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In vitro study of antioxidant and antibacterial properties of Phyllanthaceae and Rubiaceae plant families from Simeuleu Island, Aceh, Indonesia

Marlin Megalestin Raunsai, Ahmad Fathoni, Andria Agusta*
Research Center for Chemistry, National Research and Innovation Agency (BRIN), Building 452, PUSPIPTEK, Serpong, Banten, Indonesia

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ABSTRACT

Plants are still the preferred solution to solve several of the main problems the world is facing today, such as antibiotic resistance and free radicals. The plant chemical compounds have the potential as antibacterial and antioxidant agents. Therefore, four plants representing Phyllantaceae family (*Glochidion varians* Miq. *Glochidion zeylanicum* (Gaertn.) A.Juss) and two plants from Rubiaceae family (*Uncaria lanosa* Wall. and *Uncaria cordata* (Lour.) Merr.) were collected from Simeulue island, Aceh. We obtained 32 extracts from several plants with various solvents, such as hexane, dichloromethane, ethyl acetate, and methanol. The methods applied to investigate antibacterial activities were Thin Layer Chromatography (TLC) - bioautography and Minimum Inhibitory Concentration (MIC). Meanwhile, TLC - bioautography and Microdilution Assay were utilized to analyze antioxidant capacity against 1,1-diphenyl-2-picrylhydrazyl (DPPH). The results of antibacterial activity show 32 extracts displayed very strong to weak activity against *Staphylococcus aureus* and *Escherichia coli*. Among all extracts, methanol extracts of *G. varians* (stem and leaf), methanol extracts of *G. zeylanicum* (stem and leaf), methanol and ethyl acetate extracts of *U. cordata* (leaf), and ethyl acetate extract of *G. varians* (leaf) indicate potent antioxidant activity with the range of antioxidant activity index (AAI) values of 2.16 -11.73. Furthermore, all extracts exhibit moderate to weak antibacterial evaluation. The methanol extract of *G. varians* stem has the highest MIC value of 128 µg/ml against *S. aureus.* The methanol extracts of *G. varians* stem and leaf and methanol extract of *G. zeylanicum* leaf against *E. coli* have MIC value of 256 µg/ml, whereas other extracts have >256 µg/ml MIC value. To sum up, four plants from Phylantaceae and Rubiaceae families have the potential to be expanded as antioxidant agents rather than antibacterial agents.

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Keywords: antibacterial, antioxidant, medicinal plants, Phyllanthaceae, Rubiaceae, Simeuleu

Corresponding author:
Andria Agusta
Research Center for Chemistry, National Research and Innovation Agency (BRIN)
Building 452, PUSPIPTEK, Serpong, Banten, Indonesia
Email: andria.agusta@lipi.go.id

INTRODUCTION

Health problems in Indonesia are related to the high rate of diseases caused by free radicals and frequent infections caused by pathogenic bacteria. Radiation, stress, and alcohol can increase free radicals in the human body (Jayachitra & Krithiga, 2012). This condition can cause various diseases such as diabetes, cancer, and atherosclerosis which are generally associated with protein degradation, lipid peroxidation, and DNA oxidation (Jayathilake et al., 2016).

Various infectious diseases are still one of the leading health problems around the world, including in Indonesia. Infectious diseases can be caused by multiple microorganisms, one of which is bacteria. Several reasons for discovering natural antibiotics are the misuse and overuse of antibiotics and the resistance of bacteria to conventional antibiotics (Pancu et al., 2021). In addition, the high production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) in the body due to external factors such as radiation, alcohol, and cigarette smoke results in high rates of chronic and degenerative diseases (Xu et al., 2017). The use of natural antioxidants had minimal side effects compared to synthetic antioxidants (Lourenço et al., 2019). The above problems have urged many scientists to study the discovery of antioxidant and antibacterial agents from plants (Lourenço et al., 2019; Pancu et al., 2021; Xu et al., 2017).

In this study, plants were investigated their antioxidant and antibacterial properties using multiple scientific approaches. The plants studied were four plant species from the Phyllantaceae family, namely *G. varians* Miq., *G. zeylanicum* (Gaertn.) A.Juss and two plants from the Rubiaceae family, namely *U. lanosa* Wall. and *U. cordata* (Lour.) Merr. from Simeulue, Aceh, Indonesia. *G. zeylanicum* is known to have potential as anti-tumor, anti-cancer, antioxidant and anti-aging agents (Duangjan et al., 2019; Sharma et al., 2011; Tanaka et al., 2004). Meanwhile, *U. cordata* has anti-hyperglycemic and antimicrobe antivities (Abdullah, 2016; Syafriana et al., 2021). No reports have been found regarding the biological activity of *G. varians* and there are still limited reports for *U. lanosa* and *U. cordata*. Research on the plant bioactivity from Simeuleu has not been widely studied. Therefore, the results of this study will be the initial data for further investigation.

MATERIALS AND METHOD

Materials

Two plants from the Phyllanthaceae family *(Glochidion varians* Miq., *Glochidion zeylanicum* (Gaertn.) A.Juss) and two plants from Rubiaceae (*U. lanosa* Wall. and *Uncaria cordata* (Lour.) Merr.) were collected from Simeulue, Aceh, Indonesia. The plant identification was carried out at Herbarium Bogoriense, Research Center for Biology-BRIN.

Methods

Extraction

The plant materials used were stems and leaves. The materials were dried under sunlight, then powdered and extracted using organic solvents. Serial organic solvents were used with different polarities, such as n-hexane, dichloromethane, ethyl acetate, and methanol. Plant samples were macerated three times for each solvent, and then the obtained filtrate was combined and concentrated by a rotary evaporator.

Antioxidant assay using TLC bioautography

The antioxidant test using the TLC bioautography method refers to Dewanjee et al. (2015). The free radical used for measuring free radical scavenging was 1,1-diphenyl-2-picrylhydrazyl (DPPH). The mobile phases used were Hexane: Ethyl Acetate = 3: 2 (hexane extract), Dichloromethane: Methanol = 10: 1 (dichloromethane and ethyl acetate extracts), and Chloroform: Methanol: Water = 6: 4: 1 (methanol extract).

IC_{50} and AAI determination of active extract

The microdilution assay was used to determine the absorbance using Spectrophotometry (Thermo Scientific Varioskan Flash) as described before (Wulansari et al., 2017). The absorbance data obtained were then calculated IC_{50} value and AAI (Antioxidant Activity Index) value in Microsoft Excel program. The equation 1 was used to calculate the AAI (Scherer & Godoy, 2009):

$$AAI = \frac{\text{The final concentration of DPPH in the reaction } (\mu g/ml)}{IC_{50}\ (\mu g/ml)} \quad (1)$$

The inhibitory concentration (IC) was calculated using the equation 2 (Scherer & Godoy, 2009):

$$IC\ (\%) = \frac{(A_o) - (A_s)}{(A_o)}\ x\ 100\ \% \quad (2)$$

Ao and As are the absorbances of the negative control and the sample at different concentrations, respectively.

Antibacterial assay using TLC bioautography

Antibacterial test on 32 extracts using the TLC bioautography method refers to Dewanjee et al. (2015). The bacterial isolates used were *E. coli* and *S. aureus* from Indonesian Culture Collection (InaCC), Research Center for Biology-BRIN, Cibinong, Bogor. Chloramphenicol was used as a positive control. This test was carried out on a TLC plate (Silica gel GF254, Merck) by dot blot and elution methods. The growth of bacteria was stained using iodonitrotetrazolium chloride (INT, Sigma). Antibacterial activity is indicated by the formation of white zones on purple background on the plate.

Minimum inhibitory concentration determination

The MIC value of active extracts was determined by the microdilution method in 96-microwell plate (Thermo Scientific, China) as described before (Wulansari et al., 2017). Chloramphenicol (Sigma-Aldrich, Germany) was used as the positive control. Each test was performed in triplicate. The range of final concentrations of the extract was 4 – 512 µg/ml. The MIC was determined as the lowest concentration where the clear wells were observed.

RESULTS AND DISCUSSION

A total of 4 plants collected from Simeulue, Aceh, was obtained 32 extracts using several solvents. This research was limited to explore the antioxidant and antibacterial activities of the extracts. Table 1 reveals the extracts and their numbering according to the numbering on TLC plates.

Antioxidant assay using TLC bioautography

In the antioxidant activity, the color-changing from purple to yellowish-white on the purple background of the TLC plate represents a qualitatively potential sample to scavenge DPPH free radical. Catechin, as a positive control, is a plant secondary metabolite that has antioxidant activity because of its phenolic groups (Coșarcă et al., 2019).

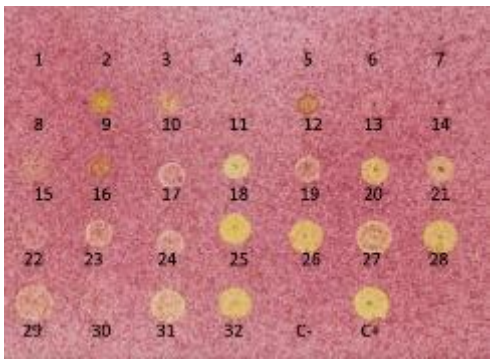


Figure 1. TLC spotting plate of plant extracts against DPPH. C+: Catechin and C-: Methanol. Numbers of 1 - 32 are extracts that comply with Table 1

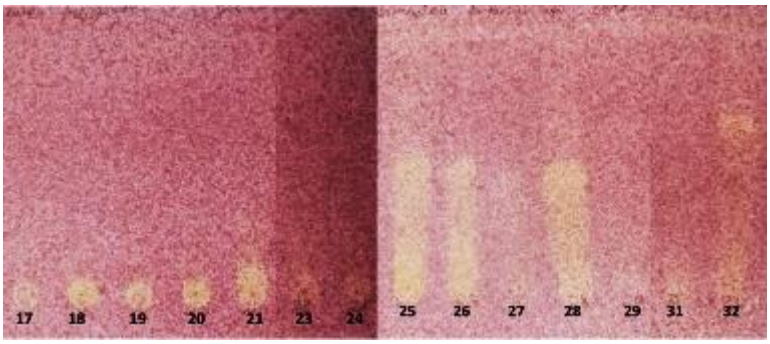


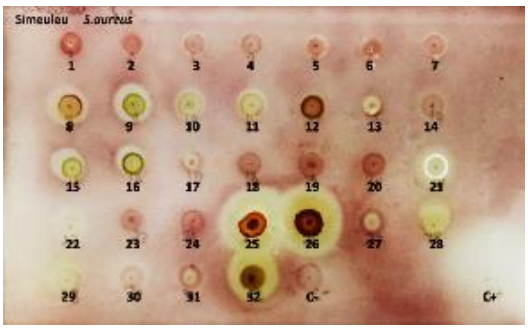
Figure 2. Eluted TLC plate of plant extracts against DPPH. All used eluents were dichloromethane: ethyl acetate = 5:1 (number 17 – 24) and chloroform: methanol: water = 6:4:1 (number 25 – 32). Numbers of 17 - 32 are extracts that comply with Table 1

In the TLC dot blot result, 14 from 32 samples are qualitatively potential as antioxidant, such as sample 17, 18, 19, 20, 21, 23, 24, 25, 26, 27, 28, 29, 31, 32 (Figure 1). To determine which compounds contributed to the antibacterial activity in each extract, a similar test was carried out on the eluted TLC plate of extracts (Figure 2). Samples were then tested by the microdilution method for the quantitative antioxidant assay. Antioxidant compounds can reduce the purple chromogen radical to yellow hydrazine and can be measured at a wavelength of 515 – 520 nm (Musa et al., 2013). The AAI value is used to classify antioxidant properties of plant extracts, whether the extract is categorized as weak, medium, or strong. Samples with AAI values < 0.5 are weak, 0.5 - 1.0 are moderate, 1.0 - 2.0 are strong, and > 2.0 are very strong (Siraichi et al., 2013).

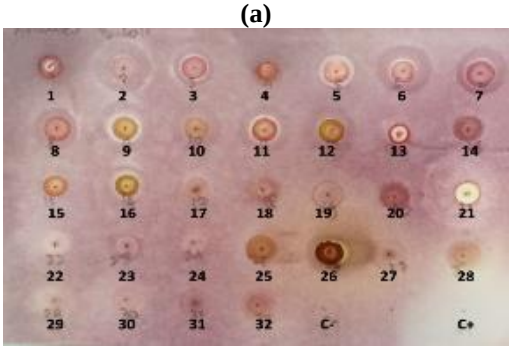
The first and second highest AAI values of the plant extracts from Simeulue are methanol extracts of *G. varians* stem and leaf, and followed by methanol extracts of *G. zeylanicum* leaf and *U. cordata* leaf, and ethyl acetate extract of *G. zeylanicum* leaf, namely 11.73, 7.08, 5.71, 5.26 and 2.16, respectively. These samples are categorized as having very strong antioxidant activity against DPPH. Study reports related to the antioxidant effect of *G. varians* have not been widely found, so that it is the potential to be developed further. Several studies reported that *G. zeylanicum* contain resveratrol 4'-methyl ether, glycitin, catechin, flavanol glucosides and megastigmane glucosides considered for the antioxidant properties (Duangjan et al., 2019; Otsuka et al., 2001; Otsuka et al., 2003).

Antibacterial assay using TLC bioautography

The bacteria used were *S. aureus* representing Gram-positive bacteria, and *E.coli* representing Gram-negative bacteria. Both bacteria are used because they are easy to cultivate and are pathogenic bacteria that often infect humans.



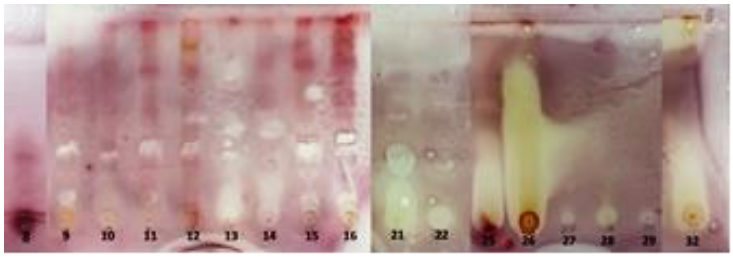
(a)



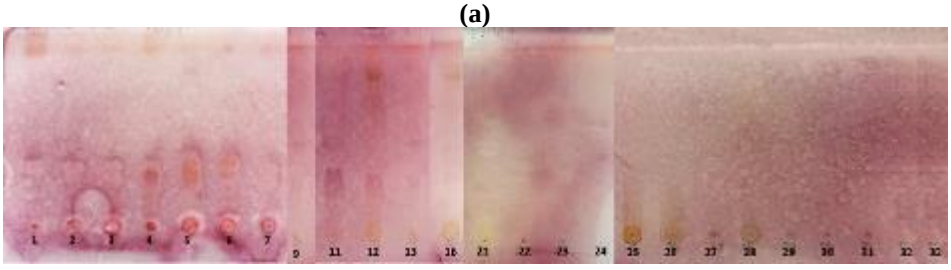
(b)

Figure 3. Antibacterial TLC-bioautography of plant extracts against (a) *S.aureus*, and (b) *E.coli*. Numbers from 1 to 32 are extract numbers that comply with Table 1

The result in figure 3a denotes a total of 15 extracts, specifically samples of 8, 9, 10, 11, 13, 15, 16, 21, 22, 25, 26, 27, 28, 29, 32 qualitatively potential as antibacterial agents against *S. aureus*. On the other hand, Figure 3b shows 21 samples such as 1, 2, 3, 4, 5, 6, 7, 9,11, 12, 13,16, 21, 22, 23,24, 25, 28, 29, 30 and 32 slightly inhibited the growth of *E. coli.* Figures 4a and 4b indicate the compound that is responsible for that bioactivity.



(a)



(b)

Figure 4. Developed TLC plate of antibacterial bioautography against a) *S.aureus* and b) *E. coli.* All used eluents were dichloromethane = 5:1 (number 1 – 8), dichloromethane: ethyl acetate = 10:1 (number 9 – 16), dichloromethane: ethyl acetate = 5:1 (number 21 – 24), and chloroform: methanol: water = 6:4:1 (number 25 – 32). Numbers from 1 to 32 are extracts that comply with Table 1

The next step was determining MIC (Minimum Inhibitory Concentration) value with four concentrations, such as 256, 128, 64, and 32 µg/ml. According to Kuete (2010), the MIC value > 625 µg/ml is categorized as weak, 100-625 µg/ml is classified as moderate, and <100 µg/ml is categorized as strong against antibacterial.

Table 1. Antioxidant and antibacterial activities of plant extracts from Simeuleu

No.	Plant Species	Part of Plant	Solvent	Antioxidant Activity			Antibacterial Activity	
				IC_{50}	AAI	Category of AAI value	MIC against *S. aureus* (µg/ml)	MIC against *E. coli* (µg/ml)
1	*G. varians*	Stem	n-Hexane	NT	NT	NT	NT	NT
2		Leaf		NT	NT	NT	NT	NT
3	*G. zeylanicum*	Stem		NT	NT	NT	>256	NT
4		Leaf		NT	NT	NT	>256	NT
5	*U. lanosa*	Stem		NT	NT	NT	>256	>256
6		Leaf		NT	NT	NT	NT	>256
7	*U. cordata*	Stem		NT	NT	NT	NT	>256
8		Leaf		NT	NT	NT	>256	>256
9	*G. varians*	Stem	Dichloromethane	34.576	0.88	Moderate	**256**	>256
10		Leaf		43.670	0.70	Moderate	>256	NT
11	*G. zeylanicum*	Stem		NT	NT	NT	>256	NT
12		Leaf		NT	NT	NT	>256	NT
13	*U. lanosa*	Stem		NT	NT	NT	>256	NT
14		Leaf		NT	NT	NT	>256	>256
15	*U. cordata*	Stem		125.420	0.24	Weak	**256**	>256
16		Leaf		NT	NT	NT	>256	>256
17	*G. varians*	Stem	Ethyl Acetate	73.498	0.41	Weak	NT	>256
18		Leaf		14.202	2.16	**strong**	NT	>256
19	*G. zeylanicum*	Stem		NT	NT	NT	NT	NT
20		Leaf		38.556	0.79	Moderate	NT	NT
21	*U. lanosa*	Stem		44.143	0.69	Moderate	>256	>256
22		Leaf		NT	NT	NT	>256	>256
23	*U. cordata*	Stem		19.105	1.60	Strong	NT	>256
24		Leaf		6.445	4.77	**strong**	>256	>256
25	*G. varians*	Stem	Methanol	2.620	1.17	**strong**	**128**	**256**
26		Leaf		4.341	7.08	**strong**	**256**	**256**
27	*G. zeylanicum*	Stem		6.422	4.78	**strong**	>256	>256
28		Leaf		5.377	5.71	**strong**	>256	256
29	*U. lanosa*	Stem		45.357	0.67	Moderate	**256**	>256
30		Leaf		NT	NT	NT	>256	>256
31	*U. cordata*	Stem		84.818	0.36	Weak	>256	>256
32		Leaf		5.835	5.26	**strong**	>256	>256

Remark: NT: Not tested. It is determined by the negative result of the TLC bioautography test. Classification of antibacterial activity for extracts based on MIC value: Strong < 100 µg/mL < Moderate < 625 µg/mL < Weak (Kuete, 2010). Classification of antioxidant activity for Extracts based on AAI value: Very strong > 2.00 > Strong > 1.00 > Moderate > 0.50 > Weak (Scherer & Godoy, 2009).

The MIC values of *S. aureus* and *E. coli* can be seen in Table 1. The highest MIC value against *S. aureus* was methanol stem extract of *G. varians* (sample 25) with the MIC value of 128 µg/ml (Table 1). Whereas, antibacterial assay against *E. coli* indicates methanol extracts of *G. varians* stem and leaf, and *G. zeylanicum* leaf (samples 25, 26, 28) having highest MIC values of 256 µg/ml, respectively. Reports about the bioactivity and chemical content of *G. varians* have not been found as well as leaf extract of *G. zeylanicum* In the previous study, *G. zeylanicum* root extract is known to have antibacterial

potency against several bacteria such as *E. coli*, *Bacillus subtilis*, and *Flavobacterium tegecticola* (Sharma et al., 2010).

CONCLUSION

Plant extracts of *G. varians* and *G. zeylanicum* (Phyllanthaceae family) and *U. lanosa* Wall., and *U. cordata* (Rubiaceae family) from Simeulue, Aceh, Indonesia had different levels of antioxidant and antibacterial activity. This study exhibited seven plant extracts having very strong antioxidant activity compared to other extracts. Meanwhile, three extracts were known to have moderate activity against *S. aureus* and *E. coli*. The interesting fact from this study is that the methanol extract of *G.varians* stem has the most superior antioxidant and antibacterial activity, but no reports have been found regarding the bioactivity and chemical components of *G. varians* (Phyllanthaceae). The development study of four plants from the Phyllanthaceae and rubiaceae family from Simeuleu is necessary because of its potential antioxidant and antibacterial properties.

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