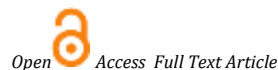


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Research Article

Formulation, Evaluation and Antibacterial Effect of Herbal Toothpaste using leaves and flowers *Acmella ciliata*

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Oral hygiene, an integral part of the body's general well-being, it is the practice of keeping the mouth clean and disease free by using tooth paste, mouth wash etc. Plants have been a great source of medical agents for many years and even be considered the origin of modern medicine. Phytochemicals the secondary metabolites of the plants are active ingredients that possess therapeutic properties that are considered as a medicine or drug for therapeutic purposes for the curing of diseases and improving human health. The main objective of this study is to formulate and develop an herbal toothpaste from herbal source and to evaluate its antibacterial activity. The herbal toothpaste containing leaves and flowers of *Acmella ciliata*, clove, honey and leaves of guava as the herbal ingredients. Different types of formulations (F1-F3) were formulated using Calcium carbonate as cleaning and polishing agent, Glycerine as humectant, Sodium lauryl sulphate, Sodium chloride as a stain remover from the teeth, Honey. The three formulations were evaluated by various parameters like colour, taste, fragrance, consistency, pH, shape maintenance, threading property, moisture content and foaming character. The herbal toothpaste having aqueous extract of herbal source are tested for antibacterial activity against *K. pneumoniae*, *S. aureus*, *P. aeruginosa* and *S. pyogenes*. The result shows that the bacteria were found to be more sensitive to the formulated toothpaste as seen by zone of inhibition. Further studies would benefit from a uniform method of assessment for clinical effectiveness of plaque and gingivitis and safety of the formulated herbal toothpaste.

Keywords: Phytochemicals, *Acmella ciliata*, herbal toothpaste, antibacterial activity

INTRODUCTION

Plants are the medical agents; its use has been done since ancient times and may even be considered the origin of modern medicine. The secondary metabolites which are present in the plant is called phytochemicals, which are active ingredients that possess therapeutic properties that are considered as a medicine or drug¹. Oral hygiene is an integral part of the body's general well-being, which begins with clean teeth which is performed as a daily morning routine. The cleaning of one's teeth is a cultural habit that is followed from generation to generation and is usually performed and it is considered as an indispensable component of oral².

Chewing sticks are the traditional method of cleaning the teeth to maintain oral hygiene³. Toothpaste, toothbrush mouthwash etc. are commonly used as products which improve oral hygiene⁴. Toothpaste is a gel or paste dentifrice used with a toothbrush as an accessory to clean and maintain the health of teeth in order to enhance oral hygiene as an irreplaceable agent in effective home care system⁵. One of the most common infectious diseases encountered by many individuals are Dental caries and Periodontal diseases at different stages of their life time due to poor oral hygiene. Dental caries, commonly known as tooth decay includes the cavity formation, eruption of enamel, swollen gums, bleeding gums, formation of hollow black eruption on the surface of the teeth. Periodontal diseases can lead to destruction of ligament,

cementum, gingiva and alveolar bone. Plaque is the main etiological causes for the gingival inflammation⁶.

Acmella ciliata is a genus of family: ASTERACEAE is a medicinal cum ornamental plant widely distributed to tropical and subtropical regions of the world⁷. *Acmella ciliata* a spice which have natives as a folk medicine to treat anemia, scurvy, a remedy for toothache, cough, throat and gum infection and as an anesthetic, analgesic, antiseptic and antimicrobial agent^{8,9,10}. It has various pharmacological activities like antioxidant, anti-inflammatory, anti-helminthic, antifungal, antimalarial, insecticidal, hepatoprotective, antimutagenic property, immunomodulatory activity and as an analgesics all of which are attributed to the presence of an important bioactive compound named as 'spilanthol', an alkylamide^{11,12}. *Psidium guajava* L. commonly known as guava has been proven to be successful in treating conditions such as diarrhoea, dysentery, gastroenteritis, hypertension, diabetes, caries, pain alleviation, cough, mouth ulcers, and liver damage inflammation. Guava have antibacterial, antidiabetic, antiviral, antioxidant and anti-inflammatory activities. Its leaves contain many compounds which act as fungistatic and bacteriostatic agents and also high content of important antioxidants and have a radio-protective ability¹³. One of the most valuable spices *Syzygium aromaticum* also known as Clove is the aromatic dried flower buds of a perennial tree belonging to Myrtaceae family. It has antimutagenic, antioxidant, antiulcerogenic, antithrombotic, antiparasitic, antibacterial

and anti-inflammatory properties¹⁴. Several studies shows the antifungal, antiviral and antibacterial effects of clove¹⁵. Honey is a natural product with a complex chemical composition. In addition to other phytochemicals compounds it contains several constituents of small amounts, such as minerals, free amino acids, proteins, vitamins, enzymes, organic acids, flavonoids, phenolic acids and other organic acids^{16,17}. Till now honey is not only considered as food or a sweetener, but it is also used as a medicine for stimulating healing of wound, regeneration of tissue, and alleviating gastrointestinal disorders, gingivitis, therapy for burns, asthma, infected wounds, and skin ulcers and various other pathologies in human and animal^{18,19,20}. The antimicrobial, antiviral, antifungal, anticancer, and antidiabetic activity of honey are shown in several studies^{21,22}.

MATERIALS AND METHODS

Collection of plant materials

Leaves and flowers of toothache plant - toothache plant s identified by Botanical Survey of India as *Acmella ciliata* (kunth) Cass. (Syn.: *Spilanthes ciliata* Kunth) - belongs to the family ASTERACEAE reference No.: BSI/SRC/5/23/2022/Tech/626. Plant is collected from Palakkad. Clove, honey and leaves of guava were also collected from Palakkad.

Preparation of sample/herbal extract

Herbal sources are washed, dried and powdered with the help of blender. 20gm of each powdered sample is mixed with 500ml of distilled water and boiled individually. The extracts are filtered separately and are dried to form crude powder. The extract was stored in Eppendorf tubes in the refrigerator at 4°C until further use.

Chemical reagents

All the chemicals used in this study are Calcium carbonate, Glycerine, Sodium lauryl sulphate, Sodium chloride.

Preliminary phytochemical analysis of plant extract

The extracts were subjected to the preliminary phytochemical analysis by following the methods of Trease and Evans (1996).

Test for Proteins

Biuret test: 0.5ml of plant extract was taken in a test tube followed by 40% NaOH and 10% CuSO₄. Violet pink colour development indicated the presence of proteins.

Test for Carbohydrates

Benedict's test: 0.5ml of plant extract and 0.5ml of Benedict's reagent were heated for 2 minutes in water bath. The presence of carbohydrates (disaccharides) was shown by the formation of a red precipitate.

Test for Phenols

Ferric chloride test: 0.5ml of plant extract was taken in a test tube added ferric chloride solution drop by drop. Blue green (dirty green) colour development indicated the presence of phenols.

Test for Tannins

Ferric chloride test: 0.5ml of plant extract was taken in a test tube added ferric chloride solution drop by drop. Blue black colour development indicated the presence of tannins.

Test for Saponin: 0.5ml of plant extract was taken in a test tube followed by 0.5ml 10% led acetate. The presence of saponin was shown by the formation of a bulky white precipitate.

Test for Glycosides: To 0.5 ml of plant extract add 2 ml of glacial acetic acid and 1 ml of 5% ferric chloride solution. This was treated with 1 ml of concentrated H₂SO₄. A brown ring at the interface indicates the presence of glycosides. The presence of glycosides was shown by the formation of a reddish-brown colour.

Test for Starch

Iodine Test: 0.5ml of plant extract was taken in a test tube followed by 0.5 ml of iodine solution, 0.5ml of potassium iodide and 3.5ml of distilled water. The presence of starch was shown by the formation of a blue colour.

Test for Terpenoids: To 0.5 ml of plant extract add 1 ml of chloroform and 0.5ml of Conc.H₂SO₄. A brown ring at the interface indicates the presence of terpenoids.

Test for Alkaloids

Mayer Test: 0.5 ml of leaf extract, 2 drops of chloroform, and 1 drops of Meyer reagent were added. A positive alkaloid reaction resulted in the production of white deposits.

Test for Flavonoids: 0.5ml of plant extract was taken in a test tube followed by 0.5ml of 2% ammonia. The presence of flavonoids was shown by the formation of a yellow colour.

Method of preparation of different formulations of herbal toothpaste

The required quantity of ingredients was weighed and taken in a beaker. Calcium carbonate, glycerine, sodium lauryl sulphate, sodium chloride and honey were mixed in water. This solution was added into a beaker containing herbal ingredients and triturated well until a paste consistency is formed. Table 1 shows plant extracts and composition of chemicals in different formulations.

Table 1: Chemical Composition of Formulation

Sl. No.	Concentration	Quantity in (gm)		
		F1	F2	F3
1.	Clove	2	2	2
2.	Guava	2	2	2
3.	<i>Acmella ciliata</i> - leaves	2	1.5	1
4.	<i>Acmella ciliata</i> -flowers	1	0.5	-
5.	Calcium carbonate	20	20	20
6.	Glycerine	5ml	5ml	5ml
7.	Sodium lauryl sulphate	1	1	1
8.	Sodium chloride	0.1	0.1	0.1
9.	Honey	0.5ml	0.5ml	0.5ml
10.	Distilled water	25ml	25ml	25ml

Evaluation of herbal toothpaste

Physical Examination

- **Colour-** The visual colour was checked for the formulated toothpaste for evaluation of its colour.
- **Odour-** Odour was found by smelling the formulated herbal toothpaste.
- **Taste-** Taste was checked manually by tasting all the three formulations.

pH determination

pH of formulated herbal toothpaste was determined by using pH paper. 1g of toothpaste was dissolved into 9 ml of water and shaken vigorously then pH of aqueous solution was observed.

Shape retention

After packing the herbal toothpaste in a squeezable bottle shape retention was tested by squeezing the toothpaste out from the tube and put entirely of a tooth brush and allowed to stand for 10 seconds, the state of the toothpaste was evaluated based on the below-described criteria;

- A. Shape of the toothpaste is maintained.
- B. Shape of the toothpaste is almost maintained.
- C. The toothpaste cannot maintain its shape on the toothbrush.

Threading property

The threading property of formulated herbal toothpaste when it was squeezed out on the entirety of a toothbrush and slowly pulled up was evaluated based on the below-described criteria.

- A. The formulated toothpaste can be put on a toothbrush smoothly without threading.
- B. The formulated toothpaste can be put on a toothbrush smoothly, though it causes slight threading.
- C. The formulated toothpaste cannot be put on toothbrush smoothly because of severe threading.

Foaming character

1 gm of formulated herbal toothpaste was poured into the test tube (height 15 cm. diameter 2 cm) and water is used to adjusted the volume of the liquid up to 10 ml. Tube was stopped and shaken twice in a second length wise, motion for 16 second. Allowed it to stand for 15 minutes and height of the foam produced by the three formulation was measured. The experiment was repeated for three times and the average was taken.

Moisture content

Toothpaste (approximately 2.7gm) weighed in a Porcelain dish and dried it in the oven. Weight was taken for each 10 minutes (observed for 1 hour). The loss of weight is recorded as percentage of moisture and is calculated by the formula:

% Moisture = [(Original sample weight – dry sample weight) / Original sample weight]* 100

Antibacterial activity of formulated herbal toothpaste

The well diffusion method was used to evaluate the antibacterial activity of the formulated toothpaste. The formulated toothpaste was tested for antimicrobial activity against *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Streptococcus pyogenes*^{23,24}. 15 ml of Muller Hinton Agar were poured onto the surface of the sterile petri plate as a basal layer. After solidification, 70 µl of the indicator bacterial strains (10⁸ cfu) were swabbed (*K.pneumoniae*, *S.aureus*, *Paeruginosa* and *S.pyogenes*) uniformly on the surface of MHA plate and allowed to dry for 5 minutes. Four wells of 7 mm were bored in the inoculated media with the help of sterile cork-borers and 10mg of each formulation was placed in the wells. Standard reference antibiotic (Gatifloxacin-GAT5mcg) was used as the positive controls and Dimethyl Sulfoxide (DMSO) serves as the negative control. It was then incubated at 37°C for 18-24 hours. After incubation, plates were observed for the formation of a clear zone around the well which corresponds to the antibacterial activity of tested compounds. The zone of inhibition (ZOI) was observed and measured in mm.

RESULTS AND DISCUSSION

Plants have secondary metabolites called phytochemicals. The results of qualitative phytochemical analysis of the plant extract were shown in Table 2. Based on the phytochemical screening of the *Acmella ciliata* - leaves extract shows the presences of Proteins, Tannins, Saponins, Terpenoids and Flavonoids. Instead of Tannins Phenols is present in the *Acmella ciliata* -flowers extract. Proteins, Carbohydrates, Tannins, Saponins, Glycosides and Terpenoids are present in the Guava leaves and Clove extract. The formulated herbal toothpaste was evaluated for physicochemical parameters such as consistency, colour, odour, taste and pH and the results were shown in Table 3. The formulated toothpaste is Semi-solid in consistency, brown in colour and better in taste. Compared to F2 and F3 F1 is slightly strong, F2 is moderate and F3 is mild. Formulation 1 and 2 is slightly acidic in nature and F3 is neutral in pH. As shown in table 4, the herbal toothpaste has features as it exhibits good shape retention and threading property effect. Height of the foam was produced by different formulation was more than 3cm every time, this indicates stable foam. The average height of the foam is given in the table 5. Moisture content estimation of formulated toothpaste of all formulations was shown in the Fig 1. After an hour approximately 0.5mg of weight was reduced. The percentage of moisture content was shown in table 6. The well diffusion method was used to evaluate the antibacterial activity of the formulated toothpaste. The formulated toothpaste was tested for antibacterial activity against *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Streptococcus pyogenes*. Fig 2. In MHA plate 70 µl of each indicator bacterial strains were treated against 10mg of different formulations of herbal toothpaste. The zone of inhibition (ZOI) was observed and measured in mm after the incubation at 37°C for 18-24 hours. The formulated toothpaste F1 exhibited highly significant effect towards *Staphylococcus aureus* and *Pseudomonas aeruginosa* as seen by the zone of inhibition (5mm) as like the standard reference antibiotic (Gatifloxacin-GAT5mcg), results are given in the table 7.

Table 2: Phytochemical screening of extracts (+ Presence - Absence)

Sl. No.	Phytochemicals	<i>Acmella ciliata</i> - leaves	<i>Acmella ciliata</i> -flowers	Guava leaves	Clove
1.	Proteins	+	+	+	+
2.	Carbohydrates	-	-	+	+
3.	Phenols	-	+	-	-
4.	Tannins	+	-	+	+
5.	Saponins	+	+	+	+
6.	Glycosides	-	-	+	+
7.	Starch	-	-	-	-
8.	Terpenoids	+	+	+	+
9.	Alkaloids	-	-	-	-
10.	Flavonoids	+	+	-	-

Table 3: Evaluation of physical characteristics of herbal toothpaste

Toothpaste Formulation	Consistency	Colour	Odour	Taste	pH
F1	Semi-solid	Brown	Slightly strong	Better	Slightly acidic
F2	Semi-solid	Brown	Moderate	Better	Slightly acidic
F3	Semi-solid	Brown	Mild	Better	Neutral

Table 4: Evaluation of Toothpaste on Different Parameter

Sl. No.	Evaluation parameter	Grade based on the evaluation criteria			Reference grade
		F1	F2	F3	
1.	Shape retention	A	A	A	A
2.	Threading property	A	A	A	A

Table 5: Foaming Character

Sl. No.	Toothpaste Formulation	Height of Foam (cm)
1.	F1	4.5
2.	F2	5.7
3.	F3	6.0

Table 6: Percentage of Moisture Content

Sl. No	Formulation	Percentage of Moisture Content
1.	F1	22.91
2.	F2	28.07
3.	F3	28.00

Table 7: Antibacterial activity of formulated toothpaste against *K.pneumoniae*, *S.aureus*, *Paeruginosa* and *S.pyogenes*

Sl. No.	Organism	Zone of inhibition in mm				
		F1	F2	F3	Disc (GAT-5mcg)	DMSO
1.	<i>Klebsiella pneumoniae</i>	5	4	5	7	Nil
2.	<i>Staphylococcus aureus</i>	5	3	4	5	Nil
3.	<i>Pseudomonas aeruginosa</i>	5	4	4	5	Nil
4.	<i>Streptococcus pyogenes</i>	4	4	4	9	Nil

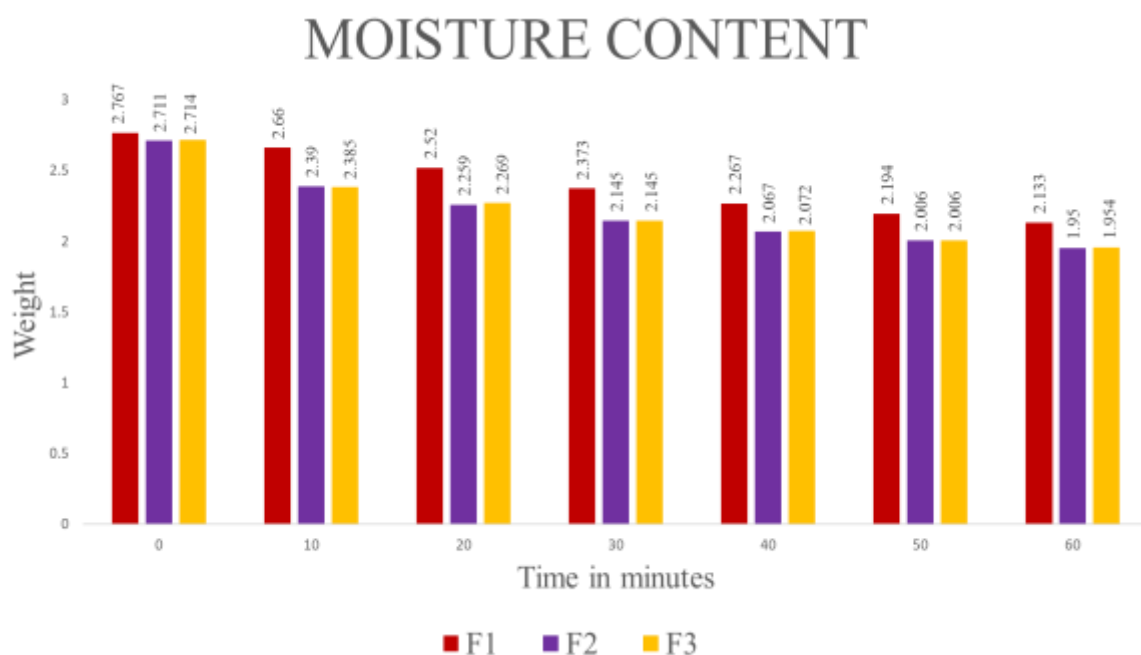


Figure 1: Moisture Content



Figure 2: Antibacterial activity

CONCLUSION

Herbal toothpastes have a main role in maintaining oral hygiene and also preventing dental caries. The formulated herbal toothpaste was successfully evaluated using different standard parameters including antibacterial properties. The formulation showed antibacterial effects against *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Streptococcus pyogenes*. The formulated toothpaste can improve the maintenance of oral hygiene to prevent dental caries and periodontal disease. Future research would benefit

from a uniform method of assessment for clinical effectiveness of plaque and gingivitis using these products. The oral mouth wash can also be developed.

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