

## FORMULATION OF ANTIMICROBIAL LIQUID HAND SOAP FROM CRUDE LEAF EXTRACTS OF *Muntingia calabura* AGAINST SELECTED SKIN PATHOGENS

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### ABSTRACT

Hand hygiene represents a critical measure for preventing disease transmission. This study determined the antimicrobial activity and physical properties of a liquid hand soap formulated from crude extracts of *Muntingia calabura* leaves using an experimental design. The plant samples were extracted with three extracting solvents: methanol, ethyl acetate, and water. The percent yields and retention factor (Rf) values of secondary metabolites were determined for the different extracts. The results indicated that the aqueous extract yielded the highest percentage, while the methanolic extract contained the highest diversity of secondary metabolites among the tested extracts. These findings suggest that solvent selection should be based on the study objectives, whether maximizing extraction yield or identifying diverse secondary metabolites. The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the extracts were also determined as the basis for soap formulation. The methanolic extract was selected for soap production because it had the lowest MIC value. The formulated liquid hand soap demonstrated antimicrobial activity against *Staphylococcus aureus* and *Candida albicans* in agar well diffusion assays. Moreover, the recorded organoleptic properties, pH, foam stability percentage, homogeneity, and skin sensitivity of the formulated liquid hand soap were all within standards and an acceptable range. These results suggest that liquid hand soap formulated from *M. calabura* leaf extracts exhibits promising antimicrobial potential.

**Keywords:** Methanolic extract, Minimum Bactericidal Concentration (MBC), Minimum Inhibitory Concentration (MIC), skin sensitivity test, zone of inhibition

### INTRODUCTION

#### Background of the Study

Skin infections are estimated to affect 1.8 billion people at any given time. Skin infections in tropical and resource-poor nations are frequently caused by bacteria, fungi, viruses, or parasites (World Health Organization, 2023). These pathogens pose a threat to public health, especially because they are common causes of diseases that affect numerous individuals worldwide. In the Philippines, skin diseases are also a threat to the local public health, given that this issue is associated with high prevalence. According to the WHO (2019), skin disease is one of the top 50 causes of mortality in the Philippines for 2018 and contributed to 3,718 fatalities in 2019.

Statistical data indicate that skin infections remain a major health problem in the Philippine healthcare system, driven by factors such as overcrowding, poor sanitation, and inadequate healthcare in both urban and rural areas. This represents a global health issue, with millions of people affected by skin diseases annually. These illnesses, which can be bacterial, viral, or fungal in origin, are often chronic, highly transmissible, and difficult to contain, especially in densely populated areas.

Regarding the plant selected for this study, *Muntingia calabura* (commonly known as *Aratiles* or *Jamaican cherry*) was chosen for its traditional use in folk medicine for health

benefits and its unexplored potential as an antimicrobial liquid hand soap. Despite existing research on this plant, its use as an antimicrobial liquid hand soap remains understudied.

A significant research gap exists in current scientific knowledge about the antimicrobial potential of products derived from *M. calabura* extracts. Although Dayana et al. (2022) explored the plant's phytochemical properties and antimicrobial activity, their study was restricted to methanol extracts, neglecting the potential of ethyl acetate and water extracts. The present study seeks to address this limitation by including methanol, ethyl acetate, and water in the extraction process. Furthermore, Dayana et al. (2022) suggested additional research using different solvents and assay methods to fully explore the plant's potential.

Liquid soap is used for hand washing due to its ease of use, absence of microbial spread, and moisturizing characteristics. Bar soap, on the other hand, can easily spread germs and should not be used in public places due to its potential for germ growth (Minnesota Department of Health, 2019). From this, formulating liquid hand soap from *M. calabura leaf* crude extracts can address various needs and offer benefits that commercially available liquid hand soap may not. The various antimicrobial soaps on the market often contain synthetic chemicals that can harm both the environment and public health. According to Shmerling (2019), triclosan, which is a common component of antimicrobial products, contributes to bacterial resistance to antibiotics.

### Statement of the Problem

This research study determined the antimicrobial and physicochemical activities of the formulated liquid hand soap prepared from the crude extracts of *M. calabura leaves*. This was conducted from January 2025 until March 2025. Specifically, it answered the following questions:

1. What are the percent yields of the methanol, water, and ethyl acetate extracts of *M. calabura leaves*?
2. What are the secondary metabolites detected in the crude extracts of *M. calabura leaves* and their Rf values?
3. What are the Minimum Inhibitory Concentrations (MIC) and Minimum Bactericidal Concentrations (MBC) of the crude extracts of *M. calabura leaves* that can inhibit the growth of *C. albicans*, *S. aureus* and *P. aeruginosa*?
4. What is the antimicrobial activity of the formulated liquid hand soap prepared from the crude extracts of *M. calabura leaves*, in terms of Zone of Inhibition?
5. What are the physical properties of the formulated liquid hand soap prepared from the crude extracts of *M. calabura leaves* in terms of organoleptic property, pH, foam stability percentage, homogeneity property, and skin sensitivity?

## METHODOLOGY

### Research Design

The study utilized an experimental design. This involved the extraction of secondary metabolites using methanol, water, and ethyl acetate, followed by the assessment of antimicrobial activity using Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC), and Zone of Inhibition. The physical characteristics of the formulated soap, including organoleptic properties, pH, foam stability, homogeneity property, and skin irritation index, were also analyzed. The study utilized various control groups, replications, and randomization.

## Study Site and Sample Collection

The study was conducted at the Center for Natural Sciences Research Laboratory of Saint Mary's University, Bayombong, Nueva Vizcaya. The plant sample used in this study was *Muntingia calabura*. The *M.calabura* leaves were obtained from Purok 7, Santa Monica, Echague, Isabela. The collection site is free from known sources of pollution, such as vehicle emissions, toxic chemicals, heavy metals, and similar contaminants.

## Specimen Identification

The plant specimen of the study was submitted to the Department of Forestry and Agroforestry of Nueva Vizcaya State University, Bayombong, Nueva Vizcaya, for certification of its botanical or taxonomic identity.

Bacterial specimens, including *Candida albicans*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*, were obtained from the CNS-SMU culture collection.

After securing Bureau of Animal Industry (BAI) clearance, the laboratory mice (*Mus musculus*) were purchased and examined in the Philippine Institute of Traditional and Alternative Health Care (PITAHC), Dalan Na Pagayaya, Regional Center, Carig Sur, Tuguegarao City, Cagayan.

## Data Gathering Procedure

### *Collection and Preparation of Plant Sample*

Fresh and healthy *M. calabura* leaves were collected on February 12, 2025, at 11:00 a.m. About 1 kilogram of leaves was placed in clean plastic bags and brought to Bacarra Street, Solano, Nueva Vizcaya, for cleaning and drying. The leaves were washed under running water to remove contaminants, drip-dried, and then oven-dried at 45 °C until crisp. The dried leaves were ground into a fine powder in a blender and stored in a sterile, sealed beaker.

### *Crude Extraction of the plant material*

*M. calabura* leaves were extracted using the maceration method adapted from Djima et al. (2021) with slight modifications. One hundred grams of dried and ground leaves were soaked separately in 1000 mL of ethyl acetate, methanol, and distilled water for 24 hours. The mixtures were filtered using Whatman No. 4 filter paper, and the filtrates were concentrated to a syrupy consistency using a rotary evaporator at 50 °C. The concentrated extracts were weighed to determine yield and diluted for serial dilutions to test the Minimum Inhibitory Concentrations (MICs).

### *Determination of Percent Yield*

The mass of the collected *M.calabura* leaves was recorded prior to solvent extraction of the samples. After the extraction, the volume of the crude extract was also noted. The percent yield of each extracting solvent was calculated based on the standard formula: Percent Yield = (Weight of Dried Extract/ Weight of Dried Plant Material) x 100

### *Phytochemical screening of plant material*

The phytochemical screening of plant extracts was performed to identify the following metabolites: coumarins, flavonoids, alkaloids, phenols, terpenoids, tannins, and glycosides, present in the extracts following standard protocols according to Guevara et al. (2005). Thin Layer Chromatography (TLC) was performed to determine the R<sub>f</sub> values of the secondary metabolites detected in each extract.

### *Determination of Minimum Inhibitory Concentrations (MIC) and Minimum Bactericidal Concentrations (MBC)*

The MIC and MBC were determined according to the protocols of Swebocki (2023), with slight modifications.

The test organisms were inoculated into 10 mL of fresh Mueller-Hinton Broth (MHB) and incubated at 37°C for 18-24 hours. After incubation, the test organisms were standardized. The crude extracts were dissolved in MHB to achieve the set maximum concentration. Subsequently, 50 µL of MHB was dispensed into wells 2-10 of a 96-well microtiter plate. Column 11 remained empty, and 100 µL were added to column 12 as a sterility control. Positive control was added to column 1. The positive control used against *S. aureus* and *C. albicans* was Gentamycin, whereas Oxacillin was used as a positive control against *P. aeruginosa*. The crude extract solution was added to column 11, followed by two-fold serial dilutions down to column 2 to achieve a concentration range of 2000 µg/ml to 3.9 µg/ml. The inoculum (50 µL) was added to wells 1-11. Five replicates were performed in well plates. The plates were covered, labeled, and incubated at 37°C for 24 hours. After 24 hours, 20 µL of *Scientific Phygene* MTT reagent was added to the wells, and color development was observed after 2 hours (Vipra et al., 2013). The MIC value is determined at the well where no color change occurs. A pale yellow color in the well after incubation indicates no bacterial growth, while a blue to purple color signifies bacterial growth due to the formation of formazan (Hanina Mohd Noor et al., 2013).

#### *MBC Assay*

For the MBC assay, the contents from wells showing no bacterial growth or no color change following the MIC assessment were plated directly onto Mueller-Hinton Agar (MHA) plates and incubated for 24 hours at 37 °C. After incubation, the Mueller-Hinton Agar (MHA) plates were observed for bacterial growth. The Minimum Bactericidal Concentration (MBC) is defined as the concentration at which no bacterial colonies are observed. The MBC value was determined by examining the agar plates before and after incubation to confirm the presence or absence of microbial growth.

#### *Formulation of liquid hand soap*

The liquid hand soap base was formulated following the procedures of Ordoyo and Sepe (2019). One hundred milliliters (100 mL) of coconut oil and forty milliliters (40 mL) of pure glycerin were heated separately, then combined and stirred at 60 °C. A lye-water solution was prepared by dissolving 30 grams of potassium hydroxide (KOH) in 100 mL of distilled water. The lye-water solution was gradually mixed with the heated coconut oil and then combined with the glycerin. The mixture was stirred continuously for approximately 3 to 4 hours to form a soap paste.

To formulate a 100 ml liquid soap, 30 grams of soap paste were dissolved in 100 ml of distilled water. Afterward, it was neutralized and preserved with 1 gram of borax decahydrate. Citric acid was added to the soap base until the pH was within the accepted range of 4 to 10. Then, 50 mg of the methanolic extract of *M. calabura* leaves was added to the soap base, which was mixed homogeneously before being transferred into a clean bottle for storage and labeling. This concentration of extract was based on the MIC and MBC values.

#### *Zone of Inhibition of the formulated liquid hand soap using agar diffusion test*

The antimicrobial activity of the formulated *M. calabura* liquid hand soap was evaluated using the agar diffusion (Kirby-Bauer) method, as described by Jain et al. (2016) with modifications. Standardized cultures of *C. albicans*, *S. aureus*, and *P. aeruginosa* (0.5 McFarland) were inoculated onto Mueller-Hinton Agar (MHA). Three equidistant wells were made for the test soap, a positive control (commercial antibacterial handwash, Brand X; Kaliyadan et al., 2014), and a negative control (soap base). Each well received 50 µL of the respective test

substance. The setup was replicated five times and incubated at 37°C for 24 hours. After incubation, the zones of inhibition (ZOI) were measured in millimeters using a digital Vernier caliper, and the diameters were recorded.

#### *Physical properties of the formulated liquid hand soap*

The parameters for determining the physical properties of the formulated liquid hand soap are based on the methods of Dewi and Mardhiyani (2021), with slight modifications, including a skin-sensitivity test with a 72-hour post-application observation period.

**Organoleptic Test:** An organoleptic test was conducted by observing the physical form of the liquid hand soap preparations using the senses. The formulated liquid hand soap was evaluated for color and odor.

**pH Test:** pH was measured with a pH meter using a 10% sample solution prepared by dissolving 1 mL of the sample in 9 mL of distilled water. The pH meter electrode, rinsed with distilled water, was immersed in the solution, and the pH value was recorded once the reading stabilized.

**Foam Stability Test:** A 1 mL sample was placed in a test tube, followed by the addition of 10 mL of distilled water. The tube was shaken, and the initial foam height was measured using the foam stability formula. After allowing the tube to stand for five minutes, the foam height was measured again.

**Homogeneity Test:** A 1 mL sample of the liquid hand soap was spread on an acetate plastic surface and examined for the presence of coarse particles by touch. The sample was considered homogenous if no coarse particles or texture were present on the surface.

**Skin-sensitivity test:** Ten mice were divided into treated (Tr) and control (Cr) groups of 5 each. After shaving the dorsal area and allowing 24 hours, the treated group received a liquid hand soap application, while the control group received sterile water. The area was covered for one hour, then rinsed and examined for irritation immediately and at 24, 48, and 72 hours post-application. Skin reactions, including erythema and edema, were evaluated and scored using the Draize scoring system (Ankomah et al., 2022).

#### **Treatment of Data**

The percent yield of each extract and the Rf values of the detected secondary metabolites were calculated using standard formulas. Additionally, MIC values were recorded and interpreted according to the standard of Aligiannis (2001). The results of the antimicrobial activity of the formulated liquid hand soap were presented as the mean±SD (Standard Deviation) of five replicates. Standard ranges were also used to evaluate the product's physical properties, including organoleptic properties, pH, foam stability, homogeneity, and skin sensitivity.

#### **Ethical Considerations**

The study was approved and monitored by the Saint Mary's University Research Ethics Board (SMUREB) of Saint Mary's University, 2nd Floor, Rev. John Van Bauwel Hall, SMU Main Campus, Ponce Street, Don Mariano Marcos, Bayombong, 3700 Nueva Vizcaya, Philippines, with contact information via email at [reb@smu.edu.ph](mailto:reb@smu.edu.ph) and cellphone at 09177053041. The study was given an animal research permit with reference number AR-2024-0501 by the Bureau of Animal Industry at Visayas Avenue, Diliman, Quezon City, through the Institutional Animal Care and Use Committee of the Philippine Institute of Health Care (PITAHC) – Cagayan Valley Herbal Processing Plant at Carig Sur, Tuguegarao City, Cagayan.

## RESULTS AND DISCUSSIONS

### Section 1. Percent Yields

**Table 1**  
*Percent Yields of the Different Extracts*

Solvent	Mass of dry extract (g)	Mass of dry plant material (g)	Percent yield (%)
Ethyl Acetate	9.04	100	9.04
Methanol	12.43	100	12.43
Water	27.88	100	27.88

*Dry weight of the plant is measured before extraction, and the weight of the extract is the weight after the extraction process*

Table 1 presents the percent yield of *M. calabura* leaves obtained with three different extraction solvents. Based on the data shown above, the aqueous extract has the highest percent yield with 27.88%. It is then followed by a methanol-ethyl acetate extract of at least 9.04 %.

According to Al Ubeed et al. (2022), the percent yield, or phytochemical extraction efficiency, is strongly influenced by factors such as temperature, extraction duration, agitation, and pressure. This means that the percent yield is affected by a wide range of factors, which can also be explored for future studies. Moreover, Zhang et al. (2018) show that ethanol and methanol are widely used as extracting solvents. However, in this study, ethyl acetate and water were also explored and showed considerable percent yields.

Hence, water can be used as an extraction solvent when aiming for a high percent yield, and ethyl acetate is the least recommended due to its lowest yield based on the obtained data.

### Section 2. Rf Values and Secondary Metabolites Detected from *M. calabura* leaf extracts

**Table 2**

*Rf Values of Detected Secondary Metabolites from M. Calabura Extracts*

Secondary Metabolites	Extracting Solvents		
	Methanol	Ethyl Acetate	Aqueous
Essential Oils	0.86	0.91	-
Fatty acids	0.92	-	-
Triterpenes & Sterols	0.22	-	-
Phenols	0.65	0.94	0.85
Anthraquinones	0.65	0.92	-
Anthrones	-	0.93	-
Tannins	0.65	0.96	-
Flavonoids	0.83	0.93	0.89
Alkaloids	0.37	0.92	-
Glycosides	0.91	0.91	0.98

*Each type of extract may have more than one secondary metabolite*

Table 2 presents the Retention Factor (RF) values of the detected secondary metabolites using three different extracting solvents for *M. calabura* leaves. Methanol extract yielded the most secondary metabolites among the three extraction solvents, followed by Ethyl Acetate extract, which did not detect fatty acids, triterpenes, or sterols, and Aqueous extract, which yielded the least, detecting only three secondary metabolites. The detected Rf values of the secondary metabolites reveal their polarity and can serve as a reference for identifying the metabolites.

The study by Priya and Yasmin (2022) found that *M. calabura* extract contained flavonoids, steroids, steroidal/triterpenoidal compounds, and their glycosides. Moreover, Sowanja et al. (2023), detected flavonoids, phenols, tannins, and saponins from the roots, fruits, and flowers of *M. calabura* using methanol as an extracting solvent. In line with this, the same secondary metabolites were also detected in a methanol extract prepared from *M. calabura* leaves. *M. calabura* leaf extracts show a significant amount of essential oils, which may be due to the aromatic scent of *M. calabura*. According to Hochma et al. (2021), polyphenols, such as flavonoids and phenolic acids, are secondary metabolites known for their strong antimicrobial activity. Alkaloids from plant extracts can prevent bacterial growth and enzymes through various mechanisms (Barati & Chahardehi, 2023; Yan et al., 2021). These large groups of secondary metabolites can be used to formulate various antibacterial or antimicrobial products, given that alkaloids are an important component of many antimicrobial drugs (Cushnie et al., 2014). Flavonoid glycosides are shown to be antioxidant compounds (Mohammed et al., 2024) and exhibit antibacterial activities comparable to those of available antibiotics, suggesting their potential as antibiotics (Tagousop et al., 2018; Zou & Fang, 2021). According to Yang et al. (2022), anthraquinones are secondary metabolites that possess antibacterial, antiulcer, anticancer, anti-inflammatory, and antiviral activities. Lastly, tannins, alkaloids, saponins, glycosides, steroids, terpenoids, flavonoids, phlobatannins, anthraquinones, and reducing sugars are among the secondary metabolites found in plants with medicinal properties (Agidew, 2022).

Thus, the presented data indicate that methanol is an efficient extraction solvent for detecting a greater number of secondary metabolites from *M. calabura*.

### Section 3. Minimum Inhibitory Concentrations (MIC) values and Minimum Bactericidal Concentrations (MBC)

**Table 3**

*MIC and MBC Values of M. Calabura Leaf Extracts*

Plant Extracts	Antimicrobial Activity					
	MIC (ug/ml)			MBC		
	SA	CA	PA	SA	CA	PA
<b>Methanol</b>	500	500	0	0	0	0
<b>Ethyl</b>	1000	2000	0	0	0	0
<b>Aqueous</b>	0	0	0	0	0	0

SA= *S. aureus*; CA= *C. albicans*; PA= *P. aeruginosa*

≤500ug/ml = strong inhibitor; 600 -1500ug/ml =moderate inhibitors; ≥1600ug/ml =weak inhibitors (from Aligiannis, 2001)

Table 3 presents the MIC and MBC values of the different extracts against the three test organisms. Based on the data, methanol extract is the recommended extract to be used for soap formulation as it has the lowest and most consistent MIC values compared to ethyl acetate and aqueous extracts. The final MIC value shown in the table was determined through the mode of values from the replicates. Moreover, wells with no bacterial growth were evaluated through MBC to determine if the extracts had a bactericidal effect. Thus, the results revealed that methanol extract has antimicrobial activities against the two test organisms.

On the other hand, the obtained MIC from the extracts did not have bactericidal activity due to observed microbial growth after streaking the wells that did not have a color change in MIC. This means that the set concentrations of the plant extracts are not potent enough to kill microbes, rather can only be considered bacteriostatic. Bactericidal agents are often considered a far superior or better choice than bacteriostatic agents, however, this view may vary with clinical situations, especially in treating numerous infections particularly those that are related

to gram-positive bacteria (Pankey & Sabath, 2004). Based on the study of Ishak et al., (2024), there are cases where bacteriostatic agents are considered clinically equal degree with bactericidal agents. According to the classification of Aligiannis (2001), the MIC value of methanolic extract with 500ug/ml is considered a strong inhibitor.

Since the soap formulation of this study utilized the data obtained from MIC and MBC, the methanolic extract which showed lower MIC values was used as the active ingredient for formulating the antimicrobial soap.

#### Section 4. Antimicrobial Activity of Formulated Liquid Hand Soap

**Table 4**

##### Zone of Inhibition of Test Substances

Test Substances	Zone of Inhibition					Mean ± SD
	Replicates (mm)					
	1	2	3	4	5	
<i>Staphylococcus aureus</i>						
Formulated Liquid Soap (FLS)	23.0	22.6	23.7	24.9	23.5	23.54±0.87
Brand X (+control)	17.6	16.9	15.4	17.1	17.5	16.75±0.89
Soap base (- control)	0	0	0	0	0	0
<i>Candida albicans</i>						
Formulated Liquid Soap (FLS)	18.9	15.5	13.7	18.6	15.5	16.675±2.24
Brand X (+control)	16.0	14.1	12.7	15.9	15.0	14.675±1.38
Soap base (- control)	0	0	0	0	0	0
<i>Pseudomonas aeruginosa</i>						
Formulated Liquid Soap (FLS)	0	0	0	0	0	0
Brand X (+control)	0	0	0	0	0	0
Soap base (- control)	0	0	0	0	0	0

<10 mm - inactive; 10-13mm - partially active; 14-19 active; >19mm - very active (from Guevara et al. 2005)

Table 4 shows that the formulated liquid soap can inhibit the growth of *S. aureus* and *C. albicans*, with mean standard deviations of 23.54±0.87 and 16.675±2.24, respectively. The results revealed that the formulated liquid soap is comparable to the positive control and shows a higher Zone of Inhibition. The negative control showed no inhibition, confirming that the soap base did not affect the product's antimicrobial activity. Lastly, *P. aeruginosa* was not inhibited by any of the test substances.

Based on the standard of zones of inhibition by Guevara et al. (2005), the formulated liquid hand soap showed very strong inhibition against *S. aureus* and strong antifungal activity against *C. albicans*. However, it has no inhibition against *P. aeruginosa*. This shows that the formulated liquid hand soap is more potent in inhibiting Gram-positive bacteria and fungi, but not against highly resistant Gram-negative bacteria. *P. aeruginosa* possesses a complex biofilm that contributes to its pathogenicity and resistance to a wide range of antibacterial agents (Tuon et al., 2022). This structure may explain why both the formulated liquid hand soap and the positive control cannot inhibit its growth. According to Nagoba et al. (2017), *P. aeruginosa* is resistant to common antiseptics and disinfectants.

Hence, the formulated liquid hand soap is comparable to the antimicrobial activity of the positive control and can be a potential alternative to commercially available liquid hand soap, especially against skin pathogens such as *S. aureus* and *C. albicans*.

### Section 5. Physical Properties of Formulated Liquid Soap

**Table 5**

Physical Characteristics of Formulated Liquid Hand Soap

Parameters	Results
Color	Yellow-Brown
Odor	None
pH	7.7±0
Foam Stability	63.11%
Homogeneity Property	Homogenous

**Figure 1**

Formulated Liquid Hand Soap



Figure 4 has a yellow-brown color with no odor. It has a transparent and thin consistency. As presented in Table 5, the pH level is within the standard pH level for liquid hand soap which is around 4-10. Moreover, the foam stability falls within the accepted range of 60-70%. Lastly, the liquid soap is homogenous because coarse particles are absent and the consistency is smooth and fine.

According to Nova et al. (2025), formulating soaps that possess a good balance between different physical properties and within accepted range is important for achieving a safe and quality soap. Moreover, the evaluation of the physical properties of formulated liquid soaps should include pH level as it is one of the important bases in assessing soaps that are healthy for the skin. This is also because pH level is usually associated with skin irritation and scaling (Odinga et al., 2024).

Hence, the formulated liquid hand soap showed good quality and is accepted within the standard ranges based on the physical properties it presented.

**Table 6**

Skin Sensitivity Test

Time Interval	Irritation Index ± SD		Interpretation
	Tr	Cr	
1 hour	0	0	No erythema or edema
24 hours	0	0	No erythema or edema
48 hours	0	0	No erythema or edema
72 hours	0	0	No erythema or edema

Interpretations were based on Draize Scoring System

## Figure 2

Images of the Shaved Area of Treated Mice

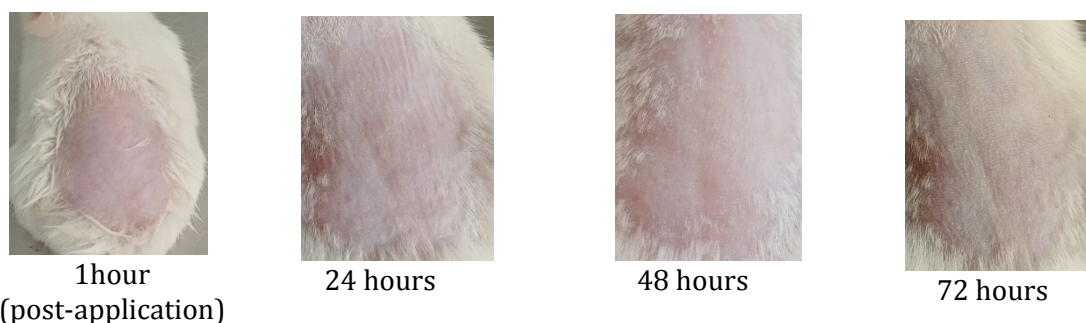


Table 6 and Figure 5 show that no erythema or edema was observed from the formulated liquid hand soap over the 72-hour post application observation. The results present that the formulated liquid hand soap was non-irritating to the skin of mice.

According to Asghar et al. (2024), a skin irritation test ensures that the formulated product possesses ingredients that are harmless to the skin and are safe to use for some time. This type of test helped in evaluating the skin sensitization of the product. Prolonged exposure to soaps can cause changes to pH balance and overall skin health (Lakshmi et al., 2010), which means that it is essential to observe the irritability or possible effect of the soap product before its commercialization. Hence, the absence of skin irritation in the mice might be attributed to the physical properties of the soap that are within the accepted range or standard quality of a liquid hand soap.

Hence, the formulated liquid hand soap showed no skin irritation on the shaved skin of mice as per observations conducted at 24, 48, and 72 hours post-application.

## CONCLUSIONS AND RECOMMENDATIONS

### Conclusion

This study determined the antimicrobial activity of the formulated liquid hand soap prepared from the crude leaf extracts of *M. calabura* against selected skin pathogens. The results revealed that the methanol extract was the most efficient among the three solvents in detecting a wide range of secondary metabolites. The methanol extract showed acceptable and consistent MIC values that can be considered for product formulation. However, the methanol extract did not exhibit a bactericidal effect as shown by its non-remarkable MBC values, hence, it can only be characterized as bacteriostatic. The formulated liquid hand soap, Botaniwash with the crude extract from *M. calabura* as an active ingredient, showed active antimicrobial activity against *S. aureus* and *C. albicans* with Zones of Inhibitions higher than those of the positive control. Botaniwash showed no skin irritation in animal models and met the standard ranges based on its physical properties. Therefore, the methanolic crude extract from the leaves of *M. calabura* is a potential source of active ingredients in formulating antimicrobial soaps.

### Recommendations

1. Utilize the findings of the study for product upscaling and further optimize the physical properties of the soap for commercial and pharmaceutical applications.

2. Explore other dosage forms such as creams, and ointments, utilizing the antimicrobial activity of the plant sample.
3. Utilize other plants that are suitable as alternative ingredients for the soap base to serve as moisturizers, foam boosters, and scents for the formulated product.
4. Evaluate the long-term stability and conduct a shelf-life analysis of the formulated liquid hand soap to improve its potential for commercialization.
5. Evaluate the acceptability of the formulated liquid hand soap based on regulatory and industry standards as well as other relevant guidelines to ensure product safety, efficacy, and marketability.

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