Evaluation of Antibacterial Properties of *Citrus amblycarpa* (Hassk.) Ochse Peel Extract

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ABSTRACT

Limau (*Citrus amblycarpa* (Hassk.) Ochse) has traditionally been utilized in Balinese medicine for its therapeutic properties. Its peel contains bioactive compounds such as flavonoids, alkaloids, and saponins that have been associated with antioxidant and antibacterial activities. This study aimed to evaluate the antibacterial potential of sequential extracts of *C. amblycarpa* peel against four pathogenic bacteria. Dried peel powder was sequentially extracted using n-hexane, ethyl acetate, methanol, and water for 72 hours. Each fraction was concentrated with rotary evaporation at 50°C. The antibacterial activity was assessed by the minimum inhibitory concentration (MIC) method against *Escherichia coli*, *Salmonella typhimurium*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. The results showed that none of the extracts demonstrated inhibitory activity against the tested bacteria. This absence of activity may be related to the loss of volatile antibacterial compounds during the drying process of the plant material. In conclusion, sequential extract of *C. amblycarpa* peel exhibited no detectable antibacterial effect against common pathogenic bacteria. Despite the negative findings, this study provides baseline data for future investigations, contributes to the scientific understanding of local herbal resources, and emphasizes the importance of optimizing extraction methods to preserve bioactive compound for the development of herbal based antimicrobials.

Keywords: Antibacterial activity, Citrus amblycarpa, Pathogenic bacteria, Peel extract, Phytopharmaceuticals

INTRODUCTION

Limau (*Citrus amblycarpa* (Hassk.) Ochse) is a local citrus species that has long been utilized in traditional Balinese medicine (Kusumawati, I G A, Putra, I M W, Yogeswara, 2020). Various parts of this plant, particularly the peel, contain bioactive compound such as flavonoid, alkaloid, and saponin, which play important roles in biological activities, including antioxidant and antibacterial effects (Ecevit et al., 2022; Nascimento et al., 2000; Shahraki et al., 2023). The use of natural products as potential source of antibacterial agents has gained considerable attention in recent decades due to the increasing resistance of pathogenic bacteria to conventional antibiotics (Gaziano et al., 2019; Wado et al., 2022).

Citrus peels are considered an agricultural by-product with significant potential as a natural source of bioactive compounds (Kusumawati, I G A, Putra, I M W, Yogeswara, 2020). Extraction of these peels, including those of *C. amblycarpa*, is expected to yield active substances capable of inhibiting the growth of pathogenic microorganisms (Anggraeny et al., 2024). In this context, evaluating the bacterial activity of *C. amblycarpa* peel extract is an essential step toward assessing its pharmacological potential as a raw material for the development of herbal based antimicrobial or phytopharmaceuticals.

However, the biological activity of plant extracts is highly dependent on the extraction method, including the choice of solvent and processing conditions (Kusumawati & Yogeswara, 2016; Navarrete-Carriola et al., 2024). Sequential extraction using solvents of varying polarity such as n-hexane, ethyl acetate, methanol, and water aims to obtain a wide range of chemical constituents (Nawaz et al., 2020; Wado et al., 2022). Nonetheless, the observed antibacterial activity may be influenced by several factors, including the degradation or loss of volatile compounds during the drying process of plant materials

(Oikeh et al., 2020).

Thereofere, this study aimed to evaluate the antibacterial activity of sequential extracts of *C. amblycarpa* peel against four pathogenic bacteria, namely *Escherichia coli*, *Salmonella typhimurium*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. The findings of this research are expected to provide baseline data for future studies on the bioactivity potential of *C. amblycarpa* peel and to offer insights into the optimization of extraction methods to preserve bioactive compounds for the development of herbal based antibacterial agents.

METHODS

Plant collection

Fruit of *Citrus amblycarpa* were collected from Kalpataru Garden, Denpasar, Bali, Indonesia (8°40'17.8"S 115°15'09.9"E). The plant species was taxonomically identified and authenticated by the Herbarium Bogoriense, National Research and Innovation Agency (BRIN), Indonesia. The specimen was verified as *C. amblyarpa* (family Rutaceae) and assigned the identification number B-3020/II.6.2/IR.01.02/8/2024.

Citrus amblycarpa peel powder preparation

Fresh fruits were thoroughy rinsed under running water, manually peeled, and cut into uniform pieces measuring approximately 1x0.5 cm using stainless steel scissors (Gómez-Mejía et al., 2023). The peel samples were subsequently dried in a hot air oven at 50°C for 48 hours on aluminum foil. After drying, the peels were finely ground using an electric grinder to obtain a homogeneous powder. The resulting powdered *Citrus amblycarpa* peels were then subjected to the extraction process.

Plant extract preparation

The powdered *Citrus amblycarpa* peels were subjected to maceration using four different solvents, n-hexane, ethyl acetate, methanol, and distilled water at a 1:10 (w/v) ratio. The extraction procedure followed the sequential cold maceration method described by (Pagi & Patel, 2017). The four resulting extracts, namely the hexane extract fraction (KLH), ethyl acetate extract fraction (KLE), methanol extract fraction (KLM), and aqueous extract fraction (KLA), were subsequently analyzed to characterize their antibacterial activities.

Determination of antibacterial activities

Four bacterial strains (*Escherichia coli*, *Salmonella typhimurium*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*) were used in this study. All microorganisms were cultured in Muller Hinton Broth at 37°C for 20 hours. Each microbial culture was then incorporated into Muller Hinton Agar and diluted with sterile distilled water to achieve a final concentration of 10^6 CFU/mL. Sample concentrations ranging from 50 to 200 mg/mL were prepared by dissolving the extracts in DMSO to reach a final concentration of 330 mg/mL. Subsequently, 50 µL of each extract was introduced into wells on the agar plates. The plates were then incubated at 37°C for 24 hours. Chloramphenicol served as the positive control, while DMSO was used as the negative control (Agung Yogeswara et al., 2022; Jeyaseelan et al., 2012).

RESULTS AND DISCUSSION

The flavonoid content present in plant extracts exhibits notable bioactivities, particularly antibacterial and antioxidant properties. The interaction among flavonoid, alkaloids, and saponins has been shown to inhibit the growth of pathogenic bacteria (Oikeh et al., 2020). Oikeh et al., (2020) reported that fresh *Citrus sinensis* extracts, which contain high levels of phenolic and flavonoid compounds, demonstrated stronger inhibitory effect against pathogenic bacteria compared to dried extracts.

In contrast, in the present study, none of the extract fractions exhibited inhibitory activity against *Escherichia coli*, *Salmonella typhimurium*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* (Table 1). Similarly, Sweidan et al., (2023) reported that butanol and ethyl acetate extracts of dried pomegranate peel showed no inhibitory effect against pathogenic bacteria. This lack of activity may be attributed to the use of dried plant material (simplicial), as the drying process likely resulted in the loss of volatile compounds responsible for antibacterial activity (Oikeh et al., 2020). These results emphasize the importance of optimizing extraction and processing conditions to preserve thermolabile and volatile phytochemicals that contribute to antimicrobial efficacy.

Table 1. Antibacterial activity of *Citrus amblycarpa* peel extract fractions

Sample	MIC			
	Escherichia coli	Salmonella thyphimurium	Pseudomonas aeruginosa	Staphylococcus aureus
n-hexane fraction	0±0.00	0±0.00	0±0.00	0±0.00
ethyl acetate fraction	0±0.00	0±0.00	0±0.00	0±0.00
Methanol fraction	0±0.00	0±0.00	0±0.00	0±0.00
Distilled water fraction	0±0.00	0±0.00	0±0.00	0±0.00

Overall, the absence of detectable antibacterial activity in the extracts of *Citrus amblycarpa* peel underscores the critical role of extraction parameters and sample preparation in determining the biological potential of plant dried materials. The findings suggest that volatile and heat sensitive compounds, which may possess antibacterial properties, could have been lost during the drying process (Oikeh et al., 2020). Future studies should therefore focus on optimizing extraction conditions, including the use of fresh materials and low temperature or solvent free extraction techniques, to better preserve these active constituents. Moreover, advanced metabolomic profiling and bioassay guided fractionation are recommended to identify specific compounds responsible for antibacterial activity. Such approaches would not only deepen the understanding of *C. amblycarpa*'s phytochemical diversity but also support its potential application in the development of natural antimicrobial agents and phytopharmaceutical products.

CONCLUSION

The present study investigated the antibacterial potential of sequential solvent extracts of *Citrus amblycarpa* (Hassk.) Ochse peel against common pathogenic bacteria, including *Escherichia coli*, *Salmonella typhimurium*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. None of the tested extracts demonstrated inhibitory effects, suggesting that the drying process or extraction condition may have led to the loss of volatile and thermolabile bioactive compounds responsible for antibacterial activity.

Despite the absence of observable activity, the findings provide important baseline data for future investigations into the bioactive potential of *C. amblycarpa*. Further studies employing optimized extraction techniques, such as cold pressing, hydrodistillation, or supercritical CO_2 extraction, as well as metabolomic and bioassay guided analyses, are warranted to isolate and characterize active constituents. This research contributed to the scientific understanding of local plant resources and underscores the importance of methodological refinement in the development of effective herbal based antibacterial agents and phytopharmaceutical products.

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