FORMULATION AND EVALUATION OF HERBAL MOUTHWASH CONTAINING NATURAL INGREDIENTS FOR ANTI-MICROBIAL ACTIVITY

Praveenkumar V. Vijapur*,1, Laxman S. Vijapur1, Pooja Yaragattimath1, Govindraj Raibagi1, Yogesh Mallapur1 and Bheemareddy Desai1

1Department of Pharmaceutics, BVVS Hanagal Shri Kumareshwar College of Pharmacy, Bagalkot, Karnataka, India.

ABSTRACT
Keeping a decent oral cleanliness supports keeping our general wellbeing in a great shape. The mouth is a fundamental piece of the stomach, and consequently any oral sickness will straightforwardly or by implication influence the GI plot as well. Neem was used as traditional medicine for several disease including cleaning of oral cavities. Banana peel can act as gentle exfoliator for our teeth. Orange peels to help whiten teeth. curcumin in turmeric can prevent gingivitis, or gum disease. It helped remove plaque, bacteria, and inflammation comparably well with traditional mouthwashes, when used properly. Clove have antibacterial and antifungal properties it is widely used in toothpaste preparations. Cinnamon bark and nutmeg seeds have anti-inflammatory property. The objective of study is find out the efficacy of these plant extract against caries inducing microorganisms. further formulating mouthwash which has efficiency to inhibit the growth of the microorganism.

KEYWORDS: Neem, Banana peel, orange peel, Clove, Cinnamon bark, Nutmeg seeds, Herbal mouthwash, Antimicrobial.

INTRODUCTION
The world oral health report, 2003, highlighted oral health as an integral and essential component of general health. Most of the chemical products contains an antiseptic that plays an important role in controlling plaque accumulation. The vehicle for delivery of chemical
agents with antiplaque action are toothpaste, mouth washes, spray, irrigators, chewing gums and varnishes. However, mostly accepted method of delivering the antimicrobial agents after toothpaste is mouth wash. Mouth wash are an antiseptic solution which is used to reduce the microbial load in the oral cavity.

Natural mouthwashes may offer significant advantages over the chemical ones. If such mouthwashes can be formulated which can be easily prepared and used safely by people at home using natural products, it may leads to improvement in the general dental health of the population. In this study the various natural ingredients and materials are used. Then the thin layer chromatography was done to check the quality of materials used. Then the physical evaluation, ph determination, stability study, thin layer chromatography, antimicrobial study was studied. By this study it was concluded that the developed herbal mouthwash possess significant, therapeutically efficacious, suitable vehicle for drug delivery in low cost but definitely with high potential. Hence there is need for increased usage of herbal preparation to avoid the adverse effects. This study is an attempt to outline such natural substances, which may be used as effective mouthwashes.

Almost all chemical mouthwashes contain alcohol and fluoride which is toxic to our body in overdoses. Hence, most herbal mouthwashes are safe alternative to pregnant women, peoples with dry mouth, diabetic and to children. The purpose of this study was to determine the prevalence of mouthwash use and not only the type of mouthwash but quantity of mouthwash to be taken for use is also important and also this study was performed to evaluate the efficacy and safety of herbal mouthwash for human medicines.[1]

Various herbal products and their extracts such as Guava, Pomegranate, Neem, Propolis, Tulsi, Green Tea, Cranberry, Grapefruit etc, have shown significant advantages over the chemical ones. Natural mouthwashes may offer significant advantages over the chemical ones. If such mouthwashes can be formulated which can be easily prepared and used safely by people at home using natural products, it may lead to improvement in the general dental health of the population.[2]

Herbal mouthwash becomes more popular they work without alcohol, artificial preservatives, flavor, or colors.[3]
As it contains natural herbs that have natural cleansing and healing property to teeth and gums. Many herbal mouthwashes contain herbs with anti-microbial property such as Neem, Yavanisatva, Nagavalli, Gandha purataila, Pilu, Bibhitaka, Ocimum, Echinacea, Chameli leaves, etc. Manuka honey, which is rich in flavonoids, increased the glycoprotein production in the ethanol model of gastric damage. Manuka honey was the most effective antioxidant and antibacterial honey compared to both acacia honey and wild carrot honey, possibly because of its high phenol content.\[4\] Guava (*Psidium guajava*) as a mouthwash for swollen gums and ulceration of the mouth and also for bleeding gums.\[5\] Extracts from neem inhibit the growth of *S. mutans* and used in the treatment of periodontitis.\[6\] Aloe vera mouthwash, mixture of *Terminalia chebula* and cinnamon, mixture of *Staphysagria, Chamomilla, Echinacea, Plantago, Ocimum*, and *cistus* extracts, mouthwashes with turmeric, neem and triphala, mixture of Acacia Arabica, Punica granatum, Chameli leaves, Glycyrrhiza glabra and neem shows significant reduction of plaque indices and gingival scores comparatively to chlorhexidine mouthwash and can be a better alternative to chlorhexidine mouthwash.\[7-9\]

**MATERIALS AND METHODS**

**MATERIALS**

Neem leaves, banana peel, orange peel, turmeric root, clove, cinnamon bark and nutmeg seeds were collected from market in Bagalkot Double distilled water was used throughout the study. Mueller Hilton agar was purchased from HIMEDIA.

**METHODOLOGY**

**Preparations of aqueous extract from different plant sources**

The collected plant sources were washed with double distilled water 2-3 times. The washed plant sources were dried at room temperature in shade. After drying they were crushed and the powder was stored for further use. 10 grams of orange peel and clove powder were weighed and were taken in 250 ml conical flask which was containing 100 ml of double distilled water. The flask was closed with the aluminium foil and flask was kept in dark place undisturbed for 24 hours. Later the mixture was filtered through muslin cloth and again filtrate was filtered using Whatmann filter paper grade no.1. The extract was stored in ambered colour glass container for further use at 4°C. 10 grams of cinnamon, turmeric, neem and nutmeg powder were weighed and were taken in 250 ml Erlenmeyer flask which was containing 100 ml of 70% ethanol. The flask was closed with the aluminium foil and flask was kept in dark place undisturbed for 24 hours. Later the mixture was filtered through
muslin cloth and again filtrate was filtered using Whatmann filter paper grade no.1. The extract was stored in ambered color glass container for further use at 4°C. 10 grams of banana powder was weighed and were taken in 250 ml Erlenmeyer flask which was containing 100 ml of 70% iso-propyl alcohol. The flask was closed with the aluminium foil and flask was kept in dark place undisturbed for 24 hours. Later the mixture was filtered through muslin cloth and again filtrate was filtered using Whatmann filter paper grade no.1. The extract was stored in ambered colour glass container for further use at 4°C. All the above obtained extracts were further dried by keeping it in hot air oven for 3 days at 40°C. The dried powders were stored in closed container and were kept in cool place for further use.

FORMULATION OF HERBAL MOUTHWASH

Required quantity of neem, turmeric, cinnamon, banana peel and nutmeg powders were weighed and mixed with sufficient quantity of 70% ethanol and menthol in a beaker, mixed properly until all the powders are dissolved in the solvent. Propylene glycol was added to the above mixture for the complete solubility (solution 1). In another beaker required quantity of clove and orange peel powders were mixed with required quantity of double distilled water and to this solution sodium saccharin was added and mixed properly (solution 2). Lastly beaker containing solution 2 was added to beaker containing solution 1 which was stirred continuously to get a solution, to this solution sodium benzoate was added as preservative and sodium lauryl sulfate was added as a foaming agent.

Table No. 01: Composition of herbal mouthwash formulations with different plant extracts.

<table>
<thead>
<tr>
<th>Sr.No</th>
<th>Ingredients (50 ml)</th>
<th>F1 (gm)</th>
<th>F2 (gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Neem</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>2</td>
<td>Banana peel</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>3</td>
<td>Orange peel</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>4</td>
<td>Clove</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>5</td>
<td>Cinnamon</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>6</td>
<td>Turmeric</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>7</td>
<td>Nutmeg</td>
<td>--</td>
<td>0.1</td>
</tr>
<tr>
<td>8</td>
<td>Sodium Saccharin</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>9</td>
<td>Menthol</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>10</td>
<td>Propylene glycol</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>11</td>
<td>Sodium Lauryl Sulfate</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>12</td>
<td>Sodium Benzoate</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>13</td>
<td>Ethanol</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>14</td>
<td>Double distilled water</td>
<td>qs</td>
<td>qs</td>
</tr>
</tbody>
</table>
CHARACTERIZATION OF HERBAL MOUTHWASH

Physical evaluation
Physical parameter such as color, odour, taste and consistency were examined by physical examination.\[1\]

pH determination
The pH of the herbal mouthwash formulations was determined by pH meter by taking mouthwash in beaker and pH of the formulations were recorded.\[10\]

Determination the Viscosity
Ostwald viscometer was cleaned by the water and ethanol and dried. Mouth wash was placed in viscometer and pull it by pipette until the small bulb is full. Let the liquid to flow through the capillary tube with run time when the liquid reaches the mark shown on the viscometer and then stopped time when the liquid reaches the bottom mark and viscosity was recorded by the below formula.\[11\]

\[
\frac{\eta_1}{\eta_2} = \frac{t_1d_1}{t_2d_2}
\]

\(\eta_1\) is viscosity of liquid 1.
\(\eta_2\) is viscosity of water 0.891 poise.
\(t_1\) flow time of liquid 1.
\(t_2\) flow time of water.
\(d_1\) density of liquid 1.
\(d_2\) density of water 0.997 g/cm\(^3\).

Anti-microbial activity study
Antibacterial activity of the herbal mouthwash was determined using a well diffusion method. Standard strains of Escherichia coli ATCC25922 and Staphylococcus aureus ATCC29213 were used and a disk of Amikacin was used as a control or for comparison. The organisms were inoculated in peptone water and the turbidity obtained was matched with 0.5 McFarland standards. This was inoculated as a lawn culture on Muller Hinton agar. A sterile filter paper disk impregnated with the herbal mouthwash and a commercially available amikacin disk were placed on it. Both the plates were incubated at 37°C for 24 hours. After 24 hours, the plates were examined for the zone of inhibition which was measured in millimeters.\[9\]
RESULTS

Physical Parameters

Physical parameter such as color, odor, taste and consistency were examined by physical examination.

pH Determination

The pH of the F1 formulation was found to be 6.1 and F2 was found to be 6.3 as the oral cavity is having pH around 6.4, this pH range of the formulation is suitable for oral disorder.

Viscosity determination by Ostwald viscometer

Viscosity determination was done by using Ostwald viscometer. The viscosity of F1 formulation was found to be 1.425cps and relative viscosity was found to be 1.70. The viscosity of F2 formulation was found to be 1.25cps and relative viscosity was found to be 1.46.

Anti-microbial activity

In this work the formulated mouth washes have been investigated for antimicrobial activity against *E. coli* and *S. Aureus* (Fig-1,2) the result showed that the formulated herbal mouth washes exhibited antimicrobial activity. While the F1 formulation shows less antimicrobial activity than F2 formulation shown in (Table No. 02). The formulated mouth washes are only effective against gram positive organism as compared to gram negative organism.

Table No. 02: Antimicrobial activity of F₁ and F₂

<table>
<thead>
<tr>
<th>Sl No.</th>
<th>Organisms</th>
<th>F1 (mm)</th>
<th>F2 (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Staphylococcus aureus</em> ATCC29213</td>
<td>18</td>
<td>20</td>
</tr>
<tr>
<td>2</td>
<td><em>Escherichia coli</em> ATCC25922</td>
<td>No Zone</td>
<td>No Zone</td>
</tr>
</tbody>
</table>

Fig No:- 01 Antimicrobial activity of Staphylococcus aureus.
DISCUSSION

The current investigation focused on the formulation of mouth wash comprising of different plant extract from clove, neem, banana peel, turmeric, orange peel, cinnamon and nutmeg which was effective against *Staphylococcus aureus* and *E.coli*, a common pathogenic bacteria causing dental caries. The study is important since it focuses on developing herbal product which can be detrimental to pathogen growth, without having any adverse effect on human health and hence can be better alternative than the commercially available chemical solutions and formulations.

The pH of prepared herbal mouthwash was measured by using pH paper. The pH of the F1 formulation was found to be 6.1 and F2 was found to be 6.3 as the oral cavity is having an acidic ph around 5.5, this ph range of the formulation is suitable for oral disorder.

Viscosity determination was done by using Ostwald viscometer. The viscosity of F1 formulation was found to be 1.425cps and relative viscosity was found to be 1.70. The viscosity of F2 formulation was found to be 1.25cps and relative viscosity was found to be 1.46.

In this work the formulated mouth washes have been investigated for antimicrobial activity against *E. coli* and *S. Aureus*. the result showed that the formulated herbal moth washes exhibited antimicrobial activity. While the F1 formulation shows less antimicrobial activity than F2 formulation.
CONCLUSIONS
Herbal mouthwash formulations can be prepared by using different plant sources with varying concentrations. The pH of the herbal mouthwash formulations was in the range of 6.1 to 6.3 which lies in the normal pH range of the oral cavity. All the herbal mouthwash formulations showed good viscosity and were in the standard range. Antimicrobial activity of both the formulation was done by well diffusion method and found that the prepared formulation was effective against gram positive microorganism as compared to gram negative microorganism.

ACKNOWLEDGEMENT
The authors would like to thank HSK, college of pharmacy Bagalkot for providing all the needed facilities to perform the study.

REFERENCES
7. Salwa K. And Khidir A.M. Hassan, ‘‘Liquorice Mouth Washes As Treatment For Mouth Ulcer’’ IAJPS, 2018; 05(02): 916-21.