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Baccaurea lanceolata Pericarpium as a Potential Natural Antibacterial Agent Against *Staphylococcus epidermidis*

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ABSTRACT

Introduction: *Natural plant-based compounds have gained increasing attention as alternative antibacterial agents, particularly in response to rising antibiotic resistance.* ***Aims***: *This study aimed to assess the antibacterial activity of limpasu (Baccaurea lanceolata) pericarpium extracts against S. epidermidis and to relate their effectiveness to flavonoid and phenolic contents.* ***Result:*** *The antibacterial activity and phytochemical composition of limpasu pericarp extracts were evaluated against S. epidermidis. Extracts were prepared using solvents of varying polarities: n-hexane, ethyl acetate (EtOAc), and methanol (MeOH). Antibacterial activity was assessed via the disc diffusion method at concentrations of 10%, 20%, 40%, and 80%. The EtOAc extract exhibited the strongest inhibitory effect, with inhibition zones increasing dose-dependently, reaching up to 24.33±1.63 mm at 80%. The n-hexane extract showed the least antibacterial activity, whereas MeOH extract exhibited moderate inhibition. Phytochemical analysis revealed that the EtOAc extract had the highest total flavonoid content (29.16 ± 0.49 mg quercetin/g extract) and total phenolic content (2.12 ± 0.11 mg GAE/g extract), correlating with its superior bioactivity.* ***Conclusion:*** *These findings suggest that limpasu pericarpium, particularly its EtOAc extract, holds promise as a natural antibacterial agent against S. epidermidis, possibly due to its high phenolic and flavonoid contents.*

KEYWORDS: *Baccaurea lanceolata*, pericarpium, antibacterial, *S. epidermidis, phenol and flavonoid content.*

INTRODUCTION

Natural products derived from medicinal plants have long played a significant role in the development of therapeutic agents, especially in the treatment of chronic and infectious diseases. Many modern drugs have their origins in traditional remedies, with plant-derived compounds offering a diverse array of bioactive constituents (Aware et al., 2022; Chaachouay & Zidane, 2024). Among these, polyphenolic compounds such as flavonoids and tannins have been extensively studied for their antioxidant, anti-inflammatory, and antimicrobial properties (Kumar & Pandey, 2013; Nijveldt et al., 2001; Zeinali et al., 2017). As the global burden of drug-resistant infections continues to rise, there is a growing interest in exploring lesser-known medicinal plants for their potential pharmacological benefits (Li et al., 2019). However, the

increasing emergence of multidrug-resistant bacteria has created an urgent need to discover new antibacterial agents from natural sources. Therefore, this study focuses on investigating the antibacterial activity of *Baccaurea lanceolata*, a lesser-known medicinal plant, to evaluate its potential as a source of novel antimicrobial compounds.

Baccaurea lanceolata, a lesser-known species belonging to the Phyllanthaceae family, is a tropical plant distributed mainly in Southeast Asia, including Indonesia, Malaysia, and Thailand. It is commonly referred to as limpasu in local languages. Traditionally, the fruit, leaves, and stems of this plant have been used by local communities to treat a variety of ailments, including fever, gastrointestinal disturbances, and skin wounds (Noor & Asih, 2018; Uluk et al., 2001). Despite its long-standing use in ethnomedicine, scientific studies on the pharmacological activities of *B. lanceolata* remain limited, and its bioactive constituents are not yet fully characterized.

One of the most pressing health challenges today is the emergence and spread of antibiotic-resistant bacteria (Vuong et al., 2016). *Staphylococcus epidermidis*, a coagulase-negative Staphylococcus species commonly found on human skin, has become increasingly recognized for its role in nosocomial infections, particularly in immunocompromised patients and those with implanted medical devices. The development of resistance to conventional antibiotics in *S. epidermidis* and other pathogens necessitates the search for novel antibacterial agents, particularly from natural sources (Jubair et al., 2021; Li et al., 2019).

Plant-derived polyphenolic compounds have shown great promise in this context. These compounds exert antimicrobial effects through multiple mechanisms, including disruption of bacterial cell walls, interference with nucleic acid synthesis, and inhibition of critical microbial enzymes (Zamzani & Triadisti, 2020). In particular, flavonoids are known to possess broad-spectrum antibacterial activity, along with antioxidant, antidiabetic, and anti-inflammatory effects (Narain et al., 2019; Bakar et al., 2014).

Several species within the *Baccaurea* genus have demonstrated notable pharmacological properties. For instance, *B. courtallensis* has been reported to exhibit anti-inflammatory and antioxidant activities (Babu & Surekha, 2019; Jasim A et al., 2019), while *B. macrocarpa* shows both antioxidant and antibacterial effects (Erwin et al., 2019; Zamzani & Triadisti, 2019). *B. ramiflora* has also been shown to possess antifungal activity (Pan et al., 2015). These biological activities are largely attributed to the presence of secondary metabolites such as flavonoids, tannins, and saponins (S. Fitriansyah et al., 2018).

Despite the promising activities reported for other *Baccaurea* species, limited data exist regarding the phytochemical content and antibacterial potential of *B. lanceolata*, particularly its fruit pericarp. Therefore, this

study aims to investigate the antibacterial activity of *B. lanceolata* pericarp extracts against *S. epidermidis* and to evaluate their flavonoid and phenolic contents. By identifying bioactive compounds and linking them to antibacterial effects, this research may contribute to the discovery of novel natural agents for combating antibiotic-resistant infections.

METHODS

Chemicals and Instrumentation

This study has used analytical grade n-hexane, ethyl acetate (EtOAc), and methanol (MeOH), Folin-Ciocalteu's phenol reagent, distilled water. *S. epidermidis* (ATCC 12228), Clindamycin, and nutrient agar (Oxoid). Centrifugation was done using a centrifuge (MSE, UK), and a digital weighing scale (Ohaus, USA) was used in weighing operations. Absorbance was measured with a UV-Vis spectrophotometer (UV 1800, Shimadzu, Japan).

Sampel Preparation and Extraction

The process of preparing simple drugs and performing the extraction stages was carried out following the methods outlined by Zamzani & Triadisti, (2021). The pericarp of *limpasu* fruit was washed, cut into small pieces, and air-dried at 18–19 °C for 10 days. The dried material was ground to obtain 1.5 kg of powder. The powder was sequentially macerated with n-hexane, ethyl acetate, and methanol (15 L each), with solvent replacement every 5 days. Each extract was filtered and the solvent was removed under ambient aeration to yield the respective n-hexane, ethyl acetate, and methanol fractions (Zamzani & Triadisti, 2021).

Phytochemical analysis

The phytochemical composition of each extract was determined using standard phytochemical methods described by Harborne (1998), including alkaloid, flavonoid, saponin, steroid/triterpenoid, and tannin.

Determination of Total Phenolic Content (TPC)

The total polyphenol content was assessed using the Folin–Ciocalteu method, as modified from the procedure described by Singleton & Rossi method. Five hundred microlitres of the extract was added to two hundred and fifty microlitres of Folin-Ciocalteu reagent and 4 mL of methanol then vortexed. Seven hundred and fifty microlitres of Na_2CO_3 was added to the mixture after 8 min reaction time. Thereafter, the mixture was vortexed thoroughly. The absorbance of the resulting blue color was measured at 765 nm against a blank after 30 min of incubation at room temperature. All samples and reading were prepared and measured in triplicate. Gallic acid was used as the standard, and TPC was expressed as milligram of gallic acid equivalent (GAE) per gram extract (Singleton & Rossi, 1965).

Table 1. Result of phytochemical screening of limpasu pericarpium extract

Phytochemical	Test	N-Haxane	EtOAc	MeOH
	Dregendorff's	+	+	+
Alkaloids	Mayer's	+	+	+
	Bourchardat's	+	+	+
Flavonoids	Mg + HCL	-	+	+
Saponins	Froth test	+	+	+
Steroids/Terpenoids	Liebermann-Burchard	+	+	-
Tannins	$FeCl_3$	-	-	+

Determination of Total Flavonoid Content (TFC)

The TFC of each sample were determined using the colorimetric method Bahorun method with minor modification. Briefly, 250 and 500 µL of extract solution was diluted with 2 mL distilled water and mixed with 150 µL of 5% $NaNO_2$. After 5 min, 150 µL of 10% $AlCl_3$ was added and then incubated for 5 min and 150 µL of 1M NaOH was subsequently added. The absorbance was measured immediately at 510 nm. Quercetin was used as the standard, and TFC was expressed as milligram of quercetin equivalent (QE) per gram extract with the similar formulation was used for calculation (Bahorun et al., 1996).

Antibacterial activity

The antibacterial activity of limpasu pericarpium extracts was evaluated using the agar well diffusion method, as previously described by Zamzani & Triadisti method. Extracts were tested at four different concentrations: 10%, 20%, 40%, and 80% (v/v). Sterile Mueller–Hinton agar plates were inoculated with *Staphylococcus epidermidis* using the lawn culture method. Wells (6 mm diameter) were made in the agar using a sterile borer, and 100 µL of each extract concentration was loaded into individual wells. A disc impregnated with clindamycin was used as the positive control, while a disc loaded with 10 µL of DMSO served as the negative control. The plates were incubated at 37 °C for 24 hours, after which the diameter of the inhibition zones (in mm) surrounding each well or disc was measured. The antibacterial activity was determined based on the size of the clear zones formed, indicating bacterial growth inhibition. Each treatment was conducted in triplicate to ensure reproducibility (Zamzani & Triadisti, 2020)..

RESULTS AND DISCUSSION

The preliminary phytochemical analysis of limpasu pericarpium extracts revealed the presence of key phytochemical components, as outlined in Table 1.

The results of TPC and TFC are presented in Table 2. EtOAc extract has the highest TPC and TFC value of 29.16 ± 0.49 mg GAE/g and 2.12 ± 0.11 mg QE/g, respectively.

Several previous investigations have highlighted limpasu as a notable source of phenolic and flavonoid compounds. For intstance Bakar et al., (2014) demonstrated

Table 2. TFC and TPC of limpasu pericarpium extract

Extract	TFC (mg quercetin/ g extract)	TPC (mg GAE/ g extract)
n-Hexane	-	-
EtOAc	29.16 ± 0.49	2.12 ± 0.11
MeOH	11.37 ± 0.52	1.113 ± 0.4

that the ethanolic extract of the flesh possessed the highest total phenolic content (4.81 mg GAE/g extract) compared to the pericarp and seed parts. Supporting this, Fitriansyah et al., (2019) reported that the ethanol extract of limpasu leaves exhibited exceptionally high levels of both phenolics and flavonoids, amounting to 1.402 g GAE/100 g extract (14.02 mg GAE/g) and 46.006 g QE/100 g extract (460.06 mg QE/g), respectively. These findings reinforce the role of limpasu as a rich reservoir of polar secondary metabolites. Moreover, they emphasize the critical influence of plant part selection and solvent polarity on the efficiency of phytochemical extraction. The substantial TFC and TPC values observed in the ethyl acetate extract of the pericarp in this study further indicate that semi-polar solvents like EtOAc are effective for targeting bioactive constituents, making them valuable for downstream bioactivity-guided fractionation.

Using the disc diffusion methodology, the antibacterial activities of the limpasu pericarpium extract. The zones of inhibition were noted and the results are presented in Figure 1.

Currently, the extensive use of antibiotics has led to a gradual rise in drug-resistant bacterial infections, significantly compromising the effectiveness of antibacterial treatments and posing serious risks to patient safety. Therefore, significant research efforts have been focused on exploring herbal plants because they represent a potentially new class of natural antibiotics with broad antibacterial spectrum, rare adverse reactions, and low tendency to generate drug resistance.

The antibacterial assay revealed that the EtOAc extract exhibited the strongest activity, with inhibition zones increasing from 18.50±1.08 mm at 10% to 24.33±1.63 mm at 80%, surpassing clindamycin at higher concentrations. The MeOH extract showed moderate activity (11.83–21.83 mm), while the n-hexane extract was the least active (4.17–8.33 mm). All extracts showed a concentration-dependent effect, suggesting that polar and semi-polar compounds contribute significantly to the antibacterial activity of limpasu pericarpium.

The findings reported by S. Fitriansyah et al., (2018) highlight the promising antibacterial potential of limpasu fruit ethanol extract. Among the tested bacterial strains, the ethanol extract exhibited the highest inhibitory activity against *Bacillus subtilis, Staphylococcus aureus, Pseudomonas*

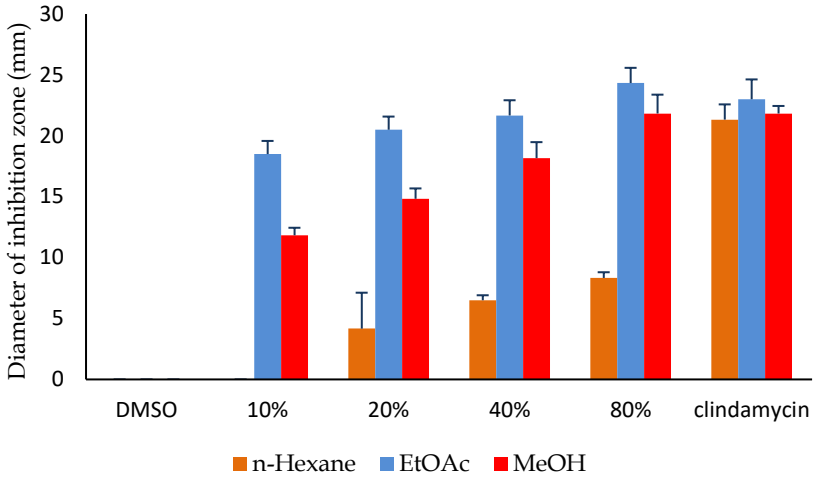


Figure 1. The inhibition zone of limpasu pericarpium extract against *S. epidermidis*

aeruginosa, Escherichia coli, and Propionibacterium acnes, with a minimum inhibitory concentration (MIC) of 2.5% (b/v). The corresponding inhibition zone diameters were 6.87 mm, 7.60 mm, 7.94 mm, 8.80 mm, and 10.29 mm, respectively. Furthermore, extracts from the fruit peel of limpasu demonstrated a concentration-dependent antibacterial activity against *P. acnes*, with the 20% extract showing strong inhibition (zone of inhibition: 18.76 mm). Higher concentrations (40–100%) exhibited very strong antibacterial effects, with inhibition zones ranging from 23.23 to 27.33 mm (Ulpah et al., 2024).

Phytochemical analysis indicates that the extract of limpasu pericarpium has the following metabolite compounds: alkaloids, flavonoids, saponins, tannins, and steroids or terpenoids (Table 1). Alkaloids are nitrogenous heterocyclic compounds, almost exclusively obtained from the plant kingdom and noted to possess antibacterial activity. This activity may arise from the disruption of cell wall construction, modification of cell membrane permeability, bacterial metabolism suppression, as well as blocking the formation of nucleic acids and proteins (Chi et al., 2018; Yan et al., 2021). Phenolic compounds, an integral part of the human diet, provide various health advantages due to their unique chemical properties, which drive a range of biological activities. In terms of antibacterial effects, plant-derived polyphenols attack bacterial cells through several mechanisms. These include interacting with proteins and bacterial cell walls, disrupting cytoplasmic functions and membrane permeability, inhibiting energy metabolism, and inducing DNA damage or preventing nucleic acid synthesis (Duggirala et al., 2014; Léonard et al., 2016). At the DNA level, the planar structure and hydrophobic core of polyphenols allow them to intercalate into the DNA helix during processes like replication, recombination, repair, and transcription (Borges et al., 2013). Furthermore, the hydroxyl groups in phenolic compounds facilitate hydrogen bond formation with nucleic acid bases. Phenolics

also affect synthetic pathways by inhibiting enzymes such as topoisomerase and DNA gyrase (Mora-Pale et al., 2015). Additionally, they can bind with metals like Cu2+, altering DNA stability. The inhibitory mechanism depends on the structural characteristics of the polyphenols and the specific bacterial species. The hydrophilic or hydrophobic properties of these molecules, which are determined by their action sites, underscore the importance of the amphipathic nature of phenolic compounds in their antibacterial activity (Oulahal & Degraeve, 2022). The hydrophobic properties of terpenoid compounds allow them to interact with the lipophilic regions of membranes and mitochondria, leading to structural damage. Additionally, these hydrophobic characteristics can disrupt the synthesis of bacterial lipid membranes, including proteins and enzymes on the membrane surface (Mahizan et al., 2019). This disruption increases membrane permeability and results in protein leakage from bacterial cells, ultimately reducing the overall protein content (Siddiqui et al., 2024). It is believed that the electronegativity of conjugated aldehydes increases antimicrobial activity through interference with electron transfer and interactions with biomolecules like proteins and nucleic acids. Regarding ketones, there is a link between the presence of an oxygen functional group and greater antimicrobial activity in some terpenoid compounds (Kamatou et al., 2013; Siddiqui et al., 2024). Through these mechanisms, terpenoids exhibit antibacterial activity.

CONCLUSION

The study showed that the antibacterial effect of the pericarp extract of *Baccaurea lanceolata* (limpasu) is particularly strong for the ethyl acetate (EtOAc) fraction which has the strongest activity against Staphylococcus epidermidis and exhibited a dose-dependent response in the widening of inhibition zones. This remarkable biological activity is associated with high total flavonoid (29.16 ± 0.49 mg quercetin/g) and phenolic (2.12 ± 0.11 mg GAE/g) concentrations, suggesting that these phytochemicals provide its antimicrobial activity. Thus, the pericarp extracts of limpasu also showed potential as natural antibacterial agents.

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