Kuroshio Science 18-1, 152-155, 2024

Symposium Proceedings

Formulation, physicochemical evaluation, and microbiological analysis of herbal ointment from dried leaves of Piper betle (L.) (buyo)

Tribiana, E.* and Tapas, W.
Natural Science Department, Faculty, Catanduanes State University, Philippines

Abstract

Piper betle is an indigenous herbal plant in the Philippines that holds robust anti inflammatory, antimicrobial, and antioxidant compounds, making it a potentially holistic remedy for diverse skin conditions. This study formulated an herbal ointment from the dried leaves of *Piper betle* and tested it for physicochemical evaluation which includes odor, color, texture, and spreadability. To test the absence of bacterial contamination, the samples were tested for *Salmonella* species. Buyo leaves were collected in Bato, Catanduanes and sun dried for 24 hours (3 consecutive days), cut into small pieces and milled into fine powder. Using 96.38% USP grade ethanol as solvent, the powdered Buyo leaves were extracted via Soxhlet apparatus, followed by rotary evaporator to extensively remove the solvent. To formulate the ointment, fusion method was employed to mix the Buyo extract using an improvised boiler with petroleum jelly (to prevent transepidermal water loss), beeswax (to provide solidity), menthol (activate the cold-sensitive receptors in the skin) and eucalyptus oil. The four ingredients were mixed while under the steam of water bath at 65degC, then removed from the water bath, allowing it to partially congeal. Varying formulations were produced using varied proportions of Buyo extract (Treatment A, B, and C). Results revealed that all formulated ointments had an acceptable odor, good color, very good texture and good spreadability. pH values ranged from 7.8 to 8.2 and exhibited no significant differences. In terms of physical attributes, there were no significant differences in terms of odor, color, texture, and spreadability. Microbiological analysis was performed by sending the samples to BIOTECH-UPLB which confirmed the absence of *Salmonella* species. *Piper betle* leaves can be formulated into herbal ointment with varying concentrations and physicochemical characteristics.

Keywords: Buyo, ointment, formulation, *Piper betel*, physicochemical

INTRODUCTION

Amidst the lush geography of the Philippines, *Piper betle* stands as one of the botanical repositories, categorized by a remarkable reservoir of therapeutic potential enhanced with anti-inflammatory, antioxidant, and antimicrobial compounds (Silalahi, 2020). This botanical plant, locally known as Buyo, surpasses its taxonomic organization to undertake the layer of cultural significance. Its versatile applications range from treating halitosis to mitigating respiratory diseases, upholding its integral role in traditional healing practices.

In the province of Catanduanes, *Piper betle* dwells in a preeminent position as a valued source of medicinal efficacy, embedded within the tapestry of local healing traditions. The formulation of this botanical specimen into an ointment serves as evidence to the seamless incorporation of ancient healing practices with modern-day scientific approaches, presenting a practical and culturally resonant modality for the application of its therapeutic points.

Amidst the global perturbation highlighted by the 2022 Global Antimicrobial Resistance and Use Surveillance System (GLASS) report, the pursuit of herbal alternatives takes on heightened significance. In the face of the imminent threat of antimicrobial resistance that threatens to compromise conventional pharmacological options, *Piper betle* arises as a safe and effective antimicrobial product. This study assumes an essential role in the ongoing fight against antimicrobial resistance, aiming to reveal and standardize the potential of

*Correspondence. E-mail: tribianaestrella680@gmail.com

Piper betle in countering prevalent bacterial pathogens that instigate dermatological infections.

Budiman et al (2018) explored the promising potential of *Piper betle* L. extract as a natural alternative for treating acne vulgaris, a prevalent skin inflammation that is becoming increasingly resistant to conventional antibiotics. This study developed an antibacterial cream and tested it against *Staphylococcus aureus* and *Propionibacterium acnes* using the disc diffusion method which reported to have a minimum inhibitory concentration (MIC) of the extract against *S. aureus* and *P. acnes* of 4.5% and 4.0% respectively. The cream formulation that exhibited the utmost antibacterial activity was observed in formulation containing 7% surfactant (inhibition zones of 10.21 ± 1.2 mm against *S. aureus* while 15.2 ± 1.6 mm against *P. acnes)*. With the aim to explore more effective products, this study explores the advantages of ointment formulation.

Ointments offer several advantages over creams, particularly in the context of antibacterial formulations. Due to their greasy and occlusive nature, ointments provide a superior barrier against moisture loss and demonstrate deeper penetration of active moiety into the skin, hence enhancing antibacterial activity. The greasy texture of ointments allows adherence to the skin, prolonging contact time to ensure the therapeutic effect (Marohom et al., 2016). Enhanced penetration and prolonged antibacterial activity of ointments represents valuable therapeutic option against skin conditions.

This research highlighted the formulation of herbal ointment derived from the desiccated leaves of *Piper betle*, a vital step needed to extract the plant into its pharmacological source. This study required a rigorous formulation and evaluation of physicochemical attributes of the ointment as the finished product. Simultaneously, the study employed strict microbiological testing after the herbal formulation, in accordance with the need for pioneering products capable of fighting antimicrobial resistance (AMR).

This research sought not only to produce an herbal ointment but to represent a paradigmatic shift in the treatment of dermatological infections. It presented a strategic response to the urgent challenge for innovative products amidst an increasingly alarming antimicrobial resistance. Botanical plants like *Piper betle* can be competently developed to redefine the landscape of dermatological options. As the global health experts grapple with the challenges caused by antimicrobial resistance, this study represents the healing potentials found within nature.

Objectives:
Formulate an herbal ointment from the dried leaves of *Piper betle*.

- Evaluate the physicochemical characteristics of Buyo ointment, including odor, color, texture, and spreadability.
- Test for the absence of bacterial contamination, specifically *Salmonella species*.

MATERIALS AND METHODS

Collection and Preparation of Buyo Leaves

Fresh Buyo leaves were meticulously harvested from the landscapes of Bato, Catanduanes. The leaves underwent a systematic sun-drying process for 72 hours (about 3 days) across three consecutive days to optimize dehydration while preserving their botanical integrity. Subsequently, the desiccated leaves were precision-cut into small fragments and finely milled into a powdered form, ensuring a homogeneous texture.

Extraction of Buyo Leaves

The extraction of bioactive compounds from the powdered Buyo leaves was precisely facilitated. Using a Soxhlet apparatus, the powder was subjected to exhaustive extraction with 96.38% USP grade ethanol as the solvent. This meticulous extraction process was followed by the application of a rotary evaporator to eliminate the solvent thoroughly, ensuring a concentrated and purified Buyo extract.

Formulation Process

This research was designed to produce 3 different formulations: treatment A, treatment B, and treatment C. The formulation of the herbal ointment commenced with the fusion method; a technique known for its efficiency in combining diverse ingredients. An improvised boiler provided the controlled environment needed for this delicate process. The key components, namely the Buyo extract, petroleum jelly (serving as the ointment base for both barrier protection and prevention of transepidermal water loss), beeswax (for solidity), menthol (activating cold-sensitive receptors for a refreshing sensation), and eucalyptus oil (providing additional relief for muscle and joint discomfort), were meticulously combined and exhibited in the table 1.

Ointment Fusion and Congealing

The combined reagents underwent a carefully-monitored fusion process under the steam of a water bath, maintaining a constant temperature of 65° C. As the consistency improves, the mixture was promptly removed from the water bath, allowing it to partially congeal. During this phase, constant stirring was employed to ensure uniform distribution and integration of the Buyo extract.

Table 1. Formulation of Buyo ointment

TREATMENT	COMPOSITION (%w/w)
A	25ml Buyo extract, 100g petroleum jelly, 25g beeswax, 10mg menthol, and 1ml eucalyptus oil.
B	25 ml Buyo extract, 100g petroleum jelly, 26g beeswax, 13mg menthol, and 1ml eucalyptus oil.
C	25 ml Buyo extract, 100g petroleum jelly, and 25g beeswax.

Gradual Addition of Extract

As the ointment began to congeal, the concentrated Buyo extract was gradually introduced into the mixture. This meticulous process was performed with utmost monitoring to achieve an even mixture of the herbal extract with the ointment base. The gradual addition allowed for optimal integration, ensuring the preservation of the extract's therapeutic properties.

The study ensured the extraction of potent bioactive compounds from *Piper betle* and the seamless integration into an herbal ointment in order to create a functional and novel therapeutic solution for various skin conditions.

RESULTS AND DISCUSSION

Physicochemical Characteristics

The study implemented varying formulations denoted as Treatment A, Treatment B, and Treatment C, each comprising distinct proportions of Buyo extract and supplementary ingredients. Notably, all formulated ointments, irrespective of their compositions, exhibited commendable attributes. They uniformly demonstrated an acceptable odor, a visually appealing color, a remarkable pleasing texture, and excellent spreadability. This consistency across formulations suggests that the herbal ointment maintains its sensory and tactile qualities regardless of minor compositional adjustments.

pH Values

In scrutinizing the pH values of the formulated ointments, a narrow range of 7.8 to 8.2 was observed. Notably, this range remained consistent across all formulations, with no statistically significant differences detected. This uniformity in pH values is indicative of the stability of the herbal ointments and suggests that variations in formulation do not exert a substantial influence on the acidity or alkalinity of the product.

Further statistical analysis using ANOVA in MS Excel, with a significance level set at $p < 0.05$, revealed significant

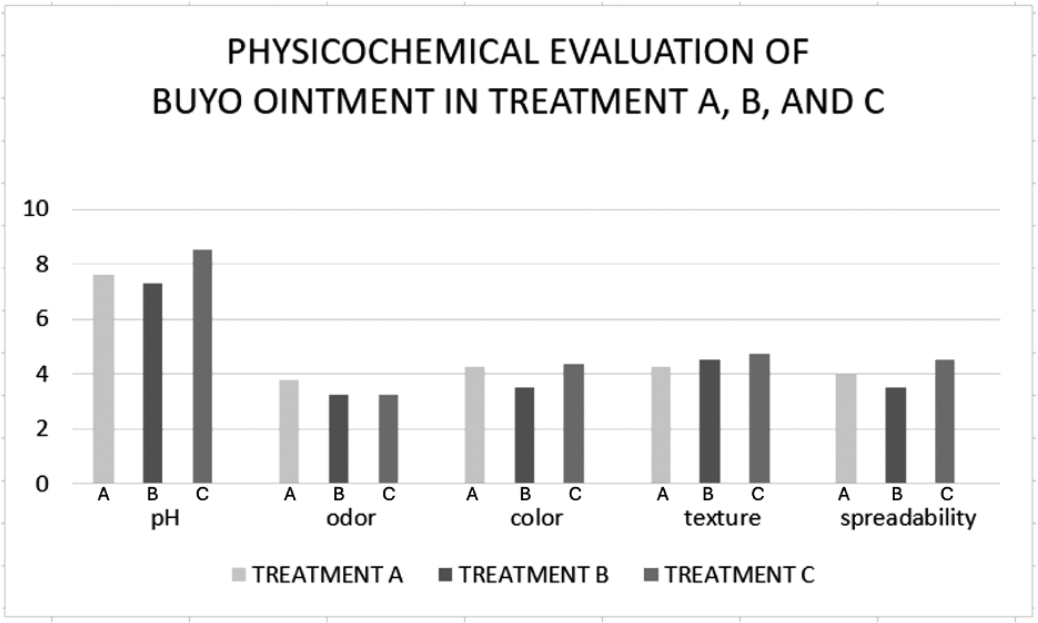

Fig. 1. Results of physicochemical characteristics of Buyo ointments

Table 2. Summary of Analysis of Variance (ANOVA) of significant differences among the level of acceptability of the treatments of *Piper betel* ointment

VARIABLE	COMPUTED VALUE	P-VALUE	REMARKS
pH	0.863	0.55	not significant
odor	7.7	0.0029	significant
color	11	0.00076	significant
texture	17.69	0.00012	significant
spreadability	14.06	0.00304	significant

differences in the physicochemical attributes of the ointments. Specifically, distinctions were identified in terms of odor, color, texture, and spreadability. The observable variances, although exhibiting statistical significance, manifested as slight deviations in terms of acceptability.

Microbiological Analysis

A critical facet of the study involved subjecting the formulated ointments to a microbiological analysis conducted at BIOTECH-UPLB. The results conclusively confirmed the absence of *Salmonella species* in all samples. The meticulous transit protocols adhered to during sample transportation underscore the reliability of the microbiological findings. The absence of *Salmonella species* signifies the effectiveness of the extraction and formulation processes in ensuring the sterility and viability of the herbal ointments.

The consistent sensory attributes and pH stability observed across all formulations affirmed the robustness of the herbal ointment derived from *Piper betel* leaves. While statistical differences in certain physicochemical attributes were identified, their practical implications in terms of overall product acceptability merit further consideration. The absence of *Salmonella species* underscores the efficacy of the extraction and formulation procedures, reinforcing the potential of *Piper betle* as a safe and viable ingredient in dermatological formulations. These results collectively advocate for the continued exploration of *Piper betle*-derived products, emphasizing their potential in herbal dermatological applications.

CONCLUSION

This study concludes that *Piper betle* leaves can be successfully formulated into herbal ointments, offering a novel and efficacious alternative in skincare. Microbial analysis ensured the ointment's freedom from contamination, paving the way for its development as a powerful herbal remedy. This formulation holds significance in redefining skincare practices, especially in the context of rising antimicrobial resistance. The study serves as a baseline for future antibacterial research, contributing to the development of drugs combating resistant microbial strains and aligning with the global need for sustainable and culturally-integrated healthcare solutions.

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