**RESEARCH PAPER**

# Antibacterial Properties of Topical Herbal Formulations Containing Euphorbia neriifolia, Diplazium esculentum, and Coleus blumei Extracts

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**ABSTRACT**
Bacterial skin infections remain a common health concern in tropical regions like the Philippines. To address the growing resistance to traditional antibiotics, this study developed and evaluated herbal products (soap, cream, and lotion) infused with KaPaMa extracts from Euphorbia neriifolia (Karimbuaya), Diplazium esculentum (Pako), and Coleus blumei (Mayana). The antibacterial activity of the herbal products at 0.2%, 0.4%, and 0.8% concentrations was evaluated using the disc diffusion assay against Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, and Acinetobacter baumannii. Results revealed that the lotion formulation demonstrated the strongest antibacterial activity against all tested bacterial species, with 0.4% concentration with the largest zones of inhibition. Cream formulations also demonstrated antibacterial effects, particularly the 0.8% concentration. Soap formulations showed minimal antibacterial activity at all concentrations comparable to the soap base. The findings of this study show that KaPaMa herbal cream and lotion can fight common skin infections. This could be used as an alternative skincare product using locally abundant plants.

**Keywords:** *antibacterial activity, Coleus blumei, Diplazium esculentum, Euphorbia neriifolia, herbal formulation, topical skincare products*
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## INTRODUCTION

Bacterial skin diseases are prevalent tropical places since the hot and damp environment helps bacteria grow (Bowden *et al.*, 2020; Bucayu *et al.*, 2022). These diseases are big public health issues, particularly as bacteria become more resistant to drugs. The Institute for Health Metrics and Evaluation (2021) reported that antimicrobial resistance (AMR) caused 56,700 deaths in the Philippines in 2019. Because of this resistance, conventional treatments do not work making people stay sick longer, which leads to paying more for health care and causing more deaths.

To fight against AMR, the use of plants has become a more favored way to explore treatments for bacterial diseases. Plant-based substances seem to be a promising solution to combat these harmful bacteria (Cushnie *et al.*, 2020). In many rural and indigenous communities, plant-based cures are still seen as affordable, accessible, and safer medicine, mostly for skin issues. This tradition of using plants comes from hundreds of years of experience, with newer studies in labs supporting these plants' healing power.

*Euphorbia neriifolia* (Karimbuaya), *Diplazium esculentum* (Pako), and *Coleus blumei* (Mayana) are some of the herbs that have been routinely used traditionally. *E. neriifolia* has been used for anti-inflammatory and wound-healing purposes, for which other studies have also reported its antibacterial properties (Amtaghri *et al.*, 2023). Similarly, *D. esculentum* has also exhibited antimicrobial activity attributed to its flavonoids and phenolic components (Semwal *et al.*, 2021). Furthermore, *C. blumei* is often used for treating wounds and skin infections. Its leaves contain compounds that can fight against microbes (Salazar-Aranda *et al.*, 2011).

While past research has shown that *E. neriifolia, D. esculentum, and C. blumei* each have properties that can kill bacteria, not much is known about how they work together in skincare products like soaps, creams, and lotions. It is essential to study their combined effects to make effective plant-based skin products that can fight bacteria (Kareru *et al.*, 2010). Hence, this study investigated the effectiveness of these mixed herb skincare formulas using Karimbuaya, Pako, and Mayana (KaPaMa) at stopping bacteria growth.

## MATERIALS AND METHODS

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**Collection and Processing of Plant Samples**
Fresh leaves were collected in different parts of Cagayan Province. The plant samples were processed following the Philippine Council for Health Research and Development (PCHRD) standard operating procedure on the Collection and Processing of Terrestrial Plant Samples for Drug Discovery and Development. After collection, plant identification was conducted at the Department of Agriculture, Regional Field Office 02, Tuguegarao City.

Only healthy, undamaged leaves were selected. Leaves showing insect damage, microbial growth, or signs of nutrient deficiency were discarded. Samples were placed in large black plastic bags or sacks with minimal compression to allow air circulation and prevent fungal growth. Upon arrival in the laboratory, the leaves were washed with running tap water followed by distilled water and were blot-dried using clean absorbent paper.

Air-drying was done for 24 h in a shaded, well-ventilated area at room temperature. This was followed by oven drying using a forced draft oven at 40-50°C for 48 h until brittle. The dried samples were ground using a plant grinder to a fine powder and stored in properly labeled glass bottles at 4°C until extraction.

**Preparation of Crude Extract**
For each plant sample, 40 g of ground powder were soaked in 200 ml of 95% ethanol. The mixture was subjected to shaking for 2.5 h to ensure thorough extraction, after which it was stored undisturbed for 24 h at room temperature. The samples were shaken again for 30 min before filtration. Filtrates were stored in tightly closed, labeled reagent bottles at 4°C. The ethanol extracts were then concentrated using a rotary evaporator under reduced pressure to obtain the crude extracts (Azwanida, 2015). Each extract was properly labeled with the plant name and date of extraction.

Development of Soap, Cream, and Lotion

**Soap Formulation**
Coconut oil and lye solution were combined and stirred continuously. Propylene glycol was added during mixing, and the mixture was allowed to cool before incorporating the crude plant extracts at concentrations of 0.2%, 0.4%, and 0.8%. The soap mixture was poured into molds. Six protocols were evaluated to identify the optimal base formulation prior to extract addition.

**Cream Formulation**
Phase B ingredients (purified water, glycerin, triethanolamine) were mixed and heated to 85-90°C. In a separate container, Phase A (stearic acid, cetyl alcohol, glycol distearate, mineral oil, isopropyl myristate) was heated to the same temperature until fully melted. Phase A was slowly added to Phase B with continuous stirring to form an emulsion. The emulsion was allowed to cool in a water bath, with gentle stirring every five minutes. At approximately 45°C, Phase C (citric acid) was added to adjust the pH. Crude plant extracts were then added in specified concentrations while continuously mixing. Five formulations were tested to determine the best base before extract addition.

**Lotion Formulation**
Aloe vera gel, mineral oil, and coconut oil were mixed and heated to 85-90°C until fully dissolved and phase separation occurred. The mixture was cooled to approximately 35°C before adding the plant extracts at concentrations of 0.2%, 0.4%, and 0.8%. Continuous mixing ensured proper dispersion of the extracts. Five lotion base protocols were evaluated prior to extract addition.

**Antibacterial Assay Using Disc Diffusion Method**
The antibacterial activity of the formulated KaPaMa soap, cream, and lotion at 0.2%, 0.4%, and 0.8% concentrations including their respective negative controls (formulations without extracts) was assessed using the disc diffusion method following the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2020).

Pure cultures of *Staphylococcus aureus*, *Escherichia coli, Pseudomonas aeruginosa,* and *Acinetobacter baumannii* were prepared. A sterile swab was dipped into each inoculum and uniformly spread on the surface of Mueller-Hinton agar plates. Sterile filter paper discs were impregnated with 100 µl of each test formulation and placed onto the agar surface. Plates were incubated at 37°C for 18-24 h. Zones of inhibition around each disc were measured in millimeters to evaluate antibacterial efficacy.

**RESULTS AND DISCUSSION**
The antibacterial activity of the KaPaMa herbal formulations soap, cream, and lotion was evaluated in vitro using the disc diffusion method against *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa,* and *Acinetobacter baumannii.* Across all tested concentrations (0.2%, 0.4%, and 0.8%), the soap formulations exhibited no substantial antibacterial activity, with inhibition zones consistently measuring 6 mm for *S. aureus, E. coli,* and *A. baumannii*, and only 8.4 mm for *P. aeruginosa*, matching the results of the negative control. This lack of efficacy suggests that either the active compounds in the plant extracts were degraded or deactivated during the saponification process, or their concentrations were insufficient to elicit a measurable antibacterial response. Nadaroglu *et al.* (2020) and Seetah *et al.* (2021) had previously shown that saponification of thermolabile phytochemicals can lead to a significant loss of their bioactivity.

On the other hand, creams had stronger antibacterial effects in moderation to strong stages, particularly at bigger extract levels. Against *S. aureus, P. aeruginosa*, and *A. baumannii*, the 0.8% cream displayed the maximum activity of 18.2 mm, 8.5 mm, and 9.8 mm, respectively. Additionally, against *E. coli*, the inhibition zones got slightly smaller (12.9 mm). Oddly, the 0.2% cream displayed more activity than the 0.4% cream against the majority of the bacteria, implying a non-linear dose-response relationship for an antibacterial agent. The

differences may suggest that the bioactive compounds interact in complex ways that may either potentiate or reduce each other's effects based on their concentrations (Nasim *et al.*, 2022).

The lotion formulations exhibited the highest antibacterial activity. The 0.4% lotion had the highest inhibition zones, producing the largest zones of inhibition against *S. aureus* (27.1 mm), *P. aeruginosa* (17.9 mm), and *A. baumannii* (16.2 mm). This has been found to be significantly more effective than both lower and higher concentrations. This non-linear trend implies that the effectiveness of an antibacterial formulation may be due to the ideal balance of active ingredients. The lotion outperformed both the soap and cream; one possible reason for this is that the water and olein base may allow for better extract dispersion and skin permeability, resulting in improved performance results (Kareru *et al.*, 2010).

The observed antibacterial effects of KaPaMa products, especially for lotion and cream, can be attributed to the known components of the plants. Several studies have shown that each of the plants contain compounds that are known to have antibacterial activity (Salazar-Aranda *et al.*, 2010; Semwal *et al.*, 2021; Amtaghri *et al.*, 2022).

The results of this study suggest that herbal lotions and creams made with KaPaMa have antibacterial effects that can stop bacteria from growing and spreading. For soaps, the formula needs to be changed to keep the active ingredients from breaking down during the manufacturing process (Chua, 2023). The results of this research show that it is possible to combine traditional medicinal plants with modern antibacterial treatments that are scientifically made. Future studies should focus on finding the best concentrations of extracts, optimizing formulations for improved spreading, and identifying the active ingredients that have an antibacterial effect.

## CONCLUSION

The results show that KaPaMa herbal lotions and creams are good plant-based antibacterial options to prevent infections caused by *S. aureus, E. coli, P. aeruginosa,* and *A. baumannii*. However, it is recommended to improve KaPaMa soap formulation to stop the breakdown of heat-sensitive plant chemicals.

## DECLARATIONS

Competing Interests / COI Statement

The authors declare that they have no competing interests.

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**Authors' Contributions**

Jinky Marie T. Chua conceptualized the study, supervised all experimental procedures, led data analysis and interpretation, and prepared the manuscript draft. Julius T. Capili contributed to the methodology design, validation of laboratory processes, and critical revision of the manuscript. Nikko Alexander S. Pacquing assisted in sample preparation, laboratory experimentation, data curation, and manuscript editing. All authors reviewed and approved the final version of the manuscript.

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