




Original Research Article *Pharmaceutics and Drug Delivery*

Development of emulgel formulation from *Markhamia tomentosa* leaf extract: Characterization and *in vitro* antimicrobial activity against skin isolates

Bukola Aminat Oseni¹, Shunammite Tofunmi Osekita¹, Mutiat Bolanle Ibrahim² , Nwamaka Henrietta Igbokwe³, Chukwuemeka Paul Azubuike¹

¹Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, University of Lagos, Lagos State, Nigeria.

²Department of Pharmacognosy, Faculty of Pharmacy, University of Lagos, Lagos State, Nigeria.

³Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmacy, University of Lagos, Lagos State, Nigeria.

*Corresponding author:

Bukola Aminat Oseni, PhD
Department of Pharmaceutics
and Pharmaceutical
Technology, Faculty of
Pharmacy, University of Lagos,
Lagos State, Nigeria.

abadebayo@unilag.edu.ng

Received: 03 March 2024

Accepted: 21 April 2024

Published: 28 May 2024

<https://ajpps.org>

DOI

10.25259/AJPPS_2024_009

Quick Response Code:



ABSTRACT

Objectives: Skin infections are mild to severe infections often treated with antimicrobial agents. There is an increase in resistance to antimicrobial agents necessitating the search for new medicines. This study developed an emulgel formulation of *Markhamia tomentosa* leaf extract and evaluated its antimicrobial activity against skin isolates.

Materials and Methods: A successive extraction of *M. tomentosa* leaves in five solvents – n-hexane, dichloromethane, ethyl acetate, ethanol, and distilled water was carried out. The activity of the extracts against skin isolates of *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Bacillus subtilis* was carried out. The distilled water extract was formulated into emulgel, characterized, and evaluated for antimicrobial activity.

Results: The n-hexane and dichloromethane extracts of *M. tomentosa* were inactive against all organisms at concentrations used. The ethanol extract was active against *S. aureus* and *S. epidermidis* at 200 mg/mL. The ethyl acetate and distilled water extracts were active against *S. epidermidis* and *B. subtilis* at 100 and 50 mg/mL, respectively. All emulgel formulations of distilled water extract of *M. tomentosa* leaves were brown, smooth, and easy to spread on the skin and had a pH of 4.73–5.55. The formulation containing the distilled water extract was more viscous (7784cP) than the blank formulation (6328cP). The formulation was active against *S. epidermidis*.

Conclusion: The distilled water extract of *M. tomentosa* leaves was the most active against *S. epidermidis* and *B. subtilis*. Its emulgel formulation had desirable physicochemical properties for application to the skin. Further studies can be carried out toward its development for the treatment of skin infection caused by organisms investigated.

Keywords: *Markhamia tomentosa*, Emulgel formulation, Antimicrobial agents, Skin infections, Antimicrobial resistance

INTRODUCTION

Skin and soft-tissue infections (SSTIs) are caused by the microbial invasion of the skin and underlying soft tissues which can result in mild to life-threatening conditions.^[1] SSTIs occur in about 24.6/1000 persons annually; about 7–10% of hospitalized patients are diagnosed with SSTIs, representing the third most common diagnosis in the emergency setting after chest

This is an open-access article distributed under the terms of the Creative Commons Attribution-Non Commercial-Share Alike 4.0 License, which allows others to remix, transform, and build upon the work non-commercially, as long as the author is credited and the new creations are licensed under the identical terms.

©2024 Published by Scientific Scholar on behalf of American Journal of Pharmacotherapy and Pharmaceutical Sciences

pain and asthma.^[2] SSTIs are caused by Gram-positive, Gram-negative, or sometimes mixed infections with most infections caused by the Gram-positive organisms – *Staphylococcus aureus* and Streptococci sp.^[3] Antibiotics are administered orally, topically, or intravenously depending on the severity of the infection for the treatment of SSTIs. However, the emergence of resistance among Gram-positive and negative organisms to antibiotics makes the treatment of SSTIs more challenging.^[1] This has necessitated the continuous search for new antimicrobial agents.

Medicinal plants represent a source of novel and effective pharmacologically active agents. Over the years, they have been used as a readily available, low-cost alternative to orthodox medicines in the treatment of various ailments including infectious diseases.^[4] According to the World Health Organization, over 80% of people in sub-Saharan Africa rely on medicinal plants for their primary health-care needs.^[5]

Markhamia tomentosa (Benth.) K. Schum. ex Engl. (*Bignoniaceae*) is a shrub of about 15 m high found in tropical West Africa and Asia.^[6] They are commonly known as Echeru in Igbo, Ogie-ikhimwim in Edo, Akoko and Oruru in Yoruba, Nigeria. The plant is used traditionally in the treatment of pain, diarrhea, scrotal elephantiasis, inflammation, and cancer.^[7] The leaf extracts of the *M. tomentosa* plant have been scientifically shown to exhibit antimalarial, antiulcer, antioxidant, anti-inflammatory, analgesic, and antimicrobial activity against some *Candida* spp., Gram-negative and Gram-positive bacteria.^[6,8] *M. tomentosa* leaf extracts have been shown to contain bioactive compounds, namely, phenolic nuclei, saponins, terpenes, flavonoids, and steroids.^[9] Furthermore, two naphthoquinone compounds isolated from the stem bark of *M. tomentosa* showed antiprotozoal activity against *Leishmania donovani*, *Plasmodium falciparum*, and *Trypanosoma brucei rhodesiense*.^[10]

Medicinal plants such as *M. tomentosa* have gained popularity due to the increasing scientific evidence of their therapeutic activity; however, challenges such as non-solubility, and instability during storage and administration, delivery to non-target sites, affect their efficacy and limit their use.^[11] Incorporation of herbal drugs in a suitable drug delivery system enhances solubility, minimizes physical and chemical drug degradation, ensures drug delivery to target sites, protects from toxicity, enhances pharmacological activity, and aids in the sustained delivery of the active pharmaceutical ingredient.^[11]

Emulgels are delivery systems that have become popular for the delivery of hydrophobic drugs.^[12] It is a drug delivery system with a mixture of emulsion and gel. It has been considered a novel type due to its unique characteristics such as the ability to incorporate either a hydrophilic or hydrophobic drug.^[13] Furthermore, the emulgel formulation

combines the benefits of emulsion and gels such as controlled and enhanced release of the drug.^[13] Emulgels are easily removable, spreadable, greaseless, transparent, non-staining, and emollient, have a long shelf life, esthetically appealing, and hence promote patient acceptability and compliance. They are most widely used for the delivery of many drugs like analgesics, anti-inflammatory, anti-acne, and antifungals.^[12]

This study is aimed at developing an emulgel formulation containing *M. tomentosa* leaf extract and evaluating its antimicrobial activity against skin isolates.

MATERIALS AND METHODS

Materials

The solvents; n-hexane, dichloromethane, ethyl acetate, and ethanol were purchased from CDH, India. Distilled water was obtained from the laboratory. Mueller–Hinton Agar (MHA) was purchased from Oxoid Ltd, UK. Gentamicin injection, Carbopol 934 (Himedia, USA), liquid paraffin, Span 20 was purchased from Fengchen, China. Tween 20 and Tween 80 were purchased from Merck, Germany. Propylene glycol, methylparaben, propylparaben, and triethanolamine (TEA) were obtained from Sigma Aldrich, USA.

Plant collection

Fresh leaves of *M. tomentosa* (Benth) K. Schum ex Engl. were collected from Oke-Igbo, Ondo state, Nigeria, in January 2023. The leaves were taken to the Herbarium of the Department of Botany, University of Lagos for taxonomic identification and authentication. An herbarium specimen was deposited, and voucher number LUH 9862 was obtained.

Organisms

The skin isolates, namely – *Staphylococcus epidermidis*, *Bacillus subtilis*, and *S. aureus* were provided by the Department of Pharmaceutical Microbiology and Biotechnology, University of Lagos.

Extraction of *M. tomentosa* leaves

The *M. tomentosa* leaves were air-dried at room temperature (23°C ± 2°C) for 8 days and blended. A successive extraction of the *M. tomentosa* leaves was carried out in five solvents, namely, n-hexane, dichloromethane, ethyl acetate, ethanol, and distilled water in their order of increasing polarity. The powdered leaf material (1 kg) was macerated in 2 L of n-hexane for 48 h at room temperature with intermittent shaking. The mixture was filtered and evaporated to dryness using a rotary evaporator (Buchi, Switzerland) at a temperature of 40°C to obtain the n-hexane extract. The marc was air dried at room temperature to completely remove the

n-hexane solvent. The dried marc obtained after extraction with n-hexane was macerated in 2.5 L of dichloromethane for 48 h at room temperature. The dichloromethane extract was obtained by filtration and evaporated to dryness using the rotary evaporator. The dry marc was subjected to the maceration process using ethyl acetate, ethanol, and distilled water, respectively. The mixtures were filtered and evaporated to dryness to obtain the extracts.

Antimicrobial activities of the extracts

Test for zone of inhibition

Different concentrations (200, 100, and 50 mg/mL) of the various extracts were prepared using distilled water (for the distilled water and ethanolic extract) or 0.1% Tween 20 in distilled water (for n-hexane, dichloromethane, and ethyl acetate extract) as the solvent. These solvents were used as the negative control while gentamicin (20 µg/mL) was used as the positive control. A culture suspension of the Bacteria (*S. epidermidis*, *B. subtilis*, and *S. aureus*) was prepared and incubated for 3 days. Molten MHA of 25 mL was mixed with 1 mL of each of the organism cultures, transferred into Petri dishes and allowed to solidify. A hole of 12 mm diameter was bore at the center of each quadrant of the Petri dish and 1 mL of the extract was introduced into the hole. The plates were allowed to stand for 4 h and incubated at 37°C for 24 h. The zones inhibited were measured for each plate.^[14]

Determination of minimum inhibitory concentration (MIC)

The MIC for the extracts that showed a zone of inhibition against the organisms tested was determined. A stock concentration of 10.24 mg/mL was prepared for each extract used. A successive 1 in 2 dilution of the stock concentration was carried out to obtain thirteen working concentrations (0.0025–10.24 mg/mL) for each extract. A mixture of the molten agar and the various concentrations of the extract were allowed to solidify, and the organisms were inoculated

on each of the plates. The plates were incubated at 37°C and examined after 24 h. The lowest concentration of the extract where there was inhibition of the growth of the organism was chosen as the MIC.^[14]

Preparation of emulgels

Seven emulgel formulations were prepared, as shown in Table 1. The gel portion of the emulgel was prepared by dissolving carbopol-934 in cold water with constant stirring at a moderate speed until a uniform mixture was formed. The pH of the gel was adjusted to 6–6.5 using TEA. For the emulsion component, Tween 80 was dissolved in distilled water to prepare the aqueous phase of the emulsion while for the oil phase of the emulsion, span 20 was dissolved in liquid paraffin. Methylparaben and propylparaben were dissolved in propylene glycol and the extract was dissolved in ethanol. The propylene glycol and extract solutions were mixed with the aqueous phase. The aqueous and the oil phases were heated in a water bath at 70°C separately. The oil phase was added dropwise to the aqueous phase with continuous stirring for 10 min. The emulsion was allowed to cool at room temperature. The gel and emulsion portions were mixed at equal portions stirring moderately and consistently after each addition to avoid air entrapment.^[15]

Characterization of emulgels

Determination of organoleptic properties

The formulations were inspected visually for their color, homogeneity, and consistency. The feel of the emulgels was tested on the skin to check for their smoothness or roughness.

Determination of pH

The pH of each of the emulgels was measured using a Mettler Toledo pH meter (Mettler-Toledo, Switzerland). The electrode was inserted into the formulation and the pH reading was recorded.

Table 1: Composition of the emulgel formulation.

| Ingredients | F1 | F2 | F3 | F4 | F5 | F6 | F7 |
|-------------------------|------|------|------|------|------|------|------|
| Extract (g) | 2.50 | 2.50 | 2.50 | 2.50 | 0.50 | 2.50 | - |
| Carbopol (g) | 0.25 | 0.50 | 0.75 | 0.50 | 0.75 | 0.25 | 0.75 |
| Liquid paraffin (mL) | 3.75 | 3.75 | 3.75 | 2.50 | 2.50 | 2.50 | 2.50 |
| Tween 80 (mL) | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 |
| Span 20 (mL) | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Propylene glycol (mL) | 3.50 | 3.50 | 3.50 | 3.50 | 3.50 | 3.50 | 3.50 |
| Methylparaben (g) | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 |
| Propylparaben (g) | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 |
| Ethanol (mL) | 15 | 15 | 15 | 15 | 15 | 15 | 15 |
| Distilled water to (mL) | 50 | 50 | 50 | 50 | 50 | 50 | 50 |

F: Formulation

Spreadability study

The spreadability test on the emulgel was carried out using a wooden block with a glass slide attached to it and another glass slide with a fixed weight attached to it. On the glass slide that is attached to the wooden block, 1 g of emulgel was placed at the center of the slide in a 2 cm diameter of circle. The second glass slide was carefully placed over it with a fixed weight of 200 g on the slide for 5 min. The increase in diameter of the emulgel was noted.^[16]

Determination of viscosity

The viscosity of the emulgel was measured using a Brookfield viscometer (Ametek, USA) with spindle RV-05 at 10, 20, 30, 40, and 50 rpm at $25 \pm 0.5^\circ\text{C}$.

Centrifugation test on emulgels

The stability of the emulgel formulations was determined by centrifugation at 4000 rpm for 10 min. The formulations were examined visually for creaming or phase separation.

Evaluation of the antimicrobial activity of the emulgels

The method of determination of the zone of inhibition for the extracts was used. The molten agar of 25 mL was mixed with 1 mL of each of the susceptible organism cultures (*S. epidermidis* and *B. subtilis*), transferred into Petri dishes and allowed to solidify. A hole of 12 mm diameter was bore at the center of each quadrant of the Petri dish using a cork borer and the hole was filled with the emulgel. The plates were allowed to stand for 4 h and the inoculated plates were incubated at 37°C for 24 h. The zones of inhibition were measured in millimeters using a ruler for each plate. The emulgel without the extract was used as the negative control while gentamicin injection (5%) was used as the positive control.

Statistical analysis

All determinations were carried out in triplicate and the results were expressed as mean \pm standard deviation. Statistical difference between the mean values was determined by one-way analysis of variance using the GraphPad® Prism 7 software (GraphPad Software, La Jolla, CA). $P < 0.05$ was considered significant.

RESULTS

Yield of *M. tomentosa* extract

The yield of the various extracts obtained from the leaves of *M. tomentosa* is shown in Table 2. The n-hexane and distilled water extract had the lowest and highest yield, respectively.

Evaluation of the antimicrobial activity of *M. tomentosa* extract

The activities of the five extracts of *M. tomentosa*, namely, n-hexane, dichloromethane, ethyl acetate, ethanol, and distilled water against three organisms, namely, *S. aureus*, *S. epidermidis*, and *B. subtilis* are presented in Table 3a-e. The n-hexane and dichloromethane extract were inactive against the three organisms at all concentrations employed. The ethyl acetate extract exhibited activity against the three bacteria strains. The ethanol extract showed activity against *S. epidermidis* and *S. aureus* while the distilled water extract was active against *B. subtilis* and *S. epidemidis*.

The MICs of ethyl acetate, ethanol, and distilled water extracts that showed antimicrobial activity against some of the organisms tested are represented in Table 4. The distilled water extract was active against *B. subtilis* and *S. epidermidis* at the lowest concentration and therefore, more potent than the ethyl acetate and ethanolic extract.

Characterization of emulgels of *M. tomentosa* extract in distilled water

The emulgel prepared using the distilled water extract of the *M. tomentosa* leaves is shown in Figure 1. The physicochemical properties and pH of the emulgels are presented in Table 5 and Figure 2, respectively. All emulgel formulations were brown and had a smooth texture with a pH between 4.73 and 5.55.

Rheology study on emulgels

The viscosity of the distilled water *M. tomentosa* extract emulgel formulation with the desirable properties and lower concentration of the extract (F5) and the formulation devoid of the extract (F7) at different speeds is presented in Figure 3. An increase in the speed caused a reduction in the viscosity of both formulations (with or without the extract). The formulation containing the extract is more viscous than the formulation devoid of the extract.

Table 2: Percentage yield of the leaves extract of *Markhamia tomentosa*.

| Extract | n-Hexane | Dichloromethane | Ethyl acetate | Ethanol | Distilled water |
|-----------|----------|-----------------|---------------|---------|-----------------|
| Yield (%) | 1.13 | 1.55 | 1.34 | 1.17 | 2.44 |

Table 3a: Zone of inhibition of the n-hexane extract of *Markhamia tomentosa* leaves against test organisms.

| Organisms | Average zone of inhibition (mm) for n-hexane extract (n=3) | | | | |
|-----------------------|--|-----------|----------|----------|-----------------------|
| | 200 mg/mL | 100 mg/mL | 50 mg/mL | n-hexane | Gentamicin (20 µg/mL) |
| <i>B. subtilis</i> | - | - | - | - | 26.33±3.79 |
| <i>S. epidermidis</i> | - | - | - | - | 24.33±0.57 |
| <i>S. aureus</i> | - | - | - | - | 23.00±1.00 |

-: No zone of inhibition, *B. subtilis*: *Bacillus subtilis*, *S. epidermidis*: *Staphylococcus epidermidis*, *S. aureus*: *Staphylococcus aureus*, n: number of replicates

Table 3b: Zone of inhibition of the dichloromethane extract of *Markhamia tomentosa* leaves against test organisms.

| Organisms | Average zone of inhibition (mm) for dichloromethane extract (n=3) | | | | |
|-----------------------|---|-----------|----------|-----------------|-----------------------|
| | 200 mg/mL | 100 mg/mL | 50 mg/mL | dichloromethane | Gentamicin (20 µg/mL) |
| <i>B. subtilis</i> | - | - | - | - | 26.33±3.79 |
| <i>S. epidermidis</i> | - | - | - | - | 24.33±0.57 |
| <i>S. aureus</i> | - | - | - | - | 23.00±1.00 |

-: No zone of inhibition, *B. subtilis*: *Bacillus subtilis*, *S. epidermidis*: *Staphylococcus epidermidis*, *S. aureus*: *Staphylococcus aureus*, n: number of replicates

Table 3c: Zone of inhibition of the ethyl acetate extract of *Markhamia tomentosa* leaves against test organisms.

| Organisms | Average zone of inhibition (mm) for ethyl acetate extract (n=3) | | | | |
|-----------------------|---|------------|----------|---------------|-----------------------|
| | 200 mg/mL | 100 mg/mL | 50 mg/mL | Ethyl acetate | Gentamicin (20 µg/mL) |
| <i>B. subtilis</i> | 18.67±0.57 | 16.00±1.00 | - | - | 26.33±3.79 |
| <i>S. epidermidis</i> | 18.33±1.15 | 14.33±0.57 | - | - | 24.33±0.57 |
| <i>S. aureus</i> | 20.00±1.00 | 10.67±0.57 | - | - | 23.00±1.00 |

-: No zone of inhibition, *B. subtilis*: *Bacillus subtilis*, *S. epidermidis*: *Staphylococcus epidermidis*, *S. aureus*: *Staphylococcus aureus*, n: number of replicates

Table 3d: Zone of inhibition of the ethanolic extract of *Markhamia tomentosa* leaves against test organisms.

| Organisms | Average zone of inhibition (mm) for ethanolic extract (n=3) | | | | |
|-----------------------|---|------------|----------|---------|-----------------------|
| | 200 mg/mL | 100 mg/mL | 50 mg/mL | Ethanol | Gentamicin (20 µg/mL) |
| <i>B. subtilis</i> | - | - | - | - | 26.33±3.79 |
| <i>S. epidermidis</i> | 20.67±0.57 | 13.33±1.53 | - | - | 24.33±0.57 |
| <i>S. aureus</i> | 13.67±0.57 | - | - | - | 23.00±1.00 |

-: No zone of inhibition, *B. subtilis*: *Bacillus subtilis*, *S. epidermidis*: *Staphylococcus epidermidis*, *S. aureus*: *Staphylococcus aureus*, n: number of replicates

Table 3e: Zone of inhibition of the distilled water extract of *Markhamia tomentosa* leaves against test organisms.

| Organisms | Average zone of inhibition (mm) for distilled water extract (n=3) | | | | |
|-----------------------|---|-----------|------------|-----------------|-----------------------|
| | 200 mg/mL | 100 mg/mL | 50 mg/mL | Distilled water | Gentamicin (20 µg/mL) |
| <i>B. subtilis</i> | - | - | 19.67±0.57 | - | 26.33±3.79 |
| <i>S. epidermidis</i> | - | - | 20.67±0.57 | - | 24.33±0.57 |
| <i>S. aureus</i> | - | - | - | - | 23.00±1.00 |

-: No zone of inhibition, *B. subtilis*: *Bacillus subtilis*, *S. epidermidis*: *Staphylococcus epidermidis*, *S. aureus*: *Staphylococcus aureus*, n: number of replicates

Stability of emulgels

After the centrifugation test, none of the emulgel formulations showed signs of creaming or phase separation as a form of physical instability.

Evaluation of the antimicrobial activity of emulgel formulation

The zones of inhibition of the formulated emulgel on the active organisms are presented in Table 6. The emulgel

Table 4: MIC of ethyl acetate, ethanol, and distilled water extract of *Markhamia tomentosa* leaves against test organisms.

| Organism | MIC (mg/mL) | | |
|-----------------------|---------------|---------|-----------------|
| | Ethyl acetate | Ethanol | Distilled water |
| <i>S. aureus</i> | 5.12 | 10.24 | - |
| <i>B. subtilis</i> | 1.28 | - | 0.32 |
| <i>S. epidermidis</i> | 5.12 | 10.24 | 0.64 |

-: No growth inhibition observed, *S. aureus*: *Staphylococcus aureus*, *B. subtilis*: *Bacillus subtilis*, *S. epidermidis*: *Staphylococcus epidermidis*, MIC: Minimum inhibitory concentration

Table 5: Physicochemical characterization of *Markhamia tomentosa* emulgel formulations.

| Formulation code | Color | Texture | Spreadability (cm/5 min) |
|------------------|-------------|---------|--------------------------|
| F1 | Light Brown | Smooth | 8.00±0.11 |
| F2 | Light Brown | Smooth | 5.00±0.15 |
| F3 | Light Brown | Smooth | 4.90±0.05 |
| F4 | Dark Brown | Smooth | 5.20±0.25 |
| F5 | Light Brown | Smooth | 5.10±0.11 |
| F6 | Dark Brown | Smooth | 6.90±0.01 |
| F7 | White | Smooth | 5.70±0.13 |

F1, F2 and F3: 0.5, 1 and 1.5% carbopol, respectively, 7.5% liquid paraffin, 5% extract, F4 and F6: 1 and 0.5% carbopol, respectively, 5% liquid paraffin, 5% extract; F5: 1.5% carbopol, 5% liquid paraffin, 1% extract, F7: 1.5% carbopol, 5% liquid paraffin

formulation was active against *S. epidermidis* but inactive against *B. subtilis*.

DISCUSSION

SSTIs are one of the common diagnoses in hospital emergencies often treated with antibiotics; however, the increased incidence of resistance of causative organisms to antibiotics has been a cause for concern and necessitated the exploration of alternative sources of antibiotics. *M. tomentosa* plant has been shown to possess antimicrobial properties, for example, the ethyl acetate, dichloromethane, and methanol leaves extracts of the plant exhibited antioxidant and antimicrobial activities.^[17] The antimicrobial activity of extracts of *M. tomentosa* against SSTIs-causing bacteria has not been fully explored. In this study, the antimicrobial activity of *M. tomentosa* leaves extracts against three skin isolates was determined. The extract that was most sensitive to the test micro-organisms was developed into a herbal topical emulgel formulation.

Antimicrobial activity of *M. tomentosa* extracts

The antimicrobial activity of the five extracts used in this study was evaluated using the zone of inhibition analysis.

The zone of inhibition test is a quantitative method used clinically to measure antibiotic resistance and to test the ability of a substance to inhibit microbial growth. This analysis can be used as a measure of the sensitivity of microorganisms to a particular antibiotic and to discover new ones.^[18] The n-hexane and dichloromethane extracts had no zone of inhibition on any of the organisms used at 50, 100, and 200 mg/mL concentrations [Table 3a and b]; this shows that they had no antimicrobial activity against the test organisms at the concentrations utilized. The ethyl acetate extract [Table 3c] was active against all bacteria tested while the ethanolic extract [Table 3d] was active against the *Staphylococcus* spp. tested. This is in line with the findings of Aladesanmi *et al.*, 2007.^[19]

The distilled water extract was active against *S. epidermidis* and *B. subtilis* at 50 mg/mL concentration [Table 3e], higher concentrations showed no activity against the organisms. In most cases, a higher concentration of a medicinal agent causes a higher therapeutic activity, in this case, activity was observed at a lower concentration. This may be because the bioavailability of the extract is dependent on its concentration and pH.^[20] The standard antimicrobial agent – gentamicin was utilized in this study because it has broad activity against many Gram-negative organism and when combined with other antibiotics, exhibits antimicrobial activity against some Gram-positive organisms such as *S. aureus*.^[21] Gentamicin exhibited antimicrobial activity against the bacteria tested at concentrations as low as 0.02 mg/mL.

According to Eucast definitive Document Methods for the determination of susceptibility of bacteria to antibiotics, MIC is the lowest concentration of antimicrobial drug that will inhibit the visible growth of a microorganism after incubating overnight at 37°C.^[22] The MIC is a measure of the sensitivity of the medicinal agent to the test organism, the lower the MIC, and the more sensitive the medicinal agent. The ethyl acetate extract of *M. tomentosa* leaves was active against *S. epidermidis*, *S. aureus*, and *B. subtilis*; however, it is more sensitive to *B. subtilis* having a MIC of 1.28 mg/mL than the *Staphylococcus* spp. tested whose MIC was 5.12 mg/mL [Table 4]. The ethanolic extract was active against only *S. epidermidis* and *S. aureus* having similar potency with an MIC of 10.24 mg/mL [Table 4]. The distilled water extract exhibited the highest sensitivity to *S. epidermidis* (MIC of 0.64 mg/mL) and *B. subtilis* (MIC of 0.32 mg/mL) [Table 4]. Therefore, the distilled water extract was chosen for formulation into emulgel.

Physicochemical evaluation of *M. tomentosa* emulgels

The emulgel formulations of *M. tomentosa* distilled water extract were assessed for their organoleptic properties. The color of the formulations ranges from light brown (F1, F2, F3, and F5) to dark brown (F4 and F6) [Table 5]. The color

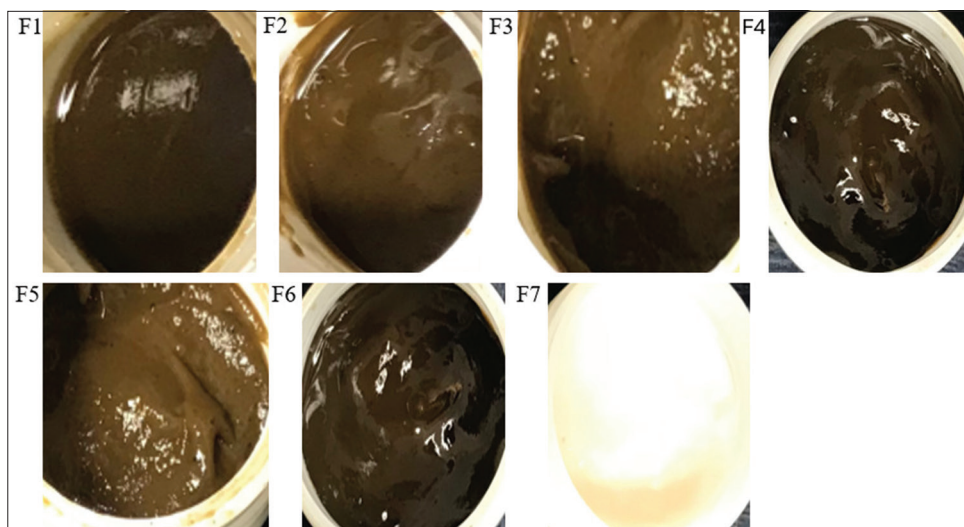


Figure 1: Emulgel formulation of *Markhamia tomentosa* extract in distilled water. F1, F2, and F3: 0.5, 1, and 1.5% carbopol, respectively, 7.5% liquid paraffin, 5% extract, F4 and F6: 1 and 0.5% carbopol, respectively, 5% liquid paraffin, 5% extract, F5: 1.5% carbopol, 5% liquid paraffin, 1% extract, F7: 1.5% carbopol, and 5% liquid paraffin. F: Formulation.

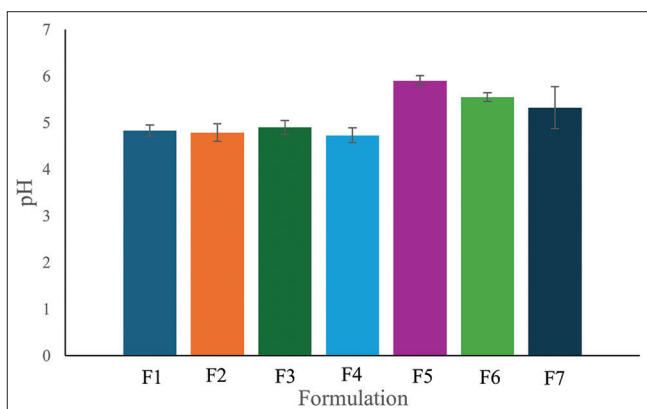


Figure 2: pH of *Markhamia tomentosa* emulgels. F1, F2, and F3: 0.5, 1, and 1.5% carbopol, respectively, 7.5% liquid paraffin, 5% extract, F4 and F6: 1 and 0.5% carbopol respectively, 5% liquid paraffin, 5% extract, F5: 1.5% carbopol, 5% liquid paraffin, 1% extract, F7: 1.5% carbopol, and 5% liquid paraffin.

observed was due to the *M. tomentosa* introduced into the formulation. The dark brown color observed in F4 and F6, may be due to the reduction in the quantity of liquid paraffin and carbopol in these two formulations; formulation F7 appeared white due to the absence of extract in the formulation. All the emulgels had a smooth feel on the skin and were easily washable after application. The pH of the emulgels falls within 4.73–5.55 [Figure 2]. In general, topical formulations are developed to have a similar pH as the intact human skin of 4–6 to prevent skin irritation and maintain a healthy skin environment.^[23] All emulgels fall within the specified range; hence, the formulations can be said to be suitable for application to the skin.

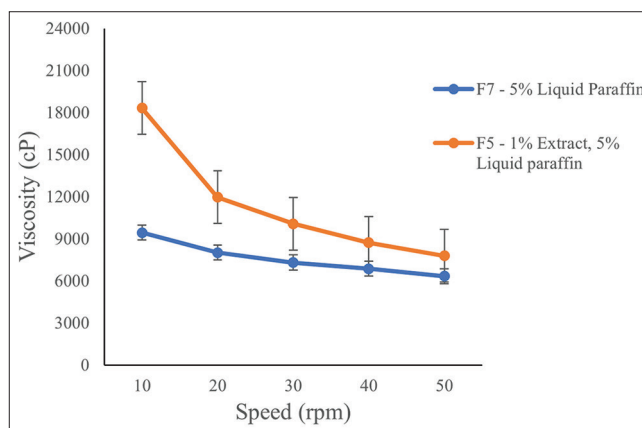


Figure 3: Viscosity of *Markhamia tomentosa* emulgel formulation (F) at different speeds.

The spreadability of a topical formulation is an important property in the development of an ideal formulation. It is used to determine the extent to which a formulation will spread on application to the skin to enhance skin penetration and bioavailability of the active ingredient. Formulations with high spreading value depict better spreadability and skin penetration ability.^[24] All emulgel formulations were easily spreadable but F1 and F6 were very spreadable. This can be attributed to a reduction in the concentration of the thickener and gelling agent – Carbopol (0.5%) in both formulations when compared with the other formulations (1 or 1.5% Carbopol).

The resistance of a fluid to deforming under shear force is measured by its viscosity. It is frequently interpreted as flow behavior or pouring resistance. Viscosity is described as a

Table 6: Zone of inhibition of *M. tomentosa* emulgel formulation on test organisms.

| Organisms | Zone of inhibition (mm) of emulgel formulation of <i>M. tomentosa</i> distilled water extract | | | | | | |
|-----------------------|---|------------|------------|------------|------------|------------|------------|
| | F1 | F2 | F3 | F4 | F5 | F6 | F7 |
| <i>S. epidermidis</i> | 23.17±0.29 | 19.50±0.50 | 25.17±0.29 | 20.33±0.29 | 23.67±0.58 | 25.33±0.28 | 15.17±0.29 |
| <i>B. subtilis</i> | - | - | - | - | - | - | - |

–: Means no zone of inhibition observed, F1, F2 and F3: 0.5, 1 and 1.5% carbopol, respectively, 7.5% liquid paraffin, 5% extract, F4 and F6: 1 and 0.5% carbopol, respectively, 5% liquid paraffin, 5% extract, F5: 1.5% carbopol, 5% liquid paraffin, 1% extract, F7: 1.5% carbopol, 5% liquid paraffin, F: Formulation.

fluid's internal resistance to flow. An increase in the viscosity of a formulation together with other factors increases its stability.^[25] An increase in viscosity was observed in the emulgel formulation with the extract (F5) when compared with the emulgel without the extract (F7) [Figure 3]. Therefore, the extract causes an increase in the viscosity of the formulation. Furthermore, a decrease in the viscosity was observed in both formulations following an increase in the shear stress from 10 rpm to 50 rpm. This is an attribute of a non-Newtonian shear-thinning fluid whose viscosity decreases with increased stress.^[26] Therefore, the amount of shear to be used during the preparation of the emulgel should be optimized to produce a stable product with desired fluidity for easy application. In addition, the viscosity of a gel is inversely proportional to its spreadability. An increase in spreadability will infer a low viscosity of the gel or product. This was observed in the F5 and F7 formulations, the latter was more spreadable [Table 5] and less viscous [Figure 3] than the former. Higher viscosity promotes stability of the gel; however, this can cause poor spreadability and poor skin penetration of the formulation; therefore, there is a need for a balance in viscosity and spreadability to obtain an ideal product.^[27]

Antimicrobial evaluation of emulgel

Antimicrobial screening was carried out on all the formulations to check if the extracts would retain their antimicrobial activity after formulating as an emulgel. All six formulations inhibited the growth of *S. epidermidis* but not *B. subtilis*. Formulations F1, F3, F5, and F6 had a higher zone of inhibition than the extract alone while the zone of inhibition of F2 and F4 is the same as the extract for *S. epidermidis* [Table 6]. The formulation containing 1% extract (F5) had similar zones of inhibition to those containing 5% extract (F1, F3, and F6). The formulation without the extract also showed the least zones of inhibition against *S. epidermidis* which suggests an inherent activity. All formulations showed no activity against *B. subtilis* whereas the extract was active against the organism. This may be attributed to the interaction between the extract and the excipients which might have hindered the activity of the formulation against the test organism. A systematic change in the excipient and the utilization of different excipients might retain the activity of the extract in the formulation.

CONCLUSION

The ethyl acetate, ethanol, and distilled water extracts of *M. tomentosa* leaves were active against *S. aureus*, *S. epidermidis*, and *B. subtilis*. The distilled water extract was the most susceptible to *S. epidermidis* and *B. subtilis*, hence used in the formulation of the emulgel. The emulgel prepared was light to dark brown, of smooth feel, easy to spread, suitable for application to the skin, and active against *S. epidermidis*. Therefore, an herbal topical formulation of *M. tomentosa* extract was developed for the treatment of skin infections caused by *S. epidermidis*. Further characterizations and studies need to be done to ascertain its suitability for development as a topical antibiotic.

Acknowledgment

We thank Mr. Abdulrahman Usman of the Department of Pharmaceutical Microbiology and Biotechnology, University of Lagos for providing the microbial organisms utilized in this study.

Ethical approval

The Institutional Review Board approval is not required.

Declaration of patient consent

Patient's consent was not required as there are no patients in this study.

Financial support and sponsorship

None.

Conflicts of interest

There are no conflicts of interest.

Use of artificial intelligence (AI)-assisted technology for manuscript preparation

The authors confirm that there was no use of artificial intelligence (AI)-assisted technology for assisting in the writing or editing of the manuscript and no images were manipulated using AI.

REFERENCES

- Kaye SK, Petty LA, Shorr AF, *et al.* Current epidemiology, etiology, and burden of acute skin infections. *Clin Infect Dis.* 2019;68:S193-S199. doi:10.1093/cid/ciz002
- Ki V, Rotstein C. Bacterial skin and soft tissue infections in adults: A review of their epidemiology, pathogenesis, diagnosis, treatment and site of care. *Can J Infect Dis Med Microbiol.* 2008;19:173-184. doi:10.1155/2008/846453
- Esposito S, Noviello S, Leone S. Epidemiology and microbiology of skin and soft tissue infections. *Curr Opin Infect Dis.* 2016;29:109-115. doi:10.1097/QCO.0000000000000239
- Dubale S, Kebebe D, Zeynudin A, *et al.* Phytochemical screening and antimicrobial activity evaluation of selected medicinal plants in Ethiopia. *J Exp Pharmacol.* 2023;15:51-62. doi:10.2147/JEP.S379805
- World Health Organization. Traditional medicine strategy 2014-2023. Geneva: World Health Organization; 2013. p. 1-76. Available from: <https://www.who.int/publications/item/9789241506096> [Last accessed on 2024 Feb 15].
- Ibrahim MB, Kaushik N, Sowemimo AA, *et al.* Review of the phytochemical and pharmacological studies of the genus *Markhamia*. *Pharmacogn Rev.* 2016;10:50-59. doi:10.4103/0973-7847.176547
- Ibrahim M, Kaushik N, Sowemimo A, *et al.* Antifungal and antiproliferative activities of endophytic fungi isolated from the leaves of *Markhamia tomentosa*. *Pharm Biol.* 2017;55:590-595. doi:10.1080/13880209.2016.1263671
- Bankole AE, Adekunle AA, Sowemimo AA, *et al.* Phytochemical screening and *in vivo* antimalarial activity of extracts from three medicinal plants used in malaria treatment in Nigeria. *Parasitol Res.* 2015;115:299-305. doi:10.1007/s00436-015-4747-x
- Borokini TI, Omotayo F. Phytochemical and ethnobotanical study of some selected medicinal plants from Nigeria. *J Med Plant Res.* 2012;6:1106-1118. doi:10.5897/JMPR09.430
- Adebajo AC, Famuyiwa FG, John JD, *et al.* Activities of some Nigeria medicinal plants against *Aedes aegypti*. *Chin Med.* 2012;3:151-156. doi:10.4236/cm.2012
- Sarangi MK, Padhi S. Novel herbal drug delivery system: An overview. *Arch Med Health Sci.* 2018;6:171-179. doi:10.4103/amhs.amhs_88_17
- Talat M, Zaman M, Khan R, *et al.* Emulgel; an effective drug delivery system. *Drug Dev Ind Pharm.* 2021;47:1193-1199. doi:10.1080/03639045.2021.1993889
- Shailendra KS, Ashutosh B, Bipin KN. Emulgel: Magnifying the application of topical drug delivery. *Indian J Pharm Biol Res.* 2017;5:25-33. doi:10.30750/ijpbr.5.1.4
- Balouiri M, Sadiki M, Ibensouda SK. Methods for *in vitro* evaluating antimicrobial activity: A review. *J Pharm Anal.* 2016;6:71-79. doi:10.1016/j.jpaha.2015.11.005
- Jain A, Deveda P, Chauhan J. Development of antifungal emulsion based gel for topical fungal infection. *Int J Pharm Res Dev.* 2011;2:18-25.
- Patel A, Patel J. Mucoadhesive micro emulsion based prolonged release vaginal gel for anti-fungal Drug. *Am J Pharm Tech Res.* 2012;2:650-661.
- Temdie RJ, Fotio AL, Metchi FD, *et al.* Protective activity of *Markhamia tomentosa* (Benth.) K. Schum. (Bignoniaceae) methanol leaves extract against galactosamine/lipopo- lysaccharide-induced acute liver injury in mice. *J Biosci Med.* 2020;8:74-89. doi:10.4236/jbm.2020.810008
- Barnard RT. The zone of inhibition. *Clin Chem.* 2009;65:819. doi:10.1373/clinchem.2018.299800
- Aladesanmi AJ, Iwalewa EO, Adebajo AC, *et al.* Antimicrobial and antioxidant activities of some Nigerian medicinal plants. *Afr J Tradit Complement Altern Med.* 2006;4:173-184. doi:10.4314/ajtcam.v4i2.31206
- Li Y, Du Y, Zhou C. Effects of pH on antioxidant and antimicrobial properties of tea saponins. *Eur Food Res Technol.* 2009;228:1023-1028. doi:10.1007/s00217-009-1014-3
- Gentamicin. In: LiverTox: Clinical and research information on drug-induced liver injury. Bethesda, MD: National Institute of Diabetes and Digestive and Kidney Diseases; 2012. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK548706> [Last accessed on 2024 Apr 17].
- European Committee for Antimicrobial Susceptibility Testing (EUCAST). EUCAST Definitive Document: Terminology relating to methods for the determination of susceptibility of bacteria to antimicrobial agents. *Clin Microbiol Infect.* 2000;6:503-8.
- Okoro NK, Oseni BA, Okubanjo OO, *et al.* Development of diclofenac and capsaicin emulgel for the management of inflammation in rheumatoid arthritis. *Trop J Nat Prod Res.* 2023;7:3246-3252.
- Soliman SM, Abdel-Malak NS, El-Gazayerly ON, *et al.* Formulation of micro emulsion gel systems for transdermal delivery of celecoxib: *In vitro* permeation, anti-inflammatory activity and skin irritation tests. *Drug Disc Ther.* 2010;4:459-471.
- Maheshwar M. Measurement of viscosity. *Int J Res Pharm Chem.* 2018;8:69-77.
- Barman PC, Kairi RR, Das A, *et al.* An overview of non-Newtonian fluid. *Int J Appl Sci Eng.* 2016;4:97-101. doi:10.5958/2322-0405.2016.00011.3
- Deuschle VC, Deuschle RA, Bortoluzzi MR, *et al.* Physical chemistry evaluation of stability, *in vitro* antioxidant and photoprotective capacities of topical formulation containing *Calendula officinale* L. leaf extract. *Braz J Pharm Sci.* 2015;5:63-75. doi:10.1080/03639045.2021.1993889

How to cite this article: Oseni BA, Osekita ST, Ibrahim MB, *et al.* Development of emulgel formulation from *Markhamia tomentosa* leaf extract: Characterization and *in vitro* antimicrobial activity against skin isolates. *Am J Pharmacother Pharm Sci* 2024;9.