

Formulation and Evaluation of Herbal Mouth Wash

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Abstract: *The importance of oral and dental hygiene has been known from the beginning of civilization to the 21st century. Patients and dentists are faced with a wide variety of mouthwash products containing a variety of active and inactive ingredients. Making an informed decision about the suitability of a particular product for a particular patient can be a complex task. Many popular herbal products help fight plaque and periodontal disease, but they have only been used for a short time and only as an adjunct to other oral hygiene measures such as brushing and flossing. Natural mouthwashes may offer significant advantages over chemical mouthwashes. If a mouthwash can be developed using natural products that can be easily prepared at home and is safe to use, it could lead to an improvement in people's overall dental health. A variety of natural ingredients and materials have been used in this study. Thin layer chromatography was then performed to confirm the quality of the materials used. Subsequently, physical evaluation, pH measurement, stability studies, thin layer chromatography and antibacterial studies were carried out. The study concluded that the developed herbal mouthwash is a significant, therapeutically effective and suitable drug delivery agent with undoubtedly high potential, albeit at a low cost. Therefore, increased use of herbal preparations is necessary to avoid side effects. This study is an attempt to outline natural substances that can be used as effective mouthwashes.*

Keywords: Herbal, Mouthwash, Gingivitis, Antimicrobial

I. INTRODUCTION

Many bacterial species present in human saliva have been identified and are responsible for different dental problems, especially dental caries. Because of the constant influx of nutrients through saliva, food intake, warm temperatures, and moisture, these microorganisms grow easily and produce dental plaque in the mouth environment. Volatile sulphur molecules are the primary cause of unpleasant mouth odour. Plaque-associated oral disease affects a large proportion of the population and is regarded as one of the leading causes of tooth loss across the worldwide. In comparison to chemical products, the importance of herbs is highly safer. Medicinal herbs have long been used to treat ailments due to their antibacterial, antifungal and antiviral properties against human infections. Dentists usually use mouth rinse as antibacterial agent before performing oral surgery on patients because it helps to sanitise the surface of inflammatory gums and teeth, preventing further contamination from other pathogens.

Plants Profile:

Bael Leaves :

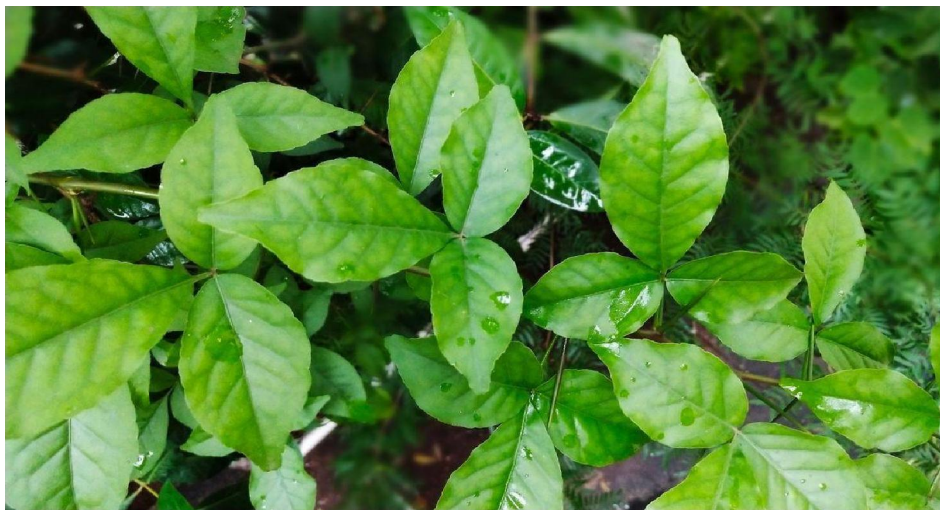


Fig.1 Bael leaves

Biological source:

It consists of green fresh leaves of *Aegle marmelos*, belonging to family Rutaceae.

Organoleptic Characterists:

Colour- Green or Dark green.

Taste- Bitter, Sweet. Odour Aromatic.

Chemical constituents:

Bael includes a variety of chemical constituent such as alkaloids, tannins, essential oils, gums, resins, coumarin, and others like skimmianine, aeglin, rutin, sitosterol A, flavone which makes it effective for a variety of disease. The drug also contains carbohydrates (11- 17%), protein, and volatile oil. The pulp also contains good amount of vitamins C and A. Other alkaloids reported in the drug are angelenine, marmeline and dictamine.

Uses:

- Bael might be beneficial in managing diarrhoea.
- Action is attributed to mucilage. Leaves contain alkaloids and are considered useful in diabetes.
- The oil obtained from seeds possesses antibacterial, antiprotozoal and antifungal and anti- Cancerous properties.
- The root of bael is one of the constituents of well-known Ayurvedic preparation Dasmula.
- In large doses it may lead to abortion, therefore it can be used as abortifacient agent and hence it should not be used in pregnant women.
- Bael might even reduce the thyroid hormone levels. Therefore, it may be helpful for conditions with elevated thyroid levels like hypothyroidism.

Tulsi Leaves:



Fig.2 Tulsi leaves

Biological source:

Tulsi consists of fresh and dried leaves of *Ocimum sanctum* Linn belonging to family Labiatae.

Organoleptic Characterists:

Colour-Green.

Taste Slightly pungent taste.

Odour-Aromatic.

Chemical Constituent:

Tulsi leaves contain bright, yellow coloured and pleas-ant volatile oil (0.1 to 0.9%). It contains approximately 70% eugenol, carvacrol (3%), and eugenol-methyl-ether (20%). It also contains caryophyllin. Seeds contain fixed oil with good drying properties. The plant is also reported to contain alkaloids, glycosides, saponin, tannins, an appreciable amount of vitamin C and traces of maleic, citric, and tartaric acid.

Uses:

- Oil of tulsi has been found to have a great effect against formaldehydes or ancillary induced arthritis.
- The leaves of Tulsi have expectorant properties and juice is effectively applied in catarrhal bronchitis and chest troubles and throat.
- Tulsi consumed along with Triphala is used in preparation of optic drops which is very essential for the eye treatment.

- Consuming Tulsi juice or syrup is helpful to reduce stomachache and cramps.
- Tulsi is consumed as a perspiration inducing agent in fevers caused by malaria by extraction of its roots.
- Tulsi is extensive qualities also include acting against Streptococcus mutans, the organism which is responsible for inducing decay in the tooth. It is also be utilized as a supplement to inorganic mouth washes for treating bad breath, gum disease and mouth ulcers.
- Tulsi is also used to treat respiratory problems.
- Tulsi might have a potential to strengthen the kidney. The juice of Tulsi leaves, when taken with honey, might help in expelling kidney stones through urine.
- The oil obtained from the tulsi plant might possess antibacterial activity against organisms like Pseudomonas aeruginosa, S.aureus, and Bacillus pumius.
- Tulsi might be used as an antiemetic (prevents vomiting)..

Eucalyptus



Fig.3 Eucalyptus

Biological source:

It consist of fresh leaves of Eucalyptus globulus, belong to family Myrtaceae.

Organoleptic Characterists:

Colour - Blue-green.

Odour - Aromatic and camphoraceous.

Taste Pungent and Camphorous.

Chemical Constituent:

The constituent present in eucalyptus are p-cymene, a-pinene: 10/27antity of sesquiterpenes like ledol, aromadendrene; aldehydes, ketones, and alcohols It also has polyphenolic acids like ferulic acid, caffeic acid, gallic acid; flavonoids such as eucalyptin, hyperoside, rutin, monoterpenes, epilobulol, Cineole and traces of phellandrene.

Uses:

- Topically, eucalyptus may be used as an antiseptic reducing the risk of infection and promoting healing.
- Eucalyptus oils may be found in mouth rinses and toothpastes because it's a natural bacteria fighter.
- Eucalyptus leaves and oil provide an aroma that can be useful in dealing with stress and fatigue.
- Rubbing oil from the leaves into the muscles and joints has been known to temporarily relieve pain. Below is a recipe for making your own relaxation massage oil that is great for a soothing massage.

- Applied directly to the skin, the plant is used for treating minor aches and pains. It is also an ingredient in several over-the-counter rubs to be used as cold treatments.
- Eucalyptus oil is added to cough and cold medicines, dental products, antiseptics and used directly to treat fevers.
- In both fresh and dried form, leaves of eucalyptus are used in medicinal teas.
- Topically, eucalyptus may be used as an antiseptic reducing the risk of infection and promoting healing.

Liquorice Root



Fig.4:- Liquorice root

Biological source:

Liquorice consists of peeled and unpeeled roots, stolons, stem of *Glycyrrhiza glabra* linn belong to family Leguminosae.

Organoleptic Characterists:

Colour Externally yellowish brown & Internally yellowish Colour.

Odour - Faint& Characteristics.

Taste Sweet.

Chemical Constituent:

Glycyrrhizine (6-8%) [Sweet instant 50 time more than sucrose].

Liquiritin and isoliquiritin are responsible for yellow colour.

Glucose, sucrose, asparanin, gum, protein, fats, resins, traces of tannin.

Glycyrrhizinic acid are produces glycyrrhitinic acid and glycyrrhitic on hydrolysis.

Uses:

- Expectorant, demulcent, flavouring agent, anti-inflammatory, anti spasmodic, relaxing stress.
- Bronchial problem, cold, bronchitis, cough, anti- Pyretic.
- It is also used in the treatment of gastric and duodenal ulcers.
- Liquorice root extract can help reduce the growth of *Streptococcus mutans* bacteria in the mouth. This, in turn, lessens environmental acidity around the teeth and helps to prevent dental cavities from forming.
- It supports the healthy function of kidney, liver and bladder.
- Liquorice helps to regulate fat metabolism in liver possess glycaemic moderating effect.
- It helps to increase energy.
- It has antioxidant property and protect the skin from sun induced skin damage.
- It is essential in hair health by preventing hair loss and dandruff prevention.
- It is a potent healing agent for tuberculosis.

Aim and Objective:

Aim: Formulation and Evaluation of Herbal Mouth Rinse from Bael, Tulsi, Eucalyptus and Liquorice for Better Oral Hygiene.

Objective: The main objective of herbal mouth rinse are as follows:

- To formulate and evaluate the effectiveness of herbal mouth rinse in reducing the oral bacterial count.
- To reduce the plaque accumulation and gingival inflammation.
- To have prolonged effect of the formulation.
- To formulate a cost-effective and affordable product.

Experimental work:

Extraction process-

1. The leaves of mature plants were collected and washed 2-3 times with sterile water to remove dust and dirt.
2. The leaves were spread in container trays and kept at room temperature for 4-5 days in shade.
3. After 5 days, the dried leaves were taken and powdered by using sterile mixer under aseptic condition.
4. The pulverized leaves are transferred to air-tight sterile container jars.
5. 100ml of sterile distilled water was taken in four conical flask (250 ml), the pulverised leaves were weighed and suspended in distilled water under sterile Condition.
6. The preparation was heat sterilised at 40°C for 5-10 mins and was kept for incubation at 37°C for 72h.
7. After incubation, the extracts were filtered with the help of a sterile Whattmann filter paper no: 1 and a funnel under lab condition.
8. The filtered extracts are boiled vigorously again to kill the bacterial spores, which will
9. prevent from contamination.
10. The extracts after heating is ready to use for the formulation of mouth rinse.

Formulation of Herbal Mouth Wash:

Table No. 1: Formulation of Herbal mouth rinse

Sr. No.	Ingredients	Role	F1	F2	F3
1	Beal leaves	Active	4ml	5ml	6ml
2	Tulsi leaves	Active	3ml	4ml	5ml
3	Eucalyptus	Active	7ml	7ml	7ml
4	Liquorice	Sweetener	2ml	3ml	4ml
5	Salt solution	Preservative	5ml	7.5ml	10ml
6	Distilled water	Vehicle	29ml	23.5ml	18ml
7	Total volume	-	50ml	50ml	50ml

Procedure for Preparation of herbal mouth wash Containing Extract:

- a) Measured quantity of each extract was taken.
- b) Extract were taken mixed thoroughly in mortar and pestle with small quantity of water. All the ingredients were triturated and mixed properly taking care to avoid lump formation.
- c) Finally, water added to make final volume and salt solution was added for its preservative as well as flavouring effect and formulated products packed in well closed container and evaluated for different formulation parameters.

Evaluation of Herbal mouth wash:

a) Colour and odour:

Physical parameters like odour and colour were examined by visual examinations

b) Measurement of pH:

pH of prepared herbal mouth rinse was measured by using digital pH meter kncp- ELICO-LI- 120. The pH meter was calibrated using standard buffer solutions of pH 7, 9.2 about 1 ml of mouth rinse was measured and dissolved in 50ml of distilled water and its pH was measured.

b) Homogeneity:

All the mouth rinse formulations were set aside on a platform and were tested for homogeneity by visual inspection. They were tested for their appearance and presence of any lump, flocculates or aggregates also.

c) Test for microbial growth in formulated herbal mouth wash:

The formulated mouth rinse 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.

d) 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- 5°C, finally the samples kept under accelerated study were withdrawn on monthly intervals and were analyzed.

e) In vitro antibacterial activity:

In vitro antibacterial activity was performed on isolated colonies of Streptococcus aureus. The Agar well diffusion technique was used for determining the zone of inhibition and minimum inhibitory concentrations (MIC). The strains of S. aureus were inoculated in prefabricated agar plate. Plates were dried and three wells were made with the help of 6 mm. agar well cutter. 20 µl, 40 µl, 60 µl of prepared mouth rinse 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. Commercial antibiotic ampicillin (50 mg/ml) was used as positive control for S. Aureus. The zone of inhibition (mm) was recorded for each plate and compared with control.

II. RESULT AND DISCUSSION

Table no. 2: Evaluation parameter herbal mouth wash.

Formulation	Colour	Odour	Homogeneity	pH
F1	Light brown	Pleasant odour	Good	6.1
F2	Light brown	Pleasant odour	Good	6.3
F3	Light brown	Pleasant odour	Good	6.2

Table No. 3: Result of agar well diffusion antibacterial assay.

Organism	Zone of inhibition (mm)		
	20 μ	40 μ	60 μ l
Staphylococcus Aureus	10	12	13
Standard ampicillin	12	13	15

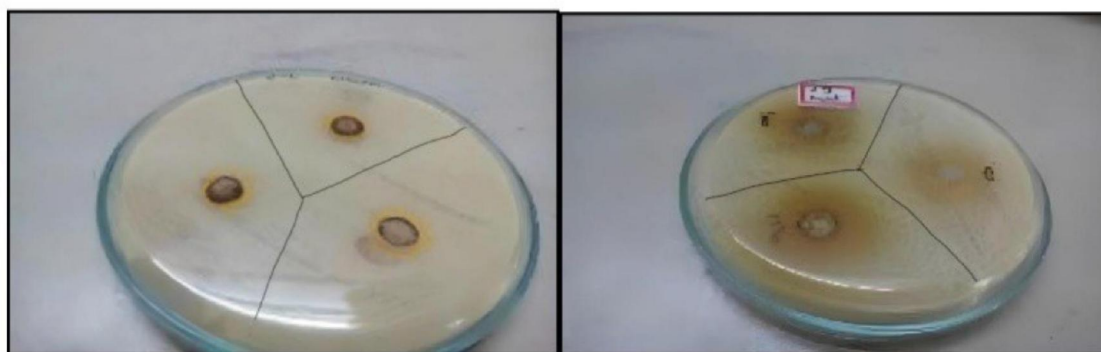


Fig. 5: Agar diffusion method for assessment of anti-bacterial study.

Table No. 4: Stability study.

Temperature	Evaluation parameter	Observation (Month)			
		0	1	2	3
3-5°C	Visual Appearance	Light brown	Light brown	Light brown	Light brown
	Phase Separation	Nil	Nil	Nil	Nil
	Homogeneity	Good	Good	Good	Good
	Odour	No change	No change	No change	No change
	pH	6.1	6.1	6.3	6.2

III. DISCUSSION

Among the different formulations, the best formulation of herbal mouth wash right now is F3. This mouth wash is fresh, effective at combating oral bacteria, and it also contains herbal ingredients. It is stable for long-term storage in terms of pH, Colour, and odour. The formulation's pH was determined to be 6.2. Because the skin has an acidic pH of roughly 5.5, the formulation's pH range is appropriate for oral problems. The formulation was free of microorganisms because they did not produce any microbial growth when introduced in agar media. This mouth rinse is made entirely from herbs and contains no additives, unlike other brands on the market. Stability studies for physical and chemical change were conducted on the formulation. There were no significant differences in the formulation's qualities.

When used with daily brushing and flossing, antimicrobial ingredients bael, tulsi eucalyptus and liquorice extracts have been reported to reduce plaque and gingivitis when used in mouth rinses. The antibacterial activity of different concentrations of mouth rinse was determined using the agar diffusion method. The inhibition result for S.aureus was determined to be 13 mm for 60 μ l, 12 mm for 40 μ l, and 10 mm for 20 μ l, respectively. These results indicated that the herbal mouth rinse has considerable antibacterial action and that the new preparation can prevent bacterial development in the oral cavity. Because the relationship between oral microbial load and dental diseases is well recognized, this herbal mouth rinse aids in stimulating proper oral health.

IV. CONCLUSION

A significant effort has been made to combine the active ingredients of several extracts to create an effective poly herbal mouth wash formulation that is alcohol-free. Bael, tulsi, liquorice and eucalyptus extracts were utilized in this study because of their reported anti-bacterial properties. The zone of inhibition results indicated that this herbal mouth wash was an effective plaque inhibitor. As a result, they can be used in conjunction with mechanical therapy to treat plaque-induced gingivitis.

The current study has a significant impact on the development of an effective and low-cost herbal oral health intervention for low-income areas. The natural herbs included in this composition have shown to help with dental hygiene and foul breath. These herbs have been recognized for years and decades to work wonders, as evidenced by several research findings. A person can simply rinse his or her mouth with this herbal mouth wash and avoid a wide range of oral health disorder.

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