
Antibacterial Property of Carabao Mango (*Mangifera indica linn*) Ethanolic Seed Extract as a Topical Ointment against *Staphylococcus aureus*

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Abstract—*MANGIFERA INDICA Linn*, commonly known as Carabao Mango, is known to possess antimicrobial activity. The study determined the antibacterial activity of the seed kernel of Carabao Mango ethanolic extract when formulated as a topical ointment. Experimental test design was used in the study. Three concentrations were formulated (1%, 2%, 5%) which were then subjected to paper disc diffusion assay to determine the antibacterial activity of the ointment. It was further subjected to organoleptic tests and skin irritability testing. Through the paper disc diffusion assay, the results showed an average mean of zone of inhibition (ZOI) against *Staphylococcus aureus* for the ointments with concentration 1% and 2% was 6 millimeters and for 5% it was 19 millimeters. This indicated that 1% and 2% ointments have an inactive inhibitory activity as compared to positive control (Mupirocin) which had 44 millimeters, indicating a very active inhibitory activity. The 5% ointment had a higher ZOI compared to the other two concentrations, indicating active inhibitory activity. However, it was still lower compared to the positive control. Using One-Way ANOVA and Tukey HSD multiple comparisons, the researchers obtained a p-value of 1.000 and .07658 for the comparison of the different concentration and the positive control (Mupirocin). The result indicated a significant difference between the groups. In conclusion, the Carabao mango ethanolic seed extract when formulated as a topical ointment showed an antibacterial property against *S. aureus*, at 5% concentration.

Keywords— Carabao mango, *Mangifera indica Linn*, ethanolic seed extract, antibacterial ointment, *Staphylococcus aureus*

I. INTRODUCTION

The primary purpose of skin is to act as a barrier between the internal environment of the body, protecting against allergens, chemicals, UV radiation, germs, chemicals, and water loss. (Benson ,2012). As the body's largest and most accessible organ, it usually harbors a diverse range of bacteria (Liu, 2014). Although many bacteria reside on the skin, they are

normally unable to establish an infection. However, once there are breaches in the skin, such wounds and scratches, bacterial pathogens can now enter and the risk of bacterial skin infection is increased (Rehmus, 2022).

Common bacterial infections can be eradicated using commercially accessible antibiotics. One way is by applying a topical antimicrobial, such as Mupirocin (Williamson et al., 2017). The advantages of using topical antibacterial over systemic antimicrobials include a higher concentration at the target site, fewer systemic adverse effects, and lower incidence of antimicrobial resistance (Punjataewakupt et al., 2019). However, the use of synthetic topical antimicrobial agents carries the risk of contact irritation (Kreft, & Wohlrab, 2022).

One of the most promising sources of antibacterial are from plant materials. Compared to synthetic compounds they are also considered safer due to their natural origin, especially when it comes to skin irritability. Carabao mango (*Mangifera indica*), a juicy stone fruit belonging to the family of Anacardiaceae and the national fruit of the Philippines, has been found to have antimicrobial properties for treating infections caused by bacteria and fungi (Awad El-Gied et al., 2012). One of its commonly used parts was the fruit; however, its seed, which is commonly considered as waste, has also shown a good antibacterial activity against pathogenic bacteria (Ahmed, 2015; Bernal-Mercado et al., 2018; Raju et al., 2019). In research conducted by Sarabia et al. (2013), the ripe seed extract of Carabao mango showed the highest inhibitory action when tested for its antimicrobial property against *Staphylococcus aureus*.

Different parts of mango exhibit antibacterial activity. Mango leaves have been reported to have various medicinal effects like antioxidant, antimicrobial, anthelmintic, antidiabetic, and antiallergic, etc. (Hannan et al., 2013). In a comparative study of mango's antibacterial activities of the

leaf, seed, and stem bark, it revealed that the seed extract has the highest antibacterial activity against Gram-positive bacteria *Staphylococcus aureus* (*S. aureus*) and Gram-negative bacteria *Escherichia coli* (*E. coli*). Furthermore, it showed that the ethanolic seed extract of mango has a more potent antibacterial activity than the extracts of the other parts at the same concentration (Osei-Djarbeng et al., 2020). According to a study by Yadav, the antibacterial activity of mango is due to the bioactive compounds in the different mango parts, with mangiferin being one of its major compounds, which is highly found on the peel and seed (2022). Aside from that mango seed kernels are also a rich source of phenolic compounds and flavonoids such as gallic acid, ellagic, pyrogallol, chlorogenic, catechin, protocatechuic, cinnamic, and catechol in which are also said to be responsible for antimicrobial property. They are said to prevent microorganisms by inhibiting extracellular microbial growth and by avoiding oxidative phosphorylation (Sen et al., 2020). It also contains myricetin caffeine, coumaric, sinapic acid, ferulic acid, salicylic, kaempferol quercetin, and tannin, which showed potent tyrosinase inhibitor and chelating activity (Melo et al., 2019). All these components are said to contribute to the antibacterial activity of *M. indica*.

Another bioactive study of *M. indica* against bacteria isolated from urine samples also showed the aqueous and ethanolic seed kernel extract good antibacterial activity against *S. aureus*. In a study conducted by Sarabia (2013), mango fruit and mango seeds underwent comparative experimental antimicrobial property testing. The data showed a significant difference in the activity of mango fruit extracts against *S. aureus* and *P. aeruginosa*, and more so in the mango seed. The ripe fruit extracts were inactive against *P. aeruginosa* while ripe and unripe seed extracts were active with the ripe seed extract presenting the highest inhibitory action. Toxic components were not detected in the seed kernel (Stuart, 2019). Taking into consideration the current risk of skin irritability on synthetic antibacterial ointments, the search for new or alternative agents that are safer on the skin, cheaper, good quality and with fewer side effects is necessary. The researchers believe that Carabao mango seed extract has the potential to be an alternative agent for topical antibacterial agents for bacterial skin infections.

This study, therefore, aims to focus on the antibacterial property of Carabao Mango (*M. indica*) seed extract when formulated as a topical ointment. The discovery of a new topical antibacterial agent will help develop herbal alternative treatments in the Philippines and lower the cost of topical antibiotics by using alternative sources of medicine that are endemic in the country.

II. METHODS

Experimental design was used in this study. The experiment was done by comparing the antibacterial activity of the different concentrations of Carabao Mango (*M. indica*) topical ointment and Mupirocin as the positive control, using the paper disc diffusion method. The ointment is also tested on different physicochemical evaluations and determines its skin irritability property.

A. Collection and Authentication of Plant Sample

Carabao Mango (*M. indica*) seed kernels were the plant material used in this study, specifically the seeds of ripe Carabao Mango. The mango seeds were collected as waste from a local food stall here in Tuguegarao. The plant materials collected were taken to the Department of Agriculture, Carig, Tuguegarao City, for authentication.

B. Preparation of mango seed kernel

Preparation of the plant material (ripe mango seed kernels) was adopted from Garga et al (2020) and Okpala & Gibson-Umeh (2013). 200 g of mango seed was collected and washed thoroughly with clean water to remove excess dirt and other unnecessary plant parts. After washing, the seeds were cracked manually to remove the shells and hulls. The collected kernels were air dried for 3 weeks until they were completely dry. Dried mango seed kernels were pounded into a fine powder using a mortar and pestle and passed through sieve no.20. The powder was packaged into clean polythene bags or containers, labeled accordingly, and stored for future use. The powdered mango kernel was used for extraction.

C. Preparation of Plant Extract

The mango seed kernel was extracted using 95 % ethanol. The powdered 200g of ripe mango seed kernel was transferred into a 1000 ml beaker. 300 ml of 95% ethanol was added in the beaker containing the powder. The mixture was stirred properly and macerated for 48 hours. It was then filtered using Whatman's No.1 filter paper to remove the insoluble materials. After removing insoluble materials, the solvent was evaporated by using a rotary evaporator. The extract produced was used to prepare Mango seed kernel topical ointment (Sarabia et al., 2013; Okafo et al., 2020).

D. Preparation of Ointment

The seed after the extraction process was formulated as ointment by different concentrations 1%, 2%, and 5%. The United States Pharmacopeia Chapter (1151) USP 31/ NF 26 was the source of the master formula used to make the ointment. United States Pharmacopoeia white ointment USP (4) was created using white wax, and white petrolatum with the addition of cetostearyl alcohol as a stabilizer.

TABLE I. MANGO SEED EXTRACT OINTMENT FORMULATION

Ingredients	Master Formula	Reduced Formula
Cetostearyl Alcohol	30 g	5 g
White Wax	50 g	10 g
White Petrolatum	920 g	185 g
	1000 g	qs.ad 200 g
Extract (%)	Prepared Mango Kernel Extract (g)	Ointment Base q.s. ad (g)

A white ointment base is based on the United States Pharmacopoeia done in the study of Demilew, Adinew & Asrade, (2018).

E. Determination of Antibacterial susceptibility

The antibacterial assay of this study was conducted by the Central Analytical Laboratory of Cagayan State University Andrews Campus. The Kirby Bauer Disc Diffusion Susceptibility Protocol was followed (Aguinaldo et al., 2005). Mueller-Hinton agar plates were prepared and properly labelled for each organism to be tested. Each MH agar plate was inoculated with the test organism. The formulated Mango seed ointment was impregnated into Whatman paper disks at a diameter of 6mm with a volume of 0.02 g each. Impregnated disks were placed with the positive disks in equidistant points on inoculated Mueller-Hinton agar plates. Each ointment was tested in three trials with triplicates for each organism. All test plates were incubated at 35 C for 18-24 hours to achieve optimal growth. The inhibition zones were determined in millimeters (mm) and interpreted descriptively as show in the table II below, adopted from Aguinaldo et al., (2005).

TABLE II. ZONE OF INHIBITION INTERPRETATION

Zone of Inhibition	Qualitative Interpretation
< 10 mm	Inactive
10 - 13 mm	Partially Active
14 - 19 mm	Active
> 19 mm	Very Active

F. Quality Control Evaluation of Ointment

Mango kernel ointment was tested for physical appearance, color, texture, phase separation, and homogeneity. These characteristics were evaluated through visual observation of five experts (Sabale et al., 2011; Maru & Lahoti, 2019). pH was measured using a digital pH-meter and spreadability was computed using the following formula:

$$S = \frac{M * L}{T}$$

Where:

S = Spreadability, (g.cm/s)

M = Weight put on the upper glass slide

L = Length moved by the glass slide (cm)

T = Time (s) taken to separate the slide from each other.

G. Skin Irritation Test

Fifteen (15) male Sprague Dawley rats were used for the skin irritation test as described by the OECD guidelines 404 (Maru, & Lahoti, 2019; Ankomah, et al, 2022). 50 mg of each formulation was applied over one square centimeter area of the whole and abraded skin of the Sprague Dawley rat. At the end of testing, the animals were observed after 24 hours, 48 hours and 72 hours and observed for any signs of edema and erythema.

TABLE III. UNITED STATES PHARMACOPOEIA (USP) ORGANOLEPTIC PARAMETER STANDARDS

Tests	Reference Results
Organoleptic Parameters	
Physical appearance	Opaque
Texture	Smooth, non-gritty
Odor	Pleasant

Tests	Reference Results
Phase separation	No sign of phase separation
Homogeneity	Homogeneous
pH	4-6 ± 0.195
Spreadability	97.32 ± 4.53
Skin Irritation Study	No sign of erythema or edema

Table 3 shows the standards to be used based on the United States Pharmacopoeia (USP) used in the journal article (Maru & Lahoti, 2019).

TABLE IV. GRADING OF SKIN IRRITATION

Mean Score Range	Qualitative Interpretation	
Erythema	No erythema	0
	Very slight erythema (barely perceptible)	1
	Well defined erythema	2
	Moderate to severe erythema	3
	Severe erythema to eschar formation preventing grading of erythema	4
Edema	No edema	0
	Very slight edema (barely perceptible)	1
	Slight edema (edges of area well defined by definite raising)	2
	Moderate edema (raised approximately 1 mm)	3
	Severe edema (raised more than 1 mm and extending beyond area of exposure)	4

Table 4 shows the grading of skin reaction based on the OECD guidelines used in the journal article (Gatne et al.,2015).

H. Data Analysis

The mean of the zone of inhibition of the test ointment is computed. One-way Analysis of Variance (ANOVA) with a 0.01 level of significance was used to identify if there were any significant differences among the ointment formulation.

I. Ethical Considerations

The researchers made sure that the standards set by the Bureau of Animal Industry (BAI) and Institutional Animal Care and Use Committee (IACUC) was followed and taken into consideration in handling the rats.

III. RESULTS AND DISCUSSION

TABLE V. EVALUATION OF THE PHYSICO-CHEMICAL CHARACTERISTIC OF MANGO KERNEL TOPICAL OINTMENTS

Mean Score Range	1% Carabao Mango Seed Ointment	2% Carabao Mango Seed Ointment	3% Carabao Mango Seed Ointment
No. of Days	1 7	1 7	1 7

Mean Score Range	1% Carabao Mango Seed Ointment		2% Carabao Mango Seed Ointment		3% Carabao Mango Seed Ointment	
Color	Whitish brown	Whitish brown	Light brown	Light brown	Brown	Brown
Odor	Slightly sweet	Slightly sweet	Moderately sweet	Moderately sweet	Moderately sweet	Moderately sweet
Texture	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth
Phase Separation	No separation	No separation	No separation	No separation	No separation	No separation
Homogeneity	Homogenous	Homogenous	Homogenous	Homogenous	Homogenous	Homogenous
pH	6.80	6.33	5.84	5.10	5.36	5.03
Spreadability g.cm/s	60.75	51.35	49.13	49.45	41.90	43.08

Table V shows the results based on the assessment parameters evaluated by faculty members of pharmacy. The results were able to meet the set standards by the United States Pharmacopeia, USP. Aside from the slight change in pH and spread ability after 7 days, no other changes were observed. This indicates that the formulation can be considered stable.

TABLE VI. RESULT OF SKIN IRRITABILITY TEST FOR ERYTHEMA FOR THE DIFFERENT TREATMENTS

Treatments	After 24 hours	Interpretation	After 48 hours	Interpretation	After 48 hours	Interpretation
Positive control (Formaldehyde)	3	Moderate to severe erythema	2.6	Moderate to severe erythema	2	Slight erythema
Negative control (Distilled water)	0	No erythema	0	No erythema	0	No erythema
1% Carabao Mango Seed Ointment	0	No erythema	0	No erythema	0	No erythema
2% Carabao Mango Seed Ointment	0	No erythema	0	No erythema	0	No erythema
3% Carabao Mango Seed Ointment	0	No erythema	0	No erythema	0	No erythema

Table VI shows the results of positive and negative control as well as the different concentrations of formulated mango ointment when subjected to skin irritability testing. Only the positive showed an effect on the skin of the rats, with it having a well-defined erythema, while the different

concentrations and the negative control showed no effect or signs of erythema at all.

TABLE VII. RESULT OF SKIN IRRITABILITY TESTS FOR EDEMA FOR THE DIFFERENT TREATMENTS

Treatments	After 24 hours	Interpretation	After 48 hours	Interpretation	After 48 hours	Interpretation
Positive control (Formaldehyde)	3	Moderate to severe edema	2.3	Moderate to severe edema	1	Very Slight edema
Negative control (Distilled water)	0	No edema	0	No edema	0	No edema
1% Carabao Mango Seed Ointment	0	No edema	0	No edema	0	No edema
2% Carabao Mango Seed Ointment	0	No edema	0	No edema	0	No edema
3% Carabao Mango Seed Ointment	0	No edema	0	No edema	0	No edema

Table VII shows the results of positive and negative control as well as the different concentrations of formulated mango ointment when subjected to skin irritability testing. Again, only the positive showed an effect on the skin of the rats, with slight edema this time, while the different concentrations and the negative control showed no effect or signs of edema at all.

TABLE VIII. ANTIBACTERIAL ACTIVITY OF THE DIFFERENT TREATMENTS AGAINST STAPHYLOCOCCUS AUREUS

Treatments	Interpretation	After 48 hours
Positive control (Mupirocin)	44	Very Active
Negative control (Distilled water)	6	Inactive
1% Carabao Mango Seed Ointment	6	Inactive
2% Carabao Mango Seed Ointment	6	Inactive
3% Carabao Mango Seed Ointment	19	Active

Table VIII shows a zone of inhibition pattern that mupirocin has the highest mean of 44 millimeters which indicates very active activity against Staphylococcus aureus. The 1%, 2% ointments and negative control showed a mean of 6 millimeters, indicating that its inactive when used against S. aureus. However, 5% Carabao Mango Kernel Ointment has shown a mean of 19 millimeters which indicates that it is active against S. aureus at that concentration.

TABLE IX. ANTIBACTERIAL ACTIVITY OF THE DIFFERENT TREATMENTS AGAINST STAPHYLOCOCCUS AUREUS

Treatments	F- value	p- value	Decision
1% Carabao Mango Seed Ointment	945.053	.000	Reject Ho
2% Carabao Mango Seed Ointment			
3% Carabao Mango Seed Ointment			
Positive control (Mupirocin)			
Negative control (Distilled water)			

Table IX shows that there is a significant difference between positive control (Mupirocin) and the formulated ointments. For concentrations 1% and 2%, there was no significant difference between it and the negative control (distilled water), indicating that the antibacterial activity of the two concentrations is not that different to the distilled water. However, at 5% concentration, a significant difference was shown. This indicates that at that level of concentration, it does possess a antibacterial property against the bacteria *S.aureus*.

For the antibacterial activity of the formulations, concentrations' 1% and 2% exhibited inactive inhibitory activity, however, for the 5% formulation an active inhibitory activity was observed. In a study conducted by Sarabia et.al (2013), on the antibacterial activity of Carabao Mango seed, the ripe seed extract exhibited a very active inhibitory activity against *S. aureus*. Comparing that to the results of this research, a decrease in inhibitory activity was observed. In research by Awad El-Gied et. al. (2015), they also formulated an ointment using a different variety of *M. indica* seed extract. Their formulated ointments also exhibited active inhibitory activity, with 10% concentration having the highest result. However, compared to the extracts they have tested alongside with the formulations, the results were lower. One possible explanation for this might have something to do with the amount and kind of ointment base used in the formulation.

In a study by Pawar & Nabar, (2010), where they tested the effect of plant extracts formulated in different ointment bases on MDR strains, the results showed that petroleum jelly or white petrolatum, Hydrophilic ointment, Aloe gel, and oil base did not exhibit any zone of inhibition towards the resistant organism, due to reduced diffusible property of the formulated ointment. Polyethylene glycol base, Carbopol base and honey showed good antibacterial property. The bases were non-greasy, water-soluble and water washable and thus, was selected for formulation of the plant extract in the form of an ointment. For this study, we used petroleum jelly as an ointment base, however a non-MRSA strain was used. Taking this into consideration there was a reduction in the diffusible property of the formulated ointment, however as a non-MRSA strain was used it was still able to exhibit an inhibitory activity although lower than what was expected.

This research, therefore, is subjected to some limitations. First, the focus of the study is to determine the antibacterial activity of Carabao Mango Seed extract when formulated as an ointment. The diffusible property of the formulated ointment is not tested. Testing the diffusible property can help in determining the appropriate ointment base to be used for this extract for it to exhibit it maximum effect. Second, the bacteria used in the study was only *Staphylococcus aureus*, due to it being a common cause of bacterial skin infection. Additionally, what was used was a non-MRSA strain. It is therefore unclear whether the formulated ointment will also work on a different bacteria strain or in a different bacterium.

IV. CONCLUSION

In terms of antibacterial property, only the 5% concentration showed an active inhibitory activity against *Staphylococcus aureus*. However, compared to the positive control (*Mupirocin*) which is available in the country, it's inhibitory effect is still much lower. Still, we, the researchers therefore conclude that the Carabao Mango (*M. indica*) ethanolic seed extract when formulated as an ointment, at 5% concentration, does possess antibacterial properties against *S. aureus*.

V. RECOMMENDATIONS

Based on the results of this research study, the following are recommended:

- To formulate Carabao Mango (*M. indica*) ethanolic extract ointment at higher concentrations.
- To test the antibacterial property of the formulated Carabao Mango (*M. indica*) Kernel Ointment at higher concentrations.
- To formulate an ointment using a different ointment base.
- To test the diffusible property of the ointment.

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