

Formulation and Evaluation of Herbal Toothpaste from *Achyranthes Aspera*

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Abstract—*Achyranthes aspera* extract was used in the formulation of a herbal toothpaste, which was assessed for stability, antibacterial activity, and physicochemical characteristics. Glycerin and sorbitol were employed as humectants, carboxymethyl cellulose and guar gum as thickeners, and calcium carbonate as an abrasive. Alkaloids, flavonoids, tannins, saponins, and phenolic substances were found using phytochemical screening. With a neutral pH of 7.03 ± 0.21 , good spreadability, moderate foaming ability, and an acceptable moisture level, the formulation had a smooth, uniform appearance. At 10 mg/mL, the antibacterial activity against *Staphylococcus aureus* demonstrated a maximal inhibition zone of 6 mm. 30-day stability tests showed no discernible alterations. According to the study, toothpaste made from *Achyranthes aspera* is a reliable and secure herbal dental care product.

Keywords— *Achyranthes aspera*, herbal toothpaste, antibacterial activity, stability

I. INTRODUCTION

Poor oral hygiene can lead to plaque buildup, dental cavities, gingivitis, and halitosis. Oral health is a crucial aspect of overall health. In everyday oral care, toothpaste—a semisolid dose form—is frequently used to clean teeth, eliminate plaque, and uphold good oral hygiene. Semisolid formulations, such as pastes and gels, are appropriate for oral hygiene products because they offer enhanced patient compliance, localised drug administration, and simplicity of application. Interest in herbal-based toothpastes has grown as worries about the negative effects of synthetic components have grown. Herbal remedies are typically regarded as safe for long-term usage and have antibacterial, anti-inflammatory, antioxidant, and analgesic qualities. Rich in alkaloids, flavonoids, saponins, and tannins with antibacterial action and safety, *Achyranthes aspera*, also referred to as Apamarga, is a traditional medicinal herb.



Fig.1 Herbal Toothpaste

A. Types of Toothpaste

- **Fluoride toothpaste:** Strengthens tooth enamel and prevents dental caries.
- **Whitening toothpaste:** Contains mild abrasives or chemical agents to remove surface stains.
- **Sensitive toothpaste:** Formulated with desensitizing agents such as potassium nitrate.
- **Tartar control toothpaste:** Helps prevent tartar formation using pyrophosphates or zinc compounds.

- **Children's toothpaste:** Contains lower fluoride concentration with mild flavors for safe use.
- **Natural toothpaste:** Prepared using plant-based ingredients with minimal synthetic additives.
- **Gum health toothpaste:** Supports gingival protection and reduces inflammation.
- **Breath-freshening toothpaste:** Helps control halitosis and improves oral freshness.
- **Charcoal toothpaste:** Used for stain removal and oral cleansing; long-term safety remains under study.

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B. Ideal Properties of Herbal Toothpaste.

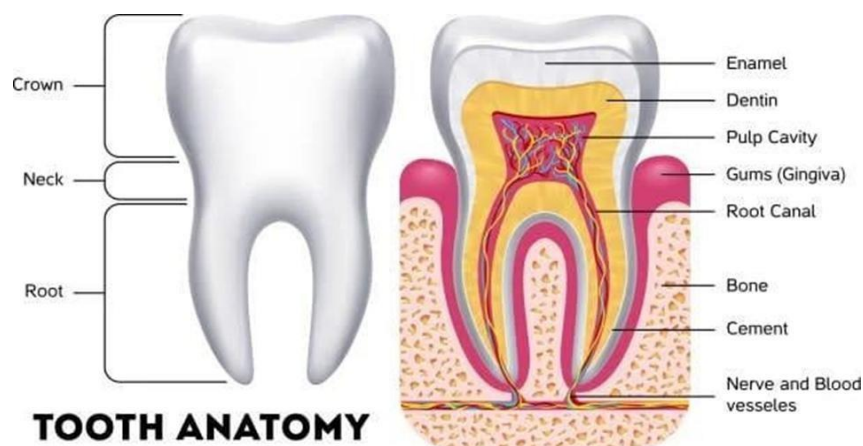
- To promote frequent use, it should taste good.
- Must result in a suitable and sufficient foaming action.
- Should have a consistent, appealing colour and look.
- It must be safe for oral tissues and non-irritating.
- Should be stable both physically and chemically while being stored.
- To lower oral bacteria, it should have antimicrobial activity.

C. Applications and Uses of Toothpaste.

- Eliminates dental plaque and food particles from teeth and gums.
- Strengthens tooth enamel, which aids in preventing dental cavities.
- Lowers the chance of developing gum disease, including gingivitis.
- Keeps breath fresh and reduces mouth odour.
- Helps lessen the sensitivity of teeth.
- Enhances the appearance of teeth and gets rid of surface stains.
- Aids in regulating the production of tartar.
- Encourages dental health and general oral hygiene.

D. Structure and composition of Buccal Cavity.

The first segment of the digestive system is the buccal cavity, or mouth, which is divided from the nasal cavity by the palate and leads to the pharynx and oesophagus. The teeth, tongue, lips, salivary glands, and buccal mucosa are all included. The oral cavity is separated into the oral vestibule, which is the area between the teeth, lips, and cheeks, and the oral cavity proper, which is mostly filled by the tongue.



The buccal mucosa lines the lips and cheeks, while the lips themselves are soft, muscular structures. Taste, speech, and swallowing are all accomplished by the tongue, a muscular organ. Adults typically have 32 teeth, which are the hardest body structures. They are made up of the crown, neck, root, enamel, and pulp cavity.

Fig.2 Anatomy of Tooth.

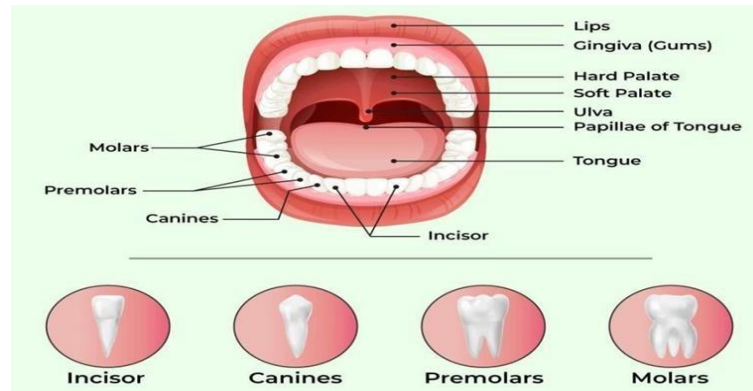


Fig.3 Buccal Cavity.

E. Types of Teeth.

Humans have four types of permanent teeth, each with a specific function:

- **Incisors** – They have sharp edges for cutting and biting food, and they are found at the front (four in the upper jaw and four in the lower jaw).
- **Canines** – One pointy tooth per quadrant, adjacent to the incisors, is used to tear and shred food. Because of their location beneath the eyes, they are also referred to as "eye teeth."
- **Premolars (Bicuspid)** – With flat, ridged surfaces for crushing and grinding, they are positioned between canines and molars and exhibit characteristics of both.
- **Molars** – found towards the back of the mouth, where it is mostly used for grinding and chewing. About 90% of chewing is handled by the twelve teeth that adults typically have, including four wisdom teeth.

F. Layers of Teeth.

A tooth has four main layers:

- **Enamel** – The hydroxyapatite outer layer, which is strong and impervious to chemical damage and wear, cannot regenerate.
- **Dentin** – The yellowish, porous bulk layer that lies beneath enamel and transmits sensations to the pulp is tougher than bone but softer than enamel.

- **Pulp** – The deepest portion, which contains blood vessels, nerves, and connective tissue, is in charge of dentin development, sustenance, and temperature and pain perception.
- **Cementum** – a thin layer that covers the root and uses the periodontal ligament to secure the tooth to the jawbone.

G. *Function of Teeth.*

- Cut, tear, and grind food into smaller pieces to aid in chewing and facilitate swallowing and digestion.
- Molar teeth are utilised for grinding, while incisors and canines are used for biting and shredding food.
- Contribute significantly to speech by assisting with clear word pronunciation.
- Maintain the form of the jaw and cheekbones by supporting the facial structure.
- Improve look, which helps to create a confident and cheery smile.
- Chewing correctly can improve digestion and general health.
- The ability to speak and eat might be impacted by missing or broken teeth.

II. AIM AND OBJECTIVES

A. *Aim:*

Formulation and evaluation of herbal antibacterial toothpaste from *Achyranthes aspera* L.

B. *Research Objectives:*

1. Preformulation Studies:

- Collect and authenticate *Achyranthes aspera* leaves.
- Prepare fine powder of the leaves.
- Perform phytochemical screening of the leaves.

2. Post-Formulation Studies:

- Formulate herbal toothpaste using *Achyranthes aspera*.
- Evaluate the antibacterial activity of the formulated toothpaste.

C. *Evaluation Parameters:*

1. Organoleptic Properties:

- Color
- Odor
- Taste
- Texture

2. Physicochemical Properties:

- pH determination
- Spreadability

- Foaming ability
- Moisture content
- FTIR analysis
- Stability studies
- Antibacterial activity

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III.LITERATURE OF REVIEW

A. REVIEW OF WORK DONE OF FORMULATION AND EVALUATION OF ANTIBACTERIAL HERBAL TOOTHPASTE FROM EXTRACT OF ACHYRANTHES ASPERA.L

- **M. Sekar et al. (2016):** Effective antibacterial action against both Gram-positive and Gram-negative bacteria was demonstrated by the formulated polyherbal toothpaste. Though more research is required to determine its safety and effectiveness, it is thought to be safer than synthetic toothpaste.
- **N.J.S. Ariffin et al. (2016):** Oral health is enhanced with herbal toothpaste prepared with natural ingredients. provides more therapeutic potential, fewer adverse effects, and safer, environmentally friendly substitutes for chemical-based treatments.
- **V. Lakshmi et al. (2018):** The medicinal herb Achyranthes Aspera has several therapeutic uses. To find active chemicals and comprehend mechanisms of action, more study is encouraged.
- **D. Gautam et al. (2020):** Herbal toothpaste had antibacterial action against E. coli, preserved oral hygiene, and reduced dental cavities. Comparable to commercial herbal toothpastes like Colgate Vedshakti and Dabur Meswak, it is safer and has less adverse effects.
- **R. Telrandhe et al. (2017):** In dental studies, herbal toothpaste is more palatable and safer than synthetic formulations. Comparable to commercial goods, the produced toothpaste maintained oral cleanliness and shown antibacterial action.
- **Dr. P. Abhang et al. (2024):** Flavonoids, alkaloids, and phenolics give Achyranthes Aspera leaf extract its potent antioxidant and broad-spectrum antibacterial properties. promotes additional therapeutic research and supports its possible usage in natural therapies.
- **A.K. Yadav et al. (2025):** Achyranthes Aspera and Curcuma longa were used in a herbal ointment that demonstrated good stability, a smooth texture, and non-irritating qualities. The best-performing group showed promise for preventing infections and repairing wounds.

B. Drug Profile



Fig. 4 *Achyranthes Aspera*

- Biological Source:**

A common herb in tropical and subtropical areas, *Achyranthes Aspera* Linn is a member of the *Amaranthaceae* family. Traditional medicine uses the entire plant because its roots, leaves, and seeds all have therapeutic qualities. Bioactive substances like alkaloids and saponins, which support its pharmacological actions, are thought to have medicinal promise.

- Morphological Characteristics:**

The erect, annual or perennial herb *Achyranthes Aspera* reaches a height of one to two meters. Its stem is angular, rough, and frequently reddish. The leaves are simple, opposite, elliptical or oval, and have fine hairs all over them. The tiny greenish-white blossoms are grouped on long, thin spikes. The plant produces tiny, brown, lens-shaped seeds and stiff, pointed bracts that stick to clothing or animal fur.

- Chemical Constituents:**

Achyranthes Aspera supplies essential chemical components such as alkaloids, saponins, flavonoids, tannins, and glycosides in herbal toothpaste formulations. These substances have astringent, antibacterial, and anti-inflammatory qualities that support tooth infection prevention, gum inflammation reduction, and oral hygiene. Their presence increases the herbal toothpaste's total medicinal efficacy.

C. Taxonomical Classifications of *Achyranthes Aspera*:

TABLE I

TAXONOMICAL CLASSIFICATION OF *ACHYRANTHES ASPERA*

Kingdom	Plantae
Subclass	Caryophyllide
Order	Caryophyllales
Family	<i>Amaranthaceae</i>
Genus	<i>Achyranthes</i>
Species	<i>Aspera</i>

D.Uses

- Its leaf extract is used to treat cough, asthma and bronchitis.
- The root is used as an emetic and purgative in traditional practices.
- It helps in managing skin diseases, wounds and insect bites due to its antimicrobial action.
- The seeds are believed to be useful in urinary disorders and kidney stones.
- It has antifertility and contraceptive properties when taken in specific doses.
- Decoctions made from the plant are used to relieve toothache and gum problems.
- It is used as a blood purifier and to treat gastrointestinal disorders.
- It helps in the treatment of rheumatism, piles and dysentery.

E.EXICIPIENT PROFILE

- Calcium Carbonate

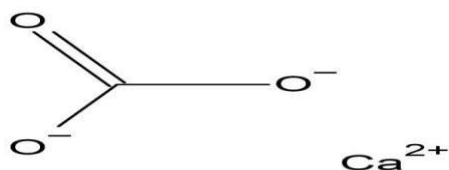


Fig.5 Chemical Structure of Calcium Carbonate

Table II

CHEMICAL PROPERTIES OF CALCIUM CARBONATE

Molecular formula	CaCO ₃
Molecular Weight	100.0869 g/mol
IUPAC Name	Calcium Carbonate
Functional category	Antacid, Calcium Supplement
Physical State	Odorless, Crystalline Solid
Appearance	Odorless Powder
Odor	Odorless
Melting point	825°C

Uses

Antacid: Used to neutralize stomach acid in indigestion, heartburn and acid reflux.

Calcium Supplement: Provides dietary calcium, essential for bone and teeth health.

Filler in Tablets: Acts as a diluent or bulking agent in pharmaceutical tablets.

Agriculture: Used to neutralize acidic soil (liming agent) and improve soil quality.

- GLYCERINE

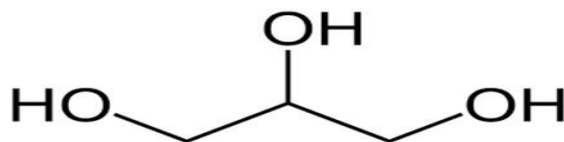


Fig.6 Structure of Glycerine

TABLE III

PROPERTIES OF GLYCERINE

Molecular formula	C ₃ H ₈ O ₃
Molecular weight	192.09G/mol
IUPAC name	Propane-1,2,3-triol
Function category	Solvent,sweeten,humectant
Color	Colorless
Odor	Odorless
Melting point	18 ⁰ C
Boiling point	290 ^{0C}
Viscosity	1400mPas

Uses

- Employ as a solvent, plasticizer, humectant, emollient, coating for paper, sweetener, antifreeze for vehicles and fluid for gas meters
- It is additionally utilized in the production of nitroglycerin, cosmetic soaps, liqueurs, inks, lubricants, adhesives and pharmaceuticals.

- SORBITOL

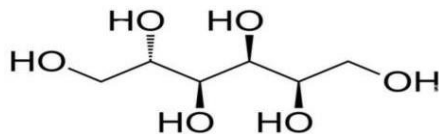


Fig.7 Structure of Sorbitol

TABLE IV

PROPERTIES OF SORBITOL

Molecular formula	C ₆ H ₁₄ O ₆
Molecular weight	182.17g/mol
IUPAC name	Hexane-1,2,3,4,5,6-hexol
Function category	Sweetener, humectant and laxative
Color	Colorless
Odor	Odourless
Melting point	95-100°C (203-212°F)
Viscosity	200-400m Pa.s at 20°C

Uses

- Sorbitol is used as a humectant in toothpaste, helping to retain moisture and prevent the paste from drying out.
 - It acts as a sweetening agent in sugar-free syrups, tablets and chewing gums.
 - In pharmaceuticals, sorbitol serves as a binder and plasticizer in tablet formulations.
 - It is used as an osmotic laxative in liquid form to relieve constipation.
- SODIUM LAURYL SULFATE

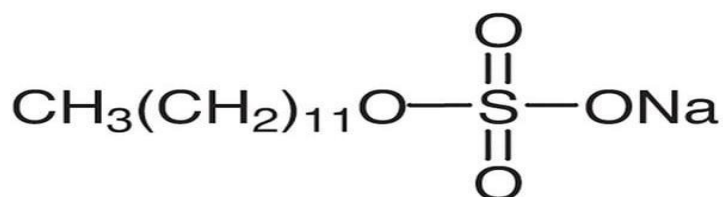


Fig.8 Structure Sodium Lauryl Sulfate

TABLE V

PROPERTIES OF SODIUM LAURYL SULFATE

Molecular formula	NaC ₁₂ H ₂₅ SO ₄
Molecular weight	288.38 g/mol
IUPAC name	Sodium lauryl sulfate
Function category	Function category
Color	White to pale yellow
Odor	Faint, characteristic
Melting point	204-207 ⁰ C
pH	7.5 to 10.0

Uses

- Sodium lauryl sulphate is used as a foaming agent in toothpaste to help spread the paste and improve cleaning.
- It acts as a detergent in shampoos and soaps, helping to remove oils, dirt and grease from skin and hair.
- In pharmaceutical creams and gels, SLS is used as an emulsifying agent to mix oil and water components.
- It functions as a cleansing agent in household products like dishwashing liquids and floor cleaners.

- GUAR GUM

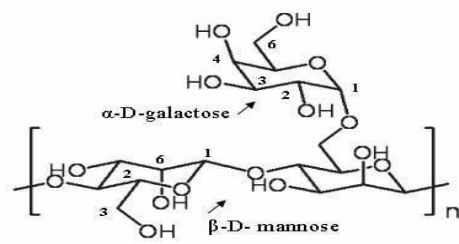


Fig.9 Structure of Guar Gum

TABLE VI

PROPERTIES OF GUAR GU

Molecular weight	1to8million Daltons (g/mol).
Function category	Thickener, stabilizer and emulsifier
Color	off-white, or cream-colored
Odor	Earthy or "beany" odor

Uses

- **Thickening Agent:** Used in food products like ice creams, sauces and soups to increase thickness and improve texture.
- **Stabilize:** Helps maintain uniform consistency in dairy products such as yogurt and beverages.
- **Binder:** Used in tablets and cosmetics to hold ingredients together.
- **Industrial Application:** Utilized in paper, textile and oil drilling industries to control viscosity and improve quality.
- **Dietary Fiber Source:** Acts as a soluble fiber, aiding digestion and promoting gut health.

- SODIUM BENZOATE

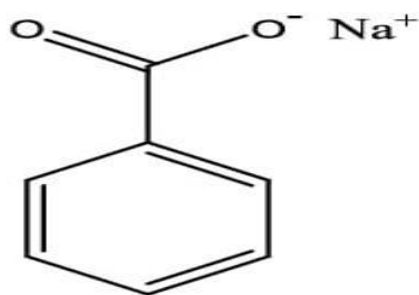


Fig.10 Structure of Sodium Benzoate

TABLE VII

PROPERTIES OF SODIUM BENZOATE

Molecular formula	C ₇ H ₅ NaO ₂
Molecular weight	144.1g/mol
IUPAC name	Sodium benzoate
Function category	Antimicrobial preservative, corrosion inhibitor, and lubricant
Color	White or off-white
Melting Point	450°C-475°C
Odor	Odorless
pH	2.5- 4.0

Uses

- **Food Preservative:** Commonly used in foods and beverages (like soft drinks, jams, pickles and sauces) to prevent the growth of bacteria, yeast and fungi.
- **Pharmaceutical Industry:** Used as a preservative in liquid medicines, cough syrups and ointments to extend shelf life.
- **Cosmetic Products:** Added to creams, lotions, shampoos and toothpaste as an antimicrobial agent to prevent spoilage.
- **Industrial Applications:** Used in antifreeze, corrosion inhibitors and other chemical.
- **CARBOXYMETHYL CELLULOSE**

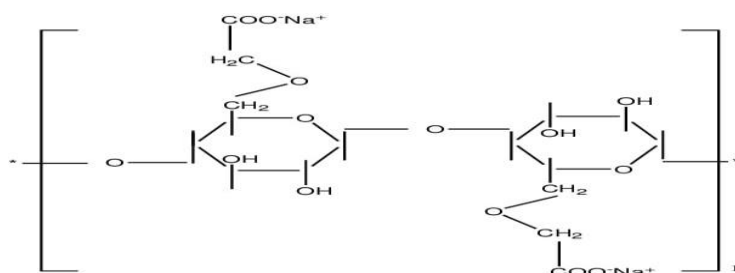


Fig.11 Sturcture of Carboxymethyl Cellulose

TABLE VIII

PROPERTIES OF CARBOXYMETHYL CELLULOSE

Molecular formula	C ₈ H ₁₅ NaO ₈
Molecular weight	262 g/mol
IUPAC name	2,3,4,5,6,pentahydroxyhexanal;acetate
Function category	Hickening, stabilizing, emulsifying agent
Color	White or off-white
Odor	Odourless
pH	7-9

Uses

- **Thickening Agent:** Used in food, pharmaceuticals and cosmetics to increase viscosity and provide a smooth texture.
- **Stabilizer:** Prevents separation of ingredients in products like ice creams, sauces and syrups.
- **Binder:** Acts as a binding agent in tablets, toothpaste and ceramics to hold ingredients together.
- **Film-forming Agent:** Used in coatings, capsules and packaging materials for controlled release or protection.
- **Suspending Agent:** Helps keep solid particles evenly distributed in liquid formulations, such as in syrups or paints.

- SACCHARIN SODIUM

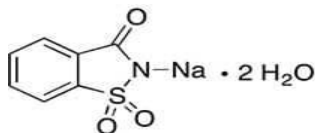


Fig.12 Structure of Saccharin Sodium

TABLE IX

PROPERTIES OF SACCHARIN SODIUM

Molecular formula	$C_7H_4NNaO_3S \cdot 2H_2O$
Molecular weight	205.17 g/mol
IUPAC name	2H-1λ6,2-benzothiazol-1,1,3trione
Function category	Artificial, non-nutritive sweetener.
Color	White or off-white
Odor	Odorless
Melting point	228-230°C
pH	6.0-7.5

Uses

- **Artificial Sweetener:** Used as a non-nutritive sweetener in food and beverages such as soft drinks, jams, jellies and desserts for people who want to reduce sugar intake.
- **Pharmaceutical Formulations:** Added to syrups, chewable tablets and lozenges to mask the bitter taste of active drug ingredients.
- **Dental Products:** Used in toothpaste and mouthwashes to provide sweetness without promoting tooth decay.
- **Dietary Products:** Common in “sugar-free” or “low-calorie” foods, especially for diabetic patients.
- **Cosmetic Industry:** Included in products like lipsticks and flavored lip balms to improve taste appeal.
- **Laboratory and Research Uses:** Employed in studies as a standard preservative or as a model compound for testing antimicrobial activity.

- PEPPERMINT OIL

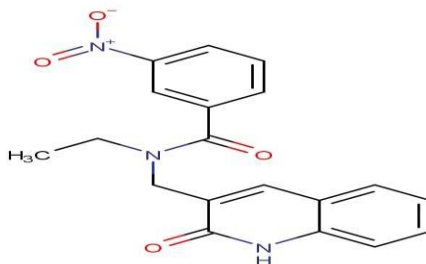


Fig.13 Stucture of peppermint oil 1%

TABLE X
PROPERTIES OF PEPPERMINT OIL 1%

Molecular Formula	$C_{62}H_{108}O_7$
Molecular weight	965.5 g/mol
Function category	Medicinal and therapeutic agent, particularly for gastrointestinal issues.
Color	Colourless to pale yellow
Odor	Sharp odor
Boiling point	197–204°C
Viscosity	Watery viscosity
pH	5.0-6.5

Uses

- **Oral Care:** Acts as a natural flavouring and antibacterial agent in toothpaste and mouthwash, helping to reduce bad breath and oral bacteria.
- **Skin Cooling and Soothing:** Provides a cooling effect that helps soothe irritation, itching or minor burns when applied topically in diluted form.
- **Headache Relief:** When applied to the temples, the menthol in peppermint oil helps relieve tension headaches by promoting relaxation and improving blood flow.
- **Digestive Aid:** Used in topical formulations or aromatherapy to relieve symptoms of indigestion, nausea and bloating.
- **Antimicrobial and Antifungal Properties:** Helps inhibit the growth of certain bacteria and fungi, making it useful in cosmetic and pharmaceutical preparations.
- METHYL PARABEN

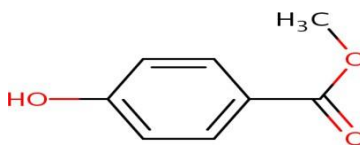


Fig 14 Structure of Methyl Paraben

TABLE XI
PROPERTIES OF METHYL PARABEN

Molecular formula	$C_8H_8O_3$
Molecular weight	152.15 g/mol
IUPAC Name	methyl 4-hydroxybenzoate
Function category	Antimicrobial preservative
Color	Colorless to off-white

Odor	Odorless
Boiling point	275°C
Melting Point	125-128°C

Uses

- **Preservative in Cosmetics:** Prevents the growth of bacteria and fungi in products like creams, lotions and makeup.
- **Pharmaceutical Preservative:** Used in medicines (syrups, ointments) to enhance shelf life by preventing microbial contamination.
- **Food Additive:** Acts as a preservative in certain packaged foods and beverages to maintain freshness.
- **Personal Care Products:** Found in shampoos, deodorants and toothpaste to prevent spoilage.
- **Topical Formulations:** Helps stabilize creams, gels and ointments by preventing microbial growth.

IV MATERIALS AND METHODOLOGY

A. Materials

The drug chemicals and equipment used in the project research are given in the Table XII and Table XIII. All the materials used were of analytical grade and procured either as gift samples or purchased.

TABLE XII
EQUIPMENT AND INSTRUMENTS

Sr. No	Equipment/Instrument Name	Model/Company
1	pH meter	Rolex Scientific Engineers Model 9027#2290 Cross Road No. 8 Kacha Bazar, Ambala Cantt- 133001, India.
2	Stirrers	Electrocraft (India) PVT.LTD.Mumbai-400 063
3	Weighing balance	
4	Hot air oven	LSC-358 Lab line

TABLE XIII

LIST OF MATERIALS

Sr. No	Materials	Manufacturer
1	<i>Achyranthes Aspera</i> powder	Local field of Belgaum, Karnataka
2	Calcium carbonate	MWIT Turkewadi
3	Glycerine	MWIT Turkewadi
4	Sorbitol	MWIT Turkewadi
5	Sodium lauryl Sulphate	MWIT Turkewadi
6	Carboxyl methyl Cellulose	MWIT Turkewadi
7	Peppermint oil	MWIT Turkewadi
8	Methyl paraben	MWIT Turkewadi
9	Guar Gum	MWIT Turkewadi
10	Sodium Benzoate	MWIT Turkewadi
11	Saccharin Sodium	MWIT Turkewadi

B. Methodology

- FORMULATION STUDY

The first stage in creating a stable and efficient dosage form is preformulation testing. The physicochemical and phytochemical characteristics of the chosen herbal extracts were ascertained in this study through preformulation evaluation of the herbal toothpaste. To guarantee stability and efficacy, factors like appearance, solubility, pH, and excipient compatibility were evaluated. This study served as the foundation for the effective formulation of herbal toothpaste and supplied crucial information for the selection of ingredients.

- Collection of *Achyranthes Aspera*.L:

The *Achyranthes Aspera* plants powder was collected from local field of Belgaum Karnataka, India.

Fig.15 *Achyranthes Aspera*.L

- METHOD OF PREPARATION OF HERBAL TOOTHPASTE

Achyranthes aspera powder and calcium carbonate were sifted and properly mixed to create the herbal toothpaste. To create a smooth gel, the binder (CMC) was added gradually after the humectant was separately dissolved in distilled water. After incorporating the powder combination into the gel, SLS, preservative, sweetener, and flavouring oil were added. After homogenising the paste to a smooth, lump-free consistency, it was put into sterile, sealed containers and kept in a dry, cool location.

TABLE XIV
LIST OF INGREDIENTS AND THEIR USES

Sr. No.	Ingredients	Use
1	<i>Achyranthes Aspera</i>	Herbal antibacterial agent
2	Calcium carbonate	Mild abrasive
3	Glycerine	Humectant
4	Sorbitol	Sweetener and humectant
5	Sodium lauryl Sulphate	Foaming agent
6	Guar Gum	Thickener
7	Sodium benzoate	Preservative
8	Carboxymethyl Cellulose	Stabilizer
9	Saccharin sodium	Sweetener
10	Peppermint oil	Flavouring and refreshing agent
11	Methyl paraben	Antimicrobial preservative
12	Distilled water	solvent/vehicle

- PHOTOCHEMICAL SCREENING

1. Test for alkaloids

a) Mayer's Test- After adding a few drops of Mayer's reagent (potassium mercuric iodide solution) to a test tube containing one millilitre of test solution, a cream-coloured precipitate was seen.

b) Dragendorff's Test- A few drops of Dragendorff's reagent (potassium bismuth iodide solution) were added to a test tube containing one millilitre of test solution, and the presence of a reddish brown precipitate was monitored.

2. Test for tannins

a) FeCl₃ Test- In test tubes, about 0.5 mg of dried powdered samples were cooked in 20 ml of water before being filtered. After adding a few drops of 0.1% ferric chloride solution, the colouration was checked for brownish green or blue-black hues.

3. Test for cardiac glycosides

a) Keller Killian Test- 0.4 ml of glacial acetic acid with a trace amount of ferric chloride was added to 1 ml of test solution in a test tube. After carefully adding 0.5 ml of concentrated sulphuric acid to the test tube's side, watch for the acetic acid layer to turn blue.

b) Salkowski Test- A clean, dry test tube was filled with one millilitre of the test solution, two millilitres of chloroform, and a few drops of sulphuric acid. After giving it a good shake and letting it stand for a while, look for a reddish-brown hue at the interface.

4. Test for Steroids

a) Liebermann Buchard test- A few drops of acetic anhydride were added to a 1 ml test solution, which was then boiled, cooled, and concentrated sulphuric acid was poured from the test tube's sides. A brown ring at the intersection of the two layers and a green layer in the upper layer were then observed.

1. Test for Terpenoids

a) Salkowski Test- A clean, dry test tube was filled with one millilitre of the test solution, two millilitres of chloroform, and a few drops of sulphuric acid. After giving it a good shake and letting it stand for a while, look for a reddish-brown hue at the interface.

2. Detections of Saponins

a) Foam Test- 20 millilitres of distilled water plus 1 gramme of powder For fifteen minutes, shake the test tube. Alkaloids are indicated by a persistent foam (1-2 cm for more than 10 minutes).

3. Detection of Flavonoids

a) Alkaline Reagent Test- Flavonoids are indicated by a yellow hue that turns colourless when 5g of powder, 5 ml of methanol extract, 2 ml of 10% NaOH, and diluted HCl are combined.

b) Shinoda Test- Add 0.5 grammes of powder, 5 millilitres of ethanol, and magnesium ribbon to extract, then gradually add concentrated HCl. The flavonoids are indicated by pink, red, or crimson hues.



Fig.16 Photochemical screening of *Achyranthes Aspera*

- Formulation of antibacterial toothpaste:

Calcium carbonate, CMC, guar gum, and sodium lauryl sulphate were precisely weighed in order to make 30 g of herbal toothpaste. To create a smooth, stable base, CMC and guar gum were dissolved in distilled water while being constantly stirred. Next, a humectant mixture of sorbitol (10%) and glycerine (20%) was applied gradually. To create a homogenous paste, *Achyranthes aspera* extract was added gradually, then calcium carbonate. In addition to sodium benzoate and methyl paraben (0.2%) as preservatives, saccharin sodium as a sweetener, and peppermint oil (1%) for flavour, sodium lauryl sulphate (2%) was added for foaming. The smooth, uniform toothpaste was then put into appropriate containers for storage and additional testing after distilled water was added to bring the weight down to 30 g.

TABLE XV
FORMULATION TABLE FOR HERBAL TOOTHPASTE

Ingredients	F1	F2
<i>Achyranthes aspera</i>	3.0 gm	3.0 gm
Calcium carbonate	10.0 gm	10.0 gm
Glycerine 20%	4.7 ml	4.7 ml
Sorbitol 10%	2.3 ml	2.3 ml
Sodium lauryl Sulphate 2%	1.0 gm	1.0 gm
Guar Gum	0.45 gm	0.45 gm
Sodium benzoate	0.6 gm	0.6 gm
Carboxymethyl Cellulose	2.0 gm	2.0 gm
Saccharin sodium	0.2 gm	0.2 gm
Peppermint oil 1%	0.3 gm	0.3 gm
Methyl paraben 0.2%	0.2 gm	0.2 gm
Distilled water	q.s.	q.s.
Total Quantity	30gm	30gm

Q.S. =Quantity Sufficient

C. EVALUATION OF HERBAL TOOTHPASTE

•ORGANOLEPTIC PROPERTIES:

a. PHYSICAL EXAMINATION

1. **Colour:** The color of the toothpaste formulation was evaluated visually by placing a small quantity of the sample on a white background and observing under natural daylight to determine its uniformity and appearance.
2. **Odour:** The odour of the formulation was assessed by the sensory smelling method. A small amount of toothpaste was taken in a clean container and the smell was evaluated by gently sniffing at room temperature.

3. **Taste:** The taste of the toothpaste was determined organoleptically by placing a small amount of the formulation on the tongue and observing the initial and after-taste characteristics. Proper hygienic precautions were taken during the process.
4. **Texture:** The texture of the toothpaste was examined by taking a small amount of the formulation between the fingers and rubbing gently to feel its smoothness and consistency. The sample was also spread on a glass slide to check for the presence of any coarse particles.



Fig.17 Herbal Toothpaste of Achyranthes Aspera

- Physicochemical Properties

1.pH Determination:

A digital pH meter that has been calibrated was used to measure the pH of the herbal toothpaste. One gramme of toothpaste was dissolved in one hundred millilitres of boiling and cooling distilled water to create a fifty percent suspension. Following careful stirring, the pH was measured in triplicate after stabilisation, and the average result was presented.



Fig.18 pH Meter

2.Spreadability:

The Spreadability was measured by placing 2 g of toothpaste between glass slides, pressing for 1 minute, removing excess, and applying a 20 g weight. The time for the top slide to move 3.2 cm was recorded in triplicate, and the average was calculated. The Spreadability (S) was calculated using the following formula:

$$\text{Spreadability(s)} = \text{ML/T}$$

Where,

M =Weight tied to upper slid

L =Length of glass slides

T =Time taken to separate the slides

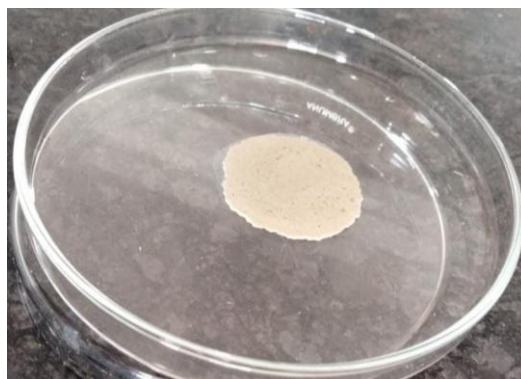


Fig.19 Spreadability Test

3.Foaming Ability:

The foaming ability was determined by mixing 1 g of toothpaste with a measured volume of distilled water in a 50 mL graduated cylinder. The initial volume was noted, the cylinder was shaken 10 times, and the final foam volume was recorded after stabilization.

The foaming power of the formulation was calculated using the following formula,

$$\text{Foaming Power} = V_1 - V_2$$

Where,

V_1 = Volume (mL) of foam with water after shaking

V_2 = Initial volume (mL) before shaking

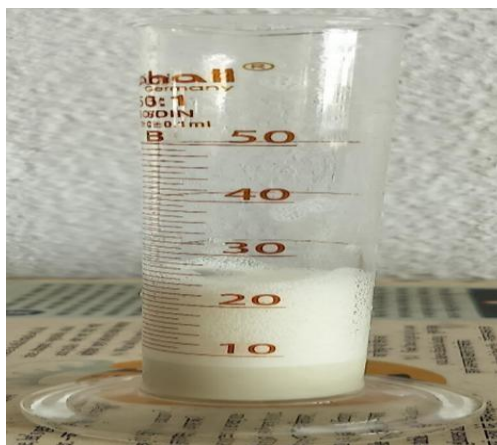


Fig.20 Foaming Ability

4.Moisture Content:

The moisture content was determined by weighing 5 g of toothpaste in a petri dish and drying it in a hot air oven at 105 °C until constant weight. The sample was then cooled in a desiccator

and reweighed to calculate moisture loss.

The percentage of moisture content was calculated using the following formula,

$$\text{Moisture Content (\% by mass)} = \frac{M_2}{M_1} \times 100$$

Where,

M_1 = Mass (g) of the sample taken for the test

M_2 = Loss of mass (g) on drying

The toothpaste was packed in a tube and stored for 45 days at three conditions, 5°C. (Cold), room Temperature & 40°C (warm). After Storage, the tube was cut open and checked to see if any liquid had separated from the toothpaste.



Fig.21 Moisture Content

5.FTIR Test:

Functional groups related to *Achyranthes aspera* bioactive substances, such as alkaloids, flavonoids, saponins, and phenols, were detected in the FTIR spectrum. The absence of significant peak shifts or losses suggests that the extract and excipients are not chemically incompatible. The integrity, stability, and compatibility of the formulation in the semi-solid dosage form were therefore validated by FTIR analysis.

6.Stability Study:

For thirty days, the stability of the herbal toothpaste formulation (F1) was assessed at room temperature ($25 \pm 2^\circ\text{C}$, $60\% \text{ RH} \pm 5\%$). On days 0, 15, and 30, samples kept in airtight containers were evaluated for appearance, colour, aroma, pH, spreadability, and foaming ability. Odour was assessed organoleptically, and appearance, smoothness, and colour changes were observed visually. A calibrated pH meter with a 50% suspension in boiled and cooled distilled water was used to measure the pH. The glass slide method was used to assess spreadability, while the measuring cylinder method was used to assess foaming ability. To assess formulation stability over time, all observations were documented.

7.Antimicrobial Activity:

The Biocyte Microbiological Testing Centre in Sangli used the agar well diffusion (cup plate) method to assess the antibacterial activity. Petri plates were filled with sterile Antibiotic Assay Medium No. 19 ($\text{pH } 6.1 \pm 0.2$) that had been inoculated with *Escherichia coli* (ATCC 8739) and *Staphylococcus*

aureus* (ATCC 6538). 100 μ L of the test sample (10 mg/mL), standard, and DMSO as a negative control were added to wells (6 mm). After pre-incubating the plates at 2–8 °C for diffusion, they were incubated for 24–48 hours at 30–35 °C. A zone reader was used to measure zones of inhibition in order to evaluate antibacterial activity.

Fig.22 Antimicrobial Activity



V RESULT AND DISCUSSION

A. FORMULATION OF HERBAL TOOTHPASTE:

Calcium carbonate was used as an abrasive and a sorbitol–glycerin mixture as humectants to successfully create the herbal toothpaste with 3% w/w *Achyranthes aspera* extract. Methyl paraben and sodium benzoate served as preservatives, and CMC and guar gum supplied the appropriate viscosity. Peppermint oil supplied flavour and aroma, sodium saccharin improved palatability, and sodium lauryl sulphate helped foaming and cleanliness. The formulation was suitable for regular oral hygiene since it had a smooth, uniform appearance, a pleasant flavour, and an appropriate pH.

B. EVALUATION TESTS FOR HERBAL TOOTHPASTE:

Several chemical and physiological tests were used to evaluate the formulation characteristics of the antimicrobial herbal toothpaste, and the findings were recorded.

C. PHYTOCHEMICAL SCREENING OF *ACHYRANTHES ASPERA*:

Alkaloids, glycosides, flavonoids, terpenoids, steroids, saponins, tannins, quinones, and carotenoids were found in *Achyranthes aspera* using phytochemical screening. The plant's medicinal potential and traditional use for anti-inflammatory, antioxidant, antibacterial, and antidiabetic properties are supported by these bioactive substances.

The results of the same are mentioned in Table XVI

TABLE XVI

Phytochemical Screening of *Achyranthes Aspera*

Sr. No.	PHYTOCHEMICAL TEST	INFERENCE
1	Alkaloids Test	+
2	Flavonoids Test	+
3	Saponins Test	+
4	Tannins Test	+
5	Phenolic Compounds Test	+
6	Glycosides Test	+
7	Terpenoids Test	+
8	Steroid Test	+

(+) indicates presence whereas (-) indicates absence of the phytochemical

D. ORGANOLEPTIC PROPERTIES

1. PHYSICAL APPEARANCE

Physical Evaluation of Formulated Herbal Toothpaste The formulated herbal toothpaste was visually examined for basic physical parameters under hygienic conditions and we get following results mention table no.XVII

TABLE XVII

PHYSICAL APPEARANCE

Sr. No.	Evaluation Parameter	Observation
1	Colour	Grayish-Green
2	Odour	Pleasant herbal fragrance
3	Taste	Mild and agreeable
4	Texture	Smooth and uniform

Discussion: The formulated herbal toothpaste containing *Achyranthes aspera* extract was evaluated for its physical appearance and organoleptic properties. The paste exhibited a grayish-green color, which is consistent with the natural color of the herbal extract and other excipients. It had a pleasant herbal fragrance, attributed to the addition of peppermint oil and a mild, agreeable taste, enhanced by sodium saccharin. The texture was smooth and uniform, indicating proper incorporation of thickeners (CMC and guar gum) and abrasives (calcium carbonate).

E. PHYSICOCHEMICAL ANALYSIS

1. DETERMINATION OF pH

The prepared formulation is examined by digital pH meter and we get following results mention Table XVIII.

TABLE XVIII

DETERMINATION OF pH

Sr. No.	Sample Name	pH
1	F1	7.03 ± 0.21

± SD (n=3)

Discussion: The pH of the formulated herbal toothpaste (F1) was measured using a digital pH meter and found to be 7.03 ± 0.21, indicating a neutral range. A neutral pH is desirable for toothpaste formulations as it is gentle on oral tissues, helps maintain the natural oral environment and reduces the risk of enamel erosion or irritation of the gums.

2. Spreadability

The prepared formulation is examined by glass plate method and we get following results mention Table XIX

Calculation:

Spreadability it can be calculated by using formula:

$$\text{Spreadability (S)} = \frac{M \times L}{T}$$

$$S = \frac{20 \times 3.2}{2.8}$$

$$= 22.8 \text{ g.cm/sec}$$

Where,

M=weight tied to upper slides(20g)

L=length of glass slides(3.2cm)

T=time taken to separate the slides(22.8sec)

TABLE XIX

DETERMINATION OF SPREADIBILITY

Sr.No.	Sample Name	Spreadability
1	F1	22.65±0.93g.cm/sec

± SD (n=3)

Discussion: The herbal gel formulation F1 showed a spreadability of 22.65 ± 0.93 g.cm/sec, indicating it can be easily and uniformly applied on the skin. The low standard deviation reflects consistent gel consistency. This value suggests that F1 has good topical application properties, ensuring patient comfort and effective drug delivery.

3.Foaming Ability

The foaming capacity of the formulated herbal toothpaste was measured and the results are presented in Table XX.

TABLE XX
FOAMING ABILITY

Sr.No.	Sample Name	Foaming Ability
1	F1	25 ± 0.5 ml

± SD (n=3)

Discussion: One crucial toothpaste factor influencing user perception and paste dispersion is foaming ability. With a foaming capacity of 25 ± 0.5 mL, the herbal toothpaste formulation F1 demonstrated moderate, steady foam that was adequate for efficient cleansing and spreading. The moderate level is ideal to guarantee cleaning without causing irritation, and the low standard deviation indicates constant foaming.

4.Moisture Content:

The moisture content of the formulated herbal toothpaste was determined to assess physical stability and shelf life. The results are presented in Table XXI

TABLE XXI
MOISTURE CONTENT

Sr. No.	Sample Name	Moisture Content
1	F1	3.2 ± 0.08 %

± SD (n=3)

Discussion: Herbal toothpaste's stability, texture, and shelf life are all significantly impacted by its moisture content. With a low moisture content of $3.2 \pm 0.08\%$, Formulation F1 demonstrated homogeneity and little water presence. F1 is appropriate for long-term storage and efficient usage because of its low moisture content, which also helps to inhibit microbiological growth, preserve consistency, and improve product stability.

5.FTIR Test:

The prepared formulation is examined by FTIR Method and we get following results mention table XXIII

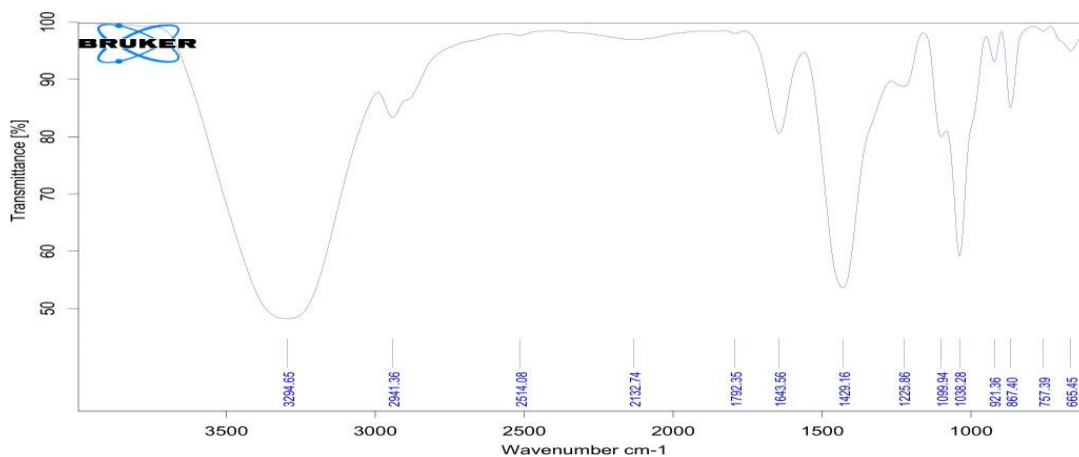


Fig.23 FTIR Test

TABLE XXIII

FTIR Spectral data of *Achyranthes Aspera*

Functional Group	Standard Band Frequency (cm ⁻¹)	Obtained Band Frequency (cm ⁻¹)
N–H stretching	3200 – 3500	3294.65
C–H stretching	2850 – 2960	2941.36
O–H stretching (acidic)	2500 – 2600	2514.08
C≡N stretching	2100 – 2260	2132.74
C=O stretching	1650 – 1750	1643.56
C=C stretching	1450 – 1600	1429.16
C–O stretching	1200 – 1300	1225.86
C–N stretching	1000 – 1100	1099.94, 1038.28
C–H bending (aromatic)	700 – 900	929.43, 867.40, 757.39
C–Cl stretching	600 – 700	665.45

Discussion: The presence of distinctive functional groups in the sample was verified by the FTIR spectra. Along with aromatic C–H bending and C–Cl stretching, peaks showed N–H, aliphatic C–H, O–H, C≡N, C=O, C=C, C–O, and C–N stretching vibrations. Overall, the FTIR analysis supported the compound's chemical structure and purity by confirming the anticipated functional groups.

6. Antibacterial Activity of Sample-1 against *Staphylococcus aureus*

The antibacterial potential of the formulated herbal toothpaste (Sample-1) was evaluated against *Staphylococcus aureus* using the zone of inhibition method. The results are summarized in Table XXIV

TABLE XXIV
ANTIBACTERIAL ACTIVITY OF STAPHYLOCOCCUS AUREUS

Sr. No.	Sample	Concentration	Zone of Inhibition (mm)
1	Control	-	-
2	Standard Streptomycin	1 mg/ml	25
3	Sample-1	5 mg/ml	2
		10 mg/ml	6

Discussion Due to its herbal components, Sample-1 demonstrated antibacterial action against *S. aureus*, although even at larger concentrations, it was far less effective than streptomycin. The zone of inhibition was found to increase in a dose-dependent manner. Sample-1 can help with oral hygiene and germ control, which supports its usage as a mild antibacterial agent in herbal toothpaste even though it is less effective than traditional antibiotics.

7. Stability Testing:

To evaluate the stability of the formulated herbal toothpaste (F1), various physical and chemical parameters were assessed at room temperature ($25^{\circ}\text{C} \pm 2^{\circ}\text{C}$) over a period of 30 days. The results are summarized in Table XXV

TABLE XXV
STABILITY TESTING OF F1 AT ROOM TEMPERATURE ($25^{\circ}\text{C} \pm 2^{\circ}\text{C}$, $60\% \pm 5\%$)

Evaluation Parameter	Duration		
	0 Days	15 Days	30 Days
Colour	Grayish-Green	No Change	No Change
Odour	Pleasant herbal fragrance	Pleasant herbal fragrance	Pleasant herbal fragrance
Taste	Mild and agreeable	Mild and agreeable	Mild and agreeable
Texture	Smooth, uniform	Smooth, uniform	Smooth, uniform
pH	7.03 ± 0.21	7.03 ± 0.21	7.03 ± 0.21
Moisture Content	$3.2 \pm 0.08 \%$	$3.2 \pm 0.08 \%$	$3.2 \pm 0.08 \%$
Spreadability	$22.65 \pm 0.93 \text{ g.cm/sec}$	$22.65 \pm 0.93 \text{ g.cm/sec}$	$22.65 \pm 0.93 \text{ g.cm/sec}$

$\pm$ SD (n=3)

Discussion: For thirty days, the stability investigation of herbal toothpaste formulation F1 was carried out at room temperature ($25 \pm 2^{\circ}\text{C}$, $60 \pm 5\%$ RH). The colour, flavour, texture, and fragrance all stayed consistent and smooth throughout the investigation, with no discernible variations. Important indicators of good physical and chemical stability included spreadability ($22.65 \pm 0.93 \text{ g.cm/sec}$), moisture content ($3.2 \pm 0.08\%$), and pH (7.03 ± 0.21). Overall, F1 showed adequate stability in ambient circumstances, which qualifies it for long-term usage and safe storage.

VI CONCLUSION

As a natural, stable, and efficient oral care product, the current study effectively developed and assessed a herbal toothpaste containing 3% w/w *Achyranthes aspera* extract. Sorbitol and glycerin were utilised as humectants, calcium carbonate as an abrasive, and CMC combined with guar gum to provide the right viscosity and consistent texture. Peppermint oil boosted flavour and acceptance, whereas sodium benzoate and methyl paraben extended shelf life.

Bioactive components including alkaloids, flavonoids, saponins, tannins, glycosides, terpenoids, and steroids with antibacterial, anti-inflammatory, and antioxidant qualities were found through phytochemical screening. Organoleptic analysis revealed a pleasing taste, texture, fragrance, and appearance. Physicochemical analyses showed that it was stable and suitable for oral use with an acceptable pH (7.03 ± 0.21), good spreadability (22.65 ± 0.93 g·cm/sec), moderate foaming ability (25 ± 0.5 mL), and low moisture content ($3.2 \pm 0.08\%$).

The extract's chemical integrity was verified by FTIR analysis, and its involvement in oral hygiene was supported by modest, dose-dependent antibacterial activity against *Staphylococcus aureus*. Physical, chemical, or functional parameters did not significantly alter throughout 30 days of stability testing at room temperature. All things considered, the herbal toothpaste showed favourable organoleptic, physicochemical, antibacterial, and stability properties, suggesting that it is a secure and promising natural substitute for synthetic toothpaste for efficient oral hygiene.

VII SUMMARY

A sorbitol–glycerin mixture was used as a humectant and calcium carbonate as an abrasive to successfully create the herbal toothpaste with 3% w/w *Achyranthes aspera* extract. Methyl paraben and sodium benzoate served as preservatives, CMC and guar gum as thickeners, sodium lauryl sulphate as a foaming agent, and peppermint oil with sodium saccharin enhanced taste and acceptance. The toothpaste had a mild flavour, a pleasant herbal scent, a smooth texture, and a consistent grayish-green hue.

Its antibacterial and antioxidant activity was supported by phytochemical screening, which verified the presence of bioactive substances like alkaloids, flavonoids, saponins, tannins, glycosides, terpenoids, and steroids. A neutral pH (7.03 ± 0.21), good spreadability (22.65 ± 0.93 g·cm/sec), moderate foaming ability (25 ± 0.5 mL), and low moisture content ($3.2 \pm 0.08\%$) were found in the physicochemical examination, guaranteeing steady and efficient functioning.

The formulation's chemical integrity was confirmed by FTIR analysis. Its use as a natural antibacterial

agent for everyday dental care is supported by antibacterial experiments against *Staphylococcus aureus*, which revealed modest to moderate, concentration-dependent action. Excellent stability and shelf life were confirmed by stability testing conducted over a 30-day period at room temperature, which revealed no appreciable changes in any parameters. For preserving oral hygiene, the composition turned out to be a secure, reliable, and efficient natural substitute for synthetic toothpaste.

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