

Original Article

Influence of Stearic Acid and Triethanolamine on the Physical Properties and Antibacterial Efficacy of *Ocimum basilicum* L. Anti-acne Cream against *Staphylococcus epidermidis*

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Abstract: Acne vulgaris represents a significant dermatological concern, with *Staphylococcus epidermidis* identified as a key pathogenic contributor. While *Ocimum basilicum* L. (basil) leaves contain bioactive compounds including flavonoids, eugenol, and tannins that demonstrate promising antibacterial properties, the successful translation of these natural antimicrobials into effective topical formulations remains critically dependent on appropriate excipient selection. Despite the growing interest in botanical-based acne treatments, there exists a significant knowledge gap regarding how emulsifying agents, particularly stearic acid and triethanolamine (TEA), influence both the physical stability and therapeutic efficacy of herbal cream formulations. This study investigated the effects of varying concentrations of stearic acid and TEA on the physical characteristics, stability, and antibacterial activity of basil leaf extract cream formulations against *S. epidermidis* ATCC-12228. Extracts obtained via maceration in 96% ethanol were incorporated into cream formulations (F0–F4), which were subsequently evaluated for organoleptic properties, homogeneity, spreadability, adhesion, pH, stability using a thermal cycling test, and antibacterial activity via disc diffusion. Stability assessment revealed notable differences across formulations. Although all formulations maintained consistent pH values and exhibited uniform microscopic homogeneity after cycling, variations in spreadability and adhesion indicated differing degrees of structural stability. Formulations F3 and F4 showed minimal changes across cycles, demonstrating superior resistance to thermal stress, whereas F0 exhibited significant instability in both spreadability and adhesion. Antibacterial testing showed that the formulation containing 20% basil extract (F4) produced the largest inhibition zone (11.83 ± 0.77 mm). Beyond its higher extract content, F4's superior antibacterial performance is attributed to its more stable structural matrix, which likely enhanced the release and bioavailability of active phytochemicals such as eugenol and flavonoids, thereby promoting more efficient diffusion into the agar medium. Overall, the findings demonstrate that stearic acid and TEA concentrations substantially influence both the physical stability and antibacterial efficacy of basil-based cream formulations. The optimal stability and enhanced antimicrobial activity observed in F3 underscore their potential as promising candidates for topical anti-acne product development.

Keywords: anti-acne cream; basil leaves; formula optimization

1. INTRODUCTION

Acne vulgaris remains one of the most prevalent dermatological conditions worldwide, affecting approximately 85% of adolescents and young adults, with significant psychological and social impacts on affected individuals [1]. The pathogenesis of acne involves multiple factors including sebum overproduction, follicular hyperkeratinization, microbial colonization, and inflammatory responses [2], [3]. Among the microbial contributors, *Staphylococcus epidermidis* has emerged as a

significant pathogenic agent in acne development, contributing to biofilm formation and inflammatory processes within the pilosebaceous follicles [4], [5], [6]. Current therapeutic approaches predominantly rely on synthetic antimicrobial agents such as topical antibiotics and systemic treatments [7]. However, extensive use of these conventional antimicrobials has precipitated alarming rates of bacterial resistance, with studies reporting resistance rates exceeding 50% in some populations [8]. Furthermore, synthetic anti-acne medications frequently produce adverse effects ranging from skin irritation and dryness to more severe systemic complications, catalyzing an urgent need for alternative therapeutic strategies with improved safety profiles and reduced propensity for resistance development [9], [10].

Ocimum basilicum L., commonly known as sweet basil, represents a promising botanical alternative for acne management due to its rich phytochemical profile. This aromatic herb from the Lamiaceae family contains bioactive compounds including flavonoids, eugenol, linalool, tannins, and saponins that exhibit multifaceted antimicrobial mechanisms such as disruption of bacterial cell membrane integrity and interference with essential metabolic processes [11], [12]. Recent investigations have demonstrated potent antibacterial activity of basil leaf extracts against both Gram-positive and Gram-negative bacteria, with particular efficacy against *Staphylococcus* species [13], [14], [15]. The polyphenolic compounds in basil, especially eugenol, exert bactericidal effects through multiple mechanisms, potentially reducing the likelihood of resistance development compared to single-target synthetic antibiotics [16]. Additionally, the anti-inflammatory properties of basil constituents offer therapeutic benefits by addressing the inflammatory component of acne pathogenesis, presenting opportunities for more comprehensive disease management [17].

Despite the demonstrated antimicrobial potential of basil extracts, successful translation into effective topical pharmaceutical preparations requires careful consideration of formulation parameters. The efficacy, stability, and therapeutic performance of dermatological creams are profoundly influenced by the selection and concentration of excipients, particularly emulsifying agents that determine the structural integrity and functional characteristics of the final product [18]. Stearic acid and TEA constitute critical components in oil-in-water emulsion creams, serving as the primary emulsifying system in many dermatological preparations. Stearic acid functions as the lipophilic component and consistency enhancer, contributing to cream texture and spreadability, while TEA reacts with stearic acid to form soap-like emulsifiers in situ, facilitating stable emulsion formation and influencing formulation pH [19], [20]. The ratio and concentration of these excipients fundamentally determine not only the physical characteristics of the cream but may also significantly impact the bioavailability and therapeutic activity of incorporated botanical extracts [19].

The interaction between formulation excipients and herbal active ingredients represents an understudied area with substantial implications for therapeutic outcomes. Emulsifying agents can influence the solubilization, dispersion, and release kinetics of phytochemical constituents, thereby affecting their availability for antimicrobial action at the target site [21]. Additionally, pH modulation resulting from varying stearic acid and TEA concentrations may impact both the chemical stability of pH-sensitive phytochemicals and the ionization state of antimicrobial compounds, factors that can substantially alter their membrane permeability and biological activity [22], [23], [24]. Furthermore, physical properties such as spreadability and adhesion directly influence product acceptability and patient compliance [25]. Despite the recognized importance of excipient optimization, there exists a conspicuous gap in the literature regarding how stearic acid and TEA concentrations specifically influence both the physicochemical properties and antimicrobial efficacy of botanical anti-acne preparations, impeding the rational design of optimized herbal topical products.

This study systematically investigated the influence of varying concentrations of stearic acid and TEA on the physical properties and antibacterial efficacy of *Ocimum basilicum* L. extract cream formulations against *Staphylococcus epidermidis*. The research employed comprehensive evaluation encompassing physicochemical characterization, stability assessment, and antimicrobial activity testing to establish clear relationships between excipient concentrations and both formulation quality attributes and therapeutic performance. The findings provide evidence-based guidance for optimal excipient selection in the development of basil-based anti-acne preparations, contributing to the

broader effort of developing effective, safe, and patient-acceptable botanical alternatives for acne management while establishing a methodological framework for similar investigations with other botanical antimicrobials.

2. MATERIALS AND METHODS

2.1. Materials

This study utilized fresh basil (*Ocimum basilicum* L.) leaves as the primary botanical material, with 96% ethanol serving as the extraction solvent. The microbiological assessments employed *Staphylococcus epidermidis* ATCC-12228 as the test organism, cultured on nutrient agar medium. The cream formulations were prepared using pharmaceutical-grade excipients including stearic acid and TEA as the primary emulsifying system, cetyl alcohol as a consistency enhancer, glycerin as a humectant, and distilled water as the aqueous phase vehicle. All materials met standard specifications for pharmaceutical research applications.

2.2. Sample collection, authentication and preparation

Fresh basil leaves displaying vibrant green coloration were selected as the plant material for this investigation. The samples were collected from Belatung Village, Lubuk Batang Subdistrict, OKU Regency, South Sumatra Province, Indonesia. Botanical identification and authentication of the basil leaves were conducted at the Faculty of Mathematics and Natural Sciences, University of Sumatera Utara, to ensure taxonomic accuracy and species confirmation (Identification No. 1120/MEDA/2025).

The sample preparation process commenced with wet sorting of the fresh basil leaves, followed by thorough washing under running water to remove surface contaminants and debris. The cleaned leaves were subsequently subjected to solar drying under natural sunlight for five days. Following the drying process, dry sorting was performed to eliminate any residual impurities from the dried plant material, ensuring the quality of the simplicia. The final processing stage involved mechanical pulverization using a laboratory blender, followed by sieving through a 60-mesh screen to obtain a uniform powder with standardized particle size suitable for extraction procedures. This systematic preparation methodology ensured consistency in the physical characteristics of the plant material and optimized the subsequent extraction efficiency.

2.3. Extraction

The extraction process employed the maceration method using 96% ethanol as the solvent as described by Rosa et al. [26]. A total of 789 grams of basil leaf powder was placed in a maceration vessel with 96% ethanol at a 5:1 (v/w) ratio. The vessel was sealed and maintained at room temperature for 24 hours with periodic agitation to optimize extraction efficiency. A two-stage filtration process was implemented to separate the extract from solid residue. Initial filtration through sterile gauze removed large particles, followed by secondary filtration using Whatman No. 52 filter paper for complete clarification. The macerate was collected in amber-colored containers and protected from light to prevent degradation of photosensitive compounds. The maceration process was repeated until the filtrate appeared clear, indicating complete extraction of bioactive constituents. The combined macerates were concentrated using water bath evaporation under controlled temperature until a thick, viscous extract of consistent density was obtained. The extract yield was recorded for standardization purposes, and the concentrated extract was stored appropriately prior to formulation development.

2.4. Phytochemical screening

Comprehensive phytochemical screening was conducted to identify the major classes of bioactive compounds present in the basil leaf extract using standard qualitative methods as described by Ayu et al. [27]. For flavonoid detection, approximately 10 mg of extract was dissolved in 5 mL of ethanol, followed by the addition of magnesium powder and several drops of concentrated hydrochloric acid.

The development of an orange to reddish coloration was considered indicative of flavonoid presence, confirming a positive result according to the established protocol.

Phenolic compound identification was performed by boiling 0.5 g of extract in 20 mL of distilled water, followed by filtration of the resulting solution. A few drops of 0.1% ferric chloride solution were then added to the filtrate, and the appearance of a greenish-brown or bluish-black coloration confirmed the presence of phenolic compounds. For alkaloid detection, 2 mL of the sample was treated with five drops of Dragendorff's reagent, with the formation of an orange precipitate serving as confirmation of alkaloid presence in the extract.

Triterpenoid screening involved adding Liebermann-Burchard reagent to approximately 2 mL of the extract solution, where a color change to red or violet indicated a positive result for triterpenoid compounds. Finally, saponin detection was accomplished by vigorously shaking 0.5 g of extract with 5 mL of distilled water, with the formation of persistent foam confirming the presence of saponins. These qualitative screening tests provided preliminary confirmation of the diverse phytochemical profile of the basil leaf extract, validating its potential as a source of bioactive compounds for pharmaceutical applications.

2.5. Anti-acne cream formulation

The basil leaf extract cream was prepared using the hot emulsification method to ensure optimal homogeneity and stability, following the formula described in Table 1 [28]. The formulation process involved two distinct phases prepared simultaneously under controlled temperature conditions. The oil phase, comprising stearic acid and cetyl alcohol, was heated in a suitable container until complete melting and uniform dispersion were achieved. Concurrently, the aqueous phase consisting of TEA, glycerin, and distilled water was heated in a separate vessel. Both phases were maintained at 70°C to ensure thermal equilibrium and facilitate emulsion formation. Once both phases reached the designated temperature, the oil phase was carefully transferred into a preheated porcelain mortar to maintain temperature consistency throughout the emulsification process. The aqueous phase was then gradually incorporated into the oil phase in small increments while applying continuous, unidirectional stirring using a pestle. This systematic addition method prevented phase separation and ensured the formation of a stable oil-in-water emulsion. Following complete incorporation of both phases and achieving a uniform, homogeneous base cream, the concentrated basil leaf extract was introduced slowly into the formulation. Stirring was continued methodically until the extract was thoroughly dispersed throughout the cream matrix, resulting in a smooth, uniform final product with consistent color and texture. The prepared cream was then transferred to appropriate containers and allowed to cool to room temperature before subsequent evaluation and testing procedures.

Table 1. The formula of *Ocimum basilicum* anti-acne cream

Materials	F0 (%)	F1 (%)	F2 (%)	F3 (%)	F4 (%)
Basil leaf extract	-	5	10	15	20
TEA	2	2	2.5	3	4
Stearic Acid	15	15	14.5	14	13
Cetyl alcohol	2	2	2	2	2
Glycerine	8	8	8	8	8
Distilled water ad	100	100	100	100	100

2.6. Physical characteristic evaluation

2.6.1. Organoleptic

This evaluation was carried out using the five senses, including the observation of the odor, color, and texture of the preparation [29].

2.6.2. Homogeneity

The homogeneity test of the cream is conducted to ensure that all components are evenly distributed throughout the formulation. The test is performed by weighing 1 g of the cream and spreading it on a glass plate. A cream preparation is considered homogeneous if no particles are clumped together or remain unmixed [30].

2.6.3. Spreadability

The spreadability test of the cream measures how easily and quickly the cream spreads on the skin surface. About 1 g of the cream was placed on a glass plate, then a 200 g weight was added and left for one minute. After this period, the diameter of the cream spread was measured [30].

2.6.4. pH

The pH test of the cream is important to ensure the stability, effectiveness, and skin tolerability of the product. This test is conducted to determine the acidity or alkalinity of the cream. The pH was measured using a pH meter. The electrode tip was immersed completely into the cream solution, and the pH value obtained was recorded. The acidity level of the cream should be within the skin's pH range of 4.5–8 [31].

2.6.5. Adhesion

The adhesive capacity of the cream formulations was evaluated through a standard detachment test procedure as described by Hidayati et al. [32]. A 0.5-gram sample of each formulation was applied to a glass slide and allowed to equilibrate for five minutes under controlled conditions. Following this equilibration period, an 80-gram weight was attached to the slide assembly, and the duration required for complete separation of the two glass slides was measured using a stopwatch. This elapsed time served as the quantitative indicator of the formulation's adhesive properties.

2.6.6. Stability

The cycling test method was employed to evaluate the stability of the cream formulation under alternating temperature conditions. The testing protocol consisted of storing the cream at 4 °C for 24 hours, followed by storage at 40 °C for the subsequent 24-hour period. Physical changes in the cream were monitored at three-cycle intervals, with assessments encompassing organoleptic properties, homogeneity, pH levels, spreadability, and adhesive capacity [33].

2.7. Antibacterial evaluation

The antibacterial efficacy of the formulated creams was evaluated against *Staphylococcus epidermidis* using the disc diffusion method. The bacterial culture was standardized to 0.5 McFarland standard, corresponding to approximately 1.5×10^8 CFU/mL, ensuring consistency across all assays. The standardized bacterial suspension was evenly inoculated onto nutrient agar plates using sterile cotton swabs to achieve uniform lawn growth. Sterile paper discs measuring 6 mm in diameter were placed onto the inoculated agar surface, and predetermined amounts of each test cream formulation were applied to the discs. Control samples were included for comparative analysis, including clindamycin as positive control, and distilled water as negative control. The petri dishes were sealed and incubated at 37°C for 24 hours under aerobic conditions. Antibacterial activity was assessed by measuring the diameter of clear inhibition zones formed around each disc, indicating areas where bacterial growth was prevented. Zone diameters were measured in mm using digital calipers, with all measurements performed in triplicate to ensure statistical reliability and reproducibility of the results.

2.8. Data analysis

All experimental data were presented as mean \pm SD, with each measurement conducted in triplicate. Statistical analysis was performed using GraphPad Prism software version 10.0.3,

employing One-Way and Two-Way ANOVA, Tukey's and SLD Fisher test. Statistical significance was established at a probability level of $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1. Extraction

The maceration of basil (*Ocimum basilicum* L.) leaves using 96% ethanol demonstrated a relatively moderate extraction efficiency, yielding 100.6 grams of thick extract from 789 grams of dried simplicia powder, corresponding to a 12.75% yield. This extraction efficiency falls within the acceptable range for polar solvent extraction of aromatic herbs from the Lamiaceae family. The yield percentage indicates that approximately one-eighth of the dried plant material consists of extractable polar and semi-polar phytochemical constituents. The extraction yield of 12.75% obtained in this investigation represents a notably successful recovery of phytochemical constituents from basil leaf material and significantly exceeds the minimum threshold established by the Indonesian Herbal Pharmacopoeia, which specifies that basil leaf extracts should yield greater than 5.6%. This substantial margin above the pharmacopeial requirement indicates that the maceration methodology employed, utilizing 96% ethanol as the extraction solvent with a 5:1 solvent-to-material ratio and 24-hour extraction period with periodic agitation, achieved efficient solubilization and recovery of bioactive compounds from the plant matrix. Furthermore, the 12.75% yield compares favorably with previously reported extraction efficiencies for basil leaves using comparable methodologies and solvents, surpassing the yields of 9.2% to 11.14% documented in recent studies [34], [35]. This superior extraction performance may be attributed to several factors including the quality of the starting plant material [36], appropriate harvesting timing ensuring optimal phytochemical accumulation [37], proper post-harvest processing [38], and effective particle size reduction that maximized surface area for solvent penetration [39]. The relatively high yield suggests that the extraction conditions successfully accessed both readily extractable surface compounds and more deeply sequestered intracellular constituents, ensuring adequate concentrations of the diverse phytochemical classes. This extraction efficiency is particularly significant for the formulation development phase, as higher yields translate to more efficient utilization of botanical raw materials, improved cost-effectiveness for potential commercial production, and greater consistency in achieving target extract concentrations across formulation batches, all critical considerations for developing viable botanical therapeutic products.

3.2. Preliminary phytochemical screening

The phytochemical screening of the basil leaf extract employed five distinct chemical tests, each based on specific reaction mechanisms that identify particular classes of secondary metabolites, as summarized in Table 2.

Table 2. Phytochemical components of *Ocimum basilicum* leaf extract.

Compound class	Reagents	Observation	Remarks
Flavonoids	Mg powder + HCl	Color changed to red-black	+
Phenolic	FeCl ₃	Color changed to greenish-black	+
Alkaloids	Dragendorff	Orange precipitate formed	+
Terpenoid	Liberman-Burchard	Color changed to reddish-yellow	+
Saponin	Distilled water + HCl	Stable foam formed	+

The detection of flavonoids through the Shinoda test relies on a reduction reaction where magnesium metal reacts with concentrated hydrochloric acid to generate nascent hydrogen. This powerful reducing agent targets the carbonyl group at the C4 position in the flavonoid C-ring structure, converting it into a more reactive flavylum ion or anthocyanidin derivative. The reduction

process creates extended conjugation across the aromatic ring system, producing characteristic colored compounds [40]. In the basil extract, this reaction produced an intense black coloration, which, while typically expected to be orange to reddish, may indicate a high concentration of flavonoids or the presence of specific flavonoid subclasses such as anthocyanins or highly conjugated flavones with extensive hydroxylation patterns.

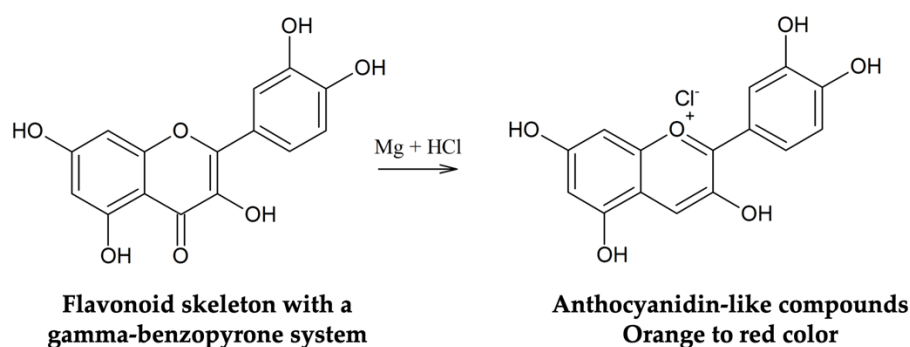


Figure 1. Schematic illustration of Shinoda test principle for flavonoids detection

The ferric chloride test for phenolic compounds exploits the ability of phenolic hydroxyl groups to form colored coordination complexes with ferric ions. When the reagent is added to the extract, Fe^{3+} ions interact with electron-rich hydroxyl oxygen atoms on aromatic rings. The phenoxide ions, formed through partial deprotonation, donate electron pairs to the ferric ion through coordinate covalent bonds, creating ferric-phenolate complexes with the general formula $[\text{Fe}(\text{ArO})_n]^{3-n}$. These complexes exhibit characteristic colors due to ligand-to-metal charge transfer transitions [41], [42]. The basil extract produced a greenish-black or black coloration, which is characteristic of polyphenolic structures, particularly catechols containing ortho-dihydroxybenzene groups or tannins with multiple adjacent hydroxyl groups. The intensity of this coloration suggests substantial phenolic content in the extract, confirming the presence of phenolic acids and other hydroxylated aromatic compounds.

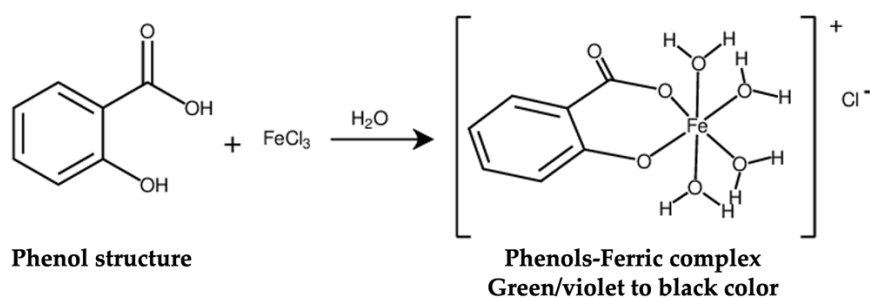
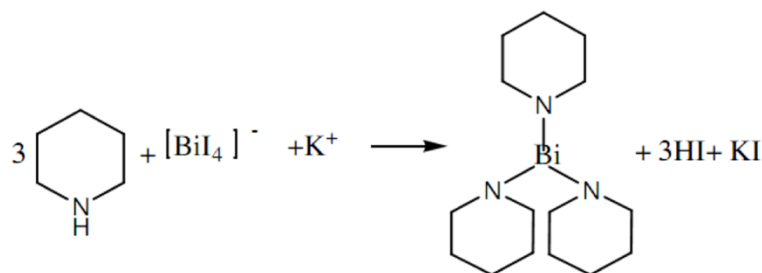


Figure 2. Schematic illustration of ferric chloride test principle for phenol detection

Alkaloid detection using Dragendorff's reagent is based on precipitation reactions between nitrogen-containing alkaloid molecules and potassium bismuth iodide. The reagent consists of bismuth subnitrate and potassium iodide dissolved in acidic solution, forming the complex anion $[\text{BiI}_4]^-$. Alkaloids contain tertiary, quaternary, or protonated nitrogen atoms that carry positive charges under acidic conditions. These positively charged nitrogen centers electrostatically attract the negatively charged bismuth iodide complex, resulting in the formation of insoluble coordination complexes that precipitate as orange to reddish-brown colored particles [41]. The positive result observed in the basil extract, evidenced by orange precipitate formation, confirms the presence of alkaloid compounds, though these are not typically the dominant secondary metabolites in species from the Lamiaceae family.



Alkaloid-Bi (metal) complex formation
Orange to reddish color

Figure 3. Schematic illustration of Dragendorff test principle for alkaloid detection

The Liebermann-Burchard test for terpenoids involves treating the sample with a mixture of acetic anhydride and concentrated sulfuric acid, which acts as a powerful dehydrating agent and catalyst. The mechanism proceeds through multiple steps beginning with the removal of water molecules from hydroxyl groups present in terpenoid structures, creating double bonds and carbocations. The sulfuric acid then oxidizes certain positions on the terpenoid skeleton while sequential dehydration reactions create extended conjugated polyene systems consisting of alternating single and double bonds. Simultaneously, acetic anhydride acetylates remaining hydroxyl groups, stabilizing reactive intermediates. The formation of these highly conjugated chromophores produces intense colored compounds through electronic transitions in the extended π -electron systems [41]. The basil extract exhibited a reddish-yellow coloration, indicating the presence of triterpenoids, which is particularly significant as the genus *Ocimum* is renowned for its rich content of monoterpenoids and sesquiterpenoids such as eugenol, linalool, and camphor. This positive result confirms the retention of these volatile and semi-volatile compounds despite the extraction and concentration processes.

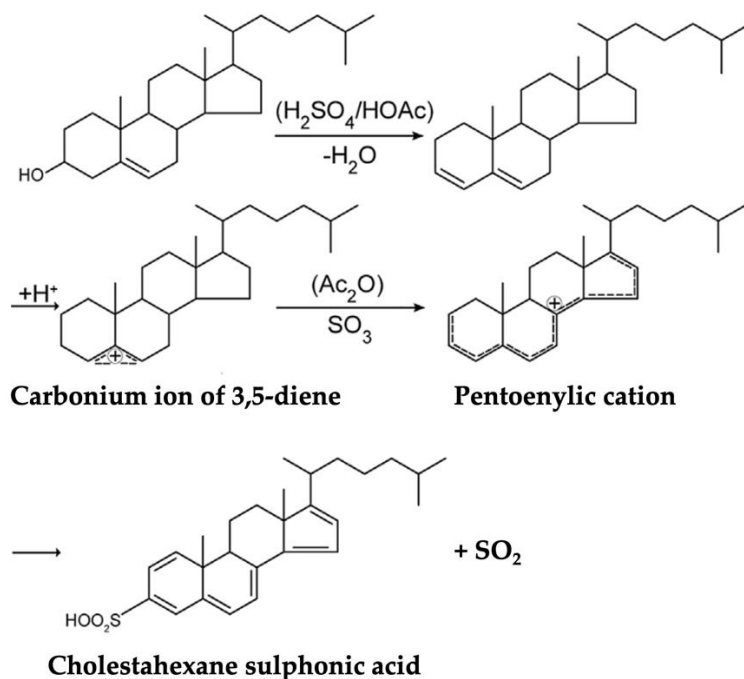


Figure 4. Schematic illustration of Liebermann-Burchard test principle for terpenoid detection

The foam test for saponins represents a physical rather than chemical detection method, based on the surfactant properties inherent to these glycosidic compounds. Saponins possess an

amphiphilic molecular structure with hydrophilic sugar moieties and hydrophobic steroid or triterpenoid aglycone portions. When the extract is mixed with water and vigorously shaken, saponin molecules migrate to the air-water interface where the hydrophobic aglycone orients toward air bubbles while the hydrophilic sugar portion orients toward the aqueous phase [43]. This molecular orientation dramatically reduces surface tension from approximately 72 mN/m in pure water to 30-40 mN/m, facilitating the incorporation of air into the solution and creating numerous small bubbles [44], [45]. The saponin molecules form stable monomolecular films around these air bubbles through their amphiphilic arrangement, creating elastic films that resist bubble coalescence and prevent rapid foam collapse. The basil extract produced stable, persistent foam, confirming the presence of saponins with sufficient concentration and appropriate structural characteristics to maintain foam stability for an extended period.

The comprehensive positive results across all five phytochemical screening tests demonstrate the diverse chemical composition of the basil leaf extract obtained through ethanolic maceration. The selection of 96% ethanol as the extraction solvent proved appropriate for recovering this broad spectrum of secondary metabolites, as this solvent polarity effectively extracts polar flavonoids and phenolic compounds, moderately polar terpenoids and alkaloids, as well as amphiphilic saponins. These complementary approaches provided comprehensive preliminary characterization of the phytochemical diversity present in the basil extract, validating the quality of both the plant material and the extraction methodology employed in this investigation.

3.3. Anti-acne cream formulation

The development of basil leaf extract anti-acne cream formulations employed a systematic approach to investigate the influence of varying stearic acid and TEA concentrations on cream properties. Five distinct formulations were prepared, designated as F0 through F4, with F0 serving as the base formulation control containing no basil extract. The formulation strategy involved incrementally increasing the basil leaf extract concentration from 5% in F1 to 20% in F4, while simultaneously adjusting the emulsifying system components to maintain emulsion stability. As the extract concentration increased across formulations, TEA content was proportionally elevated from 2% in F0 and F1 to 4% in F4, while stearic acid concentration was correspondingly reduced from 15% in F0 and F1 to 13% in F4. This inverse relationship between the emulsifier components represents a deliberate formulation optimization strategy to accommodate the increasing presence of the botanical extract while maintaining appropriate emulsion characteristics [19]. The remaining excipients, including cetyl alcohol at 2%, glycerin at 8%, and distilled water added to 100%, remained constant across all formulations to isolate the effects of the primary variables under investigation.

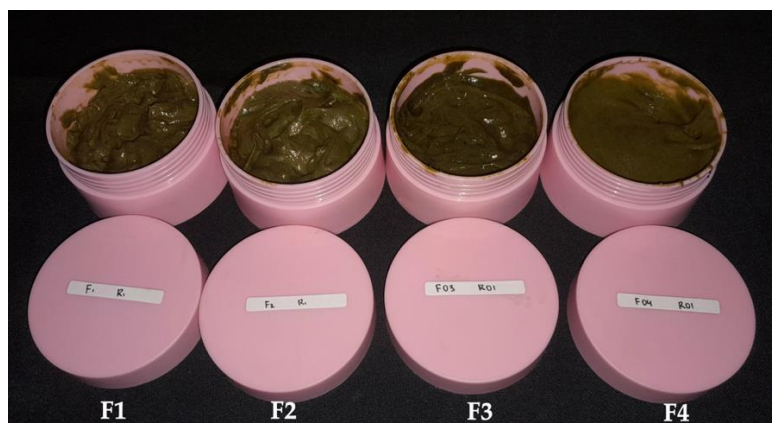


Figure 5. Visual representation of anti-acne cream of *Ocimum basilicum*

Organoleptic evaluation, described in Table 3 and visualized in Figure 5, provided immediate qualitative assessment of the formulations' sensory characteristics, revealing distinct differences

between the control and extract-containing formulations. Formula F0, lacking basil extract, exhibited a white color and was completely odorless with a thick consistency, representing the baseline characteristics of the emulsion system without botanical active ingredients. In contrast, formula F1 through F4 all displayed a characteristic dark green color attributable to the chlorophyll and other pigmented phytochemicals present in the basil leaf extract, with color intensity visually correlating with extract concentration. These formulations also possessed a characteristic herbal odor typical of basil, confirming successful incorporation of the plant material and retention of volatile aromatic compounds. Regarding consistency, formulations F0 through F3 all exhibited thick cream textures suitable for topical application, while F4 demonstrated a slightly liquid consistency. This rheological shift in F4 can be attributed to the combined effects of the highest extract concentration introducing additional aqueous and soluble components, coupled with the lowest stearic acid content and highest TEA concentration, collectively reducing the structural rigidity of the cream matrix [46].

Table 3. Organoleptic evaluation of *Ocimum basilicum* anti-acne cream.

Formula	Color	Odor	Consistency
F0	White	Odorless	Thick
F1	Dark green	Characteristic	Thick
F2	Dark green	Characteristic	Thick
F3	Dark green	Characteristic	Thick
F4	Dark green	Characteristic	Slightly liquid

Homogeneity testing assessed the uniformity of component distribution throughout each formulation, a critical quality parameter for ensuring consistent dose delivery and therapeutic performance. All five formulations (Table 4), from F0 through F4, demonstrated excellent homogeneity with no coarse particles observed upon visual and tactile examination. This universal achievement of homogeneous distribution indicates that the hot emulsification method employed, combined with thorough mixing during preparation, successfully dispersed all components uniformly throughout the cream matrix regardless of extract concentration or emulsifier ratio variations. The absence of phase separation, grittiness, or particle aggregation across all formulations confirms appropriate compatibility between the basil extract and the cream base components, suggesting that the phytochemical constituents dissolved or dispersed effectively within the emulsion system without precipitation or crystallization phenomena that could compromise product quality [32].

Table 4. Homogeneity evaluation of *Ocimum basilicum* anti-acne cream.

Formula	Observation	Remarks
F0	No coarse particles observed	Homogeneous
F1	No coarse particles observed	Homogeneous
F2	No coarse particles observed	Homogeneous
F3	No coarse particles observed	Homogeneous
F4	No coarse particles observed	Homogeneous

Spreadability testing (Figure 6a) evaluated the ease with which the cream formulations could be distributed across a surface, simulating application to skin and providing quantitative assessment of rheological properties. The test was conducted using two weight loads: 0 grams representing minimal applied force and 200 grams representing moderate pressure during application. Under the 0-gram condition, spreadability values ranged from 4.23 cm to 4.83 cm across formulations, with relatively minimal variation. Formulation F0 exhibited a spreadability of 4.27 ± 0.93 cm, while F1 showed 4.73 ± 0.40 cm, F2 demonstrated 4.23 ± 0.40 cm, F3 achieved 4.63 ± 0.35 cm, and F4 displayed 4.83 ± 0.33

cm. Under the 200-gram load condition, spreadability values increased substantially across all formulations due to the shear-thinning behavior typical of cream emulsions. Formulation F0 spread to 6.17 ± 0.93 cm, F1 reached 6.73 ± 0.45 cm, F2 achieved 6.23 ± 0.40 cm, F3 spread to 6.57 ± 0.38 cm, and F4 demonstrated the highest spreadability at 6.57 ± 0.16 cm. Interestingly, the spreadability values did not show a simple linear correlation with increases or decreases in the stearic acid–TEA (TEA) combination. Although F1 and F4 showed higher spreadability consistent with reduced stearic acid and increased TEA, intermediate formulations (F2 and F3) did not follow this trend. This non-linear pattern suggests that spreadability was influenced by multiple interacting factors beyond emulsifier concentration alone. First, stearic acid and TEA participate in complex co-emulsifier interactions that influence internal phase structuring; small concentration shifts may alter crystal packing or lamellar organization, resulting in unpredictable viscosity changes. Second, the presence of basil extract introduces additional solutes that may interact with the emulsifier matrix, affecting water binding, droplet mobility, and cream consistency. Third, microstructural factors—such as partial coalescence, droplet size distribution, and the extent of fatty-alcohol network formation—can cause formulations with similar emulsifier ratios to exhibit different rheological responses. These combined effects likely contributed to the non-linear spreadability behavior observed across the formulations [19], [46]. Notably, F4 exhibited the lowest standard deviation under load conditions, suggesting more consistent rheological behavior, likely attributable to its more fluid consistency as noted in organoleptic testing.

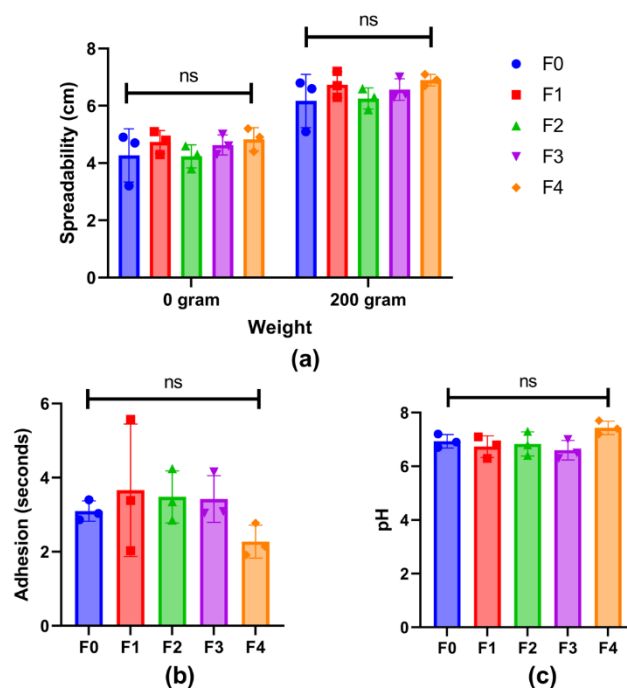


Figure 6. Physical properties of anti-acne cream of *Ocimum basilicum*. (a) Spreadability, (b) Adhesion, and (c) pH. Data were presented as mean \pm SD ($n=3$), and statistically analyzed using One-Way ANOVA, Tukey's test. Ns = $p>0.05$; * = $p<0.05$

Adhesion testing (Figure 6b) measured the duration that cream formulations remained in contact with a surface before complete detachment, providing insight into the substantivity and residence time of the product on skin. The adhesion values displayed considerable variation across formulations, ranging from 2.14 to 3.66 seconds. Formulation F0 demonstrated moderate adhesion of 2.50 ± 0.75 seconds, while formulations F1, F2, and F3 showed enhanced adhesion with values of 3.66 ± 0.27 seconds, 3.48 ± 0.70 seconds, and 3.41 ± 0.60 seconds respectively. Interestingly, F4 exhibited the lowest adhesion at 2.14 ± 0.36 seconds, comparable to the control formulation. This pattern

suggests that moderate concentrations of basil extract combined with balanced emulsifier ratios promote stronger interfacial interactions and improved adhesive properties, potentially through contributions from the extract's polysaccharide and saponin components that enhance surface binding. However, the substantially reduced adhesion in F4 can be attributed to its more liquid consistency and altered emulsion structure resulting from the highest TEA and lowest stearic acid concentrations, which decrease cream viscosity and structural cohesion, thereby reducing the time required for gravitational forces to overcome adhesive forces [19], [46].

The pH evaluation across formulations (Figure 6c) revealed a progressive alkaline shift correlated with increasing TEA concentration, a predictable outcome given the basic nature of this tertiary amine emulsifier. Formulation F0 exhibited a slightly acidic to neutral pH of 6.93 ± 0.25 , falling within the acceptable range for topical skin preparations. Formulation F1 showed a pH of 6.73 ± 0.40 , representing a slight decrease possibly influenced by acidic components in the basil extract [47]. Interestingly, formulation F3 exhibited a decreased pH of 6.60 ± 0.36 , despite containing more TEA than both F1 and F2. This deviation from the expected trend suggests that the final pH of the formulation was influenced by additional physicochemical interactions beyond TEA concentration alone. One likely explanation is that the increased TEA in F3 participated more extensively in soap formation with stearic acid, producing additional TEA stearate. This process consumes free TEA, thereby limiting its capacity to elevate pH. Additionally, the basil extract contains weakly acidic compounds such as phenolics and flavonoids; their interaction with the emulsifier matrix may be affected by formulation microstructure, leading to greater acid–base neutralization in F3. Structural factors—such as droplet distribution, internal phase organization, and water-binding capacity—may further influence the amount of free, unbound TEA available to contribute to pH elevation. In contrast, formulation F4 showed a distinct increase in pH to 7.43 ± 0.25 , consistent with its substantially higher TEA content. TEA in this formulation exceeds what is needed for complete neutralization of stearic acid, leaving more free TEA in the aqueous phase and resulting in a mildly alkaline product. Although this pH remains within acceptable limits for topical use, it may have implications for the stability of pH-sensitive phytochemicals and the skin's acid mantle [48]. While the pH of 7.43 remains within generally acceptable limits for topical products, this alkaline shift may have implications for the stability of pH-sensitive phytochemical constituents in the extract and could potentially affect the ionization state and antimicrobial activity of certain bioactive compounds [49], [50]. The skin's natural pH ranges from approximately 4.5 to 6.5, and formulations closest to this physiological range, such as F1, F2, and F3, would theoretically provide better compatibility with the skin's acid mantle and potentially enhance tolerability during prolonged use [51].

Statistical analysis of the physicochemical parameters demonstrated that variations in TEA and stearic acid concentrations did not produce significant differences in the primary physical properties of the formulations. Spreadability, adhesion, and pH values showed no statistically significant variation ($p > 0.05$) across F0–F4, indicating that the emulsifier ratio adjustments did not substantially alter the creams' rheological behavior or acid–base characteristics. These findings suggest that within the concentration ranges tested, TEA and stearic acid functioned primarily to support emulsion formation rather than to modulate measurable physical attributes, with all formulations maintaining comparable performance. Consequently, the differences observed between formulations were attributed more to physical stability under stress conditions than to inherent physicochemical property variations.

The cycling test evaluation of five *Ocimum basilicum* anti-acne cream formulations (F0–F4), presented in Figure 7, employed a rigorous protocol involving three complete temperature cycles, with each cycle alternating between storage at 4°C and 40°C for 24-hour periods. This assessment methodology examined three critical physical parameters—spreadability, adhesion, and pH—alongside microscopic evaluation of homogeneity to provide a comprehensive understanding of

formulation stability under thermal stress conditions. The spreadability assessment, displayed in Figure 7a, revealed distinct performance patterns among the formulations, with F0 demonstrating the most variable behavior across thermal cycles. Under minimal loading conditions of 0 grams, F0 exhibited spreadability values ranging from approximately 4.5 to 6.5 cm², with statistically significant differences observed between cycles 1 and 2. This variation suggests that F0 experienced rheological modifications during temperature fluctuations, potentially indicating instability in its structural composition or ingredient interactions. In contrast, formulations F1 through F3 maintained relatively consistent spreadability profiles, with most cycle-to-cycle comparisons showing no significant differences. Formulation F4, while displaying one instance of significant variation, generally preserved acceptable spreading characteristics throughout the testing period. When subjected to higher loading conditions of 200 grams, all formulations demonstrated enhanced spreadability as expected, yet the relative stability patterns remained consistent with those observed under lower loading, further confirming the robust physical stability of F1 through F4.

The adhesion testing in Figure 7b revealed more pronounced instability patterns compared to spreadability measurements, providing additional insight into the formulations' responses to thermal stress. Formulation F0 again exhibited the most significant changes, with adhesion time increasing from approximately 3 seconds in cycle 1 to 4.5 seconds in cycle 2, representing a statistically significant modification in intermolecular interactions or structural properties. This pattern was similarly observed in formulations F1 and F2, which both demonstrated significant differences between cycles 1 and 2, suggesting an initial adaptation period during which thermal cycling induced alterations in adhesive characteristics. Interestingly, adhesion times generally increased from cycle 1 to cycle 2 before stabilizing by cycle 3, indicating that these formulations underwent an equilibration process following initial thermal exposure. Formulations F3 and F4, however, maintained consistent adhesion properties throughout all cycles with no significant differences detected, demonstrating superior structural integrity and resistance to thermal stress-induced modifications. The consistently lower adhesion times observed in F4 throughout all cycles represent a potentially advantageous characteristic for practical application, as reduced adhesion facilitates easier spreading and may improve patient compliance and user experience.

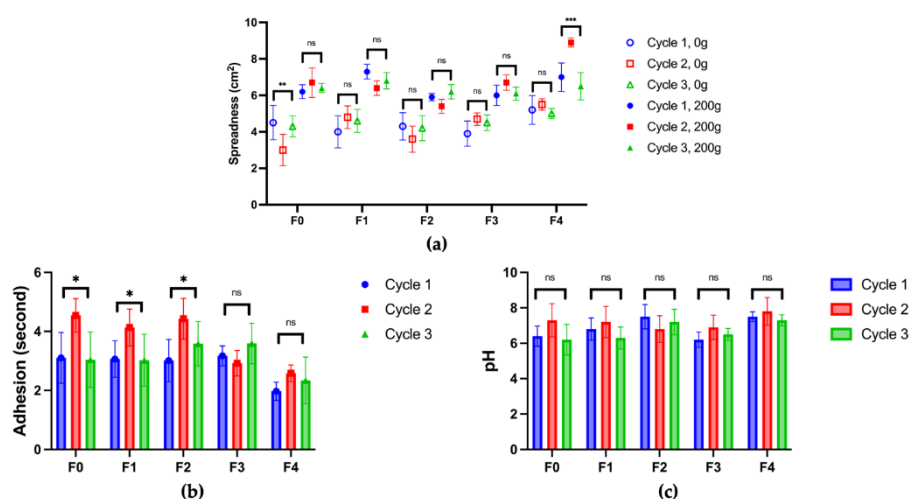


Figure 7. Stability evaluation of anti-acne cream of *Ocimum basilicum* using cycling test. (a) Spreadability, (b) Adhesion, and (c) pH. Data were presented as mean \pm SD ($n=3$), and statistically analyzed using Two-Way ANOVA, LSD Fisher. Ns = $p>0.05$; * = $p<0.05$; ** = $p<0.01$; *** = $p<0.001$.

Despite the variations observed in spreadability and adhesion parameters, the pH stability assessment, illustrated in Figure 7c, provided remarkably consistent results across all formulations, indicating robust chemical stability throughout the thermal cycling process. All five formulations

maintained pH values within the physiologically appropriate range of 6 to 8 across all three cycles, with no statistically significant differences detected between any cycle comparisons. This exceptional pH stability demonstrates that none of the formulations underwent significant chemical degradation, hydrolysis reactions, or other processes that would alter their acid-base equilibrium during temperature fluctuations. The maintenance of stable pH is particularly critical for anti-acne formulations, as pH directly influences the antimicrobial activity of active ingredients, affects skin compatibility, and determines the irritation potential of the product. The uniform pH stability across all formulations suggests that the buffering systems and chemical compositions employed were sufficiently robust to withstand thermal stress without compromising the formulations' chemical integrity.

The microscopic homogeneity evaluation, summarized in Table 5, complemented the physical and chemical assessments by confirming the absence of coarse particles in all formulations throughout all three thermal cycles. This finding indicates that the cream matrices remained uniformly dispersed without experiencing phase separation, particle aggregation, or crystallization phenomena that commonly occur in unstable emulsion systems subjected to temperature stress. The preservation of homogeneity is essential for ensuring consistent dosing accuracy, smooth application properties, and optimal delivery of active ingredients to the target site. When considered alongside the pH stability data, the homogeneity results suggest that while some formulations experienced physical property modifications affecting spreadability and adhesion, these changes did not compromise the fundamental structural integrity or chemical stability of the cream systems.

Table 5. Stability evaluation of homogeneity of anti-acne cream of *Ocimum basilicum* using cycling test.

Formula	Cycle 1	Cycle 2	Cycle 3	Remarks
F0	No coarse particles observed	No coarse particles observed	No coarse particles observed	Homogeneous
F1	No coarse particles observed	No coarse particles observed	No coarse particles observed	Homogeneous
F2	No coarse particles observed	No coarse particles observed	No coarse particles observed	Homogeneous
F3	No coarse particles observed	No coarse particles observed	No coarse particles observed	Homogeneous
F4	No coarse particles observed	No coarse particles observed	No coarse particles observed	Homogeneous

The comprehensive analysis of all stability parameters leads to the conclusion that formulations F3 demonstrated superior overall stability profiles, making them the most suitable candidates for commercial development. These formulations maintained consistent performance across spreadability and adhesion measurements while preserving excellent homogeneity and pH stability throughout the thermal cycling regimen. The minimal variation observed in F3 across all testing parameters suggests these formulations would maintain consistent performance characteristics throughout their shelf life under various storage conditions encountered in real-world distribution and use. Conversely, formulation F0 exhibited the greatest susceptibility to thermal stress, displaying significant variations in both spreadability and adhesion parameters that raise concerns about its long-term stability and performance consistency. The intermediate formulations F1 and F2, while showing some thermal sensitivity in adhesion properties, demonstrated adequate stability in other parameters and could potentially serve as acceptable alternatives depending on specific product requirements. Based on these findings, formulations F3 and F4 warrant further investigation through

accelerated stability testing and real-time stability studies to confirm their long-term stability profiles and establish appropriate expiration dating for commercial distribution.

3.4. Antibacterial efficacy evaluation

The antibacterial efficacy evaluation against *Staphylococcus epidermidis* ATCC-12228 using the disc diffusion method demonstrated a clear dose-dependent relationship between basil extract concentration and antimicrobial activity (Figure 8). Distilled water, used as the negative control for the assay, produced no inhibition zone, confirming the absence of baseline antibacterial activity. Likewise, formulation F0, which served as the negative control within the formulation set and contained only the cream base without extract, exhibited no inhibitory effect across all replicates. The lack of antibacterial activity in both negative controls verifies that neither the aqueous medium nor the cream base contributes to microbial inhibition. Therefore, the antimicrobial effects observed in formulations containing basil extract can be confidently attributed to the bioactive phytochemical constituents of the extract rather than to any excipients present in the base formulation.

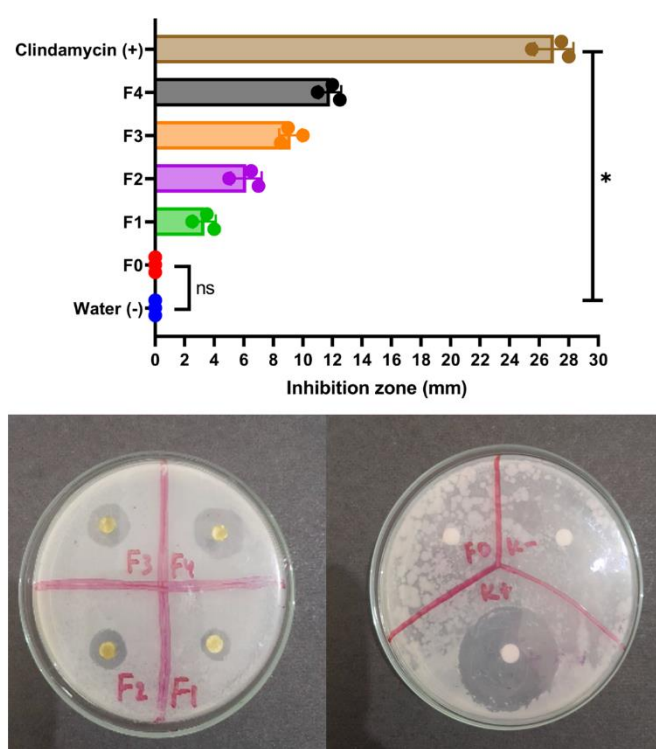


Figure 8. Antibacterial efficacy of *Ocimum basilicum* anti-acne cream against *S. epidermidis*. Data were presented as mean \pm SD (n=3), and statistically analyzed using One-Way ANOVA, Tukey's test. Ns = $p > 0.05$; * = $p < 0.05$

Formulations containing basil extract demonstrated progressively increasing antibacterial activity corresponding to extract concentration increments. Formulation F1, containing 5% extract, produced modest inhibition zones measuring 3.33 ± 0.77 mm. While this result confirms detectable antimicrobial activity at the lowest extract concentration tested, the small zone diameter and relatively high standard deviation suggest weak and somewhat inconsistent antibacterial efficacy. Formulation F2, with doubled extract concentration at 10%, demonstrated substantially improved activity with inhibition zones of 5.0, 6.5, and 7.0 mm, averaging 6.50 ± 0.50 mm. This approximate doubling of inhibition zone diameter relative to F1 indicates a proportional concentration-response relationship within this range, suggesting that the antimicrobial phytochemicals are being effectively released from the cream matrix and maintaining biological activity.

Formulation F3, containing 15% extract, exhibited further enhanced antimicrobial performance with inhibition zones measuring 8.5, 9.0, and 10.0 mm, yielding a mean of 9.17 ± 0.76 mm. This

represents a nearly threefold increase compared to F1 and demonstrates that increasing extract concentration continues to enhance antibacterial potency within this formulation range. The most striking results were observed with Formulation F4, containing the highest extract concentration at 20%, which produced inhibition zones of 11.0, 12.0, and 12.5 mm, averaging 11.83 ± 0.77 mm. This formulation demonstrated the most potent antibacterial activity among all tested concentrations, with inhibition zones approaching half the diameter of the positive control. The relatively low standard deviation across F4 replicates indicates consistent and reproducible antimicrobial performance despite this formulation's altered physical properties noted in the physicochemical characterization.

The positive control using clindamycin, a standard topical antibiotic commonly employed in acne therapy, produced substantial inhibition zones measuring 25.5, 27.5, and 28.0 mm, with a mean of 27.0 ± 1.32 mm. This robust activity confirms the susceptibility of the *S. epidermidis* test strain to conventional antimicrobial agents and validates the sensitivity of the assay methodology. While the basil extract formulations demonstrated considerably lower absolute inhibition zone diameters compared to clindamycin, with F4 achieving approximately 44% of the positive control's activity, this comparison must be interpreted within appropriate pharmaceutical and microbiological contexts that account for the fundamental differences between purified synthetic antibiotics and complex botanical extracts. Understanding these differences becomes particularly important when examining how the formulation components themselves influenced the observed antibacterial performance.

Although extract concentration served as the primary determinant of antibacterial potency, the emulsifier system components, specifically stearic acid and TEA, contributed indirectly to the observed inhibition profiles by modifying the release behavior of phytochemicals from the cream matrix. Stearic acid, a long-chain fatty acid widely used as a viscosity-enhancing and structuring agent in oil-in-water creams, does not possess intrinsic antibacterial activity at cosmetic formulation levels but significantly influences matrix density, occlusivity, and diffusion pathways [52], [53], [54]. Higher stearic acid content in formulations F1 through F3 generally increases matrix rigidity, which may slow the diffusion of active phytochemicals into the agar medium during disc diffusion testing, whereas the lower stearic acid content in F4 reduces structural density and potentially facilitates more rapid release of active compounds. This relationship may partially explain why F4, despite exhibiting compromised physical characteristics in stability testing, still produced the highest antibacterial activity, as the softer matrix likely allowed more efficient diffusion of basil extract constituents into the surrounding medium. The influence of formulation excipients extends beyond stearic acid to include the emulsifying and pH-modifying effects of TEA.

TEA functions as both an emulsifier through soap formation with stearic acid and as an alkalizing agent, and while it does not exhibit antimicrobial properties at cosmetic-use concentrations, it influences antibacterial outcomes indirectly through several mechanisms. Increasing TEA concentration raises the formulation pH, which may alter the ionization state or solubility of basil phytochemicals such as phenolics and flavonoids, potentially enhancing their diffusion or bioavailability [55]. Additionally, higher TEA concentrations produce smaller, more stable emulsion droplets, which may influence the partitioning and release rate of hydrophilic and moderately lipophilic antibacterial compounds [56], [57]. In formulation F4, the higher TEA content likely increased the fluidity of the formulation, promoting greater migration of active compounds to the agar disk surface during diffusion. Thus, while extract concentration remains the dominant factor, the modulation of stearic acid and TEA levels influenced the release dynamics of active compounds and played a secondary but meaningful role in determining antibacterial outcomes. These formulation-related observations lead to broader considerations regarding the comparative performance of botanical extracts versus conventional antibiotics.

From a practical antimicrobial efficacy perspective, inhibition zones exceeding 10 mm are generally considered indicative of moderate to good antibacterial activity in topical formulation screening [58]. By this criterion, Formulation F4 meets this threshold, while F3 approaches it with zones averaging over 9 mm. These results suggest that both F3 and F4 possess clinically relevant antibacterial potential against *S. epidermidis*, one of the key bacterial species implicated in acne pathogenesis through biofilm formation and inflammatory mediator production. However, formulations F1 and F2, with zones below 7 mm, would likely be considered to possess weak to moderate activity insufficient for standalone therapeutic applications, though they might contribute to overall formulation efficacy through complementary mechanisms such as anti-inflammatory or antioxidant effects from the phytochemical constituents.

The demonstrated antibacterial activity of the basil extract formulations, particularly F3 and F4, provides compelling evidence supporting the therapeutic potential of *Ocimum basilicum* as a botanical antimicrobial agent for topical anti-acne applications. The clear concentration-dependent relationship validates the rational formulation development approach and suggests that further optimization balancing extract concentration, physical properties, and antimicrobial efficacy could yield formulations with clinically meaningful therapeutic performance while avoiding the resistance development concerns associated with conventional antibiotic therapies.

4. CONCLUSION

This study demonstrates that variations in stearic acid and TEA concentrations did not produce statistically significant differences in the primary physical properties of the *Ocimum basilicum* L. extract cream formulations, including spreadability, adhesion, and pH. However, these components did influence the physical stability of the creams, as reflected in differences in consistency, viscosity behavior, and structural integrity over time. All formulations met acceptable quality criteria for topical use, exhibiting uniform homogeneity and pH values within the appropriate dermatological range (6.60–7.43). Antibacterial testing against *Staphylococcus epidermidis* confirmed a clear concentration-dependent effect of basil extract, with inhibition zones increasing progressively from F1 (3.33 mm) to F2 (6.50 mm) and F3 (9.17 mm), and reaching the highest activity in F4 (20% extract) at 11.83 ± 0.77 mm. The absence of inhibition in F0 verified that antibacterial effects originated solely from the phytochemical constituents of the basil extract. Although F4 demonstrated the strongest antimicrobial activity, its reduced physical stability and compromised consistency suggest practical limitations for topical use. In contrast, formulation F3 offered the most favorable balance between substantial antibacterial efficacy and stable physicochemical characteristics. Overall, these findings support the potential of basil extract as a botanical antimicrobial agent for anti-acne formulations. Future studies should include evaluation against *Cutibacterium acnes*, determination of minimum inhibitory concentrations, extended stability assessments, and clinical testing to further optimize formulation parameters and advance development toward evidence-based topical acne treatments.

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References

- [1] D. L. Guguluş *et al.*, "The Epidemiology of Acne in the Current Era: Trends and Clinical Implications," Jun. 01, 2025, *Multidisciplinary Digital Publishing Institute (MDPI)*. doi: 10.3390/cosmetics12030106.
- [2] H.-M. Yan, H.-J. Zhao, D.-Y. Guo, P.-Q. Zhu, C.-L. Zhang, and W. Jiang, "Gut microbiota alterations in moderate to severe acne vulgaris patients," *J Dermatol*, vol. 45, no. 10, pp. 1166–1171, Oct. 2018, doi: <https://doi.org/10.1111/1346-8138.14586>.

- [3] C. C. Motosko, G. A. Zakhem, M. K. Pomeranz, and A. Hazen, "Acne: a side-effect of masculinizing hormonal therapy in transgender patients," *British Journal of Dermatology*, vol. 180, no. 1, pp. 26–30, Jan. 2019, doi: 10.1111/bjd.17083.
- [4] T. Coenye, K.-J. Spittaels, and Y. Achermann, "The role of biofilm formation in the pathogenesis and antimicrobial susceptibility of *Cutibacterium acnes*," *Biofilm*, vol. 4, p. 100063, 2022, doi: <https://doi.org/10.1016/j.bioflm.2021.100063>.
- [5] M. Fournière, T. Latire, D. Souak, M. G. J. Feuilleley, and G. Bedoux, "Staphylococcus epidermidis and cutibacterium acnes: Two major sentinels of skin microbiota and the influence of cosmetics," Nov. 01, 2020, MDPI AG. doi: 10.3390/microorganisms8111752.
- [6] M. M. Brown and A. R. Horswill, "Staphylococcus epidermidis-Skin friend or foe?," *PLoS Pathog*, vol. 16, no. 11, Nov. 2020, doi: 10.1371/JOURNAL.PPAT.1009026.
- [7] Y. Li, X. Hu, G. Dong, X. Wang, and T. Liu, "Acne treatment: research progress and new perspectives," 2024, *Frontiers Media SA*. doi: 10.3389/fmed.2024.1425675.
- [8] G. Muteeb, M. T. Rehman, M. Shahwan, and M. Aatif, "Origin of Antibiotics and Antibiotic Resistance, and Their Impacts on Drug Development: A Narrative Review," Nov. 01, 2023, *Multidisciplinary Digital Publishing Institute (MDPI)*. doi: 10.3390/ph16111615.
- [9] H. J. Kim and Y. H. Kim, "Exploring Acne Treatments: From Pathophysiological Mechanisms to Emerging Therapies," May 01, 2024, *Multidisciplinary Digital Publishing Institute (MDPI)*. doi: 10.3390/ijms25105302.
- [10] M. D. Oudenhoven, M. A. Kinney, D. B. McShane, C. N. Burkhart, and D. S. Morrell, "Adverse Effects of Acne Medications: Recognition and Management," *Am J Clin Dermatol*, vol. 16, no. 4, pp. 231–242, 2015, doi: 10.1007/s40257-015-0127-7.
- [11] N. S. Azizah *et al.*, "Sweet Basil (*Ocimum basilicum* L.)—A Review of Its Botany, Phytochemistry, Pharmacological Activities, and Biotechnological Development," Dec. 01, 2023, *Multidisciplinary Digital Publishing Institute (MDPI)*. doi: 10.3390/plants12244148.
- [12] M. H. Shahrajabian, W. Sun, and Q. Cheng, "Chemical components and pharmacological benefits of Basil (*Ocimum basilicum*): a review," *Int J Food Prop*, vol. 23, no. 1, pp. 1961–1970, Jan. 2020, doi: 10.1080/10942912.2020.1828456.
- [13] M. Perwitasari, R. Anindita, M. Uzia Beandrade, D. Dwi Nathalia, W. Nurani Hasmar, and I. Kurnia Putri Sekolah Tinggi Ilmu Kesehatan Mitra Keluarga, "Aktivitas Antibakteri Ekstrak Etanol dan Minyak Atsiri Daun Kemangi (*Ocimum sanctum*) Terhadap Pertumbuhan *Staphylococcus aureus*, *Salmonella thypii* dan *Eschericia coli* Anti-Bacterial Activity of Etanolic Extract and Essential Oil of Basil (*Ocimum sanctum*) on Growth *Staphylococcus aureus* *Salmonella thypii* and *Eschericia coli*," 2023.
- [14] N. Zdolec *et al.*, "Antimicrobial Properties of Basil (*Ocimum basilicum* L.), Sage (*Salvia officinalis* L.), Lavender (*Lavandula officinalis* L.), Immortelle (*Helichrysum italicum* (Roth) G. Don), and Savory (*Satureja montana* L.) and Their Application in Hard Cheese Production," *Hygiene*, vol. 4, no. 2, pp. 135–145, Jun. 2024, doi: 10.3390/hygiene4020010.
- [15] S. Dunca *et al.*, "Antibacterial Activity of *Ocimum basilicum* L. Extracts Grown in Aquaponic Conditions Against Gram-positive and Gram-negative Species," in 2022 *E-Health and Bioengineering Conference (EHB)*, 2022, pp. 1–4. doi: 10.1109/EHB55594.2022.9991296.
- [16] Debao Niu *et al.*, "Multi-target antibacterial mechanism of eugenol and its combined inactivation with pulsed electric fields in a hurdle strategy on *Escherichia coli*," *Food Control*, vol. 106, p. 106742, 2019, doi: <https://doi.org/10.1016/j.foodcont.2019.106742>.
- [17] H. Takeuchi, C. Takahashi-Muto, M. Nagase, M. Kassai, R. Tanaka-Yachi, and C. Kiyose, "Anti-inflammatory Effects of Extracts of Sweet Basil (*Ocimum basilicum* L.) on a Co-culture of 3T3-L1 Adipocytes and RAW264.7 Macrophages," *J Oleo Sci*, vol. 69, no. 5, pp. 487–493, 2020, doi: 10.5650/jos.ess19321.
- [18] A. Kovács *et al.*, "Effects of formulation excipients on skin barrier function in creams used in pediatric care," *Pharmaceutics*, vol. 12, no. 8, pp. 1–15, Aug. 2020, doi: 10.3390/pharmaceutics12080729.

- [19] E. Wdiyati *et al.*, "The Effect Of Stearic Acid And TEA (Tea) On Physieal And Chemical Properties Of Cosmetic Emulsion Using Coconut Oil As Raw Material," *International Journal of Applied Chemistry*, vol. 11, no. 3, pp. 343–349, 2015, [Online]. Available: <http://www.ripublication.com>
- [20] C. Pereira-Leite, M. Bom, A. Ribeiro, C. Almeida, and C. Rosado, "Exploring Stearic-Acid-Based Nanoparticles for Skin Applications—Focusing on Stability and Cosmetic Benefits," *Cosmetics*, vol. 10, no. 4, Aug. 2023, doi: 10.3390/cosmetics10040099.
- [21] W. Liao, E. Dumas, S. Ghnimi, A. Elaissari, and A. Gharsallaoui, "Effect of emulsifier and droplet size on the antibacterial properties of emulsions and emulsion-based films containing essential oil compounds," *J Food Process Preserv*, vol. 45, no. 12, p. e16072, Dec. 2021, doi: <https://doi.org/10.1111/jfpp.16072>.
- [22] L. Gaohua, X. Miao, and L. Dou, "Crosstalk of physiological pH and chemical pKa under the umbrella of physiologically based pharmacokinetic modeling of drug absorption, distribution, metabolism, excretion, and toxicity," *Expert Opin Drug Metab Toxicol*, vol. 17, no. 9, pp. 1103–1124, Sep. 2021, doi: 10.1080/17425255.2021.1951223.
- [23] I. Fernando and Y. Zhou, "Impact of pH on the stability, dissolution and aggregation kinetics of silver nanoparticles," *Chemosphere*, vol. 216, pp. 297–305, 2019, doi: <https://doi.org/10.1016/j.chemosphere.2018.10.122>.
- [24] O. Ozbek, D. E. Genc, and K. O. Ulgen, "Advances in Physiologically Based Pharmacokinetic (PBPK) Modeling of Nanomaterials," Aug. 09, 2024, *American Chemical Society*. doi: 10.1021/acsptsci.4c00250.
- [25] A. Storm, E. Benfeldt, S. E. Andersen, and J. Serup, "A prospective study of patient adherence to topical treatments: 95% of patients underdose," *J Am Acad Dermatol*, vol. 59, no. 6, pp. 975–980, 2008, doi: <https://doi.org/10.1016/j.jaad.2008.07.039>.
- [26] Y. Rosa *et al.*, "Persea americana leaf extract promotes wound healing by inhibiting NF-KB1," *J Appl Pharm Sci*, vol. 15, no. 3, pp. 160–173, 2025, doi: 10.7324/JAPS.2025.218012.
- [27] D. S. Ayu *et al.*, "Perbandingan uji mukolitik ekstrak dan fraksi daun lamtoro (*Leucaena leucocephala* (lam) de wit) halus dan kasar secara in vitro," *Jurnal Ilmiah Sain dan Teknologi*, vol. 2, no. 4, pp. 12–20, 2024.
- [28] D. Apriani, E. Halimatusadyah, and K. Krismayadi, "Uji formulasi krim secara in-vitro pada ekstrak daun kemangi terhadap P.acnes dan S.epidermidis," *Health Sciences and Pharmacy Journal*, vol. 8, no. 1, pp. 56–66, Apr. 2024, doi: 10.32504/hspj.v8i1.1011.
- [29] M. Y. Wardhana, C. AR, and T. Makmur, "Daya terima konsumen terhadap produk olahan minuman serbuk dari limbah biji nangka (*Arthocarpus heterophilus*)," *MAHATANI: Jurnal Agribisnis (Agribusiness and Agricultural Economics Journal)*, vol. 5, no. 1, p. 89, 2022, doi: 10.52434/mja.v5i1.1766.
- [30] M. Tari and O. Indriani, "Formulasi Dan Uji Stabilitas Fisik Sediaan Krim Ekstrak Sembung Rambat (*Mikania micrantha* Kunth)," *Jurnal Ilmiah Multi Science Kesehatan*, vol. 15, no. 1, pp. 192–211, 2023.
- [31] L. Ilmaknun and N. C. Endriyatno, "Formulasi dan penentuan nilai spf krim minyak tamanu (*Calophyllum inophyllum* l.) dengan variasi konsentrasi asam stearat dan trietanolamin," *Forte Journal*, vol. 4, no. 1, pp. 122–133, 2024, doi: 10.51771/fj.v4i1.758.
- [32] N. Hidayati, P. Fadillah Amanda, and A. Setiawansyah, "Utilization of Avocado Leaves (*Persea americana* Mill) Ethanol Extract in Acne Spot Gel Formulation," *Indonesian Journal of Cosmetics*, vol. 2, no. 1, pp. 1–8, 2024.
- [33] N. Lumentut, H. J. Edi, and E. M. Rumondor, "Formulasi dan Uji Stabilitas Fisik Sediaan Krim Ekstrak Etanol Kulit Buah Pisang Goroho (*Musa acuminata* L.) Konsentrasi 12.5% Sebagai Tabir Surya," *Jurnal MIPA*, vol. 9, no. 2, p. 42, Mar. 2020, doi: 10.35799/jmuo.9.2.2020.28248.
- [34] K. Krismayadi, E. Halimatusadyah, D. Apriani, and M. Fitri Cahyani, "Standaridisasi Mutu Simplisia dan Ekstrak Etanol Daun kemangi (*Ocimum x africanum* Lour.)," vol. 3, no. 2, pp. 67–81, 2024.

- [35] Agung Budi Prasetyo, M. F. Imawati, and Angga Rahabistara Sumadji, "Pengaruh metode maserasi dan soxhletasi terhadap kadar flavonoid ekstrak etanol daun kemangi (*Ocimum basilicum* L)," *Jurnal Ilmiah Manuntung: Sains Farmasi Dan Kesehatan*, vol. 8, no. 2, pp. 317–321, Dec. 2022, doi: 10.51352/jim.v8i2.641.
- [36] S. Abouzeid *et al.*, "Favorable Impacts of Drought Stress on the Quality of Medicinal Plants: Improvement of Composition and Content of Their Natural Products," in *Environmental Challenges and Medicinal Plants: Sustainable Production Solutions under Adverse Conditions*, T. Aftab, Ed., Cham: Springer International Publishing, 2022, pp. 105–131. doi: 10.1007/978-3-030-92050-0_4.
- [37] D. H. Ryu, J. Y. Cho, S. H. Yang, and H. Y. Kim, "Effects of Harvest Timing on Phytochemical Composition in Lamiaceae Plants under an Environment-Controlled System," *Antioxidants*, vol. 12, no. 11, Nov. 2023, doi: 10.3390/antiox12111909.
- [38] A. Setiawansyah *et al.*, "Impact of post-harvest process and methanol polarity on the content of niazirin, flavonoids, phenols, and antioxidant activity index of *Moringa oleifera* Lam leaves," *Food and Humanity*, vol. 5, p. 100767, 2025, doi: <https://doi.org/10.1016/j.foohum.2025.100767>.
- [39] A. Setiawansyah *et al.*, "FT-IR-based fingerprint combined with unsupervised chemometric analysis revealed particle sizes and aqueous-ethanol ratio alter the chemical composition and nutraceutical value of *Daucus carota*," *Nat Prod Res*, 2024, doi: 10.1080/14786419.2024.2376351.
- [40] S. Herlina, A. Setiawansyah, and N. Hidayati, "Aerobe Fermentation Enhanced Antioxidant Activity Index of Citrus limon Leaves," *Journal of Food and Pharmaceutical Science*, vol. 12, no. 2, pp. 80–89, 2024, [Online]. Available: www.journal.ugm.ac.id/v3/JFPA
- [41] N. R. Farnsworth, "Screening plants for new medicine," in *Biodiversity*, S. Staff and E. Wilson, Eds., Washington DC: National Academy of Sciences Press, 1988, ch. 9, pp. 83–97.
- [42] N. R. Perron and J. L. Brumaghim, "A review of the antioxidant mechanisms of polyphenol compounds related to iron binding," *Cell Biochem Biophys*, vol. 53, no. 2, pp. 75–100, Mar. 2009, doi: 10.1007/s12013-009-9043-x.
- [43] H. Petkova, E. Jarek, M. Doychinov, M. Krzan, and E. Mileva, "Synergy in Aqueous Systems Containing Bioactive Ingredients of Natural Origin: Saponin/Pectin Mixtures," *Polymers (Basel)*, vol. 14, no. 20, Oct. 2022, doi: 10.3390/polym14204362.
- [44] A. Zdziennicka, K. Szymczyk, B. Jańczuk, K. Wojciechowski, and E. Kobylska, "Wetting Properties of a Saponin-Rich Aqueous Soapwort Extract," *Molecules*, vol. 30, no. 16, p. 3413, Aug. 2025, doi: 10.3390/molecules30163413.
- [45] J. Penfold *et al.*, "Saponin Adsorption at the Air–Water Interface—Neutron Reflectivity and Surface Tension Study," *Langmuir*, vol. 34, no. 32, pp. 9540–9547, Aug. 2018, doi: 10.1021/acs.langmuir.8b02158.
- [46] A. Rahma Mudhana and A. Pujiastuti, "Pengaruh Trietanolamin Dan Asam Stearat Terhadap Mutu Fisik Dan Stabilitas Mekanik Krim Sari Buah Tomat," *Indonesian Journal of Pharmacy and Natural Product*, vol. 4, no. 2, pp. 113–122, 2021, [Online]. Available: <http://jurnal.unw.ac.id/index.php/ijpnp>
- [47] J. Lee and C. F. Scagel, "Chicoric acid found in basil (*Ocimum basilicum* L.) leaves," *Food Chem*, vol. 115, no. 2, pp. 650–656, Jul. 2009, doi: 10.1016/j.foodchem.2008.12.075.
- [48] Monice M Fiume *et al.*, "Safety Assessment of TEA and TEA-Containing Ingredients as Used in Cosmetics," *Int J Toxicol*, vol. 32, no. 3_suppl, pp. 59S–83S, May 2013, doi: 10.1177/1091581813488804.
- [49] Parvesh Devi, Sushila Singh, Seema Sangwan, Promila Dalal, and Monika Moond, "Effect of pH on Antioxidant and Phytochemical Activities of *Mulhatti* Roots (*Glycyrrhiza glabra* L.)," *J Agric Sci Technol A*, vol. 10, no. 5, Oct. 2020, doi: 10.17265/2161-6256/2020.05.005.
- [50] M. Friedman and H. S. Jürgens, "Effect of pH on the Stability of Plant Phenolic Compounds," *J Agric Food Chem*, vol. 48, no. 6, pp. 2101–2110, Jun. 2000, doi: 10.1021/jf990489j.

- [51] M. Lukić, I. Pantelić, and S. D. Savić, "Towards optimal ph of the skin and topical formulations: From the current state of the art to tailored products," Aug. 01, 2021, MDPI AG. doi: 10.3390/cosmetics8030069.
- [52] Yusuf Supriadi and Nurbik Khoirin, "Formulation and Evaluation of Grape Seed Oil (Vitis Vinifera, L) Facial Cream with Variations in The Concentration of Stearic Acid as an Emulsifier," *Journal of Health Sciences and Medical Development*, vol. 1, no. 01, pp. 20–30, Aug. 2022, doi: 10.56741/hesmed.v1i01.32.
- [53] A. D. Maru and S. R. Lahoti, "Formulation and evaluation of moisturizing cream containing sunflower wax," *Int J Pharm Pharm Sci*, vol. 10, no. 11, p. 54, Nov. 2018, doi: 10.22159/ijpps.2018v10i11.28645.
- [54] E. Gore, C. Picard, and G. Savary, "Complementary approaches to understand the spreading behavior on skin of O/W emulsions containing different emollientss," *Colloids Surf B Biointerfaces*, vol. 193, p. 111132, Sep. 2020, doi: 10.1016/j.colsurfb.2020.111132.
- [55] N. Sari, E. Samsul, and A. C. Narsa, "Pengaruh Trietanolamin pada Basis Krim Minyak dalam Air yang Berbahan Dasar Asam Stearat dan Setil Alkohol," *Proceeding of Mulawarman Pharmaceuticals Conferences*, vol. 14, pp. 70–75, Dec. 2021, doi: 10.25026/mpc.v14i1.573.
- [56] L. Wang, M. Dekker, J. Heising, V. Fogliano, and C. C. Berton-Carabin, "Carvacrol release from PLA to a model food emulsion: Impact of oil droplet size," *Food Control*, vol. 114, p. 107247, Aug. 2020, doi: 10.1016/j.foodcont.2020.107247.
- [57] T. Dapčević Hadnađev, P. Dokić, V. Krstonošić, and M. Hadnađev, "Influence of oil phase concentration on droplet size distribution and stability of oil-in-water emulsions," *European Journal of Lipid Science and Technology*, vol. 115, no. 3, pp. 313–321, Mar. 2013, doi: 10.1002/ejlt.201100321.
- [58] A. L. Marlon Bayot and B. N. Bragg Affiliations, *Antimicrobial Susceptibility Testing*, StatPearls. Internet: StatPearls Publishing, 2024. [Online]. Available: <https://www.ncbi.nlm.nih.gov/books/NBK539714/?report=printable>

