International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 4 July-Aug 2024, pp: 1110-1120 www.ijprajournal.com ISSN: 2456-4494

Formulation and Evaluation of Herbal Mouthwash

Sukanya S. Kad*, Priyanka A. Panmand, Pranali M. Lendave
Department of Pharmaceutics, Indrayani Vidya Mandir's Krishnarao Bhegade Institute of Pharmaceutical Education and Research, Talegaon Dabhade, Pune, 410507, India.

Date of Submission: 01-08-2024 Date of Acceptance: 10-08-2024

ABSTRACT: Medicinal plant or herbs are considered to have rich source of ingredients which can be used in drug development. The formulation of herbal mouthwash is a natural approach to oral hygiene that utilizes plant-based ingredients to create a safe and effective mouthwash. Herbal mouthwash is intended to provide a range of benefits, including anti-bacterial, anti-inflammatory, and anti-plaque effects, as well as freshening breath and promoting healthy gums and teeth.The formulation of herbal mouthwash offers an alternative to chemical-based oral hygiene products that can sometimes cause irritation and sensitivity. With the growing interest in natural remedies and alternative health practices, the formulation of herbal mouthwash offers a promising solution for those seeking a more natural approach to oral hygiene. The main objective of this literature work is to prepare and evaluate a herbal mouthwash and check its effectiveness against microorganisms of oral cavity.The prepared mouthwash was evaluated against pathogens and was found effective for controlling and demolishing microbial growth in mouth. Further physiochemical properties were tested for mouthwash and it possesses a good antibacterial activity. Next, studies on physical evaluation, pH measurement, stability, foam test, density, viscosity, and antibacterial activity were conducted.

KEYWORDS: Herbal Mouthwash, Tulsi extract, Guava extract, Oral hygiene, Antibacterial property.

I. INTRODUCTION

Goal of herbal mouthwash

1) To enhance dental hygiene.
2) It aids in the management of dental plaque.
3) It is applicable to gum diseases.
4) Used to eradicate bacteria in the mouth.
5) It masks bad breath and freshens the breath.
6) It's crucial to use mouthwash to avoid gum disease.
7) It is utilized for cleaning septic tanks.
8) It reduces inflammation and pain.
9) Managing halitosis and mucositis.[1]

Advantages of Herbal Mouthwash

1) The use of herbal mouthwash has grown advantage over chemical mouthwashes due to their non-irritant and non-staining properties and it does not contain alcohol.
2) They have very minimal or no side effect and theyare less harmful.
3) All herbal mouthwashes do not contain alcohol and/or sugar.
4) Herbal mouthwashes is gentle for even the most sensitive mouth.
5) Herbal mouthwashes has naturally antibacterial property.
6) It contain no harsh additives.
7) Herbal mouthwash doesn't cause dry mouth.
8) It is highly in demand.
9) It keeps your mouth healthy.[1]

Disadvantages of the mouthwash:

1) A lot of mouthwashes contain alcohol, which increases tooth sensitivity. Alcohol can cause the oral mucosa to dissolve and the oral microbiome, or natural habitat in the mouth, to be disturbed, making the teeth more sensitive.
2) Infants under the age of six should avoid using mouthwash.
3) Since mouthwash sometimes contains an excessive amount of alcohol, it might cause canker.
4) Mouthwash has the potential to harm some oraltissues as well as stain and darken teeth.[2]

Types of mouthwash

1. Fluoride mouthwash:

Fluoride in mouthwashes contains salt which help protect the teeth from cavities and cavities. Since fluoride can also be found in toothpaste and water, it's advisable to require care when using this type of mouthwash since intake of an excessive amount of fluoride isn't good for your overall health.eg. ColgateFluoriGard.

2. Antiseptic mouthwash:

This is the foremost common mouthwash. This mouthwash usually contains alcohol and

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 4 July-Aug 2024, pp: 1110-1120 www.ijprajournal.com ISSN: 2456-4494

typically utilized by people with mouth infections to stop bacterial growth. This is often also helpful for peoplewho have halitosis or bad breath. This is often used alongside the proper brushing of teeth and flossing tohelp forbid bacteria that cause mouth infections and stinky breath.eg. Equate Antiseptic Mouthwash.

3. Cosmetic mouthwash:

A mouthwash that doesn't do anything to your overalloral care but is just how to freshen your breath or mask bad breath.eg. Himalaya Herbals Complete Care Mouthwash.

4. Natural mouthwash:

Natural mouthwash could also be a mouthwash that does what other sorts of mouthwash do except the ingredients are natural. It is also a popular option as an alcohol-free mouthwash. Their ingredients are safer to use as compared to other sorts of mouthwash [3]Eg. Nature's Answer, PerioBrite, Mouthwash with Xylitol, Coolmint.

Aim: Formulation and Evaluation of herbal mouthwash

Objectives:

1. To develop formulation of herbal mouthwash:

The present results therefore offer a greater use for traditional use of herbal mouth wash.

2. Safety:

Herbal mouthwash was safe and there was neitherreport of adverse reactions

3. Effectiveness:

The objective of present work is to formulate and evaluate herbal mouthwash and to evaluate its effectiveness against microbial load of oral cavity.

4. To maintain oral hygiene:

Oral health is important as overall health. Now-a- days people may faces more oral problems like periodontal disease, sore throat, gingivitis, plaqueand so on. For maintaining good oral health various formulations are formulated.

5. Prevention, control and reduction of oral infection:

It can reduce the plaque growth in your mouth, decrease your chances of developing gum disease, and prevent tooth decay.

6. To reduce side effects by promoting herbal use:

The use of herbs in dentistry should be based on evidence of effectiveness and safety. Herbal medicines, derived from botanical sources, have beenapplied in dentistry for a long history to inhibit microorganisms, reduce inflammation, soothe irritation, and relieve pain

7. Herbal medicines:

Ayurvedic medicines give a holistic approach toward entire human beings. It can maintain the balance between general and oral health as well as an environment which is in this era necessary for the well-being of humans

II. MATERIALS AND METHOD OF EXTRACTION

A. Tulsi (Ocimum sanctum)

The herb is useful in teeth disorders. Its leaves, driedin the sun and powdered, can be used for brushing teeth. It can also be mixed with mustered oil to make a paste and used as toothpaste.[4]

Kingdom: plantae Order: lamiales Family: lamiaceaeGenus: ocimum Species: sanctum

Parts used: Leaves, Seeds and Roots.

Chemical constituents: Volatile Oil-0.8%

1) Eugenol, nerol, eugenol methyl ether.
2) Caryophyllene, terpinene-4-ol-decyladehyde
3) Camphor and carvacrol
4) Essential oils, ascorbic acid, carotene, calcium, phosphorus and insoluble oxalates.
5) It also contains terpenes, mucilage, fixed oil andfatty acids. [5]

Uses of Tulsi plant:

- In reduction of oral malodour.
- Anticancer activity of O. sanctum: Tulsi has been shown to possess an excellent anticancer activity
- As an antioxidant, it also has the ability to scavenge highly reactive-free radicals
- Modulates immunity: The alcoholic extract of Tulsi modulates immunity, thus promoting immune system function
- As an antiulcer agent: The essential oil of Tulsi possesses antiulcer activity due to its lipoxygenase inhibitory, histamine antagonistic, and antisecretory effect.
- As analgesic, antipyretic, and anti-inflammatory: Methanolic extract and aqueous

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 4 July-Aug 2024, pp: 1110-1120 www.ijprajournal.com ISSN: 2456-4494

suspension of Tulsi acts a COX-2 inhibitor, proving its anti-inflammatory property.[6]

Collection plant: The collected leaves were thoroughly washed with tap water to avoid dusts and other unwanted materials accumulated on the leavesfrom their natural environment. The dust free leaveswere shade, dried at room temperature. After 4-5 days for obtaining aqueous extract, the properly dried leaves were then grinding into the fine powder by using the grinding machine than the powder material of Tulsi leaves were weighed properly.[7]

Preparation of aqueous extract of Ocimum sanctum (leaves)

The extract of leaves was obtained in sufficient quantity by using distilled water. In this process firstly 20 g powdered leaves of ocimum sanctum were placed in 200 ml of beaker and 100 ml ofdistilled was poured into beaker after addition of water kept for overnight at the room temperature approximately 22 hrs. for thorough mixing and also complete elucidation of active materials to dissolve in the respective solvent then, extract was filtered by using muslin cloth followed by Whatman no 1 filter paper then the green color filtrate was obtained, after done this process filtrate was dried. Finally, the residues were collected and used for the experiment.[7]

B. Guava (Psidium guajava)

The origin of guava is Psidium Guajava. It belongs to the Myrtaceae family. Shape: Guava fruits are usually 4 to 12 centimetres (1.6 to 4.7 inches) long and round or oval in shape, depending on the species.

[8] Guava leaf extracts and essential oil from the stem have the ability to scavenge hydrogen peroxide, superoxide anion and inhibit the formation ofhydroxyl radical.[9]

Kingdom: Plantae Order: Myrtales Family: MyrtaceaeGenus: Psidium

Species: Psidium guajava [10]

Chemical constituents:

1) **Phenolic compounds:** These are powerful antioxidants that have health-promoting properties.
2) **Flavonoids:** Such as quercetin, avicularin, apigenin, guaijaverin, kaempferol, and myricetin, which have been studied for their anticancer, antidiabetic, and antioxidant activities.
3) **Tannins:** Known for their antimicrobial properties.
4) **Terpenoids:** Which can produce relaxation effects.
5) **Saponins:** Compounds with potential health benefits.
6) **Vitamins:** Including ascorbic acid (vitamin C).

Uses of guava leaves:

- Antibacterial properties
- Anti-inflammatory effects, Reduces dental plaque, Relief from toothache
- Prevents oral cancer: Some studies suggest that compounds present in guava leaves may have anti-cancer properties, which could potentially help prevent oral cancer. However, more research is needed to establish this claim conclusively.

Collection of plant: We collected guava leaves from Botanical Garden and kept for sundry for 3 days. After sun drying grind it to coarse powder for further extraction process.[12]

Extraction process of guava leaves

Preparation of hot water extract Hot water extract was prepared by plain decoction method. For this 25 gm of leaf powder was taken into a beaker containing 200 ml of sterile double distilled water. This was heated in water bath till the volume of the extract reduced to less than 50 ml. The liquid was filtered using Whatman's filter paper no.1.[12]

C. Peppermint Oil

Peppermint is a hybrid mint, a cross between watermint and spearmint. Indigenous to Europe and the Middle East, the plant is now widely spread and cultivated in many regions of the world. It is occasionally found in the wild with its parent species.[13]

Kingdom: Plantae Order: Lamiales Family: LamiaceaeGenus: Mentha

Species: M.xpiprerita [13]

Chemical constituents :

1) essential oil including menthol, menthone, menthofuran, 1,8-cineole, and menthyl acetate.[14]
2) 1,8-cineole, limonene, beta-pinene and beta-caryophyllene.[15]

Uses of Peppermint Oil:

- antioxidant
- anti-inflammatory
- Anti-bacterial properties: potentially inhibit the growth of various Gram-positive and Gram-

International Journal of Pharmaceutical Research and Applications
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negative bacteria.

- Peppermint oil is promoted for topical use (applied to the skin) for problems like headache, muscle aches, joint pain, and itching.[16]

III. METHODS OF PREPARATION

Table 3.1: Formulation of mouthwash

Srno.	Ingredients	Formulation (50 ml)	Uses
1.	Guavaleaves extract	25 ml	Antibacterial antioxidants, anti- inflammatory agents
2.	Tulsi leaves extract	5ml	Antimicrobial, anti- oxidant, anti-inflammatory
3	Peppermint oil	1 ml	Flavouringagent Reducing pain, freshbreath
4.	Propylene glycol	7.5 ml	Thickener,Sweetener
5.	Sodium benzoate	0.1 gm	Preservative
6.	Tween -80	0.5 ml	Surfactant
7.	glycerol	5.96 ml	Humectant
8.	Ethanol	1 ml	Co-solvent
9.	Water	q.s	vehicle

- STEP 1: Wash all the apparatus and weigh all the ingredients properly.
- STEP 2: Fresh guava leaves were collected and air dried for 10 days. The dried leaves were then crushed and churned in a blender to form a coarse powder. The powder was collected in an airtight container and stored in a cool, dry place,away from sunlight.
- STEP 3: The guava leaf powder was boiled at 90 °C for 15-20 minutes and now the leaves are boiled and filtered through a filter paper.
- STEP 4: -In one beaker Add ethanol and peppermint oil.
- -Take another beaker and add glycerol, propylene glycol, Tulsi extract, tween-80 with warm water and guava extract and dilute the solution.
- STEP 5: Add Beaker -1 into beaker-2. Then add sodium benzoate. Add FDA approved green colorant and make up the volume up to required quantity by purified water.
- STEP 6: The Prepare mouthwash store and kept in tight and close container.[17]

IV. EVALUATION

Physicochemical evaluation of extractGuava

1. **Test for Phenols and Tannins**. Extract was mixed with 2 mL of 2% solution of FeCl3. A blue-green or black coloration indicated the presence of phenols and tannins.[18]
2. **Test for Saponins**. Extract was placed in a test tube and shaken vigorously. The formation of stable foam was taken as an indication for the presence of saponins.[18]
3. **Test for reducing sugar**: Take 1ml or 1gm of plant sample in a test tube and add 10ml deionized water then add few drops of Fehling solution (1ml Fehling solution A and B) and heat at 100℃ in a water bath. Brick red precipitate in bottom showed positive result.[19]
4. **Test for Cardiac Glycosides (Keller Killiani test)**: Approximately 5 ml of each plant extract was evaporated at 40°C and the residue was collected. A few mg of residue was suspended in 5 ml water. 2 ml of glacial acetic acid containing one drop of ferric chloride solution was added to it. This solution was underplayed with 1 ml of conc. sulphuric acid. A brown ring at the interface indicated the presence of deoxy sugar, a characteristic of cardiac glycosides.[20]
5. **Test for alkaloids (Wagner's test)**: Six drops of Wagner's reagent were added to 2 mL of crude extract. The Wagner's reagent was prepared by mixing iodine in potassium iodide solution. The formation of brown or reddish precipitate indicates the presence of alkaloids.[21]
6. **Test for Flavonoids Shinoda Test (for guava extract)**: To the 2 ml of extract s in the test tube, magnesium ribbon fragment were added plus concentrated Hydrochloric acid droop wise. The formation of a pink coloration indicated the presenceof flavonoids.[22]

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 4 July-Aug 2024, pp: 1110-1120 www.ijprajournal.com ISSN: 2456-4494

Tulsi

1. **Test for phenol:** 2 ml of alcohol and 2-3 drops offerric chloride solution was added to 1 ml of crude extract, blue green or black coloration indicated the presence of phenols.[23]
2. **Test for tannins:** Few drops of basic lead acetate was added in the sample solution, if brown bulky precipitate is found it means tannin are present in testsample.[24]
3. **Test for Saponins**: 1 ml of distilled water was added to1ml of extract and shaken vigorously. a stable persistent froth indicates the presence of saponins.[25]
4. **Test for reducing sugar**: To 1ml of crude extractadded 1 ml of Felhing's A solution and 1ml of Felhing's B solution. Formation of red colour indicates the presence of sugar.[26]
5. **Test for glycosides**: 0.5ml of glacial acetic acid and 3 drops of 1% aqueous ferric chloride was added to 1ml of each extract. The cardiac glycosides in the extract were determined by the appearance of brown ring at the interface.[27]
6. **Test for alkaloids:** 1ml of sample was added to 20µl of Dragendroff's reagent (gram's iodine). Formation of orange colour indicates the presence ofalkaloids.[28]
7. **Test for flavonoid (Shinoda test)**: Taken the alcoholic sample extract in the test tube and 5-10 drops of hydrochloric acid added in the sample. Thensmall pieces of magnesium added in tubes. Reddish pink or brown colour was indicated the presence of flavonoids.[29]

Evaluation of mouthwash

1. **Physical parameters:** like odour and color were examined by visual examinations.
2. **pH:** pH of prepared herbal mouthwash was measured by using digital pH meter. The pH meter was calibrated using standard buffer solution about 1ml of mouthwash was weighed and dissolved in 50mlof distilled water and its pH was measured.[30]
3. **Determination of Antibacterial Property of Mouthwash:** Twenty-four hour freshly prepared cultures of Streptococcus mutans and E. coli are used that are prepared from slant cultures of Streptococcus mutans and E. coli. This is done by: nutrient broth isprepared; pH is tested and sterilized. Using a sterilized loop, some inoculum is transferred from old culture to the newly prepared nutrient broth in the laminar. These subcultures are now placed in incubators at 37 °C for 48 h. Petri dishes are filled with 15 mL agar media as above and allowed to get cooled. Using a marker, each Petri dish is divided into two halves. 0.05 mL of Streptococcus mutans isadded to one Petri dish and 0.05 mL of E. coli is added to the other. After adding the inoculum, the inoculum is spread by the spreader. The Petri dishes are kept in the incubator at 37 °C for 5 min. The Petri dishes are taken out and placed in the laminar. Using the borer, wells are created in the inoculum-applied agar media on each of the halves of the Petri dishes. This method is known as the bore well method. 0.05mL of the refrigerated mouthwash sample is applied in one half and 0.05 mL of ambient temperaturemouthwash is placed in the other half. This process is repeated respectively for the Streptococcus mutans and E. coli Petri dishes. The Petri dishes are now kept in the incubator at 37 °C for 24 h. After 24 h, Petri dishes are looked for inhibition zones around the wells [31]
4. **Test for stability:** The purpose of the stability test is to make sure that the mouthwash formulations can keep the same properties throughout the long term before conducting an antimicrobial test. Prior to antimicrobial testing, many mouthwash formulationsundergo stability testing. This test evaluated the mouthwash formulation's visual appearance, physical separation, and homogeneity. The mouthwash was then stored at temperature at 30°C while the appearance was assessed on each 10 days up to 1 month and the results were recorded.[32]
5. **Viscosity:** Viscosity of the mouthwash was determined with the help of digital viscometer at 100 rpm with the spindle 6. The results for the viscosity of all the prepared formulations has been described inthe table 9.6. [33]
6. **Foam test:** The foam ability of the product was evaluated by taking small amount of preparation with water in measuring cylinder initial volume was noted and then shaken ten times. Final volume of foam wasnoted. [34]

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 4 July-Aug 2024, pp: 1110-1120 www.ijprajournal.com ISSN: 2456-4494

V. RESULT AND DISCUSSION

1. Phytochemical test:

Table 5.1: Phytochemical test of extract

Active constituent	Guava extract	Tulsi extract	Observation
Tannins andphenols	Present	Present	Green precipitate
saponins	Present	Present	Stable foam
Reducingsugar	Absent	Present	Red colour
glycosides	Present	Absent	Brown colour
alkaloids	Present	Present	Red brownprecipitate
flavonoids	Present	Absent	Guava extract: pink colour & Tulsi extract:yellow precipitate

Fig 5.1: Phytochemical Test Guava Extract

Fig 5.2: Phytochemical test of Tulsi extract

2. Physical parameter:

Table 5.2: Physical parameter of Herbal Mouthwash

Parameters	Formulation
Colour	Dark brown
Taste	Slightly bitter
Odour	Minty

3. pH Stability analysis: It showed a colour whichdetected the pH range between 5-6 by comparing, it with standard pHcolour range.

Table 5.3: pH analysis of Herbal Mouthwash

Day of measurements	pH of sample	pH of standard
0 th day	5-6	5-6
15 th day	5-6	5-6

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 4 July-Aug 2024, pp: 1110-1120 www.ijprajournal.com ISSN: 2456-4494

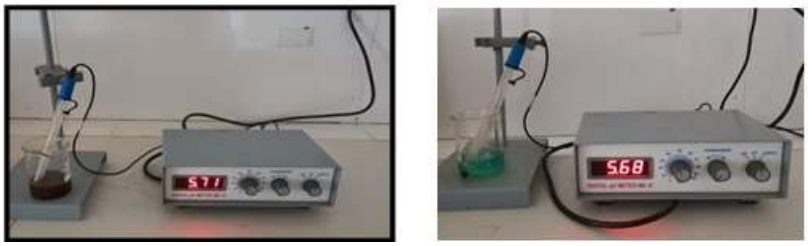

Fig 5.3: pH Analysis of Mouthwash

4. Antibacterial activity:

Table 5.4: Antibacterial Activity of Mouthwash

Organism	Zone of inhibition
Streptococcus mutans	8 mm
E. coli	6 mm

Fig 5.4: Zone of Inhibition of E. Coli by Herbal Mouthwash

5. Stability analysis:

Table 5.5: Stability Analysis of Herbal Mouthwash

Temperature and humidity	Evaluation parameter	Observation (in DAYS)			
		Initial	10	20	30
30℃±2℃/ 60% ± 5% RH	Visual appearance	Dark brown	Dark brown	Dark brown	Dark brown
	Phase separation	Nil	Nil	Nil	Nil
	Homogeneity	Good	Good	Good	Good
	pH	5	5	5	5

6. Foam test: Final volume of foam of formulation was noted to around 1 ml.

Fig 5.5: Foam test of herbal Mouthwash

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 4 July-Aug 2024, pp: 1110-1120 www.ijprajournal.com ISSN: 2456-4494

7. Viscosity:

Table 5.6: Viscosity of Herbal Mouthwash

Formulations	Viscosity (in Centipoise)
Herbal mouthwash	1.690
Standard	2.352

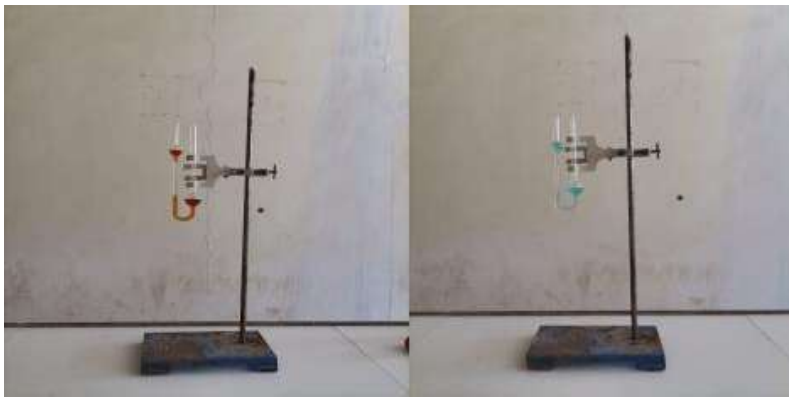

Fig 5.6: viscosity of herbal Mouthwash by Ostwald viscometer

8. Density:

Table 5.7: Density of herbal Mouthwash

Formulations	Density (gm/ml)
Herbal mouthwash	1.060
Standard	1.062

Fig 5.7: Density of herbal Mouthwash

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 4 July-Aug 2024, pp: 1110-1120 www.ijprajournal.com ISSN: 2456-4494

9. Label:

Fig 5.8: Label of herbal mouthwash

CONCLUSION

The present liquid herbal mouthwash can work in long way to help people to get rid of bad breath and many oral disorders. Besides we can be rest assured and take comfort in the fact that there aren't any unhealthy ingredients present in this preparation. The physicochemical evaluation results confirm that the colour and odour of present herbal formulation is acceptable with a pleasant odour and a better after effects. The results of zone of inhibition also confirmed that this herbal mouth rinses was found to be a potent plaque inhibitor, and were preferred by the patients for its taste, convenience of use and test duration in their mouth after rinsing. Thus, these can be used as an adjunct to mechanical therapy for treating plaque induced gingivitis. Present study has an important impact in order to create an effective and inexpensive herbal oral health intervention for low social economic communities. However, this study was short-term study so long term studies are required with larger.

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