

# ANTIOXIDANT AND ANTIBACTERIAL ACTIVITIES

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## ANTIOXIDANT AND ANTIBACTERIAL ACTIVITIES OF NON-POLAR TO POLAR SOLVENT EXTRACTS OF *Sarang Banua* (*Clerodendrum fragrans* Vent Willd) LEAVES

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### ABSTRACT

*Sarang banua* plant, known as *Clerodendrum fragrans* Vent Willd, is a division of the Verbanaceae family found in Simalungun district of Indonesia, which is traditionally used as medicinal plants. The antioxidant and antibacterial activities of the *C. fragrans* leaf extract was prepared by dissolving it in non-polar to polar solvents and treated by DPPH (1,1-diphenyl-2-picrylhydrazin) and diffusion methods. The results showed that the ethanol (polar) extract of the *C. fragrans* leaves had the highest antioxidant activity than ethyl acetate (semi-polar) and *n*-hexane (non-polar) extracts, which had IC<sub>50</sub> values of 22.37 ppm, 27.26 ppm, and 88.77 ppm respectively. Vitamin C as a positive controlled with the IC<sub>50</sub> value of 20.18 ppm. Furthermore, the ethyl acetate (semi-polar) extract of the *C. fragrans* leaves had the greatest inhibition value on Gram-positive bacteria of *S. aureus* (90.0%) with inhibitory zones of 11.25 ± 0.2 mm, meanwhile the *n*-hexane (non-polar) extract had the greatest inhibition against Gram-negative bacteria of the *E. coli* (77.21%) with inhibitory zones of 10.5 ± 0.3 mm.

**Keywords:** *Clerodendrum fragrans* Vent Willd, Antibacterial, Antioxidant, Polarity of solvent, Medicinal plant of Indonesian.

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### INTRODUCTION

It is known that Genus *Clerodendrum* (Family of Verbanaceae) has more than five hundred species which are semi-woody shrubs and widespread in the tropics regions. The root, stem and leaf extracts of the *Clerodendrum* species have been used traditionally for the treatment of asthma, cataracts, malaria, blood, skin and lung diseases, rheumatism, inflammatory, cough, genital infections, skin diseases, anti-diabetic, anti-hypertensive, sedative, antibacterial, analgesic and antioxidant<sup>1,2</sup>. One species of the *clerodendrum* is found in Raya Usang village, Simalungun district, North Sumatera Indonesia, which is typically shrubs and grows to around 2.5-3.0 m high, it has whitish pink flowers and the flower petal is reddish purple in color and 10-15 mm in length, the leaves are simple and opposite, oval and its length 6-25 cm and 5-25 cm wide and the edges of the leaves are wavy. Local people call it “*Sarang banua*” as shown in Fig. -1. Base on the herbarium determination carried out by the “Herbarium Bogorinse” Botanical Field of the Biology Research Center-LIPI Bogor, the plant is classified as a type of the *Clerodendrum fragrans* Vent Willd which is a division of the Verbanaceae family and has been stored as herbarium form “Herbarium Bogoriense” in June 2017. Generally, the local community uses it as traditional medicinal plants.

The medicinal plants are known as pharmaceutical sources and rich in active secondary metabolites such as alkaloids, steroids, flavonoids, tannins, saponins and phenol compounds, which are available at low cost and easily decomposed. For instance, *Clerodendrum volubile* plant could be used as an analgesic and containing chemical compounds such as saponins, tannins, anthraquinones, flavonoids, alkaloids, phenols, terpenes and glycosides<sup>2</sup>, steroidal glycoside. Furthermore, alkaloid β-solanin from the ethanol extract of *Solanum blumei* Nees ex Blume fruit has toxicity and anticancer for L<sub>1210</sub> leukemia cells<sup>3,4</sup>. Secondary metabolites of the plants can be obtained through solvent extraction based on the solubility and polarity of the metabolites. For instance, normal-hexane, ethyl acetate and ethanol solvents which have different polarities, namely non-polar, semi polar and polar with dielectric constant (20°C) of 1.90, 6.02 and 22.40

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respectively<sup>5</sup> could be used as the extraction solvents. Selection of the solvent in the maceration process is based on the principle of “likes dissolve likes” solubility, meaning that the polar compound only dissolves in polar solvents, and vice versa for semi polar and non-polar compounds.



Fig.-1: Sarang banua (*Clerodendrum fragrans* Vent Willd).

Qualitative phytochemicals analysis of medicinal plant extracts i.e. *Boerhaavia diffusa* Linn, *Terminalia bellerica* Linn and *Tribulus terrestris* Linn using polar and non-polar solvents showed different chemical contents. The solvent is one of the most important factors affecting the chemical composition and biological activity of the plant extracts<sup>7,8</sup>. A number of the *Clerodendrum* plant extracts have antioxidant activity against DPPH-free radical scavenging activity and antibacterial<sup>9-10</sup>. The antioxidant of the extracts was treated by DPPH (1,1-diphenyl-2-picrylhydrazin) method<sup>11-13</sup> and antibacterial activity tests carried out by disc diffusion methods<sup>14,15</sup>.

The secondary metabolites of alkaloid, steroid and flavonoid found in the *n*-hexane extract of the *C. fragrans* leaves, alkaloids, steroids, saponins, and tannins found in the ethyl acetate extracts and in addition alkaloids, triterpenoids, flavonoids, saponins, tannins and quinines found in ethanol extracts of the *C. fragrans* leaves<sup>16</sup>. Antioxidant and antibacterial activity tests of the *C. fragrans* extracts that using various solvent polarities need to carry out in order to find out fractions with a high antioxidant and antibacterial activities which are useful for developing and usage of the *C. fragrans* local plants for the medical ingredient.

## EXPERIMENTAL

### Collection of Plant Sample

Plant sample selected was the *Sarang banua* (*C. fragrans* Vent Willd) fresh leaves, obtained from Raya Usang village, Dolok Masihul sub-district, Simalungun district, North Sumatera, Indonesia. The sample was identified by the "Herbarium Bogoriense" in the Bogor LIPI-Biology Research Center.

### Sample Extraction

A total of 10 kg freshly *C. fragrans* Vent Willd leaves were washed clean with bi-distilled water, drained, dried in air and protected from direct sunlight and mechanically grounded to a simplicia powder (1.250 kg). The simplicia powder of the *C. fragrans* leaves was macerated with polar solvents, namely *n*-hexane solvent (non-polar), followed by ethyl acetate (semi polar) and ended with ethanol solvent (polar). Total of 1000.0 g of simplicia powder of the *C. fragrans* leaves were macerated with *n*-hexane solvent, filtered and the filtrate obtained was concentrated in a rotary evaporator vacuum to obtain concentrated *n*-hexane extracts. The pulp portion was macerated with ethyl acetate solvents, filtered and the filtrate was concentrated to obtain concentrated ethyl acetate extracts. Furthermore, the pulp portion was re-macerated with ethanol solvents, filtered and the filtrate was concentrated to obtain concentrated ethanol extracts. The maceration processes lasted for 48 hours, with three times solvent additions. Finally, *n*-hexane, ethyl acetate and ethanol extracts concentrated of the *C. fragrans* leaves were prepared respectively<sup>16</sup>.



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**Antioxidant Assay**

The DPPH method (1,1-diphenyl-2-picrylhydrazyl) was used for the antioxidants test. The solution of vitamin C as a positive control provided with concentrations of 5, 10, 25 and 50 ppm. Furthermore, making DPPH 0.4 mM solution was carried out by dissolving 15.7 mg DPPH in methanol p.a. to a volume of 100 mL solution.

**Preparation of Sample Test and Positive Controlled Solutions.** 2

An extract stock solution was provided by dissolving each 5.0 mg of *n*-hexane, ethyl acetate and ethanol extracts of the *C. fragrans* leaves in methanol p.a. until the volume of solution is 10.0 mL. Then, each extract solution series was made by piping 50, 100, 250 and 500  $\mu$ L extract stock solutions into a 5 mL tube to obtain extract solutions of concentrations of 5, 10, 25 and 50 ppm.

Then, 1.0 mL of DPPH 0.4 mM solution was added to each tube and methanol p.a. to a solution volume of 5 mL.

As a positive control, a solution of vitamin C with concentrations of 5, 10, 25 and 50 ppm was put into a 5.0 mL test tube, then added 1.0 mL DPPH 0.4 mM and methanol p.a. to a solution volume of 5.0 mL. As a negative control, methanol solution p.a. and 1.0 mL of DPPH 0.4 mM solution in a 5.0 mL test tube.

All test tubes used were wrapped in aluminum foil and incubated at 37°C for 30 minutes, then measured absorbance with UV-Vis spectrophotometer at a wavelength of 517 nm<sup>11, 12</sup>. Antioxidant testing of the sample was carried out in triplets.

The inhibition percentage was calculated by the following formula:

$$\text{Inhibition (\%)} = [(A - B)/A] \times 100\% \quad (1)$$

Where, A is the absorbance of the controlled negative solutions (DPPH solution without the sample), B is the absorbance of the DPPH solution in the presence of the sample (extract/ascorbic acid).

The inhibition concentration 50 (IC<sub>50</sub>) value is the concentration of antioxidants (ppm) which can inhibit 50% of free radicals. The IC<sub>50</sub> value is obtained from the intersection of the line between 50% of the resistance power with the axis of concentrations, then put into the equation below<sup>13</sup>:

$$Y = a + bX \quad (2)$$

where Y = 50, and the value of X indicates IC<sub>50</sub>.

**Antibacterial Assay**

Before analysis test carried out, glassware was sterilized in an oven at 100°C for 2 hours. Metal wares were sterilized with a spray-mounted light, and heat-resistant appliances and the high-temperature medium were sterilized in an autoclave at 121°C and 2 atm for 15 minutes. Before a laminary tube is used, that is sterilized with UV light for 15 minutes and blown for 10 minutes.

A total of 3.8 g Mueller Hinton Agar (MHA) was mixed homogeneously with 100 mL aquabidest in a beaker glass and then poured into a Petri dish as a medium.

*Escheria coli* dan *Staphylococcus aureus* were reconstructed before using in antibacterial test. The bacteria were cultured on a bend sterilized agar then incubated for 24 hours at 37°C. The bacterial suspension was spread using a sterile cotton swab on nutrient agar plat. The turbidity of the bacterial culture was adjusted to 0.5 McFarlan Standard then incubated for 24 hours<sup>19</sup>. Each *normal*-hexane, ethyl acetate and ethanol extracts were dissolved at concentrations of 0%, 1.25%, 2.50%, 5% and 10%. Chloramphenicol discs 30 g (Oxoid)<sup>20</sup> was used as a positive controlled. The inhibition zone of each extract is measured in mm<sup>17-19</sup>.

**RESULTS AND DISCUSSION****Antioxidant Assay** 2

Antioxidant assay of *n*-hexane, ethyl acetate and ethanol extracts of *C. fragrans* leaves was carried out by DPPH method using UV-Vis at a wavelength of 517 nm.

The antioxidant activity of the extract can be seen from the scavenging ability of extracts against DPPH free radicals, which can be measured from DPPH color changes from purple to yellow. The reduced

DPPH purple color, resulting in a smaller absorbance value, which shows the stronger scavenging activity of the extract. Ethanol extract of *C. fragrans* had the highest scavenging activity (93.2%), followed by ethyl acetate (88.5%) and *n*-hexane extract (35.0%). The scavenging activity of vitamin C (positive control) was 97.2% (Table-1 and Fig.-2). The greater the concentration of extract, the higher the scavenging activity.

The Inhibition Concentration 50 (IC<sub>50</sub>) value is the concentration of antioxidants (ppm) which can scavenge 50% of free radicals. A compound is said to have very strong antioxidant activity value if the IC<sub>50</sub> value is less than 50 ppm, the strength of antioxidant value for IC<sub>50</sub> is 50-100 ppm and the antioxidant is considered moderate if the IC<sub>50</sub> value ranges from 101-150 ppm, and the antioxidant is classified as weak if the IC<sub>50</sub> value ranges from 151-200 ppm<sup>20</sup>. The results showed that ethanol extract of the *C. fragrans* leaves had the lowest IC<sub>50</sub> value (22.37 ppm) than ethyl acetate extract (27.26 ppm) and *n*-hexane extract (88.77 ppm), which relatively close to the IC<sub>50</sub> vitamin C value as a positive controlled (20.18) (Table-1). It shows that ethanol extract of the *C. fragrans* leaf has the highest antioxidant activity value followed by ethyl acetate and *n*-hexane extracts, which are relatively close to the antioxidant activity of the Vitamin C.

The smaller the IC<sub>50</sub> value indicates the higher the antioxidant activity value. The antioxidant activity of the ethanol extract and ethyl acetate of the *C. fragrans* leaves found at the category of very strong which were located at a value of <50, while *n*-hexane extracts found at the strong antioxidant category at the 50-100 region. The IC<sub>50</sub> value of vitamin C as a positive controlled was 20.18 ppm considered as a very strong antioxidant category (Table-1 and Fig.-2). The study found that the findings are highly likely similar to the findings found in the antioxidant activity test of the ethanol extract of walnut *Juglans regia* L green husk which is higher than ethyl acetate and *n*-hexane extracts and contain more total polyphenols and flavonoids<sup>7</sup> and *Eugenia uniflora* L's ethyl acetate extract has a higher antioxidant activity value than *n*-hexane and water fractions<sup>15</sup>.

Table-1: Scavenging Activity of the *Clerodendrum fragrans* Vent Willd Leaf Extracts on DPPH

Leaf Extracts of <i>C. fragrans</i>	Concentration (ppm)	Absorbance	Scavenging Activity on DPPH (%)	IC <sub>50</sub> (ppm)	Category of Antioxidant
<i>n</i> -Hexane Extract	5	0.452	15.5	88.77	Strong Antioxidant
	10	0.424	20.7		
	25	0.413	22.8		
	50	0.229	35.0		
Ethyl Acetate Extract	5	0.234	54.5	27.26	Very Strong Antioxidant
	10	0.105	80.4		
	25	0.086	83.9		
	50	0.060	88.5		
Ethanol Extract	5	0.223	58.3	22.37	Very Strong Antioxidant
	10	0.122	77.2		
	25	0.063	88.2		
	50	0.036	93.2		
Vitamin C	5	0.198	93.86	20.18	Very Strong Antioxidant
	10	0.153	72.08		
	25	0.056	89.41		
	50	0.015	97.26		

The use of types of solvents at different polarity levels were carried out to obtain extracts that contain unknown compounds, such as in the ethyl acetate extract of the leaves of *Clerodendrum phlomidis* L. found steroids, alkaloids, phenols, amino acids, and no saponins, sugars, flavonoids and catechins<sup>21</sup>, meanwhile in ethanol extract found steroids and phenols. However, the crude ethanol extract of the *Cleodendrum philippinun* leaves contained alkaloids, saponin, glycoside, phenolics, tannin, flavonoid, fixed oil and fats<sup>22</sup>. The ethanolic extract of roots of *Clerodendrum phlomidis* L has the highest free



radical scavenging activity than the other three extracts, namely petroleum ether, chloroform and ethyl acetate<sup>21</sup>. The polar solvent exhibited the highest amount of bioactive compounds<sup>23</sup>.

The antibacterial activity value of the *n*-hexene (non-polar), ethylacetate (semi-polar) and ethanol (polar) extracts of the *C. fragrans* leaves at a concentration of 1.25% to 10% indicated by the diameter of the inhibition zone against *Staphylococcus aureus* and *Escherichia coli* (Table-2).

The ethyl acetate (semi-polar) extract of the *C. fragrans* has the largest inhibition zone ( $11.25 \pm 0.2$  mm) than the polar extract of the ethanol ( $9.40 \pm 0.1$  mm) and non-polar *n*-hexane ( $9.0 \pm 0.1$  mm) against *Staphylococcus aureus* (Table-2). Likewise the *Escherichia coli*, the ethyl acetate extract has the largest inhibition zone ( $14.8 \pm 0.5$  mm) than then-hexane ( $10.5 \pm 0.3$  mm) and ethanol extracts ( $10.2 \pm 0.2$  mm).

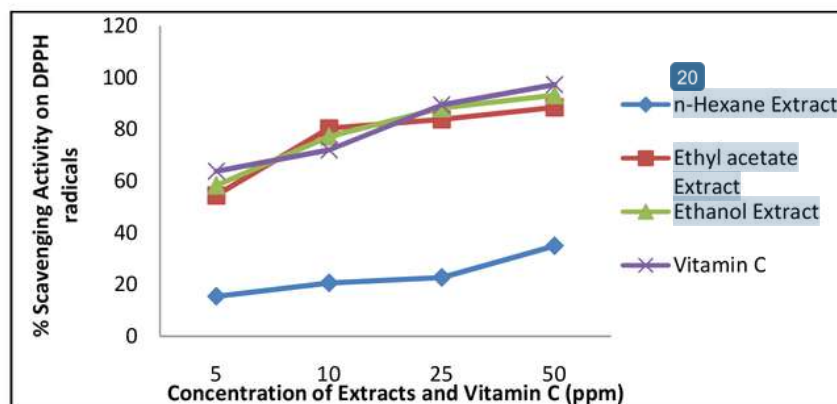


Fig.-2: Scavenging Activity of *C. fragrans* Extracts Leaves On DPPH Radicals.

Table.-2: Zone of Inhibition and Inhibition Power Leaf Extracts of the *C. fragrans* Against Bacteria (mm).

Bacteria	Plant Extracts	Antibacterial Activity					Inhibition Power (%)
		Chloramphenicol Disc (Oxoid)	The diameter of zone of inhibition (mm)				
			The concentration of Extract (%)				
			1,25%	2,5%	5%	10%	
<i>S. aureus</i>	<i>n</i> -Hexane Extract	19.8	8.40 ± 0.4	8.75 ± 0.2	8.9 ± 0.1	9.0 ± 0.1	45.45
	Ethyl Acetate Extract	12.5	9.0 ± 0.2	9.3 ± 0.4	9.7 ± 0.2	11.25 ± 0.2	90.00
	Ethanol Extract	13.4	7.9 ± 0.5	8.6 ± 0.6	8.9 ± 0.4	9.40 ± 0.1	70.15
<i>E. coli</i>	<i>n</i> -Hexane Extract	13.6	7.5 ± 0.5	8.9 ± 0.5	9.8 ± 0.2	10.5 ± 0.3	77.21
	Ethyl Acetate	24.2	7.6 ± 0.2	9.2 ± 0.2	10.1 ± 0.2	14.8 ± 0.5	61.16
	Ethanol Extract	20.3	7.5 ± 0.2	8.4 ± 0.1	10.1 ± 0.5	10.2 ± 0.2	50.25

Based on the inhibitory power of chloramphenicol positive control compounds, it was found that the greatest inhibition of *C. fragrans* leaf extract against *S. aureus* was on ethyl acetate extract (90.0%) followed by ethanol (70.15%) and *n*-hexane extracts (45.45%), but for *E. coli*, the greatest inhibition was found in *n*-hexane extract (77.21%) followed by ethyl acetate (61.16%) and ethanol extracts (50.25%).

The ethyl acetate (semi-polar) extract of the *C. fragrans* leaves has the greatest inhibition value on Gram-positive bacteria of *S. aureus* (90.0%), meanwhile *n*-hexane (non-polar) extract has the greatest inhibitory

power against Gram-negative bacteria of the *E. coli* (77.21%). In the *n*-hexane extract of the *C. fragrans* found secondary metabolites such as alkaloid, steroid and flavonoid metabolites, in the ethyl acetate extract found alkaloids, steroids, saponins and tannins and in ethanol extract found alkaloids, triterpenoids, flavonoids, saponins, tannins and quinines<sup>16</sup>.

In contrast, it was found that methanol (polar) extract of the *Curcuma aeruginosa* rhizome is active against Gram-negative bacteria the *E. coli* and not active against the Gram-positive *S aureus* bacteria<sup>15</sup> and furthermore it was found that the non polar (pet.ether) fraction of the *Brassica nigra* seed has stronger antibacterial activity than crude methanol extract against the *S. aureus* bacteria<sup>24</sup>. Ethanol extract of *Crinum asiaticum* also has antibacterial activity against the *S. aureus* and the *E. coli* bacteria<sup>25</sup>. The results indicated that the solvent polarity of *n*-hexane, ethyl acetate and ethanol extracts of the *C. fragrans* leaves are associated with the antioxidant and antibacterial activities and type of the bacteria.

### CONCLUSION

The use of suitable solvents in the extraction process of leaves of *C. fragrans* was important to consider to obtain extracts fractions which have high antioxidant and antibacterial activity for developing local *C. fragrans* Vent Willd plants as antioxidant and antibacterial medicinal ingredients.

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