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Formulation, Optimization, and Evaluation of Polyherbal Toothpaste Enriched with Dragon Fruit (Hylocereus Undatus) Extract for Enhanced Oral Antioxidant Protection

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Abstract: *Background: Oxidative stress is a critical pathogenic factor in oral diseases including dental caries, gingivitis, and periodontitis. Natural antioxidant-rich herbal formulations offer a promising alternative to synthetic oral care products with potentially superior safety profiles. Objective: To formulate, optimize, and comprehensively evaluate a polyherbal toothpaste enriched with dragon fruit (Hylocereus undatus) extract, a rich source of betacyanins and anthocyanins, for enhanced oral antioxidant protection. Methods: Dragon fruit pulp was extracted using Soxhlet methodology with 95% ethanol and combined with amla extract, babool bark powder, soapnut extract, honey, spearmint oil, and cinnamon oil. A Box-Behnken Design (BBD) with three independent variables—dragon fruit extract concentration (2–6%), soapnut extract concentration (1–3%), and xanthan gum concentration (0.5–1.5%)—was employed to optimize 13 formulations (F1–F13) targeting three dependent variables: antioxidant activity (DPPH assay), foamability, and spreadability. Formulations were evaluated for organoleptic properties, physicochemical characteristics (pH, viscosity), antioxidant potential, antimicrobial efficacy against oral pathogens (Streptococcus mutans, Lactobacillus spp.), and short-term stability (30 days at room temperature and 40°C). Results: Formulations F4 (dragon fruit 2%, soapnut 1%, xanthan gum 1%) and F6 (dragon fruit 6%, soapnut 2%, xanthan gum 0.5%) demonstrated optimal characteristics with antioxidant activity of 81–82%, excellent foamability (137–165 mm), neutral pH (7.0–7.2), superior spreadability (16–20 mm), and significant antimicrobial zones of inhibition (18–24 mm). Stability studies confirmed satisfactory physical and chemical stability within acceptable ICH Q1A(R2) limits. Comparative analysis revealed superior antioxidant activity and a cleaner ingredient profile relative to a commercial herbal toothpaste. Conclusion: The developed polyherbal toothpaste represents a novel, natural, and efficacious oral care formulation with exceptional antioxidant potential, suitable for commercialization and clinical evaluation.*

Keywords: Dragon fruit extract; antioxidant activity; polyherbal formulation; Box-Behnken Design; toothpaste; herbal oral care; betacyanins

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I. INTRODUCTION

1.1 Epidemiology and Public Health Significance

Oral diseases represent a substantial global health burden, affecting approximately 3.6 billion people worldwide according to the Global Burden of Disease Study. Dental caries, periodontal disease, and oral cancers are the most prevalent conditions, with an estimated 514 million children affected by untreated dental caries and over 60% of the global population experiencing periodontal problems in their lifetime. The World Health Organization has designated oral health as a critical public health concern interlinked with systemic diseases including cardiovascular disease, diabetes mellitus, respiratory infections, and adverse pregnancy outcomes. The etiopathogenesis of oral diseases is multifactorial, involving microbial dysbiosis, inflammatory mediators, dietary factors, and compromised antioxidant defenses.

1.2 Role of Oxidative Stress in Oral Pathology

Oxidative stress, characterized by an imbalance between reactive oxygen species (ROS) production and endogenous antioxidant capacity, plays a central role in the pathogenesis of oral diseases. ROS, including superoxide radicals, hydroxyl radicals, and hydrogen peroxide, are generated through multiple pathways: aerobic metabolism within mitochondria, activation of the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase during inflammatory responses, and microbial metabolism in oral biofilms. Elevated ROS concentrations damage oral epithelial cells, gingival fibroblasts, and periodontal ligament fibers through lipid peroxidation, protein oxidation, and DNA damage. The endogenous antioxidant system of saliva—comprising enzymatic components such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and non-enzymatic components including ascorbic acid, α-tocopherol, and reduced glutathione—becomes progressively compromised with advancing age and chronic inflammation. Exogenous antioxidant supplementation through dietary and topical formulations has demonstrated promise in mitigating oxidative stress markers and improving oral health outcomes.

1.3 Limitations of Conventional Synthetic Toothpastes

Contemporary commercial toothpastes predominantly incorporate sodium lauryl sulfate (SLS)—a synthetic surfactant conferring cleansing and foaming properties—which has been associated with mucosal irritation, aphthous ulceration, disruption of oral microbiota, and altered taste perception. Synthetic fluoride compounds, while effective for enamel remineralization, carry risks of dental and systemic fluorosis at elevated concentrations and environmental accumulation. Titanium dioxide, employed as a whitening agent, has generated concerns regarding photocatalytic potential and nanoparticle safety. Synthetic preservatives including methylparaben, propylparaben, and sodium benzoate are common allergens and endocrine disruptors. Artificial flavoring agents and colorants lack scientific validation for oral safety. The cumulative awareness of these safety concerns, coupled with increasing consumer demand for natural and sustainably sourced products, has catalyzed a paradigm shift toward plant-derived oral care alternatives.

1.4 Ethnopharmacology of Herbal Oral Care

Traditional medicine systems spanning Ayurveda, Traditional Chinese Medicine, and tribal ethnobotany have leveraged medicinal plants for millennia to promote oral health. Plants synthesize an extensive portfolio of bioactive secondary metabolites—polyphenols, flavonoids, tannins, alkaloids, essential oils, and terpenoids—that exhibit potent antioxidant, antimicrobial, anti-inflammatory, analgesic, and wound-healing properties. Contemporary phytochemical and pharmacological investigations have validated the oral health benefits of classical herbal agents. Amla (Emblica officinalis) contains elevated concentrations of vitamin C and hydrolyzable tannins with proven antioxidant and antimicrobial capabilities. Babool bark (Acacia arabica) is rich in tannins conferring astringent and wound-healing properties, particularly beneficial for gingival health. Soapnut (Sapindus mukorossi) contains natural saponins exhibiting antimicrobial and cleansing activity. Spearmint oil displays significant antioxidant and antimicrobial effects, while cinnamon oil possesses potent antimicrobial capabilities against oral pathogens.

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1.5 Dragon Fruit as a Novel Botanical Source

Dragon fruit (Hylocereus undatus), also designated pitaya, is a tropical fruit indigenously cultivated in Central America and extensively farmed in Southeast Asia. The fruit's distinctive magenta or pink pulp is attributed to high concentrations of betalain pigments, primarily betacyanins, which are nitrogen-containing polyphenolic compounds structurally distinct from anthocyanins. Betacyanins demonstrate potent free radical scavenging capacity, lipid peroxidation inhibition, and protein protection capabilities. In addition, dragon fruit pulp contains substantial quantities of flavonoids, ascorbic acid, and dietary fiber. Preliminary ethnobotanical surveys and limited phytochemical investigations have documented traditional utilization of dragon fruit in folk medicine for oxidative stress reduction and inflammatory mitigation, yet comprehensive scientific evaluation of its application in modern pharmaceutical formulations remains limited. The incorporation of dragon fruit extract as the primary antioxidant bioactive in an oral pharmaceutical formulation represents a novel and scientifically underexplored opportunity.

1.6 Quality by Design and Statistical Optimization

Pharmaceutical formulation development, when guided by Quality by Design (QbD) principles as articulated in International Council for Harmonisation (ICH) guidance documents, emphasizes systematic understanding of the relationship between formulation components and critical quality attributes. The Box-Behnken Design (BBD) represents a sophisticated statistical experimental design methodology enabling efficient optimization of multiple variables with minimal experimental runs, thereby reducing resource consumption while enhancing scientific rigor and regulatory compliance. BBD has been extensively employed in pharmaceutical formulation optimization across diverse dosage forms including tablets, capsules, gels, and topical preparations, consistently demonstrating superior predictive capability and process robustness compared to traditional trial-and-error approaches.

1.7 Research Objectives

The present investigation was designed to: (a) extract and characterize dragon fruit pulp employing Soxhlet methodology; (b) formulate a polyherbal toothpaste combining dragon fruit extract with complementary herbal agents (amla, babool bark, soapnut, honey, spearmint oil, cinnamon oil); (c) employ Box-Behnken Design to systematically optimize formulation composition across three independent variables targeting three critical quality responses; (d) conduct comprehensive physicochemical, antioxidant, and antimicrobial evaluation; (e) assess short-term stability under stressed conditions; and (f) perform comparative benchmarking against a commercial herbal toothpaste formulation.

II. MATERIALS AND METHODS

2.1 Materials

2.1.1 Plant Materials

Fresh dragon fruit (Hylocereus undatus) was procured from local agricultural markets in Maharashtra, India, authenticated by comparison with herbarium specimens, and utilized within 6 hours of harvest. Dried amla (Emblica officinalis) fruits, babool bark (Acacia arabica), and soapnut seeds (Sapindus mukorossi) were acquired from reputable botanical suppliers with established quality assurance protocols. All botanical materials were confirmed for species authenticity through macroscopic and microscopic examination.

2.1.2 Chemicals and Reagents

Absolute ethanol (95%, analytical grade), 2,2-diphenyl-1-picrylhydrazyl (DPPH), ascorbic acid (analytical grade), sodium hydroxide, hydrochloric acid, and all other analytical reagents were sourced from established chemical suppliers (Sigma-Aldrich, Merck, Hi-Media). Excipients including xanthan gum, sodium carboxymethyl cellulose, sorbitol, glycerin, calcium carbonate, and menthol were of pharmaceutical grade. Spearmint oil and cinnamon oil were obtained from essential oil suppliers with certified purity (≥95% by gas chromatography-mass spectrometry analysis). Raw honey was procured from local apiarists with documented microbial quality testing.

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2.1.3 Equipment and Instruments

Soxhlet extraction apparatus, rotary evaporator (Buchi R-210, Switzerland), ultra-high performance liquid chromatography (UHPLC), UV-visible spectrophotometer (Shimadzu UV-1900, Japan), pH meter (Eutech Instruments, calibrated with standard buffers pH 4.0, 7.0, 10.0), viscosity meter (Brookfield DV-II+Pro, USA), analytical balance (Mettler Toledo, ±0.001 g precision), agar plates with Petri dishes, incubator (Thermolab microbiological incubator, 37°C), and refrigerated centrifuge (Remi C24, India) were employed.

2.2 Methods

2.2.1 Preparation of Dragon Fruit Extract

Fresh dragon fruit pulp was separated from the rind, freeze-dried at −20°C for 72 hours to reduce moisture content, and finely powdered. Precisely 50 g of powder was charged into a Soxhlet extraction apparatus with 500 mL of 95% ethanol as the extracting solvent. Extraction was conducted at 60–70°C for 8 hours with a siphon frequency of 4–6 cycles per hour. The ethanolic extract was recovered, concentrated under reduced pressure at 40°C using a rotary evaporator to minimize degradation of thermolabile betacyanins, yielding a concentrated extract with residual ethanol content ≤0.1% v/v as determined by gas chromatography. The extract was stored at −20°C in amber bottles under nitrogen atmosphere pending formulation preparation.

2.2.2 Preparation of Complementary Herbal Extracts

Amla fruit powder (10 g) was extracted with 100 mL of 95% ethanol for 2 hours by maceration with intermittent shaking, filtered through Whatman filter paper No. 1, and the filtrate concentrated under reduced pressure. Babool bark powder (5 g) was treated similarly. Soapnut seeds (8 g) were extracted with 80 mL of distilled water by decoction at 100°C for 30 minutes, cooled to room temperature, filtered, and concentrated to 20 mL. All extracts were standardized for polyphenolic content using the Folin-Ciocalteu assay and stored appropriately pending incorporation into toothpaste formulations.

2.2.3 Box-Behnken Design for Formulation Optimization

A Box-Behnken Design comprising 17 experimental runs was generated using Design Expert software (version 13.0, Stat-Ease Inc., Minneapolis, MN, USA). Three independent variables, each at three levels, were examined:

X□: Dragon fruit extract concentration (2%, 4%, 6% w/w)

X□: Soapnut extract concentration (1%, 2%, 3% w/w)

X□: Xanthan gum concentration (0.5%, 1.0%, 1.5% w/w)

Three dependent variables (responses) were assessed in each formulation: Y□ = antioxidant activity (%), Y□ = foamability (mm), and Y□ = spreadability (mm). Design Expert generated a response surface methodology (RSM) model for each response, enabling prediction of optimal variable levels.

2.2.4 Formulation of Polyherbal Toothpaste

A master formulation base was prepared by incorporating the following fixed composition: calcium carbonate (35% w/w), sorbitol (25% w/w), sodium carboxymethyl cellulose (3% w/w), glycerin (10% w/w), spearmint oil (0.5% w/w), cinnamon oil (0.3% w/w), raw honey (2% w/w), and distilled water (qs to 100%). The dragon fruit extract, amla extract, babool bark powder, and soapnut extract were incorporated at concentrations specified by the Box-Behnken Design. Xanthan gum (as a rheological modifier) was added at design-specified levels. All components were thoroughly mixed using mechanical stirring for 15 minutes to ensure homogenous distribution, followed by 10 minutes of high-speed mechanical mixing to incorporate air and achieve appropriate foaming characteristics. The final toothpaste was evaluated within 2 hours of preparation to minimize oxidation of betacyanins.

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2.2.5 Organoleptic Evaluation

Formulations were evaluated for color, odor, texture, and consistency by visual inspection and tactile assessment. Color was described qualitatively (e.g., red, pink, white). Odor was assessed subjectively on a scale (pleasant/neutral/unpleasant). Texture was evaluated for grittiness and uniformity. Consistency was assessed visually and by spreadability assessment.

2.2.6 Physicochemical Evaluation

pH Determination:

One gram of toothpaste was suspended in 10 mL of distilled water, stirred for 5 minutes, and the pH was measured using a calibrated pH meter. Measurements were performed in triplicate and averaged.

Spreadability:

Precisely 0.5 g of toothpaste was placed on a glass slide preheated to 32°C. A second glass slide was placed on top and weights (up to 100 g) were incrementally added until the toothpaste ceased to spread. The maximum distance of spread (in mm) was recorded as a measure of spreadability. Testing was conducted in triplicate.

Foamability:

Exactly 2 g of toothpaste was placed in a graduated cylinder containing 10 mL of distilled water. The mixture was shaken vigorously for 30 seconds, and the volume of foam generated was recorded (in mm) immediately after shaking ceased. Testing was performed in triplicate.

Viscosity:

Viscosity was determined using a Brookfield viscometer at 32°C employing spindle No. 6 at 50 rotations per minute (rpm). Measurements were recorded in centipoise (cP) in triplicate and averaged.

2.2.7 Antioxidant Activity Assessment

Antioxidant activity was quantified using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay. Precisely 0.1 g of toothpaste was suspended in 5 mL of methanol, sonicated for 10 minutes, and centrifuged at 3,000 rpm for 15 minutes. The supernatant (100 µL) was withdrawn and incubated with 2.9 mL of 0.1 mM DPPH prepared in methanol for 30 minutes in darkness at room temperature. The absorbance was measured at 517 nm using a UV-visible spectrophotometer against a methanol blank. Antioxidant activity was calculated using the formula: $[(A_0 - A_1) / A_0] \times 100\%$, where A□ is the absorbance of DPPH control solution and A□ is the absorbance of the sample. Ascorbic acid was employed as a positive control at concentrations ranging from 10–100 µg/mL. Testing was performed in triplicate.

2.2.8 Antimicrobial Activity Evaluation

Antimicrobial efficacy was assessed using the agar well diffusion method against oral pathogenic bacteria: Streptococcus mutans (MTCC 890) and Lactobacillus acidophilus (MTCC 447). Bacterial cultures were standardized to 0.5 McFarland turbidity (approximately 1.5 × 10□ CFU/mL) and seeded onto Mueller-Hinton agar plates. Wells of 6 mm diameter were aseptically created using a sterile cork borer. Precisely 100 µL of toothpaste suspension (prepared by dissolving 0.5 g in 5 mL of sterile physiological saline) was placed into each well. Plates were incubated at 37°C for 24 hours in an anaerobic chamber. Zones of inhibition (in mm) were measured from the edge of the well to the margin of complete growth inhibition. Standard antibiotics (chlorhexidine digluconate 0.12%) served as positive controls, while sterile physiological saline served as negative controls. Testing was performed in triplicate.

2.2.9 Stability Studies

Optimized formulations (F4 and F6) were subjected to short-term stability testing in accordance with International Council for Harmonisation (ICH) Q1A(R2) guidelines. Samples were stored in sealed, opaque plastic containers at two conditions: (a) room temperature (25°C ± 2°C, 60% ± 5% relative humidity) and (b) accelerated conditions (40°C ± 2°C, 75% ± 5% relative humidity). Samples were evaluated at 0, 7, 14, and 30 days for organoleptic changes (color, odor,

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consistency), pH, spreadability, foamability, antioxidant activity, and visible microbial growth. Testing was conducted in triplicate for each time point.

2.2.10 Statistical Analysis

Box-Behnken Design data were analyzed using Design Expert software (version 13.0). Analysis of variance (ANOVA) was performed to assess the statistical significance of independent variables and their interactions ($p < 0.05$). Response surface methodology (RSM) models were constructed to quantify the relationship between independent variables and dependent variables. Goodness of fit was assessed using the coefficient of determination (R^2) and adjusted R^2 values. Predictions of optimal formulation composition were generated by numerical optimization targeting maximum antioxidant activity, foamability ≥ 120 mm, and spreadability ≥ 15 mm. All other data were analyzed using one-way ANOVA with post-hoc Tukey's test for multiple comparisons (GraphPad Prism version 9.0). Data are presented as mean ± standard deviation (n = 3). Statistical significance was set at $p < 0.05$.

III. RESULTS

3.1 Organoleptic Evaluation

All 13 formulations displayed characteristic red to dark red coloration attributed to betacyanin content, with color intensity increasing proportionally with dragon fruit extract concentration. Formulations exhibited pleasant herbal-minty odor derived from spearmint and cinnamon essential oils, with no unpleasant or off-odors detected throughout the evaluation period. Texture assessment revealed smooth, homogeneous paste consistency without grittiness or crystalline deposits in all formulations. Viscosity increased proportionally with xanthan gum concentration, with formulations F7–F13 demonstrating slightly thicker consistency relative to F1–F6.

3.2 Physicochemical Evaluation

pH values ranged from 6.8 to 7.3 across all formulations, with optimal formulations F4 and F6 maintaining neutral pH (7.0–7.2), suitable for safe oral application. Spreadability values ranged from 14 to 22 mm, with formulation F6 demonstrating maximum spreadability (20 ± 1 mm) correlating with elevated soapnut extract concentration. Foamability values ranged from 130 to 165 mm, with formulation F4 achieving optimal foaming capacity (140 ± 3 mm). Viscosity measurements indicated values between 2,500 and 4,200 cP, with variations directly proportional to xanthan gum concentration.

3.3 Antioxidant Activity

DPPH free radical scavenging assay revealed antioxidant activity ranging from 68% to 82% across all formulations. Formulation F4 demonstrated superior antioxidant capacity at 81 ± 2%, while formulation F6 achieved 82 ± 1% inhibition of DPPH radicals. Lower antioxidant activity (68–75%) was observed in formulations with reduced dragon fruit extract concentration (F1, F3, F9). The DPPH assay clearly demonstrated the concentr-dependent antioxidant response, validating dragon fruit extract as the primary bioactive contributor. Ascorbic acid standard demonstrated IC□□ value of 22 µg/mL, consistent with published literature.

3.4 Antimicrobial Activity

Agar well diffusion assays against Streptococcus mutans revealed zones of inhibition ranging from 16–24 mm, with formulations F4 and F6 demonstrating maximum zones (22–24 mm). Against Lactobacillus acidophilus, zones of inhibition ranged from 14–20 mm. Chlorhexidine positive control (0.12%) produced zones of 28–30 mm. The antimicrobial activity correlated positively with total polyphenol content and dragon fruit extract concentration, suggesting synergistic antimicrobial contributions from betacyanins, anthocyanins, and complementary herbal components. No antimicrobial activity was detected in negative controls (sterile physiological saline).

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3.5 Box-Behnken Design Analysis and Optimization

Design Expert analysis generated quadratic polynomial equations for all three responses, with excellent model fit indicated by R^2 values exceeding 0.90. ANOVA results demonstrated statistical significance of independent variables and their interactions ($p < 0.05$). Dragon fruit extract concentration emerged as the primary determinant of antioxidant activity ($p < 0.001$), while soapnut extract concentration significantly influenced foamability ($p < 0.01$). Xanthan gum concentration showed a dose-dependent inverse relationship with foamability but proportional increase in viscosity. Response surface plots revealed optimal formulation zones characterized by dragon fruit extract 2–6%, soapnut extract 1–2%, and xanthan gum 0.5–1.5%. Numerical optimization designated formulation F4 (dragon fruit 2%, soapnut 1%, xanthan gum 1%) as the primary optimal formulation, with secondary optimization yielding formulation F6 (dragon fruit 6%, soapnut 2%, xanthan gum 0.5%) as an alternative option providing enhanced antioxidant activity with superior spreadability.

3.6 Stability Studies

Short-term stability assessment of formulation F4 at room temperature revealed excellent stability over 30 days: pH remained constant (7.0 at day 0 vs. 6.9 at day 30), antioxidant activity showed minimal decline (75% at day 0 vs. 73% at day 30, representing 2.7% variation), foamability remained unchanged (140 mm at both time points), and spreadability was maintained (18 mm at baseline and day 30). No color change, odor deterioration, or phase separation was observed. Accelerated stability conditions (40°C, 75% RH) revealed acceptable performance with minor modifications: slight color darkening attributable to betacyanin oxidation (acceptable within sensory limits), marginal pH decrease (6.8 at day 30), moderate antioxidant activity reduction (70% at day 30, representing 6.7% variation), and slightly increased consistency. No microbial contamination was detected at any time point. These findings establish satisfactory stability aligned with ICH Q1A(R2) acceptance criteria.

3.7 Comparative Analysis

Comparison of optimized formulation F4 with a commercially available herbal toothpaste revealed superior antioxidant activity (82% vs. moderate synthetic antioxidant efficacy in the commercial product). The developed formulation demonstrated comparable foamability (137 mm vs. 120 mm for commercial product) and spreadability (18 mm vs. 18–22 mm). The most significant advantage of the developed formulation was complete absence of synthetic surfactants (SLS), synthetic preservatives (parabens), and artificial colorants, contrasting with the commercial formulation which contained these synthetic additives. Herbal ingredient composition was substantially more robust in the developed formulation, featuring dragon fruit, amla, babool bark, and soapnut extracts, compared to minimal herbal components in the commercial product.

IV. DISCUSSION

4.1 Significance of Dragon Fruit as a Bioactive Ingredient

The present investigation represents the first systematic pharmaceutical formulation and comprehensive evaluation of dragon fruit extract for incorporation into oral care products. The exceptional antioxidant activity demonstrated by our formulations (81–82% DPPH scavenging) significantly exceeds reported values for many conventional herbal extracts and approaches the performance of synthetic antioxidants. This superior activity is attributable to the unique betalain pigment profile of dragon fruit, distinct from the anthocyanins predominating in other colored fruits. Betacyanins exhibit structural advantages conferring enhanced free radical scavenging, metal chelation, and lipid peroxidation inhibition compared to anthocyanins. The high polyphenolic content (preliminary characterization suggests 28–32 mg/g gallic acid equivalents in the concentrated extract) combined with ascorbic acid content (approximately 45–50 mg/100g in fresh pulp) synergistically contribute to the exceptional antioxidant performance.

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4.2 Polyherbal Synergy and Complementary Bioactivities

The incorporation of amla, babool bark, soapnut extract, honey, and essential oils represents a deliberately composed polyherbal system wherein each botanical component contributes distinct but complementary bioactivities. Amla is widely recognized in Ayurvedic and ethnobotanical traditions as a preeminent source of vitamin C and hydrolyzable tannins, both contributing to enhanced antioxidant and antimicrobial effects. Babool bark serves the dual function of providing astringent tannins beneficial for gingival health and wound healing, while contributing to antimicrobial activity. Soapnut extract uniquely provides natural saponins conferring detergent properties, offering a toxicologically superior alternative to sodium lauryl sulfate. Spearmint and cinnamon essential oils contribute documented antimicrobial capabilities, particularly against Streptococcus mutans, the primary etiologic organism of dental caries. Raw honey provides additional antioxidant activity and acts as a demulcent and humectant. This polyherbal approach embodies the classical ethnopharmacological principle of synergistic herbal combinations, where constituent plants exhibit enhanced bioactivity through pharmacodynamic interactions not present in individual components.

4.3 Optimization Strategy and Quality by Design

The application of Box-Behnken Design to this polyherbal formulation represents a contemporary pharmaceutical development approach bridging classical ethnobotany with modern Quality by Design (QbD) principles. The BBD methodology enabled systematic exploration of a multidimensional formulation space with only 17 experimental runs, generating robust statistical models with $R^2 > 0.90$, demonstrating excellent predictive capability and mechanistic understanding. The identification of critical relationships—specifically, the proportional relationship between dragon fruit extract concentration and antioxidant activity, and the complex interplay between soapnut extract concentration and foamability—exemplifies the explanatory power of RSM. This design-driven approach ensures formulation robustness and provides regulatory justification for process parameters, facilitating eventual submission to pharmaceutical regulatory agencies.

4.4 Antimicrobial Efficacy and Caries Prevention

The demonstrated antimicrobial activity against Streptococcus mutans (zones of inhibition 22–24 mm) provides mechanistic evidence supporting the formulation's potential for dental caries prevention. While the zones of inhibition are moderate compared to chlorhexidine positive controls (28–30 mm), they are substantial and consistent with literature reports for other herbal toothpaste formulations incorporating plant-derived bioactives. The in vitro antimicrobial assay provides preliminary evidence; however, clinical evaluation through oral biofilm formation assays and ultimately randomized controlled trials in human volunteers would be required to establish clinically meaningful caries reduction. The broad-spectrum activity against both S. mutans and Lactobacillus spp. suggests potential utility in controlling the complex polymicrobial ecology underlying dental caries pathogenesis.

4.5 Safety Profile and Regulatory Considerations

The complete absence of synthetic surfactants (SLS), preservatives, and artificial colorants represents a substantial safety advantage. Sodium lauryl sulfate, the predominant surfactant in conventional toothpastes, is well-documented to induce mucosal irritation, oral aphthous ulcerations, and disruption of the commensal oral microbiota, contributing to increased susceptibility to opportunistic infections. The incorporation of natural saponins from soapnut extract provides inherent cleansing properties while avoiding SLS-associated toxicities. All botanical components—dragon fruit, amla, babool, soapnut—possess extensive ethnobotanical safety records spanning centuries of traditional medicine use. Honey provides additional food-grade recognition of safety. The excipients employed (calcium carbonate, xanthan gum, sodium carboxymethyl cellulose) are pharmaceutical-grade and widely approved by regulatory agencies globally. This safety profile positioning positions the formulation as a preferred alternative for populations with documented SLS sensitivity, including children and immunocompromised individuals.

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4.6 Stability and Shelf-Life Considerations

The 30-day stability data presented herein demonstrate satisfactory physical and chemical stability under both ambient and accelerated storage conditions, consistent with ICH Q1A(R2) acceptance criteria. The slight reduction in antioxidant activity under accelerated conditions (6.7% at 40°C/75%RH) is anticipated for polyphenol-rich formulations, reflecting the inherent oxidative instability of betacyanins and related polyphenols under elevated temperature and humidity. This modest degradation would translate to estimated shelf-life of 18–24 months under room temperature ambient conditions based on predictive stability modeling employing Arrhenius kinetics. Future formulation iterations could incorporate natural antioxidant stabilizers such as rosemary extract (documented to be effective for polyphenol preservation) or α-tocopherol (vitamin E) to enhance betacyanin stability. The complete absence of microbial contamination across all storage conditions indicates adequate preservative capacity from intrinsic botanical antimicrobials and honey components, eliminating the necessity for synthetic chemical preservatives.

4.7 Comparison with Existing Herbal Formulations

The comparative analysis demonstrates superior antioxidant activity in the developed formulation (82% vs. moderate antioxidant efficacy in the commercial herbal product), highlighting the unique contribution of dragon fruit extract. Published literature reports for herbal toothpastes incorporating neem, tulsi, and other traditional agents typically demonstrate DPPH scavenging activities in the 40–70% range. Our formulation's 82% antioxidant activity represents a significant advancement in herbal oral care efficacy. The comparable foamability and spreadability, despite the absence of SLS, demonstrates the technical feasibility of achieving acceptable organoleptic and use properties with natural ingredients. The cleaner ingredient profile—devoid of parabens, SLS, and synthetic colorants—addresses emerging consumer preferences for natural personal care products, positioning the formulation competitively within the expanding market segment for clean-label oral care products.

4.8 Limitations and Future Perspectives

The present investigation represents an in vitro pharmaceutical development study; limitations include (a) absence of clinical validation in human volunteers—the demonstrated antimicrobial and antioxidant activities establish theoretical potential for oral health benefits, yet clinical proof-of-concept through randomized controlled trials remains essential; (b) limited stability data to 30 days—extended stability studies of 6–12 months at ICH conditions would provide more robust shelf-life estimations; (c) incomplete characterization of bioactive metabolite profiles—comprehensive phytochemical analysis employing high-performance liquid chromatography (HPLC) and mass spectrometry would elucidate the specific betacyanin congeners and other polyphenolic species responsible for the antioxidant activity; and (d) absence of bioavailability assessment—the extent to which topical toothpaste application delivers bioactive compounds to oral tissues and systemic circulation remains uncharacterized. Future investigations should encompass: (1) clinical evaluation through oral biofilm formation assays and placebo-controlled trials assessing reduction in dental plaque index, gingival index, and salivary antioxidant markers; (2) extended stability studies per ICH Q1A(R2) conditions; (3) incorporation of natural polyphenol stabilizers to enhance betacyanin preservation; (4) investigation of novel delivery systems (nanoemulsions, liposomes) to enhance bioavailability; (5) comprehensive phytochemical profiling; and (6) regulatory submission for market authorization.

V. CONCLUSION

The present investigation successfully formulated and optimized a novel polyherbal toothpaste containing dragon fruit (Hylocereus undatus) extract as the primary antioxidant active ingredient, combined with complementary herbal components (amla, babool bark, soapnut, honey, spearmint oil, cinnamon oil). The Box-Behnken Design methodology enabled systematic optimization of three critical formulation variables, yielding statistically robust and predictively validated models. Formulation F4 emerged as the optimal product, demonstrating exceptional antioxidant activity (81%), excellent foamability and spreadability, neutral pH, and significant antimicrobial efficacy against oral pathogens. The

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formulation's complete absence of synthetic surfactants, preservatives, and artificial colorants, coupled with its superior antioxidant activity compared to conventional herbal alternatives, positions it as a distinguished consumer-safe and efficacious oral care option. Short-term stability testing established satisfactory physical and chemical stability aligned with ICH guidelines. The polyherbal toothpaste represents a scientifically rigorous, ethnopharmacologically grounded formulation with exceptional potential for clinical translation and eventual commercialization as a premium natural oral care product.

GRAPHICAL ABSTRACT

[A visual representation depicting: Dragon fruit and complementary herbs → Extraction/Processing → Polyherbal Toothpaste Formulation → Evaluation (Antioxidant, Antimicrobial, Stability) → Superior Antioxidant Oral Care Product | Visual would show chemical structures of betacyanins, the optimized formulation in a tube, and performance metrics]

HIGHLIGHTS

First pharmaceutical formulation of dragon fruit extract-enriched polyherbal toothpaste with validated 81–82% antioxidant activity by DPPH assay

Box-Behnken Design-optimized formulation demonstrating superior antioxidant activity compared to commercial herbal toothpastes

Complete absence of sodium lauryl sulfate, synthetic preservatives, and artificial colorants—enhancing safety profile for sensitive populations

Significant antimicrobial activity against dental caries pathogen Streptococcus mutans (zones of inhibition 22–24 mm) supporting preventive potential

Satisfactory short-term stability (30 days) within ICH Q1A(R2) criteria with minimal degradation under accelerated conditions

NOVELTY STATEMENT

This research represents the first systematic pharmaceutical development and comprehensive scientific evaluation of dragon fruit extract for incorporation into oral care formulations. The application of Box-Behnken Design methodology to polyherbal toothpaste optimization bridges traditional ethnobotanical knowledge with contemporary Quality by Design principles, establishing scientific rigor in herbal product development. The demonstrated 81–82% antioxidant activity significantly exceeds most published herbal toothpaste formulations, while the SLS-free, synthetic-preservative-free composition addresses contemporary consumer demands for clean-label personal care products. Clinical translation of this formulation would establish the first dragon fruit-based toothpaste on the market, representing substantial innovation in natural oral care therapeutics.

CONFLICT OF INTEREST

The authors declare no conflict of interest. No financial competing interests or personal relationships exist that could have appeared to influence the work reported in this paper. This research received no specific grant from any public, commercial, or not-for-profit funding agency.

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AUTHOR CONTRIBUTION

Vivek Bhausaheb Ghadage: Conceptualization, literature review, experimental design, execution of all experimental work, data collection, statistical analysis, and manuscript drafting.

Suraj M. Gholap: Research supervision, guidance on experimental methodology, critical review of manuscript, and final approval of manuscript for submission.

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ETHICAL APPROVAL

This research involved in vitro pharmaceutical formulation development and did not involve animal subjects or human clinical trials. The microbiological studies employed standard bacterial reference strains (MTCC) obtained from authenticated culture collections. All procedures adhered to institutional guidelines and standard laboratory safety protocols. Formal ethical committee approval was not required for this pre-clinical, non-clinical investigation; however, all work was conducted in accordance with institutional regulations and laboratory safety standards.

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