Evaluation of herbal handwash formulation

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Received 24 April 2007; Accepted 21 July 2008

Abstract
A herbal handwash was prepared using extracts of leaves of Terminalia catappa Linn., Couroupita guianensis Aubl. and rinds of Garcinia indica Choisy. The antibiotic sensitivity test of the prepared herbal handwash against skin pathogens was checked using Disc diffusion method and results were compared with the commercially available antiseptic soap. The results showed that the herbal handwash gave larger inhibition zone than the commercial antiseptic soap against Staphylococcus aureus and Pseudomonas aeruginosa. The efficacy of the herbal handwash was evaluated using Glove Juice method which revealed that the herbal handwash is efficient in reducing higher number of organisms from the hands than the commercial antiseptic soap. Hence, due to the higher antimicrobial activity these plant materials can be used in the preparation of herbal handwash on commercial scale.

Keywords: Skin pathogens, Staphylococcus aureus, Pseudomonas aeruginosa, Terminalia catappa, Couroupita guianensis, Garcinia indica, Herbal handwash.

IPC code; Int. Cl.— A61K 8/97, A61Q 19/10, A61K 36/00, A61P 31/00, A61P 31/04

Introduction
Skin being the most exposed part of our body requires protection from skin pathogens. Nosocomial infections have emerged as a critical issue in hospital care outcome, resulting in extended hospitalization and substantial morbidity and mortality. The hands of Health Care Workers (HCWs) are the primary routes of transmission of multidrug resistant pathogens and infection to patients. Hence, it brings up the use of antiseptics for hand washing purposes. Many of the chemical antiseptics are now available in market as alcohol based sanitizers, chlorox headache products, etc. These soaps or solutions help 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 resistance among pathogens. Organisms such as Staphylococcus aureus, Pseudomonas spp., Klebsiella pneumoniae and Proteus vulgaris are some of the causative agents of skin infections. The study conducted by French and Phillips showed that the resistance of organisms to chemical antiseptics have led to outbreaks.

Terminalia catappa Linn. (Indian Almond Tree) leaves possess antioxidant, sudorific as well as antibacterial properties and is used to cure leprosy. They also contain phytochemicals such as tannins, kaempferol and quercetin. Couroupita guianensis Aubl. (Cannon-ball tree) leaves are rich in phytochemicals and reported to contain quercetin, saponins and tryptanthrin. The tree is also rich in providing anthocyanin, flavonoids, volatile constituents like eugenol and farsenol. Similarly, rinds of Garcinia indica Choisy commonly known as Kokam are rich in organic acids mainly hydroxycitric acid and other component garcinol. It also contains in minute quantities of malic acid, citric acid and tartaric acid. The rinds are used as spice whereas the fruit is antihelminthic, cardiotonic and useful in piles, dysentery, tumours, pains and heart complaints.

Materials and Methods
The plant materials were collected from the college garden during spring and were verified at Botany Department, Mithibai College, Mumbai. The pathogens were recovered from the hands of HCWs. Amongst the isolated pathogens Staphylococcus aureus and Pseudomonas aeruginosa were selected for the study. Methanol extract of the plant materials was prepared and further used for preparation of handwash. Ten grams of each plant materials were added separately to 100 ml methanol solution (9 parts methanol: 1 part water). This mixture was heated in water bath at 60°C for 60 minutes. The contents were filtered and the filtrate was used as methanol extract.

The herbal handwash was prepared by adding 4 ml of the methanol extract of combined plant materials in 6 ml of distilled water. To the final volume of 10ml, 3 g of Sodium Lauryl Sulphate (SLS) was added as per the
requirement of standard procedure for preparation of handwash. The solution was made homogenous under room temperature and used for the antibacterial screening.

The screening of antibacterial sensitivity of the plant extracts against pathogens was performed using disc diffusion method as per standard procedures\textsuperscript{12}. Test cultures used were \textit{S. aureus} and \textit{P. aeruginosa}. Sterile filter paper discs of 6 mm were loaded with 10 µl of herbal handwash. It was taken care that the sterile discs completely absorb the handwash. In similar ways, discs of commercial antiseptic soap and SLS were prepared. The discs, when completely dried were placed on Mueller Hinton agar, which was previously inoculated with the test cultures.

The efficacy of the herbal handwash was determined using Glove Juice method\textsuperscript{13}. The test determines the efficacy of the product in reduction of organisms. The product is applied to hands, gloves are donned, sampling solutions are integrated inside the glove and bacterial levels are tested by extracting samples at regular interval of time. Higher the log reduction, the more effective is the test product. \textit{S. aureus} and \textit{P. aeruginosa} were used as test cultures for this method.

**Results**

The results of disc diffusion method showed that the handwash prepared from methanol extract of the combined plant materials had greater activity than the activity of the commercially available hand sanitizer. Disc of SLS was also maintained as control. The zone size obtained for SLS disc showed that the antibacterial activity of herbal handwash is not solely due to the use of 30% SLS but a result of combined activity of active components. The average result of ten experiments is presented in Table 1 with standard deviations.

The herbal handwash was capable of reducing 3 log CFU/ml count of organisms from the hands of Health Care Workers (HCWs) as compared to the commercial antiseptic soap which gave a reduction of 1 log CFU/ml. The average result of ten experiments is presented in Table 2 along with standard deviation. The statistical analysis was carried out using standard deviation method.

**Discussion**

The fruits of \textit{T. catappa} are widely used for medicinal purposes. The leaves of this plant are also rich in various compounds such as flavonoids, polyphenols, alkaloids, etc. The main constituent is tannins. Tannins are water soluble polyphenols that are commonly found in two categories hydrolysable and condensed tannins\textsuperscript{14}. Tannins are present in many foods. Tannins have been reported to be bacteriostatic or bactericidal against \textit{Staphylococcus aureus}\textsuperscript{15}. They act on the membranes of the organisms.

Quercetin is one of the main flavonoids. It is a yellow, crystalline structure that inhibits the release of histamine and other inflammatory mediators. It is also used as anti-allergic, anti-histaminic and anti-inflammatory. Quercetin is not soluble in water causing it to be poorly absorbed nutrient. It also acts as an antioxidant\textsuperscript{16}. The rinds of \textit{G. indica} are rich in organic acids such as (−) hydroxycitric acid, which has

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**Table 1: Antibiotic sensitivity result of herbal handwash, standard soap and SLS**

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Zone of Inhibition in mm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Herbal handwash</td>
</tr>
<tr>
<td><strong>Staphylococcus aureus</strong></td>
<td>25 ± 2</td>
</tr>
<tr>
<td><strong>Pseudomonas aeruginosa</strong></td>
<td>14 ± 2</td>
</tr>
</tbody>
</table>

**Table 2: Average count of organism for ten samples with standard deviation**

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Average count of organisms in CFU/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td><strong>Staphylococcus aureus</strong></td>
<td>6.32×10⁶ ± 0.6</td>
</tr>
<tr>
<td><strong>Pseudomonas aeruginosa</strong></td>
<td>2.1×10⁷ ± 0.31</td>
</tr>
</tbody>
</table>

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potential effect on bacteria and is a potent inhibitor of ATP citrate lyase 17.

In the present context the plants under study are rich in these varied compounds and hence are more effective against skin pathogens. The methanol extract is efficient in extracting the phytochemicals and acids which act on the pathogens.

The main ideology behind combining the plant materials is to observe the additive effect of the active constituents of different plants. The combination proves to be beneficial and hence it is used in preparation of a herbal handwash. The herbal soap prepared was checked for its efficacy using disc diffusion method. The results clearly prove that the herbal soap thus prepared is far more active than the commercial antiseptic soap. It can be stated that the active compounds in the soap prepared are more effective in killing or removing organisms than the chemicals used in soaps. Thus, these compounds can be extracted and incorporated in soap bases in order to prepare superior antiseptic soap with less or no side effects. Hence, a new way can be found to combat antibiotic resistance of pathogenic organisms and provide safe and healthier living through germfree hands. Although the removal is not 100% but a major number can be reduced.

Conclusion

The results suggest that the constituent of the various extracts of T. catappa, C. guianensis and G. indica and their combinations are capable of giving superior inhibition than the commercially available antiseptic soaps against skin pathogens. This might be rational basis for use of herbs in preparation of handwash and use of these compounds in making of antiseptic soaps in place of chemicals.

References