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"Effect of pH on Extracellular Enzyme Activity of Endophytic fungi isolated from Centella asiatica"

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KEYWORDS

Centella asiatica, Endophytic fungi, bioactive compound, and of extracellular enzymes.

ABSTRACT:

Endophytic fungus prevalent in medicinal plants was isolated and has demonstrated potent biogenic activity. Endophytic fungi were isolated from the medicinal plant Centella asiatica. Bioactive compounds from the isolates were produced in the in vitro conditions. Further, the secretion of extracellular enzymes by endophytic fungi was investigated. Twelve fungi were isolated from the leaves, shoots, and roots of Centella asiatica, out of which four isolates, namely, Penicillium chrysogenum isolate EF_S2, Trichoderma harzianum isolate EF_R1, Fusarium oxysporum isolate EF_L4, and Phoma sp. Isolate EF_L5 was found to be a potent biogenic agent for the ability to produce bioactive compounds, secondary metabolites, and secretion of extracellular enzymes. The phylogenetic identification of the endophytic fungi was performed, the type of secondary metabolite produced and was phytochemically analyzed the extracellular enzymes were screened. These results revealed that the Centella asiatica-based endophytic fungi can produce extracellular enzymes that have great promise for therapeutic uses in clinical microbiology and usage in biotechnological applications.

1 Introduction

Several medical practices and ethnic therapies make use of plants. India has a long history of using herbal remedies, and it has significantly improved the development of contemporary pharmaceutical research as well as Ayurveda and Siddha (Dahanukar and Kulkarni, 2000; Nasim *et al.*, 2022). Based on experience and traditional remedies, plants have been utilized as medicine for thousands of years, and their ability to heal both moderate and chronic illnesses continues to get significant interest. Globally, there is an increased interest in plant study, and a substantial amount of data has been gathered to demonstrate the immense potential of medicinal plants that are employed in many conventional medical practices (Vaidya and Devasagayam, 1997; Okunang *et al.*, 2011).

The *Centella asiatica* (CA) is one of the most significant and valuable perennial Indian herbs, growing in temperate and tropical wetlands (Mahlangu and Tai, 2022). Usually growing in tropical wetland habitats, this plant is endemic to humid warm areas worldwide and is widely known as pennywort or gotu kola. This occurs in India's temperate and tropical regions (Biswas *et al.*, 2022). Over hundreds of years, plant extracts have been used in medicine to treat a variety of diseases, including leprosy, varicose ulcers eczema, psoriasis, lupus, and female genitourinary tract disorders (Prakash *et al.*, 2017). Additionally, *Centella asiatica* is regularly included in anti-inflammatory, anti-ageing, and antioxidant creams in the cosmetics industry and is used as a brain tonic to enhance memory as well as learning function (Wang *et al.*, 2021). This herb's abundance in triterpenoids, flavonoids, vitamins, tannins, polyphenols, and volatile oil constituents is what gives it its therapeutic qualities (Kunjumon *et al.*, 2022). These components are known as bioactive compounds. Although the entire plant has these chemical components, the leaves have the highest concentration of them (Ragupathi *et al.*, 2022).

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Endophytes are microbial species that live in symbiotic relationships with species of plants (Ashitha *et al.,* 2019). These species can be either bacterial or fungal. Due to their symbiotic connection, endophytes have a variety of roles, such as safeguarding their host plants from pathogenic species, improving the plant's resistance to various abiotic and biotic stresses, generating hormones that promote growth like auxin and cytokinin, and promoting plant growth and development by solubilizing potassium and phosphate (Chowdhury and Bae, 2018; Singh *et al.,* 2019; Morales-Cedeño *et al.,* 2021). Furthermore, endophytes can generate bioactive chemicals, which are used as raw materials in a variety of industries, including the food, pharmaceutical, fragrance, and cosmetics domains (Olanrewaju *et al.,* 2017; Mushtaq *et al*., 2022). Numerous medicinal plants' leaves, stems, roots, and fruits have all been found to have bacterial and fungal endophytes (Kumar *et al.,* 2020; Husseiny *et al.,* 2021). The present study aimed to isolate and identify endophytic fungi from Centella asiatica, a medicinal plant known for its pharmacological potential. In addition, the study sought to analyze fungal extracts to evaluate their biochemical properties, with a particular emphasis on enzymatic activities under controlled in vitro conditions. This investigation provides insights into the potential applications of fungal endophytes in biotechnological and pharmaceutical fields, particularly through their ability to produce bioactive compounds and enzymes with various therapeutic and industrial benefits.

Material and Methods

1.1. Plant material and chemicals used

Healthy Centella asiatica plants were collected in the post-monsoon season from the Botanical Garden of Karnatak University, Dharwad, India. The plant was taxonomically identified and authenticated by a botanical survey of India, Coimbatore. The chemicals used in the study, including Tween 80, ethanol, hydrochloric acid, and sodium hydroxide, were of high analytical grade. The fungal media, Potato Dextrose Agar (PDA) was obtained from Himedia, India.

1.2. Surface sterilization of Centella asiatica explant

The selected healthy plants of *Centella asiatica* with a length of approximately 10 to 20 cm long shoots along with rhizomes were used for the isolation of endophytic fungi. surface sterilization method for the isolation of endophytic fungi was carried out as described by Rakotoniriana *et al.* (2008) and Schulz *et al.* (1998) With minor modifications. The plant samples were washed with running tap water, followed by a rinse with 250 ml of sterile distilled water with the addition of 2 to 4 drops of tween 80, and the explants were placed in 75% ethanol for 1 min. Later, the *Centella asiatica* sample was soaked in the 4% sodium hypochlorite for 5 minutes, following a repeated wash with distilled water, and followed by washing with 75% ethanol and sterile distilled water. The effectiveness of surface sterilization was tested by the method of Bhardwaj *et al*. (2015).

1.3. Isolation of Endophytic fungi from *Centella asiatica*

The sterilized explant of *Centella asiatica* was cut into 1x1 cm in size and placed on the PDA at 28 °C for 7 to 10 days. After the growth occurred from the explant, it was removed from the mother plate and inoculated separately on the potato dextrose agar to obtain a pure culture.

1.4. Characterization of endophytic fungi

The endophytic fungi isolated from Centella asiatica were initially identified by their colony morphology, Viz, colony color, texture, shape, spores and pigmentation. microscopic analysis of structures like spores and hyphae further supported species-level identification, forming the basis for molecular characterization and further functional studies. Further, 18S rRNA sequencing was done at Unigenome (Ahmedabad, India). The selected fungal DNA was isolated and quality was evaluated on 1.8% agarose gel, further, isolated DNA was amplified with 18s rRNA specific primer (18S_18A and 18S 1200R) using Veriti 96 well Thermal Cycler. Later, the PCR amplicon was purified and subjected to Sanger sequencing. Finally, the nucleotide sequence of the isolates was checked by BLAST analysis using the NCBI server (http://blast.ncbi.nlm.nih.gov/Blast.cgi), and a phylogenetic tree was constructed by the neighbor-

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joining method using MEGA X software (Tamura *et al*., 2013).

1.5. Small-scale production of endophytic fungal metabolites

The fungal secondary metabolites extraction was carried out as stated by (Andriy *et al*., 2017) and (Emmanuel *et al*., 2022) and with slight modifications. Endophytic fungal isolates were inoculated into 1000 mL Erlenmeyer flasks containing 500 mL PDB and kept at room temperature for 21 days under stationary conditions with occasional shaking. The mycelium was separated from the broth. The mycelium was rinsed with distilled water to remove residual media components, The metabolite from the fungus mycelium was extracted using ethyl acetate as an organic solvent. The extract was concentrated by eliminating the solvents under reduced pressure at 35-40 °C using rotary evaporation and the resultant compound was air dried to yield the crude metabolite and preserved at 4 ºC until further use and the pH of the media was monitored at regular intervals of time every 7 days.

1.6. Phytochemical screening of the fungal metabolites

The fungal extracts were subjected to various chemical tests to detect the presence of different phytochemical constituents: Alkaloids, Flavonoids, phenols, saponin, steroids, tannin, and terpenoids by the method (Bhardwaj *et al* 2015, Gouda *et al*., 2016. Galindo-Solís and Fernández, 2022 and Chandran *et al*., 2020).

1.6.1. Alkaloids.

The fungal cell suspension was opted for the bioassay alkaloids adding 2N hydrochloric acid and treated with a few drops of Mayer's reagent. The creamish precipitate indicates the presence of alkaloids.

1.6.2. Flavonoid

Flavonoid activities were analysed with 1 ml of fungal crude extract with the addition of 20% NaOH, the color changes to yellow, and with the addition of acid, the color disappears which indicates the presence of flavonoid.

1.6.3. phenols.

Endophytic fungi's response to phenols was tested with a 1:5 ratio of fungal extract and distilled water, respectively, after a thorough mix, a few drops of a 5% ferric chloride solution were added. The dark green color indicated the presence of phenolic compounds.

1.6.4. saponin

The saponin bioassay was performed, with the fungal extract which was vigorously shaken with distilled water and was allowed to stand for 10 minutes for frothing formation. emulsion indicated the presence of saponins.

1.6.5. steroids

A Libermann-Burchard reaction was conducted to test the reaction of steroids. A fungal extract was treated with 1 ml of chloroform, followed by the addition of acetic anhydride and a few drops of concentrated sulphuric acid. formation of blue green ring indicated the presence of steroids.

1.6.6. Tannin.

The tannin activity was analyzed by treatment of ferric chloride with fungal crude extract the appearance of a bluish-black color, which faded upon the addition of a small amount of dilute H_2SO_4, followed by the formation of a yellowish-brown precipitate, indicating the presence of tannins.

1.6.7. Terpenoids

Likewise, terpenoids were tested with crude fungal extract mixed with 2 ml of chloroform and sulphuric acid. The formation of a reddish-brown coloration at the interface indicated the presence of terpenoids.

1.7. Production of extracellular enzymes from endophytic fungal isolates.

The extracellular enzyme production by the endophytic fungi was tested by the method of (Maria *et al*., 2005). The endophytic fungus produces extracellular enzymes that decompose the dissolved or suspended substrate in the agar medium, which is subsequently incubated for 5 to 7 days at room temperature, resulting in a clean zone surrounding the fungal colonies.

1.6.1. Amylase activity

The activity of amylase was measured by growing the fungi on PDA with 0.2% soluble starch. After 5 days of the incubation period, the dishes were flooded with iodine (1%) and potassium iodide (2%).

1.6.2. Lipase activity

The fungi were grown on peptone agar medium supplemented with 1% olive oil, which was separately sterilized before being added to the media and incubated for five days to evaluate the activity of lipase.

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1.6.3. Protease activity

Protease activity was assessed by culturing the fungus on a PDA, placed to melted agar after being supplemented with 1% casein in 50 mM tris HCL buffer.

1.6.4. Cellulase Carboxymethyl cellulose (CMC) activity.

To assess CMC activity, 0.5 g of agar media was utilized. Following five days of incubation, the plates were submerged in 0.2% Congo red for 30 minutes, followed by the addition of 1 M sodium chloride for 30 minutes.

2. Results

2.1. Surface sterilization of CA explant

The effectiveness of the surface sterilization of the CA plants was tested by inoculating the homogenized mixture of last rinsed water on PDA; after two weeks of incubation, no growth was observed as shown in figure 1.

2.2. Isolation of endophytic fungi from CA

After 7 to 10 days of incubation, 26 isolates were obtained from *Centella asiatica*, through the primary screening process. Depending on the growth rate, finally, twelve isolates were taken for further study, out of which seven isolates (EF_L1-L7) were isolated from the leaf of the CA, followed by four endophytic fungi from the shoot (EF_S1-S4) and one isolate (EF_R1) from the root of the CA.

2.3. Characterization of endophytic fungi

Four endophytic (EF_S1, EF_R1, EF_L4, and EF_L5) fungi were chosen based on their phytochemical activity and extracellular enzyme potential. The colony of EF_S1 isolate was found to be powdery and blueish green in color and yellow exudate diffusing from the PDA; SEM images exhibit the filamentous hyphae with conidia (Fig. 2A). The EF_R1 colony was slightly white to green in color, forming spherical spores, and the multi nucleated, highly branched network was observed under microscopic view (Fig. 2B). The EF_L4 isolate was cottony-whitish from the front and lightish purple on the backside of the colony, and kidney-shaped oval microconidia were observed under a microscope (Fig. 2C). Lastly, the EF_L5 isolate was found to be round and velvety wrinkled; it was observed with septate hyphae with conidia and pycnidia in a microscope (Fig. 2D). Further, all four isolates were confirmed by the 18S rRNA sequence, and they were identified as *Penicillium chrysogenum* isolate EF_S1 (Fig. 2A), *Trichoderma harzianum* isolate EF_R1 (Fig. 2B), *Fusarium oxysporum* isolate EF_L4 (Fig. 3A), and *Phoma* sp. Isolate EF_L5 (Fig. 3B).

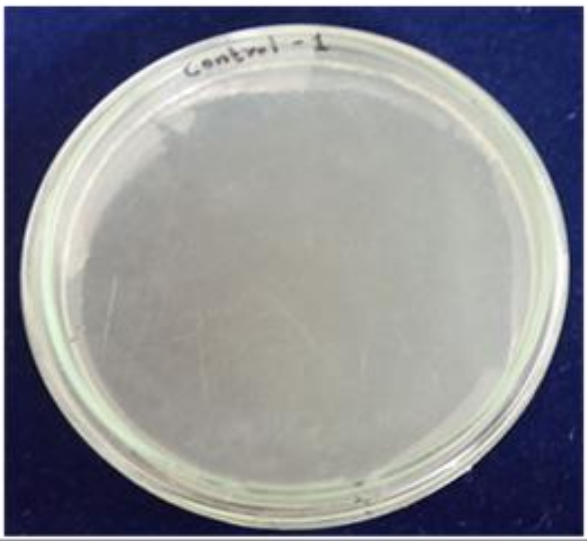

Figure 1: control for surface sterilization test

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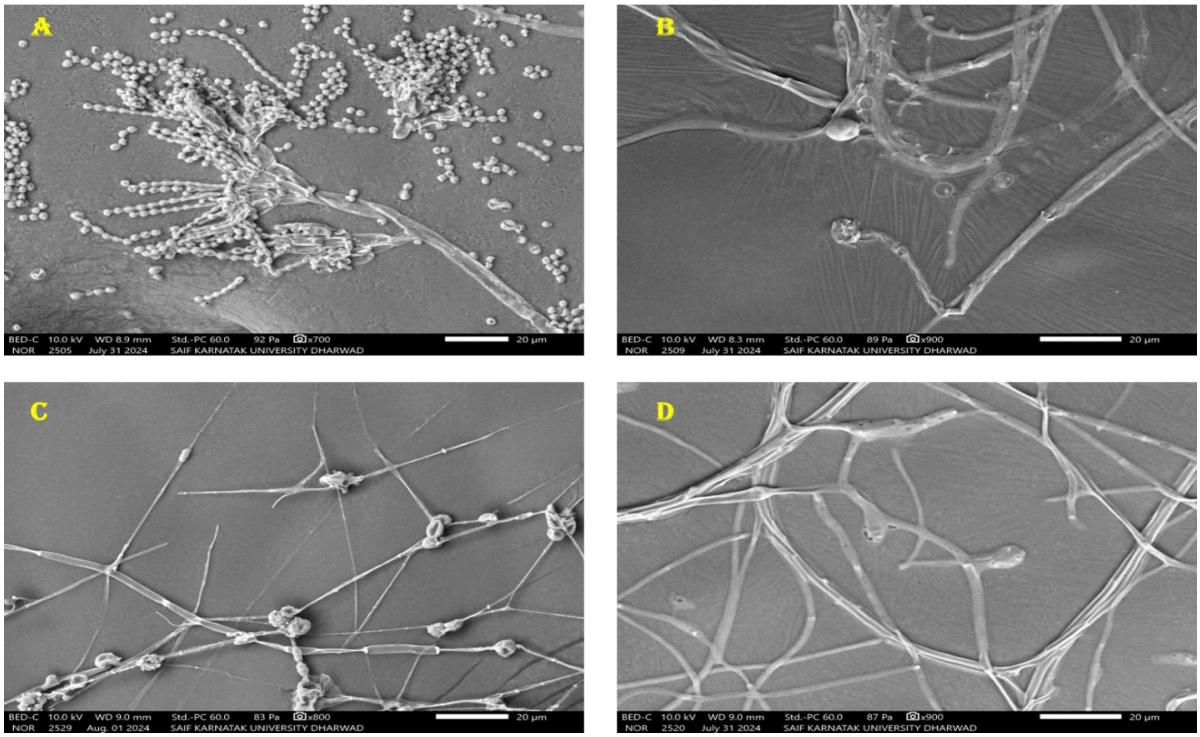

Figure 2. Scanning electron microscopy images of endophytic fungi *P. chrysogenum* isolate EF_S1 (A), *T. harzianum* isolate EF_R1 (B), *F. oxysporum* isolate EF_L4 (C), and *Phoma* sp. Isolate EF_L5 (D).

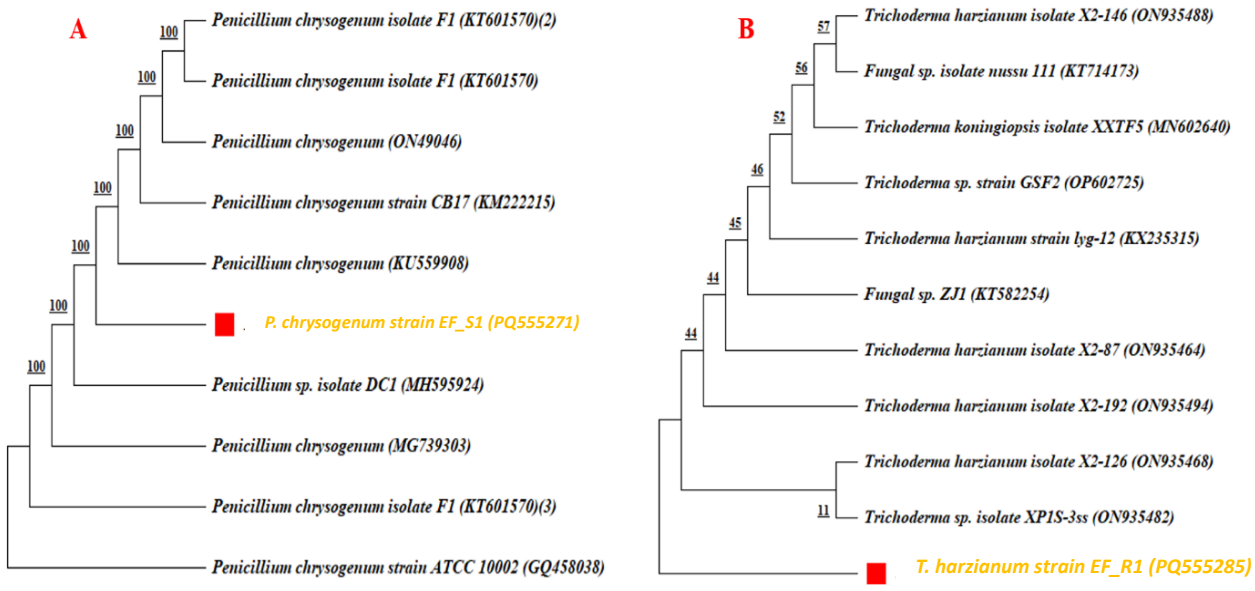

Figure 2. Phylogeny connection of P. chrysogenum (A), T. harzianum (B) based on the 18S rRNA sequence amongst similar sequences retrieved from the NCBI server.

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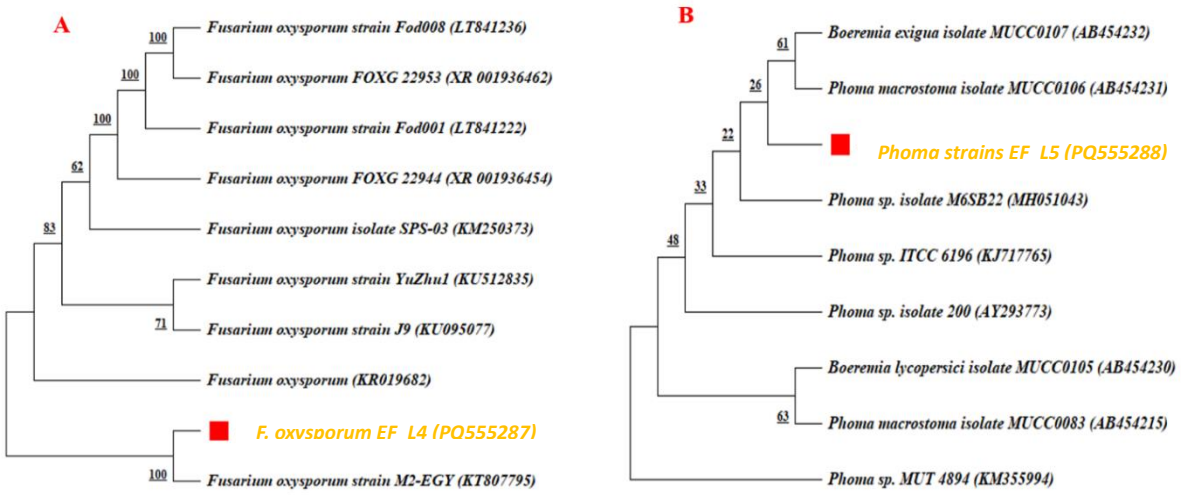

Figure 3. Phylogeny connection of F. oxysporum (A) and Phoma. (B) based on the 18S rRNA sequence amongst similar sequences retrieved from the NCBI server.

2.4. Small-scale production of endophytic fungal metabolites

Secondary metabolites from the endophytic fungi were grown in PDB, resulting from small-scale production in response to pH oscillation to monitor the metabolic activity of the fungal isolates during growth. The pH of the culture media was tabulated at 7-day intervals, to assess the metabolic activity of fungal isolates during growth. Through the production of metabolic by-products such as organic acids or alkaline compounds, fungi can change the pH in culture media and increase it significantly depending on other factors indicative of nutrient utilization processes together with secondary metabolites. The variation in pH amongst the isolates suggests that an individual has a unique metabolic profile and growth dynamics.

Lower pH values indicate more acidic environments, which may be connected to primary or secondary metabolite production, while higher pH values suggest alkaline by-products. The isolates maintaining neutral pH could be at a different stage of growth or producing rarer pH-shifting exudates. These differences play an important for selecting isolates for specific industrial or pharmaceutical applications, depending on the desired pH conditions for product formation or bioactivity.

This outcome suggests different nutrient utilization pathways, with potential implications for biotechnological applications like bioremediation or the production of specific bioactive compounds. Based on the unique morphology and different pH values, 4 isolates are used for molecular characterization. These are identified as P. *chrysogenum* isolate EF_S1, *T. harzianum* isolate EF_R2, *F. oxysporum* isolate EF_L4, and *Phoma* sp. L5_R1 isolates were not influenced by the media pH (Table 1).

Table1. pH evaluation of the endophytic fungi before and after metabolite production.

Fungal metabolite	pH Before production	pH After production
EF_L1	5.2	7.5
EF_L2	5.2	6.0
EF_L3	5.2	7.0
F. oxysporum isolate EF_L4	5.2	8.5
Phoma sp. EF_L5	5.2	7.0

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EF_L6	5.2	6.0
EF_L7	5.2	7.0
P. chrysogenum EF_S1	5.2	6.0
EF_S2	5.2	7.0
EF_S3	5.2	7.0
EF_S4	5.2	6.0
T. harzianum isolate EF_L6	5.2	8.0

2.5. Phytochemical screening of the fungal metabolites

Phytochemical assays were performed on twelve fungal isolates to assess their capacity for the production of bioactive compounds. The findings revealed a spectrum of phytochemical activity among the isolates, indicative of their diverse metabolic capabilities. Notably, four isolates—*Penicillium chrysogenum* EF_S1, *Trichoderma harzianum* EF_R2, *Fusarium oxysporum* EF_L4, and *Phoma sp*. EF_L5—underwent molecular identification. These isolates demonstrated particularly pronounced phytochemical activity, thereby suggesting their substantial potential for the generation of valuable secondary metabolites. This observation underscores their relevance for future research and potential applications within therapeutics in biotechnological domains.

Table 2. Phytochemical activities of Endophytic fungi.

Fungal isolate	Alkaloids	flavonoid	Phenols	Tannins	Terpenoids	Steroids	Saponins
EF_L1	+	+	+	+	+	-	-
EF_L2	+	+	+	+	+	-	-
EF_L3	-	-	-	-	-	-	-
***F. oxysporum* isolate EF_L4**	+	+	+	+	+	-	-
***Phoma* sp. EF_L5**	+	+	+	+	+	-	-
EF_L6	+	+	+	+	+	-	-
EF_L7	+	+	+	+	+	-	+
***P. chrysogenum* EF_S1**	+	+	+	+	+	-	+
EF_S2	+	+	+	+	+	-	-
EF_S3	+	+	+	+	+	-	+
EF_S4	+	+	+	+	+	-	-
***T. harzianum* isolate EF_R1**	+	+	+	+	+	-	+

2.6. Production of extracellular enzymes

Enzyme assays were conducted on 12 fungal isolates to evaluate their amylase, cellulase, lipase, and protease activities, as measured by halo zone formation (including fungal growth) over 3 to 4 days. The halo zones were recorded in millimeters (mm), indicating the

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enzymatic degradation of specific substrates. Among the isolates, four were molecularly identified: *Penicillium chrysogenum EF_S1, Trichoderma harzianum EF_R1, Fusarium oxysporum EF_L4, and Phoma sp. EF_L5*

Amylase Activity: The highest amylase activity was observed in *P. chrysogenum* EF_S1 (60 mm), followed by *T. harzianum* isolate EF_R1 and *F. oxysporum* isolate EF_L4 50 mm respectively, and rest EF_L1, EF_L7, and EF_S1, all with 46 mm. The lowest was found in EF_S4 (25 mm).

Cellulase Activity*: P. chrysogenum* EF_S1 (50 mm) has greater cellulase activity, followed by F. *oxysporum* EF_L4 (48 mm). Other isolates had moderate to low activity, and EF_L3 displayed no cellulase activity.

Lipase Activity: *P. chrysogenum* EF_S1 exhibited the highest lipase activity (47 mm), followed *by F. oxysporum* EF_L4 (45 mm). EF_L3, EF_L6, and EF_L2 had low to moderate activity.

Protease Activity: *P. chrysogenum* EF_S1 demonstrated the highest protease activity (55 mm), and EF_L2 and EF_L3 showed no activity across all enzymes tested. The enzyme activity of prominent fungi is shown in Fig 2 and Table 3.

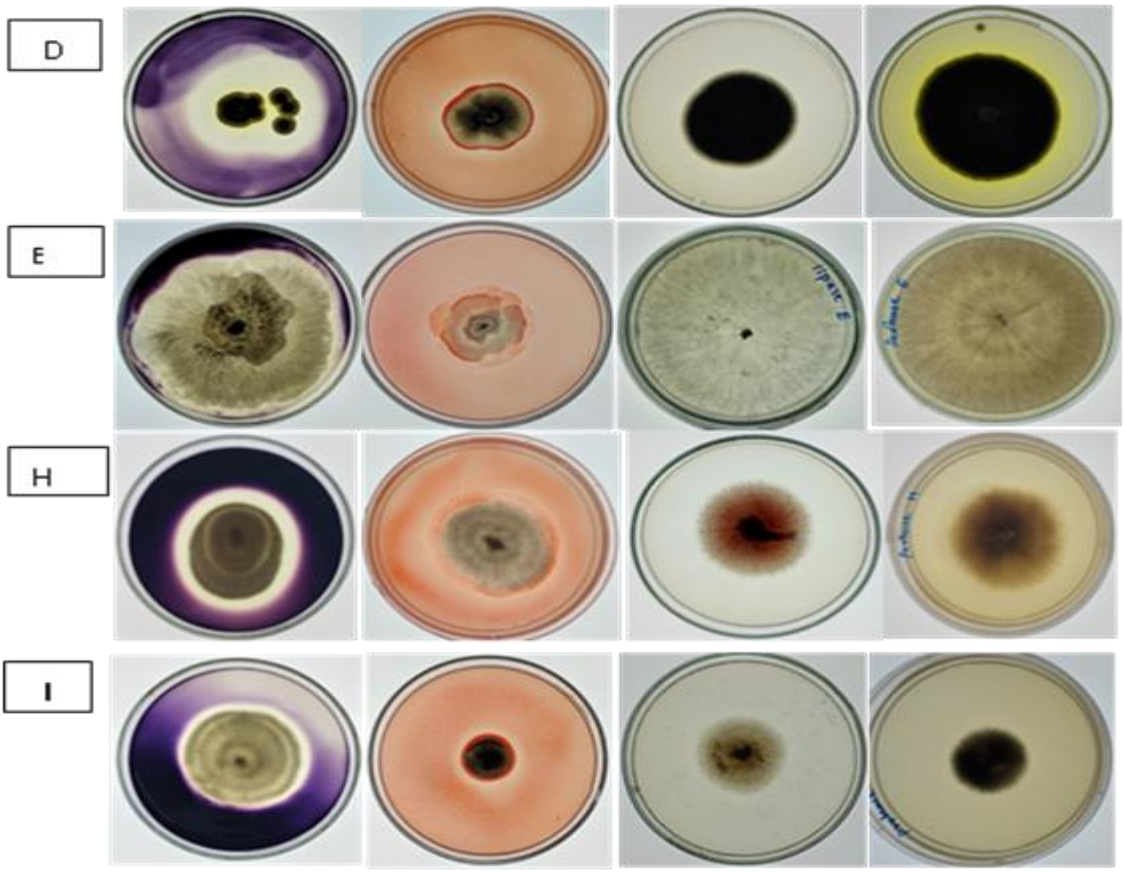

Figure 3. *The enzyme activities for amylase, cellulase, lipase, and protease are shown in Figure 2, with panels D, E, H, and I representing the respective enzymes. The halo zones illustrate the varying enzymatic activity across the isolates, with strong activity observed in Penicillium chrysogenum EF_S1, Trichoderma harzianum EF_R1, Fusarium oxysporum EF_L2, and Phoma sp. EF_L5.*

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Table 3. Extracellular enzyme production ability of endophytic fungi

Isolates	Halos zone measurement including the fungal growth 3 to 4 days in (mm)			
	Amylase assay	**Cellulase assay**	**Lipase assay**	**Protease assay**
EF_L1	46mm	47mm	22mm	20mm
EF_L2	40mm	37mm	28mm	-
EF_L3	20mm	-	-	-
F. oxysporum isolate EF_L4	50 mm	48mm	45mm	47mm
***Phoma* sp**. EF_L5	46mm	35mm	38mm	35mm
EF-L6	25mm	29mm	27mm	30mm
EF_L7	28mm	37mm	30mm	25mm
P. chrysogenum EF_S1	55mm	50mm	47mm	55mm
EF_S2	46mm	40mm	40mm	45mm
EF_S2	30mm	40mm	25mm	20mm
EF_S3	46mm	46mm	22mm	25mm
T. harzianum isolate EF_R1	50 mm	48mm	35mm	40mm

Discussion

The potential medical benefits of endophytic fungi, which reside within healthy plant tissues, have garnered significant interest (Jia *et al.*, 2016). These fungi produce a variety of beneficial compounds and establish symbiotic relationships with their host plants (Vishwakarma *et al*., 2024). According to Chandel *et al*. (2024), they are promising sources of plant-based extracts with considerable therapeutic value, including bioactive compounds with antibacterial, anticancer, and stress response properties. In the current study, we isolated prominent endophytic fungi from parts of Centella asiatica in the Dharwad region of India. A total of twelve isolates were evaluated based on their biological characteristics, among which four endophytic fungi—*Penicillium chrysogenum* isolate EF_L2, *Trichoderma harzianum* isolate EF_L6, *Fusarium oxysporum* isolate EF_S2, and *Phoma sp.* isolate EF_R1—were molecularly characterized. These isolates demonstrated their efficacy in producing fungal secondary metabolites, bioactive compounds, and enhanced extracellular enzyme activity.

Endophytic fungi are the most promising biogenic agents in the modern era, they proved their capability in various aspects like biofertilizer (Mishra *et al*., 2015; Nath *et al*., 2015), plant growth-promoting microbes (Chutima and Lumyong, 2012), phosphate solubilization (Spagnoletti *et al*., 2017; Sati and Pant, 2018), volatile compound (Lugtenberg *et al*., 2016), stress tolerance (Dastogeer and Wylie, 2017), disease resistance (Harrach *et al*., 2013; Nassimi and Taheri, 2017), biocontrol agents (Wilson 1995). During our study, we have opted for sodium hypochlorite-based surface sterilization, which damages the tissue and helps to develop an effective surface sterilization method, which was developed by Rodrigues (1994). We have achieved 80 percent colonization after 10 days of incubation in the PDA plates and this type of pattern of

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the colonization of Centella asiatica plant is well documented and reported from different countries (Frohlich *et al*., 2000; Gamboa and Bayman 2001).
Molecular characterization showed that these species were *P. chrysogenum* (Toghueo and Boyom, 2000; Fierro *et al*., 2022; Shaaban *et al*., 2023), *T. harzianum* (Tseng *et al*., 2020; Morais *et al*., 2022; Yao *et al*., 2023), *F. oxysporum* (Abro *et al*., 2019; Asim *et al*., 2022), and *Phoma* sp. (Kim *et al*., 2019; Soltani *et al*., 2022; Kumar *et al*., 2023), and these are the most promising endophytic fungi isolated from various medicinal value plants. many endophytes that were isolated more often along with several species that were isolated yet have unusual occurrences defined the endophytic community in CA. Research on endophytic fungi in plants have shown a typical distribution (Petrini 1991; Rodrigues and Petrini 1997). In extracellular enzymatic study, Using the agar plate technique, the potency of isolated fungal endophytes for extracellular enzymatic synthesis was qualitatively assessed. For every investigated enzyme, 40% of the isolated fungus showed variable degrees of enzyme activity. Among of all the enzymes that were evaluated, three-thirds of the fungal isolates capacity to generate three enzymes. The hydrolytic enzymes released by endophytes are employed in applications in biotechnology to enhance the processing of proteins and the breakdown of polysaccharides (El-Esawi *et al*., 2019). Hydrolytic enzyme functions, including those of amylase, CMCase, flavonoid, gelatinase, and saponin, are associated with hyper parasitic activity and facilitate the penetration of plant cells by fungi (Kim and Chung, 2004). Such enzyme reactions also improve the ability to resist that is produced through systematic approaches (Hallmann *et al*., 1997). According to Kavamura *et al.* (2013), endophytic fungal enzymes may stimulate growth in plants by reducing the incidence of plant diseases driven on by pathogens that circulate in the soil.

3. Conclusion

The investigation concludes that a diverse array of endophytic fungi, capable of secreting enzymes, The observed pH change during the metabolite production by fungal isolates reflects their varied metabolic activities. The isolates that caused a drop in pH to the acidic range likely produce organic acid or other acidic metabolites which may indicate active nutrient metabolism and potential for producing secondary metabolites with pharmaceutical applications. while alkaline shifts indicate the secretion of basic compounds like ammonia. These pH changes influence enzyme activity and the production of bioactive compounds. Monitoring these shifts provides insights into the metabolic potential of each isolate, guiding their application in the biotechnology and pharmaceutical industries. Control of pH could optimize the production of valuable metabolites. significant enzyme activity observed among these isolates highlights their potential for various applications, particularly following thorough isolation and characterization. The ability of these endophytic fungi to produce extracellular enzymes suggests exciting opportunities for their use in therapeutic contexts, especially in clinical microbiology, where they could contribute to the development of new antimicrobial agents or enzymes with therapeutic properties. Moreover, the biotechnological applications of these enzymes could extend to industries such as agriculture, food processing, and bioremediation, where their capabilities can be harnessed for enzyme-based solutions. Overall, the promising findings of this study emphasize the need for further research into the specific mechanisms of action and potential applications of these endophytic fungi, paving the way for innovative uses in both medicine and industry.

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