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Antibacterial Potential of Linot Honey, Yellow Honey, and Black Honey Extracts against Methicillin-Resistant *Staphylococcus aureus* (MRSA): Inhibition Study on Wound Infections

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ABSTRACT

The emergence of antibiotic-resistant bacteria has drawn significant attention. There is a need to develop new treatment methods using natural products that are safer for both the environment and humans. The aim of this research is to identify the phytochemical content of linot honey (Heterotrigona itama), yellow honey, and black honey, and to evaluate their in vitro antimicrobial activity against methicillin-resistant *Staphylococcus aureus* (MRSA). Standard qualitative methods were used to analyze the phytochemical content of the honey. The antibacterial activity of the honey was tested using the Kirby-Bauer method. Data were analyzed using one-way ANOVA and post-hoc tests. The ethyl acetate fraction of linot honey showed the highest inhibition zone against methicillin-resistant *Staphylococcus aureus*, with a zone of 38.67±0.30 mm. This study indicates that the ethyl acetate fraction of linot honey has high antibacterial activity against these bacteria in vitro. The study supports the use of linot honey as an alternative treatment for various bacterial skin infections.

Keywords: Antibacterial activity, Honey, Methicillin-resistant, *Staphylococcus aureus*

INTRODUCTION

colonized or infected by healthcare-associated pathogens, including Methicillin-resistant Staphylococcus aureus (MRSA).The spread of MRSA in both hospitals and communities is a significant challenge for doctors. Staphylococcus aureus's significance in patient wounds must be assessed for each patient. Staphylococcus aureus can invade wounds or cause infection (Eriksson et al., 2022; Yates et al., 2009). The emergence of MRSA in infected wounds poses a significant problem because topical and systemic antibiotics may not be sufficient to eradicate MRSA. Clearing MRSA from chronic wounds is generally difficult, even if appropriate antibiotics are used. A recent pilot study investigated the possibility of eradicating MRSA in chronic wounds of outpatients (Malka et al., 2023; Schaumburg et al., 2022).

Indonesia has various honey types influenced by weather, insects, and nectar sources (Ridhwan, 2022). The Aceh region itself is one of the honey-producing areas. Several types of honey are produced in the Aceh region, such as *linot* honey, yellow honey, and black honey. The people of Aceh have used honey as a traditional medicine for generations. Honey is often used to treat wounds with excellent results (Giritoy & A., 2021).

Therefore, our study wanted to determine the activity of linot honey, yellow honey, and black honey against bacteria that cause wound infections,Methicillin-resistant Staphylococcus aureus in vitro by taking extracts with solvents as a test.

This studywill also determine the most influential solvents from linot honey, yellow honey, and black honey on bacteria that cause wound infections. Methicillin-resistant Staphylococcus aureus causes infections inchronic wounds.

MATERIALS AND METHODS

This type of research is laboratory experimental research using a Completely Randomized Design (CRD) with Posttest Only

Phytochemical Screening Tests.

Phytochemical screening tests were carried out on three types of honey, namely linot honey, Control Group Design method. This study used methicillin-resistant Staphylococcus aureus with 3 treatments and 3 repetitions.

Phytochemical Screening Test

The concentration of linot honey, yellow honey, and black honey is made for phytochemical tests at the Chemistry Laboratory of the Faculty of Mathematics and Natural Sciences (FMIPA) Syiah Kuala University for phytochemical tests. The method used to extract linot honey, yellow honey, and black honey is the maceration method.

Kirby Bauer Test

In general, there are three types of honey, each with 3 solvents, namely polar (ethanol), semi semi-polarity acetate), and non-and polar (n-hexane). Muller Hinton Agar (MHA) plates were used, measuring 5/6 mm (blank disks), dipped in honey with three solvents. Incubate the germs for 1x24 hours, repeating approximately three times. Antibacterial activity was tested using the Kirby-Bauer paper disc method.

Antimicrobial Effect Sensitivity Test

Methicillin-resistant Staphylococcus aureus isolation was taken with a measuring pipette of 0.1 mL placed in a petri dish and spread flat using a spread plate. Then, discs of linot honey, yellow honey, and black honey with various solvents that have been prepared are placed in petri dishes that have previously been isolated in the space provided. The petri dish was wrapped in brown paper and incubated for 24 hours at 37o Celsius. The diameter of the resulting inhibition zone was measured.

Statistical analysis

The one-way ANOVA test was then carried out based on the data obtained to see whether there were significant differences between the treatment groups

RESULTS AND DISCUSSION

Yellow honey and black honey with a concentrationof 100% or pure honey. Qualitatively, the secondary metabolites found are presented in Table 1 below.

Tabel 1.
Phytochemical Screening Results

Parameter	Linot Honey	Yellow Honey	Black Honey
Saponin	-	-	-
Tanin	+	-	-
Alkaloid	+	-	-
Flavonoid	+	+	+
Steroid	-	-	-

The results of the phytochemical screening of linot honey showed that it contained secondary metabolites in the form of tannins, alkaloids, and flavonoids. The results of phytochemical screening of yellow honey and black honey only found flavonoid content.

Inhibition Zone Measurement

The diameter of the inhibition zone around the disc was measured to the nearest millimeter using a ruler and classified as i) no activity (-); ii) 6-10 mm (+); iii) 11-20 (++) and; iv) 21-30 mm (+++). Figure 1 shows the ethyl acetate fraction, and the positive control shows the most expansive zone of inhibition against MRSA bacteria.

Figure 1. Inhibition zone of linot honey against MRSA bacteria; ethanol fraction (P1P), ethyl acetate fraction (P1S), n-hexane fraction (P1N), pure linot honey (P1M) and positive control (K+)

The diameter of the inhibition zone formed by linot honey in various solvents against MRSA bacteria is presented in Table 2.

Table 2.
Diameter of the inhibition zone of linot honey against MRSA bacteria

Parameter	Mean±SD	Inhibition Zone
Ethanol	10.70±0.12	(+)
Ethyl acetate	38.67±0.30	(++++)
n-hexane	7.24±0.23	(+)
Pure	14.51±0.55	(++)
CP	35.18±0.65	(++++)

*PC (Positive Control) uses linezolid 30 µg

The highest average inhibition zone was formed by linot honey using the ethyl acetate fraction, namely 38.67 ± 0.30 mm, and the lowest inhibition zone was formed by linot honey using the n-hexane fraction, namely 7.24 ± 0.23 mm.

Diameter Of The Inhibition Zone Of Yellow Honey Against MRSA Bacteria

The diameter of the inhibition zone formed by yellow honey in various solvents against MRSA bacteria is presented in Table 3.

Table 3.
Diameter Of The Inhibition Zone Of Yellow Honey Against MRSA Bacteria

Parameter	Mean±SD	Inhibition Zone
Ethanol	6.05±0.05	(+)
Ethyl acetate	6.05±0.04	(+)
n-hexane	6.05±0.04	(+)
Pure	6.02±0.02	(+)
PC	35.18±0.65	(++++)

*PC (Positive Control) uses linezolid 30 µg

The positive control formed the highest mean inhibition zone, namely 35.18 ± 0.65 mm, while the ethanol fraction was 6.05 ± 0.05 mm, the ethyl acetate fraction and the n-hexane fraction were 6.05 ± 0.04 mm. Pure honey produces an inhibition zone of 6.02 ± 0.02 mm.

Diameter of The Inhibition Zone of Black Honey Against MRSA Bacteria

The diameter of the inhibition zone formed by black honey in various solvents against MRSA bacteria is presented in Table 4.

Table 4.
Diameter of the inhibition zone of black honey against MRSA bacteria

Parameter	Mean±SD	Inhibition Zone
Ethanol	21.60±0.25	(+++)
Ethyl acetate	23.42±0.10	(+++)
n-hexane	8.74±0.07	(+)
Pure	18.30±0.27	(++)
PC	35.18±0.65	(++)

*PC (Positive Control) uses linezolid 30 µg

The highest mean inhibition zone was formed by the positive control, namely 35.18 ± 0.65, followed by the ethyl acetate fraction, namely 23.42 ± 0.10. The lowest inhibition zone was formed by the pure n-hexane fraction, namely 8.74 ± 0.07 mm

There are developments in the useof honey as a therapy that has antibacterial potential, which is used as an alternative to face thethreat of bacterial resistance to antibiotics (Salsabila et al., 2022). Honey is known to have bactericidal and bacteriostatic activity against Gram-positive and Gram-negative bacteria (Rostinawati, 2009). Using honey as a combination therapy is also known to reduce bacterial antibiotic resistance (Kaligis et al., 2020).

This study assessed the antibacterial activity of various honeys with various solvent fractions against Gram-positive bacteria, namely MRSA. The assessment is carried out by measuringthe diameter of the inhibition zone formed. The inhibition zone is a clear area around the well of thetest bacterial growth medium that does not grow bacteria (Martyarini et al., 2011). The formation of an inhibition zone (clear zone) indicates the antibacterial activity of honey against the test bacteria. The diameter formed by the inhibition zone indicates the strength of the antibacterial activity of honey. The wider the clear zone formed, the stronger the bioactive compounds inhibit bacterial growth (Putri et al., 2016; Mami et al., 2021; Syaukani & E., 2017).

Antibacterial Activity of Honey Against MRSA Bacteria

Laboratory studies have revealed that honey is effective against Methicillin-resistant Staphylococcus aureus (MRSA). 100% inhibitionwas observed in MRSA compared with antibiotic usealone. A synergistic effect is achieved when honey and antimicrobial agents treat Gram-negative and positive bacteria (Almasaudi et al., 2017). In this study, it was found that thehoney that produced the highest zone of inhibition against MRSA bacteria was the ethyl acetate fraction of linot honey (38.67 ± 0.30) mm, followedby the ethyl acetate fraction of black honey (23.42 ± 0.10) mm and the ethanol fraction of black honey(21.60 ± 0.25) mm. The ethyl acetate fraction of Linot honey even produced a higher inhibition zone than the positive control using 30 µg linezolid (35.18 ± 0.65) mm ($p < 0.001$).

Linot honey has been proven vitro to havestrong antibacterial activity in accelerating wound healing. The advantage of linot honey compared tostinging bee honey is that linot honey has a more potentantimicrobial effect (Afriliah et al., 2022). Linot honey has potential as an antimicrobial agent through its bacteriostatic (protein production, DNA replication, cellular metabolism) and bactericidal (inhibiting cell wall synthesis) functions (Ma'ruf et al., 2018; Yaacob et al., 2018).

The limitation of this research is that the study focused solely on MRSA, a single Gram-positive bacterium, limiting the generalizability of the findings to other bacterial strains, including Gram-negative bacteria. The study did not explore the potential effects of different honey concentrations, which could influence the observed antibacterial activity. Furthermore, the specific bioactive compounds responsible for the antibacterial properties of the honey were not identified, leaving a gap in understanding the mechanisms behind their effectiveness. Future research should address these limitations by including in vivo studies, a broader range of bacterial strains, and detailed chemical analyses to isolate and identify the active components in honey.

CONCLUSION

There are differences in the inhibitory power of linot honey, yellow honey, and black honey against bacteria that cause wound infections, Methicillin-resistant Staphylococcus aureus, where the results of the inhibition test on linot honey gave superior results compared to the other two honeys

REFERENCES

Afriliah, Nurlaila, Wintari, Taurina, & A., M. (2022). Karakterisasi Simplisia Madu Kelulut (Heterotrigona itama) sebagai Bahan Baku Sediaan Obat Penyembuhan Luka. *Majalah Farmasi Dan Farmakologi*, *26*(3). [Crossref], [Publisher]

Almasaudi, B., S., & Al., E. (2017). Antimicrobial effect of different types of honey on Staphylococcus aureus. *Saudi Journal of Biological Sciences*, *24*(6). [Crossref], [Publisher]

Eriksson, Elof, & Al., E. (2022). *Chronic wounds: Treatment consensus Wound Repair and Regeneration 30.2.* [Crossref], [Publisher]

Giritoy, & A., M. (2021). *Efektivitas topikal madu terhadap granulasi luka diabetes melitus*. [Publisher]

Kaligis, C. J., Nangoy, E., & Mambo, C. D. (2020). Uji efek anti bakteri madu hutan dan madu hitam terhadap bakteri Staphylococcus aureus, Escherichia coli, dan Pseudomonas aeruginosa. *EBiomedik*, *8*(1). [Crossref], [Publisher]

Ma'ruf, Muhammad, & Al., E. (2018). Madu lebah kelulut (Trigona Spp.) dalam aktifitas terhadap bakteri Staphylococcus aureus resisten. *Jurnal Skala Kesehatan*, *9*(1). [Publisher]

Malka, Marcin, & Al., E. (2023). The Pathogen Isolates in

Chronic Wound Infections in Poland. *Polish Journal of Microbiology*, *72*(2). [Crossref], [Publisher]

Mami, S., H., Sabuna, A. C., & N., J. (2021). Ekstrak Etanol Daun Ajeran Antibakteri terhadap Staphylococcus aureus. *Jurnal Biosains*, *7*(1). [Crossref], [Publisher]

Martyarini, A., S., & Najatullah, N. (2011). *Efek madu dalam proses epitelisasi luka bakar derajat dua dangkal*. [Publisher]

Putri, AD, V., & Al., E. (2016). Uji daya hambat jamur endofit rimpang lengkuas (Alpinia galanga L.) terhadap pertumbuhan bakteri Escherichia coli dan Staphylococcus aureus. *Ebiomedik*, *4*(2). [Crossref], [Publisher]

Ridhwan, M. (2022). Cairan Dari Perut Linot (Trigona sp): Artikel Review. *Jurnal Serambi Akademica*, *10*(6). [Publisher]

Rostinawati, T. (2009). Aktivitas antibakteri madu amber dan madu putih terhadap bakteri Pseudomonas aeruginosa Multiresisten dan Staphylococcus aureus resisten metisilin. *Fakultas Farmasi, Universitas Padjadjaran*. [Publisher]

Salsabila, Azzahra, H., & F., S. P. (2022). Studi Literatur Aktivitas Senyawa Aktif Antibakteri Madu terhadap Methicillin- Resistant Staphylococcus aureus (MRSA). *Bandung Conference Series: Pharmacy*. [Publisher]

Schaumburg, Frieder, & Al., E. (2022). Chronic wounds in Sierra Leone: pathogen spectrum and antimicrobial susceptibility. *Infection*, *50*(4). [Crossref], [Publisher]

Syaukani, & E. (2017). Perbandingan daya hambat madu seulawah dengan madu trumon terhadap Staphylococcus aureus secara in vitro. *BIOTIK: Jurnal Ilmiah Biologi Teknologi Dan Kependidikan*, *3*(1). [Publisher]

Yaacob, M., & Al., E. (2018). Stingless bee honey and its potential value: A systematic review. *Food Research*. [Crossref], [Publisher]

Yates, Christopher, & Al., E. (2009). Wound chronicity, inpatient care, and chronic kidney disease predispose to MRSA infection in diabetic foot ulcers. *Diabetes Care*, *32*(10). [Crossref], [Publisher]