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Antimicrobial Activity of Endophytic Fungi Isolated from Ethnomedicinal Plant *Phyllanthus reticulatus* Poir.

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ABSTRACT*: The objective of the present study was to isolate Endophytic Fungi from ethnomedicinally important plant Phyllanthus reticulatus Poir. [Kirganelia reticulata (Poir.) Baill.] and to evaluate antimicrobial activities against human pathogens. A total of seven endophytic fungi were isolated and identified from the leaves and stem of Phyllanthus reticulatus Poir. Viz., Geotrichum candidum, Cylindrocladium sp. Fusarium sp. Cladosporium cladosporioides sp., Mucor pusillus, Rhizopus sp. and Alternaria alternata. The cell free ferment broth was subjected to antimicrobial assay against human pathogenic microbes such as Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus and Candida albicans. The results of the present study showed that Cladopsporium cladosporioides has antibacterial activity against Pseudomonas aeruginosa and Fusarium sp. is found to be effective against Escherichia coli. While other endophytic fungi tested against human pathogens did not show any effectiveness. This study has proven that Phyllanthus reticulatus Poir. is a very good source of endophytic fungi with potential to produce bioactive compounds having antibacterial and antifungal effect.*

KEYWORDS: *Antimicrobial, Endophytic fungi, Phyllanthus reticulatus Poir.*

I. INTRODUCTION

Endophytes are microorganisms that are present in living tissues of various plant parts establishing natural relationship without apparently causing any symptom of diseases [1, 2]. These endophytes protect their hosts from infectious agents and adverse conditions by securing bioactive secondary metabolites [3, 4]. Endophytes are recognized as rich sources of bioactive metabolites of multifold importance in medicine, agriculture [5]. Many endophytic fungi have been reported to produce novel antibacterial, antifungal, antiviral, anti-inflammatory, antitumor, and other compounds belonging to the alkaloids, steroid, flavonoid, and terpenoids derivatives types [6]**.** Fungal endophytes protect their hosts from contagious agents and withstand at adverse conditions by discharging active metabolites [7]. Endophytic fungi are a good source of antibiotics. Natural products from entophytic microbes have been observed to inhibit or kill wide variety of harmful disease causing agents. Endophytic fungi are also capable to produce antimicrobial metabolites. The production of Hypericin, Anaphthodianthrone derivative and Emodin are believed to be the main precursor of hypericin [8]. A compound polyketidecitrinin produced by endophytic fungus *Penicillium janthinellum* from fruits of *Melia azeadirachta*, presented 100 % antibacterial activity against *Leishmania* sp. [9]. These creatures were drawing a great attention after the discovery of fungi *Taxus brevifolia*, producing the anti-cancer drug taxol [10]. Endophytic fungi are also reported from *Avicennia officinalis* [11].

Medicinal plants play a crucial role in providing primary health care to human populations, since the dawn of civilization. The knowledge of medicinal plants has been accumulated from different medicinal systems such as Ayurveda, Unani, and Siddha. During the last few decades, there has been an increasing interest in the study of these medicinal plants has been witnessed in different parts of the world mainly due to many problems associated with synthetic drugs and with the emergence of multi-drug resistant pathogens [12]. Medicinal plants contain a wide variety of radical scavenging molecules such as phenolic compounds, quinones, coumarins, lignins, tannins, alkaloids, amines, vitamins, terpenoids communities produce similar therapeutic products and other endogenous metabolites [13-15]. It was assumed that medicinal plants and their fungal endophytic organisms that exist in the tissues of living plants are potential resources of novel natural products for exploitation in pharmaceutical and agricultural industries [16]. *Phyllanthus reticulatus* Poir. belongs to the family – Euphorbiaceae commonly known as karnelli, synonym: *Kirganelia reticulata* (Poir.) Baill. It is an erect or straggling shrub; branch slender**,** drooping. Leaves upto 3 x 1.5cm, elliptic-orbicular, rounded at both ends, glabrescent; petioles 3 mm long; stipules minute. Flowers in axillary fascicles; petioles filiform. Calyx lobes 5, 3 inner often larger. Stamens 5, in two series; the outer short and free; the inner three slightly larger and

connate. Ovary 5- many celled; ovules 2 per cell, superposed. Fruit baccate, 6 mm in diameter, globose, purple - black when ripe. Flowering September- April [17].

Phyllanthus reticulatus is an ethnomedicinal plant and leaf extraction showed antidiabetic, antiplasmodial, hypocholesterolemic, antimicrobial, hepatoprotective activity, analgesic and anti-inflammatory activity [18]. Hence, the present study dealt with the isolation of endophytic fungi from both leaf and stem segments of *Phyllanthus reticulatus* and their antimicrobial activity against selected human pathogens were studied.

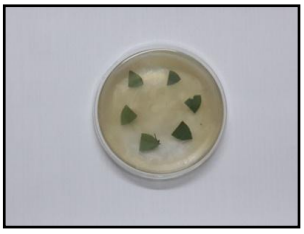
II. MATERIALS AND METHODS

2.1 Plant collection and identification

Healthy mature leaves and stems of medicinal plant *Phyllanthus reticulatus* were collected from different places of Udupi District of Karnataka State in plastic bags. The collected plant material was authenticated by the Taxonomist Dr. K. Gopalakrishna Bhat, Department of Botany, Poornaprajna College, Udupi, Karnataka, India. Fresh plant materials were used for isolation of endophytic fungi and evaluation of their antimicrobial activities.

2.2 Isolation of endophytic fungi

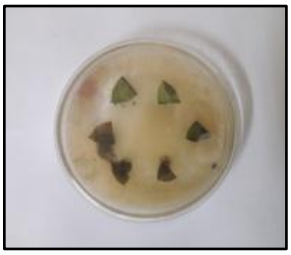
The plant materials were rinsed gently in running tap water to remove dust and debris. After proper washing, the leaf and stem is cut into small pieces of 0.5-1cm in length. Highly sterile conditions were maintained during the isolation of endophytes. All the work was performed in the laminar air hood. Sterile glass wares and mechanical things, such as scissors, forceps, scalpel, and blades were used. The isolation of endophytic fungi was done according to the standard method [19]. The surface sterilization was done by sodium hypochlorite (NaOCl) and 95% ethanol. Finally the leaves were washed in sterile distilled water for 2 minutes. The plant material was blotted on sterile blotting paper. In each Petri dish 5-6 segments were placed on PDA medium. Each Petri dish containing PDA is supplemented with antibiotics Penicillin G 100 units per ml and streptomycin 100 microgram per ml. The Petri dishes were sealed with parafilm and incubated at 27 $\pm 2^0$ C for 4-6 weeks at dark. Most of the fungal growth was expected within two weeks of inoculation. The incubation period for each fungus was recorded and the growth of endophytic fungi was observed daily.



Leaf segments of *P. reticulatus* inoculated on PDA medium

2.3 Identification of endophytic fungi

The isolated fungi were identified based on the morphology of surface texture, pigmentation and spores at the hyphal tips which were used to identify the endophytic fungi at species level using standard manual [20, 21]. The microscopic examination was also done to study their reproductive spores [22]. The identified fungal isolates from the plant tissue segments were sub cultured in a Petri dish containing sterile PDA media. To preserve as a pure culture, the endophytic fungi was inoculated in PDA slant and incubated at 4^oC.



Endophytic fungal growth on PDA medium

2.4 Calculation of colonizing frequency

Colonizing frequency was calculated as per standard procedure [23].

$$\text{Colonization frequency} = \frac{\text{Number of segments colonized by fungi}}{\text{Total number of segments observed}} \times 100$$

2.5 Relative Percentage Occurrence (RPO) of Different Fungi was calculated with slight modification [24]

$$\text{RPO} = \frac{\text{Density of colonization of one group}}{\text{Total Density of colonization}} \times 100$$

2.6 Screening for antimicrobial activity

150 ml of Potato Dextrose Broth was prepared in 250 ml flasks and autoclaved at 15 lbs psi for 20 min. The medium was inoculated with various fungi culture and incubated at 28±1°C in the incubator for 1-2 weeks. After 9 days the culture medium is centrifuged at 10000 rpm for 30 minutes. After centrifugation, the supernatant was collected and subjected to antimicrobial screening by Agar diffusion technique [25].

2.7 Antibacterial Activity of Test organisms

Three human pathogenic bacteria such as *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa* and one fungal pathogen like *Candida albicans* were used for antimicrobial assay. All the test microbial strains were obtained from the Culture Collection Centre, IMTECH, Chandigarh (India).

2.9 Antimicrobial screening by Agar Well Diffusion Technique

Antimicrobial activity of culture supernatants of endophytic fungi were tested by Agar well Diffusion Method using Mueller Hinton Agar Medium. All the overnight culture of test microbes were inoculated into Mueller Hinton Agar plates using sterile cotton swabs. About 5 mm size wells were made using sterile cork borer and 200 µl of culture supernatants added into separate wells. All the plates were observed for zone of inhibition after incubation at 37°C for 24 hours for bacterial pathogens and at 30°C for 48 hours for fungal pathogens. Sterile Sabouraud Dextrose Broth is used as negative control. The antimicrobial activities were analysed by presence or absence of inhibition zones [26].

III. RESULTS

In the present study, 12 leaflets and 12 stem segments of ethnomedicinal plant *P. reticulatus* was processed for isolation of endophytic fungi. A total of 7 endophytic fungi *Geotrichum candidum, Cylindrocladium* sp., *Fusarium* sp., *Mucor pusillus, Cladpsporium cladosporioides, Alternaria alternata, Rhizopus* sp. were isolated which is shown in Table 1. Colonization frequency percentage (% CF) and Relative Percentage Occurrence (RPO) are also given in Table 2 and 3 and Figure 1 respectively. The seven endophytic fungi were subjected for antimicrobial activity against pathogenic bacteria viz., *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli* and fungal pathogens like *Candida albicans. Cladpsporium cladosporioides* had shown antimicrobial activity against *Pseudomonas aeruginosa* and showed inhibition zone of 12 mm. *Fusarium* sp. is found to be effective against *Escherichia coli* and showed inhibition zone of 10 mm. Whereas other endophytic fungal forms tested for antimicrobial sensitivity did not show any effectiveness against the pathogenic forms (Table 4).

Table1: Endophytic fungi isolated from different parts of *P. reticulatus* Poir.

Sl. No.	Plant Parts	Endophytic fungi	Class
1	Leaf	*Geotrichum candidum*	Deuteromycetes
2	Leaf	*Cylindrocladium* sp.	Deuteromycetes
3	Leaf	*Fusarium* sp.	Hyphomycetes
4	Leaf	*Mucor pusillus*	Zygomycetes
5	Stem	*Cladpsporium cladosporioides*	Hyphomycetes
6	Stem	*Alternaria alternata*	Ascomycetes
7	Leaf	*Rhizopus* sp.	Zygomycetes

Table 2: Percentage Colonizing frequency (% CF) of Endophytic fungi isolated from *P. reticulatus*

Sl. No.	Endophytic fungi	Isolate from	Total no. of segments observed	No of segments colonized	% Colonization Frequency (%CF)
1	*Geotrichum candidum*	Leaf	12	2	16.6
2	*Cylindrocladium* sp.	Leaf	12	2	16.6
3	*Fusarium* sp.	Leaf	12	3	25
4	*Mucor pusillus*	Leaf	12	1	8.3
5	*Cladpsporium cladosporioides*	Leaf	12	2	16.66
6	*Alternaria alternata*	Stem	12	5	41.6
7	*Rhizopus* sp.	Stem	12	4	33.33

Figure 1: Percentage Colonizing Frequency (%CF) of Endophytic fungi isolated from P. *reticulatus* Poir.

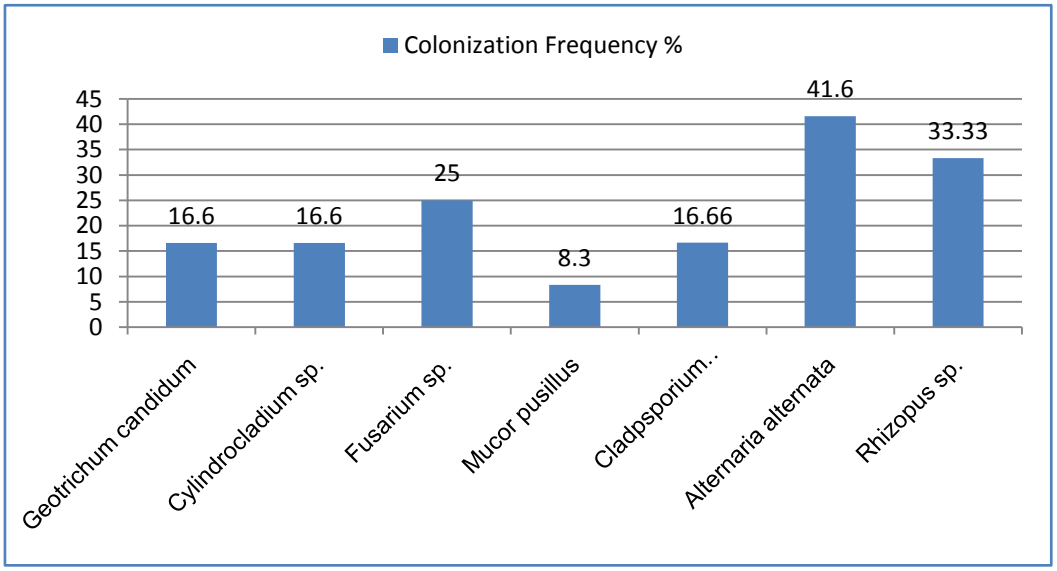


Table3: RPO of endophytic fungi isolated from *Phyllanthus reticulatus* Poir.

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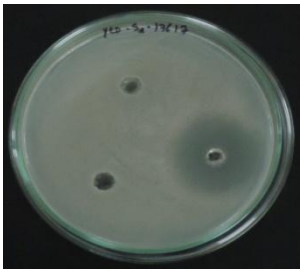
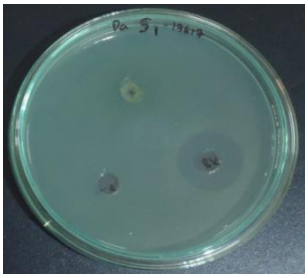
Sl. No.	Name of the Endophytic fungi	Isolate from	RPO (%)
1	*Geotrichum candidum*	Leaf	10.52
2	*Cylindrocladium* sp.	Leaf	10.52
3	*Fusarium* sp.	Leaf	15.78
4	*Mucor pusillus*	Leaf	5.26
5	*Cladpsporium cladosporioides*	Leaf	10.52
6	*Alternaria alternata*	Stem	26.31
7	*Rhizopus* sp.	Stem	21.05

Table 4: Evaluation of anti microbial activities

Endophytes isolated from *Phyllanthus reticulatus*	Zone of inhibition			
	E. coli	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Candida Albicans*
Geotrichum candidum	-	-	-	-
Cylindrocladium sp.	-	-	-	-
Fusarium sp.	+	-	-	
Mucor pusillus	-	-	-	-
Cladpsporium cladosporioides	-	-	+	-
Alternaria alternata	-	-	-	-
Rhizopus sp.	-	-	-	-

("+": Presence of inhibition zone & "-": Absence of inhibition zone)





Zone of inhibition (a) (b)
a. *Cladosporium cladosporioides* *b. Fusarium* sp.

IV. DISCUSSION

A total of seven endophytic fungi were isolated from the medicinal plant *Phyllanthus reticulatus* Poir. belongs to different classes. *Geotrichum candidum, Cylindrocladium* sp. belong to the class Deuteromycetes,, *Fusarium sp and Cladpsporium cladosporioides,* belong to the class Hyphomycetes, *Alternaria alternata* belongs to the class Ascomycetes, *Rhizopus* sp.and *Mucor pusillus* belong to the class Zygomycetes. Colonizing Frequency percentage (CF %) of *Alternaria alternata* (41.6 %) was found to be highest, whereas *Mucor pusillus* showed lowest CF % (8.3 %). *Alternaria alternata* has exhibited highest RPO (26.21%) and *Mucor pusillus* showed the lowest RPO (5.26 %). The isolated endophytic forms were subjected for antimicrobial sensitivity test against pathogenic microbes such as *Pseudomonas aeruginosa, Escherichia coli.* and *Candida albicans. Cladpsporium cladosporioides* was the most effective endophytic fungus against *Pseudomonas aeruginosa* and zone of inhibition was 12 mm. *Fusarium* sp. *w*as found to be effective against *Escherichia coli* and zone of inhibition was 10 mm. Whereas other endophytic fungal forms tested for anti microbial sensitivity test did not show any effectiveness to the tested pathogenic forms . Generally endophytic fungi are widely distributed in all plant domains. It produces valuable plant secondary metabolites and exploited for medical, agricultural and industrial uses [27]. Plants having an ethnobotanical history or used by local indigenous people for any therapeutic purpose will provide the best opportunities to isolate novel endophytic fungi and to make use of them to produce novel bioactive products [28].

V. CONCLUSION

The secondary metabolites present in endophytic fungi *Cladosporium cladosporioides* and *Fusarium* sp. may act as potential antimicrobial agents. *Phyllanthus reticulatus* Poir. harbors several endophytic fungi which produce biologically active antimicrobial substances with selective antimicrobial properties. A total of 7 endophytic fungi were isolated from the medicinal plant *Helicteres isora* L. [29].The natural bioactive compounds obtained especially from the endophytic fungi have been largely unexplored. Efforts must be made to ensure safe, effective and affordable treatments for wide range of diseases by traditional methods which use locally available medicinal plants. The scientific and authentic researches on these aspects are to be done in order to exploit traditional knowledge of ethnomedicinal plants.

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REFERENCES

[1]. Sandhu SS, Suneel K, Aharwal RP, Shukla H and Rajak RC. Endophytic Fungi: As A Source of Antimicrobials Bioactive Compounds. *World J Pharm Pharmace Sci.* 2014a; 3(2): 1179-1197.
[2]. Strobel G and Daisy B. Bioprospecting for microbial endophytes and their natural products. *Microbio Mol Biol Rev.* 2003; 67: 491-500.
[3]. Carroll GC and Carroll FE. Studies on the incidence of coniferous needle endophytes in the Pacific Northwest. *Can J Bot.* 1978; 56: 3032-3043.
[4]. Strobel GA. Microbial gifts from rainforests. *Can J Plant Pathol.* 2002; 24:14-20.
[5]. Tan RX, Zou WX. Endophytes: A rich source of functional metabolites. *Nat Prod Rep,* 2001; 18 (4): 448- 459.
[6]. Yu H, Zhang L, Li L, Zheng C, Guo L, Li W, *et al.* Recent developments and future prospects of antimicrobial metabolites produced by endophytes. *Microbiol Res,* 2010; 165 (6*):* 437-449.
[7]. Strobel G, Daisy B. Bioprospecting for microbial endophytes and their natural products. *Microbiol Mol Biol Rev* 2003: 501-502.
[8]. Kusari S, Spiteller M. Are we ready for industrial production of bioactive plant secondary metabolites utilizing endophytes?, *Nat Prod Rep,* 2011; 28*:* 1203-1207.
[9]. Marinho MR, Rodrigues-Filho E, Moitinho R, Santos LS. Biologically active polyketides produced by *Penicillium janthinellum* isolated as an endophytic fungus from fruits of *Melia azedarach*. *J BrazilChem Soci,* 2005; 16*:* 280-283.
[10]. Pandi M, Senthil Kumaran R, Choi Kim HJ, Muthumary J. Isolation and detection of taxol- an anticancer drug produced from *Lasiodiplodia theobromae*, an endophytic fungus of the medicinal plant *Morinda trifolia*. *Afr J Biotechnol,* 2011; 10: 1428-1435.
[11]. Kaul S, Ahmed M, Zargar K, Sharma P, Dhar MK. Prospecting endophytic fungal assemblage of digitalis lanata Ehrh. (Foxglove) as a novel source of digoxin: a cardiac glycoside. *Biotechnology,* 2013; 3*:*335-340.
[12]. Gritto MJ, Nandagopalan V, Doss A. Ethno-botanical study on the traditional healers in Pachamalai hill of Eastern Ghats, Tamilnadu, South India. *J Med Plants Study,* 2015; 3 (2): 80-85.
[13]. Kähkönen MP, Hopia AI, Vuorela HJ, Rauha JP, Pihlaja K, Kujala TS, et al. Antioxidant activity of plant extracts containing phenolic compounds. *J Agric Food Chem,* 1999; 47 (10):3954-3962.
[14]. Zheng W, Wang SY. Antioxidant activity and phenolic compounds in selected herbs. *J Agric Food Chem,* 2001; 49 (11):5165-5670.
[15]. Cai Y, Luo Q, Sun M, Corke H. Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. *Life Sci*, 2004; 74 (17): 2157-2184.
[16]. Kaul S, Ahmed M, Zargar K, Sharma P, Dhar MK. Prospecting endophytic fungal assemblage of digitalis lanata Ehrh. (Foxglove) as a novel source of digoxin: a cardiac glycoside. *Biotechnology* 2013; 3: 335-340.
[17]. Bhat GK. Flora of Udupi. *Indian Natural Inchara,* Chitpadi, Udupi. pp 578.
[18]. Basha NS, Ogbaghebriel A, Yemane K, Zenebe M. Isolation and screening of endophytic fungi from Eritrean traditional medicina lplant *Terminalia brownii* leaves for antimicrobial activity. *Int J Green Pharm,* 2012; 6: 40-44.
[19]. Petrini O. Taxonomy of endophytic fungi of aerial plant tissues. In: Microbiology of the phylosphere, Edited by NJ Fokkenna, Van Den J, Heuvel. Cambridge University Press, Cambridge. 1986; pp175-187.
[20]. Joseph C, Gilman. A mannual of soil fungi. 2nd edition. Biotech Books, Delhi. 2001.

[21]. Barnett HL, Hunter BB. Illustrated Genera of Imperfect Fungi. APS press. St. Paul Minneesota, USA. 1998.
[22]. Anitha D, Vijaya T, Pragathi D, Reddy NV, Mouli KC, Venkateswarulu N, et al. Isolation and characterization of endophytic fungi from endemic medicinal plants of Tirumala hills. *Int J Life Sci Biotechnol Pharm Res*, 2013; 2(3*)*: 367-373.
[23]. Suryanarayanan TS, Venkatesan G and Murali TS. Endophytic fungal communities in leaves tropical forest trees: Diversity and distribution patterns. *Current Science*, 2003;85 (4): 489-492
[24]. Suryanarayanan, TS, Thennarasan S. 'Temporal variation in endophyte assemblages of *Plumeria rubra* leaves'. *Fungal Diversity,* 2004; 15: 197-204.
[25]. Sutjaritvorakul T, Whalley AJ, Sihanonth P, Roengsumran S. Antimicrobial activity from endophytic fungi isolated from plant leaves in *Dipterocarpus* forest at Viengsa district Nan province. Thailand. *J Agric Tech*, 2011; 7: 115-121.
[26]. Roy S, Banerjee D. Isolation of antimicrobial compound by endophytic bacteria from *Vinca rosea*. *Int J Curr Res,* 2010; 5: 4751.
[27]. Aly AH, Debbab A, Kjer J, Proksch P. Fungal endophytes from higher plants: a prolific source of phytochemicals and other bioactive natural products. *Fungal Diversity,* 2010; 41: 1-16.
[28]. Strobel GA. Rain forest endophytes and bioactive products. *Crit Rev Biotechnol*, 2002; 2: 315-33.
[29]. Gayathri Pai, Chandra M. Screening of Phytochemicals and Isolation of Endophytic Fungi from Medicinal plant *Helicteres isora* L. *J Pharm (IOSRPHR),* 2017; 7 (12): 01-05.

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