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ARTICLE

FORMULATION AND ANTIBACTERIAL EVALUATION OF AN ANTI-ACNE CREAM CONTAINING ESTERIFIED STINGLESS BEE (*Trigona laeviceps*) HIVE WASTE EXTRACT AGAINST *Cutibacterium acnes* AND *Staphylococcus aureus*

[*Formulasi dan Evaluasi Antibakteri Krim Anti Jerawat yang Mengandung Ekstrak Limbah Sarang Lebah Tanpa Sengat (Trigona laeviceps) Teresterifikasi Terhadap Cutibacterium acnes dan Staphylococcus aureus*]

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ABSTRACT

Acne vulgaris is a chronic inflammatory skin disorder commonly associated with the proliferation of pathogenic bacteria such as *Cutibacterium acnes* and *Staphylococcus aureus*. The increasing prevalence of antibiotic resistance has encouraged the exploration of natural resources as alternative antibacterial agents. This study aimed to formulate and evaluate an anti-acne cream containing esterified extract of stingless bee hive waste from *Trigona laeviceps* and to investigate its antibacterial activity against acne-causing bacteria. Stingless bee hive waste was extracted using ultrasound-assisted extraction with 96% ethanol, followed by sonochemical esterification using ethanol and propanol in the presence of sulfuric acid catalyst. The esterified extracts were characterized using FTIR and GC–MS analyses. Antibacterial activity of the esterified extracts was evaluated using the agar well diffusion method at concentrations of 10%, 15%, and 20% (w/v). The most active extract was subsequently formulated into an oil-in-water cream and evaluated for physicochemical properties, including pH, spreadability, adhesion, viscosity, homogeneity, and antibacterial activity. FTIR spectra confirmed successful esterification through the appearance of ester carbonyl absorption bands at approximately 1730–1740 cm^{-1}. GC–MS analysis revealed the presence of major bioactive compounds such as methyl oleate, ethyl oleate, squalene, lanosterol, cycloartenol, and 1-monooleoylglycerol. Ethanol-derived ester exhibited the strongest antibacterial activity, particularly at 20% concentration, with inhibition zones of 1.81 ± 0.10 cm against *C. acnes* and 1.94 ± 0.17 cm against *S. aureus*. The cream formulations showed good homogeneity, acceptable pH values, appropriate spreadability, and enhanced adhesion properties. The 20% cream formulation demonstrated the highest antibacterial activity against *S. aureus* with an inhibition zone of 1.64 ± 0.06 cm. In conclusion, esterified stingless bee hive waste, particularly ethanol-derived ester, possesses promising antibacterial potential and can be successfully formulated into a stable anti-acne cream for topical application.

Keyword: anti-acne cream; antibacterial activity; esterification; stingless bee hive waste; *Cutibacterium acnes*; *Staphylococcus aureus*

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ABSTRAK

Jerawat vulgaris adalah gangguan kulit inflamasi kronis yang umumnya dikaitkan dengan proliferasi bakteri patogen seperti Cutibacterium acnes dan Staphylococcus aureus. Meningkatnya prevalensi resistensi antibiotik telah mendorong eksplorasi sumber daya alam sebagai agen antibakteri alternatif. Studi ini bertujuan untuk merumuskan dan mengevaluasi krim anti-jerawat yang mengandung ekstrak teresterifikasi dari limbah sarang lebah tanpa sengat dari Trigona laeviceps dan untuk menyelidiki aktivitas antibakterinya terhadap bakteri penyebab jerawat. Limbah sarang lebah tanpa sengat diekstraksi menggunakan ekstraksi berbantuan ultrasonik dengan etanol 96%, diikuti dengan esterifikasi sonokimia menggunakan etanol dan propanol dengan adanya katalis asam sulfat. Ekstrak teresterifikasi dikarakterisasi menggunakan analisis FTIR dan GC–MS. Aktivitas antibakteri ekstrak teresterifikasi dievaluasi menggunakan metode difusi sumur agar pada konsentrasi 10%, 15%, dan 20% (w/v). Ekstrak yang paling aktif kemudian diformulasikan menjadi krim minyak dalam air dan dievaluasi sifat fisikokimianya, termasuk pH, daya sebar, adhesi, viskositas, homogenitas, dan aktivitas antibakteri. Spektrum FTIR mengkonfirmasi keberhasilan esterifikasi melalui munculnya pita serapan karbonil ester pada sekitar 1730–1740 cm^{-1}. Analisis GC–MS mengungkapkan keberadaan senyawa bioaktif utama seperti metil oleat, etil oleat, skualen, lanosterol, sikloartenol, dan 1-monooleoilgliserol. Ester yang berasal dari etanol menunjukkan aktivitas antibakteri terkuat, terutama pada konsentrasi 20%, dengan zona inhibisi 1,81 ± 0,10 cm terhadap C. acnes dan 1,94 ± 0,17 cm terhadap S. aureus. Formulasi krim menunjukkan homogenitas yang baik, nilai pH yang dapat diterima, daya sebar yang sesuai, dan sifat adhesi yang lebih baik. Formulasi krim 20% menunjukkan aktivitas antibakteri tertinggi terhadap S. aureus dengan zona inhibisi 1,64 ± 0,06 cm. Kesimpulannya, limbah sarang lebah tanpa sengat yang telah diesterifikasi, khususnya ester yang berasal dari etanol memiliki potensi antibakteri yang menjanjikan dan dapat berhasil diformulasikan menjadi krim anti-jerawat yang stabil untuk aplikasi topikal.

Kata kunci: *krim anti jerawat; aktivitas antibakteri; esterifikasi; limbah sarang lebah tanpa sengat; Cutibacterium acnes; Staphylococcus aureus*

INTRODUCTION

Acne vulgaris, or acne vulgaris, is a chronic inflammatory disease of the pilosebaceous unit with a high prevalence worldwide and a major problem in cosmetic dermatology. Tan & Bhate, (2014) report that acne vulgaris affects more than 640 million individuals worldwide and is among the eight most prevalent diseases globally. In Indonesia, the prevalence of acne continues to increase, particularly among adolescents and young adults who actively use cosmetics and skincare products (Sudarsono *et al*., 2025). Tropical environmental factors, exposure to pollution, stress, diet, and the use of comedogenic cosmetics are known to contribute to increased acne severity (Bettoli & Araviiskaia, 2018). These conditions indicate that acne vulgaris is not only a skin health problem but also affects the quality of life and psychosocial well-being of sufferers.

The pathogenesis of acne vulgaris involves a complex interaction between follicular hyperkeratinization, increased sebum production, inflammation, and colonization of pathogenic bacteria on the skin. *Cutibacterium acnes* plays a role in triggering inflammation through the production of lipase enzymes that hydrolyze triglycerides into free fatty acids, thereby increasing the release of pro-inflammatory mediators such as interleukin-1β and tumor necrosis factor-α (Mayslich & Grange, 2021). Furthermore, *S. aureus* can also trigger skin inflammation through biofilm formation and the production of toxins that damage skin tissue (Di Domenico *et al*., 2019). The use of synthetic antibiotics such as clindamycin, erythromycin, and tetracycline remains the mainstay of acne vulgaris therapy, but long-term use can lead to bacterial resistance and skin irritation(Armillei *et al*., 2024). Several studies have shown that Cutibacterium acnes resistance to topical antibiotics has increased significantly in the past decade due to irrational antibiotic use (Zu *et al*., 2025).

The rise in antibiotic resistance has driven the development of natural products as safer and more sustainable alternative anti-acne agents. Stingless bees possess extensive pharmacological activity due to their diverse bioactive secondary metabolites (Ng *et al*., 2025). However, most research focuses on honey and propolis, while stingless bee hive waste has not been optimally utilized. *Tetragonula* spp. hive waste is known to contain flavonoids, phenolic compounds, terpenoids, and organic acids, which have potential as natural antibacterial agents (Yusuf *et al*., 2024). Phenolic compounds can also cause protein denaturation and inhibit bacterial enzyme activity,

triggering microbial cell death (Shinde *et al*., 2025). Furthermore, terpenoids have the ability to increase bacterial membrane permeability, leading to leakage of intracellular components (Erguden, 2019).

Despite their potent biological activity, natural phenolic compounds generally have low stability and hydrophilic properties, which can limit penetration of bacterial lipid membranes and the skin's stratum corneum. Therefore, chemical modification through esterification can be used to enhance the effectiveness of natural biologically active compounds. Esterification is the process of forming esters through hydroxyl group substitution, which can increase the lipophilicity and stability of bioactive compounds (Erguden, 2019). In this study, the esterification process was carried out using ethanol and propanol to produce methyl ester and propyl ester derivatives from stingless bee nest waste extract. The selection of ethanol and propanol was based on differences in carbon chain length, which is thought to affect the lipophilicity, bacterial membrane penetration, and antibacterial activity of the esterified compounds. Propyl ester derivatives are thought to be more hydrophobic than methyl esters, potentially enhancing interactions with bacterial membrane lipids and enhancing antibacterial activity (Echeverría *et al*., 2017). Furthermore, ester modification has also been reported to enhance the penetration of active ingredients in topical preparations and improve the stability of cosmetic formulations (Azmi *et al*., 2022).

Although the antibacterial properties of honey, propolis, and other stingless bee products have been widely reported, the utilization of stingless bee nest waste (*Trigona laeviceps*) remains poorly explored. In addition, studies on the esterification of stingless bee nest waste using ethanol and propanol and its application in topical anti-acne cream formulations are still lacking. We hypothesized that esterification would enhance the lipophilicity of bioactive compounds, thereby improving their antibacterial activity against *C. acnes* and *S. aureus.* Therefore, this study aimed to prepare ethanol- and propanol-esterified stingless bee nest waste extracts, characterize the resulting ester derivatives, formulate them into anti-acne cream, and evaluate their physicochemical properties and antibacterial activity.

MATERIALS AND METHODS

Materials

Waste material from stingless bee nests of Tetragonula laeviceps was collected from a stingless bee cultivation area located in Gunungpring, Magelang, Central Java, Indonesia. Ethanol 96% analytical grade, ethanol analytical grade, absolute ethanol, concentrated sulfuric acid (H_2SO_4), and ethyl acetate were purchased from Merck. Nutrient Agar (NA) medium was obtained from Oxoid, while sterile physiological saline solution (0.9% NaCl) was supplied by Otsuka Pharmaceutical. The antibacterial assay employed bacterial strains of *Staphylococcus aureus* ATCC 25923 and *Cutibacterium acnes* obtained from the Microbiology Laboratory, Diponegoro University, Indonesia. Ciprofloxacin (Sigma-Aldrich, St. Louis, MO, USA) was used as the reference antibacterial agent.

The extraction process utilized an ultrasonic bath sonicator (Branson 2510, Danbury, CT, USA). Solvent evaporation was performed using a rotary evaporator (Heidolph Laborota 4000, Germany). Sterilization and incubation procedures were conducted using an autoclave (Hirayama HV-85, Japan) and incubator (Memmert IN55, Germany), respectively. Functional group analysis was carried out using Fourier Transform Infrared Spectroscopy (FTIR Cary 630, Agilent Technologies, USA), while chemical constituent profiling was performed using Gas Chromatography–Mass Spectrometry (GC–MS QP 2010 SE, Shimadzu, Japan).

Extraction of Stingless Bee Nest Waste

Extraction of stingless bee nest waste was conducted using ultrasound-assisted extraction with ethanol 96% as the extraction solvent. Approximately 250 g of powdered nest waste was immersed in ethanol at a ratio of 1:10 (w/v). The extraction mixture was subjected to ultrasonic treatment at 40 kHz and 40°C for 60 minutes. Ultrasound-assisted extraction was selected because acoustic cavitation generated during sonication can disrupt plant and resin matrices, thereby

improving solvent penetration and enhancing the release of intracellular bioactive compounds (Mushtaq *et al*., 2025; Shen *et al*., 2023). After sonication, the extract was filtered to separate insoluble residues from the filtrate.The combined filtrates were concentrated under reduced pressure using a rotary evaporator at 40–45°C. The resulting semi-solid extract was further thickened using a water bath at 60°C until a viscous extract was obtained.

Esterification of Stingless Bee Nest Waste Extract

The esterification reaction was performed using a sonochemical approach to enhance reaction efficiency and shorten processing time. Briefly, 15 g of concentrated extract was dissolved in 100 mL of alcohol solvent according to treatment variation, namely ethanol and propanol. Subsequently, 2 mL of concentrated sulfuric acid was added as an acid catalyst. The reaction mixture was exposed to ultrasonic irradiation at 40 kHz and maintained at 40°C for 60 minutes. Sonochemical esterification was employed because ultrasonic energy is known to accelerate molecular interactions, improve mass transfer, and increase ester conversion efficiency compared to conventional heating methods (Semenova *et al*., 2025). Following completion of the reaction, liquid-liquid partitioning was performed using ethyl acetate to separate the ester-rich organic layer from the aqueous layer containing residual acid and polar impurities. The organic phase was collected and concentrated using rotary evaporation to obtain the esterified extract. Ethanol ester and propanol ester were then stored in airtight containers at 4°C before further analysis.

Characterization of Esterified Extracts

Characterization of the esterified products was conducted using FTIR spectroscopy to identify structural modifications resulting from the esterification reaction. The spectra were recorded within a scanning range of 4000–400 cm^{-1}. Successful ester formation was indicated by the appearance of a carbonyl ester absorption band around 1735 cm^{-1}, accompanied by changes in hydroxyl (–OH) and C–O stretching vibrations. Further chemical profiling was performed using GC–MS analysis to identify dominant volatile and semi-volatile constituents present in the esterified extracts (Cai *et al*., 2026).

Antibacterial Activity Assay

Antibacterial activity was evaluated using the agar well diffusion technique. Bacterial suspensions of *S. aureus* and *C. acnes* were prepared and adjusted to match the turbidity of a 0.5 McFarland standard. The bacterial cultures were uniformly spread onto sterile Nutrient Agar surfaces using sterile cotton swabs.

Subsequently, 5 µL of esterified extract solutions at concentrations of 10%, 15%, and 20% (w/v) were introduced into each well. Ciprofloxacin was used as the positive control, whereas sterile solvent served as the negative control. The inoculated plates containing *S. aureus* were incubated aerobically at 37°C for 24 hours. Meanwhile, plates inoculated with *C. acnes* were incubated under anaerobic conditions at 37°C for 48 hours. Antibacterial activity was determined by measuring the diameter of the inhibition zones using a digital caliper. The agar diffusion assay is widely recognized as a reliable preliminary method for screening antibacterial activity of natural products and bioactive extracts (Balouiri *et al*., 2016). The best antibacterial results against ethanol and propanol esters were continued in the manufacture of cream preparations. Statistical analyses were conducted using IBM SPSS Statistics version 26.0 (IBM Corp., Armonk, NY, USA), and statistical significance was established at $p < 0.05$. Differences in antibacterial activity were analyzed using a two-way analysis of variance (Two-Way ANOVA) to determine the effects of esterification type (ethanol and propanol), extract concentration (10%, 15%, and 20%), and the interaction between these factors on the inhibition zone diameter against *C. acnes* and *S. aureus*.

Preparation of Anti-Acne Cream

The anti-acne cream formulation was prepared using the conventional emulsification method following slight modifications from previous semisolid cream formulations (Choudhari *et al*., 2023). The oil phase, consisting of stearic acid and cetyl alcohol, was heated at 70 ± 2°C until completely melted. Simultaneously, the aqueous phase containing purified water, propylene glycol, Nipagin, and carbomer was heated separately at the same temperature. Carbomer was allowed to hydrate completely before mixing. Subsequently, the aqueous phase was gradually added into the oil phase under continuous stirring using a homogenizer until a homogeneous emulsion was formed. Triethanolamine (TEA) was added dropwise to neutralize the carbomer and improve cream consistency. After the base cream reached room temperature, the esterified extract of stingless bee nest waste was incorporated according to formulation concentration (10%, 15%, and 20%) and mixed continuously until uniform cream preparations were obtained. The resulting formulations were stored in airtight containers for further evaluation.

Table 1. Formulation of Anti-Acne Cream Containing Esterified Ethanol Extract of Stingless Bee Nest Waste (*Formulasi Krim Anti Jerawat yang Mengandung Ekstrak Esterifikasi Etanol dari Limbah Sarang Lebah Tanpa Sengat*).

Ingredients (*Kandungan*)	**Base**	**F1 (10%)**	**F2 (15%)**	**F3 (20%)**
Esterified extract	–	2 g	3 g	4 g
Cetyl alcohol (2%)	0.4 g	0.4 g	0.4 g	0.4 g
Stearic acid (2%)	0.4 g	0.4 g	0.4 g	0.4 g
Propylene glycol (15%)	3 g	3 g	3 g	3 g
Carbomer (1%)	0.2 g	0.2 g	0.2 g	0.2 g
Nipagin (0.2%)	0.04 g	0.04 g	0.04 g	0.04 g
Purified water	16 mL	14 mL	13 mL	12 mL
Triethanolamine (TEA)	1–3 drops	1–3 drops	1–3 drops	1–3 drops

Evaluation of Physical Characteristics of Cream

The physical characteristics of the cream formulations were evaluated through organoleptic observation, homogeneity, pH, spreadability, adhesiveness, viscosity, and stability testing according to standard cosmetic and pharmaceutical semisolid evaluation procedures (Sim *et al*., 2020).

Antibacterial Activity Test of Cream Formulation

The antibacterial activity of the cream formulations was evaluated using the agar well diffusion method against *S. aureus* ATCC 25923 and *C. acnes*. Bacterial suspensions equivalent to 0.5 McFarland standard were uniformly spread onto Nutrient Agar media. Wells with a diameter of 6 mm were prepared aseptically, and approximately 0.1 g of cream sample was introduced into each well. Ciprofloxacin cream was used as a positive control, while the cream base without extract served as a negative control. The inoculated plates were incubated at 37°C for 24 hours for *S. aureus* and under anaerobic conditions for 48 hours for *C. acnes*. Antibacterial activity was determined by measuring the diameter of the inhibition zone (Esmael *et al*., 2020).

RESULTS

A total of 250 g of stingless bee nest waste was extracted using 96% ethanol at a sample-to-solvent ratio of 1:10 (w/v) under ultrasonic-assisted extraction for 60 min. The extraction process produced a viscous extract with a yield of 32.76% after the solvent was removed using a vacuum evaporator at 50°C. Subsequently, 15 g of the concentrated extract was subjected to esterification by reacting it with 100 mL of monohydric alcohol, in the presence of 2 mL of concentrated H_2SO_4 as an acid catalyst. The resulting ethyl and propyl ester derivatives were further characterized and evaluated for their antibacterial activity.

Characteristics of compounds in ethanol and propanol esters

The FTIR spectra of the ethyl ester and propyl ester extracts from stingless bee nest waste showed characteristic absorption bands indicating successful esterification. Broad absorption around 3200–3500 cm^{-1} corresponded to hydroxyl (–OH) groups, while strong peaks at approximately 2920 cm^{-1} and 2850 cm^{-1} indicated aliphatic C–H stretching vibrations. The appearance of a distinct absorption band near 1730–1740 cm^{-1} confirmed the presence of ester carbonyl (C=O) groups formed during the esterification process. Absorption bands within 1600–1450 cm^{-1} represented aromatic C=C vibrations from phenolic and flavonoid compounds, whereas peaks in the 1300–1000 cm^{-1} region were associated with C–O stretching of ester groups. Compared to the ethyl ester spectrum, the propyl ester showed stronger absorption intensity in the aliphatic and ester regions, suggesting greater hydrophobic modification and higher ester formation efficiency.

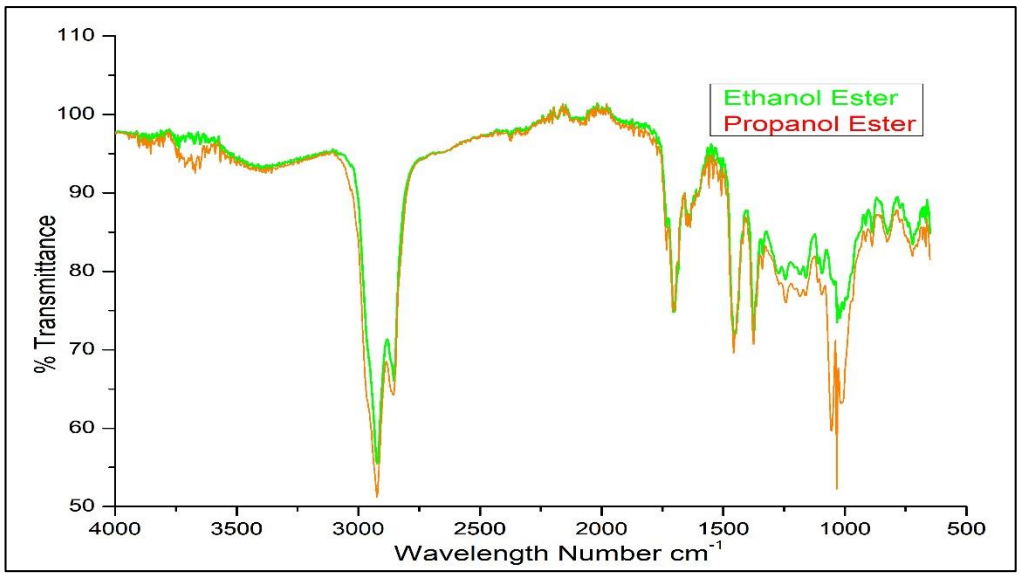


Figure 1. FTIR Spectra Comparison of Ethanol Ester and Propanol Ester Derived from Stingless Bee Nest Waste Extract (*Trigona laeviceps*) (*Perbandingan Spektrum FTIR Ester Etanol dan Ester Propanol yang Diperoleh dari Ekstrak Limbah Sarang Lebah Tanpa Sengat* (*Trigona laeviceps*)).

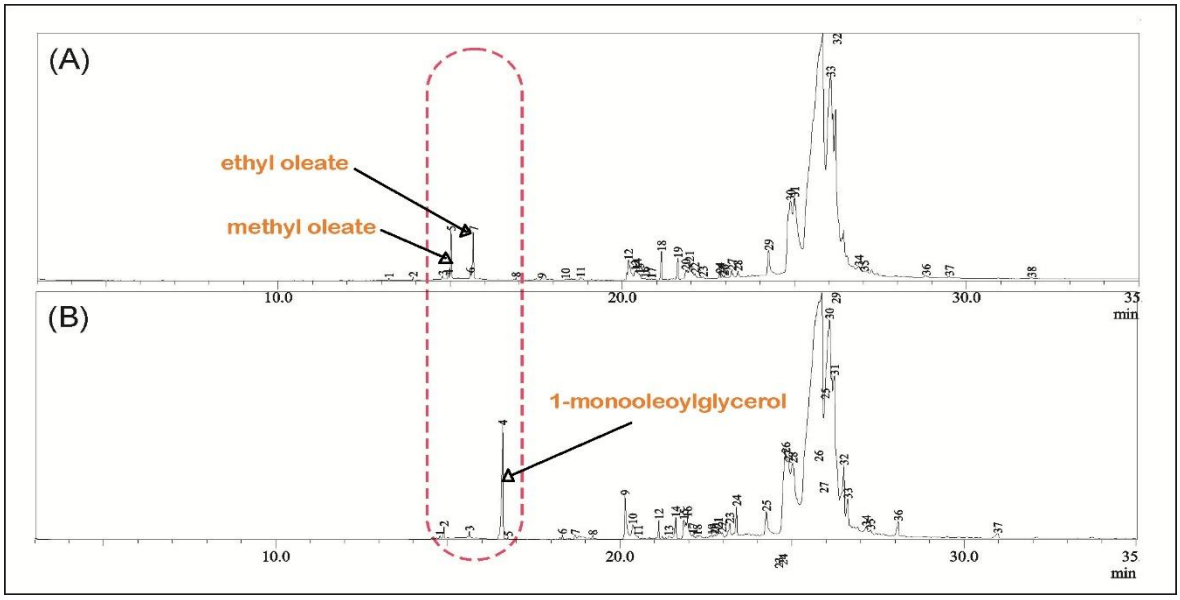


Figure 2. GC–MS Chromatogram of Ethyl Ester (A) and Propyl Ester (B) Derived from Stingless Bee Nest Waste Extract (*Trigona laeviceps*).

The chromatograms in **Figure 2** show the differences in chemical composition between the ethyl ester and propyl ester derivatives from the stingless bee hive waste extract. Chromatogram (A), representing the ethanol ester derivative, shows dominant peaks at retention times of 15.02 and 15.06 minutes, corresponding to methyl oleate and ethyl oleate, respectively. These peaks indicate that the esterification process successfully converted the fatty acid constituents into ester derivatives. Meanwhile, chromatogram (B), representing the propyl ester derivative, shows a dominant peak identified as 1-monooleoylglycerol within a retention time range of 16.58 minutes. These results indicate that the use of propanol promotes the formation or retention of monoacylglycerol compounds during the esterification process. Both chromatograms also show major peaks at retention times of around 24-27 minutes, corresponding to nonpolar compounds such as squalene, lanosterol, cycloartenol, and tetraconate derivatives. This finding indicates that propanol enhances the hydrophobic characteristics of the resulting ester compound due to its longer alkyl carbon chain. Overall, the results of this study indicate that the type of alcohol used during esterification affects the metabolite profile of stingless bee hive waste extract, particularly lipid-derived compounds that may contribute to antibacterial activity and influence the physicochemical characteristics of anti-acne cream formulations.

Comparative Antibacterial Activity of Ethanol and Propanol-derived Esters

The antibacterial activity data indicate that esterified extracts prepared using ethanol exhibited consistently higher inhibition zones than those obtained with propanol, particularly at 20% concentration against *C. acnes* and *S. aureus*. A clear concentration-dependent increase in activity was observed, suggesting that higher levels of esterified bioactive compounds enhance antimicrobial efficacy. This trend is strongly associated with the chemical composition of the ethanol-derived ester, which contains methyl oleate, squalene, tetracosanoate, lanosterol, and cycloartenol. Fatty acid esters such as methyl oleate are known to disrupt bacterial membranes by increasing permeability and inducing leakage of intracellular components, while squalene and sterol derivatives contribute to membrane destabilization and interference with microbial metabolic pathways. In contrast, the propanol ester, although containing lanosterol, cycloartenol, squalene, and 1-monooleoylglycerol, showed relatively lower activity, indicating differences in extraction efficiency and bioactive compound functionality.

Table 2. Inhibition Zone Diameter of Esterified Stingless Bee Hive Waste Against *Cutibacterium acnes* and *Staphylococcus aureus* (*Diameter Zona Inhibisi dari Limbah Sarang Lebah Tanpa Sengat yang Diesterifikasi Terhadap Cutibacterium acnes dan Staphylococcus aureus*).

Sample (*Sampel*)	**Antibacterial against** (*Antibakteri melawan*)			
	Cutibacterium acnes		*Staphylococcus aureus*	
	Concentration (*Konsentrasi*)	**Inhibition Zone** (*Zona hambat*) **(cm))**	**Concentration** (*Konsentrasi*)	**Inhibition Zone** (*Zona hambat*) **(cm))**
Stingless bee hive waste extract (*Ekstrak limbah sarang lebah tanpa sengat*)	10%	0.55 ± 0.13	10%	0.65 ± 0.12
	15%	0.66 ± 0.14	15%	0.74 ± 0.11
	20%	0.73 ± 0.18	20%	0.85 ± 0.14
Ciprofloxacin (K+)		2.31 ± 0.22		1.62 ± 0.25
Ethanol-derived ester (*Ester turunan etanol)*	10%	1.64 ± 0.20	10%	1.65 ± 0.20
	15%	1.73 ± 0.12	15%	1.81 ± 0.14
	20%	1.81 ± 0.10	20%	1.94 ± 0.17
Ciprofloxacin (K+)		2.42 ± 0.52		1.84 ± 0.41
Propanol- derived ester (*Ester turunan etanol)*	10%	1.46 ± 0.25	10%	1.47 ± 0.02
	15%	1.55 ± 0.14	15%	1.65 ± 0.02
	20%	1.57 ± 0.13	20%	1.76 ± 0.03
Ciprofloxacin (K+)		2.81 ± 0.29		1.57 ± 0.17

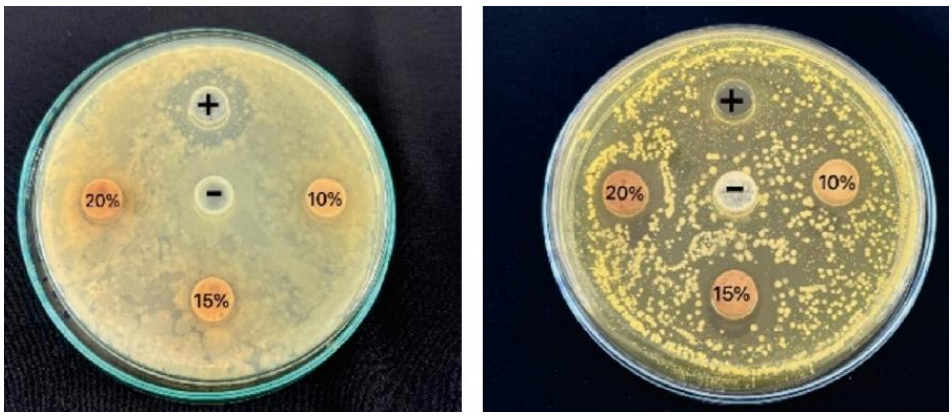
Physicochemical Characterization and Antibacterial Activity of the Cream Formulation

Table 3. Physical Characteristics of Anti-Acne Cream Containing Ethanol Ester from Stingless Bee (*Trigona laeviceps*) Nest Waste. *(Karakteristik Fisik Krim Anti Jerawat yang Mengandung Ester Etanol dari Limbah Sarang Lebah Tanpa Sengat (Trigona laeviceps)).*

Sample (*Sampel*)	**Spreadability** (*Kemampuan menyebar*) **(cm)**	**pH**	**Adhesion Time** (*Waktu Adhesi*) **(s)**	**Viscosity** (*Viskositas*) **(%)**	**Homogeneity** (*Homogenitas*)
Base	5.78 ± 0.08	6.64 ± 0.01	1.26 ± 0.04	83.7	Homogeneous
F1 (10%)	5.59 ± 0.04	5.71 ± 0.01	1.38 ± 0.03	82.9	Homogeneous
F2 (15%)	5.38 ± 0.05	5.02 ± 0.02	1.49 ± 0.03	87.0	Homogeneous
F3 (20%)	5.03 ± 0.07	5.32 ± 0.03	1.55 ± 0.02	84.2	Homogeneous

Table 4. Antibacterial Activity of Cream Formulations Containing Ethanol Ester Against Acne-Causing Bacteria (*Aktivitas Antibakteri Formulasi Krim yang Mengandung Ester Etanol Terhadap Bakteri Penyebab Jerawat*).

Bacteria (*Bakteri*)	**Concentration** (*Konsentrasi*)	**Inhibition Zone** (*Zona Hambat*) **(cm)**
Cutibacterium acnes	10%	1.04 ± 0.02
	15%	1.02 ± 0.00
	20%	1.02 ± 0.01
	Ciprofloxacin (K+)	1.70 ± 0.11
Staphylococcus aureus	10%	1.43 ± 0.02
	15%	1.55 ± 0.08
	20%	1.64 ± 0.06
	Ciprofloxacin (K+)	1.71 ± 0.03



(a) (b)

Figure 3. Inhibition zones of *C. acnes* (a) and *S. aureus* (b) following treatment with various concentrations of cream ethanol esters derived from stingless bee (*Trigona laeviceps*) hive waste, including negative control (DMSO) and positive control (ciprofloxacin) (*Zona inhibisi C. acnes (a) dan S. aureus (b) setelah perlakuan dengan berbagai konsentrasi krim ester etanol yang berasal dari limbah sarang lebah tanpa sengat (Trigona laeviceps), termasuk kontrol negatif (DMSO) dan kontrol positif (ciprofloxacin)*).

Physical characterization data clearly showed that increasing the concentration of esterified stingless bee (*Trigona laeviceps*) nest waste significantly affected the properties of the cream. Spreadability decreased from 5.78 ± 0.08 cm (base) to 5.03 ± 0.07 cm (F3), indicating that higher extract concentrations improved the internal consistency of the formulation, likely due to stronger intermolecular interactions within the cream matrix. Conversely, adhesion time increased from 1.26 ± 0.04 s after increasing the active compound content to 1.55 ± 0.02 s at 20% concentration. This indicates an increased skin retention capacity, which is beneficial for topical anti-acne effectiveness. The pH values remained within or close to the acceptable physiological range of the skin. Although F2 showed the lowest pH, this was likely due to incomplete separation of the acidic compounds after esterification. All formulations were homogeneous, indicating good dispersion of the active ingredients. One-way ANOVA analysis confirmed that variations in spreadability, pH, and adhesion time were statistically significant ($p < 0.001$), indicating that extract concentration is a determining factor in modulating the physicochemical performance of the cream system.

DISCUSSION

In **Figure 1**, the appearance of a sharp absorption band around 1730–1740 cm^{-1} confirms the presence of carbonyl ester groups (C=O), indicating successful esterification of the bioactive constituents. This peak is characteristic of esterified fatty acids such as methyl oleate and ethyl oleate, which are commonly identified in extracts containing natural lipids. Previous studies on stingless bee propolis and beeswax residues also reported similar carbonyl ester absorptions associated with fatty acid esters and glyceride compounds (Pastor & Dolaševi, 2025).The stronger carbonyl intensity observed in propyl ester derivatives indicates higher ester conversion efficiency and greater incorporation of aliphatic ester groups. Similar effects, enhanced by aliphatic hydrocarbons, have been reported in propolis extracts rich in fatty acid esters and terpenoid constituents(Quero *et al*., 2025). The stronger peak intensity in the propyl ester spectrum indicates increased hydrophobicity due to the longer carbon chain of propanol.

The superior performance of ethanol-derived esters compared to propanol-derived esters, based on the data in **Table 2**, can be explained by differences in solvent polarity, chemical compound, and esterification behavior. Ethanol, being more polar and having a shorter carbon chain, facilitates more efficient extraction and esterification of semi-polar bioactive compounds, resulting in higher yields of active esters such as methyl oleate. These smaller, somewhat polar esters generally exhibit better diffusion through bacterial cell walls and biofilms, thus enhancing antibacterial activity. Conversely, propanol, with its longer carbon chain and lower polarity, tends to produce more

hydrophobic esters that may have reduced solubility and slower diffusion in aqueous biological environments, thus limiting their interaction with bacterial cells. Fatty acid esters with shorter alkyl chains often exhibit stronger antibacterial effects due to better membrane penetration (Kim *et al*., 2023). The bioactivity of terpenoids and sterols is influenced by their chemical environment and solubility, which is directly affected by the extraction solvent (Masyita *et al*., 2022). Effective antimicrobial activity depends on the type of compound that interacts with and disrupts microbial membranes, a process enhanced by optimal polarity and molecular size. Therefore, the higher antibacterial activity observed with ethanol-derived esters is likely due to a combination of favorable ester structure enhancement and increased bioavailability of the active compound. These antibacterial results have similar activity to the study using honey at a concentration of 50% observed for 48 hours (Jilo, 2024).

The antibacterial activity of the ethanol- and propanol-esterified stingless bee nest waste extracts increased as the extract concentration increased against both *C. acnes* and *S. aureus*. The 20% ethanol ester formulation exhibited the largest inhibition zones (1.81 ± 0.10 cm against *C. acnes* and 1.94 ± 0.17 cm against *S. aureus*), indicating stronger antibacterial activity than the propanol ester formulation. Two-Way ANOVA analysis showed that the type of esterification solvent, extract concentration, and their interaction significantly affected the inhibition zone diameter 0.0182 ($p < 0.05$). These findings suggest that ethanol esterification produced more effective antibacterial compounds than propanol esterification, particularly at the highest concentration tested.

The observed trends in the results of the ethanol ester formulations are consistent with established principles in semisolid formulations as shown in **Table 3**. Increasing the concentration of natural bioactive compounds tends to alter the rheological and application properties of the system. Higher extract levels are known to reduce spreadability due to increased viscosity and the development of a more structured internal network within the cream matrix (Serra *et al*., 2025). Furthermore, increased adhesion is generally associated with better interaction between the formulation and the skin surface, leading to longer residence times and potentially better therapeutic performance. The presence of phenolic compounds and other polar compounds can further contribute to microstructural modification, affecting the mechanical stability and consistency of the formulation (Xiao *et al*., 2025). An optimal topical formulation must achieve a balance between adequate spreadability and sufficient adhesion to ensure ease of application and effectiveness. Overall, these findings reinforce that the incorporation of esterified stingless bee hive waste plays a dual role in enhancing functional bioactivity while modulating key physicochemical characteristics of the cream system.

The antibacterial activity observed in **Table 4** is consistent with recent advances in topical antimicrobial formulations, where efficacy is influenced not only by the intrinsic activity of the bioactive compound but also by formulation factors that control release and diffusion. The obtained zones of inhibition are within the moderate range generally reported for semisolid systems containing natural extracts, such as nanoemulsions, due to increased penetration and bioavailability(Rahma *et al*., 2025). Formulation components, including emulsifiers and internal structure, significantly influence activity against pathogens such as *S. aureus*. However, increased viscosity in cream systems can limit diffusion in agar-based assays, resulting in moderate zones of inhibition despite strong intrinsic antimicrobial properties (Djerri *et al*., 2025). Furthermore, higher extract loadings often result in larger zones of inhibition, while more stable and cosmetically acceptable formulations tend to exhibit moderate but consistent activity (Ardhany *et al*., 2021). Overall, these findings indicate that the ethanol ester-based cream formulation of stingless bee hive (*Trigona laeviceps*) waste has significant antibacterial potential.

CONCLUSION

This study demonstrated that esterification successfully modified the chemical characteristics and enhanced the antibacterial potential of stingless bee hive waste extract derived from *Trigona laeviceps*. FTIR and GC–MS analyses confirmed the formation of ester compounds and identified several bioactive constituents, including methyl oleate, ethyl oleate, squalene, lanosterol, and cycloartenol, which may contribute to antibacterial activity. The ethanol-derived ester showed stronger inhibitory effects against *C. acnes* and *S. aureus* compared to the propanol-derived ester. Furthermore, incorporation of the ethanol ester into cream formulations produced homogeneous and physically stable preparations with acceptable pH, spreadability, adhesion, and viscosity properties, while the 20% formulation exhibited the highest antibacterial activity. These findings indicate that esterified stingless bee hive waste has promising potential as a natural anti-acne topical agent. Future studies should further investigate the active compounds, antibacterial mechanisms, formulation stability, skin safety, and in vivo anti-acne efficacy to support the development of pharmaceutical and cosmetic products based on stingless bee hive waste bioresources.

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AUTHOR CONTRIBUTIONS

AFM: Conceptualization, Methodology, Antibacterial Testing, Data Analysis, Writing the original draft, Review-writing, and editing. MS: Synthesis, Characterization, Data Analysis, Writing the original draft, Review-writing, and editing. YP: Conceptualization, Methodology, Characterization, Data Analysis, Writing the original draft, Review-writing, and editing.

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