

Phytochemical analysis, extracellular enzymes and antioxidant activity of endophytic fungi from *Cymbopogon citratus* L.

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Abstract

A comparative study of radiation characteristics of a polarized switchable microstrip planar array of triangular patch antenna printed on synthesized LiTiMg ferrite substrate with a normal magnetic bias field has been done and reporting here. Radiation patterns and some important characteristics of proposed array antenna have been compared with the same geometry printed on RT duroid and silicon. 61% miniaturization and high quality factor are some advantages of using LiTiMg ferrite compare to RT duroid. With the biasing of external magnetic field perpendicular to the ferrite substrate arise some tunable behavior which has been elaborated by the generation quasi TEM, magnetostatic and spin waves. In this analysis spin wave exchange term (ω_r) which depends upon the static internal field (H_{ex}), has also included in the dispersion formula because the wavelength of microwave approach the inter-atomic distance of ferrite material which is the main cause of generation of spin waves in such types of layered structures.

Keywords: *Fusarium oxysporum*, *Cymbopogon citratus*, bhadra wildlife sanctuary, total phenolic content

Introduction

Endophytes are generally all microorganisms living within plant tissues without any symptoms to their hosts [1]. They are considered as a novel source for acquiring enzymes with unique prospects as they are easy to handle and cultivate with faster growth rate and high yielding abilities [2]. Endophytic fungi have been extensively studied for their ability to produce several extracellular enzymes for obtaining nutrition from their host, hydrolysis of food substances and are also involved in eliciting defence mechanisms against pathogens [3]. Such enzymes include pectinases, cellulases, lipases, laccase, amylases, chitinases, and proteinases [4, 5]. Although hydrolases are typically isolated from soil-borne fungi such as *Aspergillus* sp., *Penicillium* sp. and *Rhizopus* sp., endophytes producing these enzymes present an interesting alternative [6]. Colonization of host plants by endophytic fungi helps host plants adapt to biotic and abiotic stress factors [7]. A large number of bioactive metabolites of endophytic fungi have been extracted and characterized over 12 years. These belong to diverse groups like alkaloids, steroids, terpenoids, peptides, polyketones, flavonoids, quinols, phenols, xanthenes, chinones, isocumarines, benzopyranones, tetralones, cytochalasines, perylene derivatives, furandiones, depsipeptides and enniatines. Some of these compounds represent novel structural groups, e.g., the palmarumycins and a new benzopyroanone [8]. Several studies have reported that many plant secondary metabolites have been isolated from endophytic fungi, thereby, using these organisms as an alternative source for plant metabolites [9]. Isolation of endophytic fungi from medicinal plants may result in the production of biologically active metabolites for a large commercial scale as they are easily cultured in laboratory and fermenter instead of harvesting plants and affecting the environmental biodiversity [10, 11]. Pharmaceutically essential plants can be used as a source of new potential endophytic

fungi, as their beneficial characteristics can also be found in their endophytic community [12]. According to Tan and Zou, endophytic microbes can produce bioactive compounds with similar properties with the host due to evolutionary genetic exchange [13]. Despite this potential, many medicinal plants remain unexplored regarding their endophytic composition. *Cymbopogon citratus* L. is one such medicinal plant (Lemongrass) whose endophytic fungal community is not completely explored [14]. It belongs to the family Graminae. It is a stemless perennial grass with numerous stiff tillers from the short rhizomatous rootstock, making large tussocks [15]. The essential oils produced are used in food, perfumery and soap manufacture, and pharmaceutical products [16]. It is an important aromatic grass species native to India and Tropical Asia [17]. Grass yields essential oil called "lemongrass oil" or "citronella oil" which is used as the pesticide and in preservations [18]. Some reports show that lemongrass oil has antifungal and anti-cancer properties [14]. Scientists found that lemongrass caused programmed cell death in cancer cells [19]. Therefore, we focus on isolating endophytic fungi from *C. citratus* and evaluating their phytochemical constituents and extracellular enzymes.

Materials and methodology

Collection of plant samples and isolation of endophytic fungi

The plant samples were collected in the month of September 2016, in Bhadra wildlife sanctuary, Western Ghats, Karnataka. The collected plant samples were washed thoroughly and surface sterilized by immersing them in 75% ethanol (1 min), 2.5% sodium hypochlorite solution (NaOCl) (v/v) for 1 min and 75% ethanol for 30 sec. They were cut into 5x5mm segments after drying and placed on sterile Potato Dextrose Agar (PDA) amended with 50 mg/L Streptomycin. The plates were incubated at $27 \pm 2^\circ\text{C}$ for 15 days, and the fungi emerging from the explants were sub-

cultured on to fresh PDA plates, and pure cultures were obtained [20].

Identification of endophytic fungi

The isolated endophytic fungi were stained with lactophenol-cotton blue. The structure of fruiting bodies and spores was observed under a microscope at 40x resolution and identified using standard manuals Barnett and Hunter, 1972 [21].

Extraction of secondary metabolites

The mycelia of endophytic fungal isolates were cultured on Potato Dextrose Broth (PDB). The pure culture of endophytic fungi was inoculated in a 1000 mL conical flask containing 500 ml of broth medium on a rotary shaker for 15-22 days at $25\pm 2^\circ\text{C}$. The culture filtrates were filtered through a Whatman filter paper and the filtrate was extracted twice with an equal volume of ethyl acetate and air-dried for further analysis [22].

Phytochemical analysis

EtOAc extract of endophytic fungal isolates were evaluated for its phytochemicals like alkaloids, tannins, cardiac glycosides, saponins, terpenoids, amino acids, phenolic compounds, flavonoids and quinones qualitatively using the standard protocols.

Alkaloids (Wagner's reagent)

About 1 ml plant extract was treated with 4-5 drops of Wagner's reagent and observed for the formation of a reddish-brown precipitate. Wagner's reagent is a mixture of 1.27 g of iodine and 2 g potassium iodide in 100 ml of water [23].

Tannins

The fungal crude extract was treated with alcoholic FeCl_3 reagent. A bluish-black colour, which disappears on the addition of a drop of dilute H_2SO_4 was followed by the formation of yellowish-brown precipitate [24].

Cardiac glycosides (Keller Kellani's test)

To 2 ml of fungal extract, 5 ml glacial acetic acid and a drop of ferric chloride were added. The mixture was then carefully overlaid with 1 ml of concentrated H_2SO_4 . Brown ring formation at the interface indicates deoxy sugar being the characteristic of cardenolides [25].

Phenols

About 2 mL of fungal extract was taken and mixed with aqueous 5% FeCl_3 and observed for deep blue or black colour formation [26].

Flavonoids

About 0.5 mL of crude extract was taken in a test to which 5-10 drops of diluted HCl and a small piece of zinc were added and the solution was boiled for a few minutes. Reddish pink or brown colour precipitate indicates the presence of flavonoids [27].

Amino acids

About 2-5 drops of ninhydrin solution was added to 2 ml of extract and placed in a water bath for 1-2 min. The presence of amino acids was indicated by the formation of a purple colour [28].

Saponins (foam test)

About 6 ml of water was added to 2 ml of extract in a test tube. The mixture was shaken vigorously and observed for the formation of persistent foam confirming saponins presence [29].

Terpenoids (Salkowski's test)

About 1 ml of chloroform was added to 2 ml of extract which. A reddish-brown precipitate is formed immediately on the addition of a few drops of concentrated HCl. [30].

Test for Quinones

About 2mL of the fungal extract was treated with concentrated HCl. Formation of yellow precipitate or colouration indicated the presence of quinones [31].

Extracellular enzyme production

EtOAc extract of endophytic fungal isolates were evaluated for its extracellular enzymes like amylase, cellulose, tyrosinase, protease, chitinase and laccase were qualitatively analyzed using the standard protocols.

Amylase production

The endophytic fungi producing amylase were screened by inoculating on glucose yeast extract-peptone (GYP) agar medium (glucose 1g, yeast extract 0.1g, agar 15 g, distilled water 1L and pH 6) which included 1% soluble starch. After growth, the plates were flooded with a mixture of 1% iodine and 2% potassium iodide. The amylase production is indicated by a clear zone surrounding the colony [32].

Cellulase production

The endophytic fungi producing cellulase were screened by inoculating on GYP agar medium supplemented with 0.5% Nacarboxy- methylcellulose. Petri plates were incubated at $28\pm 2^\circ\text{C}$ and after the growth was observed flooded with 1% Congo red dye for 20 minutes and destained with 1N NaCl solution for 15 minutes. The cellulose enzyme was evaluated by the presence of a light yellow area around the colony of the fungus [33].

Tyrosinase production

The endophytic fungi producing tyrosinase were screened by inoculating on tyrosine agar medium (peptone 5 g, beef extract 3 g, agar 20 g, L-tyrosine 5 g and pH 7). The brown colour around the colony indicated the presence of tyrosinase enzyme [34].

Protease production

The endophytic fungi producing protease were screened by inoculating on GYP agar medium supplemented with 1% casein and pH 6.5 and incubated for seven days. The clear zone around the colony indicated the presence of the protease enzyme [35].

Chitinase production

Chitinase production was estimated by chitin agar medium (yeast extract 1.5 g, chitin 2.0g, agar 20 g and distilled water 1 L). Plates were inoculated with test cultures and then incubated at 26°C up to 72 h. The production of chitinase enzyme is indicated by a clear zone around the culture [36].

Laccase production

Laccase production was performed using GYP agar medium supplemented with 0.005% 1-naphthol. The blue colour indicated the laccase enzyme due to oxidation of 1-naphthol [37].

Total phenolic content

Total phenol content (TPC) was determined using the spectrophotometric method. About 0.5 mL of ethyl acetate extract in the concentration of 1 mg/mL was prepared and mixed with 2.5 mL of Folin-Coicalteu's reagent and 2.5 mL of 7.5% NaHCO₃. Blank was prepared with ethyl acetate without the fungal extract and the test tubes were incubated at 45°C for 45 min. The absorbance was determined using a spectrophotometer at 740 nm. Tannic acid (10-100 mM) was used as the standard for calibration and a linear graph was constructed and distilled water was used for setting blank. The TPC expressed as mg Tannic acid equivalent (mg TAE)/g of dry weight [38].

1, 1-diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging activity

Briefly, 40 µL of crude fungal extracts with different concentrations (0.05 – 2 mg/mL) were mixed with 200 µL of 50 µM DPPH solution in methanol. Then, the mixture was immediately shaken before it is incubated in the dark condition at room temperature for 15 min. The absorbance was measured at 517 nm. Ascorbic acid with concentrations of 5 – 80 µg/mL was used as a standard while ethyl acetate is used as a control was. The percentage of inhibition activity of the fungal extracts was calculated using the following equation:

$$\text{radical scavenging activity (\%)} = \frac{[(A_1 - A_2) \times 100]}{A_1}$$

Where, A₁ = Absorbance of control

A₂ = Absorbance of sample [39].

Molecular characterization of the endophytic fungi

The fungal strain with significant antioxidant and total phenolic content were characterized by molecular methods where genomic DNA was extracted according to the protocol described by Arora *et al.* (2019). The primers ITS1 (5'-TCCGTAGGTGAA-3') and ITS4 (TCCTCCGCTTC-3') were used to amplify the ITS region. The PCR products were subsequently purified and sequenced in two directions on an ABI 3700 automated sequencer. The analysis was performed with the basic sequence alignment BLAST program run against the database (National Centre for Biotechnology information website- <http://www.ncbi.nlm.nih.gov>). The determined sequence was aligned using Clustal X. The sequences were submitted to GenBank and the respective accession number was obtained [40].

Results

Isolation of endophytic fungi

A total of seventeen endophytic fungi were isolated from different parts of *C. citratus* and named as CCL1-4 (*C. citratus* Leaf isolates), and CCR1-4 (*C. citratus* Root isolates). The frequencies of fungal colonization were higher in leaf samples with 37.5 % compared to roots with 33.3 % colonization.

Identification of endophytic fungi

The endophytic fungi were identified as *Alternaria* sp., *Amorphotheca* sp., *Aspergillus fumigatus*, *Aspergillus* sp., *Bipolaris* sp., *Circinella* sp., *Cladosporium* sp., *Colletotrichum Curvularia* sp. *Cylindrocladium* sp. *Fusarium* sp., *Fusarium oxysporum*, *Fusarium solani*, *Mycelia Sterilia*, *Mucor* sp., *Nigrospora oryzae* and *Penicillium* sp.

Phytochemical analysis

The phytochemical analysis was carried out for 17 endophytic fungal crude extracts. Among the seventeen isolates, seven isolates showed significant results in the production of phytochemical constituents. Alkaloids, carbohydrates, cardiac glycosides, phenols, saponins, terpenoids, flavonoids, quinones, were present in *Fusarium oxysporum*. Phenol production was seen in more than 82% of the fungal isolates, 76% of the isolates were positive for flavonoids, 70% isolates were positive for terpenoids. Quinones and cardiac glycosides were seen only in 41 % of the fungal isolates (Table 1).

Extracellular enzyme production

Amylase, cellulase, laccase, lipase, chitinase, protease and tyrosinase activities were assayed. Among the seventeen isolates tested, 70.5% demonstrated amylase activity, 58.8% Protease and laccase activity, 41.1% lipase and chitinase activity and only 29.4% showed positive for cellulase activity (Figure 1) (Table 2).

Total phenolic content (TPC)

Among the seventeen endophytic fungi screened, 82% isolates produced phenolic content. A wide range in the production of total phenolic concentrations was recorded in endophytic fungal extracts, as shown in Figure 2. The values varied from 14.6 to 65.5 mg TAE/g of dry weight. The highest concentration of phenols was observed in the extract of *F. oxysporum* (65.5±0.53 mg TAE) followed by *F. solani* (50.3±0.24 mg TAE). Whereas *A. fumigatus*, *Bipolaris* sp., *Cladosporium* sp., *Cylindrocladium* sp., *N. oryzae* and *Penicillium* sp., extracts contained considerably least concentration of phenolic content.

DPPH radical scavenging activity

The isolates were screened for the antioxidant potential by DPPH assay. The positive results were noted as a colour change from purple to yellow. More than 80% of the fungal isolates showed radical scavenging ability. Among the isolates screened *F. oxysporum* and *F. solani* showed a high antioxidant capacity value of 64% whereas *A. fumigatus*, *Bipolaris* sp., *Cladosporium* sp., *Cylindrocladium* sp., *N. oryzae* and *Penicillium* sp., showed the least antioxidant activity (11-25%). Ascorbic acid was taken as standard showing 98% scavenging activity (Figure 3).

Table 1: Qualitative analysis of phytochemicals from the ethyl acetate extracts of endophytic fungi

Sl.no.	Culture code	Endophytic fungi	Alkaloids	Tannins	Cardiac glycosides	Phenols	Amino acids	Saponins	Terpenoids	Quinones	Flavonoids
1	CCL1	<i>Alternaria</i> sp.	+	+	-	-	-	-	+	+	+
2	CCL2	<i>Amorphotheca</i> sp.	+	+	+	+	-	-	+	+	+
3	CCL3	<i>Fusarium oxysporum</i>	+	+	+	+	-	+	+	+	+
4	CCL4	<i>Aspergillus</i> sp.	+	-	+	+	-	-	+	+	+
5	CCL5	<i>Bipolaris</i> sp.	+	-	-	+	-	+	-	+	+
6	CCL6	<i>Circinella</i> sp.	+	-	+	-	-	+	+	-	+
7	CCL7	<i>Cladosporium</i> sp.	+	+	+	+	-	+	-	-	+
8	CCL8	<i>Colletotrichum</i> sp.	+	-	+	+	+	+	+	-	+
9	CCL9	<i>Curvularia</i> sp.	+	-	-	-	+	-	+	-	-
10	CCR1	<i>Cylindrocladium</i> sp.	-	-	-	+	-	-	-	-	-
11	CCR2	<i>Fusarium</i> sp.	-	+	-	+	+	-	+	-	+
12	CCR3	<i>Aspergillus fumigatus</i>	+	-	-	+	+	-	+	+	+
13	CCR4	<i>Fusarium solani</i>	+	+	+	+	-	+	+	+	-
14	CCR5	Mycelia Steriilia.	-	+	-	+	-	+	+	-	+
15	CCR6	<i>Mucor</i> sp.	-	-	-	+	-	+	-	-	+
16	CCR7	<i>Nigrospora oryzae</i>	-	+	-	+	+	+	+	-	-
17	CCR8	<i>Penicillium</i> sp.	-	+	-	+	-	+	-	-	+

*CCL- *Cymbopogon citratus* Leaf, CCR- *Cymbopogon citratus* Root. + represents Positive result, - represents Negative result

Table 2: Qualitative analysis of extracellular enzymes from the ethyl acetate extracts of endophytic fungi

Sl.no.	Culture code	Endophytic fungi	Amylase	Cellulase	Tyrosinase	Protease	Chitinase	Laccase
1	CCL1	<i>Alternaria</i> sp.	+	-	-	-	-	-
2	CCL2	<i>Amorphotheca</i> sp.	+	-	+	-	+	-
3	CCL3	<i>Aspergillus fumigatus</i>	+	+	-	-	-	-
4	CCL4	<i>Aspergillus</i> sp.	+	-	+	-	+	-
5	CCL5	<i>Bipolaris</i> sp.	-	-	-	+	-	+
6	CCL6	<i>Circinella</i> sp.	+	-	+	+	+	+
7	CCL7	<i>Cladosporium</i> sp.	-	-	+	+	+	+
8	CCL8	<i>Colletotrichum</i> sp.	+	+	+	+	+	+
9	CCL9	<i>Curvularia</i> sp.	+	+	-	-	-	-
10	CCR1	<i>Cylindrocladium</i> sp.	-	-	-	-	-	-
11	CCR2	<i>Fusarium</i> sp.	+	+	-	-	-	-
12	CCR3	<i>Fusarium oxysporum</i>	+	-	+	+	+	+
13	CCR4	<i>Fusarium solani</i>	+	-	+	+	+	+
14	CCR5	Mycelia Steriilia.	+	-	-	+	-	+
15	CCR6	<i>Mucor</i> sp.	-	-	-	+	-	+
16	CCR7	<i>Nigrospora oryzae</i>	+	+	-	+	-	+
17	CCR8	<i>Penicillium</i> sp.	-	-	-	+	-	+

*CCL- *Cymbopogon citratus* Leaf, CCR- *Cymbopogon citratus* Root. + represents Positive result, - represents Negative result

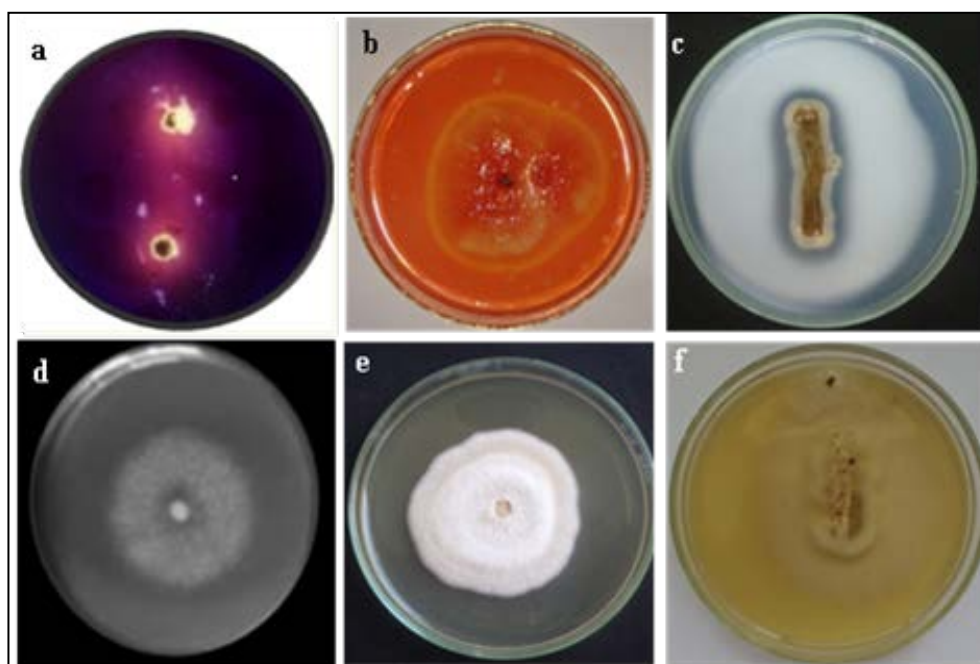


Fig 1: Qualitative analysis of extracellular enzymes from endophytic fungal isolates **a**-amylase, **b**-cellulase, **c**-protease, **d**-laccase **e**-lipase **f**-chitinase

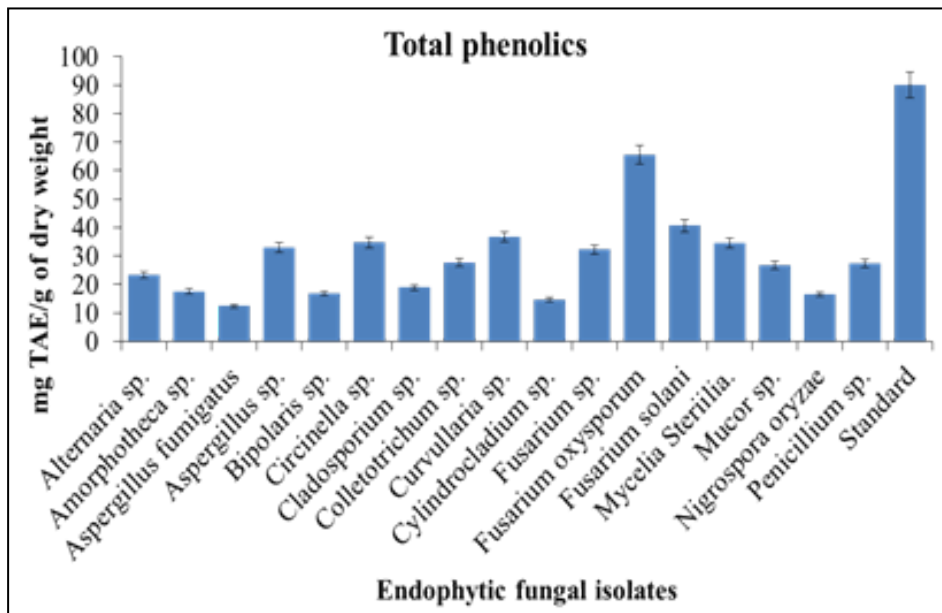


Fig 2: Total phenolic content of the ethyl acetate extracts from endophytic fungal isolates

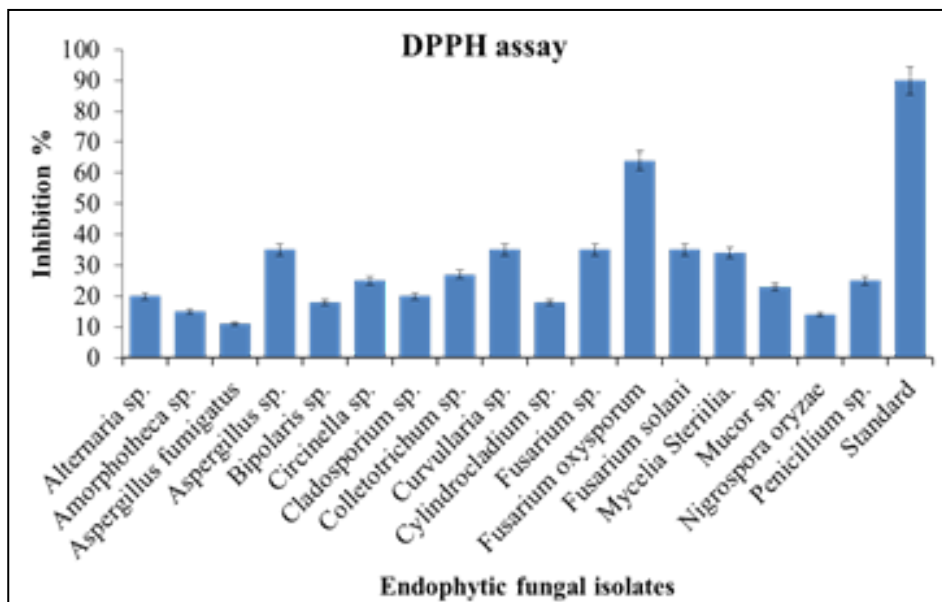


Fig 3: Antioxidant activity of ethyl acetate extracts from endophytic fungal isolate

Molecular identification

Molecular identification of isolate CCL3 and sequence analysis showed 100% homology with *Fusarium oxysporum*, MG745375 is the accession number obtained from the NCBI Genbank.

Discussion

Cymbopogon citratus is an important medicinal plant recognized for their wide variety of phytochemicals and phenolic compounds such as flavonoids with antioxidant potential [17]. A wide variety of vigorous phytochemicals such as flavonoids, alkaloids, and saponins have been isolated and identified in different plants of citratus family [16]. In the present work, 17 endophytic fungal strains were isolated from *C. citratus* and screened for phytochemical and extracellular enzymes. Similar work has been reported by Mani *et al.*, in 2018 in which endophytic fungi *Curvularia australiensis* and *Alternariacitrimacularis* from *Aegle marmelos* were found positive for phytochemicals like flavonoid, phenols, saponins, cardiac glycosides,

terpenoids, quinones, carbohydrates, alkaloids and phenols, and also showed highest antioxidant properties [39]. *Trichoderma viride* isolated from *Hybanthus enneaspermus* produced phytochemical compounds such as terpenoids, tannins, *Alternaria alternata* produced alkaloids, terpenoids, tannins, phenols and flavonoids was observed [41].

N. sphaerica has potential as an antioxidant agent due to the significant result on both antioxidant and polyphenolic properties compared to the other species [47]. All the nineteen endophytic fungi isolated from *Solanum tuberosum* L. produced amylase, while cellulase and tyrosinase were recorded in more than 50% of the isolated species, whereas laccase and protease and manganese peroxidase were shown by a few taxa. None of the isolated fungi produced chitinase [48]. The ethyl acetate extract of the *Nigrospora* sp. isolated from a medicinal plant *Ginkgo biloba* displayed a potent antioxidant activity by DPPH radical scavenging assay with an IC_{50} value of $9.28\mu\text{g/ml}$ [49]. About fourteen endophytic fungi from *S. miltiorrhiza* Bge.f.alba were screened for phytochemicals. *Fusarium proliferatum* yielded all nine

phytochemicals, including saponins, phenol, flavonoids, cardiac glycosides, steroids, tannins, alkaloids, anthraquinone and terpenoids. It also exhibited stronger antioxidant activities by FRAP and DPPH method [50]. The ethanol extracts of *Aspergillus niger* and *Fusarium oxysporum* isolated from *Crotalaria pallida* yielded tannins, flavonoids, terpenoids, phenol and saponins. It also exhibited potent antioxidant activity against FRAP and DPPH radicals with an EC₅₀ value of 7.25 and 6.41 µg/mg, respectively [51]. Thirteen different endophytic fungi were isolated *Tabebuia argentea*. The ethyl acetate extracts from *Aspergillus niger* and *Alternaria alternata* yielded saponins, phenolic compounds, anthraquinones, steroids, cardiac glycosides and tannins. They also showed the strongest antioxidant capacity, having the highest phenolics levels [52]. The endophytic fungi associated with *Cymbidium aloifolium* were subjected to the production of extracellular enzymes in which 93% produced phosphatase, 80% cellulase, 70% amylase, 63.33% protease, 30% pectinase, 23.33% lipase and 10% laccase [53]. A total of 12 different species of endophytic fungi were recorded in *Adhatoda vasica*, *Costus igneus*, *Coleus aromaticus* and *Lawsonia inermis* majority of the endophytic fungi showed the positive for amylase, cellulase, laccase, lipase, protease [54]. *Penicillium citrinum* BSL17 isolated from *Boswellia sacra* showed significantly higher amounts of glucosidases (62.15±1.8 µM-1min-1mL) and cellulases [55]. Fifty fungal strains, isolated from medicinal plants (*Alpinia calcarata*, *Bixa orellana*, *Calophyllum inophyllum* and *Catharanthus roseus*) were screened for extracellular enzymes such as amylase, cellulase, laccase, lipase, pectinase and protease on solid media. Sixty-four percent of fungi screened for enzymes showed positive for lipase, 62% for amylase and pectinase, 50% showed for lipase, 32% showed for cellulase, 30% for laccase and only 28% showed positive for protease [56]. A total of one hundred and twelve endophytic fungi isolated from six medicinal plants collected in Western Ghats of Karnataka were subjected for extracellular enzyme production, of which 29% were positive for amylase, 28% cellulase, 18% pectinase, 40% asparaginase and none for laccase activity [57]. Among the eleven endophytic fungi, which were associated with the stems of *Phragmanthera capitata* (Loranthaceae), revealed the presence of flavonoids, anthroquinones, tannins, phenols, steroids, coumarins and terpenoids and absence of alkaloids and saponins in all the extracts [58]. All the 21 endophytes isolated from *Eugenia jambolana* Lam. were screened for phytochemicals and were found positive for alkaloids, saponins, flavonoids, terpenes and phenols and 36% of isolates showed high phenolic content exhibited potent antioxidant activity [59]. *Phomopsis* sp. and *Xylaria* sp. isolated from *Emblica officinalis* showed the highest antioxidant activity and also had higher levels of phenolics [60].

Conclusion

In recent years fungal endophytes have received particular attention due to their ability to produce metabolites of pharmacological interest. Therefore, the isolation, identification and conservation of endophyte species along with the study of their metabolites are of great importance. In the present investigation, 17 endophytic fungal isolates were screened for the presence of extracellular enzymes such as amylase, cellulase, laccase, lipase and protease. 76%

of the isolates were found positive for flavonoids, 70% for terpenoids, Quinones and cardiac glycosides were seen in 41% of the fungal isolates. For the extracellular enzymes production, 70.5% demonstrated amylase activity, 58.8% protease and laccase activity, 41.1% lipase and chitinase activity and only 29.4% showed positive for cellulase activity. The TPC and antioxidant activity of the fungal cultures ranged from 14.29 to 14.6 to 65.5 mg TAE/g of dry weight and 11-60% of scavenging respectively. The endophytic isolate of *Fusarium oxysporum* showed the strongest antioxidant capacity, having the highest phenolics concentration. It was noted that fungal endophytes isolated from *C. citratus* yield pharmaceutically important compounds. The antioxidant potential might be directly linked to these phenolic and flavonoid compounds present in the ethyl acetate extract of *F. oxysporum*. Thus, endophytic fungal isolates, especially *F. oxysporum*, has proven to be a promising source of natural products with great chemical diversity.

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References

1. Tanney JB, Seifert KA. Mollisiaceae: An overlooked lineage of diverse endophytes. *Studies in Mycology*, 2020.
2. Donald J, Roy M, Suescun U, Iribar A, Manzi S, Pélissier L *et al.* A test of community assembly rules using foliar endophytes from a tropical forest canopy. *Journal of Ecology*, 2020.
3. Panke-Buisse K, Cheng L, Gan H, Wickings K, Petrovic M, Kao-Kniffin J, *et al.* Root fungal endophytes and microbial extracellular enzyme activities show patterned responses in tall fescues under drought conditions. *Agronomy*. 2020; 10(8):1076.
4. Jagannath S, Konappa N, Lokesh A, Dasegowda T, Udayashankar AC, Chowdappa S, *et al.* Bioactive compounds guided diversity of endophytic fungi from *Baliospermum montanum* and their potential extracellular enzymes. *Analytical Biochemistry*. 2020; 14:114024.
5. Dos Santos RMG, Rodrigues-Fo E, Rocha WC, Teixeira MFS. Endophytic fungi from *Melia azedarach*. *World journal of Microbiology and Biotechnology*. 2003; 19(8):767-770.
6. Lee JM, Tan WS, Ting ASY. Revealing the antimicrobial and enzymatic potentials of culturable fungal endophytes from tropical pitcher plants (*Nepenthes* spp.). *Mycosphere*. 2014; 5(2):364-377.
7. González-Teuber M, Vilo C, Guevara-Araya MJ, Salgado-Luarte C, Gianoli E. Leaf resistance traits influence endophytic fungi colonization and community composition in a South American temperate rainforest. *Journal of Ecology*. 2020, 108(3):1019-1029.
8. Tan RX, Zou WX. Endophytes: a rich source of functional metabolites. *Natural product reports*. 2001; 18(4):448-459.

9. Priti V, Ramesha BT, Singh S, Ravikanth G, Ganeshiah KN, Suryanarayanan TS, *et al.* How promising are endophytic fungi as alternative sources of plant secondary metabolites?. *Current Science*. 2009; 97(4):477-478.
10. Caruso G, Golubkina N, Tallarita A, Abdelhamid MT, Sekara A. Biodiversity, ecology, and secondary metabolites production of endophytic fungi associated with Amaryllidaceae crops. *Agriculture*. 2020; 10(11):533.
11. Christian N, Sedio BE, Florez-Buitrago X, Ramírez-Camejo LA, Rojas EI, Mejía LC *et al.* Host affinity of endophytic fungi and the potential for reciprocal interactions involving host secondary chemistry. *American Journal of Botany*. 2020; 107(2):219-228.
12. He W, Megharaj M, Wu CY, Subashchandrabose SR, Dai CC. Endophyte-assisted phytoremediation: mechanisms and current application strategies for soil mixed pollutants. *Critical reviews in biotechnology*. 2020; 40(1):31-45.
13. Tan RX, Zou WX. Endophytes: a rich source of functional metabolites. *Natural product reports*. 2001; 18(4):448-459.
14. Matasyoh JC, Wagara IN, Nakavuma JL. Chemical composition of *Cymbopogon citratus* essential oil and its effect on mycotoxigenic *Aspergillus* species. *African Journal of Food Science*. 2011; 5(3):138-142.
15. Bassolé IHN, Lamien-Meda A, Bayala BOLC, Obame LC, Ilboudo AJ, Franz C, *et al.* Chemical composition and antimicrobial activity of *Cymbopogon citratus* and *Cymbopogon giganteus* essential oils alone and in combination. *Phytomedicine*. 2011; 18(12):1070-1074.
16. Koba K, Sanda K, Guyon C, Raynaud C, Chaumont JP, Nicod L *et al.* *In vitro* cytotoxic activity of *Cymbopogon citratus* L. and *Cymbopogon nardus* L. essential oils from Togo. *Bangladesh Journal of Pharmacology*. 2009; 4(1):29-34.
17. Nguefack J, Dongmo JL, Dakole CD, Leth V, Vismer HF, Torp J *et al.* Food preservative potential of essential oils and fractions from *Cymbopogon citratus*, *Ocimum gratissimum* and *Thymus vulgaris* against mycotoxigenic fungi. *International journal of food microbiology*. 2009; 131(2-3):151-156.
18. Rauber CDS, Guterres SS, Schapoval EE. LC determination of citral in *Cymbopogon citratus* volatile oil. *Journal of Pharmaceutical and Biomedical Analysis*. 2005; 37(3):597-601.
19. Chen Z, Ye X, Qingkui G, Wenliang Q, Wen Z, Ning W, *et al.* Anticancer activity of green synthesised AgNPs from *Cymbopogon citratus* (LG) against lung carcinoma cell line A549. *IET nanobiotechnology*. 2018; 13(2):178-182.
20. Shah S, Shrestha R, Maharjan S, Selosse MA, Pant B. Isolation and characterization of plant growth-promoting endophytic fungi from the roots of *Dendrobium moniliforme*. *Plants*. 2019; 8(1):5.
21. Tibpromma S, Hyde KD, Bhat JD, Mortimer PE, Xu J, Promputtha I, *et al.* Identification of endophytic fungi from leaves of *Pandanaceae* based on their morphotypes and DNA sequence data from southern Thailand. *MycKeys*. 2018; (33):25.
22. Akpotu MO, Eze PM, Abba CC, Nwachukwu CU, Okoye FB, Esimone CO. Antimicrobial activities of secondary metabolites of endophytic fungi isolated from *Catharanthus roseus*. *Journal of Health Sciences*. 2017; 7(1):15-22.
23. Danmalam UH, Hanwa UA, Abdurrazak A, Hassan-Maidoki AL, Ambi AA. Phytochemical analysis of some *crotalaria* species growing in Samaru-zaria, Nigeria for the presence of pyrrolizidine alkaloids. *Trends sci technology J*. 2017; 2(2):1035-1037.
24. Desire MH, Bernard F, Forsah MR, Assang CT, Denis, ON. Enzymes and qualitative phytochemical screening of endophytic fungi isolated from *Lantana camara* Linn. Leaves. *Journal of Applied Biology and Biotechnology*. 2014; 2(06):1-6.
25. Jassal PS, Sharma Monika. Evaluation of antioxidant, antibacterial, antihemolytic, and phytochemical properties of *Ficus benjamina*, *Ficus infectoria*, and *Ficus krishnae*. *Evaluation*. 2019, 12(3).
26. Unuigbo C, Enahoro J, Erharuyi O, Okeri HA. Phytochemical analysis and antioxidant evaluation of lemon grass (*Cymbopogon citratus* DC.) Stapf leaves. *Journal of Applied Sciences and Environmental Management*. 2019; 23(2):223-228
27. Sarkar M. Investigation of *In-vitro* antioxidant potential, Brine shrimp lethality and Thrombolytic activity in *Ficus mollis vahl* Leaves along with Phytochemical Screening, 2018.
28. Agu KC, Okolie PN. Proximate composition, phytochemical analysis, and *in vitro* antioxidant potentials of extracts of *Annona muricata* (Soursop). *Food science & nutrition*. 2017; 5(5):1029-1036.
29. Escribano J, Cabanes J, Jiménez-Atiénzar M, Ibañez-Tremolada M, Gómez-Pando LR, García-Carmona F, *et al.* Characterization of betalains, saponins and antioxidant power in differently colored quinoa (*Chenopodium quinoa*) varieties. *Food chemistry*. 2017; 234:285-294.
30. Malik SK, Ahmed M, Khan F. Identification of novel anticancer terpenoids from *Prosopis juliflora* (Sw) DC (Leguminosae) pods. *Tropical Journal of Pharmaceutical Research*. 2018; 17(4):661-668.
31. Eghbaliferiz S, Emami SA, Tayarani-Najaran Z, Iranshahi M, Shakeri A, Hohmann J *et al.* Cytotoxic diterpene quinones from *Salvia tebesana* Bunge. *Fitoterapia*. 2018; 128:97-101.
32. Benabda O, M'hir S, Kasmi M, Mnif W, Hamdi M. Optimization of protease and amylase production by *Rhizopus oryzae* cultivated on bread waste using solid-state fermentation. *Journal of Chemistry*, 2019.
33. Gilpin GS, Andrae AS. Comparative attributional life cycle assessment of European cellulase enzyme production for use in second-generation lignocellulosic bioethanol production. *The International Journal of Life Cycle Assessment*. 2017; 22(7):1034-1053.
34. Harir M, Bellahcene M, Baratto MC, Pollini S, Rossolini GM, Trabalzini L *et al.* Isolation and characterization of a novel tyrosinase produced by Sahara soil actinobacteria and immobilization on nylon nanofiber membranes. *Journal of biotechnology*. 2018; 265:54-64.
35. Sharma KM, Kumar R, Panwar S, Kumar A. Microbial alkaline proteases: Optimization of production parameters and their properties. *Journal of Genetic Engineering and Biotechnology*. 2017; 15(1):115-126.

36. Shaikh SS, Wani SJ, Sayyed RZ, Thakur R, Gulati A. Production, purification and kinetics of chitinase of *Stenotrophomonas maltophilia* isolated from rhizospheric soil, 2018.
37. Wang F, Xu L, Zhao L, Ding Z, Ma H, Terry N *et al.* Fungal laccase production from lignocellulosic agricultural wastes by solid-state fermentation: a review. *Microorganisms*. 2019; 7(12):665.
38. Sembiring EN, Elya B, Sauriasari R. Phytochemical screening, total flavonoid and total phenolic content and antioxidant activity of different parts of *Caesalpinia bonduc* (L.) Roxb. *Pharmacognosy journal*. 2018, 10(1).
39. Tešanović K, Pejin B, Šibul F, Matavulj M, Rašeta M, Janjušević L *et al.* A comparative overview of antioxidative properties and phenolic profiles of different fungal origins: fruiting bodies and submerged cultures of *Coprinus comatus* and *Coprinellus truncorum*. *Journal of food science and technology*. 2017; 54(2):430-438.
40. Mani VM, Soundari AJPG, Preethi K. Enzymatic and phytochemical analysis of endophytic fungi on *Aegle marmelos* from Western Ghats of Tamil Nadu, India. *Int J Life Sci Pharma Res*. 2018; 8(1):1-8.
41. Sheeba H, Ali MS, Anuradha V. Bioactive compounds and antimicrobial activity of fungal crude extract from medicinal plants. *Journal of Pharmaceutical Sciences and Research*. 2019; 11(5):1826-1833.
42. Ayob FW, Mohamad J, Simarani K. Antioxidants and phytochemical analysis of endophytic fungi isolated from a medicinal plant *Catharanthus roseus*. *Borneo Journal of Sciences and Technology*. 2019; 1(2):62-68.
43. Danagoudar A, Joshi CG, Ravi SK, Kumar HGR, Ramesh BN. Antioxidant and cytotoxic potential of endophytic fungi isolated from medicinal plant *Tragia involucrata* L. *Pharmacognosy Research*. 2018; 10(2):188.
44. Gunasekaran S, Sathiavelu M, Arunachalam S. *In vitro* antioxidant and antibacterial activity of endophytic fungi isolated from *Mussaenda luteola*. *J Appl Pharm Sci*. 2017; 7(8):234-238.
45. Joseph B, Priya RM. Bioactive compounds from endophytes and their potential. *American journal of biochemistry and molecular biology*. 2011; 1(3):291-309.
46. Karadeniz F, Burdurlu HS, Koca N, Soyer Y. Antioxidant activity of selected fruits and vegetables grown in Turkey. *Turkish Journal of Agriculture and Forestry*. 2005; 29(4):297-303.
47. Ayob FW, Mohamad J, Simarani K. Antioxidants and phytochemical analysis of endophytic fungi isolated from a medicinal plant *Catharanthus roseus*, 2019.
48. Yasser M, Mousa ASM, Ai MAMT. Molecular identification, extracellular enzyme production and antimicrobial activity of endophytic fungi isolated from *Solanum tuberosum* L. in Egypt. *Egypt Biosciences Biotechnology Research Asia; Bhopal*. 2019; 16(2):135-14.
49. Pawle G, Singh SK. Antimicrobial, antioxidant activity and phytochemical analysis of an endophytic species of *Nigrospora* isolated from living fossil *Ginkgo biloba*. *Curr Res Environ Appl Mycol*. 2014; 4(1):1-9.
50. Li YL, Xin XM, Chang ZY, Shi RJ, Miao ZM, Ding J *et al.* The endophytic fungi of *Salvia miltiorrhiza* Bge. *f. alba* are a potential source of natural antioxidants. *Botanical Studies*. 2015; 56(1):1-7.
51. Govindappa M, Bharath N, Shruthi HB, Santoyo G. *In vitro* antioxidant activity and phytochemical screening of endophytic extracts of *Crotalaria pallida*. *Free Radicals and Antioxidants*. 2011; 1(3):79-86.
52. Sadananda TS, Nirupama R, Chaithra K, Govindappa M, Chandrappa CP, Vinay Raghavendra B *et al.* Antimicrobial and antioxidant activities of endophytes from *Tabebuia argentea* and identification of anticancer agent (lapachol). *J Med Plants Res*. 2011; 5(16):3643-3652.
53. Shubha J, Srinivas C. Diversity and extracellular enzymes of endophytic fungi associated with *Cymbidium aloifolium* L. *African Journal of Biotechnology*. 2017; 16(48):2248-2258.
54. Amirita A, Sindhu P, Swetha J, Vasanthi NS, Kannan KP. Enumeration of endophytic fungi from medicinal plants and screening of extracellular enzymes. *World J Sci Technol*. 2012; 2(2):13-19.
55. Khan AL, Al-Harrasi A, Al-Rawahi A, Al-Farsi Z, Al-Mamari A, Waqas M *et al.* Endophytic fungi from Frankincense tree improves host growth and produces extracellular enzymes and indole acetic acid. *PloS one*. 2016; 11(6):e0158207.
56. Sunitha VH, Nirmala Devi D, Srinivas C. Extracellular enzymatic activity of endophytic fungal strains isolated from medicinal plants. *World Journal of Agricultural Sciences*. 2013; 9(1):1-9.
57. Uzma F, Konappa NM, Chowdappa S. Diversity and extracellular enzyme activities of fungal endophytes isolated from medicinal plants of Western Ghats, Karnataka. *Egyptian journal of basic and applied sciences*. 2016; 3(4):335-342.
58. Ladoh-Yemeda CF, Nyegue MA, Ngene JP, Benelesse GE, Lenta B, Wansi JD *et al.* Identification and phytochemical screening of endophytic fungi from stems of *Phragmanthera capitata* (Sprengel) S. Balle (Loranthaceae). *Journal of Applied Biosciences*. 2015; 90:8355-8360.
59. Yadav M, Yadav A, Yadav JP. *In vitro* antioxidant activity and total phenolic content of endophytic fungi isolated from *Eugenia jambolana* Lam. *Asian Pacific journal of tropical medicine*. 2014; 7:S256-S261.
60. Nath A, Raghunatha P, Joshi SR. Diversity and biological activities of endophytic fungi of *Embllica officinalis*, an ethnomedicinal plant of India. *Mycobiology*. 2012; 40(1):8-13.