endophytes (Clay et al, 2002) [11].

Endophytic fungi within host tissues are termed as a chemical synthesizer. Specifically, endophytes harboured by the medicinal plants are recognized as of industrial importance. Many endophytic fungi are found to possess antibacterial activity. A detailed review on the biodiversity and biopotency of endophytes

was reviewed by Mishra et al., (2015). A detailed review on the endophytes serving as a reservoir of antibacterial compound is studied by Deshmukhet al., (2015) [12].

Table I: Endophyte Infection Rate of fungi recorded in Ormocarpum cochinchinense

S.No	Species	Leaf	Stem	Root
1	Acremoniumstrictum	1.96	-	1.51
2	Alternariaaltenata	-	-	1.51
3	Aspergillus flavus	-	1.11	-
4	Aureobasidium pullulans	-	2.22	-
5	Chaetomiumglobosum	0.98	1.11	-
6	Myrothecium sp	-	1.11	2.27
7	Fusarium oxysporum	1.96	1.11	-
8	Humicolagrisea	-	6.66	6.81
9	Oidiumsp.	2.94	-	-
10	Phomasp.	0.98	1.11	0.07
11	Trichocladiumsp.	-	1.11	1.51
12	Ulocladium botrytis	-	-	1.51
	Non sporulating colony	7.8	1.11	1.51

Table 2: Relative Density of Colonization of endophytic fungal isolates recorded for Ormocarpum cochinchinense

S.No	Species	Leaf	Stem	Root
1	Acremoniumstrictum	7.69	-	7.69
2	Alternariaaltenata	-	-	7.69
3	Aspergillus flavus	-	3.84	-
4	Aureobasidium pullulans	-	7.69	-
5	Chaetomiumglobosum	3.84	3.84	
6	Myrothecium sp	-	3.84	11.53
7	Fusarium oxysporum	7.69	3.84	-
8	Humicolagrisea	-	23.07	34.61
9	Oidiumsp.	11.56	-	-
10	Phomasp.	3.84	3.84	3.84
11	Trichocladiumsp.	-	3.84	7.69
12	Ulocladium botrytis	-	-	7.69
	Morpho species	30.76	3.84	7.69

Table 3: Antibacterial activity (Zone of inhibition) recorded for the extracts of endophytic fungi

Sl.No.	Species	Acremoniumstrictum			Phomasp.			Myroecium sp.		
		15µl/ disc	20µl/ disc	25µl/ disc	15µl/ disc	20µl/ disc	25µl/ disc	15µl/ disc	20µl/ disc	25µl/ disc
1	Bacillus subtilis(MTCC121)	12	14	18	-	8	10	8	10	12
2	Escherichia coli (MTCC443)	8	10	12	-	-	-	-	-	-
3	Klebsiella pneumoniae (MTCC1320)	-	-	-	-	8	8	-	-	-
4	Staphylococcus aureus (MTCC96)	12	16	16	8	10	10	6	8	8
5	Streptococcus mutans (MTCC 890)	10	14	14	8	10	10	9	10	12

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