Formulation and Evaluation of Anti-Microbial Herbal Hand Wash Gel Containing Aqueous Extract of Sapindus Mukorossi

Jagtar Singh*, Mohit Sharma, Thakur Prava Jyoti, Ritik Kaundal, Ritika Behl, Suryadev Tripathi

Faculty of Pharmaceutical Sciences, PCTE Group of Institutes, Near Baddowal Cantt, Ferozepur Road, Punjab 142021, India

*Corresponding author
Jagtar Singh, Faculty of Pharmaceutical Sciences, PCTE Group of Institutes, Near Baddowal Cantt, Ferozepur Road, Punjab 142021, India

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Abstract

Background: Herbal medicines and food products have extensively been looked upon for decades due to their biocompatible nature and the least adverse effects. At the same pace, plant-based cosmetics and toiletries are capturing the market due to their eco-friendly nature and compatibility with human skin. The marketed soaps and hand washes are based on toxic fatty acid salts, surfactants, and perfumes that are not only harmful to the skin but to the environment as well. The commonly used surfactant in soaps and hand washes, sodium lauryl sulfate makes the skin dry and sensitive. Moreover, the water getting polluted by SLS when entering the water bodies, makes the aquatic life vulnerable. So, it becomes important to formulate such hand washes that are biocompatible, without compromising their cleansing action and anti-microbial properties. This piece of work explores the formulation of an anti-bacterial hand wash having a good cleansing action to serve the purpose of maintaining personal hygiene in a biocompatible and eco-friendly way.

Results: Herbal hand wash gel was prepared and evaluated using plant extracts, having potent anti-microbial action (minimum inhibitory concentration of 1.4cm and 3.7 cm for S. aureus and P. aeruginosa respectively).

Conclusion: Formulation of plant based hand wash products by replacing the synthetic surfactants with natural saponins and antimicrobial extracts can be useful for regular use on skin, while reducing the toxic load on environment as well.

Background

The Covid-19 pandemic has increased awareness regarding the significance of hand hygiene in restricting infections. The way society has adopted the appropriate use of hand washes and hand sanitizers, it not only played an important role in reducing the Covid-19 infection but the transmission of other contagious diseases as well [1].

The marketed formulations of hand washes are synthetic surfactant-based, out of which the sodium lauryl sulfate is most widely used. These surfactants disturb the epidermal barrier and make the skin dry and sensitive. Moreover, these surfactants along with other synthetic perfumes, foaming agents, preservatives, and artificial colors when entering the water bodies and soil, makes the environment toxic. It necessitates the formulation development of such hand wash formulations, having biocompatible properties to the human skin and the environment without compromising the cleansing action and anti-microbial properties [2].

The use of traditional herbal products among south-east Asian countries has been looked upon by the west and a tremendous amount of work has been done to characterize the therapeutic potential of various herbs [3].

In this study, the surfactant properties of soap nut extract and anti-microbial properties of neem oil have been explored and incorporated to formulate a gel-based hand wash.

Sapindus Mukorossi

The soap nut tree ( Sapindus mukorossi) is a deciduous tree commonly found and grown In-Gangetic plains. The pericarp or the shell of the fruit contains saponins (10%) having anti-microbial, anti-tumor, and surfactant properties [4]. The major phytoconstituents include tri-terpenoidal saponins like tirucullane, oleanane (Sapindoside A and B), and dammarane. Due to its satisfactory detergency and anti-microbial properties, the extract of soap nuts was utilized to formulate the hand wash, being an excellent replacement for synthetic surfactants [5].

Azadirachta Indica

Neem is the most widely used traditional herb used in India for ages, for its anti-microbial, antifungal, anti-hyperglycaemic, and anti-cancer properties [6]. The cold pressed oil of neem seeds contains azadirachtin, a potent antimicrobial agent. Apart from this, various isoprenoids like azadirone, gedunin, nimbin and salalin have antibacterial and antifungal action. Thus, neem oil was used as an antimicrobial agent in the hand wash formulation [7].
The viscosity of marketed hand wash gel was evaluated in a similar manner to the viscosity of the hand wash gel at a temperature 25°C. The viscosity of marketed hand wash gel was evaluated in a similar way.

Methods
Preparation of Aqueous Extract of Sapindus Mukorossi
The extract of *Sapindus mukorossi* was prepared as reported by Chen et al. 2021, but with few modifications. The dried shells of *Sapindus mukorossi* (soap nuts) were de-seeded and washed. 100 g of shells were ground to form a coarse powder and soaked in 200 ml distilled water [8]. The resultant mixture was agitated on a rotary shaker for 6 hours and boiled on the heating mantle at 60°C for 20 minutes. The extract was allowed to cool and later centrifuged at 3200 rpm for 10 minutes. The extract was then filtered and stored till the next procedure.

Preparation of Hand Wash Gel
Using a laboratory mixer, 1 g of carbopol-940 and 2 g of glycol stearate were dissolved in 70 ml of soap nut extract. 10 ml of aloe vera gel, 5 ml of glycerine, and 1 ml of *Azadirachta indica* oil were added while continuously agitating the mixture at 500 rpm. Distilled water q.s was added to make up the volume up to 100 ml. pH was measured using a digital pH meter and a sufficient amount of citric acid solution was added to adjust the pH of the hand wash gel to 7.4.

Evaluation
Physical Evaluation
The color, texture, odor, appearance, and homogeneity of the herbal hand wash gel were evaluated by physical examination [9].

Viscosity
The viscosity of the herbal hand wash gel was evaluated using a Brookfield viscometer [2]. 100 ml of prepared hand wash gel was taken in a beaker. The spindle number LV-3 was selected for the measurement of viscosity. The readings were noted upon the successive increase in rpm of the spindle and its effect on the viscosity of the hand wash gel at a temperature 25°C. The viscosity of marketed hand wash gel was evaluated in a similar way.

Spreadability
One drop of herbal hand wash gel was placed on a glass slide. Another glass slide was kept over it and left aside for 5 minutes. The diameter up to which the formulation was spread, was measured in cm [9]. The same procedure was followed for the marketed formulation.

pH
1 ml of herbal hand wash gel was mixed with 100 ml distilled water. This solution was then analyzed using a previously calibrated digital pH meter [10]. The pH of the marketed formulation was analyzed in the same manner.

Irritancy
The herbal hand wash gel was applied to the hands till absorbed. The skin was observed for 1 hour for any signs of irritancy, redness, itching or discomfort, etc. The same process was repeated for the marketed formulation [10].

Foam Height
1 ml of herbal hand wash gel was dissolved in 100 ml of distilled water and poured into a 500 ml measuring cylinder. With the help of the palm, the mouth of the measuring cylinder was covered tightly and the cylinder was shaken to 25 strokes. The measuring cylinder was kept still and the height of the foam formed inside the measuring cylinder was noted [11].

Foam Retention
1 ml of herbal hand wash gel was dissolved in 100 ml of distilled water inside a 500 ml measuring cylinder. The cylinder was shaken for 25 strokes and foam height was measured after every 1 minute till 5 minutes. An ideal formulation should have a foam retention time above 5 minutes [11].

Cleaning Action
A piece of wool weighing 10 g was taken and dipped in oil. A solution of 1 ml herbal hand wash gel and 100 ml water was prepared and the prepared piece of wool was placed in this solution. This solution was then shaken for 4 minutes [12]. The piece of wool was taken out gently, dried, and weighed. The same procedure was followed for the evaluation of the marketed hand wash gel. The cleansing action (equation 1) was calculated using the formula:

\[
\text{Cleaning Power} = 100 \times \frac{\frac{W - W_0}{W_0}}{C}
\]

Where
- \( T \) = Weight of hand wash gel
- \( C \) = Weight of marketed hand wash gel

In Vitro Antimicrobial Activity
To prepare the culture media, 5g of premixed nutrient agar media was dissolved in 100 ml of distilled water and autoclaved at 121°C at 15psi for 15 minutes, the sterilized media was then poured into sterilized petri plates and the media was allowed to cool and gel. The bacterial inoculums of *Pseudomonas aeruginosa* and *Staphylococcus aureus* were introduced onto the separate agar plates using the pour plate method. One disc was taken and dipped into the herbal hand wash gel and allowed to absorb the formulation. The disc was then gently placed onto the inoculated agar plates. The as a control to compare the effectiveness of the herbal formulation. The inoculated agar plates were then incubated for 48 hours at 37°C inside the incubator and a zone of inhibition on the bacterial culture was noticed for both the strains [12].

Stability
The herbal hand wash gel was added in small containers and one sample each was kept at 25°C, 37°C, and 40°C separately for one week. The samples were then subjected to evaluation to all the parameters mentioned above [13].

Table 1: Materials required to prepare herbal hand wash

<table>
<thead>
<tr>
<th>Sr. no.</th>
<th>Ingredients</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Soap nut extract</td>
<td>70% v/v</td>
</tr>
<tr>
<td>2</td>
<td>Aloe vera gel</td>
<td>10% v/v</td>
</tr>
<tr>
<td>3</td>
<td>Glycerine</td>
<td>5% v/v</td>
</tr>
<tr>
<td>4</td>
<td>Carbopol-940</td>
<td>1% w/v</td>
</tr>
<tr>
<td>5</td>
<td><em>Azadirachta indica</em> oil</td>
<td>1% v/v</td>
</tr>
<tr>
<td>6</td>
<td>Glycol stearate</td>
<td>2% w/v</td>
</tr>
<tr>
<td>7</td>
<td>5% Citric acid solution</td>
<td>Sufficient to adjust the pH</td>
</tr>
<tr>
<td>8</td>
<td>Distilled water</td>
<td>Q.s. 100 ml</td>
</tr>
</tbody>
</table>
Results
The physicochemical and biological evaluation was done for the marketed and the prepared formulation and the results obtained are tabulated in Table 2.

Table 2: Comparison of evaluation parameters of prepared formulation and marketed formulation.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Evaluation Parameters</th>
<th>Prepared formulation</th>
<th>Marketed formulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Colour</td>
<td>Orange</td>
<td>White</td>
</tr>
<tr>
<td>2.</td>
<td>Odour</td>
<td>Characteristic</td>
<td>Floral</td>
</tr>
<tr>
<td>3.</td>
<td>Appearance</td>
<td>Opaque</td>
<td>Opaque</td>
</tr>
<tr>
<td>4.</td>
<td>Texture</td>
<td>Smooth</td>
<td>Smooth</td>
</tr>
<tr>
<td>5.</td>
<td>Viscosity (at 100rpm)</td>
<td>360cp</td>
<td>435cp</td>
</tr>
<tr>
<td>6.</td>
<td>Spreadability (cm)</td>
<td>4cm</td>
<td>3.7cm</td>
</tr>
<tr>
<td>7.</td>
<td>pH</td>
<td>7.4</td>
<td>8.5</td>
</tr>
<tr>
<td>8.</td>
<td>Irritancy</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>9.</td>
<td>Foam height</td>
<td>2.6cm</td>
<td>3.8cm</td>
</tr>
<tr>
<td>10.</td>
<td>Foam retention</td>
<td>12.5 ml</td>
<td>17.2 ml</td>
</tr>
<tr>
<td>11.</td>
<td>Cleansing action</td>
<td>32%</td>
<td>35%</td>
</tr>
<tr>
<td>12.</td>
<td>Zone of inhibition</td>
<td>For <em>P</em>. aeruginosa = 1.4cm For <em>S</em>. aureus = 3.7 cm</td>
<td>For <em>P</em>. aeruginosa = 1.5cm For <em>S</em>. aureus = 3.4 cm</td>
</tr>
<tr>
<td>13.</td>
<td>Stability</td>
<td>Stable</td>
<td>Stable</td>
</tr>
</tbody>
</table>

Discussions
All the parameters were found to be excellent and it can be concluded that the prepared formulation had comparable antimicrobial properties with the marketed formulation, having skin-friendly as well as environment-friendly ingredients. The plant extracts used in the concentration specified in this study inhibited the growth of microbes that are usually found on the samples taken from hands. Also, the cleansing action of the hand wash gel on the piece wool showed promising results.

Conclusions
The findings of this study are promising enough to prove the anti-microbial properties of *Azadirachta indica* and *Sapindus mukorossi*. The prepared herbal hand wash gel possessed excellent cleansing action, spreadability, viscosity, and foaming, with no irritation on the skin. This formulation is free from synthetic surfactants, alcohol, and preservatives and was found to be stable, effective, and eco-friendly. The plant extracts and essential oils can be further explored for their additional antimicrobial, emollient and fragrant properties to formulate hand washes and various other cosmetics of significant human as well as environmental significance.

Abbreviations
W/V: Weight/Volume, RPM: Revolutions per minute

Declarations
Ethics Approval and Consent to Participate
Not applicable

Consent for Publication
Not applicable

Availability of Data Materials
All the data generated and interpreted while conducting this study were included in the article.

Competing Interests
The authors have no competing interests to declare that are relevant to the content of this article.

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Authors’ Contributions
All the authors had equal contribution in the research methodology, formulation development, evaluation of the study results and preparation of the manuscript. All authors read and approved the final manuscript.

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