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FORMULATION AND EVALUATION OF TRIHERBA GMC: A POLYHERBAL COSMETIC SOAP CONTAINING GUAVA LEAVES, MANJISTHA ROOTS, AND CUCUMBER PEELS

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ABSTRACT

The present study aimed to formulate and evaluate a polyherbal cosmetic soap using guava leaves (*Psidium guajava*), manjistha roots (*Rubia cordifolia*), and cucumber peel (*Cucumis sativus*). Herbal soaps are gaining attention as safer alternatives to synthetic soaps due to their skin-friendly nature and reduced risk of irritation. The selected plant materials were chosen based on their reported antimicrobial, antioxidant, anti-inflammatory, soothing, and skin-protective properties. Herbal extracts were prepared separately using maceration with suitable hydroalcoholic solvents. A polyherbal soap was formulated by the cold process method using coconut oil, sunflower oil, castor oil, and cocoa butter as the oil base, with sodium hydroxide as the alkali. The prepared soap was cured and evaluated for organoleptic properties, pH, foam characteristics, moisture content, free alkali content, total fatty matter, antimicrobial activity, phytochemical profile, antioxidant activity, anti-inflammatory activity, skin irritation, \ and stability. The formulated soap exhibited a smooth and uniform appearance with a pleasant odour and good hardness. The pH was found to be within the acceptable range for topical application. Foam properties, total fatty matter, and free alkali content indicated good cleansing ability and skin compatibility. The soap showed effective antimicrobial activity against both Gram-positive and Gram-negative bacteria. Phytochemical screening confirmed

the presence of bioactive constituents, and antioxidant and anti-inflammatory assays demonstrated appreciable activity. No skin irritation was observed, and the formulation remained stable under normal storage conditions. The results indicate that the developed polyherbal soap is safe, effective, and suitable for regular cosmetic use.

KEYWORDS: Polyherbal soap; Herbal cosmetics; *Psidium guajava*; *Rubia cordifolia*; *Cucumis sativus*; Cold process formulation.

INTRODUCTION

Soaps are widely used cleansing agents that play an important role in maintaining personal hygiene and skin health. Many commercially available soaps contain synthetic surfactants and additives that may disturb the skin barrier, leading to dryness, irritation, and sensitivity with prolonged use. This has increased interest in herbal and polyherbal cosmetic formulations that offer effective cleansing along with improved skin compatibility.

Polyherbal soaps combine multiple medicinal plants to provide enhanced benefits through synergistic action. Guava leaves are known for their antimicrobial, antioxidant, and wound-healing properties. Manjistha roots are traditionally used for anti-acne activity, inflammation control, and improvement of skin tone. Cucumber peel provides cooling, soothing, hydrating, and antioxidant effects, making it suitable for cosmetic applications.

The present research was undertaken to formulate a tri-herbal soap using guava leaves, manjistha roots, and cucumber peel and to evaluate its physicochemical properties, biological activities, safety, and stability. The study aims to develop a herbal soap that is effective, skin-friendly, and suitable for regular use.

SCIENTIFIC BASIS FOR POLYHERBAL SOAP FORMULATION

Soaps are widely used cleansing agents prepared through the saponification reaction, in which oils or fats react with sodium hydroxide to form fatty acid salts and glycerol. The amphiphilic nature of soap molecules enables effective removal of dirt, oil, and microorganisms from the skin surface. However, many conventional soaps are alkaline and may disturb the natural skin barrier, leading to dryness, irritation, and sensitivity on prolonged use. This creates a need for mild, skin-compatible soap formulations with additional protective benefits.

Herbal soaps have gained importance due to their use of plant-based ingredients with established medicinal and dermatological properties. Unlike synthetic soaps, herbal soaps are generally free from harsh chemicals and provide added functional benefits such as antimicrobial activity, antioxidant protection, soothing effects, and support for skin healing. When multiple herbal ingredients are combined, the formulation is referred to as a polyherbal soap. The polyherbal approach is based on the principle that different plant constituents act synergistically, enhancing overall efficacy and offering broader skin benefits compared to single-herb formulations.

Consideration of Skin Compatibility

The skin acts as a protective barrier between the body and the external environment. Frequent exposure to harsh cleansing agents can disrupt this barrier, resulting in increased water loss, irritation, inflammation, and susceptibility to microbial invasion. Therefore, soaps intended for regular use should possess suitable pH, adequate fatty matter, and minimal free alkali to ensure effective cleansing without compromising skin integrity. Herbal and polyherbal soaps are particularly relevant in this context, as they combine cleansing action with bioactive compounds that support normal skin physiology.

Selection of Herbal Components

The present research focuses on a tri-herbal combination comprising guava leaves (*Psidium guajava*), manjistha roots (*Rubia cordifolia*), and cucumber peel (*Cucumis sativus*), selected based on their documented skin-related benefits.

Guava leaves are known to possess antimicrobial, antioxidant, anti-inflammatory, and wound-healing properties. These activities are attributed to the presence of phenolic compounds, flavonoids, tannins, and terpenoids, which contribute to protection against microbial growth and oxidative stress when applied topically.

Manjistha roots are traditionally valued for their detoxifying, anti-acne, anti-inflammatory, and complexion-enhancing effects. The roots contain anthraquinone derivatives and related phytoconstituents that support wound healing, reduce inflammation, and help control acne-causing microorganisms.

Cucumber peel is rich in water, vitamins, flavonoids, phenolic compounds, and phytosterols, providing hydrating, cooling, soothing, and antioxidant effects. It is widely used in cosmetic

formulations for reducing irritation, improving skin texture, and promoting a refreshed skin appearance.

The combination of these three herbal components is expected to provide complementary and synergistic effects, offering antimicrobial protection, antioxidant support, soothing action, and improved skin compatibility within a single soap formulation.

MATERIALS AND METHODS

Guava leaves, manjistha roots, and cucumber peels were collected, cleaned, shade dried, and powdered. Guava leaf extract was prepared by maceration using 70% ethanol. Manjistha root extract was obtained using hydroalcoholic maceration followed by concentration with a rotary evaporator. Cucumber peel extract was prepared by maceration using 80% methanol and concentrated under reduced pressure.

The polyherbal soap was formulated by the cold process method. Coconut oil, sunflower oil, castor oil, and cocoa butter were melted and mixed. Sodium hydroxide solution was prepared separately and added to the oil phase with continuous stirring. Herbal extracts were incorporated at the trace stage, followed by addition of essential oil. The mixture was poured into molds, allowed to solidify, and cured for four weeks.

The formulated soap was evaluated for organoleptic properties, pH, foam height, foaming index, foam retention, moisture content, free alkali content, and total fatty matter. Antimicrobial activity was assessed using the agar well diffusion method. Phytochemical screening, antioxidant activity (H_2O_2 scavenging), anti-inflammatory activity (egg albumin method), skin irritation test, and stability studies were also performed using standard procedures.

EXTRACTION OF HERBAL CONSTITUENTS

1. **Guava leaves:** Guava leaves were air dried at room temperature for seven days, then ground into a fine powder and sieved to obtain a uniform sample. Guava leaf extract was obtained by macerating 50g of powdered leaves in 150ml of 70% ethanol for 24 hours over three days. The collected filtrate was evaporated and the extract was cooled in an airtight container with desiccant before analysis.[1]
2. **Manjistha roots:** The roots of Rubia cordifolia were collected and washed thoroughly with the help of water and then it was chopped into small pieces and then it was shade

dried for one week and then it was ground by using electric grinder to get a fine powder. The extraction process was carried out using maceration. A 30:70 aqueous ethanolic solution (30% water and 70% ethanol) was used for extraction. A measured quantity of powdered material was subjected to 3-4 liters of solvent and kept at room temperature in the dark for 3 days. The mixture was then filtered by using Whatman filter paper. The resulting extract was concentrated by using a rotary evaporator to remove the ethanol and obtain the final product and stored in an amber-colored bottle for further use.[2]

3. **Cucumber peels:** Fresh and mature cucumbers were washed under running water to remove the dirt and peeled manually using a peeler. Peels were shade dried at room temperature for 5 days and then ground into fine powder using laboratory grinder. The powder was extracted with 80% methanol using maceration method for 72 hours with occasional agitation and the mixture was filtered using Whatman filter paper No. 1 and then the filtrates were concentrated under reduced pressure using a rotary evaporator to obtain the dry extract stored in airtight bottles at 5°C until further use.[3,4]



1 (a) **1 (b)** **1 (c)**

Fig. 1: (a) guava leaves (*Psidium guajava*), (b) manjistha roots (*Rubia cordifolia*), and (c) cucumber peels (*Cucumis sativus*).

Phytochemical Analysis

a) **Test for Flavonoids (alkaline reagent test):** Take 2ml of extract solution and add few drops of 10%NaOH solution to it. Upon addition of dilute HCl intense yellow colour becomes colourless which indicates presence of flavonoids.

b) **Test for Phenols:** Take 2ml of extract solution and bluish black colour appears upon addition of 0.5% Fecl3 solution which indicates presence of phenols.

c) **Test for Tannins (gelatin test):** Take 2ml of extract solution and add gelatin solution to it, which was prepared by dissolving the gelatin powder in water by applying gentle heat on a water bath. Formation of white precipitate indicates presence of tannins.

d) **Test for Alkaloids (iodine test):** Take 2 to 3ml of extract solution and add few drops of iodine solution to it, blue colour forms and disappears on heating and reappears upon cooling indicating presence of alkaloids.

e) **Test for Saponins (Foam test):** Take few ml of extract solution into a test tube and shake it vigorously for certain time. Saponins can be indicated by formation of foam layer.

f) **Test for Steroids (Libermann-Buchard test):** Add 2ml of acetic anhydride to 0.5g of methanolic extract and add 2ml of H2SO4. Change of colour from violet to blue indicates presence of steroids.[5]

g) **Test for Cardiac glycosides (Legal's test):** To the extract solution add pyridine, few drops of sodium nitroprusside and few drops of NaOH solution. Appearance of pink or red color indicates the presence of cardiac glycosides.

h) **Test for Anthraquinone glycosides (Borntrager test):** Add 2ml of H2SO4 to 2ml of extract solution, boil them, filter, add equal volume of chloroform and shake it vigorously, organic layer was separated and pinkish-red color develops on the aqueous layer upon addition of ammonia which indicates presence of anthraquinone glycosides.[6]

Formulation Approach

The polyherbal soap formulation is designed using a cold-process method, in which selected oils are saponified with sodium hydroxide to form a solid soap base. Concentrated extracts of guava leaves, manjistha roots, and cucumber peel are incorporated during the trace stage to ensure uniform distribution of herbal constituents. This approach allows preservation of bioactive compounds while achieving adequate hardness, stability, and cleansing efficiency of the soap.

SOAP FORMULATION

Table 1: Ingredients and their functional roles in Triherba-GMC polyherbal soap.

Ingredients	Uses
Coconut oil	Hydration, Wound healing, Reduce irritation
Sunflower oil	Moisturizing, Skin protective property, Prevent premature aging
Castor oil	Reduce acne, Moisturises, Soothes sunburns
Cocoa butter	reduce skin ageing, improve skin quality, reduce the appearance of scars, wrinkles
NaOH	Lye

Water	Aqueous vehicle
Guava leaf extract	Wound care, Wound healing
Manjistha root extract	Improve skin tone, Skin whitening, Reduce hyperpigmentation
Cucumber peel extract	Reduce puffiness, Skin aging, Cooling effect, Treat acne, Big pores
Essential oil	Aroma

Table 2: Composition of polyherbal soap formulations (F1 - F4).

Ingredients	F1	F2	F3	F4
Coconut oil (ml)	22	4	11	11
Sunflower oil (ml)	10	10	17	14
Castor oil (ml)	4	22	8	11
Cocoa butter	2	2	2	2
Sodium hydroxide (g)	6	6	6	6
Water	11	11	11	11
Guava leaf extract	4	4	4	4
Manjistha root extract	2	2	2	2
Cucumber peel extract	1.5	1.5	1.5	1.5
Essential oil (lemon)	2-3 drops	2-3 drops	2-3 drops	2-3 drops

1. **Preparation of lye solution:** 6 g of NaOH was dissolved in 11 mL of distilled water and gentle heat is applied to let it get dissolved completely.
2. **Preparation of oil base:** coconut oil, sunflower oil, castor oil and cocoa butter were weighed and kept in water bath until it melts completely.
3. **Mixing of the lye solution to the oil base:** Gently add the lye solution to the oil base with continuous stirring.
4. **Addition of herbal extracts:** The herbal extracts (Guava, manjistha and cucumber) were added to the above mixture and stir continuously until they get blended completely. Add few drops of lemon essential oil for fragrance.
5. **Pour into molds:** The soap formulation was poured into a suitable mold and allow it to cool at room temperature until complete solidification.



Fig. 2: Polyherbal soap formulation poured into moulds and allowed to set.

6. **Remove from molds:** The solidified product was removed from the mold after a period of 48 hours.
7. **Curing period:** The soap was cured for 4 weeks to lose residual moisture and reach the optimal firmness.[7]

Evaluation Parameters

To assess the quality, safety, and suitability of the formulated polyherbal soap for topical application, standard evaluation parameters are employed. These include organoleptic characteristics, pH determination, foam properties, total fatty matter, free alkali content, antimicrobial activity, phytochemical screening, and skin irritation testing. These parameters collectively help confirm that the formulation meets cosmetic standards and is mild, effective, and safe for regular use.[1]

EVALUATION OF POLYHERBAL SOAP

Four formulations (F1-F4) were initially designed for soap preparation. Among them, only **F1** resulted in a well-formed, solid soap with acceptable physical characteristics. The remaining formulations (F2-F4) did not produce a clear or stable soap mass and were therefore excluded from further evaluation.



Fig. 3: Optimized polyherbal soap formulation (F1) after solidification.

The successful formation of soap in F1 may be attributed to an appropriate balance of oils and alkali concentration, which facilitated complete saponification. In contrast, the failure of F2-F4 to yield a stable soap suggests inadequate saponification, possibly due to imbalance in oil composition or alkali-oil ratio. Hence, F1 was selected as the optimized formulation for further evaluation studies.

1. **Organoleptic assessment:** The colour and texture were evaluated by visual observation and touch respectively while the odour was assessed by smelling the formulation.[7]

2. **pH:** A soap solution was prepared by dissolving 5g of soap in 100ml of water and its pH was measured using the digital pH meter.

3. **Foam properties**

a) **Foam height:** A 1% w/v soap solution was transferred to a graduated cylinder, shaken for a fixed number of strokes, and the foam height is measured immediately after shaking.[5]

b) **Foaming index:** Serial dilutions of soap solution are shaken in calibrated tubes; the lowest concentration producing a standard foam height of 1 cm after 15mins is used to calculate the foaming index by using the formula, foaming index = 1000/a, where a is volume of soap solution which shows foam height of 1cm.[8]

c) **Foam retention time:** Foam height of the 25ml 1% soap solution is recorded at predetermined time intervals after shaking 10 times in a 100ml measuring cylinder; the time taken for foam to disappear is foam retention time.[5]

4. **Moisture content:** 1g of sample was weighed accurately and placed in a pre-weighed petridish. It was dried in a hot air oven for 2 hours at 105°C. The weight of the dish along with the dried soap was measured to determine the moisture content.[9]

5. **Anti-microbial assay (agar - well diffusion method procedure):** The medium was prepared using following table 3.

Table 3: Composition of nutrient agar medium.[10]

Component	Quantity (1000 mL)
Beef extract	10.0 g
Peptone	10.0 g
Sodium chloride	5.0 g
Distilled water	1000 ml
Agar	1-2 %

The dissolved medium was autoclaved at 15 lbs pressure at 121°C for 15 minutes. The autoclaved medium was mixed well and poured into petriplates while still molten. Petriplates containing the medium were seeded with 24hr culture of bacterial strains [Gram positive bacteria- staphylococcus aureus (ATCC 25923) and Gram negative bacteria- Escherichia coli (ATCC 25922)]. Wells were cut and the formulated herbal soap solution was added. The plates were then incubated at 37°C for 24 hours. The antimicrobial activity was assayed by measuring the diameter of the inhibition zone formed around the well.[11]

6. **Free alkali content:** 2g of soap sample was weighed accurately and placed in a beaker. Then 30ml of purified water was added and the mixture was kept for boiling in water bath

for 30 minutes. Then the volume of solution was adjusted to 50ml and 1ml phenolphthalein indicator was added. The solution was immediately titrated with 0.1M HCl until it turns colourless.

7. **Total Fatty Matter (TFM):** The total fatty matter of soap was measured by treating it with acid in hot water to obtain free fatty acids. 2g of the prepared soap was dissolved in 30ml of distilled water and heated. Then, 4ml of 15% H2SO4 was added and heating was continued until clear solution obtained. To solidify the fatty acids, 1.4g of beeswax was added and the mixture was allowed to form a cake. Then the cake was removed, blotted to remove moisture and weighed to determine the TFM.[9]
8. **Skin irritation test:** The skin irritation potential of the polyherbal soap was assessed by applying it to the skin for 30 minutes. After washing the area was examined for any signs of itching, rashes or redness through visual inspection and the sensory evaluation.[7]
9. **Anti-oxidant Test (H2O2 Scavenging test):** The hydrogen peroxide scavenging activity was determined using the ascorbic acid method. A 40 mM hydrogen peroxide solution was freshly prepared in 0.1 M phosphate buffer (pH 7.4). Ascorbic acid was used as the standard, and the test sample was prepared in distilled water at the required concentration. The reaction mixture consisted of 0.6 mL of hydrogen peroxide solution, 3.3mL of phosphate buffer and 0.1 mL of standard or test sample solution, while the control contained hydrogen peroxide and phosphate buffer. A blank without hydrogen peroxide was used for baseline correction. All mixtures were incubated at room temperature for 10 minutes, after which the absorbance was measured at 230 nm using a UV–Visible spectrophotometer. The percentage hydrogen peroxide scavenging activity was calculated using the formula

$$Percentage\ inhibition = \frac{A(control) - A(test)}{A(control)} \times 100$$

Where, A(control) and A(test) are Absorbance of control and test respectively.[12]

10. **Anti-inflammatory test (Egg albumin method):** This test is done using a reference drug which is diclofenac (+v e control), test (soap solution) and a negative control. Reference drug is prepared by dissolving 0.2g of diclofenac in 20ml of distilled water, mixed using a vortex and serial dilution were done until 0.01ppm is obtained. Similarly, it is done for the soap solution(test) and 0.01ppm is taken into consideration. Reaction mixture is prepared by adding 2.8ml of phosphate buffer saline(pH=6.4), 0.2ml of egg albumin in 3 test-tubes. Now add 2ml of 0.01ppm soap solution(test), 2ml of 0.01ppm diclofenac solution (+ve

control), 2ml of water (-ve control) and check the absorbance using UV-Visible spectrophotometer under 660nm.

$$Percentage\ inhibition = \frac{A(control) - A(test)}{A(control)} \times 100$$

Where, A(control) and A(test) are Absorbance of control and test respectively.[13]

11. **Stability test:** The formulated polyherbal soap was stored at **room temperature** and observed periodically for any changes in **colour, odour, texture, hardness, and surface appearance**. The **melting point** was also determined, and the absence of significant changes was considered indicative of formulation stability.

RESULTS AND DISCUSSION

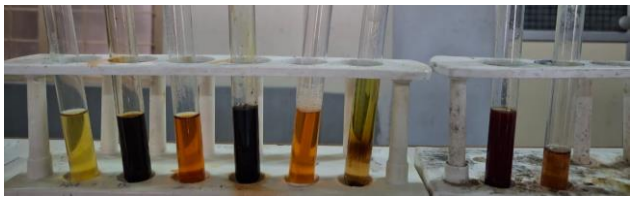
1. Extractive Value

Table 4: Extractive values of selected herbal materials.

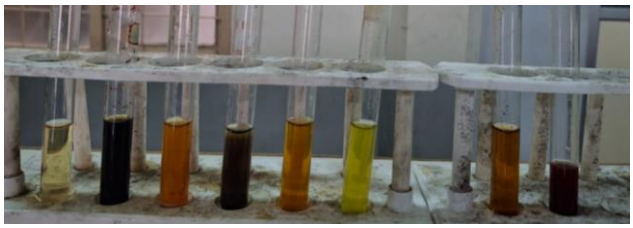
Plant material	Part used	Solvent used	Weight of dried powder (g)	Weight of dried extract (g)	Extractive value (%)
Guava (*Psidium guajava)*	Leaves	70% Ethanol	136	18.64	12.13
Manjistha (*Rubia cordifolia)*	Roots	70% Ethanol	80	11.28	11.10
Cucumber (*Cucumis sativus*)	Peels	80% Methanol	60	6.79	11.80

Extractive value reflects the ability of a solvent to dissolve and extract bioactive constituents from plant materials. In the present study, guava leaves exhibited the highest extractive value, followed by manjistha roots and cucumber peels. The higher extractive value of guava leaves may be attributed to the presence of polar phytoconstituents such as flavonoids, phenolic compounds, and tannins, which show good solubility in hydroalcoholic solvents. Manjistha roots showed moderate extractive value due to the presence of anthraquinones and related compounds, whereas cucumber peels exhibited comparatively lower extractive value, which may be due to their high moisture content and lower concentration of extractable solids. These findings support the suitability of hydroalcoholic solvents for extracting phytoconstituents intended for topical formulations.

2. Preliminary Phytochemical Screening



(a)



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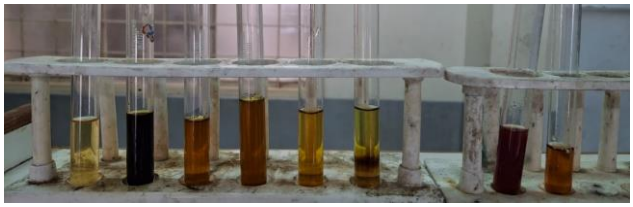

(c)

Fig. 4: preliminary phytochemical screening of herbal extracts (a) guava leaf extract (*Psidium guajava*), (b) manjistha root extract (*Rubia cordifolia*), and (c) cucumber peel extract (*Cucumis sativus*)

Table 5: Preliminary phytochemical screening of herbal extracts.

Phytoconstituent	Test Performed	Guava leaf extract	Manjistha root extract	Cucumber peel extract
Flavonoids	Alkaline reagent test	+	+	+
Phenols	Ferric chloride test	+	+	+
Tannins	Gelatin test	+	+	-
Alkaloids	Iodine test	+	+	-
Saponins	Foam test	+	-	-
Steroids	Libermann-Buchard test	+	-	+
Cardiac glycosides	Legal's test	+	-	+
Anthraquinone glycosides	Borntrager's test	-	+	-

'+' indicates presence & '-' indicates absence of phytochemicals in the herbal extracts

Preliminary phytochemical screening confirmed the presence of various bioactive constituents in the selected herbal extracts. Guava leaf extract showed the presence of flavonoids, phenols, tannins, alkaloids, saponins, steroids, and cardiac glycosides, indicating broad antimicrobial and antioxidant potential. Manjistha root extract contained flavonoids,

phenols, alkaloids, and anthraquinone glycosides, which are known for their anti-inflammatory and antimicrobial properties. Cucumber peel extract demonstrated the presence of flavonoids, phenols, steroids, and cardiac glycosides, contributing to its antioxidant and skin-soothing effects. The variation in phytochemical composition among the extracts highlights their complementary nature and justifies their use in a polyherbal soap formulation.

3. Formulation Outcome



Fig. 5: Optimized polyherbal soap formulation (F1) after solidification.

Four formulations (F1-F4) were prepared using different proportions of oils. Among these, only formulation F1 resulted in a stable, solid soap with acceptable physical characteristics. Formulations F2, F3, and F4 failed to produce a clear and stable soap mass and were therefore excluded from further evaluation. Hence, all evaluation studies were carried out using the optimized formulation F1.

The successful formation of the optimized soap formulation can be attributed to the balanced proportion of oils, particularly the higher content of coconut oil, which facilitates efficient saponification and contributes to hardness. Coconut oil, rich in lauric and myristic acids, promotes rapid soap formation and structural integrity. In contrast, excessive castor oil or reduced coconut oil content in other formulations may have interfered with proper solidification, resulting in soft or unstable soap masses. Additionally, the use of a constant sodium hydroxide concentration for varying oil compositions may have influenced the extent of saponification. These factors collectively explain the observed variation in formulation performance.

The polyherbal soap formulation was evaluated for organoleptic, physicochemical, functional, antimicrobial, phytochemical, and safety parameters to assess its quality, stability, and suitability for topical application. The results obtained from various evaluation tests are discussed below.

4. Organoleptic Evaluation

Table 6: Organoleptic characteristics of polyherbal soap.

Parameter	Observation
Colour	Dark brown
Surface appearance	Smooth and uniform
Cracks	Absent
Texture	Firm
Odour	Pleasant
Hardness	Good

Uniform appearance and absence of cracks indicate proper saponification and good formulation stability. Pleasant odour improves consumer acceptability, while sufficient hardness ensures resistance to breakage during handling and storage.

5. pH Determination



Fig. 6: pH determination of polyherbal soap solution.

The pH of the soap solution was found to be **7.92**, which lies within the acceptable range of **7.5–9.0** for topical soap formulations.[14]

The slightly alkaline pH is characteristic of soap-based products and supports effective cleansing while remaining safe for regular skin use. The value also indicates proper saponification with minimal risk of irritation.

6. Foam Properties

a) Foam Height



Fig. 7: foam height measurement of polyherbal soap.

The soap produced a foam height of **5.9 cm**, which is within the acceptable limit of **>3 cm.**[14] Adequate foam height reflects good cleansing efficiency and acceptable surfactant performance.

b) Foaming Index



Fig. 8: foaming index determination of polyherbal soap.

Table 7: Foaming index of polyherbal soap.

Volume of decoction in the test tube (millilitres)	Height of foam (centimeters)
1	0
2	0
3	0.4
4	**1.0 (A)**
5 - 10	>1.0

The foaming index of the soap was calculated as **250**.

A foaming index greater than 100 indicates satisfactory foaming capacity, likely due to the presence of saponins and fatty acid salts.

c) Foam Retention Time



Fig. 9: Foam retention study of polyherbal soap.

Table 8: Foam retention properties

Time (minutes)	Foam height (centimeters)
0	3.2
5	1.5
10	0.9
15	0.7
30	0.3
45	0.1
60	0

Foam retention was observed for a prolonged period of **60 minute**s, with gradual reduction in foam height.

Good foam retention >7 minutes[14] indicates stable foam formation, contributing to improved cleansing efficiency during application.

7. Moisture Content



Fig. 10: Moisture content determination of polyherbal soap.

The moisture content of the soap was found to be 8% within acceptable limits (<15%)[14]

Lower moisture content enhances shelf stability and reduces the risk of microbial contamination during storage.

8. Free Alkali Content



Fig. 11: Free alkali content determination of polyherbal soap.

Free alkali content was found to be **0.174%** below the permissible limit (<2%).[15]

Low free alkali content indicates complete saponification and ensures that the soap is non-harsh and safe for skin application.

9. Total Fatty Matter (TFM)



Fig. 12: Total fatty matter (TFM) determination of polyherbal soap.

The TFM value of the soap was found to be **85%** within the acceptable range of >60%.[16]

Higher TFM values indicate better moisturizing and cleansing properties. The obtained value confirms that the formulation falls under good-quality toilet soap category.

10. Antimicrobial Activity (Cup Plate Method)

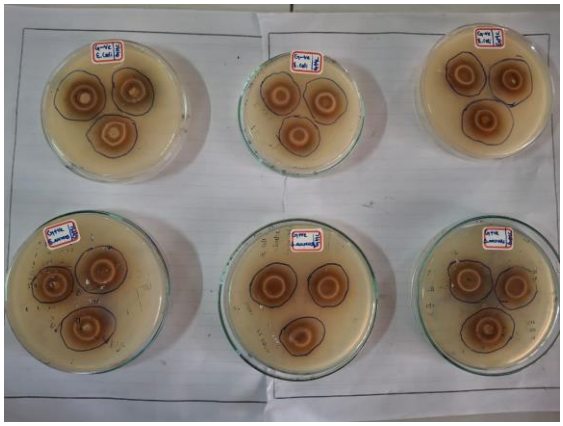

Fig. 13: antimicrobial activity of polyherbal soap against *Staphylococcus aureus* and *Escherichia coli.*

The polyherbal soap exhibited clear zones of inhibition against both Gram-positive and Gram-negative bacteria, indicating effective antibacterial activity. Consistent inhibition across replicate plates reflects good reproducibility of the assay. Slightly greater susceptibility of Gram-positive bacteria may be related to their simpler cell wall structure, while inhibition of Gram-negative bacteria suggests effective diffusion of active constituents through the outer membrane.

Table 9: Antimicrobial activity of polyherbal soap.

Organism	Plate	Cup 1 (mm)	Cup 2 (mm)	Cup 3 (mm)	Mean ± SD (mm)
S. aureus (Gram +ve)	1	30	25	24	**26.3 ± 3.21**
S. aureus (Gram +ve)	2	29	25	33	**29.0 ± 4.00**
S. aureus (Gram +ve)	3	27	35	30	**30.6 ± 4.04**
E. coli (Gram -ve)	1	30	31	28	**29.6 ± 1.53**
E. coli (Gram -ve)	2	29	24	24	**25.6 ± 2.89**
E. coli (Gram -ve)	3	31	33	29	**31.0 ± 2.0**

The antibacterial activity may be attributed to phytoconstituents such as flavonoids, tannins, and phenolic compounds present in the herbal ingredients.

11. Skin Irritation Test

Table 10: Skin irritation study.

Parameter	Observation
Irritation	Absent
Redness	Absent
Itching	Absent

No signs of irritation were observed following topical application, indicating that the formulation is safe for routine cosmetic use.

12. Anti-oxidant Test (H2O2 Scavenging test)



Fig. 14: hydrogen peroxide (H_2O_2) scavenging activity test of polyherbal soap.

Table 11: Hydrogen peroxide (H_2O_2) scavenging activity by ascorbic acid method.

Sample	Absorbance (230 nm)	H_2O_2 Scavenging Activity (%)
Control	0.94	-
Ascorbic acid (Standard)	0.17	81.91
Test sample	0.26	72.34

The formulation demonstrated effective hydrogen peroxide scavenging ability, indicating antioxidant potential. This activity may be associated with phenolic and flavonoid constituents present in the polyherbal formulation.

13. Anti-inflammatory test (Egg albumin method)



Fig. 15: anti-inflammatory activity of polyherbal soap by egg albumin denaturation method.

Table 12: Protein denaturation by egg albumin method.

Sample	Absorbance (660 nm)	% Inhibition
Negative control	0.11	-
Positive control (Diclofenac)	0.026	76.36%
Test sample (Soap formulation)	0.041	62.73%

The formulated soap exhibited appreciable inhibition of protein denaturation in the egg albumin method, indicating anti-inflammatory potential. This activity is likely due to the presence of bioactive phytoconstituents that help stabilize proteins and reduce inflammatory responses.

14. Stability test



Fig. 16: Melting point determination of polyherbal soap.

The soap formulation remained stable at room temperature, and the melting point was observed in the range of **80-100°C**, indicating good thermal stability and suitability for storage under normal conditions.

Table 13: Consolidated Evaluation Results of Polyherbal Soap Formulation.

S. No.	Parameter evaluated	Method / Basis	Result obtained	Acceptable / Reference limit	Interpretation
1	Organoleptic properties	Visual and manual inspection	Uniform, firm, pleasant odour	Acceptable	Indicates proper formulation and saponification
2	pH (1% w/v solution)	Digital pH meter	7.92	7.5–9.0	Suitable for topical application
3	Foam height	Cylinder shake method	5.9 cm	>3 cm	Adequate foaming ability
4	Foaming index	Serial dilution method	250	>100	Satisfactory foam production
5	Foam retention time	Time-based observation	60 min	>7 min	Stable foam formation
6	Moisture content	Loss on drying	8%	<15%	Good shelf stability
7	Free alkali content	Acid–base titration	0.174%	<2%	Mild and non-irritant
8	Total fatty matter (TFM)	Acidification method	85%	>60%	Good-quality soap base
9	Antimicrobial activity	Cup plate method	Effective against Gram +ve and Gram -ve	Zone of inhibition	Antibacterial potential
10	Phytochemical profile	Qualitative tests	Flavonoids, phenols, tannins present	Presence desirable	Bioactive constituents
11	Antioxidant activity	H_2O_2 scavenging assay	Significant activity	Compared with standard	Free radical scavenging
12	Anti-inflammatory activity	Egg albumin denaturation	Inhibition observed	Compared with standard	Protein stabilization
13	Skin irritation	Topical application	No irritation observed	Should be absent	Safe for use
14	Stability	Storage observation	Stable at room temperature	No physical change	Formulation stability
15	Melting point	Thermal analysis	80–100 °C	Typical range	Thermal stability

Overall, the evaluation results confirm that the formulated polyherbal soap possesses acceptable physicochemical properties, antimicrobial efficacy, antioxidant and anti-

inflammatory potential, and good safety and stability profiles, supporting its suitability as a herbal cosmetic product.

CONCLUSION

The present study successfully formulated and evaluated TRIHERBA GMC, a tri-herbal polyherbal soap using guava leaves, manjistha roots, and cucumber peel. The soap showed acceptable physicochemical properties, good foaming ability, high total fatty matter, and low free alkali content, indicating suitability for topical application. The formulation demonstrated effective antimicrobial, antioxidant, and anti-inflammatory activities, supported by the presence of bioactive phytoconstituents. The absence of skin irritation and good stability further confirm its safety and quality. Overall, the developed polyherbal soap shows strong potential as a natural, safe, and effective cosmetic cleansing product.

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