JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

Formulation and development of toothpaste from achyranthes aspera and psidium guajava plant extract and its antibacterial activity against plaque forming oral microbiota.

Miss Minal Chetule, Prof A. Gawai Sir, Dr. S. Mhaske

Student

Satyajeet College Of Pharmacy , mehkar

Abstract:

Numerous bacteria and other plaque-forming oral microbiota are involved in plaque formation, and environmental factors within the oral cavity contribute to the formation of complex biofilms that include stages of development. The currently available methods for plaque and black stain prevention, such as dental scaling, scraping, and chemical use, have been associated with a number of drawbacks. The primary goal of this study is to use the herbal component to effectively remove oral germs and black stains with anti-caries properties. Before being used for extraction, Achyranthes aspera stems and Pisidium guajava leaves were thoroughly cleaned with distilled water. The antibacterial activities were extracted using decoction. The primary component of these extracts was used to create a herbal toothpaste formulation. All evaluation tests, including pH, spreadability, and abrasiveness, were found to be within acceptable limits.

The developed toothpaste containing Achyranthes aspera and Psidium guajava extract may be suitable for daily use and prevent the formation of black stains.

Keywords: plaque-forming oral microbiota, decoction, complex biofilm, anti-caries, and black stain.

1. Introduction

The formulation and development of toothpaste from Psidium guajava and Achyranthes aspera plant extracts represents a promising advancement in oral care, particularly due to its potential antibacterial properties against plaque-forming oral microbiota. Achyranthes aspera, a member of the Amaranthaceae family, has been traditionally recognized for its medicinal benefits, including its effectiveness in maintaining oral hygiene. Similarly, Psidium guajava is known for its rich phytochemical content and strong antimicrobial properties.

Research indicates that the phytochemicals present in both Psidium guajava and Achyranthes aspera, such as

flavonoids, exhibit significant antibacterial activity against various chromogenic bacteria associated with dental issues, including plaque formation and black stain development on teeth. The aim of this study is to harness these natural compounds to create a herbal toothpaste that not only promotes oral health but also serves as an alternative to conventional toothpaste formulations that may contain synthetic additives.

The development process involves careful formulation and standardization to ensure efficacy and safety. By integrating Psidium guajava and Achyranthes aspera extracts into a toothpaste base, this research focusing on its ability to inhibit the growth of harmful oral bacteria while providing a natural solution for dental care. Through this innovative approach, the study aims to contribute to the growing field of herbal dentistry, emphasizing the importance of utilizing natural resources for improved oral health outcomes.

1.1. Background and Rationale

➢ Introduction

Dental caries, otherwise known as tooth decay, is one of the chronic diseases of people worldwide and individuals are susceptible to throughout their life. Dental plaque, a unique microbial biofilm ecosystem comprising a diverse microbial community, has a primary role in its pathogenesis. Exchange of information among single-cell organisms, the cell-to-cell communication through small chemical molecules, has been termed quorum sensing (QS).
Microbial processes like biofilm formation, virulence factor expression and bioluminescence are examples of QS-control. Recently, anti-virulence therapies have drawn attention as a new strategy to combat microbial infections, and in that context bacterial QS represents a promising therapeutic target. Even though toothbrushes and toothpastes have found wide usage, natural methods of teeth cleaning using chewing sticks from a range of plants have been practiced for thousands of years in Asia, Africa, the Middle East and America. Further, a number of clinical studies confirmed the efficiency of chewing sticks in removing dental plaque due to the collective effect of their mechanical cleaning, salivation enhancement and the action of leached- out antimicrobial substances. The leaching bioactive compounds exhibit number of reactions in the oral environment including their antimicrobial action on pathogens, mouth refreshment by imparting fragrance in the mouth, bad odor elimination and stimulation of taste buds. Hence, many traditional cultures use only herbal chewing sticks instead of plastic-bristle brushes.

❖ Achyranthes aspera, an ethanomedicinal herb, is reported to be useful in the indigenous system of medicine, for the treatment of renal dropsy, bronchial affections and leprosy, and it has also been credited with abortifacient, contraceptive, cardiac stimulant, astringent, diuretic, alterative, antiperiodic as well as purgative properties. They are also used as an antidote for snake bites and cure for women as well as child diseases, venereal diseases, malarial fever, asthma, hypertension and pneumonia . This ethanomedicinal herb is also reported to have many pronounced activities including hepatoprotective, cancer chemopreventive, anti-inflammatory, anti-arthritic, thyroid-stimulating, anti-peroxidative, reproductive function stimulative, immunomodulatory, wound healing, antioxidant, gastroprotective, vaginal contraceptive and anxiolytic activities. Beneficial effects of this herb are reported to extend to oral hygiene maintenance and caries prevention by folkloric tradition in many parts of India including Dharwad district of Karnataka and in parts of Tamil Nadu, southern India, where in number of villages, people use its stem and root as teeth cleaning natural toothbrush. In other parts, the ash from the burnt plant often mixed with mustard oil and a pinch of salt is used as a tooth cleaning powder. It is believed to relieve pyorrhea and toothache . The radical scavenging activity of the plant towards the free radical formation occurring during the periodontal

disease activity was due to the presence of phenolic compounds in the plant extract which was very evident in the studies conducted in the past. The two main radicals targeted by these phenolic compounds were namely 2,2 diphenyl-1-picrylhydrazyl and superoxide which are the main culprits responsible for the destruction of periodontal tissues during the disease process.A. aspera in its gel form was used a local drug therapy for the protection of periodontal tissues from the pathogens as it is very effective in management of inflammation in tissues.An animal study was

conducted by Kumar et al [17] in which the alcoholic extract of the plants was used, the plant extract exhibited stimulation of immune system via the proliferation of T-lymphocytes. The regeneration potential of the plant extract is also helpful in many ways. Due to the phenolic component present in the plant extract it is highly effective in regeneration of the lost tissues so it contributes majorly in the dealing with wounds . The phenolic compounds lead to formation of a film around the exposed wound tissue causing prevention of loss of fluid from the tissues as well as formation of a chemical barrier . The film also acts as a physical barrier by providing insulation to the wound area. This property of the plant helps in better healing of the treated sites.

GUAVA LEAF: - VERSATILE INGRDIENT Guajava leaves tea and aqueous extracts of guajava leaf has been recommended for pre-diabetes by FOSHU (foods for specified health uses) in Japan. Guajava extracts with water and guajava tea inhibited the invitro activities of maltase, sucrose and alpha amylase according to dose given . Guava leaves have several chemical constituents such as coumarins, essential oils, flavonoids, triterpenes and ellagitannins which are known to have antimicrobial properties. The leaves of Psidium guajava tree have a long history of medicinal uses that are still employed today. Guava leaves and fruit juice has also been tested in treatment of infantile diarrhoea and the results showed that, those who were treated with guava recovered at 3 days. Guava leaf has been studied for its anti-cancer activity. It contains components like secondary plant metabolites with certain polyphenols with potential intrinsic antioxidant, anti-inflammatory, and antiviral properties . Several guava components have been postulated as having anticancer effects in vitro, and the most frequently reported are ascorbic acid(vitaminC),flavonoids (apigenin),and lycopene.

Toothpaste formulations incorporating guava leaf extract (Psidium guajava) are gaining attention due to the numerous health benefits attributed to this plant. The rationale for using guava leaves in toothpaste primarily stems from their rich phytochemical composition and proven antimicrobial properties.

Guava (Psidium guajava) has long been recognized in various cultures for its health benefits. The leaves have been used traditionally for their antibacterial, anti-inflammatory, and antioxidant properties. In many regions, guava leaves are utilized in herbal remedies to treat ailments ranging from diarrhea to oral infections. This historical use underscores the potential of guava leaves as a natural ingredient in dental care products, particularly toothpaste.

Scientific Validation

Recent studies have validated the traditional claims regarding guava leaves. Research indicates that extracts from these leaves possess bioactive compounds that exhibit significant antimicrobial activity against common oral pathogens, such as Staphylococcus aureus and Escherichia coli 12. These findings support the rationale for incorporating guava leaf extract into toothpaste formulations aimed at enhancing oral hygiene.

- Guava Leaf Extract

-Nutritional and Phytochemical Profile

Guava leaves are abundant in various bioactive compounds such as flavonoids, phenols, carotenoids, and essential oils, including quercetin and catechin. These compounds are known for their antioxidant, anti-inflammatory, and antimicrobial properties. The presence of these phytochemicals suggests that guava leaves can effectively combat oral pathogens and promote overall oral health.

-Antimicrobial Properties

Research indicates that guava leaf extracts exhibit significant antibacterial activity against common oral pathogens such as Streptococcus mutans , Escherichia coli , and Staphylococcus aureus , which are associated with dental caries and periodontal diseases. This antimicrobial efficacy makes guava leaf extract a valuable ingredient in toothpaste formulations aimed at reducing plaque formation and gingivitis.

➢ **Rationale for Use :-** The rationale for using A. aspera in toothpaste formulation includes: Natural Antibacterial Activity: The flavonoids and phytochemicals present in A. aspera provide natural antibacterial properties that can help combat harmful oral bacteria.

- Safety Profile: Unlike many synthetic dental products, herbal formulations are often perceived as safer with fewer side effects, making them suitable for regular use, particularly among children.

- Cultural Relevance : The use of herbal remedies is deeply rooted in many cultures, providing an alternative that aligns with traditional practices while leveraging modern scientific validation.

-The rationale for using Psidium Gualava leaf

Natural Alternative to Chemical Ingredients : Herbal formulations using guava leaf extract provide a greener alternative, harnessing natural ingredients that are less likely to cause adverse effects while still delivering effective oral care.

-Enhanced Oral Hygiene : Formulations containing guava leaf extract not only help in controlling bacterial growth but also possess antioxidant properties that may protect gums from oxidative stress. Studies have shown that toothpaste with guava extract can enhance the overall effectiveness of oral hygiene routines by reducing microbial load and improving gum health.

1.2. Research Objectives

➢ Research Objective for Toothpaste Formulation from Achyranthes Aspera and Psidium Guajava Plant Extract

The primary objective of this research is to formulate and develop a toothpaste using extracts from the Achyranthes aspera and Psidium guajava plant, assessing its antibacterial activity against plaque-forming oral microbiota, particularly targeting bacteria such as Streptococcus mutans and Enterobacter ludwigi .

- Herbal toothpaste are as effective as non-herbal (conventional) dentifrices in the control of plaque and gingivitis.
- Optimize the formulation by varying concentrations of key ingredients like calcium carbonate and glycerin .

➢ Research Gap

- Comparative studies with conventional agent.

2. Literature Review :

Literature Review on the Formulation and Development of Toothpaste from Achyranthes Aspera Plant Extract and Its Antibacterial Activity Against Plaque Forming Oral Microbiota

➢ Introduction

Achyranthus aspera, a medicinal plant known for its diverse therapeutic properties, has garnered attention for its potential application in dental care, particularly in formulating herbal toothpaste. This review focuses on the formulation of toothpaste using A. aspera extracts and its effectiveness against oral microbiota responsible for plaque formation.

➢ Phytochemical Composition

The phytochemical profile of A. aspera includes flavonoids, triterpenoids, saponins, and alkaloids, which contribute to its antibacterial properties. Studies have shown that the plant's

extracts exhibit significant antimicrobial activity against common oral pathogens like Streptococcus mutans and Enterobacter ludwigi. The minimum inhibitory concentration (MIC) for these bacteria has been reported to be as low as 2.5%, indicating a strong potential for use in dental formulations.

➢ Formulation of Toothpaste

Recent research has focused on developing a herbal toothpaste incorporating A. aspera extract aimed at preventing dental black stains caused by chromogenic bacteria. In one study, an optimized formulation was created using calcium carbonate and glycerin in specific ratios (30% each) to enhance stability and efficacy . 5 trial batches were tested to identify the optimal formulation that would not only be effective but also comparable to standard treatments like chlorhexidine mouthwash in terms of antibacterial activity .

➢ Antibacterial Activity

The antibacterial efficacy of A. aspera extracts has been well-documented. In vitro studies have demonstrated that methanolic extracts possess significant antibacterial properties against plaque-forming bacteria. The extracts have been shown to inhibit bacterial growth effectively, making them suitable candidates for inclusion in oral hygiene products .

➢ Historical and Traditional Uses:

Historically, various parts of A. aspera have been utilized in traditional medicine for oral health maintenance. The stems were used as natural toothbrushes, while the leaves and roots were employed in tooth powders and pastes. This traditional knowledge supports the modern scientific exploration of the plant's benefits in dental care.

➢ Conclusion : The formulation of toothpaste from Achyranthus aspera plant extract presents a promising avenue for enhancing oral health through natural products. Its established antibacterial properties against plaque-forming bacteria make it a valuable ingredient in developing effective dental care products. Continued research into optimizing formulations and understanding the mechanisms of action will further solidify its role in modern dentistry.

➢ References : Development and Characterisation of an Optimised Toothpaste of Achyranthes aspera plant extract for the Effective Protection from Dental Black Stain forming Chromogenic bacteria species .

2.1Description :- Habit and Growth Form- Achyranthes aspera typically exhibits an erect and multi-branched structure, although it can occasionally
sprawl. The stems are often woody at the base and can be up to 2 meters tall .

Stem Characteristics

- The stems are quadr-angular , hairy, and exhibit longitudinal grooves. Nodes are prominent and can appear swollen. The stem color is generally green but may have violet or pink hues at the nodes.

- The leaves are simple, opposite, and sessile, with shapes ranging from oval to elliptic. They measure approximately 4 to 9 cm in length and 2 to 4 cm in width. The upper leaf surface is dark green and hairy, while the underside is paler with a silvery sheen .

Flowers and Inflorescence

- Achyranthes aspera produces small, greenish-white flowers that may have purple-red tinges. These flowers form dense terminal spikes that can elongate up to 60 cm. Initially upright, the flowers bend downwards as they mature . Each flower lacks petals but has two sharp, pointed scales.

Fruits and Seeds

- The plant bears small one-seeded capsules that are orange to reddish-purple or straw-brown in color. These fruits are designed to adhere easily to animals and clothing for seed dispersal . The seeds themselves are smooth, cylindrical, and shiny, measuring about 2 to 3 mm long.

➢ **Pharmacological Importance** : Beyond its ecological role, Achyranthes aspera holds significant ethnopharmacological value. It has been traditionally used for various medicinal purposes, including:

- Anti-inflammatory
- Antioxidant
- Antimicrobial
- Analgesic properties

Research into its phytochemical composition reveals the presence of compounds such as alkaloids, flavonoids, saponins, and triterpenoids, which contribute to its therapeutic potential. However, further studies are needed to assess its safety and potential toxicity.

In summary, Achyranthes aspera is a versatile plant with both ecological significance and medicinal applications. Its distinctive morphology and adaptability make it an interesting subject for both botanical study and pharmacological research.

A.Aspera Plant

➢ Overview of Achyranthes aspera for Toothpaste Formulation

Achyranthes aspera, commonly known as Apamarg, is a medicinal plant recognized for its diverse therapeutic properties, particularly in dental care. This plant has garnered attention for its antibacterial activity against oral pathogens, making it a promising candidate for formulating herbal toothpaste aimed at combating plaque-forming bacteria.

➢ Phytochemical Composition: The plant contains various phytochemicals, including:

- Flavonoids : Known for their antioxidant and antibacterial properties.

-Triterpenoid saponins : Contributing to its antimicrobial effects.

- Alkaloids and phenolic compounds : Associated with the inhibition of pathogenic bacteria like Streptococcus mutans and Enterobacter ludwigi .

Flavonoids :- It contains quercetin and kaempferol which are more or less same ,similar effectiveness antibacterial activity potential both are against caries. The antibacterial mechanism of quercetin is mainly by the destruction of bacterial cell wall integrity and inhibition of nucleic acid synthesis also denature protein to disrupt plasma membrane and destroying bacterial cell wall and cell membrane.

Kaempferol mainly functions at the molecular level of bacteria and acts as antibacterial agent, quercetin and kaempferol are more or less similar in structure except for one additional OH- group in quercetin.

3. Plaque forming oral microbiota :-

Dental plaque is a complex biofilm composed of various microorganisms that adhere to tooth surfaces. This biofilm plays a significant role in oral health, as its composition can influence the development of dental diseases such as caries and periodontal disease.

➢ Composition of Dental Plaque : Microbial Diversity

Dental plaque houses a diverse array of microorganisms, including:
-Bacteria : Key genera include Streptococcus , Actinomyces , Lactobacillus , Bacteroides , and Fusobacterium . These bacteria are essential for plaque formation and contribute to its structural integrity .

- Fungi : Species like Candida are also present, although they are less dominant than bacteria .

-Other Microorganisms : The plaque can contain archaea and viruses, adding to its complexity.

- Biofilm Structure

The structure of dental plaque is not random; it is organized into communities that evolve through ecological succession. Early colonizers, such as Streptococcus species, create a foundation for further microbial attachment, leading to a climax community that includes both health-associated and pathogenic species.

- Mechanisms of Plaque Formation :-

Dental plaque is a complex biofilm formed by various bacteria in the oral cavity. The mechanism of plaque formation involves several key bacterial species that play distinct roles at different stages of biofilm development.

Key Bacteria Involved in Plaque Formation

1. Initial Colonizers:- Streptococcus mutans : This bacterium is crucial in the early stages of plaque formation. It utilizes the enzyme glucansucrase to convert sucrose into a sticky polysaccharide called dextran, which helps it adhere to tooth surfaces and form the initial layer of plaque .

- Actinomyces species : These bacteria also contribute significantly to the early biofilm, often co-aggregating with Streptococcus species .

2. Intermediate Colonizers: - Fusobacterium nucleatum : Acts as a bridge between early and late colonizers, facilitating the adhesion of other bacteria and playing a role in the structural complexity of the plaque .

- Prevotella intermedia : Commonly found in healthy gingival crevices, it can also contribute to periodontal disease when conditions change.

3. Late Colonizers: - Porphyromonas gingivalis : This bacterium is associated with periodontal disease and can invade deeper tissues, promoting inflammation and further plaque development.

- Aggregatibacter actinomycetemcomitans : Known for its role in aggressive periodontitis, it can alter local environments to favor its survival and growth.

- Treponema denticola and Tannerella forsythia : These are also significant late colonizers that contribute to the pathogenicity of dental plaque.

Stages of Plaque Development

The formation of dental plaque occurs in several stages:

1. Acquired Pellicle Formation : Salivary proteins coat the tooth surface, providing a substrate for bacterial attachment.

2. Reversible Adhesion : Initial weak interactions allow bacteria to attach temporarily.

3. Irreversible Attachment : Stronger adhesion-receptor interactions lead to permanent attachment.

4. Co-adhesion : Secondary colonizers attach to already adhered bacteria, increasing community complexity over time.

Environmental Factors -The growth and stability of dental plaque are influenced by several factors:

1. pH Levels : Plaque thrives in a slightly acidic to neutral pH (6.7 - 8.3), which is maintained by saliva .

2. Nutrient Availability : Saliva provides essential nutrients that support bacterial growth, including amino acids

and glycoproteins .

4. Materials and Methods :- Material collection: A. aspera and Psidium guajava plants were obtained from farms and local places in Mehkar, Buldhana district, Maharashtra. Calcium Carbonate (food grade), Sodium Benzoate, Sodium Lauryl Sulfate, Glycerin, Menthol Crystal, and Industrial Solvent were obtained from the laboratory of Satyajeet College of Pharmacy Mehkar.

Extraction Process: The stems of Achyranthes aspera were dried for one week and then ground into a fine powder, with about 50 gm of the powder utilised in the decoction procedure. Also, fresh guava leaves were grinded into powder.

A. Aspera: 50gm of powder was taken into a conical flask of (500)ml with the addition of 300ml of industrial solvent for the maceration process and allowed to stand for 30 minutes, then add 200ml of distilled water for decoction and heat the mixture of hydro- alcoholic solution at 30 degrees Celsius for 4 to 5 hours in order to reduce the liquid about 1/4th of its final volume for almost 125ml and then allowed to cool it and then Filter the liquid through Whatman filter paper no. 4 in a beaker of 250 ml, then divide and pour the liquid into different clean petri plates in order to evapourate the liquid and to get a more concentrated form of liquid on a heated plate at 30 degrees Celsius, and the extracted volume is around 18 ml.

B. Guava leaf: The decoction method is accomplished by using distilled water. 30 gram of weighed powder of leaves were taken into a 500-ml beaker, and 200 ml of distilled water

was added and boiled at 30 degrees Celsius for 2 to 3 hours to minimise the volume of liquid. Allow to cool and strain through Whatman filter paper no. 4, then separate and pour that liquid into different petri plates for evapouration on a hotplate at 30 degrees Celsius until all of the liquid is evapourated and a semi-solid extract is produced.

Toothpaste Preparation :- Preparation of menthol solution – In 50ml beaker 0.8gm of menthol crystal were added in purified water(40ml) then gently heated at 10 degree celsius until crystal completely dissolve.

Trituration Method Use : In a mortar, the binder (CMC sodium) and abrasive agent (CaCO3) were first mixed with addition of liquid phases (glycerin, purified water, plant extract) with proper mixed for blending and finally sodium benzoate and saccharine sodium were blended to create uniform paste.Then material like menthol soln and surfactant, followed by gentle trituration.

5. Toothpaste Formulation :

Sr.no	Ingredients	F3	F4	Uses
1.	A.aspera extract	6ml	6ml	Anti-caries agent
2.	Psidium guajava extract	0.3gm	0.3gm	Anti-caries agent
3.	Calcium carbonate	35gm	44gm	Abrasive agent
4.	Glycerin	27ml	31ml	Humectant
5.	SLS	1.05g	1.5gm	Foaming agent

6.	CMC Sodium	2.8gm	2.8gm	Thickening agent
7.	Sodium benzoate	0.35gm	0.35gm	Preservative
8.	Saccharin sodium	0.35gm	0.2gm	Sweetening agent
9.	Menthol	q.s	q.s	Flavouring agent
10.	Distilled water	q.s	q.s	Vehicle
		×	√	

Formulation no.4

6. Preliminary Phytochemical test of both plant extracts.

Chemical Constituents	Test	Inference of (A.aspera)	Inference of (Psidium guajava)

1.Carbohydrates	Molish test Fehlings test	Presence of reducing sugar	Presence of reducing sugar
2.Alkaloids	Meyer test Hager test	Presence of alkaloids	Presence of alkaloids

3.Proteins	Biuret test	Absence of proteins	Absence of proteins
4.Tannins	Ferric chloride test	Absence of tannins	Absence of tannins
5.Resins	Acetone water test	Absence of resins	Absence of resins
6.Cardiac Glycosides	Baljet test	Presence of cardiac glycoside	Presence of cardiac glycosides
7.Anthraquinone glycoside	Borntrager test	Presence of anthraquinone glycoside	Absence of anthraquinone glycoside
8.Cyanogenetic glycoside	Grignard test	Absence of cyanogenetic glycoside	Absence of cyanogenetic glycoside
9. Coumarin	Alkali test	Absence of coumarin	Absence of coumarin
10.Flavnoids	Lead acetate test	Presence of flavnoid	Presence of flavnoid
11.Lipids	Spot test	Absence of lipids	Absence of lipids

7. Evaluation parameters

1. Physical Examination • Colour- Formulated toothpaste was evaluated for its colour. • The visually colour was checked.

- Odour- Odour was found by smelling the product.
- Taste- Taste was checked manually by tasting the formulation

2. Abrasiveness Extrude the content 15-20 cm long on the butter paper, repeat the same process for at least ten collapsible tubes. Press with the contents of the entire length with fingertip for the presence of sharp and hard edged abrasive particles. Toothpaste shall not contain such particles.

3. Determination of spreadability

In this method slip and drag characteristic of paste involve. Formulated paste (2g) placed on the ground slide under study. The formulated paste placed like sandwich between this slide and

another glass slides for 5min to expel air and to provide a uniform film of the paste between slides. Excess of the paste was scrapped off from the edges. The top plate was then subjected to pull of 80g with the help of string attached to the hook and time (sec) required by the top slide to cover a distance of 7.5cm was noted. A short interval indicated better spreadability.

Formula was used to calculate spreadability: S=M× L /T Where,\ S= Spreadability M= Weight in the pan (tied to the upper slide)L= Length moved by the glass slide T=Time (sec) taken to separate the upper slide from the ground slide.

4. Homogeneity

The toothpaste shall extrude a homogenous mass from the collapsible tube or any suitable container by applying of normal force at 27±20C. in addition bulk of contents shall extrude from the crimp of container and then rolled it gradually.

5. Foaming

The foamability of formulated toothpaste evaluated by taking small amount of formulation with water in measuring cylinder initial volume was noted and then shaken for 10 times. Final volume of foam was noted Determination of froth power Foaming power = V1–V2 V1- Volume in ml of foam with water. V2- Volume in ml of water only.

6. Stability

The stability study was performed as per ICH guideline. The formulated paste was filled in collapsible tube and stored at different temperature and humidity conditions, 25oC± 2oC / 60% ± 5% RH, 30o C ± 2oC / 65% ± 5% RH, 40oC ± 2oC / 75% ±5% RH for the period of three months and studied for appearance, pH and spreadability.

7. Determination of moisture and volatile matter

5 g of formulation placed in a porcelain dish containing 6-8 cm in diameter and 2-4 cm depth in it. Dry the sample in an oven at 105 degree Celsius. (Calculation) By mass = 100MI/M MI-Loss of mass (g) on drying M-Mass (g) of the material taken for the test.9

8. Moisture content

Toothpaste (10 gm) weighted in a Porcelain dish and dried it in the oven at 105 o C. It was cooled in a desiccater. The loss of weight is recorded as percentage moisture content and calculated by the given formula. % Moisture = Original sample weight – dry sample weight/ Original sample weight

9. Organoleptic evaluation

Organoleptic evaluation (colour, taste) was done by sensory and visual inspection.

10. pH ph of formulated herbal toothpaste was determined by using pH paper. 2g of toothpaste placed in small test tube. Allow the 5ml of boiled and then cooled water. Stir vigorously to make suspension.

11. Fragrance test

It was based on individual observation for its acceptability.5 people were asked for acceptability of fragrance and their opinion was taken. And fragrance was evaluated based on the below- described criteria; A) The fragrance was good, as good as the fragrance of reference toothpaste.
B) The fragrance was not so good but comparable to the reference toothpaste. C) The fragrance of the toothpaste was poor than the reference toothpaste.

12. Shape retention

Tooth paste was squeezed out from the tube and put entirely of a tooth brush and the state of the toothpaste after it was allowed to stand for 10 seconds was evaluated based on the below- described criteria; A) Shape just after the toothpaste is squeezed out on the toothbrush is maintained. B) Shape just after the toothpaste is squeezed out on the toothbrush is almost maintained. C) The toothpaste squeezed from the toothbrush and can not maintain its shape.

13. Changes on shell before and after brushing

Before Brushing

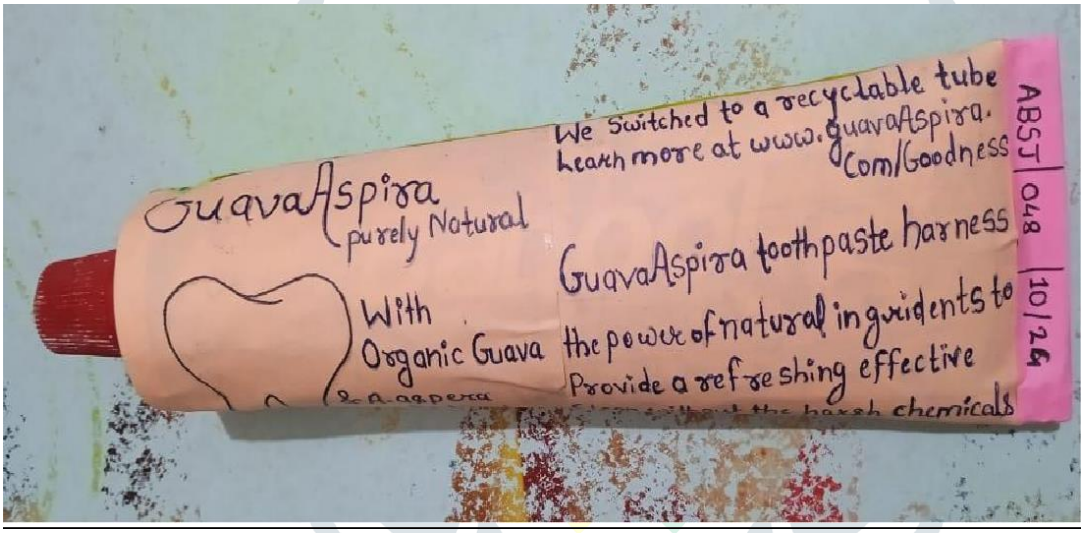
After Brushing

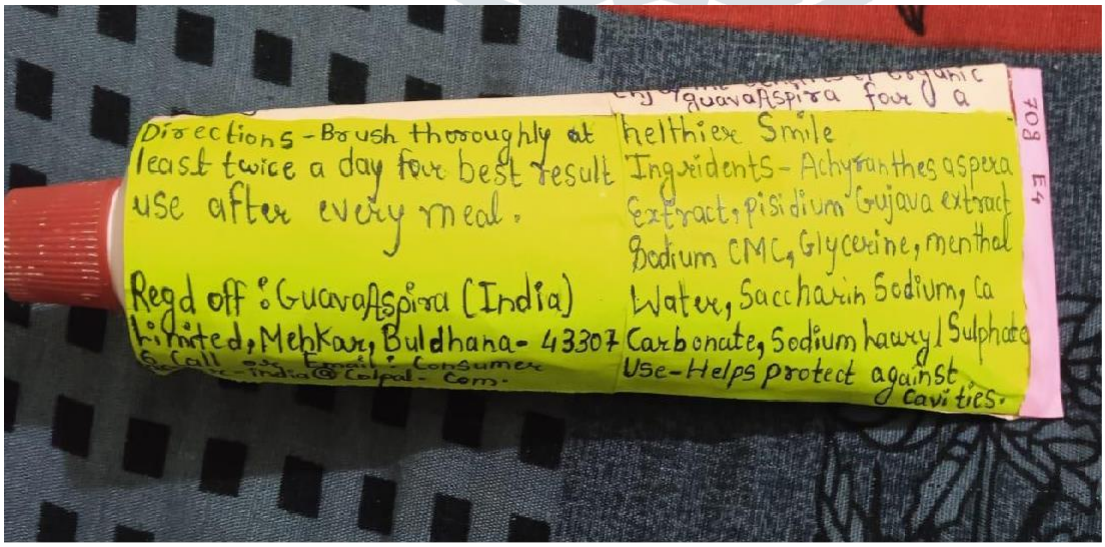
8. Result and Discussion

Evaluation test

Sr.no	Parameters	Observations
1.	Homogenicity	Good
2.	Moisture content	14%
3.	Extrudability	Extrudable
4.	Smoothness	Smooth
5.	Colour	Light brown

6.	Odour	Characterstics
7.	Taste	Sweet
8.	Stability	Stable
9.	Spreadability	Easily
10.	Abrasiveness	Good abrasive
11.	Foambility	Good
12.	pH	8





➢ **Labelling**

9. Recommendations for future research

➢ The formulation and development of toothpaste using Achyranthes aspera (A. aspera) plant extract, particularly focusing on its antibacterial properties against plaque-forming oral microbiota, presents a promising area for future research. Here are several key recommendations:

1.Determination of total flavnoid content by spectroscopy. 2.Determination of minimum inhibitory concentration.

3. Clinical trial for validation.

4. Long-term Safety and Stability Studies

- Objective : Evaluate the long-term safety and stability of toothpaste formulations.

- Methods : Conduct stability testing under various environmental conditions and assess any potential cytotoxic effects on oral tissues.

- Outcome : Ensure that formulations remain effective over time without compromising safety for consumers .

10. Conclusion : The goal of this preparation was to incorporate herbal antibacterial agents instead of chemical agents, and it was accomplished. The resulting product, toothpaste, passed all of the tests performed to evaluate it. This research will likely change people's attitudes towards herbal ingredients and personal care products, potentially influencing their preference for herbal toothpaste.

Acknowledgement : I am very much thankful to Prof. Anand Gawai Sir and Dr. Shivshankar D. Mhaske Principle at Satyajeet college of pharmacy Mehkar , Buldhana, Maharashtra, India. for his continuous support, guidance and encouragement.

Refrences :

[1] https://colab.ws/articles/10.1016%2Fj.sajb.2023.01.024

[2] https://medicopublication.com/index.php/ijfmt/article/download/12412/11413/23658

[3] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5767990/

[4] https://crsubscription.com/journals/pharmacy/pharmaceutical-science2/articles/2022/%5B1-19%5D-FORMULATION-AND-PHYTOCHEMICAL-STANDARDIZATION.pdf

[6] https://www.sciencedirect.com/science/article/abs/pii/S2210803323002208

[7] https://www.semanticscholar.org/paper/Development-and-Characterisation-of-an-Optimised-of-Kk-S./3cf685cfc427ff52723bad99857c73886536d301

[8] https://www.researchgate.net/publication/362109032_Formulation_and_comparative_standardization_of_ toothpaste

[9] https://wjcmpr.com/index.php/journal/article/view/237

[10] https://bepls.com/beplssplissue(II)%202022/100.pdf

[11] https://www.mdpi.com/1999-4923/14/10/2219

[12] https://www.researchgate.net/publication/344725993_ANTIBACTERIAL_ACTIVITY_OF_Achyranthes_aspera_EXTRACT_AGAINST_ORAL_PATHOGENS_-AN_in_vitro_STUDY

[13] https://journals.lww.com/japtr/fulltext/2016/07040/evaluation_of_antibacterial_activity_of.8.aspx

[14] https://core.ac.uk/download/pdf/236008879.pdf

[15] https://amb-express.springeropen.com/articles/10.1186/s13568-019-0744-2

[16] https://www.tjnpr.org/index.php/home/article/view/4584

[17] https://www.sciencedirect.com/science/article/abs/pii/S0254629923000248

[18] https://jddtonline.info/index.php/jddt/article/view/3344

[19] https://jidc.org/index.php/journal/article/download/34780366/2643/141652

[20] https://dergipark.org.tr/tr/download/article-file/581606

[21] https://onlinelibrary.wiley.com/doi/full/10.1002/mbo3.1271

[22] https://www.scielo.br/j/bdj/a/QBNyXHX9xYN9wPFhDyP9j8R/

[23] https://www.ijcmas.com/9-6-2020/Magdalene%20C.%20Igboama,%20et%20al.pdf

[24] https://www.ipinnovative.com/journal-article-file/16295

[25] https://patents.google.com/patent/US6149894A/en

[26] https://www.silverson.com/us/resource-library/application-reports/manufacture-of-toothpaste

[27] https://www.researchgate.net/publication/8101979_Formulation_ingredients_for_toothpastes_and_mouth washes.

[28] https://www.biotech-asia.org/vol21no2/potential-role-of-medicinal-plants-and-their- phytochemicals-against-plaque-forming-oral-microbiota/

[29] https://bmcoralhealth.biomedcentral.com/articles/10.1186/1472-6831-6-s1-s14

[30] https://www.nature.com/articles/sj.bdj.2016.865

[31] https://www.sciencedirect.com/topics/immunology-and-microbiology/oral-microbi

[32] https://www.researchgate.net/publication/362109032_Formulation_and_comparative_standardiz ation_of_toothpas

[33] http://publish.plantnet-project.org/project/plantinvasivekruger/collection/collection/synthese/details/ACYAS

[34] https://en.wikipedia.org/wiki/Prickly_chaff_flower

[35] https://www.worldfloraonline.org/taxon/wfo-0000516177

[36] https://bmccomplementmedtherapies.biomedcentral.com/articles/10.1186/s12906-019-2745-

[37] https://d-nb.info/1206636750/34

[38] https://www.ijpp.org.in/html-article/17726

[39] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8066327/

[40] https://www.researchgate.net/publication/320728048_Formulation_and_Evaluation_of_Herbal_Toothpaste_Compared_With_Marketed_Preparation

[41] https://www.researchgate.net/publication/380004264_FORMULATION_AND_EVALUATION_OF_A_HERBAL_MEDICATED_TOOTHPASTE_USING_QBD_APPROACH_Rabindra_K_Nanda_Design_QBD_analytical_methods_for_evaluation_of_a_herbal_toothpaste

[41] Shrishail M. Ghurghure , Priyanka R. Surwase, Mahewash A. Pathan, Parikshit D. Shirure.Design and Development of Tooth Paste Containing Alcoholic Extract of Psidium Guajava Leaf. American journal of pharmtech research. 2019; 9(02): 2249-3387.

[42] Shagun Malik, Amit Bhardwaj, Shilpa Kamra. Achyranthes aspera: An Ancient Treasure. Bulletin of Environment, Pharmacology and Life Sciences. 2022 : 523-526.

[43] S.Arya and R. Usha. Bioprospecting and exploration of phytochemicals as quorum sensing inhibitors against cariogenic dental biofilm. Journal of pure and applied microbiology(JPAM). 2024;18(1):100-117.

[44] Grace spatafora, Yihong Li, Xuesong He, Annie Cowan and Anne C.R.Tanner. The evolving microbiome of dental caries. Multidisciplinary digital publishing institute(MDPI).2024,12(1),121.

[45] Xinyi Li, Yanmei Liu, Xingyou Yang,Chengwen Li, Zhangyong Song.The oral microbiota: Influencing factors,pathogenesis ,and Interventions. National centre for Biotechnology Information(PMC).2022 Apr 29;13:895537.