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Research Article

"Formulation, Evaluation and Comparative Assessment of an Alcohol-Free Herbal Mouthwash Containing *Leea macrophylla* Leaf Extract: Phytochemical, Antimicrobial and Stability Studies"

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Abstract

The present research was designed to formulate and test an alcohol free herbal mouth wash using an extract from the leaves of Leea macrophylla. The phytochemical screening was conducted after macerating the leaves in 70% ethanol. Four different formulations (F1 to F4) of mouthwash were prepared and tested for appearance, color, odor, taste, homogeneity, pH, viscosity, specific gravity, antimicrobial activity and short-term stability. The phytochemical screening revealed the presence of alkaloids, flavonoids, phenolics, tannins, glycosides, steroids and terpenoids. The pH of all formulations were found to be acceptable ranging from 6.12 ± 0.05– 6.48 ± 0.03. The activity of the plant extract was found to be enhanced with the rise in concentration. But F4 yielded maximum inhibition zone against Streptococcus mutans, Staphylococcus aureus and Candida albicans and exhibited slight turbidity, sedimentation and high bitter taste. F3 demonstrated inhibition zones of 16.67 ± 0.58, 15.00 ± 1.00 and 12.67 ± 0.58 mm against the respective microorganisms. It has been chosen to be the optimized formulation due to its good antimicrobial activity, good taste, physical stability and homogeneity. After 90 days stability, no significant changes in physical appearance, odor, uniformity or precipitation were noted. The preliminary data indicated that L. macrophylla leaf extract could be a potential herbal source for formulation of mouthwash. There is a need for more standardization, safety testing and clinical evaluation.

Keywords: *Leea macrophylla*, herbal mouthwash, antimicrobial activity, oral hygiene, phytochemical screening, formulation and evaluation

Introduction

Good oral health is a key aspect of overall good health and quality of life. Dental caries, gingivitis, periodontitis and halitosis can occur as a result of inadequate oral hygiene, which leads to the formation of dental plaque composed of a variety of microbes in the oral cavity. Trained members of the dental team can be of great help in controlling dental plaque, but brushing the teeth and flossing may not be enough to completely clean the interdental spaces and inaccessible areas. Antimicrobial mouthwashes therefore are well used as adjuncts to mechanical oral hygiene as they can spread the active ingredients in the oral cavity and can aid in decreasing the growth of microorganisms and the inflammation of the gums [1].

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Antimicrobial ingredients like chlorhexidine, cetylpyridinium chloride and essential oils are often a part of commercial mouthwashes. Chlorhexidine has been widely used for controlling plaque and gingivitis, but, when used for a long time, it can cause tooth staining, change in taste, irritation to the mouth and increased calculus formation [2]. The restrictions have helped the growth of herbal mouthwashes that have bioactive compounds derived from plants. Herbal mouthwashes could have antimicrobial, antioxidant and anti-inflammatory properties and offer good plaque control whilst being more acceptable to the patient. Clinical research has provided some evidence that herbal mouthwashes can be beneficial as adjunctive treatment to conventional oral hygiene, however, the effectiveness of using them will depend on the plant, concentration of the extract and the composition of the mouthwash [3].

Leea macrophylla Roxb. ex Hornem. (family Vitaceae) is an ethnomedicinal plant found all over India, in Bangladesh, Nepal and other parts of South and Southeast Asia. Various components of the plant have been traditionally used for various wound, inflammation, pain, fracture and oral ailments. The phytochemical studies on the plant report the presence of phenolic compounds, flavonoids, tannins, saponins, glycosides, alkaloids and others secondary metabolites in the plant [4]. The members of these constituents are reported to possess antimicrobial, antioxidant, anti-inflammatory and wound healing activities [4,5] which may account for the action of L. macrophylla.

L. macrophylla extracts have been previously found to have antimicrobial activity against a few Gram-positive bacteria and the yeast Candida albicans [5]. The leaf extract also has been found to possess antibacterial and antioxidant activity, suggesting its potential use as a pharmaceutical and oral care product [6]. But, standardized herbal mouthwash containing L. macrophylla leaf extract has yet to be thoroughly researched.

Combining L. macrophylla leaf extract into an alcohol-free mouthwash could be a convenient, patient-friendly method to introduce its phytoconstituent(s) to the tooth, gingiva and other oral surfaces. However, it should have the following properties: appearance, taste, pH value, viscosity, homogeneity, antimicrobial activity and storage stability. Hence, the current study is designed to prepare various concentrations of L. macrophylla leaf extract as herbal mouthwash, to assess the physicochemical properties, phytochemical constituents, antimicrobial activity and stability of the herbal mouthwash for short term.Thus, the aim of the present study was to prepare different concentrations of L. macrophylla leaf extract as herbal mouthwash and to assess the physicochemical properties, phytochemical constituents, antimicrobial activity and stability of the herbal mouthwash for short term. The optimized formulation will also be tested to compare it with a marketed herbal mouthwash.

2. Materials and Methods

2.1 Materials

Leea macrophylla leaves were picked from [location, district and state in India]. Organic chemicals, such as glycerin, polysorbate 80, sodium benzoate, sodium saccharin, peppermint oil, ethanol and other analytical-grade chemicals were purchased from approved sources. All the formulation study was done using purified water. A commercially available herbal mouthwash was bought from a local

pharmacy and served as the reference mouthwash.

Streptococcus mutans, Staphylococcus aureus and Candida albicans were cultured with the help of microbial culture at [name of microbiology laboratory/culture collection centre]. For antimicrobial testing Mueller – Hinton agar and Sabouraud dextrose agar were used.

2.2 Collection and Authentication of Plant Material

Leaves of Leea macrophylla were harvested [month and year] when they were fresh, healthy and disease free. The plant material was verified by a qualified Pharmacognosist or Botany expert of [name of institution]. A voucher specimen, with the departmental number [insert voucher number] was deposited in the herbarium at the Department for future reference.All the leaves collected were cleaned with running water to get rid of dirt and other foreign materials. They were left to dry in the shade at room temperature for about 10–15 days, and checked for any possible fungal contamination. The leaves were dried, ground in a mechanical grinder and sieved through an appropriate sieve. All the powdered material was stored in an airtight container for extraction.

2.3 Preparation of *Leea macrophylla* Leaf Extract

About 100 g of the powdered leaves was macerated in 70% ethanol to get the extraction. The powder from the plant was put in a clean, closed container and 1,000 mL of hydroalcoholic solvent was added to it. This was left at room temperature for 72 hours, shaken up occasionally.

The extract was then filtered in muslin cloth and then Whatman No. 1 filter paper. The remnant was again subjected to extraction with the new solvent to get the maximum amount of phytochemicals. Filtrates were concentrated together by using a rotary evaporator at temperature below 45°C. This concentrated extract was then dried and kept in an air-tight container in the dark (amber coloured) at 4ºC for further use.

The percentage extraction yield was calculated using the following equation:

Extraction yield (%)=Weight of dried extract Weight of plant powder×100\text{Extraction yield (\%)}= \frac{\text{Weight of dried extract}}{\text{Weight of plant powder}}\times100Extraction yield (%)=Weight of plant powderWeight of dried extract×100

2.4 Preliminary Phytochemical Screening

Qualitative phytochemical screening for the presence of major classes of secondary metabolites was done using the prepared extract of the leaves. Alkaloids, flavonoids, phenols, tannins, saponins, glycosides, carbohydrates, proteins, steroids and terpenoids were standard chemical tested. The results were reported as present (+) or absent (−) depending on the colour change or the formation of the precipitate.

2.5 Formulation of Herbal Mouthwash

Four different formulations of mouthwash were formulated with varying concentrations of Leea macrophylla leaf extract (F1, F2, F3, and F4).

Ingredient	F1	F2	F3	F4	Function
Leea macrophylla leaf extract	0.5 g	1.0 g	1.5 g	2.0 g	Herbal active ingredient
Glycerin	10 mL	10 mL	10 mL	10 mL	Humectant
Polysorbate 80	1 mL	1 mL	1 mL	1 mL	Solubilizer
Sodium benzoate	0.1 g	0.1 g	0.1 g	0.1 g	Preservative
Sodium saccharin	0.05 g	0.05 g	0.05 g	0.05 g	Sweetener
Peppermint oil	0.05 mL	0.05 mL	0.05 mL	0.05 mL	Flavouring agent
Purified water	q.s.	q.s.	q.s.	q.s.	Vehicle
Final volume	100 mL	100 mL	100 mL	100 mL	—

The proper amount of leaf extract was diluted in about 40 mL of purified water. Polysorbate 80 was slowly added, stirring it continuously. Sodium benzoate and sodium saccharin were each dissolved in a small amount of warm purified water, and then added individually to the extract dispersion.

Continuous mixing was used for adding glycerin. A small amount of polysorbate 80 was added to peppermint oil prior to addition. Purified water was used to bring the volume to 100 mL. All the formulations were well mixed and filtered through muslin cloth and poured into an appropriately labelled, amber-coloured bottle.

2.6 Evaluation of Herbal Mouthwash

2.6.1 Organoleptic characteristics

Visual assessments of colour, appearance, odour, taste, clarity and visible particles/precipitation were conducted for the formulations.

2.6.2 Homogeneity

An appropriate volume of each formulation was placed in a clear glass container and uniformity, phase separation, sedimentation and aggregation were observed.

2.6.3 Determination of pH

The pH of each formulation was measured by a calibrated digital pH meter at room temperature. Each measurement was conducted three times and the average value of each set are presented as mean ± SD.

2.6.4 Determination of viscosity

Viscosity measurements were performed at 25±2∘C25\pm2^\circ\text{C}25\pm2∘C with a Brookfield viscometer equipped with an appropriate spindle and rotational speed, and for three times each formulation.

2.6.5 Determination of specific gravity

Specific Gravity bottle was cleaned and dried, and then weighed with the mouthwash. Specific gravity was obtained by comparing the weight of equal volume of the mouthwash and purified water.

Specific gravity=Weight of mouthwashWeight of equal volume of water\text{Specific gravity}= \frac{\text{Weight of mouthwash}}{\text{Weight of equal volume of water}}Specific gravity=Weight of equal volume of waterWeight of mouthwash

2.7 Antimicrobial Activity

An antimicrobial activity was assessed by agar-well diffusion method of the formulations.

Bacterial cultures were done on Mueller–Hinton agar while Candida albicans were cultured on Sabouraud dextrose agar. The microbial suspensions were standardized and the same amount of each was spread on the surface of each of the agars.

Aseptically prepared wells, with a diameter of about 6 mm, were prepared. An equal amount of each of the formulations was added to the wells. Mouthwash base (without plant extract served as negative control and the marketed herbal mouthwash served as a positive control.

Plates of bacteria were allowed to grow at 37±2∘C37 \pm 2^\circ\text{C}37 \pm 2^\circ\text{C} for 24 hours. The cultivation of plates with C. albicans was done for 24–48 hours at 28–30∘C28–30 ∘C28–30 ∘C. The diameter of the inhibition zone (mm) was used to measure the antimicrobial activity. Three replicates were done for each experiment.

2.8 Comparison with Marketed Mouthwash

The optimized formulation was then evaluated in terms of appearance, pH, viscosity and specific gravity and antimicrobial activity when compared to the market product mouthwash which is a herbal product. The formulation that was found to have acceptable physicochemical properties and comparable/superior antimicrobial activity was thought to be suitable for further stability testing.

2.9 Short-Term Stability Study

The optimized formulation was kept in wells in amber coloured bottles in the following conditions:

- Refrigerated condition: 4±2∘C4\pm2^\circ\text{C}4±2∘C
- Room temperature: 25±2∘C25\pm2^\circ\text{C}25±2∘C
- Accelerated condition: 40±2∘C40\pm2^\circ\text{C}40±2∘C

All samples were tested at the beginning and following 15, 30, 60 and 90 days. The colour, odour, appearance, pH level, viscosity, homogeneity, precipitation and antimicrobial activity were noted.

2.10 Statistical Analysis

All of the experiments were repeated thrice and the values were presented as the mean ± standard deviation. One way analysis of variance (ANOVA) was used to analyse the differences between the formulations followed by an appropriate post hoc test. A p value of $< 0.05p<0.05p<0.05$ was regarded as being statistically significant.

2. Materials and Methods

2.1 Materials

The fresh leaves of Leea macrophylla were picked from the [Location] which is located in [District & State] of India. Other chemicals (Analytical grade) were picked up from authorized suppliers: Glycerin, polysorbate 80, sodium benzoate, sodium saccharin, peppermint oil, ethanol. All the study was conducted with purified water. A local pharmacy was used to get a commercially available herbal mouthwash to use as the reference product.

Streptococcus mutans, Staphylococcus aureus and Candida albicans were cultured in the microbiology laboratory or culture collection centre. Antimicrobial evaluation was done by Mueller–Hinton and Sabouraud dextrose agars.

2.2 Collection and Authentication of Plant Material

The leaves of Leea macrophylla were gathered during [month and year] that were fresh, healthy and disease-free. Authenticating plant by a qualified botanist or a pharmacognosist of [name of institution]. A voucher specimen, with the voucher number [insert voucher number] was lodged in the institutional herbarium.

All collected leaves were washed with running water to wash soil and foreign materials from the leaves. These were left at room temperature to be shade dried for about 10-15 days. Dried leaves were ground with mechanical grinder, sieved with proper sieve and was kept in an air-tight container. Following the standard procedure for medicinal plant materials, the collection, processing and storage of the plant material was carried out [7].

2.3 Preparation of *Leea macrophylla* Leaf Extract

Using maceration method 1,000 mL of 70% ethanol was used to extract about 100 g of the powdered leaves. This mixture was kept under observation for a period of 72 hours in a covered container at room temperature, shaking the mixture once in a while. It was filtered using muslin cloth and then Whatman No.1 filter paper. The plant residue was re-extracted with the fresh solvent in order to get an optimum phytoconstituent recovery [7,8].

The filtrates were merged and further concentrated by rotary evaporator at a temperature below 45°C. The extract was concentrated, dried, and kept at 4°C, in an air-tight amber coloured bottle for future use.

The percentage extraction yield was determined by dividing the amount extracted by the total amount extracted in 100% in the same way as in the first two years:

$$\text{Extraction yield (\%)} = \frac{\text{Weight of dried extract}}{\text{Weight of plant powder}} \times 100$$

2.4 Preliminary Phytochemical Screening

Qualitative phytochemical screening of the hydroalcoholic leaf extract was done using the standard phytochemical screening tests [8,9]. Alkaloids, flavonoids, tannins, phenolic compounds, saponins, glycosides, carbohydrates, proteins, steroids and terpenoids were screened in the extract. Observations were made on the presence (+) or absence (−).

2.5 Formulation of Herbal Mouthwash

Four formulations with different concentrations of leaf extract of Leea macrophylla were prepared: F1, F2, F3 and F4. The base for the mouthwash was prepared using the pharmaceutically acceptable excipients that are commonly used in herbal oral care products [10].

Table 1. Composition of *Leea macrophylla* herbal mouthwash

Ingredient	F1	F2	F3	F4	Function
Leea macrophylla leaf extract	0.5 g	1.0 g	1.5 g	2.0 g	Herbal active ingredient
Glycerin	10 mL	10 mL	10 mL	10 mL	Humectant
Polysorbate 80	1 mL	1 mL	1 mL	1 mL	Solubilizing agent
Sodium benzoate	0.1 g	0.1 g	0.1 g	0.1 g	Preservative
Sodium saccharin	0.05 g	0.05 g	0.05 g	0.05 g	Sweetening agent

Peppermint oil	0.05 mL	0.05 mL	0.05 mL	0.05 mL	Flavouring agent
Purified water	q.s.	q.s.	q.s.	q.s.	Vehicle
Final volume	100 mL	100 mL	100 mL	100 mL	—

The amount of leaf extract that was required was spread out in about 40 mL of purified water. Polysorbate 80 was added little-by-little, while stirring. Sodium benzoate and sodium saccharin were added separately to the extract dispersion as solutions in a small amount of warm purified water. The continuous mixing was used to incorporate glycerin. A small amount of polysorbate 80 was added to peppermint oil prior to adding. Purified water was added to the volume to 100 mL in the final volume. All the formulations were prepared thoroughly, filtered through muslin cloth and placed in labeled amber coloured bottles [10].

2.6 Evaluation of Herbal Mouthwash

Organoleptic and physicochemical evaluation of the prepared formulations was conducted by standard procedures for a liquid oral-care formulation [10,11].

2.6.1 Organoleptic characteristics

The formulations were checked for appearance, colour, odour, taste, clarity and visible particles/precipitation.

2.6.2 Homogeneity

Each formulation was put in a clear glass bottle and inspected for uniformity, aggregation, phase separation and sedimentation.

2.6.3 Determination of pH

A calibrated digital pH meter was used to measure the pH at room temperature. The Measurement was performed three times and the results were presented as the mean ± SD.

2.6.4 Determination of viscosity

A Brookfield viscometer with appropriate spindle and rotational speed was used to measure the viscosity at 25\pm2^\circ\text{C}25\pm2∘C25\pm2∘C at 3 different times for each formulation.

2.6.5 Determination of specific gravity

An empty, dry specific-gravity bottle was weighed, and then filled with the mouthwash. Specific gravity was determined by comparing the weight of formulation with the weight of the same volume of purified water.

$$\text{Specific gravity} = \frac{\text{Weight of mouthwash}}{\text{Weight of equal volume of water}}$$

2.7 Antimicrobial Activity

An agar-well diffusion method [12] was used to determine the antimicrobial activity. Standardized suspensions of S. mutans, S. aureus and C. albicans were prepared and standardized to about 0.5 McFarland turbidity. Bacterial cultures were made in Mueller – Hinton agar and C. albicans in Sabouraud dextrose agar. The microbial suspensions were evenly distributed on the respective even surface of the agar.

Aseptically prepared wells of about 6 mm diameter were prepared. Equal amounts of each of the formulations were added to the wells. The marketed mouthwash was used as the

positive control and the base of the mouthwash without the plant extract was used as the negative control.

The bacterial plates were then incubated at 37 ± 2°C for 24 hours. Candida albicans was inoculated onto plates and were incubated for 24–48 hours at 28–30°C. The antimicrobial activity was assessed based on the extent of inhibition zone (mm). Each experiment was conducted three times [12,13].

2.8 Comparison with Marketed Mouthwash

The optimized formulation was evaluated and compared with the marketed herbal mouthwash in terms of appearance, pH, viscosity, specific gravity and antimicrobial activity. The formulation with acceptable physicochemical properties and satisfactory antibacterial effect was chosen for the stability test.

2.9 Short-Term Stability Study

The conditions of stability testing were chosen based on the already established principles of stability testing [14]. Samples were initially checked and after 15, 30, 60 and 90 days. Changes in appearance, color, odor, uniformity, precipitation, pH, viscosity and antimicrobial activity were noted.

2.10 Statistical Analysis

The experiments were repeated three times, and data expressed as mean ± standard deviation. The one-way analysis of variance (ANOVA) and Tukey's multiple comparisons (Tukey's HSD) tests were used to compare the various formulations. A value of $p<0.05p<0.05p<0.05$ was deemed as statistically significant.

3. Results and Discussion

3.1 Extraction Yield and Characteristics

Table 2. Characteristics of *Leea macrophylla* leaf extract

Parameter	Observation
Weight of dried leaf powder	100 g
Extractive solvent	70% ethanol
Extraction method	Maceration
Weight of dried extract	12.64 g
Percentage yield	12.64%
Colour	Dark greenish-brown
Odour	Characteristic herbal odour
Appearance	Semisolid extract
Consistency	Smooth and slightly sticky

The leaves of Leea macrophylla yielded 12.64 g of dried extract from 100 g of leaf powder, which yielded 12.64% extraction. The extract was dark greenish brown and had a distinct herbal smell. From the observed yield, it was determined that 70% ethanol is appropriate for extracting polar and moderately non-polar constituents from the leaves.

3.2 Preliminary Phytochemical Screening

Table 3. Phytochemical screening results

Phytochemical constituent	Test performed	Observations	Result
Alkaloids	Dragendorff's test	Orange-brown precipitate	+

Flavonoids	Alkaline reagent test	Yellow colour disappearing after acid addition	+
Phenolic compounds	Ferric chloride test	Bluish-green colour	+
Tannins	Ferric chloride test	Greenish-black colour	+
Saponins	Foam test	Persistent foam formation	+
Glycosides	General glycoside test	Characteristic colour reaction	+
Carbohydrates	Molisch's test	Violet ring at the interface	+
Proteins	Biuret test	No violet colour	−
Steroids	Salkowski test	Reddish-brown colour at the interface	+
Terpenoids	Salkowski test	Reddish-brown colour	+

Present: (+); Absent: (−).

Phytochemical screening revealed the presence of alkaloids, flavonoids, phenolics, tannins, saponins, glycosides, carbohydrates, steroids and terpenoids. The proteins were not detected. The potential antimicrobial and antioxidant activity of the formulation is due to phenolics, flavonoids and tannins.

3.3 Organoleptic Evaluation

Table 4. Organoleptic characteristics of mouthwash formulations

Parameter	F1	F2	F3	F4
Appearance	Clear	Clear	Clear	Slightly turbid
Colour	Light brown	Brown	Dark brown	Dark greenish-brown
Odour	Pleasant	Pleasant	Pleasant	Strong herbal
Taste	Acceptable	Acceptable	Moderately bitter	Bitter
Homogeneity	Homogeneous	Homogeneous	Homogeneous	Slight sedimentation
Precipitation	Absent	Absent	Absent	Slightly present

The appearance, odour and homogeneity of the F1 to F3 were good with no precipitation seen. F4 was slightly turbid, sedimented and had a relatively bitter flavor. Increasing concentration of the herbal extract was correlated to the increase in colour intensity and bitterness.

3.4 Physicochemical Evaluation

Table 5. Physicochemical evaluation results

Formulation	pH	Viscosity (mPa·s)	Specific gravity
F1	6.48 ± 0.03	1.42 ± 0.04	1.021 ± 0.002
F2	6.37 ± 0.04	1.53 ± 0.03	1.026 ± 0.003

F3	6.25 ± 0.03	1.66 ± 0.05	1.031 ± 0.002
F4	6.12 ± 0.05	1.78 ± 0.04	1.035 ± 0.003

Values are expressed as mean ± SD, n = 3.

The range of pH values for all the formulations was 6.12 to 6.48. With increasing extract concentration, there was a slight decrease in pH. The formulations were found to be within the normal oral pH range and were thus deemed to be suitable for oral application. As the concentration of the extract increased, its viscosity and specific gravity increased gradually.

3.5 Antimicrobial Activity

Table 6. Zones of inhibition produced by the formulations

Test sample	*S. mutans* (mm)	*S. aureus* (mm)	*C. albicans* (mm)
Mouthwash base	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
F1	9.67 ± 0.58	8.33 ± 0.58	7.33 ± 0.58
F2	13.00 ± 1.00	11.67 ± 0.58	9.67 ± 0.58
F3	16.67 ± 0.58	15.00 ± 1.00	12.67 ± 0.58
F4	18.33 ± 0.58	16.67 ± 0.58	14.33 ± 0.58
Marketed mouthwash	19.67 ± 0.58	18.33 ± 0.58	16.00 ± 1.00

Values represent mean inhibition-zone diameter ± SD, n = 3.

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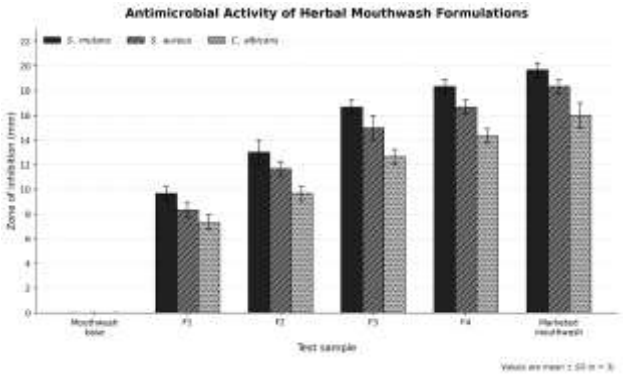

Figure 1 Antimicrobial Activity of Herbal Mouthwash Formulations

The antimicrobial activity of the L. macrophylla extract was found to enhance with increased concentration. The experimental formulations that gave the greatest inhibition zones were the F4. But F3 also exhibited good antimicrobial activity and superior clarity, taste and physical stability when compared to F4. The base of the mouthwash did not show any inhibition signifying that the activity observed in the mouthwash was mainly due to the plant extract. The marked mouth wash exhibited a slight larger inhibition zone as compared with the experimental formulations. Although, the activity of F3 and F4 indicated the possible application of L. macrophylla extract in herbal oral-care formulation.

3.6 Selection of the Optimized Formulation

Table 7. Comparison used for formulation selection

Selection parameter	F1	F2	F3	F4
Physical appearance	Good	Good	Good	Fair

Taste acceptability	Good	Good	Acceptable	Poor
Homogeneity	Good	Good	Good	Fair
Antimicrobial activity	Low	Moderate	High	Highest
Physical stability	Good	Good	Good	Fair
Overall assessment	Not selected	Not selected	Selected	Not selected

The formulation F3 was chosen as an optimum formulation due to its appropriate antimicrobial activity, physical appearance, homogeneity, taste and stability. The antimicrobial activity of F4 was found to be more potent, but its bitterness, turbidity and slight sedimentation decreased the acceptability.

3.7 Short-Term Stability of F3

Table 8. Stability results for optimized formulation F3 at 25 ± 2°C

Parameter	**Initial**	**15 days**	**30 days**	**60 days**	**90 days**
Appearance	Clear	Clear	Clear	Clear	Clear
Colour	Dark brown	No change	No change	No change	Slightly darker
Odour	Pleasant	No change	No change	No change	No change
Homogeneity	Good	Good	Good	Good	Good
Precipitation	Absent	Absent	Absent	Absent	Absent
pH	6.25	6.24	6.22	6.19	6.16
Viscosity (mPa·s)	1.66	1.66	1.67	1.68	1.70

The optimized formulation showed good physical stability at room temperature for 90 days. There was no significant change in the appearance, odour, homogeneity or precipitation. There was a small change in pH, viscosity and colour.

Conclusion

The present study proved the potentiality of Leea macrophylla leaf extract to develop an alcohol free herbal mouth wash. Four concentrations of the extract were formulated and tested on their organoleptic properties, pH, viscosity, specific gravity, homogeneity, antimicrobial activity and short-term stability.

The formulation with the best physicochemical properties, antimicrobial activity, taste acceptability and stability was F3. The antimicrobial activity was comparatively higher in F4 but the bitterness and turbidity with a little sedimentation caused a decrease in acceptability. The optimized formulation F3 showed good activity against the three tested microorganisms Streptococcus mutans, Staphylococcus aureus and Candida albicans, and was physically stable in the short-term stability study.

The results indicate the potential use of Leea macrophylla leaf extract as a herbal raw material in oral-care products. However, studies such as quantitative phytochemical standardization, minimum inhibitory concentration assays, microbial-limit tests, long-term stability and oral-mucosal safety and clinical evaluation are required to ensure that the formulation could be used therapeutically or commercially.

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