# Formulation And Evaluation of Herbal Toothpaste Containing Azadirachta Indica (Neem) Extract for Prevention of Dental Caries and Maintenance of Oral Hygiene

Ms. Janakwade Aishwarya Ramesh[1], Mr. Gavhane Sandeepan. D[2], Ms. Suryawanshi Anagha A[3], Ms. Kunte Chandrakala Devidas[4]

[1,4]*Research Scholar, Department of Pharmaceutics, Kandhar College of Pharmacy, Kandhar, Nanded, Maharashtra, India-431714*

[2]*Associate Professor. Department of Pharmaceutics, Kandhar College of Pharmacy, Kandhar, Nanded, Maharashtra, India-431714*

[3]*Assistant Professor, Department of Pharmaceutics, Kandhar College of Pharmacy, Kandhar, Nanded, Maharashtra, India-431714*

***Abstract*—The present study aimed to formulate and evaluate a herbal toothpaste containing Azadirachta indica (Neem) extract for the prevention of dental caries and maintenance of oral hygiene. Increasing concern regarding the long-term use of synthetic oral care products and their associated adverse effects has generated significant interest in herbal alternatives possessing antimicrobial and therapeutic properties. Neem has been traditionally used in Ayurveda for oral hygiene due to its well-documented antibacterial, antifungal, anti-inflammatory, and anti-cariogenic activities. Neem leaves were collected, authenticated, shade dried, powdered, and extracted using ethanol by Soxhlet extraction. Nine toothpaste formulations (F1–F9) were prepared using varying concentrations of Neem extract along with calcium carbonate, glycerin, sorbitol, sodium carboxymethyl cellulose, sodium benzoate, peppermint oil, and purified water. The prepared formulations were evaluated for organoleptic characteristics, physicochemical parameters, phytochemical screening, antimicrobial activity, anti-cariogenic activity, and stability studies according to ICH guidelines. The extraction process yielded 17.12% extract, indicating efficient recovery of phytoconstituents. All formulations demonstrated acceptable color, consistency, homogeneity, and pH suitable for oral use. Phytochemical screening confirmed the presence of alkaloids, flavonoids, tannins, polyphenols, and terpenoids. The optimized formulation (F5) exhibited significant antimicrobial activity against Streptococcus mutans, Staphylococcus aureus, and Candida albicans. Anti-cariogenic evaluation showed 74.8% inhibition of biofilm formation, demonstrating effective plaque prevention potential. Stability studies confirmed that the formulation remained stable under both room temperature and accelerated storage conditions without significant changes in physicochemical properties.**

**The findings suggest that Neem-based herbal toothpaste is a safe, stable, and effective natural formulation with promising potential as an alternative to conventional synthetic toothpaste for maintaining oral hygiene and preventing dental caries.**

***Index Terms*—Neem, Herbal Toothpaste, Azadirachta indica, Dental Caries, Oral Hygiene, Antimicrobial Activity, Herbal Formulation.**

## I. INTRODUCTION

### 1.1 Oral Cavity and Dental Disorders

The oral cavity is an essential part of the human body that plays a fundamental role in mastication, speech, digestion, and overall health maintenance. It consists of several anatomical structures including teeth, tongue, gingiva, palate, salivary glands, buccal mucosa, and supporting periodontal tissues. Among these structures, teeth are considered one of the most important components responsible for food breakdown, maintaining facial structure, and supporting normal speech function. The oral cavity also serves as the primary entry point for food and

microorganisms, making oral hygiene a critical factor in maintaining both oral and systemic health (Petersen & Ogawa, 2016).

Teeth are highly mineralized calcified structures embedded within the alveolar bone of the jaws. Anatomically, a tooth consists of four major structural components: enamel, dentin, cementum, and pulp. Enamel forms the outermost protective layer and is considered the hardest tissue in the human body due to its high mineral content. Beneath enamel lies dentin, a calcified tissue that provides structural support and transmits sensory stimuli. Cementum covers the tooth root and anchors the tooth to the periodontal ligament, while the pulp contains blood vessels, connective tissue, and nerve fibers responsible for nourishment and sensory function (Fejerskov & Kidd, 2015). The surrounding periodontal tissues provide mechanical support and protection to the teeth and play an important role in maintaining oral stability. Maintaining oral hygiene is essential for preventing microbial colonization, plaque accumulation, dental decay, and inflammatory conditions affecting oral tissues. Proper oral hygiene practices such as tooth brushing, flossing, and regular dental cleaning help remove food debris and bacterial biofilms that accumulate on tooth surfaces. Poor oral hygiene often leads to microbial proliferation, resulting in several oral diseases that can compromise both oral function and general health. In recent years, increasing evidence has shown that oral diseases may contribute to systemic disorders including cardiovascular disease, diabetes mellitus, and respiratory infections, emphasizing the importance of preventive oral healthcare (Nazir, 2017).

One of the most common oral diseases worldwide is dental caries, a chronic multifactorial disease characterized by progressive demineralization of tooth enamel and dentin caused by acid-producing bacteria. The primary microorganism associated with dental caries is Streptococcus mutans, which metabolizes dietary carbohydrates and produces organic acids capable of dissolving tooth minerals. If left untreated, dental caries may progress to pulp infection, severe pain, and eventual tooth loss (Selwitz et al., 2007). Gingivitis is another common oral disorder characterized by inflammation of the gingival tissues caused primarily by plaque accumulation along the gum line. Clinical symptoms include redness, swelling, tenderness, and bleeding during brushing. Although gingivitis is generally reversible, failure to manage the condition may lead to more severe periodontal diseases (Chapple et al., 2015). Periodontitis represents an advanced inflammatory disease affecting the supporting structures of teeth, including periodontal ligaments and alveolar bone. It usually develops as a progression of untreated gingivitis and results in destruction of connective tissue attachment, gum recession, tooth mobility, and eventual tooth loss. Periodontal disease has also been associated with systemic inflammatory disorders and chronic metabolic diseases (Kinane et al., 2017).

Dental plaque formation is one of the primary initiating factors responsible for many oral diseases. Plaque is a structured microbial biofilm consisting of bacteria, salivary proteins, polysaccharides, and food debris that adheres strongly to tooth surfaces. Continuous plaque accumulation provides an environment favorable for bacterial growth and acid production, leading to caries and gingival inflammation. Effective plaque control is therefore essential for maintaining oral health (Marsh, 2006). Halitosis, commonly referred to as bad breath, is another frequently encountered oral condition that significantly affects social confidence and quality of life. The condition primarily results from the accumulation of volatile sulfur compounds produced by anaerobic bacteria present on the tongue surface, periodontal pockets, and dental plaque. Poor oral hygiene, periodontal disease, food retention, and microbial imbalance are major contributors to halitosis. Proper oral care products with antimicrobial activity play an important role in preventing this condition (Porter & Scully, 2006). The increasing prevalence of oral diseases worldwide highlights the necessity for safe, effective, and preventive oral hygiene products. Conventional toothpaste formulations often contain synthetic antimicrobial agents, surfactants, preservatives, and chemical additives which may produce undesirable side effects after prolonged use. Therefore, growing interest has shifted toward herbal oral care formulations that provide effective antimicrobial protection with improved safety and better long-term patient compliance.

### 1.2 Toothpaste and Dentifrices

Toothpaste is a semi-solid dentifrice formulation designed for cleaning and maintaining oral hygiene

through regular brushing of teeth. It is one of the most commonly used oral care products worldwide and plays an important role in preventing dental diseases by removing food debris, plaque, and microbial deposits from tooth surfaces. The term dentifrice generally refers to preparations such as toothpaste, tooth powders, gels, or creams that are used in conjunction with a toothbrush for cleaning, polishing, and preserving the health of teeth and surrounding oral tissues. In addition to mechanical cleaning, modern toothpaste formulations are often designed to provide therapeutic benefits including prevention of dental caries, reduction of plaque accumulation, control of gingivitis, desensitization of teeth, whitening effects, and maintenance of fresh breath (Lippert, 2013).

Dentifrices play a central role in daily oral hygiene maintenance by facilitating the removal of microbial biofilms and preventing the accumulation of plaque, which is one of the primary causes of dental caries and periodontal diseases. Regular use of toothpaste improves oral cleanliness by reducing bacterial colonization and neutralizing harmful acids produced by microorganisms such as Streptococcus mutans and other cariogenic bacteria. Therapeutic dentifrices containing antimicrobial or anti-inflammatory agents may further contribute to reducing gingival inflammation, preventing tartar formation, and maintaining overall oral health. Effective dentifrice formulations also help strengthen tooth enamel and reduce enamel demineralization caused by acidic metabolites generated by oral bacteria (Joiner, 2010).

Conventional toothpaste formulations contain a variety of functional ingredients designed to provide cleaning, stability, preservation, and therapeutic action. Abrasive agents such as calcium carbonate, hydrated silica, and dicalcium phosphate help remove plaque and surface stains. Humectants including glycerin, sorbitol, and propylene glycol prevent drying and maintain moisture content. Surfactants such as sodium lauryl sulfate (SLS) generate foam that assists in dispersion and cleaning action. Fluoride compounds, particularly sodium fluoride and sodium monofluorophosphate, are incorporated to strengthen enamel and prevent dental caries. Preservatives such as parabens prevent microbial contamination, while flavoring agents, sweeteners, binders, and coloring agents improve patient acceptability and product stability (Van Loveren, 2013).

Although synthetic toothpaste formulations are widely used, prolonged exposure to certain chemical ingredients has raised concerns regarding safety and adverse effects. Triclosan, previously used as an antimicrobial agent, has been associated with disruption of endocrine function and development of bacterial resistance after repeated exposure. Sodium lauryl sulfate, a commonly used surfactant, may cause irritation of oral mucosa, dryness, burning sensation, and recurrent aphthous ulcers in sensitive individuals. Parabens, used as preservatives, have generated safety concerns due to their potential estrogenic activity and possible long-term toxicological effects. Excessive exposure to fluoride beyond recommended limits may result in dental fluorosis, enamel discoloration, skeletal fluorosis, and other toxic manifestations, particularly in children. Increasing awareness regarding these limitations has encouraged the search for safer natural alternatives, including herbal dentifrices formulated using medicinal plant extracts with antimicrobial, anti-inflammatory, and anti-cariogenic properties (McCullough & Farah, 2008).

The growing concern regarding the safety of synthetic oral care ingredients has created significant interest in herbal toothpaste formulations that can provide effective oral hygiene while minimizing chemical exposure. Herbal dentifrices offer a promising alternative by utilizing plant-derived bioactive compounds capable of preventing microbial growth, reducing inflammation, controlling plaque formation, and maintaining long-term oral health without significant adverse effects.

### 1.3 Herbal Oral Care Products

Herbal oral care products have gained substantial attention in recent years due to increasing consumer awareness regarding the potential side effects associated with prolonged use of synthetic chemicals in conventional dental formulations. Herbal products are preparations formulated using medicinal plants or plant-derived bioactive constituents that possess therapeutic properties beneficial for maintaining oral hygiene and preventing oral diseases. The use of medicinal plants for oral care has been documented for centuries in traditional systems of medicine such as Ayurveda, Traditional Chinese Medicine, and Unani medicine, where plant materials such as neem twigs, clove, miswak, babool, peppermint, tulsi, and licorice were commonly used for cleaning teeth, strengthening

gums, and preventing bad breath. With growing interest in natural healthcare, herbal oral care products have emerged as promising alternatives to synthetic dentifrices and mouthwashes (Palombo, 2011).

The global demand for herbal dental formulations has increased significantly due to changing consumer preferences toward natural and chemical-free personal care products. Modern consumers increasingly seek safer oral hygiene products that provide therapeutic benefits while minimizing exposure to synthetic preservatives, surfactants, artificial sweeteners, and antimicrobial agents. The herbal toothpaste market has expanded rapidly as individuals become more conscious of long-term oral health and product safety. Rising awareness regarding antibiotic resistance, chemical sensitivity reactions, and adverse effects associated with synthetic oral care ingredients has further accelerated the popularity of herbal dentifrices in both developed and developing countries (Khan et al., 2021). One of the major advantages of herbal oral care products is their relatively low toxicity profile compared with conventional formulations containing synthetic chemicals. Herbal ingredients generally contain naturally occurring phytochemicals such as flavonoids, tannins, alkaloids, terpenoids, essential oils, and polyphenols that exhibit antimicrobial, anti-inflammatory, antioxidant, and wound-healing properties. Unlike synthetic antimicrobial agents such as triclosan and strong detergents such as sodium lauryl sulfate, herbal ingredients are less likely to cause irritation, mucosal dryness, allergic reactions, or disturbance of normal oral microflora when used regularly. This improved safety profile makes herbal toothpaste particularly attractive for long-term daily use (Prasanth, 2011).

Patient compliance is another important factor contributing to the growing acceptance of herbal dental products. Many herbal formulations possess naturally pleasant flavors and aromas derived from plant essential oils such as peppermint, clove, cinnamon, and eucalyptus, improving user acceptability and encouraging regular oral hygiene practices. Additionally, consumers often perceive herbal products as safer, healthier, and more compatible with traditional health practices, resulting in better adherence to daily oral care regimens. The absence of harsh chemicals and reduced incidence of adverse reactions further improves patient satisfaction, especially among children and individuals with sensitive oral mucosa (Taheri et al., 2011). Environmental sustainability has become another major advantage favoring herbal oral care products over synthetic formulations. Conventional toothpaste production often involves petroleum-derived surfactants, synthetic preservatives, artificial coloring agents, and chemical manufacturing processes that may contribute to environmental pollution during production and disposal. In contrast, herbal formulations utilize biodegradable plant-based ingredients that are renewable, environmentally friendly, and associated with lower ecological burden. Sustainable cultivation of medicinal plants, reduced chemical waste generation, and lower environmental toxicity make herbal oral care products more compatible with modern green pharmaceutical and eco-friendly manufacturing practices (Dureja et al., 2012).

The growing demand for natural healthcare products, increasing awareness of chemical safety concerns, improved patient acceptance, and emphasis on sustainable manufacturing have collectively accelerated research into herbal dentifrices. Herbal toothpaste formulations therefore represent a promising approach for maintaining oral hygiene while providing effective antimicrobial protection, reduced toxicity, and long-term environmental sustainability.

### 1.4 Azadirachta indica (Neem)

Azadirachta indica A. Juss., commonly known as Neem, is one of the most widely recognized medicinal plants in traditional Indian medicine and has been extensively used in Ayurveda for the prevention and treatment of various infectious and inflammatory disorders. Neem is often referred to as the “Village Pharmacy” because almost every part of the plant including leaves, bark, seeds, flowers, fruits, and roots possesses therapeutic value. Due to its broad spectrum antimicrobial, anti-inflammatory, antioxidant, and immunomodulatory properties, Neem has gained significant attention in pharmaceutical, cosmetic, and oral healthcare formulations.

Table 1. Botanical Description of Azadirachta indica (Neem)

| Parameter | Description |
|---|---|
| Scientific Name | Azadirachta indica A. Juss. |
| Common Name | Neem |

| Family | Meliaceae |
|---|---|
| Plant Type | Evergreen medicinal tree |
| Height | 12–20 meters |
| Leaves | Pinnate, alternate, bright green, serrated margins |
| Flowers | Small, white, fragrant flowers |
| Fruits | Smooth olive-like drupe containing seed |
| Seeds | Rich source of neem oil and limonoids |
| Bark | Rough, fissured, grayish-brown |

Neem grows rapidly in tropical and subtropical climates and is highly resistant to drought conditions.

Table 2. Taxonomical Classification of Azadirachta indica

| Taxonomic Rank | Classification |
|---|---|
| Kingdom | Plantae |
| Division | Magnoliophyta |
| Class | Magnoliopsida |
| Order | Sapindales |
| Family | Meliaceae |
| Genus | Azadirachta |
| Species | Azadirachta indica |

Table 3. Geographical Distribution of Neem

| Region | Distribution |
|---|---|
| India | Widely distributed throughout all states |
| South Asia | Pakistan, Bangladesh, Sri Lanka, Nepal |
| Southeast Asia | Thailand, Indonesia, Myanmar |
| Africa | Nigeria, Kenya, Sudan, Ethiopia |
| Middle East | Iran, Saudi Arabia |
| Other Tropical Regions | Australia, Latin America |

Neem is predominantly cultivated in warm climatic conditions and is considered native to the Indian subcontinent.

Table 4. Traditional Medicinal Importance of Neem in Ayurveda

| Ayurvedic Application | Traditional Use |
|---|---|
| Oral hygiene | Cleaning teeth using neem twigs |
| Dental infections | Treatment of gingivitis and mouth ulcers |
| Skin disorders | Treatment of acne, eczema, fungal infections |
| Wound healing | Antiseptic and tissue healing agent |
| Anti-inflammatory | Used in inflammatory disorders |
| Antipyretic | Management of fever |
| Blood purification | Detoxification and immune support |
| Antiparasitic | Treatment of intestinal worms |
| Hair care | Control of dandruff and scalp infections |

In Ayurveda, Neem is considered a natural detoxifying and antimicrobial herb with significant value in maintaining oral and systemic health.

### 1.5 Phytochemical Constituents of Neem

Neem contains numerous biologically active phytoconstituents responsible for its therapeutic activity.

Table 5. Major Phytochemical Constituents of Azadirachta indica

| Phytochemical | Chemical Class | Reported Biological Role |
|---|---|---|
| Nimbidin | Limonoid | Anti-inflammatory, antibacterial |
| Nimbin | Triterpenoid | Antifungal, antimicrobial |
| Azadirachtin | Tetranortriterpenoid | Antimicrobial, insecticidal |
| Gedunin | Limonoid | Antimalarial, antifungal |
| Flavonoids | Polyphenolic compounds | Antioxidant activity |
| Alkaloids | Nitrogen-containing compounds | Antimicrobial action |
| Polyphenols | Phenolic compounds | Free radical scavenging |
| Tannins | Polyphenolic compounds | Astringent and antibacterial effect |
| Terpenoids | Isoprene derivatives | Anti-inflammatory activity |

The presence of multiple phytoconstituents contributes significantly to the medicinal and antimicrobial properties of Neem.

1.6 Pharmacological Activities of Neem

Neem exhibits a broad spectrum of pharmacological activities due to its complex phytochemical composition.

Table 6. Pharmacological Activities of Azadirachta indica

| Pharmacological Activity | Mechanism / Therapeutic Effect |
|---|---|
| Antibacterial Activity | Inhibits growth of oral pathogens including Streptococcus mutans and Staphylococcus aureus |
| Antifungal Activity | Effective against Candida albicans and other fungal species |
| Anti-inflammatory Activity | Reduces inflammatory mediators and gingival swelling |
| Antioxidant Activity | Neutralizes free radicals and reduces oxidative damage |
| Anti-plaque Activity | Prevents formation of bacterial biofilm on teeth surfaces |
| Anti-cariogenic Activity | Reduces acid-producing bacteria responsible for dental caries |
| Wound Healing Activity | Accelerates repair of damaged oral tissues |
| Immunomodulatory Activity | Enhances natural defense mechanisms |

These pharmacological properties make Azadirachta indica a promising herbal candidate for the formulation of herbal toothpaste intended for oral hygiene maintenance and prevention of dental diseases.

## II. MATERIALS AND METHODS

This section describes the materials used and the experimental procedures adopted for the formulation and evaluation of herbal toothpaste containing Azadirachta indica (Neem) extract for oral hygiene maintenance and prevention of dental caries.

2.1 Materials

Fresh leaves of Azadirachta indica (Azadirachta indica) were collected and used as the main herbal ingredient. Other excipients required for toothpaste formulation included calcium carbonate as an abrasive agent, glycerin and sorbitol as humectants, sodium carboxymethyl cellulose (CMC) as a binder, sodium benzoate as a preservative, peppermint oil as a flavoring agent, saccharin sodium as a sweetener, and purified water as the vehicle. Analytical-grade ethanol and distilled water were used during extraction and evaluation studies.

2.2 Authentication of Plant Material

The collected Neem leaves were thoroughly washed, shade dried, and authenticated by a qualified botanist from the Department of Botany. A voucher specimen number was assigned and preserved for future reference. Proper botanical identification ensured authenticity and reproducibility of the research work.

2.3 Preparation of Neem Extract

The collected leaves were cleaned and dried under shade for 7–10 days to prevent degradation of phytoconstituents. The dried leaves were pulverized into coarse powder using a mechanical grinder. Extraction was carried out by Soxhlet extraction using ethanol as solvent for 6–8 hours. The obtained extract was concentrated using rotary evaporation and stored in an airtight container for formulation studies.

2.4 Formulation of Herbal Toothpaste

The toothpaste base was prepared by mixing calcium carbonate, glycerin, sorbitol, sodium CMC, and purified water under continuous stirring. Predetermined concentrations of Neem extract were incorporated into the base followed by addition of sodium benzoate, saccharin sodium, and peppermint oil. The mixture was homogenized until a smooth and uniform paste was obtained.

2.5 Formulation Design

Nine formulations (F1–F9) were prepared using varying concentrations of Neem extract and glycerin to optimize the final formulation. The concentration of Neem extract ranged from 2–6%, while all other excipients were kept within acceptable pharmaceutical limits.

Table 7: Composition of Herbal Toothpaste Formulations (F1–F9)

| Ingredients | Function | F1 | F2 | F3 | F4 | F5 | F6 | F7 | F8 | F9 |
|---|---|---|---|---|---|---|---|---|---|---|
| Neem Extract (%) | Active antimicrobial agent | 2 | 2 | 2 | 4 | 4 | 4 | 6 | 6 | 6 |
| Calcium Carbonate (%) | Abrasive agent | 30 | 30 | 30 | 30 | 30 | 30 | 30 | 30 | 30 |
| Glycerin (%) | Humectant | 10 | 15 | 20 | 10 | 15 | 20 | 10 | 15 | 20 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Sorbitol (%) | Moisturizer/Sweetening agent | 15 | 15 | 15 | 15 | 15 | 15 | 15 | 15 | 15 |
| Sodium CMC (%) | Binder/Thickening agent | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| Sodium Benzoate (%) | Preservative | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 |
| Saccharin Sodium (%) | Sweetener | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |
| Peppermint Oil (%) | Flavoring agent | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 |
| Purified Water | Vehicle | q.s. | q.s. | q.s. | q.s. | q.s. | q.s. | q.s. | q.s. | q.s. |

2.6 Evaluation Parameters

The prepared formulations were evaluated for organoleptic properties including color, odor, texture, and appearance. Physicochemical studies included pH determination, viscosity, spreadability, foamability, abrasiveness, moisture content, and homogeneity. Phytochemical screening was performed to confirm the presence of active constituents. Antimicrobial activity was tested against Streptococcus mutans, Staphylococcus aureus, and Candida albicans using agar well diffusion method.

2.7 Stability Studies

Stability studies were performed according to ICH guidelines under room temperature (25 ± 2°C/60% RH) and accelerated conditions (40 ± 2°C/75% RH) for a period of three months. Parameters such as pH, color, odor, viscosity, and physical stability were monitored periodically to evaluate formulation stability.

## III. RESULTS AND DISCUSSION

3.1 Extraction Yield

The ethanolic extraction of dried Azadirachta indica leaves produced a dark green semisolid extract with a characteristic bitter odor. The extraction process yielded a satisfactory amount of extract, indicating efficient recovery of bioactive phytoconstituents from the plant material.

Table 1. Extraction Yield of Azadirachta indica Leaves

| Parameter | Observation |
|---|---|
| Weight of dried leaf powder | 250 g |
| Weight of extract obtained | 42.8 g |
| Percentage yield | 17.12 ± 0.36 % |

Discussion

The percentage yield obtained from Neem leaves was found to be 17.12%, which indicates effective extraction of active phytoconstituents using ethanol as the extraction solvent. The relatively high extraction efficiency may be attributed to the presence of polar compounds such as flavonoids, tannins, alkaloids, limonoids, and polyphenolic compounds. Similar extraction yield values have been reported in previous phytochemical investigations of Neem leaves, confirming that ethanolic extraction is suitable for recovering therapeutically active compounds.

3.2 Organoleptic Characteristics

All toothpaste formulations were visually evaluated for color, odor, appearance, consistency, and homogeneity.

Table 2. Organoleptic Evaluation of Toothpaste Formulations

| Batch | Color | Odor | Appearance | Consistency |
|---|---|---|---|---|
| F1 | Light Green | Mint | Smooth | Soft |
| F2 | Light Green | Mint | Smooth | Soft |
| F3 | Green | Mint | Smooth | Moderate |
| F4 | Green | Mint | Smooth | Moderate |
| F5 | Green | Pleasant Mint | Smooth | Uniform |
| F6 | Dark Green | Pleasant Mint | Smooth | Uniform |
| F7 | Dark Green | Pleasant Mint | Smooth | Thick |
| F8 | Dark Green | Pleasant Mint | Smooth | Thick |
| F9 | Dark Green | Pleasant Mint | Smooth | Highly Thick |

Discussion

All nine formulations showed acceptable organoleptic characteristics without any visible phase separation or grittiness. The green coloration intensified with increasing concentration of Neem extract, confirming successful incorporation of plant constituents into the formulation. Formulations containing higher glycerin concentration demonstrated smoother consistency and better spreadability. Among all batches, formulation F5 exhibited optimum consistency, pleasant odor, and excellent homogeneity, making it most acceptable for further evaluation.

3.3 Physicochemical Parameters

Physicochemical evaluation was performed to determine formulation quality, stability, and suitability for oral use.

Table 3. Physicochemical Evaluation of Toothpaste Formulations

| Batch | pH | Viscosity (cP) | Spreadability (cm) | Foamability (mL) | Moisture Content (%) |
|---|---|---|---|---|---|
| F1 | 6.62 ± 0.03 | 25500 ± 120 | 5.8 ± 0.2 | 38 ± 1 | 2.8 ± 0.1 |
| F2 | 6.70 ± 0.04 | 26120 ± 140 | 6.0 ± 0.1 | 39 ± 1 | 2.7 ± 0.1 |
| F3 | 6.74 ± 0.02 | 26850 ± 150 | 6.1 ± 0.2 | 40 ± 2 | 2.6 ± 0.1 |
| F4 | 6.82 ± 0.03 | 27240 ± 160 | 6.3 ± 0.2 | 41 ± 1 | 2.5 ± 0.1 |
| F5 | 6.89 ± 0.02 | 28120 ± 130 | 6.5 ± 0.1 | 42 ± 1 | 2.4 ± 0.1 |
| F6 | 6.91 ± 0.04 | 28850 ± 170 | 6.6 ± 0.2 | 42 ± 1 | 2.3 ± 0.1 |
| F7 | 6.96 ± 0.03 | 29420 ± 180 | 6.7 ± 0.1 | 43 ± 1 | 2.2 ± 0.1 |
| F8 | 7.02 ± 0.02 | 30110 ± 150 | 6.8 ± 0.1 | 43 ± 1 | 2.1 ± 0.1 |
| F9 | 7.08 ± 0.03 | 30950 ± 190 | 6.9 ± 0.2 | 44 ± 2 | 2.1 ± 0.1 |

Discussion

All formulations demonstrated pH values between 6.6 and 7.1, which are considered safe and compatible with the oral cavity. Viscosity gradually increased with increasing glycerin concentration due to improved binding and humectant properties. Spreadability remained within acceptable limits, ensuring easy extrusion and application during brushing. Moisture content remained low enough to prevent microbial contamination and preserve formulation stability. F5 showed the most balanced physicochemical profile and was selected as the optimized formulation.

3.4 Phytochemical Screening Results

Phytochemical screening confirmed the presence of major bioactive constituents responsible for therapeutic activity.

Table 4. Phytochemical Screening of Optimized Formulation (F5)

| Phytoconstituent | Result |
|---|---|
| Alkaloids | +++ |
| Flavonoids | +++ |
| Tannins | ++ |
| Polyphenols | +++ |
| Terpenoids | ++ |
| Glycosides | ++ |

Discussion

The phytochemical analysis confirmed successful incorporation of Neem bioactive compounds into the toothpaste formulation. Flavonoids and polyphenols contribute antioxidant activity, while tannins and terpenoids are associated with antimicrobial and anti-inflammatory effects. These constituents are likely responsible for the therapeutic benefits observed in antimicrobial and anti-cariogenic evaluation.

3.5 Antimicrobial Activity

The antimicrobial activity was evaluated against common oral pathogens.

Table 5. Antimicrobial Activity of Optimized Formulation (F5)

| Microorganism | Zone of Inhibition (mm) | Standard Drug | Standard Zone (mm) |
|---|---|---|---|
| Streptococcus mutans | 21.4 ± 0.6 | Chlorhexidine | 29.2 ± 0.5 |
| Staphylococcus aureus | 18.8 ± 0.5 | Chlorhexidine | 27.5 ± 0.4 |
| Candida albicans | 17.6 ± 0.4 | Fluconazole | 24.8 ± 0.5 |

Discussion

The optimized formulation exhibited significant antimicrobial activity against tested microorganisms. The highest activity was observed against Streptococcus mutans, the primary bacterium responsible for dental caries. The antimicrobial action may be attributed to Neem phytoconstituents such as nimbidin, nimbin, and azadirachtin, which disrupt microbial cell wall integrity and inhibit bacterial growth.

3.6 Anti-Cariogenic Activity

The anti-cariogenic activity was evaluated by measuring inhibition against Streptococcus mutans biofilm formation.

Table 6. Anti-Cariogenic Activity

| Sample | Biofilm Inhibition (%) |
|---|---|
| Control | 12.5 ± 1.2 |
| Commercial Toothpaste | 82.6 ± 2.1 |
| F5 Herbal Toothpaste | 74.8 ± 1.8 |

Discussion

The herbal toothpaste formulation showed strong anti-cariogenic activity with 74.8% inhibition of biofilm formation. This suggests effective suppression of plaque-forming cariogenic bacteria. Although slightly lower than commercial toothpaste, the herbal formulation offers the advantage of reduced synthetic chemical exposure and lower toxicity risk during long-term use.

3.7 Stability Study Results

Stability studies were performed according to ICH guidelines under room temperature and accelerated conditions for three months.

Table 7. Stability Study of Optimized Formulation (F5)

| Time | pH | Color | Odor | Viscosity (cP) | Stability |
|---|---|---|---|---|---|
| Initial | 6.89 | Green | Mint | 28120 | Stable |
| 1 Month | 6.87 | Green | Mint | 27980 | Stable |
| 2 Months | 6.84 | Green | Mint | 27740 | Stable |
| 3 Months | 6.81 | Slight Dark Green | Mint | 27560 | Stable |

Discussion

The optimized formulation remained physically and chemically stable throughout the storage period. Only minor reductions in pH and viscosity were observed, which remained within acceptable pharmaceutical limits. No microbial growth, phase separation, odor deterioration, or significant color changes were detected. These results indicate good formulation stability and suitability for long-term storage.

## IV. CONCLUSION

The present investigation successfully developed and evaluated a herbal toothpaste formulation containing Azadirachta indica (Neem) extract for oral hygiene maintenance and prevention of dental caries. Nine formulations were prepared and systematically evaluated for organoleptic properties, physicochemical characteristics, phytochemical composition, antimicrobial activity, anti-cariogenic potential, and formulation stability. Among the prepared formulations, batch F5 demonstrated the most desirable characteristics with acceptable pH, suitable viscosity, good spreadability, adequate foamability, and excellent homogeneity. Phytochemical screening confirmed the presence of important bioactive constituents including flavonoids, alkaloids, tannins, polyphenols, terpenoids, and glycosides, which contribute significantly to the therapeutic activity of Neem. The optimized formulation exhibited considerable antimicrobial activity against major oral pathogens including Streptococcus mutans, Staphylococcus aureus, and Candida albicans, indicating its potential to control microbial growth associated with oral infections.

The anti-cariogenic study demonstrated effective inhibition of biofilm formation and plaque-producing microorganisms, suggesting its role in reducing dental caries development. Stability studies performed according to ICH guidelines confirmed that the formulation remained physically and chemically stable throughout the study period without significant

changes in pH, viscosity, color, or odor. Overall, the study confirms that Neem-based herbal toothpaste can serve as a safe, effective, and eco-friendly alternative to conventional synthetic dentifrices. The formulation offers significant potential for long-term oral healthcare and warrants further clinical evaluation for commercial application and large-scale production.

## REFERENCES


[1] P. E. Petersen and H. Ogawa, "Prevention of dental caries through the use of fluoride—The WHO approach," *Community Dentistry and Oral Epidemiology*, vol. 44, no. 6, pp. 557–564, 2016.

[2] O. Fejerskov and E. Kidd, *Dental Caries: The Disease and Its Clinical Management*. Hoboken, NJ, USA: Wiley-Blackwell, 2015.

[3] M. A. Nazir, "Prevalence of periodontal disease and the association with systemic diseases," *International Journal of Health Sciences*, vol. 11, no. 2, pp. 72–80, 2017.

[4] R. H. Selwitz, A. I. Ismail, and N. B. Pitts, "Dental caries," *The Lancet*, vol. 369, no. 9555, pp. 51–59, 2007, doi: 10.1016/S0140-6736(07)60031-2.

[5] L. C. Chapple, F. Van der Weijden, C. Doerfer, *et al.*, "Primary prevention of periodontitis," *Journal of Clinical Periodontology*, vol. 42, Suppl. 16, pp. S71–S76, 2015, doi: 10.1111/jcpe.12366.

[6] D. F. Kinane, P. G. Stathopoulou, and P. N. Papapanou, "Periodontal diseases," *Nature Reviews Disease Primers*, vol. 3, Art. no. 17038, 2017, doi: 10.1038/nrdp.2017.38.

[7] P. D. Marsh, "Dental plaque as a biofilm and a microbial community—Implications for health and disease," *BMC Oral Health*, vol. 6, Suppl. 1, Art. no. S14, 2006, doi: 10.1186/1472-6831-6-S1-S14.

[8] S. R. Porter and C. Scully, "Oral malodour (halitosis)," *BMJ*, vol. 333, no. 7569, pp. 632–635, 2006, doi: 10.1136/bmj.38954.631968.AE.

[9] F. Lippert, "An introduction to toothpaste—Its purpose, history and ingredients," *Monographs in Oral Science*, vol. 23, pp. 1–14, 2013, doi: 10.1159/000350456.

[10] Joiner, "Tooth colour: A review of the literature," *Journal of Dentistry*, vol. 38, Suppl. 2, pp. e17–e23, 2010, doi: 10.1016/j.jdent.2010.03.002.

[11] Van Loveren, "Toothpastes," in *Monographs in Oral Science*, vol. 23, 2013, pp. 24–37, doi: 10.1159/000350465.

[12] M. J. McCullough and C. S. Farah, "The role of alcohol in oral carcinogenesis with focus on oral cancer risk factors," *Australian Dental Journal*, vol. 53, no. 4, pp. 302–305, 2008.

[13] C. G. Jones, "Chlorhexidine: Is it still the gold standard?" *Periodontology 2000*, vol. 15, no. 1, pp. 55–62, 1997, doi: 10.1111/j.1600-0757.1997.tb00105.x.

[14] E. A. Palombo, "Traditional medicinal plant extracts and natural products with activity against oral bacteria: Potential application in the prevention and treatment of oral diseases," *Evidence-Based Complementary and Alternative Medicine*, vol. 2011, Art. no. 680354, 2011, doi: 10.1093/ecam/nep067.

[15] S. A. Khan, N. Fatima, and P. Singh, "Herbal formulations in oral healthcare: Current perspectives and future opportunities," *Journal of Herbal Medicine*, vol. 28, Art. no. 100438, 2021.

[16] M. Prasanth, "Antimicrobial efficacy of different toothpastes and mouthrinses: An in vitro study," *Dental Research Journal*, vol. 8, no. 2, pp. 85–94, 2011.

[17] J. B. Taheri, S. Azimi, N. Rafieian, and Z. Akhavan, "Herbs in dentistry," *International Dental Journal*, vol. 61, no. 6, pp. 287–296, 2011, doi: 10.1111/j.1875-595X.2011.00064.x.

[18] H. Dureja, D. Kaushik, M. Gupta, V. Kumar, and V. Lather, "Cosmeceuticals: An emerging concept," *Indian Journal of Pharmacology*, vol. 44, no. 2, pp. 143–147, 2012, doi: 10.4103/0253-7613.93847.

[19] K. Biswas, I. Chattopadhyay, R. K. Banerjee, and U. Bandyopadhyay, "Biological activities and medicinal properties of Neem (*Azadirachta indica*)," *Current Science*, vol. 82, no. 11, pp. 1336–1345, 2002.

[20] R. Subapriya and S. Nagini, "Medicinal properties of Neem leaves: A review," *Current*

*Medicinal Chemistry – Anti-Cancer Agents*, vol. 5, no. 2, pp. 149–156, 2005.

[21] M. A. Alzohairy, "Therapeutics role of *Azadirachta indica* (Neem) and their active constituents in diseases prevention and treatment," *Evidence-Based Complementary and Alternative Medicine*, vol. 2016, Art. no. 7382506, 2016, doi: 10.1155/2016/7382506.

[22] M. R. S. M. Pai, L. D. Acharya, and N. Udupa, "Evaluation of antiplaque activity of *Azadirachta indica* leaf extract gel," *Journal of Ethnopharmacology*, vol. 90, no. 1, pp. 99–103, 2004, doi: 10.1016/j.jep.2003.09.035.

[23] M. A. Alzohairy, "Therapeutics role of *Azadirachta indica* and their active constituents in disease prevention and treatment," *Evidence-Based Complementary and Alternative Medicine*, vol. 2016, Art. no. 7382506, 2016, doi: 10.1155/2016/7382506.