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# Antibacterial Activity Test Ethanol Extract Leaf Ageratum conyzoides Linn against Staphylococcus aureus and Escherichia coli Bacteria

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**Abstract** : *Ageratum conyzoides* Linn (Bandotan) which is known as a weed plant can be used as a traditional medicine. The ethanol extract of *A. conyzoides* leaf contains secondary metabolite compounds, like flavonoids, alkaloids, and antibacterial saponins. The aim of this research is to test the antibacterial activity of ethanol extract of *A. conyzoides* leaf. The first step of the analysis is extracting secondary metabolite compounds with 96% ethanol solvent and tested their antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* by jell diffusion Kirby-Bauer method to know their inhibitory activity. The inhibitory activity at concentrations of 200 mg/ml, 150 mg/ml, 100 mg/ml, 5 mg/ml against *S. aureus* and *E. coli* were 8.7mm, 7.1mm, 7.9mm, 6.64mm, and 10.9 mm, 7.8mm, 7.33mm, 6.89mm. The results show us that ethanol extract of *A. conyzoides* leaf has antibacterial activity against *S. aureus* and *E. coli* in medium category.

Keywords : A. conyzoides leaf, ethanol, Kirby bauer, Staphylococcus aureus, Escherichia coli.

## Introduction

Ageratum conyzoides Linn is a plant which come from tropical America. It is considered as a weed plant in Indonesia. Although it is often labeled by people as weeds plants, but it has many advantage, for

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example as a traditional herb for skin diseases, wound's covering, ophthalmic, colic, diarrhea, dysentery, and fever. This plant has a protection against anxyolytics, pedicosis, and the damage which is produced by arsenic exposure <sup>[1]</sup>. The other benefits of *A. conyzoides* L plants are as stimulant, tonic, antipyretic, antitoxic, hemostatic, anti-inflammatory, emenagog, diuretic, and kaiminatit, and can be used as a vegetal insecticide <sup>[2]</sup>. According to <sup>[3]</sup> *A. conyzoides* Linn contains secondary metabolites such as flavonoids, alkaloids, benzofuran, chromen, chromon, cumarin, essential oils, and tannins so this plant has many benefits and the one of them is as antibacterial agent. Since science and technology have fast development, the researchers required to be able to explain the advantages of the secondary metabolite compounds scientifically, especially for medicine, so people can use this plant in traditional usage responsibility <sup>[4]</sup>. Alkaloids have the ability to inhibit the action of enzymes to synthesize bacterial proteins which disrupt bacterial metabolism <sup>[5]</sup> and can damage the peptidoglycan component in bacterial cells which make the cell wall layer is not fully formed and causes cell's death<sup>[6]</sup>. It is important for researchers to do more exploration about the phytochemical's contents of *A. conyzoides* Linn to know the active composites's existence and activity of active compounds in it, so that its usage as a medicine will be more widespread.

## Experimental

#### **Preparation and Extraction**

The researchers got *A. conyzoides leaves* from Purwokerto area. Those leaves had smoothen and then they are macerated by 96% ethanol that has been distilled for 1x24 hours. The product of maceration will be concentrated by a rotatory evaporator.

#### **Phytochemical Test**

#### Flavonoid test

The mixture of 0.1 g of *A. conyzoides* leaves extract, 0.1 mg of magnesium powder, 0.4 mL of amyl alcohol and 4 mL of alcohol. The presence of flavonoids are indicated by the formation of red, yellow or orange on the amyl alcohol layer<sup>[7]</sup>.

#### **Alkaloid Test**

*A.conyzoides* leaf extract 0.1 g was dissolved in a few drops of 2N sulfuric acid. This tests used three alkaloid reagents, Dragendorff reagents, Meyer reagents and Wagner reagents. The test results are positive if it has formation of orange red sediment in Dragendorff reagent, the formation of yellowish white sediment with the Meyer reagent and the formation of brown sediment with Wagner reagent<sup>[7]</sup>.

## Saponin test

The mixture of 0.1 g of *A. conyzoides* leaf extract and 10 mL of aquades is poured into the reaction tube and then the mixture will be shaken for 30 seconds. After that, the mixture is observed by researchers to know something happen with the mixture. When solid foam have formed (not lost for 30 seconds), that indicates the presence of saponins<sup>[8]</sup> Antibacterial test

Antibacterial test used jell diffusion Kirby Bauer method against *S. aureus* and *E. coli* bacteria. All treatments were performed aseptically and sterilely. The test was performed using NA and NB with 0.55 cm paper disk. The treatments are negative control, positive control and some variety of extract concentration. The negative control treatment used aquades, and positive control used ampicillin 0.01 mg/ml. The extract concentrations were 200 mg/ml, 100 mg/ml, 50 mg/ml, and 5 mg/ml. The disk paper was immersed in each control treatment and various concentrations of *A. conyzoides* extract. After that, the disc paper which contains the sample was placed on the surface of the bacteria smeared NA medium, and the inoculation was performed using tweezers. The bacteria were incubated for 24 hours at 28°C. The diameter of the inhibit zone formed is measured by the vernier calipers.

**Results and Discussion** 

Extraction process of ethanol extract of *A. conyzoides* leaves yielded 7.52%. Phytochemical test is a test which conduct to determine the content of metabolic compounds in ethanol extract of *A. conyzoides* leaves.

Test	Reaction	Results	Reader	Description
Alkaloid	Meyer	white sediment	white sediment	+
	Wagner	brown sediment	brown sediment	+
	Dragendorf	orange red sediment	orange red sediment	+
Flavonoid	HCl + Mg, + Amyl Alcohol	formation of red	formation of red	+
Saponin	HCl 2N	solid foams formed	solid foam is formed	+

Table 1. The results of phytochemical test of ethanolic extract of A.conyzoides leaf

Based on phytochemical test, the ethanol extract of A. conyzoides leaf contains alkaloids, flavonoids and saponins. The content of these secondary metabolite compounds make the A. convzoides has antibacterial activity. According to <sup>[9]</sup> alkaloids can disrupt the components of peptidoglycan bacteria cells, so that the cell wall layer not formed intactly and cause cell's death. Saponins have molecules which can attract water (hydrophilic) and molecules which can dissolve fat (lipophilic), so it can reduce the surface tension of cells which eventually lead to the destruction of bacteria. Flavonoids have been known have antibacterial activity. Flavonoids have many structures, and among these structures, which have antibacterial activity are *apigenin*, galangin, pinocembrin, ponciretin, genkwanin, sophoraflavanon G and its derivatives, naringin and naringenin, epigallocatechin gallate and derivatives, luteolin and luteolin 7-glucoside, quercetin, 3-omehylquercetin and various quercetin glycosides, kaempferol and their derivatives and other flavones such as flavone glycosides, isoflavones, flavonols, flavonol glyosides and chalcones. A study which is conducted by <sup>[10]</sup> showed that 5hydroxyflavanones and 5-hydroxyisoflavanones in one, two or three addition of hydroxyl groups at 7, 2'and 4'positions inhibited the growth of Streptococcus mutans and Streptococcus sobrinus. According to <sup>[11]</sup> flavonoids are bactericidal because flavonoids not kill bacterial cells but inhibit bacterial aggregation and reduce the Colony Forming Units (CFU). Flavonoids have antibacterial effects because they inhibit nucleic acid synthesis, interfere cytoplasmic membrane function and bacterial energy metabolism.

Treatment		zones of inhibition (mm) ± Standard deviation		
		Escherichia coli	Staphylococcus aureus	
1.	K (+)	$12.21\pm0.574$	$11.79 \pm 0.303$	
2.	K (-)	-	-	
3.	5 mg/ml	$6.89 \pm 0.047$	$6.64\pm0.057$	
4.	50 mg/ml	$7.33 \pm 0.048$	$7.10\pm0.054$	
5.	100 mg/ml	$7.80\pm0.068$	$7.90\pm0.062$	
6.	200 mg/ml	$10.9 \pm 0.167$	$8.70 \pm 0.115$	

 Table 2. Inhibition Zone of A. conyzoides ethanol extract leaves on bacterial growth of Staphylococcus aureus and Escherichia coli

Based on the data, the average of inhibition zone diameter of *Escherichia coli* and *Staphylococcus aureus* at concentrations of 5 mg/ml of 6.89 and 6.64 mm, smaller than the concentration of 50 mg/ml of 7.33 and 7.1 mm. Inhibit zone of 100 mg/ml ethanol extract are 7.8 and 7.9 mm. The largest inhibition zone diametre is at 200 mg/ml of ethanol extract, there are 10.9 and 8.7 mm. The inhibition zones of *Eschericia coli* and *Staphylococcus aureus* have a difference because every bacteria have different stage of tolerance against some active compounds <sup>[12]</sup>. Determination of ethanol extract concentration of *A. conyzoides* leaves greatly influences the formation of the inhibition's zone. According to<sup>[13]</sup>, if the the concentration is high, so the inhibitory power is high too. That condition caused by the higher the concentration contains more content of antibacterial active ingredient.

According to the results of the study <sup>[14]</sup>, antibacterial activity test showed that ethanol extract of *A. conyzoides* leaf with Soxhletation method had antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* with KHM value was 12, 5 mg/ml and 25 mg/mL, this at a concentration of 5 mg/ml still has a little antibacterial activity, so it could be a correction and development of more recent research.

Stout in <sup>[15]</sup> describes anti-bacterial groups based on their inhibitory extents into 4 groups, there are lowactivity (<5 mm), medium (6-10 mm), strong (11-20 mm) and very strong (antibacterial) > 20 mm). However, the inhibition zone produced by the ethanol extract of *A. conyzoides* leaves is not as large as the inhibitory power generated by ampicillin.

Ampicillin has used due to ampicillin is a type of penicilin antibiotics whose have inhibitory mechanisms cell wall's formation and the permeability of cell membranes <sup>[16]</sup>. Ampicillin also has an activity against Gram-positive and Gram-negative bacteria. Ampicillin is a broad-spectrum antibiotic <sup>[17]</sup>.

## Conclusion

The results of the antibacterial test showed inhibitory activity in concentrations of 200 mg/ml, 150 mg/ml, 100 mg/ml, 5 mg/ml against *S. aureus* and *E. coli* bacteria respectively are 8.7mm, 7.9mm, 7.1mm, 6.64mm, and 10.9mm, 7.8mm, 7.33mm, 6.89mm. This result shows us that the ethanol extract of *A. conyzoides*'s leaves have antibacterial activity against *S. aureus* and *E. coli* in the medium category.

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