ANTIBACTERIAL ACTIVITY OF BINAHONG RHIZOME ETHANOL EXTRACT (*Anrederacordifolia* (Tenore) Steen) AGAINST *Salmonella typhi*

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Abstract

Background. *Anredera cordifolia is one of plants used as traditional medicine. It has been carried out a study about antibacterial activity of ethanolic extract of A. cordifolia rhizomes against Salmonella typhi and the phytochemical screening. The aims of this study are to find out the Minimum Bactericidal Concentration (MBC) of ethanolextract of A. cordifolia rhizomes against Salmonella typhi and to identify the chemical subtances of the ethanol extract found in the rhizomes.*

Method*.The A. cordifoliarhizomes were extracted using maceration method with 70% $^{v}/_{v}$ ethanol as solvent. The antibacterial activity was tested using liquid dilution method with varied concentration of the extract (50,49, 48, 47, 46, and 45% $^{w}/_{v}$) to determine MBC. Chromatography method was used to identify the chemical substances of the extract.*

Result*. The result showed that the MBC of A. cordifolia rhizomes extract was 50% $^{w}/_{v}$. The result of the phytochemical screening with tube test and thin layer chromatography showed that the extract of Anredera cordifolia rhizomes contained flavonoids, polyphenols, and saponin.*

Conclusion*.The binahong rhizome ethanol extract had antibacterial activity against S. typhi with 50% $^{w}/_{v}$of MBC and contained flavonoids, polyphenols, and saponin.*

Keywords : *Anredera cordifolia, antibacterial, Salmonella typhi*

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INTRODUCTION

Infection is an invasion and propagation of microorganisms which occured in human tissue that clinically may not be visible or may cause local cellular injury due to competitive metabolism, toxins, intracellular replication or antigen-antibody response (Dorland, 1998).Infections are caused by microorganisms such as bacteria, viruses,fungi, protozoa.These organisms can invade the whole body or partial body (Gibson, 1996).

Typhoid fever, one of infection disease, is an acute fever caused by *Salmonellatyphi.S. typhi* is a gram negative rod-shaped bacteria. These organisms can survive longer dry and frozen environment, sensitive to chlorination and pasteurization temperature of 63^{O}C (Jawetz *et al.,* 1996). Antibacterial compounds are not always effective to kill or inhibit its growth.This may be due to the bacteria has been resistant to specific antibacterial drugs (Prahasto, 2001). Therefore, it needs to explore other agents as alternative antibacterial substances. They can be explored from animal, plant, microorganism etc as sources of medicinal compounds.

Binahong *(Anredera cordifolia* (Tenore.) Steen.) is one of the potential of medicinal plants that can cure various kinds of diseases traditionally. Binahong can be used to help the healing process of severe disease such as inflammatory bowel disease and typhoid fever. The activity of Binahong to cure various types of this disease is closely related to the active compounds contained in them such as flavonoids, alkaloid, and saponins. Flavonoids act by interfering the functions of microorganisms such as bacteria and viruses (Manoi, 2009).

Based on the description, it is necessary to perform the antibacterial activity assay of Binahong rhizome against *S. typhi* and identify the chemical contents by phytochemical screening. It is aimed to get scientific evidence of Binahong antibacterial activity, especially against *S.typhi.*

METHODS

1. Preparation of Binahong Rhizome Ethanol Extracts

Binahong rhizomes were obtained from Merapi Farma on May 18, 2011 in the form of wet roots. The rhizomes were cleared of debris by running water and then drained and oven dried at 40°C.The dried binahong rhizome was smoothed by grinding into rough powder. The powder approximately 600 grams was macerated with 70% ethanol and stirred at 400 rpm rotation speed. Furthermore, it was filtered by a Buchner funnel and the filtrate was evaporated with waterbath to obtain the rhizome extract.

2. Preparation of bacterial suspension

One loop of bacteria from the stock was suspended in 1 ml BHI broth medium and incubated for 18-24 hours at 37°C.After that,100µl of its was added into the which have been filled 1 ml of BHI. Then this mixture was incubated for 4-8 hours at 37°C. Then, it was diluted with 0.9% NaCl to get the turbidity as same as McFarland standard (10^{8}CFU/ml).The solution was diluted again until the bacteria concentration was 10^{6}CFU/ml with BHI DS medium.The suspension formed was called bacterialsuspension.

3. Antibacterial activity test

Test antibacterial activity of binahong rhizome ethanol extract was conducted to determine the lowest concentration of the sample solution that killed *Salmonella tyhpi.*The series of sample solution concentrations were 50, 49, 48, 47, 46,and 45%$^{w}/_{v}$. The 0.5 ml of 10^{6}CFU/ml of bacterial suspension *Salmonella typhi* was put into each test tubes containing 0.5 ml of test

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Table I. Antibacterial Activity of Ethanol Rhizome Extract Against *S. typhiwith* Liquid Dilution Method

Treatment (% w/v)	Observation					
	Replication I		Replication II		Replication III	
	T / C	Colony	T / C	Colony	T / C	Colony
50	T	-	T	-	T	-
49	T	+	T	+	T	+
48	T	+	T	+	T	+
47	T	+	T	+	T	+
46	T	+	T	+	T	+
45	T	+	T	+	T	+
K1	C	-	C	-	C	-
K2	C	-	C	-	C	-
K3	C	-	C	-	C	-
K4	T	-	T	-	T	-
K5	T	+	T	+	T	+

Discription :

T : the solution is turbid + : there are colonies on solid media

C : the solution is clear - : there is no colony on solid media

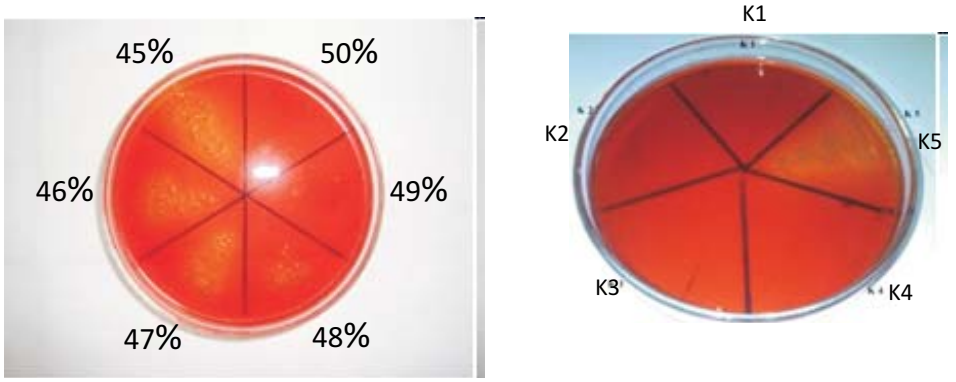

Figure 1. Antibacterial Activity of Ethanol Extract against Salmonella typhi Binahong Rhizome

solution in various concentrations.Then they were incubated at 37°C for 18-24 hours.

The antibacterial activity test of ethanol extract of rhizome binahong performed with the liquid dilution method.The principle of the liquid dilution method is to dilute the test solution and then adding a suspension of bacteria in liquid media.The advantages of this method is the test solution can be mixed with a suspension of bacteria in the medium, resulting in the possibility of an equitable distribution of the test solution of bacteria and medium. The other advantages of this method are more economical medium and not influenced by medium thickness

(Pratiwi, 2008). Parameters used to test the antibacterial activity is the Minimum Bactericidal Concentration (MBC). MBC is the lowest level that can kill bacteria.

4.Phytochemical screening

a.Preliminary Test

Rhizome ethanol extract of binahong was dissolved in distilled water, then heated for 30 minutes in the boiling water bath and filtered using filter paper.When the result solution was yellow to red, it indicated the presence of chromophore-containing compounds (flavonoids, anthraquinone, etc.) with a hydrophilic group (phenolic acids, sugars and so on).When it was added KOH solution (3 drops), the color would become more intensive (Harborne, 1987).

b. Alkaloids identification.

Binahong rhizome ethanol extract was heated in a large test tube with 1% HCl (10 ml) for 10 minutes on a boiling water bath.The suspension was filtered through cotton wool and put in test tube. The solution was divided into two equal lots and then dropped by Dragendorff reagent solution for first tube and Meyer reagent solution (3 drops) for the second.If both reactants are formed sneak's, it showed the presence of alkaloid (Sastrohamidjojo, 1996)

c.Saponin identification

Binahong rhizome ethanol extract plus 10 ml distilled water, was covered, shaked vigorously for 30 seconds, then the tube was placed in an upright position for 30 minutes. When the froth arised as high as 3 cm from the surface and when a solution of 2N HCl was added to form a stable foam, it indicated the presence of saponin (Robinson, 1995).

d. Polyphenols identification

Binahong rhizome ethanol extract was heated with distilled water for 30 minutes in boiling water bath. After it has been cold, it was added 3 drops of $FeCl_3$ solution.If there was a green-blue color, it indicatedthe presence of polyphenols (Harborne, 1987).

e. Flavonoids identification

Binahong rhizome ethanol extract was dissolved in distilled water.Then extract dripped and dried on paper filter.The next paper steamed with ammonia. A positive result means of intensive yellow flavonoid (Robinson, 1995).

f. Tannins identification

Binahong rhizome ethanol extract was heated with distilled water for 30 minutes on waterbath, then filtered. Filtrate plus 1% gelatin solution, if deposits arise, it indicated the presence of tannin or tannic substances (Harborne, 1987).

g. Thin Layer Chromatography

TLC (Thin Layer Chromatography) is a separation method of components based on the differences in adsorption or partition on stationary phase by the movement of the solvent (mobile phase). Selection of solvent is depend on the polarity of chemical substances which are separated (Suharman and Noble, 1995). Identification of chemical compounds in the ethanol extract of binahong rhizome was conducted on flavonoids, saponins, and polyphenols. Applying sample solution was done manually with a capillary tube to the stationary phase silica gel 60 $F_{254.}$ After the spots dried, put in vessel development and eluted. Elution was performed with a distance of 8 cm. After elution was complete, the paper allowed to dry and examined under UV_{254nm} and $UV_{365\ nm}$ light and sprayed with a suitable reagent (Wagner, *et al,* 1996).

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RESULT AND DISCUSSION

The binahong rhizome ethanol extract had antibacterial activity against *S. typhi* with 50% w/v of MBC. The result of the phytochemical screening with tube test and thin layer chromatography showed that the extract of Anredera cordifolia rhizomes contained flavonoids, polyphenols, and saponin.This results can be explained as the following description.

1. Preparation of 70% Ethanol Extract of Rhizome Binahong

The moisture content of simplicia rhizomes ofbinahong was 6.69%. These provided a good fit of a drying process. The simplicia of rhizome should provide water content of less than 8% (Anonymous, 1985). The weight of dry powder in this study was 600 grams. Extraction process was then performed using the maceration method and obtained 91.63 of grams extract (15.27% of rendemen).

2. Antibacterial Activity

The antibacterial activity against *S. typhi* used variation of final concentrations of extracti.e. 45%, 46%, 47%, 48%, 49%, 50% $^{w}/_{v.}$The MBC is 50% $^{w}/_{v.}$ Table I and Figure 1 show the result of antibacterial activity.

Table I and Figure 1 show that *S. typhi* colonies grew after treatment with 40 to 49% $^{w}/_{v}$ of extract concentration. There was no colony at treatment with 50% $^{w}/_{v}$extract. The turbidity of 50% $^{w}/_{v}$ testing solution was not due to bacterial growth but due to the solution testing dark colour. Therefore, it can be concluded that the ethanol (70% v/v) extract of Binahong rhizome had antibacterial activity against *S. typhi* with MBC 50% $^{w}/_{v.}$ This activity was more little than 70% ethanol of leaf extract. Its MBC against *S. typhi* was 20% $^{w}/_{v}$ (Pradita, 2011). Moreover, the rhizome activity was greater than rod extract, because the rod ethanol extract only inhibited (didn't kill) the *S. typhi* growth at 75%$^{w}/_{v}$ (Puspaningrum, 2011).

3. Phytochemical Screening Results by Tube Test

To know the content of the compound contained in the ethanol extract of binahong rhizome, phytochemical screening was carried out. The flavonoid test showed more intensive yellow of the sample droplet after giving the ammonia vapor. This color indicates that 70% ethanol extract of Binahong rhizome contains flavonoids. The yellow color arised because of the formation of the quinoids tructure of B ring containing conjugated double bonds which are longer and planar so as to fluorescent (Robinson, 1995). The polyphenols test showed a bluish green color and indicated that the ethanol extract of rhizome binahong contains polyphenols.The reaction between polyphenol compounds and chlorides could form the green, purple, blue or black complex compounds (Geissman, 1962). The saponin test obtaineda little stable foam after shaking, this may be due to the saponin was in small amounts. The foam indicated that glycosides have the abilityforming foam in water that hydrolyzed into glucose and other compounds (Rusdi,1990).The test of the presence of alkaloids performed by adding the Dragendorff reagent and Meyer in 70% ethanol extract of rhizome binahong.The extract was mixed with HCl and then boiled, filtered and added reagents Meyer/Dragendorff. The purpose of the HCl addition is for the alkaline alkaloid extraction, because the alkaline alkaloid usually can be extracted with a solvent containing the acid (Harborne, 1987). The results did not show any obvious sediment but the color of the solution became more turbid.This suggests that the ethanol extract of rhizome binahong did not contain alkaloids. More over, the tannins test indicated the absence of sediment in the solutionafter added with 1% gelatin, it means that 70% ethanol extract of rhizome Binahong contained tannin. Tannins will precipitate the

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protein in the gelatin.Tannins react with gelatin to form solid copolymer is not soluble in water (Harborne, 1987). These results are summarized in Table II.

results showed that after ammonia vapor was passed, there were more patches of bright yellow compared to the pale yellow ofethanol extract of binahong rhizome samples. At 254 nm UV light,

Table II. Results Phytochemical Screening Method Tubes With 70% Ethanol Extract Binahong Rhizome *(Anredera cordifolia (Tenore) Steen.)*

	Phytochemical test	Reagent	Result
1	Preliminary	KOH	(+)More intense color There is a chromophore group
2	Flavonoids	Ammonia vapor	(+) Yellow orange
3	Polyphenols	FeCl3	(+) Green blue
4	Saponin	-Shaking - HCl	(+) Arises froth (+) Stable froth
5	Alkaloids	- Dragendorf - Meyer	(-) No sediment (-) No sediment

4. Phytochemical Screening Results by Thin Layer Chromatography

a) Flavonoids

there were patches of blue, and UV366 nmalso gave a blue color.This suggests that the ethanol extract of rhizome binahong contained flavonoids at 0.71 of Rf. The TLC data was summarized Table III.

Table III. TLC results of flavonoids Assay of Ethanol Extract Binahong Rhizome

Excerpts	Rf	Detection			Information
		$UV_{254\ nm}$	$UV_{366\ nm}$	Visible light	
Quersetin	0, 75	Yellow	Blue fluorescence	Yellow	(+)
Ethanol extract of Binahongrhizome (5% w / v)	0, 53	Blue fluorescence	Blue fluorescence	Yellow	(-)
	0.71	Blue fluorescence	Blue fluorescence	Yellow	(+)

According to Markham (1998), the presence of flavonoids was appointed to the extinction spots under UV light at 254 nm, dark yellow, green or blue fluorescence at UV365 nm and with a spray reagent $AlCl_3$ will form a yellow color. Flavonoid compounds in the ethanol extract of rhizome of ethanol were identified by using mobile phase of ethyl acetate: methanol: formic acid (95:5:0,5) and the stationary phase silica gel F_{254}, a comparator quersetin. The TLC

b) Polyphenols

The identification of the group of polyphenol compounds in the ethanol extract of rhizome binahong used mobile phase n-butanol: acetic acid: water (4:1:5) $^{v}/_{v}$ and the stationary phase silica gel F_{254} with a comparator quersetin. It used $FeCl_3$ spray reagent to detect the present of polyphenols. According to Wagner *et al*. (1996), polyphenols with the stationary phase

silica gel F_{254} can be determined by the UV extinction at 254 nm and there was a blue fluorescence in UV 366 nm,where as after the $FeCl_3$ spraying there is a thin green spots.In this test, spot of polyphenols appears at Rf 0.75. The TLC data was summarized in Table IV.

Table IV.TLC Detection of Polyphenols of Binahong Rhizome Ethanol Extract

Excerpts	Rf	Detection			Information
		UV 254 nm	UV 366 nm	Visible light	
Gallat acid	0.72	Extinction	Blue fluorescence	Green gray	(+)
Ethanol extract of Binahong rhizome (5% w / v)	0.75	Extinction	Blue fluorescence	Thin green	(+)

c) Saponin

The identification of saponin used chloroform: methanol (95: 5) $^{v}/_{v}$ as mobile phase and silica gel F_{254}as stationary phase. Saponin glycosides can be detected by spraying with vanillin-sulfuric acid or anisaldehid-sulfuric acid. It will provide the color blue to violet blue or sometimes in the form of patches of red, yellow, blue, purple, green or a yellow brown in visible light. At UV light 254 nm, spot outages do not occur and at 365 nmUV light, there is no fluorescence (Wagner, *et al,*1996).

The test results indicated the presence of saponin in the ethanol extract of binahong rhizome which is characterized by appearing blue-purple spots after spraying witha nisaldehid-sulfuric acid, while in the U V 254 nm and UV 366 nm there was no spot. The TLC data of saponins were summarized in Table V and the chromatograms were showed on Figure 2.

Table V.Test results of TLC Rhizome Ethanol Extract Saponin Binahong

Excerpts	Rf	Detection			Information
		$UV_{254\ nm}$	$UV_{366\ nm}$	Visible (anisaldehide-H_2SO_4)	
Ethanol extract of Binahong rhizome (5% w / v)	0.15	-		H_2SO_4)	(+)
	0.20	-		Purple	(+)
	0.22	Extinction	Blue Fluorescence	Blue purple	(-)
	0.45	Extinction	-	Dark Green	(-)
	0.55	-	-	Purple	(+)
	0.68	Extinction	-	Purple	(-)
	0.75	Extinction	Blue	Yellow	(-)

Based on the tube test and the TLC result, it can be concluded the ethanol (70% $^{v}/_{v}$) extract of Binahong rhizome contained flavonoids, polyphenols and saponins. At least, one of those has antibacterial activity against *S. thypi*. The number and differences of components having antibacterial activity and their quantities affect the high of antibacterial activity.

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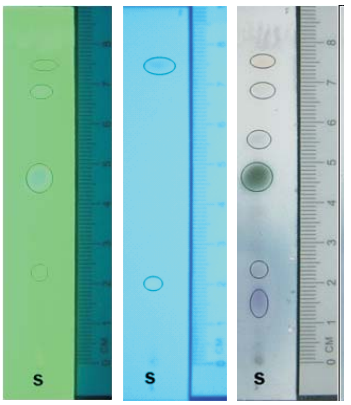

Figure 2. Chromatograms of Saponins identification at (A) 254 nm, (B) 366 nm, (C) anisaldehide-H_2SO_4

In the other study, the activity of 70% $^{v}/_{v}$ ethanol extract of Binahong leaves against *S. thypi* (Pradita, 2011) has MBC at 20% $^{w}/_{v}$ with chemical contents such asflavonoids, polyphenols, saponins and alkaloids. It can be seen that the binahong rhizome ethanol extract contents are different from binahong leaf. There was no alkaloids in rhizome. Based on the MBC value, the activity of rhizome (MBC = 50% $^{w}/_{v}$) was less than leaf (MBC = 20% $^{w}/_{v}$) against *S. typhi*, but the rod has no MBC (Puspaningrum, 2011). In this study, it hasn't known yet whether the alkaloids in leaf affects its activity against *S. typhi*. Therefore, it has to be conducted antibacterial activity study of alkaloid and the other contents in binahongleaf. Binahong leaf ethanol (70% $^{v}/_{v}$) extract has antibacterial activity not only against *S. typhi*, but also *Shigella flexneri* with MBC 15% $^{w}/_{v}$ (Andreani, 2011) and *Candida albicans* with MBC 42% $^{w}/_{v}$ (Hermila, 2011).

4. Chemical contents relationship of 70% ethanol extract Binahong rhizome With Antibacterial Activity

The chemical contents of the 70% ethanol extract binahong rhizome could be identified in this studi were flavonoid, polyphenol and saponin. Flavonoids are compounds included in the polyphenolic class, but not all polyphenols is flavonoids. Flavonoids are natural phenolic compounds present in most of the plant species. Flavonoids are simple phenolic monocyclic compounds. Phenolic compounds have aromatic ring feature with one or two hydroxyl groups (Harborne, 1987). Flavonoid compounds can cause protein denaturation, so the metabolic processes of bacteria were disturbed.T his damage is irreversible or can not be repaired again. In addition to flavonoid, phenolic compounds also can damage cell membranes, then the cell wall permeability will be changed and stimulate cell growth inhibition or kill cells (Pelczar and Chan, 1986). The last compound, saponin, has antibacterial activity with involves the formation of complexes with sterols in the plasma membrane thus destroys the cell membrane semipermeability leading to cell death (Hostettmann and Marston, 1995).

This study has the limitation because it has not known yet which one of the compoundsthat can inhibit or kill *S. typhi* growth. Therefore, it has to be conducted the study of antibacterial activity of each compound found in binahong rhizome.

CONCLUSION

1. The 70% ethanol extract binahong rhizome has antibacterial activity against *Salmonella typhi* with 50% $^{w}/_{v.}$ of MBC.
2. The 70% ethanol extract binahong rhizome contains flavonoids, polyphenols, and saponins.

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