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A Comparative Study of Randu Honey Antimicrobial Activity from Several Regions in Java

Nindya Pramesti Wardani[1], Riesta Primaharinastiti[2]*, Achmad Toto Poernomo[2], Alfi Khatib[3]
[1]Master Program of Pharmaceutical Sciences, Faculty of Pharmacy, Universitas of Airlangga, Surabaya, Indonesia
[2]Department of Pharmaceutical Sciences, Faculty of Pharmacy, Universitas Airlangga, Surabaya, Indonesia
[3]Kulliyyah of Pharmacy, International Islamic University Malaysia, Kuantan, Malaysia

*Corresponding author: riesta-p-h@ff.unair.ac.id
Orcid ID: 0000-0003-1052-7765

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Abstract

Background*: Randu honey is monofloral honey sourced from a type of plant nectar. The geographical location of randu (Ceiba pentandra) as the source of nectar is one factor that influences the antimicrobial activity of random honey. This research used randu honey from several regions in Java such as Sidoarjo (RSH), Pusat Perlebahan Nasional Bogor (RBH), Kediri (RKH), and Malang (RMH).* ***Objective****: To compare the antimicrobial activity of several random honeys (RSH,RBH,RKH, and RMH) against Gram-negative Escherichia coli ATCC 25922, Gram-positive Staphylococcus aureus ATCC 6538, methicillin-resistant Staphylococcus aureus (MRSA) ATCC 33592, and Candida albicans ATCC 10231.* ***Methods****: This study used well diffusion and dilution antimicrobial test methods. The diameter of the inhibition zone formed by the well diffusion method was measured using a Vernier caliper. The diffusion method was used as a screening test before determining the quantitative minimum inhibitory concentration (MIC) using serial dilution at a ratio1 of 2 (v/v). Streptomycin and Ketoconazole were used as positive controls. Nutrient broth and Sabouraud broth were incubated at 37°C for 24 h (antibacterial tests) and 25°C for 48 h (antifungal test), respectively.* ***Results****: The well diffusion test revealed that all random honey samples could inhibit the test bacteria and fungi with the appearance of an inhibition zone. Diameter inhibition zone ranged from 14.66±0.52 mm to 27.86±0.43 mm. The MICs of RSH,RBH,RKH, and RMH ranged from 3.12% to 25% against all test bacteria and fungi.* ***Conclusion****: The results of this study showed randu honey from Bogor (RBH) has the highest antimicrobial activity based on diffusion and dilution tests.*

Keywords*: antimicrobial activity, dilution, diffusion, MIC, randu honey*

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INTRODUCTION

Randu honey is a monofloral type of honey sourced from the dominance of nectar from a random plant (*Ceiba pentandra*). Randu plants grow widely in Asia, especially in Indonesia, Philippines, and Malaysia. The total area of the plantation was 250,500 hectares, and the honey yield ranges from 52,358.74 to 540,227.27 kg/year. Randu honey is a type of honey that is widely produced in Indonesia, especially in the Java region, where around 75% of the total honey is produced by beekeepers in Java (Badan Pusat Statistik, 2018). Randu honey is harvested from farms located around random forests during the flowering season. The best season for harvesting randu honey is from May to October during the flowering period because random nectar content will be abundant. The existence of beekeeping in random forests can help pollination and increase the productivity of random honey by approximately 20-40% (Basuki, 2018).

Randu honey has the physical characteristics of a clear yellowish-brown color, sticky sweet taste with a slightly sour taste, and distinctive aroma. The main substances in randu honey are sugar and other compounds such as water, protein, vitamins, free amino acids, and volatile organic compounds as minor components (Burgut, 2020). These compounds are known to be active compounds that exert antimicrobial activity through different mechanisms. The antimicrobial mechanism of hydrogen peroxide in honey is reactive and can break bonds in the outer membrane of bacteria until lysis. Phenolic compounds found in high amounts in honey contribute to antimicrobial activity via membrane dysfunction and binding to bacterial DNA (Almasaudi, 2021).

The chemical composition determines the quality of randu honey and its antibacterial activity. The antibacterial activity of randu honey is influenced by several factors, including the biochemical profile of the randu plant nectar used as a food source for honey-producing bees. The biochemical profile of nectar is qualitatively and quantitatively influenced by plant genetics and physiology, environmental factors (climatic conditions), soil characteristics, and pollinator bee typology (Kocsis et al., 2022). Dezmirean et al. (2017) and Tomczyk et al. (2019) conducted research on the influence of geographic origin, plant source, and polyphenolic substances on the antimicrobial properties of honey.

Currently, much research on randu honey is limited to its antibacterial activity, such as research regarding the benefits of randu honey as an antimicrobial was carried out by Djakaria et al. (2020) who successfully reported the antimicrobial activity of randu honey from *Apis dorsata* bees from Sumbawa, Riau, Belitung and *Apis cerana* from Sukabumi, Bogor, Banyuwangi against *Propionibacterium acnes*. Research by Hasan et al. (2020) showed that randu honey from Riau has potential as an antimicrobial against *Staphylococcus aureus* and *Eschericia coli*. The growth of *Staphylococcus aureus* and *Eschericia coli* can also be inhibited by administering honey from Bandung (Dewi et al., 2017).

In this research, a comparative study will be carried out on the antimicrobial activity of randu honey from several regions in Java with different geographical conditions such as Sidoarjo (RSH), Bogor National Beekeeping Center (RBH), Kediri (RKH), and Malang (RMH). This location was chosen according to geographical conditions for the growth of randu plants, such as the altitude of the area (Bogor at an altitude of 1600 m above sea level, Malang 760 m above sea level, Sidoarjo 20 m above sea level, and Kediri 350 m above sea level), rainfall, temperature, and air humidity (Widodo et al., 2017). The selection of sampling locations was based on the location of the honey bee farm. Honey bee farms in Bogor and Kediri are managed by the government; therefore, there is guidance regarding the quality of the honey produced. The Malang honey bee farm is owned by a company that has national standards, whereas the Sidoarjo honey bee farm is owned by an individual. This difference can be observed in its influence on the antibacterial activity of randu honey against Gram-negative *E. coli* ATCC 25922, Gram-positive *S. aureus* ATCC 6538, methicillin-resistant *S. aureus* (MRSA) ATCC 33592, and *C. albicans* ATCC 10231.

MATERIALS AND METHODS

Materials

Randu honey samples were obtained from beekeepers at Pusat Perlebahan Nasional Bogor (RBH), Karang Ploso Malang (RMH), Sumber Podang Kediri (RKH), and Sidoarjo (RSH) in May 2022, during the harvest season, as shown in Figure 1. All the samples were stored in amber glass at 4°C until further processing. Nutrient agar (NA) (E.Merck) was used as culture and antibacterial activity test media, Sabouraud Dextrose agar (SDA) (E.Merck) was used as culture and antifungal activity test media, ketoconazole 2%(*w/v*) (Genero) and streptomycin injection 200 mg/mL (Meiji) as positive control, NaCl 0.9% p.a (E.Merck), test microbes *E. coli* ATCC 25922, *S. aureus* ATCC 6538,

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methicillin-resistant *S. aureus* (MRSA) ATCC 33592, *C. albicans* ATCC 10231 were obtained from the Faculty of Agriculture Muhammadiyah University Jember.

Tools

Autoclave vertical type stream sterilizer (HL-340 series), micropipette (Eppendorf® research plus), vortex (IKA® maximix II), incubator (Memmet IN110®), analytical balance (Sartorius Type BP22IS®), UV-Vis spectrophotometer (Lambda EZ201 Perkin Elmer), vernier caliper (Jason).

Method

In this study, antimicrobial activity was tested using well diffusion and dilution methods to determine the ability of randu honey to inhibit (static) pathogenic microorganisms. The diffusion method was used to determine the sensitivity of test microorganisms to the randu honey, while the dilution method was used to determine the minimum inhibitory concentration (MIC) of randu honey. The test microorganisms were selected to determine the inhibitory power of randu honey against the growth of gram-negative bacteria (*E. coli* ATCC 25922), gram-positive bacteria (*S. aureus* ATCC 6538), resistant bacteria that often cause nosocomial infections (methicillin-resistant *S. aureus* (MRSA) ATCC 33592), and yeast that causes opportunistic infections (*C. albicans* ATCC 10231).

Preparation of antimicrobial test media

The antimicrobial test media used were divided into those for the antibacterial and antifungal tests. Antibacterial test media were prepared by dissolving 28 g NA powder in 1 L distilled water. Meanwhile, 65 g of SDA was weighed and dissolved in 1 L of distilled water in a different container for the antifungal test media. Each medium was magnetically stirred until the solution became clear. Each medium was then filled separately in a 12 mL reaction tube as a base layer and 8 mL as a seed layer, covered with cotton, and sterilized at 121°C for 15 min.

Preparation of test microbes

The preparation was initiated by regenerating the test microbes. First, one Ã–se of each test microbe (*E. coli* ATCC 25922, *S. aureus* ATCC 6538, MRSA ATCC 33592) was streaked onto NA slant agar medium and incubated at 37 Â °C for 24 h. *C. albicans* ATCC 10231 was streaked onto SDA agar slant medium and incubated at 25°C for 48 h. The culture results in the form of colonies on slanted agar were used to prepare the inoculum. A total of 10 mL of 0.9% saline solution was added to slanted agar medium containing colonies of *E. coli* ATCC 25922, *S. aureus* ATCC 6538, MRSA ATCC 33592, and *C. albicans* ATCC 10231. These were vortexed until the test microbes were separated from the medium (marked by the presence of turbidity). The turbidity was measured at a wavelength of 580 nm until the transmittance reached 25%. A test microbial inoculum was obtained with a bacterial count range of 10^7-10^9 cfu/mL (Kemenkes RI, 2020).

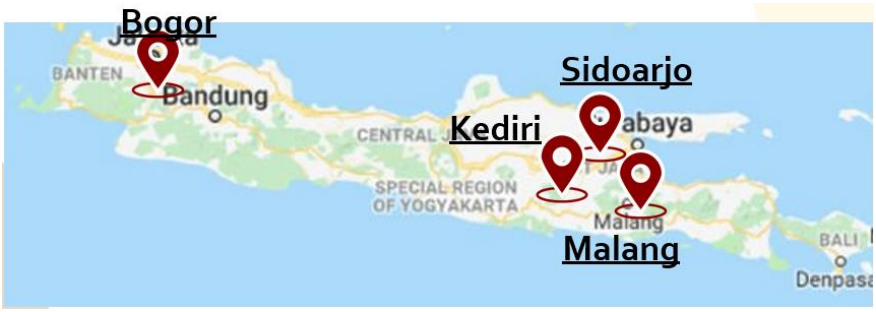

Figure 1. Sampling location of randu honey (RBH, RMH, RKH, RSH)

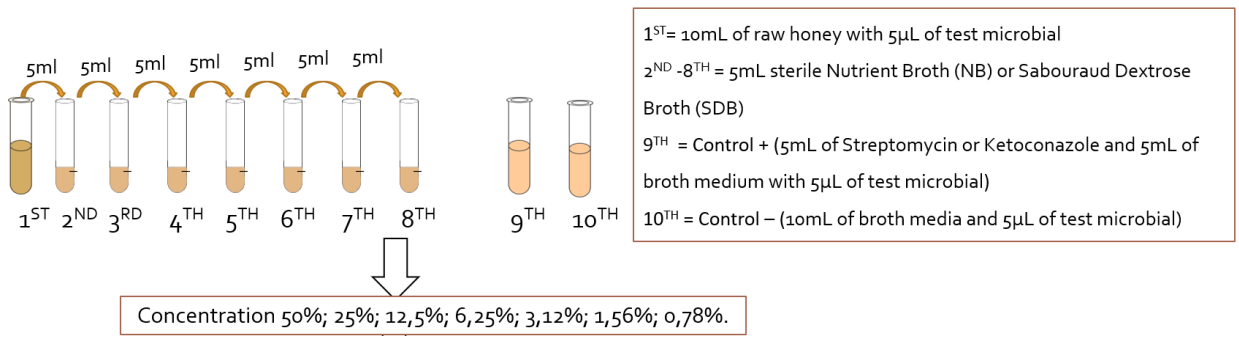

Figure 2. Serial dilution method with ratio 1:2 (v/v)

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Antimicrobial activity test

Well diffusion test

The antimicrobial activity test was performed using the diffusion method described by Irfanah (2018), with modifications. A well-diffusion technique was used in this study. This technique was carried out by filling a hole measuring 7.50 mm in diameter with a random honey sample solution. According to Anand et al. (2019), the diffusion method can provide better results than the other methods. This is because, in the well diffusion method, the test substance has more contact with the medium, so more of it diffuses and interacts with the test microbes. The working principle is the diffusion of active antimicrobial compounds in honey into media containing the test microorganisms.

First, 12 mL of NA as the base layer medium was poured into a sterile Petri dish. The test bacterial inoculum was pipetted 5µL and put into an 8mL seed layer. The mixture of the seed layer medium and bacterial inoculum was vortexed. The seed layer was then poured on top of the solidified base layer. Once the agar was solid, holes were created using a ring (diameter: 7.50 mm). The medium was perforated in five holes consisting of 100µl of randu honey with three replications, one positive control, and one negative control. Finally, the media was incubated at 37°C for 24 h to test antibacterial activity and at 25°C for 48 h to test antifungal activity. The diameter of the inhibition zone formed around the well was measured using a caliper with an accuracy of 0.05 mm. The diameter of the inhibition zone was considered a measure of antibacterial activity. The diameter of the inhibition zone exhibited a linear relationship with the antimicrobial activity of the samples. An inhibitory zone diameter of less than 7 mm is defined as a no-obstacle zone (Banerjee *et al*., 2022).

Dilution test

The minimum inhibitory concentration (MIC) was determined using the serial dilution method at a ratio of 1:2 (v/v) in 10 sterile reaction tubes. Liquid medium (broth) was used for dilution. The first tube contained 10 mL of randu honey to which 5µL of the test microbial inoculum was added, and the second to eighth tubes were filled with 5 mL of sterile Nutrient Broth (NB) medium for bacterial MIC and Sabouraud Dextrose Broth (SDB) for fungal MIC. Approximately 5 mL of randu honey in the first tube was pipetted into the second tube using a micropipette. The solution was centrifuged until homogeneous and a 50% solution was formed. The same procedure was performed up to the eighth tube, and all extract concentrations were obtained in a ratio of 1:2 to obtain concentrations of 50%, 25%, 12.5%, 6.25%, 3.12%, 1.56%, and 0.78%, respectively (Figure 2). The ninth tube contained 5mL Streptomycin or Ketoconazole as a positive control and 5mL mL broth media with 5µL inoculum. The 10th negative control tube contained 10 mL broth medium and 5 µL test microbial inoculum. All tubes were incubated at 37°C for 24 h for bacteria and at 25°C for 48 h for fungi (Kemenkes RI, 2020). After incubation, each tube was vortexed and the transmittance was immediately measured using a spectrophotometer. Transmittance measures the amount of light passing through a sample (Akinduti et al., 2019). Transmission through a sample solution can be easily measured by measuring the intensity of the incident and transmitted light.

The dilution method aims to determine the smallest concentration of randu honey that can inhibit the growth of the test microorganism, or, , plays a role in determining the minimum inhibitory concentration (MIC). The presence or absence of growth of test microorganisms was observed by measuring turbidity using a spectrophotometer at a wavelength of 580 nm. Incubation results that show turbidity indicates that there is growth of the test microorganisms, whereas a clear sample means that the active antimicrobial compounds in randu honey from Bogor (RBH), Malang (RMH), Kediri (RKH), and Sidoarjo (RSH) can inhibit the growth of the test microorganisms.

Statistical analysis

This research used two-way Analysis of Variance (ANOVA) statistical analysis via the IBM SPSS (Statistical Package for Social Sciences) version 26 application to determine whether there were significant differences in the antimicrobial activity produced by randu honey samples.

RESULTS AND DISCUSSION

Antimicrobial activity test results using the diffusion method

The test result is said to be positive if a clear area is formed around the sample (Figure 3). This clear area shows that the growth of microorganisms is inhibited; therefore, it is called the inhibition zone. Observations of the inhibition zone were adjusted according to the growth temperature of the test microbes. Incubation was carried out at 37 °C according to the growth temperature of the mesophyll bacteria and 32 °C according to the growth temperature of the fungi. An inhibition zone appeared after 24 h of incubation in the antibacterial activity test and after 48 h in the antifungal test.

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The results of the antimicrobial activity test by diffusion showed that each sample of randu honey inhibited the growth of the test microbes. The formation of the inhibition zone varied in size. Overall, the inhibition zone formed was between 11.57 ± 0.67 and 27.86 ± 0.43 mm, indicating that all randu honey samples had potent antimicrobial activity against the test microbes.

In Table 1, it can be seen that randu honey from Bogor (RBH) produces an inhibitory zone diameter between 17.59 ± 0.13 mm and 27.86 ± 0.43 mm. The inhibition zone for MRSA ATCC 33592 and *C. albicans* ATCC 10231 was more than 20 mm; therefore, it was included in the very strong inhibitory category based on the classification by Abu-Zaid et al. (2022).Randu honey from Malang (RMH) forms an inhibitory zone diameter of 15.90 ± 0.57 to 21.58 ± 0.56 mm, which is included in the strong inhibitory category. The RMH sample exhibited the highest antimicrobial activity against *S. aureus* (ATCC 6538). Randu honey from Sidoarjo (RSH) and Kediri (RKH) also showed strong inhibitory power, with a range of inhibitory zone diameters of 14.66 ± 0.52 – 20.91 ± 0.29 mm, respectively, and 11.57 ± 0.67 - 17.51 ± 0.57 mm. These two randu honey samples had the highest inhibitory power against *C. albicans* ATCC 10231 compared with the other tested microbes.

Compared to other other randu honey samples, the randu honey sample from Bogor (RBH) had the highest inhibitory activity against *C. albicans* ATCC 10231. In contrast, the antimicrobial activity produced by the RKH sample was the lowest among the four tested microbes. This is in accordance with the two-way ANOVA statistical test, which gives a value of F = 83.386 > F table (2.25) and a significance value of 0.000 < α = 0.05, indicating that there is a significant difference in the diameter of the inhibition zone between the randu honey groups and test microbes. Randu honey with the highest antimicrobial activity was tested through post-hoc multiple comparisons, obtaining the largest mean difference in samples RBH (21.1642) and *C. albicans* ATCC 10231 (21.2350). It can be concluded that there is a match between the observation results and the statistical analysis.

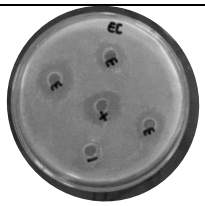
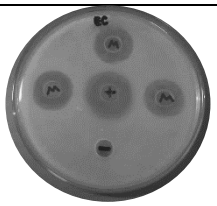
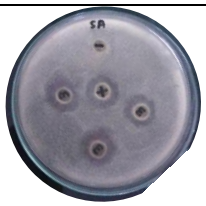
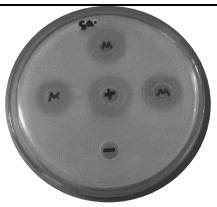
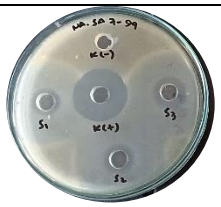
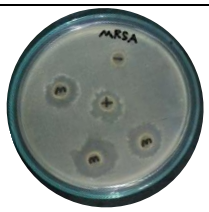
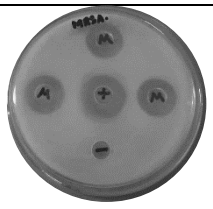
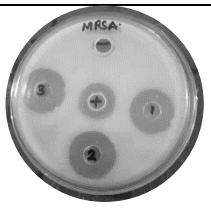
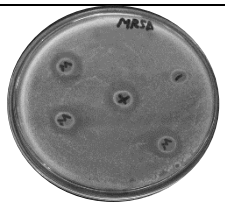
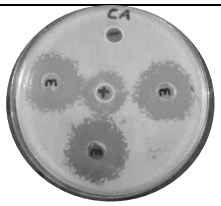
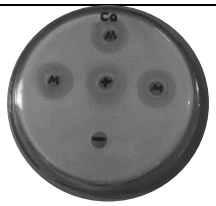
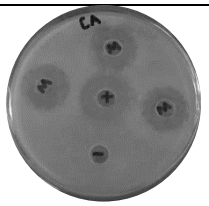
Test microbes	RBH	RMH	RKH	RSH
Eschericia coli ATCC 25922 (EC)				
Staphylococcus aureus ATCC 6538 (SA)				
MRSA ATCC 33592				
Candida albicans ATCC 10231 (CA)				

Figure 3. Results of well diffusion test (M= sample replication, + = positive control, - = negative control)

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Table 1. Diameter of inhibition zone randu honey samples

Test Microbes	Diameter of Inhibition Zone (mm)					
	RBH	**RMH**	**RSH**	**RKH**	**Positive Control**	**Negative Control**
Escherichia coli ATCC 25922	17.59±0.13	17.90±0.57	16.05±0.29	13.73±0.71	25.80	0.00
Staphylococcus aureus ATCC 6538	18.24±0.36	21.58±0.56	17.94±0.24	15.13±1.35	27.35	0.00
MRSA ATCC 33592	20.97±1.03	20.58±0.73	14.66±0.52	11.57±0.67	18.30	0.00
Candida albicans ATCC 10231	27.86±0.43	18.66±0.74	20.91±0.29	17.51±0.57	25.19	0.00

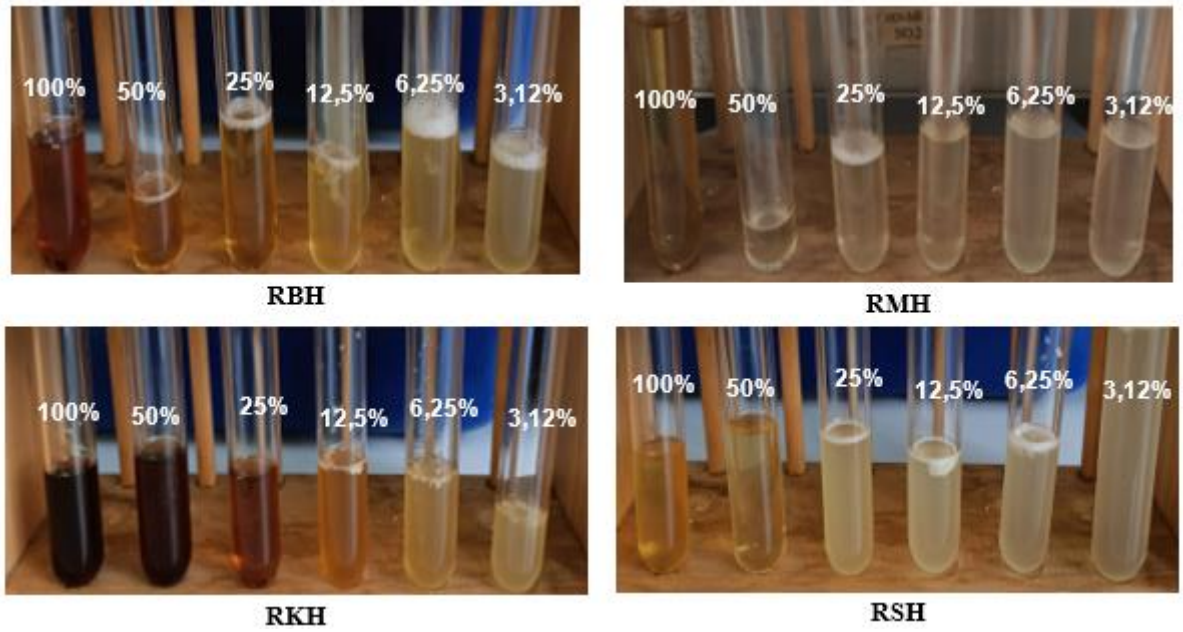

Figure 4. Results of dilution test against *Escherichia coli* ATCC 25922 with 100%; 50%; 25%; 12.5%; 6.25%; 3.12% (v/v) concentration of randu honey

Antimicrobial activity test results using the dilution method

MIC was visually observed as the smallest concentration that did not cause turbidity. The results of the dilution tests are shown in Figure 4.

The sample with the highest transmittance value in Table 2 is the positive control, which contains media and antibiotics to suppress the growth of the test microorganisms. As a result, there is no turbidity and more light can pass through the solution, resulting in a high transmittance percentage. The opposite is true for negative controls. Meanwhile, if we observe the transmittance at each concentration of randu honey, the higher the concentration of the randu honey sample, the higher the transmittance produced because the number of test microorganisms that grow decreases. A high concentration of randu honey indicates that it contains more antimicrobial compounds, therefore the inhibitory power for the growth of microorganisms is higher. The MIC value is the lowest concentration of randu honey that can inhibit microbial growth, at concentrations less than the MIC (bold numbers in Table 2) there is no inhibitory effect (Vaou et al., 2021). This is because the transmittance produced at this concentration is already lower than that produced by the negative control, which only contains the medium and test microbes without randu honey.

From the data in Table 2, it can be seen that the minimum inhibitory concentration produced by each sample of randu honey using the turbidimetric method was in the concentration range of 25% to 3.12% (v/v). The average transmittance of the MIC of the four samples was 31.70 ± 0.63%. u The MIC of RKH sample against *S. aureus* ATCC 6538 was 25% (v/v), and the other samples were 12.5% (v/v). The MICs of RKH, RMH, RSH, and RBH samples against MRSA ATCC 33592 were 6.25%, 12.5%, 25%, and 3.12% (v/v). Meanwhile, the inhibitory ability of RKH, RMH, RSH, and RBH randu honey samples against *C. albicans* ATCC 10231 was at a concentration of 25%, 12.5%, and 3.12% (v/v). The MIC value shows RKH, RMH and RSH honey had moderate antibacterial power according to the Kuete (2010) classification which differentiates antibacterial power into 3 levels: strong (<100µg/mL), moderate (100- 625µg/mL) and weak (>625µg/mL). Based on the MIC data, it can be said that the most

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potent inhibitory power is exhibited by the RBH sample with a concentration of 3.12%, which is considered to have strong inhibitory ability against pathogens.

Overall, the randu honey sample from Bogor (RBH) showed the highest ability to inhibit the growth of *C. albicans* ATCC 10231, both using diffusion and dilution methods. Based on research by Irish et al. (2021), honey with antimicrobial activity that depends on hydrogen peroxide is more effective in inhibiting dermatophyte fungi and Candida species. This suggests that these randu honey samples may have a broader spectrum and may be valuable antifungal agents.

Table 2. %Transmittance of randu honey sample against test microbes

	Concentration %(v/v)	**Average of % transmittance**			
		Escherichia coli ATCC 25922	*Staphylococcus aureus* ATCC 6538	MRSA ATCC 33592	*Candida albicans* ATCC 10231
RKH	100%	78.40 ± 0.55	88.16± 0.29	80.09± 0.15	73.51± 0.47
	50%	57.57 ± 1.01	64.23 ± 0.59	71.21 ± 0.21	48.69 ± 0.50
	25%	37.70 ± 1.10	**37.99 ± 0.73**	58.09 ± 0.43	**27.09 ± 0.41**
	12.50%	**28.64 ± 0.93**	25.10± 0.24	40.67± 0.83	19.53± 0.35
	6.25%	23.45 ± 0.58	19.36 ± 0.54	**32.07 ± 0.24**	17.24 ± 0.36
	3.12%	19.34± 0.99	15.13 ± 0.22	23.99 ± 0.17	11.93 ± 0.41
	1.56%	14.36 ± 0.98	13.15 ± 0.74	17.14 ± 0.28	9.97 ± 0.12
	0.78%	11.61 ± 1.23	10.66 ± 0.27	12.12± 0.45	7.01± 0.23
	Control -	25.71 ± 0.06	25.83 ± 0.10	25.84 ± 0.09	20.74 ± 0.13
	Control +	95.47 ± 0.06	95.37 ± 0.38	90.41± 0.06	97.46± 0.20
RMH	100%	67.96 ± 1.07	80.16± 0.18	72.88± 0.46	89.04± 0.65
	50%	51.86 ± 0.67	66.09± 0.27	57.25± 0.35	72.47± 0.63
	25%	46.17 ± 1.00	46.71 ± 0.97	44.89 ± 0.12	53.87 ± 0.35
	12.50%	34.86± 0.52	**31.48 ± 0.56**	**28.98± 0.31**	**36.57± 0,51**
	6.25%	**27.21 ± 1.04**	20.07 ± 0.91	21.32 ± 0.34	23.11 ± 0.27
	3.12%	19.03 ± 0.86	14.34± 0.59	16.03± 0.19	18.03± 0.06
	1.56%	9.98 ± 0.84	9.25± 0.59	14.00± 0.32	14.90± 0.29
	0.78%	8.56 ± 0.47	7.31 ± 0.60	10.02± 0.30	11.09± 0.21
	Control -	25.67 ± 0.13	25.52 ± 0.27	25.81 ± 0.16	20.84 ± 0.05
	Control +	95.57 ± 0.09	95.02 ± 0.19	90.47 ± 0.07	97.24 ± 0.11
RSH	100%	64.65 ± 1.42	70.03± 0.80	65.29± 0.46	80.64± 0.44
	50%	47.23 ± 1.25	59.73 ± 0.73	52.93 ± 0.19	62.23 ± 0.28
	25%	**32.53 ± 0.82**	40.17± 0.48	**33.75± 0.82**	49.96± 0.68
	12.50%	24.99 ± 0.16	**30.35 ± 1.28**	25.06 ± 0.34	**37.02 ± 0.13**
	6.25%	18.37 ± 0.49	23.18± 0.26	18.92± 0.11	19.93± 0.32
	3.12%	13.87 ± 0.38	17.34 ± 0.42	14.93 ± 0.28	17.36± 0.60
	1.56%	10.01 ± 0.63	12.90 ± 0.57	12.02 ± 0.10	11.98 ± 0.19
	0.78%	6.66 ±1.15	9.99 ±0.23	10.70 ±0.16	10.14 ±0.44
	Control -	25.64 ± 0.10	25.70 ± 0.12	25.81 ± 0.06	20.83 ± 0.11
	Control +	95.57 ± 0.07	95.63 ± 0.13	90.58 ± 0.11	97.17 ± 0.15
RBH	100%	71.37 ± 0.57	85.31 ± 0.18	84.86 ± 0.38	83.08 ± 0.59
	50%	61.96 ± 0.17	70.30 ± 0.45	65.68 ± 0.37	72.79 ± 0.69
	25%	48.41± 0.15	46.11± 0.15	57.77 ± 0.53	56.94± 0.61
	12.50%	**37.04± 0.23**	**32.43± 0.40**	48.34 ± 0.39	50.28 ± 0.39
	6.25%	23.16 ± 0.24	20.20 ± 0.38	31.33 ± 0.43	41.03 ± 0.67
	3.12%	17.40 ± 0.45	15.30 ± 0.51	**26.05 ± 0.69**	**28.03 ± 0.68**
	1.56%	13.13 ± 0.15	13.17 ± 0.33	16.05 ± 0.29	19.01 ± 0.44
	0.78%	9.80± 0.24	9.79± 0.23	7.02 ± 0.75	13.28± 0.51
	Control -	25.77 ± 0.06	20.80 ± 0.08	25.65 ± 0.07	25.09 ± 0.14
	Control +	90.61± 0.04	97.19 ± 0.12	95.63 ± 0.05	94.91 ± 0.59

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Brudzynski (2020) said that hydrogen peroxide is the main antimicrobial agent in honey because it is capable of producing an inhibitory power (MIC) in the range of 10–10000µg/ml. The reactive hydrogen peroxide in randu honey can break the bonds of the microbes' outer membrane, resulting in lysis of the microbes. Therefore, factors that influence the production and breakdown pathways of hydrogen peroxide also influence the antimicrobial activity.

Clearwater et al. (2018) stated that water content is one of the factors that influences the formation of hydrogen peroxide. Their research found that hydrogen peroxide levels in honey harvested between May and August 2006 (rainy season) in the Czech Republic were higher than those in honey harvested in July (summer). This is because the water content in honey increases, and water is needed as a reactant for the formation of hydrogen peroxide by the enzyme glucose oxidase. This is one of the factors that causes the RBH sample to have a higher inhibitory power than the other samples. The geographical conditions of the area of origin of the RBH sample, the Pusat Perlebahan Bogor, are located in an area with rainfall of approximately 3500–4000 mm per year. This rainfall is higher than that in Sidoarjo (1300–1700 mm per year), Malang (1596 mm per year), and Kediri (1652 mm per year).

It was found that even though the samples came from the same type of honey (monoflora honey) from randu plants and were harvested at the same time because the harvest location had different geographical conditions, the antimicrobial activity produced could also be different. In addition to being influenced by geographical conditions, climate, and water availability, it can also be influenced by the nutrition of plant nectar sources and bee entomological factors (Abu-Zaid et al., 2022). In the case of honey that relies on hydrogen peroxide, such as randu honey, antimicrobial activity is related to the stability of the enzyme glucose oxidase, the enzyme responsible for the production of hydrogen peroxide (Almasaudi, 2021).

CONCLUSION

In conclusion, all samples of randu honey from several regions in Java, in this case Sidoarjo (RSH), Bogor National Beekeeping Center (RBH), Kediri (RKH), and Malang (RMH), had active antimicrobial activity against *E. coli* ATCC 25922, *S. aureus* ATCC 6538, methicillin-resistant *S. aureus* (MRSA) ATCC 33592, *C. albicans* ATCC 10231. The RBH sample showed strong inhibitory activity, with a minimum inhibitory concentration (MIC) of 3.12%. The other three honey samples, namely RMH, RKH, and RSH honey, had moderate inhibitory power. Further research is needed to identify the active antimicrobial ingredients in randu honey.

AUTHOR CONTRIBUTIONS

Conceptualization, N.P.W., R.P., A.T.P.; Methodology, N.P.W., R.P., A.T.P.; Software, N.P.W.; Validation, N.P.W., R.P.; Formal Analysis, N.P.W.; Investigation, N.P.W.; Resources, N.P.W.; Data Curration; N.P.W., R.P., A.T.P.; Writing - Original Draft, N.P.W., R.P., A.T.P.; Writing - Review & Editing, N.P.W., R.P., A.T.P.; Visualization, N.P.W.; Supervision, R.P., A.T.P.; Project Administration, N.P.W., R.P., A.T.P.; Funding Acquisition, N.P.W.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

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