

Assessing the Impact of *Terminalia arjuna* Plant Extracts on the Growth and Sporulation of *Trichoderma harzianum*, *Aspergillus niger* and *Penicillium chrysogenum*

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Abstract

This research investigates the impact of ten medicinal plant extracts on the growth and sporulation of prominent industrial fungal strains-*Trichoderma harzianum*, *Aspergillus niger*, and *Penicillium chrysogenum*. The phytochemical analysis revealed a diverse array of bioactive compounds, hinting at potential antimicrobial and antioxidant properties. Particularly, *Terminalia arjuna* consistently exhibited positive effects on fungal growth, implying a lower degree of antifungal activity, whereas *Phyllanthus emblica* showed variable impacts. These findings were supported by radial growth rates, highlighting *Terminalia arjuna*'s consistent stimulation of fungal growth. Sporulation indices and spore production further affirmed substantial sporulation in *Terminalia arjuna*. Comparative analyses with previous studies emphasized the unique characteristics of this extract. In conclusion, *Terminalia arjuna* shows distinct characteristics compared to other samples, indicating the need for additional investigation, suggests promising applications in both industrial and medicinal contexts, warranting further exploration for optimal utilization.

Keywords: Medicinal Plants, Sporulation, *Trichoderma harzianum*, *Aspergillus niger*, *Penicillium chrysogenum*, *Terminalia arjuna*

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INTRODUCTION

The induction of growth of fungal sporulation by plant extracts refers to the process of promoting or enhancing the formation and release of fungal spores using extracts derived from plants. Fungal sporulation is an important reproductive process for many types of fungi, and it plays a crucial role in their life cycle and dispersal. Plant extracts can contain various compounds that may affect fungal growth and development, including phytohormones, secondary metabolites, and other bioactive substances. When these plant extracts are applied to fungal cultures, they can stimulate or modulate the production and release of fungal spores. This induction of sporulation can be beneficial for research purposes, such as studying the fungal life cycle or obtaining a higher yield of spores for experimentation.

The intricate interplay between medicinal plants and fungi, characterized by bioactive compounds, holds significant promise for therapeutic applications. This investigation explores ten medicinal plants acknowledged for their historical use in traditional medicine and well-documented bioactive profiles. *Chlorophyta*, *Phyllanthus emblica*, *Withania somnifera*, *Plantago ovata*, *Hordeum vulgare*, *Rhizophora mangle*, *Mucuna pruriens*, *Azadirachta indica*, *Asparagus racemosus* and *Terminalia arjuna* boast diverse phytochemical compositions, forming a robust foundation for interactions with fungal growth. *T. arjuna* is recognized for its notable hydrolipidemic properties. It is believed that the inotropic effects of *T. arjuna* can be attributed to its saponin glycosides, whereas the antioxidant activity and vascular amplification activity are associated with its flavonoids/phenolics. These characteristics validate the plant's potential to serve a cardio-protective function.¹⁻³ The primary focus of this study is to unveil the specific impacts of these plant extracts on the induction of growth and sporulation among selected fungi. Fundamentally, the induction of growth signifies the stimulation and enhancement of fungal development, while sporulation refers to the reproductive process involving the formation of spores. The highly complex process of fungal sporulation is influenced by both endogenous and exogenous factors.⁴

The fungi under this research include *Aspergillus niger*, *Penicillium chrysogenum*, and *Trichoderma harzianum*, which are significant industrial fungi. Many enzymes, including pectinases and amylases, as well as organic acids, such as citric acid, are produced by *Aspergillus niger* and used for biotransformations and waste treatment.⁵ *Penicillium chrysogenum* secondary metabolites have noteworthy and beneficial pharmacological activities, including cytotoxic, antifungal, antibacterial, and other miscellaneous activities. Many plant interaction effectors have been identified through research on *Trichoderma*, including small (20-30 nucleotide long) non-coding RNAs, secondary metabolites (lactones, peptaibols, polyketides, terpenes, trichothecenes, VOCs, and phytohormones), and proteins (ceratoplatanins, glycoside hydrolases, hydrophobins, and small secreted cysteine-rich proteins).⁶ *Trichoderma's* resistance to a wide range of toxic chemicals, both organic and inorganic, as well as its ability to boost plant tolerance to stress factors in xenobiotic-contaminated environments and offer total protection without the need for chemical pesticides, make it a promising candidate for use as the foundation of new phytoremediation technologies.⁷ In the realm of biological control, which involves utilizing natural enemies of pests and pathogens, fungi like various species of *Trichoderma* have proven successful in controlling soil-borne pathogens and plant-feeding nematodes.^{8,9}

This investigation is not solely of academic interest; it holds substantial implications for various industries, especially the pharmaceutical and agricultural sectors. By comprehending how plant extracts influence fungal growth, this research seeks to contribute to the establishment of a compound that helps to induce the growth and sporulation of industrially important fungi and hence increase their valuable products. In conclusion, this study aims to shed light on the intricate dynamics between medicinal plant extracts and industrial fungi, with potential outcomes leading to the development of new fungal growth promoting agents and the promotion of sustainable agricultural practices, plant pathology, and pharmaceutical research, underscoring the versatility of natural resources.

METHODOLOGY

Test Samples and organisms

Medicinal plants used as test samples in the study were: *Chlorophyta*, *Phyllanthus emblica*, *Withania somnifera*, *Plantago ovata*, *Hordeum vulgare*, *Rhizophora mangle*, *Mucuna pruriens*, *Azadirachta indica*, *Asparagus racemosus* and *Terminalia arjuna*.

Test Microorganism and growth media

The clinical and pharmacological relevance of *Aspergillus niger* ATCC 6275, *Trichoderma harzianum* ATCC 90237, and *Penicillium chrysogenum* ATCC 66564 was considered. The suspension was made and cultured on potato dextrose broth, and the culture was incubated at 27°C for 24-48 hours. Using 0.5 McFarland standards, cell density was adjusted to 1×10^6 cfu/mL.

Preparation of extracts

To prepare extracts, the plant was air-dried at 50°C, ground into powder, and dissolved in sterile water, methanol, and petroleum ether (1:5 ratio). The mixture was placed in a covered 250 mL beaker and subjected to a 50°C hot water bath for 4 hours. After incubation, the extract was filtered using Whatman paper, and the filtrate was collected in a 50 mL beaker. After discarding residue, the filtrate was retained, and dried at 50°C, yielding a semi-solid state. The weight of this sample was measured, recording the yield.¹⁰

Preliminary phytochemical analysis

The extracts underwent initial phytochemical screening to identify various chemical groups of compounds. The following tests were conducted to analyse the phytochemical composition of the medicinal plant extracts. Alkaloids (Dragendorff's Test),¹¹ Flavonoids (Alkaline Reagent Test),¹² Saponins (Foam Test),¹³ Steroids (Lieberman-Burckhardt test),¹⁴ Test for Glycosides (Prakash and Vedanayaki),¹⁵ Test for Terpenoid (Salkowski's test),¹⁶ Tannins (Braymer's test),¹⁷ Carbohydrates (Molisch's Test),¹⁸ Mucilage Test (Bhatia *et al.*),¹⁹ Starch (Iodine test), Protein (Biuret test), Phenols (Katalinic *et al.*).²⁰

Fungal growth measurement

Point inoculation method

To evaluate fungal growth using the point inoculation method, control plates were prepared without test samples, involving a single point inoculation on 90 mM Petri plates. The plant extracts, which showed almost negative results for antifungal activity are taken for this experiment. The positive control comprised 10% methanol extracts in water (samples with potential fungal growth) mixed with PDA liquid medium at <40°C before solidification, followed by a single point inoculation on 90 mM Petri plates. For the negative control, 10% methanol extracts in water (two samples without potential fungal growth) were mixed with PDA liquid medium at <40°C before solidification and single-point inoculation on 90 mM Petri plates. Incubation at room temperature for 7 days followed, with subsequent measurement and tabulation of colony growth coverage for comparison with control plates.²¹

Radial growth rate

Harvested spores for inoculation were treated with 0.1% (v/v) Tween 80 from previously grown cultures at room temperature, and the spore density was adjusted to 1×10^5 cfu/mL. Agar plates, each containing 20 mL of agar medium with 10% methanol extracts (dissolved in water), underwent inoculation at a single point using a pipette (10 μ l), while control plates were maintained without any test samples. All plates were then incubated at room temperature for 7 days. The radial growth rates of the colonies were measured in millimetres on day 1, day 3, day 5, and day 7 of incubation.²¹

Sporulation index and spore production

Sporulation,²² determined spores per gram of substrate in the culture medium. Recovery involved incubating agar medium for 5-7 days, using an electromagnetic stirrer in 0.01% Tween 80 solution for 30 minutes. For counting, a 1/1000 dilution used 0.01% Tween. In counting, 10 μ l of the suspension was placed on a Haemocytometer, covered, and observed at 40X under an optical microscope. Valid counts ranged from 10 to 30 spores per field. The average spores per unit cell were used to calculate spores per 1 mL.²³

$$\text{No. of spores per cubic mM suspension} = \frac{\text{Avg of no. of spores counted} \times \text{dilution (1000)}}{\text{No. of smallest square counted (64)}} * 4000$$

RESULTS

Phytochemical analysis results

Methanol extracts from diverse medicinal plant samples exhibited varying outcomes, revealing the presence or absence of specific chemical compounds. The phytochemical analysis of ten distinct medicinal plant extracts was conducted to elucidate their composition and potential bioactive constituents. The findings, outlined in Table 1, reveal a diverse array of phytochemicals across the examined samples.

The examination of phytochemicals in a range of medicinal plant extracts aimed to investigate their potential bioactive components. Alkaloids were present in all extracts, suggesting potential antimicrobial properties. Glycosides exhibited variability, being entirely absent in *Phyllanthus emblica*, *Plantago ovata*, *Mucuna pruriens*, *Azadirachta indica*, *Asparagus racemosus*, and *Terminalia arjuna*. Steroids were identified in specific extracts, indicating potential anti-inflammatory effects. Terpenoids, found in multiple extracts, contributed to antioxidant and antimicrobial attributes. Proteins were notably present in extracts of, *Phyllanthus emblica*, *Hordeum vulgare*, *Rhizophora mangle*, and *Terminalia arjuna*, hinting at potential nutritional benefits. Flavonoids, observed in *Chlorophyta*, *Withania somnifera*, *Plantago ovata*, and *Mucuna pruriens*, are associated with diverse therapeutic effects. Saponins, tannins, phenols and carbohydrates displayed varying patterns among the extracts. All the ten medicinal plants exhibit negative results for mucilage test and the starch is present only in *Withania somnifera*. The comprehensive phytochemical profile highlights the diverse array of bioactive compounds in these medicinal plants, suggesting their potential influence on fungal growth and sporulation, crucial for industrial applications. These findings provide valuable insights into the pharmacological potential of the investigated plant extracts,

Table 1. Phytochemical analysis of the sample extracts

TESTS	<i>Chlorophyta</i>	<i>Phyllanthus emblica</i>	<i>Withania somnifera</i>	<i>Plantago ovata</i>	<i>Hordeum vulgare</i>	<i>Rhizophora mangle</i>	<i>Mucuna pruriens</i>	<i>Azadirachta indica</i>	<i>Asparagus racemosus</i>	<i>Terminalia arjuna</i>
Alkaloid	present	present	present	present	present	present	Present	present	present	present
Glycoside	present	Absent	Partially present	Absent	Partially present	present	Absent	Absent	Partially present	Absent
Steroid	Absent	Absent	Absent	Absent	Absent	present	Absent	present	Absent	present
Terpenoid	Absent	present	present	Absent	Partially present	present	Absent	present	Partially present	Absent
Proteins	Absent	present	Absent	Absent	present	present	Absent	Absent	present	present
Flavanoid	present	Absent	present	present	present	Absent	Present	Absent	Absent	Absent
Saponin	present	Absent	Absent	Absent	Absent	present	Absent	present	Absent	Absent
Tannin	present	Absent	present	Absent	Absent	present	Absent	present	Absent	Partially present
Carbohydrate	Absent	Absent	present	present	present	Absent	Present	Absent	Absent	present
Mucilage Test	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	present
Starch	Absent	Absent	present	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Phenol	present	present	present	Absent	Absent	Absent	Absent	present	Absent	Absent

prompting further exploration in both industrial and medicinal contexts (Table 1).

Fungal growth measurement

Point inoculation method

This study aimed to evaluate the impact of medicinal plant extracts on the growth of *Trichoderma harzianum* (*T. harzianum*), *Aspergillus niger* (*A. niger*), and *Penicillium chrysogenum* (*P. chrysogenum*) utilizing the Point Inoculation Method (Table 2). The outcomes, illustrating growth percentages and spreadability on agar plates on Day 3, Day 5, and Day 7 as percentage for two experiments (n = 1, n = 2), yield valuable insights into the potential antifungal properties of these extracts (Figure 1 to

Figure 3). In terms of *P. chrysogenum* growth, both Control and *Terminalia arjuna*-treated samples consistently displayed 100% spreadability by Day 7. *Mucuna pruriens* exhibited slower growth, while *Chlorophyta*, *Plantago ovata*, and *Phyllanthus emblica* showcased progressively slower rates, culminating in minimal growth by Day 7 (Figure 4).

Concerning *A. niger* growth, the Control achieved 95% spreadability by Day 7, but *Phyllanthus emblica* demonstrated robust growth, *Terminalia arjuna* exhibited good growth, *Mucuna pruriens* displayed moderate growth, and *Chlorophyta* showed slower growth (Figure 5). For *T. harzianum* growth, both Control and *Terminalia arjuna*-treated samples consistently displayed robust growth (95-100% spreadability by Day 7).

Table 2. Results of the impact of medicinal plant extracts on *Trichoderma harzianum* (*T. harzianum*), *Aspergillus niger* (*A. niger*), and *Penicillium chrysogenum* (*P. chrysogenum*) using the Point Inoculation Method

Growth/spreadability of <i>P. chrysogenum</i> in (%)						
Experiment	n = 1			n = 2		
	Day 3	Day 5	Day 7	Day 3	Day 5	Day 7
Control	50	70	100	50	70	90
<i>Terminalia arjuna</i>	50	75	100	50	75	100
<i>Mucuna pruriens</i>	40	75	100	40	65	100
<i>Chlorophyta</i>	10	30	70	10	40	70
<i>Plantago ovata</i>	10	20	50	20	30	50
<i>Phyllanthus emblica</i>	-	5	10	-	5	10

Growth/spreadability of <i>A. niger</i> on agar plate (%)						
Experiment	n = 1			n = 2		
	Day 3	Day 5	Day 7	Day 3	Day 5	Day 7
Control	30	75	95	30	60	80
<i>Phyllanthus emblica</i>	50	80	100	40	80	100
<i>Terminalia arjuna</i>	40	80	90	45	70	85
<i>Mucuna pruriens</i>	10	20	50	10	30	40
<i>Chlorophyta</i>	5	10	30	5	10	30

Growth/spreadability of <i>T. harzianum</i> on agar plate (%)						
Experiment	n = 1			n = 2		
	Day 3	Day 5	Day 7	Day 3	Day 5	Day 7
Control	40	75	95	40	80	100
<i>Terminalia arjuna</i>	45	80	95	40	85	95
<i>Phyllanthus emblica</i>	-	5	10	-	5	10
<i>Chlorophyta</i>	60	85	100	50	80	90
<i>Plantago ovata</i>	5	5	10	5	5	10
<i>Asparagus racemosus</i>	-	-	10	-	-	10

Phyllanthus emblica exhibited minimal growth, *Chlorophyta* showcased robust growth, *Plantago ovata* displayed minimal growth, and *Asparagus racemosus* demonstrated minimal to moderate growth (Figure 6).

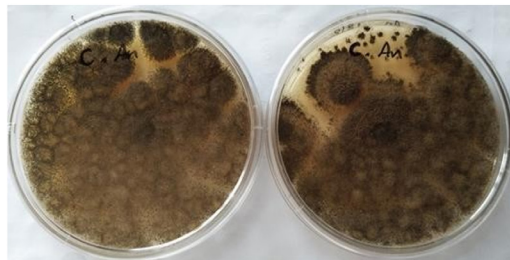
In summary, distinct plant extracts manifested diverse effects on fungal growth. *Terminalia arjuna* consistently demonstrated

positive effects suggesting it to be less antifungal, while the impacts of *Phyllanthus emblica* and *Chlorophyta* were varied. These findings provide valuable insights into the fungal proliferation while using the plant extracts.

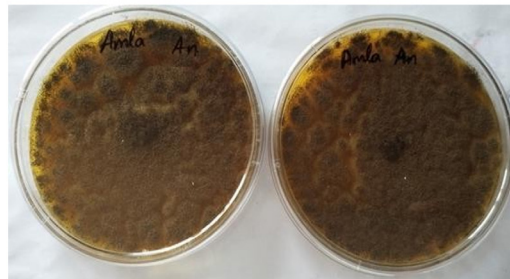
Radial growth rate

The investigation into medicinal plant extract effects on *Trichoderma harzianum*, *Aspergillus niger*, and *Penicillium chrysogenum* involved assessing four-day radial growth. Differences in growth rate were evident (Figure 7 to Figure 9). Control growth of *P. chrysogenum* increased from Day 1,3,5 to 7 were 8 ± 2 mM, 25 ± 2 mM, 62 ± 2 mM, and 78 ± 2 mM, respectively, while *Terminalia arjuna* and *Mucuna pruriens* showed potential robust growth than control. In the case of radial Growth of *T. harzianum*, the control showed progressive growth of 12 ± 2 mM, 45 ± 2 mM, 68 ± 3 mM and 80 ± 5 mM, while *Terminalia arjuna* and *Chlorophyta* extracts stimulated *T. harzianum* growth, surpassing the control. For the radial Growth of *A. niger* the control showed as 8 ± 1 mM, 33 ± 1 mM, 42 ± 2 mM and 72 ± 2 mM, while *Terminalia arjuna* and *Phyllanthus emblica* extracts slightly elevated growth.

Terminalia arjuna consistently stimulated radial growth across all fungi. Fungal plates treated with *Terminalia arjuna* extract promoted better

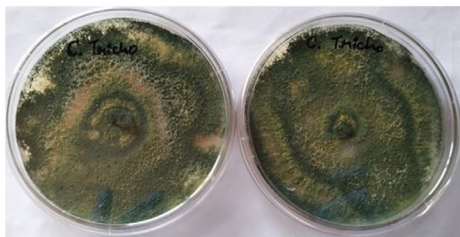


Growth of *A.niger* on Control plate (without any test sample) on 7th day



Growth of *A.niger* with 10% *Phyllanthus Emblica* on 7th day

Figure 1. Growth of *A. niger*

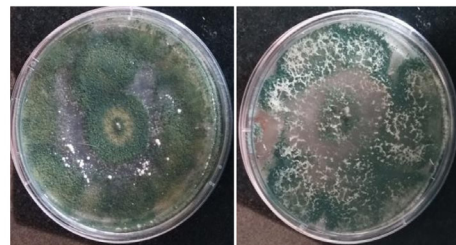


Growth of *T.harzianum* on Control agar plate (without any test sample) on 7th day

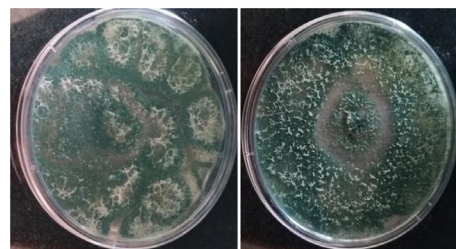


Growth of *T. harzianum* with 10% *Terminalia Arjuna* on 7th day

Figure 2. Growth of *T. harzianum*



Growth of *P. chrysogenum* on Control agar plate (without any test sample) on 7th day



Growth of *P. chrysogenum* with 10% *Terminalia Arjuna* on 7th day

Figure 3. Growth of *P. chrysogenum*

radial colony growth than the control for *A. niger*, *T. harzianum*, and *P. chrysogenum*. These changes imply potential applications of these plant extracts in modulating the growth and sporulation of industrially significant fungi (Table 3).

Sporulation index and spore production

Phyllanthus emblica and *Terminalia arjuna* samples demonstrate robust sporulation for *A. niger* (good to excellent), while *Mucuna pruriens* and *Chlorophyta* samples exhibit fair to poor sporulation (Table 4). *Terminalia arjuna* and *Chlorophyta* samples show noteworthy sporulation for *T. harzianum* (good to excellent),

while *Phyllanthus emblica*, *Plantago ovata*, and *Asparagus racemosus* exhibit inconsistent results. *Terminalia arjuna* sample displays commendable sporulation for *P. chrysogenum* (good), whereas *Mucuna pruriens* and *Chlorophyta* exhibit fair sporulation. *Plantago ovata* demonstrates poor sporulation.

Based on the findings of the study, it can be concluded that *Terminalia arjuna* exhibits superior growth and relative sporulation across all three fungi tested. This suggests that *Terminalia arjuna* shows a positive effect on the growth of these fungi. Therefore, *Terminalia arjuna* holds potential for further investigations and studies.

Table 3. Results showing the radial growth rate of fungi

Scientific Name	Day 1	Day 3	Day 5	Day 7	Day 1	Day 3	Day 5	Day 7
Radial Growth of <i>P. chrysogenum</i> in (mm)								
Control	8 ± 2	25 ± 2	62 ± 2	78 ± 2	9 ± 2	25 ± 2	60 ± 2	80 ± 2
<i>Terminalia arjuna</i>	9 ± 2	27 ± 2	60 ± 2	82 ± 2	8 ± 2	26 ± 2	60 ± 2	82 ± 2
<i>Mucuna pruriens</i>	8 ± 5	23 ± 5	58 ± 5	82 ± 5	9 ± 5	25 ± 5	59 ± 5	80 ± 5
Radial Growth of <i>T. harzianum</i> treated with test samples								
Control	12 ± 2	45 ± 2	68 ± 3	80 ± 5	11 ± 2	43 ± 2	68 ± 3	75 ± 5
<i>Terminalia arjuna</i>	14 ± 2	48 ± 2	70 ± 3	85 ± 5	14 ± 2	44 ± 2	70 ± 3	85 ± 5
<i>Chlorophyta</i>	14 ± 2	46 ± 2	69 ± 3	85 ± 5	12 ± 2	45 ± 2	68 ± 3	85 ± 5
Radial Growth of <i>A. niger</i> treated with test samples								
Control	8 ± 1	33 ± 1	42 ± 2	72 ± 2	7 ± 1	35 ± 1	45 ± 2	70 ± 2
<i>Terminalia arjuna</i>	7 ± 1	35 ± 1	45 ± 2	75 ± 2	8 ± 1	32 ± 1	43 ± 2	72 ± 2
<i>Phyllanthus emblica</i>	6 ± 1	32 ± 1	45 ± 2	70 ± 2	6 ± 1	30 ± 1	38 ± 2	68 ± 2

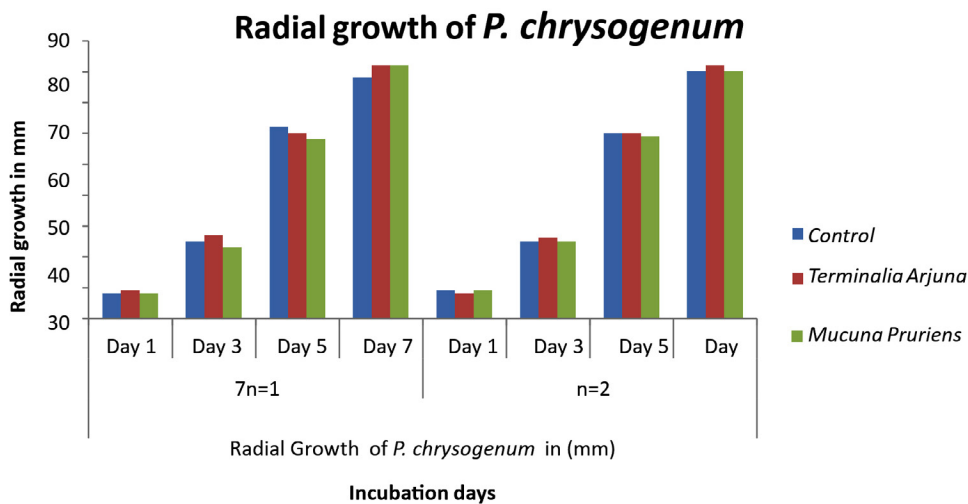


Figure 4. Growth of *P. chrysogenum*

DISCUSSION

Trichoderma harzianum, *Aspergillus niger*, and *Penicillium chrysogenum* are well-known fungi, and their growth and sporulation are of interest in various fields such as agriculture, pharmaceuticals, and biotechnology. Based on the findings of experiments in the current study, it can be inferred that *Terminalia arjuna* stands out among all the samples and warrants further

investigation. This conclusion is drawn from the absence of notable inhibitory activity against *A. niger*, *T. harzianum*, and *P. chrysogenum* in the case of *Terminalia arjuna*.

The examination of phytochemicals revealed a diverse array of bioactive compounds within the medicinal plant extracts, including alkaloids, glycosides, steroids, terpenoids, proteins, flavonoids, saponins, tannins, and carbohydrates. The presence of alkaloids in all extracts suggests

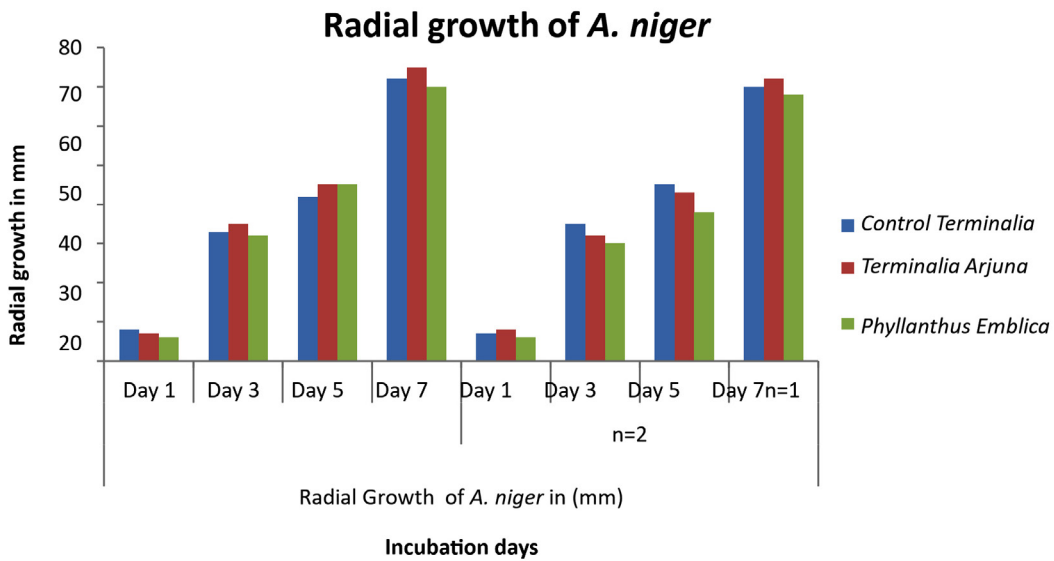


Figure 5. Growth of *A. niger*

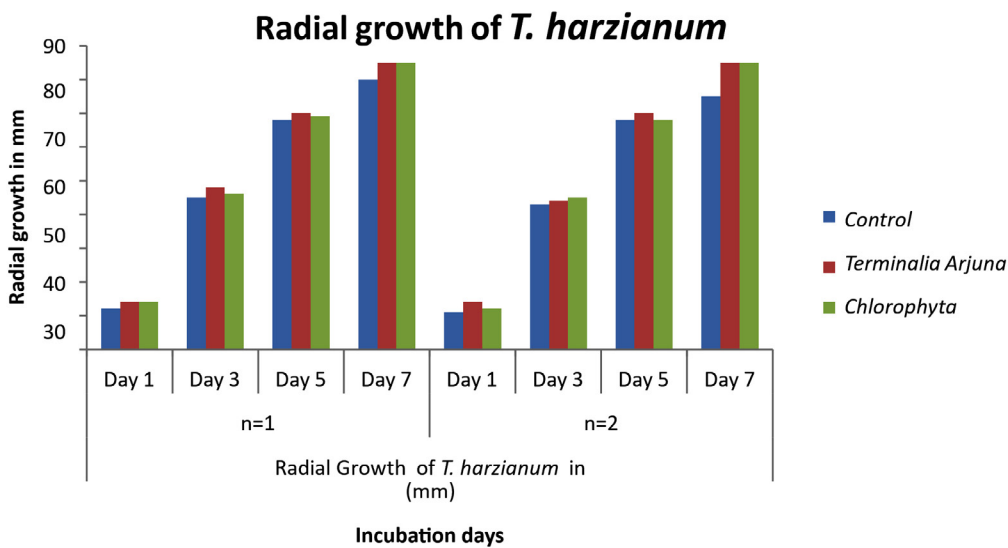


Figure 6. Growth of *T. harzianum*

potential antimicrobial properties, while the involvement of terpenoids and flavonoids suggests contributions to antioxidant and antimicrobial characteristics. These compounds hold promise for influencing fungal growth and sporulation, positioning the plant extracts as potential candidates for various industrial applications.

The results from the Point Inoculation Method showed varied effects of medicinal plant extracts on the growth of *Trichoderma harzianum*, *Aspergillus niger*, and *Penicillium chrysogenum*. Notably, *Terminalia arjuna* consistently exhibited positive effects, implying a lower degree of antifungal activity, while the impacts of *Phyllanthus Emblica* varied. This finding is particularly significant in industrial contexts where precise control of fungal growth is pivotal. The analysis of radial growth rates further supported these observations, indicating that *Terminalia arjuna* consistently stimulated radial growth across all fungi. Fungal plates treated with *Terminalia arjuna* extract facilitated superior radial colony growth compared to the control for *A. niger*, *T. harzianum*, and *P. chrysogenum*. These outcomes suggest potential applications of these plant extracts in influencing the growth and sporulation of fungi that hold industrial significance.

The evaluation of sporulation indices and spore production indicated commendable sporulation in *Terminalia arjuna* for all three fungi. In contrast, *Phyllanthus emblica* and *Chlorophyta* exhibited inconsistent results in terms of sporulation. This implies that the *Terminalia arjuna* extract may exert a less inhibitory effect on fungal sporulation when compared to other extracts.

The diversity, composition, and abundance of fungi found in sapwood align with the findings of Evans et al.²⁴ and Tejesvi et al.²⁵ a significant presence of *Trichoderma* isolates in *T. gileri* and *Terminalia arjuna*, respectively. The antifungal activity of *Terminalia arjuna* was assessed in a study, the methanol extract of *T. arjuna* leaves exhibited moderate activity against *Microsporum canis*, but showed no significant activity against *Aspergillus flavus*. The methanol extract of *T. arjuna* fruit displayed non-significant activity against *Aspergillus flavus*, *Microsporum canis*, and *Fusarium solani*. However, no antifungal activity was observed with the methanol extract of *T. arjuna* bark. Additionally, the extracts from *T. arjuna* leaves, stem bark, and fruit in dichloromethane (DCM) demonstrated no antimicrobial activity.²⁶

Table 4. Results showing Sporulation Index and Spore Production

Organism	Sample	No. of spores per microscopic field	Sporulation capacity (Number of spores/mL)
<i>A. niger</i>	Control	95	5.9*10 ⁹
	<i>Phyllanthus emblica</i>	112	7.0*10 ⁹
	<i>Terminalia arjuna</i>	102	6.3*10 ⁹
	<i>Mucuna pruriens</i>	25	1.5*10 ⁹
	<i>Chlorophyta</i>	12	0.75*10 ⁹
<i>T. harzianum</i>	Control	95	5.9*10 ⁹
	<i>Terminalia arjuna</i>	92	5.7*10 ⁹
	<i>Phyllanthus emblica</i>	-	-
	<i>Chlorophyta</i>	93	5.8*10 ⁹
	<i>Plantago ovata</i>	6	0.37*10 ⁹
<i>P. chrysogenum</i>	Control	81	5.0*10 ⁹
	<i>Terminalia arjuna</i>	94	5.8*10 ⁹
	<i>Mucuna pruriens</i>	62	3.8*10 ⁹
	<i>Chlorophyta</i>	40	2.5*10 ⁹
	<i>Plantago ovata</i>	25	1.5*10 ⁹
	<i>Phyllanthus emblica</i>	-	-

Some of the identified fungal endophytic species have the potential to be tested as biocontrol agents for combating *H. brasiliensis* diseases. Previous studies on the use of biological control against Hevea diseases²⁷⁻²⁹ have focused

on different approaches and have not specifically explored the use of fungal endophytes as antagonistic agents. In future antagonistic assays, it would be worthwhile to consider three genera: *Colletotrichum*, *Pestalotiopsis*, and *Trichoderma*.

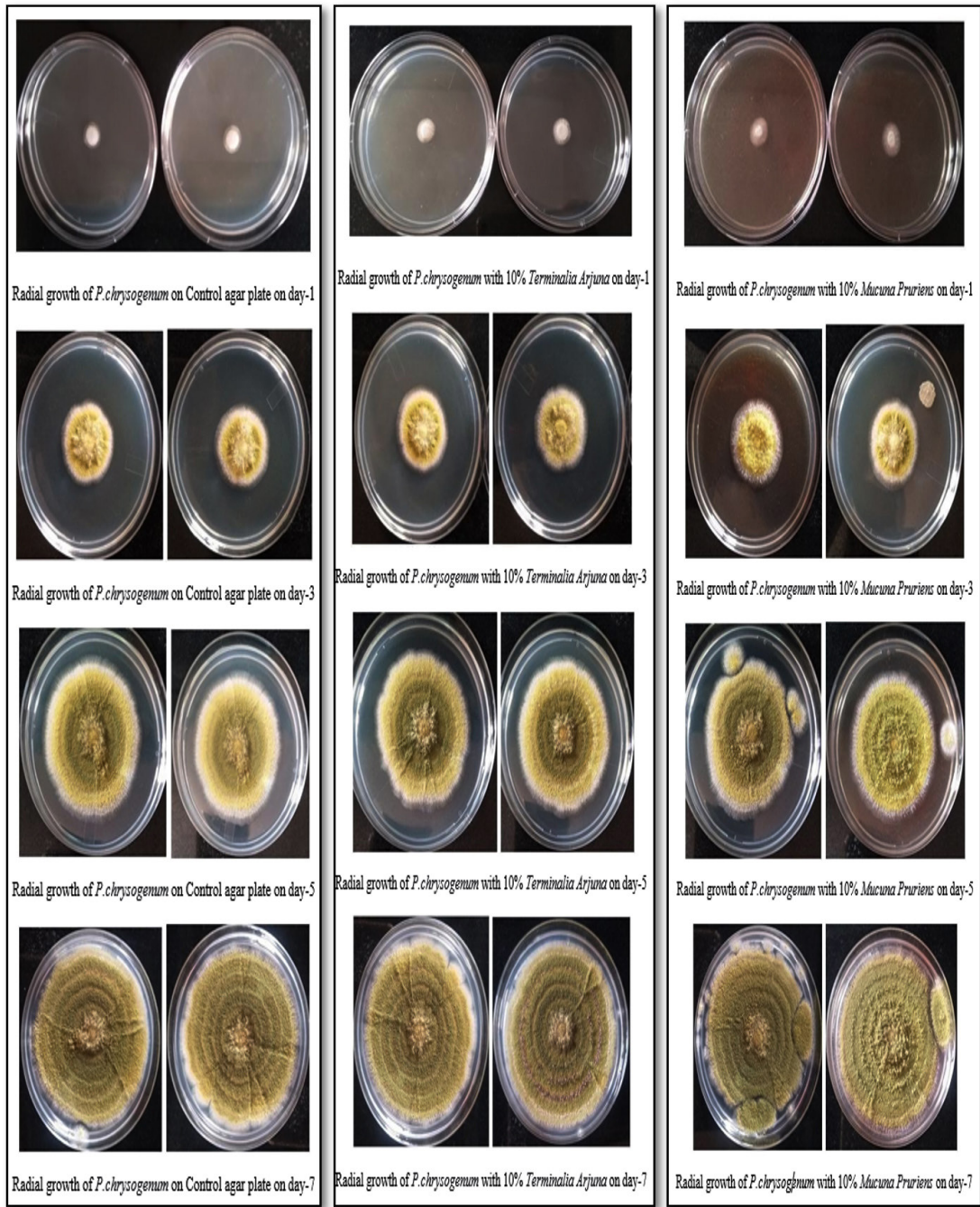


Figure 7. Radial growth of *P. chrysogenum*

These genera hold promise for their potential effectiveness in combating Hevea diseases and warrant further investigation as biocontrol candidates.

Certain strains of *Trichoderma*, such as *T. harzianum*, *T. stromaticum*, and *T. asperellum*, have demonstrated their ability to act antagonistically against fungal diseases.³⁰⁻³² *Trichoderma* employs various mechanisms to protect its host, including direct parasitism, antibiosis, nutrient competition, enhanced plant growth, and induced resistance.³³⁻³⁷

These attributes, combined with its strong competitiveness, make *Trichoderma* species highly recommended for further assessment of their biocontrol potential.

The comprehensive findings suggest that while the *Terminalia arjuna* extract is not entirely devoid of antifungal properties, it may exhibit a lesser inhibitory effect on fungal growth and sporulation compared to other extracts. This has potential implications for applications in both industrial and medicinal contexts, where

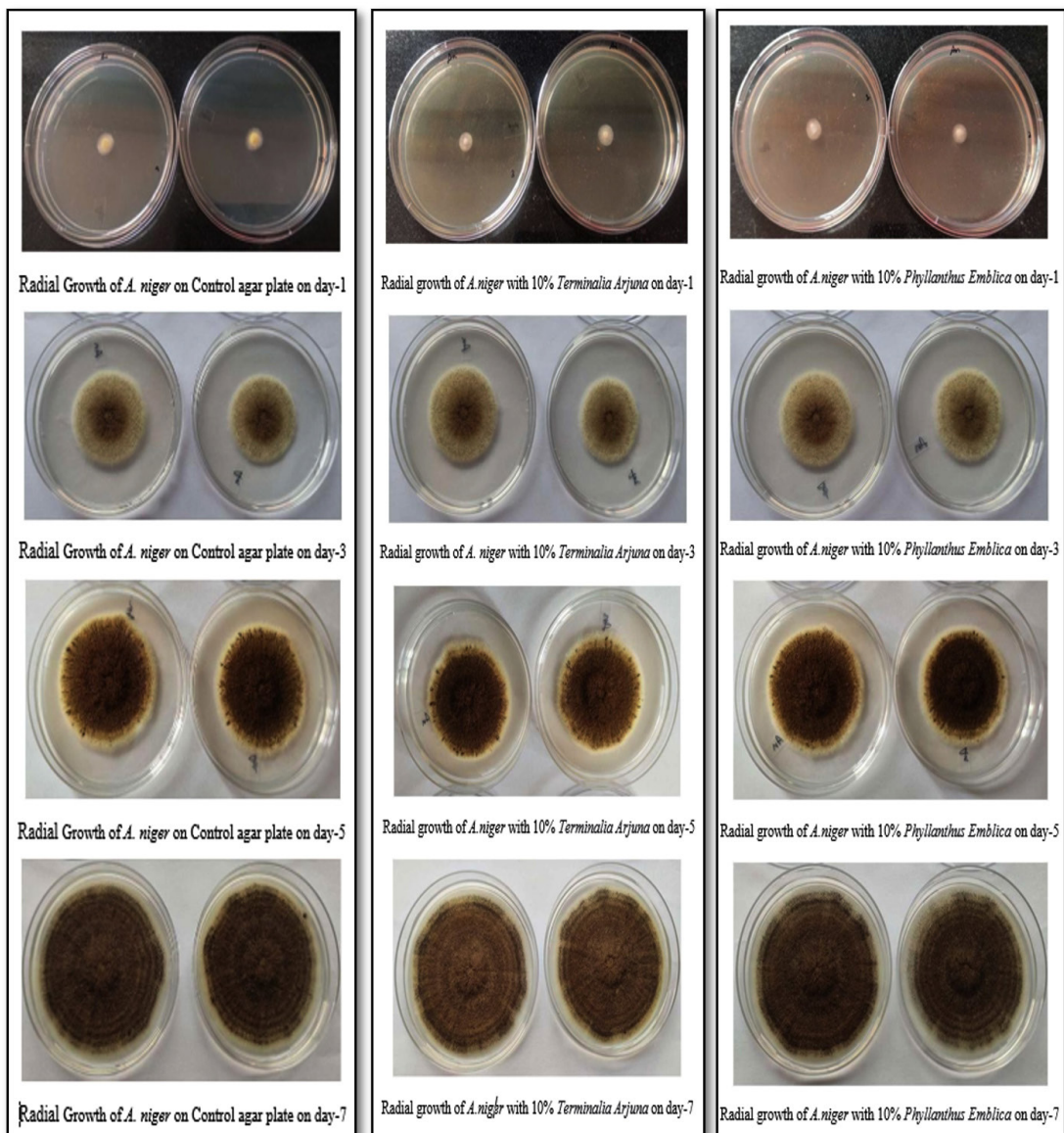


Figure 8. Radial growth of *A. niger*

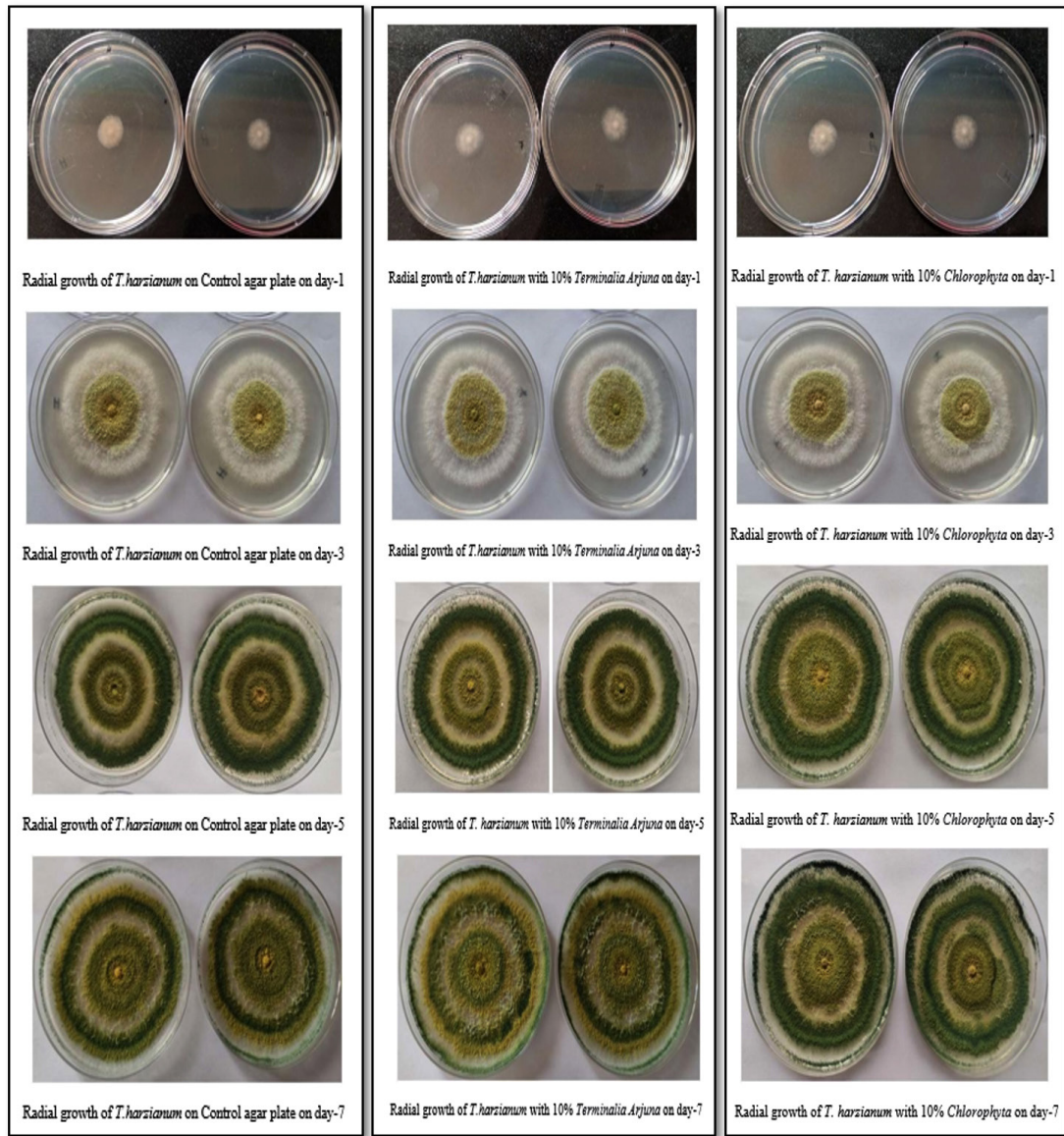


Figure 9. Radial growth of *T. harzianum*

maintaining specific levels of fungal growth may be desirable. Further investigation and validation of these findings are essential to fully exploit the pharmacological potential of medicinal plant extracts in specific applications.

CONCLUSION

The empirical exploration into the impact of medicinal plant extracts on the growth and sporulation of industrially significant fungal

strains yields valuable insights. Phytochemical analysis uncovers a diverse range of bioactive compounds, indicating potential applications in antimicrobial, antioxidant, and nutritional domains. Findings from the Point Inoculation Method and radial growth rates consistently indicate positive effects of *Terminalia Arjuna*, suggesting lower antifungal activity compared to alternative extracts. Sporulation indices confirm commendable sporulation in *Terminalia Arjuna*, contrasting with variable results in other extracts.

A comparative examination with previous studies underscores the distinctive attributes of *Terminalia Arjuna*. While not entirely devoid of antifungal properties, *Terminalia Arjuna* may exert a milder inhibitory effect on fungal growth, holding potential implications for industrial and medicinal purposes. Further research is essential to fully exploit its pharmacological potential.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

Both authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

This article does not contain any studies with human participants or animals performed by any of the authors.

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