***Antioxidant Activity Test Of Endophyte Fungi Extract From Kayu Jawa
(Lannea coromandelica (Houtt.) Merr.) Roots Using Dpph Method (2,2-
Diphenyl-1-1-Picrylhydrazyl)***

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ABSTRACT

Indonesia has abundant and diverse natural resources from land and sea. These biological natural resources can be explored as medicinal materials well-sourced from plants, animals, or microorganisms. Endophytic fungus is a microbial resource that grows in plant tissues that can produce compounds that have the same efficacy as the host plant. Zulfa (2016) has successfully isolated the endophytic fungi from kayu Jawa (Lannea coromandelica) roots and extracted the extracts with given code A11KA, A11KB, A12KC, A12KD, A21KK, A22KJ, AP12A, AP13L, AP21C, and AP32I. Other activities in these extracts besides antibacterial activity need to be tested. So this study was conducted to test the antioxidant activity of the extract with the DPPH method and used vitamin C positive control. Fifteen samples were obtained in this study, they are MeOH A11KA; MeOH A11KB; MeOH A12KC; MeOH A12KD; MeOH A21KK; MeOH AP12A; MeOH AP13L; MeOH AP12C; MeOH AP32I; EA A11KA; EA A12KC; EA AP32I; EA AP12A; EA AP13L; EA A22KJ from methanol fractions (MeOH) and ethyl acetate fraction (EA). The antioxidant activity test results showed that only six samples were potential as antioxidants. Value of AAI (Antioxidant Activity Index) EA A12KC; EA A11KA; MeOH A11KA; EA AP32I; MeOH AP21C; EA A22KJ and vitamin C are 2.27 (very strong), 2.07 (very strong), 1.78 (strong), 1.19 (strong), 0.81 (medium), 0.53 (medium) and 11.67 (very strong) respectively. The result of antioxidant analysis using GC-MS showed only EA A11KA showing the presence of phenol compound, the largest phenol compound in this sample was phenol, 2,4-bis (1,1-dimethyl ethyl).

Keywords: AAI (Antioxidant Activity Index), DPPH, endophytic fungi extract, Jawa wood (*Lannea coromandelica* (Houtt.) Merr.).

INTRODUCTION

Indonesia is a country rich in biodiversity. This abundant natural wealth is a blessing from Allah SWT. Antioxidants are compounds that can resist the occurrence of rancidity and inhibit oxidation reactions in materials containing fats or oils. (1) In addition, antioxidants are compounds that can inhibit reactive oxygen species / reactive nitrogen species and also free radicals so that antioxidants can prevent diseases associated with free radicals such as carcinogenesis, cardiovascular and aging. (2) The human body does not have an excessive amount of antioxidant reserves, so if there is excessive free radical exposure, the body needs exogenous antioxidants. (3)

Javanese wood (*Lannea coromandelica*) is one of the traditional medicinal plants used by people in South Sulawesi, because of its properties which are believed to be very effective for treating internal and external wounds such as vomiting blood and accelerating wound healing. Based on phytochemical studies, the bark of the Kayu Jawa plant has been reported to contain compounds of carbohydrates, steroids, alkaloids, cardiac glycosides, terpenoids, tannins, and flavonoids..(4) Several pharmacological studies have also been reported by researchers from India and Bangladesh that the methanol extract of the bark of the Javanese wood has biological activities such as antibacterial, antioxidant, analgesic., hypotensive activity, wound healing activity. (5)The data obtained from the chemical screening of Prawirodiharjo and Manik et al showed that the bark of the Javanese wood contains flavonoid compounds, saponins, and tannins. It has been reported that certain saponins and flavonoids can stabilize lysosomal membranes both in vivo and in vitro, while tannins and saponins can bind cations, thereby stabilizing erythrocyte membranes and other biological macromolecules. Phytochemical screening results Ethanol extract from wood roots also contains alkaloids, carbohydrates, flavonoids, triterpenoids, steroids, tannins, glycosides, saponins, and proteins. (6)

Endophytic molds are molds that live in plant tissues and can form colonies in plant tissues without harming the host plant. Endophytic molds can produce the same metabolites as their host plants. (7). Each higher plant can contain several endophytic microbes. Endophytic microbes are microorganisms that live in healthy plant cells and help the host plant to produce secondary metabolites. (8). Therefore, the metabolites produced by endophytes often have the same activity as the host plant. A small number of endophytes can also help the host plant in adapting to its environment. (9)

The latest research conducted by Zulfa (2016) has succeeded in isolating the endophytic mold of Javanese wood roots and carrying out extractions so that the extract is obtained with code A11KA, A11KB, A12KC, A12KD, A21KK, A22KJ, AP12A, AP13L, AP21C, and AP32L.

Of these 10 extracts, it is necessary to test for other activities other than antibacterial activity. The antioxidant activity was tested using the DPPH (1,1-Diphenyl-2-picrylhydrazil) method. The DPPH method provides information on the reactivity of the tested compound with a stable radical. DPPH gives strong absorption at a wavelength of 517 nm with a dark purple color. This research is expected to add new knowledge about the endophytic extract of Javanese wood roots as a new source of natural antioxidants so that it can save test materials and can be an effort to explore Indonesia's natural wealth without exploiting its biodiversity (10).

METHODS

The antioxidant activity test was carried out in vitro using the DPPH method. DPPH (2,2 diphenyl-1-picrihydrazyl) is a radical compound that is stable. DPPH is used to determine antioxidant activity through its ability to scavenge free radicals. Antioxidant activity is measured based on the electron transfer carried out by antioxidants. Initially, DPPH which is dark purple gives absorption at a wavelength of 517 nm but after being reduced, DPPH will turn into a diphenyl picryl hydrazine compound whose color will gradually fade to yellow, and the absorption value will be proportional to the number of electrons received. While the antioxidant comparison used is Vitamin C, the compound has good free radical inhibitory activity. Vitamin C has 4 hydroxyl groups that can react directly to donate one electron to form semihydroascorbate compounds that are unreactive (11). The next step is a disproportionate reaction of semihydroascorbate compounds to form dehydroascorbate which is then degraded to oxalic acid and threonic acid. (12)

RESULTS AND DISCUSSION

Based on the results that have been obtained in this research.

Results of Phytochemical Screening of Javanese Wood Root Endophytic Extracts from the Ethyl Acetate Fraction

Phytochemical screening was carried out to identify the content of secondary metabolites extracted in the ethyl acetate fraction of Javanese wood roots so that secondary metabolites could potentially have antioxidant activity. The compounds analyzed included alkaloids, flavonoids, saponins, tannins, phenols, steroids, and glycosides. The results of phytochemical screening carried out on the extract Javanese wood root endophytic from ethyl acetate fraction (EA) showed that all extracts contained secondary metabolite compounds including flavonoids, saponins, phenols, and tannins.

Table 1. Results of Phytochemical Screening of Javanese Wood Root Endophytic Extracts from the Ethyl Acetate Fraction

Test Compound	RESULTS					
	EA A12KC	EA A11KA	EA AP321	EA AP13L	EA AP12A	EA A22KJ
Alkaloids	-	-	-	-	-	-
Flavonoids	+	+	+	+	+	+
Saponins	+	+	+	+	+	+
Glycoside	-	-	-	-	-	-
Triterpenoids	-	-	-	-	-	-
Phenol	+	+	+	+	+	+
Tannins	+	+	+	+	+	+

Phytochemical Screening Java Wood Plant Root Endophytic Extract Methanol Fraction (MeOH)

Phytochemical screening was carried out to identify the content of secondary metabolites extracted in the methanol fraction of Javanese wood roots so that secondary metabolites could potentially have antioxidant activity. The compounds analyzed included alkaloids, flavonoids, saponins, tannins, phenols, steroids, and glycosides. On extract Endophytic root of Java wood from the methanol (MeOH) fraction showed the content of secondary metabolites, namely flavonoids, saponins, glycosides, phenols, and tannins. Generally, the secondary metabolites obtained are polar so that they are extracted in the solvent used is methanol. The secondary metabolites found in plants are usually alkaloids, flavonoids, saponins, quinones, tannins, and steroids/triterpenoids. These natural antioxidants are usually found in fruits, vegetables, and woody plants. (13)

Table 2. Results of Phytochemical Screening of Javanese Wood Root Endophytic Extracts from the Mertanol Fraction

Test Compound	RESULTS			
	MeOH A11KA	MeOH A12KC	MeOH AP21C	MeOH AP321
Alkaloids	-	-	-	-
Flavonoids	+	+	+	+
Saponins	+	+	+	+
Glycoside	+	+	+	+
Triterpenoids	-	-	-	-
Phenol	+	+	+	+
Tannins	+	+	+	+

Table 3. Results of Phytochemical Screening of Javanese Wood Root Endophytic Extracts from the Mertanol Fraction Endophytic Extracts from the Mertanol Fraction

Compound Test	RESULTS
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	MeOH A21KK	MeOH A12KD	MeOH A11KB	MeOH AP13L	MeOH AP12A
Alkaloids	-	-	-	-	-
Flavonoids	+	+	+	+	+
Saponins	+	+	+	+	+
Glycoside	+	+	+	+	+
Triterpenoids	-	-	-	-	-
Phenol	+	+	+	+	+
Tannins	+	+	+	+	+

The results of the phytochemical screening of both ethyl acetate (EA) and methanol (MeOH) fractions showed that the secondary metabolites identified in the two fractions were flavonoids, saponins, phenols, and tannins. These secondary metabolites are natural antioxidant compounds.

Qualitative Antioxidant Testing with Thin Layer Chromatography (TLC) Method

Java wood root endophytic extract was spotted on a TLC plate using a capillary tube.
(14)

<p>A. EA AP12A B. EA A11KA C. EA A12KC</p>

<p>A. MeOH AP12C B. MeOH A21KK C. MeOH A11KA</p>

<p>A. EA AP13L B. EA AP321 C. EA A22KJ</p>

Figure 1. The results of qualitative antioxidant testing using the Thin Layer Chromatography (TLC) method

The elution process was carried out by inserting the TLC plate into a chamber containing the eluent and saturating it. The eluent is allowed to propagate until it reaches the previously marked plate boundary. After completion, the TLC plate was removed from the chamber. The TLC plate was then dried and sprayed with 0.1 mM DPPH solution. The spots on the TLC plate that have antioxidant activity will turn white and yellow on a purple background. (15)

Quantitative Antioxidant Activity Test

Table 4. Results of Antioxidant Activity Test of Javanese Wood Root Endophytic Extract from EA A12KC

Concentration (ppm)	Absorbance Average	IC50 (ppm)	AAI
06.25	0.178472	17.48	02.27 (AAI > 2.0 or Very Strong)
12.05	0.163194		
25	0.134028		
50	0.082639		
100	0.011		
DPPH form	0.303472	-	-

Test Quantitative testing of antioxidant activity was carried out using the DPPH method (2,2-diphenyl-1-picrylhydrazyl). The choice of using this method is because it is a simple, easy, fast, and sensitive method and only requires a small sample to evaluate the antioxidant activity

of natural compounds. The following are the results of the quantitative antioxidant activity test.
(16)

Table 5. Results of Antioxidant Activity Test of Javanese Wood Root Endophytic Extract from EA A11KA

Concentration (ppm)	Absorbance Average	IC50 (ppm)	AAI
06.25	0.196528	24.62	0.08333 (AAI>1-2 Strong)
12.05	0.169444		
25	0.129861		
50	0.072		
100	0.098		
DPPH form	0.288194	-	-

In this study, this A11KA EA sample may act as a pro-oxidant. It is proven by a decrease in absorbance at a concentration of 100 ppm, this is because at a concentration of 100 ppm this sample is already above the threshold for absorbing DPPH (Nurhidayah, 2009). This shows that the maximum concentration that can be used as an antioxidant is brought to 100 ppm, so the concentration series is changed to 10-50 ppm, which can be seen in the table below.

Table 6. Test Results of Antioxidant Activity of Javanese Wood Root Endophytic Extract from EA A11KA Concentration of 10-50 ppm

Concentration (ppm)	Absorbance Average	IC50 (ppm)	AAI
06.25	0.18542	22.03	0.097222222 AAI 1-2 Strong
12.05	0.16389		
25	0.12986		
50	0.09167		
100	0.008		
DPPH form	0.29236	-	Strong

Table 7. Test Results of Antioxidant Activity of Javanese Wood Root Endophytic Extract from MeOH A11KA

Concentration (ppm)	Absorbance Average	IC50 (ppm)	AAI
10	0.24444	19.10	02.07 AAI>2.0 or Very Strong
20	0.19375		
30	0.14792		
40	0.10625		
50	0.07153		
DPPH form	0.4	-	Very strong

Table 8. Results of Antioxidant Activity Test of Javanese Wood Root Endophytic Extract from EA AP321

Concentration (ppm)	Absorbance Average	IC50 (ppm)	AAI
06.25	0.26319	33.16.00	01.19 AAI 1-2 Strong
12.05	0.24792		
25	0.22569		
50	0.16319		
100	0.012		
DPPH form	0.39375	-	Strong

Table 9. Results of Antioxidant Activity Test of Javanese Wood Root Endophytic Extract from MeOH AP21C

Concentration (ppm)	Absorbance Average	IC50 (ppm)	AAI
06.25	0.25833	49.09.00	0.05625 AAI 0.5-1 Medium
12.05	0.23542		
25	0.21111		
50	0.1375		
100	0.062		
DPPH form	0.30694	-	Currently

Table 10. Results of Antioxidant Activity Test of Javanese Wood Root Endophytic Extract from EA A22KJ

Concentration (ppm)	Absorbance Average	IC50 (ppm)	AAI
06.25	0.28611	74.77	00.53 AAI 0.5-1 Medium
12.05	0.26181		
25	0.23472		
50	0.19861		
100	0.10972		
DPPH form	0.30694	-	Currently

Table 11. Results of Antioxidant Activity Test of Javanese Wood Root Endophytic Extract from EA AP13L

Concentration (ppm)	Absorbance Average	IC50 (ppm)	AAI
06.25	0.30764	80.89	00.49 AAI < 0.5 Weak
12.05	0.29236		
25	0.27083		
50	0.24097		
100	0.17292		
DPPH form	0.39653	-	Weak

Table 12. Results of Antioxidant Activity Test of Javanese Wood Root Endophytic Extract from MeOH A12KC

Concentration (ppm)	Absorbance Average	IC50 (ppm)	AAI
06.25	0.27847	87.98	00.45 AAI < 0.5 Weak
12.05	0.27153		
25	0.24583		
50	0.20903		
100	0.13125		
DPPH form	0.28472	-	Weak

Table 13. Test Results of Antioxidant Activity of Javanese Wood Root Endophytic Extract from MeOH A21KK

Concentration (ppm)	Absorbance Average	IC50 (ppm)	AAI
06.25	0.30347	106.49.00	00.37 AAI < 0.5 Weak
12.05	0.29722		
25	0.28681		
50	0.25556		
100	0.17917		
DPPH form	0.30556	-	Weak

Table 14. Results of Antioxidant Activity Test of Javanese Wood Root Endophytic Extract from EA AP12A

Concentration (ppm)	Absorbance Average	IC50 (ppm)	AAI
06.25	0.29306	107.13.00	00.37 AAI < 0.5 Weak
12.05	0.28333		
25	0.26528		
50	0.22847		
100	0.16528		
DPPH form	0.30347	-	Weak

Table 15. Results of Antioxidant Activity Test of Javanese Wood Root Endophytic Extract from MeOH A12KD

Concentration (ppm)	Absorbance Average	IC50 (ppm)	AAI
06.25	0.27708	116.05.00	00.34 AAI < 0.5 Weak
12.05	0.27292		
25	0.25208		
50	0.22361		
100	0.16528		
DPPH form	0.29028	-	Weak

Table 16. Results of Antioxidant Activity Test of Javanese Wood Root Endophytic Extract from MeOH A12KB

Concentration (ppm)	Absorbance Average	IC50 (ppm)	AAI
06.25	0.28056	143.59.00	00.28 AAI < 0.5 Weak
12.05	0.27708		
25	0.27292		
50	0.24583		
100	0.19306		
DPPH form	0.28542	-	Weak

Table 17. Test Results of Antioxidant Activity of Javanese Wood Root Endophytic Extract from MeOH AP321

Concentration (ppm)	Absorbance Average	IC50 (ppm)	AAI
06.25	0.27361	147.84	00.27 AAI < 0.5 Weak
12.05	0.26875		
25	0.25694		
50	0.22569		
100	0.19097		
DPPH form	0.28958	-	Weak

Table 18. Results of Antioxidant Activity Test of Javanese Wood Root Endophytic Extract from MeOH AP13L

Concentration (ppm)	Absorbance Average	IC50 (ppm)	AAI
06.25	0.28264	199.01.00	00.20 AAI < 0.5 Weak
12.05	0.27778		
25	0.26875		
50	0.25486		
100	0.21597		
DPPH form	0.29514	-	Weak

Table 19. Test Results of Antioxidant Activity of Javanese Wood Root Endophytic Extract from MeOH AP12A

Concentration (ppm)	Absorbance Average	IC50 (ppm)	AAI
06.25	0.27292	412.96	00.10 AAI < 0.5 Weak
12.05	0.26944		
25	0.26528		
50	0.25764		
100	0.24306		
DPPH form	0.29097	-	Weak

Table 20. Vitamin C Antioxidant Activity Test Results

Concentration (ppm)	Absorbance Average	IC50 (ppm)	AAI
2	0.21528	03.39	0.504861111 AAI > 2.0
4	0.18472		

Concentration (ppm)	Absorbance Average	IC50 (ppm)	AAI
6	0.13958		or Very Strong
8	0.10069		
10	0.098		
12	0.041		
DPPH form	0.38194	-	

IC value₅₀ defined as the concentration of the test compound that can reduce free radicals by as much as 50%. The smaller the IC. value₅₀ the higher the free radical scavenging activity (17). IC value₅₀ was obtained from the linear regression equation while the AAI (Antioxidant activity index) value was determined by comparing the DPPH concentration used in the test (ppm) with the IC value₅₀ obtained from each extract. The AAI value needs to be known to classify the antioxidant properties of the extract. If the value of $AAI < 0.5$ is a weak antioxidant, $0.5 < AAI < 1$ is moderate, $1 < AAI < 2$ is strong, and $AAI > 2$ is strong (18). Quantitative testing of antioxidant activity consisted of 15 extracts, namely 6 from the ethyl acetate fraction (EA A12KC; EA A11KA; EA AP321; EA AP12A; EA AP13L, and EA A22KJ) while from the methanol fraction (MeOH) there were 9 extracts (MeOH A11KB; MeOH A12KC; MeOH A12KD; MeOH AP321; MeOH A21KK; MeOH AP21C; MeOH AP13L; MeOH AP12A; MeOH A111KA) along with Vitamin C control (synthetic) were carried out with various concentration series using the DPPH method, then the absorbance was measured by UV-Vis spectrophotometry.

A quantitative antioxidant test was carried out using a UV-Vis spectrophotometer. This quantitative test was carried out to determine the remaining absorbance of DPPH after adding an endophytic extract of Javanese wood roots. The decrease in the absorbance value of DPPH at a wavelength of 515 nm indicates that the compound has antioxidant activity (19). The decrease in DPPH absorbance was measured against the control, namely the absorbance of DPPH in methanol pro-analysis without an additional sample. The color change from purple to yellow indicates a decrease in DPPH absorbance. The amount of DPPH absorbance is inversely proportional to the concentration of the added extract.

The absorbance value obtained can then be calculated as the percentage value of DPPH radical inhibition (% inhibition). Then obtained a linear regression curve and its equation, with concentration as the x-axis and absorbance as the y-axis. IC value₅₀ can be calculated from the previously obtained linear regression equation by replacing y with 50 in the equation. IC value₅₀ is defined as the concentration of the test compound that can reduce free radicals by as much as 50%. The smaller the IC. value₅₀ the higher the free radical scavenging activity.

The AAI (Antioxidant activity index) value was obtained by comparing the concentration of DPPH used in the test with the IC₅₀ value which is obtained. Quantitative testing of antioxidant activity of this Javanese root endophytic extract in as many as 15 samples, along with positive control of vitamin C was carried out with various concentration series using the DPPH method, then the absorbance was measured using UV-Vis spectrophotometry. The following table and diagram of the antioxidant activity test results from all samples.

Table 21. Antioxidant Activity Test Results of All Samples and Vitamin C

NO	SAMPLE	IC ₅₀ VALUE (ppm)	AAI VALUE	DESCRIPTION
1	EA A12KC	17.09	02.27	Very strong
2	EA A11KA	19.10	02.07	Very strong
3	MeOH A11KA	22.26	0.095833333	Strong
4	EA AP321	33.16.00	01.19	Strong
5	MeOH AP21C	49.08.00	0.05625	Currently
6	EA A22KJ	74.77	00.53	Currently
7	EA AP13L	80.89	00.49	Weak
8	MeOH A12KC	87.98	00.45	Weak
9	MeOH A21KK	106.49.00	00.37	Weak
10	EA AP12A	107.13.00	00.37	Weak
11	MeOH A12KD	116.05.00	00.34	Weak
12	MeOH A11KB	143.59.00	00.28	Weak
13	MeOH AP321	147.84	00.27	Weak
14	MeOH AP13L	199.01.00	00.20	Weak
15	MeOH AP12A	412.96	00.10	Weak
16	Vitamin C	03.39	0.504861111	Very strong

Diagram 1. Antioxidant Activity Test Results of All Samples Based on AAI Nilai Values

The results obtained from antioxidant testing using a UV-Vis spectrophotometer are that two samples have a very strong AAI, two samples have a strong AAI, two samples have a moderate AAI and nine samples have a weak AAI.

While IC₅₀ and AAI vitamin C as comparison are 3.39 ppm and 11.67, indicating that vitamin C has a very strong antioxidant activity because it has AAI above 2. and AAI of 17.09 ppm and 2.27 so that this sample is in the category of very strong antioxidant. This shows that vitamin C has a stronger antioxidant activity than the whole sample. The compounds contained in the Javanese root endophytic extract are an accumulation of polar, semi-polar, and non-polar compounds. When the extract is partitioned in stages, the synergistic function between the compounds will be reduced because the components contained in the extract have been separated.

The curve is obtained using linear regression in the Microsoft Excel 2013 data processing application. The y coefficient in the linear equation is 50 is the IC₅₀ coefficient, while the x coefficient in this linear equation is the concentration of the extract to be sought, where x obtained is the amount of concentration required to can reduce 50% of DPPH radical activity. The value of R² describes the linearity of concentration to % inhibition. The value of R² which is close to +1 (positive value) indicates that as the concentration of the extract increases, the antioxidant activity also increases. This is related to the number of secondary metabolites dissolved in the extract and has antioxidant activity.

Antioxidant Analysis Using GC-MS

In this study, the samples analyzed by gas chromatography were six samples, namely EA A12KC; EA A11KA; MeOH A11KA; EA AP321; MeOH AP21C, and EA A22KJ. The reason for choosing these six samples was because their antioxidant activity was categorized as very strong, strong, and moderate. Antioxidant compounds that can be analyzed by GC-MS are phenolic compounds because they are volatile compounds. Of the six samples, which showed the presence of phenolic compounds was the EA A11KA sample. The other samples did not show any phenolic compounds that could be analyzed by GC-MS. This is indicated by the absence of a class of phenolic compounds in the library data of the other five samples. This is presumably due to the presence of phenolic compounds in the other five samples in small concentrations (20). Therefore, further purification of the compound (isolation) is necessary to obtain pure compounds from the five samples. The largest phenolic compound detected in the EA A11KA sample was phenol,2,4-bis (1,1-dimethyl ethyl), with the chemical formula C₁₄H₂₂O, molecular weight (BM) 206, then had a similarity of 83% and a retention time of 18 minutes.

CONCLUSION AND SUGGESTIONS

Based on the results obtained in this study, it can be concluded that fifteen endophytic extracts of Javanese wood roots tested using DPPH have various antioxidant activities. The sample that has the strongest antioxidant activity is the EA A12KC sample, with an AAI value of 2.27 so it is categorized as a very strong antioxidant. The results of the analysis using GC-MS found that the compounds that play a role in the antioxidant activity of the EA A11KA sample are phenolic group compounds with the name phenol, 2,4-bis (1,1-dimethyl ethyl).

It is recommended that this research be continued to isolate bioactive compounds from samples that have very strong antioxidant activity.

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