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Development and Evaluation of Antibacterial Herbal Toothpaste containing *Eugenia caryophullus, Acacia nilotica and Mimusops elengi*

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Abstract

The present work dealed with development and evaluation of herbal anti-bacterial toothpaste containing clove, babul and borsali as herbal ingredients. Different types of formulations (F1 - F9) were formulated using calcium carbonate as abrasive and sorbitol solution (70 %) as humectant in varied concentrations based on factorial design. All the formulations were evaluated for various parameters like dryness, color, appearance, consistency, wash ability, pH, spread ability and foaming power. In optimized Formulation (F7), the aqueous extracts of clove (powder of clove buds of Eugenia caryophullus), babul (powder of babul bark of Acacia nilotica) and borsali (powder of borsali bark of Mimusops elengi) were loaded and herbal toothpaste was prepared. This Formulation showed good color, appearance, consistency, wash ability, pH, spreadability and foaming capacity. The formulation showed very good anti-microbial profile during microbial assay. This optimized formulation was safe to use for dental care.

Keywords: Clove budpowder, Borsali bark powder, Babul bark powder, Microbial assay

Contents

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1. Introduction

The anatomic part of tooth consists of crown, root, enamel, dentine and pulp [1]. Many problems associated with the dental care are bad breath, tooth decay, gum (periodontal) disease, tooth sensitivity, calculus, dental caries and dental plaque [2]. Plenty of herbal as well as synthetic ingredients are reported to have remarkable beneficial effects on various dental problems mentioned above. They are Clove, neem, sunthi, mentha, tomar, pippali, aloe vera, kapoor, babul, borsali. According to the constituents they play different role through different mechanisms like providing freshness, Antibacterial which kill or retard the growth of *Streptococcus mutans*&*Lactobacillus acidophilus*,the main causative organism of dental cavity, by providing protective layer over teeth and/or local anaesthetic to reduce dental pain.Toothpaste is a paste or gel dentifrice used with a toothbrush as an accessory to clean and maintain the aesthetics and health of teeth [3]. Main objectives of the toothpaste are delivering a range of preventive and therapeutic agents such as fluoride, metal salts and pyrophosphate for calculus inhibition, to reduce

plaque growth, to treat dentine hypersensitivity and providing oral hygiene. Cleaning of the surface of the teeth is the primary function of a dentifrice when used with a toothbrush. A dentifrice helps in the removal of food particles, reduction of superficial plaque or stain, polishing of tooth surface and refreshing mouth breath. Therapeutics & cosmetic functions may be desired, such as whitening, bleaching, desensitizing, inhibition of plaque formation and protection against periodontal problems. Dentifrices are disperse systems. They consist of water and water soluble liquids, oils and both soluble and insoluble solids. As such they are dispersions of solids in a liquid vehicle. Important characteristic of toothpaste are consistency, abrasiveness, appearance, foaming, taste, stability and safety. Most commonly ingredients used in toothpaste formulation are Active pharmaceutical ingredients, abrasives, humectants, detergents, binders, sweeteners, preservatives and antioxidants. List of the ingredients used in toothpaste are shown in Table 1 [3]. Historically the need and desirability of cleaning the teeth paralleled the recognition of the necessity to maintain bodily cleanliness. Many of the materials used and recipes suggested, however, contained materials capable of damaging the teeth and gums. The modern world therefore had a real social, medical and aesthetic need for well formulated, safe and effective dentifrices. Toothpaste is the daily used material for cleansing the teeth and to provide freshness to mouth. The rationale behind making herbal toothpaste is to fight against the bacteria that cause problems regarding to teeth like gum, dental cavity and gingivitis.

The present work deals with development and evaluation of herbal toothpaste. The Plant materials used for the formulations were aqueous extract of flower bud of clove (*Eugenia caryophyllata*), powder of bark of babul (*Acacia nilotica*) and hydro alcoholic extract of powder of bark of Borsali (*Mimusops elengi)*. The plants have been reported as good anti-microbial agents in the studies. The present work also dealed with microbial study of these herbal actives on Gram positive and Gram negative bacteria. Clove has good analgesic, antibacterial and local anaesthetic activity. It is used in dental pain and teeth hypersensitivity. Babul and Borsali have good antibacterial effect on both, gram positive and gram negative, spectrum. The plants have been reported as good anti-microbial, local anaesthetic and anti-bleeding agents in the studies. Natural remedies are more acceptable in the belief that they are safer with fewer side effects than the synthetic ones.

2. Materials and Methods

Materials

Clove bud powder, babul bark powder and borsali bark powder were purchased from the ayurvedic shop of Ahmedabad. Calcium carbonate, glycerine, sorbitol solution (70%), carboxy methyl cellulose, sodium lauryl sulphate, methyl paraben and sodium saccharine were issued from the laboratory of pharmaceutical technology, KBIPER, Gandhinagar. During the microbial assay autoclave (electroquip, Ahmedabad) & hot air oven (Nova instrumentation pvt ltd, Ahmedabad) were used for maintaining sterile condition and culture of nutrient agar bored with bacteria were incubated using incubator(ambassador). Distilled water was used throughout the study.

Methods

Preparation of Herbal Extract

The net quantity of 5 g,7.5 g and 1 g of clove bud powder, babul bark powder and borsali bark powder were transferred in a 250 ml conical flask respectively. To that distilled water was added to make volume 100 ml and allowed to completely moisten. Refluxed on the water bath at temperature not exceed 60^0C for at least 1 hr. Allowed to cool at room temperature. Filtered and evaporated to dryness on waterbath.Then dry extract was stored in air tight zip-bag for further use. 1%, 7.5%, 1% aqueous extract of dried clove, babul and borsali were selected as antibacterial, as it is reported to have antibacterial and analgesic activity at this concentration [15-17].

General procedure for preparation of Toothpaste Base

For designing of the experimental work, 3^2 factorial designs were used. Factors selected were conc. of Abrasive and concentration of Humectant and levels chosen were low (-1), medium (0) and high (+1) and were shown in Table 2. Different formulations of the toothpaste base were developed according to the Table 3. All the powder materials were passed through 60# sieve. Accurate quantity of Carboxy methyl cellulose was weighed and transferred into clean Mortar Pestle. $1/4^{th}$ quantity of distilled water was added with continuous trituration till gel was formed. Glycerin and preservatives were added to the Gel. Sorbitol, Sodium Saccharine and remaining quantity of water were taken in a separate beaker. Above solution was added to the Gel. Mixed well. Calcium carbonate was added slowly to above mixture. Mixed well. Dried extract of actives prepared previously was incorporated after proper hydration in the boiling water and mixed well with the base with continuous trituration(for the evaluation of the base, the actives were replaced with same quantity of water). SLS was dissolved in minimum quantity of water and added to above mixture. The product was weighed and packed in air-tight container.

Evaluation of Formulations

Preliminary Test

Drying tendency

General Evaluation of tooth paste was done on the basis of drying tendency of the prepared bases (F1 – F9) at room temperature for a week and selected batches were evaluated for further evaluation parameters.

Then following tests were performed for the selected batches of toothpaste base.

Organoleptic Characters

Selected batches were characterised on the basis of organoleptic characters like appearance, colour, texture, after taste and extrudability.

Physicochemical Parameters

Determination of grittiness

The paste was extruded about 15 to 20 mm length from collapsible tube of each sample on a butter paper. Then all the samples were tested by pressing it along its entire length by a finger for the presence of hard and sharp edged abrasive particles.

Determination of pH

The net quantity of 5 gm of sample was accurately weighed and placed in a 150 ml beaker. To this 45 ml of freshly boiled and cooled water was added at 27°C. It was stirred well to make a thorough suspension. The pH was determined within 5 minutes by using pH meter (Sartorius CP124S, Swisser instrument, India).

Determination of foaming power

About 5gm of sample was accurately weighed and placed in a 100ml glass beaker. To this10ml of water was added and the beaker was covered with a watch glass and allowed to stand for 30 minutes. This operation was carried out to disperse the toothpaste in water. The contents of the beaker were stirred with a glass rod and the slurry was transferred to a 250ml graduated measuring cylinder, during this transfer ensured that no foam was produced and no lump paste went into the measuring cylinder. The residue left in the beaker was transferred with further portion of 5-6 ml of water to the cylinder. The content of cylinder was adjusted to 50ml by adding sufficient water and the content has to be maintained at 30°C. Stirred the contents of the cylinder with a glass rod to ensure a uniform suspension. As soon as the temperature of the content reached 30°C, the cylinder was stoppered and 12 complete shakes were given to it. The cylinder was allowed to stand for 5 minutes and the volume of foam with water (V_1) and water only (V_2) was noted for all samples.

Determination of foaming power:

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

V_1 - Volume in ml of foam with water.

V_2 - Volume in ml of water only.

Performance Evaluation

Organoleptic characters

After incorporation of herbal actives final batch wascharacterised on the basis of organoleptic characters like appearance, colour, texture, after taste and extrudability [18].

Microbial study

The antibacterial activities of different formulations were determined by modified agar well diffusion method. In this method, nutrient agar plates were seeded with 0.2 ml of 24 h broth culture of *S.aureus*. The agar plates were allowed to solidify. A sterile 8 mm borer was used to cut wells of equidistance in each of plates. 0.5 ml of formulations or herbal extract was introduced into the well. The plates were incubated at 37^0C for 24 hours. The antibacterial activity was evaluated by measuring the zones of inhibition (in mm) [19].

3. Results and Discussion

The optimization of the toothpaste bases was done on the basis of drying tendency. Table No.5 showed drying tendency of batches F1 - F9. Among these batches F1, F4, F7, F8, F9 were selected for the further evaluation. Characterization of the selected batches was done on the basis of different organoleptic characters like color, appearance, pourabilityand surface and after taste feelings. The data are shown in Table 6. Formulation F1, F4, F8 and F9 shown good appearance but have chalky feeling therefore formulation F7 was met the criteria of acceptable formulation.

Evaluation of selected and marketed formulation was done on the basis of grittiness, pH and foaming power parameters. Those were shown in Table 7 comparative study indicates high foaming capacity of marketed product. Formulation F7 has highest foam capacity among prepared batches. So optimized formulation was F7. Herbal extracts of active ingredients were evaluated for different parameters like pH, specific gravity and color as shown in Table 8.

Microbial study of herbal extracts and herbal toothpaste was evaluated by determination of zone of inhibition in nutrient agar plates seeded with broth culture of *S.aureus and E. coli*. Table 9 shown zone of inhibition of reported concentration of herbal extract on *S.aureus* (Gm +ve) &*E.coli* (Gm-ve) bacterial species. All the extracts and product showed readily inhibition. Figure 1(A-E) showed antimicrobial activity on *S.aureus* (Gm+ve) and Figure2 (A-E) showed antimicrobial activity on *E.coli* (Gm –ve).

Table 1. List of the ingredients used in toothpaste [3]

Sl no	Ingredients	Concentration (% w/w)	Materials Used
1	API	Up to 5	Clove, Neem, Sunthi, Mentha, Tomar, Pippali, Aloe vera, Kapoor
2	Abrasive	20-40	Precipitated Calciun Carbonate Calcium Phosphate Dicalcium Phosphate dihydrate Anhydrous dicalcium phosphate Calcium pyrophosphate Sodium metaphosphate Ammonium phosphate dibasic Hydrated alumina, Silica
3	Humectants	20-40	Glycerin, Poplyethylene glycol Propylene glycol Sorbitol solution (70%)
4	Water	20-40	Distilled water
5	Foaming agent	1-2	Sodium Lauryl Sulphate Sodium Lauryl Sarcosinate Sodium Lauryl Sulphoacetate Magnesium Lauryl Sulphate Monoglyceride Dioctyl-Na Sulphosuccinate
6	Binder	Up to 2	Natural gum : Gum tragacanth, Acasia,Carrageenans Cellulose derivatives :CMC, MC, Hydroxyehylcelullulose Starch ether Synth. Resins : Ehtylenoxide polymer Carbopol (carboxy vinyl polymer)
7	Flavors	Up to 2	Spearmint, peppermint, wintergreen, cinnamon mint
8	Sweetener	Up to 2	Saccharin solution (0.05-0.3%) Chloroform, Sodium cyclamate, sorbitol
9	Color	< 1	Titanium dioxide
10	Preservatives	0.25 – 1.0	Methyl parahydroxy benzoate (0.15%) Propyl parahydroxy benzoate (0.02%) Sodium benzoate, Trclosan Methyl paraben, Propyl paraben

API- Active Pharmaceutical Ingredient, CMC-Carboxy Methyl Cellulose, MC- Methyl Cellulose, %w/w- Percentage weight by weight

Table 2. Level based design of the batches as per factorial design

Factor(2)	Concentration of Abrasive	Concentration of Humectant(Sorbitol solution)
Levels(3)		
Low	-1 (20%)	-1 (30%)
Medium	0 (30%)	0 (40%)
High	+1 (40%)	+1 (50%)

Table 3. Developed batches from F1-F9 as per factorial design

Batches	Abrasive	Humectant
F1	-1	-1
F2	0	-1
F3	1	-1
F4	-1	0
F5	0	0
F6	1	0
F7	-1	1
F8	0	1
F9	1	1

Table 4. Composition of the batches: (all quantity in %w/w)

Ingredients	Quantity taken per 100 gm paste (in grams)								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
Carboxy methyl cellulose	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Calcium carbonate	20.0	30.0	40.0	20.0	30.0	40.0	20.0	30.0	40.0
Glycerin	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Sorbitol solution (70%)	25.0	25.0	25.0	35.0	35.0	35.0	45.0	45.0	45.0
Sodium Saccharine	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Methyl Paraben	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Propyl Paraben	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
SLS	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Combined Herbal extract	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Flavor	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.
Purified Water	35.0	25.0	15.0	25.0	15.0	5.0	15.0	5.0	-
Color	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.

+ SLS- Sodium Lauryl Sulphate, q.s. - Quantity Sufficient

Table 5. Evaluation of the toothpaste base on the basis of drying tendency

Batches	Dryness test
F1	Not dried
F2	Dried
F3	Dried
F4	Not dried
F5	Dried
F6	Dried
F7	Not dried
F8	Not dried
F9	Not dried

Table 6. Evaluation of the toothpaste base on basis of organoleptic characters.

Batches	Organoleptic character				
	Color	Appearance	Extrudability	Texture	After Taste
F1	White opaque	Creamy	Easy	Smooth	Chalky
F4	White opaque	Paste	Easy	Smooth	Chalky
F7	White opaque	Paste	Easy	Smooth	Slightly Chalky
F8	White opaque	Paste	Easy	Smooth	Chalky
F9	White opaque	Paste	Easy	Smooth	Chalky

Table 7. Evaluation of the toothpaste bases and marketed formulation on the Basis of different Evaluation parameter

Batches	Grittiness	pH	Foam capacity (ml)
F1	Absent	8.56	39
F4	Absent	8.70	38
F7	Absent	8.50	45
F8	Absent	8.56	40
F9	Absent	8.51	38
Amar	Absent	7.00	52
Vicco	Absent	7.00	52
Amar	Manufacture and marketed by: amar remedies ltd. Mfg date: 09/2011 Exp. Date: 08/2013		

Table 8. Characteristic of herbal extracts

Sl no.	Aqueous extract	Color	pH	Specific gravity
1	Clove	Light brown	6-8	1.019
2	Babul	Dark brown	7-8	0.899
3	borsali	Dark brown	7-8	0.911

Table 9. Microbial study of the Herbal extract & Herbal toothpaste.

Sl.No.	Microorganism	ZONE OF INHIBITION(mm)				
		Aqueous extract of Clove (1%)	Aqueous extract of Babul (7.5%)	Hydro alcoholic extract of Borsali (1%)	Combined Extract	Herbal toothpaste Formulation
1	*Staphylococcus aureus*	1	3	3	2	2
2	*Escherichia coli*	1	2	3	3	2

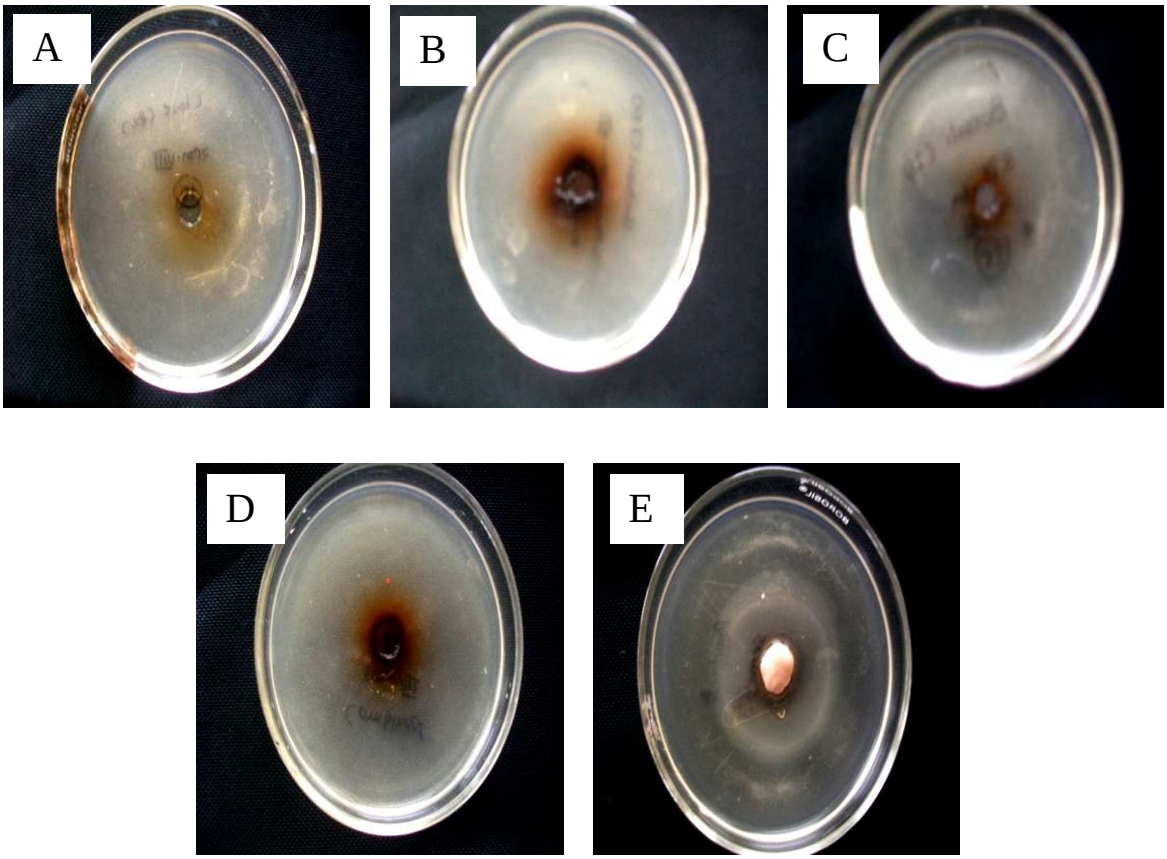

Fig.1.*Microbial Study on S.aureus* of (A)aqueous extract of clove, (B)aqueous extract of babul, (C)aqueous extract of borsali, (D)combined extract and (E)product.

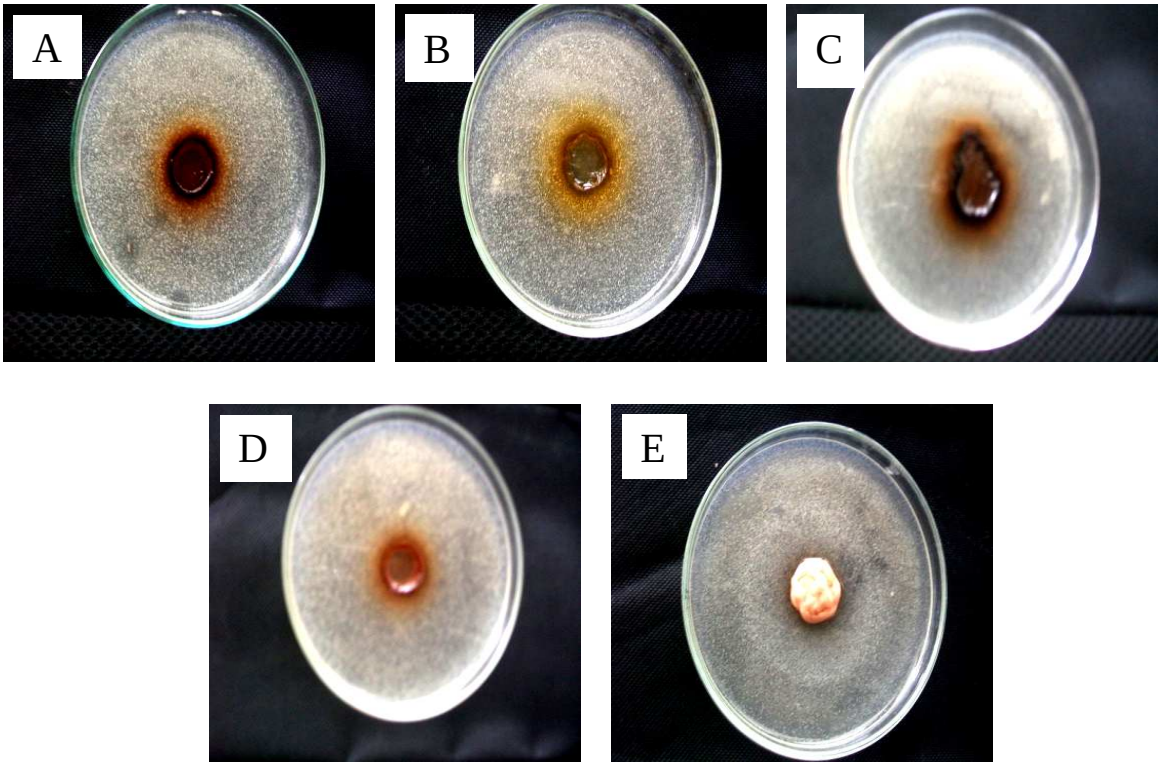

Fig 2. *Microbial Study on E.coli* of (A)aqueous extract of babul, (B)aqueous extract of clove, (C)aqueous extract of borsali, (D)combined extract and (E)product.

4. Conclusion

Herbal formulations have growing demand in the global market. Natural remedies are more acceptable in the belief that they are safer with fewer side effects than the synthetic ones. It is a very good attempt to establish the herbal toothpaste containing hydro alcoholic extract of flower bud of clove (Eugenia caryophullus), bark powder of babul (Acacia nilotica) and bark powder of borsali (Mimusops elengi).The study revealed that the developed single herbal formulation F7 consisting 1% clove extract, 1% borsali and 7.5% babul was comparatively better than later other formulation. They can be used for antibacterial activity.

5. Acknowledgement

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