FORMULATION AND EVALUATION OF HERBAL HAND WASH

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ABSTRACT  
Microbial infection has emerged as a critical issue in children and hospital care outcome, which can leads to substantial morbidity and mortality. Unhygienic hands of health care workers are the primary routes of transmission of infection directly to patients and in children it can lead to several serious health issues. So that, it brings up the use of antiseptic for hand washing purposes. There are several commercial antiseptic available in market having chemical sanitizers as a base which has some disadvantages, adverse and side effects. Their frequent and long time use can lead to some side effects and skin irritation.

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Madhuca indica is one of the most widely used and well-documented medicinal plants in the world. Present study aimed to formulate effective, safe and nontoxic herbal hand wash using barks of Madhuca indica emphasis on safety and efficacy and to avoid the risk posed by synthetic antimicrobials. Disc diffusion method was utilized for evaluation of the antimicrobial activity against skin pathogens of the prepared herbal hand. Its efficacy was checked and compared with the standard commercial hand wash. Results revealed that Madhuca indica based formulation was more efficient in reducing the number of organisms from hands than the commercial antiseptic soaps thus it can be used as an antiseptic soap based handwash with less or no side effects.

KEYWORDS: Herbal hand wash, Formulation, Evaluation, Madhuca indica Extracts, pathogens, Staphylococcus aureus, Pseudomonas aeruginosa.

INTRODUCTION  
Madhuca commonly known as Mahua or butternut tree, 17m high with a large top.[1] It belongs to family sapotaceae. The bark is recommended for phlegm and in rheumatism bark flakes are mildly heated and tied on joints. The bark is a good treatment for itch, swelling, fractures and snake-bite poisoning.[2] Preliminary phytochemical studies of stem bark with
ethanol, water and chloroform extract indicated the presence of various chemical constituents like terpenoids, proteins, starch, anthraquinone glycosides, phenolic compounds, mucilage, cardiac glycosides, tannins and saponins. It is commonly used for the treatment of snakebite as antidote for southern part of Tamilnadu, India. it is utilized in the treatment diarrhoea, a cup of infusion of bark is taken orally by the tribals. Besides the stem bark it is used in chronic tonsilitis, leprosy and fever. Few more pharmacological actions of stem bark of Madhuca indica like antioxidant activity, wound healing activity, antimicrobial activity, anti diabetic, anti-inflammatory, anticancer activity and anti epileptic activities were reported in literature.

The Hands are primary mode of transmission of microbial and infections, hygiene of hands is therefore the most important thing to avoid the transmission of harmful germs and prevent the infections. Hand hygiene is the single most important, simplest, and least expensive means of preventing nosocomial infections. Hand-washing is critical in food production, food service and also important in healthcare setting, homes and day care preparations.

Plant extracts and their products have been used for centuries in traditional and Ayurvedic medicines, food, cosmetics, natural dyes, detergent, as a functional food and in the treatment of various diseases. The advantage of using herbal drugs is that they are cheap, easily available and having less side effects in comparison to chemical products.

Therefore research has been increased tremendously towards making natural products with improved quality yet less in cost and no side effect over chemical agents.

In the present context, Madhuca indica had been selected in preparing herbal hand wash. Mahua bark were extracted with alcohol to assure maximum leaching of all the phytochemicals which show their effect on skin pathogens and ensure maximum antimicrobial efficiency. Madhuca indica extract hand wash soap was then prepared, evaluated against skin pathogens and checked for its efficacy.

MATERIALS AND METHODS

Plant collection
Samples of Madhuca indica barks were collected during October (2015) from area adjoining forests of Bhopal (M.P.).
Preparation of plant extracts
The plant has been selected on the basis of its Folk use of the plant. Dried barks of the investigated plant (200 g) were subjected to hot continuous extraction with (500 ml) 95% methanol (30-40°C) in a Soxhlet apparatus for 6 hours. After complete extraction the solvent was evaporated and concentrated to dry residue. % yield was calculated for each extract after drying under vacuum. The residue free solvent (12.5 g) was suspended in distilled water (40 ml) to be used for further study.

Preparation of Madhuca indica hand wash soap
The herbal hand wash was prepared by mixing 4 ml of the suspended water extract (1.25g /4 ml w/v) to 3 g of sodium lauryl sulphate (SLS).[20] Extracts of lemon grass was added for fragrance. The solution was mixed well, made homogenous under room temperature and further utilized for the screening of the activity. Blank soap was prepared as per the previous procedure without herbal extract. The developed formula was compared to standard soaps commercially available in Indian Pharma market. The commercial herbal soap contains green tea, aloe and lemon. In comparison newly developed formula contains extracts of Madhuca indica bark, ginger extract and extract of lemon grass.

Antimicrobial assay
Some gram positive, gram negative and fungal spp. was selected for the study. Anti-microbial activity of the hand wash soaps was investigated by the disc diffusion method.[21,22] Tested organisms used were S.aureus, P.aeruginosa, Bacillus subtilis, Escherikia coli and fungal spp Candida albicans. Nutrient agar was utilized as a culture medium.

It was performed using 24 hours incubation (for bacterial culture) and 48 hours (for fungal culture) at 37°C in 20 ml of agar medium. Bacterial and fungal inoculums were spread over the plates containing agar medium using a sterile cotton swab in order to get a uniform microbial growth on both control and test plates. The extracts were dissolved in ethylene glycol and sterilized by filtration under aseptic conditions; empty sterilized discs (what man no. 5, 6 mm diameter) were impregnated with 100µl of each of the extracts of different concentration and left to dry under laminar flow cabinet and placed on the agar surface. Paper disk moistened with ethylene glycol was placed on the seeded Petri dish as a vehicle control. Standard discs containing chloramphenicol (10µg/ml), gentamicin (10µg/ml) and fluconazole (10µg/ml) were used as reference control. All Petri dishes were sealed with sterile laboratory paraffin to avoid contamination and eventual evaporation of the test samples. The dishes were
left for 30 minutes at room temperature to allow the diffusion of test drugs and kept for incubation on 37°C.

**In vivo efficacy study Experimental design**

Five volunteer groups (n=5) were involved for *in vivo* efficacy study of four different soaps (*Madhuca indica* hand soap formulation, blank soap hand wash, commercial standard hand soap, commercial herbal hand soap). Volunteers with damaged or wounded skin and those who were on antimicrobial therapy were excluded.

**Evaluation**

**Organoleptic evaluation**

Organoleptic evaluation (colour, odour) was done by sensory and visual inspection and compared to the marketed handwash.

**Fragrance test**

It was based on individual observation for its acceptability. 5 people were asked for acceptability of fragrance and their opinion was taken. And fragrance was evaluated based on the below-described criteria;

A). Fragrance was good, as good as the fragrance of reference hand wash.

B). Fragrance was not so good but comparable to the reference hand wash.

C). Fragrance of the toothpaste was poor than the reference hand wash.

**Bacteriological study**

Point inoculation method as mentioned by Miles and Mishra[23], was used for viable count of different bacterial flora.

**RESULTS AND DISCUSSION**

Preliminary antimicrobial activity screening tests observations were shown in Table 1. *Madhuca indica* soap formulation proved to be beneficial with excellent activity against all the tested microorganisms. A significant (*p*<0.05) value was found against *S. aureus*. Hence it was encouraging to be used as a potent antiseptic in the preparation of herbal soap hand wash.[24]

All the observation data for *in vitro* antimicrobial activity evaluation of *madhuca indica* soap is presented in Table 2. *Madhuca indica* soap formulation had significant antimicrobial activity against all test organisms than the commercial hand soaps. No significant values were
obtained from the blank soap which is indicative of; antibacterial activity of herbal hand wash is solely due to the activity of active components. The activity of test soap against *Staphylococcus aureus* was of significant interest (*p*<0.05). Our skin contains large numbers of microorganisms, mainly Gram-positive. *Staphylococcus aureus* is one of these natural flora, which is commonly found on the hands, face and in deep layers of the skin. *Staphylococcus aureus* is ubiquitous and is not easily washed and eliminated by routine washing and scrubbing even with some antiseptic soap.[25,26] Hence the activity of the *Madhuca indica* herbal soap against *Staphylococcus aureus* was very remarkable.

Table 1 Antimicrobial Activity of *Madhuca indica* Inhibition zone diameter (mm) ± SD*.

<table>
<thead>
<tr>
<th>Test organisms</th>
<th>Inhibition Zone (mm) ± SD*</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>18.6 ± 0.53**</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>11.8 ± 0.53</td>
</tr>
<tr>
<td><em>Bacillus subtilis</em></td>
<td>12.6 ± 0.80</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>10.8 ± 0.46</td>
</tr>
<tr>
<td><em>Candida albicans</em></td>
<td>8.60 ± 0.88</td>
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</tbody>
</table>

*: Values calculated as means ± SD (n=3); **: Results are significant (*p*<0.05).

Table 2: *In Vitro* Antimicrobial activity Evaluation of *Madhuca indica* Hand Wash Soap

<table>
<thead>
<tr>
<th>Test organisms</th>
<th>Zone of Inhibition (mm)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Madhuca indica soap</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>+</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>+</td>
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</tbody>
</table>

+: presence of microbial growth. All Values are means of triplicates.

Table 3: Reduction in Hands’ microbial count.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Bacterial flora reduction</th>
<th>Reduction</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Control (cfu/ml)</td>
<td>Test (cfu/ml)</td>
</tr>
<tr>
<td><em>Madhuca indica</em> soap formulation</td>
<td>27.55 ± 10⁸</td>
<td>5.85 ± 10⁷</td>
</tr>
<tr>
<td>Blank soap</td>
<td>23.86 ± 10⁷</td>
<td>88 ± 10⁷</td>
</tr>
<tr>
<td>Commercial Herbal soap</td>
<td>173 ± 10⁶</td>
<td>58 ± 10⁵</td>
</tr>
<tr>
<td>Lifebuoy</td>
<td>23.34 ± 10⁷</td>
<td>69 ± 10⁶</td>
</tr>
</tbody>
</table>

Table 3 and Fig. 1 (a-d) indicates growth reduction in microbial count obtained after the use of *Madhuca indica* herbal soap formulation, commercial herbal soap and blank soap. Highest
reduction was observed by *madhuca indica* herbal soap followed by commercial herbal soap, lifebuoy and blank soap. The percent of reduction in the count shown by *madhuca indica* herbal soap was 80%, while it was found to be to be least in case of control blank soap (67%). The commercial herbal antiseptic soaps showed more reduction than arrow wash (74%). A percentage reduction in bacterial count was calculated using the formula given below.

\[
\text{% Reduction} = \frac{\text{Control (cfu/ml)} - \text{Test (cfu/ml)}}{\text{Control (cfu/ml)}} \times 100 \quad \text{(eq. 1)}.
\]

The significant \((p<0.05)\) activity of the formulated hand wash may be due to presence of tannins and phenolics in the methanolic extract.\(^{[27]}\) Tannins are reported to be bacteriostatic or bactericidal against *Staphylococcus aureus* due to its action on the membranes of the microorganisms.\(^{[28]}\) The results of the screening of the antimicrobial activity showed that the natural antimicrobials agents are present in the extract and hand wash prepared by them were far more active than the synthetic antimicrobials used in commercial antiseptic soap. The fragrance of the formulated hand wash was found pleasant due to presence of lemon grass extract.

**CONCLUSION**

In the present study, active constituents (phenolics, tannins, flavonoids) were present in the methanolic extract of *Madhuca indica* bark shown superior inhibition against various gram positive, negative and fungal skin pathogens than synthetic antimicrobials agents present in the commercially available antiseptic liquid soaps. Therefore, these compounds could be extracted and incorporated in soap bases in order to prepare superior antiseptic soap with significant activity having less or no side effects. Hence, a new way can be found to combat antibiotic resistance of these organisms and provide safer and healthier living through microbial free hands, although the removal is not possible 100%, but a significant number can be reduced with natural economic and safe *madhuca indica* soap. Formulation can also be routinely used for improving hygiene of healthy children and adults. Further pharmacological evaluations, toxicological studies and possible isolation of the therapeutic antibacterial from this plant are the future challenges.
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REFERENCES

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