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DEVELOPMENT AND EVALUATION OF FISH BONE–DERIVED HYDROXYAPATITE BASED REMINERALIZING TOOTHPASTE

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ABSTRACT

The main aim of the present work was to develop a remineralizing toothpaste using hydroxyapatite derived from tuna fish bone as the key active ingredient to promote enamel remineralization and improve oral health. Hydroxyapatite is the major inorganic component of tooth enamel and plays an important role in repairing enamel defects, reducing demineralization, and enhancing tooth strength. Fish bone-derived hydroxyapatite is a natural, biocompatible, and cost-effective alternative to synthetic hydroxyapatite and supports sustainable utilization of biological waste. Hydroxyapatite was prepared from tuna fish bone by cleaning, alkaline treatment with sodium hydroxide, drying, and calcination in a muffle furnace, followed by grinding and sieving to obtain fine powder. The prepared hydroxyapatite was characterized using X-ray diffraction (XRD) and Fourier Transform Infrared Spectroscopy (FTIR) to confirm its composition. The toothpaste was prepared by incorporating hydroxyapatite along with suitable excipients such as carboxymethyl cellulose, sodium lauryl sulphate, glycerine, xylitol, sodium benzoate, peppermint oil, and distilled water. The prepared toothpaste formulations were evaluated for organoleptic characteristics, pH, viscosity, homogeneity, spreadability, and antimicrobial activity. The results showed that all formulations exhibited acceptable organoleptic properties, neutral pH suitable for oral use, good viscosity, proper spreadability, and uniform consistency. To confirm the remineralization potential of the developed toothpaste, Scanning Electron Microscopy (SEM) analysis was conducted on tooth samples. The SEM images demonstrated surface changes

indicating the deposition of hydroxyapatite crystals and partial restoration of the enamel surface, confirming the remineralization effect of the formulated toothpaste.

KEYWORDS: Toothpaste, Hydroxyapatite, Fish bone, SEM, Remineralzation, FTIR, XRD.

INTRODUCTION

Teeth

Teeth play a fundamental role in essential physiological functions such as mastication, speech, and facial aesthetics. As highly mineralized structures, they are designed to withstand significant mechanical forces while maintaining structural integrity throughout life. Each tooth is composed of three major hard tissues—**enamel**, **dentin**, and **cementum**—and a soft connective tissue called the **pulp**, which contains nerves, blood vessels, and cellular components critical for tooth vitality. Enamel, the outermost layer, is the hardest tissue in the human body and primarily consists of tightly packed hydroxyapatite crystals. Beneath the enamel lies dentin, a less mineralized but resilient tissue that provides elasticity and supports the enamel. The root surface is covered by cementum, which anchors the tooth to the surrounding alveolar bone through the periodontal ligament. Despite their durability; teeth are continuously exposed to chemical, microbial, and mechanical challenges within the oral environment. Among these challenges, **dental caries**—commonly known as tooth decay—is one of the most widespread chronic diseases affecting children and adults globally. The primary etiological agents are acidogenic and acid uric bacteria, particularly *Streptococcus mutans* and *Lactobacillus* species, which metabolize fermentable carbohydrates to produce organic acids. These acids diffuse into the enamel, causing a progressive loss of mineral content known as **demineralization.** If this process exceeds the natural repair mechanism—**remineralization**, mediated by sal**i**va and minerals such as calcium, phosphate, and fluoride—micro structural damage advances. All of our teeth serve an important role, and it is crucial to take proper care of them in order to chew, bite and tear in a normal manner.

There are four different types of teeth: incisors, canines, premolars and molars.

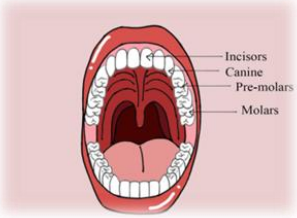


Fig. No. 1: DIFFERENT TYPES OF TEETH.

Root

The root is the part of the tooth embedded in the jawbone that holds the tooth in place. It includes the root canal, cementum, periodontal ligament, nerves, blood vessels, and alveolar bone.

Neck

The neck (dental cervix) is the region between the crown and root. It forms the junction of enamel and cementum and includes the gums, pulp, and pulp cavity.

Crown

The crown is the visible part of the tooth. It consists of enamel (outer protective layer) and dentin (inner mineralized layer providing support).

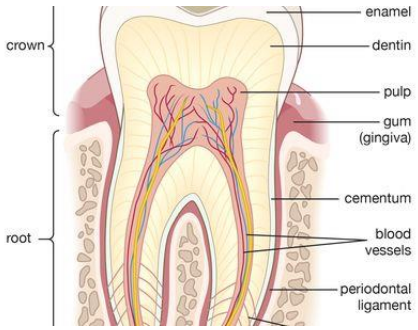


Fig. No. 2: STRUCTURE OF TEETH.

Dental caries

Dental caries is influenced by poor oral hygiene, low saliva flow, frequent sugary food intake, and limited dental care. Prevention includes proper brushing, healthy diet, professional dental care, and use of fluoride or hydroxyapatite toothpaste to promote enamel remineralization.

Oral Health

Oral health is essential for overall well-being and quality of life. Conditions like dental caries, plaque, and gum disease are common. Good oral hygiene, proper diet, and preventive care help maintain healthy teeth and gums.

Toothpaste

Toothpaste is widely used with a toothbrush to remove plaque, food particles, and stains. It contains abrasives, humectants, surfactants, flavoring agents, and active ingredients such as fluoride to help prevent dental caries and maintain oral hygiene.

Hydroxyapatite Toothpaste

Hydroxyapatite toothpaste contains a mineral similar to natural tooth enamel. It helps remineralize enamel, repair micro-defects, reduce tooth sensitivity, and improve oral health. It is a safe and biomimetic alternative to fluoride.

MATERIALS AND METHODS

5. METHODOLOGY

5.1 The materials used

Table No. 1: List of material.

SL.NO	NAME	SUPPLIER
1.	Carboxy Methyl Cellulose	NICE CHEMICALS(P)LTD
2.	Sodium Lauryl Sulphate	NICE CHEMICALS(P)LTD
3.	Xylitol	MOLYCHEM
4.	Hydroxyapatite Powder	Obtained from tuna fish bone
5.	Glycerine	NICE CHEMICALS(P)LTD
6	Sodium Benzoate	NICE CHEMICALS(P)LTD
7	Peppermint Oil	MOLYCHEM
8	Distilled Water	LAB

METHOD

5.3.1 COLLECTION OF MATERIALS

Tuna fish samples were collected from the local market.

Fig. No. 3: TUNA FISH.

5.3.2 PREPARATION OF HYDROXYAPATITE FROM TUNA FISH BONE

Tuna fish (*Thunnus albacares*) were collected from the local market. The fish bones were separated, cleaned, and thoroughly washed. The cleaned bones were then boiled in water for 2 hours to remove excess flesh. After boiling, the bones were soaked in 0.1% NaOH solution for 3 hours. They were subsequently washed with distilled water to remove residual NaOH and to adjust the pH to neutral (pH7). The muffle furnace was preheated to 100 °C, after which the dried bones were transferred into a crucible and placed inside the furnace. The temperature was gradually increased at a rate of 10 °C every 5 minutes until 150 °C and maintained at this temperature for 5 minutes. The temperature was then further increased gradually up to 900 °C for calcination, after which the furnace was switched off and allowed to cool naturally. After cooling, the calcined bones were transferred to a mortar and triturated to reduce particle size. The resulting powder was sieved through an 80-mesh sieve to obtain a uniform particle distribution. The tuna fish bone powder was finally analysed using X-ray diffraction (XRD) and Fourier transform infrared spectroscopy (FTIR) to confirm the presence of hydroxyapatite.

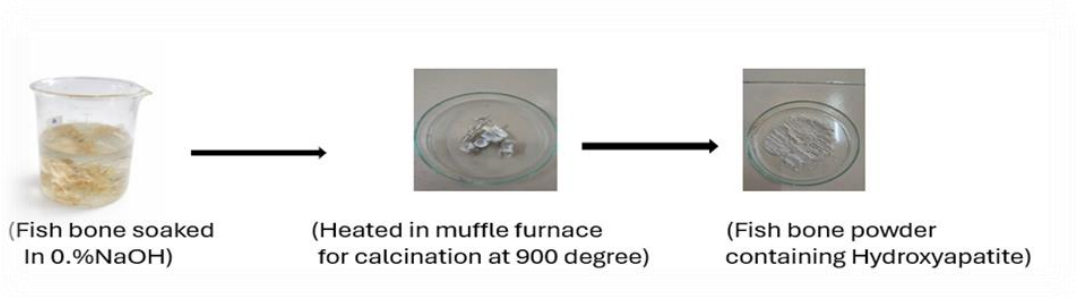


Fig. No. 4: PREPARATION OF HAP FROM.

FISH BONE

FTIR SPECTRUM INTERPRETATION

FTIR spectrum of sample

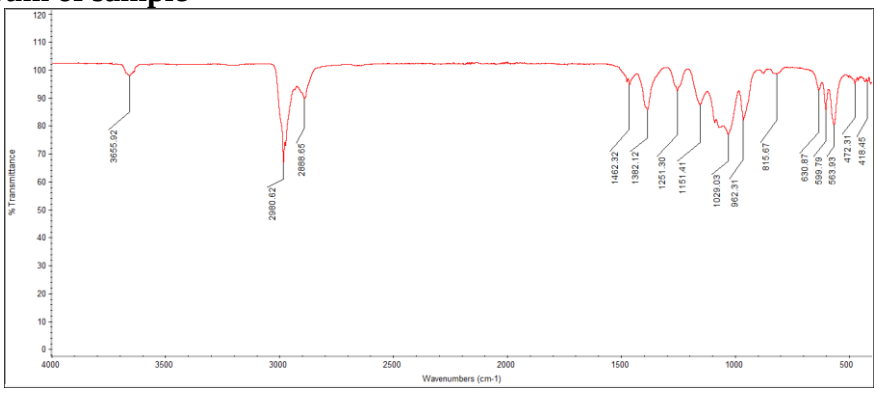


Fig. No. 5: FTIR spectrum of sample.

FTIR spectrum of Hydroxyapatite

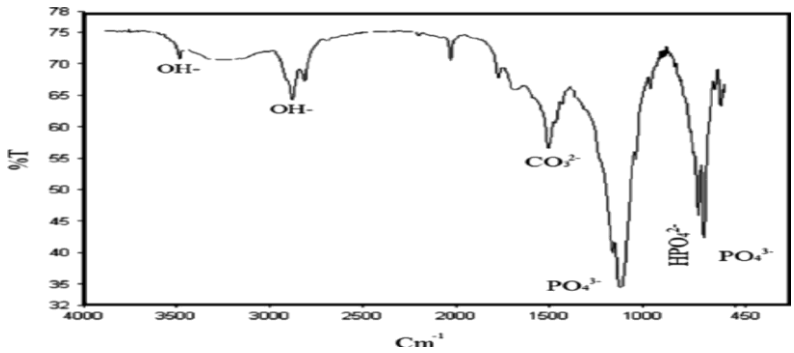


Fig. No. 6: FTIR Spectrum of Hydroxyapatite.

Characteristic FTIR Peaks of Hydroxyapatite

Table No. 3: Charactistic FTIR peaks.

Functional group	Wavenumber (cm^{-1})	Presence
O–H stretching	~3570–3600	Hydroxyl group
O–H libration	~630	Hydroxyl vibration
PO_4^{3-} stretching	~960–1100	Phosphate group
PO_4^{3-} bending	~560–605	Phosphate bending
CO_3^{2-} (if carbonate substituted)	~1410–1460	Carbonate group

Peaks Observed in the Sample Spectrum

- 3665 cm^{-1} → O–H stretching
- 2980 & 2888 cm^{-1} → C–H stretching (possible organic contamination or adsorbed molecules)
- 1462 cm^{-1} → CO_3^{2-} carbonate band
- 1382 cm^{-1} → carbonate vibration
- 1251–1151 cm^{-1} → phosphate stretching
- 1029 cm^{-1} → PO_4^{3-} asymmetric stretching
- 962 cm^{-1} → PO_4^{3-} symmetric stretching
- 815 cm^{-1} → phosphate vibration
- 630 cm^{-1} → O–H libration vibration (hydroxyapatite characteristic)
- 599 & 563 cm^{-1} → PO_4^{3-} bending modes
- 472 & 418 cm^{-1} → phosphate lattice vibration

Comparison between sample and standard hydroxyapatite

Table No. 4: Comparison between sample and standard hydroxyapatite.

Hydroxyapatite Peak	Sample Peak	Interpretation
~3570 cm^{-1}	3665 cm^{-1}	O–H stretching present
~630 cm^{-1}	630.87 cm^{-1}	O–H libration vibration confirmed
~1020–1100 cm^{-1}	1029 cm^{-1}	PO_4^{3-} stretching
~960 cm^{-1}	962 cm^{-1}	PO_4^{3-} symmetric stretch
~560–600 cm^{-1}	563 & 599 cm^{-1}	PO_4^{3-} bending
~1410–1460 cm^{-1}	1462 cm^{-1}	CO_3^{2-} substitution

OBSERVATION

All the major functional group peaks of hydroxyapatite (OH^- and PO_4^{3-}) are present in the sample spectrum. Minor peaks around 2980–2888 cm^{-1} suggest possible organic residues or adsorbed impurities.

RESULT

Hence the FTIR spectrum of the sample closely matches the characteristic peaks of Hydroxyapatite, confirming the presence of hydroxyapatite in the sample. The presence of carbonate bands indicates carbonate-substituted hydroxyapatite.

XRD INTERPRETATION

XRD Peaks of Standard Hydroxyapatite

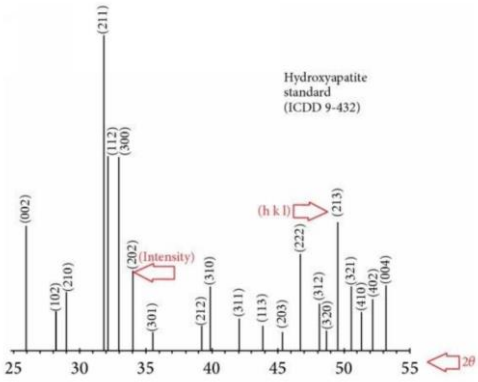


Fig. No. 7: XRD Peak.

Standard XRD Peaks of Hydroxyapatite

The characteristic diffraction peaks of hydroxyapatite occur at the following **2θ values (Cu Kα, λ = 1.5406 Å)**:

Table No. 5: XRD.

2θ (°)	Plane (hkl)
~25.9	(002)
~31.7	(211)
~32.2	(112)
~32.9	(300)
~34.0	(202)
~39.8	(310)
~46.7	(222)
~49.5	(213)
~53.1	(004)

The most intense peak normally appears around **31–33°** corresponding to the **(211), (112), and (300)** planes.

XRD Peaks of Sample

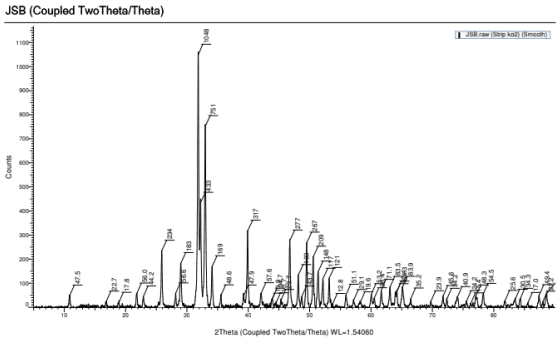


Table No. 6: XRD.

SL.NO	NAME	SUPPLIER
1.	Carboxy Methyl Cellulose	NICE CHEMICALS(P)LTD
2.	Sodium Lauryl Sulphate	NICE CHEMICALS(P)LTD
3.	Xylitol	MOLYCHEM
4.	Hydroxyapatite Powder	Obtained from tuna fish bone
5.	Glycerine	NICE CHEMICALS(P)LTD
6	Sodium Benzoate	NICE CHEMICALS(P)LTD
7	Peppermint Oil	MOLYCHEM
8	Distilled Water	LAB

The **strongest peak occurs around 31–33°**, which matches the standard hydroxyapatite peak.

Comparison Between Standard and Sample

Table No. 7: Comparison between standard and sample.

Standard Peak	Sample Peak	Observation
25.9°	~25.8°	Match
31.7°	~31.7°	Strong peak match

32.2°	~32.1°	Match
32.9°	~32.9°	Match
34°	~34°	Match
39.8°	~39.8°	Match
46.7°	~46.7°	Match
49.5°	~49–50°	Match
53°	~53°	Match

Interpretation

- The major diffraction peaks of the sample coincide with the standard peaks of hydroxyapatite.
- The intense peak around 31–33° confirms the hexagonal apatite crystal structure.
- The presence of several sharp peaks indicates good crystallinity of the sample material.
- No additional strong peaks from other calcium phosphate phases (such as β-TCP or CaO) are observed.

RESULT

The XRD pattern of the sample matches well with the standard pattern of Hydroxyapatite confirming that the sample successfully contains crystalline hydroxyapatite.

PREPARATION OF TOOTHPASTE

10g of carboxymethyl cellulose were taken in a mortar, and 9 mL of distilled water was added. The mixture was triturated until a uniform gel consistency was obtained. Separately, 3 g of sodium lauryl sulphate was taken in a beaker, and 8 mL of glycerine was added and mixed thoroughly to form a smooth paste. This paste was then transferred into the mortar containing the carboxymethyl cellulose gel and mixed well. Subsequently, 0.3 g of xylitol and 0.5 g of sodium benzoate were added and mixed uniformly. Hydroxyapatite powder was then incorporated in varying quantities (5 g, 10 g, and 15 g). Two drops of peppermint oil were added, and the final weight was adjusted to 30 g using distilled water. The prepared formulation was finally packed in an airtight container.[15]

Fish bone-derived toothpaste was prepared according to the formula and composition as outlined in table no.8

Table No. 8: Formulation of toothpaste.

INGREDIENTS	F1	F2	F3
Hydroxyapatite	5g	10g	15g
Carboxy Methyl Cellulose	1.2g	1.0g	0.8g

Sodium Lauryl sulphate	1.5g	1.5g	1.5g
Sodium Benzoate	0.1g	0.1g	0.1g
Xylitol	1g	1g	1g
Glycerine	8ml	7ml	6ml
Peppermint oil	0.15ml	0.15ml	0.15ml
Distilled Water	q.s to 30g	q.s to 30g	q.s to 30g
Total weight	30g	30g	30g

EVALUATION OF TOOTHPASTE

The toothpaste was assessed based on the specified parameters, following the standard evaluation procedure meticulously.

Organoleptic evaluation

The toothpaste obtained underwent scrutiny for its organoleptic characteristics, including colour, odour and consistency through visual observation and touch.[16]

Determination of pH

pH measurement was done using a pH meter, which is calibrated by using buffer phosphate solution with pH 10, buffer phosphate pH 7.0 and buffer phosphate pH 4.0 by dipping pH electrodes into the buffer solution. Once calibrated, the pH measurement of the distilled water is ready to use. Weigh 1 gram of hydroxyapatite toothpaste (HAp) from the bones of the tuna and dilute it with 10 ml of distilled water. Dip the pH electrodes into the prepared paste that has been diluted until the needle on the monitor shows a stable number, and read the pH on the monitor.[17]

Viscosity

An approximate amount of each formulation is transferred to a beaker, where the viscosity of the formulation is evaluated using a Brookfield Viscometer with spindle number S64 at 10 rpm.[18]

Homogeneity Test

Weigh 0.1g of toothpaste and then placed between two layers of a glass slide to observe homogeneity. If there were no coarse grains on the glass slide, then the toothpaste was declared homogeneous, while the presence of coarse grains indicates that the toothpaste was not homogeneous.[19]

Foaming test

The test for Foam formation was carried out by preparing a solution of 1% of each of the concentrations of hydroxyapatite (Hap) toothpaste from tuna bones, dissolving them in water. The solute was poured into a measuring glass and covered with aluminium foil. The solute was then shaken, and foam was formed. Then, the height of the foam was measured at 0 minutes to 5 minutes.[20]

Spreadability test

The common method for measuring the spreadability is the parallel-plate method that has many variations. During the measurement using the parallel-plate method, 0.2g of toothpaste prepared for the test is placed between two glassslides of uniform length. A weight of 100g is placed on the top of the slide for 1 minute. Then the diameter of the toothpaste between the plates is measured.[21]

In this case, spread ability is determined by the formula

$Si = d2 \times \pi/4$

Where, Si- Spreading area (mm2) depending on mass.

d- Spreading area diameter (mm).

Microbial limit test

Antimicrobial activity is determined by the cup plate method. Agar plates were inoculated with a standardised inoculum of the test microbes (Staphylococcus aureus) and incubated at 37 °C. Then, filter paper disks containing the formulation and the standard were aseptically transferred to the petridishes using forceps. The petridishes were incubated under suitable conditions for 24 hours, and the zone of inhibition was measured in millimetres.[22]

Enamel remineralization assessment

The remineralization potential of the HAP toothpaste will be evaluated via SEM to observe morphological changes on the enamel surface.

RESULTS AND DISCUSSION

Preparation of hydroxyapatite based remineralizing toothpaste

Three formulation of hydroxyapatite based remineralizing toothpaste: F_1, F_2 and F_3 were prepared.

valuation Tests

All the prepared formulations (F_1, F2 and F3) of polyherbal cream were subjected for the following evaluation parameters.

Organoleptic Evaluation

Sl.No.	Evaluation Parameters	Observations		
		F1	F2	F3
1.	Visual appearance	White	White	White
2.	Texture	Smooth	Smooth	Smooth
3.	Odour	Characteristic	Characteristic	Characteristic

All five formulations of polyherbal cream were evaluated for physical evaluation and the results were shown in the Table No 9.

6.2.2 Evaluation of pH

Table No. 10: Evaluation Of Ph.

Sl.No.	Formulations	pH value
1.	F1	7.08
2.	F2	7.09
3.	F3	7.12

The pH of all the three formulations was recorded by digital Ph meter and the results of pH were shown in Table no: 10.

6.2.3 Viscosity

Brookefield viscometer was used to measure the viscosity of each of the three antiageing cream formulations. The result of viscosities is shown in the Table No 11.

Table No. 11: Evaluation Of Viscosity.

Sl.No.	Formulations	Viscosity(cps)
1.	F1	2148
2.	F2	3671
3.	F3	4751

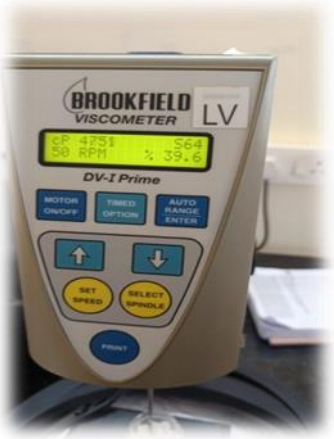


Fig. No. 10: Test for viscosity.

Viscosities of all the formulations were noted and found in the range of 2148-4751 cps at 10 rpm as shown in table no:11. All the formulations were having acceptable range of viscosities.

Homogeneity test

All the formulations were found to be homogeneous and free from grittiness. This was confirmed by visual examination and by touch.

Foamability test

The toothpaste formulation exhibited **excellent foaming ability**, producing a foam height of 4.5-**7 cm**, indicating good surfactant activity and effective cleansing property.

Table No. 12: Foamability test.

Sl no.	Formulations	Foaming height(cm)
1.	F_1	4.5 cm
2.	F_2	6cm
3.	F_3	8cm

Fig. No. 11: Foaming test.

Spreadability test

Table No. 13: Spreadability studies.

Formulation	Diameter (cm)	Time (sec)	Spreadability (mm^2)
F1	4.5	300	1589.625
F2	4	300	1256
F3	3.4	300	907.46

Spreadability of the formulations is shown in table no:13

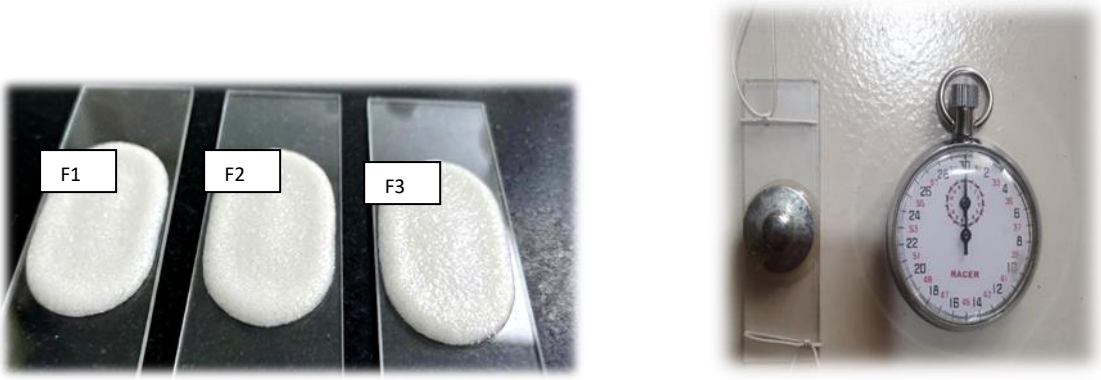


Fig No. 12: Spreadability test.

All formulations are spreadable and F1 has higher spreadability.

Antimicrobial activity

Optimised formulation F1 was selected and subjected to microbial study by using cup plate method and zone of inhibition was determined.

Fig No. 13: ZONE OF INHIBITION.

Optimised formulation F1 was selected and subjected to microbial study by using cup plate method and zone of inhibition was determined.

Table No. 14: Zone of inhibition.

Formulation	Zone of inhibition
F1	12
Standard	16

The antimicrobial test demonstrated significant activity against acne causing bacteria(Staphylococcus aureus) and found the zone of inhibition for the formulation F1 is close to that of marketed formulation.

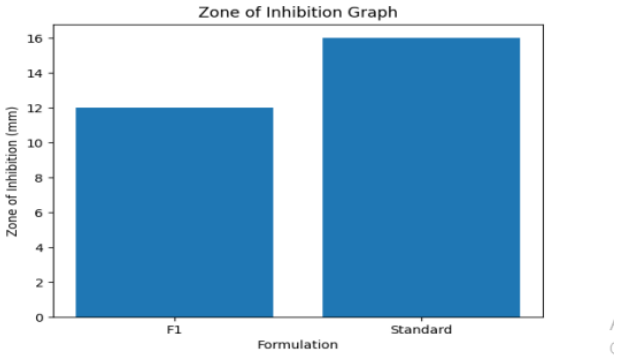
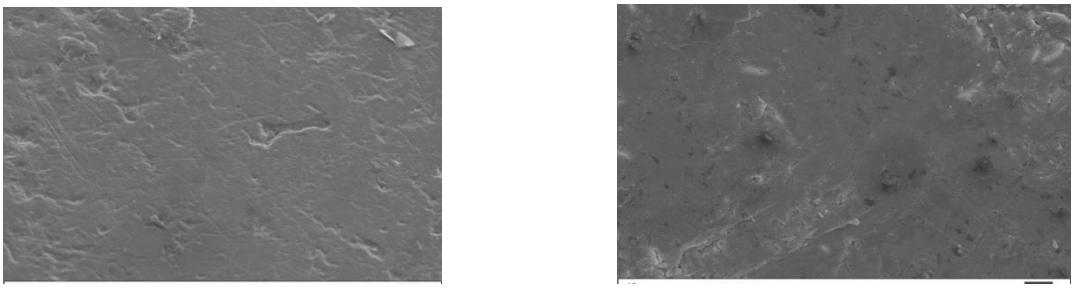
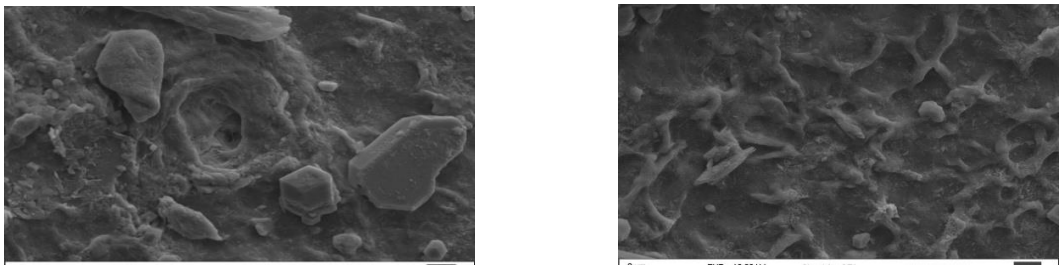


Fig. No. 14: Zone of inhibition graph.

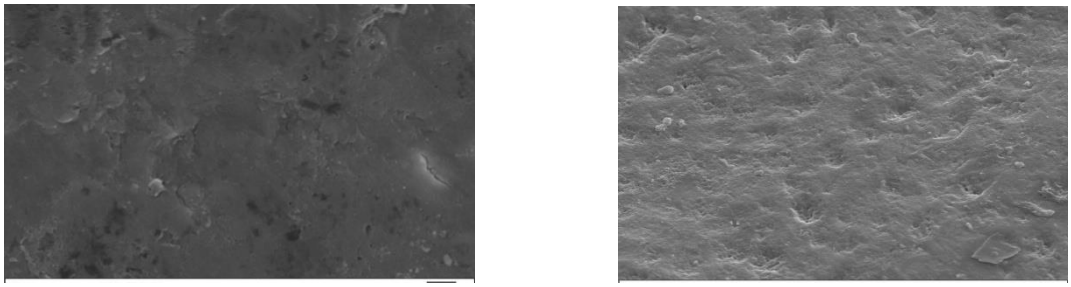
Enamel Remineralization assessment using SEM test



SEM image of Normal toothpaste



SEM image of demineralized toothpaste



SEM image of remineralized toothpaste

Fig. No. 15: Evaluuation test for remineralization.

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