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Research Article

Secondary Metabolite Identification and Antibacterial Activity of Ethanol Extract of Coconut Husk (*Cocos nucifera L.*) *Against Escherichia coli* ATCC 25922

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Abstract: Secondary metabolites are bioactive compounds commonly found in plants and have potential as natural antibacterial agents. Coconut coir *(Cocos nucifera Linnaeus)*, an abundant agricultural by-product, is known to contain various secondary metabolites such as flavonoids, tannins, terpenoids, and phenolic compounds, which have been reported to possess antibacterial properties. *Escherichia coli* is a pathogenic bacterium that frequently causes human infections and has shown increasing resistance to several antibiotics, highlighting the need for alternative antibacterial sources. Inconsistencies in previous studies regarding the antibacterial activity of coconut coir against *E. coli* indicate the necessity for further investigation. This study aimed to identify the secondary metabolite content and evaluate the antibacterial activity of ethanol extract of coconut coir against *Escherichia coli* ATCC 25922. This study was an in vitro laboratory experimental study with a descriptive–experimental approach. Identification of secondary metabolites was conducted qualitatively using phytochemical screening of the ethanol extract of coconut coir. Antibacterial activity was assessed using the disc diffusion method with a *post-test only control group design* against *Escherichia coli* ATCC 25922. The phytochemical screening results demonstrated that the ethanol extract of coconut coir contained flavonoids, tannins, terpenoids, and polyphenols. However, the antibacterial activity test showed no inhibition zones at all tested concentrations of the ethanol extract against *Escherichia coli* ATCC 25922. These findings indicate that although the ethanol extract of coconut coir contains several secondary metabolites, it does not exhibit antibacterial activity against *Escherichia coli* ATCC 25922 when tested using the disc diffusion method.

Keywords: Antibacterial Activity; Coconut Husk; *Escherichia Coli* ATCC 25922; Flavonoids; Secondary Metabolites.

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1. Introduction

Secondary metabolites are chemical compounds synthesized by plants that play an important role in defense mechanisms against microbial attacks. Various classes of secondary metabolites, such as flavonoids, tannins, terpenoids, and phenolic compounds, have been reported to exhibit diverse biological activities, including antibacterial effects. Therefore, the identification of secondary metabolites constitutes a crucial initial step in phytochemical research to evaluate the antibacterial potential of natural materials (Harborne, 1998).

Indonesia possesses exceptionally rich biodiversity and represents a promising source of natural antibacterial compounds, one of which is the coconut plant *(Cocos nucifera Linnaeus)*. Coconut husk, which has not yet been optimally utilized, has been reported to contain various secondary metabolites involved in plant defense mechanisms against microorganisms. The secondary metabolites present in coconut husk, including flavonoids, tannins, terpenoids, and phenolic compounds, have potential to be developed as antibacterial agents (Harborne, 1998).

Previous studies have demonstrated that ethanol extracts of coconut husk contain flavonoids, terpenoids, tannins, and polyphenols. In addition, the phenolic content and biological activity of coconut husk have been reported to be influenced by the maturity level of the raw material (Sari & dkk, 2021; Umarudin, 2023). However, empirical evidence regarding the antibacterial activity of coconut husk extracts against Gram-negative bacteria remains inconsistent. Several studies have reported the absence of inhibition zones against *Escherichia coli*, including extended-spectrum β-lactamase (ESBL)-producing strains, even when relatively high extract concentrations were used (Nugraha & Hendrayana, 2020).

Bacterial infections remain a global health problem, contributing substantially to high rates of morbidity and mortality. One of the most common pathogenic bacteria causing human infections is *Escherichia coli*. This bacterium is a Gram-negative bacillus belonging to the family Enterobacteriaceae and normally exists as a commensal flora in the gastrointestinal tract; however, certain strains are pathogenic and capable of causing various clinical manifestations, including diarrhea (Hutasoit, 2020; Umarudin, 2023).

The treatment of *Escherichia coli* infections generally relies on antibiotics; however, irrational use has led to an increasing prevalence of antibiotic resistance. The World Health Organization (WHO) has reported that more than 20% of *Escherichia coli* isolates in various countries are resistant to first-line antibiotics such as ampicillin and cotrimoxazole, as well as to second-line antibiotics including fluoroquinolones (Organization, 2022). Furthermore, the emergence of the pandemic clone *Escherichia coli* O25b/ST131, which is resistant to multiple classes of antibiotics—including β-lactams, fluoroquinolones, and aminoglycosides—has further exacerbated this problem (Jawetz et al., 2016). A review by Nurjanah et al. also indicated a high level of *Escherichia coli* resistance to β-lactam antibiotics, although several antibiotics still exhibit limited sensitivity (Nurjanah et al., 2020).

Based on the potential secondary metabolite content of coconut husk and the variability of previous findings regarding its antibacterial activity against *Escherichia coli*, further research is required to obtain more consistent scientific evidence. Therefore, this study aims to identify secondary metabolites and to evaluate the antibacterial activity of ethanol extracts of coconut husk *(Cocos nucifera Linnaeus)*, specifically the tall (inner) or green varieties grown in Kupang City, against *Escherichia coli* ATCC 25922 using the disc diffusion method.

2. Materials and Method

Research Design

This study employed an in vitro laboratory experimental design aimed at identifying secondary metabolites and evaluating the antibacterial activity of ethanol extracts of coconut husk *(Cocos nucifera Linnaeus)*. Secondary metabolite identification was conducted using a qualitative descriptive approach through phytochemical screening to detect flavonoids, tannins, terpenoids, and polyphenols. Antibacterial activity testing utilized a *post-test only control group design* with the disc diffusion method, involving treatment groups with various extract concentrations, a positive control using a standard antibiotic, and a negative control using the solvent. The assessment parameter was the diameter of the inhibition zone against *Escherichia coli* ATCC 25922.

Study Location and Period

The study was conducted from July to November 2025 at the Microbiology Laboratory, Faculty of Medicine and Veterinary Medicine, Nusa Cendana University.

Materials and Samples

The coconut husk used in this study was obtained from mature coconuts of a local tall variety (green coconut) collected from community-owned coconut plantations in Manulai 2 Subdistrict, Alak District, Kupang City, East Nusa Tenggara, Indonesia. The bacterial test strain used was *Escherichia coli* ATCC 25922, obtained from the Center for Health Laboratory (Balai Besar Laboratorium Kesehatan), Surabaya. Chloramphenicol was used as the positive control in the antibacterial assay, while sterile distilled water served as the negative control.

Preparation of Coconut Husk Extract

The coconut husk was air-dried and then ground into a fine powder. Extraction was performed using the maceration method with 70% ethanol as the solvent. The powdered coconut husk was immersed in the solvent for 72 hours with periodic stirring. The resulting filtrate was filtered and concentrated using a rotary evaporator to obtain a viscous extract.

Ethanol-Free Test

An ethanol-free test was conducted to ensure the absence of residual ethanol in the extract. The test was performed qualitatively using a specific chemical reaction, and the extract was considered ethanol-free if no color change was observed according to the test indicator.

Identification of Secondary Metabolites

Secondary metabolite identification was carried out qualitatively through phytochemical screening to detect the presence of flavonoids, tannins, terpenoids, and polyphenols. Each test was based on specific reactions indicated by color changes or precipitate formation. The results of the phytochemical tests were recorded as positive (+) or negative (–).

Confirmation and Preparation of the Test Bacteria

Bacterial confirmation was performed to ensure the purity and characteristics of the test bacteria through observation of colony morphology and Gram staining. The bacterial suspension was prepared by adjusting the turbidity to a 0.5 McFarland standard to ensure a uniform inoculum concentration.

Antibacterial Activity Assay

The antibacterial activity assay was conducted using a *post-test only control group design* with the disc diffusion method. The ethanol extract of coconut husk was tested at seven concentrations: 50%, 25%, 12.5%, 6.25%, 3.125%, 1.56%, and 0.78%. Mueller–Hinton Agar plates were inoculated with a suspension of *Escherichia coli* ATCC 25922, after which paper discs impregnated with the extract, positive control (chloramphenicol), and negative control (sterile distilled water) were placed on the agar surface. The plates were then incubated at 37 °C for 18–24 hours.

3. Results and Discussion

Research Results

Coconut Husk Extraction

Coconut husk *(Cocos nucifera Linnaeus)* of the green coconut variety obtained from Manulai II, Kupang City, was dried, ground, and sieved to obtain 819 g of powdered material. The powder was extracted using the maceration method with 70% ethanol for 72 hours with periodic stirring. The filtrate was then filtered and concentrated using a rotary evaporator at 40–50 °C, yielding 151.92 g of dry extract.

Ethanol-Free Test

The results of the ethanol-free test indicated that no characteristic color change from orange to bluish-green occurred after the addition of potassium dichromate ($K_2Cr_2O_7$) and sulfuric acid (H_2SO_4). Instead, a mixed color resulting from the interaction between the extract solution and the reagents was observed, indicating that the ethanol extract of coconut husk *(Cocos nucifera Linnaeus)* was free from residual ethanol.

Identification of Secondary Metabolite Compounds

Phytochemical screening revealed that the ethanol extract of coconut husk *(Cocos nucifera Linnaeus)* contained secondary metabolites, including flavonoids, tannins, phenolic compounds, and terpenoids.

Bacterial Confirmation Test

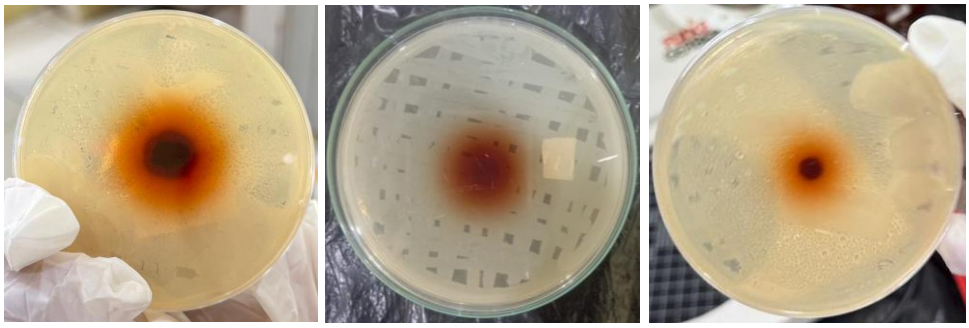
Bacterial confirmation using Gram staining showed pink-colored, rod-shaped cells, indicating that the sample was positive for Gram-negative bacteria consistent with the characteristics of *Escherichia coli*.

Antibacterial Activity Assay

The antibacterial activity of the ethanol extract of coconut husk *(Cocos nucifera Linnaeus)* against *Escherichia coli* ATCC 25922 was evaluated using the disc diffusion method at the Laboratory of the Faculty of Medicine and Veterinary Medicine, Nusa Cendana University. The extract was tested at concentrations of 50%, 25%, 12.5%, 6.25%, 3.125%, 1.56%, and 0.78%, with sterile distilled water as the negative control and chloramphenicol and amoxicillin as positive controls, each performed in triplicate. The results showed that none of the extract concentrations produced inhibition zones, indicating no antibacterial activity against *E. coli* ATCC 25922. Amoxicillin also did not produce an inhibition zone, whereas chloramphenicol produced a clear zone of inhibition, confirming that the testing conditions and methodology were appropriate.

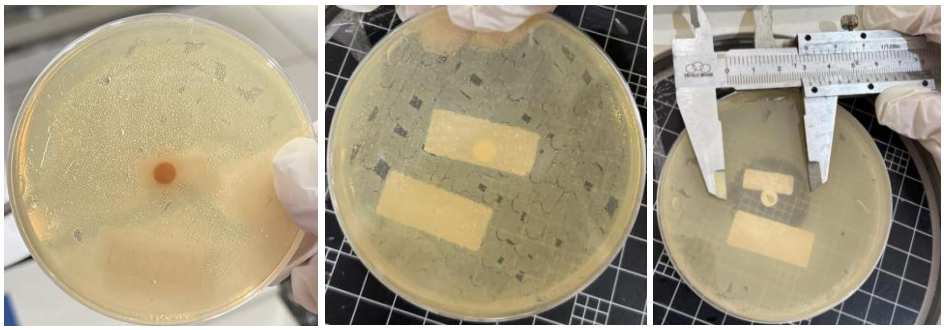
Table 1. Results of Phytochemical Screening.

No	Compounds	Results
1	Terpenoid	 Positive (+) (Reddish brown)
2	Tanin	 Positive (+) (Blackish-green)
3	Flavonoid	 Positive (+) (Orange)
4	Phenol	 Positive (+) (Blackish-green)

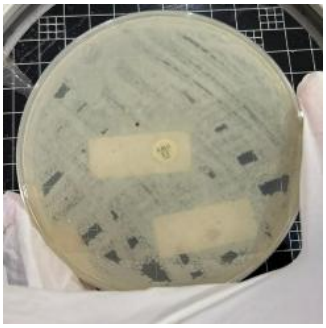


Concentration 50% Concentration 25% Concentration 12.5%

Concentration 6.25% Concentration 3.125% Concentration 1.56%



Concentration 0.78% Kontrol (-) Control (+) Chloramphenicol



Control (+) Amoxicillin

Figure 2. Antibacterial Activity Results.

Table 2. Results of Inhibition Zone Diameter Measurement of Ethanol Extract of Coconut Husk *(Cocos nucifera Linnaeus)* Against the Growth of *Escherichia coli.*

	Jamming Zone Diameter (mm)				
Extract Concentration	**Replika 1**	**Replika 2**	**Replika 3**	**Standard Average Deviation**	**Classification**
50%	0	0	0	0	None
25%	0	0	0	0	None
12,5%	0	0	0	0	None
6,25%	0	0	0	0	None
3,125%	0	0	0	0	None
1,56%	0	0	0	0	None
0,78%	0	0	0	0	None
Kontrol (-)	0	0	0	0	None
Control (+) amoxicillin	0	0	0	0	None
Control (+) chloramphenicol	31,45	31,05	31,15	0,21	Strong

Discussion

The coconut husk raw material used in this study underwent a significant reduction in mass during the drying and grinding processes, with an overall processing efficiency from fresh husk to fine powder of 10.5%. This reduction was primarily attributed to the high initial moisture content and the fibrous structure of coconut husk, which is rich in lignin, resulting in not all fractions being mechanically pulverized. This efficiency value remains within the range reported in the literature, indicating that the material preparation method employed was methodologically reasonable and acceptable. Air-drying at room temperature was selected to minimize the degradation of heat-sensitive phenolic and flavonoid compounds, although it resulted in lower drying efficiency compared to oven-drying methods (Arziyah et al., 2025; Rahayu et al., 2022).

The extraction process utilized 70% ethanol via the maceration method for 72 hours with regular agitation to maximize the solubilization of bioactive compounds from the coconut husk matrix. This ethanol concentration was chosen due to its semi-polar nature, enabling the extraction of a wide range of secondary metabolites, including flavonoids, tannins, and phenolic compounds. Solvent evaporation using a rotary evaporator at a low temperature (40–50 °C) successfully produced a viscous extract that was subsequently dried to a crystalline form, a characteristic reported to be associated with the high tannin and lignin content of coconut husk (Aniar et al., 2022; Tinasy & Wijayati, 2024).

The ethanol-free test using $K_2Cr_2O_7$ and H_2SO_4 reagents showed no color change of the solution from orange to green. This finding indicates that Cr(VI) ions were not reduced to Cr(III), allowing the conclusion that the extract was free from residual ethanol. This result ensures that subsequent antibacterial testing was not influenced by the antimicrobial activity of ethanol, but rather reflected the effects of the phytochemical components of the extract alone (Lobo et al., n.d.).

Phytochemical screening results revealed the presence of secondary metabolites, including flavonoids, tannins, phenolic compounds, and terpenoids, in the ethanol extract of coconut husk. These findings are consistent with previous reports regarding the polyphenolic content of coconut husk. However, the screening performed was qualitative in nature and therefore did not provide information on the actual levels or concentrations of the active compounds. This limitation is particularly important when interpreted in relation to the antibacterial assay results, as the qualitative presence of bioactive compounds does not necessarily correlate directly with detectable biological activity (Hutasoit, 2020; Organization, 2022; Purwaningrum, 2021).

Gram staining confirmed that the test bacterium was a Gram-negative bacillus, consistent with the characteristics of *Escherichia coli*. The cell wall structure of Gram-negative bacteria, which includes an outer membrane containing lipopolysaccharides (LPS), serves as a major barrier to the penetration of antibacterial agents, particularly large or complex phytochemical compounds. In addition, *E. coli* possesses effective efflux pump systems that actively expel various toxic substances before they reach intracellular targets (Madigan et al., 2018).

The antibacterial activity assay using the disc diffusion method showed no formation of inhibition zones at all tested extract concentrations. This finding indicates that the ethanol extract of coconut husk was unable to inhibit the growth of *Escherichia coli* ATCC 25922 under the experimental conditions employed. The absence of detectable antibacterial activity may be explained by a combination of factors, including the limited diffusion of high–molecular-weight polyphenolic compounds in agar media, the structural barrier posed by the lipopolysaccharide (LPS) layer, and the activity of efflux pump systems in Gram-negative bacteria. These findings are consistent with previous reports indicating that coconut husk extracts tend to be more effective against Gram-positive bacteria than Gram-negative bacteria (Delcour, n.d.; Lobo et al., n.d.; zhi et al., 2015).

The positive control amoxicillin also did not produce an inhibition zone, suggesting resistance of *E. coli* to β-lactam antibiotics, potentially mediated by β-lactamase production or alterations in membrane permeability. In contrast, chloramphenicol, used as an alternative positive control, produced a clear inhibition zone, confirming that the test conditions, culture medium, and bacterial viability were appropriate. Thus, the absence of inhibition zones produced by the extract cannot be attributed to procedural errors, but rather to the biological characteristics of the test bacterium and the physicochemical properties of the extract.

Overall, this study demonstrates that although the ethanol extract of coconut husk contains secondary metabolites that theoretically possess antibacterial potential, the extract

did not exhibit inhibitory activity against *Escherichia coli* ATCC 25922 when evaluated using the disc diffusion method. These results highlight the importance of considering the concentration of active compounds, the antibacterial testing method employed, and the physiological characteristics of the target bacterium when assessing the antibacterial potential of natural products.

4. Conclusion

This study demonstrates that the ethanol extract of coconut husk *(Cocos nucifera Linnaeus)* of the green coconut variety originating from Kupang City contains secondary metabolites, including flavonoids, tannins, terpenoids, and polyphenols, as indicated by phytochemical screening, yet does not exhibit antibacterial activity against *Escherichia coli* ATCC 25922 in the disc diffusion assay. These findings indicate that the presence of secondary metabolites is not necessarily directly correlated with antibacterial activity, particularly against Gram-negative bacteria with complex cell wall structures, and they further highlight the limitations of the disc diffusion method in detecting the antibacterial potential of natural product extracts. This study contributes empirical data on the phytochemical profile and the limited antibacterial activity of coconut husk, and therefore future research is recommended to employ broth dilution methods to determine minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values, as well as to include Gram-positive bacteria as comparators to evaluate the influence of cell wall structural differences and to optimize the potential utilization of coconut husk as a natural antibacterial source.

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