

CHEMICAL CHARACTERIZATION AND ANTIMICROBIAL ACTIVITY OF COCONUT SHELL EXTRACT

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Abstract

India is a country with a vast range of trees and medicinal plants, which vary depending on the environment and geographic location. However, their potential uses in biomedical and other fields have been overlooked due to the lack of applicability analyses, appropriate and targeted development, and compositional analyses. Coconut and cashew plants are cultivated on approximately 2.14 million hectares of land in India, producing 11.7 million tons of agricultural material annually. Certain byproducts derived from these plants have antifungal and antibacterial properties, which could offer a natural solution for situations where chemical components are required. Fungal infections are more common in humid, hot weather, and these plant byproducts can be used as an antibacterial gel or oil for medical purposes. To extract these elements and achieve the required purity while preserving their properties, an appropriate system must be established. Because microbial illnesses are becoming more common, especially in tropical regions, these agro-waste products provide a biocompatible and sustainable substitute for synthetic antibacterial drugs. This study examines the compositional features, method of extraction, and antibacterial activity of derivatives from coconut shells. The creation of standardized pharmaceutical formulations is prioritized in order to maximize therapeutic potential while maintaining bioactivity. The results point out how these natural byproducts can be used to make biomedical goods that are safe, environmentally friendly, and efficient.

Keywords: Antibacterial gel; Antifungal properties; Biomedicine; Biowaste; Plant Byproducts

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Introduction

Depending on the environment and geographical location, INDIA has a vast range of fruits, vegetables, and other edibles. Large varieties of naturally occurring plants and components with significant therapeutic significance result from this. In the absence of applicability analysis, proper and targeted development and compositional assessments these products are neglected for their biomedical and other applications. (Adelere *et al*, 2020).

In developing nations like India, access to high-quality medical care is easier in urban than in rural areas. However, there are disadvantages to utilizing synthetic medications, such as the possibility of allergies and other unwanted effects. Nonetheless, people in rural areas frequently turn to the readily accessible natural healing resources in their particular area (Umaru *et al.*, 2023).

Hot and humid weather appears to be more conducive to fungal infections. special kinds of oils which can be extracted from plants such as COCONUT can be used for antifungal and microbial applications. This would provide a natural remedy for applications where we are dependent upon chemical materials (Gowthaman & Thangavel, 2022).

In the Indian economy, the agriculture industry occupies a major role among other industries. In terms of employment, the agriculture industry provides a living for about 151 million people. Infections of the skin, hair, or nails, including ringworm, eczema, and other common fungal infections, have become an increasing healthcare concern in India within the last ten years. On the other hand, we can state that: In India, coconut plants are grown on about 2.14 million hectares of land, producing 11.7 million tons of crop material annually (Govardhan *et al.*, 2023). These plants waste which are shown in Figure 1 possess antifungal or therapeutic qualities.



Figure 1. Agro-industrial waste

The extracts were prepared via Soxhlet extraction and subjected to phytochemical screening to identify key bioactive compounds. The agar disc diffusion method was used to assess the antibacterial activity against specific bacterial

strains (*Staphylococcus aureus*). Strong antifungal activity was shown by the data, which showed noticeable zones of inhibition, especially in the coconut shell methanol extracts (Balouiri, 2016). Preliminary compositional analysis done by GCMS and LCMS confirmed the presence of phenolics, flavonoids, and tannins, which are likely responsible for the observed bioactivity. The kingdom of plants is an incredible source of possible medications, and the value of medicinal plants has been receiving attention in recent years (Bezerra & Koblit, 2025). Plant-based medications are widely accessible, reasonably priced, effective, safe, and few adverse effects. The most obvious option for studying the current need for therapeutically effective novel medications, such as anticancer drugs, antibacterial drugs, and antihepatotoxic compounds, is to look at the plants that have been selected for medicinal use over thousands of years. According to the World Health Organization (WHO), medicinal plants are the best source of a broad variety of pharmaceuticals. In wealthy countries, nearly eighty percent of the population uses traditional medicines, which are composed of compounds derived from medicinal plants (Esquenazi *et al.*, 2002).

Methodology

Oil will be extracted from the Agro-industrial waste. For this condensation method will be used. First cleaning will be done after this these shells are kept in closed chamber and heating is given to the chamber. one storage pot is kept at bottom for oil collection. As heat is given temperature will be increased and oil will be extracted from shell and collected in collector. This collected oil is further used with evaporation method for removal other material such as water vapors. Once oil is extracted analysis of its compositions is done. After the analysis is done suitable base material is added into it for and gel formation. Some additives will be added into it for enhancement of its properties and applications in cosmetics and creams (Rangari *et al.*, 2017).

As shown in Figure 2, Once the cleaning process, the coconut shells were crushed into little bits and allowed to dry in the sun. After that, the material was pyrolyzed in a closed furnace with oxygen limitation at 300-600°C. A water-cooled condenser was used to condense the resultant vapors, creating a liquid mixture of water, tar, and bio-oil. A separating funnel was used to remove the bio-oil layer (coconut shell oil), and filtration was used to purify it (Sarkar & Wang, 2020).

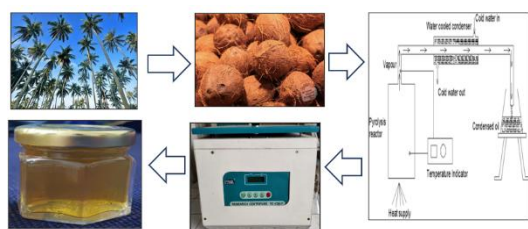


Figure 2. Synthesis Process

Characterization and Identification

Some basic phytochemical tests can be used to analyze extracted oils. Mass spectroscopy can be used for qualitative analysis and the identification of chemicals contained in oils. Samples in any state, such as solid, liquid, or gaseous, are subjected to Gas Chromatography-Mass Spectroscopy (GCMS). While liquid chromatography mass spectroscopy (LCMS) is used for separation, identification, quantification, and the investigation of chemical and structural features, it is also frequently used for monitoring, drug delivery systems, and purity analysis (Shiny & Remadevi, 2014).

A complete view of the phytochemical complexity of coconut shell extract is given by the combination of GC-MS and LC-MS investigations. LC-MS found bioactive polyphenols with known intracellular targets, whereas GC-MS identified hydrophobic membrane-active substances.

1. Gas Chromatography-Mass Spectroscopy (GCMS)

The chemical components of a sample combination can be isolated and their presence or absence, as well as their quantity, can be determined by using GC, a separation science technique. GC detectors provide limited information, usually in two dimensions, containing the response of the detector and the retention time of the analytical column. Identification is the process of comparing the length of time that peaks remain in a sample to standards of known substances that are analysed with the same method (Dhanya *et al.*, 2018).

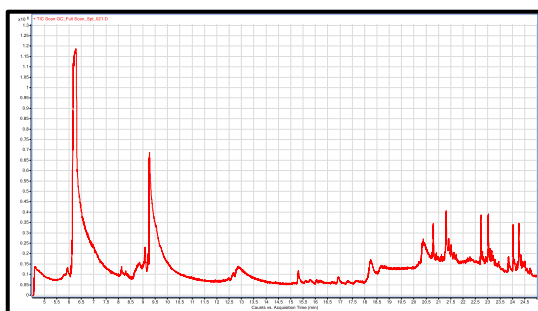


Figure 3. GC-MS full scan Spectra

Results were obtained by analysing the compounds which are present in the essential oils extracted from agro-industrial waste. Eighteen chemicals were identified as major ingredients by the chromatogram as shown in Figure 3. These substances were recognized using the peak region and RT (Moraes *et al.*, 2017). The research identified the following components to be present.

The phytochemical components of the *Cocos nucifera* Linn were examined utilizing GCMS analysis in the current experiment. The components of the dried, ripe coconut shell (*Cocos nucifera* Linn) that were identified are tabulated in the Table 1.

Table 1. compounds identified from chromatogram

Sr. No.	Retention time	Peak	Name
1	5.919	95	ETHANONE
2	6.177	94	PHENOL
3	8.127	57	CARBONIC ACID, OCTADECYL ESTER
4	12.642	124	3-AMINOMETHYL-3-5-5-TRIMETHYL HEXANOL
5	12.867	138	2-METHOXY-6 METHYL PHENOL
6	15.299	71	DI-METHANOL
7	15.599	123	CYCLOHEXANOL,5-METHYL-2-IMETHYL ACETATE
8	15.790	109	METHANOL,O-ISOBUTENYRYL
9	16.913	85	2-OCTAN-1-OL,3-7-DIMETHYL,ISOBUTYRATE
10	17.324	152	5-T-BUTYL-HEXA-3,5-DIEN-2-ONE
11	17.696	122	2-ISOPROPYLIDENE-5-METHYLHE-4-ENAL
12	20.367	154	FORMIC ACID,2,6-DIAMETHOXY PHENYL ESTER
13	20.777	168	EICOSANE
14	21.297	69	GERANANYL ISOVALERATE
15	22.701	99	TETRADECANE
16	22.905	113	BICYCLO, HEXANE-6—CARBOXYLIC ACID
17	23.824	149	PHYTHALIC ACID, BUTYL TETRADECYL ESTER
18	24.244	167	HEPTACOSANE

Long-chain alkanes (e.g., eicosane, Heptacosane), esters (e.g., phthalic acid and carbonic acid esters), phenolic compounds (e.g., phenol, methoxy-phenol), and different alcohols were among the 18 bioactive compounds found by the GC-MS study. These substances are well-known for their aromatic, antibacterial, hydrophobic, and antioxidant qualities. Esters imply industrial use as plasticizers or additives, while alkanes suggest a protective, wax-like function. Alcoholic and phenolic components suggest possible applications in food preservation, cosmetics, and medications (Shirahigue & Ceccato-Antonini, 2020).

Among all compounds eight major compounds having medicinal activities. The activities that are identified and available for the components are shown in Table 2.

2. Liquid chromatography mass spectroscopy (LC-MS)

A powerful analytical technique, liquid chromatography-mass spectrometry (LC-MS) can be used to separate, identify, and quantify known and unknown substances as well as provide the

structure and chemical characteristics of different molecules. It separates materials based on their interactions with the liquid phase solvent and the solid particles in a closely packed column (Fardhyanti & Damayanti, 2017).

The graphs below display the coconut shell oil's LC-MS chromatogram, while Figure (4-12) displays the mass spectrum of the chemicals found. It was noted that the various peaks were acquired at various retention times.

Table 2. Identified activities of components from GCMS chromatogram

Name Of Compound	Molecular Formula	Molecular Weight	Biological Activity
PHENOL	C ₆ H ₅ O ₂	94.11	ANTI-INFLAMMATORY,ANTI-AGAING,ANTI-BACTERIAL,ANTI-VIRAL
CARBONIC ACID, OCTADECYL ESTER	H ₂ CO ₃ C ₃₆ H ₇₂ O ₂	62.03 536.95	ANTIINFLAMMATORY IMPROVING SKIN HEALTH
METHANOL	CH ₃ OH	32.04	USED IN PERFUMES,RESINS AND PHARMACEUTICALS
FORMIC ACID	HCOOH	46.03	USED IN PHARMACEUTICS
TETRADECANE	C ₁₄ H ₃₀	198.39	ANTIBACTERIAL,ANTIFUNGAL
HEPTACOSANE	C ₂₇ H ₅₆	380.7	ANTIBACTERIAL,ANTIFUNGAL
PHATALIC ACID	C ₈ H ₆ O ₄	166.14	ANTIMICROBIAL
PHENYL ESTER	C ₈ H ₈ O ₂	136.15	ANTIINFLAMMATORY

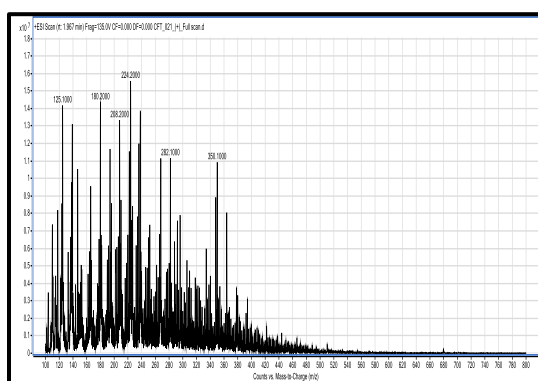


Figure 4. LCMS spectra at Retention time 1.9

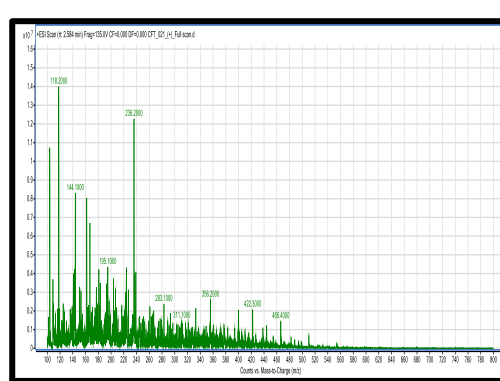


Figure 6. LCMS spectra at Retention time 2.58

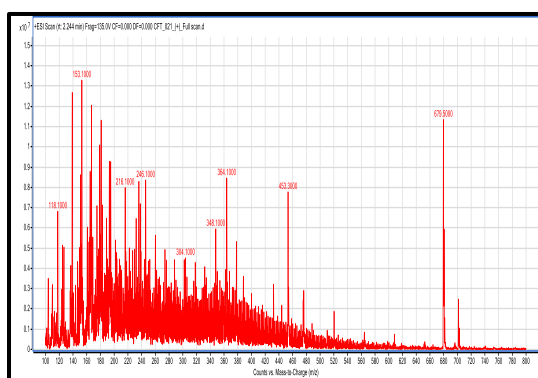


Figure 5. LCMS spectra at Retention time 2.24

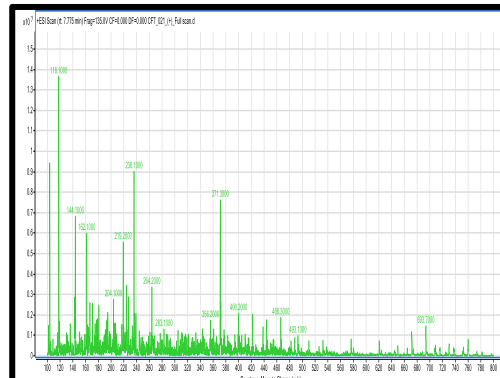


Figure 7. Spectra at Retention time 7.75

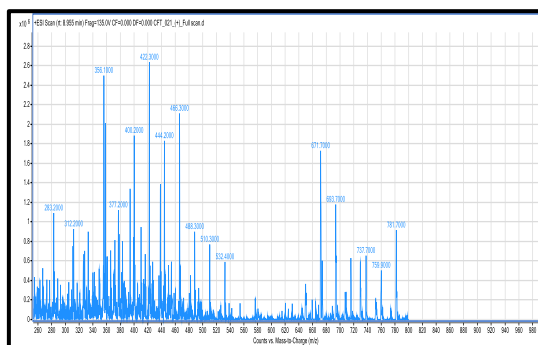


Figure 8. Spectra at Retention time 8.9

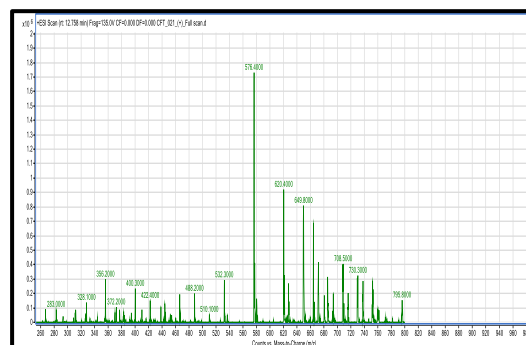


Figure 10. Spectra at Retention time 12.75

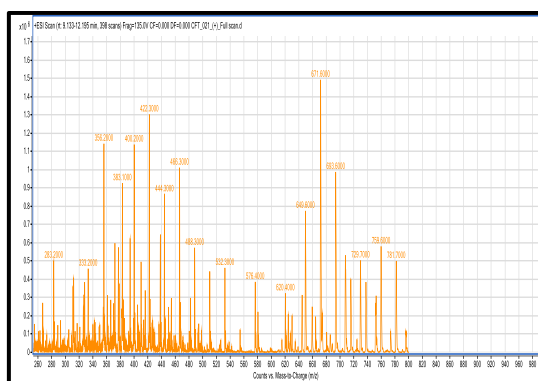


Figure 9. Spectra at Retention time 9.13

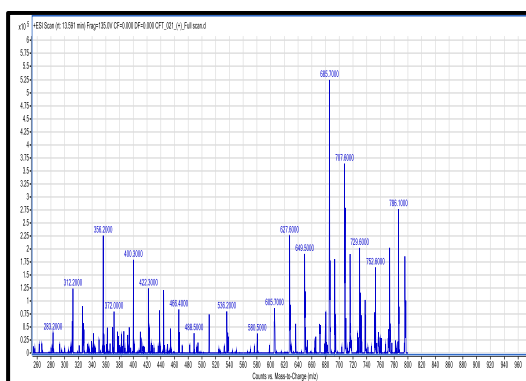


Figure 11. Spectra at Retention time 13.59

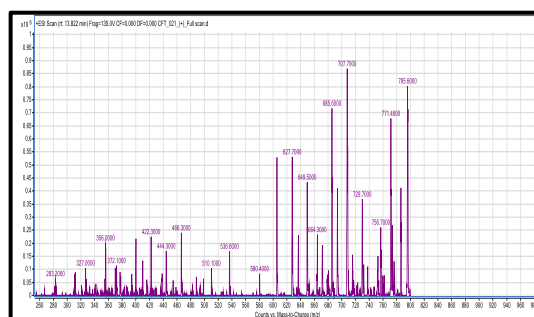


Figure 12. Spectra at Retention time 13.82

Table 3. Compounds identified with LCMS analysis

Sr.	ESI- m/z	ESI+ m/z	Compound Name
1	133	224	Malic acid / isoflavonoid fragment
2	137	153	Small phenolic acid (tentative)
3	195	118	Genipin metabolite fragment
4	123	118	Genipin derivative
5	151	422	Gallic acid moiety / saponin
6	137	671	Triterpenoid saponin
7	289	576	Catechin/ epicatechin
8	249	685	Glycosylated saponin
9	331	707	Glucogallin/ saponin glycoside

LCMS chromatograms with retention time and m/z ratio were used to analyse the results. The significant chemicals found using chromatograms are shown in the Table 3 (Vijayalakshmi *et al.*, 2015).

The LC-MS analysis of the sample was performed in both negative (ESI-) and positive (ESI+) electrospray ionization modes to maximize detection of a broad range of bioactive compounds. The chromatographic and mass spectral data revealed nine distinct peaks with unique retention times and corresponding mass-to-charge (m/z) values. Polyphenolic substances such as saponins, galloyl derivatives, and catechins are present and may have antibacterial, anti-inflammatory, and antioxidant properties. These results correlate with

studies of bioactive components found in plant-derived materials such roots, seed shells, and herbal extracts (Panzella *et al.*, 2020).

Antibacterial activity

Natural resources offer a viable path for the creation of new antimicrobial drugs because of their capacity to concurrently adjust structural integrity, enzyme function, genetic processes, and communication networks. These substances also provide a sustainable and environmentally beneficial method of treating microbial resistance, particularly when made from renewable resources like coconut shell oil.

The antibacterial efficacy of coconut shell extract was assessed against bacteria using the agar well diffusion method and broth microdilution for MIC determination.

Nutrient agar was used for the well diffusion test. To obtain fresh inoculums, the stock culture of the test organisms was sub cultured using sterile nutrient broth. Before usage, the test organisms were prepared for suspension using 0.85% saline. The investigation employed nutrient agar. A newly

prepared nutrient agar was seeded with 1 ml of a known quantity of inoculum (cfu/ml) of the test organism in order to assess the sample's antibacterial activity against the test organisms using the well diffusion method. Using a cork borer, the 8 mm diameter wells were carved. After that, each well was filled with sample-roughly 100 μ l. Every plate was incubated for 24 hours at 37°C. the zone of inhabitation around each well was measured (Pritha & Karpagam, 2018).

Following Test Bacteria were used for these studies

1. *Escherichia coli* ATCC25922
2. *Staphylococcus aureus* ATCC25293
3. *Pseudomonas aeruginosa*
4. *Salmonella typhi*
5. *Bacillus subtilis*
6. *Micrococcus luteus*

Results were obtained by measuring zone of inhabitation against all the bacteria listed above are shown in Figures (13-18) below.

Coconut shell is known to be rich in phenolic compounds, long-chain alkanes, and other secondary metabolites, many of which have been



Figure 13. Zone of inhabitation against *E. coli*



Figure 15. Zone of inhabitation against *Pseudomonas*



Figure 14. Zone of inhabitation against *S. aureus*

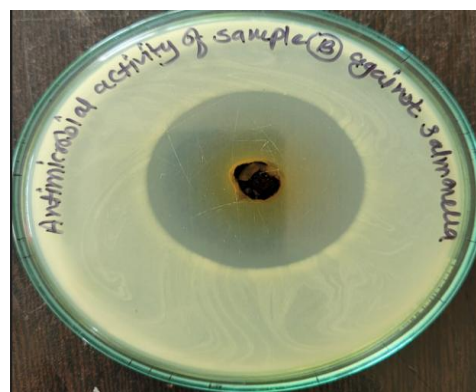
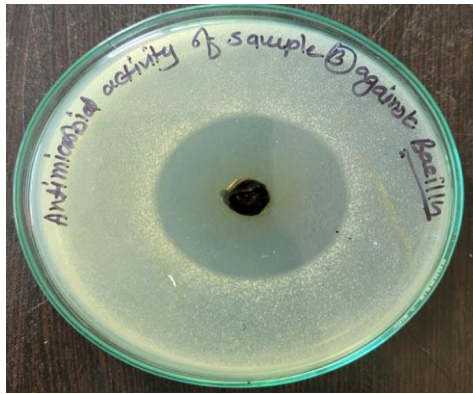


Figure 16. Zone of inhabitation against *Salmonella*

Figure 17. Zone of inhibition against *Bacillus*Figure 18. Zone of inhibition against *M. luteus*

reported to exhibit antimicrobial activity. The findings suggest that coconut shell extract holds promise as a natural antimicrobial agent, with potential applications in pharmaceutical, cosmetic, and food preservation industries (Saklani *et al.*, 2025). Broad-spectrum antibacterial activity is demonstrated by the natural reservoir and strong carrier of catechins and phenolic compounds found in coconut shell oil. Lipophilic alkanes and bioactive polyphenols work together to give coconut shell oil its multimodal action, which includes biofilm suppression, enzyme inhibition, and membrane disruption. Its biodegradability, natural origin, and antioxidant-rich profile make it a viable option for formulations of sustainable antimicrobials (Nor *et al.*, 2023).

Table 4. Zone of inhibitions of coconut shell oil against bacteria

Bacteria	Zone of inhibition in diameter mm
Escherichia coli	15 mm
Staphylococcus aureus	20 mm
Pseudomonas aeruginosa	35 mm
Salmonella typhi	25 mm
Bacillus subtilis	18 mm
Micrococcus luteus	18 mm

Zone of inhibitions are measured and tabulated as shown in Table 4. The largest inhibition zones were observed against *Staphylococcus aureus* ATCC25293 and *Pseudomonas aeruginosa*. *Staphylococcus aureus* is gram positive bacteria are found on skin and mucous membrane. It is considered as normal flora on skin and behave as opportunistic pathogen that often cause hospital and community acquired infections in immunocompromised person. Results suggesting that the extract possesses potent activity against both Gram-positive and Gram-negative bacteria.

1. Minimum Inhibitory Concentration (MIC)

The sample is soluble in water. The dilutions were prepared by using phosphate buffer solution pH 7.2. The dilution scheme with bacterial suspension was made as per Table 5. The serial dilutions were prepared for each concentration after vigorous mixing. 0.1 ml from each tube was transferred to sterile petri plate and sterile molten (temp 400c) nutrient agar was poured. After solidifying, the plates were incubated at 37°C for 24 hrs. to check the colony count (Drugkar *et al.*, 2022).

The sample is effective against *Staphylococcus aureus* ATCC25293 at concentration 50 %. The sample is 99.97% effective against *Staphylococcus*

Table 5. Dilution scheme

Sample quantity in ml	Phosphate buffer pH 7.2	Concentration of sample %	Bacterial Suspension in ml	Total volume used
1	0	100	1	0.1
0.75	0.25	75	1	0.1
0.50	0.50	50	1	0.1
0.25	0.75	25	1	0.1
0.12	0.88	12	1	0.1

aureus ATCC25293 at concentration 25 % and 99.7 % effective against 12 % concentration. Colony count can be observed at 25 % concentration in Figure 19.

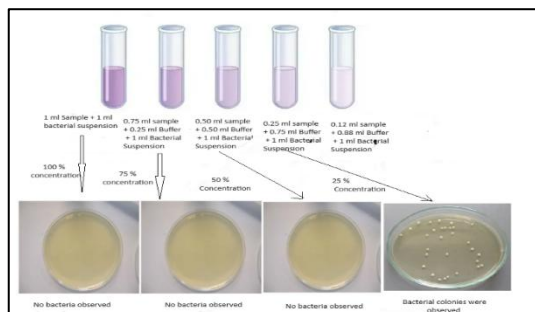


Figure 19. Colony count at different concentrations of sample

Observed values in Table 6 and 7 showed the complete inhibition of bacterial growth was observed, with no colonies detected across all dilutions. At 25% concentration, a very low bacterial count was observed. Because the bioactive components of coconut shell extract are polar, different portions of the extract exhibit variable levels of antibacterial activity. The antibacterial activity of polar fractions, especially those that were extracted with water or methanol, was noticeably higher. polar phytochemicals, such as flavonoids, phenolic acids, and catechins, have increased bioavailability and water solubility and easily pass into microbial cell environments. These substances have functional groups like hydroxyl and carboxyl moieties that help them form hydrogen bonds and interact electrostatically with membrane proteins, nucleic acids, and microbial enzymes.

The current research demonstrates the significant antibacterial efficacy of crude coconut

shell extract against specific bacterial strains. Even though conventional control antibiotics were not used at this point, the findings offer a solid framework for further fractionation and compound characterization research. To confirm and measure the relative potency of the extracted bioactive compounds, comparison analyses with well-established antibacterial agents will be carried out in later stages. According to these results, coconut shell holds promise as a natural source of antibacterial compounds for future pharmaceutical research.

Conclusion

This study serves as an overview for the identification and standardization of this specific species of plant and validates its historical use as an herbal remedy for a range of ailments. Novel medicinal chemicals may be synthesized from this plant for future application.

The LC-MS analysis of coconut shell extract revealed a chemically diverse phytochemical profile, predominantly composed of phenolics, flavonoids, and glycosides. Notably, key bioactive compounds such as catechin and glucogallin were tentatively identified, which are well-known for their antioxidant and antimicrobial potential. The presence of such metabolites provides strong justification for the traditional and emerging uses of coconut shell in therapeutic applications. The function of compound polarity in maintaining antimicrobial activity is reinforced by the polar extracts' lower minimum inhibitory concentration (MIC) values and larger zone of inhibition. Thus, the enhanced bioactivity of the polar coconut shell extract fractions indicates their potential as a rich source of natural antimicrobial agents.

Table 6. Bacterial count of *S. aureus* with different concentration of sample

Concentration of sample	Count cfu/ml					% inhibition of bacteria
100 %	Nil	Nil	Nil	Nil	Nil	100%
75 %	Nil	Nil	Nil	Nil	Nil	100%
50%	Nil	Nil	Nil	Nil	Nil	100%
25%	30	1	Nil	Nil	Nil	99.97%
12%	106	51	0	Nil	Nil	99.7 %

Table 7. Minimum Inhibitory Concentration of sample against *S. aureus*

Concentration of sample	100%	75 %	50%	25%	12%
Interpretative Category	S	S	S	R	R
Bacterial count after exposure cfu/ml	Nil	Nil	Nil	3×10^1	3×10^2

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