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Formulation and Evaluation of Herbal Toothpaste

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ABSTRACT
Herbal toothpaste containing natural ingredients is gaining popularity in oral dental care. A detailed study on toothpaste formulated from ethanolic leaf extract of Piper betel and Averrhoa bilimbi was performed to obtain an overview of the key parameters namely pH, microbial load, spreadability, extrudability and sensory attributes to create a more effective formulation and stable performance. This study suggests that the composition of herbal-based toothpaste with natural ingredients is as good in terms of results. Averrhoa bilimbi possesses anti-bacterial and anti-inflammatory properties. Piper betel is renowned for its antimicrobial and analgesic activity. The results indicated that the herbal toothpaste possesses favorable physical properties, appropriate pH levels, and spreadability. In conclusion, the herbal toothpaste containing leaf extracts of Averrhoa bilimbi and Piper betel holds promise as an alternative oral care product.

Keywords: Herbal toothpaste, Evaluation, Herbal ingredients, Leaves of Averrhoa bilimbi, Piper betle leaf, Antibacterial.

I. INTRODUCTION

For many years in India, herbs have been utilized to treat and prevent illnesses, promote overall health and extend one's life while enhancing its quality. Ayurveda, a natural healing system, originated in India over 3000 years ago. The term Ayurveda is derived from the Sanskrit words Ayur, meaning "life", and Veda, meaning "science" or "knowledge". Ayurveda's central idea is that illnesses are caused by unconscious dissatisfaction or stress. Therefore, Ayurveda recommends certain lifestyle changes and natural remedies to restore balance to the body, mind, spirit, and environment[1].Chronic gum disease can lead to tissue damage and the more severe form, Periodontitis, if left untreated[2].Medicinal plants are used to prevent and control diseases, reducing side effects. However, herbal remedies are challenging due to limited understanding of their mechanism of action and side effects[3].

Dental plaque is the main cause of gingivitis and tooth decay, which continue to be major health problems worldwide. Oral diseases affect the overall quality of life and are associated with chronic illnesses and diseases. There is considerable evidence linking poor oral health to chronic conditions such as diabetes. Dental caries and periodontal disease are among the most preventable diseases globally. Therefore, proper oral hygiene practices, such as brushing, flossing, and regular dental check-ups, are essential for maintaining good oral health[4]. Poor oral health is linked to diseases like cardiovascular diseases, rheumatoid arthritis, and osteoporosis[5].According to the World Health Organization (WHO), around 80% of the world's population depends on traditional herbal medicines. Traditional medicine is widely used to prevent, diagnose, and treat various diseases. It is easily accessible, particularly in developing and developed countries[6].

1.1 Oral health

According to the WHO Global Oral Health Status Report (2022), oral diseases are a major concern for nearly 3.5 billion people around the world, with three out of four affected individuals residing in middle-income countries. The report estimated that over 2 billion people suffer from permanent tooth decay, while 514 million children are affected by decay of primary teeth.

- Dental caries (tooth decay)
- Toothache
- Tartar
- Gingivitis
- Cavities
- Chipped tooth
- Sensitive tooth
- Gum disease

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- Stained teeth
- Periodontal (gum disease)
- Edentulism (total tooth loss)
- Oral cancer& Oro-dental trauma[7]

1.2 Toothpaste

Toothpaste is a gel used with a toothbrush to maintain dental aesthetics. It's not a modern invention; ancient Egyptians used a dental cream with oxen hooves, myrrh, eggshells, and pumice to remove debris from teeth. The Chinese also used toothpaste with flavorings like ginseng, herbal mints, and salt - similar to today's kinds of toothpaste. Toothpowders became popular during the industrial age in the 18th century. Dentists, doctors, and chemists developed them for the sole purpose of cleaning teeth[8].

PLANT PROFILE

AVERRHOA BILIMBI	**PIPER BETLE**
Common name: Bilimbi Kingdom: Plantae Division: Magnoliophyta Class: Dicotyledonae Order: Oxalidales Family: Oxalidaceae Genus: Averrhoa Species: bilimbi	Common name: Betel pepper Kingdom: Plantae-plants Division: Magnoliophyta Class: Magnoliopsida Order: Piparales Family: Piperaceae Genus: Piper Species: betle
Chemical Constituents: Saponins, tannins, flavonoids, citric acids, cyanidin-3-o-h- glucoside, phenolics, potassium ion and sugar[9]	Chemical constituents: Chavibetol, hydroxyl chavicol, caryophyllene, chavicol acetate, eugenol, limonene, pinene, flavonoid
Uses: Anti-diabetic, anti-microbial, anti-inflammatory, cytotoxic activities, anti-oxidant activity, anti-fertility, and anti-bacterial activity[10].	Uses: Antifungal, antimicrobial, anti-oxidative, antiulcer, analgesic activity, and neutraceutical in diabetic patients to control blood glucose levels.

Leaf of Averrhoa bilimbi

Leaf of Piper betle

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II. MATERIALS & METHODS

1. PLANT COLLECTION AND DRYING: The leaves of Piper betle and leaves of Averrhoa bilimbi were collected from various places in Kasaragod and the part collected were authenticated by botanist Mr.Biju P, Assistant professor, Dept. of Botany, Government College Kasaragod.The collected materials were cleaned to remove the adhered dust particles and were then dried in the shade at room temperature. The dried plant materials were coarsely powdered, weighed and stored in an air-tight container till use.

2. PHARMACOGNOSTIC STUDY:

2.1. Evaluation of Foreign Matter: About 100-500gm of the plant to be examined was weighed and spread out in a thin layer. The foreign matter was detected. It was separated and weighed and the percentage of foreign matter was calculated[11].

2.2. Determination of moisture content:5g of the powdered leaves of the plant were placed in a tared evaporating dish. Drying was carried out at 105˚C for two hours. The drying was continued with intermittent weighing at one-hour intervals until the difference between two successive weights was not more than 0.25%. Constant weight was reached when the two- consecutive weights after drying for 30 minutes and cooling for 30 minutes in a desiccator, showed not more than 0.01gm difference.The percentage of moisture present in the sample was calculated[11].

2.3.Total ash:2g of ground air-dried material was accurately weighed out in a crucible previously ignited for 30 minutes. The material was spread in an even layer and ignited at a temperature of more than 450˚C until it indicated the absence of carbon, cooled in the desiccator, and weighed. Calculated the content of total ash per gram of air-dried material was calculated.

2.4. Acid insoluble ash: To the total ash, 25ml of 2N HCl was added. It was boiled for 5 minutes, then rinsed with hot water. The insoluble matter was collected on filter paper, washed until neutral, and dried on a hot plate. The residue was weighed and used to calculate the content of acid-insoluble ash per gram of air-dried material.[12].

2.5. Water soluble ash: To determine water-soluble ash content: boil the sample's ash (25ml water added) for 5 mins. Collect the insoluble matter, wash and ignite (≤450°C). Subtract residues' weight from total ash, and calculate water-soluble ash content/g of air-dried material.[13].

2.6. Alcohol soluble extractive value:5 grams of air-dried plant parts were mixed with 100 ml ethanol and left to macerate for 24 hours. The mixture was filtered and 25 ml of the filtrate was evaporated and weighed to calculate the w/w ethanol-soluble extractive of the air-dried material.[11].

2.7. Water soluble extractive value: Macerated 5 grams of coarsely powdered air-dried plant with 100 ml water in a stoppered flask for 24 hours, with occasional shaking during the 1st 6 hours and allowed to stand undisturbed for another 18 hours. Filtered rapidly, then 25 ml of the filtrate was evaporated to dryness in a tared flat-bottomed shallow dish, dried at 105˚C, and weighed. Calculated W/W ethanol-soluble extractive concerningair-dried material[11].

2.8. Extraction: Powdered leaves of Piper betle and Averrhoa bilimbi are placed separately in thimble-shaped filter paper inside a glass cylinder. The cylinder is equipped with a siphon and inlet tube and a water condenser at the top. The cylinder is then inserted into a round bottom flask containing ethanol and heated in a water bath or sand bath. Solvent vapors rise and enter the cylinder through the inlet tube, dissolve the crude organic substance, and flow back into the flask through the siphon tube. Finally, the heating is stopped and the solution in the flask is collected and air-dried to obtain the extract[14,15,16].

2.9. Phytochemical Screening:

- **Chemical test for alkaloids:**[11,12]

a) Mayer's Test: To 2 ml of the above acidified extract, 1 ml of Mayer's reagent (potassium mercuric iodide) was added, shaken, and noted for the presence of a creamy precipitate.

b) Wagner's test: The acidified extract (2ml) was treated with a few ml of Wagner's reagent (solution of iodine in potassium iodide) and observed for the presence of a reddish-brown precipitate.

c) Hager's test: To 2ml of acidified extract, 1ml of Hager's reagent (saturated solution of picric acid) was added and observed for the presence of a yellow precipitate.

d) Dragendroff's test: The acidified extract (2ml) was treated with a few ml of Dragondroff's reagent (potassium bismuth iodide) and observed for the presence of orange-red precipitate.

- **Chemical test for glycosides:**[12]

a) Legal's Test: Dry extract was dissolved in pyridine. Sodium nitroprusside solution was

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added and made alkaline with sodium hydroxide solution. A pink-red color was observed.

b) Baljet's test: The few ml of the extract was treated with 1ml sodium picrate solution and a yellow to orange color reveals the presence of cardiac glycosides.

c) Liebermann Burchard's Test: To test, evaporate 5 ml of hydrolysate in a test tube and add 1 ml of dry chloroform. Mix with 2 ml of distilled acetic anhydride and a few drops of sulphuric acid. Observe for red and green color in the lower and upper portions. The color will change to blue and violet.

d) Borntrager's test: A little of the residue obtained from the hydrolysate was mixed with water and shaken with an equal volume of chloroform. The chloroform layer was separated to which dilute ammonia solution was added and shaken well and noted whether any pink color was present in the ammoniacal layer.

e) Modified Borntrager's test: The residue obtained was treated with ferric chloride and dilute HCl, for the oxidative hydrolysis of C-glycoside. Then it was extracted with chloroform. The chloroform layer was separated, and dilute ammonia solution was added and shaken. The ammoniacal layer was observed for pink color.

- **Chemical tests for Phenolic compounds and Tannins:**[9]

a) Ferric chloride test: A small quantity of the extract diluted with water was treated with dilute ferric chloride solution (5%) and observed for the presence of blue colour.

b) Gelatin test: The extract dissolved in water was filtered. To the filtrate,2% solution of gelatin containing 10% sodium chloride was added. Noted for the presence of milky white precipitate.

c) Lead acetate test: The extract dissolved in water was treated with 10% lead acetate solution. Noted for the presence of bulky white precipitate.

d) Decolorization test: The extract dissolved in water was treated with dilute potassium permanganate solution.Noted for the decolorization of potassium permanganate.

- **Chemical tests for Flavanones and Flavones:**[9]

a) Aqueous sodium hydroxide test: Aqueous sodium hydroxide solution was added to the few ml of the extract and the presence of yellow colouration of the solution was noted.

b) The filter paper was wetted with small quantity of alcoholic solution of the extract. That filter paper was exposed to ammonia vapors and noted the yellow color.

- **Chemical tests for Carbohydrate:**[9]

a) Molisch's Test: 2ml filtrate was treated withfew drops of Molisch's reagent, 2ml of concentrated sulphuric acid was added through the sides of the test tube without shaking. Observed for the presence of a violet ring at the junction of two solutions.

b) Fehling's Test:1ml filtrate treated with 1 ml each of Fehling's solutions A and B and boiled in a water bath for half an hour, then observed for the presence of red residue at the bottom of test tube.

c) Benedict's Test: The filtrate was treated with 2ml of Benedict's reagent. Then the mixture was heated in a boiling water bath for 2min and the presence of red precipitate was noted.

- **Chemical tests for Proteins and Amino acids:**[9,12]

a) Million's Test: 2ml extract was treated with a few drops of Million's reagent and observed for the presence of white precipitate, which on warming turned into a red-colored solution.

b) Biuret Test: 2ml extract was treated with 1 drop of 2% copper sulphate solution. To this 1ml of 95% ethanol was added followed by excess of potassium hydroxide solution and observed for the presence of violet colored solution.

c) Ninhydrin Test: The extract was treated with 2 drops of ninhydrin solution and heated on a water bath and then the presence of violet colour was noted.

- **Chemical test for Terpenoids:**[9]

Salkowski's Test: The extractwas dissolved in chloroform. An equal volume of concentrated sulphuric acid was added to it and noted for the appearance of red colour in the chloroform layer and greenish-yellow fluorescence in the acid layer.

- **Chemical tests for Sterol:**[9]

a) Liebermann – Burchard's test: Mixed residue with dry chloroform (1ml) and added acetic anhydride (2ml) and concentrated sulphuric acid. Observed red-green-blue-violet color change.

b) Salkowski's Test: The residue was dissolved in chloroform and equal volume of

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concentrated sulphuric acid was added to it and observed for the red colour in the lower layer.

- **Chemical tests for Saponins:**[9]

Foam or Froth Test: A small quantity of extract was diluted with 20 ml of distilled water in a graduated cylinder. The suspension was shaken for 15 minutes and waited to see if any froth was formed.

- **Chemical tests for Gums and Mucilage:**[9]

To 10 ml aqueous extract of the plant, 25 ml of absolute alcohol was added with constant stirring. Filtered and the precipitate formed was dried in air and examined for swelling properties.

3. PRE-FORMULATION STUDY:

3.1. Selection of excipients: Selected toothpaste excipients: calcium carbonate (abrasive), acacia (thickener), sodium lauryl sulphate (foaming), sodium benzoate (preservative), clove oil (flavoring) and glycerine (humectant)[17].

3.2. Drug-excipient compatibility study using FTIR spectroscopy: Drug and excipients were mixed in a 1:1 ratio, passed through a sieve 40, filled in vials, and subjected to stress conditions. API was kept under stress conditions. Samples were analyzed within two days, physical observations were done every week for up to a month, and an FTIR study was performed to assess compatibility[17].

4. PREPARATION OF TOOTHPASTE:

Dry gum method: All the solid components of the formulation are mixed in a dry mixer.The liquid components and water are gradually added to the dry mix.The mixing process is carried out till a smooth paste is formed.The remaining ingredients like the surfactants and the flavouring agents are added to the homogenous paste under vacuum[9].

INGREDIENTS	F1	F2	F3
Calcium carbonate	8.0g	8.5g	9.0g
Sodium lauryl sulphate	0.5g	1.0g	1.5g
Saccharine	0.2g	0.2g	0.2g
Clove oil	0.1ml	0.1ml	0.1ml
Acacia	2.0g	1.5g	1.0g
Glycerine	1ml	1ml	1ml
Sodium benzoate	0.1g	0.1g	0.1g
Distilled water	qs	qs	qs
Piper betle extract	1.0g	1.5g	2.0g
Averrhoa bilimbi extract	1.0g	1.5g	2.0g

Formulation of herbal toothpaste

5. EVALUATION OF HERBAL TOOTHPASTE:

5.1. Physical appearance: The prepared toothpaste formulations were inspected visually for their color, homogeneity, and consistency.

5.2. Physico-chemical evaluation:

1. Determination of pH: pH was determined using a digital pH meter that was calibrated to neutral pH by dipping the glass electrode in distilled water. The emulsion was prepared by dissolving or diluting one gram of paste in 100ml of distilled water and keeping it for 2 hours. The resulting solution or dispersion was used to take the pH reading 18.

2. Foamability: 2g of paste was taken in a 100 ml measuring cylinder containing 10 ml water. The initial volume was noted, the beaker was shaken 10 times and the final volume was noted[19].

3. Spreadability: Toothpaste spreadability can be measured using a Multimer apparatus. A generous amount of toothpaste is placed between two glass slides and a weight is placed on top for 5 minutes. Excess toothpaste is removed and the slides are pulled apart with a weight attached. The time it takes for the slides to separate over a 7.5cm distance is recorded. A shorter time indicates better spreadability.

$$S = \frac{M \times L}{T}$$

Where, S = Spreadability, M = Weight tied to the upper plate L = Length through which the gel spreads, and T = Time (in a sec) taken to separate the upper slide from the ground slide[20].

4. Extrudability: In this method, the paste was filled into an aluminum tube and sealed. The tube was weighed, placed between glass slides, and clamped. After applying weight and removing the cap, the extruded paste was weighed to calculate the percentage.[21].

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$$\text{Extrudability} = \frac{\text{Amount of paste extruded from the tube} \times 100}{\text{The total amount of paste filled in the tube}}$$

5. Anti-microbial test: In-vitro anti-bacterial study was performed by disc diffusion methodusing Muller Hinton Agar medium against pathogenic bacterial strains of Staphylococcus aureus (S. aureus, MTCC 3160) and E coli. S. aureus and E. coli initially tended to multiply in the Muller-Hinton agar plates. Then the formulated paste containing discs wasplaced over the bacterial plates and incubated at 37ºC for 24h, comparing ceftriaxone as the positive control. The diameter of the zone of inhibition (ZOI) was measured in mm[22].

6. Thin Layer Chromatography: The formulation was tested using TLC. Extracts were dotted onto the TLC plates, and various solvent systems were tested to separate bioactive components. Toluene: ethyl acetate: formic acid was used as the mobile phase. After pre-saturation for 30 min, elution was performed using the solvent systems[23].

III. RESULTS & DISCUSSIONS:

1.COLLECTION AND AUTHENTICATION OF SAMPLES:Averrhoa bilimbi and Piper betle leaves were collected from various Kasaragod locations, authenticated by Dr. Biju P, and cleaned, dried, and powdered.

2. PHARMACOGNOSTIC EVALUATION:

2.1. Evaluation of foreign matter:

Plant	**Weight of foreign matter(g)**	**% of foreign matter**
Piper betle	5.6	5.6
Averrhoa bilimbi	3.2	3.2

2.2. Determination of moisture content:

Drug	Weight of petridish + drug before drying(g)	Weight of petridish+ drug after drying(g)	Losson drying(g)	Average loss on drying(g)	Average % loss on drying (%w/w)
Piper betle	44.72 43.82 44.01	43.97 43.09 43.29	0.75 0.73 0.72	7.5 7.3 7.2	7.3
Averrhoa bilimbi	38.41 38.62 38.93	37.85 38.09 38.38	0.56 0.53 0.55	5.6 5.3 5.5	5.4

2.3. Total Ash Value:

Drug	Weight of empty petridish(g)	Weight of petridish+ drug (g)	Weight of petridish+ash (g)	Weight of total ash (g)	%yield (%w/w)	Average e % yield (%w/w)
Piper betle	44.72 43.82 44.01	46.72 45.95 46.00	44.77 43.87 44.06	0.05 0.05 0.05	2.5 2.5 2.5	2.5
Averrhoa bilimbi	38.41 38.62 38.93	40.42 40.61 40.93	38.61 38.82 39.13	0.2 0.2 0.2	10 10 10	10

2.4. Acid Insoluble Ash Value:

Drug	Weight of empty petridish(g)	Weight of petridish+acid insoluble ash(g)	Weight of acid insoluble ash(g)	Average weight of acid insoluble ash (g)	Average % yield (%w/w)
Piper betle	44.72 43.82 44.01	44.72 43.84 44.03	0.021 0.020 0.022	0.021	1.06
Averrhoa bilimbi	38.41 38.62 38.93	38.50 38.71 39.02	0.09 0.09 0.09	0.09	4.5

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2.5. Water Soluble Ash:

Drug	Weight of empty petridish(g)	Weight of petridish+water soluble ash(g)	Weight of watersoluble ash (g)	Average weight of water soluble ash (g)	Average % yield (%w/w)
Piper betle	44.72 43.85 44.00	44.76 43.89 44.04	0.015 0.015 0.015	0.015	1.75
Averrhoa bilimbi	38.41 38.62 38.93	38.59 38.81 39.12	0.19 0.19 0.19	0.019	0.50

2.6. Water Soluble Extractive Value:

Drug	Weight of empty petridish (g)	Weight of petridish+ extract (g)	Weight of extract (g)	Average weight (g)	% yield (%w/w)
Piper betle	43.2 42.3 43.2	43.39 42.48 43.40	0.195 0.185 0.205	0.195	3.9
Averrhoa bilimbi	43.2 42.3 43.2	43.69 42.80 43.68	0.49 0.50 0.48	0.49	9.8

2.7. Alcohol Soluble Extractive Value:

Drug	Weight of empty petridish (g)	Weight of petridish+ extract (g)	Weight of extract (g)	Average weight (g)	% yield (%w/w)
Piper betle	43.2 42.3 43.2	43.97 49.06 43.96	0.77 0.78 0.76	0.785	15.5
Averrhoa bilimbi	43.2 42.3 43.2	43.78 42.90 43.76	0.58 0.60 0.56	0.58	11.6

2.8. Phytochemical Screening:

CONSTITUENT	**PIPER BETLE**	**AVERRHOA BILIMBI**
Alkaloid	-	-
Glycosides	-	-
Phenols & Tannins	+	+
Flavones & Flavonoids	+	-
Carbohydrates	+	+
Proteins & amino acids	-	-
Terpenoids	+	+
Sterols	-	-
Saponins	-	+
Gums & Mucilage	-	-

2.9. Preparation of Extract:

Plant	**Color of extract**	**Consistency of extract**	**%yield (%w/w)**
Piper betle	Green	Semisolid	27
Averrhoa bilimbi	Green	Semisolid	29.4

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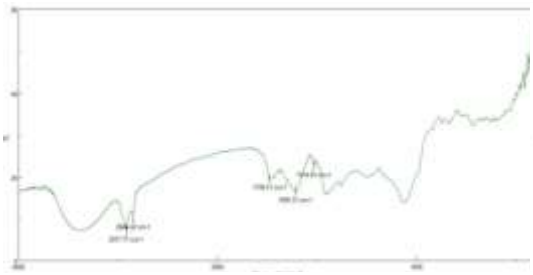
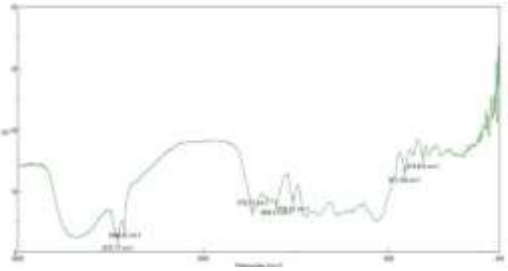
3. PRE-FORMULATION STUDY:

3.1. Drug-excipient compatibility study by FTIR:

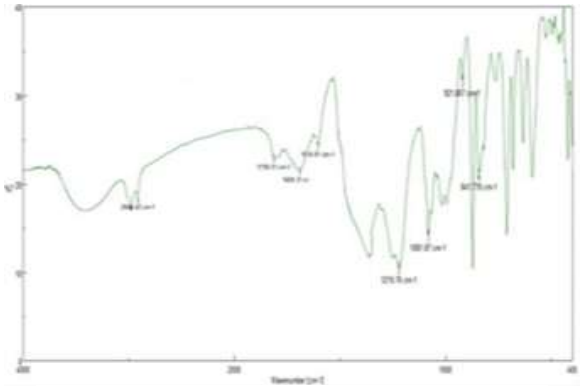
Functional group (Piper betle)	Frequency(cm^{-1}) (Piper betle)	Functional group (Averrhoa bilimbi)	Frequency(cm^{-1}) (Averrhoa bilimbi)	Functional group (Extracts +ingredients)	Frequency(cm^{-1}) (Extract+ ingredients)
O-H	3400	O-H	3400	0-H	3400
C-H	2900-2800	C-H	2900-2800	C-H	2900-2800
C=O	1800-1650	C=O	1800-1650	C=O	1800-1650
C=C	1600	C=C	1600	C=C	1600



IR Spectrum of Piper betle



IR Spectrum of Averrhoa bilimbi



IR Spectrum of extracts + ingredients

4. EVALUATION OF HERBAL TOOTHPASTE:

4.1. Organoleptic Evaluation:

Sl no	Formulation code	colour	odour	taste	texture
1	F_1	light green	characteristic	sweet	smooth
2	F_2	light green	characteristic	sweet	Smooth
3	F_3	light green	characteristic	sweet	smooth

4.2. pH, Foamability, Spreadability, Extrudability:

Formulation	pH	Foamability(ml)	Spreadability (g.cm/sec)	Extrudability (%)
F1	7.1	40	3.5	78
F2	7.2	50	4.1	80
F3	7.6	70	4.8	85

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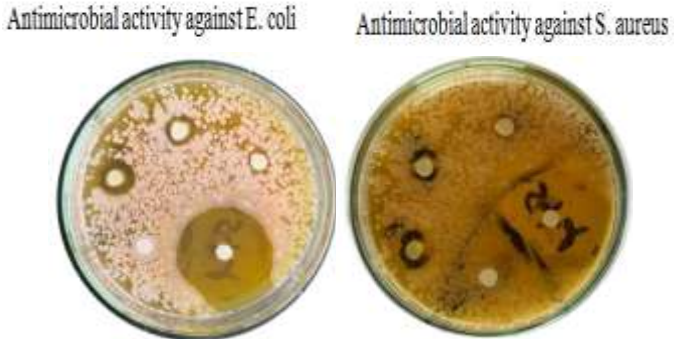
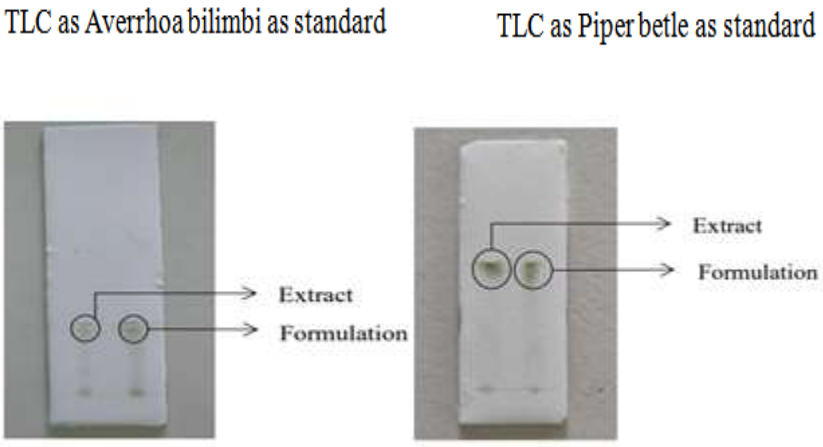
4.3. Antimicrobial Activity:

Test organism	Formulation	Zone of inhibition (mm)
E. coli	F1 F2 F3	8 14.5 23.5
S. aureus	F1 F2 F3	7 11 14



4.4. Thin Layer Chromatography:

STANDARD	FORMULATION	RF VALUE
Averrhoa bilimbi	F3 Extract	0.93 0.93
Piper betle	F3 Extract	0.90 0.90



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IV. SUMMARY & CONCLUSION:

Piper betle and Averrhoa bilimbi, two herbs from Kasaragod, have antimicrobial and digestive properties. After being dried and powdered, the leaves were evaluated for foreign matter content, ash value, moisture content, and extractive value. The extract was screened for phytochemicals and analyzed for drug-excipient compatibility using FTIR.Ethanolic extract of leaves of the plant Piper betle and Averrhoa bilimbi were formulated as herbal toothpaste by using suitable excipients.

Prepared formulations were evaluated for various physico-chemical parameters such as color, pH, spreadability, extrudability, and TLC.All the formulations have good organoleptic properties, while formulation 3 has better antimicrobial activity compared to formulation 1 and formulation 2.All the 3 formulations are in the pH range of 7.1-7.6, which is within the standard range for herbal toothpaste.F3 herbal toothpaste showed good spreadability and extrudability. It exhibited the best antimicrobial activity with 23.5mm and 14mm zone of inhibition for E. coli and S. aureus respectively. The formulation can be easily prepared using the dry gum method. It has great potential in natural remedies research and dental health.

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