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Research Article

DEVELOPMENT AND EVALUATION OF A POLYHERBAL MOUTHWASH FOR ENHANCED ORAL HYGIENE AND ANTIMICROBIAL DEFENSE

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ABSTRACT

Objective: Oral diseases have a prevalence of 3.5 billion people worldwide and a few of them use synthetic mouthwashes which lead to side effects. The purpose of this research was to design and test a polyherbal mouthwash containing Neem, Tulsi, Turmeric, Peppermint, Lemongrass, and Clove extracts for use as a natural substitute for chlorhexidine-based products.

Methods: The plant materials were extracted by cold maceration using ethanol. Three compositions (F1-F3) were developed with different concentrations of the ingredients. Phytochemical investigation revealed the presence of alkaloids, flavonoids, tannins, terpenoids, phenols, and saponins. Quantitative measurement of total phenolic content (TPC) and total flavonoid content (TFC) was also carried out. The formulations were tested for organoleptic properties, pH, viscosity, surface tension, and foaming ability. The antimicrobial activity was assessed against *Streptococcus mutans*, *Candida albicans*, *Lactobacillus acidophilus*, and *Enterococcus faecalis* by broth microdilution method. Stability testing was carried out at 25±2°C for 30 days.

Results: F3 had the greatest antimicrobial activity with minimum inhibitory concentration values that were 50–62.5% lower than F1. Clove and turmeric gave the highest TPC (58.6±2.9 and 51.3±2.6 mg gallic acid equivalents/mL) and TFC (48.9±2.4 and 45.6±2.3 mg QE/mL). The formulations kept their pH 6.4–6.7, had good viscosity (2.10–2.14 cP), and were both physically and chemically stable for 30 days.

Conclusion: The polyherbal mouthwash has the same antimicrobial efficacy as chlorhexidine, but with better safety and patient compliance, thus it can be a natural and eco-friendly oral hygiene and disease prevention solution.

Keywords: Polyherbal Formulation, Antimicrobial Activity, Phytochemical Analysis, Mouthwash, Natural Oral Care, Herbal Therapeutics.

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INTRODUCTION

Oral health functions as a vital indicator for total body wellness and shows the condition of complete health status. Despite major progress in dental science and preventive care, oral diseases persist as a leading global public health issue. The World Health Organization reports that 3.5 billion people worldwide experience dental caries, gingivitis, and periodontitis, which stand as the most widespread chronic diseases among global populations [1]. Because oral infections induce inflammation in the body, systemic disorders such as cardiovascular disease, diabetes mellitus, and respiratory infections have been connected to them [2,35].

Multiple factors contribute to the increased frequency of oral diseases, such as neglect of oral hygiene, excessive intake of sugary foods, smoking, microbial biofilm formation, and poor accessibility to preventive dental services [3,25,38]. One of the most common solutions for abrasive problems is the prescription of mouthwashes with chlorhexidine gluconate, alcohol, and fluoride compounds as additives to normal brushing and flossing. However, along with the effectiveness of these formulations against microbes, these compositions are often associated with some side effects such as discoloration of the teeth, irritation of the mucosa, change in taste, and dryness of the mouth, which, in turn, give rise to low patient compliance and limited duration of use [4,37].

Over the past 10 years, the regime of oral care has changed dramatically, basically staying closer to nature, using safe and eco-friendly products that are not of synthetic origin. The demand for these products has led to the emergence of various phytochemical and herbal-based mouthwash formulations, which are spreading their market due to their compatibility with the biological system, affordability, and least side effects [5,34]. Phytomedicinal mouthwash formulated from herbs comprises a multitude of bioactive phytoconstituents, namely, flavonoids, terpenoids, alkaloids, phenolic acids, and essential oils, that provide antimicrobial, antioxidant, anti-inflammatory, and wound-healing effects [6,7]. These phytochemicals do not work in the same way as chemical agents that are effective through one single pharmacological pathway. Their multimodal mechanisms, for example, breaking down bacterial cell walls, stopping quorum sensing, and removing oxidative stress in the mouth, synergistically enhance their antibacterial and antioxidant activities [8,22].

Increasingly, the world is acknowledging the growing benefits and diverse applications of traditional medicine, which include the use of plants such as *Azadirachta indica*(Neem), *Ocimum sanctum* (Tulsi), *Curcuma longa* (Turmeric), *Mentha piperita* (Peppermint), *Cymbopogon citratus* (Lemongrass), and *Syzygium aromaticum*. Aside from their well-recognized roles as the main ingredients in household remedies for common ailments, these six plants have also attracted the attention of modern researchers due to the fact that their unique characters are integrally relied on for the maintenance [9-12]. Neem having nimbidin and azadirachtin as its active principles shows strong anti-plaque and antibacterial activity; Tulsi and Clove are known for their anti-inflammatory, analgesic, and immunomodulatory properties; Peppermint and Lemongrass give out antimicrobial and cooling effects through menthol and citral, whereas Turmeric by virtue of its curcumin

content acts as an antioxidant and is also a wound-healing agent that can accelerate gum tissue regeneration [13,14].

The idea of using these herbs as a polyherbal formulation is a new way of developing phytopharmaceuticals. Polyherbalism is based on the synergy that occurs when multiple plant extracts are combined, which allows the bioactive compounds to support each other and to increase therapeutic effectiveness while reducing the toxic effect [15,36]. These kinds of preparations provide a complete oral care strategy that is beyond the inhibition of microbes alone but also includes the reduction of inflammation, the control of Odor, and the protection of the mucosa.

Consequently, this study is designed to develop and assess a new polyherbal mouthwash incorporating extracts of Neem, Tulsi, Peppermint, Turmeric, Lemongrass, and Clove. It involves the physicochemical characterization, stability testing, and antimicrobial screening of the prepared formulation against the pathogens chosen for oral infection, namely, *Staphylococcus aureus* and *Escherichia coli*. As a result, we are aiming to validate the scientific basis of a natural, effective, and patient-friendly alternative to synthetic mouthwashes, thus being able to make a valuable contribution to the progress of herbal therapeutics in preventive oral healthcare [17,27].

MATERIALS AND METHODS

Materials

The fresh leaves of Neem, Tulsi, Peppermint, Lemongrass, Rhizomes of Turmeric, and Flower Buds of Clove are collected form Herbal Garden. Chemicals such as ethanol, Folin-Ciocalteu reagent, aluminium chloride, sodium carbonate, gallic acid, and quercetin, were collected from Sharadchandra Pawar College of Pharmacy, Otur, Pune.

Test organisms

- Standard cultures were obtained from recognized repositories:
- *Streptococcus mutans* (primary pathogen associated with dental caries)
- *Candida albicans* (common oral fungal pathogen)
- *Lactobacillus acidophilus* (another oral flora microorganism involved in caries)
- *Enterococcus faecalis* (associated with oral infections and endodontic infections). Cultures were maintained on appropriate media at 4°C and sub-cultured 48 h before use.

Extraction process

The extraction of phytochemicals was carried out by the cold maceration method using ethanol as a solvent. For this fresh leaves of neem, Tulsi, peppermint, lemongrass, flower buds of clove, and rhizomes of turmeric were collected. Then, the collected plant materials were washed with distilled water. All the leaves of plant material were cut into small pieces to increase the surface area for extraction. The cut pieces were placed in iodine flask. 50 mL ethanol were added as a solvent for extraction. The plant material was soaked in ethanol to facilitate extraction of phytochemicals. After 24 h, the mixer was filtered to separate the plant material from ethanol. Then filtered was collected in a clean beaker, then the porcelain dish was placed on hot plate apparatus at low temperature to facilitate gentle evaporation of solvent. It is heated until the ethanol has completely evaporated. That leaves behind a concentrated aqueous extract [19,28].

Phytochemical analysis

Phytochemical analysis is crucial step before formulating polyherbal mouthwash that it reveals the presence of major classes of secondary metabolites such as alkaloids, tannins, flavonoids, terpenoids, saponin, cardiac glycosides, and phenols. These constituents are responsible for antimicrobial, anti-inflammatory, antioxidant, and astringent activities that are essential for oral hygiene and the prevention of dental plaque, gingivitis, and mouth infections. Also ensures safety and quality. Hence, the aqueous extracts of each plant sample were evaluated for the presence of various phytochemicals such as alkaloids, steroids, tannins, flavonoids, terpenoids, saponins, cardiac glycosides, and phenols by the phytochemical analysis techniques [30,39].

Qualitative phytochemical tests (preliminary screening)

Alkaloids

About 2 mL of extract was taken and 2 drops of Mayer's reagent were added. The formation of a creamy or white precipitate indicates the positive result.

Tannins

For tannins analysis, 5 mL of extract was taken and few drops of neutral 5% ferric chloride solution were added. Appearance of dark green color indicates positive result.

Flavonoids

For flavonoids analysis, drops of 20% NaOH were added to 2 mL of the extract. The yellow colors disappear on the addition of concentrated hydrochloric acid, indicating a positive result.

Terpenoids

To a 3 mL of extract, 1 mL of chloroform and 1.5 mL of concentrated sulfuric acid were added to the side of the test tube. Positive result is denoted by the presence of reddish-brown color in the interface.

Saponin

5 mL of the extract was taken in tube and 1 mL of distilled water was added and shaken vigorously, the formation of foam indicates positive result for saponins.

Cardiac glycosides

To 5 mL of the extract, 2 mL of glacial acetic acid was added and a drop-wise ferric chloride solution was added to it. Then, 1 mL of concentrated sulfuric acid was added. The formation of brown ring indicated a positive test for the presence of deoxy sugar of cardenolides. Formation of violet ring beneath the brown layer and in the acetic layer green ring might appear.

Phenols

To a 2 mL of extract, 5% alcoholic ferric chloride was added. Appearance of blue color indicates positive result for phenols.

Quantitative analysis

Determination of total phenolic content (TPC)

TPC was determined using Folin-Ciocalteu method as per standard protocol. Serial dilutions of gallic acid (50–450 µg/mL) were prepared in distilled water as calibration standards. For each extract, 1 mL of sample (appropriately diluted) was mixed with 5.0 mL of diluted Folin-Ciocalteu reagent (1:10 in distilled water) and incubated at room temperature for 3 min. Subsequently, 4.0 mL of sodium carbonate solution (75 g/L) was added, and the reaction mixture was incubated in darkness at room temperature for 90 min. Absorbance was measured at 765 nm using Ultraviolet (UV)-visible spectrophotometer against blank solution. Calibration curve was plotted using gallic acid standards, and TPC was expressed as mg/mL gallic acid equivalent (GAE) (mg GAE/mL).

Total flavonoid content (TFC)

TFC was quantified using aluminium chloride colorimetric method. Serial dilutions of quercetin standard (10–100 µg/mL) were prepared for calibration curve. To 2.0 mL of appropriately diluted extract sample 0.15 mL of sodium nitrite (1.0 moL/L) was added and mixed for 1 min. Subsequently, 0.15 mL of aluminium chloride (10% w/v) was added and mixed. After 6 min incubation, 2.0 mL of sodium hydroxide (1 moL/L) was added to each well followed by addition of 0.6 mL distilled water to bring total volume to 5.0 mL. Absorbance was immediately measured at 415 nm using UV-visible spectrophotometer [40].

Table 1: Preliminary screening of phytochemicals [30]

Sr no	Constituent	Test performed	Observation	Tulsi	Lemongrass	Clove	Turmeric	Peppermint	Neem
1	Alkaloids	Mayer's test	Formation of cream-colored precipitate	+	–	+	–	–	+
2	Tannins	Ferric chloride test	Formation of dark green color	+	–	+	–	–	+
3	Flavonoids	Alkaline reagent test	Disappearance of yellow color	+	+	+	+	+	+
4	Terpenoids	Salkowski's test	Reddish-brown color at interface	+	+	+	+	+	+
5	Saponins	Foam test	Persistent froth formation	+	+	–	–	–	+
6	Cardiac glycosides	Keller-Killiani test	Brown ring at interface (violet/green layer beneath)	+	–	–	–	–	+
7	Phenols	Ferric chloride test	Blue coloration observed	+	+	+	+	+	+

(–): Absence, (+): Present

Table 2: Roles and mechanism of phytochemicals present in polyherbal mouthwash [3,6,7]

S. no	Phytochemical	Main role in polyherbal mouthwash	How it helps in oral cavity/mechanism
1	Alkaloids	Antibacterial and plaquereducing	Many plant alkaloids have the ability to break the bacterial cell wall or hinder the body from making protein and nucleic acid, thus they help in the reduction of *Streptococcus mutans* as well as other cariogenic bacteria, which, in turn, leads to a decrease in plaque formation and the risk of caries.
2	Tannins	Astringent, antiplaque, and antiinflammatory	Tannins bind and precipitate salivary and microbial proteins, thus producing an astringent effect that not only tightens the gingival tissue but also bleeding is reduced because of it. Furthermore, bacterial adhesion and glucan formation on tooth surfaces are limited as a result of which plaque and gingivitis are diminished.
3	Flavonoids	Antioxidant, antiadhesive, and antiinflammatory	Flavonoids scavenge free radicals in gingival tissues and inhibit glucosyl transferase and other enzymes involved in biofilm formation, reducing microbial adherence and protecting against oxidative damage in periodontal tissues.
4	Terpenoids/ Terpenes	Broadspectrum antimicrobial and antiinflammatory	One of the mechanisms by which terpenes derived from essential oils act is by disruption of the microbial membranes. They also inhibit the growth of plaqueforming bacteria as well as period onto pathogens, and are able to modulate the inflammatory pathways in gingiva, which is in part responsible for fresher breath and less gingival inflammation
5	Saponins	Surfactant, antibacterial, cleansing, and foaming	Saponins are agents that reduce surface tension and generate foam, thus aiding the mechanical removal of debris and biofilm; at the same time, they raise the permeability and lysis of bacterial cell membranes, thereby facilitating the antibacterial action of the mouthwash.
6	Cardiac glycosides	Supportive antibacterial contribution	Some plant sources used in oral formulations contain cardiac glycosides which show adjunct antibacterial activity against oral microbes, though they are usually minor contributors compared with other phytochemicals and must be kept at very low, safe levels
7	Phenols/ Polyphenols	Strong antimicrobial, antioxidant, and antiplaque	Phenolic compounds (e.g., eugenol, catechins, and gallic and ellagic acids) denature microbial proteins, disrupt cell membranes, inhibit plaque-forming enzymes, and provide high antioxidant capacity that protects oral mucosa and gingiva from oxidative stress and inflammation

Formulation of mouthwash

Neem, Tulsi, Peppermint, Turmeric, Lemongrass, Clove, Honey, and Salt are mixed in a beaker, as shown in formulation Table 3. Then, slowly ethanol was added to this mixture. Then, it was stirred well and slowly added distilled water to make up the volume upto 100 mL. So to ensure uniformity and clarity, the mixture was shakes continuously. Then, the sufficient quantity of charcoal was added to the mixture for discoloration, the mixture has boiled for 2–5 min. Then, it is cooled and filter to remove any solid particles [16,20].

Table 3: Total phenolic and flavonoid content in phytochemical extract [40]

Sr no	Extract	Total phenolic content (mg gallic acid equivalent/mL)	Total flavonoid content (mg QE/mL)
1	Neem	42.5±2.1	28.4±1.4
2	Tulsi	38.7±1.9	32.1±1.6
3	Peppermint	35.2±1.8	24.5±1.2
4	Turmeric	51.3±2.6	45.6±2.3
5	Lemongrass	39.8±2.0	26.8±1.3
6	Clove	58.6±2.9	48.9±2.4

(n=3)

Evaluation of herbal mouthwash

Organoleptic characteristic

Organoleptic properties such as color, odor, taste, appearance, and texture were assessed visually and sensorially. These attributes play a key role in patient compliance and product acceptability in routine oral care. The results of organoleptic properties are shown in Table 5.

pH

pH of prepared herbal mouthwash was measured using digital pH meter. The pH meter was calibrated using standard buffer solution, and then, the pH of prepared formulations was measured. An ideal mouthwash should maintain a pH close to neutral (5.5–7.0) to avoid mucosal irritation and protect tooth enamel from demineralization [18]. The results are shown in Table 6 and graphically represented in Fig. 6.

Viscosity

Viscosity of the mouthwash was determined with the help of a Brookfield viscometer at 100 rpm with the spindle 6 [24]. The results for the viscosity of all the prepared formulations have been described in the Table 7 and graphically represented in Fig. 7.

Table 4: Formulation table [21,23]

S. No	Ingredient	Batch F1 (Low conc)	Batch F2 (medium conc)	Batch F3 (high conc)	Role of ingredient
1	Neem	1 mL	2 mL	3 mL	Anti-microbial
2	Tulsi	2 mL	4 mL	6 mL	Anti-inflammatory
3	Peppermint	3 mL	6 mL	9 mL	Cooling agent, freshener
4	Turmeric	2 mL	4 mL	6 mL	Anti-bacterial
5	Lemongrass	2 mL	4 mL	6 mL	Anti-bacterial
6	Clove	3.5 mL	7 mL	10.5 mL	Anti-inflammatory, dental analgesic
7	Ethanol	5 mL	5 mL	5 mL	Preservative
8	Salt	2 g	2 g	2 g	Osmolytic preservative
9	Honey	5 mL	10 mL	15 mL	Anti-bacterial, sweetening agent
10	Distilled water	q.s. up to 100 mL	q.s. up to 100 mL	q.s. up to 100 mL	Vehicle

Table 5: Organoleptic characteristics

Formulation	Color	Odor	Appearance	Taste	Texture
F1	Light yellow	Characteristic	Homogeneous	Minty and spicy	Liquide
F2	Yellowish-brown	Characteristic	Homogeneous	Minty and spicy	Liquide
F3	Yellowish-brown	Characteristic	Homogeneous	Minty and spicy	Liquide

Table 6: pH of various formulations

Formulation	pH
F1	6.4±0.1
F2	6.7±0.1
F3	6.7±0.1

(n=3)

Table 7: Viscosity of various formulations

Sr no	Viscosity (centipoise)
F1	2.10±0.05
F2	2.12±0.06
F3	2.14±0.06

(n=3)

Table 8: Surface tension of various batches

Sr no	Surface tension (mN/m) Millinewton per meter
F1	68.5±1.2
F2	65.8±1.1
F3	62.3±1.0

(n=3)

Table 9: Foaming ability of the various batches

Sr no	Formulation batch	Foaming height (mm)
1	F1	45±2
2	F2	52±2
3	F3	58±2

(n=3)

Table 10: Antimicrobial activity assay

Test organism	F1 MIC (%)	F2 MIC (%)	F3 MIC (%)	CHX control (%)
Streptococcus mutans	2.0	1.5	1.0	0.50
Candida albicans	4.0	3.0	1.5	0.75
Lactobacillus acidophilus	3.0	2.5	1.5	0.60
Enterococcus faecalis	2.5	2.0	1.0	0.50

MIC: Minimum inhibitory concentration

Fig. 1: Extraction of phytochemicals

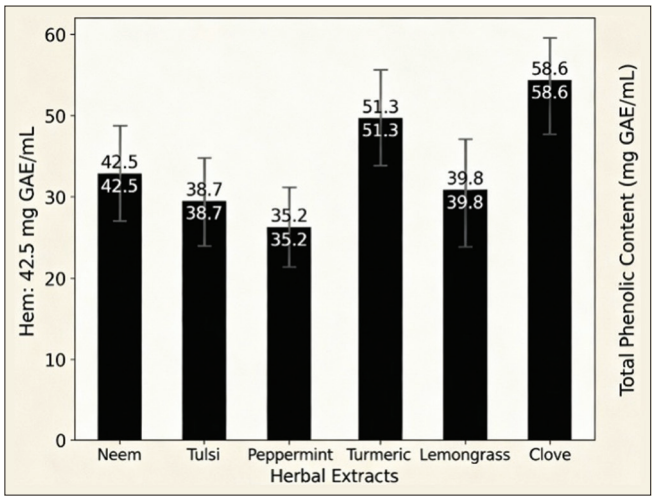

Fig. 2: Total phenolic content

Surface tension measurement

The surface tension of three separate batches of polyherbal mouthwash formulation was figured out by drop count method (Traube's stalagmometer technique) according to the set pharmaceutical standards. The temperature for this measuring was maintained at

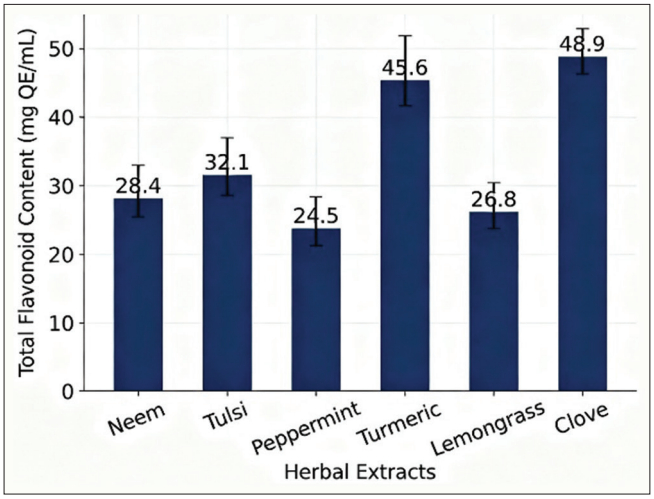

Fig. 3: Total flavonoid content

Fig. 4: Formulated polyherbal mouthwash

Fig. 5: Digital pH Meter with formulation

25±2°C to keep it consistent for all batches. The Stalagmometer was made clean with the help of chromic acid, and then, it was rinsed with distilled water and alcohol. In each batch, the apparatus was loaded with the test formulation and 10 drops were taken from a pre-weighed wide-mouthed bottle at a controlled drop rate of 10–15 drops/min. The mass of 10 drops was found by an analytical balance. For each batch, three replicate measurements were carried out. Surface tension of the polyherbal mouthwash was determined using the equation:

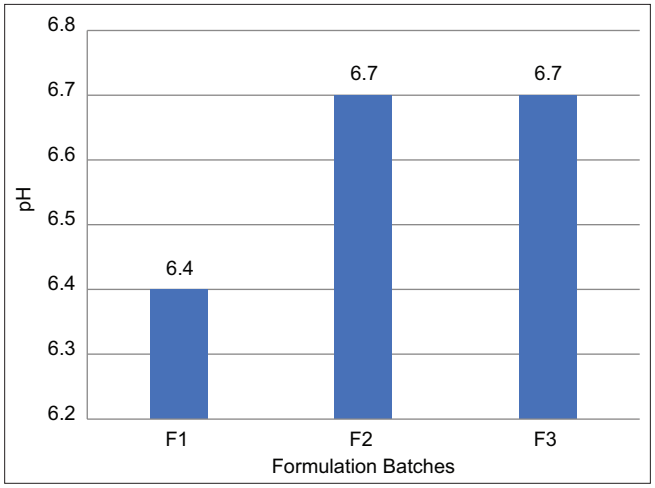

Fig. 6: pH of various formulations

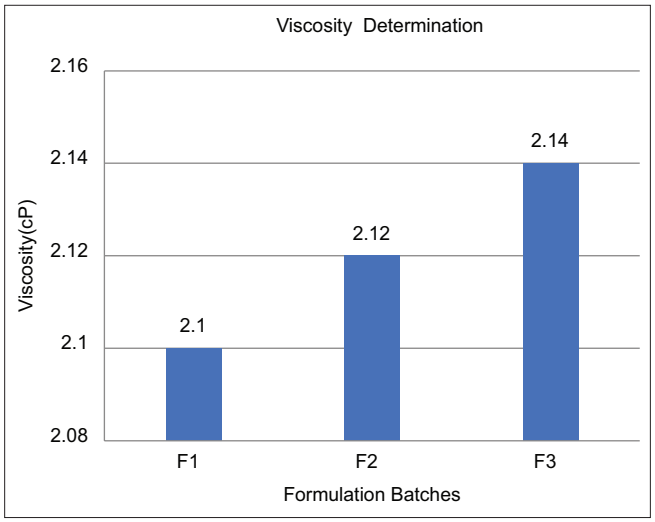

Fig. 7: Viscosity of various formulations

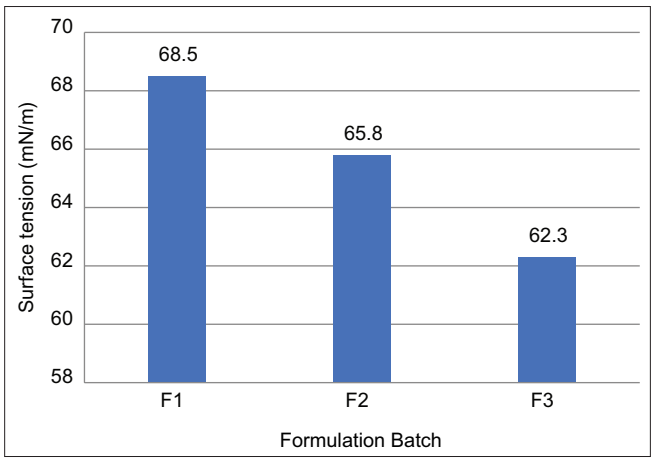

Fig. 8: Surface tension of various formulations

$$\gamma_l = \gamma_w \times \frac{w_l}{w_w}$$

where γ_l is surface tension of the formulation (N/m), γ_w is surface tension of water (72.2 N/m), w_l is weight of 10 drops of formulation (g), and w_w is weight of 10 drops of water (g). The density of each batch was

determined using a specific gravity bottle method to ensure accurate surface tension calculations [22].

Foaming ability assessment

Foaming ability was evaluated using dynamic foam analysis. Neat sample (50 mL) was stirred for 30 s at 3000 rpm using a flat paddle stirrer head, and foam height was measured immediately after stirring cessation. Measurements were repeated 3 times with fresh sample aliquots. Foam height (mm) was recorded. This parameter is important as acceptable foaming enhances cleansing action while excessive foam may cause discomfort [26].

Antimicrobial activity assay

Selected pathogens

- *S. mutans* (primary pathogen associated with dental caries)
- *C. albicans* (common oral fungal pathogen)
- *L. acidophilus* (another oral flora micro-organism involved in caries)
- *E. faecalis* (associated with oral infections and endodontic infections).

Broth microdilution method for MIC determination

The formulations (F1, F2, and F3) of the mouthwash were serially diluted two-fold with Mueller-Hinton broth for bacterial species and Sabouraud dextrose broth for *C. albicans* to obtain final concentrations ranging from 0.125% to 2.0%, with each dilution (100 µL) being placed in separate wells of a sterile 96-well microtiter plate. To each well, 10 µL of the standardized microbial inoculum (approximately 5×10^5 colony-forming unit [CFU]/mL) was added to obtain a final inoculum concentration of about 5×10^4 CFU/mL. The plates were sealed and incubated at 37°C for 24 h (bacterial strains) or 48 h (*C. albicans*) in a humidified incubator. The minimum inhibitory concentration (MIC) was determined as the lowest concentration of mouthwash that showed no visible microbial growth (no turbidity or color change) when compared to positive control wells. All the determinations were carried out in triplicate, and the results were recorded as mean values. Quality control consisted of positive control wells with inoculum and broth (without formulation) to confirm organism viability and growth, negative control wells with only broth and formulation (without inoculum) to detect medium contamination, and a standard chlorhexidine solution (0.12%) as a reference antimicrobial agent for comparison purposes [12,26].

Stability study

The stability testing of the formulated polyherbal mouthwash of optimized batch F3 was carried out for 30 days to assess its physical and chemical integrity under controlled conditions. The formulation was stored at room temperature (25±2°C) and relative humidity (60±5%), in accordance with the ICH Q1A (R2) stability testing guidelines for pharmaceutical products. Periodic evaluations were conducted at 0, 10, 20, and 30 days, and the results are presented in Table 11. Stability testing is an essential parameter to determine the shelf life, safety, and performance reliability of pharmaceutical and herbal preparations. The absence of changes in organoleptic and physicochemical properties demonstrates that the developed polyherbal mouthwash maintains ingredient compatibility and resistance to environmental degradation, ensuring product efficacy throughout storage. These findings validate the formulation's suitability for long-term use and potential for large-scale commercialization [21,26].

Statistical analysis

All the data were analyzed using GraphPad Prism software (version 8.0). Results were expressed as mean standard deviation from minimum three replicates (n=3). One-way analysis of variance was employed.

RESULT AND DISCUSSION

Quantitative phytochemical analysis

TPC

Quantitative analysis revealed significant phenolic content in all six herbal extracts. Clove extract demonstrated the highest TPC (58.6±2.9 mg GAE/mL), followed by Turmeric (51.3±2.6 mg GAE/mL), Neem (42.5±2.1 mg GAE/mL), Lemongrass (39.8±2.0 mg GAE/mL), Tulsi (38.7±1.9 mg GAE/mL), and Peppermint (35.2±1.8 mg GAE/mL). These quantitative values are critical for standardization, ensuring batch-to-batch consistency and predictable biological efficacy.

TFC

Total flavonoid analysis similarly revealed Clove extract with highest flavonoid content (48.9±2.4 mg QE/mL), followed by Turmeric (45.6±2.3 mg QE/mL), Tulsi (32.1±1.6 mg QE/mL), Neem (28.4±1.4 mg GAE/mL), Lemongrass (26.8±1.3 mg QE/mL), and Peppermint (24.5±1.2 mg QE/mL). The high flavonoid content correlates with documented antioxidant and antimicrobial properties, explaining the efficacy of these extracts in oral care applications.

Organoleptic characteristic

Organoleptic properties such as color, odor, taste, appearance, and texture were assessed visually and sensorially. All the formulation appears in light yellow to yellowish-brown in color depending on the concentration of ingredients. All the formulations have odor characteristics, show homogenous appearance, taste minty, and spicy, and have liquide texture.

pH

pH of prepared herbal mouthwash was measured using digital pH meter. The pH of all formulation shows within acceptable range, that is 6.4–6.7.

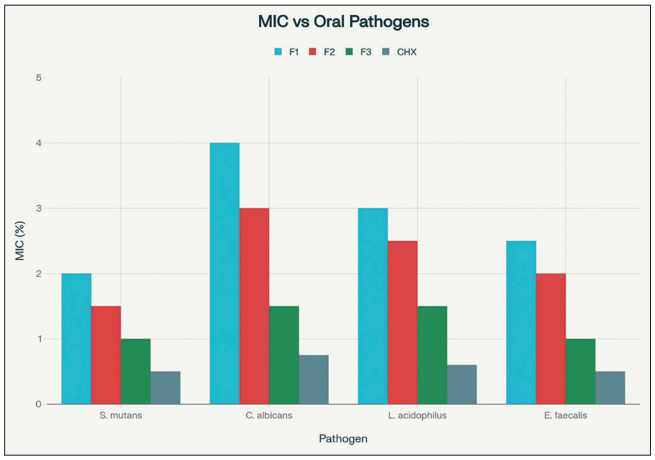

Fig. 9: Minimum inhibitory concentration of polyherbal mouthwash formulations against

Table 11: Stability study of optimized formulation F3 at temperature (25±2°C) and relative humidity (60±5%)

S. no	Parameters	Observation			
		Initial	10 days	20 days	30 days
1	Color	LightYellow	LightYellow	LightYellow	LightYellow
2	Odor	Characteristics	Characteristics	Characteristics	Characteristics
3	Consistency	Stable	Stable	Stable	Stable
4	Phase separation	Nil	Nil	Nil	Nil
5	pH	6.7±0.05	6.5±0.07	6.7±0.05	6.6±0.05

Viscosity

Viscosity of the mouthwash was determined with the help of a Brookfield viscometer. All the formulation from F1 to F3 shows viscosity within a range. As the concentration of ingredients increases viscosity of formulation also increase.

Viscosity

Viscosity measurements showed minimal but notable increases with increasing ingredient concentrations. F1 showed 2.10±0.05 cP, F2 showed 2.12±0.06 cP, and F3 showed 2.14±0.06 cP. All values represent acceptable fluid viscosity for mouthwash application, allowing ease of use while maintaining sufficient contact time with oral tissues.

Surface tension

F3 demonstrated the lowest surface tension (62.3±1.0 mN/m) compared to F1 (68.5±1.2 mN/m) and F2 (65.8±1.1 mN/m). Reduced surface tension in F3 is advantageous, as it improves spread ability and penetration into interdental spaces where plaque and pathogens accumulate, addressing a critical weakness of conventional mouthwashes [22].

Foaming ability

Foaming ability increased progressively with ingredient concentration. F1 produced foam height of 45±2 mm, F2 produced 52±2 mm, and F3 produced 58±2 mm. This acceptable foaming profile is desirable for oral care, as foam enhances physical cleansing action and retention of active ingredients on oral mucosa, improving therapeutic efficacy [26].

The least MIC values were observed for the F3 formulation, followed by F2 and F1 in the case of all the oral pathogens tested, and the values for chlorhexidine control were less than half the lowest of the samples. The MIC of F1 against *S. mutans* was 2.0% and it decreased to 1.5% for F2 (25% reduction) and 1.0% for F3 (50% reduction from F1) while the chlorhexidine control showed 0.50%. For *C. albicans*, the MIC of F1 was 4.0% and was improved to 3.0% for F2 (25% reduction) and 1.5% for F3 (62.5% lower than F1, 50% lower than F2) with the chlorhexidine control at 0.75%. In a similar way, against *L. acidophilus*, F1 exhibited 3.0%, F2 showed 2.5% (17% reduction), and F3 demonstrated 1.5% (50% lower than F1, 40% lower than F2) while the chlorhexidine control was 0.60%. For *E. faecalis*, F1 displayed 2.5%, F2 showed 2.0% (20% reduction), and F3 exhibited 1.0% (60% reduction from F1 and 50% reduction from F2) while the chlorhexidine control was 0.50%. In general, F3 formulation showed the best antimicrobial effect for all the microorganisms tested with the MIC values being 50–62.5% lower than those of F1 and as low as the chlorhexidine control.

The stepwise lowering of MIC values from F1 to F3 for all the microorganisms tested clearly showed that the formulations were optimized and the antimicrobial potency was enhanced as more ingredient concentrations were added. Most notably, for *C. albicans*, F3 was responsible for a 2.7 times lower MIC value compared to F1. In the case of bacteria, the fold changes were from 2.0 to 2.5. Thus, these findings served as evidence that the increased concentrations of herbal ingredients in F3 brought about significantly elevated antimicrobial effectiveness against the entire panel of microbial isolates. Based on the above findings, F3 batch demonstrated superior performance and was identified as the optimized formulation consequently stability study was carried out for F3 batch.

Stability study

Throughout the study, the mouthwash consistently retained its light-yellow color, characteristic odor, and uniform consistency, with no phase separation observed, confirming excellent physical stability. The pH remained nearly constant (6.5–6.7) within the acceptable oral care range (5.5–7.0) indicating that the formulation remained chemically stable and non-irritating to oral tissues.

CONCLUSION

The developed polyherbal mouthwash exhibited remarkable stability, safety, and antimicrobial effectiveness that is comparable with traditional chlorhexidine mouthwash. The blend of six medicinal herbs resulted in improved antibacterial properties, a pleasant flavor, and high patient adherence. These results indicate that the formulated mouthwash has the potential to act as a sustainable and efficient natural substitute for chemical-based oral care products.

Future perspective

The present *in vitro* findings validate strong antimicrobial efficacy against *S. mutans*, *C. albicans*, *L. acidophilus,* and *E. faecalis;* however, extensive clinical trials are necessary to verify safety, effectiveness, and patient acceptance during extended use. Moreover, toxicological assessments and studies on mucosal irritation must be conducted to ensure biocompatibility and adherence to regulatory standards. The application of nanotechnology or bioadhesive systems could improve retention and controlled release in the oral cavity. Expanding the study toward standardization, scaling-up, and regulatory approval could lead to the successful commercialization of this natural, sustainable oral care formulation [29].

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AUTHORS' CONTRIBUTIONS

Shritesh Bhoyar works on manuscript writing and supervised the study. Vrushabh Hupparage and Prasad Matte conducted the experimental work.

CONFLICTS OF INTEREST

The author reports no financial or any other conflicts of interest in this paper.

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