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Isolation and Antioxidant Assay of Artonol A from the Bark of *Artocarpus elasticus* Reinw ex Blume

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Abstract. Artocarpus species have been used as the conventional resource of medicine in Indonesia due to its abundant phenolic compounds and biological activities. The objective of this study was to examine antioxidant activity of artonol A isolated from the bark of *Artocapus elasticus* Reinw. ex Blume using 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. Extraction was conducted by maceration using methanol as a solvent and followed by a fractionation process using Column Vacuum Chromatography, column chromatography, and Preparative Thin Layer Chromatography (PTLC). The structure was determined by FTIR, LCMS, and ^{1}H NMR analysis and subsequently identified as artonol A. Artonol A showed antioxidant activity using DPPH methods with an IC_{50} value of 0.29 mM.

Keywords: *Artocarpus elasticus Reinw.* ex Blume, antioxidant activity, artonol A, 2,2-diphenyl-1-picrylhydrazyl, NMR.

INTRODUCTION

Antioxidants are stable compounds which counteract negative effects of oxidants in the body by donating one electron to inhibit oxidant compounds. Antioxidant compounds has important role in human's body by reducing the adverse effects caused by free radicals [1]. Free radicals are relatively reactive since they have unpaired electrons, requiring electrons in the surrounding molecule. Unpaired electrons enable the chain reactions that result in free radicals continuing to multiply and attack the body [2].

Artocarpus is a plant growing in tropical forests which have potential as a source of bioactive chemicals. This plant is classified as the Moraceae family with 60 genera including 1400 species. In particular, Indonesia has 20 plant species from 17 genera Moraceae [3]. Based on the results of literature studies, species of Artocarpus consists of several secondary metabolites such as terpenoids, flavonoids, stilbenoids, arylbenzofurans, and neolignans. The flavonoid group contains the majority of the Artocarpus plant constituents [4,5].

One of the valuable species from the genus Artocarpus is *Artocarpus elasticus*. Previous studies relating to the biological activities of several compounds from *A. elasticus*, including cytotoxic, anti-inflammatory, antitumor, anticancer, antibacterial, antiviral, antifungal, and antidiabetic properties have been carried out [6,7]. Additionally, a previous study on the antioxidant activity from ethanol extract of *A. elaticus* Reinw ex blume's bark revealed that the

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highest antioxidant activity obtained by the bark of its crude extract by showing IC_{50} value of 70.59 µg/mL [8]. Therefore, further research is needed on the determination of antioxidant activity and isolation of secondary metabolites from the bark of *A. elasticus* Reinw ex blume.

MATERIALS AND METHODS

Materials

General

UV/Vis data was analyzed with Agilent Technology, Cary 60, FTIR spectrum was measured using Shimadzu-Prestige 21 and ^{1}H- NMR spectra were recorded with JEOL ECZR500 operating at 500 MHz using $CDCl_3$ as a solvent. Molecular weight was obtained from LCMS data with the ESI system (Mariner Biospectrometry). Column chromatography was conducted using silica (60-230 mesh, Merck) and sephadex LH-20 (Sigma Aldrich). Preparative TLC and TLC were analyzed on GF_{254} plates (Merck). Free radical scavenging method using 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was used to determine antioxidant activity.

Plant material

The *A. elasticus* barks from Mekongga Forest in Southeast Sulawesi, Indonesia, were determined by botanists from the Research Center for Biology LIPI. The voucher specimen referring to number UHA46 was then deposited in the herbarium.

Methods

Extraction and Fractionation

Extraction was carried out by macerating 800 grams of dry simplicia in methanol solvent with the ratio of 1: 3 for 3 x 24 hours. The remaining solvent was reduced to dryness using a rotary evaporator at a temperature of 40-50°C, obtaining methanol extract (47.67 g). 30 grams of methanol extracts was then fractionated by solvent extraction method using a separating funnel with n-hexane, ethyl acetate and n-buthanol in a ratio of 1: 1 for 3 times. Each fraction was evaporated under reduced pressure to yield n-hexane (7.57 g), ethyl acetate (9.72 g), *n*-butanol (3.01 g) and residue (water, 3.04 g) fractions.

Isolation and Purification

About 7 grams ethyl acetate fraction (the active fraction) was purified using VLC (Vacuum Liquid Chormatography) with gradient elution (*n*-hexane-ethyl acetate-methanol) to provide nine main fractions. A silica gel (Kieselgel 60, 60-230 mesh) column chromatography eluting with gradient elution (*n*-hexane-ethyl acetate-methanol) was performed on Fraction 6 to obtain nine sub-fractions (SF1-SF10). After further purification of sub-fraction SF3 using sephadex LH-20 and dichloromethane-methanol (1:1) elution, three sub-fractions ($SF_{3.1}$ – $SF_{3.3}$) were acquired. $SF_{3.1}$ was then purified using TLC preparative with *n*-hexane: ethyl acetate (5:95) as the mobile phase to afford a pure compound **1** (3 mg). The characterization of compound 1 was carried out using UV/Vis, FTIR, LCMS and FT-NMR spectrometers, while the antioxidant activity was performed using 2,2-Diphenyl-1-picrylhydrazyl (DPPH) method.

Antioxidant activity assay [9]

a. DPPH Solution Preparation

In brief, 0,1 mM DPPH was prepared by carefully weighing 4.0 mg DPPH powder (Molecular Weight: 394.32) and then being dissolved in 10 mL of methanol. The solution was stored in a dark bottle and for each test a new solution was made.

b. Mother Solution Preparation and Antioxidant Activity Test

The mother liquor was prepared by weighing 4.0 mg of sample before being dissolved in 4 mL of methanol, obtaining the concentration of 1000 mg/mL. Test solutions were made with various concentrations starting from concentrations of 100 µg/mL, 50 µg/mL, 25 µg/mL, 10 µg/mL, 5 µg/mL, by pipetting 250 µL, 125 µL, 62.5 µL, 25 µL and 12.5 µL of mother liquor to a test tube. The amount of 500 µL of 0.1 mM DPPH solution was added to each sample. Methanol was used to produce the solution of 2.5 mL in total. The standard of quercetin was prepared with the same concentration as the sample. All samples then were then incubated at 37°C. After 30 minutes, the absorbance of all samples was measured using UV-Vis spectrophotometer at a wavelength of 515 nm. The absorption of DPPH solution will decrease along with the increase of sample concentration which leads to the drop of absorbance measured as well. The inhibition percentage of sample towards DPPH solution is calculated by the following equation:

$$\%\ Inhibition = \frac{(A\ control - A\ sample)}{A\ control} x\ 100\% \quad (1)$$

Moreover, the concentration (µg/mL) as the abscissa (X axis) and the % inhibition value as the ordinate (Y axis) are plotted to obtain a linear equation. The linear regression equation y = bx ± a was obtained and the values of a, b, r with the linear regression equation can be used to find IC_{50} value, where y = 50 and x as IC_{50}.

RESULTS AND DISCUSSION

The extraction process of the dried barks of *A. elasticus* yielded 5.96% of methanol extract, 25.25% of *n*-hexane fraction, 32.41% of ethyl acetate fraction, and 10.05% of *n*-butanol fraction. Extracts from different solvents can scavenge the free radicals indicating their potential as radical scavengers. The antioxidant activity of methanol extract, all fractions, and quercetin as positive control was shown at Table 1 with the highest and lowest IC_{50} value was obtained by *n*-hexane fraction (173.74 µg/mL) and *n*-buthanol fraction (76.71 µg/mL), respectively. Plant phenolics are a major group of compounds acting as primary antioxidants or free radical scavengers, therefore, the observed high free radical scavenging activity of *n*-butanol and ethyl acetate extracts may have accounted to its polarity in this study of the radical scavenging potential of the plants extract. The radical scavenging activities may be due the presence of some flavonoids with free hydroxyl group that can donate hydrogen and electron, this is agrees well with the literature reported for the antioxidant activity of *A. altilis* [10].

TABLE 1. Antioxidant Activity of extract and fractions from *A. elasticus* barks

Sample	IC_{50} (µg/mL)
Quercetin (positive control)	9.30
Methanol Extract	107.21
n-hexane fraction	173.74
Ethyl acetate fraction	90.03
n-butanol fraction	76.71

Further studies were conducted on ethyl acetate fraction. This fraction was purified by gradient elution utilizing solvents with varying degrees of polarity, ranging from 100% non-polar solvents to 100% polar solvents (*n*-hexane, ethyl acetate and methanol) and a gravity chromatography column with silica gel 60 GF254 as the stationary phase. All nine sub-fractions (SF1-SF9) were identified by Thin Layer Chromatography (TLC by observing the spots using UV light at λ 254 nm and 365 nm. These subfractions were analyzed for their antioxidant activity (Table 2). As SF6 showed potential activity and highest yield, it was selected for the next purification process. This subfraction was further subjected to Sephadex LH-20 column chromatography and Preparative Thin Layer Chromatography obtaining a yellow powder compound, which was identified as the active compound **1.** The pure compound **1** was identified using UV-Vis spectrophotometry, FTIR, LC-MS, and NMR to determine the molecular structure.

TABLE 2. Antioxidant Activity of subfractions from *A. elasticus* barks

Sample	IC_{50} (µg/mL)	Sample	IC_{50} (µg/mL)
SF1	1093.27	SF6	100.77
SF2	523.30	SF7	81.71
SF3	105.24	SF8	79.67
SF4	126.58	SF9	82.90
SF5	126.58		

UV-Vis spectra of compound **1** dissolved in methanol solvent showed absorption at maximum wavelengths of 235 nm and 280 nm. Based on these results, compound **1** was considered as typical for an aromatic system (benzene), which exhibits high absorption at wavelengths of 184 and 202 nm and has succession of absorption bands between 230 and 270 nm. The infrared spectrum of compound **1** (Fig. 1) showed a hydroxide (OH) group at a wavenumber (v) of 3417 cm-1 (stretching vibrations of hydroxyl groups that can form hydrogen bonds) and absorption peaks in the wavenumber region (v). 2922 and 2860 cm-1 which showed the presence of aliphatic groups -CH_3 and -CH-. Meanwhile, aromatic CH groups and C=O groups were presented at wavenumbers of (v) 1645, 1591, 1444 cm^{-1}, and (v)1700 cm^{-1}, respectively.

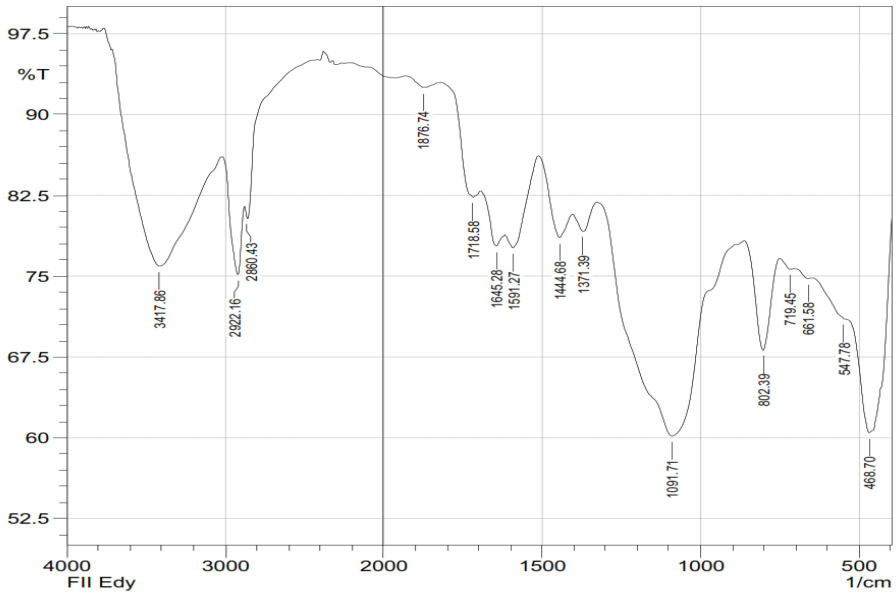

FIGURE 1. FTIR spectrum of compound **1** isolated from *A. elasticus* leaves

The data obtained from the UV-Vis spectrum and IR spectrum demonstrated the characteristic of xanthone compounds [9]. In addition, the ESI-MS data (Fig. 2) showed that compound **1** has a molecular weight *m*/*z* 352.37.

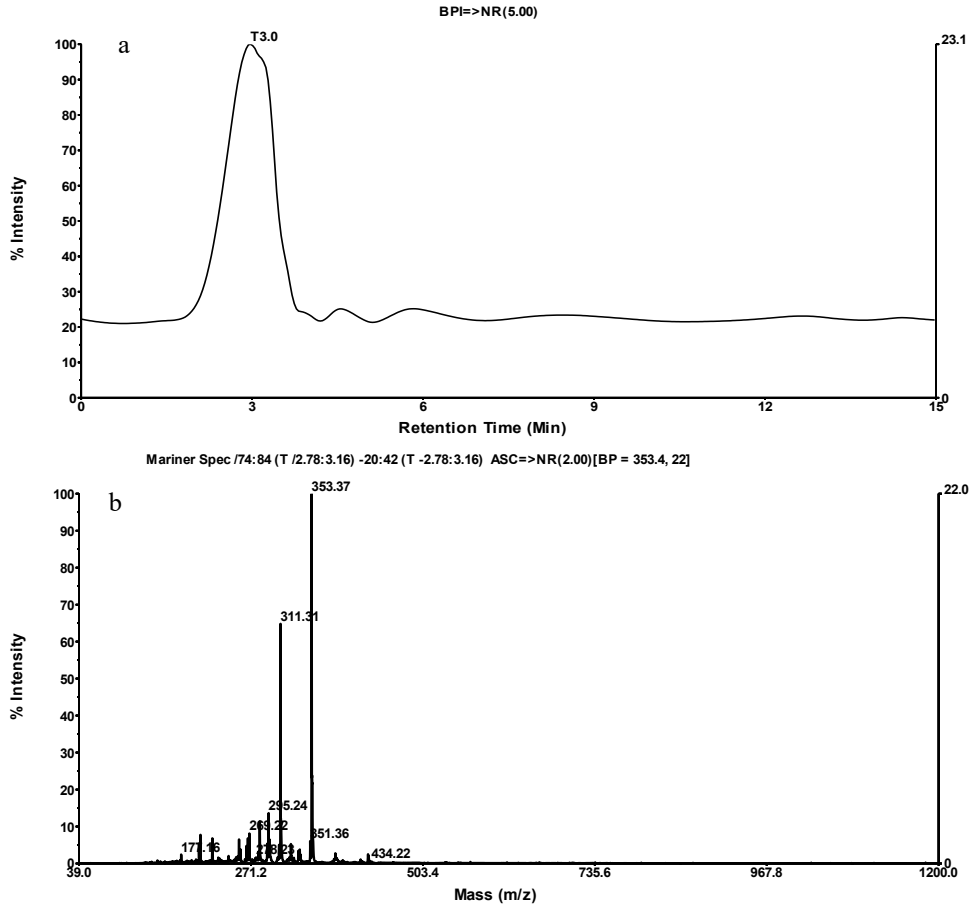

FIGURE 2. LCMS spectrum of compound **1** isolated from *A. elasticus* leaves: (a) chromatogram of compound **1**; (b) mass spectrum of compound **1**

The 'H NMR data ($CDCI_3$) reported the signals of various protons, such as 2,2-dimethylpyran ring protons, δ 1.47 (6H, s), 5.58, 6.81 (each lH, d, J = 10.1 Hz); an aromatic proton, δ 6.28 (lH, s); a hydrogen-bonded hydroxyl group proton, δ 12.36 (lH, s); isopropenyl group protons, δ 1.82 (3H, s), 4.84, 4.91 (each lH, br s); and five aliphatic protons, δ 2.61 (lH, dd, J = 10.3 and 17.4 Hz), 2.66 (lH, dd, J = 11.5 and 15.6 Hz), 2.81 (lH, m), 2.88 (lH, ddd, J = 1.8, 3.4, and 15.6 Hz), 3.15 (lH, ddd, J = 1.8, 4.0, and 17.4 Hz).

TABLE 3. ^{1}H data of compound 1 and comparison with literature

Position	δ_H (ppm, multiplicity, J(Hz))	
	Compound 1	[9,10]
1	12.36 (S, OH)	12.35 (S, OH)
2	6.28 (S)	6.28 (S)
6	2.66 (dd, J=11)	2.68 (dd, J=11.5)
	2.88 (ddd, J=1.8)	2.88 (ddd, J=1.8, 3.4
7	2.81 (m)	2.81 (m)
8	2.61 (dd, J=10.3)	2,61 (dd, J=10)
	3.15 (ddd, J=1.8)	3.15 (ddd, J=1.8, 4.0)
11	6.81 (d, J=10.8)	6.82 (d, J=10.1 Hz)
12	5.58 (d, J=10)	5.59 (d, J=10 Hz)
14	1.47 (6H, s)	1.47 (6H, S)
15	1.82 (3H, s)	1.83 (3H, s)
17	4.91 (1H, br. s)	4.92 (1H, br. s)
18	4.84 (1H, br. s)	4.85 (1H, br. s)

According to the characterization performed using 1H-NMR, UV-VIS, FTIR and LC-MS spectrometers along with supported literature [11, 12], it was concluded that compound **1** is a xanthone derivative, Artonol A.

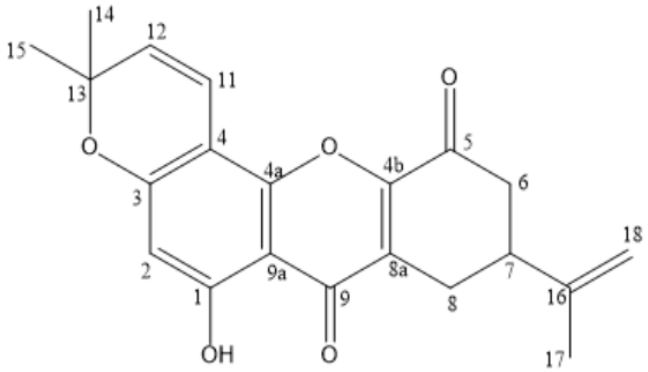

FIGURE 3. Artonol A isolated from *A. elasticus* barks.

The antioxidant effect of artonol A was evaluated using the DPPH method with the IC_{50} value of 103.20 µg/mL (0.29 mM). In this study, the antioxidant activity of artonol A was weaker than quercetin as a positive control. This is due to the different antioxidant activity mechanism of compounds with a xanthone moiety, such as artonol and a flavone moiety compunds, such as quercetin. As a prenylflavonoids, artonol A showed higher potential as antioxidant activity by the body protection mechanism from DNA damage caused by O_2^{-}. The lower oxidation potential has provided higher inhibitory effect on oxidative DNA damage [13]. Therefore, the xanthone oxidase method on artonol A will be more favorable to use since this compound may be able to prevent carcinogenesis by supressing oxidative DNA damage induced by ROS [13, 14].

CONCLUSIONS

. Ethyl acetate and *n*-butanol extracts have antioxidant activity higher than others with IC_{50} 90.03 and 76.71 µg/mL, respectively. Isolation of artonol A from *A. elasticus* barks has been successfully performed with potential to be utilized as an antioxidant agent. In addition, the extracts, and fractions from *A. elasticus* also can be developed as herbal medicine for antioxidant remedies.

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