Antibacterial Activity Study of Active Fraction from Chick Weed Plants (*Ageratum Conyzoides* L.) Against *Bacillus subtilis* and *Vibrio cholerae*

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ABSTRAK

Penelitian ini bertujuan untuk menentukan aktivitas antibakteri fraksi dari daun tumbuhan bandotan terhadap bakteri *Bacillus subtilis* dan *Vibrio cholerae* dan menentukan nilai Konsentrasi Hambat minimum (KHM) dari fraksi yang bersifat antibakteri terkuat. Penelitian dilakukan pada bulan Agustus sampai dengan November 2016. Metode yang digunakan pada penelitian ini adalah ekstraksi secara maserasi, fraksinasi fase cair-cair, pemisahan fraksi secara kromatografi kolom, uji aktivitas antibakteri dengan metode Kirby-Bauer sedangkan penentuan konsentrasi hambat minimum secara dilusi kaldu, bakteri uji yang digunakan *Bacillus subtilis* dan *Vibrio cholerae*. Hasil penelitian menunjukan bahwa ekstrak metanol dari tumbuhan bandotan aktif terhadap bakteri uji *Bacillus subtilis* dan *Vibrio cholerae*. Fraksi kolom yang menunjukkan antivitas antibakteri kuat adalah fraksi metanol dengan kode S4. Konsentrasi hambat minimum fraksi kolom S4 terhadap bakteri uji *Vibrio cholerae* sebesar 62,5 ppm, nilai tersebut menunjukkan kekuatan antibakteri setengah dari antibiotik standar streptomisin dan penisilin, sedangkan terhadap tetrasiklin menunjukkan aktivitas seperempatnya. Fraksi aktif dari tumbuhan bandotan diperoleh dari ekstrak metanol fraksi metanol dengan fraksi kolom S4 bersifat efektif sebagai antibakteri terhadap bakteri uji *Vibrio cholerae*.

Kata Kunci : *Ageratum conyzoides* L., Konsentrasi Hambat Minimum, Antibakteri, *Bacillus subtilis,Vibrio cholerae*.

ABSTRACT

The purpose of this research to determine the fractions of leafs chick weed plants which has strong antibacterial activity against bacteria of *Bacillus subtilis* and *Vibrio cholerae* and than determine of the minimum inhibitory concentration (MIC) from the strongest antibacterial fraction. The research was carried out from August to November 2016. The method used in this research was the maceration extraction, liquid-liquid phase fractionation, column fractionation chromatography, antibacterial activity test by Kirby-Bauer method while the determination of minimum inhibitory concentration by broth dilution, the test bacteria used consisted of *Bacillus subtilis* and *Vibrio cholerae*. The results showed that the extract methanol from Chick Weed plants active against bacteria test of *Bacillus subtilis* and *Vibrio cholerae*. A column fraction showing strong antibacterial antivities is a methanol fraction with S4 column code. The minimum inhibitory concentration of the S4 column fraction against the Vibrio cholerae was 62.5 ppm. The value indicating the antibacterial strength of half of the

standard antibiotics of streptomycin and penicillin, whereas tetracycline showed a quarter activity. The active fraction of the Chick Weed plants obtained from methanol extract of methanol fraction with fraction of S4 column is effective as antibacterial to Vibrio cholerae.

Keywords: *Ageratum conyzoides* L., Minimum Inhibitory Concentration, Antibacterial, *Bacillus subtilis*, *Vibrio cholera*.

INTRODUCTION

Most villagers still use natural medicine from medicinal plants that are inherited from their ancestors (Akinyemi, K.O. et al., 2006). One of the medicinal plants that has been known and used by people in the village Pampangan Ulak Depati district Ogan Ilir of South Sumatra is Chick Weed Plants (Ageratum conyzoides L) from the Asteraceae family. This plant has been proven to cure various diseases such as scarring infection, itching scars, diarrhea and inflammation of the intestines (Sukamto, 2007; Hasim, 2005)

The leaf extract of Chick Weed Plants showed that it was antibacterial to test bacteria of Salmonella typosa, Staphylococcus aureus and Eschericia coli (Ahmad, I. 2015). Test of antibacterial activity of ethyl acetate fraction of Chick Weed leaf has been done to *Staphylococcus aureus* and Eschericia coli. The results show that the ethyl acetate extract and the fractions contained therein have broad spectrum antibacterial activity but tend to be more sensitive to gram-positive bacteria (Sugara,H.T. et al., 2016).

Bacillus subtilis is a Gram-positive bacterium that can be found in water, soil, air. Vibrio cholerae, is a Gram negative bacteria that is also found in soil and water. The two bacteria are easy to contaminate foodstuffs so that they are opportunistic, therefore they are used as representative of Gram positive and Gram negative bacterial groups.

Chick Weed plants used as a medication for bacterial infection of hereditary need to be scientifically assessed. It is therefore necessary to research the active ingredient content of the plant and its biological activity. In this study we studied the active fraction of the Chick Weed plant and its potential as an antibacterial compound against Gram positive and gram negative bacteria.

MATERIALS AND METHOD

Sample of Chick Weed Plants

Chick Weed Plants was collected from Pampangan Ulak Depati district Ogan Ilir of South Sumatra. The plant sample was taken with a herbalist in the village, who used to use the plant as a traditional medicine. Subsequently the sample was prepared in the laboratory.

Extraction of Chick Weed Plants

Plant fresh Chick Weed taken leafs. Leafs are cleaned and made simplicia with mashed and dried. Dry simplicia is taken as 100 gram, inserted into the bottle by maseration method using methanol for 2 × 24 hours and filtered. Methanol was evaporated on the rotaryvacum evaporator until a methanol-crude extract was obtained.

Subculture of *Bacillus subtilis* and *Vibrio cholerae*

Bakteri Bacillus subtilis dan Vibrio cholerae diambil masing-masing sebanyak satu ose dan diinokulasikan ke dalam media NA miring (composition g/l: 5 g peptone; 3 g beef extract; 15 g bacto agar, Atlas, 2010). Kultur diinkubasi pada suhu 37ºC selama 24 jam (Lay, W. B., 1994).

Standar McFarland 0.5

Sulfuric acid (H_2SO4) 1% concentration was added as much as 85 ml into a 100 ml measuring flask, anhydrous barium chloride ($BaCl_2$) concentration of 1.175% was added 0.5 ml, then re-added (H_2SO_4) 1% concentration, up to 100 ml and shaken until mixed evenly. The solution formed was a standard McFarland 0.5 solution, and then measured the absorbance using a spectrophotometer at a wavelength of 625 nm. The readable absorbent is equivalent to a suspension containing bacteria 1.5×10^8 cells / ml (Clinical & Laboratory Standards Institute, 2009).

Suspension of*Bacillus subtilis* and *Vibrio cholerae*

Bacillus subtilis and *Vibrio cholerae* have been subcultured, each made a suspension of 0.85% NaCl (physiological saline). Each bacteria was taken as many as 1-2 loop and inoculated into aerlenmeyercapacity 50 ml containing 25 ml of physiological saline. The culture was homogenised evenly and measured its absorbance with a spectrocotometer at a wavelength of 625 nm. The absorbance value must equal the standard absorbance value of McFarland 0.5, if the absorbance value is higher than the McFarland 0.5 standard diluted by adding the physiological saline and if too low plus the bacteria again.

The antibacterial activity of methanol-crude extract from leaf chick weed plants

Test antibacterial activity using Kirby-Bauer method. The concentration of **methanol-crude** extract from leaf chick weed plantswas made 400 µg / disk or 400 µg/10µL. The concentration of antibiotics as standard consists of Streptomycin 10 µg / disk, Penicillin 10 µg/disk, Tetracycline 30 µg/disk. Standard paper discs is placed on medium Mueller Hinton Agar (composition g / l: 17.5 g casein; 3 g beef extract; 1.5 g stachsoluble; 15 g agar) (Atlas,R.M., 2010), plate in petri dish, then added methanol extract in accordance with the concentration andstandard antibiotics used. Each culture in a petri dish was incubated at 37 ° C for 2 x 24 hours. Observed the formation of clear zones and measured using a vernier caliper (Harley & Prescott., 2002).

Fractionation of methanol-crude extract from leaf chick weed plants

The fractionation is carried out by solvent partition method.The solvent used consists ofn-hexane, ethyl acetate and methanol-water. The extract of methanol-crude extract from leaf chick weed plants of15 g was dissolved in methanol and water with the ratio between methanol and water 1: 1 (50 ml of methanol and 50 ml of water). The extract in methanol-water was put into a 1-liter separation funnel, added 100 ml of n-hexane shaken for 2 hours, left separately to form two boundary planes, the n-hexane fraction (top) separated and inserted into 1 liter bottle. The residue extract in methanol-water is fractionated again using ethyl acetate. The extract in methanol-water was poured intothe separating funnel and added 100 ml of ethyl acetate, shaken for 2 hours, allowed to separate to form two boundary planes, ethyl acetate fraction (top) separated and inserted into a 1-liter bottle. The remaining fraction is the methanol fraction. Each fraction of the fraction of n-hexane, ethyl acetate, and methanol was concentrated with a rotary vacuum evaporator and in an oven at 70ºC to obtain a concentrated fraction (Salni., et al., 2010). All three concentrated fractions were tested for antibacterial activity with a concentration of 400 µg / disk. A fraction having a strong resistance of>70% against standard antibiotics was continued in the fraction separation step by column chromatography.

Separation of Antibacterial Active Fraction by Column Chromatography

The active fraction of analysis by thin layer chromatography (TLC) used various eluents to determine eluent suitable to separations in column chromatography. The active fraction with a concentration of 400 µg/10 µl is bottled on the TLC plate. The fraction obtained was column chromatographed using silica gel G60 stationary phase. Samples that have been preabsorpted prepared, are incorporated into chromatographic columns and eluted using suitable eluents. The column fractions are collected in 10 ml bottles each. The fractions of columns 1 to n are tested for antibacterial activity. Fractions having a strong antibacterial of>70% against standard antibiotics were followed by the determination of Minimum Inhibitory Concentration (MIC).

Determination of Minimum InhibitoryConcentration (MIC)

Determination of Minimum Inhibitory Concentration using liquid dilution method. The column fraction with strong antibacterial activity was made in dilution series in Mueller Hinton Broth or MHB (composition g/L: 17.5 g casein: 3 g beef extract; 1.5 g stach soluble, (Atlas,R.M., 2010)) consisting of 100 ppm; 500 ppm; 250 ppm; 125 ppm; 62,5 ppm; 31.25 ppm; 15.62 ppm; and 7.81 ppm, each of 5 ml in the test tube. Each test tube was inoculated with a suspension of test bacteria of 0.1 ml. As negative control was prepared 5 ml medium MHB plus fraction without bacteria, and as a positive control prepared 5 ml medium MHB without extract plus bacteria. All tubes were incubated at 37 °C for 2 x 24 hours. After 2x 24 hours observation of turbidity at each concentration by comparing with positive control. If the turbidity is less than the positive control then the fraction at that concentration still inhibits the test bacteria, if the turbidity is more than the positive control means the fraction at that concentration does not inhibit the test bacteria (Clinical & Laboratory Standards Institute, 2009).

RESULTS AND DISCUSSION

Extraction of Leafs Chick Weed Plants

The methanol extract of leafs chick weed plantssimplicia from Pampangan village was 16,46%. Based on the volume, the extract is classified as a major extract, and can be developed as a raw material for the manufacture of drugs. The percentage of extracts from plants with a percentage of ≥ 11% can already be developed as raw materials for the manufacture of drugs (Badan Pengawasan Obatdan Makanan RI, 2005).

Antibacterial Activity of Methanol Extract fromLeafs of Chick Weed Plant

The results of antibacterial activity of methanol extract fromleafs of chick weed plantagainst bacteria test of *Bacillus subtilis* and *Vibrio cholerae* compared with antibacterial activity of antibiotic standard (tetracycline, streptomycin, penicillin) are presented in Table 1.

Table 1. Inhibitory zone diameter of extract and antibiotics against test bacteria

No	Test material	Diameter of the inhibitory zone (cm) in the test bacteria	
		Bacillus subtilis	*Vibrio cholerae*
1	Methanol extract	1,35±0,28	1,68±0,09
2	Tetracycline	2,33±0,05	1,89±0,08
3	Streptomycin	1,47±0,13	2,16±0,20
4	Penicilin	1,68±0,10	1,59±0,23

Based on Table 1, showed that the antibacterial activity of methanol extract is more effective against *V. cholerae* compared with *B. subtilis*, this is shown in each inhibitory zone diameter between *V. cholerae* (1.68 cm) whereas in *B. subtilis* (1.35 cm). The percentage of antibacterial activity of methanol extract to *B subtilis* test bacteria had moderate to strong strength (57.94-91.84%) while to *V. cholerae* had percentage of strong category antibacterial activity (77.78-107.01%).A sample with antibacterial activity can be grouped into three categories: weak / resistant (inhibitory zone diameter <50% of standard antibiotic inhibition zone diameter), medium / intermediate (inhibitory zone diameter 50-70% antibiotic Standard), and strong / sensitive (drag zone diameter> 70% of standard antibiotics) (Chan, E.W.C., et al., 2007)..The antibacterial activity of methanol extract when compared with the antibiotic standard (tetracycline, streptomycin, and penicillin), the effectiveness of methanol extract against *B. subtilis* bacteria is still below all three antibiotics but against *V. cholerae* more effective than penicillin but less effective against tetracycline and streptomycin. Tetracycline has a wide spectrum that effectively works on Gram-positive bacteria.

Fractionation of Active Methanol Extract and Antibacterial Test

The fractionation of methanol extract fromleafs of chick weed plant was carried out using a solvent according to the polarity level ie n-hexane (non polar), ethyl acetate (semi polar), and methanol (polar). Fractionation is the process of separating the compounds contained in a plant based on the degree of polarity of the solvent used. Commonly used solvents are n-hexane (non-polar), ethyl acetate (semi-polar), and methanol and water (polar) so that the

compounds can be separated by their polarity. After each fraction was evaporated, the concentrated fraction of n-hexane was 4,23 gram, ethyl acetate 3,76 gram and methanol 6,81 gram. The results of the antibacterial activity test and the percentage of the fraction of n-hexane, ethyl acetate, and methanol with test bacteria *B. subtilis* and *V. cholerae* compared with standard antibiotic activity (tetracycline, streptomycin, penicillin) can be seen in Table 2.

Table 2. Inhibitory zone diameter of fraction and antibiotics against test bacteria

No	Test material	Diameter of the inhibitory zone (cm) in the test bacteria	
		Bacillus subtilis	*Vibrio cholerae*
1	n-hexane fraction	0.77±00	0.00±00
2	Ethyl acetate fraction	1.06±00	0.79±0,03
3	Methanol fraction	1.91±0,13	1.39±0,05
4	Tetracycline	2.13±0,18	2.11±0,05
5	Streptomycin	1,82±0,13	1,74±0,07
6	Penicilin	1.78±0,24	1.59±0,03

Table 2 shows that the methanol fraction has greater antibacterial activity (1.39-1.91 cm) than with both n-hexane and ethyl acetate fractions (0-1.06 cm). This suggests that the active compound on methanol extract is attracted or dissolved in the methanol fraction when compared to the n-hexane and ethyl acetate fractions to both *B subtilis* and *V. cholerae* test bacteria. Compared with the three standard antibiotics (tetracycline, streptomycin, and penicillin) the antibacterial activity of the methanol fraction against *V. cholerae* (1.39 cm) bacteria is still below the three standard antibiotics (1.59-2.11 cm) but against bacteria *B. subtilis* (1.91 cm) is still above the standard antibiotic penicillin and streptomycin (1.78 to 1.82 cm) while the standard antibiotic tetracycline is still under standard antibiotics (2.13 cm).

The inhibitory capacity of the methanol fraction against the *B subtilis* test bacteria had strong antibacterial activity of ≥ 70% (89.7-107.3%) compared to the three standard antibiotics (tetracycline, steptomycin, and penicillin) while the *V. cholerae* bacteria were moderate to strong (65.9-87.4%). The n-hexane fraction has a weak category (0-43.3%) against both *B subtilis* and *V. cholerae* test bacteria. The ethyl acetate fraction had weak category antibacterial activity against *V. cholerae* (37.4-49.7%) and the weak to moderate category against *B. subtilis* (49.8-59.6%). The methanol fraction with strong antibacterial activity against the antibiotic standard of tetracycline, streptomycin, and penicillin continued into the separation phase by column chromatography.

Separation of Methanol Faction with Column Chromatography and Antibacterial Test

The result of separation of methanol fraction by column chromatography was obtained seven column fraction. Antibacterial test results of methanol fraction column to test bacteria *B. subtilis* and *V. cholerae* were compared with the activity of standard antibiotics (tetracycline, streptomycin, penicillin) are listed in Table 3. Based on the results listed in Table 3 shows that the effectiveness of antibacterial fraction S4 column are more sensitive to the test bacterium *V. cholerae*compared with bacteria *B. subtilis*, it can be seen in each the diameter of inhibition zone against *V. cholerae*of 1.60 cm while the test bacteria *B. subtilis* was 0.70 cm, but when

compared with fractions of column S1 up to fraction of column S7 fraction of column S4 have greater antibacterial effectiveness. Stating if one component of the compounds separately then so can the active compound that remains inactive against one of the test bacteria (Elfita., et al., 2016).. The results shown that the fraction of S4 column gives strong antibacterial activity (≥ 70%) to *V. cholerae* test bacteria with tetracycline antibiotic, streptomycin, penicillin. Thus, the fraction of the column S4 proceed to the stage of determining the Minimum Inhibitory Concentration (MIC) against the bacteria test.that*V. cholerae.*

Table 3. Inhibitory zone diameter of columnfraction and antibiotics against test bacteria

No	Test material	Diameter of the inhibitory zone (cm) in the test bacteria	
		Bacillus subtilis	*Vibrio cholerae*
1	Column fractionS1	0.00±0.00	0.00±0.00
2	Column fractionS2	0.00±0.00	0.00±0.00
3	Column fractionS2	0.70±0.05	0.00±0.00
4	Column fractionS4	0.69±0.12	1.60±0.07
5	Column fractionS5	0.74±0.05	0.90±0.03
6	Column fractionS6	0.70±0.09	0.80±0.16
7	Column fractionS7	0.00±0.00	0.00±0.00
8	Tetracycline	2.33±0.08	2.12±0.23
9	Streptomycin	1.42±0.05	1.76±0.09
10	Penicilin	1.58±0.15	1.49±0.06

Determination of Minimum Inhibitory Concentration (MIC) of S4Column Fraction

Table 4. showsthat the smallest concentration of turbidity is less than the positive control is on the fraction of column S4. Minimum Inhibitory Concentration (MIC**)** fraction of S4 column is 62.5 ppm gave the strength of antibacterial activity half (31,25 ppm) from standard antibiotic activity of steptomycin and penicillin, while tetracycline gave activity of one quarter (15.62 ppm).

Table. 4. Minimum Inhibitory Concentration (MIC) determination resultagaint *V. cholerae*

Concentration of S4 fraction column (ppm)	Result than positive control
1000	-
500	-
250	-
125	-
62,5	-
31,25	+
15,62	+
7,81	+

Remark: + : inhibit; - : not inhibit

The antibacterial activity of methanol extract, n-hexane fraction, ethyl acetate, and column fraction indicated that the active compounds contained in the chick weed plant were concentrated in total methanol extract, then concentrated in the methanol fraction and then concentrated in the fraction of column 4 (S4). The antibiotics used as standard (tetracycline, streptomycin, penicillin) are already pure compounds that have high antibacterial effectiveness, whereas the fraction of column 4 (S4) is not a pure compound which is still a crude extract so its antibacterial properties are not yet maximal and still below standard antibiotics.

Based on the antibacterial activity of methanol extract of chick weed plant in Pampangan village, UlakDepati sub-district of OganIlir regency, to fraction of S4 column have the opportunity to be developed as a cure forvibriosis caused by *V. cholerae*. Furthermore, if the S4 columnfraction is purified then it is very likely to get the pure compounds that are responsible for providing such antibacterial activity. This indicates that the chick weed plantpotentiallyto be developed into a source of new antibiotics for infectious diseases caused by *V. cholerae*.

CONCLUTION

The extract methanol from Chick Weed plants active against bacteria test of *Bacillus subtilis* and *Vibrio cholerae*. A column fraction showing strong antibacterial antivities is a methanol fraction with S4 column code. The minimum inhibitory concentration of the S4 column fraction against the *Vibrio cholerae* was 62.5 ppm. The active fraction of the Chick Weed plants obtained from methanol extract of methanol fraction with fraction of S4 column is effective as antibacterial to *Vibrio cholerae*.

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