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FORMULATION AND EVALUATION OF HERBAL TOOTHPASTE CONTAINING *EUPATORIUM TRIPLINERVIS* LEAF EXTRACT

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Abstract

Eupatorium triplinervis (Asteraceae),popularly known as Ayapana , is widely used in folk medicine, due to its antibacterial, antifungal, analgesic, antianorexic, antiparasitic, anthelmintic and sedative properties. The present study was focused on evaluating the anti-bacterial action of the formulated herbal toothpaste containing *Eupatorium triplinervis* leaf extract and determining the best formula for preparing the toothpaste. The extraction was conducted by the soxhlet apparatus using methanol as solvent and was subjected to preliminary phytochemical screening. The antimicrobial activity of the extracts was performed and the minimum inhibitory concentration (MIC) values of the extract were reported against three organisms- *Staphylococcus aureus* (gram positive), *E. coli* (gram negative) and oral flora by agar dilution assay. Also, the antibacterial activity by agar well diffusion method was determined for the prepared toothpaste against all the above three bacteria. The phytochemical screening shows the presence of coumarins in the methanolic extract which were responsible for the antibacterial activity of the extract. The formulations shows considerable zoneof inhibition when compared with a marketed toothpaste formulation (Dant kanti). Thus, this toothpaste preparation has antibacterial properties and so can be used for gingivitis and other bacterial disorders of the oral cavity.

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1. Introduction

Any form of a plant or plant product, including leaves, stems, flowers, roots, and seeds, is considered a herb. Herbal products might comprise a single herb or a blend of many plants that are thought to provide complementary benefits. *Eupatropium triplinervis is* an ornamental fragrant erect perennial tropical American shrub with attractive leaves. It is used as stomachic, for the treatment of ulcerative colitis, oral gingivitis and as antibacterial and antifungal agent. Oral gingivitis is a gum disease that causes irritation, redness and swelling of your gingiva. Toothpaste is a paste or toothpaste dentifrice that is used in conjunction with a toothbrush to clean and maintain the appearance and health of our teeth while also promoting oral hygiene. Gingivitis, and mostly all other oral cavity issues can be treated with this tooth paste.Aim is to formulate and evaluate the herbal toothpaste containing *Eupatorium triplinervis* leaf extract. Objective is the invitro antimicrobial study of herbal toothpaste preparation against gram positive, gram negative and oral flora and compare the antimicrobial activity of formulation with a standard drug available in the market.

2. Materials and Methods

2.1 Plant material

The plant, *Eupatorium triplinervis* was collected from Sreedhareeyam Farms and Foods Ventures Pvt Ltd, Kizhakombu, Koothattukulam.

2.2 Extraction of *Eupatorium triplinervis*

Methanolic extraction of *Eupatorium triplinervis* By Soxhlet apparatus

Fresh young leaves of *Ayapana tripilnervis*was gently washed with distilled water. Shade dried at room temperature for 1week and powdered using mechanical grinder. Briefly Soxhlet extraction was carried out .The extraction continued until the solvent in the upper extraction chamber became clear. Collected the extracts and filtered using Whatsmann filter paper. From the extracts, the solvent was removed by placing in water bath at 30 -50^0 C and the resulting semisolid mass was taken. The crude extracts were stored in glass container and kept in refrigerator until further analysis.

2.3 Phytochemical analysis

It includes Test for alkaloids, carbohydrates, flavinoids, coumarins, tannins and phenolic compounds

2.4 MIC Determination of plant extract

2.4.1 Preparation of nutrient broth (Muller Hinton Agar medium)

2.4.2 Preparation of Inoculums

Inoculums were prepared by comparing to 0.5 McFarland standards. Test suspension was prepared from fresh cultures of *S.aureus, E.coli* and oral flora and inoculated in saline buffer.

SI. NO	INGREDIENTS	QUANTITY USED FOR 30g							
		F1	F2	F3	F4	F5	F6	F7	F8
1	Leaf extract(g)	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
2	Bromelain(g)	2	2	2	2	2	2	2	2
3	Charcoal(g)	5	10	5	10	5	10	5	10
4	Sodium lauryl suphate (g)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
5	p-Hydroxy benzoic acid (g)	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
6	Glycerin (ml)	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
7	Clove oil (ml)	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
8	Acacia(g)	0.5	0.5	1	1	1.5	1.5	2	2
9	Menthol(ml)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
10	Sodium saccharine (ml)	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09
11	Water (ml)	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s

2.4.3 Agar dilution Method

Each extract was mixed in medium in the selected concentration by using agar dilution method.

2.5 Preparation of toothpaste

- **Procedure**

Dissolved sodium saccharine in small quantity of water. Required quantity of acacia was weighed and placed in a mortar. To this added glycerine and mixed well. Added the aqueous solution to the gum. Triturated rapidly until uniform suspension of gum obtained, kept aside for 20 minutes to swell. To this required quantity of charcoal was added and triturated well to obtain paste. Dissolved the sodium lauryl sulphate, p-hydroxy benzoic acid, bromelain in water, add clove oil. Added the required quantity of plant extract to the paste .Then added 2 drops of menthol and weighed about 30gm of paste and filled in the tube.

2.6 Evaluation of herbal toothpaste

2.6.1 Physical evaluation

Physical appearance of the prepared herbal toothpaste was evaluated by visual perception.

2.6.2 pHmeasurement

pH measurement of the toothpaste was carried out using a digital pH meter by dipping the glass electrode completely into the toothpaste system to cover the electrode. The measurement was carried out in triplicate and an average of the three readings was recorded.

2.6.3 Spreadability

To assess the spreadability, important factors to consider include hardness or firmness of the formulation, the rate and time of shear produced upon shearing, and the temperature of the target site. The rate of spreading also depends on the viscosity of the formulation, the rate of evaporation of the solvent, and the rate of increase in viscosity with concentration that results from evaporation. The parallel-plate method was the most widely used method for determining and quantifying the spreadability of semisolid preparations.

2.6.4 Foamability

Taking small amount of formulation with water in measuring cylinder initial volume was noted and then shaken for 10times. Final volume of foam was noted.

2.6.5 Extrudability

In this method, the formulated paste was filled in a standard capped collapsible aluminum tube and sealed by crimping to the end. Applied the pressure on tube by the help of finger. The amount of the extruded paste was collected and weighed. The percent of the extruded paste was calculated.

2.7 In-vitro antimicrobial study

2.7.1 Ingredients of nutrient agarmedia

2.7.2 Antibacterial Analysis

After preparing nutrient agar medium it was sterilized by autoclaving at 121^0C for 15minutes at 15lbs pressure. After sterilization media was cooled to room temperature, then required quantity added in the 4 petri plates and allowed to solidify. After that, bacterial culture of *Staphylococcus aureus, Escherichia coli* and Oral flora was incorporated into the medium. Then, a hole with a diameter of 6 to 8 mm was punched aseptically with a sterile cork borer and a volume of the antimicrobial agent or extract solution at desired concentration was introduced into the well. Then the plates were kept for incubation.

3. Result and Discussion

3.1 Phytochemical screening of extract of *Eupatorium triplinervis*

Phytochemicals	Tests	Observation	Inference
Alkaloids	Mayer's Test	No blue colour	-
	Wagner's Test	No reddish brown precipitate	-
	Dragendorff's Test	No orange brown precipitate	-
Coumarins	Coumain Test	At the end yellow colour	+
Flavinoids	Shinoda's Test	No red colour	-
	Lead acetate Test	Yellow colour precipitate	+
Tannins	Gelatin Test	No white precipitate	-
Phenols	Ferric chloride Test	No bluish black colour	-
Carbohydrates	Molisch's Test	No violet coloured ring	-

- The presence of coumarins and flavonoids in the extract was revealed by the screening.
- The presence of coumarin in methanolic extract was responsible for the antibacterial property.
- **3.2 MIC Determination of Plant Extract**

Organism	Concentration (ug/ml)	Response
Staphyloccous aureus	Control	++
	600	+
	640	-
	680	-
	750	-
	800	-
Escherichia coli	Control	++
	1200	+
	1250	-
	1300	-
Live organism	Control	++
	3333.3	+
	5833.3	-
	6666.6	-

(+ - Presence of growth, **--** Absence of growth)

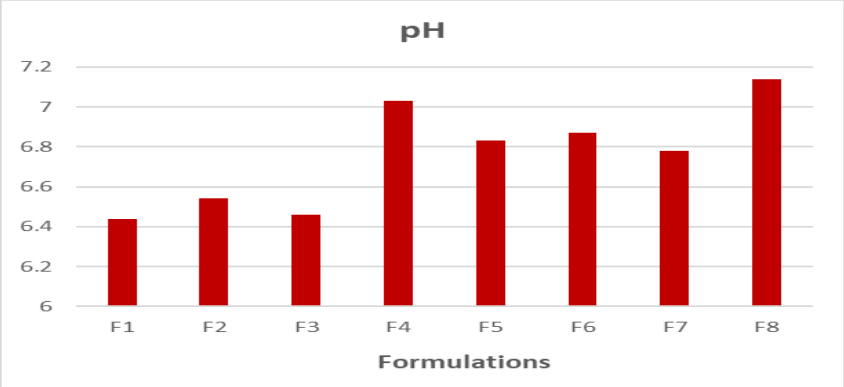
- *Eupatorium triplinervis* leaf extract was tested for antimicrobial activity against *Staphylococcus aureus, Escherchia coli,* and oral flora.

- The methanolic extract demonstrated effective activity against *Staphylococcus aureus* at 0.64mg/ml, *E.coli* and oral flora at 1.25mg/ml and 10mg/ml respectively, during MIC determination.
- The negative control, DMSO, has just a minor inhibitory effect.

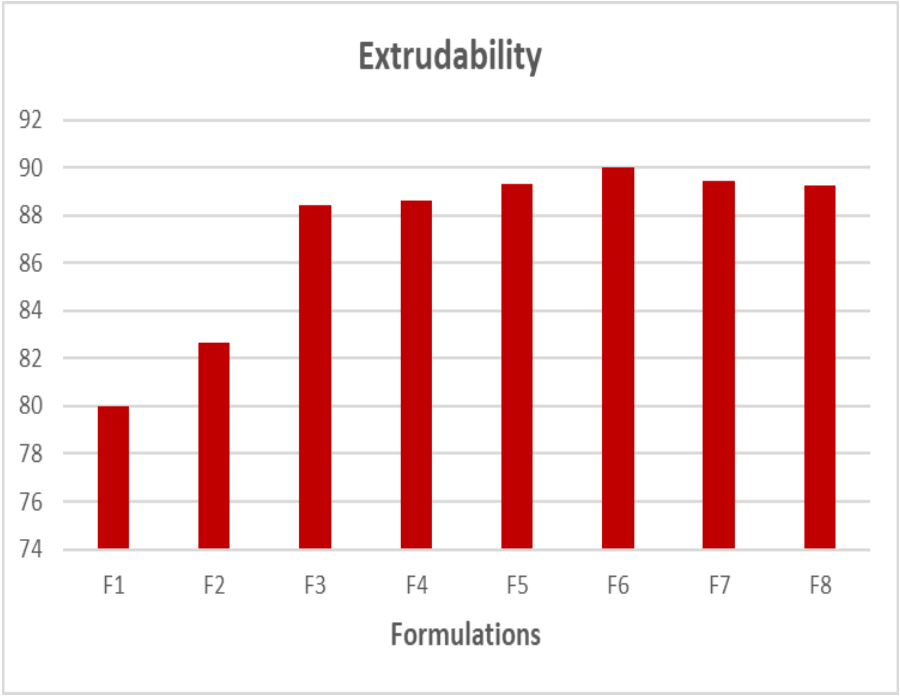
3.3 Evaluation of toothpaste

Parameters	F1	F2	F3	F4	F5	F6	F7	F8
Colour	Black	Black	Black	Black	Black	Black	Black	Black
Odour	Characteristic	Characteristic	Characteristic	Characteristic	Characteristic	Characteristic	Characteristic	Characteristic
Taste	Pleasant	Pleasant	Pleasant	Pleasant	Pleasant	Pleasant	Pleasant	Pleasant
Spreadability	5.1cm /sec	5cm/sec	4.5cm/sec	4.6cm/sec	4.2 cm/ sec	4 cm/sec	3.9cm/sec	3.8cm/sec
Foamability	Good	Good	Good	Good	Good	Good	Good	Good

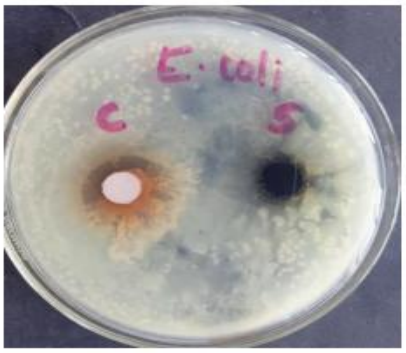
- The herbal toothpaste was black in colour and had a distinct odour as well as a pleasing flavour.
- The amount of acacia in a toothpaste formulation can impact its spreadability. The optimum formulation has good spreadability extending 4cm.
- The foaming ability of formulations was found be good, the presence of detergent (SLS) may affect the foaming power of toothpaste.

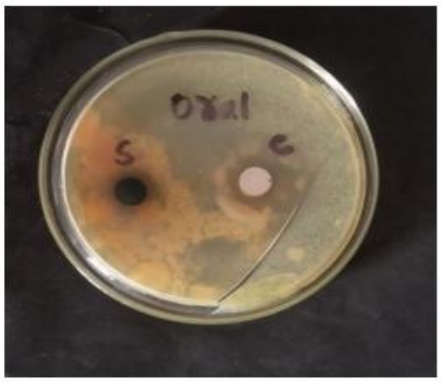


- An alkaline pH causes fewer side effects on the dental surface. In this study, the pH value ranged from 6.43 to 7.03 in the formulations.



- The extrudability was approximately 90% in the F6 formulation. It was the ability of toothpaste to extrude 90% of the content of a totally filled tube.
- It was evident that formulation F6 has comparatively good foamability, spreadability, and applicable pH range.So the F6 formulation was selected as the best antimicrobial agent against different microorganisms.





- The formulation exhibited a zone of inhibition of 20 mm against *S.aureus* and also 15 mm and 5 mm against *E. coli* and oral flora, respectively, which was slightly less than Dant Kanti. As in the results, it was shown that the prepared toothpaste has antibacterial activity, but it was slightly less than that of the marketed formulation.
- Methanol was primarily injurious to people if it was present in the formulation.
- Determination of methanol was carried out by gas chromatography coupled with mass spectrometry (GC-MS), analysing the formulation sample.
- The estimated methanol content in the formulation was 119.77 ppm which within the acceptable standard limit.

4. Conclusion

Herbal medicine is still the mainstay of more than 80% of the whole population.*Eupatorium triplinervis* leaf extract was prepared and has good antibacterial activity against *Staphylococcus aureus,Escherchia coli,* and oral flora. Also, this toothpaste can be used for gingivitis andother oral bacterial disorders. In conclusion, the use of *Ayapana triplinervis* formulation of toothpaste should be of interest in oral care products due to the presence of bioactive compounds such as coumarins and flavonoids that are capable of inhibiting the growth of microorganismscausing oral infection. The adoption of herbal toothpaste by consumers and dentists will safeguardthe side effect of oral care products containing synthetic compounds and reduce the cost oftreatment.

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