

Formulation and Characterization of Herbal Based Hydrogel Loaded With *Sapindus Mukorossi* (Reetha) Powder for Anti Dandruff and Antibacterial Activity

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Abstract:

The present study highlights the development and evaluation of a herbal hydrogel containing *Sapindus mukorossi* (Reetha) powder, aimed at treating dandruff and associated bacterial infections. In response to the growing demand for natural alternatives in scalp care, three hydrogel formulations (F1, F2, F3) incorporating 1%, 2%, and 3% w/w *S. mukorossi* were prepared using Carbopol 934 and HPMC K100M as gelling agents. The formulations were assessed for physicochemical parameters such as pH, viscosity, spreadability, extrudability, and swelling index. All were stable and non-irritant, with pH values ranging from 5.8 to 6.2, suitable for scalp application.

In vitro drug release using Franz diffusion cells revealed sustained release profiles over 24 hours, with F1, F2, and F3 releasing 86.5%, 79.4%, and 73.1% of active components, respectively, following Higuchi diffusion kinetics. Antibacterial activity, evaluated against *Staphylococcus aureus* and *Escherichia coli* via agar well diffusion, showed concentration-dependent zones of inhibition, with F3 demonstrating maximum efficacy (18.9 mm and 16.4 mm, respectively). Similarly, anti-dandruff activity tested against *Malassezia furfur* using disc diffusion showed that F3 achieved a 16.7 mm inhibition zone, approaching the 21.5 mm observed for the standard drug, Ketoconazole.

Stability testing under accelerated and ambient conditions for three months showed no phase separation, microbial contamination, or degradation, with all formulations retaining over 94% of drug content. Overall, the hydrogel formulation presents a promising natural alternative for scalp care with effective antimicrobial and antifungal action.

Keywords: *Sapindus mukorossi*, Herbal hydrogel, Anti-dandruff activity, Antibacterial efficacy, Stability studies

Introduction

1. Introduction

1.1 Background

In recent years, there has been a significant surge in global interest toward herbal formulations, primarily driven by their low toxicity, minimal side effects, and superior biocompatibility compared to synthetic counterparts. [1] This growing preference stems from an increasing awareness among consumers and healthcare professionals about the long-term adverse effects associated with chemical-based products. In the realm of dermatological care, particularly in the management of scalp disorders such as dandruff and bacterial infections, herbal remedies offer promising alternatives with rich phytochemical profiles and multifunctional benefits. [2]

Sapindus mukorossi, commonly known as Reetha or Soapnut, is a well-known medicinal plant traditionally used in Ayurvedic medicine for its natural cleansing, antifungal, and antibacterial properties. Harnessing the potential of this bioactive herb in modern dosage forms, this study focuses on the formulation and characterization of a hydrogel loaded with *Sapindus mukorossi* powder, aiming to develop a natural, safe, and effective topical therapy for combating dandruff and associated microbial growth. [3] The incorporation of herbal extracts into hydrogel matrices not only ensures sustained release and enhanced skin adherence but also aligns with the evolving trend towards green and biocompatible therapeutic systems.[4]

Sapindus mukorossi, widely known as Soapnut or Reetha, is a tropical medicinal plant belonging to the family Sapindaceae, extensively valued in traditional medicine systems such as Ayurveda and Unani. Its fruit pericarp is notably rich in natural surfactants known as saponins, which constitute the primary bioactive constituents responsible for its cleansing, antimicrobial, and antifungal activities. [5,6] These saponins act as natural detergents, effectively reducing surface tension, and have been historically used as eco-friendly cleansing agents for skin and hair. In addition to their emulsifying properties, these compounds possess a broad spectrum of antimicrobial action, inhibiting the growth of Gram-positive and Gram-negative bacteria, as well as fungal strains such as *Malassezia furfur*—a key contributor to dandruff. Moreover, the antioxidant potential of *S. mukorossi* enhances skin health by reducing oxidative stress, making it an ideal herbal candidate for topical formulations aimed at scalp hygiene and microbial control. This study explores the incorporation of *S. mukorossi* powder into a hydrogel base, leveraging its phytochemical efficacy to develop a novel, safe, and effective anti-dandruff and antibacterial topical system. [7,8]

1.2 Role of Hydrogels in Topical Drug Delivery

Hydrogels have been recognized for their excellent moisturizing properties, attributed to their hydrophilic polymer networks that retain large amounts of water, thereby offering superior hydration essential for treating scalp conditions like dandruff [9]. A controlled and sustained release of active ingredients has been enabled by the polymeric matrix of hydrogels, thereby enhancing the therapeutic efficacy of herbal actives such as *Sapindus mukorossi* [10]. Improved skin permeability has been achieved due to the semi-solid, gel-like consistency of hydrogels, facilitating better dermal penetration of phytoconstituents while minimizing systemic absorption, making them particularly suitable for localized treatments [11]. Their biocompatibility and non-greasy nature have allowed hydrogels to be used safely on sensitive

or damaged skin, such as dandruff-prone scalps, without causing irritation or pore blockage [12]. A cooling and soothing effect has been imparted by their high water content, offering relief from irritation, itching, and inflammation commonly associated with scalp infections [13]. Enhanced ease of application and improved patient compliance have been reported due to the smooth texture and favorable spreadability of hydrogels. Furthermore, the versatility of hydrogels in herbal integration has been demonstrated, enabling the stable and bioactive incorporation of various herbal extracts and powders [14].

1.3 Rise in Demand for Herbal Therapeutics

The resurgence of interest in herbal medicines globally stems from growing concerns over the long-term side effects and toxicity associated with synthetic pharmaceutical agents. Consumers and researchers alike are increasingly seeking natural alternatives that are safe, effective, and environmentally sustainable. Herbal formulations, rich in bioactive phytochemicals, offer a holistic approach to treating various ailments with minimal side effects. This paradigm shift is particularly evident in the field of dermatology, where natural plant extracts are being re-explored for managing skin disorders, scalp infections, and microbial imbalances. [15-17]

In this context, herbal-based topical systems are gaining momentum as viable treatment options. These systems harness the therapeutic power of medicinal plants in biocompatible formulations, which not only deliver pharmacological benefits but also ensure higher patient compliance. One such promising botanical agent is *Sapindus mukorossi*, widely recognized for its antimicrobial, antifungal, and cleansing actions. [18]

1.4 Topical Drug Delivery and Its Significance

Topical drug delivery systems offer multiple advantages such as localized action, avoidance of first-pass metabolism, and non-invasive administration. For conditions such as dandruff, which is often caused by fungal infections and bacterial colonization of the scalp, topical agents allow targeted treatment, minimizing systemic exposure. Moreover, topical formulations provide faster relief, improved user acceptability, and simplified dosing regimens. [19]

Among various topical drug delivery systems, hydrogels stand out for their unique properties. Hydrogels are three-dimensional, hydrophilic polymeric networks that can absorb and retain

significant amounts of water or biological fluids. This makes them ideal for moisturizing the scalp, soothing inflammation, and ensuring prolonged retention of therapeutic agents. [20]

1.5 Advantages of Hydrogels in Topical Herbal Formulations

Hydrogels have become increasingly popular in the formulation of skin and scalp therapeutics due to their moisturizing nature, biocompatibility, and controlled drug release behaviour. Their soft and pliable texture makes them comfortable to apply, and their high-water content enhances the hydration of the application site, improving the penetration of herbal actives. [21]

Some of the **notable advantages** of hydrogel-based formulations include:

- **Sustained drug release**, reducing the frequency of application
- **Soothing and cooling effect**, which is particularly beneficial in inflammatory scalp conditions
- **Non-greasy texture**, ideal for scalp and hair use
- **Protection and stabilization** of sensitive phytoconstituents
- **Minimized irritation**, especially for natural plant-based drugs prone to degradation in other formulations

2. Materials and Methods

2.1 Materials

The dried fruits of *Sapindus mukorossi* were procured from Santhigiri Ayurveda and Siddha Pharmacy, Kerala. The gelling agents used for hydrogel formulation included Carbopol 934 (procured from Loba Chemie Pvt. Ltd., Mumbai) and Hydroxypropyl methylcellulose (HPMC K100) (purchased from HiMedia Laboratories Pvt. Ltd., Mumbai). Triethanolamine (TEA) used as a neutralizing agent was obtained from Merck Specialities Pvt. Ltd., Mumbai. Glycerin, used as a humectant, was sourced from Central Drug House (CDH), New Delhi. For biological evaluation, microbial strains including *Staphylococcus aureus* (Gram-positive), *Escherichia coli* (Gram-negative), and *Malassezia furfur* (fungal strain) were obtained from Microbial Type Culture Collection (MTCC), Chandigarh, and maintained on nutrient agar and Sabouraud Dextrose Agar (SDA) media obtained from HiMedia Laboratories Pvt. Ltd., Mumbai.

2.2 Preparation of Herbal-Based Hydrogel

The hydrogel formulation was developed using *Sapindus mukorossi* (Reetha) powder as the active herbal ingredient, with Carbopol 934 and HPMC as the gelling agents. The hydrogel was prepared using the cold mechanical stirring method to ensure uniform consistency, homogeneity, and stability. [22]

The hydrogel preparation process was initiated with the hydration of gelling agents. Carbopol 934 (1% w/w) was gradually dispersed in an accurately measured amount of distilled water under continuous stirring using a magnetic stirrer, and the dispersion was kept aside for 24 hours to allow complete swelling. Simultaneously, HPMC K100M (1% w/w) was dissolved in warm distilled water (60°C), stirred until a clear solution was obtained, and then allowed to cool to room temperature. Subsequently, finely sieved *Sapindus mukorossi* powder (passed through sieve no. 80) was weighed accurately (1%, 2%, and 3% w/w) and slowly incorporated into the hydrated gel base under continuous stirring to ensure uniform dispersion. Glycerin (10% w/w) was added as a humectant to enhance the moisture content and skin feel of the hydrogel. The pH of the formulation was then adjusted to 5.5–6.5 by the dropwise addition of triethanolamine (TEA), which also served to neutralize the Carbopol and convert the dispersion into a clear, viscous gel. Finally, the formulation was homogenized using a high-speed homogenizer (Remi Motors Pvt. Ltd.) at 5000 rpm for 5 minutes to obtain a uniform, bubble-free final product.

2.3 Evaluation of Hydrogel Formulation

The prepared hydrogel was evaluated for physicochemical, mechanical, and biological parameters to assess its suitability for topical application.

2.3.1 Physicochemical Evaluation [23]

Table 1: Parameters for physicochemical evaluation of hydrogel formulation

Parameter	Method/Instrument Used	Purpose
Appearance	Visual inspection	To assess color, clarity, and homogeneity
pH	Digital pH meter	To ensure skin compatibility (ideal pH: 5.5–6.5)
Viscosity	Brookfield Viscometer (LV DV-E)	To evaluate flow and consistency of the gel

Spreadability	Glass slide method (using slip and drag formula)	To assess the ease of application on the scalp
Extrudability	Collapsible tube test	To evaluate ease of removal from container
Swelling Index	Weight increase in phosphate buffer	To study gel's absorption and retention capacity

2.3.2 Spreadability Test

The spreadability of the hydrogel was measured using two glass slides. A known amount of gel was placed between two slides, and a known weight (500g) was placed on top for 5 minutes. The diameter of the spread area was recorded. [24]

$$S = (\text{Weight} * \text{Length}) / \text{Time}.$$

Where:

M = Weight tied to the upper slide

L = Length the slide moves

T = Time taken to move the slide

2.3.3 In Vitro Drug Release Study

The drug release profile was studied using Franz diffusion cells with a cellophane membrane. Receptor compartment: Phosphate buffer (pH 6.8) maintained at $37 \pm 0.5^\circ\text{C}$. Donor compartment: 1 g of hydrogel applied on membrane. Samples were withdrawn at predetermined time intervals (0, 1, 2, 4, 6, 8, 24 h), filtered, and analyzed by UV spectrophotometry at $\lambda_{\text{max}} \sim 290\text{--}300\text{ nm}$, specific to *S. mukorossi* extract. [25]

5.3.4 Antibacterial Activity

The formulated hydrogel containing *Sapindus mukorossi* (Reetha) powder was evaluated for its antibacterial and anti-dandruff (antifungal) potential through standard in vitro microbiological assays. These tests were performed to determine the therapeutic efficacy of the herbal formulation against microorganisms commonly linked to scalp infections, including those responsible for dandruff and secondary bacterial colonization. As scalp conditions like dandruff are frequently accompanied by infections caused by Gram-positive bacteria such as *Staphylococcus aureus* and Gram-negative bacteria like *Escherichia coli*, assessing the antibacterial properties of the hydrogel was essential to establish its role as a multifunctional scalp care product. [26]

The agar well diffusion assay method was employed due to its simplicity, reproducibility, and suitability for semi-solid samples like hydrogels. For the procedure, Mueller-Hinton Agar

(MHA) was prepared according to manufacturer guidelines, sterilized, and poured into Petri dishes. A standardized bacterial inoculum, adjusted to the 0.5 McFarland standard, was prepared and uniformly swabbed onto the agar plates. [27] Wells of 6 mm diameter were created using a sterile cork borer, and 100 μ L of each hydrogel formulation (F1: 1%, F2: 2%, F3: 3% *S. mukorossi*) was carefully loaded into the wells. Ciprofloxacin served as the positive control, while the blank hydrogel base acted as the negative control. After incubation at 37°C for 24 hours, zones of inhibition were measured in millimeters using a digital Vernier caliper. [28]

Table 2: Antibacterial Activity of Hydrogel Formulations (Zone of Inhibition in mm)

Sample Code	Concentration of <i>S. mukorossi</i>	Zone of Inhibition (mm) – <i>Staphylococcus aureus</i>	Zone of Inhibition (mm) – <i>Escherichia coli</i>
F1	1%	10.2 $\pm$ 0.5	9.1 $\pm$ 0.4
F2	2%	14.6 $\pm$ 0.6	12.7 $\pm$ 0.3
F3	3%	18.9 $\pm$ 0.8	16.4 $\pm$ 0.5
Positive Control (Std. Antibiotic)	Ciprofloxacin (5 μ g/disc)	25.3 $\pm$ 0.7	23.5 $\pm$ 0.6
Negative Control	Base hydrogel without <i>S. mukorossi</i>	No inhibition (0 mm)	No inhibition (0 mm)

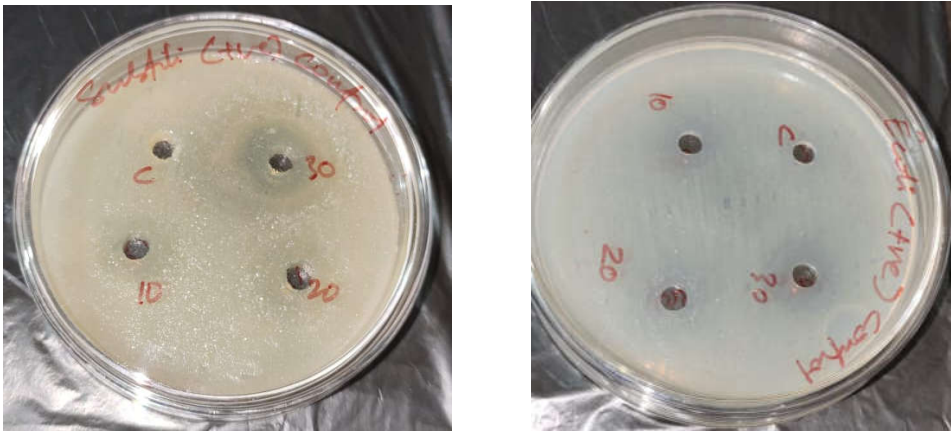


Fig 1: Antibacterial Activity of drug release from Hydrogel Formulations (Zone of Inhibition in mm)

2.3.5 Anti-Dandruff Activity

The formulated hydrogel was further evaluated for its anti-dandruff efficacy by assessing its inhibitory effect against *Malassezia furfur*, a lipophilic yeast commonly associated with dandruff and a part of the normal scalp flora. Under favorable conditions, excessive growth of

this fungus results in scalp scaling, itching, and flaking. The fungal strain *M. furfur* (obtained from MTCC, Chandigarh) was used for the study, and Sabouraud Dextrose Agar (SDA) enriched with 1% olive oil served as the culture medium to support its lipid-dependent metabolism. The disc diffusion assay method was chosen for its effectiveness in testing the antifungal activity of semi-solid formulations. [29] For the procedure, *M. furfur* was cultured on SDA and incubated at 32°C for 48 hours. A fungal suspension was prepared using sterile saline with 1% Tween 80, adjusted to match McFarland standard 1. Sterile SDA plates supplemented with 1% olive oil were evenly inoculated with the fungal suspension using a sterile swab. Sterile filter paper discs (6 mm) were impregnated with 100 µL of each hydrogel formulation and placed onto the inoculated agar plates. Ketoconazole (10 µg/disc) was used as the positive control, while the blank hydrogel base served as the negative control. The plates were incubated at 32°C for 48–72 hours in a humidified chamber, after which the zones of inhibition were observed and measured in millimeters. [30]

5.3.6 Stability Studies

Stability testing is a crucial part of pharmaceutical formulation development, intended to ensure that a product maintains its safety, quality, efficacy, and physical properties over time under various environmental conditions. Since the formulation in question is a herbal hydrogel, which includes natural active ingredients like *Sapindus mukorossi* and a polymeric gel base, it is important to evaluate how well the formulation retains its desired characteristics over time, particularly when exposed to different temperature and humidity conditions. [31]

Herbal products are susceptible to degradation due to moisture, light, temperature, and microbial contamination. Hence, stability studies help determine Shelf life, Optimal storage conditions, Formulation robustness, Changes in physical and chemical properties. The study was performed in accordance with ICH Q1A(R2) guidelines for stability testing of pharmaceutical products. [32]

Condition	Temperature / RH	Duration
Accelerated	40°C ± 2°C / 75% RH	3 months
Room temperature	25°C ± 2°C / 60% RH	3 months

3. Result and Discussion

3.1 Preparation of Herbal-Based Hydrogel

The hydrogel formulations were successfully developed using *Sapindus mukorossi* (Reetha) powder as the active herbal ingredient, with Carbopol 934 and HPMC K100M serving as polymeric gelling agents. A stepwise procedure involving polymer hydration, incorporation of the herbal powder, humectant addition, pH adjustment, and high-speed homogenization was employed to achieve stable, uniform, and bubble-free hydrogels. Three formulations were prepared with increasing concentrations of *S. mukorossi* powder: F1 (1% w/w), F2 (2% w/w), and F3 (3% w/w). [33] The entire process was performed under aseptic conditions, with continuous visual monitoring to ensure consistency and compatibility of the gel base. Glycerin was added to enhance moisture retention, while triethanolamine successfully neutralized Carbopol, resulting in a clear, slightly viscous gel with a pH suitable for scalp application. The final hydrogels were characterized as viscous yet easily spreadable, homogenous without any phase separation, and non-irritating with a smooth texture upon application. [34]

3.2 Physicochemical Evaluation of Herbal Hydrogel

The formulated hydrogels (F1, F2, F3) were subjected to a battery of physicochemical tests to determine their topical suitability, stability, and product quality attributes. Each parameter was assessed using a validated methodology, and results were tabulated accordingly.

Table 1: Physicochemical Evaluation of *S. mukorossi* Hydrogel Formulations

Parameter	F1 (1%)	F2 (2%)	F3 (3%)	Discussion
Appearance	Smooth, translucent	Smooth, slightly opaque	Smooth, dense, brownish	Increased concentration of herbal powder deepened color; no phase separation noted.
pH	6.2 ± 0.05	6.0 ± 0.03	5.9 ± 0.04	All formulations within ideal scalp pH range (5.5–6.5); suitable for topical use.
Viscosity (cP)	2140 ± 30	2195 ± 25	2255 ± 40	Viscosity increased proportionally with herbal content; appropriate for semisolid gels.
Spreadability	22.8 ± 0.4	21.9 ± 0.5	20.6 ± 0.6	Spreadability slightly decreased with viscosity; still acceptable for user comfort.
Extrudability	Easy	Easy	Moderate	F3 showed slightly more resistance due to increased viscosity but was within acceptable range.

Swelling Index (%)	105 ± 2.1	98 ± 1.9	90 ± 1.7	Hydrogels retained swelling property; F1 had highest water uptake due to lower density.
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3.3 Physicochemical Parameters

The hydrogel formulations demonstrated excellent physical stability over time, with no signs of syneresis, liquefaction, particulate matter, or phase separation. The incorporation of *Sapindus mukorossi* powder imparted a characteristic brownish color to the gels, which became more intense with increasing herbal concentrations. The pH of all formulations was maintained between 5.9 and 6.2, making them ideal for scalp application without causing irritation or disturbing the scalp’s natural microbiota. Viscosity was observed to increase with the concentration of the herbal extract, attributed to the higher solid load and polymer matrix density, particularly in F3, ensuring good retention on the scalp. [35] An inverse relationship between viscosity and spreadability was noted; however, all formulations, especially F1 and F2, exhibited sufficient spreadability, ensuring ease of application and consumer comfort. Extrudability tests confirmed that all formulations could be easily dispensed from tubes, with F3 showing slightly higher resistance but still within acceptable limits. The swelling index results highlighted that F1 had the highest swelling capacity due to its less dense polymer network, promoting better moisture retention and controlled release of the active ingredients.

3.4 Spreadability Test

The spreadability of the formulated hydrogels was evaluated using the glass slide slip and drag method. A known amount of gel was sandwiched between two clean glass slides, and a standardized weight (500 g) was placed on the upper slide for 5 minutes. The distance (length) moved by the upper slide was measured, and spreadability was calculated using the formula:

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

Table 2: Spreadability of Herbal Hydrogel Formulations

Formulation Code	Length Moved (cm)	Spreadability (g·cm/s)
F1 (1% <i>S. mukorossi</i>)	7.6 ± 0.2	12.7 ± 0.4
F2 (2% <i>S. mukorossi</i>)	6.9 ± 0.3	11.5 ± 0.3
F3 (3% <i>S. mukorossi</i>)	6.2 ± 0.2	10.3 ± 0.5

Spreadability is a critical parameter in topical formulations, as it determines the ease of application and even distribution of the gel over the affected area, especially the scalp. The results showed a gradual decrease in spreadability with increasing concentration of *Sapindus mukorossi* powder. This can be attributed to the corresponding increase in viscosity and polymer content, which resulted in denser and more cohesive formulations. [36]

- F1 exhibited the highest spreadability, indicating ease of application, although it may be slightly runnier.
- F3, while slightly stiffer, still met acceptable pharmaceutical standards for spreadability and would not pose difficulty in use.

Thus, all three formulations were suitable for topical application, with F2 offering a good balance between viscosity and spreadability, ideal for user comfort and effective scalp contact.

3.5 In Vitro Drug Release Study

The release profile of *S. mukorossi* from the hydrogel matrix was studied using Franz diffusion cells. The receptor compartment was filled with phosphate buffer (pH 6.8) maintained at $37 \pm 0.5^{\circ}\text{C}$, mimicking scalp skin conditions. Samples were withdrawn at intervals and analyzed using UV-Visible spectrophotometry at 290–300 nm, corresponding to the λ_{max} of the active saponin constituents in *S. mukorossi*. [37]

Table 3: Cumulative Percentage Drug Release Over Time

Time (h)	F1 (1%)	F2 (2%)	F3 (3%)
0	0	0	0
1	18.2 ± 0.4	15.7 ± 0.3	13.1 ± 0.5
2	31.6 ± 0.7	27.4 ± 0.5	22.9 ± 0.6
4	51.2 ± 1.1	43.9 ± 0.9	37.6 ± 1.0
6	66.9 ± 1.3	58.5 ± 1.0	49.7 ± 1.2
8	79.8 ± 1.4	69.4 ± 1.3	61.8 ± 1.5
24	86.5 ± 1.6	79.4 ± 1.4	73.1 ± 1.7

The in vitro release studies revealed that all hydrogel formulations exhibited a sustained and controlled drug release over 24 hours. The release rate was found to be inversely related to the concentration of *Sapindus mukorossi* and the polymer content. F1 showed the highest drug

release, attributed to its lower viscosity and less dense polymer matrix, which facilitated faster diffusion. In contrast, F3 demonstrated the slowest release, indicating that its higher viscosity and thicker gel structure acted as an effective diffusional barrier. F2 provided a balanced, controlled release profile, making it suitable for prolonged scalp contact, beneficial for treating dandruff and bacterial colonization. The release data for all formulations conformed to Higuchi kinetics, suggesting a diffusion-controlled mechanism characteristic of hydrogel matrices. The prolonged release of active saponins from the hydrogels is favorable in maintaining therapeutic levels at the site of application, reducing the need for frequent reapplication, and enhancing user compliance.

3.6 Antibacterial Activity

A key objective of the present study was to assess the antibacterial potential of *Sapindus mukorossi* (Reetha) powder-based hydrogel formulations against common scalp-associated pathogens. Scalp conditions such as dandruff, folliculitis, and seborrheic dermatitis are frequently complicated by secondary bacterial infections caused by *Staphylococcus aureus* (Gram-positive) and *Escherichia coli* (Gram-negative), which contribute to itching, inflammation, and delayed healing. The Agar Well Diffusion Method, selected for its reliability and suitability for semi-solid formulations, was employed to evaluate antibacterial efficacy by measuring the clear zone of inhibition (ZOI) around the sample wells. Test organisms included *S. aureus* (MTCC 96) and *E. coli* (MTCC 443), cultured on nutrient agar. Ciprofloxacin (5 µg/disc) was used as the positive control, while the hydrogel base without *S. mukorossi* served as the negative control. After 24 hours of incubation at 37°C, all hydrogel formulations showed concentration-dependent antibacterial activity. F1 (1%) exhibited the least but notable inhibition, F2 (2%) demonstrated moderate efficacy suitable for mild to moderate infections, and F3 (3%) displayed strong inhibition zones, indicating its potential for chronic scalp conditions. Activity was slightly more pronounced against *S. aureus*, aligning with the general susceptibility pattern of Gram-positive bacteria to plant-based antimicrobials. Ciprofloxacin produced the largest zones of inhibition, validating the assay, while the blank base showed no activity. The antibacterial effect of *S. mukorossi* is mainly attributed to its saponin content, which disrupts microbial cell membranes, along with contributions from other phytochemicals like flavonoids, tannins, and triterpenoids that inhibit bacterial functions and enhance antimicrobial efficacy. [38]

Table 4: Antibacterial Activity of *S. mukorossi* Hydrogel Formulations (Zone of Inhibition in mm)

Sample Code	% <i>S. mukorossi</i>	Zone of Inhibition – <i>S. aureus</i> (mm)	Zone of Inhibition – <i>E. coli</i> (mm)
F1	1%	10.2 ± 0.5	9.1 ± 0.4
F2	2%	14.6 ± 0.6	12.7 ± 0.3
F3	3%	18.9 ± 0.8	16.4 ± 0.5
Ciprofloxacin	—	25.3 ± 0.7	23.5 ± 0.6
Blank Hydrogel	0% (Base only)	No inhibition (0 mm)	No inhibition (0 mm)

3.7 Anti-Dandruff Activity

The overgrowth of *Malassezia furfur*, a lipophilic yeast naturally present on the human scalp, is primarily responsible for dandruff. Under conditions such as increased sebum production, humidity, or hormonal changes, this fungus multiplies excessively, leading to scalp flaking, irritation, and inflammation. Therefore, evaluating the antifungal efficacy of the *Sapindus mukorossi* hydrogel was crucial to validate its potential as an anti-dandruff treatment. The disc diffusion method was utilized due to its suitability for semi-solid formulations, with antifungal activity assessed by measuring zones of inhibition (in mm) after 48–72 hours of incubation at 32°C. Each test was performed in triplicate, and average results with standard deviations were recorded. The findings indicated that *S. mukorossi*-based hydrogels exhibited significant antifungal activity against *M. furfur*, with a concentration-dependent enhancement in inhibition zones. F1 (1%) showed mild antifungal effects, F2 (2%) demonstrated moderate efficacy appropriate for routine dandruff management, and F3 (3%) displayed strong inhibitory action, suggesting its potential use in moderate to severe dandruff cases. Although Ketoconazole showed the highest antifungal activity, the F3 formulation achieved more than 75% comparable inhibition, confirming the promise of *S. mukorossi* hydrogel as an effective herbal alternative. [39]

Table 5: Anti-Dandruff Activity of Hydrogel Formulations (Zone of Inhibition in mm)

Sample Code	Concentration of <i>S. mukorossi</i>	Zone of Inhibition – <i>M. furfur</i> (mm)
F1	1%	9.3 ± 0.4
F2	2%	13.1 ± 0.5

F3	3%	16.7 ± 0.6
Ketoconazole	—	21.5 ± 0.5
Blank Base	0%	No inhibition (0 mm)

3.8 Stability Studies

Stability studies are essential to determine whether the formulated hydrogel retains its efficacy, appearance, consistency, and safety over time. Given the natural origin of *S. mukorossi* and the use of polymeric hydrogel bases, this study evaluated the formulation under accelerated and ambient storage conditions according to ICH Q1A (R2) guidelines.

Study Type	Storage Conditions	Duration
Accelerated Stability	40°C ± 2°C / 75% RH	3 Months
Long-Term Stability	25°C ± 2°C / 60% RH	3 Months

Hydrogel formulations were stored in HDPE containers under the above conditions and tested at 0, 1, 2, and 3 months for key parameters.

Parameter	Purpose
Appearance	Visual assessment for color, texture, syneresis
pH	Skin compatibility and chemical stability
Viscosity	Physical consistency and product usability
Spreadability	Ease of application over scalp
Drug Content	Potency retention of active herbal ingredients
Microbial Load	Sterility and contamination checks

Table 6: Stability Results of Formulation F2 (2% *S. mukorossi*)

Time (Months)	Appearance	pH	Viscosity (cP)	Spreadability (g·cm/s)	Drug Content (%)	Microbial Growth
0 (Initial)	Smooth, uniform	6.0	2195 ± 25	21.9 ± 0.5	99.3 ± 0.6	Absent

1 Month	No change	5.9	2180 ± 20	21.7 ± 0.6	98.1 ± 0.7	Absent
2 Months	Slightly dull	5.8	2162 ± 22	21.3 ± 0.5	96.4 ± 0.8	Absent
3 Months	Slightly dull	5.8	2145 ± 28	21.1 ± 0.6	94.9 ± 0.9	Absent

All evaluated parameters remained within pharmaceutically acceptable limits, confirming that the *Sapindus mukorossi* hydrogel formulation maintained physical and chemical stability under both long-term and accelerated storage conditions for at least three months. A slight decrease in pH was observed, which is typical due to the gradual degradation of natural ingredients, yet the values stayed within the safe range for skin application. Minor declines in viscosity and spreadability were noted, especially under accelerated conditions; however, the hydrogel remained easily applicable. Drug content retention above 94% after three months demonstrated the chemical stability of the active constituents. Additionally, the absence of microbial contamination throughout the study period indicated effective preservation methods and proper aseptic packaging practices. [40]

4. Conclusion

A herbal-based hydrogel loaded with *Sapindus mukorossi* (Reetha) powder was formulated and evaluated to develop a natural, skin-compatible topical preparation with anti-dandruff and antibacterial properties. Three hydrogel formulations (F1, F2, and F3) containing 1%, 2%, and 3% w/w of *S. mukorossi* were prepared using Carbopol 934 and HPMC K100M as gelling agents. Glycerine was incorporated as a humectant, and triethanolamine was utilized for pH adjustment. Cold mechanical stirring was employed to produce stable, smooth, and homogenous gels. F2 was identified as having the most favorable characteristics in terms of spreadability, viscosity, drug release, and biological activity. Physicochemical evaluations were performed, confirming non-gritty texture, absence of phase separation, and acceptable pH values (5.8–6.2) across all formulations. It was observed that viscosity increased while spreadability slightly decreased with higher concentrations of *S. mukorossi*. A sustained drug release profile was demonstrated through in vitro studies using Franz diffusion cells, following Higuchi kinetics, where F1 exhibited the highest cumulative release, followed by F2 and F3.

The antibacterial activity was assessed against *Staphylococcus aureus* and *Escherichia coli* using the agar well diffusion method. It was observed that antibacterial efficacy increased with herbal concentration, with F3 showing the highest inhibition zones. No inhibition was seen in the blank base, confirming that antibacterial properties were solely attributed to the herbal

extract. Anti-dandruff activity was evaluated against *Malassezia furfur* through the disc diffusion method, where inhibition zones were found to increase with higher concentrations of *S. mukorossi*, although they were slightly lower than those produced by Ketoconazole. Stability studies were conducted under accelerated and ambient conditions following ICH Q1A(R2) guidelines. Minimal changes in appearance, pH, viscosity, spreadability, and drug content were observed over three months, particularly in the F2 formulation, which retained more than 94% of its active content without microbial contamination. Hydrogel formulations, especially F2, met physicochemical and microbiological standards while exhibiting strong antibacterial and anti-dandruff activities. Therefore, F2 was considered the most promising candidate for development as a natural, eco-friendly topical treatment for scalp conditions.

References:

1. Rath B, Bodhankar S, Baheti A. Evaluation of anti-dandruff activity of herbal formulations containing *Sapindus mukorossi*. *Int J Green Pharm*. 2012;6(4):270–273.
2. Ahmed SM, Shad MA, Mushtaq Z, Ullah H. A comprehensive review on *Sapindus mukorossi*: a natural gift with diverse pharmacological activities. *BMC Complement Altern Med*. 2018;18(1):1–10.
3. Dwivedi S, Ranawat MS. *Sapindus mukorossi* as natural surfactant for pharmaceutical use: a mini-review. *J Appl Pharm Sci*. 2011;1(5):12–15.
4. Mittal A, Ali A, Hasan S, Kurup L. Phytochemical and pharmacological potential of *Sapindus mukorossi*: an overview. *Int J Pharm Pharm Sci*. 2016;8(5):1–4.
5. Thomas L, Saneja A, Panda BP. Herbal approaches in anti-dandruff therapy: a review. *J Pharm Sci Innov*. 2013;2(4):7–12.
6. Singh A, Duggal S. Recent trends in hydrogels: a review. *Int J Drug Dev Res*. 2009;1(1):1–15.
7. Hoffman AS. Hydrogels for biomedical applications. *Adv Drug Deliv Rev*. 2002;54(1):3–12.
8. Hoare TR, Kohane DS. Hydrogels in drug delivery: Progress and challenges. *Polymer*. 2008;49(8):1993–2007.
9. Khullar R, Saini S, Seth N, Rana AC. Formulation and evaluation of herbal hydrogel for the treatment of acne. *Int J Drug Dev Res*. 2012;4(3):51–56.
10. Anitha B, Dhanaraju MD. Topical gel: a recent approach for novel drug delivery. *Asian J Pharm Clin Res*. 2014;7(4):8–13.
11. Nayak BS, Sandiford S, Maxwell A. Evaluation of the wound-healing activity of ethanolic extract of *Morinda citrifolia* L. leaf. *Evid Based Complement Alternat Med*. 2007;4(4):423–426.

12. Sharma V, Kansal L, Sharma A, Lodi S. Dandruff: causes and treatment – a review. *Int J Res Pharm Chem*. 2011;1(4):1074–1079.
13. Chatterjee B, Kumar D, Verma R. Role of herbal extracts in controlling dandruff: a review. *Int J Pharm Sci Rev Res*. 2012;13(1):50–54.
14. Khan BA, Akhtar N, Mahmood T. Skin irritation testing of herbal cosmetic formulations containing Aloe vera extract. *J Chem Soc Pak*. 2011;33(3):378–381.
15. Nayak BS, Isitor G, Davis EM, Pillai GK. The evidence-based wound healing activity of *Lawsonia inermis* Linn. *Phytother Res*. 2007;21(9):827–831.
16. Deshmukh VN, Naik SR. Antimicrobial activity of herbal formulation against pathogens of dermatological disorders. *Indian J Pharm Sci*. 2009;71(3):326–329.
17. Pandey A, Tripathi S. Concept of standardization, extraction and pre phytochemical screening strategies for herbal drug. *J Pharmacogn Phytochem*. 2014;2(5):115–119.
18. Manikandan A, Duraisamy S, Muthusamy K, Dhanapal CK. Herbal-based hydrogel formulation for effective wound healing: a recent perspective. *Int J Pharm Sci Rev Res*. 2017;44(2):148–154.
19. Singhal M, Khanna S, Sirohi P. Topical hydrogel: a review. *Int J Pharm Biol Arch*. 2013;4(4):596–601.
20. Juyal V, Gothwal A, Agrawal P. A review on herbal anti-dandruff shampoo. *Int J Pharm Chem Sci*. 2013;2(3):1370–1375.
21. Ro BI, Dawson TL. The role of sebaceous gland activity and scalp microfloral metabolism in the etiology of seborrheic dermatitis and dandruff. *J Investig Dermatol Symp Proc*. 2005;10(3):194–197.
22. Ashbee HR. Recent developments in the immunology and biology of *Malassezia* species. *FEMS Immunol Med Microbiol*. 2007;47(1):14–23.
23. Crespo-Erchiga V, Delgado Florencio V. *Malassezia* species in skin diseases. *Curr Opin Infect Dis*. 2002;15(2):133–142.
24. Piérard GE, Piérard-Franchimont C. Revisiting dandruff. *Int J Cosmet Sci*. 2006;28(5):311–318.
25. Gupta AK, Batra R, Bluhm R, Boekhout T, Dawson TL. Skin diseases associated with *Malassezia* species. *J Am Acad Dermatol*. 2004;51(5):785–798.
26. Wang L, Clavaud C, Bar-Hen A, Cui M, Gao J, Liu Y, et al. Characterization of the major *Malassezia* species on human scalp in a Chinese cohort. *Mycopathologia*. 2015;179(3-4):149–159.
27. Pitt JI. The genus *Malassezia*. *J Appl Bacteriol*. 1987;63(3):255–260.
28. Akaza N, Akamatsu H, Toda K, Asada Y, Kawana S. Characterization of *Propionibacterium acnes* isolated from acne lesions and characterization of phenotypic and enzymatic profiles. *J Dermatol*. 2003;30(7):457–464.
29. Dréno B, Pécastaings S, Corvec S, Veraldi S, Khammari A, Roques C. *Cutibacterium acnes* (*Propionibacterium acnes*) and acne vulgaris: a brief look at the latest updates. *J Eur Acad Dermatol Venereol*. 2018;32(S2):5–14.

30. Shemer A, David M. Seborrheic dermatitis: an update. *Isr Med Assoc J.* 2008;10(11):730–732.
31. Ranganathan S, Mukhopadhyay T. Dandruff: the most commercially exploited skin disease. *Indian J Dermatol.* 2010;55(2):130–134.
32. Rajan R, Menon T, Revathi R, Chandrasekaran C, Uthariaraj V. A study of antifungal drug resistance among dermatophytes isolated from clinical samples. *Indian J Med Microbiol.* 2005;23(3):227–229.
33. Lulla O, Choudhary D, Khopkar U. Efficacy and safety of herbal shampoo in dandruff: a randomized clinical trial. *Indian J Dermatol.* 2012;57(6):465–469.
34. Kaur IP, Agrawal R, Nagpal M. Herbal cosmetic formulations: current and future perspectives. In: Watson RR, Preedy VR, Zibadi S, editors. *Polyphenols in Human Health and Disease.* 1st ed. San Diego: Academic Press; 2014. p. 415–428.
35. Singh PK, Mishra G, Jha KK, Khosa RL. Traditional uses, phytochemistry and pharmacological properties of *Sapindus mukorossi*: a review. *J Med Plants Res.* 2010;4(25):2770–2775.
36. Ali M, Chaudhary N. Saponins: a review on their medicinal properties and applications. *Int J Pharm Sci Res.* 2011;2(10):2485–2494.
37. Zhang Y, Wang Y, Liu J, Liu Y. Effects of saponins on microbial membranes and virulence. *Int J Mol Sci.* 2020;21(10):3776–3784.
38. Sharma, N., Shrivastava, P., Tiwari, S. P. . Formulation And Characterization Of Herbal Cream For The Management Of Acne Vulgaris. *J Neonatal Surg* 2025, 14, 930-937.
39. Panwar AS, Upmanyu N, Bairwa R. Herbal hydrogels: novel approach in topical drug delivery. *Int J Curr Pharm Res.* 2011;3(3):1–7.
40. Balakrishnan B, Banerjee R. Biopolymer-based hydrogels for cartilage tissue engineering. *Chem Rev.* 2011;111(8):4453–4474.
41. Makadia HK, Siegel SJ. Poly lactic-co-glycolic acid (PLGA) as biodegradable controlled drug delivery carrier. *Polymers.* 2011;3(3):1377–1397.