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Research Article

Implementation and Evaluation of 24-Hours Matured Cold Process Anti-Acne Herbal Soap using *Ocimum sanctum* and *Mentha piperita* Peppermint Essential Oil

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Abstract

The present study focuses on the preparation and evaluation of a cold process anti-acne herbal soap matured within 24 hours using natural ingredients such as tulsi (*Ocimum sanctum*) and peppermint essential oil (*Mentha piperita*). The soap was formulated using vegetable oils including coconut oil, olive oil, and castor oil, along with additives such as sodium lactate, sugar, and salt to reduce curing time. The traditional cold process method usually requires 4–6 weeks of curing; however, this study successfully reduced the maturation time to 24 hours. The prepared soap was evaluated for physicochemical properties such as pH, hardness, foam stability, and antimicrobial activity against acne-causing bacteria like *Cutibacterium acnes* and *Staphylococcus epidermidis*. The results indicated good performance with effective antimicrobial activity, stable foam formation, and an acceptable pH for skin application. The study concludes that the developed herbal soap is a safe, efficient, and natural alternative to synthetic anti-acne soaps.

Keywords: Herbal soap, anti-acne, *Ocimum sanctum*, peppermint essential oil, cold process, antimicrobial activity.

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INTRODUCTION

Soap is a fundamental cleansing product, but growing awareness of skin sensitivity and acne has increased demand for formulations that are both effective and gentle. Herbal soaps have emerged as a promising alternative to synthetic products because plant-derived ingredients can offer antimicrobial, anti-inflammatory, and skin-soothing benefits [1]. In acne-prone skin, this approach is especially valuable because the condition is closely linked to microbial activity, excess sebum, and inflammation [2].

Acne is a common dermatological condition characterized by excessive sebum production, follicular blockage, and microbial colonization. Among the microorganisms involved, *Cutibacterium acnes* and *Staphylococcus epidermidis* play a major role in the development and progression of acne [3]. Conventional anti-acne products often contain synthetic chemicals that may lead to skin irritation, dryness, and long-term adverse effects, thereby increasing the demand for safer and more effective natural alternatives [4].

Herbal ingredients have attracted attention because many plant extracts contain bioactive compounds with antimicrobial, anti-inflammatory, and antioxidant properties [5]. *Ocimum sanctum* (tulsi) is well known for its medicinal value, particularly its antibacterial and soothing effects, while *Mentha piperita* (peppermint) essential oil adds a refreshing sensation along with antimicrobial activity. When incorporated into soap, these ingredients may help support acne-prone skin by reducing microbial load and improving user acceptability [6].

The cold process method of soap preparation is widely used due to its ability to retain the beneficial properties of natural ingredients. However, faster maturation methods are being explored to make production more practical without compromising quality. In this study, the formulation was designed to mature within 24 hours by using suitable additives such as sodium lactate, salt, and sugar, along with carefully selected oils, to produce a soap that is both functional and suitable for anti-acne use.

MATERIALS AND METHODS

Preparation of *Ocimum sanctum* Extract

Fresh tulsi (*Ocimum sanctum*) leaves were collected and thoroughly washed with clean water to remove dust and impurities. The cleaned leaves were then ground into a fine paste using a pestle and mortar. The obtained paste was filtered using filter paper to separate the liquid extract. The filtrate collected was used as the tulsi extract for further formulation.

Preparation of Lye Solution

Sugar, salt, and sodium lactate were first added to distilled water to enhance the hardness and lathering properties of the final soap. The required amount of sodium hydroxide (NaOH) was then carefully weighed and slowly added to the distilled water with continuous stirring. This step was carried out cautiously to control the exothermic reaction and prevent excessive heat generation. The prepared lye solution was then directly used for mixing with the oil phase.

Preparation of Oil Mixture

Coconut oil, castor oil, and olive oil were accurately measured using a weighing balance. These oils were transferred into a clean beaker and mixed thoroughly to obtain a uniform oil mixture. The temperature of the oil mixture was maintained between 35–40°C to ensure optimal conditions for the saponification process.

Cold Process Soap Preparation

The prepared hot lye solution was slowly poured into the oil mixture after passing through a steel filter to remove any insoluble impurities. The mixture was then blended using a hand blender until it reached the "trace" stage, which is indicated by slight thickening of the mixture. At this stage, tulsi extract and peppermint essential oil were added and mixed thoroughly to ensure uniform distribution throughout the soap base. A small amount of fragrance oil was also added to mask any residual odor of the oils [7].

Molding and 24-Hours Maturation

The prepared soap mixture was poured into suitable soap molds. Due to the addition of sugar and salt, the temperature of the mixture increased significantly, reaching approximately 70–80°C. The molds were covered with plastic wrap to retain heat and maintain proper reaction conditions. The temperature was monitored frequently to ensure consistency. The soap was allowed to mature and harden for 24 hours. After completion of the maturation period, the soap bars were removed from the molds and subjected to further evaluation tests [8]. Normally in traditional method, water and lye are in the ratio of 2:1 but in 24 hours maturing soap 1:1 ratio of water and lye is used because low amount of water will harden the soap faster than usual.

Isolation and Identification of Test Organisms

Isolation and identification of test organisms were carried out using standard microbiological techniques. Samples were collected from skin using sterile swabs and subjected to isolation procedures. The isolates were identified based on Gram staining, motility testing, and biochemical assays including catalase and indole tests. For Staphylococcus epidermidis, samples obtained from skin or clinical sources were streaked onto Nutrient Agar (NA) or Mannitol Salt Agar (MSA) plates using the streak plate method. The inoculated plates were incubated at 37°C for 24–48 hours. In the case of *Cutibacterium acnes*, samples were collected from sebaceous areas of the skin using sterile swabs. These were inoculated onto anaerobic blood agar plates or reinforced clostridial medium and incubated under anaerobic conditions at 37°C for 48–72 hours. For Propionibacterium species, samples were similarly collected from sebaceous regions of the skin and inoculated onto anaerobic blood agar. The plates were incubated anaerobically at 37°C for 48–72 hours.

Gram staining and motility test

Gram staining and motility test were performed following the method of Chanda and Rakholiya (2010) [9]. For Gram staining, a clean glass slide was taken and a drop of water was placed on it. A small amount of bacterial culture was added and spread to form a thin smear, which was then air-dried and gently heat-fixed. The smear was stained with crystal violet for 1 minute, rinsed with water, followed by the addition of iodine solution for 1 minute and rinsed again. Decolorization was carried out using alcohol or acetone for 10–20 seconds, immediately followed by washing. Safranin was then applied as a counterstain for 30–60 seconds. The slide was rinsed, blotted dry, and observed under a microscope. For the motility test, a semi-solid motility agar medium was prepared in a test tube. A sterile inoculating needle was used to pick a bacterial colony, which was then stabbed straight into the center of the medium. The tube was incubated at 35–37°C for 18–24 hours, and motility was determined based on the spread of growth away from the line of inoculation.

Biochemical tests

Biochemical tests were performed following the method of Chanda and Rakholiya (2010) [9]. For the indole test, a sterile test tube containing tryptone broth was inoculated with the test organism using a sterile loop and incubated at 35–37°C for 18–24 hours. After incubation, 0.5 mL of Kovac's reagent was carefully added to the surface of the broth to detect indole production. For the catalase test, a drop of 3% hydrogen peroxide was placed on a clean glass slide, and a small portion of the bacterial colony was transferred onto the drop using a sterile loop or wooden stick. Immediate observation was made for the formation of bubbles, which indicates a positive catalase reaction.

Antimicrobial Activity of Soap Sample (Well Diffusion Method)

The antimicrobial activity of the prepared herbal soap was evaluated using the well diffusion method. Nutrient agar medium was prepared, sterilized, and poured into sterile Petri plates, which were allowed to solidify. Fresh cultures of *Staphylococcus epidermidis*, *Cutibacterium acnes*, and *Propionibacterium* were spread uniformly over the agar surface using a sterile cotton swab to form

a lawn culture. A soap extract solution was prepared by dissolving a small quantity of the soap in sterile distilled water. Wells were created in the agar using a sterile cork borer, and a measured volume of the soap extract was added into each well. The plates were incubated at 37°C for 24 hours. After incubation, clear zones around the wells indicated antimicrobial activity, and the diameter of the zone of inhibition was measured in millimeters. Different concentrations of soap solution were prepared by dissolving 1 g, 2 g, and 3 g of soap separately in 20 mL of distilled water in three labeled test tubes to study concentration-dependent activity[10].

Foam Test

The foam test was performed to evaluate the lathering ability and foam stability of the soap. A soap solution was prepared by dissolving 1 g of soap in 100 mL of distilled water. From this, 20 mL was transferred into a measuring cylinder, which was then closed and shaken vigorously for about 10 seconds. The cylinder was placed on a flat surface, and the foam was allowed to settle. The height of the foam layer was measured immediately and again after 5 minutes to assess foam stability [11].

pH Test

The pH of the soap was determined to ensure its safety and skin compatibility. One gram of soap was dissolved in 10 mL of distilled water in a beaker and stirred until completely dissolved. The pH was then measured using a pH meter or pH indicator paper [12].

Skin Irritability Test (Patch Test)

A mild soap solution was prepared and applied to a small area of the inner forearm. The area was cleaned before application and left undisturbed for 15–20 minutes. Afterward, it was washed with clean water and observed for any signs of irritation such as redness, itching, or swelling. Observations were recorded immediately and after 24 hours to check for delayed reactions [12].

Color, Shape, and Appearance Test

After 24 hours of maturation, the soap bars were removed from the molds and visually inspected under natural light. Observations were made regarding color uniformity, shape, surface texture, and the presence of any cracks or defects. The odor and tactile properties such as smoothness and firmness were also noted [13].

Moisture Content Determination

A clean, dry weighing dish was weighed (W_1), and approximately 5 g of soap sample was added and weighed again (W_2). The sample was dried in a hot air oven at 105°C for 1–2 hours to remove moisture. After cooling in a desiccator, the final weight (W_3) was recorded to determine moisture content [14].

Consistency / Hardness Test

The soap bars were allowed to reach room temperature and then gently pressed between fingers to assess firmness. A penetrometer or simple finger pressure method was used to evaluate hardness, and observations were recorded at multiple points to ensure uniformity [15].

Washability Test

A clean surface such as a glass plate or ceramic tile was used for the test. The soap was rubbed on the wet surface, and water was added gradually to simulate washing conditions. The time required for lather formation, ease of spreading, and ability to rinse off without residue were observed and recorded [11].

RESULTS

Isolation and Identification of Test Organisms

The identification of the isolates was carried out using Gram staining, motility, and biochemical tests, and the results are presented in Figures 1–4. Gram staining revealed that Isolate 1 was Gram-positive cocci arranged in clusters, while Isolates 2 and 3 were Gram-positive rod-shaped bacteria (Figure 1). The motility test indicated that Isolate 1 was motile, as growth spread away from the line of inoculation, whereas Isolates 2 and 3 were non-motile, with growth restricted to the stab line (Figure 2). The catalase test showed positive results for all isolates, evidenced by immediate bubble formation upon the addition of hydrogen peroxide (Figure 3). The indole test showed a negative result for Isolate 1, with no red ring formation, while Isolates 2 and 3 showed positive results indicated by the formation of a red ring after the addition of Kovac's reagent (Figure 4).

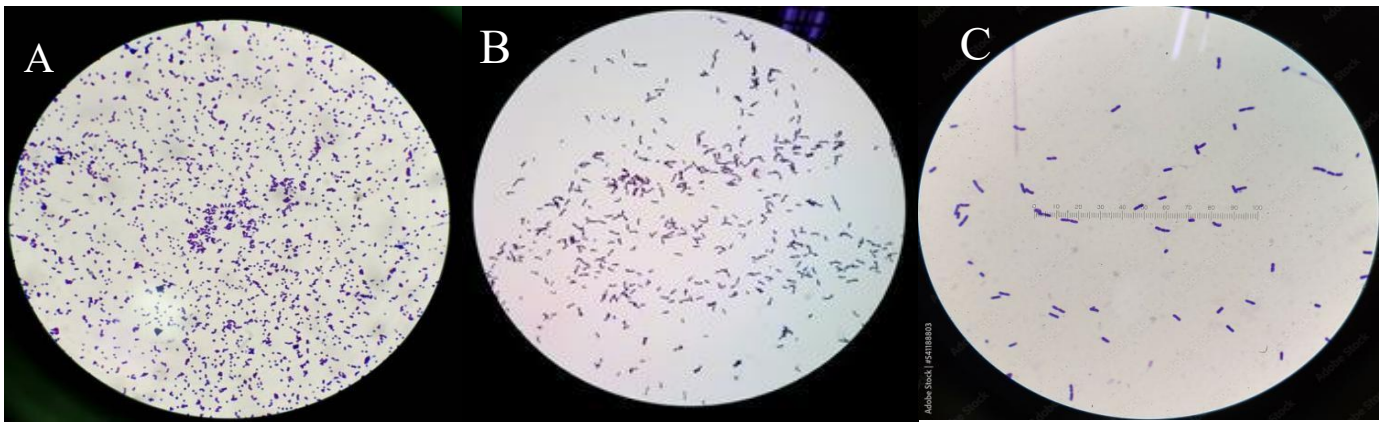


Figure 1: Gram staining of bacterial isolates showing (A) Gram-positive cocci in clusters (Isolate 1) and (B–C) Gram-positive rod-shaped bacteria (Isolates 2 and 3) observed under oil immersion (100X)

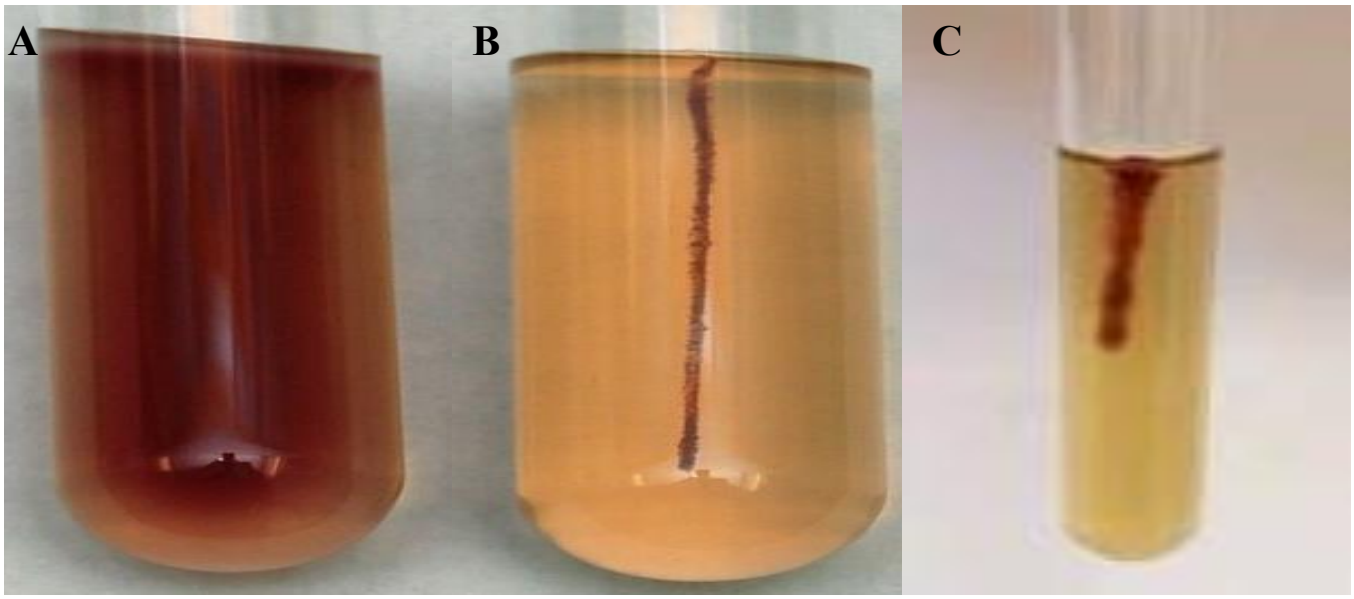


Figure 2: Motility test of bacterial isolates

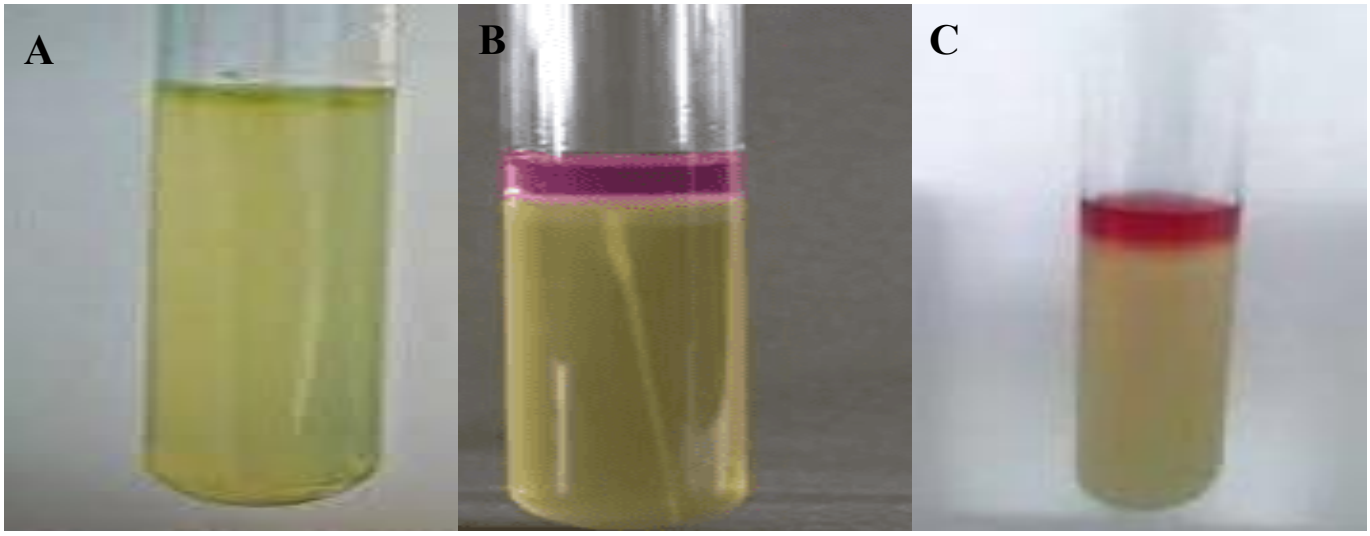


Figure 3: Indole test showing absence of red ring in (A) indicating a negative result, and presence of a red ring in (B–C) indicating positive results



Figure 4: Catalase test showing bubble formation in (A–C), indicating positive results for all isolates

Antimicrobial Activity of Soap Sample

The antimicrobial activity of the prepared herbal soap was evaluated using the well diffusion method, and the results are presented in Table 1. The soap exhibited significant antibacterial activity against all tested organisms, with inhibition zones increasing with concentration. Among the organisms tested, *Cutibacterium acnes* showed the highest sensitivity, with zones of inhibition ranging from 15–17 mm across trials. This was followed by *Staphylococcus epidermidis*, which exhibited moderate inhibition with zones ranging from 13–15 mm. The least inhibition was observed against Propionibacterium species, with zones ranging from 12–14 mm. The results were consistent across trials, indicating reproducibility. Overall, the highest concentration produced the maximum antibacterial effect, confirming a concentration-dependent antimicrobial activity of the herbal soap.

Table 1: The antibacterial activity of formulated herbal soap against *Staphylococcus epidermidis*, *Cutibacterium acnes* (formerly *Propionibacterium acnes*), *Propionibacterium* species (general)

S.no	Organism	50 mg/mL	100 mg/mL	150 mg/mL
1.	*Staphylococcus epidermidis*	13 ± 0.2	14 ± 0.1	15 ± 0.3
2.	*Cutibacterium acnes*	15 ± 0.3	16 ± 0.4	17 ± 0.2
3.	*Propionibacterium sp*	12 ± 0.2	13 ± 0.2	14 ± 0.6

Values were in triplicates expressed as mean ± standard deviation (SD)

Foam Test

The foam test results are presented in Table 2. The prepared herbal soap exhibited good lathering ability, with a high initial foam height observed immediately after shaking. The foam height showed a gradual decrease over time, from 30 cm initially to 25 cm after 5 minutes and 20 cm after 10 minutes, indicating good foam stability. The sustained foam height suggests that the formulation possesses satisfactory lathering properties, which may be attributed to the presence of sugar and castor oil that enhance foam formation and retention.

Table 2: Foam height (cm) of the prepared herbal soap at different time intervals.

Time	Foam Height(cm)
Immediately after shaking	30
After 5 minutes	25
After 10 minutes	20

pH Test

The pH of the prepared herbal soap is presented in Table 3. The formulation exhibited an initial pH of 10.0 immediately after preparation, which decreased to 8.5 after 24 hours, indicating stabilization over time. The final pH (8.5) falls within the acceptable range for skin application, suggesting mild alkalinity and the absence of excess unreacted sodium hydroxide in the formulation.

Table 3: pH of the prepared herbal soap at different time intervals

Time	pH Value
Immediately after preparation	10.0
After 24 hours	8.5

Skin Irritability Test (Patch Test)

The patch test results showed no signs of irritation such as redness, itching, or swelling on the skin after application of the soap solution. Observations made immediately and after 24 hours confirmed that the soap is safe and suitable for use on human skin, especially for acne-prone conditions.

Color, Shape, and Appearance Test

The physical characteristics of the prepared herbal soap are presented in Table 4. The soap bars exhibited a uniform light green color with a smooth surface texture. The shape was well-defined and rectangular, corresponding to the mold used, and no visible cracks or defects were observed. The soap showed good hardness and was easily removed from the mold. Additionally, a pleasant odor was noted due to the incorporation of peppermint essential oil.

Table 4: Physical characteristics of the prepared herbal soap

Parameter	Observation
Shape	Rectangular
Color	Light green
Texture	Smooth
Hardness	Firm
Unmolding	Easy

Moisture Content Determination

The moisture content of the prepared herbal soap is presented in Table 5. The formulation showed a consistent moisture content of 1.0% across all samples, indicating effective drying and proper maturation within 24 hours. The low moisture content contributes to improved hardness and enhanced shelf life of the soap.

Table 5: Moisture content (%) of the prepared herbal soap determined after drying.

S. No	Initial Weight of Dish + Soap (W_2) (g)	Final Weight after Drying (W_3) (g)	Moisture Content (%)
1	50.5	50.0	1.0
2	50.6	50.1	1.0
3	50.4	49.9	1.0

Consistency / Hardness Test

The prepared herbal soap exhibited good hardness and consistency upon manual evaluation. The bars were firm and resistant to deformation under applied pressure, indicating effective saponification and suggesting that additives such as sodium lactate and salt contributed to enhanced hardness of the formulation.

Washability Test

The prepared herbal soap demonstrated satisfactory washability characteristics. It produced lather readily, spread uniformly over the surface, and was easily rinsed off without leaving any residue, indicating good cleansing efficiency.

DISCUSSION

The present study focused on the formulation and evaluation of a 24-hour matured cold process anti-acne herbal soap using natural oils and plant-based ingredients. The aim was to develop a formulation with effective cleansing properties and antimicrobial activity against bacterial isolates associated with acne-causing microorganisms. Herbal ingredients such as *Ocimum sanctum* (tulsi) extract and peppermint essential oil were incorporated due to their well-documented antimicrobial, antioxidant, and anti-inflammatory properties. Medicinal plant extracts have been widely reported to exhibit significant antimicrobial activity and are commonly used in traditional skincare formulations[16,17].

The composition of oils played a crucial role in determining the physicochemical properties of the soap. Coconut oil contributed to cleansing efficiency and hardness due to its high lauric acid content, while olive oil provided moisturizing and skin-conditioning properties. Castor oil enhanced lather stability and foam quality due to its ricinoleic acid content. The combination of these oils resulted in a balanced formulation with desirable cleansing and moisturizing characteristics. Similar effects of oil composition on soap quality have been reported in cosmetic science studies[18].

The soap was prepared using a modified cold process method with reduced water content (1:1 ratio of water to lye) to accelerate maturation within 24 hours. Additives such as sodium lactate, sugar, and salt were incorporated to improve hardness, facilitate unmolding, and enhance foam stability. The reduced water content contributed to rapid hardening without compromising structural integrity, which is consistent with reported soap formulation approaches[19].

The antimicrobial activity of the formulated herbal soap demonstrated a clear concentration-dependent effect against the tested bacterial isolates. The highest inhibition was observed against rod-shaped isolates (15–17 mm), followed by cocci isolates (13–15 mm) and other isolates (12–14 mm). This antibacterial activity can be attributed to the presence of bioactive compounds such as eugenol, flavonoids, and phenolic constituents in *Ocimum sanctum*, along with menthol and essential oils from peppermint. These compounds exert antimicrobial effects by disrupting microbial cell membranes and interfering with enzyme activity[16,20]. The observed increase in inhibition zones with increasing concentration further confirms the dose-dependent antibacterial activity of the formulation.

Physicochemical evaluation of the soap indicated desirable characteristics. The foam test showed stable lather formation, which can be attributed to the presence of castor oil and sugar that enhance foam stability. The pH of the formulation decreased from 10.0 to 8.5 after 24 hours, indicating completion of saponification and stabilization of the soap within a skin-compatible alkaline range. Similar pH ranges have been reported for mild soaps intended for topical use[19]. The low moisture content (1.0%) contributes to improved hardness and extended shelf life. Additionally, the soap exhibited uniform appearance, firm consistency, good washability, and no signs of skin irritation, indicating its safety and suitability for topical application.

Overall, the 24-hour maturation technique proved effective in producing a stable and functional herbal soap with desirable physicochemical and antimicrobial properties. The formulation demonstrated significant antibacterial activity, stable foam, suitable pH, and good user acceptability. These findings support the potential of herbal formulations as effective alternatives to synthetic products for acne management and align with the growing interest in natural and plant-based cosmetic formulations[17].

CONCLUSION

The present study successfully developed a 24-hour matured cold process herbal soap using *Ocimum sanctum* and peppermint essential oil with suitable physicochemical properties and good antimicrobial activity against acne-causing bacteria. The formulation showed acceptable pH, stable foam, adequate hardness, non-irritant nature, and effective washability, indicating that it is safe and practical for skin use. The reduced maturation time makes the method more efficient than the traditional curing process, while the use of natural ingredients enhances its value as an eco-friendly and skin-friendly anti-acne soap. Overall, this herbal soap can serve as a promising natural alternative to synthetic anti-acne products.

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Author Contributions:

Kannahi M: Conducted and supervised the study, guided the experimental work, and revised the manuscript.

Iswarya G, Kamali S, Madhumidha S, Manimozhi B, Najibah Banu N and Santhoshini R: Performed the experimental work, collected data, and assisted in manuscript preparation.

All authors read and approved the final manuscript.

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