REV_Antioxidant activity of vacuum column chromatography fractions of ethanol extract of sarang banua (Clerodenrum fragrans vent willd) leaves

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Antioxidant activity of vacuum column chromatography fractions of ethanol extract of sarang banua (*Clerodenrum fragrans* vent willd) leaves

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Abstract. Sarang banua plants are found in Simalungun, North Sumatra, Indonesia, which have been used by the community as traditional medicinal plants. The result of plant determination, sarang banua plant is a type of *Clerodendrum fragrans* Vent Willd, including the family *Verbenaceae*. The antioxidant activity of vacuum column chromatography fractions of ethanol extract of *C. fragrans* leaves was completed. This study aims to find a pure fraction through vacuum column chromatography from the ethanol extract of *C. fragrans* leaves which has antioxidant activity. Purification of ethanol extract of *C. Fragrans* was carried out using vacuum column chromatography with silica gel 1.07733.1000 stationary phase and *n*-hexane, ethyl acetate and methanol as mobile phase.The antioxidant activity test of the fractions were carried out by DPPH free radical reduction method. The antioxidant test results using the DPPH free radical scavenging method showed that the F5 fraction had higher antioxidant activity than F4 and F3 fractions with IC_{50} values in a row 19.80; 24.65 and 84.02 ppm. The IC_{50} value of vitamin C as a positive control is 22.25 ppm. The F5 fraction has antioxidant activity higher than Vitamin C (positive control) and includes a very strong category of antioxidants.

Keywords: *Clerodendrum fragrans* Vent Willd, antioxidant activity, vacuum column chromatography fractions, ethanol extract.

1. Introduction

Sarang banua plants are found in Simalungun, North Sumatra, Indonesia, which have been used by the community as traditional medicinal plants. The result of plant determination, sarang banua plant is a type of *Clerodendrum fragrans* Vent Willd, including the family Verbenaceae [1]. The *C. fragrans* plant is a semi-wood plant, typically shrubs and the community in Simalungun says the name of the plant is "sarang banua" (Figure 1). The genus Clerodendrum (Family Verbanaceae) has more than five hundred species that have been identified which are semi-woody shrub plants and are widespread in the tropics. Root, stems, leaves extracts several species of the genus Clerodendrum have been used for the treatment of asthma, cataracts, malaria, blood, skin and lung diseases traditionally [2].

Medicinal plants contain secondary metabolites such as alkaloids, flavonoids, steroids, saponins, tannins so that they can be used as pharmacological sources that are cheap and biodegradable [3]. For example, *Clerodendrum volubile* plants contain saponins, anthraquinones, flavonoids, alkaloids, phenolic compounds, terpenes and glycosides have been used as analgesics [4]. Ethanol extract of *Solanum blumei* Nees ex Blume fruit which is a local Indonesian plant containing β-solanine glycoside alkaloid which is toxic and has anticancer activity for L_{1210} leukemia cells [5,6].

The secondary metabolites of alkaloids, triterpenoids, flavonoids, saponins, tannins and quinine were found in the ethanol extract of *C. fragrans* leaves [1] and has antioxidant and antibacterial activity of *S. aureus* and *E. coli* [7]. Purification of ethanol extract from leaves of *C. fragrans* needs to be done to find active compounds that have potential as antioxidants. This study aims to find a pure fraction through vacuum column chromatography which has antioxidant activity.

Figure 1. "Sarang banua"- *Clerodendrum fragrans* Vent Willd

2. Experimental Method

2.1. Preparation of ethanol extract of C. fragrans leaves

Fresh *C. fragrans* leaves are taken from Raya Usang village, Simalungun, Indonesia. The leaves of *C. fragrans* are dried and made into powder. Simplicia of *C. fragrans* leaf powder was extracted by maceration with solvents of different polarity [1].

2.2. Fractionation of ethanol extract of C. fragrans leaves

Purification of ethanol extract of *C. fragrans* was carried out using vacuum column chromatography with silica gel 1.07733.1000 stationary phase and the mobile phase of *n*-hexane, *n*-hexane: ethyl acetate (9: 1; 8: 2; 7: 3; 6: 4; 5: 5; 4: 6; 3: 7; 2: 8; 1: 9; 0:10), ethyl acetate: methanol (9: 1; 8: 2; 7: 3; 6: 4; 5: 5; 6: 4; 7: 3; 8: 2; 9: 1) and methanol. Column fractionation results were tested by TLC and fractions which had the same chromatogram combined.

2.4. Antioxidant test of the combined fraction of vacuum column chromatography

The antioxidant test of the combined fraction was carried out by the DPPH (1,1 diphenyl-2-picrylhydrazyl) free radical scavenging method using UV-Visible spectrophotometer at a wavelength of 517 nm. The concentration of the fraction sample solution was made in concentrations of 5, 10, 25 and 50 ppm and the concentration of DPPH solution was 0.4 mM in methanol p.a. As a positive control a solution of vitamin C with a concentration of 5, 10, 25 and 50 ppm in methanol p.a. is used. Percent of sample inhibition was calculated based on the difference in absorbance of DPPH solution with absorbance of sample solution divided by absorbance of DPPH solution multiplied by 100%. The amount of antioxidant concentration that can inhibit as much as 50% of free radicals is called the IC_{50} value. The IC_{50} value can be calculated using a linear regression equation, where Y equals 50 and X shows the IC_{50} value of the test sample [8, 9, 7, 10].

3. Result and Discussion

The results of vacuum column chromatography ethanol extract of leaves of *C. fragrans* obtained 20 fractions. The TLC test results for each column fraction are shown in Figure 2. Fractionation of vacuum column chromatography ethanol extract of *C. fragrans* leaves using column mobile phase solution which is different in polarity (increasingly polar). The next twenty column fractions are in TLC using the phase hexana solution : ethylacetate (7: 3) and hexana : ethylacetate (8: 2) to determine the purity of each fraction. Fractions that have the same chromatogram are then combined into a combined fraction. The fraction that has the same chromatogram is combined so that five combined fractions are obtained.

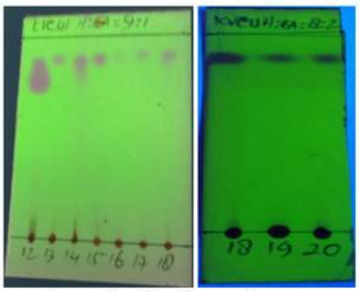

Figure 2. TLC chromatogram chromatography fraction column vacuum ethanol extract of *C. fragrans* leaf.

Results fractionation of vacuum column chromatography ethanol extract of *C. fragrans* leaves is presented in Table 1.

Table 1. Results of fractionation of chromatography of vacuum column ethanol extract of *C. Fragrans* leaf

Fraction	No Vial	Column Mobile Phase	TLC Mobil Phase	Rf	Weight (g)
F1	1-2	Hex : EtilAc (9 : 1~ 8:2)	Hex:EtilAc (7:3)	0,93	0,148
F2	3	Hex:EtilAc (7:3)	Hex:EtilAc (7:3)	0,59;0,70;0,80	0,095
F3	4-5	Hex:EtilAc (1:9)	Hex:EtilAc (7:3)	0,42;0,59;0,70;0,82	0,397
F4	6-12	EtilAc:MeOH(8:2)	Hex:EtilAc (7:3)	0,19;0,42;0,59;0,70	1,819
F5	13-20	EtilAc:MeOH (6:4 ~ MeOH)	Hex:EtilAc (8:2)	0,88	3,850

Each combined fraction was then tested for antioxidant activity to determine the most active combined fraction. Because the weight of the combined fraction F1, F2 was obtained very little (0.148 and 0.095 g), the antioxidant test was not carried out on the two fractions. The antioxidant activity test was carried out on a combined fraction of F3, F4 and F5 using the DPPH free radical reduction method. The test results of the antioxidant activity of fractions F3, F4 and F5 are presented in Table 2.

Table 2. Antioxidant activity of chromatographic fraction column of leaves *Clerodendrum fragrans* Vent Willd with DPPH scavenging method.

Vacuum Column Chromatography Fraction	Concentration (ppm)	Absorbance	Scavenging Activity on DPPH (%)	IC_{50} (ppm)	Category of Antioxidant
Fraction F3	5	0, 481	14,82	84,02	Strong antioxidants
	10	0,449	20,45		
	25	0,419	25,72		
	50	0,365	35,20		
Fraction F4	5	0, 249	55,73	24,65	Very strong antioxidants
	10	0,225	60,15		
	25	0,132	76,62		
	50	0,095	83,17		
Fraction F5	5	0,194	65,60	19,80	Very strong antioxidants
	10	0,168	70,24		
	25	0,059	89,45		
	50	0,011	98,01		

Vitamin C	5	0,213	62,13	22,25	Very strong antioxidants
	10	0,129	77,10		
	25	0,007	86,45		
	50	0,015	97,34		

The F5 vacuum column chromatography fraction showed the highest percentage of (98.01%). The higher the percentage of DPPH free radical scavenging the sample, the higher the antioxidant activity.

The antioxidant test showed that results F5 vacuum column chromatography fraction is the highest antioxidant activity with an IC_{50} value of 19.80 ppm followed by F4 fraction (24.65 ppm) and F3 fraction (97.99 ppm). Antioxidant concentration (ppm) which can reduce 50% of free radicals is called IC_{50} value (inhibition concentration 50). Compounds that have IC_{50} values below 50 ppm are grouped as very strong antioxidants, 50-100 ppm are classified as strong antioxidants and 100-150 ppm are classified as weak antioxidants [11].

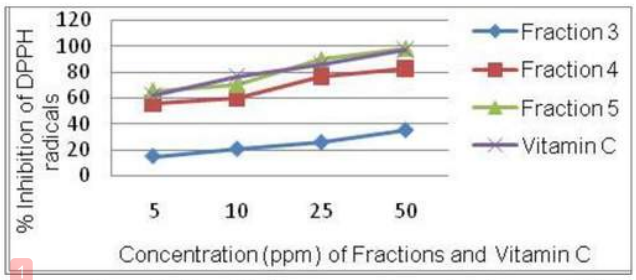

Figure 3. Scavenging activity of *C. fragrans* column fractions on DPPH radicals

The F5 vacuum column chromatography fraction has antioxidant activity higher than Vitamin C (positive control) and includes a very strong category of antioxidants. The magnitude of antioxidant activity is caused by the ability of antioxidants to reduce DPPH free radicals (Figure 3). The ability to reduce free radicals F5 vacuum column chromatography fraction was the highest (98.01%), followed by vitamin C (97.43%), F4 fraction (83.17%) and F3 fraction (32.18%) (Figure 3). The F5 vacuum column chromatography fraction from the ethanol extract of *C. fragrans* leaves had the highest scavenging activity on DPPH (98.01%), followed by vitamin C (97.43%), F4 fraction (83.17%) and F3 fraction (32.18%).

4. Conclusion

The F5 vacuum column chromatography fraction had higher antioxidant activity than F4 and F3 fractions with IC_{50} values in a row 19.80; 24.65 and 84.02 ppm. The F5 fraction has antioxidant activity higher than Vitamin C (positive control) and includes a very strong category of antioxidants.

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