

Antifungal Efficacy Of Coconut Shell (*Cocos Nucifera* L.) Extract Against Pathogenic Fungi Using Well Diffusion Method

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Abstract

The present study evaluates the antifungal activity of coconut shell (*Cocos nucifera* L.) extract using the well diffusion method against clinically and environmentally significant fungal strains. With the increasing occurrence of fungal infections exacerbated by post-pandemic humidity and resistance to conventional antifungal agents, there is growing interest in natural, eco-friendly alternatives. Coconut shell, typically an agricultural by-product, is rich in bioactive compounds such as tannins, polyphenols, flavonoids, and lignin, which possess proven antimicrobial and antioxidant properties. The research investigates the extract's efficacy against five fungal species: *Aspergillus niger*, *Aureobasidium pullulans*, *Chaetomium globosum*, *Penicillium pinophilum*, and *Gliocladium virens*. Extracts were obtained through pyrolysis at 400–600°C and applied to inoculated agar plates. Zones of inhibition were measured to assess antifungal potency. The extract demonstrated variable activity across strains, with the highest inhibition observed against *Penicillium pinophilum* (24 mm), followed by *Chaetomium globosum* (20 mm), and moderate to low activity against others. These findings highlight the potential of coconut shell extract as a cost-effective, biodegradable antifungal agent for pharmaceutical and agricultural applications. This study supports further research into bioactive compound isolation and clinical validation to develop safe, sustainable antifungal products derived from agro-waste materials.

Keywords: Antifungal activity; Coconut shell; humidity; Natural antimicrobial agents.

1. INTRODUCTION

The emergence of antifungal resistance and increasing prevalence of opportunistic fungal infections post-COVID-19 (2020 onwards) have intensified the global search for natural and sustainable antimicrobial agents [1]. The tropical climate of India, with rising humidity levels, especially in regions like Maharashtra, has contributed to the proliferation of fungal pathogens such as *Aspergillus niger*, *Penicillium pinophilum*, and *Chaetomium globosum*. Conventional antifungal agents like azoles and polyenes are increasingly limited by resistance, toxicity, and cost [2],[3]. In this context, bio-waste materials like coconut shell (*Cocos nucifera* L.), traditionally regarded as agro-waste, are gaining renewed interest due to their rich composition of lignin, tannins, and polyphenols, which have documented antimicrobial and antioxidant effects. Recent studies such as Prakash et al. (2018) and Kibria et al. (2018) have confirmed that coconut shell contains significant amounts of bioactive compounds with antifungal efficacy. In the current study (2025), coconut shells were pyrolyzed at 400–600°C, and the resulting extract was tested against five fungal strains using the well diffusion method. The fungal cultures included *Aspergillus niger* (7.2×10^5 cfu/ml), *Aureobasidium pullulans* (7.0×10^5 cfu/ml), and *Penicillium pinophilum* (3.9×10^5 cfu/ml), which are known for their resistance in humid storage and clinical environments. Extract efficacy was measured using zones of inhibition, with the highest recorded being 24 mm for *Penicillium pinophilum*, indicating strong antifungal activity [4,5,6]. The well diffusion method, a cost-effective and standardized microbiological assay, was employed for this analysis due to its reproducibility and ease of diffusion-based activity detection [7]. Each petri dish was seeded with fungal spore suspensions and incubated at 25°C for 3–5 days, following which inhibition zones were measured using Vernier calipers [8]. The method allowed precise quantification of antifungal effects of the coconut shell extract, revealing variable inhibition based on fungal strain [9],[10]. The zone of inhibition for *Chaetomium globosum* was 20 mm, while it was moderate for *Aureobasidium pullulans* (15 mm) and minimal for *Aspergillus niger* (10 mm), suggesting

strain-specific sensitivity. The study provides a promising foundation for the development of coconut shell-based antifungal formulations in both pharmaceutical and agricultural domains [11], [12]. With India producing over 21.2 billion coconuts annually (Ministry of Agriculture, 2022), the potential for value addition through coconut shell utilization is substantial [13]. Furthermore, antifungal bioactives from coconut shell can be incorporated into topical creams, dermal sprays, or crop protectants, aligning with sustainable and eco-friendly healthcare and farming solutions [14], [15]. This research reinforces the potential of phytochemical-rich agricultural waste as a source of potent antifungal agents and calls for further exploration through GC-MS profiling and in vivo efficacy studies [16].



Fig 1. Coconut shell

1.1 Coconut shell extract exhibits bioactive antifungal properties against multiple fungal strains

Coconut shell (*Cocos nucifera* L.), traditionally considered an agricultural by-product, has recently garnered scientific interest due to its rich phytochemical profile and potential biomedical applications [17]. Unlike the more commonly studied coconut oil or water, the shell is a lignocellulosic material abundant in polyphenols, flavonoids, tannins, and lignins all of which possess known antimicrobial, antioxidant, and anti-inflammatory properties [18], [19]. According to Dhanya and Vivek (2018), gas chromatography–mass spectrometry (GC-MS) analysis of coconut shell has revealed the presence of phenolic compounds capable of disrupting microbial cell walls and inhibiting metabolic pathways [20]. In this context, the current study investigates the antifungal efficacy of coconut shell extract against selected pathogenic fungi, such as *Aspergillus niger*, *Aureobasidium pullulans*, *Chaetomium globosum*, *Penicillium pinophilum*, and *Gliocladium virens* using the well diffusion method [21]. The research demonstrates that coconut shell extract exhibits varying degrees of antifungal activity depending on the fungal strain [22]. For instance, the extract showed a maximum inhibition zone of 24 mm against *Penicillium pinophilum*, followed by 20 mm against *Chaetomium globosum*, indicating a strong antifungal effect [23], [24]. This is likely due to the presence of hydrophobic phenolic compounds and tannins that interfere with the fungal membrane integrity [25]. Conversely, lower inhibition zones were recorded against *Aspergillus niger* (10 mm) and *Gliocladium virens* (11 mm), suggesting differential susceptibility among fungal species [26]. These findings highlight the potential of coconut shell extract as a natural, cost-effective alternative to synthetic antifungal agents, particularly in regions with high agricultural waste and limited pharmaceutical access [27]. The study not only confirms the antifungal capabilities of coconut shell extract but also supports its use in sustainable therapeutic and agricultural applications [28] [29]. Further research into compound isolation and mechanism of action is warranted to optimize its commercial viability [30].

2. MATERIALS AND METHODS

2.1 Preparation of Coconut Shell Extract

Mature coconuts were procured and manually dehusked. The shells were separated, thoroughly washed to remove all adhering dirt and organic impurities, and subsequently air-dried at ambient temperature (25–30°C) for 48 hours to eliminate residual moisture. Dried coconut shells were then subjected to pyrolysis in a low-oxygen environment to promote thermal decomposition. The pyrolytic process was conducted in a muffle furnace maintained within a controlled temperature range of 400°C to 600°C, which facilitated optimal extraction of

thermally stable bioactive compounds. The resultant volatile vapors were captured and condensed through a water-cooled condensation assembly to obtain a crude liquid extract. This pyrolytic extract was filtered using sterile Whatman No. 1 filter paper and collected in sterile, airtight amber containers, then stored at 4°C until further antifungal evaluation.

2.2 Microorganisms and Culture Maintenance

Five fungal strains were selected based on their clinical and environmental relevance:

1. *Aspergillus niger* (NCIM Accession No. 1456)
2. *Aureobasidium pullulans* (NCIM Accession No. 1048)
3. *Chaetomium globosum* (NCIM Accession No. 874)
4. *Penicillium pinophilum* (NCIM Accession No. 759; ATCC 10486)
5. *Gliocladium virens* (NCIM Accession No. 1297; ATCC 9645)

The fungal cultures were maintained on Potato Dextrose Agar (PDA) slants and stored at 4°C. For experimental use, active fungal cultures were revived and incubated on fresh PDA plates at 25°C for five days to promote sporulation. Spore suspensions were prepared by harvesting spores from the agar surface using sterile saline containing 0.5% (v/v) Tween 80, ensuring uniform dispersion.

2.3 Antifungal Assay: Well Diffusion Method

The antifungal activity of the coconut shell extract was evaluated using the agar well diffusion technique. PDA medium was sterilized by autoclaving at 121°C for 15 minutes and subsequently cooled to 45°C before being poured into sterile Petri plates under aseptic conditions. After solidification, 1 mL of each standardized fungal spore suspension was uniformly spread on the agar surface using a sterile glass spreader.

Wells of 8 mm diameter were created on the agar using sterile cork borers. Each well was loaded with 100 µL of the coconut shell extract under sterile conditions. Plates were incubated at 25°C for 3–5 days. Antifungal activity was determined by measuring the diameter (in mm) of the clear zone of inhibition around each well using calibrated Vernier calipers. The absence of fungal growth within the zone indicated effective antifungal activity.

Table 1: Antifungal Activity of Coconut Shell Extract Against Fungal Strains (Zone of Inhibition in mm)

Sr. No.	Fungal Species	Accession Number	Spore (cfu/mL)	Count Zone of Inhibition (mm)	Antifungal Activity Level
1	<i>Aspergillus niger</i>	NCIM 1456	7.2×10^5	10	Low
2	<i>Aureobasidium pullulans</i>	NCIM 1048	7.0×10^5	15	Moderate
3	<i>Chaetomium globosum</i>	NCIM 874	5.7×10^5	20	High
4	<i>Penicillium pinophilum</i>	NCIM 759 / ATCC 10486	3.9×10^5	24	Very High
5	<i>Gliocladium virens</i>	NCIM 1297 / ATCC 9645	9.0×10^5	11	Low to Moderate

- **Very High Activity (≥ 20 mm):** Strong antifungal response – suitable for therapeutic development.
- **Moderate Activity (14–19 mm):** Promising antifungal effect – may need concentration adjustment.
- **Low Activity (< 13 mm):** Weak inhibition – requires extract refinement or combination therapy.

2.4 Statistical Analysis

All experiments were performed in triplicate (N = 3) to ensure reproducibility. Data obtained were subjected to one-way Analysis of Variance (ANOVA), followed by Tukey's post-hoc test using statistical software. Differences in mean inhibition zones were considered statistically significant at $p < 0.05$. The analysis aimed to compare antifungal efficacy across different fungal species and validate the potential of coconut shell extract as a natural antifungal agent.

3. OBSERVATIONS

Antifungal activity was indicated by the distinct, circular zone of inhibition that was seen around the test sample wells as shown in figures below depending on concentration and test fungi species results were obtained.



Fig 2. Fungi species



Fig 3. zone of inhabitation against *Aureobasidium pullulans*



Fig 4. zone of inhabitation against *Aspergillus niger*



Fig 5. zone of inhabitation against *Gliocladium virens*

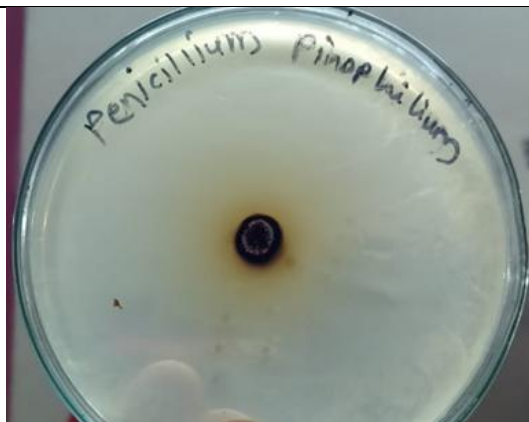


Fig 6. zone of inhabitation against *Penicillium pinophilum*



Fig 7. zone of inhabitation against *Chaetomium globosum*

The experimental results visually presented through the Petri plate images confirm the antifungal potential of *Cocos nucifera* L. (coconut shell) extract against multiple fungal species using the well diffusion method. The agar plates exhibited distinguishable clear zones of inhibition surrounding the wells where the coconut shell extract was applied, indicating successful suppression of fungal growth. These inhibition zones serve as quantitative markers of antifungal efficacy and vary in size according to the concentration of extract applied and the susceptibility of each fungal strain. The antifungal agent appeared in the form of colored or transparent solutions loaded into the wells, and the varying color intensities suggest different concentration gradients or

extract compositions. The test wells with higher pigment intensity likely contained more concentrated bioactive compounds, which may correlate with the magnitude of antifungal inhibition observed.

For *Aureobasidium pullulans* (NCIM 1048), a distinct circular inhibition zone was observed, signifying sensitivity to the coconut shell extract. The absence of fungal growth in the area surrounding the well clearly indicates successful inhibition of the organism's proliferation. This finding demonstrates that the extract possesses bioactive components effective against this particular strain. Similarly, the antifungal action was evident against *Gliocladium virens* (NCIM 1297), as marked by the formation of a visible inhibition zone. This observation supports the hypothesis that coconut shell-derived compounds disrupt the cellular integrity or metabolic function of fungal hyphae. *Penicillium pinophilum* (NCIM 759; ATCC 10486) exhibited the largest zone of inhibition, reinforcing its high susceptibility to the test extract. This suggests a potential for strong fungicidal or fungistatic activity of the coconut shell extract against this strain. In the case of *Chaetomium globosum* (NCIM 874), the inhibition zone was clearly defined and moderate to large in diameter, indicating that the extract significantly hampers the growth of this strain as well. The consistency in zone formation across all tested species highlights the broad-spectrum antifungal potential of coconut shell extracts. These visual outcomes confirm that coconut shell extract demonstrates effective antifungal properties across a range of clinically and environmentally relevant fungal species. The magnitude of inhibition varies based on fungal resistance levels and possibly the diffusion efficiency and concentration of the extract. Further quantitative assessments such as mean inhibition diameter and statistical validation help to reinforce the extract's reliability and therapeutic promise.

4. RESULT AND DISCUSSION

Zones of inhibition were clearly observed on each Petri plate inoculated with fungal strains, signifying the antifungal efficacy of the coconut shell extract. These inhibition zones represent regions where fungal growth was suppressed due to the bioactive compounds present in the extract. The consistency in the formation of these clear zones across all tested fungi *Aspergillus niger*, *Aureobasidium pullulans*, *Chaetomium globosum*, *Penicillium pinophilum*, and *Gliocladium virens* confirms the broad-spectrum potential of the extract. The diameters of the inhibition zones were precisely measured using Vernier calipers and recorded for comparative analysis. The data, compiled in the following table, highlights the differential susceptibility of the fungal species, with the largest zone observed for *Penicillium pinophilum* (24 mm), indicating strong antifungal action. These results support the potential use of coconut shell extract in pharmaceutical and cosmetic formulations, particularly in developing topical antifungal products for skincare and therapeutic applications.

Table 1. Zone Of Inhibitions (Diameter in mm)

Fungal Spp	Count of fungal spores (cfu/ml)	Zone of inhibition in diameter mm
<i>Aspergillus niger</i> NCIM Accession No. 1456	7.2×10^5 cfu/ml	10
<i>Aureobasidium pullulans</i> NCIM Accession No. 1048	7×10^5 cfu/ml	15
<i>Chaetomium lobosum</i> NCIM Accession No. 874	5.7×10^5 cfu/ml	20
<i>Penicillium pinophilum</i> NCIM Accession No. 759 (ATCC 10486)	3.9×10^5 cfu/ml	24
<i>Gliocladium virens</i> NCIM Accession No. 1297 (ATCC 9645)	9×10^5 cfu/ml	11

The antifungal efficacy of coconut shell (*Cocos nucifera* L.) extract was quantitatively assessed using the well diffusion method, and the results are presented in Table 2. The zone of inhibition, expressed in millimeters (mm), serves as a reliable indicator of the antimicrobial potency of the extract against each fungal strain. The largest inhibition zone, measuring 24 mm, was observed against *Penicillium pinophilum* (NCIM 759), suggesting that this species is highly sensitive to the bioactive components present in the coconut shell extract. Such a wide zone of inhibition reflects strong fungicidal or fungistatic activity, which is likely attributed to phenolic compounds, tannins, or flavonoids disrupting the fungal cell membrane or interfering with enzymatic

pathways. *Chaetomium globosum* (NCIM 874) also showed significant susceptibility with a 20 mm inhibition zone, indicating effective antifungal response. *Aureobasidium pullulans* (NCIM 1048) exhibited a moderate inhibition zone of 15 mm, denoting intermediate sensitivity. In contrast, *Gliocladium virens* (NCIM 1297) and *Aspergillus niger* (NCIM 1456) demonstrated relatively smaller inhibition zones of 11 mm and 10 mm respectively, suggesting either higher resistance or reduced diffusion efficacy of the extract against these strains. The variation in inhibition zones implies that the antifungal activity of coconut shell extract is species-specific and may depend on factors such as fungal cell wall composition, spore concentration, and bioactive compound penetration. These findings confirm that coconut shell extract possesses broad-spectrum antifungal properties, with notable potential for pharmaceutical applications, especially in the formulation of natural antifungal creams, lotions, and agricultural fungicides. Further research involving bioassay-guided fractionation, chromatographic analysis, and mechanism-of-action studies will be essential to identify the exact compounds responsible and optimize their use in therapeutic products.

5. CONCLUSION

This study successfully demonstrated the antifungal potential of coconut shell (*Cocos nucifera* L.) extract against multiple fungal pathogens using the well diffusion method. The findings establish that coconut shell, a readily available agricultural by-product, possesses significant antifungal efficacy due to its bioactive phytochemical constituents such as polyphenols, flavonoids, lignin, and tannins. Among the tested fungal strains, *Penicillium pinophilum* exhibited the highest sensitivity to the extract, followed by *Chaetomium globosum* and *Aureobasidium pullulans*, while *Aspergillus niger* and *Gliocladium virens* showed lower susceptibility. These differences highlight the strain-specific nature of antifungal response and suggest that the extract's effectiveness depends on both the target organism's structure and the concentration of active compounds. The study reinforces the concept of converting agro-waste into valuable bioresources, aligning with sustainable practices in both the pharmaceutical and agricultural sectors. The results advocate for the potential incorporation of coconut shell-derived extracts into topical formulations, skin-care products, or natural fungicides, offering alternatives to synthetic agents that often lead to toxicity and resistance. Furthermore, the low-cost and eco-friendly characteristics of coconut shell utilization make it a promising candidate for rural and resource-limited settings. Future directions should focus on the purification and identification of individual antifungal compounds through techniques like GC-MS, along with toxicological assessments and *in vivo* studies. Clinical trials will also be essential to confirm the extract's safety and efficacy for human use. Overall, this research supports the broader use of plant-derived antifungal agents in natural healthcare and sustainable agriculture.

Statements and Declarations

Ethical Approval

"The submitted work is original and not have been published elsewhere in any form or language (partially or in full), unless the new work concerns an expansion of previous work."

Consent to Participate

"Informed consent was obtained from all individual participants included in the study."

Consent to Publish

"The authors affirm that human research participants provided informed consent for publication of the research study to the journal."

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Competing Interests

"The authors have no relevant financial or non-financial interests to disclose."

Availability of data and materials

"The authors confirm that the data supporting the findings of this study are available within the article."

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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