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Antibacterial Activity of Esterified *Trigona laeviceps* Hive Waste Extract against Skin-Associated Bacteria

Aktivitas Antibakteri Ekstrak Limbah Sarang *Trigona laeviceps* Teresterifikasi terhadap Bakteri Terkait Kulit

Mighfar Syukur*, Ahmad Fuad Masduqi, Yuliana Purwaningsih

Department of Pharmacy, Faculty of Pharmacy, Sekolah Tinggi Ilmu Pharmasi Yayasan Pharmasi Semarang, Semarang, Central Java 50192, Indonesia

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ABSTRACT

Stingless bee (*Trigona laeviceps*) hive waste contains bioactive compounds, including flavonoids, phenolics, terpenoids, and organic acids, with potential antibacterial activity. This study aimed to enhance its antibacterial activity through esterification using methanol and ethanol. The extract was obtained by sonochemical extraction with 96% ethanol and reacted with methanol or ethanol using H_2SO_4 as a catalyst. Esterification products were characterized by FTIR and GC–MS. Antibacterial activity against *Staphylococcus aureus* and *Propionibacterium acnes* was evaluated at 10%, 15%, and 20% using the agar well-diffusion method. Alcohol variation affected the chemical profile and antibacterial activity. The ethanol ester showed the largest inhibition zones at 20%, measuring 1.97 ± 0.24 mm against *S. aureus* and 1.90 ± 0.10 mm against *P. acnes*. These findings suggest that esterification may improve the value of stingless bee hive waste as a natural antibacterial candidate.

Kata kunci:
Aktivitas antibakteri
Esterifikasi
Limbah sarang lebah
Propionibacterium acnes
Staphylococcus aureus

ABSTRAK

Limbah sarang lebah tanpa sengat (*Trigona laeviceps*) mengandung senyawa bioaktif, seperti flavonoid, fenolik, terpenoid, dan asam organik, yang berpotensi sebagai antibakteri. Penelitian ini bertujuan meningkatkan aktivitas antibakteri ekstrak melalui esterifikasi menggunakan metanol dan etanol. Ekstrak diperoleh dengan metode sonokimia menggunakan etanol 96%, kemudian direaksikan dengan metanol atau etanol menggunakan H_2SO_4 sebagai katalis. Produk esterifikasi dikarakterisasi menggunakan FTIR dan GC–MS. Aktivitas antibakteri terhadap *Staphylococcus aureus* dan *Propionibacterium acnes* diuji pada konsentrasi 10%, 15%, dan 20% menggunakan metode difusi sumuran agar. Variasi alkohol memengaruhi profil kimia dan aktivitas antibakteri. Ester etanol menunjukkan zona hambat terbesar pada 20%, yaitu 1,97 ± 0,24 mm terhadap *S. aureus* dan 1,90 ± 0,10 mm terhadap *P. acnes*. Temuan ini menunjukkan bahwa esterifikasi berpotensi meningkatkan nilai guna limbah sarang lebah tanpa sengat sebagai kandidat antibakteri alami.

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**Corresponding author:*
Mighfar Syukur (syukurmighfar.stifar@gmail.com)

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1. INTRODUCTION

Stingless bee (*Trigona laeviceps*) hive waste is commonly regarded as an unused by-product, although it may still contain bioactive compounds with pharmacological potential. Previous studies have reported that stingless bee-related materials contain flavonoids, phenolics, terpenoids, and organic acids, which are associated with antioxidant and antibacterial activities (Ng et al., 2025). Compounds such as squalene and cycloartenol from the terpenoid group, oleic acid as an organic acid, caffeic acid as a phenolic compound, and 3-methyl quercetin as a flavonoid derivative have also been reported in stingless bee nest materials or related natural matrices (Li et al., 2018; Lozano-Grande et al., 2018). These constituents provide a scientific basis for investigating stingless bee hive waste as a potential source of antibacterial compounds.

Phenolic and flavonoid compounds may exert antibacterial effects through protein binding, enzyme inhibition, and bacterial cell membrane disruption (Tuksitha et al., 2018). However, the relatively high polarity of some natural phenolic compounds may limit their diffusion through lipid-rich bacterial membranes, thereby reducing antibacterial effectiveness (Echeverría, Opazo, et al., 2017a). Chemical modification is one strategy to improve the physicochemical properties of bioactive compounds. Esterification can increase lipophilicity and chemical stability, which may enhance interaction with bacterial membranes and improve biological activity (Ju et al., 2025).

Previous studies have reported antibacterial activity of honey and bee-derived materials against *Staphylococcus aureus* and *Propionibacterium acnes*. Wild bee honey has been reported to inhibit *P. acnes*, with an inhibition zone of approximately 24 mm (Marwah et al., 2022), while other honey samples have shown antibacterial activity against acne-associated bacteria, either alone or in combination with natural ingredients (Ananda et al., 2020; Julianti et al., 2017). Various honey samples have also produced inhibition zones against *S. aureus*, with reported values ranging from approximately 15 to 25 mm depending on botanical source, bioactive composition, and concentration (Gambo et al., 2018; Nweze et al., 2016). However, most previous studies have focused on honey or unmodified bee-derived extracts, whereas the antibacterial potential of esterified stingless bee hive waste extract remains insufficiently explored.

Esterification using short-chain alcohols, such as methanol and ethanol, can produce ester derivatives with different physicochemical characteristics, which may influence membrane interaction, diffusion capacity, and antibacterial activity (Dąbrowska-Maś, 2017; Totsingan et al., 2021). This approach is relevant because *S. aureus* and *P. acnes* are important skin-associated bacteria involved in skin infection and acne inflammation and are also associated with antimicrobial resistance concerns (Belay et al., 2024; Kumar et al., 2016; Tong et al., 2015). Therefore, this study aimed to evaluate the antibacterial activity of esterification products derived from *T. laeviceps* hive waste extract using methanol and ethanol as primary alcohols. Antibacterial activity was assessed against *S. aureus* and *P. acnes* at several concentrations based on inhibition zone diameter, and the chemical profiles of the esterification products were characterized to support interpretation of their antibacterial effects.

2. METHODS

2.1. Materials and Instruments

Stingless bee (*Trigona laeviceps*) hive waste was obtained from a bee farm in Gunungpring, Magelang, Central Java, Indonesia. The extraction solvent was 96% ethanol (Merck, Darmstadt, Germany). The esterification reaction used methanol (Merck, Darmstadt, Germany), absolute ethanol (Merck, Darmstadt, Germany), concentrated sulfuric acid (H_2SO_4; Merck, Darmstadt, Germany), and ethyl acetate (Merck, Darmstadt, Germany). Antibacterial testing used Nutrient Agar medium (Oxoid, Hampshire, UK), 0.9% sodium chloride physiological solution (Otsuka, Tokyo, Japan), *Staphylococcus aureus* ATCC 25923, *Propionibacterium acnes* obtained from Universitas Diponegoro, Central Java, Indonesia, and ciprofloxacin (Sigma-Aldrich, St. Louis, MO, USA) as the positive control.

The main instruments used in this study included an ultrasonic bath sonicator (Branson 2510, Danbury, CT, USA), a rotary evaporator (Heidolph Laborota 4000, Schwabach, Germany), an autoclave (Hirayama HV-85, Saitama, Japan), an incubator (Memmert IN55, Schwabach, Germany), an FTIR spectrometer (Agilent Cary 630, Santa Clara, CA, USA), and a GC–MS system (Shimadzu QP 2010 SE, Kyoto, Japan).

2.2. Extraction of *Trigona laeviceps* Hive Waste

The extraction of stingless bee hive waste was performed using a sonochemical method with 96% ethanol as the solvent. A total of 100 g of hive waste was mixed with ethanol at a ratio of 1:10 w/v and extracted using an ultrasonic bath at 40 kHz for 60 min at 40°C, then macerated for 24 hours. This method was selected because acoustic cavitation can enhance solvent penetration into the sample matrix and accelerate the release of bioactive compounds compared with conventional extraction methods (Chemat et al., 2017; Suslick & Price, 1999).

The extract was filtered through Whatman No. 1 filter paper to separate the filtrate from the insoluble residue. The residue was re-extracted three times under the same conditions to maximize the recovery of secondary metabolites. The combined filtrates were concentrated using a rotary evaporator at 40–45°C, followed by further concentration in a water bath at 60°C until a viscous extract was obtained.

2.3. Esterification of *Trigona laeviceps* Hive Waste Extract

Esterification was performed using a sonochemical method to facilitate ester formation. A total of 15 g of concentrated extract was dissolved separately in 100 mL of methanol or ethanol according to the treatment group. Concentrated H_2SO_4, 2 mL, was then added as an acid catalyst. The mixture was sonicated using an ultrasonic bath at 40 kHz and 50°C for 1 h. Sonication was used to accelerate the reaction rate and improve reaction efficiency compared with conventional reflux methods (Maddikeri et al., 2012; Mason et al., 2011).

After completion of the reaction, the mixture was partitioned with ethyl acetate to separate the organic ester-containing phase from the aqueous phase containing residual acid and impurities. The ethyl acetate phase was collected and evaporated to obtain the concentrated esterification product.

2.4. Characterization of Esterification Products

The esterification products were characterized using FTIR spectroscopy to identify functional group changes associated with ester formation. The analysis focused on the appearance of the ester carbonyl absorption band around 1735–1740 cm^{-1} and changes in the –OH and C–O absorption regions. Further characterization was performed using GC–MS to identify the dominant compounds present in the esterification products, particularly fatty acid esters, phenolic derivatives, terpenoid-related compounds, and lipid constituents (Lozano-Grande et al., 2018).

2.5. Antibacterial Activity against *S. aureus* and *P. acnes*

Antibacterial activity was evaluated using the agar well-diffusion method. Bacterial suspensions of *S. aureus* and *P. acnes* were prepared and adjusted to the 0.5 McFarland standard. The bacterial suspension was inoculated evenly onto the surface of Nutrient Agar medium. Wells with a diameter of 6 mm were then prepared in each agar plate, and 5 μL of the test solutions at concentrations of 10%, 15%, and 20% w/v were added into the wells. The test samples included the crude extract, methanol ester, and ethanol ester.

Plates inoculated with *S. aureus* were incubated at 37°C for 24 h, whereas plates inoculated with *P. acnes* were incubated under anaerobic conditions at 37°C for 48 h. Ciprofloxacin was used as the positive control at 5% concentration, and dimethyl sulfoxide as the negative control at 2% concentration. Antibacterial activity was determined by measuring the clear inhibition zone using a digital caliper. Calculation of the inhibition zone by measuring the diameter of the clear zone minus the diameter of the well. The antibacterial activity test was carried out with 3 replications. The agar well-diffusion method was used because it is commonly applied for preliminary screening of antibacterial activity in natural products (Balouiri et al., 2016).

2.6. Statistical Analysis

Data were expressed as mean ± standard deviation. One-way analysis of variance was used to evaluate differences in inhibition zone diameter among concentrations within each treatment group. An independent-samples *t*-test was used to compare inhibition zone diameters between methanol ester and ethanol ester groups. Statistical significance was set at $p < 0.05$. Statistical analysis was performed using IBM SPSS Statistics 26.0 software.

3. RESULTS AND DISCUSSION

3.1. Extraction and Esterification Yield

Extraction of stingless bee (*T. laeviceps*) hive waste was performed using sonication-assisted extraction with 96% ethanol. A total of 100 g of hive waste was extracted with ethanol at a ratio of 1:10 w/v at 40°C for 1 h. Sonication was used to improve extraction efficiency through acoustic cavitation, which enhances solvent penetration into the sample matrix and facilitates the release of secondary metabolites (Chemat et al., 2017; Mason et al., 2011). After filtration, the residue was re-extracted under the same conditions to increase the recovery of extractable compounds. Repeated extraction can improve the recovery of polar and semi-polar constituents that may not be fully extracted during the first extraction cycle (Gori et al., 2021). The combined filtrates were concentrated under reduced pressure to obtain a viscous extract, with an average extraction yield of 32.76%.

Esterification of the hive waste extract using H_2SO_4 as an acid catalyst produced esterified fractions with yields of 77.72% for the methanol ester and 78.29% for the ethanol ester (**Figure 1**). The comparable yields indicate that both methanol and ethanol reacted effectively with esterifiable constituents in the extract under the sonochemical conditions used. Sonication may have facilitated ester formation by increasing molecular collision and mass transfer during the reaction (Chemat et al., 2017; Mehta et al., 2022).

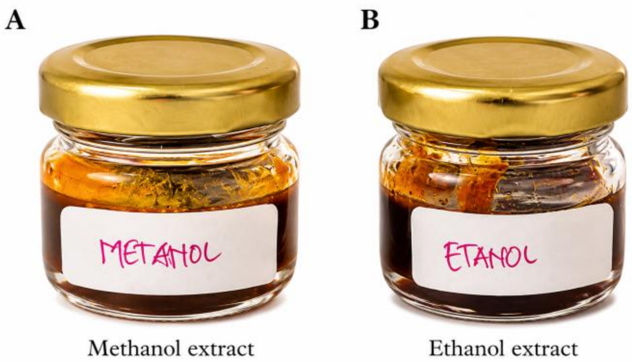


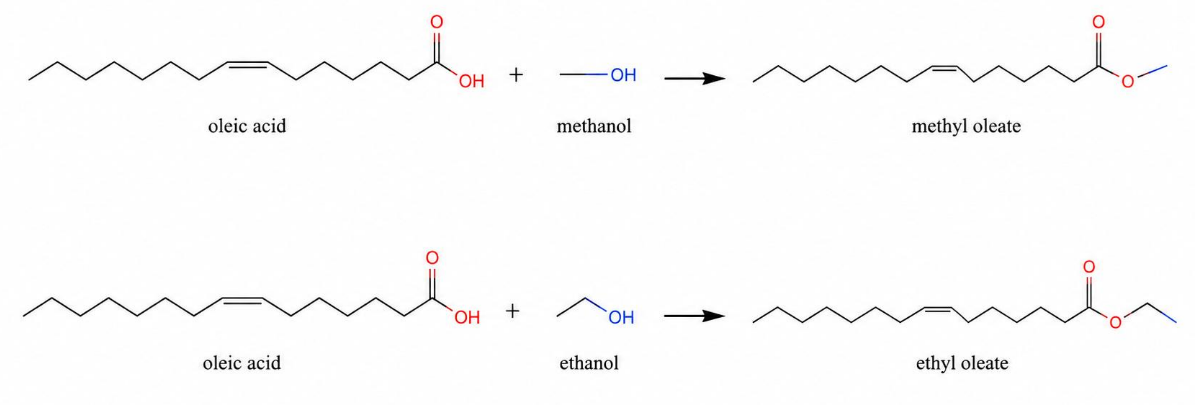
Figure 1. Results of esterification of stingless bee hive waste extract using methanol (A) and ethanol (B). The methanol and ethanol esterification products showed comparable yields, indicating that both alcohols were suitable for ester formation under the sonochemical reaction conditions.

The compounds most likely to participate in esterification include bioactive lipids containing free carboxyl groups, such as oleic and linoleic acids, as well as hydroxyl-containing constituents that may undergo structural modification. Esterification can convert free carboxyl or hydroxyl groups into ester derivatives, potentially increasing organic solvent solubility, chemical stability, and lipophilicity (Nayak & Bhushan, 2019; Wang et al., 2019). Increased lipophilicity may support interaction with bacterial membranes, although this mechanism requires further confirmation through membrane permeability or molecular assays. The proposed esterification reaction of oleic acid with methanol and ethanol is shown in **Scheme 1** as a supporting reaction scheme rather than an experimental result.

3.2. FTIR Characterization of Esterification Products

FTIR analysis was performed to identify functional group changes associated with esterification. As shown in **Figure 2**, both methanol and ethanol ester products exhibited absorption bands around 1740 cm^{-1}, corresponding to ester carbonyl stretching. Bands in the 1170–1240 cm^{-1} region were also observed and are associated with C–O stretching vibrations of ester groups. These spectral characteristics support the formation or enrichment of ester-containing compounds after esterification.

The two ester products showed generally similar FTIR profiles, although differences in peak intensity and shape were observed. The ethanol ester spectrum showed slightly stronger C–O and CH_2-related bands than the methanol ester, which may reflect the contribution of the ethyl group and its longer alkyl chain. The aliphatic C–H stretching region at 2950–2850 cm^{-1} appeared in both spectra, indicating the presence of long-chain hydrocarbon structures. Weak absorption near 3000–3010 cm^{-1} may correspond to =C–H stretching from unsaturated compounds, such as oleate derivatives or squalene, whereas the band around 720–730 cm^{-1} is consistent with long-chain CH_2 rocking vibrations (Kamairudin et al., 2021). These results are consistent with the FTIR characteristics of esterified lipid compounds and long-chain hydrocarbon constituents (Smith, 2011). However, because FTIR provides functional group information rather than definitive compound identification, GC–MS analysis was used to further characterize the chemical profile of the esterification products.



Scheme 1. Proposed esterification reaction of oleic acid with primary alcohols. Oleic acid may react with methanol or ethanol in the presence of an acid catalyst to form methyl oleate or ethyl oleate, respectively.

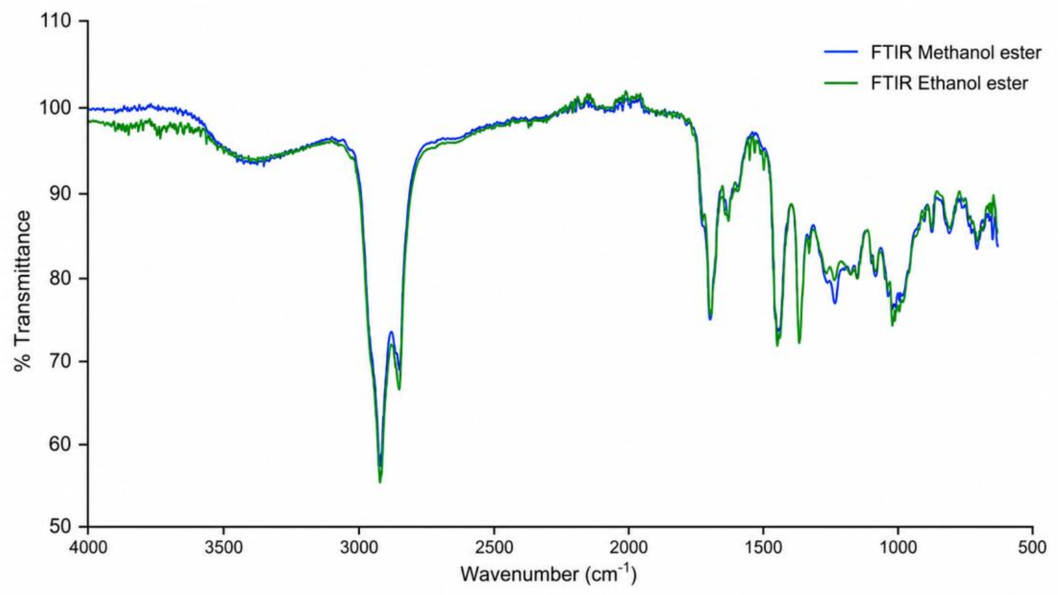


Figure 2. Comparison of FTIR spectra of methanol ester and ethanol ester. Both spectra show characteristic ester absorption bands, including C=O stretching around 1740 cm^{-1} and C–O stretching in the 1170–1240 cm^{-1} region, supporting the formation of esterified products.

3.3. GC–MS Profile of Methanol and Ethanol Esters

GC–MS analysis further supported the presence of fatty acid ester derivatives and other lipophilic compounds in both esterified fractions (**Figure 3** and **Table 1**). Methyl oleate and ethyl oleate were detected in both methanol and ethanol ester fractions, indicating the formation or presence of oleate ester derivatives after esterification. Triterpenoid- and sterol-related compounds, including squalene, lanosterol, and cycloartenol, were also detected. Cycloartenol was the dominant compound in both fractions, with relative peak areas of 49.00% in the methanol ester and 48.37% in the ethanol ester.

The detection of oleate esters is relevant because fatty acid esters have been associated with antibacterial activity through interaction with bacterial membranes and disruption of membrane integrity (Davoodbasha et al., 2018; McGaw et al., 2002). In addition, squalene, lanosterol, and cycloartenol may contribute to the biological activity of the esterified fractions because lipophilic terpenoid and sterol-like compounds can interact with microbial membranes (Nazemi et al., 2022; Pujirahayu et al., 2019). Nevertheless, the contribution of each compound to antibacterial activity cannot be determined from GC–MS data alone. Therefore, the antibacterial effects observed in this study should be interpreted as the combined activity of the esterified extract fractions rather than the effect of a single identified compound.

3.4. Antibacterial Activity against *Propionibacterium acnes*

The antibacterial activity of the crude extract, methanol ester, and ethanol ester against *P. acnes* is shown in **Figure 4** and **Table 2**. All tested samples produced measurable inhibition zones, whereas the negative control using DMSO showed no inhibition. The crude extract produced inhibition zones of 0.61 ± 0.20, 0.92 ± 0.13, and 0.99 ± 0.11 mm at concentrations of 10%, 15%, and 20%, respectively. Both esterification products produced larger inhibition zones than the crude extract, suggesting that esterification improved antibacterial activity against *P. acnes* under the test conditions.

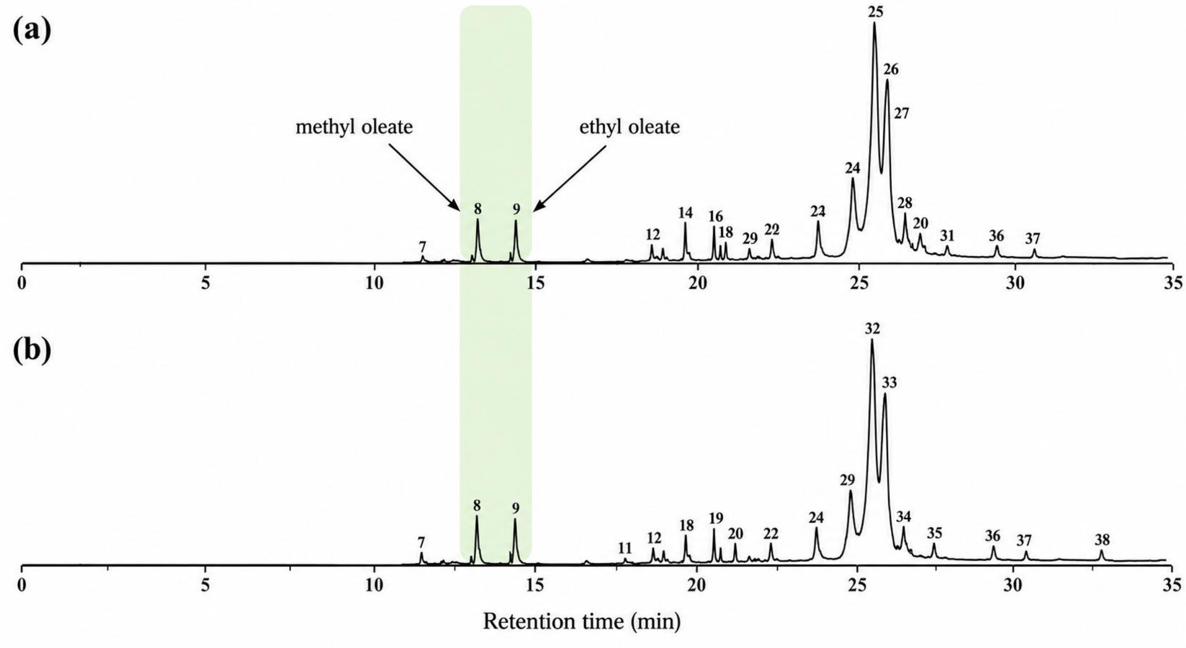


Figure 3. GC–MS chromatograms of esterification products from stingless bee hive waste extract. Chromatograms show the chemical profiles of (a) methanol ester and (b) ethanol ester fractions, indicating the presence of fatty acid esters, triterpenoid-related compounds, sterols, and long-chain hydrocarbons.

Table 1. GC–MS Profile of Compounds Detected in Methanol and Ethanol Esters from *T. laeviceps* Hive Waste Extract

Methanol Ester Peak	% Area	MW	Similarity (%)	Compound	Ethanol Ester Peak	% Area	MW	Similarity (%)	Compound
4	1.15	296	95	Methyl oleate	5	0.77	296	95	Methyl oleate
6	0.82	310	90	Ethyl oleate	7	0.84	310	90	Ethyl oleate
17	0.63	619	97	Tetratetracontane	14	0.34	304	77	Hydroginkgol
14	0.53	410	95	Squalene	18	0.42	410	95	Squalene
23	4.91	426	85	Lanosterol	19	0.35	619	96	Tetratetracontane
25	49.00	426	86	Cycloartenol	20	0.45	619	94	Tetratetracontane
26	16.82	426	93	Cycloartenol	30	5.77	426	85	Lanosterol
29	1.05	486	83	Methyl commate	31	6.67	502	81	Methyl commate
—	—	—	—	—	32	48.37	426	84	Cycloartenol

Note: Compound identification was based on GC–MS library matching and should be interpreted as tentative, particularly for compounds with lower similarity values.

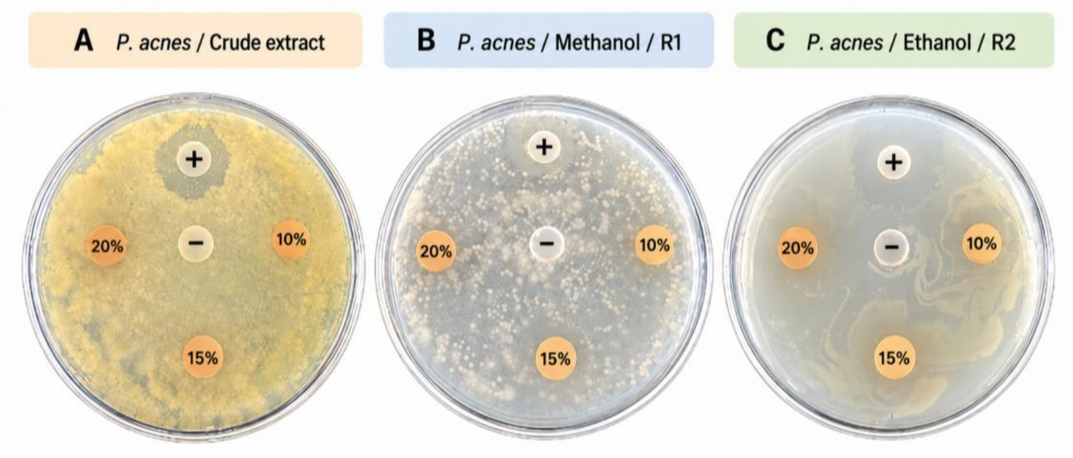


Figure 4. Growth inhibition zones of *Propionibacterium acnes* after treatment with crude extract, methanol ester, and ethanol ester of *Trigona laeviceps* hive waste extract. DMSO was used as the negative control, and ciprofloxacin was used as the positive control.

Table 2. Inhibition Zone Diameter of Crude Extract, Methanol Ester, and Ethanol Ester against *P. acnes*

Sample	Concentration	Clear Zone Diameter (mm)
Crude extract	10%	0.61 ± 0.20
	15%	0.92 ± 0.13
	20%	0.99 ± 0.11
Ethanol ester	10%	1.74 ± 0.21
	15%	1.82 ± 0.12
	20%	1.90 ± 0.10
Methanol ester	10%	1.62 ± 0.30
	15%	1.42 ± 0.08
	20%	1.41 ± 0.07
Positive control (ciprofloxacin)	—	2.31 ± 0.36
Negative control (DMSO)	—	0.00 ± 0.00

Among the esterified samples, the ethanol ester showed the highest inhibition zone against *P. acnes*, reaching 1.90 ± 0.10 mm at 20%. This value was lower than that of ciprofloxacin, 2.31 ± 0.36 mm, but higher than that of the crude extract at the same concentration. The stronger activity of the ethanol ester may be related to the formation of ethyl oleate and the presence of other lipophilic constituents, including hydroginkgol, squalene, lanosterol, and cycloartenol. Ethyl oleate may interact more effectively with lipid-rich bacterial membranes than more polar constituents, whereas alkylresorcinol-related compounds such as hydroginkgol have been associated with membrane-disruptive antimicrobial activity (Sati & Joshi, 2011; Zabolotneva et al., 2022). However, this mechanism remains inferential and should be confirmed through membrane permeability or molecular assays.

Statistical analysis showed that concentration did not significantly affect the inhibition zone diameter against *P. acnes* for either ethanol ester ($p = 0.465$) or methanol ester ($p = 0.348$). This suggests that, within the tested concentration range, increasing sample concentration did not produce a statistically significant increase in antibacterial activity. This pattern may be related to limited diffusion of lipophilic compounds in the agar medium or a plateau effect in the inhibition response. In contrast, the independent-samples *t*-test showed a significant difference between methanol ester and ethanol ester ($p = 0.0007$), indicating that ester type had a stronger influence than concentration on antibacterial activity against *P. acnes*.

3.5. Antibacterial Activity against *Staphylococcus aureus*

The antibacterial activity against *S. aureus* is presented in **Figure 5** and **Table 3**. The crude extract produced inhibition zones of 0.96 ± 0.02, 1.02 ± 0.02, and 1.45 ± 0.03 mm at concentrations of 10%, 15%, and 20%, respectively. The methanol ester produced inhibition zones ranging from 1.28 ± 0.08 to 1.65 ± 0.05 mm, whereas the ethanol ester showed the strongest activity among the tested samples, with inhibition zones of 1.75 ± 0.19, 1.90 ± 0.13, and 1.97 ± 0.24 mm at 10%, 15%, and 20%, respectively.

The ethanol ester at 20% produced the largest inhibition zone against *S. aureus*, at 1.97 ± 0.24 mm, which was numerically comparable to the positive control, 1.88 ± 0.35 mm. The ethanol ester also produced a larger inhibition zone than the methanol ester, with a difference of approximately 0.32 mm at 20%. The independent-samples *t*-test indicated a significant difference between ethanol and methanol esters ($p = 0.0003$), suggesting that ethanol esterification produced a fraction with stronger antibacterial activity against *S. aureus*. This result is consistent with the GC–MS profile, which showed the presence of ethyl oleate and other lipophilic bioactive compounds in the ethanol ester fraction.

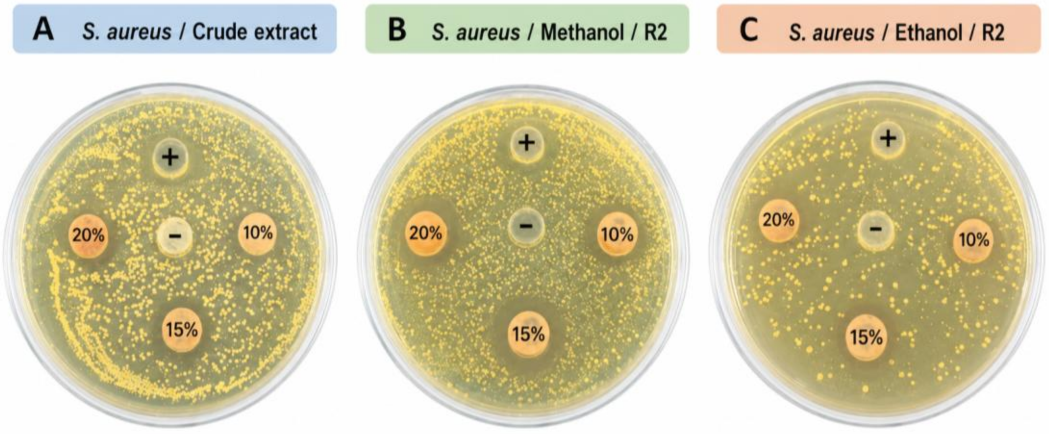


Figure 5. Growth inhibition zones of *Staphylococcus aureus* after treatment with crude extract, methanol ester, and ethanol ester of *Trigona laeviceps* hive waste extract. DMSO was used as the negative control, and ciprofloxacin was used as the positive control.

Table 3. Inhibition Zone Diameter of Crude Extract, Methanol Ester, and Ethanol Ester against *S. aureus*

Sample	Concentration	Clear Zone Diameter (mm)
Crude extract	10%	0.96 ± 0.02
	15%	1.02 ± 0.02
	20%	1.45 ± 0.03
Ethanol ester	10%	1.75 ± 0.19
	15%	1.90 ± 0.13
	20%	1.97 ± 0.24
Methanol ester	10%	1.28 ± 0.08
	15%	1.51 ± 0.13
	20%	1.65 ± 0.05
Positive control (ciprofloxacin)	—	1.88 ± 0.35
Negative control (DMSO)	—	0.00 ± 0.00

For the methanol ester, one-way ANOVA showed a significant effect of concentration on inhibition zone diameter against *S. aureus* ($p =$ 0.021), indicating that increasing concentration enhanced antibacterial activity within the tested range. However, the inhibition zones produced by methanol ester remained lower than those produced by ethanol ester. The stronger activity of the ethanol ester may be associated with increased lipophilicity, which can improve interaction with the phospholipid membrane of Gram-positive bacteria. Bioactive compounds such as phenolic derivatives, fatty acid esters, squalene, and cycloartenol may contribute to bacterial growth inhibition through disruption of cell wall and cytoplasmic membrane integrity (Echeverría, Urzúa, et al., 2017; Lobiuc et al., 2023).

Overall, the antibacterial findings indicate that esterification improved the activity of stingless bee hive waste extract against both *P. acnes* and *S. aureus*. The ethanol ester showed the most consistent antibacterial activity across the tested bacteria, particularly at 20%. However, the inhibition zones were relatively small, and the agar diffusion method may underestimate the activity of lipophilic compounds because of limited diffusion through the agar matrix. Therefore, further studies using broth microdilution, minimum inhibitory concentration, minimum bactericidal concentration, membrane permeability assays, and cytotoxicity testing are needed to confirm the antibacterial potency and safety of these esterified fractions.

4. CONCLUSION

Esterification of stingless bee (*Trigona laeviceps*) hive waste extract using methanol and ethanol produced esterified fractions containing fatty acid esters and lipophilic bioactive compounds, including methyl oleate, ethyl oleate, squalene, cycloartenol, lanosterol, and tetratetracontane, as indicated by FTIR and GC–MS analyses. Among the tested samples, the ethanol ester showed the strongest antibacterial activity, particularly at 20%, with inhibition zones of 1.97 ± 0.24 mm against *Staphylococcus aureus* and 1.90 ± 0.10 mm against *Propionibacterium acnes*. These findings suggest that ethanol-based esterification may improve the antibacterial potential of *T. laeviceps* hive waste extract, likely through increased lipophilicity and the combined activity of esterified and native bioactive constituents. However, because the antibacterial activity was evaluated only by the agar well-diffusion method and the inhibition zones were relatively small, further studies using minimum inhibitory concentration, minimum bactericidal concentration, membrane permeability assays, compound standardization, and safety evaluation are required before this material can be developed as a pharmaceutical or cosmetic antibacterial candidate.

AUTHOR CONTRIBUTIONS

Conceptualization, A.F.M. and M.S.; methodology, A.F.M. and M.S.; validation, M.S. and Y.P.; formal analysis, A.F.M. and M.S.; investigation, A.F.M. and M.S.; resources, M.S. and Y.P.; data curation, A.F.M. and M.S.; writing—original draft preparation, A.F.M. and M.S.; writing—review and editing, A.F.M., M.S., and Y.P.; visualization, A.F.M. and M.S.; supervision, M.S. and Y.P.; project administration, M.S.; funding acquisition, M.S. All authors have read and agreed to the published version of the manuscript.

INSTITUTIONAL REVIEW BOARD STATEMENT

Not applicable.

INFORMED CONSENT STATEMENT

Not applicable.

DATA AVAILABILITY STATEMENT

Data supporting the findings of this study are available upon reasonable request from the corresponding author.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

ROLE OF FUNDERS

The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

DECLARATION OF GENERATIVE ARTIFICIAL INTELLIGENCE (AI) USE

During the preparation of this manuscript, the authors used ChatGPT (OpenAI) to assist in improving the clarity, structure, grammar, and readability of the text. After using this tool, the authors thoroughly reviewed, edited, and verified the entire content to ensure that it accurately represents their own ideas, data, analyses, and interpretations. The authors take full responsibility for the integrity, accuracy, and originality of the published work.

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