EduChemia
Jurnal Kimia dan Pendidikan

Vol.10, No.1, 2025, p. 129-148
e-ISSN 2502-4787

IDENTIFICATION AND ANTIBACTERIAL ACTIVITY OF BIOACTIVE COMPOUNDS EXTRACTED FROM GADING COCONUT (*Cocos nucifera var. eburnea*) LEAF

Uswatun Hasanah[1*], Ade Heri Mulyati[1], Sandra Amalia Riyadi[1], Lusia Magdalenis Pita[1]

[1]*Department of Chemistry, Faculty of Mathematics and Natural Sciences, Pakuan University, Bogor, Indonesia*

E-mail: *uswatun.hasanah@unpak.ac.id

Received: 13 April 2025. Accepted: 30 Juli 2025. Published: 8 Agustus 2025

DOI: 10.30870/educhemia.v10i1.31974

Abstract: Traditional medicine continues to play a vital role in Indonesia's healthcare landscape, with increasing reliance on plant-derived remedies for disease prevention and treatment. Among these, the gading coconut (*Cocos nucifera* var. *eburnea*), native to Bogor, Indonesia, presents untapped potential as a source of antibacterial agents. This study aimed to identify bioactive compounds with antibacterial activity from gading coconut leaves and to evaluate the impact of extraction methods—maceration and sonication—on their yield and efficacy. Compared with maceration (5.92%), sonication produced a significantly greater extract yield (9.48%). Phytochemical screening confirmed the presence of alkaloids, flavonoids, and tannins in both extracts. Quantitative analysis revealed that the sonicated extract contained greater concentrations of total flavonoids (52.06 mg QE/g) and total phenolics (147.10 mg GAE/g). Chemical profiling using liquid chromatography–tandem mass spectrometry (LC–MS/MS) identified key antibacterial constituents, including 3-hydroxycoumarin, isoorientin, vitexin, and α-linolenic acid. Biological evaluation demonstrated that the sonicated extract exhibited stronger antibacterial activity, with minimum inhibitory concentrations (MICs) of 50 mg/mL against *Escherichia coli* and 25 mg/mL against *Staphylococcus aureus*. These findings suggest that the extraction method significantly influences both the chemical composition and antibacterial potency of gading coconut leaf extracts. Overall, this study highlights the pharmacological relevance of *C. nucifera* var. *eburnea* as a promising source of natural antibacterial agents and contributes to the scientific validation of traditional medicinal plants in Indonesia.

Keywords: *Cocos nucifera var. eburnean*; extraction; antibacterial; LC–MS/MS

Abstrak: Pengobatan tradisional terus memainkan peran penting dalam sistem kesehatan di Indonesia, dengan semakin banyak masyarakat yang beralih ke pengobatan berbasis tanaman untuk pencegahan dan pengobatan penyakit. Salah satu tanaman dengan potensi antibakteri yang menjanjikan adalah kelapa gading (*Cocos nucifera* var. *eburnea*), yang banyak ditemukan di wilayah Bogor, Indonesia. Penelitian ini bertujuan untuk mengidentifikasi senyawa bioaktif beraktivitas antibakteri dari daun kelapa gading serta mengevaluasi pengaruh metode ekstraksi—maserasi dan sonikasi—terhadap hasil ekstrak dan efektivitas biologisnya. Metode sonikasi menghasilkan rendemen ekstrak yang secara signifikan lebih

tinggi (9,48%) dibandingkan dengan maserasi (5,92%). Uji fitokimia menunjukkan adanya kandungan alkaloid, flavonoid, dan tanin pada kedua jenis ekstrak. Analisis kuantitatif mengungkapkan bahwa ekstrak hasil sonikasi memiliki kadar flavonoid total (52,06 mg QE/g) dan fenol total (147,10 mg GAE/g) yang lebih tinggi. Profil kimia menggunakan kromatografi cair-spektrometri massa tandem (LC–MS/MS) berhasil mengidentifikasi senyawa antibakteri utama, antara lain 3-hidroksikumarin, isoorientin, vitexin, dan asam α-linolenat. Uji aktivitas antibakteri menunjukkan bahwa ekstrak sonikasi memiliki potensi yang lebih kuat, dengan konsentrasi hambat minimum (KHM) sebesar 50 mg/mL terhadap *Escherichia coli* dan 25 mg/mL terhadap *Staphylococcus aureus*. Temuan ini menunjukkan bahwa metode ekstraksi secara signifikan memengaruhi komposisi kimia dan potensi antibakteri ekstrak daun kelapa gading. Secara keseluruhan, studi ini menegaskan relevansi farmakologis *C. nucifera* var. *eburnea* sebagai sumber agen antibakteri alami dan memberikan kontribusi terhadap validasi ilmiah tanaman obat tradisional Indonesia.

Kata kunci: *Cocos nucifera var. eburnean*; ekstraksi; antibakteri; LC–MS/MS

INTRODUCTION

The use of traditional medicine in Indonesia has shown a consistent upward trend, as evidenced by basic health research findings. In 2013, approximately 48% of the population in West Java utilized traditional remedies for health maintenance, increasing to 51.9% by 2018 (Central Statistics Agency, 2019). This growing interest in traditional healing reflects a broader global shift toward natural and plant-based therapies, fueled by rising public trust in herbal medicine and integrative health approaches (Saklani et al., 2025; Archana et al., 2021). Scientific research has correspondingly intensified, with over 110,000 studies on medicinal plants published between 1960 and 2019, illustrating the rapid expansion of phytomedicine as a field (Salmeron-Manzano et al., 2020).

Among the diverse medicinal plants under investigation, *Cocos nucifera* L.—the coconut tree—is widely recognized for its versatile ethnomedicinal applications. Often called the "tree of life," nearly every part of the coconut, from fruit to husk, has demonstrated therapeutic potential for various ailments (Belem-Kabre et al., 2021). In Indonesia, *Cocos nucifera* var. *eburnea*, locally known as the gading coconut, is predominantly found in rural and rice field regions and holds promise as an underexplored medicinal resource (Nor et al., 2023). The longstanding use of this variety in traditional remedies, such as those practiced by the Ngayogyakarta Hadiningrat Palace, underscores its cultural and therapeutic significance (James et al., 2019).

Empirical evidence highlights the pharmacological potential of different coconut parts. Extracts from fruit, flesh, coconut milk, oil, roots, husks, and fibers have been used to address respiratory

disorders, oral diseases, skin conditions, hypertension, and hernias (Erinle et al., 2021; Mazaya et al., 2020). Traditional applications are supported by recent research demonstrating the antimicrobial efficacy of coconut extracts; for example, Irawan et al. (2023) reported the inhibition of *Staphylococcus aureus* and *Escherichia coli* by coconut husk acetone extracts, whereas Nor et al. (2023) reported that high phenolic contents correlated with antibacterial activity in coconut coir extracts. Additionally, a 2024 clinical study confirmed the antibacterial properties of coconut oil against clinical bacterial isolates, attributing its activity mainly to lauric acid (PubMed, 2024).

Young coconut leaves and roots are traditionally employed in regions such as Lou Island, Papua New Guinea, to treat diarrhea and gastrointestinal disorders. Modern investigations corroborate these ethnomedical claims: Tayler et al. (2020) identified bioactive phytochemicals with potent antiparasitic and anticancer properties in coconut leaf extracts, and Ukaoma et al. (2024) reported significant antimicrobial activity against pathogenic bacteria, including *E. coli* and *Salmonella typhi*. These findings underscore the therapeutic potential inherent in coconut leaves.

Despite these promising data, the bioactive constituents of the gading coconut variety remain insufficiently characterized. Studies on other coconut varieties have identified important bioactive compounds, such as flavonoids, alkaloids, tannins, and phenolic compounds, which are known for their antimicrobial, antioxidant, and anti-inflammatory effects (Belem-Kabre et al., 2021; Ivan et al., 2019). It is plausible that gading coconut leaves results in similar or unique metabolites, meriting thorough phytochemical investigation.

Given its widespread cultivation in rural Indonesia and established traditional uses, the gading coconut represents an accessible and valuable botanical resource. Comprehensive phytochemical profiling using advanced analytical methods such as liquid chromatography–tandem mass spectrometry (LC–MS–MS/MS) is essential for elucidating the chemical composition of this plant. Concurrent evaluation of their biological activities, particularly their antibacterial effects, will provide critical insight into their pharmacological potential and support their integration into evidence-based herbal medicines. This study therefore aims to bridge the existing knowledge gap by exploring the phytochemical constituents and antibacterial properties of

gading coconut (*Cocos nucifera* var. *eburnea*) leaves, contributing valuable information to the expanding field of Indonesian medicinal plant research.

METHOD

Materials and Equipment

The reagents and chemicals used in this study, including 96% ethanol, quercetin standard, $AlCl_3$, sodium acetate, Folin-Ciocalteu reagent, Na_2CO_3, DPPH, chloroform, ammonia, sulfuric acid, Mayer's reagent, Dragendorff's reagent, concentrated hydrochloric acid, glacial acetic acid, hydrochloric acid, distilled water, and $FeCl_3$, were supplied by Merck (Darmstadt, Germany). The materials and equipment utilized in this study include a grinder, analytical balance, rotary evaporator, sonicator, micropipette, incubator, UV–Vis spectrophotometer, and LC–MS/MS. The materials used in this research consisted of gading coconut leaves, samples at a single time point, amoxicillin, PCA agar media, and bacterial cultures of *Escherichia coli* and *Staphylococcus aureus*.

Plant identification and taxonomy

The gading coconut (*Cocos nucifera var. eburnea*) used in this study was sent to the Indonesian Institute of Sciences (LIPI) in Bogor for species identification. The identification process involved examining the plant morphology and comparing it with herbarium collections at LIPI. Botanists verified the species on the basis of their distinct characteristics, ensuring the authenticity of the plants used in the research. Once identified, the sample was properly labeled and documented, confirming its reliability for further analysis of bioactive compounds and antibacterial potential.

Extraction of Gading Coconut Leaves

The gading coconut (*Cocos nucifera* var. *eburnea*) leaves were thoroughly washed, air-dried under direct sunlight in a controlled environment for seven days, and subsequently ground into a coarse powder to obtain simplicia. Two extraction techniques—maceration and sonication—were employed to isolate bioactive constituents.

For maceration, 250 grams of simplicia were soaked in 1250 mL of 96% ethanol at room temperature for 24 hours. The mixture was then filtered, and the residue was re-extracted with fresh ethanol. This process was repeated until the filtrate became colorless. The combined filtrates were concentrated

under reduced pressure using a rotary evaporator to obtain the macerated extract.

For the sonication method, 100 grams of simplicia were mixed with 1250 mL of 96% ethanol and subjected to ultrasonic extraction at 40 kHz for 60 minutes at room temperature. The resulting mixture was filtered, and the filtrate was concentrated using a rotary evaporator to yield the sonicated extract. Both extraction procedures were conducted in triplicate to ensure reproducibility.

The extraction yields obtained from both methods were statistically compared using an independent t test to determine significant differences in extraction efficiency.

Phytochemical Screening

Phytochemical screening was carried out to detect the presence of secondary metabolites in gading coconut (*Cocos nucifera* var. *eburnea*) leaf extracts obtained through maceration and sonication methods. All procedures followed the standard qualitative techniques described by Harborne (1996), with minor modifications. Each test was conducted using pooled extracts from three replicates for both extraction methods. The identification of each compound group involved specific reagents and visual indicators, as outlined in Table 4:

Table 4. Phytochemical tests, reagents, and positive result indicators for gading coconut leaf extracts

Compound Group	Procedure	Reagents Used	Indicators of Positive Result
Alkaloids	Extract (50 mg) was mixed with 2 mL of chloroform and 2 mL of ammonia, filtered, followed by addition of concentrated H_2SO_4. The acid layer was tested with Mayer's and Dragendorff's reagents.	Mayer's reagent, Dragendorff's reagent, concentrated H_2SO_4	White precipitate (Mayer's), orange–red precipitate (Dragendorff's)
Flavonoids	Extract was boiled in 100 mL hot water for 5 minutes, filtered, and treated with concentrated HCl.	Concentrated HCl	Development of red or orange color
Triterpenoids/Steroids	Extract was treated with glacial acetic acid and concentrated H_2SO_4.	Glacial acetic acid, concentrated H_2SO_4	Red/purple color (triterpenoids), blue/ green color (steroids)
Saponins	Extract was shaken in water, followed by addition of 2 drops of 1 N HCl, then shaken again.	1 N HCl	Stable foam persisting for ≥7 minutes

Compound Group	Procedure	Reagents Used	Indicators of Positive Result
Phenolics	Extract was treated with 1% ferric chloride solution.	$FeCl_3$ 1%	Green, red, purple, blue, or black coloration
Tannins	Extract was boiled in distilled water, cooled, then treated with $FeCl_3$.	$FeCl_3$ 1%, distilled water	Green–brown or dark green coloration

Total phenolic content

The total phenolic content was determined using the Folin–Ciocalteu method. A 0.5 mg/mL solution of each extract was mixed with 1 mL of Folin–Ciocalteu reagent and 15.8 mL of distilled water. After 8 min, 3 mL of 10% Na_2CO_3 was added. The mixture was incubated at room temperature for 2 hours, and the absorbance was measured at the maximum wavelength using a UV–Vis spectrophotometer. A calibration curve was prepared using gallic acid at concentrations of 20, 40, 60, 80, and 100 ppm. The results are expressed as mg gallic acid equivalent per gram of extract (mg GAE/g).

Total Flavonoid Content

The total flavonoid content was determined using the aluminum chloride colorimetric method. A 1 mg/mL extract mixture was combined with 0.1 mL of 10% $AlCl_3$, 0.1 mL of 1 M sodium acetate, and 2.8 mL of distilled water. After 30 minutes of incubation at room temperature, the absorbance was measured at the maximum wavelength (400–800 nm). Quercetin was used as a reference standard with the same concentration range as gallic acid. The results are expressed as mg quercetin equivalent per gram of extract (mg QE/g).

Identification with LC–MS/MS

The identification of compounds in the extract was performed at the Forensic Laboratory Center, Sentul, using an MS detector. The ion source was ESI (+), and the MS analyzer was a Q-ToF. The stationary phase used was C18 (1.8 µm, 2.1x100 mm), which was set at a column temperature of 50°C. The mobile phase consisted of water + 5 mM ammonium formate (A) and acetonitrile + 0.05% formic acid (B) in a gradient flow at 0.2 mL/min for 23 minutes. The extract was filtered through a 0.2 µm filter and injected (5 µL) into the LC–MS/MS system (Friardi Ismed et al., 2021).

Antibacterial activity

Standard amoxicillin (0.35 mg/mL) and extracts at concentrations of 50 mg/mL and 25 mg/mL were used. The media, cultured with *Staphylococcus aureus* and *Escherichia coli*, were utilized to assess the antibacterial activity of the gading coconut leaf extract using the well diffusion method. Well A was filled with sample A, well B was filled with sample B, well C was filled with the standard solution, and well D was filled with distilled water as the negative control. The media were incubated at 35°C for 24 hours. Antibacterial activity was indicated by the formation of clear inhibition zones, which were then measured and compared with the standard. The analysis was replicated in duplicate.

RESULTS AND DISCUSSION

Taxonomic classification

The taxonomic classification confirmed that the plant used in this study was *Cocos nucifera L.*, which belongs to the Arecaceae family. This confirmation, which is based on a morphological comparison with herbarium samples, ensures the accuracy and validity of the plant material used in the research. The gading coconut (*Cocos nucifera var. eburnea*) was selected for its known medicinal properties, particularly its potential bioactive compounds and antibacterial activity.

Extraction of Gading Coconut Leaves

The special treatment during the drying process was implemented by using a closed environment to reduce the moisture content, thereby minimizing the biological activity in the leaves that could degrade their active compounds. The dried leaves were then ground into a coarse powder, which increased the surface area, facilitating the dissolution of the active compounds in the solvent. The extraction methods of sonication and maceration were selected because neither process involves the use of heat, which helps prevent the degradation of heat-sensitive (thermolabile) active compounds.

The yields of the macerated and sonicated extracts were then measured, and the results are shown in Table 5.

Table 5. Comparison of the Extraction Yields and T test Analyses for the Sonication and Maceration Methods

Extract Type	Average % Yield	Replication Deviation	T test
Sonication	9.48	0.58	Significant difference
Maceration	5.92	0.58	

The yield of the sonicated extract was greater than that of the macerated extract, with a statistically significant difference indicated by the t test. This result suggests that the sonication extraction method is more efficient than maceration is. The enhanced yield obtained through sonication can be attributed to the use of ultrasonic waves during the extraction process, which generate acoustic cavitation. Acoustic cavitation creates microscopic bubbles within the solvent, which expand and collapse rapidly, leading to the disruption of cell walls in the simplicia. This mechanical disruption facilitates the release of the active compounds from the plant material, increasing their solubility in the solvent.

Although the maceration process is typically performed at room temperature, it is important to note that the maceration method employed in this study did not involve the application of external heat. Instead, the extraction was performed at room temperature with the periodic replacement of ethanol to maintain solvent efficacy. The goal was to avoid the use of heat, which could degrade heat-sensitive compounds, making the process suitable for extracting thermolabile bioactive compounds. This approach helps preserve the integrity of the plant's active compounds while allowing for the comparison of two effective extraction methods, sonication and maceration (Kibra et al., 2018).

Phenolic Compounds

The calibration curve for gallic acid was constructed by plotting the concentration against the absorbance. The standard curve for gallic acid was constructed by plotting the concentration against the absorbance, yielding the equation y = 0.0077x - 0.0073, with a r^2 value of 0.9994. This highly linear calibration curve demonstrated excellent correlation between concentration and absorbance, indicating its reliability for quantifying the total phenolic content in the sample extracts (Figure 1). The equation derived from this calibration curve serves as a reference for accurately determining the total phenolic concentration in the gading coconut leaf extracts used in this study.

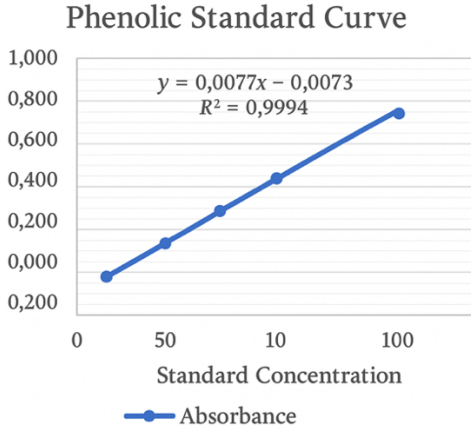


Figure 1. Phenolic standard curve

Based on the calibration curve, the total phenolic content in the macerated extract was calculated to be 136.75 mg GAE/g, whereas the sonicated extract contained 147.10 mg GAE/g. The higher phenolic content observed in the sonicated extract suggests that sonication may be a more effective extraction method for phenolic compounds than maceration is. However, statistical analysis using a t test revealed no significant difference in the phenolic concentrations between the two extraction methods. This finding indicates that while sonication results in a greater yield of phenolic compounds, the difference is not statistically significant, suggesting that both methods are comparable in terms of extracting phenolic compounds from gading coconut leaves. These findings contribute to understanding the efficiency of different extraction techniques in obtaining bioactive compounds, which may play a crucial role in evaluating the therapeutic potential of gading coconut leaves.

Flavonoid Compounds

The calibration curve for quercetin was constructed by plotting its concentration against the absorbance, resulting in the equation $y = 0.0077x + 0.0009$, with a r^2 value of 0.9963 (Figure 2). This strong linear relationship between the quercetin concentration and absorbance confirms the reliability of the calibration curve. The equation derived from this calibration curve can be used as a reference to determine the total flavonoid concentration in sample extracts, providing a highly accurate and reproducible method for quantifying flavonoid content. The calibration curve for quercetin is thus a critical tool for evaluating the flavonoid concentration in gading coconut leaf extracts, ensuring the precision and validity of the experimental results.

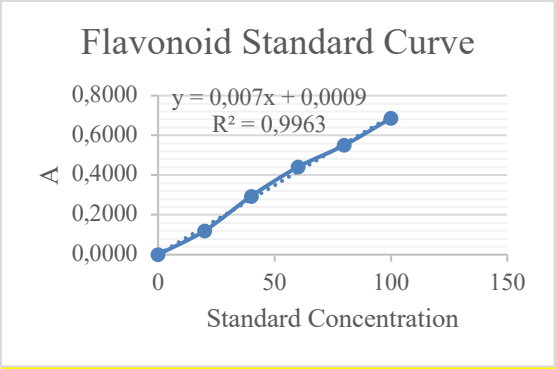


Figure 2. Flavonoid Standard Curves

The calculations revealed that the flavonoid concentration in the macerated extract was 40.67 mg QE/gram, whereas the sonicated extract presented a higher flavonoid concentration of 52.06 mg QE/gram. These results indicate that, compared with maceration, sonication is a more effective method for extracting flavonoids from gading coconut leaves. Statistical analysis using a t test revealed a significant difference between the

flavonoid concentrations of the macerated and sonicated extracts, further supporting the greater efficiency of the sonication method. This finding underscores the importance of optimizing extraction techniques to maximize the yield of bioactive compounds, particularly flavonoids, which are known for their antioxidant and potential therapeutic properties (Pardede et al., 2020; Pratama et al., 2021).

Triterpenoid identification

The secondary metabolites in the gading coconut leaf extract were tested for the presence of various bioactive compounds. The results are summarized in Table 6. Isoorientin, vitexin, and 3-hydroxycoumarin have been identified as flavonoids and phenolic compounds that have been shown to exhibit antimicrobial and antioxidant activities (Tayler et al., 2020). α-Linolenic acid, identified as both a phenolic compound and a tannin, has demonstrated antibacterial potential in previous studies (Dai et al., 2022). Taxinine and lupenone have been identified as terpenoids that have been linked to anti-inflammatory properties (Sanro Tachibana et al., 2005; Romero et al., 2016).

These findings highlight the bioactive potential of gading coconut leaf extract, supporting its potential applications in antimicrobial, antioxidant, and anti-inflammatory treatments.

Table 6. Phytochemical Composition of Gading Coconut Leaf Extracts: Presence or Absence of Bioactive Compounds

Compound	Flavonoids	Alkaloids	Tannins	Phenolic Compounds
Isoorientin	(+)	(−)	(−)	(+)
Vitexin	(+)	(−)	(−)	(+)
3-Hydroxycoumarin	(+)	(−)	(−)	(+)
α-Linolenic Acid	(−)	(−)	(+)	(+)
Taxinine	(−)	(+)	(−)	(−)
Lupenone	(−)	(+)	(−)	(−)

Antibacterial activity

Antibacterial testing was conducted to assess the ability of the coconut leaf extract to inhibit bacterial growth. The inhibition of bacterial growth is attributed to the presence of active compounds in the extract, which function as antibacterial agents. The method employed in this study was the well diffusion method. In this method, antimicrobial compounds are introduced into wells formed in a Petri

dish containing agar medium that has been inoculated with bacterial cultures. The media was then incubated for 24 hours at 35°C.

The bacterial strains used in this study were *Escherichia coli*, a gram-negative bacterium, and *Staphylococcus aureus*, a gram-positive bacterium. These bacteria were selected because of their pathogenic nature, as they are known to be harmful to humans. *S. aureus* possesses a cell wall rich in peptidoglycan and low in lipids but also contains significant amounts of polysaccharides. These characteristics make *S. aureus* an important target for antibacterial testing.

In this study, coconut leaf extracts obtained through sonication were gaded at concentrations of 50 mg/mL and 25 mg/mL. The positive control was the standard amoxicillin at 0.35 mg/mL, and the negative control was distilled water. The results of the antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* are presented in Table 7.

Table 7. Calculation of Antibacterial Effectiveness

Concentration of Extract (mg/mL)	% Effectiveness	Effectiveness Category
50 mg/mL	64,52%	Effective
25 mg/mL	42,61%	Not Effective

The antibacterial activity of the coconut leaf extract obtained via the sonication method demonstrated the inhibition of bacterial growth in *Staphylococcus aureus* and *Escherichia coli*. Clear zones of inhibition were observed around the wells filled with gading coconut leaf extract at concentrations of 50 mg/mL and 25 mg/mL. In comparison, the positive control, which consisted of amoxicillin solution at 0.3525 mg/mL, exhibited a stronger inhibitory effect, as evidenced by the formation of larger inhibition zones. On the other hand, the negative control, consisting of water, did not produce any inhibition zones, indicating that water had no effect on bacterial growth, confirming that the antibacterial activity was solely attributed to the gading coconut leaf extract.

According to Stout's (1971) categorization of antibacterial activity, as the concentration of gading coconut leaf extract increased, the inhibitory effect also increased. The antibacterial activity on *Staphylococcus aureus* and *Escherichia coli* cultures followed a similar trend. At a

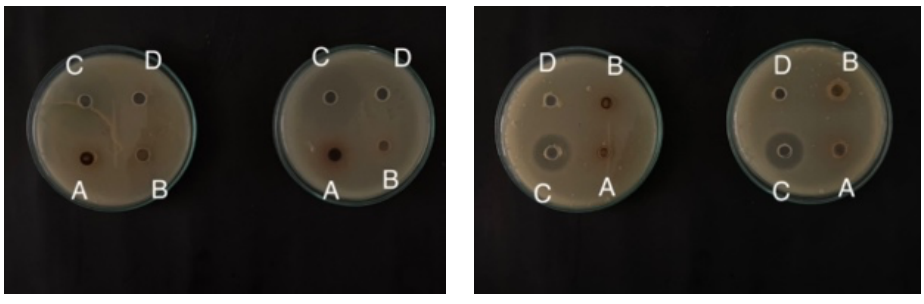
concentration of 50 mg/mL, the extract had a very strong inhibitory effect on both bacterial cultures, whereas at 25 mg/mL, the inhibitory effect was moderate against *Escherichia coli* and strong against *Staphylococcus aureus* (Dai et al., 2022). This suggests that the antibacterial properties of the extract are concentration dependent, with a stronger effect at higher concentrations (Lima et al., 2015).

The antibacterial activity observed in this study is likely due to the presence of secondary metabolites such as flavonoids, phenolic compounds, and tannins, which have been linked to antibacterial properties (Fathoni et al., 2020). The formation of inhibition zones around the wells further supports the role of these compounds in inhibiting bacterial growth (Suryani et al., 2021).

The effectiveness of the coconut leaf extract was evaluated based on the inhibition zones observed via the well diffusion method, as described by Oroh et al. (2015). The inhibition zone refers to the area around the well where bacterial growth is inhibited, indicating the antibacterial activity of the extract. The larger the inhibition zone is, the greater the antibacterial effect of the extract.

$$\%\ \text{Effectiveness:}\ \frac{\text{Inhibition zone of the sample}}{\text{Inhibition zone of the positive control}}\ x\ 100\%$$

For *Staphylococcus aureus*, the extract at 50 mg/mL was classified as effective, whereas at 25 mg/mL, it was categorized as ineffective. Similarly, for *Escherichia coli*, the extract at 50 mg/mL was effective, whereas at 25 mg/mL, it was ineffective. Amoxicillin, as a positive control, showed very strong and effective antibacterial activity against both *Staphylococcus aureus* (Figure 3a) and *Escherichia coli* (Figure 3b), demonstrating its superior antibacterial efficacy to that of the gading coconut leaf extract.



a b

Figure 3. Zone of inhibition of Sonicated Gading Coconut Leaf Extraction Sample against the Growth of *S. aureus* Bacteria (a) and *E. coli* (b).

These findings suggest that coconut leaf extract can be considered a potential alternative treatment for infections caused by *Staphylococcus aureus* and *Escherichia coli*. This finding is consistent with previous studies by Ukaoma et al. (2024), who reported that ethanol extracts of coconut leaves could inhibit the growth of bacteria such as *Acinetobacter spp*., *Bacillus cereus*, *Escherichia coli*, *Shigella dysenteriae*, *Salmonella typhi*, and *Aspergillus niger*.

The results also indicate that the inhibition zone for *Staphylococcus aureus* was larger than that for *Escherichia coli*. This is likely due to the presence of flavonoids in the sonicated extract, which are more effective against gram-positive bacteria such as *Staphylococcus aureus*. Flavonoids contain hydroxyl groups, which are polar in nature and allow them to penetrate the thicker peptidoglycan layer of gram-positive bacteria more effectively. Gram-negative bacteria, such as *Escherichia coli*, have an additional lipid bilayer membrane, which acts as a barrier, making it more difficult for polar compounds to penetrate. The hydrophilic nature of peptidoglycan in gram-positive bacteria increases their susceptibility to the inhibitory effects of flavonoids, which disrupt the cell wall, compromising its structural integrity and leading to cell lysis (Obeng-Boateng et al., 2024). This differential activity between gram-positive and gram-negative bacteria underscores the importance of flavonoids in the antibacterial mechanisms of gading coconut leaf extracts.

Identification of the Active Compounds via LC–MS

Liquid chromatography–mass spectrometry (LC–MS/MS) is a combined analytical technique that uses liquid chromatography to separate the components (Figure 4) of a sample and mass spectrometry as the detector (Harmita et al., 2019). In this analysis, two mass analyzers are used in tandem, with a collision cell placed between them. The resulting chromatogram displays peaks corresponding to different analytes, with the mass–charge (m–z) ratio plotted on the x–axis and the relative mass abundance on the y–axis. The m/z ratios represent the mass of each ion divided by its charge, which is critical for identifying the compounds present in the sample. Larger peaks indicate higher concentrations of the corresponding analytes, whereas smaller peaks reflect lower concentrations. The fragmentation of ions occurs in the collision cell, where they are subjected to

controlled collisions, breaking them into smaller fragments. These fragments provide additional structural information, which is essential for the identification of the molecular structure of a compound.

The following active compounds were identified in the gading coconut leaf extract, with their corresponding retention times, measured masses, and fragmentation masses: 3,5-dimethyladamantane-1-carboxylic acid (retention time: 4.747 minutes, measured mass: 209.1542 m/z, molecular structure: $C_{13}H_{20}O_2$) was identified as a cycloaliphatic carboxylic acid with antiviral potential. The first fragmentation mass observed was 165.12 m/z, indicating the loss of a carboxyl group (-COOH). The second fragmentation observed was 121.10 m/z, resulting from the loss of a methyl group.

Octinoxate (retention time: 9.492 min, measured mass: 291.196 m/z, molecular structure: $C_{18}H_{26}O_3$) is a cinnamate ester known for its UVB protection properties. The first fragmentation mass observed was 163.06 m/z, corresponding to the loss of the ester group. The second fragmentation peak observed was 135.05 m/z, corresponding to the loss of a C_6H_5 group.

The indole (retention time: 13.711 min, measured mass: 118.0657 m/z, molecular structure: C_8H_7N) is an alkaloid with anti-inflammatory activity. The first fragmentation mass observed was 92.05 m/z, which was the result of the loss of an NH_2 group. The second fragmentation mass observed was 64.03 m/z, corresponding to the loss of a C_2H_5 group.

Pheophorbide A (retention time: 16.263 min, measured mass: 593.2764 m/z, molecular structure: $C_{35}H_{36}N_4O_5$) is an alkaloid with anticancer, antioxidant, and anti-inflammatory properties. The first fragmentation mass observed was 361.18 m/z, corresponding to the loss of a sugar moiety. The second fragmentation mass observed was 307.10 m/z, corresponding to the loss of a porphyrin ring fragment.

Taxinine (retention time: 16.770 min, measured mass: 607.2907 m/z, molecular structure: $C_{35}H_{42}O_9$) is a diterpenoid with antifungal properties. The first fragmentation mass observed was 589.30 m/z, resulting from the loss of a hydroxyl group (-OH). The second fragmentation observed was at 533.25 m/z, indicating the loss of a C_6H_{12} fragment.

Lupenone (retention time: 17.711 min, measured mass: 425.3783 m/z, molecular structure: $C_{30}H_{48}O$) is a terpenoid with anti-inflammatory and antioxidant effects. The first fragmentation mass observed was 207.15 m/z, which corresponds to the loss of a side chain. The

second fragmentation mass observed was 189.14 m/z, corresponding to the loss of a C_2H_4O fragment.

The picogram values presented in the chromatogram reflect the concentrations of these compounds, with larger peaks corresponding to higher concentrations. The sensitivity of the LC–MS/MS technique enables the detection of these compounds even at low concentrations, making it a powerful tool for identifying bioactive metabolites in the gading coconut leaf extracts. These findings underscore the rich bioactive potential of the gading coconut leaf extract, with several compounds demonstrating significant therapeutic activities, including antiviral, anti-inflammatory, antioxidant, anticancer, antifungal, and UVB protection properties.

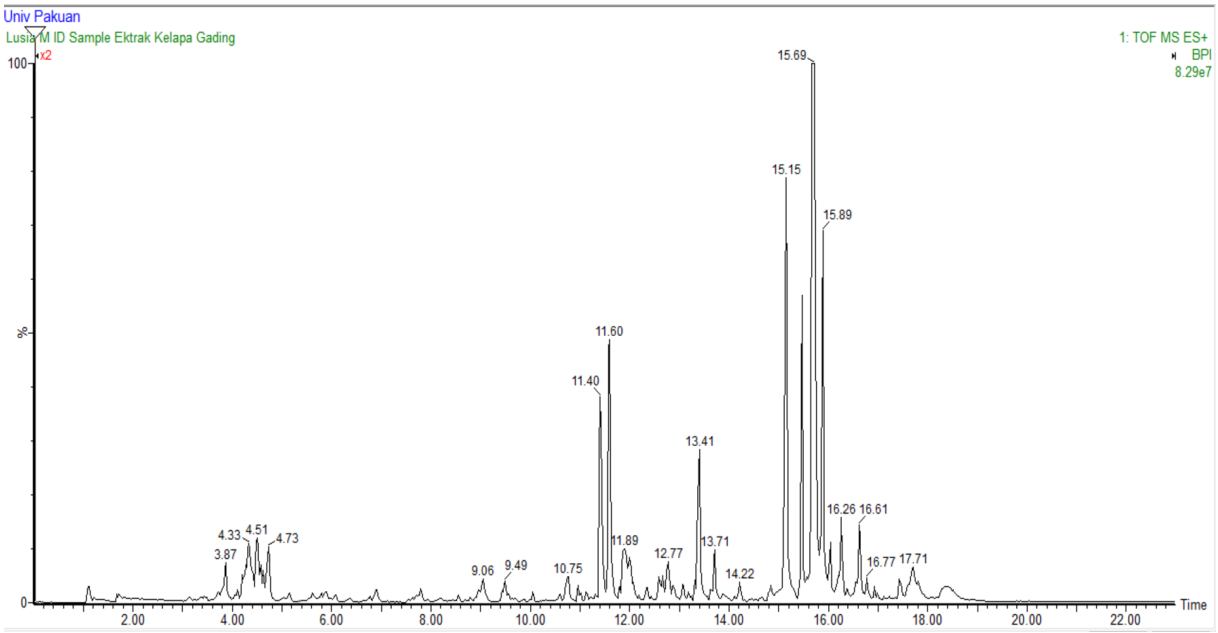


Figure 4. Base Peak Chromatogram of the Extract Sample from Coconut Leaf Sonication on the LC–MS/MS Instrument.

CONCLUSION

The extraction of *Cocos nucifera var. eburnea* leaves using sonication with 96% ethanol was found to be more efficient than maceration, yielding 9.48% with a total phenolic content of 147.10 mg GAE/g, which was not significantly different from that of maceration. However, the total flavonoid content reached 52.06 mg QE/g, which was significantly greater than that obtained through maceration. LC–MS/MS analysis revealed several compounds with potential antibacterial properties, including 3-hydroxycoumarin, isoorientin, vitexin, and

α-linolenic acid, as well as other compounds, such as 3,5-dimethyladamantane-1-carboxylic acid, octinoxate, indole, pheophorbide a, taxinine, and lupenone. Additionally, the extract demonstrated strong antibacterial activity at a concentration of 50 mg/mL against *Staphylococcus aureus* and *Escherichia coli*, with a larger inhibition zone observed for *Staphylococcus aureus*, likely due to the flavonoid content. These findings suggest that Cocos nucifera leaves have potential for further development as natural antibacterial products.

REFERENCES

Archana B., Mudavath, R.N., Enumula, V., N Ravali and Kumar, P.S. (2021). Evaluation of Antiepileptic Activity of Flowers of Cocos nucifera L. Against Experimentally Induced Convulsions in Rats. *Journal of Drug Delivery and Therapeutics*, 11(6); 159-166.

Belem-Kabre, W.L.M., Kabore, B., Compaore-Coulibaly, A., Traore, T.K., Thiombiano, E.A.M., Nebie-Traore, M., Compaore, M., Kini, F.B., Ouedraogo, S., Kiendrebeogo, M. and Ouedraogo, N. (2021). Phytochemical And Biological Investigations of Extracts From The Roots of Cocos nucifera L. (Arecaceae) And Carica papaya L. (Caricaceae), Two Plants Used In Traditional Medicine. *African Journal of Biochemistry Research*, 15(2); 28-35.

Burcu C., Hatipoglu, S.D., Donmez, A.A. and Gulacti T. (2015). Antioxidant And Anticholinesterase Activities of Lupane Triterpenoids And Other Constituents of Salvia Trichoclada. *Medical Chemistry Research*, 24(11); 6.

Chen, B., Hou, M., Zhang, B., Dang, L., Wang, Z., Liu, T. and Guo, Y. (2017). Enhancement of The Solubility And Antioxidant Capacity of A-Linolenic Acid Using An Oil In Water Microemulsion. *Food & Function*, 8(8); 9.

Dai Y, Qin C, Huang G, Qin Y, Huang Y, Huang Y, Zhao L. (2022). Linolenic Acid-Metronidazole: a Compound Relieving Drug Resistance and Inhibiting Helicobacter pylori. *Antimicrob Agents Chemother* 66:e00073-22.

D'Almeida, R.E., Molina, R.R.D.I., Viola, C.M., Luciardi, M.C., Nieto Peñalver, C., Bardón, A., Arena, M.E. (2017). Comparison of seven structurally related coumarins on the inhibition of

Quorum Sensing of Pseudomonas aeruginosa and Chromobacterium violaceum. *Bioorganic Chemistry*, 73(1); 37-38.

Erinle, B.A., Ajayi, A.O., & Osuntokun, O.R. (2021). Antibacterial properties of different husk extracts of Cocos nucifera (Linn) in South Western Nigeria. *South Asian Journal of Research in Microbiology*, 9(2), 26-30.

Farah D.S.W., Jumari, and Endah D.H. (2018). Kelapa Sebagai Komponen Bahan Ramuan Obat di Karaton Ngayogyakarta Hadiningrat dan Pura Pakualaman. *Jurnal Penelitian dan Pengembangan Pelayanan Kesehatan*, 2(1); 23-28.

Fathoni, A., Sumarlin, L.O., Putri, J.R., Fitriana, N. (2020). Antioxidant Activity of Mixed Katuk Leaf Extract and Honey. *EduChemia*, 5(2); 7275.

Friardi I., Desti, Widi N., Nurwahidatul A., Rustini Rustini, Putra, Prima P. (2021). TLC-Bioautographic and LC-MS/MS Detection of Antimicrobial Compounds from Four Semipolar Extracts of Cladonia Species. *Atlantis Press*, 40; 49-59.

Harmita, K., Harahap, Y., Supandi. (2019). Liquid Chromatography-Thandem Mass Spectrometry (LC-MS/MS). *ISFI Penerbit*, Jakarta.

Hakim, A., Mutiah, R., Aprinda, R., Suryadinata, A. and Maslakhah, F. N. (2019). Metabolite Profiling Bagian Akar, Batang, Daun, dan Biji Helianthus annuus L. Menggunakan Instrumen UPLC-MS. *Media Pharmaceutica Indonesiana*, 2(2); 64-81.

Irawan, A., Ulfah, M., Efriani, L., Hadi, I., Putra, T.A., & Puspitasari, A. (2023). Antibacterial activity of acetone coconut husk (Cocos nucifera Linn) extract against Staphylococcus aureus and Escherichia coli bacteria. *Jurnal Medisci*, 1(1).

Ivan A. Uy., Dapar, Mark Lloyd G., Aranas, Agnes T., Mindo, R.A.R., Cabrido, C.K., Torres, M.A.J., Manting, M.M.E. and Demayo, C.G. (2019). Qualitative Assessment of The Antimicrobial, Antioxidant, Phytochemical Properties of The Ethanolic Extracts of The Roots of Cocos Nucifera L. *Pharmacophore*, 10(2); 63-75.

James, J.J., D.Silva, Dylan, Saji Varghese, Jobi Xavier and Paari, K.A. (2019). Drinking Straw from Coconut Leaf: A Study of its Epicuticular Wax Content and Phenol Extrusion Properties. *Asian Journal of Plant Sciences*, 18(3); 142.

Jana Cahova, Jana Blahova, Jan Mares, Nikola Hodkovicova, Pavel Sauer, Hana Kocour Kroupova, Zdenka Svobodova, Octinoxate as a potential thyroid hormone disruptor – A combination of in vivo and in vitro data, *Science of The Total Environment*, Volume 856, Part 1, 2023, 159074.

Kibra, A.A., Kamrunessa and Rahman, M.M. (2018). Extraction And Evaluation of Phytochemicals From Green Coconut (Cocos nucifera) Shell. *Malaysian Journal of Halal Research Journal*, 1(2); 19-22.

Krettli, A.U., Aguiar, A.C.C., Andrade, I.M., Souza, N.B., and Adebayo, J.O. (2013). Antimalarial Activity of Cocos nucifera Husk Fibre: Further Studies. *Evidence-Based Complementary and Alternative Medicine*, Vol. 2013; 4-7.

Lima, E.B.C., Sousa, C.N.S., Meneses, L.N., Ximenes, N.C., Junior, Santos M.A., Vasconcelos, G.S., Lima, N.B.C., Patrocinio, M.C.A., D. Macedo and Vasconcelos, S.M.M. (2015). Cocos nucifera (L.) (Arecaceae): A phytochemical and Pharmacological Review. *Brazilian Journal of Medical And Biological Research*, 48(11); 954-957.

Malomo, S.O., Soladoye, A.O., Olatunji, L.A., Kowawole, O.M., Oguntoye, O.S., Babatunde, A.S., and Akinola, O.B. (2013). Antimalarial Activity of Cocos nucifera Husk Fibre: Further Studies. *Evidence-Based Complementary and Alternative Medicine*, Vol. 2013; 4-7.

Mazaya, G., Karseno, K., & Yanto, T. (2020). Antimicrobial and phytochemical activity of coconut shell extracts. *Turkish Journal of Agriculture-Food Science and Technology*, 8(5), 1090-1097.

Nor, M.E.M., Hussin, N., Salahuddin, M.A.H., Samah, A.H.A., Towhid, M.F., & Radzi, M.F.M. (2023). Evaluation of phenolic content and antibacterial activity of coconut (Cocos nucifera L.) shell and coir powder in different extraction solvents. *Journal of Tropical Plant Physiology*, 15(1), 9.

Obeng-Boateng, F., Kpordze, S.W., and Addy, F. (2024). In vitro antibacterial activity of Morinda citrifolia extracts against eight pathogenic bacteria species. *PLOS ONE*, 19(10), e0313003.

Pardede, A., Wardhani, R.A.A.K., Frasisca, E. (2020). Antileukemic Activity of Methanol Extract from Stem of Baccaurea macrocarpa,

Syzygium jambos, Bouea macrophylla Griff., and Diospyros discolor Willd. *EduChemia*, 5(2); 8320.

Pratama, Y., Mahardika, R.G., Adisyahputra, A. (2021). Antibacterial Activity of Nanoemulsion Stem Fraction Pucuk Idat (Cratoxylum glaucum). *EduChemia*, 6(2); 10242.

Romero-Estrada, A., Amalia M.M., Judith G.C., Silvia M.B., Maria Luisa G.R., Veronica R.L., and Laura Alvarez. (2016). Anti-Inflammatory And Antioxidative Effects of Six Pentacyclic Triterpenes Isolated From The Mexican Copal Resin of Bursera copallifera. *BMC Complementary and Alternative Medicine*, 16; 5.

Rudiana, T., Yala Merru, E.S., Hendrawati, D., Sukandar, D. (2022). Characterization and Anticancer Activity from Gaharu (Aquilaria malaccensis) Stem Bark Extract. *Educhemia*, 6(1); 10983.

Saide, A., Lauritano, C. and Adrianna Ianora. (2020). Pheophorbide a: State of the Art Assunta. *Marine Drugs*, 18(5); 9-10.

Saklani, P., Dora, K.C., Roy, S., Siddhnath, K., & Mandal, R. (2025). Total phenol and antimicrobial properties of green coconut husk extract. *Indian Journal of Animal Health*, 64(1), 169-174.

Salmeron-Manzano, Esther, Garrido-Cardenas, Jose A. and Manzano-Agugliaro, F. (2020). Worldwide Research Trends on Medicinal Plants. *International Journal of Environmental Research and Public Health*, 17(10); 3-4.

Stahl, E., Bellwon, P., Huber, S., Schlaeppi, K., Bernsdorff, F., Vallat Michel, A., Felix Mauch and Jurgen Zeier. (2016). Regulatory And Functional Aspects of Indolic Metabolism In Plant Systemic Acquired Resistance. *Molecular Plant*, 9(5); 662-681.

Suryani, N., Julaeha, E., Herlina, T. (2021). Dihydrokaempferol-3-O-Rhamnoside from Ethyl Acetate Extract of Ki Encok (Plumbago zeylanica L.) Leaves. *EduChemia*, 6(1).

Tayler, N.M., De Jesús, R., Spadafora, R., Coronado, L.M., & Spadafora, C. (2020). Antiplasmodial activity of Cocos nucifera leaves in Plasmodium berghei-infected mice. *Journal of Parasitic Diseases*, 44(2), 305-313.

Todorov L, Saso L, Kostova I. Antioxidant Activity of Coumarins and Their Metal Complexes. *Pharmaceuticals (Basel)*. 2023 Apr 26;16(5):651. doi: 10.3390/ph16050651.

Tulin Askun. (2023). Investigation of Anti-Mycobacterial Activity of Orientin and Vitexin on the Six Mycobacterium tuberculosis Strains. *European Journal of Biology*, 82(2); 129.

Ukaoma, A.A., Iwu, I.C., Udensi, J., Ukachukwu, V.I., Anyika, L.C., Nwogwugwu, N.U., Umeh, S., Kenechukwudozie, O.Q., Duru, C.C., Chinakwe, E., Nnadozie, I., & Anyanwu, C.O. (2024). Phytochemical composition and antimicrobial activity of coconut husk (Cocos nucifera): A sustainable solution to antimicrobial resistance. *African Journal of Biomedical Research*, 27(3S). https://doi.org/10.53555/AJBR.v27i3S.6963

Wang, Xiao, Shenyuan He, Li Yuan, Hong Deng, and Zhong Zhang. (2021). Synthesis, Structure Characterization, and Antioxidant and Antibacterial Activity Study of Iso-orientin−Zinc Complex. *Agricultural and Food Chemistry*, 2021(69); 3952-3962.

Ye, M., Glauser, G., Lou, Y., Erb, M. and Hu, L. (2019). Molecular Dissection of Early Defense Signaling Underlying Volatile-Mediated Defense Regulation And Herbivore Resistance In Rice. *Plant Cell*, 31, 687–698.

Zhang, Xin Zhong, Craig A. Downs, Yuting Li, Zishan Zhang, Yiman Li, Binbin Liu, Huiyuan Gao and Qingming Li. (2020). Comparison of Toxicological Effects of Oxybenzone, Avobenzone, Octocrylene, And Octinoxate Sunscreen Ingredients On Cucumber Plants (Cucumis Sativus L.). *Science of The Total Environment*, 714(2020); 1-11.

Zhao, L., Qin, C., Huang, Y., Huang Y., Huang Y., Qin Y. (2022). Linolenic Acid-Metronidazole: a Compound Relieving Drug Resistance and Inhibiting Helicobacter pylori. *Antimicrob Agents Chemother*, 66:e00073-22