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Redaksi mengundang berbagai ilmuwan dari berbagai lembaga pendidikan tinggi maupun penelitian untuk memberikan sumbangan ilmiahnya, baik berupa hasil penelitian maupun kajian ilmiah mengenai berbagai topik Kesehatan dan Imu Kedokteran.

Redaksi sangat mengharapkan masukan-masukan dari para pembaca, profesional bidang kedokteran, atau yang terkait dengan penerbitan, demi makin meningkatnya kualitas jurnal sebagaimana harapan kita bersama.

Redaksi berharap semoga artikel-artikel ilmiah yang termuat dalam Jurnal Ilmiah Kedokteran Wijaya Kusuma (JIKW) bermanfaat bagi para akademisi, peneliti dan profesional yang berkecimpung dalam dunia Kedokteran.

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Characteristics of Indonesian Wild honey and Cultivited Honey and Their Antibacterial Activity against Staphylococcus aureus and Escherichia coli

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Abstract

Indonesia's natural wealth is very abundant in the form of flora and fauna that can be developed as raw materials for medicine. Honey in Indonesia is very diverse from Sabang to Merauke. Different types of honey are influenced by regional origin, season at harvest, type of bee, type of plant source of nectar, way of life of bees (cultivated or wild), harvesting method and post-harvest handling. This study aims to determine the characteristics of forest bee honey and cultivated honey and to determine the potential of honey as an antibacterial in the treatment of infectious diseases caused by Staphylococcus aureus and Escherichia Coli bacteria. The materials used were Carisa honey samples from Wild honey [Wild Klanceng and Wild Cerana] and Cultivation [Cerana Cultivation], S. aureus and E. coli bacteria. The characteristic test method is in accordance with the Indonesian National Standard and the honey inhibition test against the growth of S. aureus and E. Coli bacteria using the diffusion method. The results showed that Wild Cerana (WC) and Wild Klanceng (WK) honey demonstrated higher water content, ash content, acidity and glucose from Cerana Cultivated (CC) honey. Carissa Honey (beside Cerana cultivated honey) had antibacterial activity against S. aureus and E. Coli at different concentrations. Wild Wild Honey has the highest antibacterial activity compared to other types of honey. Conclusion Indonesian wild honey showed weak antibacterial activity against Staphylococcus aureus. Meanwhile, honey that is cultivated does not have antibacterial activity.

***Keywords**: antibacterial, Wild honey, cultivated honey*

Original Research Article

Abstrak

Kekayaan alam Indonesia sangat melimpah berupa flora dan fauna yang dapat dikembangkan sebagai bahan baku obat. Madu di Indonesia sangat beragam dari Sabang sampai Merauke. Berbagai jenis madu dipengaruhi oleh daerah asal, musim panen, jenis lebah, jenis tanaman sumber nektar, cara hidup lebah (budidaya atau liar), cara panen dan penanganan pasca panen. Penelitian ini bertujuan untuk mengetahui karakteristik madu lebah hutan dan madu budidaya serta mengetahui potensi madu sebagai antibakteri dalam pengobatan penyakit infeksi yang disebabkan oleh bakteri *Staphylococcus aureus* dan *Escherichia coli*. Bahan yang digunakan adalah sampel madu Carisa dari madu Liar [Klanceng Liar dan Cerana Liar] dan Budidaya [Budidaya Cerana], bakteri S. aureus dan E. coli. Metode uji karakteristik madu disesuaikan dengan Standar Nasional Indonesia dan uji daya hambat madu terhadap pertumbuhan bakteri *S. aureus* dan *E. Coli* dilakukan menggunakan metode difusi. Hasil penelitian menunjukkan bahwa madu Cerana Liar (WC) dan Klanceng Liar (WK) menunjukkan kadar air, kadar abu, keasaman dan glukosa yang lebih tinggi dari madu Cerana Budidaya (CC). Madu Carissa (selain madu budidaya Cerana) memiliki aktivitas antibakteri terhadap *S. aureus* dan *E. Coli* pada konsentrasi yang berbeda. Madu Liar Liar memiliki aktivitas antibakteri paling tinggi dibandingkan dengan jenis madu lainnya. Kesimpulan Madu liar

Indonesia menunjukkan aktivitas antibakteri yang lemah terhadap *Staphylococcus aureus*. Sedangkan madu budidaya tidak memiliki aktivitas antibakteri.

Kata Kunci: antibakteri, Madu liar, madu yang dibudidayakan

INTRODUCTION

Honey is a natural product that has been widely used by people. In addition to its sweet flavour, honey also known to have antibacterial property(Arawwawala and Hewageegana, 2017). Various studies have been conducted over years to investigate the antibacterial activity of honey by determining its minimun inhibitory concentration (MIC), such as the forest honey from Australia and Manuka honey from New Zealand(Sindi et al., 2019). The antibacterial activity of honey is attributed to its active compounds, hydrogen peroxide, high osmolarity, and low pH.

Honey has been used for several kinds of wound treatments (Arawwawala and Hewageegana, 2017)(Greenwood et al., 2012)**.** Manuka honey from New Zealand has been used as standardized medical honey in various researches, yet Manuka honey is costly and not easy to be found in Indonesia. Therefore, it is necessary to conduct research about Indonesian local honey characteristic.

There are various kinds of Indonesian local honey originated from Sabang to Merauke. The diversity of local Indonesian honey could be affected from the different origins, harvest seasons, bees species, nectar source plants, bees way of life (cultivated or wild), harvest methods and honey processing methods after harvest. The different nectar sources would produce the different kinds of honey. The variety of honey could be observed physically by the difference in colour, scents and tastes. The dark-colored honey indicated that the honey is ripe and it contents less water. The darker-colored honey varieties contain higher amounts of antioxidants(Fatma et al., 2017) (Erejuwa et al., 2012).

Staphylococcus aureus and *Escherichia coli* are the most common bacteria species found in sepsis and infected wounds. The bacterial culture sensitivity test in a research discovered that the various bacteria showed the multidrug resitance characteristic in infected wounds and sepsis (Ayub, 2015). The efforts to reduce resitance and microbial production rate are slower than the growth of antibiotic resistance level (WHO, 2014). Therefore, a novel strategy is needed to treat the infections. Honey could be used as complementary medication to reduce the microbial resitance.

Until recenty, the local honey researches were limited to the honey quality assay, thus, the characteristic of cultivated and wild local honey were mostly unidentified. Therefore, this research aims for studying the local Indonesian honey (from cultivated and wild bees) characteristics and the antibacterial activity againts *staphylococcus aureus* and *Escherichia coli.*

MATERIALS AND METHODS

Study period and location

This research was conducted from April 2021 to July 2021 in Biochemistry laboratory, Biochemistry Departement, Medical Faculty, UWKS, Surabaya. Honey samples characterization were conducted in Assessment Service Unit, Faculty of Pharmacy, Airlangga University, Surabaya. Antibacterial assay was conducted in Gastroentritis and Salmonellosis laboratory, ITD, UNAIR Surabaya.

Materials

Carisa honey sample from wild bees [Wild Klanceng (WK) and Wild Cerana(WC)] and cultivated [Cultivated Cerana (CC) and Cultivated Malifera (CM)], Mueller Hinton Agar (MHA), Mueller Hinton Broth (MHB), *Escherichia coli* (*E. coli)* and *Staphylococcus aureus* (*S. aureus*) were used in this research. The equipments used for this research were analytical balance, spectrophotometer, autoclave, moisture balance, calipers, petri dish, glass bottle, volumetric flask, Beaker glass and stirrer.

Honey characterization

Honey characterization consisted of ash content, free acidity, Hydroxy Methyl Furfural, Diastase enzyme, glucose and water content analysis were determined using standard SNI

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8664-2018 methods (Badan Standardisasi Nasional, 2018).

Bacterial Isolate Rejuvenation

E. coli and *S. aureus* bacterial isolates were purchased from Gastroenteristis and Salmonellosis laboratory, ITD, UNAIR Surabaya. The isolates were cultured in NA medium using streak method and incubated for 24 h in the temperature of 37ºC.

***Mueller Hinton Agar (MHA)* Preparation**

A 19 g of MHA powder was weighed and diluted to 500 ml using distilled water in an Erlenmeyer flask. The suspension was homogenized by heating in a hot plate. Then, the medium suspension was sterilized using autoclave (121^{o}C, 15 min). The sterilized medium was poured in a petri dish (±25 ml) and allowed to solidify at room temperature (Utomo et al., 2018).

Mueller Hinton Broth (MHB) Preparation

A 21 g of MHB powder (2 g Beef infusion, 17.5 g Casein hydrolysat were diluted to 1L of distilled water. The medium was heated on a hot plate, and stirred using a magnetic stirrer. The final homogenized medium colour was clear yellow. The medium was sterilized using autoclave (121^{o}C, 15 min), and poured in a sterile micro tube aseptically in LAF (Maarisit et al., 2021).

Bacteria Inoculum Preparation

E.coli and *S.aureus* inoculation were prepared by picking one single colony from NA medium to the Muller Hinton Broth (MHB) tube and were incubated overnight in 37^{o}C. The bacterial cultures were then centrifugated (5000rpm; 5min). The supernatant was separated from the bacterial pellet (precipitate) from each tube. The pellet was resuspended with saline water and adjusted to OD 490=0.5 to be used for inoculum/suspension in every treatment (Debalke et al., 2018 and modification).

Antibacterial Assay

The 0.1 ml of bacterial suspensions were inoculated in MHA using spread method. The 20 µL of honey samples were dropped to the test discs along with the blank solution (control solution) and placed on the MHA medium aseptically. The medium were then incubated in 37^{o}C with reverse position (Debalke et al., 2018 and modification)**.**

Data Analysis

The clear zones around the test discs were measured for diameter using calipers. The data obtained were tabulated and analyzed statistically.

RESULTS

Honey Characterization

Carisa Honey WK, WC, CC and CM were tested for ash content, free acidity, Hydroxy Methyl Furfural, Diastase enzyme, glucose and water content. The characterization results were shown in Table 1.

Table 1. Honey Characteristics

Parameter	SNI 8664-2018	Wild Cerana Honey (WC)	Wild Klanceng Honey (WK)	Cerana Honey (Cultivated)
HMF (mm/kg)	Max 40	4,01 ± 0,06	6,92 ± 0,18	5,32 ± 0,11
Diastase Enzyme (DN)	Min 3	5,11 ± 0,09	4,2 ± 0,12	5,38 ± 0,08
Water Content %(w/w)	Max 22	20,10 ± 0,14	>25	18,3 ± 0,07
Ash Content %(w/w)	Max 0,5	0,46 ± 0,01	1,3 ± 0,01	0,10 ± 0,00
Acidity (ml eq/kg)	Max 50	75,7 ± 0,37	418,9 ± 8,55	61,8 ± 0,11
Glucose (%)	Min 65	18,69 ± 0,32	29,24 ± 0,06	9,29 ± 1,73

Antibacterial Assay against *E. coli* and *S. aureus*

The antibacterial assay was performed using agar diffusion method. The assay used 6 treatment groups and 4 honey samples concentration of 40%, 60%, 80% and 100%. The positive control (chloramphenicol) and negative control (distilled water) were used in this assay. Table 2 and Table 3 demonstrated the inhibition ability of honey samples against *E. coli* and *S. aureus* growth.

Table 2. The average inhibition zone of honey against *S. aureus* growth

Honey samples	The average inhibition zone against *S. aureus* (mm)				C +	C -
	40%	60%	80%	100%		
CC	0^{a}	0^{a}	0^{a}	$1,75 \pm 0,96^{b}$	$21,5 \pm 0,58^{c}$	0^{a}
WC	0^{a}	0^{a}	$2 \pm 0,82^{b}$	$4,25 \pm 1,89^{c}$	$23,5 \pm 1,73^{d}$	0^{a}
WK	$9,18 \pm 0,85^{b}$	$9,55 \pm 1,17^{b}$	$10,1 \pm 0,71^{b}$	$12,33 \pm 1,69^{c}$	$25,93 \pm 1,42^{d}$	0^{a}

Notes: abc superscript consists of different alphabets on the same row implies the significant difference (P<0,05) according to Anova test and Post Hoc LSD (CC: Cultivated Cerana; WC: Wild Cerana; Wild Klanceng; C+: positive Control; C-: Negative Control)

Tabel 3. The average inhibition zone of honey against *Escherichia coli* growth

Honey sample	The average inhibition zone against *Escherichia coli* (mm)				C +	C -
	40%	60%	80%	100%		
CC	0^{a}	0^{a}	0^{a}	0^{a}	$23.3 \pm 1,89^{b}$	0^{a}
WC	0^{a}	0^{a}	0^{a}	$3.28 \pm 0,17^{b}$	$19 \pm 1,16^{c}$	0^{a}
WK	$2.23 \pm 0,86^{a,b}$	$3.33 \pm 0,86^{b}$	$4,10 \pm 1.13^{b}$	$5.68 \pm 1,25^{b}$	$26.25 \pm 3,69^{c}$	0^{a}

Notes: abc superscript consists of different alphabets on the same row implies the significant difference (P<0,05) according to Anova test and Post Hoc LSD (CC: Cultivated Cerana; WC: Wild Cerana; Wild Klanceng; C+: positive Control; C-: Negative Control)

DISCUSSION

Cultivated honey and wild honey are two different types of honey. The cultivated honey are mostly produced from a single area with limited or even single plant variations. The single plant cultivation is frequently called monofloral cultivation. The forest honey cultivation could be produced naturally or intentionally from a wide range of species of plants, called multi-floral cultivation. In this research, Cerana Cultivated honey (CC) were produced from Avocado plant nectare. Bhal chandra et al. (2014) stated that the flowering schedule is influenced by soil type, climate, and vegetation conditions which then affect the quality and quantity of nectar secretion produced (Erejuwa et al., 2012).

Wild Cerana (WC) and Wild Klanceng (WK) honey demonstrated higher water content, ash content, acidity and glucose from Cerana Cultivated (CC) honey as shown in Table 1. These results are in accordance with research in Greece, which stated that Wild/forest multi-floral honey possesed a higher acidity to inhibit the growth of microbes in honey and loaded with minerals, such as calcium (Karabagias et al., 2018).

In the provisions of SNI 8664:2018 honey quality that the maximum HMF content is 40 mg/kg and all types of Carisa Honey have low HMF levels around 4-7.16 mg/Kg below the SNI standard. This shows that the Carissa honey sample used in this study is categorized as fresh honeyn (Boussaid et al., 2018; Sumarlin et al., 2021)

Based on the results of the research conducted, it can be seen that all honey samples have diastase enzyme activity above 3 DN (minimum SNI requirement 3), so the three honeys above are categorized as Qualified. Diastase enzyme itself is an enzyme that converts complex carbohydrates into simple carbohydrates (Adji, 2007).

The water content of honey according to SNI 8664:2018 is a maximum of 22%. Based on Table 1, it shows that each type of honey has a different moisture content, which meet the SNI requirements are wild cerena honey and cultivated cerena honey. The difference in water content of honey is related to climatic conditions and the level of maturity of honey.

Determination of ash content using the Gravimetric method with a maximum ash content of 0.5%. Cultivated Cerana Honey has an ash content of 0.1%, Wild cerena honey is 0.46% while the highest ash content is Klanceng wild honey 1.3% (Table 1). It is possible that the mineral content in Klanceng honey is the most among other honeys. The ash content in honey is influenced by the presence of minerals derived from nectar and bee food sources. According to Setya Sri Antary (2013), various minerals such as potassium (K), sodium (Na), calcium (Ca), magnesium (Mg), iron (Fe), chlorine (Cl),

phosphorus (P), sulfur (S), and iodine (I) and radium salt (Ra) contained in honey. Among these minerals, the most abundant in general are calcium, sodium and potassium (Boussaid et al., 2018)

Based on Table 1, it shows that all types of Carissa honey have low glucose levels below the SNI 8664-2018 standard, which is at least 65%. There are several factors that affect the reducing sugar content of honey, among others, water content, humidity, and harvest time. There are studies that show that the high water content in honey can stimulate yeast activity to grow and develop in honey, thus causing the fermentation process. The yeast that causes fermentation in honey is an osmophilic yeast from the genus Zygosaccharomyces, which is resistant to high sugar concentrations, so it can live and thrive in honey. Yeast in honey will degrade sugar, especially dextrose and levulose into alcohol and CO2, thus affecting the dextrose (glucose) and levulose (fructose) content of honey. (Hariyati, 2010).

The antibacterial properties of honey samples were tested using clear zone measurement around the discs from the diffusion of the antibacterial compounds in solid medium to inhibit the growth of bacteria and were referred as inhibition zone (Perdana and Setyawati, 2017). The inhibition zones were formed due to the potential of honey samples as an antibacterial agent. According to the series of data in Table 2 and 3, in 100% concentration, WC and WK samples were able to inhibit both Gram positive (+) bacteria *S. aureus* and Gram negative (-) bacteria *E. coli*, meanwhile CC sample only inhibited Gram positive (+) bacteria *S. aureus.* It could be concluded that the antibacterial activities of WK > WC > CC consecutively. The potential of honey as an antibacterial agent is attributed to its osmolarity, acidity, pH, high glucose, hydrogen peroxide, and non-hydrogen peroxide compounds such as phenolic acid and flavonoids (Aggad H, 2014)(Kwakman and Zaat, 2012) (Nolan et al., 2019). In this study, the factors that caused the honey to have the highest inhibition were the acidity, pH and high glucose factors.

Klanceng Wild honey (WK) showed the highest antibacterial activity compared to the other samples, due to the highest acidity in WK sample. The acidity in honey is caused by the presence of gluconic acid , that is formed by the reaction of glucose oxidase and glucose (Bittmann et al., 2010). The higher the glucose level is, the higher the acidity. The acidity of honey is caused by the presence of organic acids, especially gluconic acid, pyruvic acid, malic acid and citric acid, as well as inorganic ions, such as phosphate, sulfate, and chloride (Terrab et al., 2003). Honey provides an acid environment that is unfavorable for bacteria to grow and also inhibits most microorganisms activities(Brudzynski et al., 2011). With the higher acidity level in honey, the hydrogen ion concentration is increased. The enhancement in hydrogen ion concentration could interfere the proton transmembrane gradient fom bacterial cells (HARIYATI, 2010).

Cerana Cultivated (CC) honey showed weak activity against Gram-positive bacteria (+) *S. aureus.* The bioactive compounds of CC honey, phenol and flavonoids, are suspected to be at a lower level than the wild honey. Ahmed (2013) stated that total phenolic and flavonoids compounds of commonly cultivated honey were lower compared to the wild honey, that the cultivated honey was ineffective against Gram-negative bacteria (Ahmed and OthmAn, 2013). A phytochemical research in Indonesia also stated that wild honey contains more flavonoids and saponins than the forest and cultivated honey (Yelin and Kuntadi, 2019). The antibacterial mechanism of phenol is by poisoning the protoplasm, breaking and invading the cell wall, then precipitating the microbe cell protein (HARIYATI, 2010).

Table 2 and 3 show that Gram (-) bacteria *E.coli* was insensitive against antribacterial compounds from honey samples, where *E. coli* inhibition zones were narrower than *S. aureus.* It could be caused by *E. coli* as Gram-negative bacteria is equipped with complex cell wall structure, consists of peptidoglican, lipopolysaccharide and periplasmic space. The periplasmic space has more ability to hold the plasma membrane firmly. Meanwhile, *S. aureus* has a thick cell wall consists of peptidoglican only, therefore the antibacterial agent might work effectively to inhibit the bacterial growth (Nur, 2019).

CONCLUSION

Indonesian wild honey showed relatively antibacterial weak against *S. aureus* bacteria.

Cultivated honey possessed minimum antibacterial effect. Both wild honey and cultivated honey showed insignificant antibacterial activity against *E. coli.* The characteristic of honey that might contribute to the antibacterial effect was acidity level and high glucose.

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