Development and Evaluation of Polyherbal Handwash Containing *Mimosa pudica*, *Azadirachta indica* and *Glycyrrhiza glabra*.

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**ABSTRACT**

The aim of present work was to prepare formulations of polyherbal handwash from the methanolic extracts of leaves of *Mimosa pudica*, *Azadirachta indica* and *Glycyrrhiza glabra*. Formulations of polyherbal hand wash were prepared and the formulations were evaluated for physical properties like appearance, pH, viscosity, grittiness, spreadability, cleaning action, and foam height. The antimicrobial activity of prepared formulations of polyherbal hand wash was checked against skin pathogens *Bacillus subtilis*, *Staphylococcus aureus*, *Psuedomonas aeruginosa* and *Escherichia coli* by agar diffusion method. The results revealed that prepared herbal hand wash formulations showed significant zone of inhibition compared with standard (marketed formulations). So, these plant materials can be used in the preparation of polyherbal hand wash on commercially scale.

**Keywords:** Polyherbal handwash, *Mimosa Pudica*, *Glycyrrhiza glabra*, *Azadirachta indica*, antimicrobial activity.

**INTRODUCTION**

Skin being the most exposed part of our body requires protection from skin pathogens. The hands of Health Care workers (HCWs) are the primary routes of transmission of multidrug resistant pathogens and infection to the patients. Hence, it brings up the use of antiseptic for hand wash purpose. Many of the chemical antiseptics are now available in market as alcohol-based sanitizers, chlorhexidine products etc. These soaps or solutions help to reduce health care associated transmission of contagious diseases more effectively but they have some shortcomings or adverse effects. Their frequent use can lead to skin irritation and also resistant among pathogens. Organisms such as *Bacillus subtilis*, *Staphylococcus aureus*, *Psuedomonas aeruginosa* and *Escherichia coli* are some of the skin pathogens.

Hand washing is an important way to help fight the spread of disease. Hand washing removes visible dirt from hands and reduce the number of harmful microorganisms. Harmful bacteria and viruses such as, *E.coli* and *Salmonella* can be carried by people, animals or equipment and transmitted to food.

Antimicrobial properties of certain Indian medicinal plants were reported based on folklore information and only few reports are available on inhibitory activity against certain pathogenic bacteria and fungi. Use of plants as source of medicine has been inherited and is an important component of the health care system in India. In these systems of Indian medicine, most practitioners formulate and dispense their own recipes; hence this requires proper documentation and research.

*Mimosa pudica* L. (Mimosaceae) also referred to as touch me not, live and die, shame plant and humble plant is a prostrate or semi-erect shrub of tropical America and Australia, also found in India heavily a

**Figure 1: Mimosa pudica**

*Azadirachta indica* A. Juss, (Neem tree), from the Meliaceae family, also known as Margosa or Indian lilac. Various parts of the Neem tree have been used as...
traditional Ayurvedic medicine in India. Neem oil, the bark and leaf extracts have been therapeutically used as folk medicine to control leprosy, intestinal helminthiasis, respiratory disorders and constipation and also as a general health promoter. Neem leaves possesses a wide spectrum of antibacterial action against gram-negative and gram-positive microorganisms.

**Figure 2: Azadirachta indica**

Glycyrrhiza glabra L. (Fabaceae) is a native of south-east Europe and south-west Asia, including Iran. Antimicrobial activities of roots and rhizomes have been studied in previous researches, but there are a few reports about the effect of licorice leaves against microorganisms.

**Figure 3: Glycyrrhiza glabra**

**MATERIALS AND METHODS**

**Collection of materials**

The plant material Glycyrrhiza glabra, Mimosa pudica and Azadirachta indica were collected from the local market and authenticated from Department of Botany, RTMNU, and Nagpur.

**Method of extraction of plant material**

**Extraction of Azadirachta indica and Mimosa pudica:**

The leaves of Azadirachta indica and mimosa pudica were collected, air dried and powered using mixture grinder. 10 gram of coarsely powdered leaves of both plants was extracted in 200 ml of methanol by soxhlet apparatus up to 1 to 2 days after extraction the extract was filtered and the filtrate was collected and stored at room temperature.

**Method of extraction of Glycyrrhiza glabra**

10 grams powered of Glycyrrhiza glabra were extracted with 100 ml of ethanolic solution (9 parts of ethanol and 1 part of distilled water by means of extraction). The mixture was heated in water both at 60°C for 1 hour. The content was filtered thought Whatman filter paper. The filtrate was collected and stored at room temperature.

**Method of preparation of polyherbal handwash**

Herbal hand wash was prepared by using carbopol 940 as a gelling agent in 0.5%w/v concentration with distilled water over night then the swelled polymer was stirred using mechanical stirrer to ensure the uniform dispersion of polymer the pH was adjust to 7.

Azadirachta indica, Glycyrrhiza glabra and Mimosa pudica with lemon oil were measured accurately and dissolved by gentle heating. After heating keep the solution aside for some time. The required Sodium lauryl Sulphate dissolved in 10ml distilled water along with Glycerine were mixed in above aqueous phase with continuous stirring.

**Table 1: Formulation table of polyherbal handwash**

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Ingredient</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ethanolic extract of Glycyrrhiza glabra</td>
<td>-</td>
<td>20ml</td>
<td>-</td>
<td>5ml</td>
</tr>
<tr>
<td>2</td>
<td>Methanolic extract of Mimosa pudica</td>
<td>-</td>
<td>-</td>
<td>20ml</td>
<td>5ml</td>
</tr>
<tr>
<td>3</td>
<td>Extract of Azadirachta indica</td>
<td>20ml</td>
<td>-</td>
<td>-</td>
<td>10ml</td>
</tr>
<tr>
<td>4</td>
<td>Carbopol 940</td>
<td>15ml</td>
<td>15ml</td>
<td>15ml</td>
<td>15ml</td>
</tr>
<tr>
<td>5</td>
<td>Sodium lauryl sulphate</td>
<td>6gms</td>
<td>6gms</td>
<td>6gms</td>
<td>6gms</td>
</tr>
<tr>
<td>6</td>
<td>Lemon oil</td>
<td>2ml</td>
<td>2ml</td>
<td>2ml</td>
<td>2ml</td>
</tr>
<tr>
<td>7</td>
<td>Glycerin</td>
<td>10ml</td>
<td>10ml</td>
<td>10ml</td>
<td>10ml</td>
</tr>
<tr>
<td>8</td>
<td>Methyl paraben</td>
<td>0.1gm</td>
<td>0.1gm</td>
<td>0.1gm</td>
<td>0.1gm</td>
</tr>
<tr>
<td>9</td>
<td>Distilled water</td>
<td>q. s</td>
<td>q. s</td>
<td>q. s</td>
<td>q. s</td>
</tr>
</tbody>
</table>

**Figure 4: Formulation of Polyherbal Handwash (F4)**

The methyl paraben was dissolved in remaining quantity of purified water and dispersed into the extract. The swelled polymer (Carbopol 940) was stirred using a mechanical stirrer to ensure the uniform dispersion of polymer and finally added into the above mixture to form a homogenous Gel and then the required quantity of neem oil was added for fragrance. And the formulation was stored in well closed container and labelled suitably for further analysis.
Evaluation of Polyherbal Handwash

1. Organoleptic properties:
Organoleptic properties like colour, odour, and texture was carried out. Colour and texture were evaluated by visual and touch sensation respectively.\(^9\)

2. Appearance and homogeneity:
Appearance and homogeneity were evaluated by visual inspection.\(^10\)

3. Grittiness:
1ml gel was taken on fingertip and rubbed between two fingertips, then formulation was evaluated.\(^11\)

4. Spreadability:
0.5 gm of sample of polyherbal handwash was taken and passed between two slides and left for 5 minutes where no more spreading was expected. Diameter of spreader circle was measured in cm and was taken as comparative value for spreadability.\(^12\)

5. Foam height:
1 gm of sample of polyherbal handwash was taken and dispersed in 50 ml distilled water dispersion was transferred into measuring cylinder. Volume was make up to 100 ml with water. This solution was taken in 10 test tubes in the series of successive portion of 1, 2, 3....10ml and remaining volume is made up with water to 10 ml. Then the test tube was shaken for 15 sec. Then the test tube allowed to stand for 15 min. and the height of foam was measured.\(^13\)

6. pH: The pH was determined by using digital pH meter.\(^13\)

7. Antimicrobial activity:
Agar well diffusion method was performed for the determination of antimicrobial activity. After sterilization of the nutrient medium and petri dishes transfer into laminar air flow unit for aseptic transfer. 0.25µm of each bacterial inoculum were added to 25 ml of nutrient medium and poured into Petri dishes. After solidification, cups were made by using borer (5mm). 50µm of test formulation I, II, III & IV and marketed herbal handwash (standard) were added to each cup. Then the plates were incubated at 37°C for 24hrs in the incubator. After incubation, the diameter of clear zones of inhibition produced around the well and it was measured in mm compared to the standard (Marketed formulation).\(^14\)

RESULTS AND DISCUSSION

Collection and authentication of plant
The collected plant material of *Glycyrrhiza glabra*, *Mimosa pudica* and *Azadirachta indica* were identified and authenticated by Dr. N.M. Dongarwar, Department of Botany, RTMNU, Nagpur.

Formulation of polyherbal handwash
Four formulations of polyherbal gel were formulated by varying the herbal ingredients in each of the formulation as shown in table no.1.

Evaluation of polyherbal handwash
All the prepared formulations were evaluated for parameters such as colour, odour, texture, appearance, homogeneity, grittiness, spreadability, foam height and pH.

The observation reveals that the polyherbal handwash were translucent in appearance, Smooth in texture, and pH. All the formulations showed good homogeneity with absence of lumps. The developed preparations were much translucent. The results are shown in table no. 2.

Antimicrobial activity
The antimicrobial activity was studied using the well diffusion method. Out of all the formulations, formulation F3 containing all the three extracts showed the highest zone of inhibition and it was comparable with the marketed formulation against *Bacillus subtilis*, *Staphylococcus aureus*, *Psuedomonas aeruginosa* and *Escherichia coli*. The results is shown in table no.3.

Table 2: Evaluation of polyherbal handwash

<table>
<thead>
<tr>
<th>Formulations</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
<th>Marketed formulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colour</td>
<td>Green</td>
<td>Orange</td>
<td>Light green</td>
<td>Dark brown</td>
<td>Light orange</td>
</tr>
<tr>
<td>Odour</td>
<td>Lemon like</td>
<td>Lemon like</td>
<td>Lemon like</td>
<td>Lemon like</td>
<td>Pleasant</td>
</tr>
<tr>
<td>Texture</td>
<td>Smooth</td>
<td>Smooth</td>
<td>Smooth</td>
<td>Smooth</td>
<td>Smooth</td>
</tr>
<tr>
<td>Appearance and homogeneity</td>
<td>Translucent</td>
<td>Translucent</td>
<td>Translucent</td>
<td>Translucent</td>
<td>Translucent</td>
</tr>
<tr>
<td>Grittiness</td>
<td>Non-gritty</td>
<td>Non-gritty</td>
<td>Non-gritty</td>
<td>Non-gritty</td>
<td>Non-gritty</td>
</tr>
<tr>
<td>Spreadability</td>
<td>3.1</td>
<td>3.0</td>
<td>3.3</td>
<td>3.5 cm</td>
<td>3.7 cm</td>
</tr>
<tr>
<td>Foam height</td>
<td>11ml</td>
<td>9ml</td>
<td>12ml</td>
<td>14 ml</td>
<td>18ml</td>
</tr>
<tr>
<td>pH</td>
<td>7.4</td>
<td>7.2</td>
<td>6.9</td>
<td>7.9</td>
<td>7.8</td>
</tr>
</tbody>
</table>
**CONCLUSION**

Nowadays there is a lot of demand for herbal formulations in the market due to their cost-effectiveness and absence of any side effects. From the above experimental data it is clear that a polyherbal handwash formulation with herbal ingredients such as *Glycyrrhiza glabra*, *Mimosa pudica* and *Azadirachta indica* has good characteristics and also possesses a good antimicrobial activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Psuedomonas aeruginosa* has good characteristics and also possesses a good antimicrobial activity against *Escherichia coli*.

**REFERENCES**


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**Table 3: Zone of inhibition of polyherbal handwash**

<table>
<thead>
<tr>
<th>Formulations</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
<th>Marketed formulations</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bacillus subtilis</em></td>
<td>12mm</td>
<td>15mm</td>
<td>17mm</td>
<td>25mm</td>
<td>17mm</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>13mm</td>
<td>14mm</td>
<td>16mm</td>
<td>25mm</td>
<td>15mm</td>
</tr>
<tr>
<td><em>Psuedomonas aeruginosa</em></td>
<td>10mm</td>
<td>16mm</td>
<td>18mm</td>
<td>23mm</td>
<td>18mm</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>15mm</td>
<td>18mm</td>
<td>18mm</td>
<td>26mm</td>
<td>17mm</td>
</tr>
</tbody>
</table>

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