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

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**Abstract**
Seventeen endophytic fungi were isolated from leaves and roots of *Cymbopogon citratus*. The endophytic fungal extracts were prepared using ethyl acetate and evaluated for their phytochemical constituents and extracellular enzymes using standard protocols. They were also subjected to Total Phenolic Content (TPC) and antioxidant potential. 76% of the isolates were found positive for flavonoids, 70% for terpenoids, Quinones and cardiac glycosides were seen in 41%. 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 65.5 mg TAE/g of dry weight and 11-60% of scavenging respectively. Among the 17 isolates, *Fusarium oxysporum* showed the strongest antioxidant capacity, having the highest phenolics concentration. The present work reveals that the metabolites produced by endophytic fungi can be a potential source of natural antioxidants and enzymes with pharmaceutical importance.

**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, xanthones, 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 a-nd 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±2ºC 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±2°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 FeCl3 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 underlaid 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±2°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 6.5and 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 water1 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 with2.5 mL of Folin-Coicalteu's reagent and 2.5 mL of 7.5% $NaHCO_3$. Blank was prepared with ethyl acetate without the fungal extract and the test tubes were incubated at 45ºC 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 dey 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, $A_1$ = Absorbance of control
$A_2$ = 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 ITSI (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 Curvullaria sp. Cylindrocladium sp. Fusarium* sp., *Fusarium oxysporum*, *Fusarium solani*, Mycelia Steriilia, *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 Figure2. 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 | *Alternaria* sp. | + | + | - | - | - | - | + | + | + |
| 2 | CCL2 | *Amorphotheca* sp. | + | + | + | + | - | - | + | + | + |
| 3 | CCL3 | *Fusarium oxysporum* | + | + | + | + | - | + | + | + | + |
| 4 | CCL4 | *Aspergillus* sp. | + | - | + | + | - | - | + | + | + |
| 5 | CCL5 | *Bipolaris* sp. | + | - | - | + | - | + | - | + | + |
| 6 | CCL6 | *Circinella* sp. | + | - | + | - | - | + | + | - | + |
| 7 | CCL7 | *Cladosporium* sp. | + | + | + | + | - | + | - | - | + |
| 8 | CCL8 | *Colletotrichum* sp. | + | - | + | + | + | + | + | - | + |
| 9 | CCL9 | *Curvullaria* sp. | + | - | - | - | + | - | + | - | - |
| 10 | CCR1 | *Cylindrocladium* sp. | - | - | - | + | - | - | - | - | - |
| 11 | CCR2 | *Fusarium* sp. | - | + | - | + | + | - | + | - | + |
| 12 | CCR3 | *Aspergillus fumigatus* | + | - | - | + | + | - | + | + | + |
| 13 | CCR4 | *Fusarium solani* | + | + | + | + | - | + | + | + | - |
| 14 | CCR5 | Mycelia Steriilia. | - | + | - | + | - | + | + | - | + |
| 15 | CCR6 | *Mucor* sp. | - | - | - | + | - | + | - | - | + |
| 16 | CCR7 | *Nigrospora oryzae* | - | + | - | + | + | + | + | - | - |
| 17 | CCR8 | *Penicillium* 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 | *Alternaria* sp. | + | - | - | - | - | - |
| 2 | CCL2 | *Amorphotheca* sp. | + | - | + | - | + | - |
| 3 | CCL3 | *Aspergillus fumigatus* | + | + | - | - | - | - |
| 4 | CCL4 | *Aspergillus* sp. | + | - | + | - | + | - |
| 5 | CCL5 | *Bipolaris* sp. | - | - | - | + | - | + |
| 6 | CCL6 | *Circinella* sp. | + | - | + | + | + | + |
| 7 | CCL7 | *Cladosporium* sp. | - | - | + | + | + | + |
| 8 | CCL8 | *Colletotrichum* sp. | + | + | + | + | + | + |
| 9 | CCL9 | *Curvullaria* sp. | + | + | - | - | - | - |
| 10 | CCR1 | *Cylindrocladium* sp. | - | - | - | - | - | - |
| 11 | CCR2 | *Fusarium* sp. | + | + | - | - | - | - |
| 12 | CCR3 | *Fusarium oxysporum* | + | - | + | + | + | + |
| 13 | CCR4 | *Fusarium solani* | + | - | + | + | + | + |
| 14 | CCR5 | Mycelia Steriilia. | + | - | - | + | - | + |
| 15 | CCR6 | *Mucor* sp. | - | - | - | + | - | + |
| 16 | CCR7 | *Nigrospora oryzae* | + | + | - | + | - | + |
| 17 | CCR8 | *Penicillium* sp. | - | - | - | + | - | + |

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

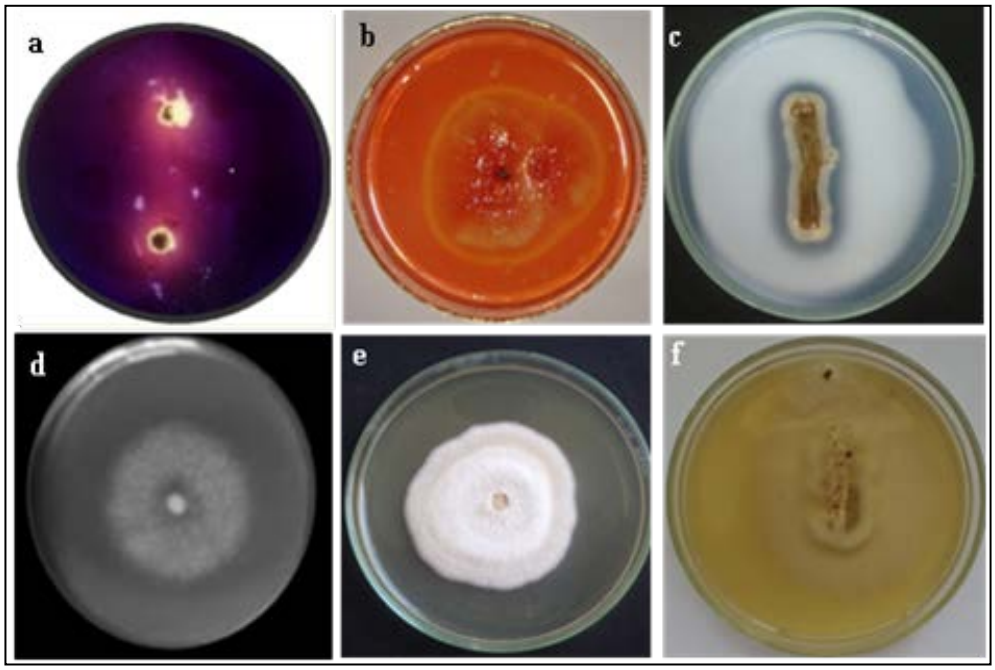


**Fig 1:** Qualitative analysis of extracellular enzymes from endophytic fungal isolates **a**-amylase**, b**-cellulase**, c**-protease**, d**-laccase **e**-lipase **f**-chitinase

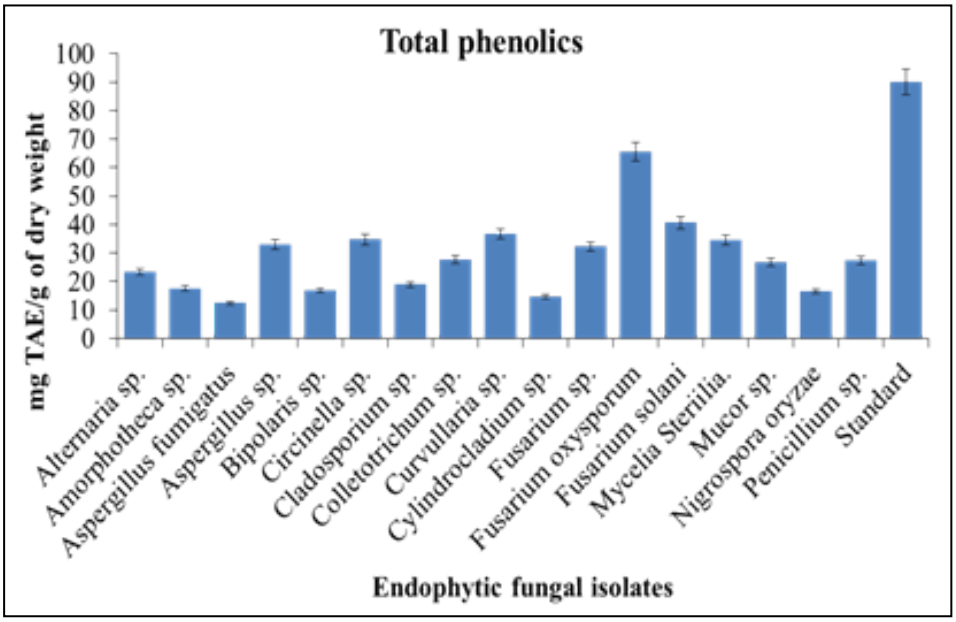


**Fig 2**: Total phenolic content of the ethyl acetate extracts from endophytic fungal isolates

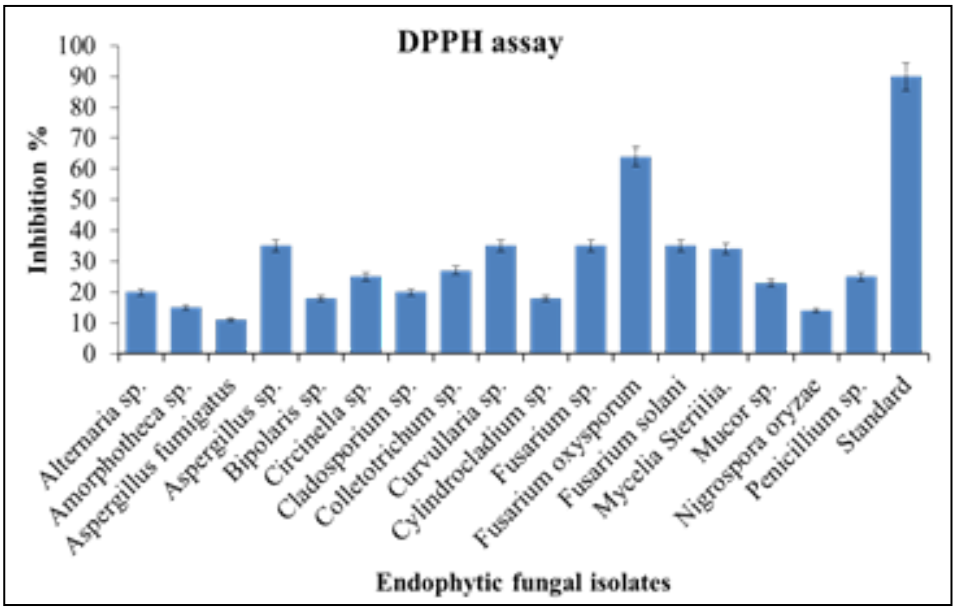


**Fig 3:** Antioxidant activity of ethyl acetate extracts from endophytic fungal isolate

**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 alternate* 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 9.28µg/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 $EC_{50}$ 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 alternate* 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 inerims* 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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