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Inhibitory Effects of Linot, Yellow, and Black Honey Extracts on Carbapenem-Resistant Klebsiella pneumoniae and *Escherichia coli* in Wound Infections

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ABSTRACT

Honey is known to have potential as an alternative treatment for wound infections caused by bacteria. This study aimed to evaluate honey's antimicrobial activity against Carbapenem-resistant Klebsiella pneumoniae and Escherichia coli in vitro. Methodology: The antibacterial activity test of honey was conducted using the disc diffusion method. The data were analyzed using one-way ANOVA and post-hoc tests. The results showed that the ethyl acetate fraction of Linot honey exhibited the highest inhibition zones against Carbapenem-Resistant Klebsiella pneumoniae and Escherichia coli; 25.64±0.20 mm and 25.07±0.05 mm, respectively. This study indicates that the ethyl acetate fraction of Linot honey possesses high antibacterial activity against these bacteria in vitro. Conclusion: This research supports using Linot honey as an alternative treatment for skin infections caused by Klebsiella pneumoniae and *Escherichia coli*.

Keywords: Inhibition zone, Linot honey, non-Linot honey, Carbapenem-Resistant, Klebsiella pneumonia, Escherichia coli

INTRODUCTION

Indonesia is rich in traditional medicinal resources, including honey, that can be utilized as alternative treatments. Honey is a natural product bees produce using flower nectar (Susanto, 2017). Honey has been used in wound treatment applications, such as burns, skin lacerations, surgical wounds, and other injuries. Among the types of honey are Linot honey and non-Linot honey. Linot and non-Linot honey are distinguished based on their origins. Linot honey refers to honey produced by wild bees that feed on nectar from Linot flowers, a specific type of plant. This honey is typically collected from forests or specific areas where these bees gather and are often considered high-quality honey with a unique taste and significant health benefits. Conversely, non-Linot honey is not produced by bees feeding on Linot flower nectar. This includes honey from bees that collect nectar from various flowers or other plants. Non-Linot honey is a more general term encompassing all types of honey other than Linot honey, with each type having its unique qualities and benefits based on its nectar source.

The compounds in honey are known to play a role in combating damage caused by oxidative stress in the body. The antimicrobial, anti-inflammatory, and antioxidant activities of honey are believed to enhance the body's immunity, function as autolytic debridement, and accelerate the wound healing process (Priscilla, 2017). The antimicrobial content in honey can also serve as a treatment for wounds. A wound is the disruption of tissue continuity due to injury or surgery. Wounds can become contaminated, leading to infection (Primadina et al., 2019). Various organisms easily colonize infected wounds on the skin surface. The occurrence of colonization depends on the cells' ability to form new protoplasm from available nutrients in the environment. Colonization can occur through several phases: the lag phase, logarithmic/exponential phase, stationary phase, and death phase (Amir, 2023). Carbapenem-resistant Klebsiella pneumoniae (CRKP) is a common bacteria causing wound infections. In 2012, culture results from inpatients at M. Jamil Hospital in Padang showed that CRKP accounted for 10.22% (28 out of 274) of the cases (David et al., 2019).

In individuals with immune disorders, neonates, and the elderly, Klebsiella pneumoniae (K. pneumoniae) can cause severe infections, potentially leading to outbreaks (Hamdani, 2022). The World Health Organization (WHO) has also identified CRKP as a major threat to public health. Therefore, bacterial resistance to carbapenems has become a global public health threat. Another bacterium frequently isolated from skin and soft tissue infections is Escherichia coli (Ekawati & Herawati, 2018). Atia et al. mentioned in their study that E. coli is the most prevalent

Gram-negative bacterium causing skin infections, accounting for up to 93.71% (n=164) of cases. Moreover, a seven-year study in Europe, Latin America, and North America reported E. coli as the most significant agent causing skin infections (Wahyudi et al., 2018).

In Indonesia, during the 2019-2020 period, E. coli was identified as a cause of surgical wound infections at Dr. M. Djamil Central General Hospital Padang, accounting for 23.1% of cases. This bacterium is frequently found as the causative agent of post-operative wound infections and ranks first among the Enterobacteriaceae group responsible for increasing healthcare costs, morbidity, and mortality rates (Sekar Feni, 2023). Moreover, the virulence profile and antibiotic susceptibility of E. coli strains isolated from skin tissue are generally high. Bacterial resistance to various drugs has steadily increased over the past few years, limiting therapeutic options. The limitations of antibiotics due to resistance have led to efforts to discover new, more sensitive, and effective antimicrobials. Antibiotic resistance has been identified as one of the greatest threats to human health in the future in both developed and developing countries (Andi Kayzar, 2023).

Research on the effectiveness of Linot and non-Linot honey in inhibiting the growth of Carbapenem-resistant Klebsiella pneumoniae (CRKP) and Escherichia coli remains scarce despite many previous studies having been conducted to test the inhibitory power of honey against bacterial growth (Pujiarti et al., 2021). Therefore, the researcher aims to investigate the activity of these three types of honey against the growth of bacteria that cause infections in chronic wounds. The research problem includes questions regarding the differences in the inhibitory power of various fractions and solvent-free honey against these two types of bacteria.

The general objective of this study is to determine the inhibitory zones formed by honey against these two types of bacteria, while the specific objective is to investigate the differences in the inhibitory power of various fractions and solvent-free honey. The benefits of this research include providing important information for both academic and practical fields, where honey could become an alternative treatment for the healing of chronic wounds. The theoretical framework explains that honey contains secondary metabolites with antimicrobial activity, which is believed to be effective in the wound-healing phase when infected by these two bacteria. The research hypothesis is that there is a difference in the inhibitory power between various fractions and types of honey against the two bacteria that cause wound infections.

RESEARCH METHOD

This study is a laboratory experimental research using a Completely Randomized Design (CRD) with a Posttest Only Control Group Design method. The study involved Carbapenem-resistant Klebsiella pneumoniae and Escherichia coli bacteria, as well as three types of honey: Linot honey, non-Linot yellow honey, and non-Linot black honey. Four solvents are used: ethanol, ethyl acetate, n-hexane, and pure honey without a solvent. Each treatment group was repeated three times. The study tested the inhibitory effects of the treatment combinations against the two types of bacteria. This research was conducted at the Microbiology Laboratory of the Faculty of Medicine, Syiah Kuala University, during February and March 2024. The research variables included honey (independent variable) and the inhibition zone formed on the petri dish against bacterial cultures (dependent variable). The study employed the disc diffusion method to measure the inhibition zones. Data analysis was then performed using One-Way ANOVA and post hoc tests to identify significant differences between the treatment groups.

Theoretical Framework

Honey contains various secondary metabolites believed to possess antimicrobial activity. The antimicrobial effects of honey operate during the wound healing stages (inflammatory, proliferative, and maturation phases), which Carbapenem-resistant Klebsiella pneumoniae and Escherichia coli have colonized.

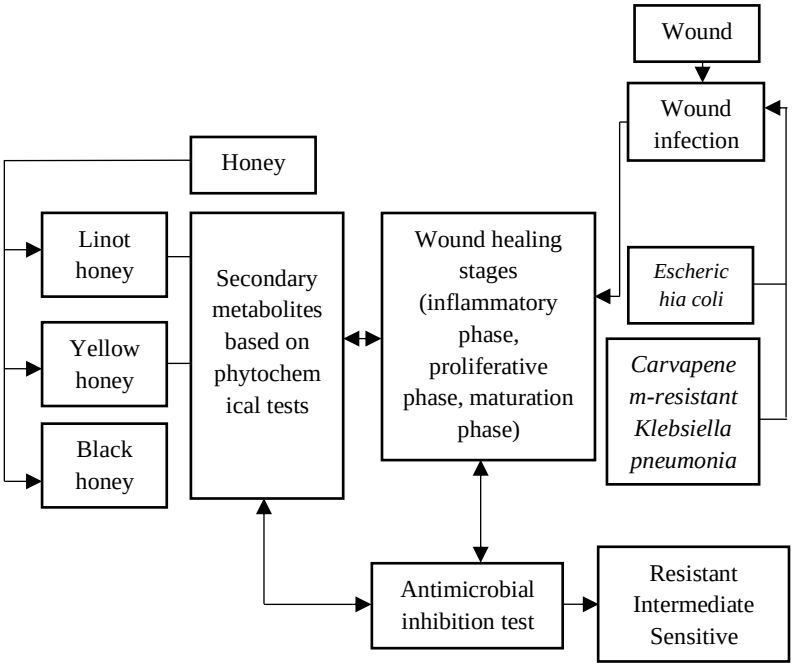

Figure 1. Theoretical Framework

The assessment of antimicrobial activity is conducted by performing an antimicrobial inhibition test, which is then interpreted into categories of resistant, intermediate, and sensitive.

Honey's Inhibition Zone Against Test Bacteria

The results of the measurements, expressed in millimeters, are classified as follows: i) <6 mm indicates no activity (-); ii) 6-10 mm indicates weak activity; iii) 11-20 mm indicates moderate activity; and iv) 21-30 mm indicates strong activity.

Differences in the Antibacterial Effectiveness of Honey

The differences in the antibacterial effectiveness of honey in this study are evaluated based on the type of

honey with the same fraction. The Shapiro-Wilk normality test shows that all numerical variable data are normally distributed. The difference in mean inhibition zones among the test groups is analyzed using one-way ANOVA, followed by post hoc analysis to determine differences between the test groups.

RESULTS AND DISCUSSION

Characteristics of Honey

The color characteristics of Linot honey, non-Linot yellow honey, and non-Linot black honey used in this study are presented in Figure 1 below—the three types of honey range in color from yellowish to dark brown. Linot honey has a brownish color, non-Linot yellow honey is yellowish, and non-Linot black honey is dark brown.

A) B) C)

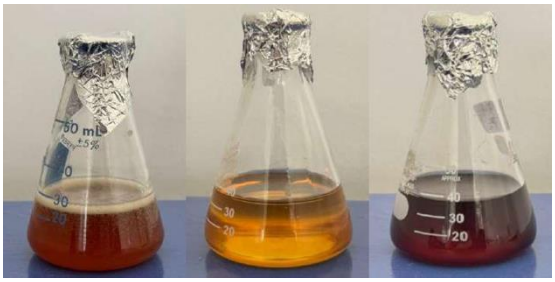

Figure 2. Macroscopic Appearance of Honey Used in This Study
(a) Linot honey, (b) Non-Linot yellow honey, (c) Non-Linot black honey

The evaluation of honey characteristics in this study is presented in Table 1. Linot honey in this study is produced by the species *Heterotrigona itama*, while non-Linot yellow honey and non-Linot black honey are produced by forest bees.

Table 1.
Characteristics of Honey

	Linot Honey	Non-Linot Yellow Honey	Non-Linot Black Honey
Color	Brownish	Yellowish	Dark brown
Taste	Sweet and sour	Sweet	Bitter
Aroma	Floral fragrance	Fragrant	Fragrant
pH	3.6	3.9	3.4

The taste test revealed that Linot honey has a sweet and sour taste, non-Linot yellow honey has a sweet taste, and non-Linot black honey has a bitter taste. The aroma test showed that Linot honey has a floral fragrance, while non-Linot yellow and black honey has a general fragrant aroma. The acidity test, measured as the potential of hydrogen (pH), indicated that non-Linot black honey has the highest acidity level with a pH of 3.4.

Phytochemical Screening Test

The secondary metabolites identified through phytochemical screening are presented in Table 2 below. The screening used qualitative methods. The results indicate that Linot honey contains three secondary metabolites: flavonoids, alkaloids, and tannins. In contrast, non-Linot yellow and black honey contains only flavonoids.

Table 2.
Phytochemical Screening Results

Parameter	Linot Honey	Non-Linot Yellow Honey	Non-Linot Black Honey
Saponin	-	-	-
Tannin	+	-	-
Alkaloid	+	-	-
Flavonoid	+	+	+
Steroid	-	-	-

Test of Inhibition Zone Differences for Ethanol Extracts of Honey Against CRKP

In the ethanol fraction, Non-Linot Yellow Honey exhibited the best inhibition zone among the three types of honey. The significance of the mean differences in the inhibition zones formed by different honey types with the ethanol fraction is presented in Table 3.

Table 3.
Differences in Antibacterial Effectiveness of Ethanol Extracts of Honey Against CRKP

Solvent	Mean ± SD	P-value
Linot Honey	6.00±0.00	<0.001
Non-Linot Yellow Honey	7.74±0.11	
Non-Linot Black Honey	6.00±0.00	
KP	11.82±0.41	

Table 3 shows a significant difference in the mean inhibition zones of honey with ethanol fractions for at least two test groups ($p<0.05$).

Table 4.
Post Hoc Test of Antibacterial Effectiveness of Ethanol Extracts of Honey Against CRKP

Bacteria	Comparison	p-value
CRKP	Linot Honey vs Non-Linot Yellow Honey	<0.001
	Linot Honey vs Non-Linot Black Honey	>0.05
	Linot Honey vs KP	<0.001
	Non-Linot Yellow Honey vs Non-Linot Black Honey	<0.001
	Non-Linot Yellow Honey vs KP	<0.001
	Non-Linot Black Honey vs KP	<0.001

The Bonferroni post hoc analysis (Table 4) indicates that the mean inhibition zones between test groups are significantly different ($p<0.05$) except for the Linot Honey vs Non-Linot Black Honey comparison ($p>0.05$).

Test of Antibacterial Activity of Ethyl Acetate Extract of Honey Against CRKP Bacteria

Linot Honey exhibited the highest inhibition zone in the ethyl acetate fraction compared to the other types of honey. The significance of the average inhibition zones formed by different types of honey with the ethyl acetate fraction is presented in Table 5.

Table 5.
The difference in Antibacterial Effectiveness of Ethyl Acetate Honey Extracts Against CRKP Bacteria

Solvent	Mean ± SD	p-value
Linot Honey	25.64±0.20	<0.001
Non-Linot Yellow Honey	7.81±0.70	
Non-Linot Black Honey	6.00±0.00	
KP	11.82±0.41	

* One Way Anova Test

Table 5 indicates a significant difference in the mean inhibition zones among Linot Honey, Non-Linot Yellow Honey, Non-Linot Black Honey, and the positive control when using the ethyl acetate fraction ($p<0.05$).

Table 6.
Post Hoc Test of Antibacterial Effectiveness of Ethyl Acetate Honey Extracts Against CRKP Bacteria

Bacteria	Comparison	P-value
CRKP	Linot Honey vs Non-Linot Yellow Honey	<0.001
	Linot Honey vs Non-Linot Black Honey	<0.001
	Linot Honey vs KP	<0.001
	Non-Linot Yellow Honey vs Non-Linot Black Honey	0.001
	Non-Linot Yellow Honey vs KP	<0.001
	Non-Linot Black Honey vs KP	<0.001

The Bonferroni post hoc analysis (Table 6) indicates significant differences in the mean inhibition zones among the test groups ($p<0.05$).

Test of Inhibition Zone Differences for n-Hexane Honey Extracts Against CRKP Bacteria

In the n-hexane fraction, Non-Linot Yellow Honey showed the best inhibition zone among the other two types of honey. The significance values for the differences in mean inhibition zones formed by various types of honey with the n-hexane fraction are presented in Table 7.

Table 7.
Differences in Antibacterial Effectiveness of n-Hexane Honey Extracts Against CRKP Bacteria

Solvent	Mean ± SD	P-value
Linot Honey	6.00±0.00	<0.001
Non-Linot Yellow Honey	6.00±0.00	
Non-Linot Black Honey	6.00±0.00	
KP	11.82±0.41	

*One Way Anova Test

Table 7 shows a significant difference in the mean inhibition zones formed by Linot Honey, Non-Linot Yellow Honey, Non-Linot Black Honey, and the positive control using the n-hexane fraction ($p < 0.05$).

Table 8.
Post Hoc Analysis of Antibacterial Effectiveness of n-Hexane Honey Extracts Against CRKP Bacteria

Bacteria	Comparison	p-value
CRKP	Linot Honey vs Non-Linot Yellow Honey	0.963
	Linot Honey vs Non-Linot Black Honey	0.996
	Linot Honey vs KP	<0.001
	Non-Linot Yellow Honey vs Non-Linot Black Honey	0.995
	Non-Linot Yellow Honey vs KP	<0.001
	Non-Linot Black Honey vs KP	<0.001

Tamhane's post hoc analysis (Table 8) indicates significant differences in the mean inhibition zones between Linot Honey, Non-Linot Yellow Honey, Non-Linot Black Honey, and the positive control with p-values <0.05.

Test of Differences in Inhibition Zones of Pure Honey Against CRKP Bacteria

In pure honey, Linot Honey demonstrated the best inhibition zone compared to the other two types of honey. The significance values for the differences in the mean inhibition zones formed by various types of pure honey are presented in Table 9.

Table 9.
The difference in Antibacterial Effectiveness of Pure Honey Against CRKP Bacteria

Solvent	Mean ± SD	P-value
Linot Honey	8.45±0.60	<0.001
Non-Linot Yellow Honey	7.16±0.33	
Non-Linot Black Honey	6.00±0.00	
KP	11.82±0.41	

Table 9 shows significant differences in the mean inhibition zones formed by Linot Honey, Non-Linot Yellow Honey, Non-Linot Black Honey, and the positive control ($p<0.05$).

Table 10.
Post Hoc Test for Antibacterial Effectiveness of Pure Honey Against CRKP Bacteria

Bacteria	Comparison	P-value
CRKP	Linot Honey vs Non-Linot Yellow Honey	0.008
	Linot Honey vs Non-Linot Black Honey	<0.001
	Linot Honey vs KP	<0.001
	Non-Linot Yellow Honey vs Non-Linot Black Honey	0.021
	Non-Linot Yellow Honey vs KP	<0.001
	Non-Linot Black Honey vs KP	<0.001

The Bonferroni post hoc analysis (Table 10) shows that there are significant differences in the mean inhibition zones between the test groups (p<0.05).

Test of Differences in Inhibition Zones of Ethanol Extracts of Honey Against E. coli Bacteria

In the ethanol fraction, Non-Linot Black Honey showed the highest inhibition zone among the three types of honey. The significance values for the differences in the mean inhibition zones formed by various types of honey with the ethanol fraction are presented in Table 11.

Table 11
Differences in Antibacterial Effectiveness of Ethanol Extract of Honey Against E. coli Bacteria

Solvent	Mean ± SD	P-value
Linot Honey	6.00±0.00	<0.001
Non-Linot Yellow Honey	6.00±0.00	
Non-Linot Black Honey	12.47±0.18	
KP	15.30±0.30	

*One Way Anova Test

Table 11 shows significant differences in the mean inhibition zones formed by Linot Honey, Non-Linot Yellow Honey, Non-Linot Black Honey, and the positive control using ethanol extract (p<0.05).

Table 12.
Post Hoc Test of Antibacterial Effectiveness of Ethanol Extract of Honey Against E. coli Bacteria

Bacteria	Comparison	P-value
E. coli	Linot Honey vs Non-Linot Yellow Honey	>0.05
	Linot Honey vs Non-Linot Black Honey	<0.001
	Linot Honey vs KP	<0.001
	Non-Linot Yellow Honey vs Non-Linot Black Honey	<0.001
	Non-Linot Yellow Honey vs KP	<0.001
	Non-Linot Black Honey vs KP	<0.001

Bonferroni post hoc analysis (Table 12) indicates significant differences in mean inhibition zones between test groups (p<0.05), except between Linot Honey and Non-Linot Yellow Honey (p>0.05).

Test of Differences in Inhibition Zones of Ethyl Acetate Extracts of Honey Against E. coli Bacteria

In the ethyl acetate fraction, Linot Honey showed the highest inhibition zone among the three types of honey. The significance values for the differences in the mean inhibition zones formed by various types of honey with the ethyl acetate fraction are presented in Table 13.

Table 13.
Differences in Antibacterial Effectiveness of Ethyl Acetate Extracts of Honey Against E. coli Bacteria

Solvent	Mean ± SD	P-value
Linot Honey	25.07±0.05	<0.001
Non-Linot Yellow Honey	7.23±0.08	
Non-Linot Black Honey	15.35±0.82	
KP	15.30±0.30	

*One Way Anova Test

Table 13 shows a significant difference in the mean inhibition zones formed by Linot Honey, Non-Linot Yellow Honey, Non-Linot Black Honey, and the positive control using ethyl acetate extract (p<0.05).

Table 14.
Post Hoc Test of Antibacterial Effectiveness of Ethyl Acetate Extracts of Honey Against E. coli Bacteria

Bacteria	Comparison	P-value
E. coli	Linot Honey vs Non-Linot Yellow Honey	<0.001
	Linot Honey vs Non-Linot Black Honey	<0.001
	Linot Honey vs KP	<0.001
	Non-Linot Yellow Honey vs Non-Linot Black Honey	<0.001
	Non-Linot Yellow Honey vs KP	<0.001
	Non-Linot Black Honey vs KP	<0.001

Post hoc Bonferroni analysis (Table 14) indicates that there are significant differences in the mean inhibition zones among all honey groups (p<0.05).

Test of Differences in Inhibition Zones of n-Hexane Extracts of Honey Against E. coli Bacteria

In the n-hexane fraction, Non-Linot Black Honey exhibited the highest inhibition zone among the types of honey tested. The significance values for the differences in the mean inhibition zones formed by various types of honey with the n-hexane fraction are presented in Table 15.

Table 15.
Differences in Antibacterial Effectiveness of Hexane Extracts of Honey Against E. coli Bacteria

Solvent	Mean ± SD	P-value
Linot Honey	6.00±0.00	<0.001
Non-Linot Yellow Honey	6.00±0.00	
Non-Linot Black Honey	6.00±0.00	
KP	15.30±0.30	

*One Way Anova Test

Table 15 shows a significant difference in the mean inhibition zones among Linot Honey, Non-Linot Yellow Honey, Non-Linot Black Honey, and the positive control using hexane fraction ($p<0.05$).

Table 16.
Post Hoc Analysis of Antibacterial Effectiveness of Hexane Extracts of Honey Against E. coli Bacteria

Bacteria	Comparison	P-value
E. coli	Linot Honey vs Non-Linot Yellow Honey	>0.05
	Linot Honey vs Non-Linot Black Honey	>0.05
	Linot Honey vs KP	<0.001
	Non-Linot Yellow Honey vs Non-Linot Black Honey	>0.05
	Non-Linot Yellow Honey vs KP	<0.001
	Non-Linot Black Honey vs KP	<0.001

Post Hoc Bonferroni analysis (Table 16) shows significant differences in the mean inhibition zones ($p<0.05$) for Linot Honey, Non-Linot Yellow Honey, and Non-Linot Black Honey compared to the positive control.

Test of Differences in Inhibition Zones of Honey Without Solvent Extracts Against E. coli Bacteria

Non-Linot Black Honey showed the highest inhibition zone among the honey types tested in the pure honey samples. The significance values for the differences in the mean inhibition zones formed by various pure honey samples are presented in Table 17.

Table 17.
Differences in Antibacterial Effectiveness of Pure Honey Against E. coli Bacteria

Solvent	Mean ± SD	P-value
Linot Honey	8.63±0.20	<0.001
Non-Linot Yellow Honey	6.00±0.00	
Non-Linot Black Honey	12.41±0.25	
KP	15.30±0.30	

*One Way Anova Test

Table 17 shows significant differences in the mean inhibition zones formed by Linot Honey, Non-Linot Yellow Honey, Non-Linot Black Honey, and the positive control ($p<0.05$).

Table 18.
Post Hoc Test of Antibacterial Effectiveness of Pure Honey Against E. coli Bacteria

Bacteria	Comparison	P-value
E. coli	Linot Honey vs Non-Linot Yellow Honey	<0.001
	Linot Honey vs Non-Linot Black Honey	<0.001
	Linot Honey vs KP	<0.001
	Non-Linot Yellow Honey vs Non-Linot Black Honey	<0.001
	Non-Linot Yellow Honey vs KP	<0.001
	Non-Linot Black Honey vs KP	<0.001

Bonferroni post hoc analysis (Table 18) indicates significant differences in mean inhibition zones ($p<0.05$) among all honey groups.

Indonesia is internationally recognized as a country rich in biodiversity, including a wide variety of stingless and stinged honey bees, resulting in diverse types of honey. Treating infections caused by antibiotic-resistant bacteria requires natural remedies (traditional medicine), generally considered safer than modern drugs (Purnomo et al., 2023). One natural substance empirically believed to have numerous benefits and is relatively safe is honey. The antibacterial activity of honey has been known since the 19th century. Honey has demonstrated various antibacterial properties against different wound pathogens. The antibacterial substances in honey work together, enhancing each other's activity and producing a synergistic effect on various bacterial pathogens (Salosso, 2021).

Honey Characteristics

Different types of honey typically originate from different flowering plants. Physical and chemical composition differences are commonly found depending on the type of honey. This variation is caused by several factors, such as climatic conditions, the maturation phase, bee species, the type of flowering plants, and the processing and storage of the product, all of which affect its quality (Triwanto et al., 2021).

Color, Taste, and Aroma of Honey

Honey is a seasonal product derived from different plants and geographical regions. Due to seasonal and environmental factors, the content of carbohydrates, volatile compounds, vitamins, minerals, phytochemicals, and other components can vary (Ardiana & Widjaja, 2022). Physical properties such as pH, acidity, viscosity, electrical conductivity, and color also depend on the type of honey. Additionally, processing methods (e.g., filtering and heating) and storage affect the composition and properties of honey. The characteristics of honey are largely determined by the type of flower that serves as the nectar source. Each flower can produce honey with different colors, tastes, and aromas (Amperawati, 2022). Using the senses, characterizing honey involves testing its taste, aroma, and color. In this study, Linot honey has a brownish color, a sour, pungent taste, and aroma. Non-Linot yellow honey has a yellowish color, a bitter taste, and a typical honey aroma. In contrast, non-Linot black honey has a dark brown color with honey's characteristic taste and aroma (Chamidah Ardila Putri, 2019).

In nature, honey can be found in various colors, ranging from light yellow to black, and in some extreme cases, it can even be green or red. The color variation in honey is generally influenced by various factors such as the nectar source (the mineral composition of the soil

where the nectar plants grow), bee habitat, production process, temperature, duration of honey storage, and more (Pramudi et al., 2021). The flower source and changes largely determine the color of honey according to seasonal transformations in the composition of the plant community throughout the year. While honey color is related to the production process, temperature, and storage conditions, the most significant determining factor is the flower source (Leksono, 2017).

Honey contains various aliphatic and aromatic acids. Aromatic acids in honey are significant contributors to its flavor. Both free and bound aromatic acids have been reported in various monofloral kinds of honey (from a single type of flower). Their presence can be used to describe the honey's floral source. Aroma, taste, and color are important quality criteria for honey. These three factors can serve as indicators of the plant source. Honey can range from very pale yellow, yellow, and reddish yellow to nearly black. This is a result of the flower nectar type, primarily associated with the presence of carotenoids, chlorophyll, and plant phenolic compounds. Pollen grains present in honey also affect its color. Additionally, dark honey is closely related to its mineral content (Ladaywa, 2019).

The color of honey is closely related to its plant origin and is an important parameter for evaluating honey quality. Honey color is generally related to sensory properties such as taste and smell and can provide information about its floral source, mineral content, and storage conditions. The mineral content of honey is reflected in its color; darker honey contains more minerals than lighter-colored honey. Generally, darker honey has higher mineral and antioxidant content than lighter honey (Thohari, 2018).

Melon honey and dark nectar honey (dark multifloral, buckwheat, heather) have been reported to exhibit higher antibacterial activity against Escherichia coli compared to light nectar honey (acacia, linden). It has also been found that long-term storage of honey at room temperature in a dark place results in resistance of Bacillus subtilis to the antibacterial action of honey and a loss of some sensitivity of Escherichia coli. A correlation between different honey colors and honey's antioxidant activity has also been observed. Farsi et al. noted that flavonoid levels are also related to the color spectrum of honey. In this study, all three types of honey contain flavonoids despite their different colors. This is suspected to be due to varying concentrations of flavonoids in the honeys. Farsi et al.'s research shows that higher flavonoid content in honey corresponds to darker honey color. Pearson correlation analysis indicates a significant positive correlation between honey color and concentrations of polyphenols and flavonoids, with increased color intensity correlating with higher levels of polyphenols and flavonoids ($p<0.001$). The increase in color intensity is strongly associated with antioxidant properties and phenolic content.

The organic acids present in honey can influence its taste and aroma. Similar to Erwan's research, honey produced by Trigona species generally has a sweet and slightly sour taste, distinguishing it from honey produced by Apis species, which has a purely sweet taste. The sour taste commonly found in Linot honey indicates that it contains ascorbic acid or vitamin C. Honey taste can also be related to the nectar source. A bitter taste in honey can be due to nectar from plants with a bitter taste, such as the Pelawan tree, which has a slightly bitter flavor. Additionally, the sweetness of honey refers to sugar content, such as sucrose—the more bitter the honey, the lower the sucrose content, and vice versa. Furthermore, a bitter taste in honey, such as in non-Linot black honey, is generally associated with high antioxidant content. Therefore, the researcher assumes that the color, taste, and aroma variations of the honeys in this study may be due to differences in nectar source characteristics and the bees producing the honey.

pH Levels

The pH level of honey highly depends on the amount of amino acids and fatty acids secreted by bees. Additionally, pH levels can affect honey's texture, taste, and shelf life. The pH value of honey may be influenced by the nectar and the pH of the soil, as well as by the relationship between plants contributing to the composition of honey. Acidity in honey is a crucial parameter in determining its quality. pH levels are an important component of honey's taste and aroma. The low pH of honey can inhibit the presence and growth of microorganisms. Honey naturally has a very acidic pH, typically between 3 and 4, which can inhibit the growth of bacteria and other spoilage organisms. Historically, formic acid has been considered a primary acid in honey. Today, it is known that honey contains various organic acids. In addition to formic acid, honey also contains oxalic acid, butyric acid, citric acid, 2,3-dihydroxybutanedioic acid, malic acid, pyroglutamic acid, lactic acid, benzoic acid, maleic acid, gluconic acid, isobutyric acid, succinic acid, pyruvic acid, α-ketoglutaric acid, and glycolic acid.

Bacteria are sensitive to the concentration of hydrogen ions in their environment, making pH an important factor in bacterial inhibition. Honey's acidic pH can denature bacterial cells, thereby hindering bacterial growth, as the optimal pH for bacterial growth is typically between 6 and 8. In this study, the highest acidity (pH) was observed in non-Linot black honey (pH = 3.4), followed by Linot honey (pH = 3.6), and non-Linot yellow honey (pH = 3.9). Honey produced by Trigona bees generally has an acidity range with pH values between 3.05 and 4.55. Rozykulyyeva et al., in their research on wild forest honey, reported an acidity level of pH 3.75±0.02 (May honey) and 3.44±0.01 (wild forest honey from Jambi). Based on this literature, it can be assumed that all three types of honey have antibacterial potential related to their acidity levels.

Nasri's research indicates that Trigona honey has varying pH values ranging from 2.99 to 3.33. The acidity of honey is significantly influenced by its water content and the composition of the plant vegetation that serves

as the bees' forage. Honey can help suppress the growth of certain bacteria through several mechanisms; for instance, high acidity reduces bacterial growth and viability, causing bacteria to die. Acidity substantially impacts bacterial growth; when pH drops to its lowest limit, bacterial cells not only cease to grow but also lose their ability to survive. High acidity in honey increases the concentration of hydrogen ions, which can disrupt the transmembrane proton gradient of bacterial cells.

Secondary Metabolite Content

In this study, Linot honey contained the highest amount of secondary metabolites compared to the other types of honey. Linot honey showed the presence of secondary metabolites such as tannins, alkaloids, and flavonoids, whereas non-Linot yellow and non-Linot black honey only contained flavonoids. The type of secondary compounds present in honey can be influenced by factors such as the vegetation of nectar sources, the geographical location or origin of the honey, and various other factors.

The activity of honey is attributed to its phenolic compounds, which have antioxidant properties that can scavenge free radicals, prevent damage to living cells, and reduce oxidative damage from reactive oxygen species. Factors like hyperosmolarity, acidity, and the ability to produce hydrogen peroxide can enhance honey's therapeutic activity. Phytochemical tests on Trigona honey reveal a range of secondary metabolites, including phenolics, saponins, tannins, flavonoids, steroids, alkaloids, and triterpenoids. Nova et al. also found that phytochemical analysis of stingless bee honey identified alkaloids, steroids, triterpenoids, saponins, quinones, and phenolics.

In *Melghat* (wild honey) samples, secondary metabolites such as glycosides, saponins, steroids, tannins, phenols, carbohydrates, proteins, and flavonoids were identified. For honey from Apis spp., compounds such as saponins, alkaloids, flavonoids, tannins, phenols, glycosides, carbohydrates, proteins, and steroids were found. Fadhmi et al. also identified terpenoids and saponins in forest honey. Adalina also discovered saponins, phenols, and flavonoids in honey samples produced by *Apis dorsata*.

Antibacterial Activity of Honey

Honey exhibits both bactericidal and bacteriostatic activities against Gram-positive and Gram-negative bacteria. Its antibacterial mechanisms include inhibiting cell wall synthesis, disruption of cell membranes, interference with protein synthesis, and inhibiting nucleic acid synthesis. Osmotic effects, acidity, and the presence of peroxide and non-peroxide compounds also influence the antibacterial properties of honey. Osmotic effects arise from honey's high sugar and low water content, creating an environment that inhibits bacterial growth. Peroxide compounds, such as hydrogen peroxide, induce oxidative stress that controls bacterial colonization in wound areas. Non-peroxide compounds, including phytochemical metabolites, contribute to honey's antioxidant and antimicrobial activities. These components work synergistically, enabling honey to combat a variety of microorganisms. The quality of the nectar source also affects the composition of honey and its antibacterial activity. In this study, the antibacterial activity of different types of honey was assessed against Gram-positive (CRKP) and Gram-negative (E. coli) bacteria by measuring the diameter of inhibition zones formed around the bacterial growth media wells.

Antibacterial Activity of Honey Against CRKP Bacteria

The study found that the ethyl acetate fraction of Linot honey exhibited the highest inhibition zone against CRKP bacteria (25.64±0.20 mm), surpassing the positive control with tobramycin 10 µg (11.82±0.41 mm) ($p<0.001$). Other studies have reported various antibacterial activities of different kinds of honey, such as Nigerian local honey with an inhibition zone of 22±3.1 mm against Klebsiella pneumoniae, and Manuka honey, showing inhibition zones of 7.47±0.82 mm and 17.4 mm, respectively. Australian *Trigona carbonaria* honey has also demonstrated broad-spectrum activity against Gram-positive and Gram-negative bacteria, with its strong acidic environment potentially contributing to its robust antibacterial properties. Additionally, honey's compounds like flavonoids, antimicrobial peptides, and hydrogen peroxide contribute to its antimicrobial activity. Linot honey, in particular, is known for its high antioxidant and anti-inflammatory activity due to its phenolic and flavonoid content. Flavonoids in honey can enhance bacterial membrane permeability and reduce cell resistance, while other uncharacterized compounds may stimulate the body's immune response to infection.

Antibacterial Activity of Honey Against E. coli Bacteria

The study identified that the ethyl acetate fraction of Linot honey produced the highest inhibition zone against E. coli (25.07±0.05 mm), followed by the ethyl acetate fraction of non-Linot black honey (15.35±0.82 mm). The ethyl acetate fraction of Linot honey showed a larger inhibition zone compared to the positive control with gentamicin 10 µg (15.30±0.30 mm) ($p<0.001$). Previous studies have also shown the antibacterial potential of honey against E. coli, with Trigona honey and wild honey demonstrating significant antibacterial activity against E. coli growth in vitro. The antibacterial potency of honey is thought to be related to its organic compound content, such as flavonoids, which work by disrupting protoplasm, damaging cell walls, and inhibiting enzyme biosynthesis. Additionally, honey's ability to convert glucose into hydrogen peroxide may inhibit E. coli growth by altering protein properties and disrupting nucleic acid synthesis in the bacteria.

CONCLUSION

There are differences in the antibacterial activity of ethanol, ethyl acetate, and n-hexane fractions, as

well as solvent-free extracts of Linot honey, non-Linot yellow honey, and black honey against Carbapenem-resistant Klebsiella pneumoniae and Escherichia coli, two bacteria causing wound infections.

REFERENCES

Amir, A. M. (2023). *Pembuatan Nutrien Dan Media Bokashi Berbahan Organik Serta Aplikasinya Pada Pertumbuhan Tanaman Hias Sirih Philodendron Hederaceum Lemon*. Fkip Unpas. [Publisher]

Amperawati, S. (2022). Bab 3 Karakteristik Fisik Hasil Pertanian. *Teknologi Pertanian*, 27. [Publisher]

Andi Kayzar, A. K. (2023). *Gambaran Tingkat Pengetahuan, Sikap, Dan Perilaku Dokter Dalam Upaya Pencegahan Resistensi Antibiotik Di Kabupaten Bone, Soppeng, Dan Wajo= Doctor's Knowledge, Attitude, And Practice In Preventing Antibiotic Resistance In Bone, Soppeng, And Wajo*. Universitas Hasanuddin.

Ardiana, M., & Widjaja, N. A. (2022). *Panduan Pola Diet Sehat Untuk Pasien Dengan Gangguan Kardiovaskular*. Airlangga University Press. [Publisher]

Chamidah Ardila Putri, C. (2019). *Uji Kandungan ß-Karoten Dan Uji Daya Terima Pada Pembuatan Minuman Instan Daun Kemangi (Sanctum Ocimum L) Dengan Jahe (Zingiber Officinale)*. Institut Teknologi Sain Dan Kesehatan Pku Muhammadiyah Surakarta.

David, S., Reuter, S., Harris, S. R., Glasner, C., Feltwell, T., Argimon, S., Abudahab, K., Goater, R., Giani, T., & Errico, G. (2019). Epidemic Of Carbapenem-Resistant Klebsiella Pneumoniae In Europe Is Driven By Nosocomial Spread. *Nature Microbiology*, *4*(11), 1919–1929. [Crossref], [Publisher]

Ekawati, E. R., & Herawati, D. (2018). Identifikasi Kuman Pada Pus Dari Luka Infeksi Kulit. *Jurnal Sainhealth*, *2*(1), 31–35. [Crossref], [Publisher]

Hamdani, M. J. (2022). *Deteksi Fenotip Isolat Bacteria Klebsiella Pneumoniae Resisten Karbapenem Penghasil Enzim Metallo Beta Laktamase Dengan Metode Tes Sinergi Disk Ganda (Ddst) Pada Pasien Infeksi Di Rsup Dr Wahidin Sudirohusodo Makassar Tahun 2019*. Universitas Hasanuddin.

Ladaywa, A. (2019). *Optimasi Kualitas Kimia & Sensori Produk Minuman Herbal Berbasis Daun Sirih*. Unika Soegijapranata Semarang. [Publisher]

Leksono, A. S. (2017). *Ekologi Arthropoda*. Universitas Brawijaya Press. [Publisher]

Pramudi, M. I., Soedijo, S., Rosa, H. O., & Aphrodyanti, L. (2021). Dasar-Dasar Ekologi Serangga. *Banjarbaru: Cv. Banyubening Cipta Sejahtera*. [Publisher]

Primadina, N., Basori, A., & Perdanakusuma, D. S. (2019). Proses Penyembuhan Luka Ditinjau Dari Aspek Mekanisme Seluler Dan Molekuler. *Qanun Medika-Medical Journal Faculty Of Medicine Muhammadiyah Surabaya*, *3*(1), 31–43. [Crossref], [Publisher]

Priscilla, L. (2017). Perbandingan Efektivitas Aplikasi Topikal Lidah Buaya (Aloe Vera), Madu (Apis Mellifera), Dan Kombinasi Keduanya Terhadap Penyembuhan Luka Sayat Pada Kulit Mencit (Mus Musculus).(Skripsi Sarjana, Universitas Hasanuddin, Makassar, Indonesia). *Skripsi, Fakultas Kedokteran Gigi, Universitas Hasanudin. Makassar*. [Publisher]

Pujiarti, R., Amin, A., Ngadianto, A., Septiana, R. M., Purba, B. A. V., & Permadi, D. B. (2021). Quality Of Three Forest Honey Types From Baduy Tribe, Lebak District, Banten Province. *Jurnal Ilmu Kehutanan*, *15*(2), 123–136. [Crossref], [Publisher]

Purnomo, H., Puspitaloka, D., Junandi, B., Juniyanti, L., & Dharmawan, I. W. S. (2023). *Pembelajaran Dari Aksi Restorasi Gambut Berbasis Masyarakat Di Indonesia Dan Asia Tenggara*. Cifor. [Publisher]

Salosso, Y. (2021). *Potensi Madu Lokal Asal Pulau Timor Sebagai Antibacteria Pada Ikan*. Deepublish.

Sekar Feni, W. (2023). *Identifikasi Bacteria Methicilin-Resistant Staphylococcus Aureus (Mrsa) Dan Vancomicyn-Resistant Staphylococcus Aureus (Vrsa) Pada Pasien Infeksi Luka Operasi (Ilo) Di Rsud Dr. H. Abdul Moeloek Bandar Lampung*. [Publisher]

Susanto, A. (2017). *Terapi Madu*. Niaga Swadaya. [Publisher]

Thohari, I. (2018). *Teknologi Pengolahan Dan Pengawetan Telur*. Universitas Brawijaya Press. [Publisher]

Triwanto, J., Herlinda, K., & Muttaqin, T. (2021). Kualitas Fisikokimia Pada Madu Dari Nektar Bunga Randu (Ceiba Pentandra) Dan Kaliandra (Calliandra Callothyrsus). *Journal Of Forest Science Avicennia*, *4*(2), 102–113. [Crossref], [Publisher]

Wahyudi, B., Setiawati, E. P., Shahib, N., & Wirakusumah, F. F. (2018). Perbedaan Antara Faktor Intrinsik Dan Ekstrinsik Pada Pasien Infeksi Nosokomial Di Bagian Bedah Dan Medikal Rsup Dr. Hasan Sadikin Bandung. *Majalah Kedokteran Bandung*, *50*(4), 235–239. [Crossref], [Publisher]