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EFEKTIVITAS *MADU HUTAN JAMBI DAN MADU KLANCENG DALAM MENGHAMBAT PERTUMBUHAN Escherichia coli DAN Klebsiella pneumoniae PENGHASIL ESBL*

Effectiveness Of Jambi Forest Honey And Klanceng Honey In Inhibiting Escherichia coli ESBL and Klebsiella pneumonia ESBL

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Abstrak

Resistensi bakteri terhadap antibiotik merupakan permasalahan global yang terjadi di seluruh dunia, baik yang terjadi di rumah sakit maupun di dalam komunitas. Resistensi terhadap antibiotik dapat memengaruhi hasil terapi, biaya terapi, penyebaran penyakit, dan lama sakit. Tujuan dari penelitian ini adalah untuk mengetahui potensi madu hutan Jambi dan Madu Klanceng dalam menghambat pertumbuhan *Escherichia coli* dan *Klebsiella pneumonia* penghasil ESBL. Penelitian ini menggunakan eksperimental laboratorium dengan pendekatan *post test with control*, dilakukan di laboratorium Bakteriologi STIKES Nasional. Hasil penelitian menunjukkan bahwa madu hutan Jambi & madu Klanceng memiliki potensi penghambatan terhadap pertumbuhan *coli* dan *K pneumoniae* ESBL yang lebih baik dibanding kontrol antibiotik (uji rutin beta-laktam) cefotaxime dan ceftriaxone tetapi tidak lebih baik dibanding ceftazidime, sedangkan terhadap kontrol positif (ciprofloxacin untuk *K pneumoniae* dan chloramphenicol untuk *E coli*) sampel madu potensi penghambatannya jauh lebih lemah

Kata Kunci
Madu Hutan Jambi
Madu Klanceng
Escherichia coli
Klebsiella pneumoniae
ESBL

Abstract

Bacterial resistance to antibiotics is a global problem that occurs around the world, both in hospitals and in communities. Antibiotic resistance can affect therapeutic outcomes, therapeutic costs, the spread of disease, and length of illness. The purpose of this study is to find out the potential of Jambi forest honey and Klanceng Honey in inhibiting the growth of ESBL-producing Escherichia coli and Klebsiella pneumonia. This study used experimental laboratory using posttest with control approach, conducted at the STIKES Nasional Bacteriology laboratory. The results showed that Jambi forest honey and Klanceng honey had better potential to inhibit the growth of ESBL-producing Escherichia coli and Klebsiella pneumonia than cefotaxime and ceftriaxone, but less than ceftazidime. On the other hand, honey samples had weaker inhibition potential against positive control (ciprofloxacin for K pneumoniae and chloramphenicol for E coli)..

Keywords :
Jambi Forest Honey
Klanceng Honey
Escherichia coli
Klebsiella pneumoniae
ESBL

INTRODUCTION

Bacterial resistance to antibiotics is a problem that occurs worldwide, both in hospitals and in the community. Research results of Fauziyah, et al (2011) showed that, in the ICU room of Fatmawati Hospital, there were more than 60% of *S. epidermidis*, *E. aerogenes*, *P. aeruginosa*, *Klebsiella sp*, and *Serratia sp*, resistant to ceftriaxone. Infection by bacteria that are resistant to antibiotics can affect the outcome of therapy, cost of therapy, spread of disease, and duration of illness.

Based on the research conducted by Hidayat (2016) related to the germ resistance pattern of patients who were admitted to the ICU and perinatalogy at Dr. H. Abdul Moeloek, it is known that *Klebsiella sp* is the main cause in patient samples from blood, urine, sputum with resistance levels of Cefixime (89%), Cefotaxime (84.2%), Ceftraxone (84%), Chloramphenicol (71%), Ceftrazidime (68%).

Antibiotic susceptibility test showed that germs have the highest resistance to penicillin, amoxicillin, and ampicillin.

The incidence of bacterial resistance to antibiotics is increasing, especially in Asia, including Indonesia. Enterobacteriaceae is the most common cause of infection, especially in the ICU and often causes resistance to third-generation cephalosporin antibiotics as it is able to produce the enzyme beta-lactamase, known as extended-spectrum beta lactamase (ESBL) (Taslim & Maskoen, 2016). Taslim & Maskoen (2016) explained that *Klebsiella pneumonia* from the Enterobacteriacea family was the most common bacteria found in isolates taken in the ICU at Cipto Mangunkusumo Hospital, Jakarta in 2011. In addition, it was found that 58.42% of the isolates was ESBL-producing. The results of this study indicate that the prevalence of beta-lactamase-producing *K. pneumoniae*, especially ESBL, is very high. It is a strong consideration that there is a need for better control of infection as well as appropriate and rational administration of antibiotics.

The increasing incidence of bacterial resistance to antibiotics encourages researchers to look for alternative treatments to find more effective, efficient and safe antimicrobials for the treatment of bacterial infections (Munfaati et al., 2015). Widespread microbial resistance to antibiotics encourages the search for antimicrobial sources from natural ingredients. Natural ingredients are known to have the potential to be further developed in treating infectious diseases (Agustiningsih, 2010). One of the natural sources that can be used as medicine is honey.

Honey is a liquid that has a sweet taste and is produced by honey bees (Apis sp) from floral nectar or other parts of plants (extra floral) (SNI 3545: 2013). The potential of honey as an antibacterial is due to several things including osmotic pressure, hydrogen peroxide, acidity (pH), and phenolic compounds such as flavonoids contained in honey (Garedew, et al., 2003).

The purpose of this study is to determine the potential of Jambi forest honey and Klanceng honey in inhibiting the growth of ESBL-producing *Escherichia coli* and *Klebsiella pneumonia*.

METHODE

The research was conducted at the STIKES Nasional Bacteriology Laboratory using experimental laboratory research using a posttest with control approach. Honey samples were obtained from Jambi forest harvested in April, while ESBL-producing *E. coli* and *K. pneumoniae* were obtained from pure cultures of patients at a hospital in Surakarta.

The materials used in this study were Gram A, B, C, and D stain, MacConkey Agar, biochemical test media, Mueller Hinton Agar, 0.9% Sodium Chloride, Mg powder, Hydrochloric Acid 2N, Dragendrof, Standard Mc Farland, Iron (III) Chloride 10%, Kovack, Barried, Potassium Hydroxide 40%, antibiotic disk of Cloramphenicol, Ciprofloxacin, cefotaxim, ceftriaxone, ceftazidim, and blank disk. The tools used in this study were glass objects, test tubes, measuring pipettes, and petri disks.

How to Make Honey Concentration

The procedure of making the concentration of Jambi Forest Honey and Klanceng honey are as following (Pratiwi, dkk 2011).

1. 100% concentration was made of 3 grams of honey and 3 ml of sterile distilled water
2. 80% concentration was made of 0.8 ml of 100% concentration and 0.2 ml of sterile aquadest
3. 60% concentration was made of 0.6 ml of 100% concentration and 0.4 ml of sterile aquadest
4. 40% concentration was made of 0.4 ml of 100% concentration and 0.6 ml of sterile aquadest
5. 20% concentration was made of 0.2 ml of 100% concentration and 0.8 ml of sterile aquadest

The Isolation of The Test Bacterial Culture

ESBL-producing *E. coli and K. Pneumonia* were obtained from the isolation of infected patients in hospital. Blood samples from diarrhea patients were fertilized using Brain Heart Infusion and Blood Pepton Broth as media, then incubated at 37°C for 24 hours. The fertilization results were isolated using Blood Agar Plate and MacConkey as media and incubated at 37°C for 24 hours. Colonies of *E. coli* and *K. pneumoniae* suspects were tested biochemicaly and cultured using Nutrient Agar media.

Phytochemical Test of Jambi Forest Honey and Klanceng Honey

Flavonoid Test

2 ml of thick extract was added with 0.5 g of Magnesium powder and 1 ml of Hydrochloric Acid 2N. Positive result is indicated by the formation of red, yellow, or orange color (Prod et al., 2012).

Alkaloid Test

3 ml of honey was added with 0.5 g of Sodium Chloride, and then filtered. The filtered filtrate was added with Hydrochloric Acid 2M and Dragendorff reagent. A positive result is indicated by the presence of violet color with orange deposits (Harahap, S.N. Situmorang, 2021).

Inhibition Test of Honey Against ESBL-producing *E coli* and *K. pneumoniae*

Inoculate samples of pure ESBL-producing *E coli* and *K. pneumoniae* aged 24 hours into Sodium Chloride 0.9%, then compare the turbidity using Mc Farland standard no. 0.5. Inoculate the bacterial suspension with Sodium Chloride into the Mueller Hinton Agar Plate medium using the flattening method. Incubation is for 15 minutes at 37°C.

Put honey-impregnated disc (20 µg), chloramphenicol-impregnated disc (30 µg) and Ciprofloxacin-impregnated disc (5 µg). Incubation temperature was 37 °C for 24 hours. Observe the formed zone of inhibition.

Data Analysis

The inhibition potential of Jambi forest honey on the growth of ESBL-producing *Escherichia coli and Klebsiella pneumoniae* was descriptively analyzed by comparing the inhibitory zone using ESBL-standard antibiotic control, first-line antibiotic control, and negative control. The radical inhibition zone formed from antibiotic control was seen in the sensitivity of the tested bacteria according to the standard (CLSI, 2018).

RESULT AND DISCUSSION

1. Phytochemical Test of Jambi Forest Honey and Klanceng Honey

   In this study, the honey that had been concentrated was tested phytochemically. The phytochemical results of Jambi Forest honey and Klanceng honey are shown on Figure

Fig. 1. Results of Phytocemical Test; A. The Flavonoids of Jambi Forest Honey (+); B. The Flavanoids of Klanceng Honey (+) B. The Alkaloids of Klanceng Honey (+)

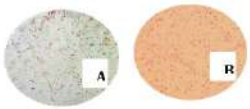
Based on Figure 1, it was known that Jambi Forest honey contains flavonoids, while Klanceng honey contained flavonoids and alkaloids.

2. The Characteristiscs of *E. coli* dan *K. pneumonia*

   Blood samples obtained from infected patients in the hospital were characterized by the following results.

   a. Gram staining

      Dispersed rod-shaped bacteria were found in gram staining, as shown on Figure 2



Fig, 2. The Results of Gram Staining: A. *Escherichia coli*; B. *Klebsiella pneumoniae*

b. The Colony on MacConkey

Colony results on MC could be seen in Figure 3.

Figure 3. Morfologi colonies at MC medium: A. *Escherichia coli*; B. *Klebsiella pneumoniae*

Escherichia. coli and *K. pneumoniae* are gram negative bacteria. Both of these bacteria have cell walls consisting of lipoproteins, phospholipids, peptidoglycan and lipopolysaccharides so that they are unable to firmly bind Gram A, faded by gram C and are able to bind gram D. On gram staining, these bacteria will appear red as shown in Figure 2 (Hamidah, dkk 2019). On the MacConkey, *E coli* and *K penumoniae* resulted in red colony (Figure 3), this is because the two bacteria were able to ferment lactose (Darna, Turnip, 2018).

c. Biochemical Test

The results of the biochemical tests for *E coli* and *K. pneumoniae* were shown in Table 1.

Table 1. The result of biochemical test for *E.coli* dan *K.pneumoniae*

No	Test	Bacteria	
		E.coli	*K.pneumoniae*
1	TSIA	Acid/Acid	Acid/Acid
2	H2S	(+)	(+)
3	Gas	(+)	(+)
4	Indole	(+)	(-)
5	Motil	(+)	(-)
6	Urea	(-)	(+)
7	Citrat	(-)	(+)
8	MR	(+)	(-)
9	VP	(-)	(-)
10	PAD	(-)	(-)
11	Glucose	(+/gas)	(+/gas)
12	Maltose	(+)	(+)
13	Mannitol	(+)	(+)
14	Sucrose	(+)	(+)
15	Lactose	(+)	(+)

Based on the biochemical tests in Table 1, the bacteria examined were true *E. coli* and *K. pneumonia*.

3. The ESBL Susceptibility Control Test

The ESBL Susceptibility Control Test was used to determine *E. coli* and K. *pneumoniae* used were resistant or sensitive to Beta-lactam antibiotics. The results of the susceptibility test are shown in Table 2.

Table 2. Result of Susceptibility Test of *E coli* dan *K pneumoniae*

Antibiotics	Sensibility Interpretation (CLSI, 2011)			Diameter Mean of Inhibition Zone (mm)	
	Sensitive	Intermediate	Resistant	*E coli*	K *pnumoniae*
Cefotaxime	-	-	≤ 27	6	6
Ceftazidime	-	-	≤ 22	10,24	13,17
Ceftriaxone	-	-	≤ 25	6	6
Chloramphenicol	≥ 18	13–17	≤ 12	29,15	-
Ciprofloxacin	≥ 21	16–20	≤ 15	-	27,03

4. Potential Test of Jambi Forest Honey and Klanceng Honey against ESBL-producing *E.coli* dan *K.pneumoniae*

The Potential Test of Jambi Forest Honey and Klanceng Honye Uji potensi Madu Hutan Jambi dan Klanceng against *E coli* is shown on Table 3

Table 3. Difussion Result of Honey Samples *against ESBL-Producing E coli*

Concentration	Mean of Radical Inhibition Zone (mm)	
	Jambi Forest Honey	Klanceng Honey
100%	9,2	9,5
80%	8,7	8,0
60%	7,6	7,5
40%,	6	6,5
20%,	6	6
K (-)	6	6

Mean	7,5	8,7

Based on Table 3, it was found that of Klanceng Honey had better inhibitory potential against ESBL-producing *E.coli* than Jambi Forest honey

4. The Potential Test of Jambi Forest Honey and Klanceng Honey against *K pneumoniae* is shown on Table 4

Table 4. Test Results of Honey Samples against ESBL-producing *K-pneumoniae*

Concentration	Mean of Radical Inhibition Zone (mm)	
	Jambi Forest Honey	Klanceng Honey
100%	8,3	8,8
80%	7,1	7,5
60%	6,7	7,1
40%,	6,4	6,8
20%,	6	6,4
K (-)	6	6
Mean	6,9	7,3

Based on Table 4, it was found that Klanceng honey had better inhibitory potential against ESBL-producing *K. pneumoniae* than Jambi Forest honey.

Preliminary test of the ESBL test control antibiotics was carried out to determine the ability of *E. coli* and *K. pneumoniae* bacteria in producing ESBL with an indicator that if the bacteria produce ESBL, the results of the disc diffusion test will show the results of no inhibition or inhibition including into the resistant category by CLSI, 2018 The controls used were Ceftazidime, Cefotaxime, and Ceftriaxone (CLSI, 2018). The results of the examination of *E. coli* and *K. pneumoniae* samples showed that the samples had experienced resistance to the three antibiotics (Table 2). Thus, it can be concluded that the bacteria are able to produce ESBL.

Klanceng honey provides better inhibitory activity of ESBL-producing *E. coli* and ESBL-producing *K. pneumoniae* than Jambi Forest Honey (Tables 3 and 4), It is because the antibacterial content in Klanceng Honey is more than in Jambi Forest Honey. Klanceng honey contains flavonoids and alkaloids, while Jambi Forest honey only contains flavonoids (Figure 1).

Flavonoids have antibacterial activity by inhibiting bacterial nucleic acid synthesis. Flavonoid compounds have A and B rings that play a role in the intercalation process, causing nucleic acid bases to accumulate and inhibit the formation of DNA and RNA. Besides, flavonoids can damage the permeability of bacterial cell walls (Nomer., et al, 2019). Flavonoids can also inhibit bacterial energy metabolism (Dwicahyani, et all 2018). Alkaloids have a working mechanism as antibacterial by disrupting the peptidoglycan constituent components in the bacterial cell wall, so that the cell wall is not completely formed and causes cell death (Marfuah., dkk, 2018).

Based on Tables 3 and 4, the results of disc diffusion show that both Jambi Forest honey and Klanceng honey have the best antibacterial potential against the tested bacteria at a concentration of 100%, while Cefotaxime and Ceftriaxon have no inhibitory potential (unable to form radical zones) against the growth of *E coli* and *K pneumonia* (Table 2).

Tables 3 and 4 show that samples of Jambi Forest honey and Klanceng honey have bacterial inhibitory ability seen from the formation of radical inhibition zones, and their inhibitory ability is weaker than ceftazidime and ciprofloxacin (*K.pneumoniae*). The inhibitory potential of honey samples against the growth *of E. coli* and *K. pneumoniae* cannot be separated from the phenol content which is antibacterial, namely flavonoids, as well as the alkaloids content in Klanceng honey (Figure 1).

Either Jambi Forest Honey or Klanceng Honey does not have inhibitory potential equal to ciprofloxacin and chloramphenicol. This can possibly occur because the antibiotic is a single dose with proven bactericidal ability. Faidiban, et al (2020) explained that ciprofloxacin is a second generation of quinolone derivative with a broad spectrum against Gram positive and gram negative. Ciprofloxacin has a working mechanism to inhibit the activity of bacterial DNA gyrase. Ciprofloxacin is effective for urinary tract infections, urethritis, typhoid and paratyphoid fever, respiratory tract infections, soft tissue infections and osteomyelitis.

Chloramphenicol is a broad-spectrum antibiotic that effectively inhibits protein synthesis by binding to ribosomes, thereby inhibiting the formation of peptide bonds (Ronal, D; Fatimali; Budiarso, 2015). The working mechanism of these two antibiotics causes their inhibitory activity is better than honey.

CONCLUSION

Based on the result of the study, it can be concluded that Jambi Forest Honey and Klanceng honey are able to inhibit the growth of ESBL-producing *E. coli* and *K. pneumoniae*. The potency of both honey is higher than cefotaxim and ceftriaxone, but lower than ceftazidime. Jambi Forest Honey and Klanceng Honey are not equal to Ciprofloxacin and Chloramphenicol.

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