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Antibacterial activity of Randu Honey against some bacterial pathogens using agar well diffusion method

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Abstract

Background: Honey's antibacterial and other therapeutic qualities have long been recognized because of its bioactive components, which include hydrogen peroxide, flavonoids, and phenolics. Randu honey is a monofloral honey that is made from the nectar of *Ceiba pentandra* and has the ability to suppress Gram-positive as well as Gram-negative bacteria. Because antibiotic resistance is on the rise, it is crucial to investigate alternative treatments like honey.
Method: This study evaluated the antibacterial activity of randu honey against *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* using the agar well diffusion method. Honey dilutions (100%–10% w/v) were prepared and incubated at 37°C for 24 hours. The resulting inhibition zones were measured to determine the Minimum Inhibitory Concentration (MIC).
Results: The antibacterial activity of randu honey increased with concentration. *Staphylococcus aureus* showed the highest sensitivity with an inhibition zone of 9.02 ± 0.67 mm at 100%, classified as moderate at 70%–100% concentrations (MIC 20%). *Escherichia coli* exhibited lower sensitivity, with inhibition zones ranging from 7.07 ± 0.56 mm at 100% to 0.18 ± 0.01 mm at 30%, categorized as moderate at 80%–100% (MIC 30%). For *Pseudomonas aeruginosa*, moderate activity was observed at 80%–100%, with inhibition zones up to 8.16 ± 0.13 mm at 100% (MIC 30%).
Conclusion: Randu honey shows promising antibacterial activity, especially against Staphylococcus aureus, with concentration-dependent effects. Its moderate activity at higher concentrations highlights its potential as a natural antibacterial agent.

Keywords: Antibacterial Activity; Randu Honey; *Ceiba pentandra*; Minimum Inhibitory Concentration; Diffusion Method

INTRODUCTION

Since ancient times, honey has been recognized as a natural product with a variety of therapeutic benefits in addition to its use as a sweetener (1). Because of honey's unique flavor and scent, complex chemical makeup, and health advantages, its use has grown in recent years. Indonesia's total honey output in 2023 was 21,392.00 liters or 9,430.62 tons. The country's per capita honey consumption ranges from 40 to 60 grams annually. Indonesia's primary honey sources are the Java Islands and Sumatra (2).

The natural liquid known as honey is often sweet and is made by bees from plant nectar (floral nectar) or other plant parts (extrafloral) (3). Blossom honey and honeydew honey are the two primary types into which honey is often divided. The nectar of flowers that bees gather is known as blossom honey. Blossom honey is further divided into two types: multifloral honey, which is made from nectar collected from a variety of plant species, and monofloral honey, which is mostly produced from a single plant species, such as randu, longan, acacia, or rambutan honey (4). However, honeydew honey is made from the sweet secretions of insects like aphids that consume plant sap rather than flower nectar. This sweet liquid is collected by bees then turn it into honey (5).

Randu honey is a type of monofloral honey derived from the nectar of the *Ceiba pentandra* tree, commonly known as the kapok or silk cotton tree. This tree is widely

found in tropical and subtropical regions, including Indonesia, where it blooms seasonally and serves as an abundant source of nectar for bees. Randu honey is distinguished by its extremely thick consistency and yellow-brown hue (6). Honey's bioactive ingredients effectively heal microbial illnesses and shield people from the possible negative effects of conventional antibiotics (7). It is believed that honey's Hydrogen Peroxide (H_2O_2) content, high osmolarity, low pH, phenolic and flavonoid components (8), methylglyoxal, and bee defensin-1 peptide (9) are what give it its antibacterial properties. Research has shown that randu honey has antibacterial properties against a range of pathogens, suggesting that it could be used as a natural remedy for bacterial illnesses. Randu honey shown strong antibacterial action against *Candida albicans, Escherichia coli, Staphylococcus aureus*, and *methicillin-resistant Staphylococcus aureus*. The Minimum Inhibitory Concentration (MIC) was 3.12% to 25%, while the inhibition zone widths varied from 14.66 ± 0.52 mm to 27.86 ± 0.43 mm (10). The MIC of randu honey against *Bacillus subtilis* was 35%, which was regarded as the Limit of Detection (LOD) (11). While randu honey suppresses the growth of *Staphylococcus aureus* and *Staphylococcus epidermidis*, variations were noted. The MIC concentrations for *Staphylococcus epidermidis* and *Staphylococcus aureus* were 40 µL (0.67 mm ± 1.15) and 80 µL (2.67 mm ± 4.62) respectively (12). Honey's distinct chemical compound makes it a promising Complementary and Alternative Medicine (CAM) option for treating infections because it has antibacterial qualities against harmful bacteria (9,13).

There are several methods available for testing antibacterial activity, such as diffusion methods, dilution methods, bioautography, time-kill curve analysis, ATP bioluminescence, and flow cytometry (14). Among these, the agar diffusion method is among the most often used methods. Despite being labour-intensive and time-consuming, the agar diffusion method is still considered the gold standard for phenotypic analysis (15). The disc diffusion method has a number of benefits over other approaches, such as simplicity, affordability, the capacity to test numerous antimicrobial compounds at once, and ease of interpreting the results. Furthermore, the MIC can also be found using this method. The gradient of antimicrobial activity in the inhibition zone or adding different doses of the test chemical to the agar are two methods used to determine MIC (14). This quantitative approach complements the qualitative antibiogram results and provides crucial information about the lowest concentration of the compound that inhibits visible bacterial growth (15).

Staphylococcus aureus is a well-known pathogen responsible for a variety of infections, including skin and soft tissue infections as well as nosocomial infections in humans (16). *Escherichia coli* is a natural component of mammalian gut flora, some strains benefit humans by preventing pathogenic colonization in the digestive system. However, certain pathogenic strains can cause foodborne illnesses and diarrhea, posing significant health risks (17). *Pseudomonas aeruginosa* is classified as an opportunistic pathogen, capable of causing a wide range of infections, particularly in individuals with compromised immune systems (18).

The increasing prevalence of antibiotic resistance underscores the urgent need to explore alternative therapeutic agents. Honey, with its complex bioactive composition, has shown significant promise as a natural antimicrobial. However, research on the antibacterial potential of specific monofloral honeys, such as randu honey derived from *Ceiba pentandra* remains limited, particularly in its application against Gram-positive (*Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*). By assessing randu honey's antibacterial activity using the agar well diffusion method and calculating its Minimum Inhibitory Concentration (MIC), this work fills this research gap. The focus on randu honey's bioactivity against several bacterial strains and its prospective use as a natural antibacterial agent is what makes this study innovative. The results are intended to assist sustainable honey production in

Indonesia, offer fresh scientific understanding of the medicinal uses of randu honey, and enhance public health.

MATERIALS AND METHOD

Materials

The materials used include: *Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 8739, and *Pseudomonas aeruginosa* ATCC 9027 sourced from the Testing Services Unit Universitas Airlangga (Surabaya, East Java, Indonesia), Mueller Hinton Agar (Oxoid), Randu Honey An-Nikmah (Blitar, East Java, Indonesia), sterile 0.9% Sodium Chloride infusion (Otsuka), sterile Water for Irrigation (Otsuka), Kanamycin Sulphate powder (Meiji), and paper filter.

Method

Antibacterial activity test using Agar Well Diffusion Method or Kirby-Bauer Assay (11,19).

a. Preparation of Mueller Hinton Agar (MHA) Media

Assays for antibacterial activity and bacterial regeneration were conducted using MHA. In all, 38 grams of MHA powder were mixed with 1000 mL of distilled water and boiled until the powder was fully dissolved. The medium was autoclaved for 15 minutes at 121°C to sterilize it.

b. Sterilization of Equipment

All equipment was wrapped in parchment paper and sterilized using an autoclave at 121°C for 15 minutes.

c. Bacterial Culture Rejuvenation

Bacterial cultures (second passage) were rejuvenated by streaking a loopful of *Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 8739, and *Pseudomonas aeruginosa* ATCC 9027 aseptically onto slanted MHA media in test tubes. The test tubes were sealed with plastic wrap and incubated at 37°C for 24 hours in an incubator.

d. Preparation of Bacterial Suspension

Approximately 10 mL of sterile 0.9% saline solution was poured into the slanted MHA media containing rejuvenated bacterial cultures *Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 8739, and *Pseudomonas aeruginosa* ATCC 9027 aseptically. The mixture was vortexed until the bacterial colonies were fully suspended. The turbidity of the suspension was adjusted to the McFarland standard of 0.5 equivalent to a bacterial concentration of 1.5×10^8 CFU/mL, by measuring the percent transmittance (25% T) using a UV-Vis spectrophotometer at a wavelength of 580 nm.

e. Preparation of Positive and Negative Controls

The positive control used was 500 µg/mL Kanamycin Sulphate solution. While the negative control used was sterile Water for Irrigation.

f. Preparation of Test Solutions

To create a stock solution with a 100% (w/v) concentration, 5 grams of randu honey were dispersed in 5 mL of sterilized water and then filtered through paper filter. Following that, the stock solution was diluted to 90%, 80%, 70%, 60%, 50%, 40%, 30%, 20%, and 10% (w/v) concentrations.

g. Antibacterial Activity Assay

The antibacterial activity was tested using the agar well diffusion method or Kirby-Bauer assay. The testing was conducted aseptically within a Biological Safety Cabinet (BSC) to ensure sterility and prevent contamination.

Approximately 30 mL of sterile MHA media was first added to sterile petri dishes as the base layer, and the mixture was let to harden. The seed layer was then made by combining 10 µL of the bacterial culture with 20 mL of sterile MHA medium. After being homogenized by vortexing, this mixture was put on top of the base layer that had set. Ten test solution concentrations, one positive

control, and one negative control were included in the well pattern that was created on the bottom of the petri dishes once the MHA media had completely set. To make wells, the media was perforated using a sterile cork-borer (8 mm) in accordance with the pattern. To create clean wells, the perforated MHA medium was carefully removed.

Then, using a sterile micropipette, 100 µL of the test honey solutions at different concentrations, positive control, and negative control were added to the matching wells. The petri dishes were incubated at 37°C for 24 hours after all the wells were filled in order to facilitate contact between the test solutions and the bacteria on the agar media. After the incubation time, a close examination was conducted of the inhibition zones that formed surrounding each well. Using the following formula, the diameter of the inhibitory zones was measured with a calliper:

$$\text{Diameter of the Inhibition Zone} = \left(\frac{D_v + D_h}{2}\right) - D_w$$

(D_v): Vertical Diameter (mm)
(D_h): Horizontal Diameter (mm)
(D_w): Well Diameter (8 mm)

The MIC is the concentration at which no inhibition zone is seen. This concentration is the smallest quantity of the test material needed to totally stop the bacterial strain's apparent growth in the experimental setup.

RESULT

The agar well diffusion method was used to assess randu honey's antibacterial activity and ascertain its MIC against *Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 8739, and *Pseudomonas aeruginosa* ATCC 9027. The findings show the diameter of the inhibitory zones created by randu honey at various concentrations, as shown in **Table 1** and **Figure 1**.

Table 1. Inhibition zones of Randu Honey against *Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 8739, and *Pseudomonas aeruginosa* ATCC 9027

Conc. (%w/v)	Average Diameter of Inhibition zone (mm) ± SD		
	S. aureus	***E. coli***	***P. aeruginosa***
100%	9.02 ± 0.67	7.07 ± 0.56	8.16 ± 0.13
90%	7. 90 ± 0.70	6.36 ± 0.13	7.30 ± 0.05
80%	6.90 ± 0.47	5.33 ± 0.14	6.23 ± 0.06
70%	5.83 ± 0.57	4.27 ± 0.19	5.18 ± 0.02
60%	4.97 ± 0.56	3.28 ± 0.20	4.39 ± 0.19
50%	3.80 ± 0.61	2.33 ± 0.18	3.33 ± 0.16
40%	2.98 ± 0.70	1.37 ± 0.08	2.25 ± 0.20
30%	1.87 ± 0.38	0.18 ± 0.01	1.11 ± 0.17
20%	1.04 ± 0.54	-	-
10%	-	-	-
C+	17.61 ± 0.43	16.39 ± 0.19	15.75 ± 0.50
C-	-	-	-

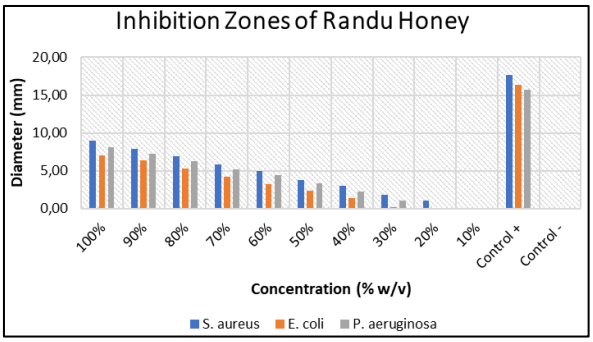

Figure 1. Inhibition zones of Randu Honey against *Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 8739, and *Pseudomonas aeruginosa* ATCC 9027

DISCUSSION

The disc or well diffusion method on agar is widely used in clinical microbiology for routine antimicrobial susceptibility testing. Mueller-Hinton Agar (MHA) is preferred for nonfastidious bacteria due to its nonselective nature, ability to absorb bacterial toxins, and enhanced diffusion of antimicrobials. It is cost-effective, reproducible, and supports flexibility in modifying antimicrobial discs (15). Antimicrobials diffuse into the agar, inhibiting bacterial growth and forming a measurable clear zone, which indicates antibacterial activity (14). Based on the diameter of the inhibition zones observed, the antibacterial

activity of the sample can be categorized as follows:

Table 2. Classification of Antibacterial Activity

Classification	Diameter of Inhibition Zone (mm)
Very Strong	> 20 mm
Strong	10-20 mm
Moderate	5-9 mm
Weak	< 5 mm
No Activity	-

Based on Diameter of Inhibition Zone (20,21)

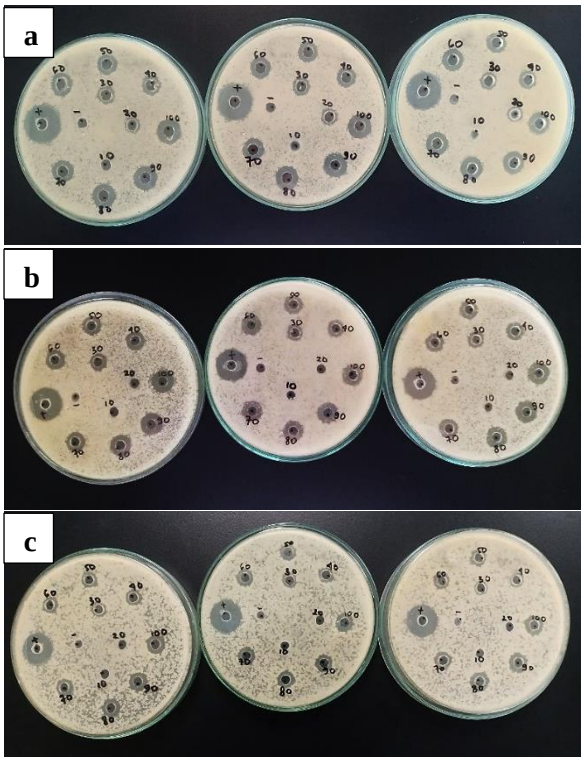

Figure 2. Antibacterial activity of Randu Honey against (a) *Staphylococcus aureus* ATCC 6538, (b) *Escherichia coli* ATCC 8739, and (c) *Pseudomonas aeruginosa* ATCC 9027

The antibacterial activity of randu honey was tested at various concentrations (10%–100%). The results show a concentration-dependent effect, where higher concentrations exhibit greater inhibition zones.

Staphylococcus aureus is classified as a Gram-positive organism, characterized by its spherical (coccus) shape, non-motility, and non-spore-forming properties. This bacterium is a facultative anaerobe that is catalase-positive and oxidase-negative. Optimal growth of *Staphylococcus aureus* occurs within a temperature range of 6.5–46°C and a pH range of 4.2–9.3, indicating its ability to survive and proliferate under a wide range of environmental conditions (16). The antibacterial activity of randu honey against *Staphylococcus aureus* was characterized by an inhibition zone of 9.02 ± 0.67 mm at a 100% concentration, which was the largest among the three bacteria tested, indicating that *Staphylococcus aureus* is the most sensitive to randu honey. The antibacterial activity was classified as moderate at higher concentrations (70%–100%) and weak at lower concentrations (20%–60%). The MIC was 20%, as no inhibition was observed at 10%.

Escherichia coli is a Gram-negative, rod-shaped bacterium from the *Enterobacteriaceae* family, It sizes 1.0-1.5 µm x 2.0-6.0 µm. It can grow in both aerobic and anaerobic conditions as a facultative anaerobe and survive in nutrient-limited environments. Biochemically, *Escherichia coli* produces indole, has limited citrate fermentation, and is urease-negative (17). The antibacterial activity of randu honey against *Escherichia coli* was lower than for *Staphylococcus aureus*, suggesting that *Escherichia coli* is less sensitive to randu honey compared to *Staphylococcus aureus*. The inhibition zones ranged from 7.07 ± 0.56 mm at 100% to 0.18 ± 0.01 mm at 30%. The antibacterial activity falls in the moderate category at 80%–100% and weak category at 40%–70%. The MIC was 30%, as no inhibition was observed at 20% and below.

Pseudomonas aeruginosa is a Gram-negative, rod-shaped, aerobic bacterium that exhibits motility through the use of flagella. It has known can infect various organs and systems, including the eyes, ears (leading to otitis externa), skin, bones, central nervous system, gastrointestinal tract, and heart

(causing endocarditis). Additionally, it is associated with infections of the urinary tract, respiratory system, and bloodstream, resulting in conditions such as bacteremia and septicaemia (18). In the case of *Pseudomonas aeruginosa*, the honey demonstrated moderate antibacterial activity. The inhibition zones ranged from 8.16 ± 0.13 mm at 100% to 1.11 ± 0.17 mm at 30%. The activity is categorized as moderate at 80%–100% and weak at 40%–70%. No activity was observed at 20% and below made 30% was assumed to be MIC.

The positive control (C+), a standard antibiotic, exhibited significantly larger inhibition zones for all bacteria, with values of 17.61 ± 0.43 mm for *Staphylococcus aureus*, 16.39 ± 0.19 mm for *Escherichia coli*, and 15.75 ± 0.50 mm for *Pseudomonas aeruginosa*. Meanwhile, the negative control (C-), presumably sterile water or a similar inert substance, showed no inhibition zones.

Honey has been found to work synergistically with various antibiotics, lowering the doses needed to inhibit bacterial growth or reversing previously acquired antibiotic resistance. Scientific evidence shows that honey has advantages over current chemotherapeutic agents, but its medical use remains underutilized due to limitations, particularly in composition and application (9).

As a highly complex substance, honey contains hundreds of compounds that exert specific and distinct effects on microorganisms. The observed effects include structural and morphological changes, alterations in bacterial membrane potential, disruptions in the bacterial cell cycle and growth, interference with bacterial metabolism, inhibition of efflux pump activity, modifications in quorum sensing, biofilm suppression, and impacts on bacterial stress response (9).

Honey's composition varies based on its botanical origin, affecting its bioactive potential and clinical use. Proper honey selection requires prior screening to quantify and profile bioactive substances (9). Indonesian randu honey offers significant therapeutic benefits, particularly in antibacterial agent and wound care. Randu honey demonstrating efficacy against various pathogens like *Candida albicans*, *Escherichia coli*, *Staphylococcus aureus*, *methicillin-resistant Staphylococcus aureus* (MRSA) (10), *Bacillus subtilis* (11), and *Staphylococcus epidermidis* (12). Its application as a wound dressing also has been shown to stimulate healing in chronic wounds of patients with uncontrolled Type 2 Diabetes Mellitus, effectively preventing allergic reactions and secondary bacterial infections (6).

Randu honey contains a rich composition of natural sugars (primarily fructose and glucose), enzymes, organic acids, vitamins, and minerals like common honeys. In addition to these common components, randu honey is also characterized by its secondary metabolites, including phenolic compounds and flavonoids (6). The levels of phenolic and flavonoids in honey are influenced by the geographical location and botanical source of nectar, as these metabolites are transferred from flower nectar to honey. Total phenolic content is also significantly affected by seasonal variations (8). Apart from geographic location, a number of environmental parameters, such as sunshine exposure, rainfall patterns, soil nutrient availability, altitude, and humidity levels, have a substantial impact on the overall phenolic content of honey. The phenolic composition of honey is significantly influenced by several environmental factors. Furthermore, changes in total phenolic content are also caused by agricultural procedures used during cultivation, including as fertilizer use, irrigation techniques, and post-harvest treatments. Together, these elements demonstrate the intricate relationship between human and natural impacts on honey's bioactive qualities. (22).

Phenolic content of randu honey in Indonesia is estimated around 309.12 ± 33.40 mg GAE/kg with flavonoid content is 47.25 ± 1.49 mg QE/100 g (23). Another study also found that the total phenolic content of randu honey was 465.9 ± 7.3 mg GAE/kg of honey, which is much greater than that of longan honey (24). Since plants with higher concentrations of phenolic compounds typically have stronger antioxidant capacity,

phenolic compounds and antioxidant activity are closely related. By mitigating the negative effects of reactive oxygen species (ROS) and scavenging free radicals, these phenolic substances help to protect against antioxidant damage. This process highlights the vital function that phenolic compounds play in promoting health and preventing cellular damage by preventing oxidative damage to human cells (24).

Honey has several phenolic components that contribute to its antibacterial qualities. The mechanism by which honey acts against bacteria varies between Gram-positive and Gram-negative microorganisms. Some studies suggest that certain cellular targets may be specifically associated with each bacterial class (9). Cell membrane dysfunction is caused by ferulic acid, which results in morphological changes; gallic acid, which causes intracellular leakage and pore formation; p-coumaric acid, which causes cell membrane disruption and binds to bacterial DNA; syringic acid, which causes cytoplasmic and nucleotide leakage due to increased membrane permeability; and caffeine, which lessens oxidative stress. Honey's antibacterial qualities are also influenced by a number of flavonoid components. DNA gyrase is inhibited by apigenin, chrysin, and kaempferol; hydrogen peroxide is promoted by catechin; peptidoglycan and ribosome synthesis is inhibited by galangin; luteolin inhibits FAS-I in mycobacteria and DNA helicases DnaB and RecBCD; myricetin inhibits DNA B helicase; and cell lysis is induced by pinocembrin (8).

CONCLUSION

This study explores the antibacterial potential of randu honey, a monofloral honey sourced from *Ceiba pentandra*, against *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*. Using the agar well diffusion method, randu honey showed antibacterial activity that increased with higher concentrations. The largest inhibition zone was observed for *Staphylococcus aureus* (9.02 ± 0.67 mm) at 100% concentration, followed by *Pseudomonas aeruginosa* (8.16 ± 0.13 mm) and *Escherichia coli* (7.07 ± 0.56 mm). The MIC was 20% for *Staphylococcus aureus* and 30% for both *Escherichia coli* and *Pseudomonas aeruginosa*. At higher concentrations (70%–100%), the honey demonstrated moderate antibacterial activity, while at lower concentrations (30%–60%), the activity was categorized as weak.

These findings highlight the potential of randu honey as a natural antibacterial agent, particularly effective against Gram-positive bacteria like *Staphylococcus aureus*. While the antibacterial effect of randu honey is less potent than standard antibiotics, as reflected in the smaller inhibition zones, it offers promising opportunities for alternative treatments, especially in addressing antibiotic resistance. Future research could delve deeper into the bioactive compounds of randu honey, its mechanisms of action, and its potential to enhance the effectiveness of conventional antibiotics.

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REFERENCES

1. Cianciosi D, Forbes-Hernández TY, Afrin S, Gasparrini M, Reboredo-Rodriguez P, Manna PP, et al. Phenolic compounds in honey and their associated health benefits: A review. Molecules. 2018;23(9):1–20.
2. BPS. Statistik Produksi Kehutanan 2022. Badan Pus Stat. 2022;32.
3. SNI. SNI 8664:2018 Honey. Standar Nas Indones Madu. 2018;27.
4. Se KW, Wahab RA, Syed Yaacob SN, Ghoshal SK. Detection techniques for

adulterants in honey: Challenges and recent trends. J Food Compos Anal. 2019;80(September 2018):16–32.
5. Bobis O, Moise AR, Ballesteros I, Reyes ES, Durán SS, Sánchez-Sánchez J, et al. Eucalyptus honey: Quality parameters, chemical composition and health-promoting properties. Food Chem. 2020;325(December 2019):126870.
6. Wahdini SI, Seswandhana MR, Vityadewi N, Ramli RN, Gabriela GC, Dachlan I. The use of Indonesian randu honey for chronic wounds in a patient with uncontrolled type 2 diabetes mellitus: A case report. Int J Surg Case Rep. 2022;95(April):107140.
7. Saranraj P, Sivasakthi S. Comprehensive Review on Honey : Biochemical and Medicinal Properties Comprehensive Review on Honey : Biochemical and Medicinal Properties. 2018;6(April).
8. Almasaudi S. The antibacterial activities of honey. Saudi J Biol Sci. 2021;28(4):2188–96.
9. Combarros-Fuertes P, Fresno JM, Estevinho MM, Sousa-Pimenta M, Tornadijo ME, Estevinho LM. Honey: Another alternative in the fight against antibiotic-resistant bacteria? Antibiotics. 2020;9(11):1–21.
10. Wardani NP, Primaharinastiti R, Poernomo AT, Khatib A. A Comparative Study of Randu Honey Antimicrobial Activity from Several Regions in Java. 2024;11(2):147–55.
11. Diyah NW, Irfanah L, Isnaeni I. Methods validation for evaluation of ceiba honey's growth inhibitory activity against Bacillus subtilis ATCC 6633. Pharmaciana. 2020;10(1):1.
12. Fadhilla RR, Diyantoro, Indriati DW, Sundari AS. Antibacterial potency of Indonesian Randu Honey against Staphylococcus sp. Malaysian J Med Heal Sci. 2020;16:67–71.
13. Panjaitan RA, Darmawati S, Prastiyanto ME. Aktivitas Antibakteri Madu Terhadap Bakteri Multi Drug Resistant Salmonella typhi dan Methicillin-Resistant Staphylococcus Aureus. Pros Semin Nas Edusainstek. 2018;1(1):70–7.
14. Balouiri M, Sadiki M, Ibnsouda SK. Methods for in vitro evaluating antimicrobial activity: A review. J Pharm Anal. 2016;6(2):71–9.
15. Salam MA, Al-Amin MY, Pawar JS, Akhter N, Lucy IB. Conventional methods and future trends in antimicrobial susceptibility testing. Saudi J Biol Sci. 2023;30(3):103582.
16. Abdilah F, Kurniawan K. Morphological Characteristics of Air Bacteria in Mannitol Salt Agar Medium. Borneo J Med Lab Technol. 2022;5(1):353–9.
17. Rahayu WP, Nurjanah S, Komalasari E. Escherichia coli: Patogenitas,Analisis, dan Kajian Risiko. IPB Press. 2018;1–151.
18. Yosias Beslar S, Norma Ethica S, Srikandi Fitria M, Rahman Ernanto A. Deteksi Bakteri Pseudomonas aeruginosa Isolat Pus Luka Berbasis Polymerase Chain Reaction dengan Target Gen Penkode Flagelin fliC. Pros Semin Nas UNIMUS. 2022;5:1–13.
19. Weinstein MP, Lewis JS. The clinical and laboratory standards institute subcommittee on Antimicrobial susceptibility testing: Background, organization, functions, and processes. Vol. 58, Journal of Clinical Microbiology. 2020.
20. Davis WW, Stout TR. Disc plate method of microbiological antibiotic assay. II. Novel procedure offering improved accuracy. Appl Microbiol. 1971;22(4):666–70.
21. Rahayuningsih SR, Patimah SS, Mayanti T, Rustama MM. Aktivitas Antibakteri Ekstrak n-Heksana Daun Mangrove (Rhizospora stylosa Griff) Terhadap Bakteri Patogen Pada Ikan Nila (Oreochromis niloticus). J Mar Res. 2023;12(1):1–6.
22. Maliníková E, Kukla J, Kuklová M, Balážová M. Altitudinal variation of plant traits: Morphological characteristics in Fragaria vesca L. (Rosaceae). Ann For Res. 2013;56(1):79–89.

23. Ustadi U, Radiati L, Thohari I. Bioactive Components of Rubber Tree Honey (Hevea Brasiliensis) and Calliandra (Calliandra Callothyrsus) and Kapok Honey (Ceiba Pentandra). J Ilmu dan Teknol Has Ternak. 2017;12(2):97–102.
24. Sulistyianingsih, Poernomo AT, Primaharinastiti R. Physicochemical Properties and Antioxidant Activity of Three Types of Monofloral Honey from Indonesia. J Farm Dan Ilmu Kefarmasian Indones. 2022;9(3):290–7.