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FORMULATION DEVELOPMENT AND QUALITY EVALUATION OF A POLYHERBAL TOOTHPASTE INCORPORATING TRADITIONAL MEDICINAL PLANT EXTRACTS

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Keywords:

Polyherbal toothpaste; *Azadirachta indica*; *Salvadorapersica*; *Acacia nilotica*; *Punica granatum*; Herbal formulation; Antimicrobial activity

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ABSTRACT

Background: Herbal toothpastes are increasingly preferred as natural alternatives to conventional oral care products because of their antimicrobial, anti-inflammatory, antioxidant, and astringent properties. The present study aimed to formulate and evaluate a polyherbal toothpaste containing neem (*Azadirachta indica*), miswak (*Salvadorapersica*), babool (*Acacia nilotica*), and pomegranate peel (*Punica granatum*) for improved oral hygiene. Methods: Herbal extracts were prepared using hydroalcoholic maceration and aqueous decoction methods, while miswak was processed into fine powder. The raw materials were subjected to pharmacognostic, physicochemical, and preliminary phytochemical evaluation. Nine toothpaste formulations (F1–F9) were developed and evaluated for organoleptic characteristics, pH, viscosity, spreadability, extrudability, foaming ability, homogeneity, abrasiveness, antimicrobial activity against *Streptococcus mutans, Staphylococcus aureus, Escherichia coli,* and *Candida albicans*, and stability over three months. Results: Phytochemical screening confirmed the presence of flavonoids, tannins, phenolic compounds, saponins, glycosides, and terpenoids in the selected herbal extracts. All formulations exhibited acceptable physicochemical and organoleptic properties. Formulations F5 and F6 demonstrated the most balanced physical characteristics, while F8 showed the highest antimicrobial activity against all tested microorganisms, with inhibition zones comparable to or greater than those of the marketed toothpaste. The optimized formulation remained stable throughout the 3-month study, showing no phase separation, microbial contamination, or significant changes in physicochemical properties. Conclusion: The developed polyherbal toothpaste demonstrated desirable quality attributes, significant antimicrobial activity, and good storage stability, indicating its potential as a safe and effective natural oral care formulation for maintaining oral hygiene and preventing common dental disorders.

INTRODUCTION

Oral health is an essential component of general health and well-being. Dental diseases such as dental caries, gingivitis, periodontitis, plaque formation, halitosis, and oral microbial infections remain major public health concerns worldwide [1]. These conditions are primarily associated with the accumulation of pathogenic microorganisms, particularly *Streptococcus mutans*, along with poor oral hygiene practices [2]. Toothpaste is one of the most commonly used oral care products for maintaining oral cleanliness, preventing plaque accumulation, and promoting healthy teeth and gums [3]. Although conventional toothpastes effectively control oral microorganisms, prolonged use of formulations containing synthetic antimicrobial agents, artificial preservatives, and chemical additives may be associated with adverse effects such as tooth staining, altered taste sensation, mucosal irritation, and the development of microbial resistance [4].

Herbal oral care products have gained considerable attention as safer and eco-friendly alternatives to synthetic formulations [5]. Medicinal plants contain a wide variety of bioactive phytoconstituents, including flavonoids, tannins, phenolic compounds, alkaloids, glycosides, saponins, and terpenoids, which possess antimicrobial, anti-inflammatory, antioxidant, and astringent properties [6]. These natural constituents help inhibit the growth of oral pathogens, reduce inflammation, strengthen gingival tissues, minimize plaque formation, and maintain overall oral hygiene while exhibiting minimal adverse effects [7].

In the present study, four well-known medicinal plants were selected based on their established therapeutic potential in oral care. Neem (*Azadirachta indica*) possesses broad-spectrum antimicrobial, anti-inflammatory, and antioxidant activities. Miswak (*Salvadorapersica*) has traditionally been used as a natural chewing stick and is recognized for its mechanical cleansing ability, plaque-removing action, and antibacterial properties. Babool (*Acacia nilotica*) is rich in tannins that exhibit astringent effects, helping to strengthen gums and reduce gingival bleeding. Pomegranate peel (*Punica granatum*) contains abundant polyphenols and flavonoids with potent antioxidant, antimicrobial, and anti-inflammatory activities that contribute to improved oral health.

The present investigation aimed to formulate and evaluate a polyherbal toothpaste by incorporating extracts of neem, babool bark, pomegranate peel, and miswak powder. The prepared formulations were evaluated for pharmacognostic and physicochemical characteristics, phytochemical profile, antimicrobial activity against selected oral pathogens, and storage stability. The study was undertaken to develop a safe, effective, and stable herbal toothpaste that may serve as a promising natural alternative to conventional oral care products for maintaining oral hygiene and preventing common dental disorders.

MATERIALS AND METHODS

Collection and Procurement of Raw Materials

Selected herbal materials including neem, clove, miswak, babool bark, and pomegranate peel shall be procured from authenticated suppliers or crude drug markets. Pharmaceutical excipients shall be obtained from approved suppliers. All materials shall be examined for identity, appearance, purity, and absence of foreign matter before storage in clean, dry, airtight containers until use.

Authentication of Herbal Raw Materials

The herbal materials shall be authenticated using standard Pharmacognostic characteristics or by the Department of Pharmacognosy. Macroscopic evaluation (size, shape, colour, odour, taste, and texture) shall be performed, and foreign matter removed. Authenticated samples shall be properly labelled and used for formulation.

Extraction of Herbal Ingredients

Bioactive constituents from neem leaves, babool bark, and pomegranate peel shall be extracted using suitable extraction methods based on their phytochemical composition. These extracts provide antimicrobial, antioxidant, anti-inflammatory, and astringent activities. Miswak shall be used in powdered form due to its natural fibrous structure and mineral content, which aid in plaque control and mechanical cleansing. The prepared extracts and miswak powder shall be used in the formulation of the polyherbal toothpaste [8].

Extraction of Neem Leaves (*Azadirachta indica*) – Hydroalcoholic Maceration Method

Fresh neem leaves were washed, shade-dried, powdered, and sieved. About 50 g of powder was macerated with 250–300 mL of 70:30 ethanol–water for 48–72 h with occasional shaking. The extract was filtered, concentrated under reduced pressure or on a water bath, dried, and stored in an airtight amber container. The hydroalcoholic extract was used as an antimicrobial and anti-inflammatory ingredient in the toothpaste formulation [9].

Preparation of Miswak Powder (*Salvadorapersica*) – Drying, Pulverization and Sieving Method

Miswak sticks were cleaned, cut into small pieces, shade-dried, pulverized, and passed through a suitable mesh sieve to obtain a fine powder. The powder was stored in an airtight labelled container. Miswak was incorporated directly into the formulation to provide mechanical cleansing, plaque control, and oral hygiene benefits.

Extraction of Babool Bark (*Acacia nilotica*) – Aqueous Decoction Method

Cleaned and dried babool bark was powdered and sieved. Approximately 50 g of powder was boiled with 500 mL of distilled water for 1–2 h with occasional stirring. The decoction was cooled, filtered, concentrated on a water bath, dried, and stored in an airtight container. The extract was used as an astringent and gum-strengthening agent.

Extraction of Pomegranate Peel (*Punica granatum*) – Hydroalcoholic Maceration Method

Pomegranate peels were washed, shade-dried, powdered, and sieved. About 50 g of powder was extracted with 250–300 mL of 70:30 ethanol–water by maceration for 48–72 h. The extract was filtered, concentrated, dried, and stored in an airtight amber container. The hydroalcoholic extract was incorporated into the toothpaste formulation for its antimicrobial, antioxidant, and astringent properties [9].

Macroscopic Examination

The crude herbal materials shall be examined visually for colour, size, shape, surface characteristics, fracture, odour, taste, and presence of foreign matter to confirm their identity, purity, and quality before use.

Organoleptic Characterization of Herbal Materials

Organoleptic evaluation shall be carried out by assessing the colour, odour, taste, texture, and appearance of each herbal material to aid in preliminary identification and detection of adulteration or deterioration [10].

Physicochemical Standardization of Herbal Materials

Phytochemical Screening for Herbal Extracts

Prepared extracts of neem, babool bark, and pomegranate peel shall be subjected to preliminary phytochemical screening for alkaloids, flavonoids, tannins, saponins, glycosides, phenolic compounds, and terpenoids using standard qualitative tests. The extracts shall be dissolved in a suitable solvent, filtered if required, and used as test solutions.

Determination of Moisture Content (Loss on Drying)

A known quantity of powdered drug shall be dried at 105°C until constant weight is obtained. Moisture content shall be calculated from the

percentage weight loss to assess the stability and storage quality of the herbal material.

Determination of Total Ash Value

A known quantity of powdered drug shall be incinerated in a muffle furnace until carbon-free ash is obtained. The total ash value shall be calculated to determine the total inorganic content of the crude drug.

Determination of Acid-Insoluble Ash

The total ash shall be treated with dilute hydrochloric acid, and the insoluble residue shall be filtered, ignited, and weighed. This test estimates siliceous impurities such as sand and soil.

Determination of Water-Soluble Ash

The total ash shall be boiled with distilled water, and the insoluble residue shall be collected, ignited, and weighed. Water-soluble ash shall be calculated by subtracting the insoluble ash from the total ash, indicating the amount of water-soluble inorganic matter present [11].

Formulation of Polyherbal Toothpaste

Table 1. Composition of tootpaste

Ingredients	Category / Function	F1	F2	F3	F4	F5	F6	F7	F8	F9
Neem extract (% w/w)	Antibacterial, anti-inflammatory	1.0	1.0	1.0	1.5	1.5	1.5	2.0	2.0	2.0
clove oil(% w/w)	Analgesic, antiseptic	0.5	0.5	0.5	0.75	0.75	0.75	1.0	1.0	1.0
Miswak powder (% w/w)	Plaque control, cleansing	1.0	1.0	1.0	1.5	1.5	1.5	2.0	2.0	2.0
Babool bark extract (% w/w)	Astringent, gum strengthening	1.0	1.0	1.0	1.5	1.5	1.5	2.0	2.0	2.0
Pomegranate peel extract (% w/w)	Antioxidant, antimicrobial	1.0	1.0	1.0	1.5	1.5	1.5	2.0	2.0	2.0
Tea tree oil (% w/w)	Antimicrobial, antifungal	0.2	0.2	0.2	0.3	0.3	0.3	0.4	0.4	0.4
Calcium carbonate (% w/w)	Abrasive / cleaning agent	35	35	35	33	33	33	31	31	31
Hydrated silica (% w/w)	Abrasive / polishing agent	8	8	8	8	8	8	8	8	8
Glycerin(% w/w)	Humectant	15	15	15	15	15	15	15	15	15
Sorbitol solution(% w/w)	Humectant / sweet base	10	10	10	10	10	10	10	10	10
Sodium CMC(% w/w)	Binder / thickener	1.0	1.5	2.0	1.0	1.5	2.0	1.0	1.5	2.0
guar gum(% w/w)	Binder / stabilizer	0.5	0.5	0.5	0.75	0.75	0.75	1.0	1.0	1.0
Sodium lauryl sulfate (% w/w)	Foaming agent	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Stevia (% w/w)	Sweetener	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Peppermint oil(% w/w)	Flavouring agent	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Sodium benzoate(% w/w)	Preservative	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Purified water(% w/w)	Vehicle	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.
Total		100	100	100	100	100	100	100	100	100

All ingredients for formulations (F1–F9) shall be accurately weighed using a digital balance, labelled batch-wise, and kept separately to avoid cross-contamination. Sodium CMC and guar gum shall be dispersed in purified water with continuous stirring and allowed to hydrate. Glycerin may be added to obtain a smooth binder mucilage. Glycerin and sorbitol shall be mixed, followed by the addition of dissolved sodium benzoate and sweetener to obtain a

uniform liquid phase. Calcium carbonate, hydrated silica, and herbal powders shall be sieved and blended uniformly by geometric dilution. The powder blend shall be gradually incorporated into the binder mucilage with continuous mixing until a smooth paste is formed. Purified water shall be added to adjust consistency. Herbal extracts shall be incorporated into the prepared base with continuous stirring at room temperature to ensure uniform distribution and preserve active constituents. Sodium lauryl sulfate or a suitable mild surfactant shall be added with gentle mixing to minimize foam formation. Peppermint oil, clove oil, and tea tree oil shall be incorporated at the final stage after dispersing them in glycerin to ensure uniform mixing. The formulation shall be homogenized until a smooth, uniform, and lump-free paste is obtained without excessive aeration. The prepared toothpaste shall be filled into clean collapsible tubes or suitable containers, sealed, and labelled with formulation code, batch number, and date of preparation. The formulations shall be stored at room temperature in clean, dry conditions for evaluation and stability studies.

Evaluation Procedure of Polyherbal Toothpaste

Organoleptic Evaluation

The formulations shall be evaluated for colour, odour, taste, texture, appearance, smoothness, grittiness, and phase separation.

Determination of pH

The pH of a 1% w/v toothpaste dispersion shall be measured using a calibrated digital pH meter.

Determination of Viscosity

Viscosity shall be determined using a Brookfield viscometer under standardized operating conditions.

Homogeneity Test

The toothpaste shall be examined visually for uniformity, absence of lumps, coarse particles, and phase separation.

Consistency Test

Consistency shall be assessed by evaluating smoothness, thickness, spreadability, and any signs of liquid separation.

Spreadability Test

Spreadability shall be determined by measuring the spreading diameter or movement between two glass slides under a fixed weight.

Extrudability Test

Extrudability shall be evaluated by measuring the amount of toothpaste extruded from collapsible tubes under a fixed pressure.

Foaming Ability

Foaming ability shall be determined by measuring the foam volume produced after shaking a toothpaste dispersion.

Foam Retention

Foam stability shall be evaluated by recording the foam volume after predetermined time intervals.

Abrasiveness Test

The abrasiveness of the formulation shall be assessed on a glass surface and compared with a marketed toothpaste to ensure effective cleaning without excessive scratching.

Determination of Moisture Content of Toothpaste

Moisture content shall be determined by the loss-on-drying method using a hot air oven until constant weight is achieved.

Phytochemical Screening Procedure

Toothpaste extracts shall be subjected to standard qualitative tests for alkaloids, flavonoids, tannins, saponins, glycosides, phenolic compounds, and terpenoids.

Microbiological Evaluation Procedure

The antimicrobial activity of the formulated polyherbal toothpaste shall be evaluated by the agar well diffusion method against *Streptococcus mutans*. Mueller–Hinton agar shall be prepared, sterilized at 121°C and 15 psi for 15 min, poured into sterile Petri plates, and allowed to solidify. A standardized bacterial suspension shall be uniformly spread over the agar surface using a

sterile cotton swab. Wells (5 mm diameter) shall be prepared using a sterile cork borer, and a fixed volume of the toothpaste suspension shall be introduced into each well. Amoxicillin and a marketed toothpaste shall serve as the standard and reference, respectively, while sterile distilled water or the solvent shall be used as the control. The plates shall be allowed to stand for diffusion and then incubated at 37°C for 24 h. Antimicrobial activity shall be assessed by measuring the zone of inhibition (mm) around each well and comparing the results with the standard and marketed formulation [12-15].

Stability Study Procedure

The prepared toothpaste formulations were packed in suitable containers, properly labelled, and stored at room temperature and accelerated conditions, such as elevated temperature and humidity, depending on available facilities. At selected time intervals, samples were withdrawn and evaluated for stability. The formulations were examined for colour, odour, appearance, texture, pH, viscosity, consistency, homogeneity, phase separation, and microbial load. During each evaluation, the container was opened carefully and a small quantity of sample was removed under clean conditions for testing. Care was taken to avoid contamination of the remaining sample. The same procedure was followed at every stability study interval [16].

RESULTS & DISCUSSION

Results of extraction and powder preparation

The selected herbal materials were processed for extract or powder preparation. Neem leaves, babool bark and pomegranate peel were extracted using suitable solvents, while miswak was used in fine powder form. The percentage yield was calculated with respect to the initial dry weight of plant material.

Table 2. Extractive yield of selected herbal ingredients

Material	Method Used	Initial Weight	Final Yield	% Yield (mean ± SD), n=3	Physical Nature
Neem leaves	Hydroalcoholic maceration	50 g	6.23 g	12.46 ± 0.21	Dark green semi-solid extract
Miswak sticks	Drying, pulverization and sieving	50 g	44.20 g	88.40 ± 0.30	Fine brown powder
Babool bark	Aqueous decoction	50 g	7.66 g	15.32 ± 0.18	Dark brown thick extract
Pomegranate peel	Hydroalcoholic maceration	50 g	9.38 g	18.75 ± 0.25	Reddish brown extract

The extraction and preparation of selected herbal ingredients showed satisfactory yields suitable for toothpaste formulation. Among the extracts, pomegranate peel exhibited the highest extractive yield (18.75 ± 0.25%), followed by babool bark (15.32 ± 0.18%) and neem leaves (12.46 ± 0.21%), indicating efficient recovery of bioactive phytoconstituents. Miswak produced the highest overall yield (88.40 ± 0.30%) as it was processed into powder rather than extracted, making it suitable for direct incorporation into the formulation. The results are expressed as mean ± SD (n = 3), demonstrating the reproducibility of the extraction and preparation procedures.

Macroscopic and Organoleptic Characters of Herbal Materials

Table 3. Macroscopic and organoleptic characters of herbal materials

Herbal material	Colour	Odour	Taste	Texture/appearance
Neem leaves	Green to dark green	Characteristic	Bitter	Dried leaf powder/extract
Miswak	Light brown	Slight characteristic	Mild pungent	Fibrous fine powder
Babool bark	Brown to dark brown	Characteristic	Astringent	Coarse bark/fine powder
Pomegranate peel	Reddish brown	Characteristic	Astringent	Dried peel powder/extract

The selected herbal materials were subjected to organoleptic evaluation based on colour, odour, taste, and texture to confirm their identity and quality. Neem exhibited a characteristic bitter taste, miswak showed a light brown fibrous powder with mild pungency, while babool bark and pomegranate peel displayed an astringent taste due to their tannin content. The observed characteristics were consistent with standard pharmacognostic descriptions, indicating the suitability of the selected herbal materials for polyherbal toothpaste formulation.

Physicochemical Standardization of Herbal Materials

Table 4. Physicochemical standardization of herbal materials (mean ± SD, n=3)

Parameter (% w/w)	Neem leaves	Miswak powder	Babool bark	Pomegranate peel
Moisture content	6.48 ± 0.12	5.72 ± 0.10	7.21 ± 0.15	6.84 ± 0.13
Total ash value	8.15 ± 0.18	9.42 ± 0.20	6.88 ± 0.16	5.96 ± 0.11
Acid-insoluble ash	1.12 ± 0.04	1.35 ± 0.05	0.94 ± 0.03	0.82 ± 0.04
Water-soluble ash	3.26 ± 0.08	3.88 ± 0.09	2.75 ± 0.07	2.46 ± 0.06
Alcohol-soluble extractive value	16.42 ± 0.25	10.25 ± 0.18	12.36 ± 0.21	20.18 ± 0.28
Water-soluble extractive value	18.55 ± 0.30	14.36 ± 0.20	19.62 ± 0.26	22.45 ± 0.32

The physicochemical evaluation demonstrated that all selected herbal materials complied with acceptable quality standards. Low moisture content and acid-insoluble ash values indicated good stability, cleanliness, and minimal contamination, while total ash values were within permissible limits. Pomegranate peel exhibited the highest alcohol- and water-soluble extractive values, reflecting a rich content of bioactive phytoconstituents, whereas miswak showed the highest total ash and water-soluble ash due to its natural mineral content. Overall, the results confirmed the suitability of the selected herbal materials for the development of a stable and effective polyherbal toothpaste formulation.

4. Preliminary Phytochemical Screening of Herbal Extracts

Table 5. Phytochemical screening of prepared herbal extracts

Phytoconstituent	Neem extract	Babool bark extract	Pomegranate peel extract
Alkaloids	+	-	-
Flavonoids	+	+	+
Tannins	+	++	++
Saponins	+	+	+
Glycosides	+	+	+
Phenolic compounds	+	++	++
Terpenoids	+	-	+

+ indicates presence, ++ indicates strongly present and - indicates absence/not detected in the preliminary test.

Preliminary phytochemical screening confirmed the presence of important bioactive constituents, including flavonoids, tannins, saponins, glycosides, phenolic compounds, and terpenoids in the selected herbal extracts, with alkaloids detected only in neem extract. These phytoconstituents are responsible for the antimicrobial, antioxidant, anti-inflammatory, and astringent properties of the extracts, supporting their suitability for incorporation into the polyherbal toothpaste formulation.

Organoleptic Evaluation of Toothpaste Formulations

Table 6. Organoleptic evaluation of polyherbal toothpaste formulations

Formulation	Colour	Odour	Taste	Texture	Appearance
F1	Light brown	Minty and herbal	Acceptable	Smooth	Uniform paste
F2	Light brown	Minty and herbal	Acceptable	Smooth	Uniform paste
F3	Light brown	Minty and herbal	Acceptable	Slightly thick	Uniform paste
F4	Brown	Minty and herbal	Acceptable	Smooth	Uniform paste
F5	Brown	Pleasant minty-herbal	Good	Smooth	Homogeneous paste
F6	Brown	Pleasant minty-herbal	Good	Slightly thick	Homogeneous paste
F7	Dark brown	Strong herbal	Acceptable	Thick	Uniform paste
F8	Dark brown	Strong herbal	Acceptable	Thick	Uniform paste
F9	Dark brown	Strong herbal	Slightly astringent	Very thick	Uniform paste

The organoleptic evaluation showed that all polyherbal toothpaste formulations possessed acceptable colour, odour, taste, texture, and appearance. Formulations with medium concentrations of herbal extracts (F5 and F6) exhibited the best overall acceptability, with a pleasant minty-herbal odour, smooth texture, homogeneous appearance, and good taste. Higher herbal concentrations produced a darker colour, stronger herbal odour, and slightly thicker consistency, indicating that moderate levels of herbal ingredients provide superior consumer acceptability.

Phytochemical Screening of Polyherbal Toothpaste Formulations

Table 7. Phytochemical Screening of Polyherbal Toothpaste Formulations

Phytoconstituents	F1	F2	F3	F4	F5	F6	F7	F8	F9
Alkaloids	+	+	+	+	+	+	+	+	+
Flavonoids	+	+	+	+	++	++	++	++	++
Tannins	+	+	+	++	++	++	++	++	++
Saponins	+	+	+	+	+	+	+	+	+
Glycosides	+	+	+	+	+	+	+	+	+
Phenolic compounds	+	+	+	++	++	++	++	++	++
Terpenoids	+	+	+	+	+	+	+	++	++

The phytochemical screening of formulations F1–F9confirmed the presence ofalkaloids, flavonoids, tannins, saponins, glycosides, phenolic compounds, and terpenoids, indicating successful incorporation of the selected herbal extracts and essential oils. These bioactive constituents are responsible for the antimicrobial, antioxidant, anti-inflammatory, astringent, and cleansing properties of the toothpaste. Among the formulations, F5–F8 exhibited a comparatively stronger phytochemical profile, with F8 showing the highest intensity due to its higher herbal content.

Physicochemical Evaluation of Formulations F1 to F9

Table 8. Physicochemical evaluation of formulations F1 to F9 (mean ± SD, n=3)

Parameter	F1	F2	F3	F4	F5	F6	F7	F8	F9
pH	7.08 ± 0.03	7.12 ± 0.02	7.16 ± 0.03	7.18 ± 0.02	7.22 ± 0.03	7.25 ± 0.03	7.29 ± 0.02	7.31 ± 0.03	7.34 ± 0.02
Viscosity (cPs)	34850 ± 120	37240 ± 135	39860 ± 145	38220 ± 130	41680 ± 150	44720 ± 160	43150 ± 145	46890 ± 165	49680 ± 175
Spreadability (g.cm/sec)	6.84 ± 0.09	6.52 ± 0.08	6.18 ± 0.07	6.42 ± 0.08	6.12 ± 0.06	5.78 ± 0.07	5.96 ± 0.08	5.62 ± 0.06	5.28 ± 0.07
Extrudability (%)	92.10 ± 0.45	90.86 ± 0.40	88.92 ± 0.52	91.20 ± 0.44	89.74 ± 0.38	87.85 ± 0.50	88.30 ± 0.48	86.94 ± 0.46	84.72 ± 0.55
Foaming ability (mL)	27.80 ± 0.35	28.60 ± 0.42	29.20 ± 0.40	30.40 ± 0.45	31.80 ± 0.38	32.50 ± 0.46	33.60 ± 0.50	34.40 ± 0.44	35.20 ± 0.52
Foam retention after 10 min (%)	78.20 ± 0.60	79.10 ± 0.55	80.40 ± 0.58	82.30 ± 0.62	84.10 ± 0.50	84.80 ± 0.56	85.40 ± 0.60	86.20 ± 0.55	87.10 ± 0.65
Moisture content (%)	22.30 ± 0.18	22.18 ± 0.16	22.05 ± 0.15	22.64 ± 0.17	22.48 ± 0.14	22.35 ± 0.16	23.10 ± 0.19	22.96 ± 0.17	22.82 ± 0.18

The physicochemical evaluation demonstrated that all formulations (F1–F9) possessed acceptable quality and stability, with pH values within the suitable range for oral use. Viscosity increased progressively with higher binder concentrations, while spreadability and extrudability showed a corresponding decrease; however, all formulations remained within acceptable limits. Foaming ability, foam retention, and moisture content were satisfactory across all batches, indicating good cleansing performance and formulation stability.

Homogeneity, Consistency and Abrasiveness

Table 9. Homogeneity, consistency and abrasiveness of formulations

Formulation	Homogeneity	Consistency	Phase separation	Abrasiveness
F1	Good	Soft and smooth	Absent	Mild
F2	Good	Smooth	Absent	Mild
F3	Good	Slightly thick	Absent	Mild
F4	Good	Smooth	Absent	Mild
F5	Excellent	Smooth and uniform	Absent	Mild
F6	Excellent	Thick but uniform	Absent	Mild
F7	Good	Thick	Absent	Mild
F8	Good	Thick and uniform	Absent	Mild
F9	Good	Very thick	Absent	Mild to moderate

The physical evaluation demonstrated that all formulations (F1–F9) exhibited satisfactory homogeneity, consistency, stability, and mild abrasiveness, with no evidence of phase separation. Increasing binder and herbal concentrations produced thicker formulations, while F5 and F6 showed the most desirable characteristics, including excellent homogeneity, smooth consistency, and suitable abrasiveness. These findings indicate that F5 and F6 are the most physically stable and suitable formulations for further evaluation.

Antimicrobial Activity

The antimicrobial activity of the prepared formulations was evaluated by agar well diffusion method. The zone of inhibition was measured in millimetres after incubation. The results are expressed as mean ± SD, n = 3.

Table 9. Antimicrobial activity of formulations expressed as zone of inhibition in mm (mean ± SD, n=3)

Sample	*Streptococcus mutans*	*Staphylococcus aureus*	*Escherichia coli*	*Candida albicans*
F1	14.20 ± 0.30	12.60 ± 0.25	11.40 ± 0.20	10.80 ± 0.25
F2	14.80 ± 0.26	13.10 ± 0.30	11.90 ± 0.25	11.20 ± 0.26
F3	15.30 ± 0.35	13.60 ± 0.28	12.20 ± 0.30	11.60 ± 0.25
F4	17.00 ± 0.32	15.10 ± 0.25	13.40 ± 0.26	12.70 ± 0.30
F5	18.40 ± 0.40	16.30 ± 0.32	14.50 ± 0.35	13.90 ± 0.28
F6	18.00 ± 0.36	16.10 ± 0.30	14.20 ± 0.30	13.70 ± 0.25
F7	19.20 ± 0.42	17.00 ± 0.35	15.10 ± 0.32	14.60 ± 0.30
F8	20.10 ± 0.45	17.80 ± 0.38	15.80 ± 0.35	15.20 ± 0.32
F9	19.60 ± 0.40	17.30 ± 0.36	15.40 ± 0.34	14.90 ± 0.30
Commercial toothpaste	17.10 ± 0.38	15.40 ± 0.32	13.80 ± 0.30	13.20 ± 0.28
Amoxicillin standard	27.30 ± 0.60	25.80 ± 0.55	24.20 ± 0.50	--
Fluconazole standard	--	--	--	24.80 ± 0.52
Negative control	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

The antimicrobial evaluation demonstrated that all polyherbal toothpaste formulations (F1–F9) exhibited antibacterial and antifungal activity against *Streptococcus mutans, Staphylococcus aureus, Escherichia coli,* and *Candida albicans*. Antimicrobial activity increased with increasing herbal extract concentration, with F8 showing the largest zones of inhibition, comparable to or better than the marketed toothpaste. Although the standard drugs produced the highest inhibition, the formulations showed significant antimicrobial efficacy, confirming the contribution of the incorporated herbal extracts and essential oils. Considering both antimicrobial performance and physicochemical properties, F5, F6, and F8 were identified as the most promising formulations.

Microbial Load Test

Table 10. Microbial load of prepared toothpaste formulations (mean ± SD, n=3)

Formulation	Total bacterial count (CFU/g)	Total fungal count (CFU/g)	Inference
F1	18 ± 2	5 ± 1	Within acceptable limit
F2	16 ± 2	4 ± 1	Within acceptable limit
F3	14 ± 1	4 ± 1	Within acceptable limit
F4	12 ± 1	3 ± 1	Within acceptable limit
F5	10 ± 1	2 ± 1	Within acceptable limit
F6	11 ± 1	2 ± 1	Within acceptable limit
F7	9 ± 1	2 ± 1	Within acceptable limit
F8	8 ± 1	1 ± 1	Within acceptable limit
F9	8 ± 1	1 ± 1	Within acceptable limit

Stability Study of Optimized Formulation

On the basis of physicochemical properties, acceptable consistency, good spreadability, satisfactory extrudability and antimicrobial activity, formulation F5 was selected as the optimized formulation for stability study. The optimized formulation was evaluated at room temperature and accelerated conditions. The results are expressed as mean ± SD, n = 3.

Table 11. Stability study of optimized formulation F5 at room temperature (mean ± SD, n=3)

Parameter	Initial	1 Month	2 Months	3 Months
Colour/appearance	Brown, uniform	No change	No change	No significant change
Odour	Pleasant minty-herbal	No change	No change	No significant change
pH	7.22 ± 0.03	7.21 ± 0.02	7.20 ± 0.03	7.19 ± 0.03
Viscosity (cPs)	41680 ± 150	41720 ± 155	41860 ± 160	41940 ± 165
Spreadability (g.cm/sec)	6.12 ± 0.06	6.10 ± 0.05	6.08 ± 0.06	6.05 ± 0.07
Extrudability (%)	89.74 ± 0.38	89.60 ± 0.40	89.42 ± 0.45	89.20 ± 0.44
Phase separation	Absent	Absent	Absent	Absent
Microbial load	Within limit	Within limit	Within limit	Within limit

The optimized formulation (F5) remained physically, chemically, and microbiologically stable throughout the 3-month stability study. No significant changes were observed in colour, odour, pH, viscosity, spreadability, extrudability, or phase separation, and the microbial load remained within acceptable limits. These findings indicate that the formulation possesses good storage stability and is suitable for long-term use as a polyherbal toothpaste.

Table 12. Stability study of optimized formulation F5 under accelerated condition (mean ± SD, n=3)

Parameter	Initial	1 Month	2 Months	3 Months
Colour/appearance	Brown, uniform	No change	Slight darkening	Slight darkening
Odour	Pleasant minty-herbal	No change	Slight decrease in aroma	Slight decrease in aroma
pH	7.22 ± 0.03	7.18 ± 0.03	7.15 ± 0.04	7.12 ± 0.04
Viscosity (cPs)	41680 ± 150	42120 ± 160	42680 ± 170	43240 ± 180
Spreadability (g.cm/sec)	6.12 ± 0.06	6.04 ± 0.06	5.96 ± 0.07	5.88 ± 0.08
Extrudability (%)	89.74 ± 0.38	89.20 ± 0.45	88.72 ± 0.48	88.20 ± 0.50
Phase separation	Absent	Absent	Absent	Absent
Microbial load	Within limit	Within limit	Within limit	Within limit

The **3**-month stability study demonstrated that the optimized polyherbal toothpaste formulation remained physically, chemically, and microbiologically stable during storage. Only minor changes were observed, including slight darkening of colour, a marginal reduction in aroma, a small decrease in pH, and slight increases in viscosity with corresponding reductions in spreadability and extrudability; however, all values remained within acceptable limits. No phase separation or microbial contamination was detected throughout the study, confirming the effectiveness of the formulation and preservative system.

CONCLUSION

A polyherbal toothpaste containing neem, miswak, babool bark, and pomegranate peel was successfully formulated and systematically evaluated. The selected herbal ingredients exhibited acceptable pharmacognostic and physicochemical quality and were rich in bioactive phytoconstituents, including flavonoids, tannins, phenolic compounds, saponins, glycosides, and terpenoids, which contribute to their antimicrobial, antioxidant, anti-inflammatory, and astringent activities.All nine formulations showed satisfactory organoleptic, physicochemical, and physical characteristics. Among them, F5 and F6 demonstrated the most balanced properties in terms of pH, viscosity, spreadability, extrudability, homogeneity, and consumer acceptability, whereas F8 exhibited the strongest antimicrobial activity against *Streptococcus mutans, Staphylococcus aureus, Escherichia coli,* and *Candida albicans*. The optimized formulation also remained stable during the 3-month stability study, with no significant changes in physicochemical properties, no phase separation, and acceptable microbiological quality. Findings indicate that the developed polyherbal toothpaste is a stable, effective, and promising natural oral care formulation with potential to reduce oral microbial load, support gum health, and improve overall oral hygiene. Further in vivo clinical studies are recommended to confirm its long-term safety and therapeutic efficacy.

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