

**FORMULATION AND EVALUATION OF HERBAL MOUTHWASH**

Dr. Suja C.*, Abhisree R. B., Anamika Vijayan, Aswathi Rajan, Fathima Bint Sadhakathulla and Sameeha Sayeed M. K.

Department of Pharmacy, Crescent College of Pharmaceutical Sciences, Payangadi, Kannur – 670358.



*Corresponding Author: Dr. Suja C.

Department of Pharmacy, Crescent College of Pharmaceutical Sciences, Payangadi, Kannur – 670358.

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ABSTRACT

The **aim** of this study is to formulate mouthwashes containing herbal extracts obtained from the leaves of *Syzygium aqueum* (watery rose apple), *Mangifera indica* (mango). The formulated mouthwashes will be evaluated for its effectiveness. **Materials and Methods:** Phytochemical screening of above mentioned plant was done. Leaf extraction was carried out for the preparation of mouthwash. Formulation and evaluation of all three formulations was done. **Result:** Phytochemical screening of herbal extracts obtained from the leaves of *Syzygium aqueum* has revealed the presence of compounds like alkaloids, carbohydrate, glycosides, saponins, tannins, flavonoid, phenols, phytosterols, fixed oil and in *Mangifera indica* leaf extracts has revealed the presence of many compounds, including, alkaloids, carbohydrate, glycosides, saponins, terpenoids, tannins, flavonoid, phenols. Three formulations of mouthwash were prepared by using *Mangifera indica*, *Syzygium aqueum*, mint oil, saccharin, sodium lauryl sulphate, glycerol, salt solution, purified water and evaluated for different parameters like colour, odour, taste, pH, viscosity, foaming ability, stability studies and antimicrobial test. **Conclusion:** Based on the results of evaluation, it shows that formulation F1 have pH 4.85 and in antibacterial study formulation F1 have better antibacterial activity when compared to other two formulations.

INTRODUCTION

Cosmetics can be defined as any product intended to be rubbed, poured, sprinkled or sprayed on, or introduced into, or otherwise applied to the human body or any part thereof for cleansing, beautifying, promoting attractiveness, or altering the appearance, includes any article intended for use as component of cosmetic.^[1]

ORAL CAVITY

Refers to the mouth. It includes the lips, the lining inside the cheeks and lips, the front two thirds of the tongue, the upper and lower gums, the floor of the mouth under the tongue, the bony roof of the mouth, and the small area behind the wisdom teeth.

Vestibule: It is the space between lips/cheeks, and the gums/teeth. The vestibule communicate with the mouth proper via the space behind the third molar tooth, and with the exterior through the oral fissure.

Mouth proper: The mouth proper lies posteriorly to the vestibule. It is bordered by a roof, a floor, and the cheeks. The tongue fills a large proportion of the cavity of the mouth proper.

Roof: The roof of the mouth proper consist of hard and

soft palate.

Hard palate is found anteriorly, it's a bony plate that separates the nasal cavity, it is covered superiorly by respiratory mucosa and inferiorly by oral mucosa.

Soft palate is the posterior continuation of the hard palate. In contrast to the hard palate, it is a muscular structure.

Cheeks: The cheeks are formed by the buccinator muscle, which is lined internally by the oral mucous membrane. The buccinator muscle contracts to keep food between the teeth when chewing, and is innervated by the buccal branches of the facial nerve.

Floor: The floor of the oral cavity consist of several structures.

Muscular diaphragm: It provides structural support to the floor of the mouth, and pulls the larynx forward during swallowing.

Tongue: Connected to the floor by frenulum of the tongue, a fold of oral mucosa.

Salivary glands and ducts^[2]

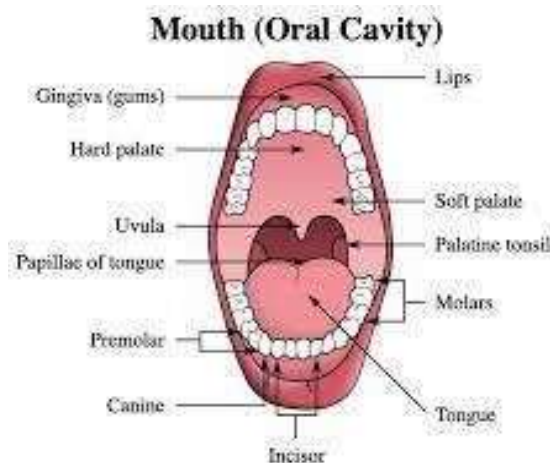


Figure no 1: Structure of oral cavity.

MOUTHWASH

Mouthwash is a medicated liquid used for cleaning the oral cavity and treating mucous membranes of the mouth, may contribute to surface softening and increased wear of dental resins and composite materials.

Types of Mouthwash

- Antiseptic
- Natural (herbal)
- Fluoride
- Cosmetic
- Total care

Advantages of mouthwash

- Fresh breath
- Helps to get rid of food and debris stuck between the teeth
- Prevent buildup of space
- Helps to fight cavities
- Whitens the teeth
- Cure canker sores

Disadvantages of the mouthwash

- Mouthwash can be dangerous to children below 6 years.
- Mouthwash can stain and darken teeth.
- It can damage some parts of the mouth.
- Many mouthwash contain alcohol that make teeth more sensitive.^[3]

Oral diseases

1. Dental Caries

Mouth ulcers are small sores that form on gums, lips, inner cheeks or palate (roof of mouth). They can be triggered by several different factors, including minor injuries, hormonal changes and emotional stress.^[8]

Caries is the most typical oral infection and illness. A persistent, contagious illness called caries is brought on

by bacteria that consume sugar to generate an acidic environment that erodes teeth. This process causes holes (cavities) in the tooth's structure over time.^[4,5]

2. Candidiasis

A candida species infection of the oral mucosa is known as candidiasis. Oral candidal infections are more common among immunocompromised people, such as the elderly, young children, HIV- positive people, cancer patients, diabetics. People who take certain treatments, like chemotherapy, inhaled steroids.^[6]

3. Gingivitis

An reversible form of gingival inflammation is gingivitis. A gentle form of periodontal disease.

There are three classifications

Plaque-induced, non-plaque-induced, and systemic diseases and medication-induced gingivitis. Poor dental hygiene/plaque formation, primary or secondary tooth emergence, and dental equipment (braces, dentures) are risk factors for gingivitis.^[7]

4. Mouth Ulcer

HERBAL MOUTHWASH

Herbal mouthwashes are designed and prepared with extracts and essential oils from phytotherapeutic plants, containing a mixture of active agents such as catechins, tannins, and sterols. The mixture of natural compounds inside the herb- or plant-derived substances usually performs gentle remedial effects.^[9]

Advantages of Herbal Mouthwash

- They have very minimal or no side effect and they are less harmful.
- All herbal mouthwashes do not contain alcohol and/or sugar.
- Herbal mouthwash is gentle for even the most sensitive mouth.
- Herbal mouthwashes has naturally antibacterial property.
- It contain no harsh additives.

- Herbal mouthwash doesn't cause drymouth.
- It is highly in demand.
- It keeps your mouth healthy.^[10]

Uses of Herbal Mouthwash

- Use of herbal mouthwash is to improve oral hygiene.
- It help to control dental plaque.
- It can be use in gum diseases.
- Used for killing germs in oral cavity.
- It freshen breath and covers badbreath.
- It is use to clean septic sockets.
- It relieve pain and inflammation.
- In treatment of Mucositis and Halitosis.
- Used in Periodontal diseases.^[11]

MATERIALS AND METHODS MANGO LEAVES

Mango (*Mangifera indica* L.) ascribed to the family Anacardiaceae. A major bio- macromolecule present in mango leaves is protein. The most active biological constituent of mango leaves is mangiferin, followed by phenolic acids, benzophenones, and other antioxidants such as flavonoids, carotenoids, quercetin, isoquercetin, ascorbic acid, and tocopherols. Mangiferin is the main contributor of most of the biological activities of mango leaves extract. Mango leaves have a great anti-microbial, antioxidant, anti-diabetic, anti-tumour, and immunomodulatory effects. Mango leave oil (MLO) contains monoterpenes, sesquiterpenes, minor quantities of other analogues, and trace amounts of non-terpenoid hydrocarbons and oxygenated hydrocarbons.^[12]



Figure no. 2: Mango (*Mangifera indica*) leaves.

WATERY ROSE APPLE LEAVES

Watery rose apple is a plant of myrtle family, Myrtaceae, constitutes over 5500 species, and *Syzygium* is considered the largest genus of the flowering plants within the family. The watery rose apple, *Syzygium aqueum*, is a traditional medicinal plant with various bioactive compounds distributed in all plant parts. These include

phenolic compounds, flavonoids, tannins, terpenoids, and essential oils. *S. aqueum* extracts and their isolated compounds showed multiple beneficial biological effects such as antibacterial, antifungal, antidiabetic, analgesic, antimalarial, antioxidant, anti-inflammatory, and anticancer activities.^[13]



Figure no. 3: Watery rose apple (*Syzygium aqueum*) leaves.

METHOD OF COLLECTION AND PREPARATION

The leaves of *Mangifera indica* (mango) and *Syzygium aqueum* (watery rose apple) were collected locally and authenticated by Dr. Ratheesh Narayanan. M. K, HOD,

Assistant Professor and Research guide, P.G Department of Botany, Payyanur College, and the leaves were sun dried for 48 hours. The dried leaves were then powdered and enclosed in a airtight container in a cool place.^[14]



Figure no. 4: Dried powder of mango (*Mangifera indica*) leaves.



Figure no. 5: Dried powder of watery rose apple (*Syzygium aqueum*) leaves.

Method of extraction: Cold maceration method was used for the extraction of *Syzygium aqueum* and *mangifera indica* leaves. Both the powder were weighed about 20 g and added to two separate stoppered conical flask then 150 ml of alcohol was added to the flask that contain *syzygium aqueum* and 150 ml water in the *mangifera*

indica containing flask it was shaken for 5-6 times in 24 hours. After 24 hours it was strained through the muslin cloth and the extract was collected. It was then transferred to petridish and placed on hot plate until the solvent gets evaporated.



Figure no. 6: Extract of *Mangifera indica* leaves.



Figure no. 7: Extract of *Syzygium aqueum* leaves.

FORMULATION OF HERBAL MOUTHWASH

We have formulated herbal mouthwash

containing extracts obtained from the leaves of *Syzygium aqueum* (watery rose apple), *Mangifera indica* (mango)

as active ingredient. Mint oil was used as a flavouring agent. Saccharin as sweetener. Sodium lauryl sulphate as surfactant and glycerol as co-surfactant. Salt was used as preservative. Purified water was the vehicle we used. Three formulations of herbal mouthwash were prepared; F1, F2, and F3. All the ingredients were weighed accurately. The weighed quantity of herbal extract was mixed with small amount of water in a mortar and

pestle, then saccharin and salt solution were added. Mint oil was added drop wise and mixed thoroughly to prevent the formation of lump. Sodium lauryl sulphate and glycerol were then added and mixed thoroughly. Finally, water was added to make up the volume up to 100 ml. The formulations were transferred to an attractive, well-closed container.^[15]

Table no. 1: Formulation design of herbal mouthwash.

Ingredients	F1	F2	F3
<i>Mangifera indica</i>	200 mg	–	100 mg
<i>Syzygium aqueum</i>	–	200 mg	100 mg
Mint oil	0.1 ml	0.1 ml	0.1 ml
Saccharin	0.1 mg	0.1 mg	0.1 mg
Sodium lauryl sulphate	0.1 g	0.1 g	0.1 g
Glycerol	8.5 ml	8.5 ml	8.5 ml
Salt (10% w/v solution)	2 ml	2 ml	2 ml
Purified water	qs to 100 ml	qs to 100 ml	qs to 100 ml

METHODOLOGY

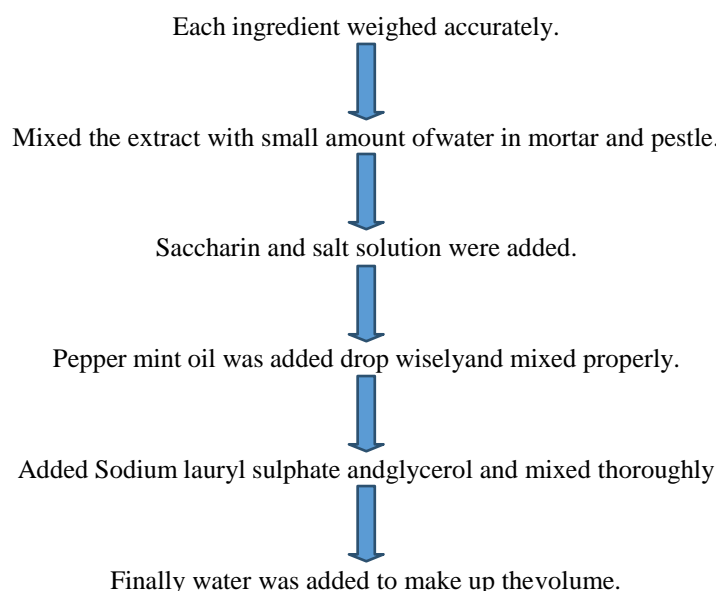


Figure no. 8: Flow chart of formulation procedure.

EVALUATION OF HERBAL MOUTHWASH

The prepared herbal mouthwash formulations (F1, F2, F3) were subjected to various evaluation test.

Physical Evaluation

The formulated herbal mouthwashes were subjected to physical evaluation for their colour odour and taste.

pH determination

pH of prepared herbal mouthwash was measured by using digital pH meter at constant temperature.

The pH meter was calibrated using standard buffer solution (Acetate buffer solution pH 4) then 70 ml of the formulation was taken in a beaker, the probe was dipped in to the beaker and its pH was measured.

Viscosity determination

Viscosity of formulated mouthwashes were measured by using ostwald viscometer. The viscometer was mounted in vertical position on a suitable stand. The formulation was filled into the viscometer up to mark A. The time required for formulation to flow from mark A to B was recorded. The viscosity was determined three times. Average time was calculated and standard deviation was found.

Foaming ability

10 ml of the formulation was mixed with 40 ml of distilled water, the mixture was transferred into 100 ml measuring cylinder. The mixture was vigorously shaken for 25 times, after which it was kept aside. The height of the foam formed was measured.

Stability studies

The prepared formulations were kept at room temperature for a period of one month, during and after this period formulations were tested for its colour, odour, and physical appearance.

Antibacterial Test

The method used for the antibacterial testing of formulated mouthwash is Cup-plate method.

Preparation of media : 2.8 g of nutrient agar was taken in a sterile conical flask, and 100 ml water was added and dissolved the nutrient agar properly, it was sterilized in an autoclave at 121°C for 15 minutes. After sterilization the culture was added, and this culture media was transferred to the petridish which was previously

sterilized. It kept for some time to get solidify.

Test: After the solidification of culture media, with the help of a sterile borer one cavity was made in each plate. The cavities were filled with formulated mouthwashes and incubated the plates at 37°C for 24 hours. After the incubation period we measured the zone of inhibition.^[15]

RESULTS AND DISCUSSION

Three formulations F1, F2, F3, of herbal mouthwashes containing leaf extract of *Mangifera indica* and *Syzygium aqueum* were prepared and evaluated.

The photographs of mouthwashes are shown below



Figure no. 9: Photograph of formulated herbal mouthwash.

Phytochemical screening of leaf extracts

- Phytochemical screening of mango leaf extracts has revealed the presence of many compounds, including, alkaloids, carbohydrate, glycosides, saponins, terpenoids, tannins, flavonoid, phenols. Phytosterols and fixed oils were absent in the mango leaf extract.
- Phytochemical screening of *Syzygium aqueum* leaf extracts has revealed the presence of compounds like alkaloids, carbohydrate, glycosides, saponins, tannins, flavonoid, phenols. Phytosterols, fixed oils

and terpenoids were absent in the *Syzygium aqueum* leaf extract.

The following evaluation tests were performed on formulated mouthwash,

Physical evaluation

The formulated mouthwashes were inspected for their colour, odour, and appearance by sensory and visual examination. Results are shown below in table 4.

Table no. 5: pH of the formulated herbal mouthwashes.

Formulation	pH ± Standard deviation
F1	4.91±0.06
F2	4.71±0.095
F3	4.64±0.092

n= 3 ± SD

Table no. 4: Physical evaluation of formulated herbal mouthwashes.

Formulation	Colour	Odour	Appearance
F1	Gold Yellow	Pleasant (Coolmint)	Clear
F2	Greenish Yellow	Pleasant (Coolmint)	Clear
F3	Gold Yellow	Pleasant (Coolmint)	Clear

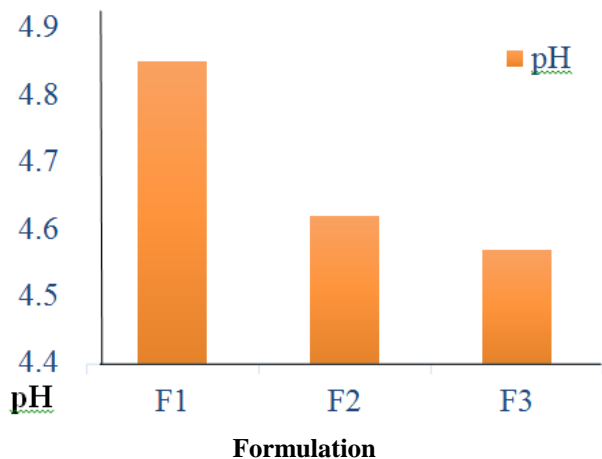


Figure no. 10: pH of the formulated herbal mouthwashes.

The pH of all formulated mouthwash was found to be within the ideal pH range for herbal mouthwash that is between 4.1 - 7.9.

Determination of pH

pH of the prepared herbal mouthwash was measured by using digital pH meter at constant temperature, and the pH values and standard deviation were found and given

in the table 5.

Viscosity

Viscosity of formulated mouthwashes were measured by using ostwald viscometer. The viscosity was determined three times. The viscosity and standard deviation were found and given in the table 6.

Table no. 6: Viscosity of the formulated herbal mouthwashes.

Formulation	Viscosity (cP) ± Standard deviation
F1	1.32 ± 0.0416
F2	1.34 ± 0.0493
F3	1.12 ± 0.0208

n = 3 ± SD

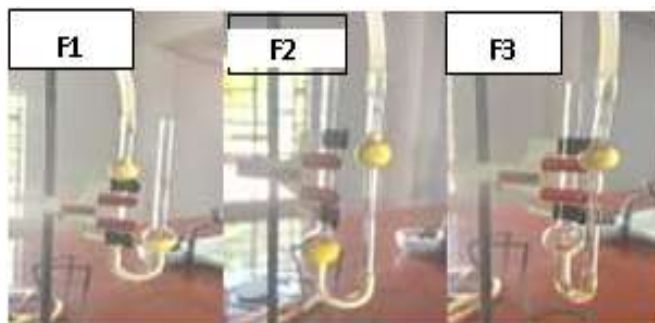


Figure no 11: Viscosity of formulated herbal mouthwashes.

Foaming ability

Foaming ability of all formulated herbal mouthwashes

were determined. Results are shown in table 7.

Table no. 7: Foam height of the formulations.

Formulation	Foam height (ml) ± Standard deviation
F1	6.6 ± 1.1547
F2	8.6 ± 1.5275
F3	6.3 ± 0.5773

n = 3 ± SD

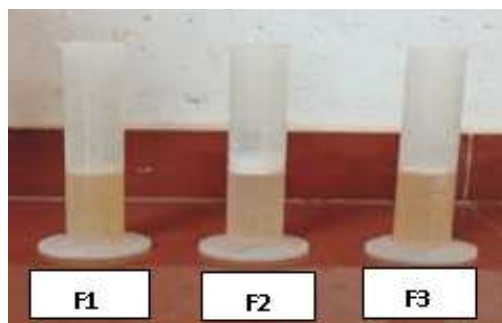


Figure no. 12: Foam height of the formulations.

Antibacterial activity

All formulated mouthwashes were subjected to

antibacterial study and zone of inhibition was determined.

Table no. 8: Determination of antibacterial activity.

Formulation	Zone of inhibition (mm)
F1	15
F2	12
F3	13

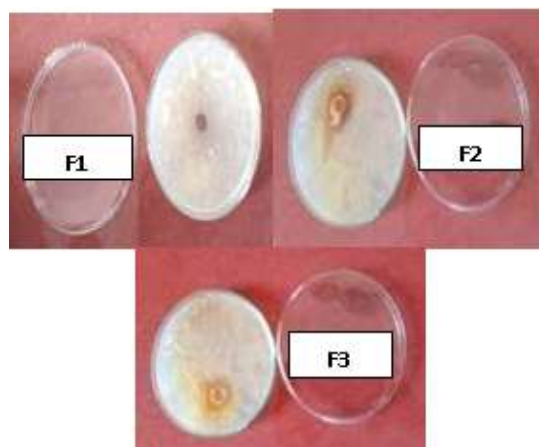


Figure no. 13: Zone of inhibition of formulations.

The zone of inhibition of F1 was found to be 15 mm which is more than the zone of inhibition for other formulations. This shows that F1 has better antibacterial activity compared to other formulations.

CONCLUSION

Oral health is important as overall health. Herbal mouthwash are preferred over chemical mouthwash as it shows less side effects and it is non-irritant, less toxic, and do not contain alcohol. Medicinal plants because of their antiviral and antibacterial action against human microorganisms and it has no or less side effects as compared to synthetic mouthwash. Herbal mouthwashes are prepared from various plant extracts.

The herbs used in this study are *Mangifera indica* and *Syzygium aqueum*. This study was aimed to formulate herbal mouthwash and to evaluate it. The formulated mouthwashes were evaluated for various parameters such as physical parameter, pH, viscosity, foaming ability, stability and anti bacterial study. Based on the results of

evaluation, it shows that formulation F1 have pH 4.85 and in antibacterial study formulation F1 have better antibacterial activity when compared to other two formulations.

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