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Research Article

Formulation of Bar Soap Containing Neem and Sulfur as Active Ingredients and Antibacterial Testing Against *Propionibacterium acnes*

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ABSTRACT

Propionibacterium acnes is one of the main bacterial contributors to acne vulgaris, a prevalent dermatological condition. The prolonged use of topical antibiotics often leads to bacterial resistance, highlighting the need for safe and effective natural alternatives. Neem and sulfur are known for their antibacterial, anti-inflammatory, and keratolytic properties, but studies on their combined application in herbal soap formulations remain limited. This study aimed to formulate an antibacterial herbal bar soap combining neem extract and sulfur and to evaluate its physicochemical characteristics and antibacterial activity against *P. acnes*. The soap was prepared through saponification of coconut, palm, and olive oils (25%:35%:40%) producing four formulations: base soap (H1), neem soap (H2), sulfur soap (H3), and combined neem–sulfur soap (H4). Each formulation was assessed for organoleptic properties, pH, moisture content, free alkali and antibacterial activity against *P. acnes* with a commercial sulfur soap as positive control.All formulations met the reference standard of solid soap with pH 8.67–9.22, moisture content 7.76–8.46 %, and free alkali <0.1%. The H4 formula showed the highest antibacterial activity with a 9.00 mm inhibition zone, surpassing the positive control (8.4 mm). The combination of neem extract and sulfur can be effectively formulated into a stable herbal antibacterial soap with significant inhibitory activity against *P. acnes*. This product shows promising potential as a natural therapeutic for acne-prone skin.

Keywords: *Acne vulgaris*; *Azadirachta indica*; antibacterial soap; *Propionibacterium acnes;* sulfur

INTRODUCTION

Acne vulgaris is a common dermatological condition characterized by inflammatory and non-inflammatory lesions driven by increased sebum production, follicular hyperkeratinization, and colonization by *Propionibacterium acnes* (Anyanwu & Okoye, 2017; Kim & Kim, 2024). *P. acnes* is an anaerobic, Gram-positive commensal that proliferates in sebum-rich microenvironments and contributes to inflammation through lipase activity and subsequent free fatty acid–mediated irritation (Chukwuemeka Paul Azubuike et al., 2015; McLaughlin et al., 2019). Conventional acne therapies, including topical and systemic antibiotics, retinoids, benzoyl peroxide, and keratolytic agents, are effective but are associated with adverse effects and the emergence of antimicrobial resistance with prolonged use (Brüggemann et al., 2021; Wylie & Merrell, 2022).

Azadirachta indica (neem) leaves contain limonoids, flavonoids, tannins, and phenolic constituents that exhibit antibacterial, anti-inflammatory, and anti-biofilm activities relevant to acne management (Ali et al., 2021). The extract exhibits potent antibacterial activity by disrupting bacterial cell membrane synthesis and inhibiting the growth of *P. acnes*, making it a promising agent against antibiotic-resistant strains (Baby et al., 2022; Silvyana et al., 2022).

Sulfur has long-standing dermatological use as a keratolytic, comedolytic, sebostatic, and antimicrobial agent, and topical sulfur formulations have been shown to reduce comedones and inflammatory lesions in acne through combined keratolytic and direct antimicrobial effects. Formulation of herbal actives into solid soap matrices presents specific challenges because saponification conditions (alkaline pH and elevated temperatures) can degrade sensitive phytochemicals and because surfactant matrices may affect active diffusion in microbiological assays (Chen et al., 2025; Kim & Kim, 2024).

Previous studies have demonstrated antibacterial activity of neem extracts and topical efficacy of sulfur separately, and several reports have described herbal soap formulations meeting physical-chemical quality standards, yet empirical data on combined neem–sulfur incorporation in a solid soap bar and its antibacterial efficacy against *P. acnes* remain limited (Adlia et al., 2019; Caesa Anjarini et al., 2023; Chen et al., 2025; Malawati et al., 2024). Addressing this gap, the present study formulated four soap variants (base, neem, sulfur, neem–sulfur) using a coconut oil:palm oil:olive oil ratio optimized for hardness and skin feel and evaluated physical-chemical quality parameters (organoleptic properties, pH, free alkali, and moisture) according to national standards alongside antibacterial testing versus *P. acnes* by agar well diffusion.

METHODS

Materials

The primary active ingredients used in this study were Neem leaf extract (*A. indica*) and Sulfur powder. The base materials for soap formulation included coconut oil, palm oil, and olive oil. Essential chemical reagents, which were of analytical grade, included Sodium Hydroxide (NaOH), Potassium Hydroxide (KOH), and Hydrochloric Acid (HCl). The solvent utilized for extraction was 96 % ethanol, while the titration process employed phenolphthalein indicator (PP) and distilled water (aquades). The antibacterial activity was tested against a pure culture of *P. acnes*, maintained on Mueller Hinton Agar (MHA) media, supplied by Merck. A commercial sulfur-based soap (Asepso brand) was used as the positive control.

The instruments employed for research activities including an analytical balance for precise mass measurements, a digital pH meter, a blender for sample homogenization, a laminar air flow cabinet, an autoclave for sterilization, and an incubator for bacterial culture. Other supporting tools included soap molds, Petri dishes, micropipettes, filter paper, and an oven for moisture content determination.

Methods

Neem Leaf Extract Preparation

Fresh neem leaves were cleaned under running water and air-dried briefly. The leaves were then cut into small pieces and homogenized using blender together with 96 % Ethanol as the extraction solvent, maintaining a 1:1 (w/v) fresh leaves to solvent ratio. The resulting mixture was filtered to obtain the crude extract filtrate, which was then stored in a refrigerator prior to its use in the soap formulation stage.

Herbal Bar Soap Formulation

The solid herbal soap was prepared via the hot saponification method with slight modification (Putri et al., 2023). The oil blend comprised coconut oil, palm oil, and olive oil in a weight ratio of 25%:35%:40%, respectively. The selection of the 25% coconut oil, 35% palm oil, and 40% olive oil ratio was based on previous studies demonstrating that this combination yields optimal hardness, cleansing ability, and mildness in solid soap formulations (Kuntom & Kifli, 1998; Ermawati et al., 2022; Putri et al., 2023). A total mass of 1000 g of the oil mixture was accurately weighed and heated to a temperature range of 40–45 °C. Concurrently, the lye solution was prepared by dissolving 147 g of NaOH pellets in 294 mL of distilled water. The NaOH solution was slowly added to the heated oil blend while continuously stirring until the mixture reached the trace phase (initial emulsification). Active ingredients were incorporated based on defined weight ratios relative to the total soap mass. Specifically, H1 served as the base soap without added actives, H2 contained 90 g neem extract (9 % w/w), H3 contained 90 g sulfur powder (9 % w/w), and H4 combined 30 g neem extract (3 % w/w) with 60 g sulfur powder (6 % w/w). These concentrations of sulfur-based topical products are permitted at 3–10 % w/w according to the U.S. FDA monograph for over-the-counter acne treatments (U.S. Food and Drug Administration, 2021). The saponified mixture was poured into molds, followed by a curing process conducted for two weeks at ambient temperature to ensure complete saponification and hardening (Putri et al., 2023).

Antibacterial Activity Assay of Herbal Bar Soap Against P. acnes

The antibacterial activity of the soap formulations was evaluated using the agar well diffusion method. A pure culture of *P. acnes* was inoculated and evenly distributed onto the Mueller Hinton Agar (MHA) medium. Wells were aseptically created in the agar medium using a sterile cork borer. The soap samples were prepared by dissolving the solid soap into distilled water to obtain a test concentration of 10 % w/v. A volume of 50 µL of each soap solution was dispensed into the respective wells. The plates were then incubated in an incubator at a temperature of 37 °C for 24 hours. The antibacterial efficacy was determined by measuring the diameter (in millimeters) of the resulting clear zone of inhibition. Distilled water served as the negative control, and a commercial sulfur-based soap (Asepso) was utilized as the positive control (Tahar et al., 2023).

Physicochemical Quality Analysis of Antibacterial Soap

The quality parameters of the solidified herbal soap were analyzed, including organoleptic evaluation, pH measurement, free alkali content, and moisture content determination.

Organoleptic Test

The Organoleptic Test, a visual and sensory assessment was performed, including the evaluation of the soap's aroma, physical shape, and color. The pH Test was conducted by dissolving 1 g of solid soap in 10 mL of distilled water (a 1:10 ratio) until a homogeneous solution was formed. The pH value of this solution was measured at room temperature using a digital pH meter that had been pre-calibrated with standard buffer solutions of pH 4.00 and pH 7.00.

Free Alkali Content Test

The Free Alkali Content Test began by heating 100 mL of 96 % ethanol to near boiling, followed by the addition of 0.5 mL of Phenolphthalein (PP) indicator. The solution was then cooled to approximately 70 °C and neutralized with 0.1 N KOH in ethanol until a stable faint pink color appeared. A 5 g sample of the solid soap was weighed and dissolved completely in the neutral ethanol on a water bath with stirring. The mixture was then titrated with 0.1 N HCl in ethanol until the pink color entirely disappeared (the titration endpoint). The volume of HCl consumed was recorded and used to calculate the percentage of free alkali.

Moisture Content Test

The Moisture Content Test was carried out using the gravimetric approach, adhering to the standard method outlined in SNI 3532:2016. A 5 g soap sample was heated in an oven set at 105 ± 2 °C for one hour. The sample was subsequently cooled in a desiccator and reweighed repeatedly until a constant mass was achieved, thereby determining the total moisture loss.

RESULTS AND DISCUSSIONS

The evaluation of the developed herbal soap formulations was conducted in two critical phases, such as ensuring product quality and safety through comprehensive physicochemical analysis, and assessing the comparative antibacterial efficacy against *P. acnes*, the primary causative agent of acne vulgaris. Four formulations were tested: a base soap (H1), neem extract (H2), sulfur powder (H3), and the novel combined neem–sulfur formulation (H4).

Physicochemical Properties of the Formulated Soap

Organoleptic and Physical Characteristics

All four formulations yielded visually appealing, solid, and hard bar soaps, conforming to standard physical quality requirements. The visual differences among the formulations are presented in **Figure 1**. The oil blend ratio employed (25% coconut oil, 35% palm oil, and 40% olive oil) was critical for achieving this hardness. Specifically, Palm oil, rich in palmitic acid (C-16), significantly contributes to the structural integrity and desirable consistency of the solid soap bar (Ermawati et al., 2022; Kuntom & Kifli, 1998; Listyani & Muna, 2024).

Figure 1. Visual comparison of herbal soap: base (H1), neem (H2), sulfur (H3), and neem–sulfur (H4)

Sensory analysis revealed distinct differences based on the incorporated active ingredients. The base soap (H1) maintained its characteristic neutral odor and white color, while the addition of neem extract (H2) resulted in a light brown color derived from the phytochemical constituents. Formulations containing elemental sulfur (H3 and H4) naturally developed a brown hue and a distinct sulfurous aroma. However, a noteworthy observation was that the combined formulation (H4) exhibited a milder sulfur odor compared to the sulfur monotherapy (H3). This sensory improvement is attributed to the complex lipophilic compounds present in the neem extract, which may partially mask the volatile sulfurous compounds, enhancing the overall consumer acceptability of this product.

Analysis of pH Value and Residual Alkali Content

The pH value and the free alkali content are the most important indicators of soap quality, affecting skin compatibility and the completeness of the saponification process. The analysis results are summarized in **Table 1**.

All formulations successfully met the specified regulatory limits, including the SNI 3532-2016 requirement for pH (6.0–11.0) and the critical limit for free alkali (<0.1%). The low residual free alkali levels (0.080%) confirm that the hot process saponification resulted in products that are safe for dermal application, minimizing the risk of caustic irritation often associated with incompletely cured soaps. The neem–sulfur herbal soap exhibited typical alkaline characteristics. The free alkali content was very low, indicating nearly complete saponification. Industry standards (ISO) recommend free alkali be below 2% in soaps (Betsy et al., 2021). This low free alkali is important, as excess alkali can cause skin irritation (Betsy et al., 2021; Nova et al., 2025).

The measured pH was 8.88. such pH values are in line with the natural soap range and generally considered safe for cleansing applications (Lukić et al., 2021). Balanced soap pH is known to be gentler to the skin than highly alkaline formulations (Nova et al., 2025). A significant finding was the marked reduction in pH in the formulations containing active ingredients, particularly H2 (neem), which registered the lowest pH (8.67) and the lowest free alkali content (0.064%). This reduction suggests an interaction between the neem extract and the residual alkali present in the soap matrix. Neem extracts, especially those prepared using ethanol, contain organic acids, phenolic compounds, and other acidic metabolites, all of which possess acidic functional groups capable of donating protons. These components likely participate in the neutralization or buffering of residual sodium hydroxide (free alkali) that remained after the primary saponification reaction. This consumption of residual base effectively lowers the overall pH of the final product. As illustrated in **Figure 2**, the mechanism shows the neutralization reaction between free alkali and organic acids derived from neem extracts. This process yields salt and water, which are relatively less basic, thereby contributing to a reduction in the overall acidity (pH).

Table 1. Physicochemical Characteristics of Herbal Soap Formulations

Formulation	pH Value	Free Alkali (%)	Moisture Content (%)
H1	9.22 ± 0.02	0.084 ± 0.004	8.03 ± 0.02
H2	8.67 ± 0.03	0.064 ± 0.007	8.46 ± 0.03
H3	9.01 ± 0.03	0.075 ± 0.005	7.76 ± 0.01
H4	8.88 ± 0.01	0.070 ± 0.002	7.87 ± 0.08

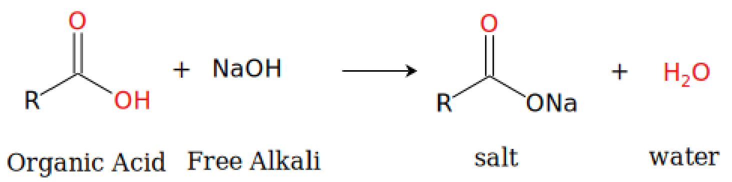

Figure 2. Neutralization mechanism of Free Alkali with Organic Acids from Neem Extract

Determination of Moisture Content and Formulation Stability

The moisture content across all four variants was narrow, ranging from 7.76 % to 8.46 %, well below the SNI maximum acceptable limit of <15%. Low moisture content is a critical determinant of product stability, as it inhibits chemical degradation kinetics and prevents the proliferation of microorganisms over the product's shelf life (Febriani et al., 2020). This resulted (<10%) is consistent with previous studies; for example, Das et al. (2024) reported 10% moisture for a neem-honey herbal soap (Das et al., 2024). Maintaining moderate moisture helps the soap harden properly while still retaining a creamy lather. In accordance with these findings, the formulated soap cured to a firm, smooth bar that did not crumble.

Observed differences between formulations were attributable to the physical properties of the incorporated active material. The sulfur formulation (H3) showed the lowest moisture content (7.76 %) because elemental sulfur is an inert, non-hygroscopic solid that increases the total dry mass fraction, thus decreasing the relative water content. Conversely, H2 (neem extract) demonstrated the highest moisture content (8.46 %). This slight increase is likely due to residual polar solvents, specifically water and ethanol, carried over from the 96 % ethanol extract during the formulation phase, which became trapped within the soap matrix.

Antibacterial Efficacy Against P. acnes

The antibacterial activity of the formulations against *P. acnes* was assessed using the agar well diffusion method. The results, presented as the mean diameter of the zone of inhibition, are summarized in **Table 2**.

The results demonstrate that all formulated herbal soaps exhibit intrinsic antimicrobial activity against *P. acnes*. The base soap (H1), containing no added active ingredient, produced an inhibition zone of 7.50 mm. This baseline inhibition is likely a consequence of the intrinsic properties of the alkaline soap matrix and the surfactant action itself, which can exert a denaturing effect on bacterial cell walls. The exact sulfur concentration in the commercial positive-control soap was not disclosed by the manufacturer, the comparison in this study was performed based solely on the observed antibacterial performance rather than the active ingredient content. Despite this limitation, the formulated soaps remain within the acceptable regulatory limits for sulfur content in topical cleansing products. According to the U.S. FDA Monograph for Over the Counter Acne Treatments, sulfur is permitted at 3–10 % w/w in topical cleansing bars, and formulations H3 (9 % w/w sulfur) and H4 (6 % w/w sulfur combined with 3 % w/w neem extract) fall within this approved range. (U.S. Food and Drug Administration, 2021).

Both formulations showed increased activity over the base soap. H2 (neem) produced a zone of 8.35 mm, and H3 (sulfur) yielded 7.96 mm. Critically, the combined formulation (H4) achieved the maximum zone of inhibition at 9.00 mm. This efficacy is higher to that of either monotherapy and surpasses the standard commercial sulfur soap used as the positive control (8.40 mm). The incremental gain provided by the combination validates the formulation, suggesting an enhanced effect upon integrating neem extract and sulfur powder.

Table 2. Diameter of Inhibition Zone of Herbal Bar Soap Against *P. acnes*

Sample	Active Ingredient	Inhibition Zone Diameter (mm)
K(-)	Distilled Water (Negative Control)	0.00 ± 0.000
K(+)	Commercial Sulfur Soap (Positive Control)	8.40 ± 0.000
H1	Base Soap (No Active)	7.50 ± 0.813
H2	Neem Extract	8.35 ± 0.566
H3	Sulfur	7.96 ± 0.406
H4	Neem Extract + Sulfur Powder	9.00 ± 0.318

The high efficacy observed in H2 (8.35 mm) is consistent with the established antimicrobial properties of *A. indica* (neem). Neem leaves are a potent source of secondary metabolites, including limonoids (such as nimbolide and mahmoodin), flavonoids, and phenolic compounds. These compounds are known to exhibit strong antibacterial activity against various pathogens, including *P. acnes* (Ali et al., 2021).

The sulfur monotherapy (H3) provided moderate anti-*P. acnes* activity (7.96 mm). Sulfur has a long history in dermatology due to its multifunctional role in treating acne. Its primary mechanisms include keratolytic and antimicrobial effects (Chen et al., 2025). The observation that H3 showed a lower inhibition zone diameter compared to H2 suggests that the keratolytic benefit of sulfur a critical in vivo therapeutic function is not fully captured in the in vitro agar diffusion assay, which mainly measures acute antimicrobial toxicity of the dissolved species. The combined formulation H4 exhibited the highest antibacterial activity, with an inhibition zone of 9.00 mm, which is 1.50 mm larger than that of the base soap (H1). This enhanced efficacy confirms the premise that the combination strategy yields a the best therapeutic outcome.

The phytochemicals in neem, known to disrupt bacterial membranes and interfere with cell wall synthesis, effectively weaken the structural integrity of *P. acnes*. This compromised bacterial state facilitates greater and more rapid uptake and action of the highly reactive sulfur metabolite, H_2S, thereby maximizing the total cytotoxic effect (Baby et al., 2022; Olson et al., 2018; Wylie & Merrell, 2022). The successful combination of neem and Sulfur delivers a comprehensive therapeutic solution for acne. Neem contributes anti-inflammatory, anti-lipid synthesis, and antibacterial actions, while Sulfur provides potent keratolytic and secondary antimicrobial properties. This synergistic, multi-pathway attack strategy is highly valuable in combating *P. acnes*, particularly in mitigating the risk of antimicrobial resistance, which is a common challenge associated with prolonged use of conventional single-agent topical antibiotics. The results of H4 position this formulation as a promising candidate for advanced, natural anti-acne dermatological products.

CONCLUSION

The combination of neem leaf extract and sulfur was successfully formulated into a herbal soap bar, demonstrating highest efficacy against *P. acnes*, the primary etiological agent of acne vulgaris. All developed formulations successfully complied with the quality standards for solid soap specified by the Indonesian National Standard (SNI 3532:2016), exhibiting favorable physicochemical characteristics including a safe pH range of 8.67–9.22, low moisture content (7.76–8.46%), and minimal free alkali content (<0.1%).

Specifically, the combination of neem–sulfur bar soap (H4) displayed the most significant antibacterial activity, resulting in an inhibition zone of 9.00 mm against *P. acnes*, which was demonstrably greater than that achieved by the commercial sulfur-based positive control (8.4 mm). This enhanced antibacterial effect is attributed to the synergistic action between the antimicrobial and anti-inflammatory properties of neem's phytochemicals and the keratolytic and antibacterial action of sulfur. In conclusion, this study validates that the incorporation of a neem–sulfur combination yields a stable herbal antibacterial soap with significant inhibitory activity against *P. acnes*, positioning this product as a promising natural therapeutic alternative for acne management..

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