



Evaluation of the antibacterial activity of *Boesenbergia rotunda* (L.) Mansf. (ឡឃ), *Eucalyptus globulus* Labill. (ឡឃឡ), and *Anacardium occidentale* L. (ឡឃឡ)

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ABSTRACT

Context: The rise of antimicrobial resistance has created an urgent need for new antibacterial agents. In Cambodia, many traditionally used plants have potential antimicrobial properties that warrant scientific evaluation. **Objectives:** This study aimed to evaluate the antibacterial activity of *Boesenbergia rotunda* (L.) Mansf., *Eucalyptus globulus* Labill., and *Anacardium occidentale* L., locally extracts against *Staphylococcus aureus* and *Pseudomonas aeruginosa* and to formulate an herbal antibacterial cream. **Methods:** Antibacterial activity was assessed by broth microdilution to determine minimum inhibitory concentrations. **Results:** Against *S. aureus*, *E. globulus* showed the strongest activity, with MICs of 0.1 and 0.05 mg/mL for the ethanol extracts and 80:20 ethanol–water extracts, respectively. *B. rotunda* showed MICs of 0.4 and 0.2 mg/mL, respectively, while *A. occidentale* showed an MICs of 0.9 mg/mL with both extracts. **Conclusion:** The selected extracts demonstrated antibacterial activity and could be used in Cambodia as active ingredients of interest in dermatology

Keywords: antibacterial, Cambodia, dermatology, medicinal plants

INTRODUCTION

The growing threat of antimicrobial resistance is significantly compromising the effectiveness of modern medicine. In response, there has been increasing interest in the use of medicinal plants as alternative sources of antimicrobial agents (Coculescu, 2009). Despite their widespread traditional use, many of these plants have not been scientifically validated for antibacterial activity, highlighting the need for further investigation (Biswas et al, 2013; Gonçalves et al, 2008). Medicinal plants are known to produce a variety of bioactive compounds, including flavonoids, tannins, alkaloids,



and terpenoids, many of which exhibit antimicrobial properties. Several studies have validated the antibacterial effects of traditional plant remedies against common skin pathogens such as *Staphylococcus aureus* and *Pseudomonas aeruginosa*. For instance, *Anacardium occidentale* L. (cashew), a member of the Anacardiaceae family native to Brazil, is widely used across Southeast Asian countries such as Thailand, Malaysia, and Indonesia. Traditional uses of its leaves, bark, roots, and stem include the treatment of allergies, coughs, stomachaches, diarrhea, and skin infections. Scientific studies have confirmed that both *Anacardium occidentale* and *Anacardium excelsum* exhibit antimicrobial activity against pathogens like *Staphylococcus aureus* and *Pseudomonas aeruginosa* (Sudjaroen et al., 2018; Mohd Ali Jinah, 2020; Celis et al., 2011). *Anacardium occidentale* L. or *svay chanti* is also documented as a Cambodian medicinal plant; its fruit has traditionally been used for cough with dyspnea, thirst and vexation (Song et al., 2020). A 2024 review of Cambodian medicinal plants also lists *A. occidentale* among Cambodian medicinal plants and identifies the bark as the plant part associated with traditional use (Oeung et al., 2024). Another notable plant is *Boesenbergia rotunda* (L.) Mansf., commonly known as *khcheay*. This member of the Zingiberaceae family is valued both for its medicinal and culinary applications. It contains various bioactive compounds such as cardamon, boesenbergin, 5,7-dihydroxy-flavone, and panduratin A. Panduratin A, in particular, has been studied for its antimicrobial activity and potential use in cosmetic formulations (Atun et al., 2018; Sritananuwat et al., 2024). The selected plants have a history of medicinal and traditional use in Cambodia and Southeast Asia. *Boesenbergia rotunda* (L.) Mansf. is traditionally used in Cambodian medicine, with its rhizome reported for digestive complaints, including flatulence, as well as fatigue and menstrual discomfort (Wang et al., 2025; Saensouk et al., 2025). Additionally, *Eucalyptus globulus* Labill. or *breg khyal*, a Myrtaceae found in Australia, is another well-documented medicinal plant. Its leaves are a major source of essential oils, which exhibit strong antimicrobial properties against both gram-positive and gram-negative bacteria (Shala & Gururani 2021; Jamil, 2017; Bachir & Benali, 2012). *Eucalyptus globulus* Labill. is recognized among medicinal and aromatic plants found in Cambodia and has been reported for febrifuge and antiseptic uses (Handa et al.,). In Cambodia, traditional medicine remains vital, with 70–80% of the population relying on it and 40–50% regularly using medicinal plants. These natural resources offer a sustainable and culturally acceptable avenue for therapeutic development. Moreover, herbal products are often perceived as safer and more environmentally friendly than synthetic alternatives (Wani et al., 2013; Cui et al., 2020). This study aims to evaluate the antibacterial activity of selected Cambodian medicinal plants and develop a herbal cream for potential use in treating bacterial skin infections. Although medicinal plants are widely used in traditional medicine in Cambodia, scientific evidence supporting the antibacterial activity of many locally used plants remains limited (Ali et al., 2011; Wang et al., 2025; Lim et al., 2022). In particular, systematic evaluation of *Boesenbergia rotunda* (L.) Mansf., *Eucalyptus globulus* Labill and *Anacardium occidentale* L. against

important bacterial pathogens is needed to better understand their potential pharmaceutical applications (Rukayadi et al., 2010; Salari et al., 2006). The increasing threat of antimicrobial resistance further emphasizes the need to explore alternative



sources of antibacterial agents (World Health Organization, 2025). Therefore, this study was designed to evaluate the antibacterial activity of selected medicinal plant extracts against *Staphylococcus aureus* and *Pseudomonas aeruginosa* and to investigate their potential incorporation into a topical herbal cream. The findings may contribute to the scientific validation of traditional medicinal plants and support the development of locally available plant-based antibacterial formulations.

MATERIAL AND METHODS

Study Location: This study was conducted at the Faculty of Pharmacy, University of Health Sciences (UHS), Phnom Penh, Cambodia. The laboratory procedures, including plant extraction and antimicrobial analysis, were performed at the designated laboratories of the Faculty of Pharmacy.

Sample collection: Fresh rhizomes of *Boesenbergia rotunda* (L.) Mansf. and fresh leaves of *Eucalyptus globulus* Labill. and *Anacardium occidentale* L. were collected from Takeo Province, Cambodia. The plant materials were authenticated at the Faculty of Pharmacy, University of Health Sciences, Phnom Penh, Cambodia, prior to extraction.

Extraction: The collected plant materials were thoroughly washed with clean water, air-dried for several days, and subsequently dried in an oven at a controlled temperature until a constant weight was achieved. The dried rhizomes and leaves were then pulverized using a milling machine to obtain fine powders. For each plant, 20 g of powdered material was macerated separately for 48 hours using either 100% ethanol or an ethanol–water mixture (80:20, v/v), with occasional stirring. The mixtures were filtered through filter paper, and the resulting filtrates were concentrated using a rotary evaporator under reduced pressure. The crude extracts were further dried in an oven (étuve) until a constant weight was obtained and stored at 4 °C until further analysis (Apongolosun et al., 2020).

Antimicrobial test: about 300 µL of each bacterial strain was inoculated into 3 mL of Nutrient Broth and incubated overnight at 37°C. The optical density (OD) of the bacterial suspension was then measured at 600 nm using a microplate reader (Thermo Scientific, USA) and adjusted to correspond with the 0.5 McFarland standard, equivalent to approximately 1.5×10^8 CFU/mL. The resulting bacterial suspension was subsequently diluted to achieve a final concentration of about 10^7 CFU/mL, representing the standard inoculum turbidity used for further assays (Nisa et al., 2024). The minimum concentration of the extract was determined following the methods of Valgas et al., 2007 and Anza et al., 2021.

Preparation of cream oil phase: the required amounts of beeswax and liquid paraffin were accurately weighed and transferred into a clean beaker. The mixture was heated on a water bath at 70–75°C until the beeswax completely melted and a uniform oily phase was obtained. The temperature was maintained to keep the oil phase warm until emulsification.



Preparation of cream queous phase: in a separate beaker, the required amount of distilled water was heated to approximately 70°C. Borax and sodium benzoate were then dissolved in the warm water. The extracts were subsequently added to the warm aqueous phase and mixed thoroughly until a homogeneous dispersion was obtained. The hot aqueous phase was slowly added to the hot oil phase with continuous stirring. The mixture was stirred vigorously using a glass rod or mechanical stirrer for 10–15 minutes to facilitate emulsification. Stirring was continued during cooling to room temperature. As the preparation cooled, it gradually thickened to form a smooth and stable water-in-oil (W/O) cream. Addition of Fragrance. After the cream had cooled to below 40°C, a small amount of natural fragrance was added and mixed thoroughly to ensure uniform distribution throughout the formulation (Karpe et al., 2024; Jadhav et al., 2023).

pH Compatibility testing: the pH of the formulated cream was measured using a digital pH meter to ensure that it remained within an appropriate range for formulation stability and efficacy (Jadhav et al., 2023).

Stability testing: the cream remained physically stable when stored at different temperatures (2 °C, 25 °C, and 37 °C) over a four-week period. No observable changes in color, odor, texture, or phase separation were detected, indicating good physical stability under various storage conditions (Jadhav et al., 2023).

Antibacterial activity of cream: the antibacterial efficacy of the formulated herbal water-in-oil (W/O) cream was evaluated using the agar well diffusion method on Mueller–Hinton (MH) agar. The test was performed against selected bacterial strains, including *S. aureus* and *P. aeruginosa*. The antibacterial activity of the formulated herbal water-in-oil cream was evaluated using the agar diffusion method on Mueller–Hinton agar. Fresh bacterial cultures were standardized to 0.5 McFarland ($\approx 1.5 \times 10^8$ CFU/mL) and uniformly spread onto agar plates. After drying, 1 g of the herbal cream was aseptically placed at the center of each plate. The plates were incubated inverted at 37 °C for 24 h to allow diffusion of the active compounds. A larger inhibition zone indicated stronger antibacterial activity, while the absence or reduction of inhibition zones in the base cream confirmed that the effect was due to the incorporated herbal extract (Bania et al, 2024).

RESULTS

Antimicrobial activity: Among the tested extracts, *E. globulus* exhibited the strongest antibacterial activity, with MIC values of 0.11 mg/mL for the ethanol (100%) extract and 0.05 mg/mL for the ethanol (80%):water (20%) extract. *B. rotunda* (also demonstrated notable antibacterial effects, showing MICs of 0.46 mg/mL and 0.23 mg/mL for the ethanol (100%) and ethanol (80%): water (20%) extracts, respectively. *A. occidentale* extracts displayed moderate inhibitory activity with MICs of 0.93 mg/mL for both solvent systems. In contrast, the controls, ethanol (EtOH) and dimethyl sulfoxide (DMSO), showed no significant antibacterial effect, with MIC



values greater than 3.75 mg/mL and 3.75 mg/mL, respectively. The positive control, erythromycin, exhibited the lowest MIC (< 0.01 mg/mL). Among the tested extracts, *E. globulus* exhibited antibacterial activity, with MIC values of >3.75 mg/mL for the ethanol (100%) extract and 0.93 mg/mL for the ethanol (80%): water (20%) extract against *Pseudomonas aeruginosa*. *B. rotunda* (showed weak or no antibacterial activity, with MIC values >3.75 mg/mL for both solvent systems against *Pseudomonas aeruginosa*. *A. occidentale* extracts demonstrated moderate antibacterial activity against *Pseudomonas aeruginosa*, with MIC values of 0.93 mg/mL for erythromycin, exhibited an MIC of 0.11 mg/mL, confirming its antibacterial activity against *Pseudomonas aeruginosa*.

Evaluation of herbal cream:

The formulated antimicrobial cream exhibited a smooth and homogeneous consistency with good spreadability and no phase separation, indicating satisfactory physical stability. The pH of the formulation was measured at 5.8, which falls within the acceptable range for topical preparations and is compatible with the natural pH of the skin. Antibacterial activity testing revealed a clear inhibition zone ranging from 15 to 20 mm against both *Staphylococcus aureus* and *Pseudomonas aeruginosa*, demonstrating the effective antimicrobial potential of the formulation.

CONCLUSION

This study may benefit the Cambodian population by providing scientific evidence on the antibacterial potential of medicinal plants that are locally available and traditionally used.

DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest.

DECLARATION OF HONOR

The authors declare on their honor that the research is genuine and that the manuscript does not contain fabricated or knowingly made-up research data.

AI ASSISTANCE DISCLOSURE

The authors used ChatGPT/GPT-5 to improve the clarity and readability of the manuscript. The authors carefully reviewed and edited the content and take full responsibility for the final text.

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