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A Systematic Study on the Endophytic Fungi of Cordia *macleodii* and their Medicinal Properties

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Abstract: ***Endophytic fungi live inside the higher plants, apparently without causing any harm to the hosts and its produce the secondary metabolites are potential antimicrobial activity. Cordia macleodii has been used to treat disease such as jaundice, flu, fever, cough, malaria, asthma, aphrodisiac, wound healing, blood purification, and several other health issues. In this study, an isolate and identify the endophytic fungi from collected from Cordia macleodii Labhaghogri and Sawari forest area of chhindwara district, Madhya Pradesh. The plant materials were taken and first washed in running tap water to remove the dust and the other debris present on the surface of explants. Cut the Segments of explants approximately 0.5 cm by sterile lancet blades and all segments are surface sterilized by agitating in 5% Sodium hypochlorite solution (1 – 2 min) followed by treatment with 70% ethanol (1 – 2 min), and then rinsed in sterile distilled water (30 sec.). 36 (leaf, stem and root samples) segments from Cordia macleodii plant are processed for the isolation of endophytic fungi. About 36 segments (12 segments of each part respectively) of the medicinal plant were screened for the isolation of the endophytic fungi. A total of 40 endophytic fungi was isolated and identified from medicinal plant Cordia macleodii. The leaf segments showed a maximum repository for endophytic fungi than the other segments. Among the 40 endophytic fungi, the predominant endophytic fungi isolated belonged to the genera of Alternaria altrnata, Curvularia spp., Mucor phoma spp., Aspergillus niger, Aspergillus flavus, and Cuvularia spp, Cladosporium sp, Fusarium spp. In this study, the majority of the fungi belonged to hyaline hyphomycetes. In this study, conclude that the isolation of endophytic fungi from medicinal plant of Cordia macleodii. To isolate the 24 endophytic fungi produce the novel bioactive compound. However, further studies are required to screen these endophytic fungi for production of novel bioactive compounds.***

Keywords: Endophytic fungi, *Cordia macleodii*, Bioactive compound. Medicinal plant, Pharmacology

1. Introduction

Cordia *macleodii* is the small tree which height range from 9 to 12 m, trunk about 50–60 cm in diameter. Thickness of Bark is 12-15 mm light green, reddish color inside, forming exudates on cut or damaged area, branchlets white tomentose. Leaves are broadly ovate, light green colored on the ventral surface and shiny dark green on the dorsal surface with numerous hairs, 20–25 cm × 15–18 cm, entire, obtuse or bluntly acuminate, somewhat sorgos above and with numerous white crystolyths, 3–5 nerved from or from near the base, base often deeply cordate. Petiole: 3.7-7.5 cm long. Leaf opposed or extra-axillary Flowers: Yellowish white in color, polygamous, subsessile, in dense paniculate terminal and axillary tomentose cymes; male flowers with a rudimentary ovary but without style or stigma. Calyx: 8 mm long, obconic, densely tomentose, ribbed; lobes short, obtuse. Corolla: Yellowish white in color, 1.6 cm long; lobes 8 by 2.5-3 mm, exceeding the tube, spathulate-oblong, obtuse, veined. Kirtikar & Basu, *et. al*., (2003). Stamens: Usually 6, exerted; filaments hairy at the base. Anthers: Anthers of male flowers large, those of hermaphrodite flowers smaller. Drupes: Subglobose, yellowish, somewhat tomentose, apiculate, seated on the broadly campanulate toothed or lobed, ribbed calyx. Bhargav Bhide, *et. al*., (2011).

The medicinal plants Cordia macleodii (Boraginaceae) is called to have medicinal properties. The leaf, stem and root extracts of these medicinal plants are used ethanopharmaceutically for their medicinal properties viz., antioxidant, anti-inflammatory, wound heeling and anti microbial activity Martin, P. *et. al.,* (1997). The present study was aimed at isolation of endophytic fungal isolates which are capable of producing novel and biologically active secondary metabolites useful for pharmaceutical purpose.

2. Methods

Collection of Plant Samples

The healthy plant parts (Leaves, stem, root) of the plant *Cordia macleodii*, were collected from Labhaghogri and Sawari forest area of chhindwara district, Madhya Pradesh. The collected plant parts were kept in sterile bags and brought to the laboratory. and processed immediately to reduce the chances of contamination.

Surface Sterilization of Plant Materials

The healthy plant segments were cleaned completely in running tap water to remove the dust particles. Then the segments were subjected to sequential immersion in surface sterilization agents such as 70% ethanol and 5% sodium hypochlorite solution for 2 minutes to remove the surface contaminants. Again it was washed thrice in sterilized distilled water to remove the sterilization agents Schulz. *et. al.,* (1993). Then all the segments were blotted and dried using sterile blotting paper.

Isolation of endophytic fungi

The endophytic fungi were isolated by imprint technique Petrini, *et. al.,* (1986). The sterilized plant segments were cut into small sections and 36 (leaf, stem, and fruit samples) segments from *Cordia macleodii*, plant are processed for the

isolation of endophytic fungi. Leaf, stem, and fruit segments were then placed on potato dextrose agar (PDA) plates added with antibiotic chloramphenicol 150 mg/l. Then the plates were incubated for 3 - 8 Days at 27^0C. The unsterilized plant segments and the finally rinsed distilled water were placed on the agar plates as a control to check for surface contaminated fungi. The fungi that grown out from the tissues was isolated and stocked. The cultures were maintained on PDA slants at 4°C for further screening Rajeswari, *et. al.,* (2016).

Purification of Endophytic Fungi

After the incubation period the fungal endophytes that emerged from the tissue were purified on PDA plates. Purification was done by transferring the hyphal tip to the fresh PDA plates. Continuous sub culturing was done to maintain the culture. Ibrahim, *et. al.* (2017).

Identification of endophytic isolates

The endophytic fungal isolates were identified up to genus level based on the morphological features such as colony morphology, pigmentation, growth pattern, spore structures, and other hyphal characteristics with the help of the standard mycological manuals. The microscopic examination was also done to study their reproductive spores. Cultures which failed to produce spores were grown on different minimal media and incubated for several weeks to months Rajeswari, *et. al.,* (2016).

Statistical analysis

Colonization Frequency (CF) of endophytes was calculated to each fungus obtained from various plant parts Suryanarayanan, *et. al.,* (2003). It is equivalent to the number of sections colonized by each endophyte divided by the total number of incubated sections expressed as its percentage.

Colonization frequency (CF%):

$$CF\% = \frac{\text{No. of individual fungi recorded}}{\text{Total no. of segments screened}} \times 100$$

3. Results

About 36 segments (12 segments of each part respectively) of the medicinal plant were screened for the isolation of the endophytic fungi. A total of 40 endophytic fungi was isolated and identified from medicinal plant *Cordia macleodii*. The leaf segments showed a maximum repository for endophytic fungi than the other segments. Among the 40 endophytic fungi, the predominant endophytic fungi isolated belonged to the genera of *Alternaria alternata*, *Cladosporium* spp., *Mucor* spp., *Aspergillus niger, Aspergillus flavus, Curvularia lunata* and *Fusarium* spp.Tables 1 showed the CF value.

The predominant endophytic fungi isolated belonged to the genera of *Aspergillus niger, Aspergillus flavus, Curvularia lunata, Fusarium* spp., *A. niger, Mucor* spp., *Alternaria alternata*, *Cladosporium* spp., Some fungi which did not produce any reproductive structure, as they produced sterile mycelia and in some cases sterile pycnidium were also grouped under mycelia sterilia. These fungi did not sporulate in spite of repeated subculturing.

Table 1: Endophytic fungi isolated from the medicinal plant, *Cordia macleodii*

Site of location	No. of samples	No. of fungi isolated	CF (%)
Leaves	12	20	16.6
Stems	12	10	8.3
Roots	12	10	8.3
Total	36	40	111

C: macleodii: Cordia macleodii, CF: Colonization

on to sporulating media (PDA, Sabouraud's dextrose agar, and tap water agar) and hence are grouped on mycelia sterilia Frequency In this study, the majority of the fungi (Table 3) belonged to hyaline hyphomycetes or group of fungi imperfecti or deuteromycetes, *Mucor* spp. was belonged to zygomycetes and. The colonization frequency was found to be 111%.

4. Discussion

This study was carried to isolation and identification of endophytic fungi from Labhaghogri and Sawari forest area of chhindwara district, Madhya Pradesh. In the study, a total of 40 fungal colonies were isolated from 36 segments. Among the 40 endophytic fungi, the predominant endophytic fungi isolated belonged to the genera of *A. alternata*, *Curvularia* spp., *A. niger, A. flavus,* and *Cladosporium* spp. These results were similar to the studies of Dhanalakshmi *et al.* (2013). Who isolated *Alternaria* spp., *Aspergillus* spp., *Bipolaris* spp., *Exophiala* spp., *Nigrospora* spp., and *Penicillium* spp. in *Moringa oleifera* and in another study of Barnabas *et al*. (2013). This reported *Aspergillus* spp., as the predominant isolate in the leaves, stem, and roots of *M. oleifera*. They belonged to hyaline hyphomycetes 40%, coelomycetes are 8.33%, dematiaceous hyphomycetes 29%, zygomycetes 12.5%, and mycelia sterilia 12.5%. These isolated are belonged to hyphomycetes (59.32%), coelomycetes (22.03%), ascomycetes (13.56%), and sterile mycelium (5.08%). Tenguria RK, *et. al.,* (2015). The same results are showed the presence of endophytic fungi in Avicennia officinalis, that isolated the endophytic fungi namely reported *Aspergillus*, *Penicillium*, *Curvularia*, *Cladosporium*, *Phoma*, and *Fusarium* species. Job N, *et. al.,* (2015).

5. Conclusion

Medicinal plants are good source for isolation of endophytic fungi that colonize the tissue without causing apparent symptoms. Endophytic organisms have received considerable attention as they are found to protect their hosts against pests, pathogens and even domestic herbivores. In this study, a total of 40 endophytic fungi were isolated from the *Cordia macleodii*, a well-known medicinal plant contains various chemical compounds. Isolation of endophytic fungi from this plant produces novel bioactive compounds.

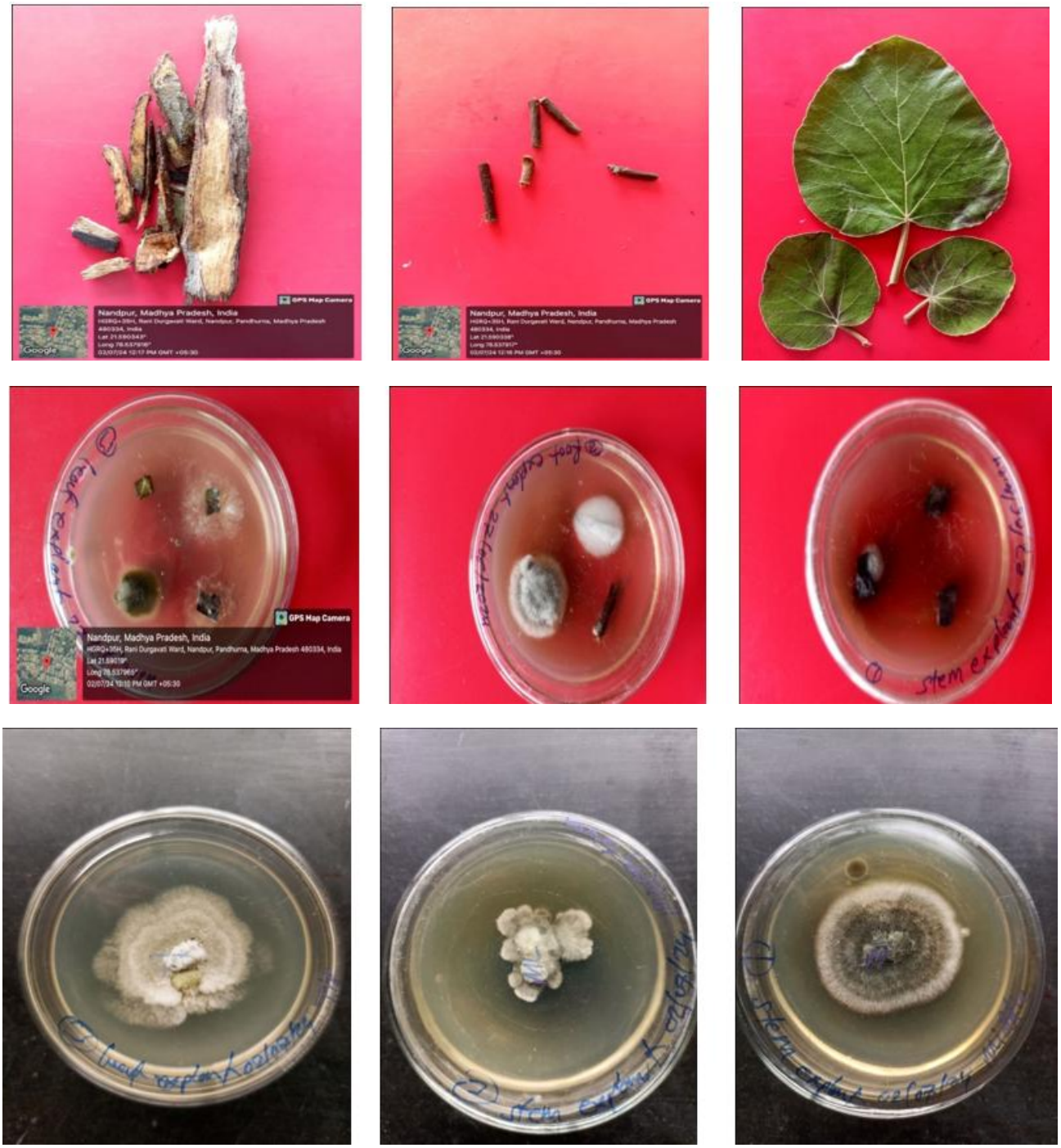

Table 2: Taxonomic position of endophytic fungi

S. No.	Isolated endophytes	Fungal class	Total (%)
1	*Alternaria alternate*	Dematiaceous hyphomycetes (deuteromycetes)	5 (12.5)
2	*Curvularia* spp.	Dematiaceous hyphomycetes (deuteromycetes)	5 (12.5)
3	*Nigrospora spp*	Dematiaceous hyphomycetes (deuteromycetes)	2 (5)
4	*Mucor*	Zygomycetes	3 (7.5)
5	*Fusarium* spp.	Hyaline hyphomycetes (deuteromycetes)	4 (10)
6	*Aspergillus flavus*	Hyaline hyphomycetes (deuteromycetes)	6 (15)
7	*Cladosporium spp.*	Hyaline hyphomycetes (Dothideomycetes)	3 (7.5)
8	*Aspergillus niger*	Hyaline hyphomycetes (deuteromycetes)	7 (17.5)
9	*Microsporium spp.*	Eurotiomycetes	2 (5)
10	Sterile forms	Mycelia sterilia	3 (7.5)
Total			40 (100)

Table 3: Percentage of isolated fungal class

S. No.	Fungal class	Total (%)
1	Dematiaceous hyphomycetes	12 (30)
2	Zygomycetes	3 (7.5)
3	Hyaline hyphomycetes	20 (50)
4	*Eurotiomycetes*	2 (5)
5	Mycelia sterilia	3 (7.5)

Table No. 4 Isolation of Endophytic fungus from medicinal plant (Cordia macleodii leaf)

S. No.	Medicinal Plant (leaf)	Section of Leaf Parts	Isolates
1	Replication - 1	1	*Fusarium* spp.
2		2	*Aspergillus niger*
3		1,3	*Curvularia* spp.
4		3	*Mucor*
5	Replication - 2	4	Sterilia form
6		3	*Microsporium spp.*
7		1,2	*Aspergillus niger*
8		1	*Curvularia* spp.
9	Replication - 3	2	*Aspergillus niger*
10		3,4	*Cladosporium spp.*
11		2	*Aspergillus flavus*
12	Replication - 4	4	*Microsporium spp.*
13		1,2	*Alternaria alternate*
14		3	*Aspergillus flavus*
15		2,3	*Nigrospora spp.*

Table 5: To Isolation of Endophytic fungus from medicinal plant (Cordia macleodii stem).

S. No.	Medicinal Plant (Stem)	Section of Stem Parts	Isolates
1	Replication - 1	Middle	*Alternaria alternate*
2		Lower Middle	No Growth
3		Upper Lower	*Aspergillus flavus*
4	Replication - 2	Middle	*Aspergillus niger*
5		Lower Middle	*Curvularia* spp.
6		Upper Lower	*Fusarium* spp.
7	Replication - 3	Middle	*Aspergillus niger*
8		Lower Middle	*Aspergillus flavus*
9		Upper Lower	Sterilia form
10	Replication - 4	Middle	*Alternaria alternate*
11		Lower Middle	*Aspergillus flavus*
12		Upper Lower	No Growth

Table 6: To Isolation of Endophytic fungus from medicinal plant (Cordia macleodii root).

S. No.	Medicinal Plant (root)	Section of Root Parts	Isolates
1	Replication - 1	Upper Lower	*Fusarium* spp.
2		Middle	*Aspergillus niger*
3		Lower Middle	No Growth
4	Replication - 2	Upper Lower	*Mucor*
5		Middle	Sterilia form
6		Lower Middle	*Curvularia* spp.
7	Replication - 3	Upper Lower	*Fusarium* spp.
8		Middle	*Cladosporium spp.*
9		Lower Middle	No Growth
10	Replication - 4	Upper Lower	*Mucor*
11		Middle	*Alternaria alternate*
12		Lower Middle	*Aspergillus flavus*

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