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Isoflavone from *Artocarpus integer* (Thunb.) Merr. and the bioactivity of antioxidants.

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ABSTRACT

Isolation of secondary metabolites heartwood of *Artocarpus integer* (Thunb.) Merr. (Moraceae) by maceration, fractionation, and purification stage have been conducted. The molecular structure of isolated compounds was determined by UV spectroscopy, IR, ¹H-NMR, ¹³C-NMR, HSQC, and HMBC. The compounds were tested antioxidant activity by DPPH method using spectrophotometer UV-Vis at λ_{\max} 500 nm with IC₅₀ values: artocarpanone (1) (29.88 $\mu\text{g/ml}$), cudraflavone C (2) (3.35 $\mu\text{g/ml}$), artocarpin (3) (4.70 $\mu\text{g/ml}$), tephrosin (4) (55.58 $\mu\text{g/ml}$), norartocarpetin (5) (2.83 $\mu\text{g/ml}$), and ascorbic acid (2.79 $\mu\text{g/ml}$) as a comparison. Tephrosin is a compound that first time discovered in this plant.

Keywords: Moraceae, *Artocarpus integer*, flavonoids, antioxidant

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INTRODUCTION

Artocarpus consists of about 50 species and spread in Indonesia, particularly in Sumatra, Java, Kalimantan, and Sulawesi. The plant also found in South Asia, Papua New Guinea and the South Pacific [1]. In Indonesia, *Artocarpus* plant known as "nangka-nangkaan" [2]. *Artocarpus* generally have characteristic are: high tree, gummy white in all part, somewhat hard sheets and arranged leaves alternate, fleshy fruit with small to large sizes, large woody and taproot [3]. Some parts of *Artocarpus* genus used as building materials [4,5] and traditional medicine [6,7]. The root of *A. heterophyllus* used to treat fever, dysentery, and malaria, the seeds for diarrhea, and leaves for medicine ulcers, fever, sores, and skin diseases [3]. *A. elasticus* bark infusion used as a contraceptive, the gum used as a cure dysentery and fever reliever [3].

In general, plants of the *Artocarpus* contain phenol compound derivatives [8,9,10] and nonphenolic [11,12]. *Artocarpus* most abundant of flavonoid group compounds compared to other [9,11]. These compounds have diverse biological activities, including anti-malarial [13,14], anti-tumor [15] and antioxidant [16,17,18].

Development of science and technology has changed the pattern of human life which impact the emergence of a wide range of degenerative diseases [19]. Air pollution and fast food can be a source of free radicals in our body. Free radicals are very unstable because they have one or more unpaired electrons that are harmful to health. In fact, the body has an enzyme superoxide dismutase (SOD) and glutathione peroxidase, but if the body's defenses down, the enzyme cannot ward of the free radical attack [6,10].

Compound that can reduce free radicals in the body is an antioxidant. The synthetic antioxidants such as butyl hydroxy toluene (BHT), butylhydroxyanisole (BHA), propyl gallate (PG) and tert-butyl hydroquinone (TBHQ) has high efficacy, but these compounds will stimulated carcinogenesis [20]. Based on this condition needed antioxidant compounds derived from natural ingredients including vitamins C and E, curcumin, curcuminoid, phenolic and flavonoids [21,22].

MATERIALS AND METHODS

Plant material

The heartwood of *Artocarpus integer* (Thunb.) Merr. was collected from North Luwu, South Sulawesi, Indonesia in 2015. The voucher specimen of the plant has been kept in the Bogoriense herbarium, Research Center for Biology, Indonesian Institute of Sciences (LIPI), Bogor, Indonesia. The plant specimens stored as collections in Bogoriense herbarium with registration number BO-1936238.

General experimental procedures

Melting points were determined on a micro-melting point apparatus and are uncorrected. UV spectra using Hewlett Packard 8543 Agilent Technologies and IR spectra were measured with Shimadzu Prestige-21. ¹H and ¹³C NMR spectra were recorded with a Bruker AM 500 spectrometer, operating at 500 MHz (¹H) and 125 MHz (¹³C). TLC analysis on precoated Si gel plates (Merck Kieselgel 60 F, 0.25 mm), VLC was carried out using Merck Si gel 60 GF, and radial chromatography with Merck Si gel 60 PF 254.

Extraction and isolation

The heartwood samples were dried, shredded and pollinated. *Artocarpus integer* heartwood powder (4.8 kg) was macerated with methanol for 72 hours, filtered and evaporated using rotary evaporator to get crude methanol extract (350 g) was obtained. The methanol extract was partitioned to obtain a fraction of n-hexane (7.63 g), chloroform (94.75 g), ethyl acetate (7.33 g), and methanol (43.69 g). Subsequently, chloroform fraction (20 g) was fractionated by vacuum liquid chromatography, eluent n-hexane/ethyl acetate ratio are 10:0, 7:3, 6:4, 5:5 and 3:7 produced 16 major fractions.

Main fraction number 10 (100 mg) was further separated by radial chromatography/centrifugal planar chromatography (KR1), eluted with chloroform:ethyl acetate in ratio 9:1 enhanced by polarity gradually yielding 8 subfractions. The fraction of 10 was carried out by radial chromatography 4 times until a mass of 133 g was obtained and then fractionated again yielding 10 subfractions. The subfraction was grouped by TLC (Thin layer chromatography) analysis resulted in 5 fractions.

Antioxidant Test

A total of 9.9 mg of DPPH was dissolved with methanol p.a. to a volume of 10 ml to obtain 0.4 mM DPPH solution. Furthermore 1.00 mg of Artocarpanone compound (1) was dissolved with methanol p.a. to a volume of 5 ml, obtained a concentration of 5000 ppm as a mother liquor. From the mother liquor pipette 1 μ l, 2 μ l, 3 μ l, 4 μ l, and 5 μ l and added with 1 ml of DPPH 0.4 mM, then it was added to volume of 5 ml in order to obtain a concentration of 1 μ g/ml, 2 μ g/ml, 3 μ g/ml, 4 μ g/ml, and 5 μ g/ml. The solution is shaken and stored at room temperature in the dark room for 30 minutes. Then measured the absorbance λ_{\max} 500 nm wavelength. The same treatment was performed for cudraflavone C (2), artocarpin (3), tephrosin (4), and norartocarpin (5).

RESULTS AND DISCUSSION

Based on the TLC result by three eluent systems, showed subfraction 108.4-6 with single and similar stains, so that after the analyzed using UV, IR, NMR, generates artocarpanone (1) (14.1 mg). With the same fractionation technique 1010.3-6 subduction was obtained cudraflavone C (2) (19.6 mg), subfraction 313.5-8 artocarpin (3) (67.4 mg), subtraction 1018.2 tephrosin (4) (3.9 mg), and subtraction 1217.5 norartocarpin (5) (11.6 mg).

Spectral measurements

Artocarpanone (1): white powder, mp 208-210 °C. UV (MeOH) λ_{\max} (nm) (log ϵ): 335, 287. IR (KBr) ν_{\max} (cm⁻¹): 3425 (OH), 2924 dan 2852 (CH aliphatic), 1637 (C=O), 1517 (C=C aromatic), 1462 (CH₂), 1381 (CH₃), 1157 (C-O). ¹H NMR (500 MHz, acetone-d₆) (ppm): 5.72 (1H, *dd*, *J* = 2.8; 13.2 Hz, H-2); 3.19 (1H, *dd*, *J* = 13.2; 17.1 Hz, H_{ax}-3); 2.74 (1H, *dd*, *J* = 2.9; 17.1 Hz, H_{eq}-3); 12.15 (1H, *s*, 5-OH); 6.04 (1H, *d*, *J* = 2,2 Hz, H-6); 6.02 (1H, *d*, *J* = 2.2 Hz, H-8); 6.46 (1H, *d*, *J* = 2.2 Hz, H-3'); 6.42 (1H, *dd*, *J* = 2.2; 8.4 Hz, H-5'); 7.30 (1H, *d*, *J* = 8.4 Hz, H-6'); 3.83 (3H, *s*, 7-OMe). ¹³C NMR (125 MHz, acetone-d₆) (ppm): 75.4 (CH, C-2); 42.6 (CH₂, C-3); 198.0 (C=O, C-4); 103.6 (C, C-4a); 159.5 (C-OH, C-5); 94.4 (C-6); 168.7 (C, C-7); 95.3 (CH, C-8); 164.6 (C, C-8a); 117.2 (C, C-1'); 156.3 (C-OH, C-2'); 103.4 (CH, C-3'); 159.5 (C-OH, C-4'); 107.8 (CH, C-5'); 128.9 (CH, C-6'); 56.14 (OCH₃).

Cudraflavone C (2): orange powder, mp 97-100 °C. UV (MeOH) λ_{\max} (nm) (log ϵ): 313, 263. IR (KBr) ν_{\max} (cm⁻¹): 3419 (OH), 2962 dan 2920 (CH aliphatic), 1649 (C=O), 1622 (C=C aromatic), 1462 (CH₂), 1359 (CH₃), 1166 (C-O). ¹H NMR (500 MHz, acetone-d₆) (ppm): 13.43 (1H, *s*, 5-OH); 6.35 (1H, *s*, H-8); 6.54 (1H, *d*, *J* = 2.3 Hz, H-3'); 6.50 (1H, *dd*, *J* = 2.3; 8.3 Hz, H-5'); 7.17 (1H, *d*, *J* = 8.3 Hz, H-6'); 3.09 (2H, *d*, *J* = 6.9 Hz, H-9); 5.11 (1H, *m*, H-10); 1.42 (3H, *s*, H-12); 1.56 (3H, *s*, H-13); 3.35 (2H, *d*, *J* = 7.2 Hz, H-14); 5.27 (1H, *m*, H-15); 1.77 (3H, *s*, H-17); 1.64 (3H, *s*, H-18). ¹³C NMR (125 MHz, acetone-d₆) (ppm): 160.0 (C, C-2); 121.5 (C, C-3); 183.0 (C=O, C-4); 105.0 (C, C-4a); 162.0 (C-OH, C-5); 111.7 (C, C-6); 162.3 (C, C-7); 93.4 (CH, C-8); 156.9 (C, C-8a); 113.0 (C, C-1'); 161.3 (C-OH, C-2'); 103.8 (CH, C-3'); 157.0 (C-OH, C-4'); 108.0 (CH, C-5'); 132.2 (CH, C-6'); 24.6 (CH₂, C-9); 122.7 (CH, C-10); 131.9 (C, C-11); 17.6 (CH₃, C-12); 25.8 (CH₃, C-13); 21.9 (CH₂, C-14); 123.3 (CH, C-15); 131.4 (C, C-16); 17.8 (CH₃, C-17); 25.7 (CH₃, C-18).

Artocarpin (3): yellow powder, mp 174-177 °C. UV (MeOH) λ_{\max} (nm) (log ϵ): 323, 280. IR (KBr) ν_{\max} (cm⁻¹): 3396 (OH), 3041 (CH aromatic), 2958 dan 2824 (CH aliphatic), 1651 (C=O), 1618 dan 1479 (C=C aromatic), 1454 (CH₂), 1352 (CH₃), 1147 (C-O). ¹H NMR (500 MHz, acetone-d₆) (ppm): 13.97 (1H, *s*, 5-OH); 6.54 (1H, *s*, H-8); 6.57 (1H, *d*, *J* = 2.3 Hz, H-3'); 6.53 (1H, *dd*, *J* = 2.3; 8.4 Hz, H-5'); 7.22 (1H, *d*, *J* = 8.3 Hz, H-6'); 3.14 (2H, *d*, *J* = 7.0 Hz, H-9); 5.14 (1H, *d*, *J* = 7.0 Hz, H-10); 1.44 (3H, *s*, H-12); 1.58 (3H, *s*, H-13); 6.60 (1H, *d*, *J* = 17.5 Hz, H-14); 6.70 (1H, *dd*, *J* = 7.1; 16.2 Hz, H-15); 2.44 (1H, *m*, H-16); 1.09 (3H, *d*, *J* = 6.8 Hz, H-17); 1.09 (3H, *d*, *J* = 6.8 Hz, H-18). ¹³C NMR (125 MHz, acetone-d₆) (ppm): 162.4 (C, C-2); 121.9 (C, C-3); 183.3 (C=O, C-4); 105.5 (C, C-4a); 159.7 (C-OH, C-5); 109.7 (C, C-6); 163.7 (C, C-7); 90.4 (CH, C-8); 157.3 (C, C-8a); 112.8 (C, C-1'); 157.2 (C-OH, C-2'); 103.8 (CH, C-3'); 161.4 (C-OH, C-4'); 108.0 (CH, C-5'); 132.3 (CH, C-6'); 24.6 (CH₂, C-9); 122.5 (CH, C-

10); 132.2 (C, C-11); 25.8 (CH₃, C-12); 17.6 (CH₃, C-13); 116.9 (CH, C-14); 142.1 (CH, C-15); 33.9 (CH, C-16); 23.1(CH₃, C-17/18).

Tephrosin (4): yellow powder, mp 194-198 °C. UV (MeOH) λ_{max} (nm) (log ε): 312, 270. IR (KBr) ν_{max} (cm⁻¹): 3444 (OH), 3072 (CH aromatic), 2964 dan 2929 (CH aliphatic), 1674 (C=O), 1596 dan 1510 (C=C aromatic), 1452 (CH₂), 1377 (CH₃), 1217 (C-O). ¹H NMR (500 MHz, chloroform-d) (ppm): 6.55 (1H, s, H-1); 6.48 (1H, s, H-4); 4.49 (1H, dd, J = 2.3; 12.6, H-6); 4.63 (1H, dd, J = 2.3; 12.1, H-6); 4.57 (1H, dd, J = 1.4; 2.4, H-6a); 6.47 (1H, d, J = 8.9, H-10); 7.72 (1H, d, J = 8.7 Hz, H-11); 6.60 (1H, d, J = 10.0, H-4'); 5.55 (1H, d, J = 10.1, H-5'); 1.38 (3H, s, H-7'); 1.44 (1H, s, H-8'). ¹³C NMR (125 MHz, chloroform-d) (ppm): 108.7 (C, C-1a); 109.3 (CH, C-1); 144.1 (C, C-2); 151.2 (C, C-3); 101.2 (CH, C-4); 148.5 (C, C-4a); 64.0 (CH₂, C-6); 76.4 (CH, C-6a); 156.8 (C, C-7a); 109.4 (C, C-8); 160.9 (C, C-9); 111.8 (CH, C-10); 128.7 (CH, C-11); 111.2 (C, C-11a); 191.5 (C, C-12); 67.6 (C-OH, C-12a); 115.5 (CH, C-4'); 128.9 (CH, C-5'); 78.1 (C, C-6'); 28.4 (CH₃, C-7'); 28.7 (CH₃, C-8'); 56.0 (OCH₃(₁)); 56.5 (OCH₃(₂)).

Norartocarpetin (5): yellow powder, mp 244-248 °C. UV (MeOH) λ_{max} (nm) (log ε): 338, 287, 264. IR (KBr) ν_{max} (cm⁻¹): 3361 (OH), 2960 dan 2924 (CH aliphatic), 1616 dan 1510 (C=C aromatic), 1454 (CH₂), 1359 (CH₃), 1163 (C-O). ¹H NMR (500 MHz, acetone-d₆) (ppm): 13.11 (1H, s, 5-OH); 7.07 (1H, s, H-3); 6.23 (1H, d, J = 2.0 Hz, H-6); 6.50 (1H, d, J = 2.0 Hz, H-8); 6.60 (1H, d, J = 2.3 Hz, H-3'); 6.57 (1H, dd, J = 2.3; 8.7 Hz, H-5'); 7.84 (1H, d, J = 8.7 Hz, H-6'). ¹³C NMR (125 MHz, acetone-d₆) (ppm): 161.9 (C, C-2); 107.6 (CH, C-3); 182.5 (C=O, C-4); 104.3 (C, C-4a); 162.4 (C-OH, C-5); 98.5 (CH, C-6); 163.8 (C-OH, C-7); 93.6 (CH, C-8); 157.9 (C, C-8a); 109.8 (C, C-1'); 158.4 (C-OH, C-2'); 103.4 (CH, C-3'); 161.6 (C-OH, C-4'); 108.2 (CH, C-5'); 130.0 (CH, C-6').

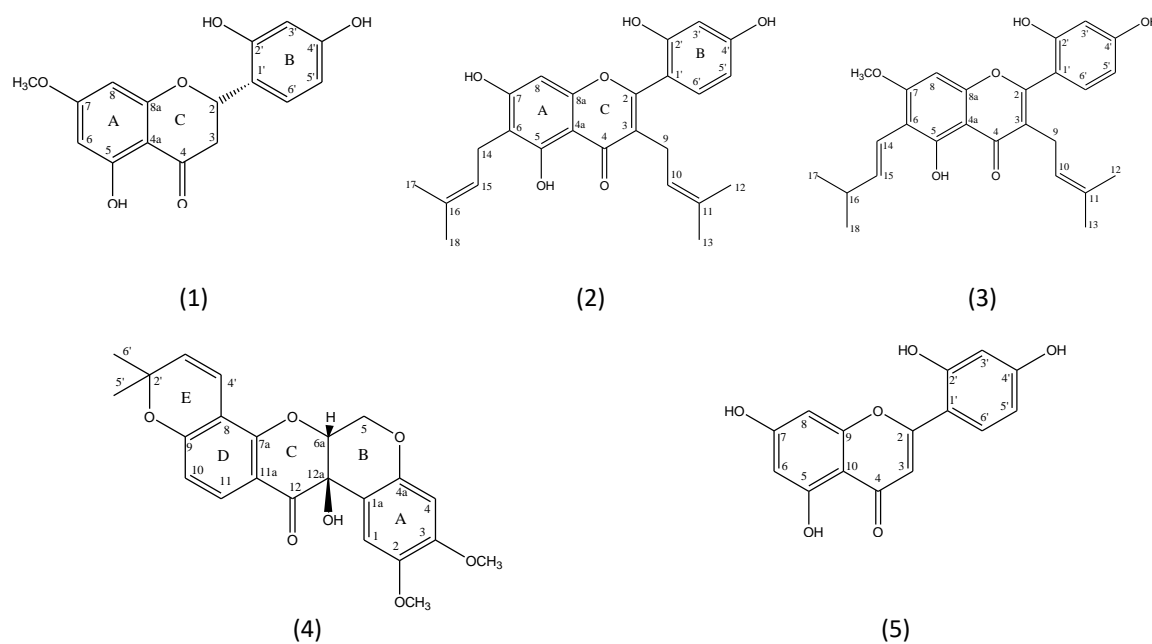


Figure 1. Isolated compounds from heartwood *Artocarpus integer* (Thunb.) Merr.

Antioxidant Activity

The antioxidant activity test by spectrophotometer method was done by reacting the sample with DPPH solution. The DPPH radicals scavenging activity was calculated according equation [23]:

$$\text{Scavenging activity} = [(A_0 - A_1) / A_0] \times 100$$

A₀ is the absorbance of the control (blank, without sample) and A₁ is the absorbance in the presence of the sample.

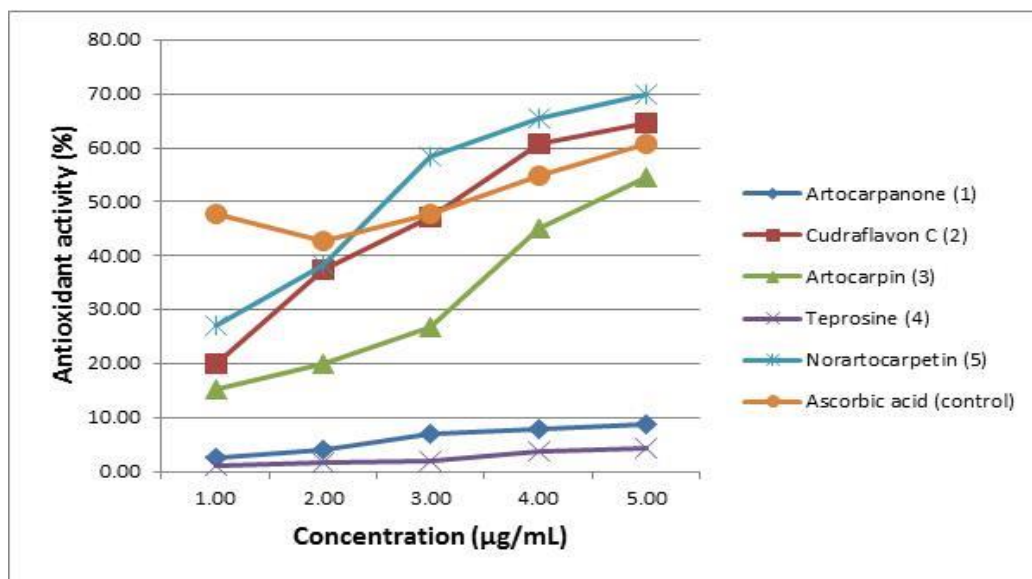


Figure 1. Graph antioxidant activity of isolated compounds heartwood of *Artocarpus integer* (Thunb.) Merr. and ascorbic acid

Table 1. IC₅₀ value of compounds heartwood of *Artocarpus integer* (Thunb.) Merr.

Compound	The linear regression equation	IC ₅₀ Value (µg/mL)
Artocarpanone (1)	$y = 1.6352x - 1.1321$ $R^2 = 0.9562$	29.88
Cudraflavone C (2)	$y = 7.8981x + 8.9172$ $R^2 = 0.559-0$	5.20
Artocarpin (3)	$y = 10.3870x + 1.1613$ $R^2 = 0.9510$	4.70
Tephrosin (4)	$y = 0.9034x + 0.2181$ $R^2 = 0.9344$	55.58
Norartocarpetin (5)	$y = 11.3250x + 17.8860$ $R^2 = 0.9426$	2.83
Ascorbic acid (positive control)	$y = 3.7869x + 39.4260$ $R^2 = 0.7238$	2.79

Categories ability of antioxidant level is: strong (IC₅₀<50 µg/ml), active (IC₅₀ 50-100 µg/ml), medium (IC₅₀ 101-250 µg/ml), low (IC₅₀ 250-500 µg/ml), and inactive (IC₅₀>500 µg/ml) [24].

All the compounds found in the heartwood of *Artocarpus integer* (Thunb.) Merr. is flavonoids group. Composition content compounds is artocarpanone (1) derivated of flavanone; while cudraflavone C (2), artocarpin (3), norartocarpetin (5) derivated of flavon; and tephrosin (4) derivated of isoflavon. The structure of these compounds shown in Figure 1. Tephrosin (4) was first time discovered in this plant, but often found in family Pabaceae [25].

The antioxidant activity of isolation compound shows that artocarpanone (1), cudraflavone C (2), artocarpin (3), norartocarpetin (5) respectively have strong antioxidant category level, while and tephrosin (4) have active category.

CONCLUSIONS

Five flavonoid derived compounds have been found from *Artocarpus integer* (Thunb.) Merr. and tested antioxidant activity. IC₅₀ value resulted are: artocarpanone (1) (29.88 µg/ml), cudraflavone C (2) (3.35 µg/ml), artocarpin (3) (4.70 µg/ml), tephrosin (4) (55.58 µg/ml), norartocarpetin (5) (2.83 µg/ml) and ascorbic acid (2.79 µg/ml) as a comparison.

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