Medical and Health Journal
Volume 5, Issue 1, 2025, pp. 71-76
e-ISSN: 2807-3541
Open Access: https://jos.unsoed.ac.id/index.php/mhj

Comparative Antibacterial Effects of Honey Bee Propolis on Gram Negative Bacteria *Escherichia coli* and Gram Positive Bacteria *Staphylococcus aureus*

Rani Afifah Nur Hestiyani[1*], Tri Okmawati Handini[2], Galuh Yulieta Nitihapsari[2], Anriani Puspita Karunia Ning Widhi[1], Wahyudin[3]

[1]Department of Microbiology, Faculty of Medicine, Jenderal Soedirman University , Purwokerto, Indonesia
[2]Faculty of Medicine, Jenderal Soedirman University , Purwokerto, Indonesia
[3]Department of Pharmacology, Faculty of Medicine, Jenderal Soedirman University , Purwokerto, Indonesia

ARTICLE INFO

Article history:
Received April 28, 2025
Revised June 3, 2025
Accepted July 11, 2025
Available online August 2, 2025

Keywords:
Antibacterial, *Escherichia coli*, propolis, *Staphylococcus aureus*

ABSTRAK

Antimicrobial resistance in pathogens such as Escherichia coli and Staphylococcus aureus poses an urgent global health challenge. Honeybee propolis is known for its promising antibacterial potential.This study aimed to compare the antibacterial effectiveness of propolis against E. coli (Gram-negative) and S. aureus (Gram-positive) using the in vitro disk diffusion method. Propolis was tested at concentrations of 6.25%, 12.5%, 25%, 50%, and 100% against both bacterial species. Disk diffusion assays were conducted on Mueller Hinton Agar, and inhibition zones were measured using a vernier caliper. Data were analyzed using one-way ANOVA. The results showed the Inhibition zone diameters increased with higher propolis concentrations. At 100% concentration, S. aureus showed a 15 mm inhibition zone, while E. coli showed only 7 mm. One-way ANOVA indicated significant differences ($p < 0.05$) in both bacteria. These findings indicate that propolis exhibits antibacterial activity against both S. aureus and E. coli, with greater effectiveness against Gram-positive S. aureus. These findings support the potential use of propolis as a natural antibacterial agent, particularly for Gram-positive infections.

1. INTRODUCTION

Antimicrobial resistance (AMR) continues to threaten the efficacy of antibiotic therapies worldwide, particularly against common pathogens such as *Escherichia coli* and *Staphylococcus aureus*. Conventional antibiotics are losing effectiveness due to increasing resistance, creating an urgent need for alternative therapeutic agents. Among the promising candidates is propolis, a natural resinous product collected by honeybees, which has long been used in traditional medicine. *Escherichia coli* and *Staphylococcus aureus* are among the most frequently implicated bacterial pathogens in community and hospital-acquired infections. *E. coli*, a Gram-negative rod-shaped, is associated with gastrointestinal and urinary tract infections, while *S. aureus*, a Gram-positive coccus, is a leading cause of skin infections, pneumonia, and bacteremia. The resistance of these bacteria to multiple classes of antibiotics exacerbates treatment difficulties and increases the risk of complications and mortality (WHO, 2022).

The structural differences between *Escherichia coli* (Gram negative) and *Staphylococcus aureus* (Gram-positive)—particularly the outer membrane of Gram-negative bacteria, which functions as a permeability barrier—can significantly influence their susceptibility to antibacterial agents. Therefore, comparing the antibacterial effects of propolis on these two distinct bacterial groups is essential for understanding its therapeutic potential (Sa-eed et al., 2023; Hossain et al., 2022;). Propolis is a resinous substance produced by honeybees (*Apis mellifera*) from plant-derived resins mixed with bee enzymes. Propolis has long been used in traditional medicine and is increasingly recognized for its antibacterial, antifungal, anti-

*Corresponding author
E-mail addresses: rani.hestiyanti@gmail.com (Rani Afifah Nur Hestiyani)

inflammatory, and antioxidant properties. The composition of propolis is influenced by various factors, including the botanical origin, time of harvest, local environment, the floral sources available to bees, climatic variations, and the species of honeybees collecting it (Hossain et al., 2022). Numerous in vitro studies have demonstrated that propolis can inhibit the growth of various Gram-positive and Gram-negative bacteria, although its efficacy varies depending on bacterial structure and strain, seasonal and regional factors of propolis (Almuhayawi, 2020; Agustin et al., 2022, Purnama et al., 2024).

The chemical composition of propolis, which includes flavonoids, phenolic acids, terpenoids, and aromatic esters, is largely responsible for its antimicrobial activity. These compounds can disrupt bacterial cell walls and membranes, inhibit protein synthesis, and impair nucleic acid replication. The antimicrobial effectiveness of propolis is influenced by various factors, such as the diversity of bioactive compounds it contains, extraction methods, concentration used, harvesting season and location, as well as the bee species producing it. The various crude propolis extracts frequently produced zones of inhibition against Gram positive bacteria *Staphylococcus aureus* than Gram negative bacteria *Pseudomonas aeruginosa*, and *Escherichia coli* test isolates.

Despite growing evidence of propolis' antimicrobial potential, direct comparative studies examining its effects on both Gram-negative and Gram-positive pathogens under controlled conditions remain limited. Therefore, this study aimed to answer the question: "Is there a difference in the antibacterial effectiveness of propolis against Gram-positive and Gram-negative bacteria?". A better understanding of these effects may support the development of propolis-based therapeutics as a complementary strategy in managing infections and mitigating antibiotic resistance.

2. METHOD

This study was conducted using a observational laboratory design (in vitro) and applied the disk diffusion method to evaluate the antibacterial effects of honeybee propolis against Gram-negative *Escherichia coli* and Gram-positive *Staphylococcus aureus*. The experimental design consisted of six treatment groups with five replications each, based on the following propolis concentrations: 6.25%, 12.5%, 25%, 50%, 100%, and a negative control (distilled water). The population and sample in this study were Gram-negative *Escherichia coli* and Gram-positive *Staphylococcus aureus* bacteria. The preparation of Gram-negative *Escherichia coli* and Gram-positive *Staphylococcus aureus* isolates and as well as the antibacterial testing of honeybee propolis was conducted at the Microbiology Laboratory of the Faculty of Medicine, Universitas Jenderal Soedirman.

The propolis used in this study was a commercially available British Propolis brand, known to contain flavonoid levels up to ten times higher than regular propolis, laboratory stock isolates of *Escherichia coli* (Gram-negative) and *Staphylococcus aureus* (Gram-positive) obtained from sepsis patients, 0.5 McFarland standard, Mueller Hinton Agar (MHA), MacConkey agar, Mannitol Salt Agar (MSA), set Gram staining, IMViC test media, H_2O_2 3%, Test latex (Scientific™ staphaurex™ Latex agglutination Test), disposable reaction cards , sterile NaCl solution, and sterile paper discs. **Equipment used:** petri dishes, Olympus light microscope, Memmert autoclave, Memmert incubator (37°C), refrigerator, glass slides, dropper pipettes, test tubes, test tube rack, 5 ml tubes, hot plate, magnetic stirrer, forceps, and vernier caliper.

Research Procedure

The honeybee propolis from the UK (commercial brand) was diluted into five different concentrations: 6.25%, 12.5%, 25%, 50%, and 100%, with distilled water (aquadest) used as a negative control. Laboratory stock isolates of *E. coli* and *S. aureus* were revived and confirmed. The *E. coli* isolate was cultured on MacConkey agar, while *S. aureus* was grown on MSA. Both

cultures were incubated at 37°C for 24 hours. Colony morphology was observed, and Gram staining was performed to confirm Gram reaction and cell shape. Further identification included:For *E. coli*: biochemical tests such as TSIA and IMViC (Indole, Methyl Red, Voges-Proskauer, and Simmon's Citrate tests). For *S. aureus*: catalase and coagulase tests. Confirmed colonies were then used for antibacterial testing against honeybee propolis. Antibacterial Activity Test using Disk Diffusion Method (Abouzeed *et al.,* 2013). The antibacterial activity was tested using the Kirby-Bauer disk diffusion method. Solidified MHA plates were prepared. Bacterial suspensions were adjusted to match the 0.5 McFarland standard (approximately 1×10^8 CFU/mL) in 5 ml of sterile NaCl. A sterile cotton swab was dipped into the bacterial suspension (*E. coli* or *S. aureus*), then inoculated onto MHA plates and allowed to dry for 5 minutes. Sterile paper discs were soaked in the respective concentrations of honeybee propolis, drained until no excess liquid remained, and allowed to stand for 30 minutes to allow absorption. The discs were then placed onto the surface of MHA plates previously inoculated with bacteria and incubated at 37°C for 18-24 hours. The clear zones of inhibition around the discs were then observed and measured using a ruler and vernier caliper.

Data Collection Technique

The diameter of the inhibition zones was measured using a vernier caliper in millimeters. Measurements were taken for each treatment replicate on the test plates. The antibacterial activity results were analyzed using one-way ANOVA to determine the significance of differences in inhibition zone diameters among various propolis concentrations.

3. RESULT AND DISCUSSION

Result

This study aimed to compare the antibacterial effects of honeybee propolis against two pathogenic bacteria, *Staphylococcus aureus* and *Escherichia coli*. The results showed that *Staphylococcus aureus* exhibited yellow colonies with a golden-yellow zone around them on Mannitol Salt Agar (MSA), indicating mannitol fermentation. Microscopically, the bacteria were Gram-positive, coccoid in shape, and arranged in clusters resembling grapes. The biochemical test showed a positive catalase result. On the other hand, *Escherichia coli* grew on McConkey Agar with pink colonies, indicating it is a non-lactose fermenter. Microscopically, the bacteria were gram-negative and rod-shaped. The IMVIC biochemical tests showed positive results for Indole and Methyl Red, and negative results for Voges-Proskauer and Citrate, which is a typical profile for *E. coli* (Table I).

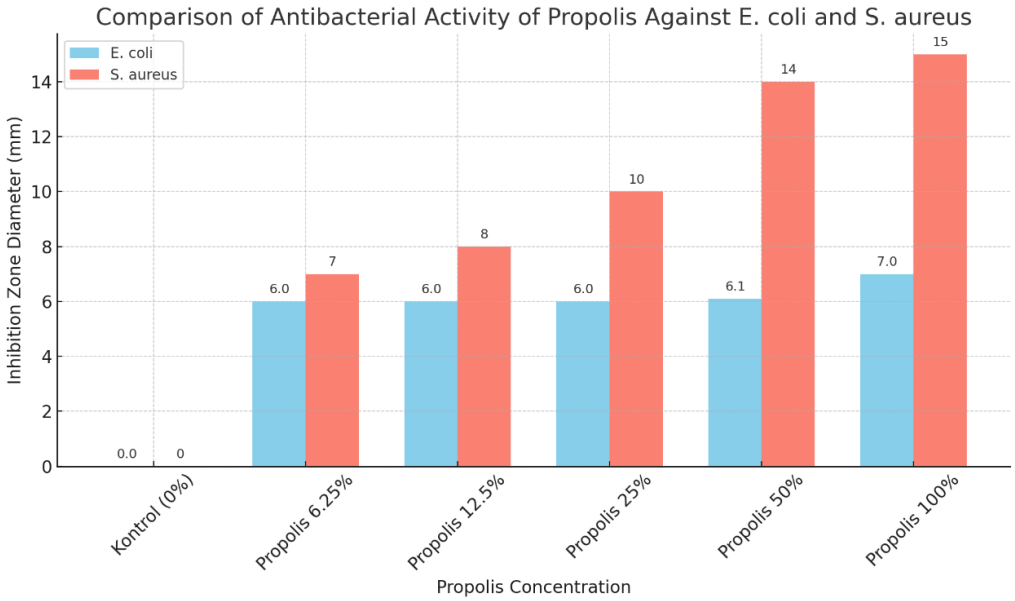
Table 1. Colony Characteristics, Cell Morphology, and Biochemical Results of *S. aureus* and *E. coli* Isolates

No	Isolate	Colony Characteristics	Cell Characteristics	Biochemical Test
1	*Staphylococcus aureus*	Grown on **Mannitol Salt Agar (MSA)**: Fermentation of mannitol changes the medium color from red to yellow, yellow colonies with a golden-yellow zone around them.	Gram-positive, coccus shape, arranged in clusters like grapes.	Catalase positive.
2	*Escherichia coli*	Grown on **McConkey Agar**: Non-fermenter, pink color on the medium.	Gram-negative, rod-shaped.	IMVIC tests : Indole positive Methyl Red positive, Voges-Proskauer negative, Citrate test negative

In the antimicrobial susceptibility testing, propolis extract showed increasing inhibitory effects with higher concentrations of the extract. At 6.25%, the inhibition zone diameters were 7 mm for *Staphylococcus aureus* and 6 mm for *Escherichia coli*, while at 100% concentration, the inhibition zone increased to 15 mm for *S. aureus* and 7 mm for *E. coli* (Table II and Picture 1). The results indicate that propolis extract has a greater antimicrobial effect on *Staphylococcus aureus* compared to *Escherichia coli*. Although both bacteria showed significant inhibition zones at higher concentrations, *Staphylococcus aureus* was more susceptible to the propolis extract at various concentrations.

Table 2. The average diameter of the inhibition zones for each concentration treatment against the Gram-negative bacterium *Escherichia coli* and the Gram-positive bacterium *Staphylococcus aureus.*

No	Concentration	Average inhibition zones diameter (mm)	
		Escherichia coli	*Staphylococcus aureus*
1	Negative control (0%)	0	0
2	Propolis 6.25%	6	7
3	Propolis 12.5%	6	8
4	Propolis 25%	6	10
5	Propolis 50%	6.1	14
6	Propolis 100%	7	15



Picture 1. Comparison of Antibacterial Activity of Propolis Against *Escherichia coli* and *Staphylococcus aureus*

Table 3 presents the mean inhibition zone diameters (in mm) of honeybee propolis at varying concentrations against *Escherichia coli* and *Staphylococcus aureus*, along with the results of one-way ANOVA analysis.

Table 3. One-way ANOVA Results of Inhibition Zone Diameters

Bacteria	p-value	Interpretation
Escherichia coli	1.82×10^{-34}	Significant difference ($p < 0.05$)
Staphylococcus aureus	0.0	Extremely significant ($p < 0.05$)

The results of this study demonstrated a clear dose-dependent antibacterial effect of propolis extract against *Staphylococcus aureus* and *Escherichia coli*. Increasing concentrations of propolis were associated with larger inhibition zones for both bacterial species. One-way ANOVA revealed statistically significant differences in inhibition zone diameters across propolis concentrations for both bacteria. For *E. coli*, the p value = 1.82×10^{-34}), while for *S. aureus*, the p value was infinitely large (p = 0.0), confirming highly significant treatment effects ($p < 0.05$).

Discussion

This study aimed to compare the antibacterial effects of honeybee propolis against *Staphylococcus aureus* and *Escherichia coli*, representing Gram-positive and Gram-negative pathogenic bacteria, respectively. The results clearly demonstrate that propolis exhibits a dose-dependent antibacterial effect, with greater efficacy observed against *S. aureus* than *E. coli*. The data affirmatively confirm this, with larger inhibition zones formed against *S. aureus* across all tested concentrations. Propolis exhibited stronger antibacterial activity against *S. aureus* than *E. coli*. At a concentration of 6.25%, the inhibition zones were 7 mm and 6 mm, respectively. At 100%, the inhibition zone for *S. aureus* reached 15 mm, while *E. coli* only showed a 7 mm zone.

The differential activity can be attributed to structural differences between Gram-positive and Gram-negative bacteria cell. The structural differences in bacterial cell walls as the key factor influencing susceptibility to propolis. *S. aureus*, being Gram-positive, has a thick peptidoglycan layer but lacks the outer membrane that characterizes Gram-negative bacteria like *E. coli*. This outer membrane serves as a selective permeability barrier, limiting the entry of hydrophobic molecules such as flavonoids and phenolic acids—the main antibacterial constituents of propolis. Consequently, *E. coli* demonstrates reduced susceptibility, as supported by the consistently smaller inhibition zones across all propolis concentrations. *S. aureus*, a Gram-positive bacterium, has a thick peptidoglycan layer that is more susceptible to the action of bioactive compounds in propolis, such as flavonoids (pinocembrin, galangin, chrysin) and phenolic acids (e.g., caffeic acid phenethyl ester). In contrast, the outer membrane of *E. coli*, a Gram-negative bacterium, acts as a permeability barrier that restricts the entry of many hydrophobic antimicrobial agents (Almuhayawi, 2020; Torres et al., 2020; Sa-eed et al., 2023).

Previous studies have similarly reported greater efficacy of propolis against Gram-positive bacteria. Bouzahouane et al. (2021) observed that Algerian propolis was more effective against *S. aureus* than *E. coli*. Sinaga et al. (2024) also reported larger inhibition zones for *S. aureus*, despite having identical MIC values for both bacteria. These congruent findings strengthen the validity of the current results and confirm that Gram classification plays a critical role in determining antibacterial susceptibility to propolis.

The effectiveness of propolis is influenced not only by bacterial characteristics but also by propolis-specific variables, including botanical origin, geographic region, seasonal factors, and extraction techniques which affect its phytochemical composition (Hossain et al., 2022). The propolis used in this study, sourced from the United Kingdom, may have a unique phytochemical profile that contributed to its selective antibacterial potency. Additionally, the method of extraction plays a crucial role in determining the efficacy of propolis. Future studies should investigate alternative extraction techniques and solvents to enhance its activity, particularly against Gram-negative bacteria. From a theoretical standpoint, the results reinforce the selective permeability barrier model, where Gram-negative bacteria inherently resist many bioactive compounds due to their outer membrane. However, the limited activity of propolis against *E. coli* also suggests that formulation enhancement (e.g., nanoparticle delivery, synergistic blending with permeabilizers or antibiotics) may be necessary to overcome this barrier. Therefore, these findings could contribute to modifying existing antibacterial theories by proposing that the efficacy of natural agents like propolis can be expanded through combinatorial or formulation-based strategies.

Moreover, evaluating the synergistic effects between propolis and standard antibiotics could provide promising strategies to overcome antimicrobial resistance. Such combinations may improve efficacy against both Gram-positive and Gram-negative pathogens. Despite the promising results, this study has limitations. The absence of a standard antibiotic control limits comparison with conventional treatments. Furthermore, minimum inhibitory concentrations (MICs) were not determined, and potential synergistic effects with antibiotics were not explored.

In summary, propolis shows significant antimicrobial potential, particularly against Gram-positive bacteria like *S. aureus*. However, its limited effect on Gram-negative bacteria such as *E. coli* highlights the need for further research into optimizing its formulation, extraction, and potential combination with other agents. These efforts are essential to support the clinical development of propolis-based antibacterial therapies.

4. CONCLUSION

This study demonstrates that honeybee propolis exhibits antibacterial activity against both Gram-positive *Staphylococcus aureus* and Gram-negative *Escherichia coli*, with a more pronounced effect on *S. aureus*. The inhibition zones increased with higher propolis concentrations, indicating a dose-dependent response. The greater susceptibility of *S. aureus* is likely due to differences in cell wall structure between Gram-positive and Gram-negative bacteria. These findings support the potential of propolis as a natural antibacterial agent, particularly against Gram-positive pathogens. Further research is needed to optimize propolis formulations for broader antimicrobial applications and to better understand its mechanisms of action.

5. REFERENCES

Abouzeed, Y.M., Elfahem, A., Zgheel, F. & Ahmed, M.O. (2013). Antibacterial In-Vitro Activities Of Selected Medicinal Plants Against Methicillin Resistant *Staphylococcus aureus* from Libyan environment. *Journal Environmental & Analytical Toxicology*, 3(6), 1-10.

Agustin, A.N., Roswati, R., Ruhdiana, T., Suci, S.E., Indriyani, W. & Gunarti, N.S. (2022). Potensi Propolis sebagai Antibakteri: Review jurnal. *Jurnal Buana Farma,* 2(4), 17–21.

Almuhayawi, M.S. (2020). Propolis as a Novel Antibacterial Agent. *Saudi Journal of Biological Sciences,* 27(11), 3079-3086.

Bouchelaghem, S. (2022). Propolis Characterization And Antimicrobial Activities Against *Staphylococcus aureus* and *Candida albicans*: A review. *Saudi Journal of Biological Sciences,* 29(4), 1936-1946.

Gunarti, N.S., et al. (2022). Potensi Propolis Sebagai Antibakteri: Review jurnal. *Jurnal Buana Farma,* 2(4), 17–21.

Hossain R, Quispe C, Khan RA, Saikat ASM, Ray P, et al. (2022). Propolis: An Update On Its Chemistry And Pharmacological Applications. *Chin Med,* 17,100.

Inggas, Made Agus M., et al. Biokimia, Biologi Molekuler Dan Aplikasinya Dalam Bidang Kesehatan. Mafy Media Literasi Indonesia Publisher, 2024, doi:10.1112/kakinaan1132.

Purnama, F.M., Hestiyani, R.A.N., Widhi, A.P.K.N. and Wahyudin, W. (2024). Aktivitas Antibiofilm Propolis Lebah Madu Terhadap Bakteri *Pseudomonas aeruginosa*. *Mandala Of Healt,h 17*(2), 308-317.

Inggas, Made Agus M., et al. Dasar-Dasar Kimia Untuk Ilmu Biomedis. Mafy Media Literasi Indonesia Publisher, 2025, doi:10.1112/kakinaan1144.

Sa-eed, A., Donkor, E.S., Arhin, R.E., Tetteh-Quarcoo, P.B., Attah, et al. (2023). In Vitro Antimicrobial Activity Of Crude Propolis Extracts And Fractions. *FEMS Microbe,* 4,1–8.

Sinaga, R., et al. (2024). Antibacterial activity of propolis from Trigona bees against *S. aureus* and *E. coli*. *Bali Medical Journal*.

Torres, E., et al. (2020). Resistance Mechanisms In *E. Coli* Against Natural Antimicrobial Compounds. PubMed.

World Health Organization (2022). WHO Global Report on Antimicrobial Resistance Surveillance.