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DEVELOPMENT OF EDIBLE SLS FREE PROBIOTIC HERBAL TOOTH GEL FOR TREATMENT OF PERIODONTITIS

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ABSTRACT:

Current research aimed to develop an edible herbal tooth gel for the treatment of periodontitis using *Vaccinium macrocarpon* fruit extract. The formulation was prepared using conventional techniques and 2^3 full factorial designs. The final formulation had a reddish-pink colour, smooth appearance, thick consistency, and high viscosity. The gel was tested for its various *in vitro*, *in-vivo*, and *ex-vivo* studies such as appearance, viscosity, spread ability, pH, foaming activity, fineness, abrasiveness, stickiness, and grittiness, homogeneity, cleaning ability, antibacterial activity and normal flora. The gel was found to have no negative effects on normal gastrointestinal flora (E. coli). The study concluded that the herbal edible tooth gel is natural, free from SLS, probiotic-loaded, and safe in the gastrointestinal tract if swallowed unintentionally. As per the outcome of the study, it was detected effective for dental care and periodontitis.

Keywords- *Vaccinium macrocarpon*, Edible tooth gel, Periodontitis, Normal flora, MIC

1. Introduction: Periodontal diseases are inflammatory conditions that affect the supporting structures of teeth, including the gingiva, bone, and periodontal ligament. They can lead to tooth loss and contribute to systemic inflammation. Chronic periodontitis predominantly affects adults, while aggressive periodontitis may occasionally occur in children. (Iacopino, 2000) The disease is initiated through a dysbiosis of the commensal oral microbiota (dental plaque), which interacts with the host's immune defences, leading to inflammation and disease. There are two types of periodontal diseases: gingivitis and periodontitis. Gingivitis is an inflammation of the gingiva caused by plaque microorganisms, which release enzymes that damage epithelial and connective tissue. (Bodet,2008) There are three types: acute, sub-acute, recurrent, and chronic. Acute gingivitis is characterized by sudden onset, short duration, and pain, while sub-acute and recurrent gingivitis are less severe stages. (Hugo, 2021) Chronic gingivitis is characterized by gingival redness, edema, bleeding, contour changes, loss of tissue adaptation to the teeth, and increased gingival crevicular fluid flow. (Borrell, 2008)

Prevention of periodontitis is crucial for maintaining gum and teeth health. A Crest pro-health regimen, including Crest gum detoxify deep clean toothpaste, Crest pro-health multi-protection mouthwash, a soft-bristled toothbrush, and Oral-B glide pro-health comfort plus floss, can help to prevent and reverse early signs of gum disease. (Board of Trustees of The American Academy of Periodontology,1999) These products kill bacteria in plaque around the gum line, provide 24-hour protection against gingivitis and plaque, and freshen breath.(Fleck, 2019) Edible tooth gel is a dental product used with a toothbrush to clean and maintain teeth's health. (Rubido, 2014) It removes plaque and food, suppresses halitosis, and prevents tooth decay and gum disease. (Giampieri, 2012) NASA invented edible toothpaste in 1987 to allow astronauts to brush their teeth at pace. (Tipton, 2012) Early oral health care is recommended for children, starting during the neonatal period. (Jain, 2008)

2. Material and Methode :

2.1 Selection of plant:

The plant, *Vaccinium macrocarpon* is belonging to *Ericaceae* family. The extract was collected and provided by SV Agrofood, India, Pawane MIDC, Navi Mumbai- 400705, India. The research focused on the fruit part of *Vaccinium macrocarpon*, which contains active phytochemicals like flavan-3-ols, A-type procyanidins, anthocyanins, benzoic acid, and ursolic acid, which can inhibit tooth decay and periodontal diseases. (Blumberg, 2013)

2.2 Solubility Analysis:

Solubility analysis was done which included the selection of a suitable solvent, to dissolve the respective drug as well as various excipients used for the fabrication of Tooth gel. About 10 mg quantity of the extract sample was added into 5 mL of water and the solubility was noted after proper shaking.

2.3 Determination of pH:

pH of 1% solution of *Vaccinium macrocarpon* extract was taken by using a pH meter model of systronics system 361.

2.4 Identification of Drug:

The IR spectrum of extract and excipients was recorded using a Fourier Transform Infra-Red spectrophotometer (Shimadzu Corp, Japan). The extract and excipients were triturated and mixed thoroughly with IR grade KBr in 1:100 proportions and IR spectrum was recorded and compared with the reference standard infrared spectra.

2.5 Method of preparation:

Precisely measured herbal cranberry extract, powdered strawberry, xylitol was homogenised into water completely (Kaye, 2017). Subsequently, the solution above was mixed with xanthan gum and HPMC E15, causing it to swell overnight. It was then thoroughly agitated, followed by incorporation of propyl and methyl paraben.(Kinane, 2001) To guarantee even mixing, magnetic stirring was used to agitate it constantly for ten minutes. The gel is subjected to a sonicator in order to eliminate air bubbles. A high-speed homogenizer was used to homogenise the gel after that. Finally, saponin was added to create foam.(Koo, 2010; Lorena, 2022)

2.6 Factorial design:

A factorial design is a method used to evaluate multiple factors simultaneously, combining different levels of factors. Factors can be qualitative or quantitative, and their levels are assigned numerical values. Factorial experiments involved all combinations of all levels of factors. The effect of a factor is the change in response caused by varying the level(s). Optimization of pharmaceutical formulations is crucial to produce a mathematical model that describes responses. This involves preparing a series of formulations and varying ingredient concentrations. (Lachke, 2004)

2.6.1 Two-level full factorial designs:

A 2^3complete factorial design was employed in the study to evaluate the effect of certain factors on response. There are high and low values for each of the k components in this two-level arrangement. It was determined that viscosity, foaming power, and spread ability were all influenced by three key parameters. Cranberry extract (X1), strawberry powder (X2) and saponin (X3) were used as three independent variables (Table no.1). Other components of the formulation stayed the same, however the amounts of these variables were determined by preliminary research and observations. (Neha, 2022) Design Expert Software 7.0 was used to analyse the data.

Table no. 1: 2^3 Full factorial Design Layout, and Their combination

Sr. No.	Batches	Factor Levels with Combinations		
		X1	X2	X3
1	F1	40	10	6
2	F2	40	5	6
3	F3	40	5	3
4	F4	10	10	3
5	F5	10	5	6
6	F6	10	5	3
7	F7	10	10	6
8	F8	40	10	3

2.7 Evaluation of Formulated Tooth Gel:

2.7.1 General appearance:

The general appearance of a prepared tooth gel formulation was determined by observing its visual identity and overall “elegance” which is necessary for consumer acceptance and for indicating trouble-free manufacturing.

2.7.2 Stickiness and grittiness:

The texture of the tooth gel in terms of stickiness and grittiness was evaluated by visual inspection of the product after mildly rubbing the tooth gel sample between two fingers. (Ogboji J. Y., 2018)

2.7.3 Determination of pH: (Ogboji J. Y., 2018)

The pH of all the tooth gel was determined using a digital pH meter. 10 gm of the weighed formulation was dispersed in 10 mL of freshly boiled and cooled water (at 27°C) to make 50% aqueous suspension and the pH was noted using a pH meter. (Indian Pharmacopoeia 2014) Indian Standard (BIS) (2001).

2.7.4 Spread ability:

A glass plate with 1 g of formulated tooth gel in the centre was covered with another glass plate. A 1 kg weight was carefully placed on the setup for 10 minutes. The weight was removed, and the gel's diameter was measured in g.cm/sec. (Ogboji J. Y., 2018)

2.7.5 Viscosity study:

The viscosity of different tooth gel formulations was determined at 370 °C using a Brookfield viscometer. The viscosity was measured by using spindle T-96. (Khullar R.

2.7.6 Determination of moisture content:

5g of tooth gel was heated in a 105°C oven for 24 hours. It was allowed to cool before being reweighed. The heating and reweighing procedure was repeated until a consistent weight was recorded in successive checks. The weight loss was used to calculate the moisture content using the formula. (Ogboji J. Y., 2018)

$$\%\, Moisture = Original\ sample\ weight - Dry\ sample\ weight \times 100\ \% \quad \ldots\ldots\ldots (1)$$

2.7.7 Extrudability test (tube test):

It is usually an empirical test to measure the force required to extrude the material from the tube. The method adopted for tooth gel formulation for extrudability is based upon the quantity in the percentage of gel and gel extruded from aluminium collapsible tube on the application of weights in grams required to extrude at least 0.5 cm ribbon of tooth gel in 10 sec. The extrudability value was calculated using the following formula: (Ashara K. C. et al, 2014, Nikumbh K. V. et al, 2013).

$$Extrudability = Weight\ applied\ to\ extrude\ tooth\ gel\ from\ tube\ (gm.) / Area\ (cm^2) \ldots. (2)$$

2.7.8 Cleaning ability:

Eggshells, high in calcium, were used to test the cleaning ability of toothpastes. A hard-boiled egg was immersed in a solution of vinegar and red food colouring, resulting in a red stain. A line was drawn along the eggshell's length, and a toothbrush was used to brush one side of the egg for 10 strokes. After rinsing and shaking off the toothbrush, a pea-sized amount of toothpaste was applied, and the egg was washed and examined for colour removal. This process was repeated for each tooth gel tested. (Ogboji J. Y., 2018)(Majeed, 2016)

2.7.9 Gritty matter:

A small amount of tooth gel was rubbed onto butter paper. The number and intensity of scratches on the butter paper were recorded as either absent or present. (Ogboji J. Y., 2018)

2.7.10 Homogeneity:

At room temperature normal force was applied on the tube containing the tooth gel at room temperature and observed if the gel extrudes homogeneously from the tube. (Ogboji J. Y., 2018)

2.7.11 Determination of Fineness:

A. Determination of Particle Feel on Butter Paper

The gel, a suspension of 15-20 cm in length, was extracted from ten tubes, tested for particles, and then subjected to ultrasonic treatment and fineness tests.

B. Determination of Particle Size on 150-micron IS Sieve

A 250-mL beaker was filled with 20g of tooth gel, water, and allowed to stand for 30 minutes. The beaker was then placed in an ultrasonic bath and subjected to ultra-sonification for 10 minutes to loosen out the constituents. The suspension was transferred to a 150micron IS sieve, washed with tap water and a wash bottle, and dried in an oven at 105 ± 20C. The sieve was then dried to remove any remaining material.(Paul, 2017)

C. Determination of Particle Size on 75-micron IS Sieve

Weighed accurately about 20 g of the tooth gel and proceed as in B, used a 75-micron IS Sieve. Then residue on the sieved dried in an oven at 105 ± 2°C. (Indian Standard, 2001)

Calculation

$$\text{Material retained on } 150-\text{Micron IS Sieve, Percent by mass} = \frac{\text{M1} \times 100}{\text{M}} \ldots (3)$$

Where M1 = massing of residue retained on the sieve, and

M = massing of the material taken for the test.

2.7.12 Determination of foaming activity:

A 100mL glass beaker was filled with 5g of tooth gel, then given 10mL of distilled water and left to stand for 30 minutes. The slurry was transferred to a 250mL graduated cylinder, rinsed, and mixed with water. The cylinder was stirred to ensure uniform suspension, sealed, and shaken 12 times. After standing for 5 minutes, the volume of foam was calculated using the Indian Standard (2001).(Nemzer,2022)

$$Foaming\ ability = V1 - V2 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (4)$$

Where,

V1 = volume in ml of foam with water

V2 = volume in ml of water only

2.7.13 Determination of Antibacterial activity

The antibacterial activity was evaluated using the well diffusion method, which involves preventing the growth of microorganisms in a zone around a hole containing a solution of the tested material. The method involves mixing and shaking diluted inoculums of 24hour-old cultures of test organisms in Muller Hinton agar medium, pouring the media into sterilized Petri dishes, filling wells with toothpaste, using methanol as a negative control, and using an antibiotic (Amikacin) as a positive control.(Listgarten, 1986; Nayak, 2016)

2.7.14 Determination of Abrasiveness

A toothpaste sample was placed on a microscope slide, rubbed 30 times with a cotton swab, rinsed and dried, and examined using a dissecting microscope. The number of scratches on the slide's surface was counted and rated on a scale of 0 (no scratch) to 5 (major scratch).(Shaheena, 2019)

2.7.15 Stability

The ICH Guidelines were followed in a one-month accelerated stability study of formulated formulations, evaluating the effects of temperature, humidity, and time on the tooth gel's appearance.

3. Results and discussion:

3.1 Characterization:

3.1.1 Solubility Determination of *Vaccinium macrocarpon*:

The solubility of Vaccinium macrocarpon was assessed in both ethanol and water, yielding soluble results in both solvents.

3.1.2 FTIR Spectroscopy:

The IR spectra of *Vaccinium macrocarpon* extract powder were analysed for functional groups, revealing potential presence of Amine, Alcoholic, Nitro, and phenolic groups. Figure 1 displayed the FTIR spectra of the extract from *Vaccinium macrocarpon*. Characteristic peaks were seen in the FTIR spectra of the *Vaccinium macrocarpon* extract at 3305.99 cm^1 (O-H stretching), 2939.52 cm¯1 (C-H stretching), 1660.71 cm¯1 (C=O stretching), and 1232.51 cm¯1 (O-R Bending).

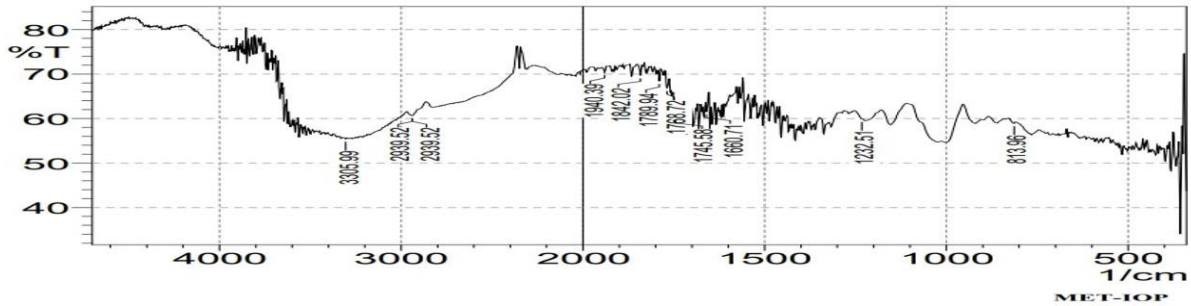


Fig. no. 1: FTIR of *Vaccinium macrocarpon* Extract

3.1.3 Minimum inhibitory concentration (MIC) study of Vaccinium macrocarpon Fruit extract:

3.1.3.1 Zone of inhibition study of Cranberry extract:

The study tested Cranberry extract with various concentrations using liquid dilution method. The minimum inhibitory concentration ranged from 31.25 mg/mL to 62.5 mg/ml. The MIC value indicated that the optimal concentration for optimum bacteria action should be between 31.5 mg/mL and 62.5 mg/ml. (Fig.2)

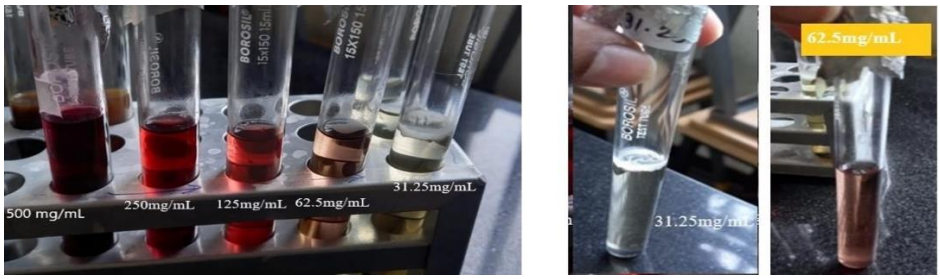


Fig. no. 2: (A) MIC of tooth gel (B) MIC range

3.2 Preparation of Tooth gel:

3.4 Criteria for optimization

The study analysed the effects of variables on responses in 8 batches designed by 2^3 factorial designs, resulting in a final optimized batch with desirability 1. The batch also predicted responses and compared predicted results with observed responses.

3.5 Optimization Study & Data Analysis of Tooth gel:

Fig. no.3: Experimental Design of the Optimization Step

3.5.1 Determination of pH

The pH of *Vaccinium macrocarpon* tooth gel was measured, with Formulation F1 being the closest to neutrality, and the pH of F1-F8 batches was considered. (Table no.2)

3.5.2 Stickiness and grittiness:

The formulations F4 to F7 showed less stickiness, whereas the formulations F8, F1 to F3 exhibited no such stickiness and smooth. (Table no.2)

3.5.3 Viscosity:

3.5.3.1 Analysis of variance (ANOVA): Viscosity

Final Equation in Terms of Actual Factors:

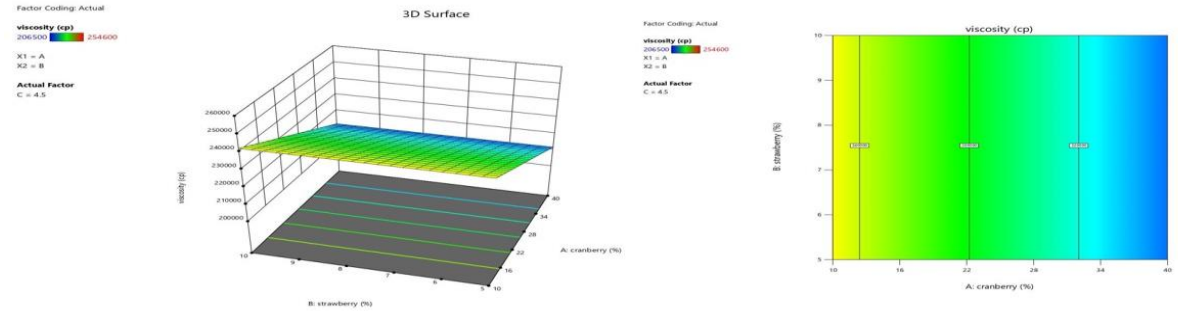
Viscosity = +2.52633E+05 -1018.33333 Cranberry……. (5)

3.5.3.1 ANOVA for selected factorial model

Response 1: Viscosity

The model's significance is determined by its F-value of 20.45, which is 0.40% likely to be noise. Significant model terms are less than 0.0500, with A being a significant term. P-values above 0.1000 are not important. Model reduction can improve the model by removing unimportant terms.

The Adjusted R^2 and Predicted R^2 show reasonable agreement, with a precision of less than 0.2. The signal-to-noise ratio is measured with a precision of ideally higher than 4, with a ratio of 6.395 suggesting sufficient signal. A 3D surface plot is used to determine the relationship between a response variable and two predictor variables. The 3D surface plot shows that the viscosity of the formulation increases with the concentration of strawberries and cranberries, with the amount of both significantly impacting the tooth gel formulation's viscosity.



(A) **(B)**

Fig. no. 4: (A) 3D Response Surface Plot showing the influence of Cranberry, Strawberry on the Viscosity of tooth gel. (B) Contour plot showing the influence of Cranberry, Strawberry on the Viscosity of tooth gel.

3.6 Analysis of variance (ANOVA): Spread ability:

Final Equation in Terms of Actual Factors:

Spread ability = Cranberry 10 +6.22500 Cranberry 40 +5.20000…………. (6)

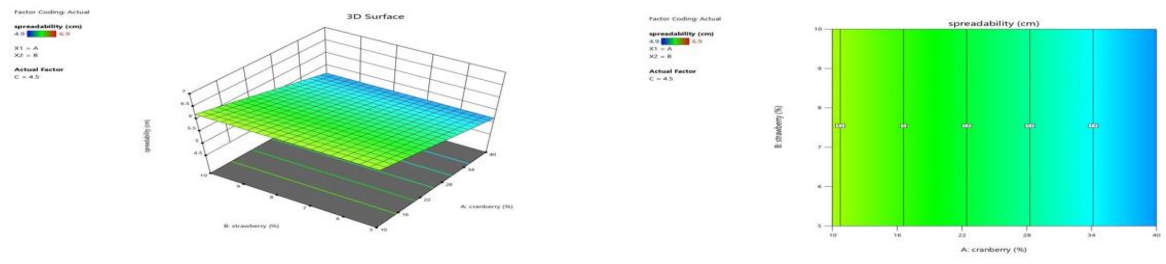
In this equation, the signs of Cranberry were positive, indicating that there was a direct relationship with spread ability. As the concentration of Cranberry increased, the spread ability increased.

3.6.1 ANOVA for selected factorial model

Response 2: Spread ability

The model's significance was determined by its F-value of 11.81, with a 1.39% chance of noise. Significant model terms were less than 0.0500, and A was a significant term. Model terms were not important if the value was greater than 0.1000. Model reduction could improve the model if there were many unimportant terms. The discrepancy between the Expected R^2 and Adjusted R^2 was greater than 0.2, indicating potential issues with the data or model. Confirmation runs were recommended for testing empirical models. The signal-to-noise ratio should have been higher than 4, and a 4.860 ratio indicated sufficient signal strength. This model could help navigate the design area effectively.

The tooth gel formulation's capacity to spread was significantly impacted by the percentage of strawberries and cranberries. The tooth gel formulation's spread ability decreased as the concentrations of cranberries and strawberries rose. The tooth gel formulation's capacity to spread was significantly impacted by the percentage of strawberries and cranberries. The tooth gel formulation's spread ability increased as the concentrations of cranberries and strawberries rose.



(A) **(B)**

Fig. no. 5: (A) 3D Response Surface Plot showing the influence of Cranberry, Strawberry on the Spread ability of tooth gel. (B) Contour plot showing the influence of Cranberry, Strawberry on the Viscosity of tooth gel.

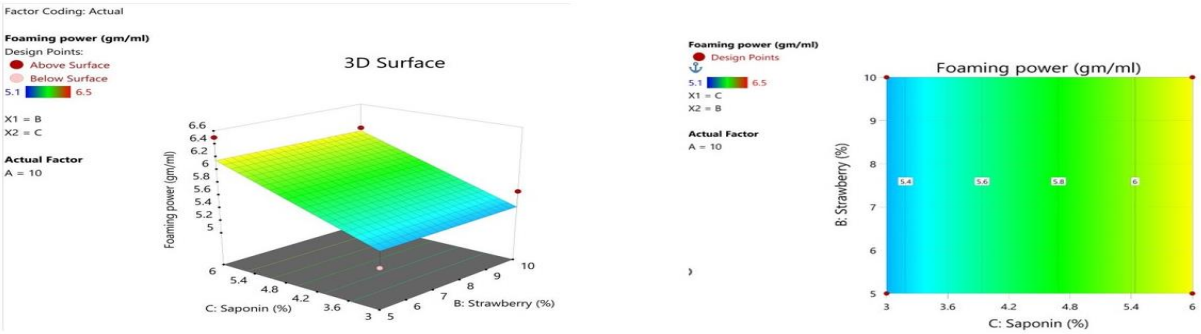
3.7 Analysis of variance (ANOVA): Foaming Power

Final Equation in Terms of Actual Factors:

$Foaming\ power\ = +4.55000\ + 0.266667\ Saponin$……………………… (7)

The model's significance was determined by its F-value of 15.36, which indicated a 0.78% probability of noise. P-values were considered significant when the model terms were less than 0.0500, with C being an important term. The Adjusted R^2 and Predicted R^2 were in agreement, with a difference of less than 0.2. The signal-to-noise ratio was measured with precision, with

a ratio of 5.543 indicating a strong signal. The amount of strawberry and saponin in the tooth gel formulation significantly affected its foaming strength, with an increase in the concentration of these ingredients.



(A) **(B)**

Fig. no.6: (A) 3D Response Surface Plot showing the influence of Strawberry, Saponin on the Foming power of tooth gel. (B) Contour plot showing the influence of Strawberry, Saponin on the Foaming power of tooth gel.

3.9 Evaluation of optimized batch:

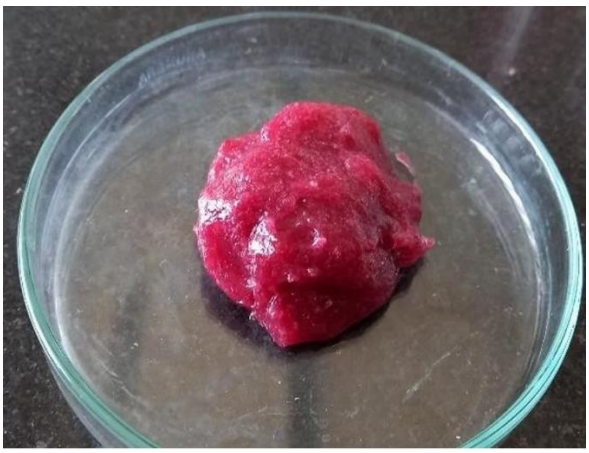


Fig. no. 7: Optimized batch of tooth gel

3.9.1 pH determination

pH of formulated tooth gel was determined by using Digital pH meter. The pH of formulated tooth gel was found to be 7.1±0.1 at room temperature.

3.9.2 Viscosity

The viscosity of tooth gel was measured using a Brookfield viscometer at different rpms, with its highest level at 2 rpm being 227175±100 cp, indicating its optimal performance.

3.9.3 Spread ability

The spread ability of the formulated tooth gel was determined with the help of spread ability apparatus and the tooth gel showed good spread ability as 5.2±0.5 cm.

3.9.4 Homogeneity

The tooth gel formulation was tested for homogeneity by rubbing it between fingers, and it was found to be uniform in nature, free of any foreign particles.

3.9.5 Wash ability

The optimized antibacterial tooth gel was observed to be easily washable and free from stain or bacteria after removal under running water.

3.9.6 Determination of Fineness

The fineness of a tooth gel was measured using a 150-micron IS sieve. The residue retained on the sieve was 0.001 grams, while the total mass of the material used was 1 gram. The percentage of material retained on the sieve was calculated to be 0.1 grams, indicating the proportion of particles larger than 150 microns, providing insight into the texture and particle size distribution of the tooth gel.

3.9.7 Determination of moisture content

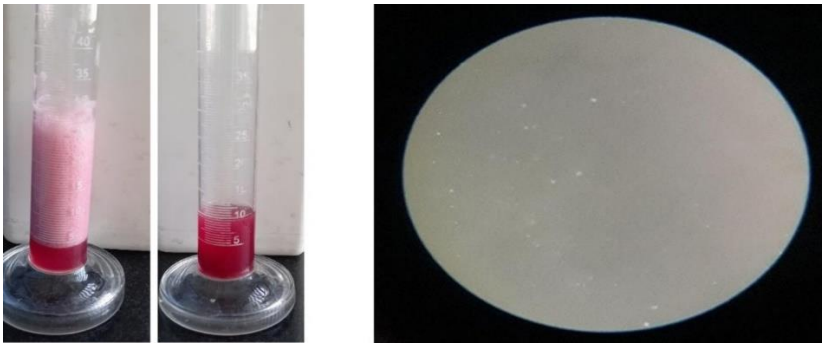
The moisture content of a tooth gel was calculated by weighing the product before and after drying, revealing that 30% of its original weight was moisture, calculated using a formula.

3.9.8 Determination of foaming activity

The foaming activity of a tooth gel was measured using a formula, resulting in a foaming ability of 6.1 millilitres. This indicates the gel's significant foaming capability, indicating its potential for a satisfactory oral hygiene experience. Further assessments may explore additional factors influencing the gel's foaming properties. (fig. no. 8)

3.9.9 Determination of Abrasiveness

The abrasiveness of formulated tooth gel was examined under a dissecting microscope and there are no scratches on the surface of the slide were found and rated on a scale of 0 (no scratches). (Fig no.8)



(A) **(B)**

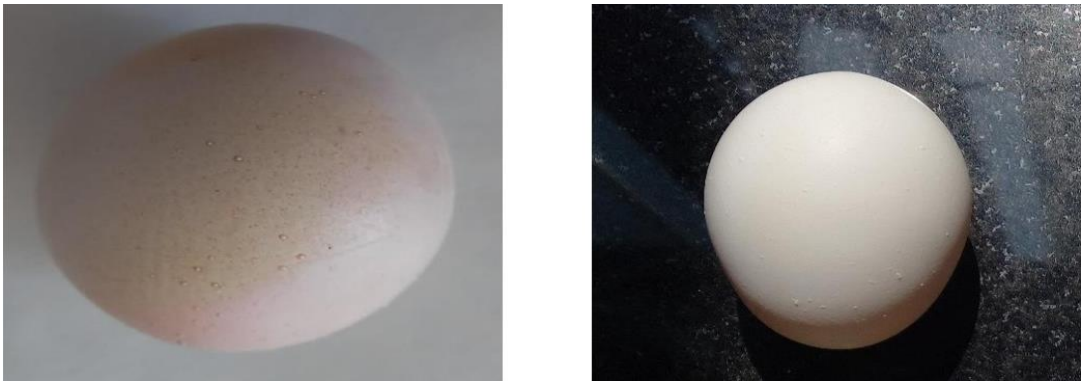
Fig. no. 8: (A) Foaming Power of Tooth gel. (B) Abrasiveness of Tooth gel

Table no. 2: The experimental factors in a factorial design along with their corresponding attributes such as stickiness, grittiness, Viscosity, Spread ability, Foaming Power, pH.

Batch	Factor1 Cranberry extract (%)	Factor 2 Strawberry powder (%)	Factor 3 Sponin (%)	Appearance	Stickiness	Grittiness	Viscosity (c.p)	Spread ability (g.cm/Sec.)	Foaming Power (gm./ml)	pH
F1	40 (+)	10 (+)	6 (+)	Thick	Non-Sticky	Smooth	218625 ± 15224.18	5.67±0.88	5.77±0.23	6.95±0.12
F2	40 (+)	5 (-)	6 (+)	Thick	Non-Sticky	Smooth	224175 ± 13415.5	5.9±0.71	5.95±0.43	6.77±0.26
F3	40 (+)	5 (-)	3 (-)	Thick	Non-Sticky	Smooth	232125 ± 20090.69	6.1±0.54	5.7±0.58	6.72±0.22
F4	10 (-)	10 (+)	3 (-)	Less Thick	Less-Sticky	Less-Smooth	242450 ± 11158.7	6.22±0.47	5.85±0.62	6.62±0.26
F5	10 (-)	5 (-)	6 (+)	Less Thick	Less-Sticky	Less-Smooth	235725 ± 19443.48	5.75±0.52	5.72±0.73	6.67±0.34
F6	10(-)	5 (-)	3 (-)	Less Thick	Less-Sticky	Less-Smooth	237966.66 ± 23171.6	5.73±0.64	5.46±0.63	6.76±0.35
F7	10 (-)	10 (+)	6 (+)	Less Thick	Less-Sticky	Less-Smooth	229650 ± 25667.97	5.6±0.84	5.65±0.77	6.75±0.49
F8	40 (+)	10 (+)	3 (-)	Thick	Non-Sticky	Smooth	211500 ± 0	5	5.1	7.1

3.9.10 Cleaning ability

The test on eggshell revealed that tooth gel effectively removed or cleaned 80% of stain eggs in single use. It indicating a high level of cleaning efficacy. (Fig. no. 9)



(A) **(B)**

Fig. no. 9: (A) Before using Tooth gel to brush the stained egg.
(B) After using Tooth gel to brush the stained egg.

3.9.11 Determination of Antibacterial activity

Antibacterial activity of tooth gel was carried out by well diffusion method. Thezones are appeared on *staphylococcus aureus* strains, A and A1, were tested for antimicrobial susceptibility. Results showed distinct zones of inhibition, with A showing a 9-millimeter zone and A1 showing a slightly larger 10-millimeter zone, suggesting differences in susceptibility or resistance profiles. (Fig. no. 10)

3.9.12 Normal Flora test

Tooth gel is safe and normal-based, but its impact on the GI tract is crucial for safety. A test was conducted to determine the formulation's effect on E. coli, and no zone was found to inhibit normal flora. (fig. no.10)

The effect of tooth gel (sample B) on normal flora (E. coli, control) was found that there is no inhibition of E. coli i.e. No zone found.

(A) **(B)** **(C)**

Fig no. 10 (A) & (C) Zone of Inhibition of Optimized Tooth Gel and (B) Normal Flora test of Optimized Tooth Gel.

3.9.13 Stability Studies

The optimized formulation showed stable performance under accelerated temperature and moisture conditions for 1 month. It maintained all properties at day 0, 15, and 30. When covered with aluminium foil and stored in collapsible tubes for 1 month, no significant changes were observed in texture, consistency, stickiness, grittiness, or pH. The formulation met the ICH guideline for 1 month of stability, demonstrating its suitability for oral use.

4. Conclusion:

The study explores the use of *Vaccinium macrocarpon* extract in tooth gel formulation, a patient-friendly dosage form that is safe for consumption and can be swallowed without systemic side effects. The natural, probiotic-loaded tooth gel is found to be effective for dental care and periodontitis, and can be used for prevention purposes to prevent dental health from infections and diseases. It is safe in the gastrointestinal tract if unintentionally swallowed.

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