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Antibacterial Activity of Ethanol Extract of Kale Leaves (*Brassica oleracea* var. sabellica) against *Escherichia coli* and *Staphylococcus aureus*

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Abstract

Inappropriate use of antibiotics can cause resistance problems in pathogenic bacteria. One alternative to overcome this is the search for antibacterial compounds by utilizing plant extracts, one of which is kale. This study aims to determine the potential of kale leaf extract as a source of antibacterial compounds against *Escherichia coli* and *Staphylococcus aureus*, and to determine the best concentration that can inhibit the growth of *E. coli* and *S. aureus* so as to determine the Minimum Inhibitory Concentration (MIC) value of kale leaf extract. Kale leaf extract was obtained by maceration method while the antibacterial activity test was carried out by agar diffusion method (Kirby Bauer). MIC against *E. coli* and *S. aureus* was determined by liquid dilution method. Kale leaf extract with a concentration of 140 mg/mL has the potential as a source of antibacterial compounds with the formation of the largest inhibition zones in *E. coli* of 3.3 mm and of 3.93 mm in *S. Aureus*. Meanwhile, the MIC value for *E. coli* was obtained from a concentration of 50% while against *S. aureus* was obtained from a 25% concentration of Kale extract, each from a dilution of the antibacterial activity concentration of 140 mg/mL.

Keywords: Antibacterial, *Brassica oleracea*, *Escherichia coli*, *Staphylococcus aureus*, MIC

Abstrak

Penggunaan antibiotik yang kurang tepat dapat menimbulkan masalah resistensi pada bakteri patogen. Salah satu alternatif untuk menanggulangi hal tersebut adalah dengan pencarian senyawa antibakteri dengan memanfaatkan ekstrak tanaman, salah satunya adalah kale. Penelitian ini bertujuan untuk mengetahui potensi ekstrak daun kale sebagai sumber senyawa antibakteri terhadap *Escherichia coli* dan *Staphylococcus aureus*, serta mengetahui konsentrasi terbaik yang mampu menghambat pertumbuhan *E. coli* dan *S. aureus* sehingga dapat menentukan nilai *Minimum Inhibitory Concentration* (MIC) ekstrak daun kale. Ekstrak daun kale diperoleh melalui metode maserasi sedangkan uji aktivitas antibakteri dilakukan dengan metode difusi agar (*Kirby Bauer)*. MIC terhadap *E. coli* dan *S. aureus* ditentukan dengan metode dilusi cair. Ekstrak daun Kale konsentrasi 140 mg/mL berpotensi sebagai sumber senyawa antibakteri dengan terbentuknya zona hambat terbesar pada *E. coli* sebesar 3,3 mm dan sebesar 3,93 mm pada *S. Aureus.* Sementara itu, nilai MIC terhadap *E. coli* diperoleh dari konsentrasi 50% sedangkan terhadap *S. aureus* diperoleh dari konsentrasi ekstrak Kale 25%, masing-masing dari pengenceran konsentrasi aktivitas antibakteri 140 mg/mL.

Kata kunci : Antibakteri, *Brassica oleracea*, *Escherichia coli*, *Staphylococcus aureus*, MIC.

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1. Introdution

Preventive and curative measures are efforts taken to maintain health (Trisnayanti, 2003). These precautions and medications are taken to avoid the risk of infection. Giving antibiotics is one option in treating infectious diseases but pathogenic microbes can become resistant if antibacterial is used irrationally. The existence of this resistance can cause problems in the treatment of infectious diseases, so that efforts are needed to develop traditional herbal medicines that can kill bacteria to avoid the occurrence of such resistance (Refdanita *et al*., 2004). One alternative in preventing this disease is to use traditional herbal ingredients. Traditional herbal concoctions until now are increasingly widely used among the people because they are part of the culture of the Indonesian people. One of the plants that can be used as traditional herbal ingredients is Kale (*Brassica oleracea* var. sabellica).

Kale contains antioxidant compounds such as glucosinolates, flavonoids, and carotenoids. Although still rare in Indonesia, but these vegetables began to be cultivated locally. Kale is very high in fiber, but has a low calorie content and fat content such as omega 3 fatty acids called alpha linolenic acid, which is small so it is good for digestion. Kale can also be used as an anti-inflammatory so that Kale can treat diseases such as Arthritis, Asthma, and Autoimmune Diseases (Ferioli *et al*., 2013).

Kale comes from the same genus Brassica as Cabbage and Broccoli. Kale comes from the Dutch language which means Cabbage Farmer. Kale's morphology is similar to Cabbage. The difference is that the true leaves of Kale are headless and the color of the leaves is green or bluish purple. Kale leaf types can be divided into two, namely Kale Curly and Kale Nero (Roni, 2016). Through this research, Kale (*Brassica oleracea* var. sabellica) extract can be obtained as a potential source of antibacterial compounds against *Staphylococcus aureus* and *Escherichia coli*.

2. The Methods

The antibacterial activity test uses the agar diffusion method (Kirby Bauer) and to determine the Minimum Inhibitory Concentration (MIC) using the liquid dilution method. This study uses curly Kale (*Brassica oleracea* var. Sabellica) leaves as an antibacterial agent and uses *S. aureus* and *E. coli* as test bacteria. The extraction process is done by maceration method.

A. Sample Preparation

3,480 grams of Kale leaves are washed clean, then cut into small pieces and then dried in the open with good air circulation and not exposed to sunlight for 13 days. This sample preparation refers to the Afrilla (2011) study which conducted a sample preparation for green betel leaf extract.

B. Kale leaf extraction (*Brassica oleracea* var. sabellica) with Maceration methods

Kale leaf extraction was carried out by maceration method using distilled ethanol 96%. 344 grams of dried Kale leaf samples were then mashed using a blender and soaked with 1,500 mL 96% ethanol in a dark bottle

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and closed, then allowed to stand for 4 x 24 hours with several stirring. Then the filter is filtered using filter paper and obtained maserate and filtrate (I), maserate (I) then macerated again and obtained maserate and filtrate (II). Then the filtrate (I) and filtrate (II) were combined and evaporated with a rotary vacum evaporator at a temperature of 50 °C and obtained the results of a thick extract. This Kale leaf extraction refers to the journal Dasopang (2017) which extracts Sangitan leaves. After that, a dilution of Kale leaf extract is carried out to get a concentration of 60 mg/ml, 80 mg/mL, 100 mg/mL, 120 mg/mL, 140 mg/mL.

C. Bacterial Rejuvenation Test

The bacterial culture of the test used in this study came from the Faculty of Medicine, University of Riau. 1 ose of *S. aureus* and *E. coli* test bacteria isolates were taken and transferred into a cup containing NA media and incubated for 24 hours. After the incubation period, the growth of test bacterial isolates was observed (Afrilla, 2011).

D. Antibacterial Activity Test

The method used to test antibacterial activity is Kirby Bauer with the following stages:

1) Preparation of Bacterial Suspension Test

Two or three bacterial colonies were suspended into a test tube containing 10 ml of physiological NaCl and then homogenized. If the suspension still looks bright (clear) can be added with some bacterial colonies and if the suspension looks turbid can be added with physiological NaCl, the suspension turbidity level is adjusted to the standard Mc Farland solution 0.5 (1x107 CFU / ml).

2) Antibaketri Activity Test of Kale Leaf Extract (Brassica oleracea var. Sabellica)

Suspension of bacteria that has been made, applied to the NA growth media using a cotton swab. Empty test disc paper that has been immersed in each kale extract concentration is placed on the surface so aseptically in laminar air flow. Then the NA media which has been filled with disk paper that has been soaked, is incubated in an incubator at 37 ° C for 24 hours. Clear zones formed, observed and measured using calipers.

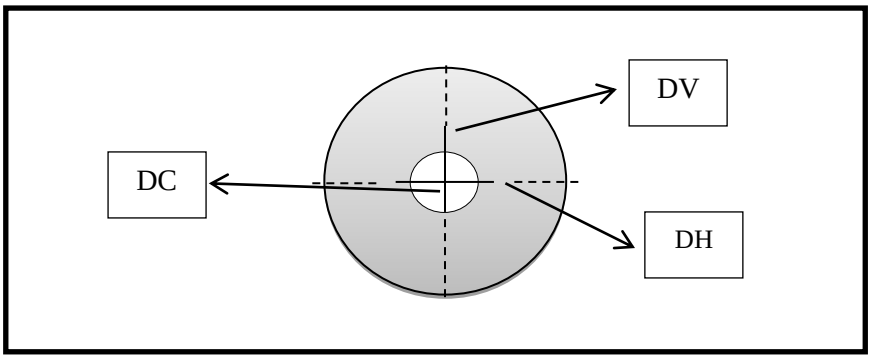

Figure 1. How to measure inhibition zones on NA media after 24 hours of observation

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The diameter of the clear zone can be measured by the formula:

Clear Zone = $\frac{(DV-DC)+(DH-DC)}{2}$

Explanation :

: Clear Zone
DV : Vertical Diameter
DH : Hotizontal Diameter
DC : Disc Diameter

E. *Minimum Inhibitory Concentration* (MIC) Test

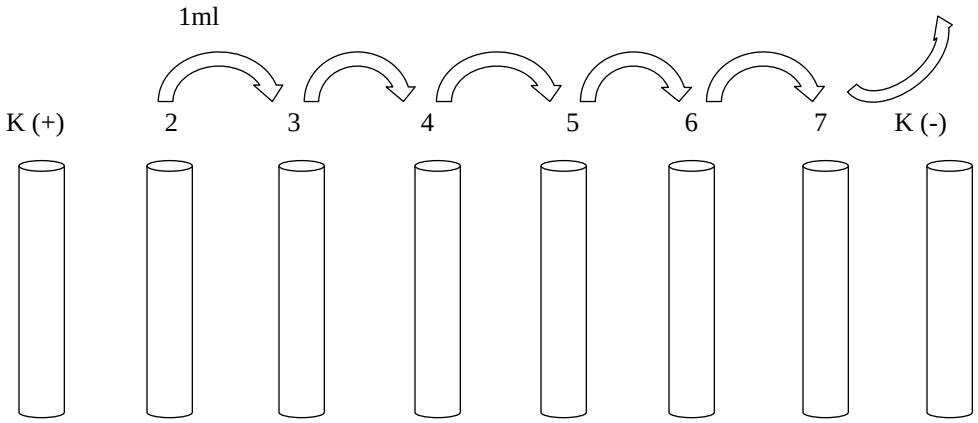

Figure 2. *Minimum Inhibitory Concentration* (MIC) Dilution Test

Explanation :
Test tube K (+) : 1 ml NB (single strenght) + 1 ml bacterial suspension
Test tube 2 : 1 ml NB (double strenght) + 1 ml kale extract + suspension bacteria
Test tube 3 : 1 ml NB (single strenght) + 1 ml dilution results tube 2 + bacterial suspension
Test tube 4 : 1 ml NB (single strenght) + 1 ml dilution results tube 3 + bacterial suspension
Test tube 5 : 1 ml NB (single strenght) + 1 ml dilution results tube 4 + bacterial suspension
Test tube 6 : 1 ml NB (single strenght) + 1 ml dilution results tube 5 + bacterial suspension
Test tube 7 : 1 ml test tube NB (single strenght) + 1 ml dilution results tube 6 + bacterial suspension
Test tube K (-) : 1 ml NB (single strenght) + 1 ml kale extract

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3. Result and Discussion

1) Antibacteri Activity Test of Kale Leaf Extract (*Brassica oleracea* var. Sabellica)

Inhibition zones formed are measured in diameter using calipers in millimeters (mm). Inhibitory zones are calculated using the formula and entered into the observation table, namely Table 1 and Table 2:

Table 1. Clear zone diameter of *E. coli*

Repetition	Inhibition Zone Diameter (mm)				
	Ectract concentration 140 mg/mL	Ectract concentration 120 mg/mL	Ectract concentration 100 mg/mL	Ectract concentration 80 mg/mL	Ectract concentration 60 mg/mL
1	3,25	2,65	2	0,85	-
2	3,35	1,55	1,45	1	-
Average Inhibition Zone Diameter (mm)	3,3	2,1	1,73	0,93	-

Source : primary data 2019

Table 2. Clear zone diameter of *S. aureus*

Repetition	Inhibition Zone Diameter (mm)				
	Ectract concentration 140 mg/mL	Ectract concentration 120 mg/mL	Ectract concentration 100 mg/mL	Ectract concentration 80 mg/mL	Ectract concentration 60 mg/mL
1	3,85	2,45	1,55S	1	-
2	4	2,25	2	1,45	-
Average Inhibition Zone Diameter (mm)	3,93	2,35	1,78	1,3	-

Source : primary data 2019

Based on Table 1. the largest inhibitory zone is produced by Kale leaf extract against *E. coli* is at a concentration of 140 mg / ml, followed by concentrations of 120 mg / mL, 100 mg / mL, 80 mg / mL, and 60 mg / ml, each of 3.3 mm, 2.1 mm, 1.3 mm, 0.93 mm, and 0. Based on Table 1., the largest inhibitory zone produced by Kale leaf extract against *S. aureus* was at a concentration of 140 mg / ml, followed by a concentration of 120 mg / ml mL, 100 mg / mL, 80 mg / mL, and 60 mg / ml in the amount of 3.93 mm, 2.35 mm, 1.78 mm, 1.3 mm and 0 mm.

In general, the inhibition zone formed shows that the average diameter of the inhibition zone has increased along with the increased concentration given. According to Lingga & Rustama (2005), the diameter of the inhibition zone formed is indicated by the presence of a clear zone which will increase with the higher concentration of the extract given. Inhibition zone is not formed at a concentration of 60 mg/ mL because the

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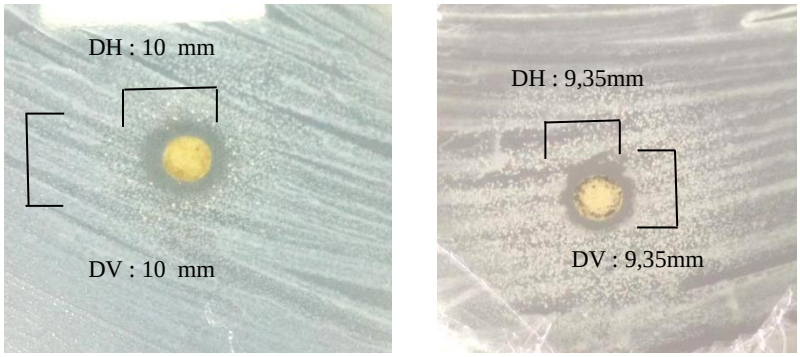
concentration is still low, so it has not been able to influence the test bacteria. According to Cowan (1999), this is caused by the concentration in the extract is still low so it has not been able to damage the cell membrane and disrupt cell physiological processes . Munifatul (2007) said that several factors also caused differences in the diameter of inhibitory zones, namely the speed of diffusion, molecular size and stability of antibacterial agents, the nature of the media used, the number of organisms inoculated, the speed of bacterial growth, the concentration of chemicals and conditions at incubation.



(A) (B)

Figure 3. Kale Leaf Extract (*Brassica oleracea* var. sabellica) Antibacterial Activity Test Concentration 140 mg/mL (A) *S. aureus* (B) *E. coli*

The inhibition zone formed (Figure 3) shows that Kale leaf extract is able to inhibit the growth of *S. aureus* and *E. coli*, this is presumably because Kale extract contains secondary metabolites that are thought to function as antibacterial compounds. According to Prihatiningtias (2005), secondary metabolite compounds which are bioactive compounds that can kill pathogens. This is the same as Hafidh's research in 2012 using extracts of Cabbage (*Brassica oleracea* L. var. Capitata) which has antibacterial activity in *S. aureus* and *E. coli*. Tahira (2013) also reported that the presence of antibacterial activity in Cabbage extract against *S. aureus*, *E. coli*, *S. epidermidis*, *Streptococcus* and *Proteus* test bacteria.

Inhibitory zone diameters of bacteria formed against *S. aureus* is greater than in *E. coli*. This shows that the concentration of Kale leaf extract given is more able to inhibit *S. aureus* (Gram positive) than *E. coli* (Gram negative). This is presumably because the structure of the cell membrane of each bacterium is different. According to Navarre & Scheewind (1999), Gram-negative bacteria have a complex outer membrane structure compared to Gram-positive bacteria because the peptidoglycan in Gram-positive bacteria is not protected by the outer membrane. According to Cetin (2011), the Gram-positive peptidoglycan layer is polar so that it is more polar easily penetrated by flavonoids which are also polar. Gram-positive cell walls are polar because they contain polysaccharides (terikoic acids) are water-soluble polymers, which function as positive ion transforations to get in and out. Ferioli (2013) says that Kale contains phenol compounds such as

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flavonoids. Flavonoids are a group of compounds that can bind to proteins that inhibit the activity of microbial enzymes, and can also disrupt metabolic activity (Widiatmojo, 2009).

2) Minimum Inhibitory Concentration Test (MIC)

MIC testing is carried out after the antibacterial activity test. The concentration of Kale leaf extract used was the extract concentration which showed the largest inhibitory zone diameter in *S. aureus* and *E. coli*, ie at a concentration of 140 mg / ml. Observation of MIC values was performed after 24 hours of visual incubation period (Table 3 and Table 4).

Table 3 MIC test results Kale leaf extract (*Brassica oleracea* var. Sabellica) of *E. coli*

Kale leaf extract concentration (*Brassica oleracea* var. sabellica)	**Result**	
	Repetition 1	**Repetition 2**
K (+)	+	+
100 %	_	_
50 %	_	_
25 %	+	+
12,5 %	+	+
6,25 %	+	+
3,125 %	+	+
K (-)	_	_

Note: (+): liquid looks turbid, meaning that bacteria is still growing. (-): the fluid in the tube begins to decrease turbidity, which means that the growth of Staphylococccus aureus begins to be inhibited. K (+): positive control containing 0.5 McFarland equivalent bacterial suspension. K (-): negative control containing kale extract with a concentration of 140 mg / mL.
Source: primary data 2019.

Table 4. MIC test results Kale leaf extract (*Brassica oleracea* var. Sabellica) of *S. aureus*

Kale leaf extract concentration (*Brassica oleracea* var. sabellica)	**Result**	
	Repetition 1	**Repetition 2**
K (+)	+	+
100 %	_	_
50 %	_	_
25 %	_	_
12,5 %	+	+
6,25 %	+	+
3,125 %	+	+
K (-)	_	_

Note: (+): liquid looks turbid, meaning that bacteria is still growing. (-): the fluid in the tube begins to decrease turbidity, which means that the growth of Staphylococccus aureus begins to be inhibited. K (+): positive control containing 0.5 McFarland equivalent bacterial suspension. K (-): negative control containing kale extract with a concentration of 140 mg / mL.
Source: primary data 2019.

Based on Table 3 and Table 4, MIC values of Kale leaf extract (*Brassica oleracea* var. Sabellica) against *E. coli* were at 50% concentration, whereas against *S. aureus* there was at 25% concentration. At a concentration of 50% of the *E. coli*, visually appeared clearer than at concentrations of 25%, 12.5%, 6.25%, and 3.125%

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whose turbidity approached the positive control tube. At a concentration of 25% of the *S. aureus* visually appeared clearer than at concentrations of 12.5%, 6.25% and 3.125%, with turbidity approaching the positive control tube. This shows that in tube number 3 with a concentration of 50% there has been an inhibition of the growth of *E. coli* and in tube number 4 with a concentration of 25% there has been an inhibition of the growth of *S. aureus*. According to Ajizah (2004), turbid tubes indicate that the source of antibacterial compounds is not able to inhibit the growth of test bacteria because the lower the concentration, the extract ability to inhibit is also lower because the number of compounds contained is also less.

One of the ideal criteria for determining an antibacterial agent is to find the smallest concentration that is still capable of killing or inhibiting a microorganism. This is shown by the ability of these antibacterial compounds to inhibit the growth of Gram-positive and Gram-negative bacteria groups (Pelczar & Chan, 2008). The ideal antibacterial compounds are compounds that in the lowest concentrations have been able to inhibit or kill groups of bacteria, both Gram-positive and Gram-positive bacteria groups. negative (Nurhasanah, 2014). In addition, the ideal antibacterial agent also has selective toxicity which means that a drug is harmful to the parasite, but does not harm the host. Often, selective toxicity is relative rather than absolute; this means that a drug which at a certain concentration can damage the parasite, but can also be tolerated by the host (Jawetz *et al*., 1987 in Rufaidah *et al*., 2010).

The difference in MIC values in *S. aureus* and *E. coli*, MIC values in *S. aureus* are smaller than *E. coli*. This is also the same as in antibacterial activity testing which shows that the biggest inhibitory zone is found in *S. aureus* (Gram positive) than *E. coli* (Gram negative). This occurs because Gram positive bacteria are more sensitive to antibacterial compounds compared to Gram negative bacteria. According to Peclzar & Chan (1988), cell walls of Gram-negative bacteria have an outer membrane membrane that envelops a thin layer of peptidoglycan. The outer structure of the peptidoglycan is a double layer containing phospholipids, proteins and lipopolysaccharides. Lipopolysaccharides are located in the outer layer and are characteristic of Gram-negative bacteria while Gram-positive bacteria have a cell wall that consists of a thick layer of peptidoglycan, which contains the theoicate and lipoteichoic compounds.

4. Conclusion

Based on the research that had been conducted, Kale leaf extract has the potency as the source of antibacterial compound with the forming of the biggest inhibition zone produced by concentration 140 mg/mL on *S. aureus* in the amount of 3,93 mm and on *E. coli* in the amount of 3,3 mm, and the value of MIC towards *S. aureus* given Kale leaf concentration extract 25% while towards *E. coli* on concentration 50% from concentration dilution of antibacterial activity 140 mg/mL.

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