OPEN ACCESS

Kashf Journal of Multidisciplinary Research
Vol: 02 - Issue 1 (2025)
P-ISSN: 3007-1992 E-ISSN: 3007-200X
https://kjmr.com.pk

HARNESSING COCONUT HUSK: SOLVENT-BASED EXTRACTION AND ITS ANTIMICROBIAL ACTIVITY AGAINST FOODBORNE PATHOGENS

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DOI: https://doi.org/10.71146/kjmr235

Article Info

Abstract

Natural sources of antimicrobial agents have gained attention as antibiotic resistance continues to threaten global health. Coconut husk, a renewable agro-industrial byproduct rich in phenolics, flavonoids, and tannins, demonstrates antimicrobial properties. This study evaluates coconut husk extracts prepared with ethanol, methanol, and water solvents for antimicrobial activity against four food borne pathogens including Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, and Salmonella typhi. The ethanol extract showed significant antimicrobial activity, particularly at 48 hours, producing the largest inhibition zones (up to 29 mm for P. aeruginosa), due to its ability to extract a broader range of bioactive compounds. Methanol and water extracts showed moderate activity but were less effective compared to ethanol. Structural changes observed in microbial cell membranes after coconut husk extraction were confirmed by SEM, which indicated disruption of cellular integrity as mode of action of the antimicrobial activity. Active compounds present in phytochemical analysis confirmed to be alkaloids, tannins, flavonoids, and phenolics. The antimicrobial activity of coconut husk extracts indicating their potential to use in food preservation reducing pathogen load and extending shelf life.

Introduction

Natural sources of antimicrobial agents have emerged as critical global health issues driven by the development of antibiotic-resistant pathogens (Ahmad et al., 2019). Given the current environmental and health problems associated with food preservatives, the food industry suffers from various challenges in ensuring food safety without overreliance on synthetic preservatives (Forough and Farhadi, 2010). Therefore, there is a growing interest in developing eco-friendly and nontoxic materials for food preservation applications (Iravani et al., 2014).

Coconut husk, the fibrous outer shell of the coconut fruit, is an abundant agro industrial by product in many phytochemicals including phenolics, flavonoids and tannins (Guzmán et al., 2009).These bioactive compounds have shown antimicrobial activity against various microorganisms (Rafique et al., 2017). This waste material can be used as a value added practice as it enhances the value as a waste material and is in accordance with the practices of the use of sustainable renewable resources for minimization of environmental pollution. The yield and effectiveness of the produced bioactive compounds in plant extraction is affected by the choice of solvent (Ahmed et al., 2016). Common solvents used to extract phytochemicals are ethanol, methanol and water, and solubility (Borase et al., 2014). Optimizing the application of coconut husk extract in food preservation and food safety is dependent on a solvent effect on coconut husk extract antimicrobial activity.

Additionally, the structural and morphological features of plant extracts could be characterised, and antimicrobial mechanisms elucidated. Scanning Electron Microscopy (SEM) is a powerful approach to the visualization of the microstructural characteristics of plant derived materials associated with their functional properties (Zamiri et al., 2011). The prospect of the antimicrobial activity of various solvents extracted from coconut husk extracts is well recognised; however there is a dearth of comprehensive research on the antimicrobial activity of said extracts against common food borne pathogens. Additionally, very few research has been done on SEM microstructural analysis of coconut husk extracts to understand how coconut husk extracts are antimicrobial.

This work aims to evaluate the antimicrobial activity of coconut husk extracts made from ethanolic, methanolic and water solvents against *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus* and *Salmonella typhi.* The study also aims to characterize the morphological features of the extracts using Scanning Electron Microscopy (SEM) to correlate structural properties and antimicrobial efficacy. Although the antimicrobial potential of coconut husk components has been highlighted in some studies, there is no systematic study on a comparison of the efficacy of different coconut husk solvent extracts, nor a correlation between structural characteristics and their efficacy. This gap in the literature is addressed by critically evaluating the antimicrobial activity of extracts of coconut husk made with ethanol, methanol, and water, and through a detailed SEM study to elucidate the structure-function relationship.

Methodology

Materials

Fresh coconut husks were obtained from the local markets of Karachi, Pakistan. Ethanol (99.5%), and Methanol (99.8%) were products of Sigma-Aldrich. Reagents such as Dragendorff's reagent, aluminum chloride and ferric chloride were purchased from Merck Millipore. Distilled water were used throughout the study. Four bacterial strains (*Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus* and *Salmonella typhi*) were procured from the Microbiology Laboratory, Ziauddin University.

Methods

The methodology involves extracting phytochemicals from coconut husk using ethanol, methanol, and water as solvents. Phytochemical analysis of the extracts is then conducted to identify active compounds that could contribute to the antimicrobial activity. The microstructural features of the extracts are observed using SEM. Standard microbiological assays for antimicrobial activity against selected foodborne pathogens are performed using extracts of 24, 48 and 72 hours.

Preparation of Coconut Husk Extract

A fine powder of coconut husk was prepared by washing, drying and grinding. Ten grams of coconut husk powder was suspended in 100 ml distilled water and placed on a stirrer to prepare an extract. The extract was filtered with 0.22µm filter paper and allowed to extract at room temperature extract.

Ethanol Extraction

Ethanol extraction was done by mixing the powdered coconut husk with ethanol in a 1:10 ratio. It was stirred and left for 24, 48 and 72 hours. The mixture was filtered and the solution collected for further analysis.

Methanol Extraction

Similar to the ethanol extraction, the powdered husk was mixed with methanol in a 1:10 ratio. The mixture was stirred and allowed to stand for 24, 48 and 72 hours before being filtered to give the methanol extract.

Aqueous Extraction

For aqueous extraction, the mixture of powdered husk and distilled water was prepared in a 1:10 ratio. The extract was collected by filtering the mixture, it was boiled for 30 minutes, cooled and the extract was boiled. The extract was left for 24, 48 and 72 hours and filtered.

Antimicrobial Activity

Activity of coconut husks extract as antimicrobial against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Salmonella typhi* was determined using agar well diffusion method. Nutrient agar plates were prepared, sterilized, and wells created for each of the extracts. 100 µl of each extract was pipetted into the wells and each of the bacterial strains was inoculated into it. The plates were incubated for 24 hours at 37°C. Antimicrobial potential was estimated and zones of inhibition were measured.

Minimum Inhibitory Concentration (MIC) Determination Using Serial Dilution

The antibacterial efficacy of the different dilutions of an extract was evaluated using the serial dilution method to determine the Minimum Inhibitory Concentration (MIC) of various dilutions of an extract. Nutrient agar plates were inoculated with standardized suspensions of *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Salmonella typhi*. Extract-impregnated discs were prepared at varying dilutions—1:2, 1:4, 1:8, and 1:16, and placed on the inoculated agar surfaces. The bacterial growth and the formation of inhibition zones (clear zones around the discs) were allowed to occur at 37°C for 24 hours on the plates. By directly measuring these zones, this method allows for direct visual and quantitative observation of antibacterial activity and provides a visual and quantitative method for extract efficacy against various bacterial strain.

Phytochemical Analysis

The presence of various bioactive compounds of the coconut husk extracts was analyzed by phytochemical analysis. The properties of the extracts that may be responsible for the reduction and stabilization are understood by this analysis. Methods described by Obidoa et al., (2010) were utilised to identify the active agents within the extract.

Scanning Electron Microscopy (SEM)

The surface morphology of the bacterial cells was estimated by scanning electron microscope (JSM-6380A, Jeol Japan) under different magnifications at a voltage of 10 kV.

Results

Extract preparation

The grinding of coconut husks powder yielded a fine brown-coloured powder with uniform particle size distribution. After extraction, the final product of coconut husk extract is shown in Figure 1.

Figure 1: Processing stages of coconut husk extract

Antimicrobial Activity of coconut husk extract

The synthesized extract exhibited significant antimicrobial activity against all four foodborne pathogens (*E. coli*, *P. aeruginosa*, *S. typhi* and *S.aureus*). The coconut husk was more effective in ethanol extracts compared to methanol and water extracts. The analysis of inhibition zones on agar plates provided evidence of significant antimicrobial activity for the ethanol extract after a 48-hour extraction period against all tested pathogens. Notably, the ethanol extract produced an inhibition zone of 19 mm against *E. coli* at 48 hours, compared to smaller zones of 15 mm and 16 mm observed at 24 and 72 hours, respectively. This trend of strongest activity at 48 hours was consistent for *Salmonella typhi*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. In this study, *Pseudomonas aeruginosa* exhibited the largest zone of inhibition showing the highest antimicrobial activity with a zone of 29 mm in 48 hours. The ethanol extract obtained after 48 hours emerged as the most promising candidate due to its consistently superior antimicrobial activity (Figure 1).

Table 1: Zone of Inhibition (mm) of coconut husk extract against Foodborne Pathogens

Microorganism	Solvents	Zone of Inhibition (mm)		
		24 hours	48 hours	72 hours
Escherichia coli	Ethanol	15	19	16
	Methanol	7	9	12
	D. Water	13	11	15
Salmonella typhi	Ethanol	20	22	21
	Methanol	15	10	10
	D. Water	13	15	21
Pseudomonas aeruginosa	Ethanol	28	29	25
	Methanol	21	15	17
	D. Water	24	17	25
Staphylococcus aureus	Ethanol	19	15	16
	Methanol	19	20	16
	D. Water	18	17	15

Figure 2 shows an experiment testing the antimicrobial activity of pathogens using coconut husk extract. Agar plates were labelled with different pathogens: *Salmonella*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. Each dish contains a solution with either ethanol, methanol, or water as solvents. Observations were made at 24, 48, and 72 hours, with a control (C) for comparison. The largest zones of inhibition represented ethanol treatment at 48 hours. The findings suggest that ethanol is the most effective solvent for extracting antimicrobial compounds from coconut husk. This experiment shows the antimicrobial properties of coconut husk extract over different solvents and periods. The clear zones of inhibition, particularly prominent in the ethanol extracts at 48 hours, indicate strong antimicrobial activity against the tested pathogens.

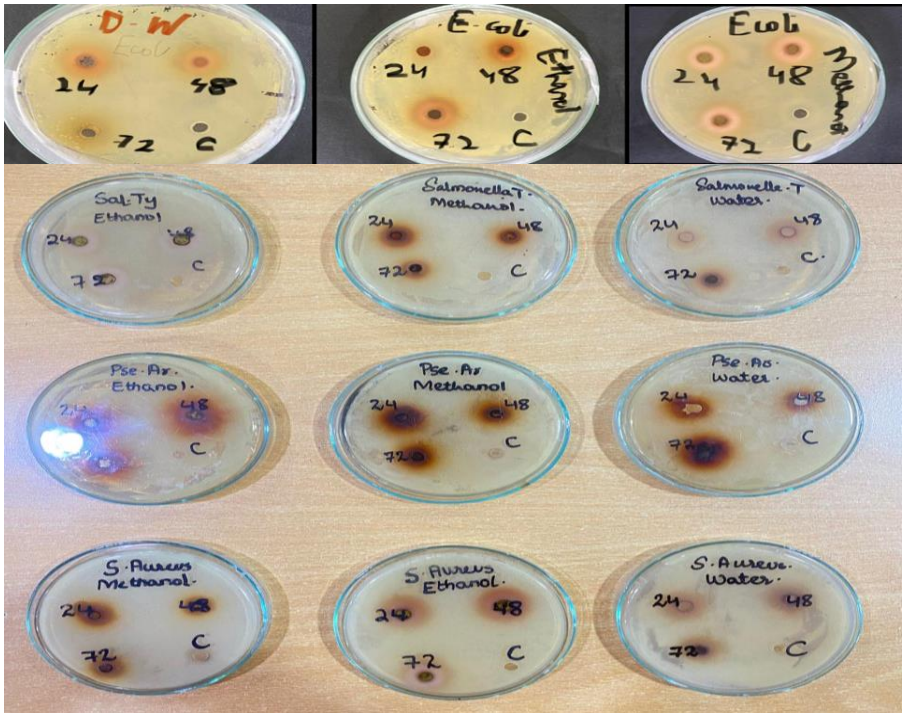

Figure 2: Agar plates showing zone of inhibition of coconut husk extract against food-borne pathogens.

Observations of MIC

The agar diffusion test results for the different bacterial strains are illustrated in Figure 3. The antibacterial effectiveness of the extract can be seen by clear zones of inhibition around the discs. The inhibition zones have variable sizes depending on the dilution factor of the extract applied. For example, in the case of *E. coli* and *S. aureus*, there are smaller zones of inhibition at higher dilutions indicating that activity is reduced at lower concentrations. However, while *P. aeruginosa* and *S. typhi* have a relatively constant zone size across different dilutions, this suggests a potentially more widespread action of the extract against these pathogens. The results show that different bacteria are sensitive to the same antibacterial agent, but in varying levels, and therefore the importance of dilution in the application of such extracts for antibacterial use. The variance that this offers is necessary for assessing optima for the effective concentration needed for microbial inhibition in practical applications.

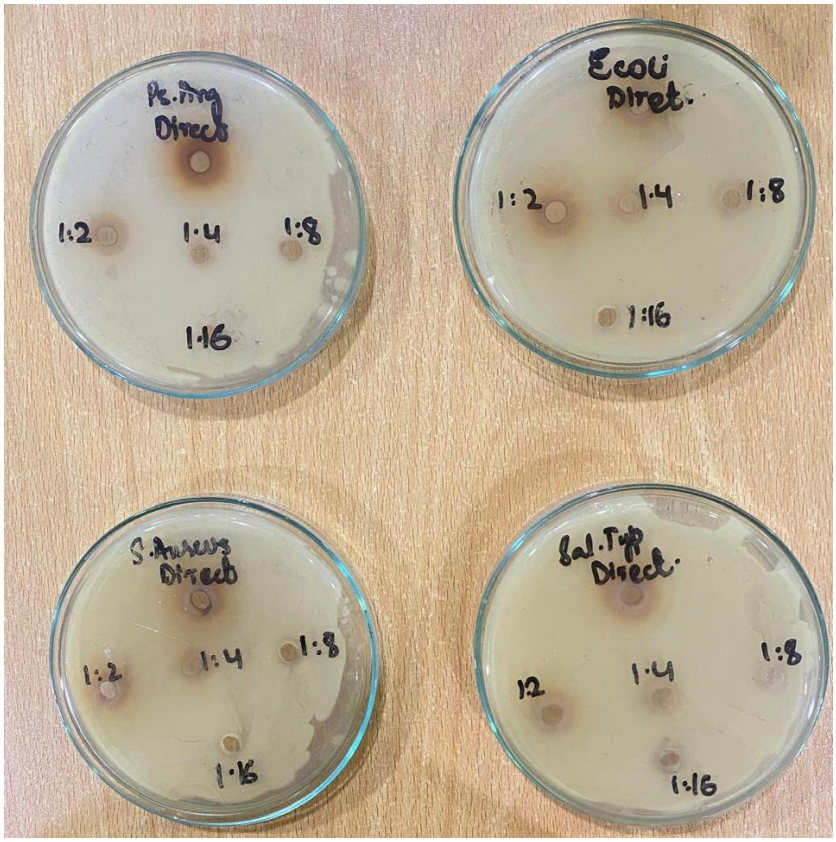

Figure 3: Zone of inhibition at different dilutions around the extract discs on agar plates inoculated with *E. coli, P. aeruginosa, S. aureus* and *S. typhi.*

Phytochemical Analysis

The phytochemical test demonstrated that the plant extract contains Alkaloids, Tannins, Saponins, Resins, Flavonoids, Steroids, Glycosides and Terpenoids. The phytochemical analysis of various coconut husk solvent extracts revealed a range of phenolic concentrations. Phenolic compounds, which are known for their potent antioxidant and antimicrobial properties, play a critical role in the reduction. The ethanol extract, with its high phenolic content, showed significant antibacterial activity against *Pseudomonas aeruginosa*, likely due to the enhanced reduction facilitated by these phenolic compounds.

Scanning electron microscopy

Morphological changes in *Pseudomonas aeruginosa* cells were observed on Scanning Electron Microscopy analysis after the treatment with the coconut husk extract. The surface of the untreated cells was densely packed with small, granular particles and sparse, rod-shaped bacteria present on the surface of the samples. There are interspersed structures between these larger, elongated structures resembling rod-shaped bacteria. They are sparsely distributed over the surface. There are also some irregular, flaky structures visible. On the other hand, treated samples showed a smoother background, and were less granular, consistent with reorganization of surface structures. The fact that these alterations occurred demonstrates that the extract exhibited an alteration in cell morphology and distribution that the cell membrane was likely disrupted, and cellular processes. The results of the observed damages imply that the coconut husk extract bioactive compounds interfere with the integrity of microbial cell membranes resulting in cell lysis. The results obtained from these SEM findings are in agreement with the antimicrobial efficacy of the extracts which therefore suggests their use as natural antibacterial agents.

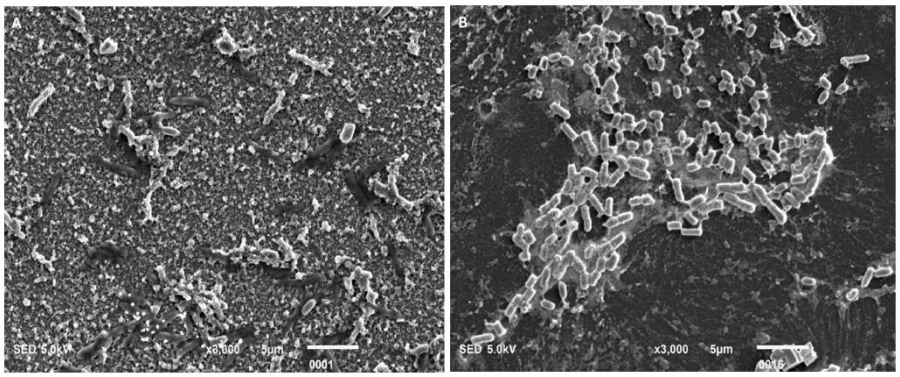

Figure 4: Morphological analysis of untreated (A) and treated (B) cells of *P. aeruginosa* with coconut husk extract

Discussion

Results from this study show the strong antimicrobial activity of coconut husk extracts prepared in ethanol, methanol, and water solvents on common food-borne pathogens *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus* aureus and *Salmonella typhi*. The ethanol extract was the most effective of the three solvents tested, followed by the methanol and water extracts. The results of the work suggest that the solvent used is an important factor in the extraction of bioactive compounds from coconut husk responsible for antimicrobial activity. Antimicrobial nature of the phytochemicals (phenolic compounds, flavonoids, and tannins) ensured the superior performance of the ethanol extract (Govarthanan et al., 2016). Ethanol as an intermediate polar solvent can extract both polar and nonpolar compounds (Rajesh et al., 2020). On the contrary, the polar solvent water can dissolve only a subset of bioactive compounds since it fails to extract nonpolar compounds in the phase transfer. In addition to being good at extracting phytochemicals, methanol is also not as good as ethanol in dissolving classes of compounds responsible for antimicrobial activity.

Scanning Electron Microscopy (SEM) was used in the analysis of the morphological characteristics of coconut husk extract. SEM images revealed a porous and fibrous microstructure that could facilitate interaction of bioactive compounds with microbial cells. Absorption of antimicrobial agents into diffusible porous materials has been reported to disrupt microbial cell membranes, thereby inhibiting cellular processes, and if the pulse is of sufficient duration, killing the microbe. Correlation between the structural differences observed among the extracts and their different antimicrobial efficacies was interpreted and the influence of morphology on antimicrobial activity was also highlighted (Li et al., 2024). .The findings

agree with previous studies which reported the antimicrobial capacity of plant extracts. In another study, Uddin et al. (2020) reported that coconut leaf extracts showed high antibacterial activity against Gram-positive and Gram-negative bacteria, which demonstrates that coconut-derived phytochemicals could be used in combating microbial infections. Also, banana peel extract studies have demonstrated antimicrobial activities making agricultural byproducts valuable sources of bioactive compounds (Goh et al., 2023).

In addition to the ethanol extract, ethanol residues might also contribute to the observed higher antimicrobial activity of the ethanol extract, which has intrinsic antimicrobial properties (Kumari et al., 2021). However, the extract ethanol concentration should probably be low and may not contribute much to the antimicrobial effect. Contrarily, the higher concentrations of active phytochemicals giving microbial inhibition are more likely the ethanol extract itself. Antimicrobial activity is seen to increase dose dependently with increasing concentrations of the extracts, indicating that there is a sufficient amount of bioactive compounds available that are necessary for effective inhibition of the microbes (Rizwana et al., 2023; Loo et al., 2018).These results imply that by optimizing the concentration of coconut husk extracts, they may improve the practical uses of the extracts in food preservation, reducing pathogen load and extending the shelf life of perishable products.

However, the study's promises are limited. There was no discussion about the variability in phytochemical content, mediated by factors such as coconut variety, maturity and environmental conditions, which may impact the consistent antimicrobial activity Also, the potential cytotoxicity of the extracts was not evaluated, since this is crucial for the safety of the food applications.

Future research should isolate and characterize the specific bioactive compounds responsible for the antimicrobial activity that was observed. Knowing these compounds would help us further understand the controlling mechanisms of microbial inhibition and will enable us to develop standardized extracts of high efficacy. In addition, the effectiveness of these extracts in real food systems and the food product sensory impact of these extracts would need to be evaluated to proceed toward commercial application.

Conclusion

The antimicrobial activity of extracts of coconut husk obtained with ethanol, methanol, and water against common foodborne pathogens is demonstrated in this study. The ethanol extract showed the highest efficacy, probably because it was much more effective at solubilizing a broader range of bioactive phytochemicals. The distinct morphological characteristics of the extracts revealed by Scanning Electron Microscopy may be responsible for their antimicrobial properties. The potential food preservation use of coconut husk as a natural antimicrobial agent is suggested by the findings. Importantly, the results are encouraging but much further work is needed to characterize the specific active compounds, understand the mechanisms of action of these extracts, and assess the safety and efficacy of these extracts for food safety.

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