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Research Article

GALLIC ACID FROM PODS OF JIRINGA (*ARCHIDENDRON JIRINGA* [JACK] I.C. NIELSEN) AND ITS ANTIOXIDANT

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ABSTRACT

Objective: This study was aimed to isolate and investigate antioxidant activity of gallic acid in pods of jiringa (*Archidendron jiringa* [Jack] I.C. Nielsen).

Methods: Pods of jiringa were extracted by maceration. Phenolic compounds were tested using $FeCl_3$. Identification of pure compound was obtained from spectra data from nuclear magnetic resonance of proton H (^{1}H NMR), NMR of carbon (^{13}C NMR), infra-red, and mass spectrometry. Antioxidant activity was investigated using 1,1-diphenyl-2-picrylhydrazyl method.

Results: The IC_{50} of gallic acid from jiringa's pods (*A. jiringa* [Jack] I.C. Nielsen) was 3.65 µg/ml. This value showed that gallic acid from jiringa's pods (*A. jiringa* [Jack] I.C. Nielsen) had high antioxidant activity.

Conclusions: Gallic acid presents in pods of jiringa (*A. jiringa* [Jack] I.C. Nielsen) and has high antioxidant activity.

Keywords: Phenolic, Antioxidant, Spectra data, Gallic acid.

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INTRODUCTION

Jiringa (*Archidendron jiringa* [Jack] I.C. Nielsen) is a leguminous tree that is found in Indonesia, Malaysia, and Thailand [1]. It is economically important with diverse uses including as a vegetable (young shoots), food flavoring agent (seeds) [2], medicine (leaves and seeds), and timber for craft work [3]. This plant usually stands up to 25 m in height with a smooth, grey colored bark. The beans measure up to 3.5 cm in diameter and 2.0 cm in thickness. Jiringa's beans are typically taken together with rice as a side dish either as raw vegetable, roasted, fried, or boiled [4,5]. Jiringa is one of the traditional medicine herbs. Seeds have been reported as a source of natural antioxidant [6] that could destroy excess free radicals and prevent oxidative damage [7]. Seeds are used to treat hypertension and diabetic disease; the old leaves burnt to obtain ashes were used against itching [6]. Pods of jiringa until now still much wasted because it is not used, so pile up into garbage.

Currently, to obtain natural antioxidants have grown rapidly because of several degenerative and aging-related diseases [7] such as cancer and cardiovascular diseases. Antioxidants have important role in human health [8]. Antioxidants prevent damage caused by free radical before they attack biological targets in cells [9,10]. Synthetic antioxidants may have toxic, carcinogenic, and negative effects to human body [11]. Ascorbic acid (Vitamin C) is one of the sources of natural antioxidants. In recent years, there has been increased interest in the use of natural phenolics as antioxidants due to their safety in nature [11,12]. Ethanolic extract pods of jiringa showed concentration phenolics and potent antioxidant activity [13].

Gallic acid is one of the phenolic compounds widely present in the plant kingdom such as *Givotia rottleriformis* Griff. [14], *Caesalpinia decapetala* [15], *Elaeagnus angustifolia* [16], *Terminalia chebula* [17], and *Crassula ovata* (Mill.) [18]. Gallic acid, a naturally occurring low-molecular-weight triphenolic compound, has been suggested to possess strong antioxidant activity in many studies [7]. Isolation of gallic acid from pods of jiringa (*A. jiringa* [Jack] I.C. Nielsen) has not been reported.

METHODS

Plant material

The pods of jiringa (*A. jiringa* [Jack] I.C. Nielsen) were collected from Namorambe village, Deli Serdang, North Sumatera, Indonesia. Authentication of plant material was carried out at the Herbarium Bogoriensis of the Research Centre for Biology, Indonesian Institute of Sciences (LIPI), Cibinong, Indonesia.

Preparation of extracts

The pods of jiringa (*A. jiringa* [Jack] I.C. Nielsen) were dried at room temperature, powdered (4.16 kg) and macerated with methanol. The resultant extracts were then concentrated to dryness in a rotary evaporator. Methanol extract (140 g) was dissolved with water. After filtration, the aqueous solution was partitioned with ethyl acetate solvent. Soluble portion of ethyl acetate solvent was collected (37.88 g). Further, ethyl acetate extract was partitioned with mixture of *n*-hexane-methanol to get extracts. The methanol extracts were considered as total phenolic (13.87 g). We use $FeCl_3$ to test phenolic compounds.

Separation of compounds

Total phenolic was subjected to column chromatography silica gel (only 10 g was subjected) and eluted with chloroform:methanol (chloroform 100%; 9:1; 8:2; 7:3; 6:4; and methanol 100%) to got six fractions.

Purification

Fraction IV (2×200 mg) purification by preparative chromatography used eluent $CHCl_3$:ethyl acetate (6:4) and eluted with methanol:ethyl acetate (1:1) with volume 400 ml. From preparative chromatography was got 6 zone and test with $FeCl_3$. Zone 4–5 cristalizated with acetone and *n*-hexane to got pure compound.

Table 1: Fraxion's from column chromatography

No.	Fraction	Number of vial	Mobile phase	Weight (mg)
1.	I	79–80	$CHCl_3$:CH_3OH=9:1	160
2.	II	81–85	$CHCl_3$:CH_3OH=8:2	290
3.	III	86–111	$CHCl_3$:CH_3OH=8:2	4830
4.	IV	112–159	$CHCl_3$:CH_3OH=7:3	2740
5.	V	160–198	$CHCl_3$:CH_3OH=6:4	440
6.	VI	199–408	CH_3OH 100%	830

Table 2: IC_{50} gallic acid from pods of *A. jiringa* (Jack) I.C. Nielsen and ascorbic acid (Vitamin C) as a standard

Sample	Concentration (μg/ml)	A1[a]	A2[b]	$\bar{A}$[c]	A[d] blank	Inhibition[e] (%)	IC_{50} (μg/ml)
Gallic acid	5	0.437	0.459	0.448	0.948	52.7	3.65
	10	0.298	0.318	0.308	0.948	67.5	
	15	0.277	0.277	0.277	0.948	70.8	
	20	0.103	0.107	0.105	0.948	88.9	
	25	0.012	0.014	0.013	0.948	98.6	
Ascorbic acid (Vitamin C)	5	0.437	0.492	0.464	0.948	50.98	2.31
	10	0.298	0.252	0.275	0.948	70.97	
	15	0.277	0.114	0.195	0.948	79.4	
	20	0.103	0.201	0.151	0.948	83.99	
	25	0.012	0.057	0.034	0.948	96.34	

[a]A1=First absorption measurements of sample, [b]A1=Second absorption measurements of sample, [c]$\bar{A}$=Mean absorption measurements of sample,

[d]A blank=Absorption measurements of blank, [e]% inhibition= $\frac{\text{A blank} - \bar{A}}{\text{A blank}} \times 100\%$. *A. jiringa: Archidendron jiringa*

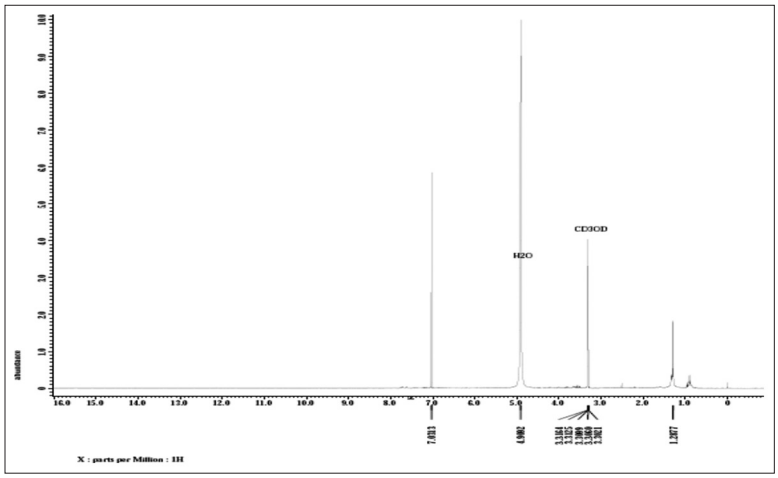

Fig. 1: Data spectra ^{1}H nuclear magnetic resonance Gallic acid from pods of *Archidendron jiringa* (Jack) I.C. Nielsen

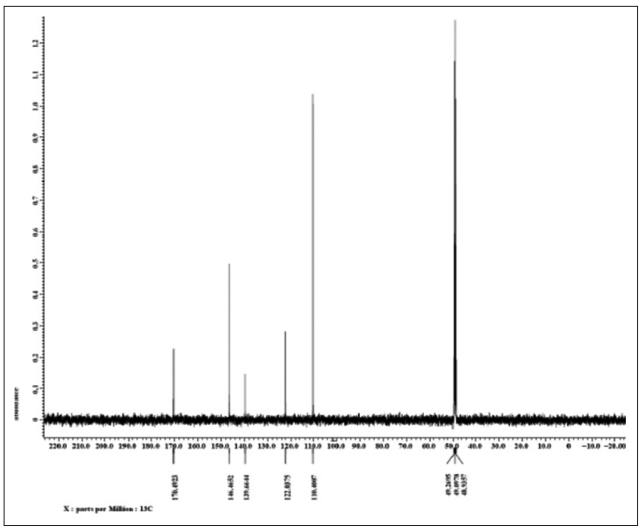

Fig. 2: Data spectra ^{13}C nuclear magnetic resonance Gallic acid from pods of *Archidendron jiringa* (Jack) I.C. Nielsen

Identification of pure compound

Structure isolated compound was determined by^{1}H nuclear magnetic resonance (NMR) and ^{13}C NMR on Joel 500 spectrometer (500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR), infrared (IR) spectra (Perkin Elmer Fourier-transform spectrometer Fourier-transform infrared and electron impact mass spectrometry [MS] mass spectra by liquid chromatography [LC]–MS/MS high-performance liquid chromatography Alliance 2659, Waters Detector Photodiode Array 2996).

1,1-Diphenyl-2-picrylhydrazyl (DPPH) method

Measurement of antioxidants by DPPH method in principle is to measure the occurrence of color fading of DPPH radicals due to the presence of antioxidants that can neutralize free radical molecules. The previously colored DPPH radicals will lose their color if there are antioxidants, because antioxidants will donate their electrons to DPPH radicals, so previously unstable radicals (due to unpaired electrons) become stable (electrons in free radicals are now paired up for donations electron from antioxidants) [19].

RESULTS

Separation of compounds from column chromatography silica gel (only 10 g was subjected) and eluted with chloroform: methanol (chloroform

100%; 9:1; 8:2; 7:3; 6:4; and methanol 100%) was gave six fractions shown in Table 1.

From fraction IV after purification by preparative chromatography use eluent $CHCl_3$:ethyl acetate (6:4) and eluted with methanol: ethyl acetate (1:1) with volume 400 ml obtained 6 zone. We got pure compound as a gallic acid at zone 4–5.

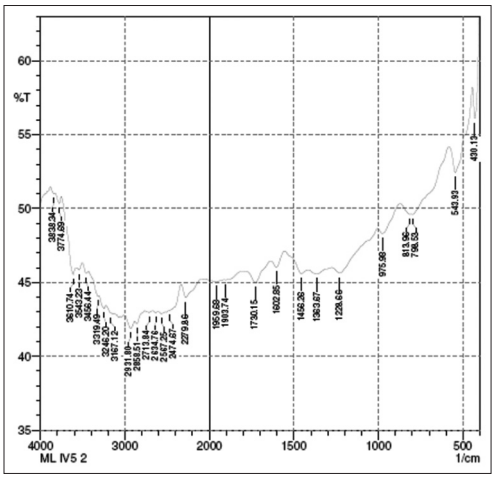

Fig. 3: Spectra data Fourier-transform infrared gallic acid from pods of *Archidendron jiringa* (Jack) I.C. Nielsen

Structure isolated compound was determined from data ^{1}H NMR, ^{13}C NMR, IR, and MS (Figs. 1-4).

We used DPPH method to evaluated antioxidant activity. The value of IC_{50} of sample as gallic acid and ascorbic acid as standard calculated from linear regression equation (Figs. 5 and 6).

DISCUSSION

Phenolic compound test

Phenolic compound test result indicated phenolic compound. Extract was added $FeCl_3$ showed black-bluish color [21].

Determination chemical structure pure compound

Pure compound was identified from data ^{1}H NMR, ^{13}C NMR, IR, and LC MS. Data ^{1}H NMR shows peaks at 1.2877 ppm indicated proton H from acid OH and 7.0313 indicated proton H at position number 2.

Data ^{13}C NMR shows peaks at 170.4923 ppm indicated atom C at position 7 conjugated to C=O; 146.4652 ppm indicated atom C at position 3 and 5; 139.664 ppm indicates atom C at position 4; 119.87 ppm indicated atom C at position 1; and 108.14 ppm indicated atom C at position 2 and 6.

Data IR shows peaks at 3319.49/cm indicated –COOH; 3167.12/cm indicated C-H; 1730.15/cm indicated =C=O; and 1602.85/cm indicated C=C.

From LC–MS data show m/z 171.02829. Mass of pure gallic acid is 170. Identification from spectra data above compared with the other reports about isolation of gallic acid [15-18].

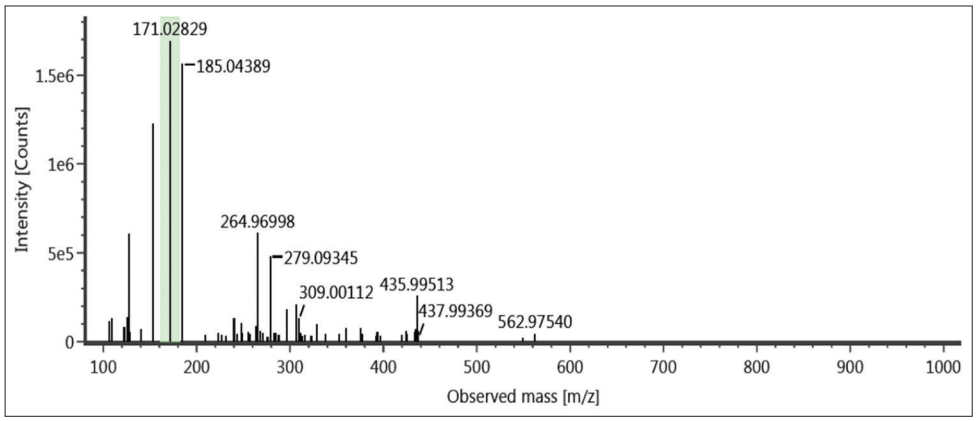

Fig. 4: Spectra data liquid chromatography–mass spectroscopy gallic acid from pods of *Archidendron jiringa* (Jack) I.C. Nielsen

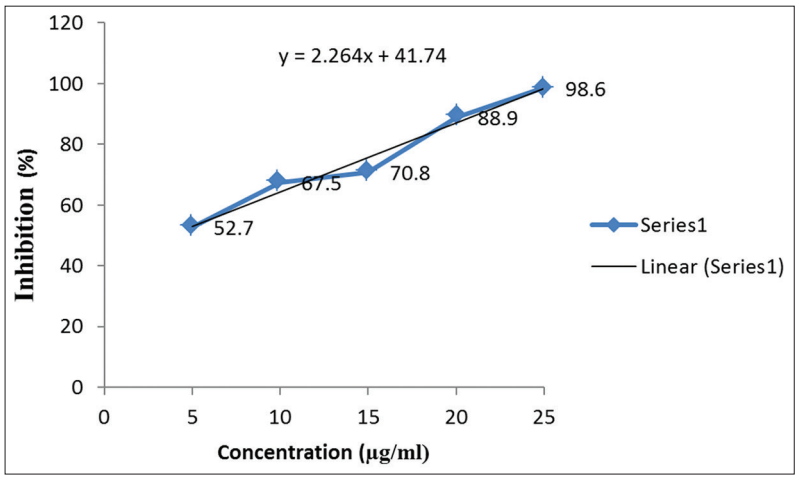

Fig. 5: Chart concentration versus % inhibition to obtain IC50 of gallic acid

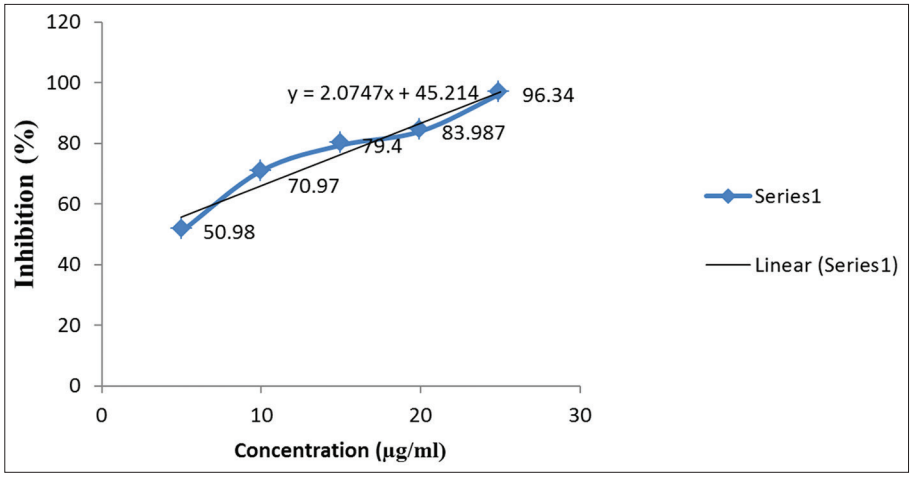

Fig. 6: Chart concentration versus % inhibition to obtain IC50 of ascorbic acid (Vitamin C) as a standard

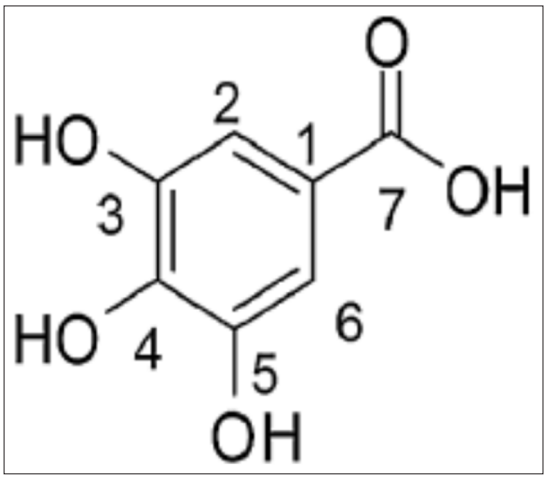

Fig. 7: Chemical structure gallic acid

Antioxidant activity

The antioxidant activity data were shown in Table 2. We used Vitamin C (ascorbic acid) as standard. We used linear regression equation from chart in Figs. 5 and 6 to get IC_{50} value.

CONCLUSION

Based on ^{1}H NMR, ^{13}C NMR, IR, and MS spectral data, pure compound isolated from *A. jiringa* (Jack) I.C. Nielsen pods (Fraction 4–5) was determined as gallic acid (Fig. 7), and it has high antioxidant activity which can be used for preventing or treating oxidative disease.

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