

ORIGINAL RESEARCH ARTICLE

Pharmaceutical formulation and evaluation of *Xylopi aethiopica* fruit-based topical ointment and cream

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Abstract

For centuries, humans have long sought plant-based remedies derived from barks, seeds, and fruits in their enduring battle against illness. Over time, traditional medicines have evolved to become more effective and safer through pharmacological validation. One key area where these remedies have shown promise is in treating skin infections, as the skin is often the first point of contact for microorganisms and particularly vulnerable to infections caused by bacteria, fungi, viruses, and parasites. Currently, topical infections are primarily treated using ointments and creams, which deliver high concentrations of antimicrobials directly to the affected area. Studies reveal that ethanolic extracts have potent antibacterial activity against *Pseudomonas aeruginosa* and *Staphylococcus* species. This effect is ascribed to moderate to high concentrations of saponins, known for their antibacterial qualities. The present study investigates the antibacterial properties of ethanolic and aqueous extracts of *Xylopi aethiopica* for the formulation of a topical ointment. To determine their minimum inhibitory concentrations (MICs), cultures of *Staphylococcus aureus* and *P. aeruginosa* were tested against the extracts, which exhibited MIC values ranging from 2.5 mg/mL to 10 mg/mL for both the ethanolic and aqueous forms. The ointments and creams formulated from these extracts demonstrated notable antibacterial activity, alongside excellent physicochemical properties, including a smooth texture, appealing appearance, uniform consistency with no phase separation, characteristic brown color, excellent spreadability, stability, and suitable pH values (6.3 for the ointment and 7 for the cream), as well as non-irritating effects on the skin. The findings confirm that *X. aethiopica* possesses effective antibacterial properties against *S. aureus* and *P. aeruginosa*, supporting its potential for further *in vivo* studies and broader therapeutic applications.

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1. Introduction

The quest for effective and natural remedies has been an enduring aspect of human civilization. Since ancient times, humans have sought medications from the bark, seeds, fruits, and other parts of plants to combat various illnesses.¹⁻⁴ *Xylopia aethiopica* (Figure 1), commonly known as the African pepper, has traditionally been used for medicinal purposes. This plant is native to West Africa⁵ and has a rich historical use in traditional medicine, particularly for its antibacterial, antifungal, and anti-inflammatory properties.⁶ *X. aethiopica* is an angiosperm belonging to the family Annonaceae that thrives in the evergreen rainforests of tropical and subtropical Africa.⁷ The plant's name originates from the Greek words “*xylon pikron*,” meaning “bitter wood.” The second part of the plant's binomial name, *aethiopica*, refers to its origin, Ethiopia;⁸ however, it currently grows most prominently as a crop in Ghana.⁹ The plant has several local names. In Ghana, it is known as “*hwentee*” in Akan, “*etso*” in Ewe, “*so*” in Ga, and “*samaamdabile*” by the Waala people in the Upper West Region of Ghana.

In contemporary medicine, skin infections remain a prevalent issue, often caused by various pathogens, including bacteria, fungi, viruses, and parasites.^{10,11} The skin, which is the largest organ and the body's first line of defense, is frequently exposed to these microorganisms. Topical infections can lead to a range of conditions, ranging from mild irritation to severe dermatological issues.^{11,12} Currently, the most used treatments for such infections are topical formulations, such as ointments and creams. These formulations deliver high concentrations of antimicrobial agents directly to the site of infection, thereby ensuring effective treatment.¹³⁻¹⁵

The present study aims to explore the potential of *X. aethiopica* in the formulation of ointments and creams. Specifically, it investigates the antibacterial properties of aqueous and ethanolic extracts of *X. aethiopica* fruit and

their efficacy when incorporated into topical formulations. The choice of *X. aethiopica* is influenced by its historical usage and the presence of bioactive compounds, such as alkaloids, glycosides, saponins, tannins, and flavonoids, which are known for their therapeutic benefits.¹⁶ Phytochemical analysis of *X. aethiopica* revealed the presence of these compounds, which contribute to its antibacterial activity. Saponins have been linked to potent antibacterial effects. This study performed phytochemical tests on aqueous and ethanolic extracts of *X. aethiopica* to identify bioactive compounds.¹⁷ The antibacterial efficacy of the extracts was tested against common skin pathogens, including *Staphylococcus aureus* and *P. aeruginosa*, through minimum inhibitory concentration (MIC) assays.¹⁸ These pathogens are known to cause significant skin infections and represent the bacterial targets that an effective topical formulation must address.^{19,20}

The formulation process involves incorporating the aqueous and ethanolic extracts into ointments and cream bases, followed by a comprehensive evaluation of these formulations.^{21,22} The evaluation criteria included assessing the texture, appearance, homogeneity, phase stability, and color of the formulations.²³ In addition, the antibacterial activity of the formulated ointments and creams was tested against selected microorganisms to confirm their efficacy.^{24,25}

The significance of this study lies in its potential to provide a natural and effective alternative for treating skin infections. By utilizing the medicinal properties of *X. aethiopica*, this study aimed to develop formulations that are not only effective but also have minimal side effects compared to synthetic antibiotics. Furthermore, this research sought to validate traditional medicinal knowledge through scientific methods, bridging the gap between traditional practices and modern medicine.²⁶ In summary, this study represents a promising step toward the development of natural antimicrobial treatments. These findings could pave the way for further research into the *in vivo* efficacy of *X. aethiopica* and its potential applications in dermatology and beyond.

2. Materials and methods

2.1. Collection of plant materials

Fresh *X. aethiopica* fruits were obtained from the Agboghloshie market in Accra. The fruits were dried and ground into a powder, which was then examined and authenticated by Mr. Peter Boateng—a Botanist at the Center for Plant Medicine Research, Mampong, Ghana—using the herbarium library at the Center for Plant Medicine Research.



Figure 1. Dried fruits of *Xylopia aethiopica*

2.2. Materials, chemicals, and reagents

Glacial acetic acid, watermelon seeds, Fehling's A and B solutions, picric paper, hydrochloric acid, hard paraffin, cetostearyl alcohol, filter paper, ferric chloride, methanol, Mueller–Hinton agar, white paraffin, concentrated sulfuric acid, wool fat, dilute ammonia, and 20% sodium hydroxide were obtained from the Chemistry and Pharmacology Laboratory of the School of Pharmacy, Central University.

2.3. Equipment and instrumentation

An analytical balance (Model ALE-223; US-Solid, USA), test tubes, beakers, funnels, wash bottles, measuring cylinders, cotton wool, spatula, Petri dishes, a hot air oven (Model DGT-G500-X; Narang medical limited, India), stirring rods, a DLC electric blender (Model-411; Dongguan Jinsen Craft Technology Co. Ltd, China), a water bath (Model-WB10C11B; Cole-Palmer, United States), an autoclave (Model CJ-18LDJ), Whatman no.1 filter papers, and a pH meter (Model – 6177M/6177EU; Jenco Instruments, United States) were used in this study.

2.4. Drying and preparation of crude extracts

The fruits obtained were weighed and dried under direct sunlight for 5 days. The weight was measured until a constant weight was achieved. The fruits were ground into a fine powder using an electric blender. The resulting powder was weighed and stored under suitable conditions. A total of 200 g of the pulverized extract was weighed and dissolved in 2 L of a water and 100% ethanol mixture (ratio of 1:1) and was allowed to stand for 48 h. Subsequently, the solution was filtered using a Whatman no. 1 filter paper, and the filtrates were evaporated in an oven at 65°C for 6 h.^{27,28}

2.5. Phytochemical analysis

Qualitative phytochemical analyses were conducted on both the ethanolic and aqueous extracts of *X. aethiopica* fruits according to standard procedures as previously described.^{16,27,29-31} Samples were screened for saponins, tannins, flavonoids, alkaloids, glycosides, and reducing sugars.

2.6. Test organisms

The test organisms, *S. aureus* and *Pseudomonas aeruginosa*, were obtained from the Microbiology Laboratory of Central University. Cultures of these test organisms were purified. These bacteria were re-cultured in a newly prepared nutrient broth by dissolving 2.6 g of nutrient broth powder in a total of 300 mL of distilled water—initially using a small portion of the water to mix the powder, followed by the addition of the remaining volume. The prepared nutrients were distributed evenly into test tubes and were autoclaved

at 121°C for 20 min. After 20 min of heating, the broth was allowed to cool before introducing the bacteria, followed by incubation at 37°C for 24 h.

2.7. Sterilization of materials

All apparatus was sterilized using the autoclave at 121°C for 15 min. The workbench was disinfected with 70% ethanol before and after use. A total of 400 mL of distilled water was autoclaved to ensure sterility. Micropipette tips were also sterilized using the autoclave.

2.8. Determination of the MIC of the plant extracts

The MIC of the extracts was determined using the tube dilution method as described. A 0.1 mL aliquot of the prepared bacterial suspension for each test organism was added to eight sterile test tubes containing nutrient broth with two-fold serial dilutions of the extracts at concentrations of 80, 40, 20, 10, 5, 2.5, 1.25, and 0.625 mg/mL. Test tubes containing an antibiotic (ciprofloxacin) with the growth medium, the growth medium with the bacterial suspension, and nutrient broth alone were used as controls. All tubes were incubated at 37°C for 24 h. The MIC was defined as the lowest concentration that prevented visible bacterial growth (turbidity) compared to the control tubes.^{18,28,32}

2.9. Preparation of topical cream and ointment of *X. aethiopica*

The ointment of *X. aethiopica* was prepared according to the master formula presented in Table 1.

2.9.1. Preparation of 10g of a 10%w/w *X. aethiopica* ointment

The herbal ointment was prepared by carefully blending 1 g of *X. aethiopica* ethanolic extract with the ointment base (Table 1) using the levigation method. The process involved first forming a smooth paste by mixing the extract with two or three times its weight in the base. An additional base was then gradually incorporated until a uniform ointment was obtained. The final product was then placed in an appropriate container and properly sealed. This same process was repeated for the aqueous extract.²²

2.9.2. Preparation of 10 g of a 10%w/w *X. aethiopica* cream

The master formula for the cream formulation is presented in Table 2. In one porcelain dish, 1.3 g of stearic acid, 0.5 g of potassium hydroxide, and 0.5 g of sodium carbonate were melted together at 70°C. In another dish, 0.6 g of glycerin, 7.1 g of water, and 1 g of ethanolic extract of *X. aethiopica* were heated to 70°C. The aqueous mixture was then added to the oil mixture while stirring continuously at 70°C. After the transfer was complete, the mixture was allowed to cool

to room temperature while being continuously stirred. It was then immediately poured into a container, capped, labeled, and stored until future use. This same process was repeated for the aqueous extract.²⁶

2.10. Antimicrobial sensitivity test on formulated *X. aethiopica* creams and ointments

The susceptibility of microbial clinical strains against the prepared aqueous and ethanolic creams and ointments, as well as standard antibiotics, was evaluated using the agar well diffusion method.^{32,33} A 0.1 mL sample of the standard culture of *S. aureus* and *P. aeruginosa* was added to sterile Mueller–Hinton agar. The mixtures were thoroughly combined, left at room temperature for 3 h, and then poured into sterile Petri dishes to solidify. Wells were created in the seeded plates using a sterile 6 mm diameter cork borer and subsequently filled with 1.2 g of the cream and ointment samples, along with antibiotics of known concentrations. The plates were incubated at 35°C for 24 h, after which the zones of inhibition were measured.³⁴

2.11. Quality evaluation tests on formulated *X. Aethiopica* creams and ointments

Several evaluation tests were conducted on the formulated aqueous and ethanolic ointments and creams to determine their consistency, durability, comfort, and acceptability. These tests included assessments of physical appearance, color, odor, texture, phase separation, stability, pH, skin irritability, and homogeneity.²⁵ A random selection of students from Central University performed these tests.

Table 1. Master formula for ointment preparation

Ingredient	Weight (g)
Extracts	1
Wool fat	0.5
Hard paraffin	0.5
Cetostearyl alcohol	0.5
White paraffin	7.5

Table 2. The master formula for cream preparation

Phase	Ingredient	Weight (g)
Aqueous	Extracts	1
	Glycerin	0.6
	Water	7.1
Oil	Stearic acid	1.3
	Potassium hydroxide	0.5
	Sodium carbonate	0.5

They visually inspected the ointment, applied a small amount to their skin, and assessed the texture by rubbing it between their thumb and index finger to determine if it was rough or smooth.

2.12. Statistical analysis

The data obtained were analyzed using one-way analysis of variance on a completely randomized design using an Excel spreadsheet (Microsoft Office Excel 2021). Statistical significance was set at $p < 0.05$.

3. Results and discussion

3.1. Phytochemical screening of *X. aethiopica* fruit extracts

The phytochemical screening result of the *X. aethiopica* fruit extracts is shown in Table 3. The ethanolic extracts possessed a rich presence of steroids, tannins, glycosides, and reducing sugars. The aqueous extracts contained saponins, tannins, and reducing sugars, but glycosides and alkaloids were absent.

3.2. The MICs of the *X. aethiopica* fruit extracts

The MICs of the fruit extracts against the microorganisms are shown in Table 4. MIC refers to the lowest concentration of an antimicrobial agent that will inhibit the visible growth of a microorganism. The ethanol extract had stronger antibacterial activity against *S. aureus* and *P. aeruginosa* compared to the aqueous fraction. The MICs for all active extracts of *X. aethiopica* ranged between 2.5 mg/mL to 10 mg/mL.

3.3. Antimicrobial sensitivity of *X. aethiopica* formulated creams and ointments

The sensitivity pattern of the bacterial isolates against the different formulated dosage forms was compared with ciprofloxacin (1 mg/mL), a standard antibiotic (Table 5). The sensitivity tests indicated that ciprofloxacin was highly effective against both Gram-positive and Gram-negative bacteria, which supports its use as a broad-spectrum

Table 3. Phytochemical analysis of *Xylopia aethiopica* fruit extracts

Phytochemicals	Extract	
	Ethanolic	Aqueous
Saponins	+	+
Tannins	+	+
Glycosides	+	–
Alkaloids	+	–
Reducing sugars	+	+

Notes: + Indicates present; – Indicates absence.

Table 4. Minimum inhibitory concentration of ethanolic and aqueous extracts of *Xylopia aethiopica* fruits

Test organism	Extract	Minimum inhibitory concentration (mg/mL)								
		80	40	20	10	5	2.5	1.25	0.625	Ciprofloxacin (1 mg/mL)
<i>Staphylococcus aureus</i>	Ethanolic	–	–	–	–	–	MIC	++	++	–
	Aqueous	–	–	–	MIC	++	++	++	++	–
<i>Pseudomonas aeruginosa</i>	Ethanolic	–	–	–	–	–	MIC	++	++	–
	Aqueous	–	–	–	MIC	++	++	++	++	–

Notes: + Indicates growth (turbid); – Indicates no growth.

Abbreviation: MIC: Minimum inhibitory concentration.

Table 5. The sensitivity pattern of formulated *Xylopia aethiopica* creams and ointments against test bacterial isolates

Test organism	Zone of inhibition (mm)				Ciprofloxacin
	Ointments		Creams		
	Ethanolic	Aqueous	Ethanolic	Aqueous	
<i>Staphylococcus aureus</i>	31	20	25	11	40
<i>Pseudomonas aeruginosa</i>	25	14	19	8	52

antibiotic. The formulated creams and ointments showed antimicrobial activity against all tested microorganisms. This was demonstrated by the observed zones of inhibition (Figure 2).

3.4. Physicochemical evaluation of formulated ointments and creams

The quality of the prepared *X. aethiopica* ointment and cream was determined based on organoleptic properties, including physical appearance, color, texture, phase separation, uniformity, and texture.^{35–39} The formulated ointments and creams were found to have a smooth texture, pale brown color for ointment and a dark brown color for cream, an elegant appearance with no phase separation, as shown in Table 6.

The presence of phytochemicals in *X. aethiopica* was identified using established phytochemical methods.¹⁶ The tested phytochemicals included tannins, saponins, glycosides, and alkaloids, each playing a vital role in wound healing and medicinal applications.^{36,37} Tannins aid in wound healing through their astringent properties, promoting tissue contraction and reducing bleeding, and possess antioxidant properties that protect tissues from oxidative damage.^{38,39} Saponins offer antimicrobial and anti-inflammatory benefits, aid in the management of wound infections and inflammation, and act as surfactants, enhancing the solubility of other active compounds.^{40,41} Glycosides, convertible into active forms in the body, exhibit anti-inflammatory, analgesic, and antioxidant properties, contributing to pain relief and inflammation reduction in wound healing.^{42,43} Alkaloids provide analgesic properties and can impact the central nervous system, aiding in pain

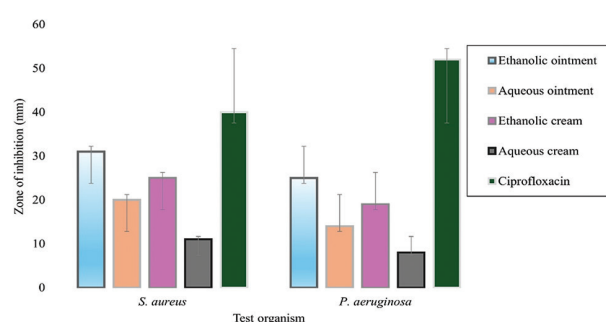


Figure 2. Antibacterial activities of the formulated *Xylopia aethiopica* creams and ointments against test bacterial isolates.

relief and patient well-being during the healing process.^{44,45} The results from two extraction methods (aqueous and ethanolic) showed that the ethanolic extraction possessed more phytochemicals, highlighting the impact of the solvent on the plant's phytochemical content.^{17,46} Further research is needed to uncover valuable data on *X. aethiopica* fruit extracts.

The results showed that the extract significantly inhibited the growth of *S. aureus* and *P. aeruginosa* (which are common bacteria causing wound infections⁴⁷) at a certain concentration,⁴⁸ with varying degrees of inhibition. It was observed that the antibacterial effect of the extract depended on the concentration and the solvent used in the extraction,^{49,50} which was evident in the MIC test performed. The MICs of the extracts recorded ranged from 2.5 mg/mL to 10 mg/mL (Table 4). Standard references used included ciprofloxacin, a broad-spectrum antibiotic that inhibits the growth of both Gram-positive and Gram-negative organisms.

Table 6. Physicochemical evaluation of formulations of *Xylopia aethiopica* ointments and creams

Physicochemical parameters	Observation	
	Ointments	Creams
Physical appearance	Opaque	Opaque
Color	Pale brown	Dark brown
Texture	Fine	Fine
Odor	Characteristic	Characteristic
Phase separation	No phase separation	No phase separation
Homogeneity	Positive	Positive
Consistency	Smooth on skin	Smooth on skin
pH	6.3	7
Spreadability (s)	5	4
Irritancy	Non-irritant	Non-irritant
Stability	Stable	Stable

There were clear zones observed around the boreholes in the agar where the ointments and creams were seeded, indicating bacterial growth inhibition. The zone of inhibition of the ethanolic ointments against *S. aureus* and *P. aeruginosa* was 31 mm and 25 mm, respectively (Table 5). The aqueous ointments produced inhibition zones of 20 mm and 14 mm against *S. aureus* and *P. aeruginosa*, respectively, as shown in Table 5. The ethanolic and aqueous creams yielded similar results to those of the ointments. The zone of inhibition for the ethanolic cream against *S. aureus* and *P. aeruginosa* was 25 mm and 19 mm, respectively, whereas the aqueous cream exhibited zones of 11 mm and 8 mm, respectively. Overall, the ethanolic formulations demonstrated greater antibacterial activity than their aqueous counterparts, with the ethanolic ointment showing the highest efficacy (Figure 2). A larger zone of inhibition was observed around the ciprofloxacin-containing disk, indicating the test organism's susceptibility to the antibiotic. Comparing the cream and the ointment, the ointment showed a greater zone of inhibition than the cream against the test organisms used.

With the aim of using *X. aethiopica* as a basis for pharmaceutical creams and ointments for wound healing, this study explored the potential of the plant's phytochemicals, such as tannins, saponins, glycosides, and alkaloids. Each of these phytochemicals plays a distinct and important role in promoting wound healing and holds promise for alternative medicine applications.⁵¹ This diverse phytochemical composition provides a comprehensive approach to wound healing by supporting both physical tissue repair and psychological well-being. The MIC obtained shows the effectiveness of the extract

at low concentrations, representing a practical advantage, such as reduced product usage and a lower likelihood of adverse reactions/side effects. These findings suggest strong potential for both product safety and commercial viability.

In summary, this research indicates a promising future for *X. aethiopica*-based pharmaceutical products in wound healing. The synergistic phytochemical combination, precise manufacturing processes, and favorable MIC results provide a strong foundation for further research. This work encourages ongoing exploration of natural remedies and innovative medical solutions.

4. Conclusion

In conclusion, the results have shown that both ethanolic and aqueous extracts of *X. aethiopica* fruits have antibacterial properties and can be used as a potential source of natural antimicrobial compounds. This research validates the historical use of *X. aethiopica* in traditional medicine by demonstrating its significant antibacterial properties against *S. aureus* and *P. aeruginosa*. Both ethanolic and aqueous extracts of the plant showed effective antibacterial activity with MIC values ranging from 2.5 mg/mL to 10 mg/mL. The development of topical ointments and creams from these extracts resulted in formulations with excellent organoleptic properties and antibacterial efficacy. These findings not only underscore the potential of *X. aethiopica* as a viable source of antimicrobial agents but also set the stage for further research into its broader therapeutic applications.

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Conflict of interest

The authors declare that they have no competing interests.

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Ethics approval and consent to participate

This study was approved by the Central University Institutional Review Board (CU-IRB), with approval number CU-IRB/PHARM/2024/012. All participants involved in the product evaluation provided verbal informed consent before participation. The study was conducted in accordance with the ethical standards outlined in the Declaration of Helsinki.

Consent for publication

Not applicable.

Availability of data

The datasets used during the current study are available from the corresponding author upon request.

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