SUPPLEMENTARY MATERIALS

Flavonoids of the leaves and heartwoods of *Artocarpus lowii* King and their bioactivities

Siti Awanis Abdullah, Shajarahtunnur Jamil*, Norazah Basar, Siti Mariam Abdul Lathiff and Norzafneza Mohd Arriffin

Department of Chemistry, Faculty of Science, Universiti Teknologi Malaysia, 81310 Johor Bahru, Johor, Malaysia

*Department of Chemistry, Faculty of Science, Universiti Teknologi Malaysia, 81310 Johor Bahru, Johor, Malaysia; Tel. No. (office): +607-5534156; (mobile): +6019-7291822; Fax No.: +607-5566162; Email: shaja@kimia.fs.utm.my

Flavonoids of the leaves and heartwoods of *Artocarpus lowii* King and their bioactivities

A new dihydrochalcone, 2',4'-dihydroxy-3,4-(2'',2''-dimethylchromeno)-3'-prenyldihydrochalcone (**1**) together with 4-hydroxyonchocarpin (**2**), isobavachalcone (**3**), 4',5-dihydroxy-6,7-(2,2-dimethylpyrano)-2'-methoxy-8- γ,γ -dimethylallylflavone (**4**), artocarpin (**5**) and cycloheterophyllin (**6**) were successfully isolated from the leaves and heartwoods of *Artocarpus lowii* King (Moraceae). The structures of these compounds were fully characterized spectroscopically and by direct comparison with published data. These compounds were tested for their antioxidant and tyrosinase inhibitory activities. Compound (**1**) displayed moderate antioxidant activity towards DPPH and tyrosinase inhibitory activities with SC₅₀ value of 223.8 μ M and IC₅₀ value of 722.5 μ M, respectively. Among the isolated compounds, cycloheterophyllin (**6**) showed the most potential antioxidant activity with SC₅₀ value of 320.0 μ M and 102.8 μ M for ABTS and DPPH radicals scavenging activities, respectively and also exhibited highest FRAP equivalent value of 4.7 ± 0.09 mM. Compound (**6**) showed tyrosinase inhibitory activity with the IC₅₀ value of 104.6 μ M.

Keywords: *Artocarpus lowii*, dihydrochalcone, flavonoids, antioxidant, tyrosinase inhibitory activity

Biological Activities

DPPH free radical scavenging assay

The free radical scavenging assay was conducted based on method described by Najihah et al. (2012) with minor modification. Briefly, 100 μ M DPPH (1 mL) dissolved in MeOH was added to the MeOH solution (3 mL) of the tested samples at different concentration. An equal volume of MeOH was added in the control test. The mixture was shaken vigorously and allowed to stand at room temperature for 30 minutes. Butylated hydroxyanisole (BHA) was used as standard reference. Then the absorbance at 517 nm was measured with Epoch microplate reader. The percentage of scavenging of DPPH was calculated using the following equation:

$$S\% = [A_{\text{blank}} - A_{\text{sample}} / A_{\text{blank}}] \times 100$$

Where A_{blank} is the absorbance value of the control reaction (containing all reagents except the test compound) and A_{sample} is the absorbance value of the test compound. The sample concentration providing 50% scavenging (SC_{50}) was calculated by plotting scavenging percentages against concentrations of the sample. All tests were carried out in triplicate and scavenging values were reported as means $\pm$ SD of triplicates.

Ferric reducing antioxidant potential (FRAP) assay

The ferric reducing antioxidant potential (FRAP) assay was carried out according to Channarong et al. (2012) and Shahwar et al. (2012) with minor modification. FRAP reagent was freshly prepared, consist of stock solution with ratio 10:1:1 of acetate buffer (300 mM), TPTZ (10 mM) in HCl (40 mM) and $FeCl_3 \cdot 6H_2O$ solution (20 mM). Sample (5 μ L), MeOH (15 μ L) and FRAP reagent (150 μ L) were added to the 96-well plates. The absorbance at 573 nm was measured after 10 minutes of incubation at 37°C. $FeSO_4 \cdot 7H_2O$ solution (0.1 mM – 1.0 mM) was used to build up calibration curves of standard antioxidant.

2,2'-Azinobis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS) Assay

The ABTS assay was conducted according to method described by Zou et al. (2011) with minor modification. ABTS and potassium persulfate were dissolved with distilled water to obtain solution with concentration of 7 mM and 4.9 mM, respectively. The two solutions were mixed and incubate in the dark for 12-16 hours at room temperature. After incubation time, the ABTS radical was added with distilled water until the absorbance is 0.7 at 734 nm. Sample (10 μ L) and ABTS solution (190 μ L) were added to the 96-well plates. The absorbance was recorded after 30 minutes incubation in the dark at room temperature. The percentage of antioxidant activity was calculated using the following formula:

$$\text{Scavenging Percentage} = \frac{\text{Abs (ABTS)} - \text{Abs (ABTS + Sample)}}{\text{Abs (ABTS)}} \times 100$$

Tyrosinase Inhibitory Activity

This assay was performed using method described by Kamken et al. (2007) and Likhitwitayawuid et al. (2001) with minor modification. The samples were dissolved in dimethyl sulfoxide (DMSO) to the final concentration 100 μ g/mL (as the stock solution). 0.1 M phosphate buffer solution (pH 6.7) and L-DOPA was added to each 96-well plates. Four wells labeled as well 1 and well 2 contained sample stock solution (40 μ L). DMSO (negative control) replaced the sample in the well 3. The plate was incubated for 10 minutes at 25°C. Then, tyrosinase enzyme was added to well 1 and well 4. The plate was then incubated again for 20 minutes at 25°C. The absorbance was measured using Epoch microplate reader at 475 nm with 700 nm as reference. The percentage of inhibition was calculated as follows:

$$\% \text{ Inhibition} = ((4 - 3) - (1 - 2)) / ((1 - 2)) \times 1$$

1: absorbance of sample solution with enzyme

2: absorbance of sample solution without enzyme

3: absorbance of blank solution without enzyme

4: absorbance of blank solution with enzyme

Figure S2. HMBC correlations of compound (**1**)

1

Elastichalcone A

Figure S3. Structures comparison of compound (**1**) and Elastichalcone A

Table S2. NMR spectral data of compound (**1**) in acetone-*d* (δ in ppm, *J* in Hz)

No	δ_{H}	δ_{C}	COSY	HMBC
1	-	113.0	-	-
2	6.35 (d, 1H, <i>J</i> = 2.4)	102.8	H-6	C-3, 4, 7''
3	-	108.3	-	-
4	-	160.3	-	-
5	8.06 (d, 1H, <i>J</i> = 8.8)	131.3	H-6	-
6	6.43 (dd, 1H, <i>J</i> = 2.4, 8.8)	107.9	H-2, 6	-
α	2.91 (t, 2H, <i>J</i> = 7.6)	29.8	H- β	-
β	3.22 (t, 2H, <i>J</i> = 7.6)	39.5	H- α	-
C=O	-	204.3	-	-
1'	-	115.5	-	-
2'	12.87 (s, 1H, OH)	150.6	-	-
3'	-	127.1	-	-
4'	-	160.8	-	-
5'	7.78 (d, 1H, <i>J</i> = 8.6)	133.3	H-6'	C-1'
6'	6.95 (d, 1H, <i>J</i> = 8.6)	115.9	H-5'	C-4'
1''	3.34 (d, 2H, <i>J</i> = 7.6)	28.7	H-2''	C-1', 2', 5'
2''	5.31 (t, 1H, <i>J</i> = 7.6)	122.8	H-1''	-
3''	-	77.5	-	-
4''	1.73 (s, 3H)	25.0	-	-
5''	1.73 (s, 3H)	17.2	-	-
6''	5.73 (d, 1H, <i>J</i> = 10.0)	126.6	H-7''	C-3, 4
7''	6.72 (d, 1H, <i>J</i> = 10.0)	117.2	H-6''	C-3, 4

8"	-	131.9	-	-
9"	1.46 (s, 3H)	27.6	-	-
10"	1.46 (s, 3H)	27.3	-	-

Spectra of 2',4'-Dihydroxy-3,4-(2'',2''-dimethylchromeno)-3'-prenyldihydrochalcone (1)

IR Spectrum of (1)

UV Spectrum of (1)

ALLM23-c1-av #1 RT: 4.61 AV: 1

T: + c Full ms [360.16-409.98]

HREIMS of (1)

¹H NMR (acetone-d, 400 MHz) of (1)

¹H NMR (acetone-d, 400 MHz) of (1) - expansion

COSY spectrum of (1)

COSY spectrum of (1) - expansion

COSY spectrum of (1) – expansion

^{13}C NMR spectrum of (1)

^{13}C NMR and DEPT Spectra of (1)

HMQC spectrum of (1)

HMQC spectrum of (1) - expansion

HMQC spectrum of (1) - expansion

HMQC spectrum of (1) - expansion

HMBC spectrum of (1)

References

- Channarong S, Jutiviboonsuk A, Korsanan S. 2012. Total Reducing Antioxidant Capacity of Thai Herbal Aromatic Powder (Ya Hom) Measured by FRAP Assay. Thai Pharmaceutical and Health Science Journal. 7(3):111-114.
- Kamkaen N, Mulsri N, Treesak C. 2007. Screening of some tropical vegetables for anti-tyrosinase activity. Thai Pharmaceutical and Health Science Journal. 2:15-19.
- Likhitwitayawuid K, Sritularak B. 2001. A new dimeric stilbene with tyrosinase inhibitory activity from *Artocarpus gomeizianus*. Journal of Natural Product. 64: 1457-1459.
- Najihah MH, Mawardi R, Gwendoline CLE, Aspollah MS, Maizatulkamal Y, Aizat MMA, Manaf MA, Rusea G. 2012. Antioxidant, antimicrobial and tyrosinase inhibitory activities of xanthones isolated from *Artocarpus obtusus* F.M. Jarret. Molecules. 17:6071-6082.
- Shahwar D, Raza MA, Bukhari S, Bukhari G. 2012. Ferric reducing antioxidant power of essential oils extracted from *Eucalyptus* and *Curcuma* species. Asian Pacific Journal of Tropical Biomedicine. S1633-S1636.
- Zou Y, Chang SKC, Gu Y, Qian SY. 2011. Antioxidant activity and phenolic composition of lentils (*Lens culinaris* var. Morton) extracts and its fractions. Journal of Agriculture Food Chemistry. 59(6):2268-2276.