

Article

Compound Profile of Methanol Extract of Katang-Katang Leaves (*Ipomoea pes-caprae*) and Evaluation of Its Antibacterial and Antioxidant Activities

Article Info

Article history :

Received November 15, 2025

Revised December 29, 2025

Accepted January 05, 2026

Published March 30, 2026

In Press

Keywords :

Ipomoea pes-caprae,
metabolit sekunder,
antioksidan,
antibakteri,
LC-HRMS

Dessy Rosye Rooy¹, Imanuel Berly Delvis Kapelle^{2*},
Rosmawaty²

¹Postgraduate Students of Chemistry Study Program, Pattimura University, Maluku, Indonesia

²Department of Chemistry, Faculty of Science and Technology, Universitas Pattimura, Ambon, Indonesia

Abstract. Infectious diseases remain a major health problem, with *Staphylococcus aureus* being an opportunistic pathogen that frequently causes skin infections. Increasing antibiotic resistance has prompted the search for safer and more sustainable natural alternatives. *Ipomoea pes-caprae*, a coastal plant traditionally used for the treatment of wounds and inflammation, is known to be rich in secondary metabolites such as flavonoids, terpenoids, alkaloids, and phenolic acids that have potential antioxidant and antibacterial properties. This study aimed to map the metabolite profile of the methanolic extract of *I. pes-caprae* leaves using LC-HRMS, to analyze its relationship with antioxidant activity measured by the DPPH method, and to evaluate its antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*. LC-HRMS analysis identified 406 compounds with Palmitic Acid as the dominant component (24.503%), including primary and secondary metabolite groups such as flavonoids (quercetin, rutin, kaempferol), phenolic acids (caffeic, ferulic, chlorogenic acid), terpenoids (caryophyllene oxide, lupeol), alkaloids, and phytohormones. The methanol extract showed moderate antioxidant activity ($IC_{50} = 136.71$ ppm) and antibacterial activity against *E. coli* (inhibition zone 9.5 mm), which is thought to originate from the synergy of various secondary metabolites through free radical scavenging mechanisms and bacterial membrane damage. These findings strengthen the scientific basis for *I. pes-caprae* as a natural source of antioxidant and antibacterial agents for the development of natural pharmaceutical products.

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Corresponding Author :

Immanuel Berly Delvis Kapelle
Department of Chemistry, Faculty of Science and Technology, Universitas Pattimura,
Ambon, Indonesia
Email : berly_mollucas@yahoo.com

1. Introduction

Infectious diseases are a major health problem in developing countries, including Indonesia, and often affect the skin. Under certain conditions, normal flora such as *Staphylococcus aureus* can transform into opportunistic pathogens and cause serious infections such as impetigo, folliculitis, erysipelas, and cellulitis [1]. The increasing bacterial resistance due to inappropriate antibiotic use has prompted the need for safer and more sustainable alternative natural therapies. Medicinal plants have long been utilized by the community and are recommended by the WHO as part of the prevention and treatment of various diseases, including chronic and degenerative diseases [2–3]. Medicinal plants are rich in secondary metabolites such as phenols, triterpenoids, alkaloids, and fatty acids that act as antioxidants and antibacterials [4–5]. Their composition is strongly influenced by solvent polarity, making solvent selection a critical factor in determining biological activity [6].

Ipomoea pes-caprae (L.) is a promising medicinal plant commonly found on sandy beaches. It has a brownish-green stem measuring 5–30 cm in length, thick, simple leaves, reddish-purple flowers, and black capsule fruits [7–8]. Traditionally, this plant has been used to treat wounds, inflammation, digestive disorders, pain, and stings from marine organisms [9–10]. Various studies have reported the anti-inflammatory, antinociceptive, antibacterial, antifungal, anticancer, anti-sunscreen, and antioxidant activities of this plant, supported by its flavonoid, terpenoid, steroid, saponin, and tannin content [11–13]. The antioxidant activity of *I. pes-caprae* is highly relevant because free radicals play a role in collagen degradation and premature skin aging. In contrast, natural plant antioxidants can protect tissues from oxidative damage [14].

Accurate identification of *I. pes-caprae* bioactive compounds requires modern analytical techniques such as LC-HRMS, which can provide structural information based on retention time and mass with high precision [15]. The use of this technique is essential for obtaining a comprehensive metabolite profile that can serve as a scientific basis for the development of natural pharmaceutical preparations [16]. Although various research results have been reported, several gaps remain. These include the limited number of studies assessing the relationship between metabolite profiles extracted from specific solvents and antioxidant and antibacterial activity, the limited use of LC-HRMS for comprehensive characterization of *I. pes-caprae* leaf metabolites, and the limited number of studies simultaneously testing antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*.

Based on these gaps, this study aims to (1) comprehensively map the secondary metabolite profiles of *I. pes-caprae* leaves extracted from methanol using LC-HRMS, (2) analyze the potential relationship between identified metabolites and DPPH antioxidant activity and antibacterial activity, and (3) compare the metabolite profiles and biological activities obtained with previous studies to strengthen the scientific basis for this plant's potential. The novelty of this study lies in the use of an integrated approach consisting of methanol extraction, metabolite characterization using LC-HRMS, antioxidant and antibacterial activity testing, and simultaneous evaluation against *S. aureus* and *E. coli* [17]. This approach is expected to provide a stronger scientific basis for the use of *I. pes-caprae* as a source of antioxidants and antibacterials for the development of natural pharmaceutical products.

2. Experimental Section

2.1. Extract Preparation

The katang-katang leaves were dried and ground into powder. The powder was gradually extracted with methanol using a maceration method. The resulting extract was filtered and concentrated using a rotary evaporator. The katang-katang leaves were dried at room temperature for 5–7 days, then ground to a fine powder. A total of 200 g of powder was extracted using 96% methanol with a material: solvent ratio of 1:10 (w/v). The maceration was carried out at room temperature (25–27°C) for 3 x 24 hours, with occasional stirring. The filtrates from each maceration were filtered and combined, then evaporated using a rotary evaporator at 60°C to obtain a thick extract. The extract was dried in a desiccator, and the yield (%) was calculated using the formula:

$$\text{Yield (\%)} = (\text{weight of thick extract}) / (\text{weight of initial sample}) \times 100\%$$

2.2. Characterization of Secondary Metabolite Compounds

The extract was analyzed using Liquid Chromatography-High Resolution Mass Spectrometry (LC-HRMS) to identify secondary metabolites, including triterpenoids, alkaloids, phenols, and fatty acids. Secondary metabolite analysis was performed using LC-HRMS. The sample was dissolved in methanol (1 mg/mL concentration) and then filtered through a 0.22 μm membrane. The equipment used was a Q-TOF (Quadrupole Time-of-Flight) LC-HRMS. Separation was performed on a C18 column (2.1 x 100 mm, 1.7 μm). The gradient program was: 5% B (0–1 minute), increasing to 95% B (1–15 minutes), holding at 95% B (15–20 minutes), then returning to the initial conditions at 22 minutes. The flow rate was 0.3 mL/minute, the column temperature was 40°C, and the injection volume was 5 μL. Ionization was performed using electrospray ionization (ESI) with positive and negative ion modes (ESI+ and ESI-). Data acquisition was performed in the m/z range 100–1200.

2.3. Antioxidant Test

Antioxidant activity was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method. A color change from purple to paler indicates antioxidant activity. The IC₅₀ value was calculated from the linear relationship between the extract concentration and the percentage of DPPH inhibition. Antioxidant activity was determined using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method. A 0.1 mM DPPH solution was prepared in methanol. The extract was tested at several concentrations, while vitamin C was used as a reference standard. 1 mL of the extract solution was mixed with 1 mL of the DPPH solution and incubated for 30 minutes at room temperature in the dark. Absorbance was measured at 515 nm using a UV-Vis spectrophotometer. The percentage of inhibition was calculated using the formula:

$$\% \text{Inhibisi} = \frac{A_0 - A_S}{A_0} \times 100\%$$

where A₀ = control DPPH absorbance, and A_S = sample absorbance. The IC₅₀ value was determined from a linear regression of extract concentration against DPPH inhibition percentage.

2.4. Antibacterial Test

The antibacterial test was conducted using the well diffusion method. The test bacteria included Gram-positive *Staphylococcus aureus* and Gram-negative *Escherichia coli*. Bacterial cultures were adjusted to 0.5 McFarland ($\approx 1 \times 10^8$ CFU/mL). The medium used was Mueller-Hinton Agar (MHA). Wells were made with a diameter of 6 mm, then filled with extracts at concentrations of 25%, 50%, 75%, and 100%, each with a volume of 50 μL. Amoxicillin was used as the positive control, and methanol as the negative control. The plates were incubated at 37°C for 24 hours, and the inhibition zone diameter was then measured. The test was performed in triplicate. Antibacterial activity was categorized based on the diameter of the inhibition zone [18].

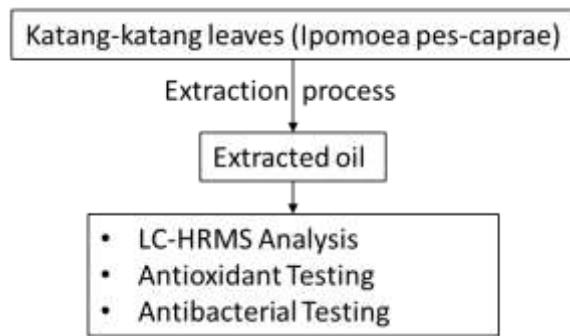


Figure 1. Research flow diagram

3. Results and Discussion

Ipomoea pes-caprae leaves (Figure 2) are known to contain a variety of secondary metabolites that can be extracted using different solvents, resulting in varying biological activities. In this study, extraction was carried out using the maceration method, which was chosen because it is commonly used to obtain most phytochemicals, is easy to apply, time-efficient, and capable of retaining thermolabile compounds [19]. Extract yield is influenced by the solvent type and extraction method [20]. Polar solvents such as methanol gave the highest yield, namely 53.2%, indicating the dominance of polar compounds in these leaves.



Figure 2. Katang-katang leaf sample

Identification of compounds in katang-katang leaf extract was performed using LC-HRMS (Liquid Chromatography–High Resolution Mass Spectrometry), which provides a more sensitive and detailed metabolomic profile, encompassing primary and minor metabolites [21]. Characterization results showed that the methanol extract contained 406 compounds, including both primary and secondary metabolites, with palmitic acid (24.503%) as the dominant component (Figures 2 and 3).

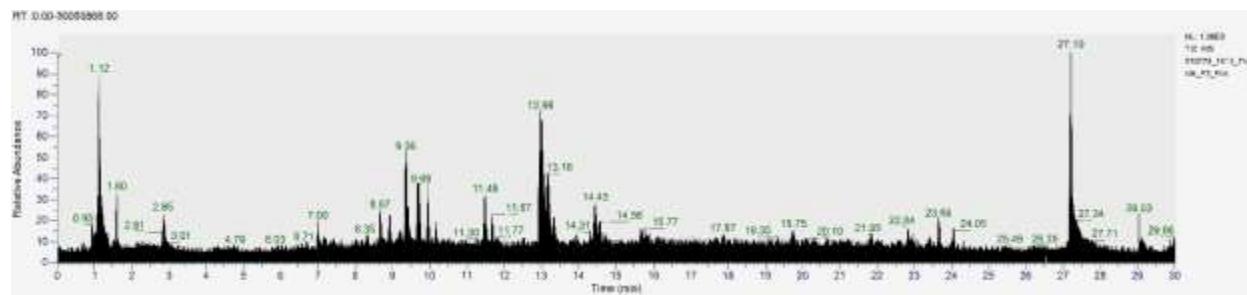


Figure 3. LC-HRMS Results of Positive Ion Methanol Extract

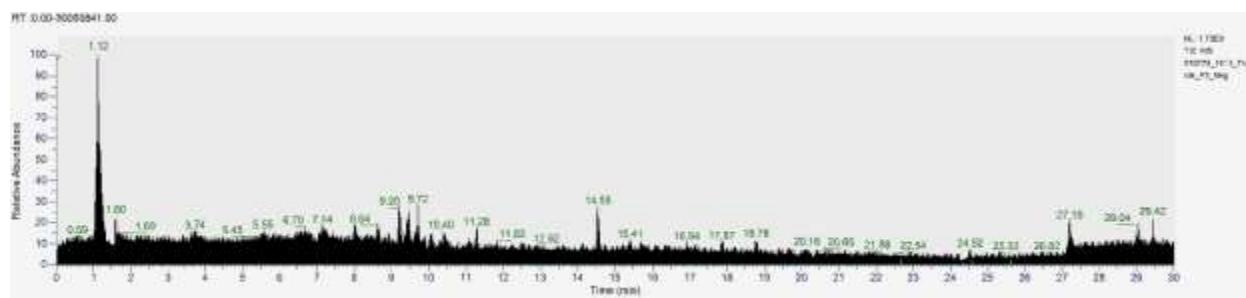


Figure 4. LC-HRMS Results of Negative Ion Methanol Extract

Metabolites detected in the methanol extract of katang-katang leaves can be grouped into two main categories. Primary metabolites include amino acids and their derivatives (asparagine, leucine, betaine, DL-stachydrine), sugars and their derivatives (glucose, maltose, arabitol), organic acids (quinic acid, malic acid, caffeic acid), lipids and fatty acids (palmitic acid, palmitoleic acid, α -linolenic acid), nucleotides and vitamins (adenine, nicotinamide), and physiological compounds (phloroglucinol, sphingosine). Secondary metabolites consist of phenolic acids and caffeic derivatives (caffeic acid, ferulic acid, chlorogenic acid, dicafeoylquinic acid), flavonoids (quercetin, rutin, kaempferol), terpenoids and sterols (caryophyllene oxide, lupeol), phytohormones (indole-3-acetic acid, jasmonic acid, abscisic acid), alkaloids and heterocyclic compounds (tropine, norharman), and aromatic/phenolic compounds (salicylic acid, pyrogallol, gingerol). These results indicate that the methanol extract of katang-katang leaves possesses a very broad chemical diversity, potentially contributing to the plant's biological activity [21].

Table 1. Dominant Components of Methanol Extract

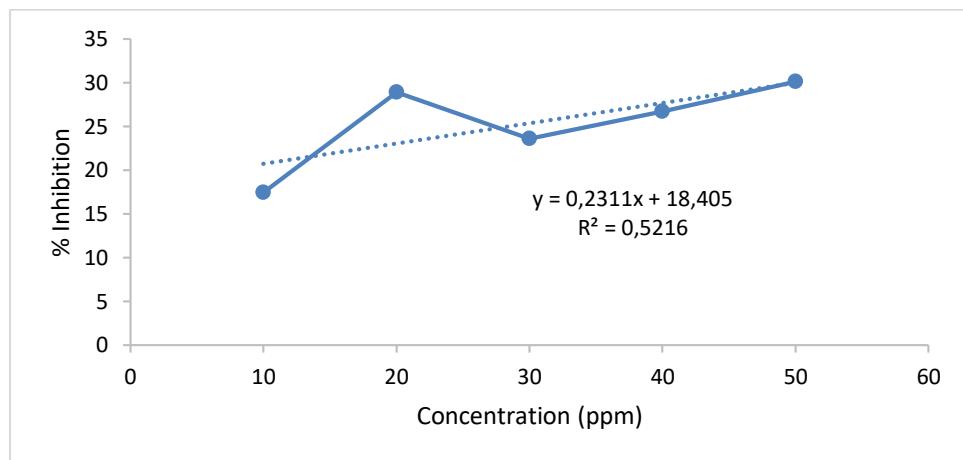
No	Nama Senyawa	Rumus Molekul	% Area
1	Palmitic Acid	$C_{16}H_{32}O_2$	24.503
2	2-Amino-1,3,4-octadecanetriol	$C_{18}H_{39}NO_3$	8.277
3	Betaine	$C_5H_{11}NO_2$	5.467
4	DL-Stachydrine	$C_7H_{13}NO_2$	5.465
5	D-(-)-Quinic acid	$C_7H_{12}O_6$	3.179
6	4,5-Dicaffeoylquinic acid	$C_{25}H_{24}O_{12}$	2.392
7	4,5-Dicaffeoylquinic acid	$C_{25}H_{24}O_{12}$	2.224
8	D-(+)-Arabitol	$C_5H_{12}O_5$	1.627
9	4,5-Dicaffeoylquinic acid	$C_{25}H_{24}O_{12}$	1.605
10	D-(+)-Malic acid	$C_4H_6O_5$	1.560

The content of secondary metabolites in *Ipomoea pes-caprae* leaves, such as alkaloids, triterpenoids, and phenolic compounds, is thought to play an important role in antioxidant and antibacterial activity [22]. Alkaloids, for example, function as bioprotective agents against neurotoxins [7, 23] and protect plants from herbivores; thus, their presence reflects a natural defense mechanism against herbivore attack [4]. The antioxidant activity of leaf extracts was evaluated using the DPPH (1,1-diphenyl-2-picrylhydrazyl) method, which is relatively easy, fast, and more sensitive than other methods [24-26].

Table 2. Antioxidant activity results

No	Concentration (ppm)	Average Absorbance	% Inhibition
1	10	0.686	17.44 %
2	20	0.591	28.88 %
3	30	0.635	23.58 %
4	40	0.609	26.71 %
5	50	0.581	30.08 %

DPPH is a purple-colored free radical with a maximum wavelength of 500–610 nm, and its absorbance is measured at 517 nm using a UV-Vis spectrophotometer [27]. DPPH solution can absorb hydrogen atoms from other compounds, such as phenolic compounds, so the purple color indicates the presence of free electrons. The DPPH antioxidant mechanism involves the transfer of electrons or hydrogen from phenolic compounds, leading to oxidation of the DPPH solution [28]. In this test, the DPPH solution oxidizes antioxidant compounds found in plant extracts. Extracts were tested at concentrations of 10–50 ppm, and the percentage inhibition (% inhibition) was calculated to assess the extract's ability to scavenge free radicals. The test results showed a line equation of $y = 0.2311x + 18.405$ with an IC_{50} value of 136.71 ppm ($\mu\text{g/mL}$), which is categorized as moderate antioxidant activity (100–150 ppm) [4]. The antioxidant activity categories of a compound based on its IC_{50} value are: <50 ppm very strong, 50–100 ppm strong, 100–150 ppm moderate, 150–200 ppm weak, and >200 ppm very weak [4].

**Figure 5.** Antioxidant activity curve of methanol

The antioxidant activity of medicinal plants is strongly correlated with the presence of phenolic compounds, because the hydroxyl group (-OH) in phenolic compounds can donate hydrogen atoms to stabilize free radicals [29–30]. Based on LC-HRMS characterization, flavonoids such as quercetin, rutin, kaempferol, trifolin, and tangeritin have the strongest antioxidant activity, primarily through the free radical scavenging mechanism using the phenolic OH group in the DPPH and ABTS assays. Phenolic acids and caffeic derivatives (including caffeic, ferulic, chlorogenic, and isoferulic acids) exhibit strong to moderate antioxidant potential through electron and proton donor mechanisms to inhibit lipid oxidation. Coumarin groups and cinnamate derivatives, such as 4-hydroxycoumarin and 4-methoxycinnamic acid, exert moderate effects primarily through free radical stabilization. Lipophilic compounds such as 6-gingerol exhibit moderate antioxidant activity and are effective in oil systems by scavenging ROS. Meanwhile, phenolic organic acids such as salicylic and gentisic acids

have moderate potency, acting as both antioxidants and anti-inflammatory agents. In general, phenolic compounds are secondary metabolites with potential as antioxidant agents because they contain hydroxyl groups (-OH) that can donate hydrogen atoms to free radicals via an electron-transfer mechanism [31].

Table 3. Antibacterial activity of methanol extract of katang-katang leaves

Bacteria	Sample concentration	Inhibition zone diameter (mm)		
		Methanol extract	Positive control	Negative control
<i>Staphylococcus aureus</i> ATCC 6538	25	-		
	50	-	29 ± 0.1	-
	75	-		
	100	-		
<i>Escherichia coli</i> ATCC 8739	25	-		
	50	-	40 ± 0.1	-
	75	-		
	100	9.5 ± 0.06		

In addition to antioxidant activity, *Ipomoea pes-caprae* leaf extract was tested for its effect on the growth of Gram-positive *Staphylococcus aureus* and Gram-negative *Escherichia coli* using the well-diffusion method [32]. The extract was tested at concentrations of 25%, 50%, 75%, and 100%, with antibacterial activity measured as the diameter of the inhibition zone (mm) on nutrient agar after incubation at 37°C for 24 hours. The test results showed an inhibition zone of 9.5 mm on *E. coli*, categorized as moderate to weak antibacterial activity according to the Riski classification [18], where <5 mm (weak), 5–10 mm (mild), 10–20 mm (strong), and >20 mm (very strong). This antibacterial activity is primarily derived from secondary metabolites in the leaves, including flavonoids (quercetin, rutin, kaempferol), phenolic acids (caffeic acid, ferulic acid, chlorogenic acid), terpenoids (caryophyllene oxide, eucalyptol, lupeol), coumarins (7-hydroxycoumarin), and unsaturated fatty acids (α-linolenic acid), which work by damaging membranes and cell walls, disrupting enzyme activity, and triggering leakage of cell contents [33].

Antibacterial effectiveness depends on the concentration of the extract; the higher the concentration, the larger the inhibition zone, due to a greater number of active substances, which increase interaction with bacterial proteins and DNA, potentially causing damage to the cell nucleus and lysis [34]. Furthermore, the type of extraction solvent affects antibacterial activity because the active compound dissolved depends on the solvent's polarity, according to the principle of like dissolves like [35]. In comparison, amoxicillin exhibits very strong activity because it inhibits bacterial cell wall synthesis [36]. The antibacterial mechanism of *I. pes-caprae* leaves involves the synergy of various classes of secondary metabolites [37]. Flavonoids such as quercetin, rutin, and kaempferol damage bacterial membranes, inhibit topoisomerase enzymes, and disrupt protein synthesis, effectively targeting *E. coli*. Phenolic acids, including caffeic acid, ferulic acid, and chlorogenic acid, disrupt membrane permeability and inactivate enzymes, thus exerting moderate to strong antibacterial effects. Terpenoids and essential oil components (caryophyllene oxide, eucalyptol, lupeol, 6-gingerol) enhance activity by disrupting cell walls and leaking cellular contents. Coumarins (7-hydroxycoumarin, 4-hydroxycoumarin) target DNA and membranes, while unsaturated fatty acids (α-linolenic acid, palmitoleic acid) act as surfactants that can lyse bacterial cell membranes.

Overall, *I. pes-caprae* leaves are a rich source of bioactive compounds, including primary and secondary metabolites such as flavonoids, phenolic acids, terpenoids, alkaloids, and phytohormones, which contribute significantly to their antioxidant and antibacterial activities. This supports the

biological potential of these leaves as a natural bioactive agent that is effective against both Gram-positive and Gram-negative bacteria, including *S. aureus* and *E. coli* [38].

4. Conclusion

This study comprehensively mapped more than 406 metabolites in *Ipomoea pes-caprae* leaves using LC-HRMS. It showed that phenolic compounds and flavonoids play a major role in antioxidant activity ($IC_{50} = 136.71$ ppm) and in antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*. Compared with previous studies, this study provides a more complete metabolite profile and biological data, thus further strengthening the potential of *I. pes-caprae* leaves as a natural source of antioxidant and antibacterial agents for the development of natural pharmaceutical products.

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