

Antibacterial Activity of thanol Extract and Ethylacetate Fraction of *Casia Alata Leaf* from Kendari-Southeast Sulawesi

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ABSTRACT

Active compound extracted from *Casia alata leaf* was obtained from methanol extract and ethylacetat fraction. Based on research, anti bacteria activity of methanol extract of *Casia alata* at concentration 6 mg/mL gave high inhibition of the growth of positive gram bacteria colonies of *B. cereus* and *S. aureus* with inhibition zone 11,0 mm and 16,1 mm and gaves negative inhibiton of negative gram bacteria colonies of *E. coli* and *S. typhi* with inhibition zone 11,6 mm dan 5,9 mm. Ethylasetat fraction showed higher zone inhibition then methanol extract. Based on research result it was show that ethylacetat fraction gaves strong inhibition of the growth of positive gram bacteria colonies of *B. cereus* and *S. aureus* with inhibition zone 18,1 mm and 14,3 mm, and gave moderate inhabitation for negative gram bacteria colonies of *coli* and *S. typhi* with inhibition zone 9,0 mm and 5,7 mm. Separation result of ethylacetat fraction using coloumn chromatography with silica gel G₆₀ F₂₅₄ as stationary pahase showed that ethylacetat fraction with the same concentration at 6 mg/mL show higher inhibition effect especially for positive gram bacteria colonies of *B. cereus* and *S. aureus* with inhibition zone 24,2 mm and 24.0 mm.

Key Word: *Casia alata*, ethylacetat ftaction, antibacteria activity.

PRELIMINARY

Ketapang cina Leaf (*Cassia alata* L.) used by people as traditional medicine to cure skin disease, infected wound, influenza, and bronchitis as well as to cure liver disease. *Cassia alata* L. is one of local plant from Kendari that interesting to be researched because it has varies chemical constituents. Based on phytochemical screening we found that methanol extracts contained several groups of secondary metabolites such as flavonoids, anthraquinones, alkaloids, saponins and polyphenols. Therefore, exploration of such plants are expected to be useful for the development of science, bioindustry, and other related field in

order to empower biodiversity in indonesia

RESEARCH METHODE

1. MATERIALS AND INSTRUMENTS

Part of plant that being used was the leaf of *Cassia alata*. The activity against gram-positive bacteria, of *B. cereus* and *S. aureus* and gram-negative bacteria of *E. coli* and *S. typhi* was being tested. Chemical materials that was being use use were petroleum ether, ethanol, n-hexane, ethylacetate, silica gel GF254, and reagents for the phytochemicals test.

This research instrument consists of *rotary evaporatory* coloumn

chrpmatgraphy electronic balance, ultra violet light, ultra sonic and other tools that commonly used in research laboratorium of organic chemistry

2. EXTRACTION

Sample was extracted by graded maceration. Before it put into macerator, simplisia was crude using grinder to enlarge it surface area. Simplicia that has been refined was put into macerator while adding the methanol until all part of simplicia are fully submerged. Submersion was done for 24 hours with three time repetition. Filtrate that was filtered were mix and then evaporate it using *rotary evaporator* with temperature is 40^o C until it became viscous extract and after that, extract was weighed. Methanol extract of *Casia alata* then being partitioned with hexan and distilled water (1:1), water layer that form was taken then being partition using ethylacetat. This ethylacetat fraction of *Casia alata leaf* than evaporate back using *rotary evaporator* so crude ethylacetat were obtained.

3. RESULT AND DISCUSSION

Antibacterial activity or inhibition activity of methanol extract and fraction ethylacetat of *C alata* of positive and negative gram bacterial activity is show in table 1.

Based on data at Table 1, methanol extract and ethylacetate fraction of *C alata leaf* at concentration of 6mg/mL show strong inhabitation against growth positive gram bacteria of *S. aureus* with inhibition z one for each are 16,1 dan 14,3 mm. as well as with bacteria *B.cereus growth*, methanol extract and ethylacetate fraction at concentration of 6mg/mL have strong inhabitation against growth of positive gram bacteria of *.B cereus* with inhibition zone for each are 11,0 dan 18,1 mm. At the concentration of 8mg/mL both methanol extract and ethylacetate fraction show reduction in effectivity when

inhibiting the growth of bacterial colonies. The formation of clear zone for each concentration of material test show that there is inhibition of bacterial colonies reactions because of the effect of anti bacterial compound in material test namely methanol extract and ethylacetate fraction of *C. alata leaf*.

The nature and strength of antibacterial compounds is determined from the size of inhibition zone against the target bacteria growth. It's based on common standard from health department (1998). According to health department, microbe/bacterial can be categorized as sensitive against bacterial from plant if it has diameter size of inhibition zone between 12-24 mm. According to strength classification of a test substance on inhibition of bacterial colonies as follow: size inhibition zone ≥ 20 mm as very strong inhibition (2) inhibition zone ≤ 10 mm until < 20 mm as strong inhibiton (3) inhibition zone ≤ 5 mm until < 10 mm as moderate inhibition (4) and inhibition zone < 5 mm as weak inhibiton (Aryanti *et al.*, 2007).

Test result of antibacterial activity with methanol extract and ethylacetate fraction of *C. alata* using bacterial gram negative of *E. coli* dan *S. typhi* each show almost no inhibition when compared to two kind bacterial target (bacterial gram positive)

Inhibition of methanol extract of *C alata* of bacterial bakteri *E coli* at the same variant concentration of 0,5 mg/mL, 2 mg/mL, 4 mg/mL, 6 mg/mL, and 8 mg/mL. each inhibition zone are 4,3 mm; 5,2 mm; 6,5 mm; 11,6 mm and 8,9 mm. For bacterial *S typhi* at the same variant concentration of 0,5 mg/mL, 2 mg/mL, 4 mg/mL, 6 mg/mL, and 8 mg/mL. show smaller inhibition each inhibition are 0,1 mm; 0,4 mm; 0,9 mm; 5,9 mm and 2,9 mm. from inhibition zone data we can concluded that methanol extract *Cassia alata* leaf show inhibition zone moderate to strong inhibiton when inhibiting the growth of *E. coli*.

Table 1. The Result of Antibacteria Activity Test of Methanol Extract and Ethylacetate Fraction of *C. alata* Leaf against Bacteria *B.cereus* and *S. aureus* Positive Gram. and Bacteria *E.coli* dan *S. typhi* Negative Gram.

Bacteria	Fraction	Concentration (mg/mL)	Methanol (mm)	explanation	Ethylacetate (mm)	explanation
1. <i>B. cereus</i>		0,5	4,9	WI	5,2	MI
		2	6,3	MI	6,3	MI
		4	7,1	MI	8,4	MI
		6	11,0	SI	18,1	SI
		8	7,7	MI	16,3	SI
2. <i>S. aureus</i>		0,5	6,5	MI	2,1	WI
		2	7,3	MI	3,6	WI
		4	9,0	MI	6,2	MI
		6	16,1	SI	14,3	SI
		8	10,4	SI	8,3	MI
3. <i>E. coli</i>		0,5	4,3	WI	1,0	WI
		2	5,2	WI	3,3	WI
		4	6,5	MI	6,3	MI
		6	11,6	SI	9,0	MI
		8	8,9	MI	7,5	MI
4. <i>S. typhi</i>		0,5	0,1	WI	0,1	WI
		2	0,4	WI	3,0	WI
		4	0,9	WI	4,2	WI
		6	5,9	WI	5,7	WI
		8	2,9	WI	4,8	WI
Positive control (tetracyclin 1%)	with diameter inhibition zone (mm) for <i>B.cereus</i> = 24,6 and <i>S. aureus</i> = 25,8					
positive control (chloramphenicol 1%)	with diameter inhibition zone (mm) for <i>B.cereus</i> = 21,1 and <i>S. aureus</i> = 20,63.					
Negative control (distilled water)	with diameter inhibition zone (mm) for <i>E.coli</i> = for <i>S. aureus</i> = 0 <i>B. Cereus</i> = 0 dan <i>S. typhi</i> = 0					

Explanation:

M = methanol

H= Hexane

E= Ethylacetate

SI = Strong inhabition

MI= Moderate Inhabition (MI)

WI= weak Inhibition

Inhibition of ethylacetate fraction of *C.alata* against *E coli* at the same variant concentration of 0,5 mg/mL, 2 mg/mL, 4 mg/mL, 6 mg/mL, and 8 mg/mL. each inhibition zone are 1,0 mm; 3,3 mm; 6,3 mm; 9,0 mm dan 7,5 mm. For bacterial *S. typhi* at the same variang concentration of 0,5 mg/mL, 2 mg/mL, 4 mg/mL, 6 mg/mL, and 8 mg/mL. show smaller inhibition each inhibiton are 0,1 mm; 0,4 mm; 3,0 mm; 5,7 mm dan 4,2

mm. Based on inhibition zone data we can concluded that ethylacetate fraction of *Cassia alata* leaf, it showed inhibition zone from weak to moderate inhibiton when inhibiting the growth of *S. typhi*. The formation of a clear zone less than 12 mm in each concentration of material test show that inhibition bacterial colonies reactions by the effect of anti bacterial compound in ethylacetate fraction did not show significance

inhibition activity to the growth of target bacterial colonies.

The important things that can be describe here is the inhibition of the growth of bacterial colonies between the fraction of methanol and ethylacetate extracts of *C. alata* leaf mainly on gram-positive bacteria that is *B.cereus*. in table 1 we can see that the inhibition of the growth of bacterial colonies from ethylacetate fraction of *C.alata* is generally higher than the inhibition of the growth of bacterial colonies from the methanol extract of *C.alata* at the same concentration of the test substance. This

was happening because the active metabolite constituent within *c. alata* leaf become stronger when inhibiting growth of bacterial colonies if it's chemical constituent is in synergy with other chemical constituent.

Separation with chromatography column, using silica gel G₆₀ F₂₅₄ as stationary phase produce several fraction and after being tested with KLT there are fraction group EA₁, EA₂ and EA₃ (See table 2) where fraction with the highest inhibition is fraction EA₁.

Table 2 Antibacterial Test Data Result Using Ethylacetate Fraction of *C. alata* leaf with concentration of 6 mg/mL

Fraction	Extract weight	Average inhibition zone			
		<i>B.cereus</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>S. thypi</i>
EA ₁	2,43	24,2	24,0	12,4	10,3
EA ₂	0,95	4,0	6,7	5,1	0,0
EA ₃	1,62	2,9	4,2	0,0	0,0
Positive control (tetracyclin 1%)		With diameter inhibition zone (mm) for <i>B.cereus</i> = 24,6 and <i>S. aureus</i> = 25,8			
Control positive (chloramphenicol 1%)		With diameter inhibition zone (mm) for <i>B.cereus</i> = 21,1 and <i>S. aureus</i> = 20,63.			
Negative control (aquades)		With diameter inhibition zone (mm) for <i>E.coli</i> = for <i>S. aureus</i> = 0 <i>B. Cereus</i> = 0 dan <i>S. thypi</i> = 0			

Table 2 show that test result of anti bacterial activity fraction EA₁ with concentration of 6 mg/mL show higher diameter in inhibition growth of bacterial colonies *B. cereus* and *S. aureus* that is 24,2 mm and 24,0 mm compared with original ethylacetate fraction of *C. alata leaf* is 18,1 mm and 14,3 mm. This is because, after being separated by chromatography, anti bacterial compound have been separated with other compound so that there would be no mutual interaction that would be weaken for each compound. this show that chemical constituent of *C. alata* become more active if used in pure compound

without any unsynergic metabolite constituent. According the term of strength of a test substance Aryanti *et al.* (2007) fraction EA₁ can be classified as very strong (inhibition zone ≥ 20 mm as very strong inhibition) against bacteria *S. aureus* and *B. cereus* as well as strong against bacteria *E.coli* dan *S. typhi* each are 12,4 and 10,3 mm, while fraction EA₂ dan EA₃ can be classified as strong to weak against all bacteria target. Based on table 1, ethylacetate fraction can inhibi the growth of bacteria *S. aureus* and *B. cereus* at strong level and can inhibiting the growth of bacteria *E. coli* and *S. typhi* at weak level. The cause of the differences in antibacterial activity of ethylacetate fraction of *C. alata* against positive gram bacterial and negative gram bacterial is

the differences in component of cell wall. The cell wall of gram positive bacteria *S. aureus* and *B. cereus* is relatively simple, consistis only of three layers: (1) the cytoplasmic membrane, (2) the peptidoglycan layer, and (3) an outer layer called the hoop. While the cell walls of the bacteria *E. coli* and *S. typhi* has layered structure and a very complex cell wall (Jawetz et al., 1986).

CONCLUSION

Based on this research the conclusions are:

1. Anti bacterial activity of methanol extract *C. alata* leaf show strong inhibition against gram positive bacteria of *S. aureus* and *B. cereus* and show moderate inhibition against the growth of gram negative bacteria of *E. coli* and *S. typhi*.
2. Anti bacterial activity of ethylacetate cassia alata leaf show strong inhibition against gram positive bacteria *S. aureus*, *B.cereus* and *E. coli*. Also show moderate inhibition against growth of gram negative bacteria namely dan *S. typhi*.

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