Journal of Neonatal Surgery
ISSN(Online): 2226-0439
Vol. 14, Issue 8 (2025)
https://www.jneonatalsurg.com

OPEN ACCESS

Evaluation Of Antifungal Activity of Coconut Shell (Cocos Nucifera L.) Using Well Diffusion Method

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Cite this paper as: Ms. Sailee Naresh Gund, Dr. Sonali Vaibhav Dhamal, Dr. Sachin S. Chavan, (2025) Evaluation Of Antifungal Activity of Coconut Shell (Cocos Nucifera L.) Using Well Diffusion Method. *Journal of Neonatal Surgery*, 14 (8), 757-763.

ABSTRACT

After the pandemic, there has been increased interest in finding alternative sources and traditional methods for improving immunity, as well as promoting good mental and physical health. Fungal infections are becoming more common due to rising temperatures and humidity levels. Recently, herbal products have gained attention in the cosmetics industry due to their natural origins, minimal side effects, and biodegradability. These herbal products extend beyond cosmetics and have applications in pharmaceuticals as well. Coconut is one of the standout options, demonstrating excellent results due to its eco-friendliness and versatile applications. Despite often being considered waste, coconut shells have substantial potential as a raw material for herbal goods. Rich in nutrients, coconut shells are recognized for their Ayurvedic medicinal uses. They contain beneficial compounds, such as activated carbon and various detoxifying agents. This study aims to analyze the antifungal properties of coconut shells with respect to various fungi. Antifungal activities are essential for preventing and treating fungal infections in humans, animals, and plants.

Keywords: *Antifungal properties, Coconut shell, herbal, humidity, biodegradable.*

1. INTRODUCTION

The COVID-19 pandemic has heightened global awareness of immunity, medical care, and preventive measures within healthcare. It has highlighted the need for effective antifungal, antibacterial, and antiviral agents, leading to increased research into naturally occurring compounds for anti-infective purposes. India, with its rich biodiversity, has integrated both modern healthcare and traditional medicine into its approach. Medicinal plants used in Ayurveda, Siddha, and other indigenous systems are being studied scientifically to evaluate their therapeutic potential. However, there are gaps in standardized scientific methodologies for assessing the viability of herbal remedies. Coconut (Cocos nucifera L.) is an underutilized natural resource known as "kalpavruksha," or "wish-fulfilling tree," in ancient Indian texts. The coconut has various by-products and wastes that are utilized across different industries. For instance, the coconut shell, which is often regarded as waste, is helpful in the textile and handicraft industries, yet its therapeutic benefits remain largely unexplored. Fungal infections continue to pose a public health challenge, especially in tropical and humid climates that facilitate fungal growth. These infections range from superficial dermal issues to serious, life-threatening systemic conditions that require antifungal treatment. The well diffusion method is a widely accepted laboratory technique used to assess the antimicrobial efficacy of natural compounds, including plant materials. This method will be employed to investigate the antifungal efficacy of coconut shell extracts, determining whether they could serve as a natural antifungal treatment. Identifying the antifungal properties of coconut shell extracts could provide a potential, cost-effective treatment option that is environmentally friendly and readily available for use.

The coconut (Cocos nucifera L.) is a versatile plant used worldwide for both nutritional and industrial purposes. Although the coconut shell is typically regarded as an agricultural by-product, it holds potential biomedical applications due to its bioactive compounds. Phytochemical studies have shown that coconut shell contains polyphenols, flavonoids, tannins, and lignins, all of which exhibit antimicrobial, antioxidant, and anti-inflammatory properties. These bioactive components may help inhibit fungal pathogens through various mechanisms, such as disrupting the fungal cell wall, inhibiting spore germination, and interfering with fungal metabolic processes. Several fungal pathogens, including Candida spp., Aspergillus spp., and dermatophytes, can cause serious infections in humans, particularly in immunocompromised individuals. Current traditional antifungal therapies include azoles, polyenes, and echinocandins. While these treatments are effective, they are often accompanied by issues such as the development of resistance, toxic side effects, and high treatment costs. Therefore, research into alternative antifungal agents derived from plant-based bioactive compounds is necessary. Through extraction and characterization, compounds derived from coconut shell can provide valuable insights into their mechanisms of action and may ultimately be developed into pharmaceutical products.

The well diffusion method is a widely used technique for assessing the antimicrobial activity of natural extracts against microbial pathogens. This method involves the diffusion of test compounds through an agar medium, leading to the formation of inhibition zones that indicate the antimicrobial effectiveness of a substance. The well diffusion method is cost-effective, reproducible, and reliable for screening antifungal activity against various microbial pathogens. In this study, we will assess the antifungal activity of coconut shell extracts using the well diffusion assay against selected fungal strains. The diameter of the inhibition zones will be measured to quantify the antifungal activity and facilitate comparative analyses with standard antifungal compounds. Several parameters can influence the effectiveness of the well diffusion method, including extract concentration, solvent polarity, diffusion capacity, and the susceptibility of fungal strains. Therefore, optimizing these parameters is crucial for obtaining accurate and reproducible results. The findings from this study will help identify potential active components responsible for antifungal activity and contribute to further pharmacological studies and their applications as therapeutic agents.

Fig 1. Coconut shell

Research into the use of coconut shells as a natural antifungal agent has significant implications for both the pharmaceutical and agricultural sectors. Natural antifungal agents derived from coconut shell materials could be developed into topical treatments for skin infections, foot fungi, and other dermatological conditions. Additionally, bioactive compounds extracted from coconut shells may be utilized in agriculture to create biocompatible antifungal coatings for crops, helping to reduce fungal spoilage and enhance food security. Future research should focus on isolating and characterizing specific bioactive compounds found in coconut shell extracts. Advanced techniques such as gas chromatography-mass spectrometry (GC-MS) and high-performance liquid chromatography (HPLC) could be employed to identify and quantify these bioactive constituents. Furthermore, in vivo studies and clinical trials will be essential to establish the safety, efficacy, and any potential side effects of using coconut shell in antifungal formulations. Integrating antifungal agents derived from coconut shells into conventional medicine could provide a sustainable and cost-effective alternative to synthetic antifungal drugs, which would particularly benefit populations with limited access to healthcare. This study aims to evaluate the experimental antifungal activity of an extract from coconut shells, prepared using the well diffusion method. The results will contribute to the expanding body of knowledge about plant-based antifungal agents and support the development of affordable and effective natural treatments for fungal diseases. The potential of coconut shells as a bioactive resource underscores the importance of sustainably managing and utilizing agricultural waste for both medical and industrial applications.

2. MATERIALS AND METHODS

The mature coconuts were collected, and the shells were thoroughly cleaned to remove all dirt and organic residues. The shells were allowed to air dry to remove excess moisture. The dried coconut shells were subjected to a pyrolytic process in a low-oxygen environment to chemically decompose the shells. Pyrolysis was carried in a controlled temperature range of 400-600°C to ensure good extraction of bioactive compounds. The vapor from the pyrolysis was cooled using a condensation system to produce a liquid extract. The liquid extract was then filtered using sterile Whatman No. 1 filter paper and stored in an airtight container at 4° C for antifungal testing.

Microorganisms and Culture Preparation

For evaluating the antifungal efficacy of the coconut shell extract, five fungal species were selected based on their pathogenic significance and common occurrence. The fungal species used in this study were:

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

Fungal strain stock cultures were preserved in Potato Dextrose Agar (PDA) slants at 4°C. For testing, fungal spores were obtained by spreading the stock cultures onto pre-prepared PDA plates. These cultures were incubated at 25°C for five days to promote mycelial growth and spore development. The spores were harvested into a spore suspension, which was created by resuspending the spores in a sterile saline solution containing 0.5% Tween 80 to ensure uniformity. The antifungal activity of coconut shell extract was assessed using the well diffusion method, a widely used technique in microbiological research for testing plant extracts, drugs, and other bioactive materials due to its simplicity and reproducibility. The growth medium for the fungal cultures consisted of the aforementioned PDA medium, which was sterilized at 121°C for 15 minutes. After sterilization, it was allowed to cool to approximately 45°C before being poured into sterile Petri dishes under aseptic conditions. Once the medium solidified, 1 mL of a standardized fungal spore suspension was evenly spread on each plate using a sterile spreader.

Sterile cork borers (8 mm) were used to create wells in the agar plates. A volume of 100 µL of coconut shell extract was then aseptically added to each well. The plates were incubated at 25°C for 3 to 5 days to allow the fungal cultures to grow in the presence of the extract. After the incubation period, antifungal activity was assessed by measuring the diameter of the zone of inhibition around each well using Vernier calipers. The presence of a clear zone of inhibition with no fungal growth indicated that the extract was effective against the respective fungal strain. Each experiment was repeated three times (N=3) to ensure the reliability and reproducibility of the results. The antifungal activity was analyzed by comparing the mean zones of inhibition for each fungal species. Data collected from the antifungal activity assays were statistically analyzed using one-way analysis of variance (ANOVA). Mean values were compared with Tukey's post-hoc test at a significance level of $p < 0.05$. Statistical software was used to analyze the data and identify significant differences in antifungal activity among the tested fungal species. The purpose of this analysis was to establish the antifungal potential of coconut shell extract and explore its potential application in controlling fungal pathogens.

3. OBSERVATIONS

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

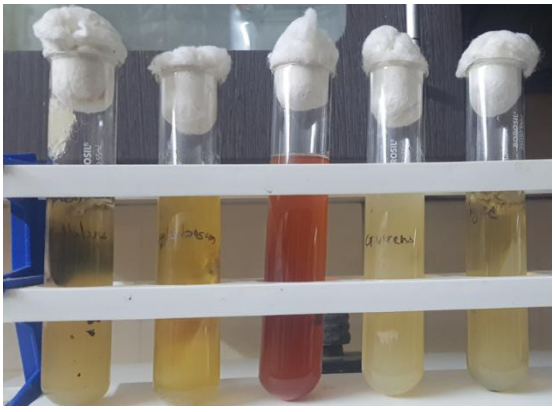

Fig 2. Fungi species

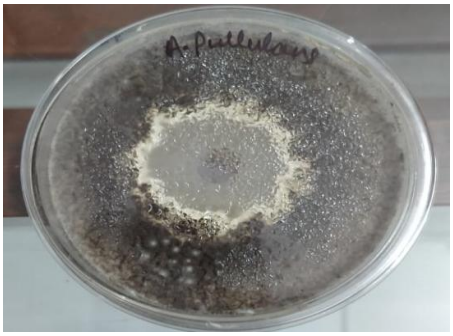

Fig 3. Zone of inhabitation against *Aureobasidium pullulans*

Fig 4. Zone of inhabitation against *Aspergillus niger*

Fig 5. Zone of inhabitation against *Gliocladium virens*

Fig 6. Zone of inhabitation against *Penicillium pinophillum*

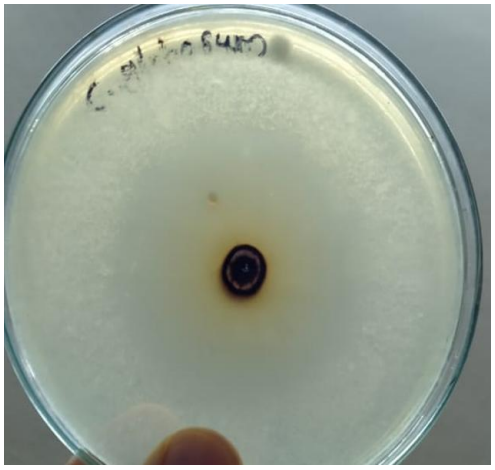

Fig 7. Zone of inhabitation against *Chaetomium globosum*

The provided figures appear to illustrate a study on the antifungal activity of coconut shell (Cocos nucifera L.) against various fungal species, utilizing a well diffusion method. Here is a clearer interpretation of the observations based on the figures: The test tubes display different levels of fungal growth along with the solutions used to assess antifungal activity. The varying colors of the solutions in the tubes suggest that the antifungal agent was present in different concentrations. There seems to be a correlation between concentration and antifungal effectiveness. The clear circular zones of inhibition—areas around the wells where no fungal growth occurred—likely indicate the antifungal activity of the agents tested against the fungi in the figures. One of the figures shows the growth of Aureobasidium pullulans in a petri dish. The clear zone surrounding the area where the antifungal solution was applied (presumably from the well) suggests that the fungal growth was inhibited. This indicates that the coconut shell extract or solution has antifungal properties against Aureobasidium pullulans. Another figure demonstrates a similar clear circular area surrounding the well, further confirming that the coconut shell extract exhibits antifungal activity. Overall, these observations suggest that coconut shell extract possesses measurable antifungal properties.

This figure illustrates a zone of inhibition around the well where the antifungal agent was applied, indicating that Gliocladium virens was inhibited by the coconut shell extract. The image also shows that the well inhibited the growth of Penicillium pinophilum, as evidenced by the surrounding zone of inhibition. Lastly, the figure demonstrates the antifungal effects of the coconut shell extract on Chaetomium globosum, again shown by the presence of a zone of inhibition. These results suggest that the extract has the potential to control or inhibit the growth of these fungal species. The consistent presence of a zone of inhibition across all figures indicates that coconut shell extract exhibits antifungal activity against multiple fungal species. The size of the zone of inhibition may vary based on the concentration of the extract used or the specific fungal species. This variation could be confirmed by measuring the diameter of each zone in relation to the concentration of the antifungal agent.

4. RESULT AND DISCUSSION

Zones of inhibition were observed on each plate where fungi were inoculated. The results indicate that this extract possesses antifungal properties, which may be beneficial for skincare and pharmaceuticals. The zones of inhibition were measured and are presented in the following table.

Table 1. Zone Of Inhibitions (Diameter in mm)

Fungal Species	**Count of fungal spores (cfu/ml)**	**Zone of inhibition in diameter mm**
Aspergillusniger NCIM Accession No. 1456	7.2 X 10^5 cfu/ml	10
Aureobasidium pullulans NCIM Accession No. 1048	7 X 10^5 cfu/ml	15
Chaetomium lobosum NCIM Accession No. 874	5.7 X 10^5 cfu/ml	20

Penicilliumpinophillum NCIM Accession No. 759 (ATCC 10486)	3.9 X 10^5 cfu/ml	24
Gliocladium virens NCIM Accession No. 1297 (ATCC 9645)	9 X 10^5 cfu/ml	11

Using the well diffusion method, we assessed the antifungal activity of coconut shell extract, as summarized in Table 1. The measurements of inhibition zones (expressed in mm) demonstrate varying antifungal effects of the extract on different fungal species. The largest inhibition zone was recorded for Penicillium pinophilum (NCIM Accession No. 759), measuring 24 mm, indicating that the coconut shell extract has strong antifungal activity. Chaetomium lobosum (NCIM Accession No. 874) exhibited an inhibition zone of 20 mm, also falling into the strong antifungal category. Aureobasidium pullulans (NCIM Accession No. 1048) showed intermediate susceptibility to the extract, with an inhibition zone of 15 mm. In contrast, Gliocladium virens (NCIM Accession No. 1297) and Aspergillus niger (NCIM Accession No. 1456) exhibited less sensitivity, with inhibition zones of 11 mm and 10 mm, respectively. The varying levels of antifungal activity observed suggest that coconut shell extract may contain bioactive compounds that target different fungal species in unique ways. These findings support the potential of coconut shell extract as a natural antifungal product that could be used in pharmaceutical and cosmetic applications to combat fungal infections. Future studies will focus on isolating and characterizing the active compounds responsible for this antifungal activity and exploring their mode of action.

5. CONCLUSION

The research study investigates the antifungal properties of coconut shell (Cocos nucifera L.) using the well diffusion method, demonstrating its potential as a natural and cost-effective antifungal agent. Coconut shells, often regarded as agricultural waste, contain bioactive compounds such as polyphenols, flavonoids, tannins, and lignins, which exhibit antimicrobial, antioxidant, and anti-inflammatory properties. These bioactive compounds can inhibit fungal growth, making coconut shell extracts a viable alternative to synthetic antifungal agents that can lead to issues of resistance and toxicity. This study evaluated the effects of coconut shell extracts on five fungal species: Aspergillus niger, Aureobasidium pullulans, Chaetomium globosum, Penicillium pinophilum, and Gliocladium virens, by measuring the inhibition zones formed around the wells containing the extracts. The findings indicated varying degrees of antifungal activity; the highest activity was noted against Penicillium pinophilum, with a 24 mm zone of inhibition, followed by Chaetomium globosum at 20 mm and Aureobasidium pullulans at 15 mm. The extract exhibited lower activity against Aspergillus niger and Gliocladium virens, with inhibition zones measuring 10 mm and 11 mm, respectively. This research underscores the potential of coconut shell as a valuable source of bioactive compounds for pharmaceutical and agricultural applications. Extracting and characterizing specific bioactive components may lead to the development of antifungal formulations based on coconut shell, which could be used in consumer skincare products, medicinal products, or agricultural applications. The positive results of this study support the sustainable use of agricultural by-products in medicine and industry, providing greener and more affordable alternatives to chemical antifungals. Future research could refine extraction methods and incorporate clinical trials to evaluate the safety, efficacy, and broader applications of these bioactive antifungals.

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