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Evaluation of Antimicrobial and Antioxidant Activities of Endophytic *Penicillium* and *Aspergillus* Species Isolated from *Curcuma caesia* Roxb. (Zingiberaceae), a Medicinal Plant of Odisha, India

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Abstract

In addition to being important sources of bioactive chemicals, medicinal plants are essential for the development of new therapeutic medicines. The capacity of endophytic fungus linked to medicinal plants to create a variety of secondary metabolites with important biological functions has drawn more attention. The antibacterial and antioxidant properties of endophytic fungi isolated from the medicinal plant Curcuma caesia Roxb. (family Zingiberaceae) were examined in this work. Based on morphological traits, three endophytic fungal isolates from the plant's rhizomes were identified as Aspergillus oryzae, Penicillium citrinum, and Aspergillus niger. This identification was verified by 18S rRNA sequencing using ITS markers. The isolates' antibacterial activity against four human pathogenic bacteria Bacillus subtilis, Escherichia coli, Staphylococcus aureus, and Pseudomonas aeruginosa was first assessed using the agar block method. P. citrinum produced inhibition zones up to 3.5 cm against the investigated pathogens, demonstrating the greatest antibacterial activity among the isolates. The disc diffusion method was used for secondary screening with ethyl acetate extracts. DPPH and DMPD radical scavenging tests were used to measure antioxidant activity. While A. oryzae demonstrated the highest activity in the DMPD testing (59.56%), P. citrinum demonstrated the highest DPPH antioxidant activity (78.61%). Additionally, all isolates showed enzymatic activity, such as tyrosinase, protease, and amylase. These results suggest that C. caesia endophytic fungus could be useful sources of bioactive compounds with possible medical uses.

1. Introduction

In recent decades, one of the biggest threats to world health has been the fast growth of antimicrobial resistance (AMR). The efficacy of traditional antibiotics has been greatly diminished by the ongoing evolution of resistance among harmful bacteria. As a result, infections that were previously manageable are now more challenging to control, which results in longer illnesses, higher medical expenses, and higher death rates. In addition to endangering clinical therapy, antimicrobial resistance presents significant global threats to the environment and public health [1]. The evolution of resistant microbial strains has been greatly aided by the overuse and abuse of antibiotics in veterinary care, human medicine, and agriculture. Antibiotic-resistant infections have also

become more prevalent due to inappropriate prescription procedures, self-medication, and the extensive use of antibiotics in livestock production.

Using current antimicrobial medications to treat infections caused by bacterial pathogens such Staphylococcus, Bacillus, Klebsiella, Enterobacteriaceae, Acinetobacter, and Pseudomonas is becoming more challenging. Finding novel antibacterial molecules from natural sources has thus become a top goal [2, 3]. One of the most prominent sources of bioactive secondary metabolites that have greatly aided in the creation of contemporary pharmaceuticals is fungus. Among these, endophytic fungi are a special class of microbes that colonise plant tissues without exhibiting any outward signs of illness. These fungi live either intracellularly or intercellularly in plant tissues, and they frequently form mutualistic interactions with their host plants [4]. By creating bioactive compounds that shield the host from infections, herbivores, and environmental stressors, endophytic fungi can improve plant life through such relationships.

A rich community of endophytic fungi that can produce a variety of biologically active secondary metabolites is known to be present in medicinal plants. According to reports, these metabolites contain antiviral, anticancer, antibacterial, antioxidant, and anti-inflammatory qualities. Endophytic fungi are regarded as a significant and mostly untapped source of new bioactive chemicals with substantial medicinal promise because of their extensive metabolic diversity [5]. Fungal endophytes have produced a number of therapeutically significant molecules, such as antibiotics and anticancer drugs, underscoring their significance in drug discovery [6]. One of the most important discoveries in the fields of microbiology and medicine, for example, is the creation of penicillin by species of Penicillium, which has historically transformed antimicrobial therapy [7].

More than 120 species of the genus Curcuma, which is a member of the Zingiberaceae family, are found in tropical and subtropical areas such as northern Australia, Malaysia, India, Southeast Asia, and Thailand [8,9]. Because of their many pharmacological characteristics, including anti-inflammatory, antibacterial, antiviral, antioxidant, and antifungal effects, members of this genus have been extensively employed in traditional medicine. Significant variety within the genus Curcuma has also been found by taxonomic and phylogenetic analyses, underscoring its significance as a group of medicinal plants [10]. Curcuma caesia Roxb., also referred to as "Kali Haldi" or black turmeric, is a significant medicinal herb that Indian indigenous and tribal cultures have long utilised. It is a perennial herb with pale yellow blooms with reddish edges and a bluish-black rhizome.

Traditionally, C. caesia rhizomes have been used to treat a variety of conditions, including fever, wounds, asthma, epilepsy, haemorrhoids, inflammation, and digestive issues [11]. Curcuma caesia rhizome extracts have been shown in earlier pharmacological research to have smooth muscle relaxant qualities, suggesting the plant's possible medicinal value [12]. Relatively little research has been done on the endophytic fungus communities connected to

Curcuma caesia, despite the plant's widespread medical potential. It is known that endophytic fungi that live inside medicinal plants produce a variety of secondary metabolites that may have strong antioxidant and antibacterial qualities. Thus, examining the endophytic fungal diversity of therapeutic plants may offer important information for finding new bioactive substances with therapeutic uses.

The goal of the current work was to isolate and identify endophytic fungi connected to Curcuma caesia rhizomes in light of the growing need for novel antibacterial agents and antioxidant chemicals. Additionally, the study intends to investigate these fungal isolates' antibacterial efficacy against specific human pathogenic bacteria as well as their potential as antioxidants. The results of this study may help identify prospective endophytic fungal strains that can produce bioactive compounds that may be used as therapeutic agents to treat oxidative stress and microbial infections.

2. Materials and methods

2.1. Plant sample collection

Curcuma caesia, a medicinal plant, was obtained from the Kalahandi area of Odisha, India, with its rhizomes completely developed and in good health. To guarantee the successful isolation of endophytic fungi, only mature, disease-free, and symptomless plant tissues were chosen. The plant samples were meticulously washed under running tap water as soon as they were collected to get rid of surface impurities, adhering soil particles, and epiphytic bacteria. To make sure that any leftover contaminants were completely removed, the cleaned samples were then rinsed three times with sterile distilled water.

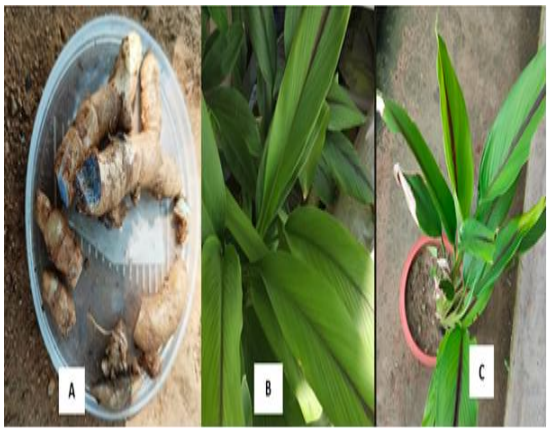


Fig. 2.1. Medicinal plant *Curcuma caesia* L. showing different plant parts. A- Rhizome, B-leaves, C-Whole plant.

After being aseptically divided into smaller pieces, the rhizomes were placed in zip-lock polyethylene bags that had already been sterilised. Before being processed further, the samples were kept at 10 °C to preserve their freshness and stop microbiological degradation. To maintain the viability of the endophytic fungus communities found in the plant tissues, all obtained plant samples were brought to the lab and processed within a day after collection.

2.2 Endophytic Fungal Isolation and Surface Sterilisation

With a few minor adjustments, the procedure outlined by Hallmann et al. [13] was used to isolate endophytic fungi. To get rid of epiphytic microbes, the gathered plant tissues were first surface sterilised. The plant parts were first cleaned with sterile distilled water after being submerged in 70% ethanol for one minute.

A sterile blade was then used to aseptically cut the tissues into small segments that measured around 2 × 2 cm for leaves, 1 cm for roots and stems, and 2 × 2 × 2 cm for rhizomes. Twenty segments from each plant organ were surface sterilised by submerging them in 70%

ethanol for one minute, then washing them three times with sterile distilled water. After five minutes of treatment with 0.5% sodium hypochlorite (NaOCl), the samples were rinsed six times with sterile distilled water to eliminate any remaining disinfectant. Following sterilisation, the segments were put on sterile filter paper and left to dry for around 12 hours in a biosafety cabinet.

In order to prevent bacterial contamination, the sterilised tissue segments were subsequently inoculated onto potato dextrose agar (PDA; Difco) medium supplemented with chloramphenicol (0.5 g/L). For seven to twenty-one days, the inoculation plates were incubated at 28 °C until fungal growth appeared from the plant tissues. on create pure cultures, emerging fungal hyphae were carefully moved on brand-new PDA plates without the addition of antibiotics. By plating the last rinse water onto PDA plates to look for microbial growth, the effectiveness of surface sterilisation was confirmed. For additional examination, distinct fungal colonies with various morphological traits were chosen.

2.3 Endophytic Fungal Microscopic Identification

The isolated endophytic fungus were first identified using both macroscopic and microscopic traits. For macroscopic identification, colony morphology, including colour, texture, growth pattern, margin, and pigmentation, was investigated. Fungal mycelia were stained with lactophenol cotton blue and placed on glass slides for microscopic inspection. For taxonomic identification, microscopic characteristics such as hyphal structure, septa presence or absence, spore type, sporangia, conidia, and the arrangement of sporangiophores or conidiophores were examined under a microscope.

2.4 Endophytic Fungal Genomic DNA Extraction and Molecular Identification

To obtain adequate fungal biomass, pure cultures of the identified fungus were inoculated into 250 mL conical flasks containing 100 mL of potato dextrose broth (PDB) and shaken at room temperature for three to seven days. According to Lee et al. (1990), genomic DNA was extracted using a slightly modified version of the CTAB technique.

The internal transcribed spacer (ITS) region of rDNA was amplified by polymerase chain reaction (PCR) using ITS-1 (TCCGTAGGTGAACCTGCG) and ITS-4 (TCCTCCGCTTATTGATATGC) primers, as reported by White et al. (1990). After being purified, the amplified PCR products were sequenced. To identify the closest sequence similarity and verify the species identity of the fungal isolates, the acquired sequences were examined using BLAST searches against publically accessible databases.

2.5 Antimicrobial Test

Selected human pathogenic bacteria from the Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Chandigarh, were used to test the fungal isolates' antibacterial activity. Pseudomonas aeruginosa (MTCC 4676), Staphylococcus aureus (MTCC 1588), Bacillus subtilis (MTCC 10403), and Escherichia coli (MTCC 2961) were among the bacterial strains utilised in the investigation. For the purpose of evaluating antibiotics, these bacterial cultures were kept on nutritional agar (NA) medium. To ensure consistent cell density throughout the test, bacterial suspensions were made in accordance with the 0.5 McFarland standard.

2.6 Initial Antimicrobial Test

Using a slightly modified version of Arya and Sati's agar block method, the antibacterial activity of the isolated endophytic fungi was first assessed [15]. After preparing Petri plates with the proper growth conditions for bacteria, a sterile cotton swab was used to evenly distribute

100 µL of the bacterial suspension across the agar surface. Using a sterile cork borer, actively growing fungal discs with a diameter of 9 mm were removed from PDA cultures and applied to the surface of the inoculated agar plates. To enable the diffusion of antimicrobial chemicals from the fungal discs into the media, the plates were covered with parafilm and stored at 4 °C for 12 hours. The plates were then incubated for 12 hours at room temperature. The plates were inspected for the development of inhibition zones surrounding the fungal discs following incubation. A ruler was used to measure the inhibitory zones' diameter in millimetres.

2.7 Extraction of Secondary Metabolites

After a preliminary screening, endophytic fungal isolates that showed positive antibiotic activity were chosen for secondary metabolite extraction using a slightly modified version of the Atalla et al. method [16]. For 21 days, each fungal isolate was cultivated in 250 mL conical flasks with 100 mL of potato dextrose broth (PDB) and kept at room temperature in static circumstances. Following incubation, muslin fabric was used to filter the fungal biomass out of the culture medium.

2.8 Mycelial Biomass and Culture Filtrate Extraction

After transferring the culture filtrate into a separating funnel, an equivalent volume of ethyl acetate was added. To separate the solvent layer, the mixture was agitated rapidly for ten minutes and then left to stand for five minutes.

After that, the organic solvent phase was gathered. The isolated mycelial biomass was cleaned of any remaining medium components using sterile distilled water in order to extract intracellular metabolites. After that, the mycelia were crushed with a crusher and pestle while ethyl acetate was present. After being filtered through muslin fabric, the crushed material was placed in a separating funnel, agitated for ten minutes, and left to undergo phase separation. Fungal metabolites were found in the ethyl acetate fraction. The filtrate and mycelial fraction extracts were mixed together and dried in a hot air oven. For additional analysis, the dried residue was dissolved in dimethyl sulfoxide (DMSO) and kept at 4 °C.

2.9 Disc Diffusion Method Secondary Antimicrobial Assay

According to Balouiri et al. [14], the disc diffusion method was used to assess the antibacterial activity of ethyl acetate extracts. 100 µL of bacterial solution that had been adjusted to the 0.5 McFarland standard was used to inoculate sterile agar plates. On the surface of the inoculated agar plates, sterile filter paper discs with a diameter of 6 mm were impregnated with 20 µL of the fungal extract. Furthermore, a sterile cork borer was used to create wells with a diameter of 6 mm, and each well received 20 µL of the crude extract. Norfloxacin (10 µg) was utilised as the positive control and DMSO and ethyl acetate as the negative controls to evaluate solvent effects.

Every experiment was carried out three times. After the bacterial strains (P. aeruginosa, S. aureus, B. subtilis, and E. coli) were incubated at 35 ± 2 °C, the diameter of the inhibitory zones was determined in millimetres.

2.10 Antioxidant Activity Screening

Two distinct assays were used to assess the fungal extracts' antioxidant potential: the DPPH free radical scavenging assay and the DMPD (N,N-dimethyl-p-phenylenediamine) assay. The average values were computed after each experiment was carried out in triplicate.

2.10.1 Assay for DPPH Radical Scavenging

Using the DPPH radical scavenging technique outlined by Brand-Williams et al. [17], the antioxidant activity of the endophytic fungal extracts was assessed.

A concentration of 1 mg/mL was used to make fungal extracts. In this experiment, 1.9 mL of 0.1 mM DPPH solution and 100 µL of fungal extract were combined and extensively vortexed. A spectrophotometer was used to detect the reaction mixture's absorbance at 525 nm following a 10-minute incubation period. The following formula was used to compute the free radical scavenging activity, which was represented as % inhibition:

DPPH Scavenging activity (%) = ($A_{control}$-A_{sample} /$A_{control}$) × 100

Where, (A_{sample}) = Absorbance of the sample

($A_{control}$) = Absorbance of the control containing all the reaction reagents except the Sample

2.10.2. Scavenging Capacity of DMPD

The DMPD (N,N-dimethyl-p-phenylenediamine) radical scavenging experiment was used to further assess the antioxidant ability of the endophytic fungal extracts. This method was slightly modified from Fogliano et al. (1999). 209 mg of DMPD were dissolved in 100 mL of distilled water to create a 100 mM DMPD stock solution. One millilitre of this stock solution was added to one hundred millilitres of 0.1 M sodium acetate buffer (pH 5.2). The stable purple-red DMPD radical cation was then produced by adding 0.2 mL of a 0.05 M ferric chloride solution. The resultant solution's absorbance was adjusted to roughly 0.900 ± 0.100 at 505 nm. At room temperature, the produced DMPD radical solution held steady for up to 12 hours.

Distilled water was used to adjust the final amount to 3.0 mL after different volumes of endophytic fungal extracts were combined with 2.0 mL of the produced DMPD radical solution for the test. To allow the radical solution and the antioxidant chemicals in the extracts to interact, the reaction mixtures were incubated at room temperature for ten minutes. A UV-visible spectrophotometer was used to detect the absorbance at 505 nm following incubation. The blank used in the analysis was sodium acetate buffer. The following formula was used to determine the extracts' radical scavenging activity, which was represented as percentage inhibition:

DMPD Scavenging activity (%) = (A_0 - A_1 /A_0) × 100

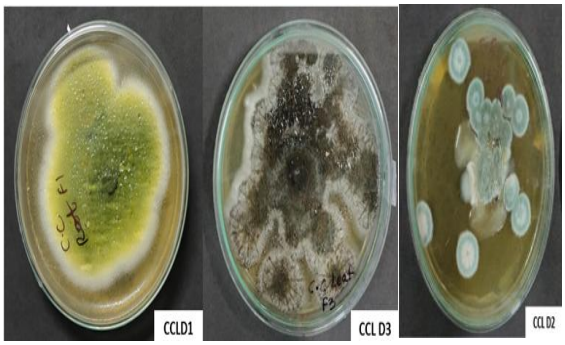
Where, A_0 =Absorbance of the initial concentration of DMPD

A1= Absorbance of the remaining concentration of DMPD

3. Results and discussion

3.1 Endophytic Fungal Isolation and Morphological Identification

Six endophytic fungal isolates, designated CCL1, CCL2, CCR1, CCLD1, CCLD21, and CCLD32, were collected from fourteen rhizome segments of Curcuma caesia. One isolation was identified as belonging to the genus Penicillium, two isolates were identified as Aspergillus species, and the remaining three isolates could not be assigned to a specific genus based on preliminary



identification based on colony morphology

and microscopic features. On potato dextrose agar (PDA), colony properties were analysed, and lactophenol cotton blue staining was used to view microscopic structures. The isolates demonstrated the richness of the endophytic fungal population by displaying discernible differences in colony colour, texture, growth rate, and sporulation patterns.

Fig. 3.1: Isolated endophytic Aspergillus species in pure culture

The isolate CCLD1 grew quickly, producing homogeneous, slightly elevated, greenish colonies with flat surfaces, regular borders, and a velvety feel. Penicillium species are characterised by brush-like penicilli with chains of spherical conidia, which are produced by septate hyphae and branched conidiophores, according to microscopic examination. Fast-growing mycelia with bluish colonies, granular surface texture, powdery sporulation, and uniform edges were characteristics of the CCLD2 isolate. Under a microscope, it displayed the characteristic Aspergillus morphology of lengthy conidiophores ending in globose vesicles with radiating phialides and rough-walled conidia. The CCLD3 isolate produced quickly growing colonies with white borders and black, powdery conidial heads; radial fissures occasionally appeared on the colony surface. Large spherical vesicles covered in biseriate phialides and darkly coloured conidia were seen under a microscope, which is compatible with characteristics of Aspergillus niger.

3.2 Identification of Molecules

The Internal Transcribed Spacer (ITS) region of ribosomal DNA was sequenced in order to confirm the taxonomic identity of the morphologically described endophytic fungal isolates. The three chosen fungal isolates' pure cultures were successfully used to harvest genomic DNA. All isolates produced distinct PCR amplicons of roughly 550–650 bp when the ITS sections were amplified using the universal primers ITS1 and ITS4.

Table 3.1: ITS sequencing-based molecular identification of endophytic fungal isolates together with their accession number

SL No.	Endophytic fungal Isolate Code	Closest match species	Host plant	Plant part	Accession number
1	CCLD2	*Penicillium citrinum*	*Curcuma caesia*	Rhizome	PX567963.1
2	CCLD1	*Aspergillus oryzae*	*Curcuma caesia*	Rhizome	PX567920.1
3	CCLD3	*Aspergillus niger*	*Curcuma caesia*	Rhizome	PX567958.1

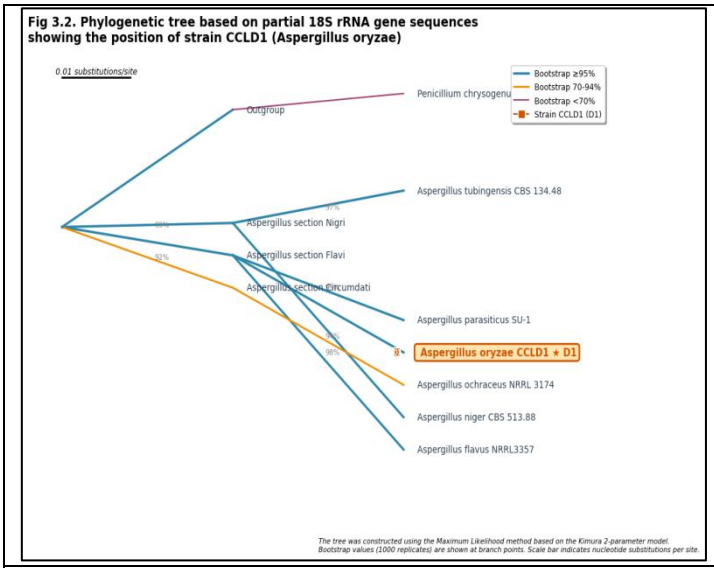


Fig 3.2: Phylogenetic tree of Aspergillus oryzae CCLD1 based on 18S rRNA sequences

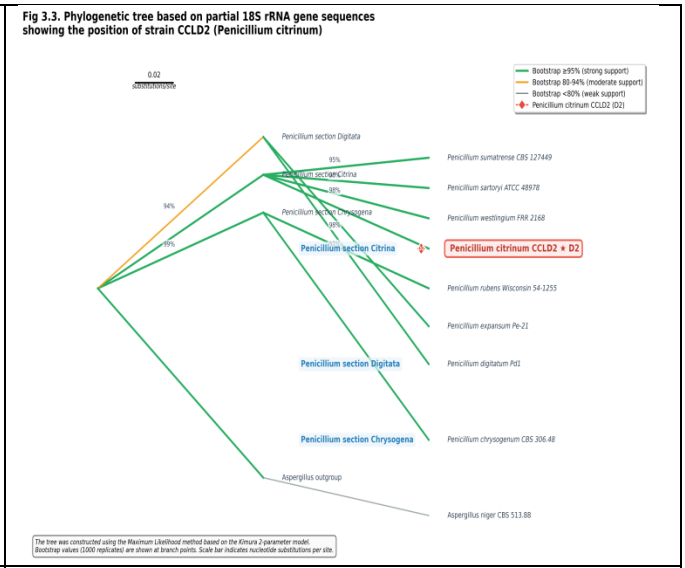


Fig 3.3: Phylogenetic tree of Penicillium citrinum CCLD2 based on 18S rRNA sequences

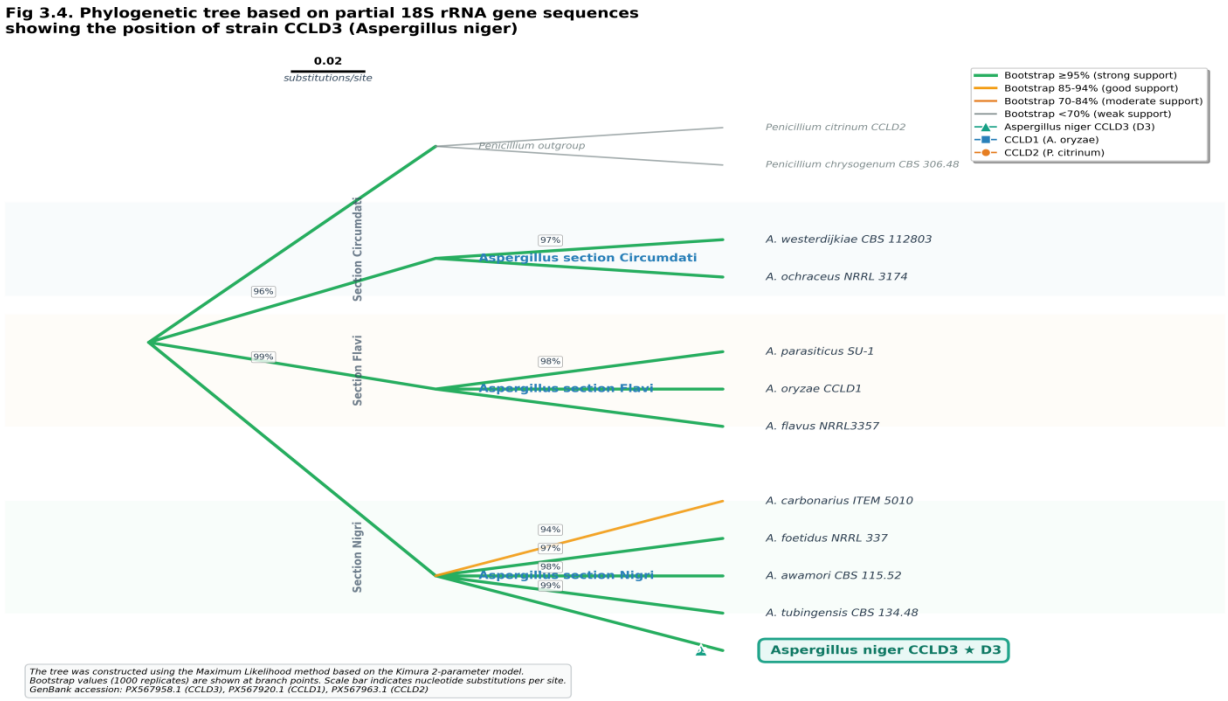


Fig 3.4: Phylogenetic tree of Aspergillus niger CCLD3 based on 18S rRNA sequences

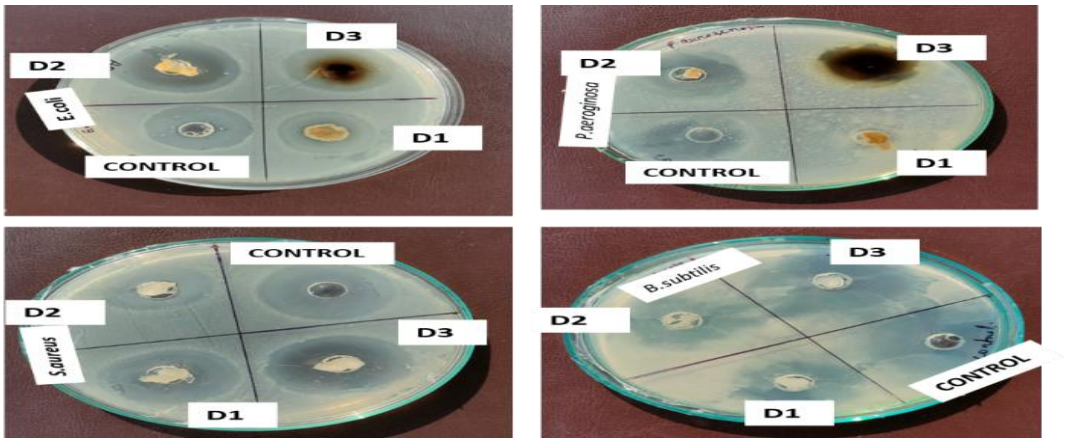
After the amplified products were sequenced, BLAST analysis was used to compare the obtained sequences to reference sequences in the NCBI GenBank database. High sequence similarity with recognised fungal species was found in the analysis, allowing for species-level identification. The isolates' identities as Penicillium citrinum (isolate CCLD2), Aspergillus oryzae (isolate CCLD1), and Aspergillus niger (isolate CCLD3) were further verified by phylogenetic analysis.

Aspergillus oryzae (PX567920.1), Aspergillus niger (PX567958.1), and Penicillium citrinum (PX567963.1) were the endophytic fungi whose ITS sequences were submitted to the NCBI GenBank (Table 3.1). These findings supported the isolates' molecular identity and provide a solid foundation for additional functional and bioactivity investigations.

3.3 Initial Antimicrobial Test

Using the agar plate diffusion method, the antibacterial activity of the three chosen endophytic fungal isolates against four human pathogenic bacteria was assessed. After 12 hours of incubation, all isolates showed quantifiable zones of inhibition, suggesting the generation of bioactive secondary metabolites with potential antibacterial properties. Inhibition zones varied in diameter from 24 to 36 mm.

Figure 3.5 shows the zone in which endophytic fungi suppress human harmful bacteria



With an inhibition zone of 36 mm against Staphylococcus aureus, Penicillium citrinum and Aspergillus niger had the strongest antibacterial activity among the isolates. Table 3.2 provides a full summary of the antibacterial screening data, including mean inhibition zone widths and standard deviations from three independent duplicates. Figure 3.5 displays representative pictures of agar plates displaying the zones of inhibition created by isolates of Aspergillus and Penicillium.

Table 3.2: Direct mycelia were used to screen the antibacterial activity of the three endophytic fungus isolates.

Sl. No.	Endophytic Fungi	Microorganisms zone of inhibition in cm (mean ±SD)			
		PA	SA	BS	EC
1	*Aspergillus niger*	3.46 ±0.03	3.6±0.1	3.33±0.06	2.6±0.08
2	*Penicillium citrinum*	3.43±0.03	3.6±0.08	3.6±0.15	2.96±0.14
3	*Aspergillus oryzae*	1.2±0.03	2.9±0.05	2.73±0.12	2.43±0.08

*PA-*Pseudomonas aeruginosa,* SA-*Staphylococcus aureus,* BS-*Bacillus subtilis,* EC-*Escherichia coli.*

These findings demonstrate the potential of the chosen endophytic fungus from Curcuma caesia rhizomes as sources of natural antimicrobial agents by confirming their ability to produce secondary metabolites with strong antibacterial activity.

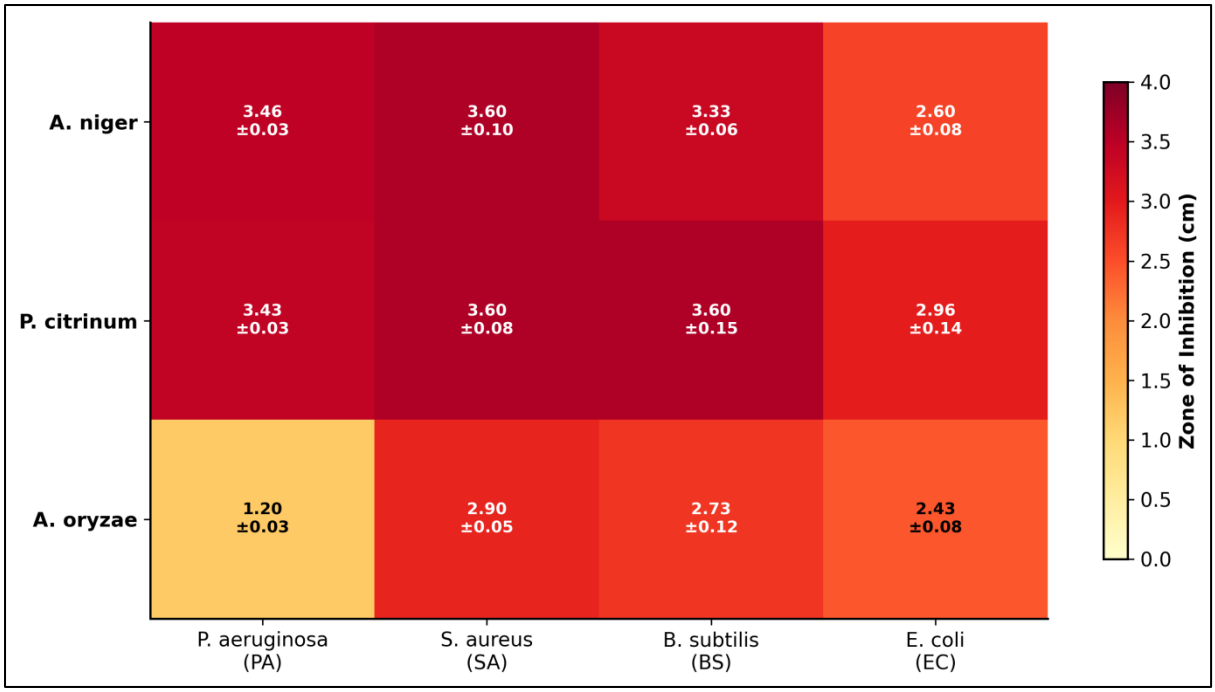


Fig. 3.6: Antibacterial Activity Heatmap

3.4 Secondary Metabolites' Antimicrobial Activity Using Well and Disc Diffusion Techniques

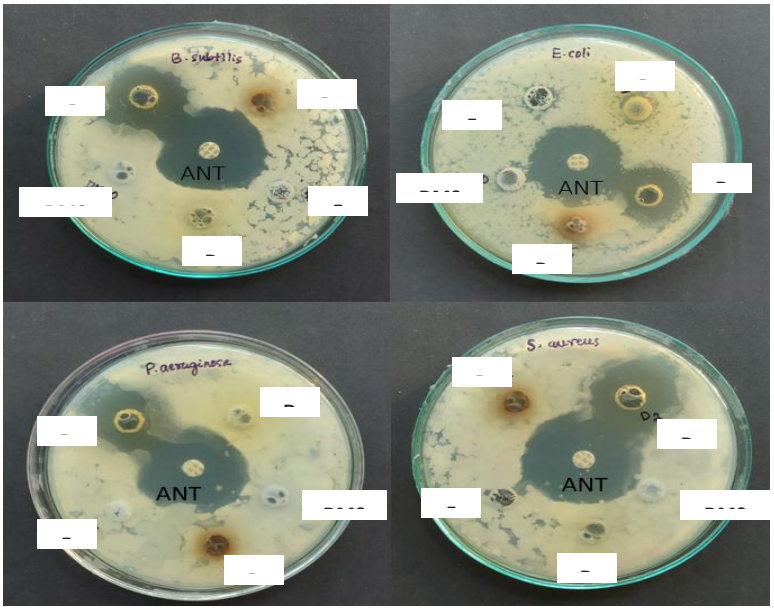
Ethyl acetate was used to extract secondary metabolites from the three endophytic fungal isolates that were chosen. For the purpose of evaluating their antibacterial properties, the crude extracts were dried and dissolved in 1 mg/µL of dimethyl sulfoxide (DMSO). The antibacterial potential was evaluated using both well diffusion and disc diffusion assays. The evaluated bacterial strains were significantly inhibited by all three isolates, which had previously been found to be bioactive in preliminary screening. The positive control was norfloxacin (10 µg).

Table 3.3: Antibacterial activity of the ethyl acetate extracts of the three endophytic fungi tested by using well diffusion method

		Microorganisms zone of inhibition in cm (mean ±SD)			
Sl. No.	**Endophytic Fungi**	**PA**	**SA**	**BS**	**EC**
1	*Aspergillus niger*	1.73±0.14	2.27±0.08	1.6±0.05	2.06±0.03
2	*Penicillium citrinum*	3.5±0.23	3.43±0.23	2.73±0.16	3.6±0.17
3	*Aspergillus oryzae*	1.13±0.33	1.76±0.12	1.3±0.03	1.43±0.03
4	Ethyl Acetate	0.9±0	0.9±0	0.9±0	0.9±0
5	DMSO	1.03±0.08	0.9±0	0.9±0	0.9±0
6	AntibioticDisc (Norfloxacin 10 mcg)	3.2±0.11	3.2±0.14	3.0±0.33	2.8±0.33

Following the application of 50 µL of each extract in the well diffusion experiment, the plates were promptly incubated. Table 3.3 summarises the zones of inhibition found against Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa, and Staphylococcus aureus. Figure 3.6 displays representative pictures of inhibitory zones. With the biggest zones of inhibition against E. coli and the other pathogens examined, Penicillium citrinum showed

the strongest antibacterial activity among the isolates, indicating its potential as a source of antimicrobial chemicals. Aspergillus oryzae, on the other hand, showed relatively less



activity, suggesting that it is the least bioactive of the three isolates.

Figure 3.6: Endophytic fungus inhibit harmful bacteria using the agar well method

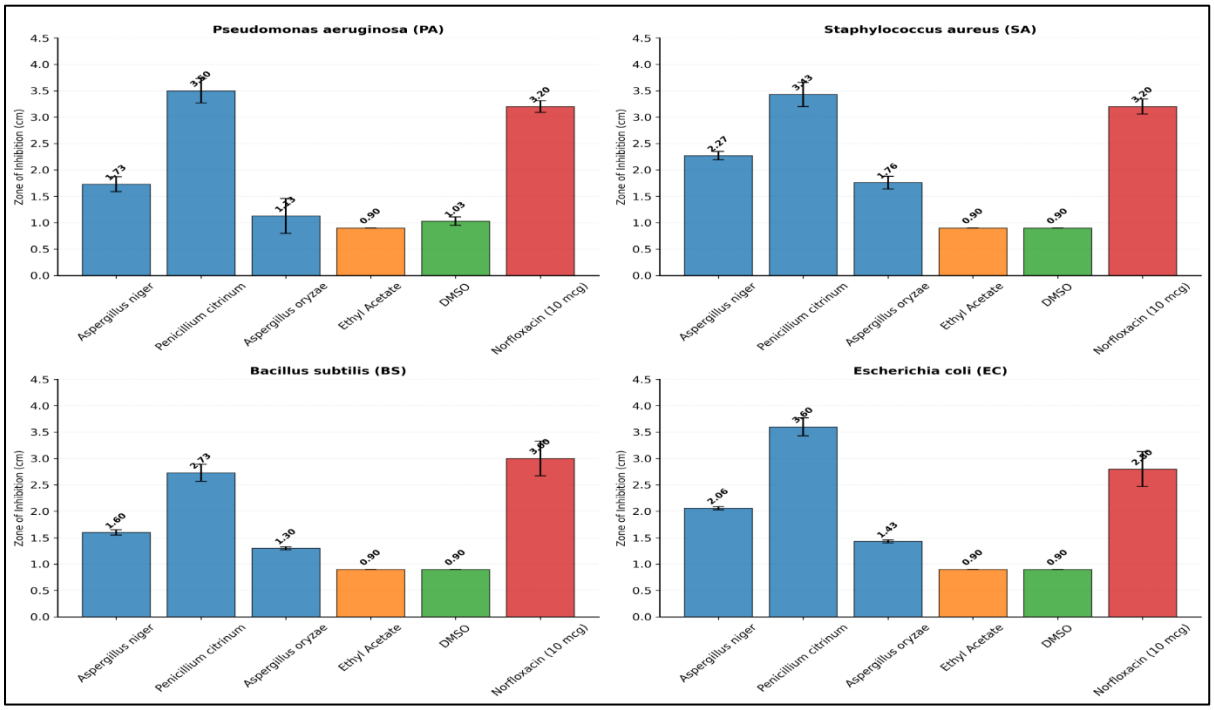


Fig. 3.7 Antibacterial Activity against individual test bacteria

These endophytic fungi's antibacterial activity persisted even after submerged culture, proving that bioactive secondary metabolites may be efficiently extracted using medium-polar solvents like ethyl acetate. These results are in line with earlier research showing the production of strong antibacterial substances by endophytic Penicillium and Aspergillus species. While Verma et al. [19]

demonstrated that Penicillium spp. isolated from medicinal plants strongly inhibited S. aureus and other pathogenic bacteria, Strobel and Daisy [18] highlighted the abundance of endophytes as sources of new antibiotics. The antibacterial activity of *Aspergillus niger* has been attributed to the production of polyketides, organic acids, and other secondary metabolites [20].

3.5 Radical Scavenging Activity of DPPH

The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay, a quick and accurate technique for assessing free radical neutralisation by biological substances, was used to evaluate the antioxidant capability of the endophytic fungal isolates [21]. A colour shift from purple to yellow, signifying the donation of electrons or hydrogen atoms by antioxidant chemicals found in the fungal extracts, indicates the decrease of DPPH radicals. Penicillium citrinum was the isolate with the highest DPPH scavenging activity (78.61%), followed by Aspergillus niger (64.5%). At 39.95%, Aspergillus oryzae showed the least amount of activity. The three isolates' secondary metabolite profiles, which include phenolic compounds, flavonoids, and other bioactive molecules with the ability to scavenge radicals, differed in terms of antioxidant activity.

Although the fungal extracts demonstrated significant free radical neutralization, their antioxidant efficiency was lower than that of standard antioxidants. These results are consistent with previous studies, where DPPH scavenging activity correlated with hydrogen-donating antioxidant compounds in microbial and plant-derived samples [22].

3.6 Radical Scavenging Activity of DMPD

The fungal extracts' antioxidant potential was further assessed using the DMPD (N,N-dimethyl-p-phenylenediamine) radical scavenging assay. The DMPD radical cation is reduced in this assay by bioactive metabolites, which results in a noticeable colour shift and indicates electron- or hydrogen-donating activity [23]. Aspergillus oryzae showed the highest DMPD radical scavenging activity in the current investigation, at 59.56%, indicating the existence of bioactive reducing chemicals and significant free radical neutralisation. Aspergillus niger had the lowest activity (31.08%), while Penicillium citrinum had moderate activity (55.3%). Figure 3.8 shows the proportion of DPPH and DMPD radical scavenging activities for the fungal isolates in comparison to ascorbic acid.

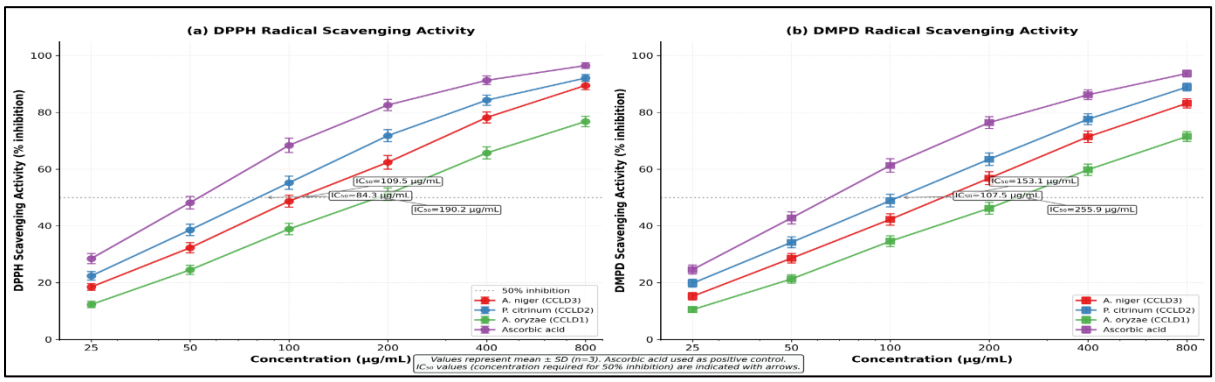


Fig.3.8. DPPH and DMPD free radical scavenging activity of Endophytic fungal strains

These results support earlier discoveries that microbial endophytes are an important source of bioactive substances with possible medicinal uses, such as antibacterial, antioxidant, and anticancer properties [18]. Aspergillus and Penicillium species have significant antioxidant potential, which emphasises their significance in bioprospecting research to find natural antioxidants for medical and industrial uses.

4. Conclusion

The current study shows that the medicinal herb Curcuma caesia, which was obtained from the Kandhamal area of Odisha, India, has endophytic Penicillium and Aspergillus species that have notable antibacterial and antioxidant properties. These fungi have been successfully isolated and identified, proving that medicinal plants are important sources of physiologically active microbes. These endophytic fungi produce bioactive compounds that have potent inhibitory effects against pathogenic bacteria, according to preliminary antimicrobial screening.

Penicillium citrinum was one of the isolates that had significant activity against Staphylococcus aureus, indicating its potential as a source for the creation of new antimicrobial drugs. Additionally, the extracts showed significant free radical scavenging activity in DPPH and DMPD tests, suggesting the existence of secondary metabolites that can lessen oxidative stress.

These results highlight the importance of endophytic fungi as natural manufacturers of bioactive chemicals in the pharmaceutical industry. To find new natural products with therapeutic promise, future research on the purification, characterisation, and mechanistic assessment of these metabolites is crucial.

Overall, this work shows that endophytic Penicillium and Aspergillus species linked to medicinal plants are prospective substitutes for synthetic antioxidants and antibacterial agents, helping to create environmentally friendly and sustainable biologically produced goods.

Conflict of interest

There are no conflicts to declare.

Supporting information

Not applicable.

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