“FORMULATION AND EVALUATION OF ANTIMICROBIAL ACTIVITY OF POLYHERBAL MOUTHWASH”

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KEYWORDS:
Mouthwash, Antimicrobial activity, poly-herbal extract.

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ABSTRACT

The extracts of three herbs namely *Azadirachta indica*, *Acacia nilotica*, *Mangifera indica*, as individual and as combination were tested for anti-microbial activity in vitro studies. The formulation was then prepared and its anti-microbial activity was compared with the marketed chlorhexidine mouthwash. The results indicate that this poly-herbal mouthwash holds promise in improving the oral hygiene and in preventing dental caries.
INTRODUCTION:
Mouthwash means that a liquid which is oral product that made to freshen the breath. It is also may kill the bacteria in the mouth or whiten the teeth. The mouthwash can cure the illness due to toothache and mouth infections. Various products such as toothpastes, gels, pastes, mouthwash, lozenges, etc. have been available for years. However, in the recent years, use of mouthwash has been on the increase as it is relatively easy to use for maintaining oral hygiene.

A number of chemical agents which have antiseptic or antimicrobial action have been used to inhibit supragingival plaque formation and the development of gingivitis. Among these are; phenolic compounds, Bis-biguanides, Pyrimidines, Quaternary ammonium compounds, Oxygenating agents, heavy metal salts. And among these agents, chlorhexidine is the most studied and effective antiseptic for plaque inhibition and prevention of gingivitis when used twice daily as mouthwash. But in oral use as a mouthwash chlorhexidine has been reported to have a number of side effects like staining on the teeth, bitter taste, high degree of alcohol content, irritation during use which restricts its general use. Other chemical antiplaque agents have been tested, none has shown equal or better results than chlorhexidine without eliciting unfavourable side effects. To overcome such side effects the world health organization (WHO) advice researchers to investigate the possible use of natural products such as herb and plant extracts. A number of clinical studies have shown the effects of using mouth washes extracted from herbs such as Sanguinarina, Myrtus communis, Qureucus infectoria and Cinnamon in the prevention of dental plaque accumulation and subsequent gingival inflammation. The herbal medicines are normally considered safer than the non-herbal medicines because the natural active ingredients present in herbal medicines are in combination with other components.

In the present study the herbs used for the preparation of extract are Azadirachta indica, Acacia nilotica, Mangifera indica. These herbs are known for their medicinal properties and are traditionally used in India.

MATERIALS AND METHODS
The following plants powder is used for the extraction purpose:
Azadirachta indica-leaves, Acacia nilotica -stem -bark , Mangifera indica- leaves. Other materials used for the extraction and in vitro evaluation are 95% v/v Ethanol, Pet ether, Distilled water, Nutrient Agar, chlorhexidine.
EXPERIMENTAL

Extraction of individual herbs

*Extraction process*

Thirty grams of powder was then extracted in ethanol [80 ml] and water[20 ml] by using Soxhlet method. Ten cycles were done. The solvent was removed by evaporation at room temperature. This extract was then used for antimicrobial activity evaluation.

*Evaluation of antimicrobial activity of individual extract*

Antimicrobial activity was evaluated by using Agar Well Diffusion Assay method against dental micro flora. 0.2 ml Dental micro flora was spread by Spread Plate Technique on sterile pre solidified Nutrient Agar Medium. The standard procedure was adopted and the zone diameters were measured from six different angles for each well and the average Zone diameter was recorded.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Extract</th>
<th>Zone of Inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Azadirachta indica</td>
<td>20</td>
</tr>
<tr>
<td>2</td>
<td>Acacia Arabica</td>
<td>18</td>
</tr>
<tr>
<td>3</td>
<td>Mangifera indica</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>Average zone of inhibition in mm</td>
<td>19</td>
</tr>
</tbody>
</table>

Results are mean of five experiments (n = 3)

The results showed that all the herbs used have antimicrobial activity.

*Extraction of Poly-herbal herbs (Poly-herbal Extract)*

Total 30g of Poly-herbal powder of selected parts of herbs in the proportion (*Azadirachta indica*, *Acacia nilotica*, *Mangifera indica* 1 : 1 : 1 respectively) was extracted using 100 ml mixture of ethanol and water). By using Soxhlet method and then filtered. The filtrate was concentrated to yield 20ml of a semisolid extract.
Table 2: Zone of inhibition shown by Poly-herbal extract of powders of herbs

<table>
<thead>
<tr>
<th>Sample used</th>
<th>Average Diameter of the Zone of inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poly-herbal extract of powders under study</td>
<td>19</td>
</tr>
</tbody>
</table>

Results are mean of five experiments (n = 3)

Antimicrobial activity was evaluated by using Agar Well Diffusion Assay against dental microflora as explained earlier. (Table no.1)

Preparation of mouthwash

The extract of Poly-herbal powders was formulated by using pharmaceutical excipients like polysorbate 80, ethyl alcohol, sodium saccharin, peppermint oil amaranth, propylene glycol into a mouthwash. The formulation was prepared using 10% w/v of poly-herbal extract, in any single dose of the formulation, to provide optimal antimicrobial activity.

Evaluation of antimicrobial activity of mouthwash

Table 3: Zone of inhibition shown by the mouthwash prepared from the Poly-herbal extract

<table>
<thead>
<tr>
<th>Sample used</th>
<th>Average Diameter of the Zone of inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouth wash (under study)</td>
<td>19</td>
</tr>
<tr>
<td>Chlorhexidine (0.2 %w/v)</td>
<td>20</td>
</tr>
</tbody>
</table>

Results are mean of three experiments (n = 3)

The study was carried out by using Agar Well Diffusion Assay method with undiluted marketed formulation of chlorhexidine (0.2 % w/v) as Positive Control and Sterile distilled water as Negative Control. The antimicrobial activity of the formulation was found to be comparable with antimicrobial activity of marketed Chlorhexidine formulation.

RESULTS

The herbs used in the formulation *Azadirachta indica*, and *Acacia nilotica* having antibacterial activity and *Azadirachta indica* widely used in oral care formulations and *Acacia nilotica* also used in maintenance of oral hygiene. *Mangifera indica* were selected because of their astringent and antioxidant properties in addition to antimicrobial activity. Extraction was done for
individual herbs and was evaluated for antimicrobial activity against dental micro flora. And the results showed that all the plants used in the study have antimicrobial activity.

CONCLUSION
The powders of the selected part of herbs were mixed in a set proportion and the Poly-herbal powder was extracted to obtain a Poly-herbal extract. The results of antimicrobial study showed that the extract prepared by using Poly-herbal powders is equally effective as antimicrobial and has advantage of consuming less solvent system and thus is also cost effective. The formulation of 10%w/v of extract in the form of mouthwash showed significant effect in terms of antimicrobial activity, comparable with the marketed synthetic drug like chlorhexidine.

REFERENCES:
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