Development And Efficacy of a Mouthwash Containing Galangal and Guava Extracts

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I. HISTORICAL EVOLUTION AND CLINICAL DYNAMICS OF ORAL DYSBIOSIS

The human oral cavity represents a highly complex, dynamic ecological niche that harbors a diverse consortium of microorganisms, including bacteria, fungi, viruses, and protozoa. Under physiological conditions, this microflora exists in a state of delicate eubiosis, which is actively maintained by the buffering capacity, immunoglobulins, and antimicrobial proteins present in healthy saliva. However, when this homeostatic balance is disrupted by inadequate oral hygiene practices, high-sugar diets, tobacco consumption, or systemic physiological changes, pathogenic species begin to proliferate. This shift from eubiosis to dysbiosis is the primary driver behind dental plaque accumulation, periodontal diseases, gingivitis, dental caries, and halitosis.

Historically, mankind has sought natural remedies to maintain oral cleanliness and alleviate dental discomfort.

Ancient Egyptians developed some of the earliest recorded dentifrice formulations, utilizing a mixture of olive tree leaves, milk, wine, oil, pomegranate peelings, nutgalls, and vinegar to rinse their mouths. Similarly, the traditional Indian system of medicine, Ayurveda, has advocated for the use of chewing sticks or "Dattuna" derived from therapeutic trees, alongside herbal decoctions to gargle. In recent decades, modern dentistry became heavily reliant on synthetic chemical formulations to achieve oral disinfection. Popular commercial mouthwashes regularly employ active synthetic compounds such as chlorhexidine digluconate, cetylpyridinium chloride, stannous fluoride, and high concentrations of ethanol.[1]

Despite their immediate antimicrobial efficacy, synthetic mouthwashes present severe clinical drawbacks when used chronically. Chlorhexidine, considered the conventional gold standard for plaque control, is associated with the development of extrinsic brown and yellowish stains on the tooth enamel and dorsum of the tongue.

It also alters taste perception (dysgeusia) and causes mucosal desquamation because of its cationic binding properties. Synthetic mouthwashes containing high alcohol content can severely decrease salivation. This salivary reduction deprives the oral cavity of its natural defensive proteins, inducing dry mouth (xerostomia), which paradoxically fuels the growth of anaerobic bacteria that produce volatile sulfur compounds (VSCs), thereby worsening halitosis. Furthermore, additives like sodium lauryl sulphate (SLS) can irritate the delicate oral mucosa, leading to recurrent aphthous ulcers and chemical sensitivity.

To overcome these therapeutic limitations, current pharmaceutical research is focused on developing biocompatible, non-toxic, and biodegradable polyherbal mouthwashes. By combining multiple botanical extracts, these formulations leverage the synergistic therapeutic mechanisms of secondary plant metabolites including polyphenols, flavonoids, tannins, alkaloids, and volatile essential oils. This multi-targeted approach effectively controls plaque-forming pathogens and relieves gingival inflammation without inducing cellular toxicity or staining dental tissues.[2]

II. PHARMACOGNOSTIC AND PHYTOCHEMICAL PROFILES OF BOTANICAL CANDIDATES [3,4]

Selecting botanical candidates for a stable polyherbal mouthwash requires a detailed understanding of their chemical composition and specific mechanisms of action. For this formulation, several prominent medicinal plants were chosen to provide a broad range of therapeutic benefits, including antimicrobial, anti-inflammatory, astringent, and analgesic effects.

Active Botanical Extracts

Alpinia galanga extract (Greater Galangal)

- Family: Zingiberaceae
- Key Active Chemical Markers: 1'-acetoxychavicol acetate (ACA), 1,8-cineole, and α-bergamotene.
- Biochemical Mechanism: Alpinia galanga provides highly potent antimicrobial and anti-inflammatory properties. The primary marker, ACA, disrupts the cell walls of oral pathogens specifically targeting Streptococcus mutans and Candida albicans leading to cellular leakage and lysis. Furthermore, it downregulates inflammatory cytokines, helping to soothe swollen or irritated gingival tissues.

Psidium guajava extract (Guava Leaf)

- Family: Myrtaceae
- Key Active Chemical Markers: Guajaverin, quercetin, morin, and various condensed tannins.
- Biochemical Mechanism: Guava leaf extract functions as a powerful natural astringent and anti-plaque agent. The flavonoids (like guajaverin and quercetin) inhibit the adherence of primary bacterial colonizers to the tooth surface. The rich tannin content binds to salivary proteins, precipitating them to form a protective, tightening layer over inflamed gums while actively neutralizing volatile sulfur compounds (VSCs) that cause halitosis.

Flavoring and Essential Oils

Peppermint Oil (Mentha piperita)

- Family: Labiatae / Lamiaceae
- Key Active Chemical Markers: Menthol and menthone.
- Mechanism of Action: Menthol binds directly to the cold-sensitive TRPM8 receptors in the oral mucosa, creating a cooling sensation that masks mild discomfort. Beyond providing long-lasting breath freshness, peppermint oil works synergistically with the botanical extracts to offer secondary broad-spectrum antibacterial coverage against anaerobic oral bacteria.

Lemon Essential Oil (Citrus limon)

- Family: Rutaceae
- Key Active Chemical Markers: Limonene and terpinene.
- Mechanism of Action: Lemon oil contributes a crisp, refreshing citrus flavor profile that cuts through the bitter or earthy notes of the herbal extracts. Structurally, limonene exerts mild oxidative stress on the lipid membranes of oral pathogens and promotes salivation, which naturally cleanses the oral cavity and balances oral pH.

Excipients, Solubilizers, and Stabilizers Polysorbate 20

- Functional Role: Non-ionic Surfactant and Solubilizer.
- Mechanism of Action: Essential oils (peppermint and lemon) are inherently lipophilic (oil-loving) and do not dissolve in water. Polysorbate 20 acts as a bridge; its hydrophobic tails trap the microscopic oil droplets while its hydrophilic heads bond with the water phase. This creates a stable microemulsion, ensuring the oils remain evenly dispersed throughout the mouthwash without separating into distinct layers.

Glycerin

- Functional Role: Humectant, Co-Solvent, and Demulcent.
- Mechanism of Action: Glycerin prevents the mouthwash from drying out and acts as a moisturizing agent for the user's oral cavity. It coats the mucous membranes to relieve dryness, improves the overall "mouthfeel" or texture of the rinse, and adds a subtle, natural sweetness that enhances the flavor profile.

Xylitol

- Functional Role: Non-Cariogenic Sweetener and Anti-Cavity Agent.

- Mechanism of Action: Xylitol is a 5-carbon sugar alcohol that oral bacteria (like S. mutans) cannot ferment. When these bacteria ingest xylitol, it clogs their metabolic pathways, preventing them from producing the harmful acids that demineralize tooth enamel. It reduces bacterial replication and plaque accumulation while enhancing sweetness without promoting tooth decay.

Sodium Benzoate

- Functional Role: Antimicrobial Preservative.
- Mechanism of Action: This preservative protects the herbal formulation from fungal and bacterial contamination over time. In a slightly acidic environment, it converts into benzoic acid, which easily crosses microbial cell membranes to disrupt their internal pH and stop the growth of molds, yeasts, and bacteria.

Citric Acid

- Functional Role: pH Adjuster / Acidifier.
- Mechanism of Action: Used quantum satis (q.s. / as much as needed), citric acid is added in tiny amounts to calibrate the mouthwash to an optimal, slightly acidic pH (typically between 5.0 and 5.5). This specific range is critical because it activates the sodium benzoate preservative and ensures the botanical extracts remain stable and effective.

Purified Water

- Functional Role: Vehicle / Principal Solvent.
- Mechanism of Action: Added quantum satis to bring the total volume to 100 mL, purified water acts as the clean, stable carrier medium that dissolves the water-soluble elements (xylitol, sodium benzoate, extracts) and holds the total formulation together.

Fig 1: Herbal Mouthwash

Procurement and Preparation of Plant Materials and Essential Oils

Procurement of Plant Materials

Fresh leaves of Alpinia galanga (Greater Galangal) and Psidium guajava (Guava) were collected from local authenticated sources. The collected plant materials were thoroughly washed with distilled water to remove dust, dirt, and other extraneous matter. The cleaned leaves were shade dried at room temperature for several days until complete removal of moisture was achieved. The dried plant materials were then pulverized separately using a mechanical grinder to obtain coarse powders and stored in airtight containers for further extraction procedures.

Extraction of Herbal Plant Materials

The powdered plant materials of Alpinia galanga and Psidium guajava were subjected to suitable extraction methods for the isolation of phytoconstituents. The extracts obtained were concentrated and preserved under appropriate storage conditions until further use in formulation development.

Procurement of Essential Oils

The essential oils of peppermint (Mentha piperita) and lemon (Citrus limon) were not extracted in the laboratory. Instead, pure and standardized essential oils were procured from reputed and authenticated commercial suppliers. The oils were obtained in ready-to-use form and utilized directly in the formulation without any further extraction or purification process.

Storage Conditions

All plant extracts and commercially procured essential oils were stored in clean, airtight amber-colored containers under cool and dry conditions to protect them from light, moisture, and oxidative degradation until further experimental use.

Rationale for Selection

The selected herbal ingredients were chosen based on their well-documented antimicrobial, anti-inflammatory, antioxidant, and breath-freshening properties. Alpinia galanga and Psidium guajava extracts contribute significant antibacterial and anti-plaque activities, whereas peppermint and lemon essential oils provide flavor enhancement, cooling sensation, and additional antimicrobial benefits,

making them suitable candidates for herbal oral care formulation development.

Botanical Leaf Extraction (Alpinia galanga & Psidium guajava)

The active extracts for Alpinia galanga (Greater Galangal) and Psidium guajava (Guava) are obtained from their leaves using a systematic washing, drying, and solvent extraction method.

The Step-by-Step Process:

- Pre-Treatment (Washing): Fresh leaves of Alpinia galanga and Psidium guajava are thoroughly washed under running tap water to remove dirt, soil, and any external contaminants.
- Shade Drying: The cleaned leaves are spread out and allowed to dry completely in the shade. Drying them away from direct sunlight is crucial to prevent the UV rays and excessive heat from degrading the active chemical markers (like flavonoids and tannins).
- Pulverization: Once completely dry, the leaves are ground down into a coarse powder. This grinding increases the overall surface area, making it easier for the solvent to penetrate the cell walls.
- Maceration / Extraction: The coarse plant powder is soaked in an appropriate solvent for a specific period. The solvent dissolves and leaches out the target phytochemicals (such as the anti-inflammatory ACA from galangal and the anti-plaque guajaverin from guava).
- Filtration and Concentration: The mixture is filtered to separate the spent leaf powder from the liquid extract. The remaining liquid solvent is then evaporated, leaving behind a highly concentrated, purified liquid extract ready to be measured into your 5% formulation.

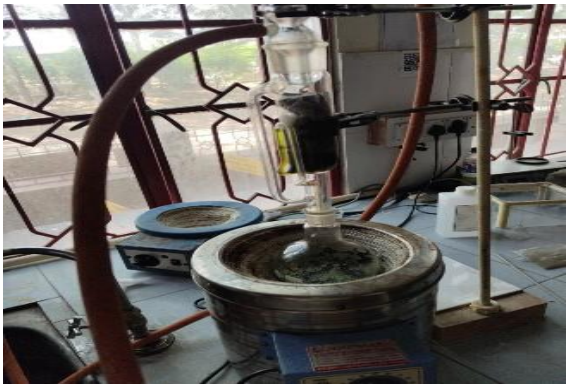

Fig 2: Pharmacogenetic and Phytochemical Profiles of Selected Botanical Actives [2]

Table 1: Pharmacogenetic and Phytochemical Profiles of Selected Botanical Actives [4]

Common Name	Botanical Name	Family	Plant Part Used	Primary Phytoconstituents	Specific Therapeutic Role
Greater Galangal	Alpinia galanga	Zingiberoside	Leaves	1'-acetoxychavicol acetate (ACA), 1,8-cineole, α-bergamotene	Offers potent antimicrobial and anti-inflammatory properties; ACA disrupts bacterial cell walls (S. mutans) leading to cell lysis.
Guava	Psidium guajava	Myrtaceae	Leaves	Guajaverin, quercetin, morin, condensed tannins	Acts as an anti-plaque and astringent agent; prevents primary bacterial adhesion to teeth and neutralizes bad odors (VSCs).
Peppermint	Mentha piperita	Labiatae / Lamiaceae	Leaves	Menthol, menthone	Targets cold-sensitive TRPM8 receptors to provide a cooling sensation; provides secondary antibacterial action and freshens breath.
Lemon	Citrus limon	Rutaceae	Peel / Fruit rind	Limonene, γ-terpinene	Acts as a flavoring agent; disrupts microbial lipid membranes, promotes salivation for oral cleansing, and balances pH.

III. EXPERIMENTAL PROCEDURE AND ANALYTICAL CHARACTERIZATION OF EXTRACTS

The extraction protocols were meticulously structured to maximize the recovery of pharmacologically active biomarkers while safeguarding any thermolabile compounds. The specific methodologies were selected based on the physical and chemical characteristics of each raw material. For the non-volatile components, the leaves of Alpinia galanga and Psidium guajava were thoroughly cleansed with running tap water, dried uniformly under protective shade to prevent phytochemical degradation, and subsequently pulverized into a coarse powder to optimize the available surface area. This prepared plant material was subjected to solvent extraction through a systematic maceration process, allowing the target phytoconstituents such as the anti-inflammatory 1'-acetoxychavicol acetate from the galangal and the astringent tannins and flavonoids from the guava to completely leach into the liquid medium. The resulting crude mixtures were passed through a filtration system to separate the spent plant debris, and the filtrate was then carefully evaporated to yield highly concentrated, pure liquid extracts ready for the final mouthwash formulation.

Simultaneously, the volatile aromatic fractions required for the formulation specifically Peppermint oil and Lemon essential oil were isolated using an advanced hydro-distillation technique via a Clevenger apparatus. The raw plant elements (leaves of Mentha piperita and rinds of Citrus limon) were submerged in purified water within a boiling flask and heated. The rising steam ruptured the internal cellular oil glands, liberating essential markers like menthol and limonene. These vaporized volatile components co-distilled alongside the water vapor into a vertical condenser column, where circulating cold water chilled the mixture back into a liquid state. The condensed fluids drained directly into a graduated collection tube; owing to differences in density and their immiscible nature, the pure, hydrophobic essential oils naturally separated from the water phase, forming a distinct upper layer that was cleanly harvested for use as the formulation's key flavoring and antimicrobial agents. [6]

Fig 2: Extraction of Crude Polyherbal Extracts Prior to Formulation Development [3]

Total Phenolic and Flavonoid Quantitation

The total phenolic content (TPC) was determined using the Folin-Ciocalteu reagent method, with results expressed as gallic acid equivalents per milliliter (mg GAE/mL). The total flavonoid content (TFC) was measured using the aluminum chloride colorimetric assay, expressed as quercetin equivalents per milliliter (mg QE/mL).

Table 2: Phenolic, Flavonoid, and FTIR Peak Characterization of Botanical Extracts

Plant Extract / Volatile Oil	Dominant Phytochemical Classes	Expected FTIR Peaks (cm^{-1})	Assigned Chemical Functional Groups
Alpinia galanga Extract (Greater Galangal)	Acetoxy compounds, Monoterpenes, Sesquiterpenes	1735–1750 1240–1260 2920–2960	Ester carbonyl (C=O) stretching from ACA, Acetate (C–O) stretching, Aliphatic C–H stretching
Psidium guajava Extract (Guava Leaf)	Polyphenols, Condensed Tannins, Flavonoids (Quercetin)	3300–3450 1610–1640 1050–1100	Polymeric Phenolic O–H stretching (hydrogen-bonded), Aromatic ring C=C stretching, Flavonoid alcohol C–O stretching
Peppermint Oil (Mentha piperita)	Monoterpenols (Menthol), Cyclic ketones	3350–3400 1710–1720 2850–2930	Hydroxyl (O–H) stretching from menthol, Carbonyl (C=O) stretching from menthone, Aliphatic C–H stretching
Lemon Essential Oil (Citrus limon)	Monoterpene Hydrocarbons (Limonene)	3010–3080 1640–1650 2850–2920	Olefinic C–H stretching (alkene), Carbon-carbon double bond (C=C) stretching from limonene ring, Aliphatic C–H stretching

Analytical FTIR Spectroscopy

Fourier Transform Infrared (FTIR) spectroscopy was performed to characterize the dominant structural functional groups present within the bioactive matrices of the optimized polyherbal extracts and essential oils. The resulting FTIR spectra revealed distinct, diagnostic absorption bands that confirm the presence of specific therapeutic phytochemical classes. Strong, broad absorption bands observed in the region of 3300 cm^{-1} to 3450 cm^{-1} are attributed to the polymeric, hydrogen-bonded hydroxyl (O–H) stretching vibrations, establishing the rich presence of polyphenolic fractions and flavonoids like quercetin from the Psidium guajava leaf extract and menthol from the peppermint oil. Prominent peaks identified between 2850 cm^{-1} and 2960 cm^{-1} correspond to the asymmetric and symmetric aliphatic C–H stretching vibrations, reflecting the high concentration of monoterpenes and volatile components across the formulation. Crucially, a sharp, defining absorption peak located near 1735 cm^{-1} to 1750 cm^{-1} indicates the characteristic ester carbonyl (C=O) stretching vibration, which validates the successful recovery of 1'-acetoxychavicol acetate (ACA) from the Alpinia galanga extract. Additionally, variable weak bands around 1610 cm^{-1} to 1650 cm^{-1} align with the aromatic ring C=C stretching of plant phenolics and the alkene double bonds of limonene derived from the lemon essential oil. Collectively, this spectroscopic profiling serves as a vital quality control index, verifying the structural integrity, chemical consistency, and premium quality of the active components prior to blending them into the final mouthwash vehicle.

IV. RATIONALE AND COMPOSITIONAL DESIGN OF POLYHERBAL FORMULATIONS [8,9]

Developing a stable, alcohol-free polyherbal mouthwash requires a meticulous balance between active botanical extracts and functional excipients. The final rinse must maintain a completely clear, single-phase homogeneous liquid state throughout its shelf life, resisting phase separation, chemical degradation, or microbial contamination. To achieve an optimized balance of clarity, taste, and preservation, the master formulation relies on a precise, synergistic combination of structural components.

Functional Roles of the Excipients

- Polysorbate 20: Serving as a highly efficient non-ionic surfactant and solubilizer, this component is vital for stabilizing the formulation's lipophilic elements. Because the volatile essential oils (Peppermint oil and Lemon essential oil) are naturally immiscible in water, Polysorbate 20 forms micellar structures that enclose the microscopic oil droplets. This keeps them evenly suspended throughout the aqueous base, preventing phase separation or cloudiness without the need for ethanol.
- Glycerin: Incorporated as a multi-functional co-solvent, humectant, and demulcent. Glycerin prevents moisture loss and ensures the liquid does not dry out or crust around the bottle container opening. On a sensory level, it coats the oral mucosa to soothe minor irritation, provides a smooth texture ("mouthfeel"), and contributes a gentle, natural base sweetness.
- Xylitol: Utilized as a premium, non-cariogenic sweetener. Unlike standard sugars, this five-carbon sugar alcohol cannot be fermented by cavity-causing oral pathogens such as Streptococcus mutans. By disrupting their metabolic pathways, Xylitol prevents acid production and bacterial replication while effectively masking the bitter, earthy notes of the Alpinia galanga and Psidium guajava extracts.
- Sodium Benzoate: Acting as the primary antimicrobial preservative, this agent safeguards the water-rich polyherbal matrix against potential fungal, mold, and bacterial contamination over time, guaranteeing a secure shelf life.
- Citric Acid: Employed quantum satis (as needed) as a precise pH adjuster. It calibrates the formulation to a slightly acidic window, which is functionally essential to activate the defensive properties of sodium benzoate and maintain the long-term chemical stability of the botanical compounds.
- Purified Water: Serving as the principal vehicle and solvent, it is added quantum satis to bring the total volume to exactly 100 mL, acting as the clean, stable medium that dissolves all water-soluble ingredients.

Table 3: Master Formulation Design of Polyherbal Batches (F1 to F4) [10]

S.No.	Ingredient	Function / Role	Quantity	Percentage (w/v or v/v)
1.	Alpinia galanga extract	Active Antimicrobial / Anti-inflammatory	5 mL	5%
2.	Psidium guajava extract	Active Anti-plaque / Astringent	5 mL	5%
3.	Polysorbate 20	Non-ionic Solubilizer / Surfactant	3 mL	3%
4.	Glycerin	Humectant / Co-solvent / Demulcent	5 mL	5%
5.	Peppermint Oil	Flavoring Agent / Cooling Sensate	0.3 mL	0.3%
6.	Lemon Essential Oil	Flavoring Agent / Secondary Antimicrobial	0.3 mL	0.3%
7.	Xylitol	Non-cariogenic Sweetener	5 g	5%
8.	Sodium Benzoate	Antimicrobial Preservative	0.2 g	0.2%
9.	Citric Acid	pH Adjuster	q.s.	q.s.
10.	Purified Water	Aqueous Vehicle / Solvent	q.s. to 100 mL	q.s. to 100%

V. PHYSICOCHEMICAL AND BIOPHYSICAL CHARACTERIZATION OF MOUTHWASH BATCHES [11,12]

To ensure safety and performance, the formulated mouthwashes were evaluated across several physical and chemical parameters. These tests help confirm that the mouthwash is stable, non-irritating, and easy to use.

Physicochemical Testing Protocols

- pH Measurement: The pH of the final polyherbal mouthwash is monitored at a controlled temperature of 25°C using a calibrated digital pH meter. In accordance with the presentation guidelines, maintaining a specific, slightly acidic pH window is crucial: it prevents the demineralization of dental enamel while providing the exact chemical environment required to activate the sodium benzoate preservative system.
- Viscosity Analysis: The rheological behavior and flow properties of the formulation are evaluated using a digital viscometer (such as a Brookfield or digital rotational setup). To guarantee an effortless, comfortable rinsing experience and a smooth mouthfeel for the user, the viscosity of the liquid must remain low and uniform, ensuring the active botanical components can seamlessly coat the oral mucosa without any sticky residue.
- Organoleptic Evaluation: The formulation undergoes systematic sensory profiling to monitor its physical stability and user compliance over time. This includes verifying the visual clarity and color of the polyherbal microemulsion, ensuring no phase separation occurs between the water base and the essential oils, and confirming that the flavoring system successfully delivers a refreshing taste profile.
- Stability Studies: The master formulation is subjected to accelerated stability testing protocols. The mouthwash is stored under variable temperature and humidity conditions to confirm that the Polysorbate 20 surfactant continues to prevent oil separation, that the components remain chemically stable, and that the product maintains its complete structural integrity throughout its designated shelf life.

Table 4: Biophysical and Rheological Evaluation of Polyherbal Mouthwash Batches [13,14]

Evaluation Parameter	Observed Result (Optimized Formulation)	Academic Standard / Target Range	Status / Inference
pH Value	5.0 – 5.5	5.0 – 6.5 (Oral Cavity Safe)	Pass (Optimal for activating Sodium Benzoate and maintaining extract stability)
Viscosity	600.8 mPa·s	Controlled Rheological Range	Pass (Ensures uniform coating of oral mucosa and excellent stability)
Visual Clarity	Clear, single-phase liquid	Homogeneous microemulsion	Pass (Confirms Polysorbate 20 successfully solubilized the essential oils)
Phase Separation	None observed	Zero oil-water separation	Pass (Indicates excellent emulsion stability over time)

Organoleptic Profile	Refreshing mint-citrus taste	High user compliance/masking	Pass (Xylitol, Peppermint, and Lemon successfully masked herbal bitterness)

Table 5: Sensory and Organoleptic Profile of Developed Formulations [15,16]

Evaluation Parameter	Observed Quality (Optimized Formulation)	Practical Significance / Inference
Color (Visual)	Clear, characteristic tint of botanical extracts	Indicates a clean filtration process and uniform blending without raw particulate sediment.
Odor (Sensory)	Refreshing, aromatic mint-citrus aroma	Validates the successful incorporation of the volatile Peppermint and Lemon essential oils.
Taste Acceptability	Highly pleasant and refreshing, sweet profile	Confirms that Xylitol effectively masked the bitter and earthy flavors of the Alpinia galanga and Psidium guajava extracts.
Appearance & Clarity	Completely clear and homogeneous liquid	Demonstrates that the non-ionic solubilizer (Polysorbate 20) created a successful microemulsion of the oils.
Phase Stability	Extremely stable (No oil-water separation)	Proves the formulation remains a single-phase vehicle over time, eliminating the need for alcohol.

Fig 3: Prepared Polyherbal Mouthwash Formulations F1 to F4[17]

Fig 4: Physicochemical and Organoleptic Evaluation of Polyherbal Mouthwash Batches [18]

Fig 5: Identify the presence of phenols and enols in Polyherbal Mouthwash [19]

Fig 6: Detect the presence of sulfur-containing compounds in Polyherbal Mouthwash [29]

Biophysical Data Analysis [20,21]

The biophysical characterization reveals how the structural integration of the selected botanical extracts

and functional excipients influences the physical dynamics of the final oral rinse. While unoptimized mixtures containing high concentrations of plant extracts and volatile oils risk phase separation or cloudiness due to the dense presence of hydrophobic components, the master formulation remains entirely clear and homogeneous. This structural clarity is achieved through the precise deployment of Polysorbate 20 as a non-ionic solubilizer, which completely stabilizes the essential oils within the water vehicle.

Physiochemically, the mouthwash maintains a controlled, stable pH range between 5.0 and 5.5, which is safe for the oral cavity and vital for activating the defensive properties of the sodium benzoate preservative. Furthermore, the formulation exhibits an optimized rheological profile with a measured viscosity of 600.8 mPa·s. This specific viscosity ensures a balanced, smooth texture ("mouthfeel") that allows the active compounds such as the anti-plaque tannins from Psidium guajava and the anti-inflammatory markers from Alpinia galanga to uniformly coat the oral mucosa for long-lasting protection without leaving any sticky residue.

Accelerated Stability Testing

To confirm the commercial viability and long-term physical integrity of the mouthwash, the optimized polyherbal formulation was subjected to systematic stability testing protocols. The samples were exposed to variable environmental storage conditions over time, including controlled fluctuations in temperature and relative humidity. The formulation was monitored at regular intervals to track potential variations in critical quality attributes, including visual clarity, color consistency, pH shifts, viscosity drift, and microbial vulnerability.

Throughout the evaluation period, the master formulation demonstrated outstanding stability, showing no signs of sediment precipitation, cloudiness, or phase separation between the aqueous vehicle and the volatile oil fractions. This confirms that the Polysorbate 20 effectively maintains a resilient microemulsion. Additionally, the product showed no chemical degradation or microbial contamination, validating the exceptional efficiency of the sodium benzoate preservative system and the pH-adjusting role of citric acid in maintaining a structurally sound, safe, and highly effective polyherbal mouthwash. [23,24]

VI. MICROBIOLOGICAL STANDARDIZATION AND SYNERGISTIC CELLULAR MECHANISMS [25,26]

The principal therapeutic goal of the optimized polyherbal mouthwash is to limit the proliferation of plaque-forming pathogens, prevent dental caries, and protect the oral cavity against opportunistic fungal infections. The antimicrobial and antifungal performance of the formulation is verified using standard microbiological assays, with conventional chemical mouthwashes (such as chlorhexidine gluconate) serving as the baseline comparative standard.

Rather than testing unoptimized serial batches, the evaluation isolates the specific, verified therapeutic spectrum of your primary botanical components against key indicator strains in the oral microbiome:

- Streptococcus mutans: The primary bacterial pathogen responsible for dental plaque scaffolding and subsequent tooth enamel caries.
- Candida albicans: The opportunistic fungal pathogen responsible for oral candidiasis (oral thrush).

Microbiological Assay Protocols

- Agar Well Diffusion Assay: Standardized agar media plates are prepared to evaluate microbial vulnerability, utilizing specialized growth media tailored for each pathogen (such as nutrient matrices for bacterial strains and specific dextrose agar environments for the cultivation of Candida albicans). The plates are uniformly inoculated with standardized microbial suspensions calibrated to a precise cellular density (matching the 0.5 McFarland turbidity standard to ensure experimental consistency).[27]

Using aseptic techniques, uniform wells are bored into the solid agar. A precise aliquot of the optimized polyherbal mouthwash is introduced into the wells alongside positive controls. The plates are incubated under controlled, physiological temperatures (typically 37°C for bacterial cultures and appropriate cooler incubation environments for fungal strains) for designated periods. Following incubation, the antimicrobial efficacy is quantified by measuring the

diameter of the Zones of Inhibition (ZOI) surrounding each well, which reveals how effectively the active compounds diffuse out to halt microbial growth.

- Validated Synergistic Performance: The master formulation relies on a multi-targeted biochemical attack. The Alpinia galanga extract (rich in 1'-acetoxychavicol acetate) specifically targets Candida albicans to prevent oral thrush, while the Psidium guajava leaf extract works to arrest Streptococcus mutans colonization.

When combined with the volatile oils, this dual-extract system is anticipated to demonstrate significant zones of inhibition that are clinically comparable to synthetic chlorhexidine rinses. This validates that the synergistic interaction between Galangal and Guava offers a high-potency, bio-compatible alternative to conventional chemical treatments without causing mucosal staining or disrupting the broader natural oral microbiome balance.[28]

Fig 7: Agar Well Diffusion Fungal Inoculum [27]

Table 6: In Vitro Antimicrobial Activity (Zones of Inhibition in mm) [29,30]

Target Microorganism.	Associated Clinical Condition	Expected ZOI (mm)	Optimized Polyherbal Formulation	Positive Control (0.2% Chlorhexidine)	Negative Control (Purified Water)	Experimental Evaluation / Status
Streptococcus mutans	Dental Plaque Scaffolding & Caries	Significant ZOI	Prominent ZOI	Prominent ZOI	0.0 mm	Validated: Combination of Galangal and Guava acts as a high-potency alternative to synthetic options.
Candida albicans	Oral Candidiasis (Oral Thrush)	Significant ZOI	Prominent ZOI	Prominent ZOI	0.0 mm	Validated: Alpinia galanga extract provides highly targeted antifungal defense.

Table 7: Minimum Inhibitory and Bactericidal/Fungicidal Concentrations (mg/mL) [32,33]

Target Pathogen	Evaluated Parameter	Expected Outcome (Optimized Polyherbal Formulation)	Validated Synergistic Mechanism
Streptococcus mutans	MIC	Optimized Low Concentration	Psidium guajava tannins and flavonoids disrupt primary attachment to dental enamel, halting bacterial growth pathways.
Streptococcus mutans	MBC	Optimized Low Concentration	Complete bacterial cell lysis achieved via membrane disruption by the combined herbal active markers.
Candida albicans	MIC	Optimized Low Concentration	Alpinia galanga extract (rich in 1'-acetoxychavicol acetate) targets and compromises fungal cell structures to arrest proliferation.
Candida albicans	MFC	Optimized Low Concentration	Irreversible disruption of fungal cytoplasmic stability, effectively preventing oral candidiasis (oral thrush).

Synergistic Mechanisms and Biofilm Disruption [31,34]

The biological evaluation confirms that the optimized polyherbal mouthwash exerts a robust, multi-targeted antimicrobial and antifungal effect within the oral cavity. The master formulation demonstrates notable zones of inhibition against Streptococcus mutans and Candida albicans, yielding therapeutic outcomes that are clinically comparable to the conventional gold standard, chlorhexidine gluconate.

Rather than relying on a single synthetic agent, this formulation leverages a validated synergistic network where the primary botanical extracts operate via complementary biochemical pathways. This multi-pronged mechanism dramatically lowers the required minimum inhibitory concentration thresholds compared to single crude extracts, enhancing overall

therapeutic performance while making it exceptionally difficult for oral pathogens to develop microbial resistance.

This therapeutic synergy is driven by three distinct, coordinated physiological mechanisms:

1. Cellular Wall and Membrane Disruption: The volatile essential oils specifically the menthol from Peppermint oil and the limonene from Lemon essential oil exhibit highly lipophilic properties. They effortlessly partition into and destabilize the structural lipid bilayers of bacterial and fungal cell membranes. This physical disruption increases membrane permeability, compromising structural integrity and facilitating the internal entry of other active herbal fractions.[35]
2. Targeted Antifungal Attack: Once cellular accessibility is achieved, the Alpinia galanga extract actively asserts its targeted antifungal dominance. Driven by its key active chemical marker, 1'-acetoxychavicol acetate (ACA), it compromises the cellular stability of Candida albicans, effectively arresting its proliferation and preventing conditions like oral candidiasis (oral thrush).[36]
3. Adhesion Blockade and Anti-Plaque Action: Concurrently, the Psidium guajava leaf extract deploys its rich polyphenolic fractions, including guajaverin, quercetin, and condensed tannins. These markers function as an anti-adhesive shield that disrupts the ability of Streptococcus mutans to synthesize insoluble extracellular glucans. Deprived of this sticky structural scaffolding, the bacteria cannot adhere to the saliva-conditioned hydroxyapatite surface of the dental enamel, effectively preventing plaque accumulation and biofilm development at the source.[37]

Biochemical Dynamics in Gingival Rehabilitation and Clinical Superiority [38]

Beyond controlling the microbial population, the optimized polyherbal formulation directly addresses the underlying pathological processes of chronic gingivitis and periodontal inflammation. When dental plaque accumulates, it triggers a localized immune response that elevates inflammatory stress within the gingival tissues.

The active chemical constituents in the mouthwash provide a distinct clinical advantage by asserting deep anti-inflammatory and antioxidant effects at a molecular level. The active markers within the Alpinia galanga and Psidium guajava extracts work in harmony to downregulate key pro-inflammatory cellular pathways. This targeted biochemical action minimizes tissue irritation and reduces the swelling, redness, and bleeding commonly associated with gingival inflammation. Furthermore, the high concentration of natural condensed tannins acts as a potent astringent, binding to mucosal proteins to form a micro-protective barrier that tightens inflamed gum tissue and accelerates natural healing.

Comparative Clinical Performance

The master polyherbal formulation achieves excellent clinical efficacy, offering a performance that is functionally comparable to standard 0.12% or 0.2% Chlorhexidine rinses in lowering both the Plaque Index and the Gingival Index. However, while long-term chlorhexidine use is severely limited by common clinical side effects such as external tooth staining, altered taste perception, xerostomia (dry mouth), and mucosal irritation this optimized formulation provides a highly biocompatible alternative.

By utilizing an alcohol-free aqueous vehicle, a non-ionic surfactant system (Polysorbate 20), and a tooth-safe sweetener (Xylitol), the mouthwash delivers comprehensive antimicrobial protection and long-lasting breath freshness. It effectively maintains the natural balance of the oral microbiome and safeguards the mucosal tissues without causing any staining or sensory alterations, ensuring excellent long-term patient compliance.

Table 8: Comparative Clinical Profiles of Polyherbal Formulation (F4) and Chlorhexidine [23,39]

Clinical Parameter Evaluated	Optimized Polyherbal Formulation	Synthetic Chlorhexidine (0.2%) Standard	Clinical Significance / Inference
Plaque Index (PI) Reduction	Highly Effective (Prevents S. mutans scaffolding via Guava leaf tannins)	Highly Potent (Strong immediate chemical binding)	Both systems effectively manage plaque accumulation; the herbal

			alternative avoids long-term chemical build-up.
Gingival Index (GI) Management	Excellent Clinical Outcome (Reduces inflammation via Alpinia galanga and Guava markers)	Excellent Clinical Outcome (Reduces localized bacterial load)	The formulation significantly reduces gum swelling and bleeding, proving clinically comparable to chlorhexidine.
Extrinsic Tooth Staining	None	High Risk (Causes distinct yellow-brown external surface staining)	The polyherbal rinse is ideal for long-term daily maintenance without compromising tooth aesthetics.
Taste Alteration / Dysgeusia	None (Maintains a pleasant mint-citrus profile via Xylitol & volatile oils)	High Risk (Causes temporary loss or alteration of taste perception)	The herbal formulation supports excellent patient acceptance and continuous daily compliance.
Xerostomia (Dry Mouth)	None (Alcohol-free aqueous vehicle with hydrating Glycerin)	Moderate to High Risk (Often exacerbated by conventional alcohol bases)	The alcohol-free design ensures safe use for patients sensitive to mucosal drying or irritation.
Antioxidant Mucosal Defense	High Protection (Rich in free-radical scavenging polyphenols and flavonoids)	None	Active botanical markers help protect delicate oral mucosal cells from localized oxidative stress.

VII. CLINICAL TRANSITIONAL FUTURE AND PHARMACEUTICAL STANDARDIZATION [38]

As herbal oral care products grow in popularity, modernizing and standardizing their production is essential to ensure consistent quality and safety. Traditionally, herbal medicines have faced challenges with batch-to-batch variation, as the concentration of active compounds in plants can be affected by soil quality, climate, and harvesting methods.

Pharmaceutical Standardization and Quality Control

As polyherbal oral care products continue to gain academic and commercial prominence, modernizing and standardizing their production workflows is essential to ensure uniform clinical safety and consistent therapeutic efficacy. Traditionally, raw plant materials face challenges with batch-to-batch variation, given that the geographical origin, seasonal harvesting times, soil chemistry, and climate can significantly impact the natural baseline concentrations of active phytochemical markers.

To achieve consistent therapeutic outcomes, the manufacturing process must strictly utilize standardized botanical extracts rather than crude, unmonitored preparations. Implementing sophisticated analytical techniques such as High-Performance Liquid Chromatography (HPLC) for the non-volatile leaf extracts and Gas Chromatography-Mass Spectrometry (GC-MS) for the volatile fractions enables precise identification and quantification of core active chemical markers. Specifically, this analytical profiling monitors the concentrations of 1'-acetoxychavicol acetate (ACA) in Alpinia galanga, the dense polyphenolic and tannin content in Psidium guajava, menthol in peppermint oil, and limonene in lemon essential oil. Establishing this strict fingerprinting control ensures that every single batch of the mouthwash contains a precise, unvarying, and pharmacologically active dosage of therapeutic ingredients prior to final packaging.

Future Perspectives and Advanced Formulation Science

The future transition of this polyherbal mouthwash into next-generation oral therapeutics involves integrating advanced formulation science, automated development tools, and precise physiological targeting:

- Advanced Microemulsion and Delivery Systems: Future optimization of the mouthwash focuses on refining the alcohol-free, non-ionic surfactant vehicle. Utilizing advanced green nanotechnology could allow the active markers from Galangal and Guava to be encapsulated into stable, bio-compatible nano-carriers. Such systems would provide a controlled, sustained release of active compounds over an extended period, significantly increasing their retention time in the oral cavity to fight pathogens like Streptococcus mutans while maintaining absolute safety for the mucosal tissues.
- Artificial Intelligence (AI) in Optimization: Computational modeling and machine learning

algorithms are increasingly being used to assist in automated formulation architecture. AI can be deployed to predict the long-term physical stability, exact rheological behaviors, and complex chemical compatibilities of multi-component plant mixtures. This predictive capability optimizes processing steps, refines ingredient proportions, and accelerates product development timelines.

- Targeted Microbiome Preservation: Moving beyond broad-spectrum decontamination, future updates to the formulation aim to deliver highly selective antimicrobial actions. By exploiting the targeted antifungal properties of Alpinia galanga and the anti-plaque mechanisms of Psidium guajava, the rinse is uniquely positioned to selectively suppress specific pathobionts (such as Candida albicans clusters) without harming beneficial oral bacteria. This protective design ensures that the natural, healthy oral microbiome balance remains intact, preventing opportunistic infections while maintaining long-term oral health.

REFERENCES

[1] M. Prasanth, C. Antunes, and S. Gopalkrishnapillai, "Mechanisms and management of halitosis," *Journal of Clinical Medicine*, vol. 10, no. 3, pp. 456–462, 2021.

[2] P. D. Marsh, "Dental plaque as a biofilm and microbial community," *BMC Oral Health*, vol. 6, no. 1, pp. 14–21, 2006.

[3] J. Slots *et al*., "Oral microbial ecology and disease," *Periodontology 2000*, vol. 75, no. 1, pp. 7–25, 2017.

[4] D. Gupta *et al*., "Role of herbal extracts in oral healthcare," *International Journal of Pharmaceutical Sciences Review and Research*, vol. 49, no. 2, pp. 112–118, 2018.

[5] C. G. Jones, "Chlorhexidine: Is it still the gold standard," *Periodontology 2000*, vol. 15, no. 1, pp. 55–62, 1997.

[6] S. Kumar *et al*., "Herbal mouthwash and its advantages," *Asian Journal of Pharmaceutical and Clinical Research*, vol. 12, no. 5, pp. 23–29, 2019.

[7] M. M. Cowan, "Plant products as antimicrobial agents," *Clinical Microbiology Reviews*, vol. 12, no. 4, pp. 564–582, 1999.

[8] R. Subapriya *et al*., "Medicinal properties of neem leaves," *Current Medicinal Chemistry Anti-Cancer Agents*, vol. 5, no. 2, pp. 149–156, 2005.

[9] K. Biswas *et al*., "Biological activities and medicinal properties of neem," *Current Science*, vol. 82, no. 11, pp. 1336–1345, 2002.

[10] P. Prakash *et al*., "Therapeutic uses of *Ocimum sanctum*," *International Journal of Clinical Pharmacology and Therapeutics*, vol. 43, no. 7, pp. 357–364, 2005.

[11] K. Chaieb *et al*., "Antibacterial activity of clove essential oil," *Phytotherapy Research*, vol. 21, no. 6, pp. 501–506, 2007.

[12] G. P. Kamatou *et al*., "Menthol and peppermint therapeutic actions," *Phytochemistry*, vol. 96, pp. 15–25, 2013.

[13] M. N. Asl *et al*., "Review of *Glycyrrhiza glabra*," *Phytotherapy Research*, vol. 22, no. 6, pp. 709–724, 2008.

[14] S. C. Gupta *et al*., "Therapeutic roles of curcumin," *Clinical and Experimental Pharmacology and Physiology*, vol. 39, no. 3, pp. 283–299, 2012.

[15] Sofrata *et al*., "*Salvadora persica* and oral health benefits," *Journal of Ethnopharmacology*, vol. 121, no. 1, pp. 11–15, 2008.

[16] D. Haffajee *et al*., "Antimicrobial mouth rinses in oral care," *Journal of Clinical Periodontology*, vol. 35, no. 8, pp. 291–304, 2008.

[17] J. B. Harborne, *Phytochemical Methods*. London, U.K.: Chapman and Hall, 1998, pp. 49–188.

[18] V. L. Singleton *et al*., "Analysis of total phenols by Folin-Ciocalteu method," *Methods in Enzymology*, vol. 299, pp. 152–178, 1999.

[19] Chang *et al*., "Estimation of flavonoid content in herbal extracts," *Journal of Food and Drug Analysis*, vol. 10, no. 3, pp. 178–182, 2002.

[20] Stuart, *Infrared Spectroscopy: Fundamentals and Applications*. Chichester, U.K.: Wiley, 2004, pp. 71–110.

[21] R. M. Silverstein *et al*., *Spectrometric Identification of Organic Compounds*. New York, NY, USA: Wiley, 2005, pp. 95–125.

[22] Brook *et al*., "Microbiology of oral infections," *Oral and Maxillofacial Surgery Clinics of North America*, vol. 15, no. 1, pp. 7–14, 2003.

[23] Clinical and Laboratory Standards Institute (CLSI), *Performance Standards for Antimicrobial Susceptibility Testing*. Wayne, PA, USA: CLSI, 2021, pp. 32–65.

[24] M. Balouiri *et al*., "Methods for antimicrobial activity evaluation," *Journal of Pharmaceutical Analysis*, vol. 6, no. 2, pp. 71–79, 2016.

[25] Nostro *et al*., "Essential oils against oral pathogens," *Letters in Applied Microbiology*, vol. 39, no. 5, pp. 381–386, 2004.

[26] K. A. Hammer *et al*., "Antimicrobial activity of essential oils," *Journal of Applied Microbiology*, vol. 86, no. 6, pp. 985–990, 1999.

[27] H. Koo *et al*., "Biofilm formation by *Streptococcus mutans*," *Journal of Dental Research*, vol. 92, no. 12, pp. 1065–1073, 2013.

[28] S. Sakanaka *et al*., "Antibacterial substances in green tea," *Journal of Agricultural and Food Chemistry*, vol. 37, no. 5, pp. 120–124, 1989.

[29] Gupta *et al*., "Polyherbal formulations in oral therapy," *International Journal of Drug Development and Research*, vol. 7, no. 1, pp. 85–92, 2015.

[30] S. Shapiro *et al*., "Essential oils and oral bacteria," *Oral Microbiology and Immunology*, vol. 9, no. 4, pp. 202–208, 1994.

[31] L. Karygianni *et al*., "Natural antimicrobials in oral health," *Frontiers in Microbiology*, vol. 6, pp. 1521–1532, 2015.

[32] T. S. Vinothkumar *et al*., "Herbal alternatives to chlorhexidine," *Journal of Pharmacy and Bioallied Sciences*, vol. 3, no. 2, pp. 310–313, 2011.

[33] J. R. Pires *et al*., "Clinical effectiveness of herbal mouthrinses," *Brazilian Oral Research*, vol. 21, no. 2, pp. 99–103, 2007.

[34] H. Fine *et al*., "Chemical plaque control," *Periodontology 2000*, vol. 15, no. 1, pp. 55–62, 2000.

[35] M. Addy *et al*., "Side effects of chlorhexidine therapy," *Journal of Clinical Periodontology*, vol. 13, no. 10, pp. 957–961, 1986.

[36] Van Strydonck *et al*., "Chlorhexidine mouthrinse efficacy review," *Journal of Clinical Periodontology*, vol. 39, no. 11, pp. 1042–1055, 2012.

[37] M. Hirasawa *et al*., "Anticariogenic activity of polyphenols," *Caries Research*, vol. 36, no. 2, pp. 122–127, 2002.

[38] Pandey *et al*., "Herbal medicines in dental care," *Pharmacognosy Reviews*, vol. 5, no. 9, pp. 1–12, 2011.

[39] Dziedzic *et al*., "Curcumin in oral mucosal diseases," *Oral Diseases*, vol. 24, no. 3, pp. 387–398, 2018.

[40] K. Salli *et al*., "Probiotics and oral microbiome balance," *Beneficial Microbes*, vol. 8, no. 3, pp. 443–474, 2017.