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Evaluation of Fungal Endophytes from *Terminalia sp.* for Extracellular Enzymes, Antioxidant, and Bioactive Metabolites

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Abstract

In an approach towards developing antifungal compounds active against plant pathogens under in vitro conditions, the present study was carried out in which enzymatic, and metabolic properties of fungal endophytes isolated from four *Terminalia sp.* were evaluated. A qualitative phytochemical analysis was performed to detect alkaloids, flavonoids, tannins, saponins, phenols, steroids, and glycosides. Screening of test fungi for bioactive metabolite, and its methanolic extract against three pathogens exhibited raised metabolic response of test fungi. The bioactive potential of fungi has been presented in terms of growth reduction (%) of pathogenic fungi, calculated based on morphological growth on solid plate culture. The findings reveal information on the biological activities of fungal endophytes isolated from *Terminalia spp*.. The in vitro analysis of methanolic extracts of endophytic fungi leads us to infer its promising antifungal capabilities against phytopathogens, and it is obvious that the aforementioned fungus produce a variety of bioactive chemicals with potential enzymatic and antioxidant activity. Overall, the study reveals that segregated fungal endophytes have enormous potential for various extracellular enzymatic properties, use in the development of new antifungal drugs, and as a therapeutic model in the agricultural and pharmaceutical industries. However, more study is needed to uncover and understand bioactive components that have a variety of biological functions and might be used for human and environmental benefits.

Keywords*:* Bioactive, Endophytes, Enzymatic activity, Fungi, Phytochemical, *Terminalia*

Introduction

In the 21st century global concern focuses on infectious diseases. 25 % of total deaths are caused by pathogenic microorganisms given by the World Health Organization (WHO). Many antibiotics are produced to remove fungal and bacterial infections, but the problem that remains is antibiotic resistance. Due to the development of multi-drug resistance microorganisms which show resistance in two or more classes of antibiotics, this kind of difficulty is displayed nowadays (1). There is a challenge for the development of a better understanding of resistance and finding newer drugs against microbial disease (WHO). Several antimicrobial assays are well-known and commonly used in microbial laboratories nowadays (2). This promotes research for novel antimicrobial agents to prevent resistance and provide relief from diseases (3).

Historically plants contribute a potential source of compounds and species like *Combretum, Terminalia, and Pteleopsis* have found profound antimicrobial effects. The second largest genus, *Terminalia* has nearly about 200

species of the family Combretaceae after Combretum. Plants from the *Terminalia* genus (Combretaceae family) are utilized as traditional medicines all over the world. *Terminalia arjuna, Terminalia belerica,* and *Terminalia chebula* are the most commonly utilized *Terminalia* species in medicine. Members of *Terminalia* are used for the treatment of Cardiovascular problems, wound healing, colds, conjunctivitis, ulcers, headaches, hypertension, jaundice, leprosy, pneumonia & skin diseases, and also for the treatment of HIV and other microbial disease (4, 5). Root, stem-bark samples of *Terminalia arjuna* contain some bioactive compounds like tannins, glycoside, alkaloids, steroids, triterpenoids, etc are known to display both pharmacological and physiological properties (6, 7). It has been found that tannin, a secondary metabolite from *Terminalia sp.* is responsible for anticancer properties (8, 9). *Terminalia* contains ingredients that help for the stimulation of the heart & also help the heart by lowering cholesterol and blood pressure. *T. arjuna* is used for bile duct disorder, asthma, scorpion stings, and poisonings whereas *T.belerica* is used for respiratory tract infections, cough, and sore throat & *T. chebula* is used for treating vaginal infections, and dysentery. In the field of Ayurvedic medicine *Terminalia belerica* in combination with *Terminalia chebula* has been used as a “Health harmonizer” & both are used for lowering cholesterol which prevents the destruction of heart tissue.

Plants and microbes endowed some natural products that have an established record of providing new pharmaceutical medicines (10). Many medicinal plants are recognized for housing endophytic fungi, which are key sources of many bioactive secondary metabolites and enzymes useful in the pharmaceutical business (11-13). Therefore, increasing efforts are made to identify and focus on endophytic fungi from medicinal plants. Fungal organisms that reside in the plant without forming disease or damage to their host, this definition of endophytic fungi includes the symbiotic interaction in which plants and endophytic fungi participate: Parasitism, Commensalism, and Mutualism (14). Endophytes have recently known as a major source of a variety of new physiologically active secondary metabolites possibly useful for human treatment, and a recent study found that 51% of compounds extracted from endophytes (15, 16). Fungi present inside the plant could be a very promising way to produce various metabolites for medicinal, agricultural, and industrial uses (17). Fungal endophytes form a mutual relationship with the plant in which the plant gives shelter & nutrients to the endophytes whereas endophytes produce bioactive substituent which increases the resistance & benefits the plant growth (18, 19).

A great number of antifungal compounds may be found in endophytic fungi isolated from plant *Terminalia* and exploring natural compounds synthesized by endophytic fungus is thought to be a strategy to eliminate resistance while also meeting the demand for the discovery of extremely cost-effective, less toxic antibiotics to treat infectious diseases caused by microorganisms (20).

Materials and Methods

Plant sample collection

Samples like leaves and barks of four *Terminalia sp.* were collected from the campus of Regional Plant Resource Centre, Nayapalli, Bhubaneswar, Odisha. The samples were taken to the laboratory, rinsed to eliminate any dirt, and air-dried. Leaf and bark samples were chopped into with sterile scalpels. Sample fragments were successively surface sterilized in 70% ethanol for 1 minute, 2.5% sodium hypochlorite for 2 minutes, and sterile distilled water 2 times for 1 minute each (21).

Isolation and purification

The inner tissues of the leaves and barks were removed, and approximately 2-3 segments were put in SD agar media and incubated. Daily observations were made un-

til endophytic fungi began to proliferate (22, 23). Following incubation, fungal colonies were collected, streaked on agar plates, and incubated at 30°C for three days, so that the microbial cells were well spaced from each other. The processes like streak plate and central inoculation were repeated 3-4 times until we got purified pathogens.

Preliminary plate test of endophytic fungi for extracellular enzymes and mineral solubilization potential

To carry out the screening of fungi for extracellular enzyme production and mineral solubilization potential, a plate test was performed by inoculating 7days old culture of fungal isolates on media specified for amylase, cellulase, xylanase, L-asparaginase, Lipase, IAA, organic acid production and Phosphate solubilization, Zinc solubilization potential (24-26). A clear zone enclosing the fungal colony formed after 3-5 days of incubation was considered an indicator of enzyme production and mineral solubilizing potential (27).

Preliminary phytochemical screening of isolated fungal extracts

SD broth was prepared for phytochemical screening of fungal isolates. Inoculation of isolated endophytic fungi into the broth and after incubation filtration occurs to separate the mycelial mat. The mat was pulverized in a pestle and mortar with ethyl acetate, methanol, and ethanol individually, and the grounded mycelia were then put into three distinct flasks and stored for 5 days (28). The extract was partitioned into ethyl acetate, aqueous ethanol, and aqueous methanol soluble fractions, and the filtrate of the above fungal isolates was taken for biochemical test. Different biochemical tests like alkaloids, phenols, flavonoids, saponins, steroids, tannins, and glycosides were done with the four samples (Ethanol, Methanol, Ethyl acetate, and Culture filtrate) to know the availability of metabolites.

Biological screening of methanolic extract of fungal endophytes

Three references of plant pathogens, i.e., *Fusarium sp*. were taken for antifungal assay. Endophytic fungi isolated from *Terminalia sp.* were examined for antifungal activity against three pathogens of *Fusarium sp.* by inoculating them using the co-inoculation method on agar media and incubated for 5- 7 days (29). Two pieces of growing mycelial disc of endophytic fungi were inoculated into SD broth & incubated for 10 days. After the incubation period, filtration occurred to separate the culture filtrate and mycelial mat through Whatmann no. 1 filter paper (30). The culture filtrate was concentrated by the Soxhlet apparatus and solvent was added to the concentrated filtrate for 72 hours. The upper layer was separated and evaporated by Soxhlet. Evaporated samples were dissolved in methanol and preparation of methanolic extract was completed. Methanolic extract samples were screened for qualitative phytochemical screening to know the availability of secondary metabolites discussed earlier. A qualitative free-radical scavenging activity test occurred with the methanolic sample and the antimicrobial activity test was done through the pour plate method to find the best test organism (31). The bioactive potential of fungi has been presented in terms of growth reduction (%) of pathogenic fungi, calculated based on morphological growth on solid plate culture.

Results and Discussions

Occurance of fungi on leaf and bark of Terminalia sp.

A total of 29 nos. of fungi have been isolated from samples collected from different species of *Terminalia.* All were characterized morphologically and evaluated for their extracellular enzyme and mineral solubilization potential.

Profiling of endophytic fungi for extracellular enzymes and mineral solubilization potential

Profiling of fungi for enzymes and sol-

ubilization potential is described in Table 1. Cellulase has been used in biofuel, agriculture, food, detergent, and also in textile industries. Cellulase is present in *Phoma sp., Penicillium sp.,* etc. (32). Among all, 6 fungi (24.13%) were found to be producers of cellulase and 11 fungi (37.93%) for xylanase activity. Almost all fungi exhibited zinc-solubilization potential but no IAA, lipase, and L-asparaginase activity could be observed in these fungi. Phosphate solubilization potential has been observed in 4 fungi (13.79%). Many fungal isolates have been observed as organic acid producers in the present study (27.58%). There is limited information about amylase enzymes from endophytic fungus (33). In the present study, 51.7% of the isolates tested positive including *Aspergillus sp., Penicillium sp., Trichoderma sp., Fusarium sp.* etc. As shown in Figure 1, amylase-producer fungi are more in number as well as Zinc solubilization potential. Another fungal extracellular enzyme is lipase which is dominantly used in the food industry. The report says that there are very less findings regarding lipolytic activity. In the present study, no lipolytic activity was found in the fungi isolated from *Terminalia sp.* Microbial L-asparaginase is secreted extracellularly and considered to be intracellular in nature (34), in the present study, no L-asparaginase activity is found positive. A report from (35) says that if tryptophan is used as a substrate then fungi are capable of synthesizing IAA. In the present study, fungi show no activity for producing IAA. Results obtained for extracellular enzyme production and mineral solubilization potential are given in Table 1 and the percentage of incidence is given in Figure 1.

Table 1: Preliminary plate screening tests of fungi for extracellular enzymes, mineral solubilization potential

Sl no	Name of fungal isolates	Extracellular activity, mineral solubilization potential								
		A	B	C	D	E	F	G	H	I
1	*Aspergillus sp. 1*	-	-	-	-	-	-	-	-	+
2	*Aspergillus sp. 2*	-	-	-	-	-	-	-	-	-
3	*Aspergillus sp. 3*	-	+	-	-	+	-	+	+	-
4	*Aspergillus sp. 4*	-	-	-	-	-	-	-	-	-
5	*Aspergillus sp. 5*	-	+	-	-	+	-	+	+	+++
6	*Aspergillus sp. 6*	-	+	-	+++	+	-	-	+	-
7	*Aspergillus sp. 7*	-	+	-	-	+	-	-	+	-
8	*Aspergillus sp. 8*	-	+	-	-	+	-	-	+	-
9	*Aspergillus sp. 9*	-	+	-	+	+	-	+	+	-
10	*Aspergillus sp. 10*	-	+	-	-	-	-	-	+	-
11	*Aspergillus sp. 11*	-	+	-	-	-	-	-	+	++
12	*Aspergillus sp. 12*	-	-	-	-	-	-	-	+	-
13	*Aspergillus sp. 13*	-	+	-	+	+	-	-	+	+
14	*Aspergillus sp. 14*	-	-	-	-	+	-	-	-	+
15	*Aspergillus sp. 15*	-	-	-	+++	+	-	-	+	-
16	*Penicilium sp. 1*	-	+	-	-	-	-	-	+	-

17	*Penicilium sp. 2*	-	-	-	-	-	-	-	+	++
18	*Penicilium sp. 3*	-	+	-	+++	-	-	-	+	-
19	*Penicilium sp. 4*	-	-	-	+	+	-	+	-	+
20	*Trichoderma sp. 1*	-	+	-	-	-	-	-	+	-
21	*Trichoderma sp. 2*	-	-	-	-	-	-	-	+	-
22	*Trichoderma sp. 3*	-	-	-	-	-	-	-	-	-
23	*Myceloid sp. 1*	-	-	-	-	-	-	-	+	-
24	*Myceloid sp. 2*	-	+	-	-	-	-	-	+	+
25	*Fusarium sp. 1*	-	+	-	-	-	-	-	+	++
26	*Fusarium sp. 2*	-	-	-	-	-	-	-	-	-
27	*Nectria sp.*	-	+	-	-	-	-	-	-	-
28	*Acladium sp.*	-	-	-	+++	+	-	-	+	-
29	*Colletotrichum sp.*	-	-	-	-	-	-	-	-	+

Abbreviations: +, (Present) ; ++, (Present Significantly); +++, (Present in excess) ; -, (Absent)

A, Indole acetic acid (IAA); B, Amylase; C, Lipase ;D, Cellulase ;E, Xylanase; F, L-asparaginase; G, Phosphate solubilisation; H, Zinc-solubilization; I, Organic acid.

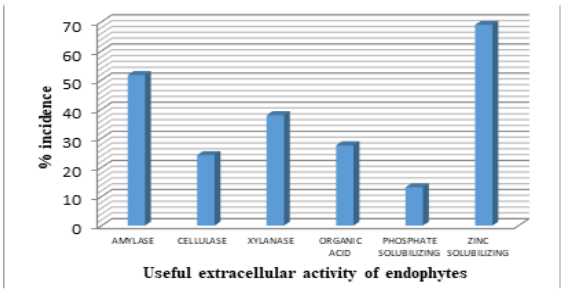

Figure 1: Incidence of fungal endophytes (%) isolated from *Terminalia sp.*

% of incidence= Fungi tested positive/Total isolated fungi × 100

Preliminary phytochemical screening of isolated fungi and plant sample

Phytochemicals, like phenols, tannins, flavonoids, saponins, glycosides, and steroids present in plant extracts which is revealed in the present study (Table 2). *T. arjuna* is a widely used medicinal plant responsible for the treatment of degenerative diseases considered a pharmacological system of medicine (36). Phytochemical screening of extract of endophytes isolated from leaf, and bark sample of four *Terminalia sp.* were completed. This was done to know the presence of bioactive metabolite. Ethanolic, Methanolic, and ethyl acetate extract of crude extract of 29 endophytes and plant samples contain alkaloids, flavonoids, steroids, phenols, saponins, tannins, and glycosides. Alkaloid, the secondary metabolite resides in plant extract which hinders the microorganism by inhibiting the enzymes involved in energy generation. *Terminalia bellerica* containing tannins in plant extract might have hampered the growth of microorganisms by precipitating the microbial protein and making nutritious proteins inaccessible to them (37). There are several reports available on the phytochemical screening of fruit & stem extract but comparatively fewer reports on leaf and bark samples. In one report, ethanolic extract contains all biomolecules, methanolic extract contains all biomolecules except tannins and saponins whereas ethyl acetate extract except saponins, steroids & tannins but in our study it shows the better result that all extract contains all biomolecules (28).

Table 2: Preliminary Phytochemical screening of isolated fungal extracts.

Name of fungal isolates	Secondary metabolites																																	
	Alkaloids				Phenols				Flavanoids				Tanins				Glycosides				Steroids				Saponins			
	M	E	EA	CF	M	E	EA	CF	M	E	EA	CF	M	E	EA	CF	M	E	EA	CF	M	E	EA	CF	M	E	EA	CF
Aspergillus sp. 1	+	+	+	-	+	+	-	+++	-	-	-	-	+	+	+	+++	+	+	+	-	-	-	-	-	-	+	-	-
Aspergillus sp. 2	-	-	-	-	+	+++	+++	-	-	-	-	+	+	+	+	-	-	+	+	-	-	-	+	-	-	-	-	
Aspergillus sp.3	+	++	-	-	+	-	++	++	-	-	-	-	+	+	+	+	-	-	+	+	+++	+++	+++	-	-	-	-	-
Aspergillus sp. 4	++	+	-	-	++	+	++	+	-	-	-	-	+	+	+	+	+	++	+	-	+	+	+	-	-	-	-	-
Aspergillus sp. 5	+	+	+	+	++	++	+	+	-	-	-	-	+	+	+	+	+	+	+	-	+	+	+	-	+	+	+	-
Aspergillus sp. 6	+	+	-	-	+	+++	++	+++	+	-	-	-	+	+	+	++	++++	++	-	+	-	+	+++	+	-	-	-	-
Aspergillus sp. 7	+	+	-	-	+	++	++	+	-	-	+	-	+	+	+	+	++	+++	+	-	-	-	-	-	-	-	-	-
Aspergillus sp. 8	+	-	-	-	+	+	+	+	-	-	-	-	+	+	+	+	++	+	+	+	-	-	-	-	-	-	-	-
Aspergillus sp. 9	+	+	-	+	+	+	+	++	-	+	-	-	+	+	-	+	++	-	-	-	++	-	++	-	-	-	-	-
Aspergillus sp. 10	+	+	-	-	+	-	+	+	-	-	-	+	+	+	+	+	++	+++	+	-	+	+	++	+	-	-	-	-
Aspergillus sp.11	+	+	-	-	+	+	+	+	-	-	-	+	+	+	+	-	-	-	-	-	+	-	+	+	-	-	-	+
Aspergillus sp. 12	-	+	-	-	+++	++	++	+	-	-	-	-	+	+	+	-	+	+	+	-	-	+	++	-	-	-	-	-
Aspergillus sp. 13	+	+	-	-	+++	+++	+++	-	-	-	-	+	+++	+	+++	+	+	+	+	+	+++	+	+	-	-	-	-	-
Aspergillus sp. 14	-	+	-	-	+	++	-	-	-	-	-	+	+	+	-	+	-	-	-	+	-	-	-	-	-	+	-	-
Aspergillus sp. 15	+	+	+	+	+	+	-	+	-	-	-	-	+	+	-	+	+	+	+	-	-	-	+	-	-	+	-	-
Penicillium sp. 1	+	+	-	+	++	+	+	-	-	-	-	-	+	+	+	+	-	-	-	-	-	+	+	-	-	-	-	+
Penicillium sp. 2	+	-	-	-	+	+	+	-	-	-	-	-	+	+	+	-	+	+	-	+	-	-	-	+	-	-	-	-
Penicillium sp. 3	-	+	-	+	+	+	+	-	-	-	-	-	+	+	+	++	+++	+	-	+	-	+	++	-	-	-	-	-
Pernicilium sp. 4	+	+	-	+	+	+	-	+	-	-	-	-	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	+
Trichoderma sp. 1	+	+	-	+	+	+	+	++	+	+	-	-	+	+	-	+	-	-	-	++	+	+	+	+	-	-	-	-
Trichoderma sp. 2	+	+	-	-	+	+	+	++	+	-	+	-	+	+	+	+	+	+	-	+	+	-	+	-	-	-	-	-
Trichoderma sp. 3	+	+	-	-	+	+	+	++	-	-	-	-	+	+	-	+	+++	+++	-	+	+	-	+	+	-	-	-	-
Myceloid sp. 1	+	+	-	-	++	+	+	+	-	-	-	-	+	+	+	+	-	-	-	-	-	+	+	+	-	-	-	-
Myceloid sp. 2	+	-	-	+	+	-	+	+	-	-	-	-	+	+	+	-	+	+	-	-	-	-	+	+	-	-	-	-
Fusarium sp. 1	-	+	-	-	+++	+	+	++	-	-	+	+	+	+	+	-	-	-	-	-	-	+	+	+	-	-	-	-
Fusarium sp. 2	-	-	-	+	+	+	-	+	-	-	-	-	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-
Nectria sp.	-	+	-	-	++	++	-	+	+	+	+	-	+	+	-	+	-	-	-	-	-	-	-	-	+	-	-	-
Acladium sp.	+	+	-	-	++	+++	+	+	-	-	-	+	+	+	-	+	-	+	-	-	+	-	++	+	-	-	-	+
Colletotrichum sp.	+	+	-	-	+	+	-	+	-	-	-	-	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-

Abbreviations: +, (Present) ; ++, (Present Significantly); +++, (Present in excess) ; -, (Absent)

M, Methanol; E, Ethanol; EA, Ethyl acetate; CF, Culture filtrate

Biological Screening of methanolic extract of fungal endophytes

Several studies have described the antioxidant properties of different parts of various medicinal plants. Antioxidant activity test, and qualitative biochemical estimation to know the best endophytic fungi for further experiment (Table 3)**.** Some positive results of the test for alkaloids, flavonoids, phenols, tannins, saponins, steroids, and glycosides were recorded. DPPH is a stable nitrogen-centered, lipophilic free radical that is extensively used to evaluate antioxidant activity in a shorter period of time than other techniques. The odd electron in DPPH is coupled with hydrogen from a free radical scavenging antioxidant, resulting in decreased DPPH. The ensuing decolorization from purple to yellow revealed a favorable result (38). After completion of the biological screening test, we selected the best fungal strain. Among all fungi, the methanolic extract of *Aspergillus sp.* 14 indicated the greatest result.

Table 3: Phytochemical and qualitative antioxidant activity test of solvent extract of 29 fungal isolates

Sl no	Fungal isolate	Alkaloids	Phenols	Flavonoids	Tannins	Glycosides	Steroids	Saponins	DPPH
1	*Aspergillus sp. 1*	+	+	-	+	-	+	+	+++
2	*Aspergillus sp. 2*	+	-	-	+	-	+	-	++
3	*Aspergillus sp. 3*	-	+	-	+++	+	+	+	++
4	*Aspergillus sp. 4*	-	-	-	+	+	+	-	-
5	*Aspergillus sp. 5*	+	-	-	+	+	+	+	-
6	*Aspergillus sp. 6*	-	+	-	+	+	+	-	+
7	*Aspergillus sp. 7*	-	-	-	+	+	+	-	-
8	*Aspergillus sp. 8*	-	+	-	+	+	-	-	++
9	*Aspergillus sp. 9*	+	+	++	+	+	+	+	+++
10	*Aspergillus sp. 10*	-	-	-	-	+	-	-	+
11	*Aspergillus sp. 11*	-	+	-	+++	+	++	-	+++
12	*Aspergillus sp. 12*	-	-	-	+	+	-	-	-
13	*Aspergillus sp. 13*	-	-	-	+	-	+	-	+
14	*Aspergillus sp. 14*	+	+	-	++	+	+	+	+
15	*Aspergillus sp. 15*	-	-	-	+	+	-	-	+
16	*Penicillium sp. 1*	+	-	-	+	+	+++	-	+
17	*Penicilium sp. 2*	-	-	-	+	+	+++	-	+++
18	*Penicilium sp. 3*	+	+	+	+	+	+	+	+
19	*Penicilium sp. 4*	+	-	+	++	+	++	+	+++
20	*Trichoderma sp. 1*	+	+	-	+	-	++	+	-
21	*Trichoderma sp. 2*	-	+	-	+++	+	+++	-	++
22	*Trichoderma sp. 3*	+	+	-	++	+	++	-	++
23	*Myceloid sp.1*	-	+	+	+	++	++	+	+
24	*Myceloid sp. 2*	-	+	-	+	++	+	-	++
25	*Fusarium sp. 1*	-	-	-	+	+	++	-	-
26	*Fusarium sp. 2*	-	-	-	+	+	+	-	++
27	*Nectria sp.*	-	-	-	+	+	-	-	++
28	*Acladium sp.*	+	-	++	-	+	+++	+	+++
29	*Colletotrichum sp.*	-	-	-	+	-	-	-	-

Abbreviations: +, (Present) ; ++, (Present Significantly) ; +++, (Present in excess) ; -, (Absent)

The solvent extract of *T. bellerica* was tested against *S. aureus* and *K. pneumonia*, which are two respiratory pathogens that indicated the greatest activity (39). The antimicrobial activity of different solvent extracts of *Terminalia sp.* was conducted against four bacteria and two viruses and the various solvent extracts were hexane, benzene, chloroform, Ethyl acetate, Acetone, Ethyl alcohol and methanolic extracts, among all the extracts studied, methanol and ethyl acetate extracts exhibited good effects against NDV, PV virus, S. aureus, and E. coli (40). In our study, the prepared methanolic sample of 29 fungi undergoes antimicrobial activity test against three *Fusarium sp.,* result denoted in Table 4**.** This was demonstrated in terms of growth reduction (%) of pathogenic fungi, calculated based on morphological growth on solid plate culture, indicating that there was an inhibition of growth of the test microbe. So *Aspergillus 14* was considered a good source of antimicrobial compounds. This finding suggested that the antimicrobial compounds generated by active fungal endophytes might have specialized applications where disease control is needed.

Table 4: Antimicrobial activity test of methanolic extract of 29 fungal isolates

Pathogens				
		D1	D2	D3
Sl no	Fungal isolate	% of growth reduction	% of growth reduction	% of growth reduction
1	*Aspergillus sp. 1*	1.45±0.77	11.83±2.305	13.3±0
2	*Aspergillus sp. 2*	10.82±1.666	17.84±0.749	16.51±2.142
3	*Aspergillus sp. 3*	13.1±4.101	18.76±0.657	17.6±1.088
4	*Aspergillus sp. 4*	1.1±0.42	12.85±0.855	5.78±1.103
5	*Aspergillus sp. 5*	12.82±1.661	1.96±0.063	16.52±0.975
6	*Aspergillus sp. 6*	7.81±2.559	1.74±0.424	19±0.219
7	*Aspergillus sp. 7*	6.68±0.417	3.38±1.640	16.5±3.344
8	*Aspergillus sp. 8*	4.35±2.877	7.88±1.725	14.63±1.845
9	*Aspergillus sp. 9*	11.5±1.195	14.59±1.364	3.66±0.049
10	*Aspergillus sp. 10*	13.36±0.841	5.63±1.675	7.32±0.919
11	*Aspergillus sp. 11*	4.35±2.863	8.99±0.141	2.55±1.52
12	*Aspergillus sp. 12*	12.8±1.93	15.52±1.067	13.53±2.375
13	*Aspergillus sp. 13*	13.9±0.378	8.07±1.774	8.88±0.41
14	*Aspergillus sp. 14*	15.4±1.209	20.69±3.549	16.4±2.59
15	*Aspergillus sp. 15*	11.93±0.806	12.64±0.205	19.43±3.146
16	*Penicillium sp. 1*	4.99±1.774	17.5±1.64	6.6±0.077
17	*Penicilium sp. 2*	9.01±3.705	1.68±0.763	1.83±0.48
18	*Penicilium sp. 3*	6.58±2.729	6.74±0.113	8.07±1.159
19	*Penicilium sp. 4*	5.49±1.873	8.03±1.491	18.6±0.806
20	*Trichoderma sp. 1*	2.9±1.272	4.92±1.195	19.2±1.131
21	*Trichoderma sp. 2*	*2.4±2.26*	10.83±1.852	5.77+1.294

22	*Trichoderma sp. 3*	9.65±4.023	8.63±0.961	6.59±0.106
23	*Myceloid sp.1*	4.7±4.101	16.8±1.944	2.48±1.195
24	*Myceloid sp. 2*	8.2±0.84	6.74±0.106	1.09±0.502
25	*Fusarium sp. 1*	12.5±0.318	21.32±4.426	11.35±0.339
26	*Fusarium sp. 2*	11.93±0.799	15.5±1.117	4.54±0.53
27	*Nectria sp.*	2.18±0.141	17.23±1.286	9.37±0.891
28	*Acladium sp.*	2.23±0.141	10.06±4.617	6.6±1.138
29	*Colletotrichum sp.*	7.58±1.074	6.9±0.113	5.79±1.23

Abbreviations: D1, *Fusarium sp.* 1 ; D2, *Fusarium sp.* 2 ; D3, *Fusarium sp.*3 ;

Conclusion

We can conclude that endophytic fungi isolated from four *Terminalia sp.* may be a valuable natural resource for producing physiologically active chemicals with significant antifungal activity against *Fusarium sp.* We also reported that the endophytic fungi are very effective in eliciting the production of secondary metabolite that acts against plant pathogens. Further research into the compounds' purity and structure is underway. It is thought that finding natural compounds formed by endophytes may be a viable strategy to overcome the problem of resistance and meet the emerging desire for highly effective, low-toxicity, and environmentally friendly antibiotics to combat infections.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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