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FORMULATION AND CHARACTERIZATION OF BROMELAIN ENRICHED SINGLE USE SOAP FOR SKIN REJUVENATION

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Abstract:

This study focuses on the formulation of single use soap for skin rejuvenation utilizing bromelain extract of Ananas comosus. The extract has measurable antioxidant and proteolytic activity. Ananas comosus contain variety of phytochemicals like saponins, tannins, steroid, flavonoids, terpenoids, phenols, alkaloids, naphthoquinone, inulin, amino acid, proteins and polyphenols, which contribute to its nutritional and medicinal value. The enzyme based herbal formulation provide a safer and eco-friendly, alternative to synthetic soap that may cause skin irritation and dryness. The collected fruit was thoroughly washed, cut and ground using a grinder followed by extraction using alcohol with centrifugation process. The centrifugation was done for 15 minutes at 3500 rpm resulting extract was then dried using desiccator. The dried extract integrated into single use soap formulation containing sodium hydroxide, stearic acid, glycerine, alcohol, propylene glycol, cocamido propyl betaine, cocodiethanol amide, methyl cellulose, carrier oils and some colouring agent and flavouring agent to enhance the visual appearance and improve its pleasant aroma and overall consumer acceptability. Bromelain which provides a gentle enzymatic exfoliation to sweep away dead skin cells without the harshness of physical scrubs. The standardized physical dimensions of soap tablet (8 mm diameter and 5 mm thickness) ensure uniformity, dose precision, and convenience for single-use applications, minimizing the risk of contamination and preserving enzyme stability. The soap was evaluated for colour, odour, texture, pH, washability, lather, foam stability, total fatty matter, saponification, dirt dispersion, alcohol insoluble matter. All evaluation parameter conducted were found to be within the standard range confirming the reliability and effectiveness of the formulation. The present study successfully concludes that the bromelain enriched single use soap is an effective and safe formulation for skin rejuvenation. Bromelain helped in gently removing dead skin cells and improved skin. The single use soap was easy to use, hygienic and prevented contamination. It was travel-friendly, light weight and easy carry. Since it was eco-friendly and free from toxic chemicals, it was safe for both skin and environment. Overall, this soap was a convenient, safe and a better option for daily skin care.

Key words: *Ananas comosus, single use soap tablets, enzymatic exfoliation, skin rejuvenation, antioxidant, proteolytic, bromelain.*

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INTRODUCTION:

Ananas comosus (L.) Merr., is a well -known and widely grow species in the family Bromeliaceae, has substantial health values from peel, fruit, crown. The fruit is also known by the name of Pinna. It has a several medicinal uses include diuretic, immune boosting, anti-aging and anti- oxidant. It is used to sea sickness and also it supports nutrient absorption. It has stress- resistant effect. (1)

The extract contains a variety of phytochemicals like: saponins, tannins, steroids, flavonoids, terpenoids, phenols and alkaloids. The fruit also has naphthoquinone inulin, amino acid, proteins and polyphenols which contribute to its nutritional and medicinal value. Phytosterols and cardiac glycosides, which have health related benefit. Pineapple also contain bromelain, an enzyme known for its anti- inflammatory and digestion supportive.(2)

The bromelain alcohol extract prepared by centrifugation method was used to prepare single use soap. The extract was found to show presence of active protease enzyme and showed low but dose-depended antioxidant activity.

MATERIALS AND METHOD:

Collections and authentication

Fig 1*: Ananas comosus*

Fig 2: Fresh fruit

Fig 3: Fruit slices

The pineapple was collected from a plant of *Ananas comosus* (L.) Merr. in a rural area of Idukki during the month of early October 2025. The fruit of the *Ananas comosus* (L.) Merr. was authenticated by Dr. Rogimon P Thomas, Associate Professor & Head Department of Botany, CMS College Kottayam (Autonomos), Kerala, India.

EXTRACTION METHOD(3)

The crown was separated from pineapple fruit. The fruit was washed thoroughly with distilled water to remove the dirt and impurities. The fruit was cut into small pieces and blended to obtain pulp. The mashed pulp was filtered using muslin cloth and the filtrate was collected. Ethanol was added to the filtrate in a ratio of 1:4 (filtrate: ethanol). The mixture was gently mixed and stored at 4-5°C for 8 hours to allow precipitation of bromelain. After incubation, the mixture was centrifuged at 3500 rpm for 15 minutes. The precipitate, which contain crude bromelain enzyme was collected. The collected precipitate was dried at 30 ± 0.5°C until a dry crude bromelain was obtained. The dried crude bromelain was stored at 4°C in an airtight container.

Fig 4: Centrifuge Apparatus


Fig 5: crude bromelain extract

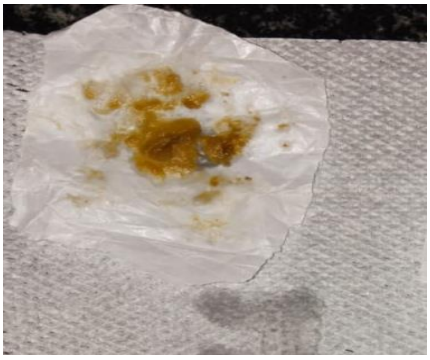


Fig 6: Bromelain extract

IDENTIFICATION OF BROMELAIN

I.Organoleptic Evaluation

Organoleptic evaluations are done for the extracts colour, odour texture also the confirmatory test was done which include gelatine liquefaction test, Milk clotting test and Spectral scanning of bromelain content.

II.Confirmatory Test:

Gelatine liquefaction Test

A gelatine solution was prepared and allowed to solidify. A few drops of the bromelain extract were then added to the solidified gelatine and incubated at room temperature or at 37 °C. After incubation, the gelatine became soft or liquefied, which indicated the presence of proteolytic activity and confirmed bromelain activity

Milk Clotting Test

In the milk clotting test, the bromelain extract was added to warm milk maintained at 37 °C. The formation of milk curdles was observed, which further confirmed the proteolytic activity of bromelain

III.Spectral Scanning of Bromelain content (4)

A bromelain enzyme solution of the same concentration was prepared using 96% ethanol as the solvent. The required amount of sample was weighed and dissolved until a homogeneous solution was obtained. The solution was then magnetic stirred for 15 minutes at room temperature (25–30 °C) to improve bromelain extraction. Before analysis, the UV–Visible spectrophotometer was calibrated using 90% ethanol as the blank. The prepared sample was transferred into a 1 mL quartz cuvette with a 1 cm optical path length, and scanning was carried out over a wavelength range of 200–800 nm to identify the characteristic absorption peak of bromelain. The bromelain content was determined based on the maximum wavelength (λmax) and the corresponding absorbance value.

IV.Determination of Protease Activity(3)

Protease activity of bromelain is measured by its ability to hydrolyse casein into tyrosine and other amino acids. The amount of tyrosine released is measured spectrophotometrically at 275 nm. Tyrosine standard solutions of 0, 20, 40, 60, 80, and 100 µg/mL (S1-S6) were prepared, and their absorbance values were measured to construct a standard calibration curve. Equal volumes of casein solution (1 mg/mL) and crude bromelain extract (10 mg/mL) were mixed and incubated at room temperature for 30 minutes. The reaction was terminated by adding trichloroacetic acid (TCA), followed by incubation at 90 ± 0.5 °C for 5 minutes. After cooling, the supernatant was collected, and its absorbance was measured at 275 nm. The concentration of tyrosine released was determined from the standard curve, and protease activity was calculated in units per milligram (U/mg) of extract. Protease activity was calculated by equation(5):

Protease Activity (U/mg) = µmol produced tyrosine / (mg extract × time (min)

V.DPPH Free Radical Scavenging Assay(6)

Sample stock was prepared by dissolving 50mg of sample in 1ml of distilled water to form 50mg/ml stock solution. This stock was used for further assay. The free radical scavenging activity of the sample was evaluated by assessing its ability to scavenge DPPH radicals. A DPPH reagent solution was prepared by mixing 100 µl of DPPH (1 mM) with 100 µl of methanol (98%). Test solutions were prepared by adding varying volumes of the sample (T1=20 µl, T2=40 µl, T3=60 µl, T4=80 µl, T5=100 µl) to the DPPH reagent solution, adjusting the final volume to 300 µl. A control sample was prepared using solvent alone, and ascorbic acid (10mM) served as the standard. After 30 minutes of incubation in the dark at room temperature, the absorbance was recorded at 515 nm. Antioxidant activity was indicated by a colour change from purple to yellow or colourless, depending on the level of radical scavenging. The IC50 value was determined from the dose-response curve.

Radical scavenging activity % = (Control OD-Test OD) ×100/ Control OD

FORMULATION OF SOAP(7)

Table 1: formulation of soap

Ingredients	**Working formula g/ml for 15 ml**
Sodium hydroxide	0.65 g
Coconut oil	3 ml
Stearic acid	1.5 g
Glycerine	0.4 ml
Alcohol	2.25 ml
Propylene glycol	1.95 ml
Cocamidopropyl betaine	1 ml
Cocodiethanol amide	1ml
Methyl cellulose	0.4 g
Curcumin	0.05 g
Bromelain	0.75 g
Castor oil	0.5 ml
Flavouring agent	0.05 ml
Distilled water	q. s to 15 ml

Method of Preparation:

Step 1: Preparation of solution A

- Distilled water was taken in a beaker.
- Sodium hydroxide was added slowly into the water with constant stirring.
- The mixture was stirred thoroughly until completely dissolved and solution was allowed to stand for 30 minutes.

Step 2: Preparation of solution B:

- A China dish was placed on water bath.
- Coconut oil, castor oil, and stearic acid were added to the China dish.
- The mixture was heated gently and mixed continuously until all the ingredients melted (70- 75 °C)

Step 3: Combination of the solutions

- Solution A was added slowly into solution B with continuous stirring.
- The mixture thickened rapidly and the China dish was covered immediately to prevent evaporation.
- Alcohol, propylene glycol was added.
- Once the mixture become clear, coco diethanolamide and cocamidopropyl betaine were added
- Methyl cellulose (pre-dispersed in glycerine) was added.
- The mixture was stirred gently to avoid bubble formation, the heat was turned off, and the mixture was allowed to cool approximately at 50°C
- In a small separate beaker, curcumin and bromelain was mixed with few drops of water to form a smooth slurry.
- The slurry was incorporated into the above solution and stirred at low temperature to protect the bromelain and maintain the vibrancy of curcumin colour.
- Required amount of pineapple flavour were added the mixture.
- The mixture was then poured into the mould with previously lubricated with PEG. (7)

EVALUATION OF SINGLE USE SOAP

I. Organoleptic Evaluation(8)

Table 2: Organoleptic Evaluation of single use soap

Sl.no	**Parameter**	**Method**
1	Colour	A white background was used to determine the colour and visualize the clarity of soap
2	Odour	Odour of sample were analysed by heating the soap sample on a hot plate
3	Shape	Sensory and visual evaluation of organoleptic properties like shape and clarity was done to obtain results

II.Determination of pH(9)

1 g of soap was dissolved in 100 mL of distilled water, stirred, and allowed to stand for 2 hours. The pH meter was calibrated (pH 4,7,9 and pH of solution was measured using the pH meter.

III.Washability Test (9)

The soap was applied on the skin and lathered. It was then rinsed with water and observed the removal.

IV.Foam height and Foam stability Test (9)

Soap was added to 100 mL water in a cylinder and shaken for10-15 minutes. The resulting foam height was measured

2 g of soap was dissolved in 50 mL distilled water. The solution was shaken to form foam. The foam was allowed to stand undisturbed for 30 minutes. The height of the foam was recorded after 30 minutes.

V.Total fatty matter Test(10)

1 g of formulated soap was weighed. It was dissolved in 150 mL of distilled water and heated gently.1 mL of alcoholic HCl was added to precipitate fatty acids. Fatty acids appeared on the surface of the solution. Sodium chloride (NaCl) was added to solidify the fatty layer and the mixture was heated again. The mixture was allowed to cool so that the fatty matter hardened. The fatty layer was carefully removed, blotted dry, and weighed. TFM was calculated using the formula:

% TFM = (Weight of fatty matter/ Weight of soap sample) × 100

VI.Saponification Test (11)

A small amount of the sample was weighed in a conical flask. A measured volume of alcoholic KOH was added and mixed well. The mixture was refluxed/heated for 30 minutes on a water bath. The solution was cooled to room temperature and 2–3 drops of phenolphthalein were added. The solution was titrated with 0.5 N HCl until the pink colour disappeared. A blank titration was performed without the sample.

Saponification Value (SV)= ((A -B) X N×56.1)/W

VII.Dirt dispersion Test (8)

A 1% soap solution was prepared using distilled water. Two drops of ink were added to the soap solution. The mixture was poured into a straight glass jar. The jar was shaken thoroughly. The mixture was allowed to settle and the dispersion of ink (dirt) was observed. Observations were recorded on how well the ink dispersed.

VIII.Alcohol insoluble matter Test (12)

5 g of the sample was accurately weighed and transferred into a clean conical flask.50 mL of 70% ethanol was added to the flask and the contents were shaken vigorously to dissolve the material completely.20 mL of warm ethanol was added and mixed thoroughly. The solution was filtered using filter paper to collect the residue. The filter paper with the residue was dried in a hot air oven at 105 °C for 1 hour. After drying, the filter paper was cooled in a desiccator and weighed accurately. The weight of the dried residue along with the filter paper was noted.

Alcohol insoluble matter equation(13):

Alcohol Insoluble Matter (%) = (Weight of dried residue (g)/ Weight of sample (g))×100

RESULT AND DISCUSSION:

IDENTIFICATION OF BROMELAIN

Bromelain extract underwent the following evaluation parameters:

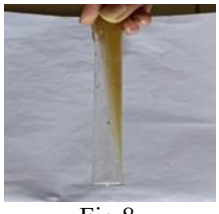
I. Organoleptic Evaluation

Table 3: Organoleptic Evaluation of Bromelain

Sl.no	Parameter	Observation
1	Colour	Yellowish-brown
2	Odour	Characteristic odour
3	Texture	Clay-like

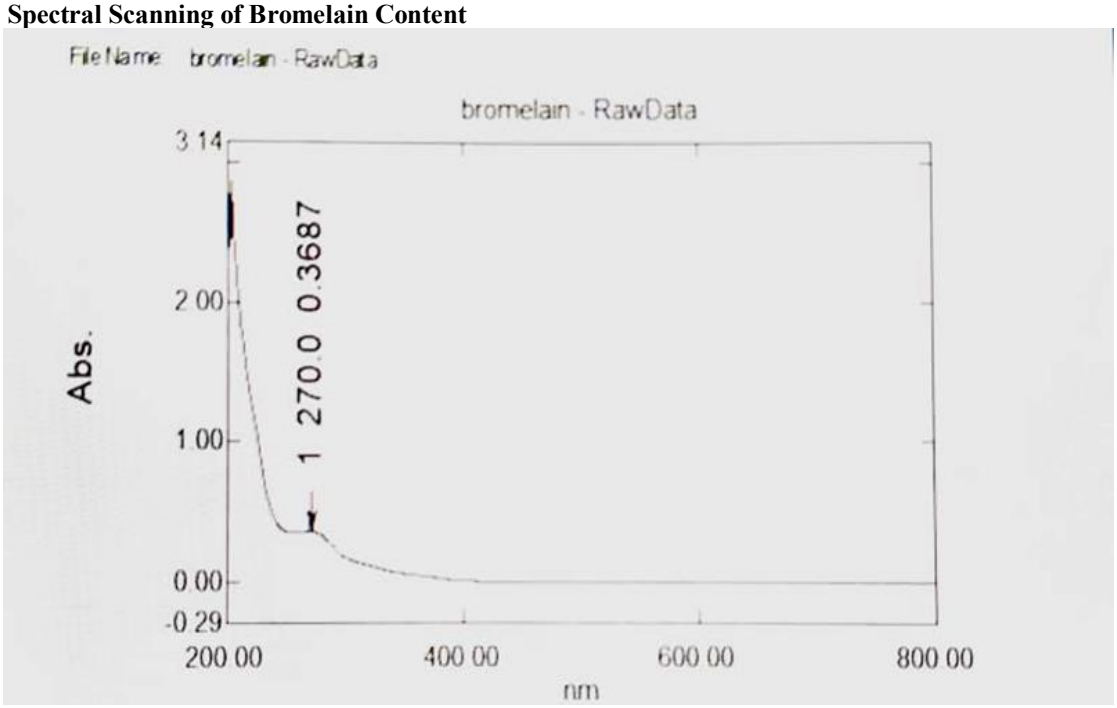
II. Confirmatory Test

Table 4: Confirmatory Test

Sl.no	Test	Observation	Inference
1	Gelatine Liquefaction test	  Fig 7 Fig 8 Solidified gelatine solution (Fig 7) After adding bromelain extract gelatine become liquified form (Fig 8)	Liquefaction confirms protease activity of bromelain

2	Milk clotting test	 Fig 9  Fig 10 Warm milk (Fig 9) After adding bromelain extract milk become curdles (Fig 10)	Confirms proteolytic activity of bromelain

III. Spectral Scanning of Bromelain Content



Graph 1: Absorption spectrum of Bromelain at 270 nm

The UV–Visible spectrophotometric analysis of the bromelain sample showed a characteristic maximum absorbance at 270 nm. This absorption is due to the presence of aromatic amino acids present in protein enzymes such as bromelain. The observed UV–Visible spectrum confirms the protein nature of the sample and supports the successful identification and confirmation of bromelain. Therefore, UV–Visible spectrophotometry is a suitable and reliable method for the confirmation of bromelain enzyme.

IV. Determination of protease activity

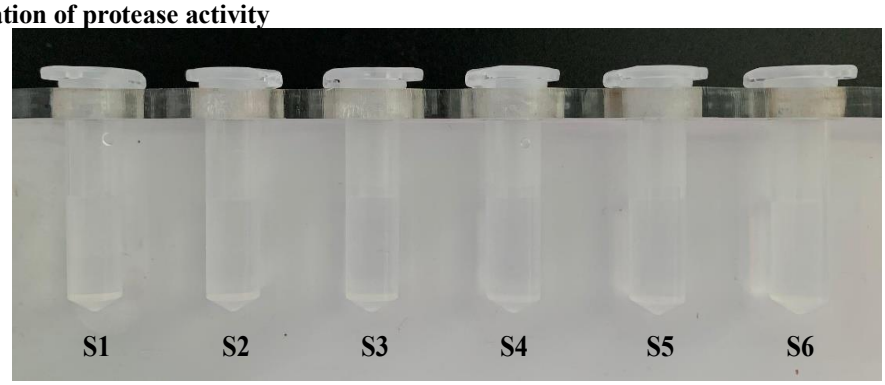
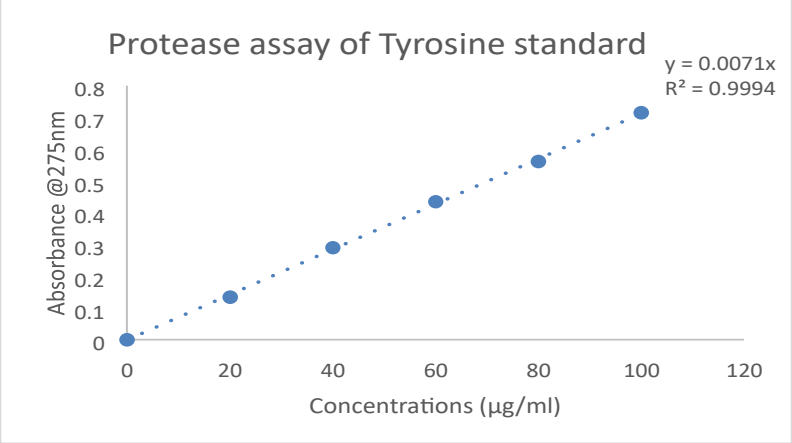


Fig 11: protease assay of standard tyrosine at different concentrations



Graph 2: protease assay of standard tyrosine at different concentrations

Fig 12: protease assay of sample

Table 5: Protease activity of crude bromelain extract (U/mg)

Sample concentration (mg/ml)	Absorbance @ 275 nm	Concentration of tyrosine from graph (µg/ml)	Protease activity (U/mg) =µmol Tyrosine/(mg enzyme*time)	Protease activity (mU/ mg)
10	0.323	45.4930	0.00084	0.84

The crude bromelain extract exhibited measurable proteolytic activity, as evidenced by its ability to hydrolyse casein and release tyrosine. Based on the tyrosine concentration obtained from the standard calibration curve, the protease activity of crude bromelain was calculated to be 0.84 mU/mg, confirming the presence of active protease enzymes in the extract. The result indicated that while crude bromelain possesses detectable protease activity.

V. DPPH Free Radical Scavenging Assay

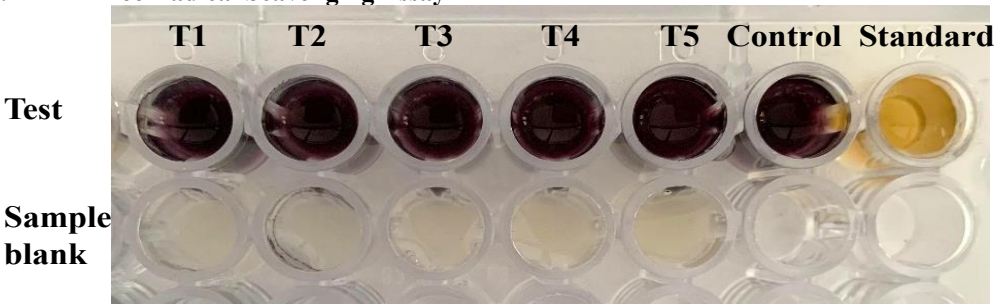


Fig 13: DPPH Free Radical Scavenging Assay of sample with standard and control

Table 6: Percentage scavenging activity of DPPH by sample

Sample Name	Sample Dose (µL)	Absorbance	Sample Blank	Corrected OD	Radicle Scavenging activity %
T1	20	1.732	0.100	1.632	4.784
T2	40	1.742	0.114	1.628	5.018
T3	60	1.758	0.135	1.623	5.309
T4	80	1.764	0.146	1.618	5.601
T5	100	1.771	0.159	1.612	5.951
Control			1.714		
Standard = 100 µl (10mM ascorbic acid)		0.125			92.707

In the DPPH free radical scavenging assay, the crude bromelain showed low but dose-dependent antioxidant activity, with a gradual increase in radical scavenging percentage from 4.78% to 5.95% as the sample concentration increased. However, the antioxidant activity did not exceed 50% at any tested concentration; therefore, the IC50 value could not be determined under the experimental conditions. The result indicates the while crude bromelain possesses detectable its antioxidant potential as assessed by DPPH assay is relatively weak at the tested concentrations.

EVALUATION OF SINGLE USE SOAP

I. Organoleptic evaluation

Table 7: Organoleptic Evaluation of single use soap

Sl. No	**Parameter**	**Observation**
1	Colour	Yellow to golden yellow
2	Odour	characteristic pineapple odour
3	Shape	cylindrical and conical form

II. Determination of pH

Table 8: pH value

Sl.no	Formulation code	Value obtained using pH meter
1	Sample soap	7.95
2	Standard soap	9

Normal pH range of soap used in skin is 7-10. The pH of bromelain enriched single use soap was found to be 7.95. Hence it was found to be skin friendly.

III. Washability Test

Fig 14: Hand washability test

The formulation was readily washable with water, indicating good washability and acceptable formulation quality.

IV. Foam height and Foam stability Test

Fig 15: Solution before shaking

Fig 16: solution after shaking

Fig 17: Foam formation

Fig 18: After 30 minutes

The foam height of the formulation was found to be 0 and foam height after shaking was found to be 7.5 cm, indicating the formation of adequate and stable foam. The persistence of foam with minimal collapse reflects good lathering and foaming ability of the formulation. Therefore, the obtained foam height value confirms satisfactory surface-active and cleansing properties.

The formulation showed good foam stability with a foam height of 7.5 cm, indicating stable foam and satisfactory foaming efficiency suitable for cleansing applications.

V. Total fatty matter Test

Fig 19: Dried fatty matter

Calculation

% TFM = (Weight of fatty matter/ Weight of soap sample) × 100

Weight of fatty matter- 0.78 g

Weight of soap sample- 1g

% TFM = 0.78/1 × 100 = 78%

The Total Fatty Matter (TFM) analysis revealed that the formulated soap possessed a TFM value of approximately 78%. According to standard classification, a TFM value greater than 76% indicates high-quality soap with good moisturizing properties and mild action on the skin. The obtained result confirms that the prepared formulation is of superior quality, skin-friendly, and suitable for regular cleansing applications.

VI. Saponification Test

Fig 20: Before titration

Fig 21: After titration (Pink colour disappear)

Table 9: Blank and sample Titration

	Content of flask	Volume of HCl (ml)	End point	Colour change
Blank	25 ml KOH+ Phenolphthalein	26.6	26.6	Pink to colourless
Sample	0.75 g of soap +25ml KOH+ Phenolphthalein	21.2	21.2	Pink to colourless

Calculation

Saponification Value (SV)= ((A -B) X N×56.1)/W

A- Blank titration value

B- Sample titration value

W- weight of the sample

N- Normality of HCl

SV= (26.2 -21.2) ×0.5 × 56.1/0.75 = 187 mg KOH/g

The saponification value was 187 mg KOH/g, which lies within the normal range of 180–220 mg KOH/g, indicating acceptable fatty acid composition and good quality of the sample.

VII. Dirt dispersion Test

Fig 22: Clear formulation before adding ink

Fig 23: Formulation after adding ink and shaking

The dirt dispersion test showed that when ink was added to the soap solution and shaken, the ink was uniformly dispersed in the liquid and did not concentrate in the foam. This indicates that the formulation has good cleansing efficiency, as the dirt particles remain suspended in the solution rather than redepositing on the surface. Hence, the formulation passed the dirt dispersion test and is considered effective for cleaning purposes.

VIII. Alcohol insoluble matter Test

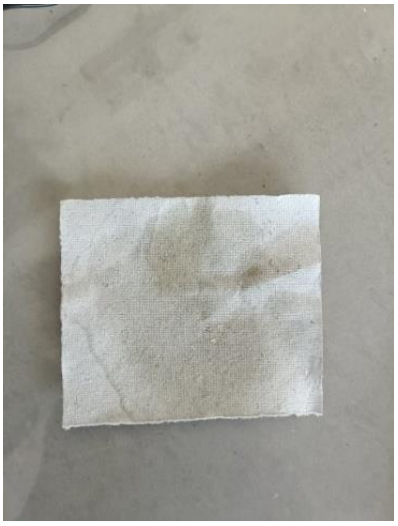


Fig 24: Dried residue

Calculation

Alcohol Insoluble Matter (%) = (Weight of dried residue (g)/ Weight of sample(g))×100

Weight of dried residue- 0.02 g

Weight of sample- 5 g

Alcohol insoluble matter (%) = (0.02/5) × 100 = 0.4 %w/w

The alcohol-insoluble matter of the prepared soap was found to be 0.4 %. Which is within the normal limit (not more than 2.5% w/w). This indicates low impurities in the soap. Hence the formulation is acceptable

SUMMARY AND CONCLUSION:

The developed single-use bromelain soap represents an innovative and practical approach to topical enzymatic cleansing and skin care. The formulation successfully integrates bromelain as the active proteolytic agent, providing potential benefits such as gentle exfoliation, removal of dead skin cells, and enhancement of skin hygiene. The incorporation of curcumin as a natural colourant not only improves the aesthetic appeal but also aligns with the growing preference for natural and biocompatible ingredients. The addition of pineapple flavour enhances sensory acceptability, reinforcing user compliance and product attractiveness. The extract shows better protease assay value & dose dependent DPPH value also. The pH determination, washability test, form height- foam stability, total fatty matter, saponification test, Dirt dispersion test and alcohol insoluble matter test for soap tabs were analysed and was within the permissible range.

The standardized physical dimensions (8 mm diameter and 5 mm thickness) ensure uniformity, dose precision, and convenience for single-use applications, minimizing the risk of contamination and preserving enzyme stability. The use of butter paper as an individual wrapping material provides a cost-effective, protective, and hygienic packaging solution suitable for maintaining product integrity during storage and handling.

From a formulation and product design standpoint, the developed bromelain soap demonstrates satisfactory physical characteristics, user-friendly design, and potential functional effectiveness. Overall, this single-use format represents a promising advancement in enzyme-based topical delivery systems, with potential applications in personal hygiene, dermatological care, and clinical settings.

Further stability, efficacy, and user acceptability studies are recommended to support large-scale production and commercialization. In future we can scale up and adapt clinical testing and personalized soap tabs for various skin types.

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