WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
Coden USA: WJPRAP Impact Factor 8.63
Volume 15, Issue 21, 1167-1186. Research Article ISSN 2277–7105

QUALITY-BY-DESIGN APPROACH FOR THE DEVELOPMENT AND PERFORMANCE EVALUATION OF A HERBAL THERAPEUTIC TOOTHPASTE

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Article Received on 28 Sept. 2026
Article Revised on 18 October 2026
Article Published on 01 Nov. 2026

https://doi.org/10.5281/zenodo.23791809

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How to cite this Article: Mr. Chetan M. Gosavi[*1], Mr. Lalitkumar A. Patil[2], Dr. Sachin N. Kapse[3], Mr. Vaibhav K. Pawar[4], Mr. Shrikrushna R. Jagadale[5], Mr. Aakash R. Chaudhari[6], Mr. Rohit R. Aghade[7], ,Mr. Yash D. Khairnar[8], Ms. Komal H. Matale[9] , Mr. Rahul. N. Patil[10] (2026). Quality-By-Design Approach For The Development and Performance Evaluation of A Herbal Therapeutic Toothpaste. World Journal of Pharmaceutical Research, 15(21), 1167–1186.

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ABSTRACT

Objective-In the present study an attempt was made to formulate Herbal medicated Toothpaste containing Psidium Guajava *Linn* (Guava) extract and was evaluated for antimicrobial activity against different microorganisms. **Method**-The leaves of Psidium Guajava Linn plant was collected and extracted with a suitable solvent. Different toothpastes were formulated by using Design of Experiment. The toothpaste was designed by using Psidium guajava Linn extract, Hydroxy Propyl Methyl Cellulose, sodium carboxy methyl cellulose, sodium saccharide, alum, calcium carbonate, sodium lauryl sulphate, clove oil, peppermint oil, glycerine, polyethylene glycol (PEG -400), methyl paraben, propyl paraben etc. **Result**-In present study formulated herbal toothpaste was evaluated for its organoleptic and physical properties such as colour, odour, taste, pH, spreadability, viscosity, moisture content, fineness, foamability etc. as per standards specified by Bureau of Indian Standards and compared with commercially marketed herbal toothpaste formulations. The herbal medicated toothpaste showed that the

formulation containing Psidium guajava *Linn* (Guava) extract have better Antibacterial activity. **Conclusion-**In the present study, the toothpaste was prepared by using various herbal ingredients which possess antibacterial, antiseptic and cooling properties. Formulation of toothpaste with Psidium guajava *Linn* (Guava) extract was acceptable and might be considered as a desirable herbal medicated toothpaste.

KEYWORDS: *Psidium guajava Linn leaves (Guava) extract, Herbal Medicated Toothpaste, Quality by Design (QBD), analytical methods for evaluation of herbal toothpaste.*

1.0 INTRODUCTION

The tooth composed of crown, root, enamel, dentine and pulp. Problems associated with teeth are bad breath, tooth decay, gum (periodontal) disease, tooth sensitivity, calculus, dental caries and dental plaque.[1] The main species involved in the development of dental caries is S. mutans, *S. aureus* and *E. coli*, *S. mutans* is an acid producing bacteria, causes fermentation of carbohydrates which results in tooth decay.[2] Oxidative stress causes disturbance in the pro-oxidant and antioxidant balance, in favour of the former, resulting in potential tissue damage.[3] Several leaves extracts, including guava leaves extract have been found to exhibit antimicrobial activity against S. mutans, *S. aureus* and *E. coli*. In present times, medicinal plants are being explored for effective source of medicines. *Psidium guajava Linn leaves are* high in lutein, zeaxanthin, lycopene and vitamin C which are antioxidants. Guava leaves are also rich in phenols, triterpenes, carotenoids, lectins, fiber, fatty acids, quercetin, anthraquinones, and cardiac glycosides.[4-5] The decoction of leaves is also effective to gargle for bleeding gums. Keeping this in view, the present study was aimed to formulate herbal medicated toothpaste to fight against the pathogenic bacteria like *S. mutans, E. Coli* and *S. aureus*, which cause problems like dental cavity, gingivitis and (periodontal) disease etc.[6]

1.1 Classification of toothpastes

While many toothpastes have multiple ingredients and additives that differentiate the dental professional's recommendations for use, one can categorize toothpastes into nine major categories based upon patient needs and desires. These broad categories of toothpastes are Caries prevention-cavity protection Anti-tartara ctivity (reduction of calculus formation) Gingivitis reduction Plaque formation reduction Re-mineralizing-calcium-phosphate-fluoride containing Cosmetic effect –tooth whitening, stain removal Reduction in tooth sensitivity (Multicare toothpastes) Natural toothpaste –natural ingredients Toothpastes for patients with dry mouth- xerostomi.

2.0 MATERIALS AND METHODS

2.1 Pre-formulation studies

2.1.1 Plant materials

Fresh plants of *P. guava leaves* were collected from Lonik and Farmers, District- Pune, Maharashtra, India inJuly2017.ThePlantleaveswereauthenticated from Botanical Survey of India, Pune. Authentication No: DC 01. It was cleaned, shade dried, and processed for further studies.

These above products were identified by various physical parameters, spectroscopic studies and chromatographic studies. Various confirmatory chemicals tests were performed to identify the presence of phyto chemicals. And thus the selected phyto chemicals were further taken to assay their Antibacterial Activity.

2.1.1 Preparation of Leaves Extract

Procedure: Guava leaves were taken and washed in order to take out impurities from them. They were shade dried for about 4 to 5 days, after proper drying, they were grounded to a fine powder which was passed through sieve no.6. The powder was packed in Soxhlet apparatus and continuous extraction process was done for about 6 hours at 50˚C with ethanol and methanol. After the extraction process, the product was collected and shade dried for 10 days and the extract was powdered. The standard toothpaste base was formulated. The powdered extracts of Guava leaves was incorporated in toothpaste. All the formulations were filled in regular metal tubes used in toothpaste. The storage in tubes was done to overcome the problem of drying of extruded toothpaste during evaluation and stability studies.[7]

2.2 Thin Layer Chromatography (TLC)

Thin-layer chromatography (TLC) is a

Chromatography technique used to separate non-volatile components. Thin-layer chromatography is performed on a sheet of glass, plastic, or aluminium foil, which is coated with a thin layer of adsorbent material, usually silica gel, aluminium oxide(alumina), or cellulose.

2.3 HPTLC Analysis

Chromatography was performed using commercially-prepared, pre-activated (110°C) silica gel60 F 254TLC plates (E Merck, Germany) using Camag HPTLC and Wincats Software. A Linomat V (Camag, Muttenz, Switzerland) automatic TLC applicator was used to apply

samples and standards onto the TLC plate under a flow of nitrogen gas. Different mobile phases containing methanol, water, toulene, acetone, n -hexane, ethyl acetate and formic acid in different concentrations were used. The mixture of toulene:acetone:formic acid (7.5:2:0.5 v/v/v) was found to be most suitable for further studies. Each TLC plate was developed to a height of about 8 cm on the stationary phase. For quantitative determination, spots were scanned using a Camag TLC Scanner 3 at 254 nm.[8]

2.4 Determination of λ_{max}

Preparation of calibration curve in ethanol

Standard curve was obtained using the standard solution in the range of 1 µg/ml to 5 µg/ml. Weigh 10mg of active ingredients (Guava leaves extract) and transfer to a 100ml volumetric flask. Add methanol ¾ volume of the flask and sonicate for 15 min. Then make up the volume upto 100ml and filter it using Whatman filter paper. Pipette out 2, 4, 6, 8, 10ml in 10ml volumetric flask and make up volume using ethanol to produce conc.in the range of 1 µg/ml to 5 µg/ml. Absorbance of these solutions were taken at 525nm, 366nm, 254nm using UV-Visible spectrophotometer (Model No.1800 Shimadzu Corporation, Tokyo, Japan) having two matched pairof quartz cells with 1 cm path length.[9]

2.5 Optimization of Herbal Medicated Toothpaste by using quality by design (QBD) experiments

A Box Behnken Design was used with 29 trial runs to study the impact of 4 factors on the 3 key response variables. In this design, 4 factors were evaluated, each at 3 levels, and experimental trials were performed at all possible 29 combination. The conc. of Calcium Carbonate, Guava Extract, Polyethylene Glycol (PEG) and Hydroxy Propyl Methyl Cellulose were selected as independent variables and Spreadability, Viscosity, Antibacterial Activity were selected as dependent variables. The resulting data was fitted into design expert software (design expert version 11) and analysed statistically using analysis of variance (ANOVA).

The data were also subjected to3-D response surface methodology to determine the influence of concentration on dependent variables. The probable runs using Box Behnken design are as shown in table 1.

Table 1: Box Behnken design for optimization of herbal medicated toothpaste.

Run Order	Calcium Carbonate (mg)	PEG (mg)	Guava extract (mg)	HPMC (mg)
1	22	9	0.75	0.8
2	26	12	0.75	1
3	26	9	0.75	0.8
4	24	9	0.75	1
5	24	12	0.9	1
6	22	9	0.9	1
7	24	12	0.6	1
8	24	12	0.75	0.8
9	24	9	0.9	0.8
10	24	9	0.75	1
11	22	9	0.75	1.2
12	24	6	0.75	0.8
13	24	6	0.6	1
14	24	12	0.75	1.2
15	24	9	0.6	1.2
16	22	6	0.75	1
17	24	6	0.75	1.2
18	24	6	0.9	1
19	24	9	0.75	1
20	26	9	0.6	1
21	24	9	0.6	0.8
22	26	6	0.75	1
23	24	9	0.75	1
24	24	9	0.75	1
25	22	12	0.75	1
26	26	9	0.9	1
27	22	9	0.6	1
28	26	9	0.75	1.2
29	24	9	0.9	1.2

2.6 Formulation of Herbal Medicated Toothpaste

Procedure

Method:-1

The binder, pre-wetted with the humectant, is disperse in liquid portion containing the saccharin and preservative and it was allowed to swell to form a homogeneous gel. The swelling gets accelerated by heat and agitation. The extract powder was added slowly to homogeneous gel and mixed in mixer until a paste formed. The flavour and detergent are added last and distributed uniformly. Excessive, aeration, particularly in the presence of detergent, should be avoided. The paste can then be milled, de-aerated and filled in tubes.

Method:-2

The binder is premixed with extract powder, which is then mixed with the liquid phase, containing humectant, preservative and sweetener into a mixer. After formation of homogeneous paste, the flavor and detergent are added, mixed, milled de-aerated and filled in tubes.[10]

3.0 EVALUATION OF HERBAL MEDICATED TOOTHPASTE

According to the guidelines, the standards were prescribed foreach evaluation test of herbal medicated toothpaste.

3.1 Physical Examination (Colour, odour, taste, smoothness)

Formulated toothpaste was evaluated for its colour.

Colour

The visually colour was checked.

Odour

The odour was found by smelling the product.

Taste

The taste was checked manually by tasting the formulation.

Smoothness

The Smoothness was tested by rubbing the paste formulation between the fingers.[11]

3.1.1 Composition

All ingredients should comply with the Indian standards. The prepared toothpaste is not composed of mono or disaccharides such as glucose, sucrose or fermentable carbohydrates.

3.1.2 Test for solubility of colours: 1gm of each sample was taken. To this 50 ml of water was added and boiled for 15 minutes and filtered. From this filtered solution, 10ml of sample was taken and 15ml of rectified spirit was added. It was refluxed for 15 minutes and filtered. The filtrate should be colourless or faintly coloured.[12]

3.1.3 Determination of Particle Size on 150-Micron IS Sieve

10gm of each sample was accurately weighed and placed in a 100ml beaker. To this 50ml of water was added and allowed to stand for 30 mins with occasional stirring until the toothpaste

was completely dispersed. This solution was passed through 150 micron Indian Standard sieve. Then the sieve was washed with running tap water. Washing should be continued until all the matters passed through the sieve. After washing, the residue remaining on sieves were collected and dried in an oven at 105°c. Particle size was determined by using following formula.[12]

Materialretainedon150-micron

IS Sieve, percent by mass= $\frac{M_1 \times 100}{M}$

Where

M_1= massing of residue retained on the sieve and M = massing of the material taken for the test.

3.1.4 Homogeneity

Determination of homogeneity was studied by Applying a normal force to containers at 27±2°C.[13]

3.1.5 Tube inertness

Tube inertness was examined by cutting the tubes and investigating internal surface for any signs of deterioration in different storage conditions including normal and heating temperature at 45±2°C for 180 days.[13]

3.1.6 Determination of sharp and edge abrasive particles

Extrude the contents 15-20 cm long on the butter paper, repeat the same process for at least ten collapsible tubes. Press with the contents of the entire length with fingertip for the presence of sharp and hard-edged abrasive particles. Toothpaste shall not contain such particles.[14]

3.1.7 Determination of pH

Dispense 10 g of the toothpaste from the container in a 50-ml beaker and add 10 ml of freshly boiled and cooled water (at 27°C) to make 50 percent aqueous suspension. Stir well to make a thorough suspension. Determine the pH of the suspension within 5min, using a pH meter.[14]

3.1.8 Determination of viscosity

Test conditions

Type of equipment–Brookfield RVDV-II +Pro spindle - small sample volume adaptor (S95) Sample quantity – 5gm The Speed of rotation of stirring element – 0.5 to 100 rpm.

Procedure

The specified quantity of prepared formulation was transferred in the beaker and was placed carefully within the adaptor. The guard leg was placed around the adaptor and the sample was stirred slowly using motor driven stirring element. The viscosity values were recorded from the display window.[15]

3.1.9 Determination of Spreadability

About1gm 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 to avoid sliding of the plate. The diameter of the paste in centimetres was measured, after 30 minutes for all samples. The experiment was repeated three times and the averages were reported for all samples.[16]

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

Where, S= Spreadability

M=Mass attached with thes lide

T=Time required to travel a distance on the slide.

3.1.10 Determination of foaming power

Weigh about 5 g of the toothpaste accurately in a 100-ml glass beaker, add 10 ml of water, cover the beaker with a watch glass and allow to stand for 30 min. Stir the contents of a beaker with a glass rod and transfer the slurry to the 250 ml graduated cylinder, ensuring that no foam (more than 2 ml) is produced and no lump paste goes into the cylinder. Repeat the transfer of the residue left in the breaker with further portions of 5 to 6 ml of water ensuring that all the matter in the beaker is transferred to the cylinder. Adjust the contents of the cylinder to 50 ml by adding sufficient water and bring the contents of the cylinder to 30˚C. Stir the contents of the cylinder with a glass rod or thermometer to ensure a uniform suspension. As soon as the temperature of the contents of the cylinder reaches 30°C, stop the cylinder and give it 12 complete shakes, each shake comprising movements in a vertical plane, upside down and vice versa. After the 12 shakes have been given, allow the cylinder to stand still for 5 minutes and read the volumes of foam. Foam power is determined by using

following formula:[17]

Foaming power, ml= $V_1 - V_2$

Where,

V_1=Volume in ml of foam with water and V_2= Volume in ml of water only.

3.1.11 Determination of moisture and volatile matter

Weigh 5 g of a sample placed in a porcelain dish containing 6-8 cm in diameter and 2- 4 cm depth in it. Dry the sample in an oven at 105˚C. Moisture and volatile matter is determined by using following formula:[18]

% by mass = 100 M1 / M

M1-loss of mass (in grams) on drying

M- Mass (in grams)of the material taken for the test.

3.1.12 Extrudability

In this method, the formulated paste were filled in standard capped collapsible aluminum tube and sealed by crimping to the end. The weights of tubes were recorded. The tubes were placed between two glass slides and were clamped. 500g was placed over the slides and then cap was removed. The amount of the extruded paste was collected and weighed. The percent of the extruded paste was calculated.

3.1.13 Test organisms

Pure cultures of bacterial isolates of (*Escherichia coli* And *Staphylococcus aureus.*

3.1.14 Antimicrobial Assay

By using agar well diffusion method antimicrobial activity was performed. For growth of *E. coli* and *S. aureus*, MaConkey agar and nutrient agar were used respectively. The agar was spread on the plate for solidification purpose. Spread 0.1µL overnight cultures of *E. coli and S. aureus* onto the plate after solidification of agar. The 100µL of cell free supernatant was added in 6mm wells. Plates were kept in refrigerator for 4 hours for diffusion puprose. Then plates were incubated aerobically at 37°C for 24 hours for *E. coli* and *S. aureus*. By recording zone of inhibition antimicrobial activity was studied.[19]

3.1.14 Stability

The toothpaste should be stable, but not to be deteriorating, ferment and segregate during normal storage conditions and usage. The stability study was performed by storing

formulations in three different temperatures and humidity conditions, At 25±2° C/60%±5°CRH, 30±2°C/65%±5°CRH and 40±2°
C/75%±5°C RH for a period of 3 months considering ICH guidelines.[20]

4.0 RESULTS AND DISCUSSION

The herbal medicated toothpaste formulated from the Guava leaves extract with natural ingredient along with other ingredients used in toothpaste preparation. At the formulation trial process various batches were prepared as per design of experiments and checked for various parameter like homogeneity, spreadability, foamability and viscosity in these batches. Those batches which did not conform to Bureau of Indian Standard were discarded. The formulated herbal medicated toothpaste shows greenish brown in colour and the optimized batch showed the good homogeneity with absence of lumps and good anti-microbial activity.

4.2 Extraction of Guava leaves powder

Table 3: Extraction method and the solvents used.

Sample	Extraction Method	Solvent use	Wt of sample (gm)	Extraction value (%w/w)
Guave Leaves powder	Soxhlet extraction	Ethanol	14.23	14.23%
Guava Leaves powder		Methanol	10.41	10.41

Table 2: Proximate Analysis.

Test	Observed value in %	Standard Value
Ash Value	7.4	NMT11%
Water soluble ash	3.0	NMT3.0%
Acid soluble ash	1.063	NMT3.0%
Alcohol Soluble extract	1.466	NMT6%
Water soluble extract	7.266	NMT12%
Losson drying	10.0	NMT10%

Table 4: Preliminary Phytochemical investigation.

Sr. No.	Chemical Test	Ethanol	Methanol
1	**Test for alkaloids** a) Dragnd orff' test b) Mayer's test c) Hager's test	 + - -	 + - -
2	**Test for Tannins** Ferric chloride test b) Lead acetate test c) Potassium Dichromate test d) DiluteHNO_3	 + + +	 + + +
3	**Test for flavonoid**	+	+

	a)Lead acetate test b)Ferric chloride test c)Sodium Hydroxide test d)Shinoda test	+ + +	+ + +
4	**Test for Steroids** a) Salkowski test b) Liebermann – Burchard Reaction	+ +	+ +
5	**Saponification test** Foam test	+	+
6	**Test for Carbohydrates** a) Molisch's test b) Fehlings test c) Benedict test	+ + -	+ + +
7	**Test for Proteins** a) Biuret test b) Millon's test	- -	+ -
8	**Test for amino acids** Ninhydrin test	-	-

Table 5: Thin Layer Chromatography.

Sr.	Mobile Phase composition	Std. Rf Value	Sample Rf Value	Compound
1.	N-Hexane & Ethyl acetate (7:3)	0.22	0.22	Quercetin

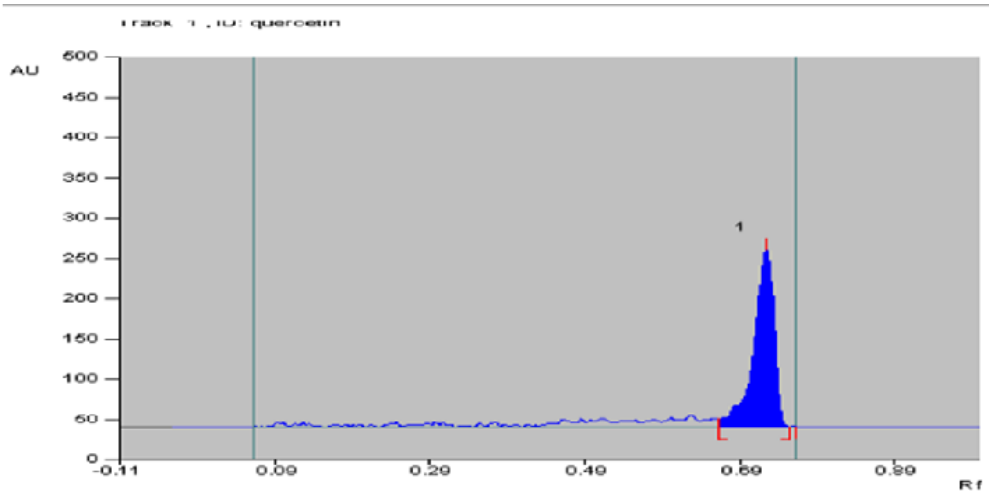


Fig. 1: Chromatogram of Standard Quercetin.

Track	Rf Value	Area
1.	0.69	5453



HPTLC Analysis.

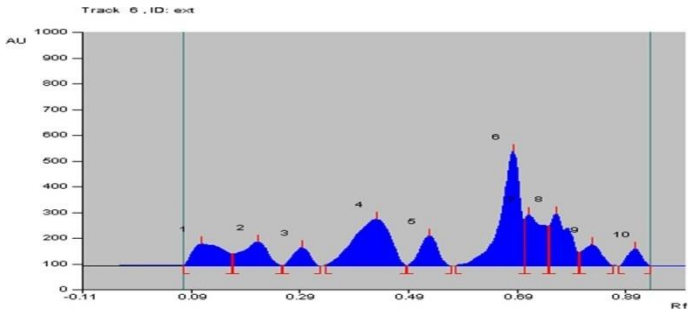


Fig.2: Chromatogram of Standard Quercetin.

Table 6: Chromatogram of Standard Quercetin.

Track	Rf Value	Area
1.	0.69	5453

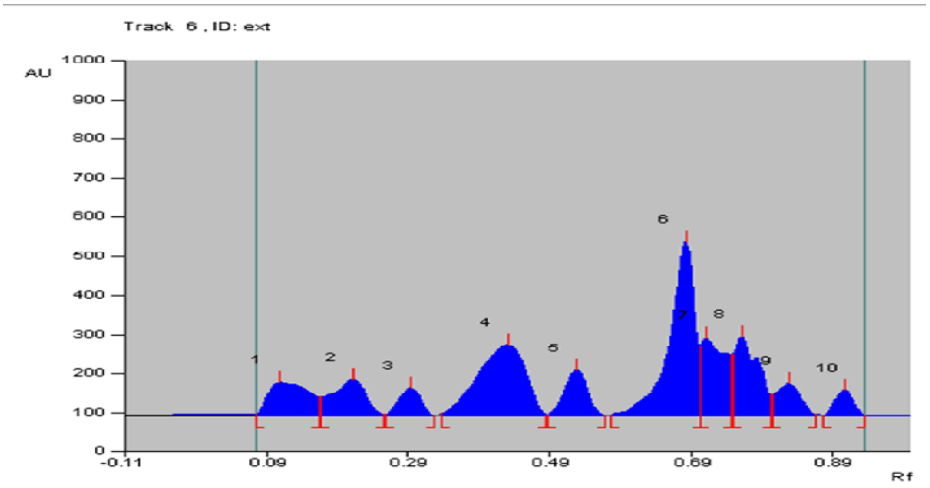


Fig. 3: Chromatogram of Standard Quercetin.

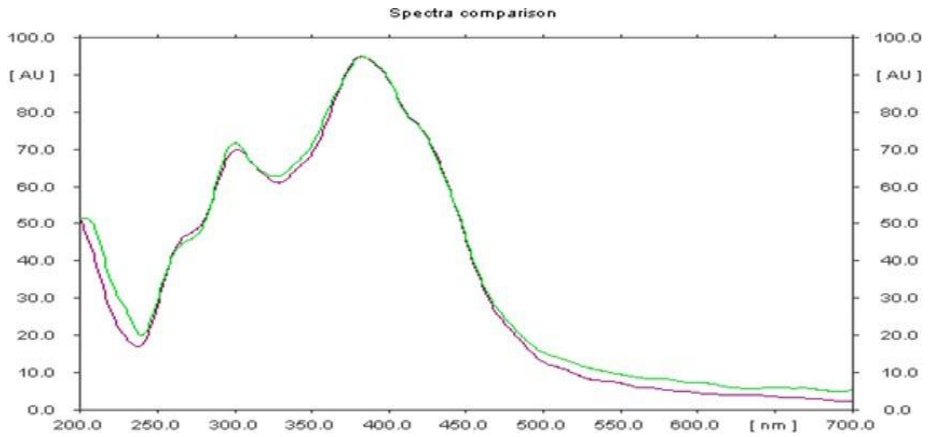


Fig. 4: Overlay Spectra of Standard Quercetin and Extract.

Table 7: Chromatogram of Standard Quercetin.

Track	Rf value	Area
1.	0.68	8450.3



Fig. 5: Developed TLC Plate.

Procedure: Leaves powder were packed in soxhlet apparatus. This packed material was extracted with solvents like ethanol and methanol. When clear solution was observed in siphon tube then the extraction was stopped. Solvent was evaporated by using rota evaporator and yield was calculated. (Table 3)

4.3 On the basis of extractive value the ethanol gave better yield (% w/w) as compared to methanol. (Table 4)

The above study indicates the presence of tannins, flavonoids, steroids, saponins and carbohydrates in the alcoholic extracts.

4.5 Calibration Curve of Guava Leaves Extract

Stock solutions (100µg/mL) of extract prepared in ethanol and different concentrations were spotted onto TLC plates in order to prepare the calibration graphs.

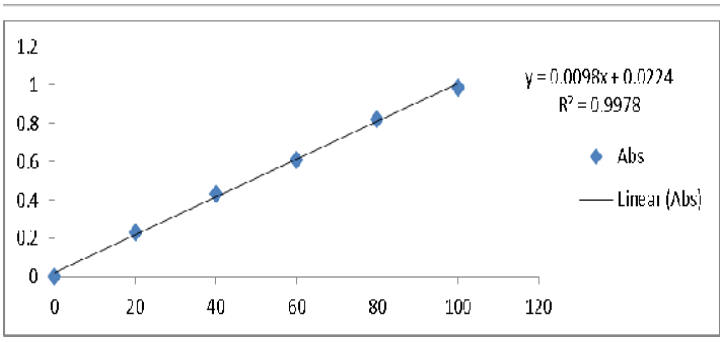
4.6 SPECTROSCOPICAL STUDY

4.7.1 FTIR study of active drug and Formulation

The use of analytical technique Fourier Transform Infrared (FTIR) Spectroscopy is an approach used in compatibility tests based on the hypothesis that same functional group frequencies change during drug-excipients interaction and if the functional groups remain unchanged means the drug and excipients are compatible with each other.

Table 8: Calibration Curve of Guava Leaves Extract.

Concentration (µg/ml)	λmax	Absorbance
0	254	0
20	254	0.214
40	254	0.424
60	254	0.612
80	254	0.822



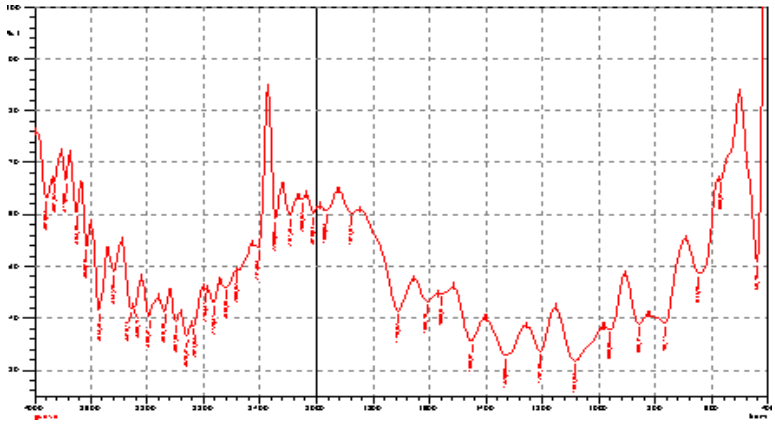


Fig. 6: FTIR spectra of active drug and Formulation.

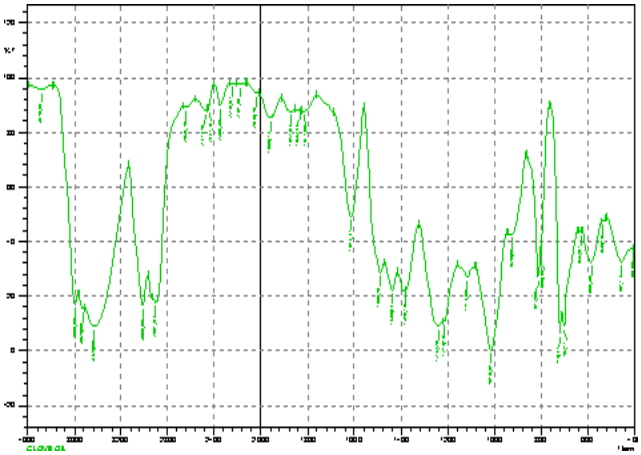


Table 9: Interpretation of FTIR of G.leaves extract.

Sr. No.	Frequency (cm^{-1})	Functional group present
1.	**765.77, 856.42**	Aromatic C-HBend
2.	1084.03	C-O
3.	1710.92	C=O
4.	2920.32	CH3, CH2
5.	3078.49	Aromatic C-H
6.	3315.74, 3288.74	N-H
7.	3543.35	O-H

Table 10: Interpretation of FTIR of clove oil.

Sr. No	Frequency (cm^{-1})	Functional group present
1.	3350, 3593.50, 3525.99	N-H
2.	3009.05	Aromatic C-H
3.	2901.04	CH_3,CH_2
4.	1614.47	C=C
5.	1016.52	C-O
6.	760, 850	AromaticCH Bend

5.0 EVALUATION OF HERBAL MEDICAT ED TOOTHPASTE

RESULTS OF OPTIMIZED BATCH (B12)

Table 11: Physical Examination.

Sr. No	Parameters	Observations
1.	Colour	Greenish
2.	Odour	Characteristic
3.	Taste	Sweet-spicy
4.	Smoothness	Smooth

Table 12: Evaluation Results.

Sr.	Parameter	Observation
1.	Particle size	≤50 µm
2.	Homogeneity	Good
3.	Tube inertness	No Deterioration

4.	Abrasiveness	Good Abrasive
5.	pH	7.8
6.	Viscosity	84210 cp
7.	Spreadability	8.7 cm/sec
8.	Foamability	62
9.	Moisturecontent	16.23 %
10.	Extrudability	91.37
11.	Antibacteria lGrowth *(S. ureus)(E. coli)*	29.7 mm 12.9
12.	Stability	Stable

Table 13: Extrudability.

Extrudability	Mean of Three Tubes
Net wt of formlation in tube (g)	13.5
Wt of toothpaste extruded (g)	12.2
Extrudability amount percentage	91.37

5.0 Anti-microbial activity

The formulated herbal toothpaste exhibited good antibacterial activity against *S. aureus* and *E. coli* as compared to the standard marketed Toothpaste. The formulation exhibited an impressive ZOI of *S. aureus* 29.7 mm and *E. coli* 12.9, whereas Standard toothpaste exhibited 18.23 and 12.3 mm ZOI. Therefore it may be concluded that formulated toothpaste has potential to exhibit better antibacterial activity.

Antimicrobial activity shown by optimized batch B12 against (a) S. aureus, (b) E. coli.

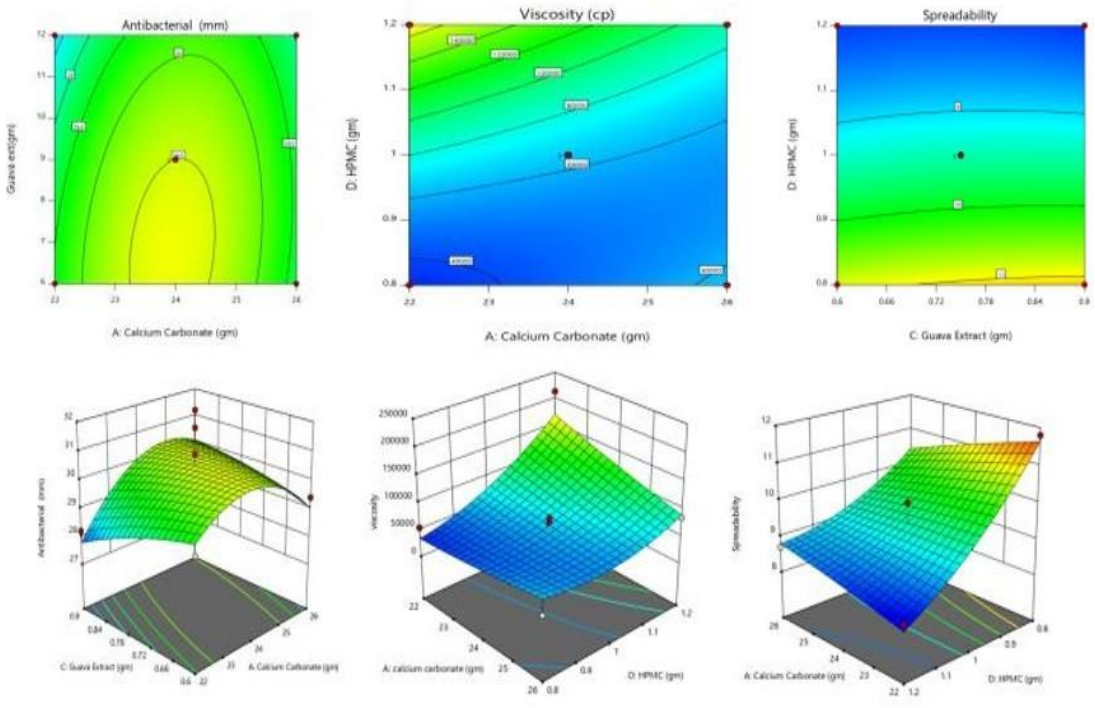


Fig.7: Response surface plot (a, b, c) Spreadability (CCVS HPMC), Viscosity (CCVS

HPMC), Antibacterial activity (Guava ext VS CC).

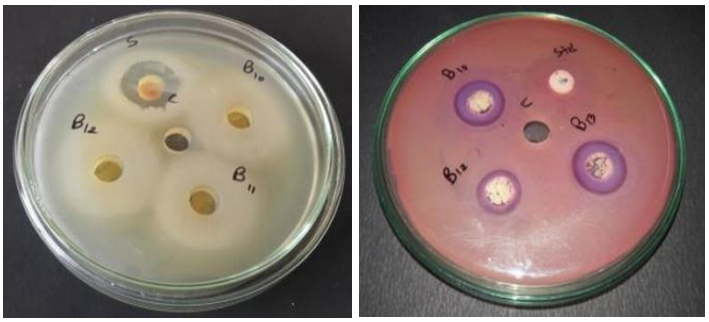


Fig. 8: Anti-microbial activity (S. aureus / E. Coli).

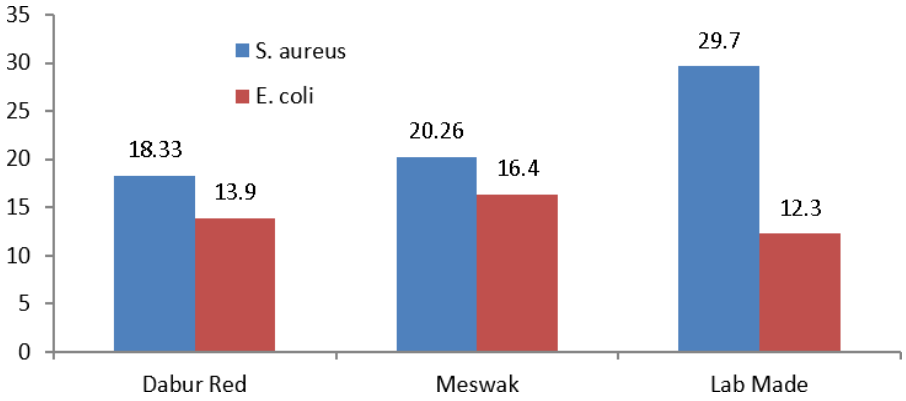


Fig. 10: Comparison of antibacterial activity of optimized batch (B12) with marketed formulations.

Table 14 A: Stability:-At25ºC±2ºC/60%±5%RH (3rd month)

Colour	Appearance	Spreadability	pH
Green	Homogeneous	8.7	7.8

Table 14 B: At 30ºC± 2ºC/ 65%±5%RH (3rd month)

Colour	Appearance	Spreadability	pH
Green	Homogeneous	8.4	7.90

Table 14 C: At40ºC±2ºC/ 75%±5%RH (3rd month)

Colour	Apperance	Spreadability	pH
Green	Homogeneous	8.22	7.84

The stability study indicated that the formulated toothpaste has a good stability.

5.1 COMPARATIVESTUDY: FORMULATED HERBAL MEDICATED TOOTHPASTE WITH MARKETED PREPARATIONS

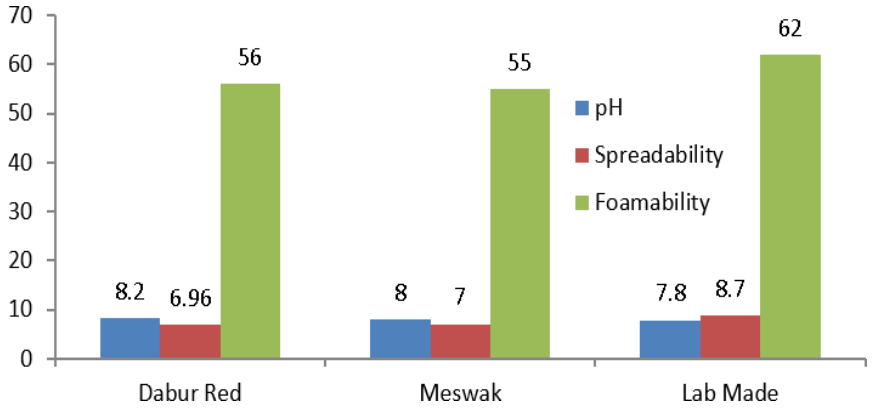


Fig. 9: Comparison between pH, Spreadability & Foamability with marketed preparations.

Table 15: % Moisture Content Camparison.

Preparation	% moisture content
Marketed preparation	25.40%
Lab Made (B12)	16.23

The above chart 5.6.1, 5.6.2 and5.7 table shows that optimized formulated herbal toothpaste is having a better physico-chemical and antimicrobial activity than the marketed preparations.

5.1 CONCLUSION

A herbal medicated toothpaste has been developed using QBD approach which shows better antibacterial activity than the marketed preparations. Optimized batch (B12) passes all the test of toothpaste given in the specification by Bureau of Indian Standards. Guava leaf powder and leaf extract which is popularly used by villagers as a tooth powder is formulated into regular toothpaste by using various ingredients commonly used in toothpaste preparation.

It also has the advantage of absence of harmful chemicals & presence of herbal powders with wide spectrum of natural compounds beneficial for teeth & oral cavity compared to conventional toothpastes. Toothpaste made from guava leaves extract is cheaper than other natural toothpastes available in the market, for example Meswak is imported from gulf countries and guava leaves are available locally. Use of guava leaves gives an additional business opportunity to the farmers, who are producing guava. The product developed can be successfully commercialised as a new herbal toothpaste which is new in India and cheaper than marketed preparations.

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