

Formulation and Evaluation of Herbal Mouthwash

Prerna Rajendra Borate, Vaishnavi Adhure, Rushikesh Hiran Bhojse, Shraddha M. Gaikwad

Siddhi College of Pharmacy, Pimpri-Chinchwad, Maharashtra, India

Abstract: *The objective of present work is to formulate and evaluate herbal mouthwash and to evaluate its effectiveness against microbial load of oral cavity. The plant materials were collected and extracted for water soluble ingredients. Prepared mouthwash was further evaluated for its physicochemical properties and antimicrobial activity. The present mouthwash possesses a good antibacterial property. The results of stability study also confirm the effectiveness of preparation. Present mouthwash is a liquid preparation which normally contains antibacterial and antiseptic agents. These solutions can be used to reduce the microbial growth in the oral cavity and may also be given for other reasons like for their analgesic action, anti-inflammatory property or anti-fungal activity.*

Keywords: Aegle Marmelos, Invitro antimicrobial activity, Radioprotective Activity.

I. INTRODUCTION

Dental plaque is a complex biofilm that accumulates on the surface of teeth, containing more than 500 bacterial species [1,2]. The dental plaque is produced by initial colonizing bacteria in the salivary film of enamel, followed by secondary colonization through antibacterial adhesion [3-5]. Prenominal diseases affect the supporting tissues of teeth. Gingivitis, the mildest form of prenominal disease is generally caused by insufficient oral hygiene. Gingivitis is characterized by inflammation and bleeding of the gum. The main cause of gingivitis is plaque that forms on the surface of teeth and gums. As a main stay of maintain oral hygiene, mechanical plaque control measures are used. Mechanical plaque control techniques are time consuming and require motivation and skill to be performed well; hence antimicrobial agents have been employed extensively as an adjunct to mechanical cleaning. Several antimicrobial chemical agents such as chlorhexidine metronidazole etc. have been used. However, these artificial drugs have unpleasant side effects, so researchers are trying to pay more attention to herbal drugs. Plants and plant's isolates demonstrates effects that are immune enhancing, anti-inflammatory, anticancer etc. [6, 7]

II. MOUTHWASH

A usually antiseptic liquid preparation for cleaning the mouth and teeth or freshening the breath .

Mouthwash, mouth rinse, oral rinse, or mouth bath[8] is a liquid which is held in the mouth passively or swirled around the mouth by contraction of the perioral muscles and/or movement of the head, and may be gargled, where the head is tilted back and the liquid bubbled at the back of the mouth.

Usually mouthwashes are antiseptic solutions intended to reduce the microbial load in the mouth, although other mouthwashes might be given for other reasons such as for their analgesic, anti-inflammatory or anti-fungal action. Additionally, some rinses act as saliva substitutes to neutralize acid and keep the mouth moist in xerostomia (dry mouth).[9][10] Cosmetic mouthrinses temporarily control or reduce bad breath and leave the mouth with a pleasant taste.[11]

Rinsing with water or mouthwash after brushing with a fluoride toothpaste can reduce the availability of salivary fluoride.

Advantages of mouthwash

- Freshens breath.
- Removes food debris.
- Cavity protection.
- Reduces sensitivity.
- Fight gum disease.

- Reduces dry mouth.
- Whitens teeth.
- Kills bacteria.



III. INGREDIENTS

1] *Aegle marmelos* as Active Pharmaceutical ingredient.



2] Cinnamon



3] Clove oil



4] Salt



5] Sodium Benzoate



6] Distilled water



Ingredient and their roles :-

Sr. no.	Ingredient	Chemical constituent	Therapeutic role
1.	Aegle Marmelos	Alkaloids Coumarins Terpenoids	Antioxidant Antidiabetic Antibacterial Laxative Anti-inflammatory Radioprotective Wound healing Antipyretic Antimicrobial
2.	Cinnamon	Cinnaldehyde Cinnamate Cinnamic acid	Antimicrobial Antioxidant Lipid-lowering Anticancer
3.	Clove oil	Eugenol Eugenol acetate	Boost immune system Reduce toothache Anticancer Antimicrobial
4.	Salt	Sodium cations Chloride anions	Cleaning incisions Dentistry Optometry Promote oral hygiene
5.	Sodium benzoate	-	Antimicrobial Preservative Lubricant
6.	Distilled water	-	Diluent Sterilizing water

Therapeutic uses of various plant parts of Aegle marmelos for treatment of different diseases :-

1. Leaves biological activity :-

Antidiabetic, anti-inflammatory, wound healing, antipyretic and analgesic antifungal activity, radioprotective, spontaneous beating and calcium-paradox of myocardial cells, wound healing properties.

2. Ripe and unripe fruits :-

Digestive, antiulcer, constipation, ailments, highly nutritive.

3. Wood :-

Source of soluble potassium and sodium compounds, 0.16%; phosphates of calcium and iron, 0.13%; calcium carbonate, 2.16%; Magnesium carbonate.

4. Leaf powder :-

Hepatoprotective, antioxidant, hypoglycemic, testicular activity.

5. Flower :-

Limonene-rich oil is used as scent and perfume scenting hair.

6. Sweet-scented flower extract :-

Used as lotion for the eyes.

7. Fruit pulp :-

Show detergent action, used as a soap substitute for washing clothes Used to make water-proofing walls mixed with lime plaster and added to cement. Added to watercolors or as protective coating for paintings.

Formulation Table :-

Sr.no.	Ingredient	Formulation-1	Formulation-2
1.	Aegle Marmelos	15%	20%
2.	Cinnamon	10%	15%
3.	Clove oil	15%	20%
4.	Salt	5%	10%
5.	Sodium Benzoate	0.2%	0.4%
6.	Distilled water	Q.S upto 50ml	Q.S upto 50ml

Method of preparation :-

Collection of Plants :-

- 1] Leaves of Aegle Marmelos .
- 2] Buds of Eugenia caryophyllus (clove).
- 3] Bark of Cinnamomum zeylanicum (cinnamon)

Were randomly collected from mature plants.

Aqueous extracts of Leaves by Shadow drying technique:-

1. The leaves of mature plants were collected and washed 3 to 5 times with tap water to remove dust and dirt.
2. The leaves were allowed to soak in already boiled water bath at 30-40°C for 10 to 15 minutes to kill microbes present in the surface of the leaves.
3. The leaves undergoes Shadow drying technique were the leaves were spread in sterile container trays and kept at ambient temperature for 5 days.
4. After 5 days, the dried leaves were taken and powdered by using sterile mixer under aseptic condition.
5. The pulverized leaves are transferred to air-tight sterile container jars.
6. 100ml of sterile distilled water was taken in conical flasks (250 ml), the pulverized leaves were weighed and suspended in distilled water under sterile condition.
7. The preparation was heat sterilized at 40°C for 5-10 minutes and was kept for incubation at 37±2°C for 72 hours.
8. After incubation, the extracts were filtered with the help of a sterile Whattmann filter paper no: 1 and a funnel under lab condition.

9. The filtered extracts are boiled vigorously again to kill the bacterial spores, which will prevent from contamination.
10. The extracts after heating is ready to use for the formulation of Mouth wash and also can be tested against the oral pathogens by using Agar well diffusion techniques.

Extraction process:

The leaves ,stem and bark were washed with sterile water, shadow dried, pulverized and stored in air-tight bottles. The Aqueous extracts were prepared by soaking the powdered leaves, stem and bark in sterile distilled water and maintained in Incubator at 37±2°C for 72 hours and were filtered using Whattmann filter paper. Equipments: Sterile Petri plates, volumetric flasks, Test tubes, Conical flask, Whattmann filter paper, mortar pestle, Incubator, Autoclave, Pippetting device, Hot air-oven.

Formulation of herbal Mouthwash :-

- 1.The herbal Mouthwash was prepared by the formula given in table .
- 2.Salt solution was made by preparing 1% w/v solution of salt in sterile water.
- 3.Then all the extracted ingredients are mixed in a fixed ratio.

Evaluation of Mouthwash :-

Sr. no.	Parameter	Observation
1.	Colour	Light Brown
2.	Odour	Slightly Aromatic
3.	PH	7.5
4.	Foaming Height	1.3

5] Test for microbial growth in formulated mouthwash-

The formulated mouthwash was inoculated in the plates of agar media by streak plate method and a control was prepared. The plates were placed in the incubator and are incubated at 37°C for 24 hours. After the incubation period plates were taken out and checked for microbial growth by comparing it with the control .

6] Stability Studies-

The formulation and preparation of any pharmaceutical product is incomplete without proper stability studies of the prepared product. This is done in order to determine the physical and chemical stability of the prepared product and thus determine the safety of the product. A general method for predicting the stability of any product is accelerated stability studies, where the product is subjected to elevated temperatures as per the ICH guidelines. A short term accelerated stability study was carried out for the period of 3 months for the prepared formulation. The samples were stored at under the following conditions of temperature as 3-50 C, 250 C RH=60%, 400 C ±2% RH= 75%. Finally the samples kept under accelerated study were withdrawn on monthly intervals and were analyzed .

7] In vitro antibacterial activity –

In vitro antibacterial activity was performed on isolated colonies of Streptococcus mutans. The Agar well diffusion technique was used for determining the zone of inhibition and minimum inhibitory concentrations (MIC). The strains of S. mutans were inoculated in prefabricated blood agar plate. Plates were dried and 4 wells were made with the help of 6 mm agar well cutter. 20 µl, 40µl, 60 µl, 80 µl of prepared mouthwash was loaded in all the respective wells. The agar plates were kept undisturbed to allow the passive diffusion of herbal mouth wash into the agar culture medium. Then the plates were incubated at 37°C for 24 hours. The zone of inhibition was calculated in mm.

III. CONCLUSION

Aegle Marmeloshas been used in Ayurveda medicine and has become admiration of modern medicine. Aegle marmelos leaves elaborates a vast range of biologically active compounds that’s structurally complex and chemically diverse. Aegle marmelos mouthwash was prepared with1% w/v solution of salt in sterile water mixing with active ingredient

and other excipients. Formulation is free from alkali components, in accordance to its evaluation parameter. Therefore based on the study research it can be concluded that Aegle marmelos effectively formulated in the form of mouthwash showing antimicrobial activity.

IV. RESULT

Preparation and evaluation of Aegle marmelos mouthwash was done. The physiochemical parameters of the prepared mouthwash were determined. The formulation exhibit good in appearance with the pH ranging from 6-8. The various parameters like test for microbial growth in formulated mouthwash, stability studies , Invitro antibacterial activity showed a positive result for the prepared formulation.

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