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Formulation Development and Quality Evaluation of Polyherbal Toothpaste "Oral S"

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Subject: Pharmaceutics

Abstract

Objective: To Formulate and Develop Polyherbal toothpaste formulation and then evaluating its antimicrobial activity.

Method: Methanol extract of Polyherbal formulations was prepared. Standard cultures of *Streptococcus mutans(MTCC 890), Streptococcus oralis(MTCC 2696) ,staphylococcus aureus(MTCC 7443), Candida albicans (MTCC 183),* and *Lactobacillus acidophilus(MTCC 10307),* gram positive was used for the study. The antibacterial tests used was the agar well diffusion method. Methanol and Gentamicin were used as the negative and positive control respectively. Marketed formulations were compared.

Also Quality control parameters like physiochemical, phytochemical and HPTLC(for identification of compounds) were done on developed polyherbal formulations.

Results: The methanol extract of Polyherbal formulation 2 showed maximum activity against *Streptococcus mutans* and minimum activity against *Candida Albicans*. Whereas Methanol extract of polyherbal formulation 1 and 3 showed lesser activity when compared with Polyherbal formulation 2 as shown in table 3 and 4. However, the activity was less than the standard Gentamicin. The extract shows increasing inhibitory activity with increase in concentration (50%-100%). Quality parameters were conducted on polyherbal formulations shown in table 5, 6 and figure 3.

Conclusion: The results of the study support the traditional application of the medicinal plants and suggest that various herbs which were used in Polyherbal formulation possess antimicrobial properties that can be used as antimicrobial agents, and toothpaste developed can be utilized to prevent various dental diseases.

Key Words: *Polyherbal toothpaste , Antimicrobial activity, Quality Parameters*

Introduction

Dental caries is an infectious microbial disease that results in localized dissolution and destruction of the calcified tissues of the teeth [1]. The untreated condition may lead to pain, tooth loss, infection and finally death in severe cases. Today, caries remains one of the most common diseases throughout the world. *Streptococcus mutans* is known as the causative bacteria in the formation of dental plaque and dental caries. The acid producing *S. Mutans* causes damage by dissolving tooth structures in the presence of fermentable carbohydrates such as sucrose, fructose, and glucose[2]. The food debris, acid, bacteria, and saliva combine in the mouth to form a sticky substance called "plaque" that adheres to the teeth [3]. Dental disease is painful, and most importantly, it has also been suggestively linked to diabetes, high blood pressure, heart disease. The pain can be worsened by heat, cold or sweet foods and drinks [4, 5]. Treatment often prevents further infection of the tooth structure. Early treatment is less painful than treatment of extensive decay. Dental caries can also cause bad breath and foul tastes. In highly progressed cases, infection can spread from the tooth to surrounding soft tissues which may lead to anedentulous mouth [6]. Antibiotics such as Amoxicillin and Gentamicin have been reported to effectively prevent dental caries in animals and humans, but they are never used clinically because of many adverse [7]. Indian medicine is one of the oldest organized systems of medicine. Its earliest concepts are set out in the sacred writings called the *Vedas*, especially in the

metrical passages of the Atharvaveda (2nd millennium BC). Recent natural remedies with the use of medicinal plants, which are good reservoirs of chemotherapeutants are being becoming as an alternative for antibiotic adverse effects such as hypersensitivity reaction, supra infections, and teeth staining[8, 9]. Despite several anticaries agents being available commercially, the search for an effective natural agent still continues. Natural products have shown to be a good alternative to synthetic chemical substances for caries prevention.

Polyherbal toothpaste formulation is the composition of *Stevia rebaudiana*. Leaves(SR)-1part , *Glycyrrhiza glabra*.Root(GG)-1part, *Azadirachta indica*.Bark(AZ)-1part, *Ocimum Sanctum*.Leaves(OS)-1part, *Terminalia belirica*. *Fruit* (TB)-1part,*Terminalia chebula*.Fruit(TC)-1part, *Piper longum*.Fruit(PL)-1part,*Curcuma longa*.Rhizome(CL)-1part, *Emblica Officinals*.*Fruit*(EO)-1part, *Acacia Arabica*.Bark (AA)-1part, *Mimusops elengi*.Bark(ME)-1part, *Quercus Infectora*.Galls(QI)-1part and *Salvadora Persica*.Bark(SP)- 1part used. For present study, all the ingredients of Polyherbal formulations toothpaste, in house formation and Marketed formulations were taken for the investigation. Hence, for the present investigation, *Streptococcus mutans, Streptococcus oralis, Streptococcus aureus, Candida albicans,* and *Lactobacillus acidophilus* are the bacterial strains selected as target organisms and screened using methanol extract. Once the antimicrobial property of polyherbal formulation extracts is screened under *in vitro* condition against oral pathogens, physiochemical parameters can be carried out for the evaluation of prepared polyherbal formulation which will be treat dental caries by external application on the caries tooth or as a preventive mouth toohpaste.

Material and Methods

Plant Material and Extraction Procedure

Different plants were collected from the local market of kari Baouli, Old Delhi and they were authenticated in Department of Botany, pharmacopoeia Laboratory of Indian medicine, Ghaziabad(U.P).

Polyherbal toothpaste formulation is the composition of *Stevia rebaudiana*. Leaves(SR)-1part , *Glycyrrhiza glabra*.Root(GG)-1part, *Azadirachta indica*.Bark(AZ)-1part, *Ocimum Sanctum*.Leaves(OS)-1part, *Terminalia belirica. Fruit*(TB)-1part,*Terminalia chebula*.Fruit(TC)-1part, *Piper longum*.Fruit(PL)-1part,*Curcuma longa.* Rhizome (CL)-1part, *Emblica Officinals.Fruit*(EO)-1part, *Acacia Arabica*.Bark (AA)-1part, *Mimusops elengi*.Bark(ME)-1part, *Quercus Infectora*.Galls(QI)-1part and *Salvadora Persica*.Bark(SP)- 1part used.

Extraction of Polyherbal Herbal Extract: The plant parts were shade-dried and powdered and used for extraction, 10grams of different powdered herbal extracts was taken in an aspirator bottle, 100mL hot sterile water (1: 1 W/V) with 1 grams of sodium chloride added and boiled until the volume was reduced to one-fourth (20ml) and the mixture was shaken occasionally at 40^{o}c with the help of stirrer. This procedure was repeated three times for three different formulation (F1,F2,F3) and polyherbal extracts were decanted and taken separately for different formulations. The extracts were filtered before using Whatman filter paper no. 2 on a Buchner funnel and was added to the base formulation.[10].

Figure 1 Polyherbal Toothpaste

Table 1: Ingredients for (base) polyherbal toothpaste formulation

Ingredients	Quantity(g)
Dicalcium Phosphate	36
Water	8
Calcium Phosphate	2
Glycerine	12.6
Gum carragean	0.7
Peppermint oil	1.05
Clove oil	0.3
Herbal extracts	10
Sodium chloride	1

Table 2 Ingredients For Herbal Extracts

F1	F 2	F 3
Stevia rebaudiana (3g)	*Stevia rebaudiana* (5g)	*Stevia rebaudiana* (4g)
Emblica Officinals(1g)	*Quercus Infectora*(1g)	*Azadirachta indica (1g)*
Terminalia chebula(1g)	*Azadirachta indica (1g)*	*Quercus Infectora*(1g)
Ocimum Sanctum(2g)	*Salvadora Persica* (1g)	*Ocimum Sanctum*(1g)
Curcuma Longa (1g)	*Glycyrrhiza glabra* (0.5g)	*Salvadora Persica* (1g)
Terminalia belirica.(1g)	*Piper longum*(0.5g)	*Acacia catechu*(1g)
Azadirachta indica (1g)	*Acacia Arabica(1g)*	*Mimusops elengi* (1g)
Total – 10g	Total – 10g	Total -10g

Microorganisms

The *invitro* screening was carried out against the human pathogens bacteria which include freeze dried form of *Streptococcus mutans(MTCC 890), Streptococcus oralis(MTCC 2696) ,Staphylococcus aureus(MTCC 7443), Candida albicans (MTCC 183),* and *Lactobacillus acidophilus(MTCC 10307)which* were purchased from Microbial Type Culture Collection (MTCC) and Gene Bank, Institute of Microbial Technology, sector 39-A, Chandigarh-160036, India. Ampules of freeze dried form of the microbes were kept in the refrigerator at 4°C till they brought to use. The inoculum size of each strain was standardized by adjusting the optical density of bacterial suspension to turbidity corresponding to spectrophotometric absorbance 0.5 at 550nm (approximately 1.5 x 10^8 CFU/mL).[11]

Screening of Antibacterial Activity

The Cup- plate method was used to evaluate the antibacterial activity.This method depends upon the diffusion of the tested material to such an extent that growth of added microorganisms is prevented entirely in a zone around the hole containing a solution of tested material. One hundred microlitres of diluted inoculums of 1.5×10^8CFU/ml of 24hours old cultures of test organisms were mixed in in Muller Hinton agar medium and shaken. Then media was poured (20-25 ml) in sterilized Petri dishes (20 × 90 mm). Wells of 5 mm diameter were punched into the agar medium and filled with formulated Polyherbal toothpaste extract. Methanol was served as negative control. Antibiotic (Gentamicin concentration 100ug/ml.) was simultaneously used as positive control. Each sample was assayed in triplicate and the mean values were observed (Fig 1). The plates were then incubated at 37^0Cfor 24 h. The antimicrobial activity was interpreted from the size of the diameter of zone of inhibition measured in mm, it was observed as the clear zones surrounding the hole evaluated by measuring the inhibition zone diameter.[12]

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The minimum inhibitory concentration (MIC) of the Polyherbal toothpaste extracts was determined by serial micro dilution method. The concentration used in experiment ranging from (100 to 5000mg/ml). Serial dilutions of Polyherbal toothpaste extracts with Gentamicin (100ug/ml) as a positive control were prepared by Muller Hinton agar broth. A direct suspension of microorganisms were prepared in 5ml sterile distilled water from a 24h old suspension in Muller Hinton agar broth. The turbidity of the suspension was adjusted to match 0.5 Mc Farland standards using a spectrophotometer at 550nm which corresponds (1.5×10^8 CFU/ml). For broth dilution tests, 0.25ml of standardized suspension of bacteria (10^8 CFU/ml) was added to each tube at final concentration of (100 to 5000mg/ml) and incubated at 37^oC. The lowest concentration of the tube which did not show any visible growth after macroscopic

evaluations was considered as the MIC. The dilution starting from the tube that did not show any visible growth were streaked on agar plates. These plates were inoculated 24h at 37^0C and observed the visible growth. The tubes containing the lowest concentration of the extract, which when streaked on the plates did not show any visible growth after 24hr was considered as the minimum Bactericidal count (MBC). The entire assay was performed in triplicates.[13]

Quality Parameters of Polyherbal Formulations
Organoleptic Evaluation:

The organoleptic characters of the samples were evaluated based on the method described by Siddiqui *et al*. Organoleptic evaluation refers to evaluation of the formulation by color, odor, taste and texture etc.[14]

Physiochemical Evaluation of the Polyherbal Formulations:

Physico-chemical investigations of Polyherbal formulations were carried out as per Bureau of Indian Standards for tooth paste. IS (6356-1993).[15]

1 **Determination of hard and sharp edged abrasive particles:**
The paste was extruded about 15 to 20 cm 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 for all samples.

2 **Determination of spread ability**:
About 1 gm of each sample was weighed and placed at the centre of the glass plate (10X10 cm) and another glass plate was placed over it carefully. Above the glass plates 2 kilogram weight was placed at the centre of the plate avoid sliding of the plate. The diameter of the paste in centimeters was measured, after 30 minutes for all samples. The experiment was repeated three times and the averages were reported for all samples.

3 **Determination of fineness:**
Place about 10 gm of toothpaste, accurately weight, in a 100 ml beaker. Add 50 ml of water & allow to stand for 30 minute with occasional stirring until the toothpaste is completely disperse. Transfer to the 150 µ IS sieve & wash by means of a slow stream of running tap water. Let the water drained from the sieve & then dry the sieve containing the residue in an oven, if there is any residue on the sieve carefully transfer it to a tare watch glass & dry it to constant mass in an oven at 105 ± 2^0C.
Calculation

a) $\frac{\text{Material retained on sieve \% by mass}}{\text{Material taken}} \times 100$

b) Sieve is now 75µ weigh accurately 10 gm of toothpaste & proceed as above & if there is any residue on the sieve. Transfer it to a tare watch glass & dry it to constant mass in an oven at 105 ± 2^0C.

4 **Determination of pH:**
Take 10 gm of toothpaste in 150 ml beaker. Add 10 ml of freshly boiled & cooled water (at 27^0C). Stir well to make a thorough suspension. Determine the pH of the suspension within 5 minutes using pH meter.

5 **Determination of foaming power:**
About 5gm of each sample was 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 ensure 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. Stir 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 and water only was noted for all samples.

6. **Determination of foaming power:**
Foaming power = $V_1 - V_2$
V_1 - Volume in ml of foam with water
V_2 - Volume in ml of water only

7. **Moisture content**
Toothpaste (10 gm) weighted and dried it in the oven at 105oC then it was cooled. The loss of weight is recorded as percentage moisture content and calculated by the given formula.

$$\%\text{Moisture} = \frac{\text{Original sample weight} - \text{dry sample weight}}{\text{Original sample weight}} \times 100$$

8. *Determination of Heavy Metals:*

Accurately weigh 2 g of the sample in a kjeldahl flask. An acid mixture of HNO_3:$HClO_4$ (4:1) was added in the flask and heated continuously till the solution becomes colorless. The sample was then transferred to a 25 ml volumetric flask and volume was made up with distilled water. A reagent blank was synchronously prepared accordingly to the above procedure. The standard of Lead (Pb) was prepared as per the protocol in the manual and then the sample was visually analyzed and compared with the standard solution of lead.

9. *Microbial purity*

a) For Total aerobic bacteria: -

the test consists of plotting a known dilution of sample on soyabean (casein digest, agar medium, and any other suitable for growth of aerobic bacteria) incubating them for the specified periods to permit the development of visual colonies for counting.

b) For Salmonella & E.coli: -

the test consists of enrichment of above bacteria form simple in a suitable culture mediums & then a selective culture medium & after incubation, streaking on selective agar plates for identification.

10. Accelerated stability studies

Toothpaste was stored at 40°C and RH 75% ± 5% for 45 days. Then Physiochemical properties were done on formulated toothpastes at zero period and then samples were withdrawn after every 9 days, total 5 samples were withdrawn. It was observed that there were no change in the formulated toothpaste.[15]

11. Preliminary phytochemical analysis:

Preliminary qualitative phytochemical analysis of all the three Polyherbal formulations were carried out as described by *Solomon Charles Ugochukwu et al*. Phytochemical analysis includes determination of alkaloids, carbohydrates, terpenoids, cardiac glycosides, flavonoids, saponins, phenols etc.[16]

High Performance Thin Layer Chromatography (HPTLC):

High performance thin layer chromatography (HPTLC) is used for the quality assessment for the evaluation of botanical materials. It allows for the analysis of a broad number of compounds present.

Procedure: Apply 10µl of Test solution on a precoated silica gel 60 F254 TLC plate (E.Merck)which was used as stationary phase , mobile Phase methanol: Dichloromethane: acetic acid(20:75:5) and of uniform thickness of 0.2 mm. Develop the plate in the solvent system to a set distance . Spray the plate with $KMNO_4$ reagent. Heat the plate at 100 – 105°C until the colour develops.

Results

The results from present study showed that methanol extract of polyherbal formulations displayed significant antimicrobial activity against all selected human oral pathogens. As per table 3, and 4, methanol extract exhibited broad spectrum of activity when compared with marketed brands of toothpastes and with standard Gentamicin. The polyherbal methanolic extract formulations show more effective when compared with marketed brands of toothpastes.

The MBC of Polyherbal methanolic extract formulation (500mg/ml) antimicrobial results showed the diameter of inhibition zone ranging from(17.8- 26.1mm) with highest inhibition zone observed against *Streptococcus mutans*(26.1mm) in F2, and lowest against *Candida albicans(17.2mm)* in F3 against human oral pathogens showed zone of inhibition which was compared with that of control (Gentamicin) used.

Also the MIC of polyherbal methanolic extract formulation (250mg/ml) antimicrobial results showed the diameter of inhibition zone ranging from(9.0- 12.8) with highest inhibition zone observed against *Streptococcus mutans*(13.1mm) in F2, and lowest against *Candida albicans(9.0mm)* in F3. The result of present research also highlights the fact that the organic solvent exhibited greater antimicrobial activity because the antimicrobial principles were either polar or non polar and they were extracted only through methanolic solvent medium.

To avoid unwanted effects of polyherbal formulation ie toothpaste it was compared with Bureau of Indian standards .So according to the standards specified by the Bureau of Indian standards IS 6356-1993, the tests were carried out on Polyherbal toothpaste formulations. Sample values were compared with standard values (specified in the Bureau of Indian standards IS 6356-1993) and are reported in (table 5). All the samples were complies with Bureau of Indian standards and were found to be of good quality.

The results of preliminary phytochemical analysis of formulated polyherbal formulation of formulation 1,2 and 3 are given in (Table-6). This shows the presence of Carbohydrate, cardiac glycosides, alkaloids, saponins,tannins, terpenoids, quinines and flavanoids.

Discussion

The diseases produced by a number of microorganisms are manifested in or about the oral cavity. Some of these diseases are of a specific nature and are produced by a specific microorganism while others are clinically specific and may be caused by any of a broad group of microorganisms. This microbial specificity or non specificity is characteristic of All pathogens isolated during present disease wherever they may occur in the body. Dental caries is caused by infection with *streptococcal, staphylococcal, Lactobacillus and Candida* species. The streptococcal and staphylococcal organisms of the beta hemolytic type that elaborate an erythrogenic toxin in the early stages of characteristic of disease wherever they may occur in the body. *S. mutans* is the main causative agent of dental caries. *Streptococcus mutans,* fermented sugars and produced acids.[17] The acids which affect this primary decalcification of enamel leads to its total destruction and the decalcification of dentin. The acids derived from the fermentation of starches and sugar lodged in the retaining centers of teeth. The streptococci are acidogenic and ferment glucose, lactose, sucrose and maltose. The major end product of fermentation is lactic acid, dextrans and levans. These compounds showed to make dental caries and other infections.These microorganisms demineralized the calcified structures (enamel, dentin) and dissolution of the organic matrix. *S.mutans, S.aureus* and *Lactobacillus acidophilus* play a major role in dental plaque formation. The causative agents of dental caries and dental plaque were isolated and identified. The results from present study showed that methanolic extract of polyherbal toothpaste formulations displayed significant antimicrobial activity against all selected human oral pathogens. As seen in table 3, and 4 when compared with marketed brands of toothpastes. The demonstration of antimicrobial activity against Gram-positive bacteria and fungal strain is an indication that the plants are a potential source for production of drugs with a broad spectrum of activities. Also study has shown that these plants extract are potentially rich source of antibacterial agents. This demonstrates their importance in traditional remedies in the rural populations. Polyherbal formulations were subjected to various quality parameters. Results of qualitative analysis for Polyherbal formulations 1,2 and 3 are given in (table-5). Loss on drying at 105^{o} C was found to be (6.6%, 6.8%,7.2%) , pH value (7.56,7.75,7,79), Foaming power was found to be (32), (28) and (30), spreadibility was found to be (3.5, 4.4, 4.6), hard and sharp edge particles were absent in the respective formulations. The results of preliminary phytochemical analysis of formulated polyherbal formulation of formulation 1,2 and 3 are given in (Table-6). This shows the presence of Carbohydrate, cardiac glycosides, alkaloids, saponins,tannins, terpenoids, quinines and flavanoids. Toothpastes has 6.2- 6.9% of moisture content, that is sufficient to prevent hardening effect. The results obtained from the foam evaluation longer time of fall indicated the denser and more stable foam. Formulation toothpastes has shown good stability, at 40^{o}C, room temperature and at 5^{o}C Separation of a liquid component is not observed at all. From the accelerated stability studies it can be concluded that formulation is stable on storage by the effect of temperature and moisture.

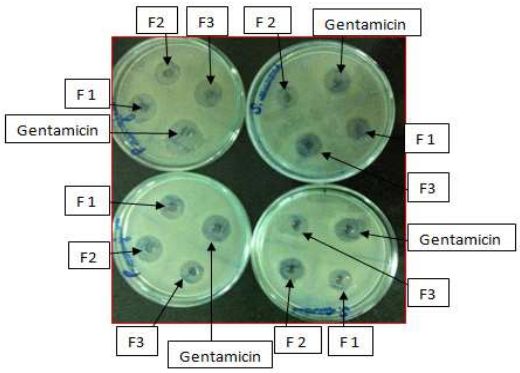


Fig.2 Formulation effect

Table 3: Antimicrobial activity of Polyherbal Formulation against human oral pathogen Zone of Inhibition , diameter(mm)

S.no	Dental Organisms	MIC	MIC mg/ml	MIC Mg/ml	MBC Mg/ml	MBC Mg/ml	MBC Mg/ml	Rf Drug
		F1mm 250mg/ml	F2mm 250mg/ml	F3mm 250mg/ml	F1 mm 500mg/ml	F2 mm 500mg/ml	F3 mm 500mg/ml	Gentamicin 100ug/ml
1)	***Streptococcus mutans***	12.6	13.0	12.7	22.1	26.1	22.3	24.5
2)	***Streptococcus oralis***	12.1	12.8	12.3	20.0	21.2	20.4	20.5
3)	***Streptococcus aureus***	11.8	12.6	11.2	19.6	20.0	19.2	22.1
4)	***Lactobacillus acidophilus***	11.7	12.5	11.3	19.4	21.0	19.6	20.7
5)	***Candida albicans***	9.4	9.5	9.0	17.2	17.8	17.4	23.7

MIC: Minimum Inhibitory Concentration, **MBC:** Minimum Bactericidal Concentration

Table 4: Comparison of formulated toothpaste vs marketed toothpaste brands

S.NO	Dental Organisms	F 1 250 Mg/ml	F 2 250 Mg/ml	F 3 250 Mg/ml	Herbodent Toothpaste 250 Mg/ml	Apollo Toothpaste 250 Mg/ml	Gentamicin 100ug/ml
1)	***Streptococcus mutans***	12.6	13.0	12.7	10.6	11.2	24.5
2)	***Streptococcus oralis***	12.1	12.8	12.3	11.5	11.8	20.5
3)	***Streptococcus aureus***	11.8	12.6	11.2	10.1	12.1	22.1
4)	***Lactobacillus acidophilus***	11.7	12.5	11.3	10.4	10.7	20.7
5)	***Candida albicans***	9.4	9.6	9.0	8.4	8.9	23.7

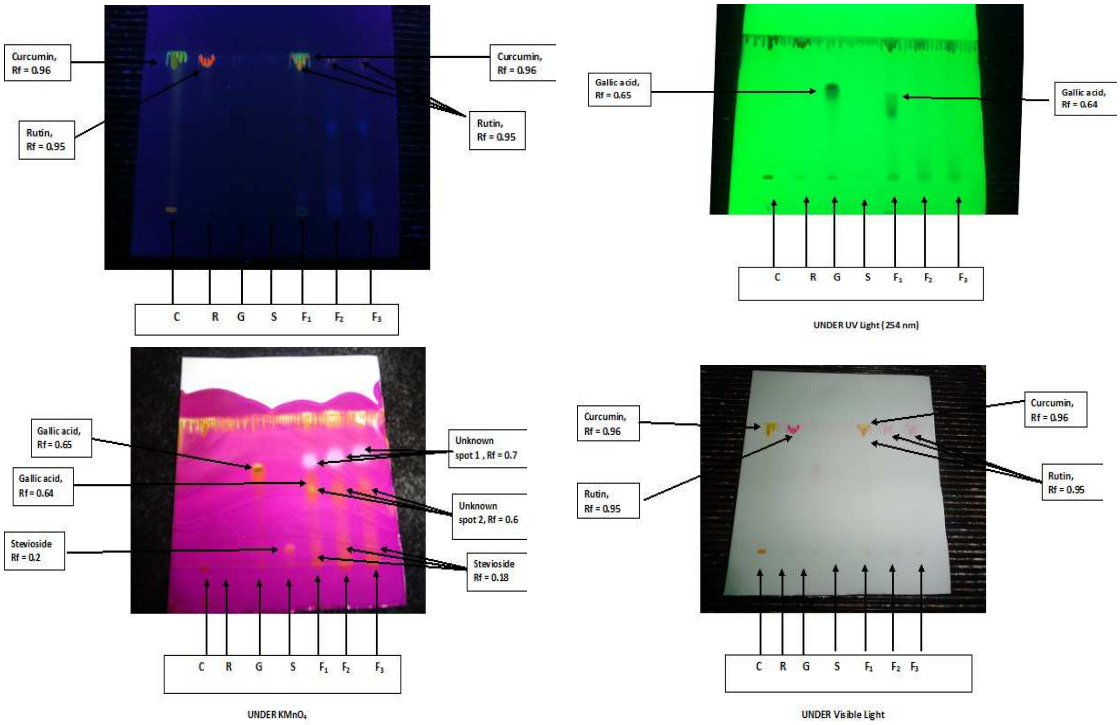
Table 5: Organoleptic, Physiochemical, and Microbiological Examination of Poly Herbal Formulations

S. No	PARAMETER	RESULTS		
1	Organoleptic character	**F 1**	**F 2**	**F 3**
	Appearance	Semi solid	Semi solid	Semi solid
	Colour	Brownish	Brownish	Brownish
	Smell	Characteristic	Characteristic	Characteristic
	Taste	Sweet	Sweet	Sweet
	Texture	Smooth	Smooth	Smooth
2	Loss in weight on drying at 105^{o}C	6.8 %	6.6%	7.2%
3	pH value	7.56	7.75	7.79
4	Foaming power	32	28	30
5	Fineness			
	Particle retained on 150 IS sieve	0	0	0
	Particle retained on 75 IS sieve	0.2	0.3	0.2
6	Heavy metals (as lead), ppm, max	Absent	Absent	Absent
7	Microbial counts:			
	Total viable counts	Absent	Absent	>1000CFU/gm
	Gram negative pathogens	Absent	Absent	Absent
8	Hard and sharp edge particles	Absent	Absent	Absent
9	Spreadability	3.5	4.4	4.6

Table 6: Phytochemical Properties of Polyherbal Formulations

Phytochemicals	Aqueousextracts			Methanolic extracts		
	F 1	**F 2**	**F 3**	**F 1**	**F 2**	**F 3**
Alkaloids	+	+	+	+	+	+
Cardiac glycosides	+	+	+	+	+	+
Carbhydrate	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+
Oxalate	-	-	-	-	-	-
Phenols	-	-	-	-	-	-
Phlobatannins	-	-	-	-	-	-
Proteins	-	-	-	-	-	-
Saponins	+	+	+	+	+	+
Sterols	-	-	-	+	+	+
Tannins	+	+	+	+	+	+
Terpenoids	+	+	+	+	+	+
Quinines	+	+	+	+	+	+

Present = + , absent = -



Figures 3: Identification of Polyherbal Formulations By HPTLC

Abbrevations

A) **C** – Curcumin B) **R** – Rutin C) **G** – Gallic Acid
D) **S** – Stevioside E) **F 1** – Formulation 1 F) **F 2** - Formulation 2
G) **F 3** – Formulation 3

Table 7: Observations

S.no	Standards	Rf value	visible	366nm	254 nm	KMNO4
1)	Rutin	0.95	+	+	+	-
2)	Curcumin	0.96	+	+	-	-
3)	Stevioside	0.2	-	-	-	+
4)	Gallic acid	0.65	-	-	+	-

Present = + , absent = -

Table 8: Observations of formulation

S.no	Formulations	UV	Rutin	Curcumin	Stevioside	Gallic acid
1)	F 1	366nm	+	+	-	-
2)	F 2	254nm	+	+	-	+
3)	F 3	$Kmno_4$	-	-	+	-

Present = + , absent = -

Conclusion

The results of the study also support the traditional application of the plant and suggest that plant extracts possess compounds with antimicrobial properties that can be used as antimicrobial agents. And the developed polyherbal toothpaste could be utilized in the treatment of various dental diseases. Further pharmacological evaluations, toxicological studies and possible isolation of the therapeutic antibacterial from this plant are the future challenges.

"Cite this article"

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