Jurnal Teknologi

Full paper

Tyrosinase Inhibitory Activity of Flavonoids from *Artocarpus Lowii* King

Shajarahtunnur Jamil*, Siti Awanis Abdullah, Siti Mariam Abdul Lathiff, Hasnah Mohd Sirat

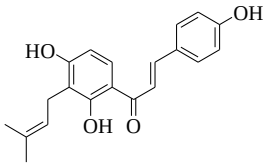
Department of Chemistry, Faculty of Science, Universiti Teknologi Malaysia, 81310 UTM Johor Bahru, Johor, Malaysia

*Corresponding author: shaja@kimia.fs.utm.my

Article history

Received :27 February 2014
Received in revised form :
21 August 2014
Accepted :15 October 2014

Graphical abstract



Abstract

Tyrosinase inhibitory activity was studied on the crude extracts and flavonoids successfully isolated from the leaves and heartwoods of *Artocarpus lowii* King. The flavonoids were fully characterized spectroscopically as isobavachalcone **(1)**, 4-hydroxyonchocarpin **(2)**, 2',4'-dihydroxy-4-methoxy-3'-prenyldihydrochalcone **(3)**, 2',4'-dihydroxy-3,4-(2",2"-dimethylchromeno)-3'-prenyldihydrochalcone **(4)**, artocarpin **(5)**, cycloheterophyllin **(6)** and 4',5-dihydroxy-6,7-(2,2-dimethylpyrano)-2'-methoxy-8-γ,γ-dimethyl allylflavone **(7)**. Tyrosinase inhibitory activity of the samples was determined against mushroom tyrosinase using ELISA microplate reader. Cycloheterophyllin **(6)** exhibited an excellent inhibitory activity against mushroom tyrosinase comparable to the standard kojic acid with the IC_{50} value of 52.5 µg/mL (88.3%).

Keywords: Tyrosinase inhibitory activity; flavonoid; *Artocarpus lowii*

Abstrak

Aktiviti perencatan tirosinase telah dikaji ke atas ekstrak mentah dan flavonoid yang berjaya diasingkan daripada daun dan batang *Artocarpus lowii* King. Struktur sebatian flavonoid telah dikenal pasti dengan menggunakan kaedah spektroskopi dan dikenali sebagai isobavachalkon **(1)**, 4- hidroksionchokarpin **(2)**, 2',4'-dihidroksi-4-metoksi-3'-prenildihidrochalkon **(3)**, 2',4'-dihidroksi-3,4-(2",2"-dimetilkromeno)-3'-prenildihidrochalkon **(4)**, artokarpin **(5)**, sikloheterofilin **(6)** dan 4',5-dihidroksi-6,7-(2,2-dimetilpirano)-2'-metoksi-8-γ,γ-dimetilalilflavon **(7)**. Aktiviti perencatan tirosinase sampel telah ditentukan terhadap cendawan tirosina menggunakan pembaca mikroplate ELISA. Sikloheterofilin **(6)** menunjukkan keputusan yang paling ketara terhadap cendawan tirosinase setanding dengan standard asid kojik dengan nilai IC_{50} 52.5 µg/mL (88.3%).

Kata kunci: Aktiviti perencatan tirosinase; flavonoid; *Artocarpus lowii*

1.0 INTRODUCTION

Plants are valuable source of new natural products. Plants and their products have been used for centuries to prevent and cure diseases. More than a quarter of all the medicines used in the world today contain ingredients derived from plants and were also used in traditional medicine [1]. Plants of Moraceae family are among the tropical plants that had shown interesting chemistry and biological activities. The family comprises about 60 genera and approximately 1400 species that form a significant element in the flora of the tropical region of Southeast Asia. The most investigated genera are *Artocarpus*, *Morus* and *Ficus* [2].

The genus *Artocarpus* comprising about 50 species is native to South and Southeast Asia, New Guinea and the South Pacific [3]. *Artocarpus* is the most commonly encountered genus found in the lowland forest of the East Coast and Southern parts of Peninsular Malaysia. Economically, the genus is of appreciable importance as a source of edible fruit, yield fairly good timber and is widely used in folk medicine. This genus is known worldwide for its edible fruits like the jackfruit (*A. heterophyllus*), miku (*A. lowii*) and breadfruit (*A. altilis*). These species are widely cultivated in Malaysia as villagers and traders commercially sell their fruits in local market. The lightwood known locally as 'terap' and the medium hardwood known as 'keledang' constitute valuable timble resources [4, 5].

Previous phytochemical studies of *Artocarpus species* had revealed that this genus is rich source of the isoprenoid-subtituted phenolic compounds, including flavonoids containing isoprenyl substituents and certain oxygenation pattern [6]. Many of the compounds have been reported to show interesting biological properties such as cytotoxic, antioxidant, antimicrobial and anti-inflammatory activities. Previous study on the leaves of *A. lowii* had successfully isolated several flavonoids including isobavachalcone **(1)**, 4-hydroxyonchocarpin **(2)** and 2',4'-dihydroxy-4-methoxy-3'-prenyldihydrochalcone **(3)** which showed strong free radical scavenging activity towards 2,2-diphenyl-1-picrylhydrazine (DPPH) radical measured by electron spin resonance (ESR) spectrometry [7]. Prenylated flavones,

named artoindonesianin A and artoindonesian B from *A. champeden* exhibited cytotoxic activity against murine leukimia (P-388) cells [8]. Investigation on *A. heterophyllus* reported that 3-prenyl luteolin as strong tyrosinase inhibitor where the prenyl existence on C-3 played an important role in tyrosinase inhibition activity [9]. In addition, bioactivity study of the wood of *A. elasticus* has led to the isolation of artelastin which was evaluated for cytotoxicity against MCF-7 human breast cancer cell line. The result showed that artelastin treatment disturbed microtubules and interfered with DNA replication of the cancer cell line [10].

On continuing our research on *Artocarpus* species, this study focused on the potential of the crude extracts and seven isolated flavonoids from the leaves and heartwoods of *A. lowii* for their tyrosinase inhibitory activity. The structural criteria of flavonoids for this bioactivity was also discussed.

■2.0 EXPERIMENTAL

2.1 Plant Material

The leaves and heartwoods of *A. lowii* were collected from Paka, Terengganu with voucher specimen number AZ 7094 was deposited at Herbarium of Universiti Kebangsaan Malaysia, Bangi.

2.2 Extraction and Isolation

The leaves (2 kg) and heartwoods (2 kg) of *A. lowii* were collected, air-dried and ground. The air dried samples were sequentially extracted by cold extraction with different polarity of solvent starting from hexane, dichloromethane and methanol. Evaporation of each solvent gave respective crude extracts. Each crude extracts were subjected to silica gel vacuum liquid chromatography to afford different fractions based on solvent polarity. The purification and isolation of the leaves crude extracts yielded four flavonoids identified as isobavachalcone **(1)**, 4-hydroxyonchocarpin **(2)** and 2',4'-dihydroxy-4-methoxy-3'-prenyldihydrochalcone **(3)** and 2',4'-dihydroxy-3,4-(2",2"-dimethylchromeno)-3'-prenyldihydrochalcone **(4)** while artocarpin **(5)**, cycloheterophyllin **(6)** and 4',5-dihydroxy-6,7-(2,2-dimethylpyrano)-2'-methoxy-8-γ,γ-dimethylallylflavone **(7)** were purified and isolated from the heartwoods crude extracts **(Figure 1)**. Their structures were spectroscopically identified by UV, IR, MS, and NMR. The isolated compounds from the leaves and heartwoods of *A. lowii* were tested for their tyrosinase inhibitory activity.

2.3 Tyrosinase inhibitory activity

This assay was performed using method described by Kamken *et al*. and Likhitwitayawuid *et al.* with minor modification [11, 12]. The crude extracts and isolated flavonoids were dissolved in dimethyl sulfoxide (DMSO) to the final concentration of 100 µg/mL as stock solution. Kojic acid was used as the positive control. A flat-bottomed 96-well plate was used and each well contained 0.1 M phosphate buffer solution (pH 6.7) and L-DOPA. Four wells labeled as well 1 and well 2 contained sample stock solution (40 µL). DMSO acted as 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. Finally, the absorbances of the tested samples were measured using ELISA microplate reader at 475 nm with 700 nm as reference. The percentage of inhibition was calculated as follows:

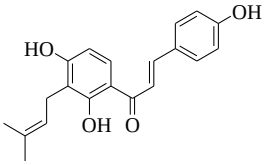
$$\% \text{ Inhibition} = ((4-3)-(1-2))/((1-2)) \times 1$$

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.

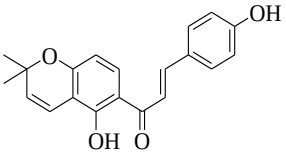
■3.0 RESULTS AND DISCUSSION

3.1 Extraction and Isolation

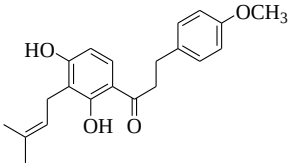
The powdered leaves and heartwoods of the plant were extracted using cold extraction method to yield hexane, dichloromethane and methanol crude extracts. Purification and isolation of the hexane crude extract of the leaves yielded two flavonoids identified as 4-hydroxyonchocarpin **(2)** [7, 13] and 2',4'-dihydroxy-4-methoxy-3'-prenyldihydrochalcone **(3)** [7]. Isobavachalcone **(1)** [7, 13] was purified and isolated from the dichloromethane crude extract while 2',4'-dihydroxy-3,4-(2",2"-dimethylchromeno)-3'-prenyldihydrochalcone **(4)** was isolated from the methanol crude extract of the leaves. Purification of the dichloromethane crude extracts of the heartwoods had successfully isolated cycloheterophyllin **(6)**. Artocarpin **(5)** [9, 14, 15] and 4',5-dihydroxy-6,7-(2,2-dimethylpyrano)-2'-methoxy-8-γ,γ-dimethylallylflavone **(7)** were successfully purified and isolated from the methanol crude extract of the heartwoods. The structures of the isolated compounds **(Figure 1)** were fully characterized on the basis of their spectroscopic data and direct comparison with published reports.



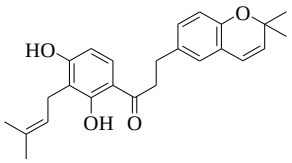
(1)



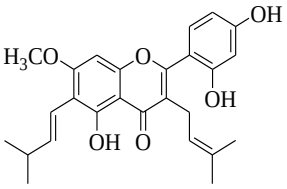
(2)



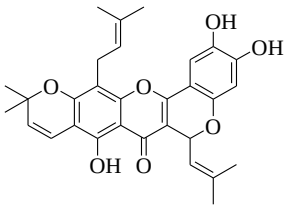
(3)



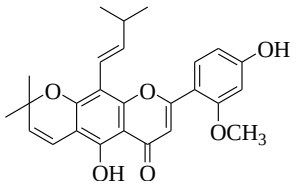
(4)



(5)



(6)



(7)

Figure 1 Chemical structures of flavonoids from the leaves and heartwoods of *Artocarpus lowii* King

3.2 Tyrosinase Inhibitory Activity

Table 1 presented the percentage inhibition at 1000 µg/mL and tyrosinase inhibition (IC_{50}, µg/mL) of six crude extracts and seven flavonoids. The dichloromethane crude extract of the heartwoods (ALBD) showed strong tyrosinase inhibition with IC_{50} value of 85.8 µg/mL (89.6%) comparable to the positive control, kojic acid with the IC_{50} value of 31.2 µg/mL (95.5%) [12]. The methanol crude extracts of the leaves (ALLM) and the heartwoods (ALBM) showed weak tyrosinase inhibitory activity with IC_{50} value of 440.0 µg/mL (65.3%) and 310.5 µg/ml (67.7%) respectively. Therefore, this plant can be a potential source of active compounds such as flavonoids and xanthones for tyrosinase inhibitory activity [11].

Table 1 Percentage and IC_{50} of tyrosinase inhibitory activity of crude extracts and isolated flavonoids

Samples	Inhibition at 1000 µg/mL (%)	IC_{50} (µg/mL)
Crude extracts		
ALLH	20.0	> 1000
ALLD	51.2	980.5
ALLM	65.3	440.0
ALBH	41.2	> 1000
ALBD	89.6	85.8
ALBM	67.6	310.5
Flavonoids		
(1)	78.5	218.8
(2)	71.2	122.5
(3)	64.5	302.8
(4)	67.8	124.3
(5)	63.1	315.0
(6)	88.3	52.5
(7)	65.2	312.5
Positive control		
Kojic Acid	95.5	31.2

Leaves of *A. lowii*: *n*-hexane crude extract (ALLH); dichloromethane crude extract (ALLD) and methanol crude extract (ALLM), heartwoods of *A. lowii*: *n*-hexane crude extract (ALBH); dichloromethane crude extract (ALBD) and methanol crude extract (ALBM). **(1)**: Isobavachalcone, **(2)**: 4-Hydroxyonchocarpin, **(3)**: 2',4'-Dihydroxy-4-methoxy-3'-prenyl, **(4)**: 2',4'-dihydroxy-3,4-(2",2"-dimethylchromeno)-3'-prenyl-dihydrochalcone, **(5)**: Artocarpin, **(6)**: Cycloheterophyllin, **(7)**: 4',5-dihydroxy-6,7-(2,2-dimethylpyrano)-2'-methoxy-8-γ,γ-dimethylallylflavone

Cycloheterophyllin (**6**) exhibited an excellent inhibitory activity against mushroom tyrosinase with the IC_{50} value of 52.5 µg/mL (88.3%). Isobavachalcone **(1)**, 4-hydroxyonchocarpin **(2)** and 2',4'-dihydroxy-3,4-(2",2"-dimethylchromeno)-3'-prenyl-dihydrochalcone **(4)** showed the potential as tyrosinase inhibitor with the IC_{50} value of 218.8 µg/mL (78.5%), 122.5 µg/mL (71.2%) and 124.3 µg/mL (67.8%) respectively. Compound **(6)** possessed 4-substituted resorcinol skeleton (1,3-benzenediol) which proved to be potent tyrosinase inhibitor as described by Shimizu *et al.* and Khatib *et al*. [16, 17]. Interestingly, compound (**6**) showed the presence of polyhydroxyl groups at ring A and ring B which contributed to the significant of tyrosinase inhibitory activity by the inhibition of melanin biosynthesis *via* interruption of the oxidative hydroxylation [17, 18]. Previous study from *A. obtusus* had resulted in the isolation of pyranocycloartobiloxanthone A which also reported to exhibit potent tyrosinase inhibitory activity due to the presence of 4-substituted resorcinol skeleton [19].

Artocarpin (**5**) showed weak tyrosinase inhibitory activity with IC_{50} value of 315.0 µg/mL (63.1%). Even though the structure has a 4-substituted resorcinol skeleton at ring B, but the presence of isoprenoid-substituents and steric hindrance at the C-3 position dramatically decreased the tyrosinase inhibitory activity [16]. Based on the previous study, compound **(5)** gave the IC_{50} value more than 459 µM in tyrosinase inhibitory activity [9]. Arung *et al*. also stated that any compounds with IC_{50} value more than 300 µg/mL did not show potent tyrosinase inhibition activity. This study showed that for flavonoids, not only a 4-substituted resorcinol skeleton but also some additional structural factors such as the presence of polyhydroxyl groups are necessary to reveal the tyrosinase inhibitory activity [16].

■4.0 CONCLUSION

Six crude extracts and seven isolated flavonoids from *A. lowii* were evaluated for their tyrosinase inhibitory activity. The results showed that cycloheterophyllin **(6)** exhibited an excellent inhibitory activity against mushroom tyrosinase comparable to the standard kojic acid with the IC_{50} value of 52.5 µg/mL (88.3%). The tyrosinase inhibitory activity of these flavonoids depends on the number and configuration of phenolic hydroxyl groups in the molecules and influenced by configuration of other substituent.

Acknowledgements

The authors acknowledge Ministry of Higher Education (MOHE) for financial support under Research University Grant number QJI30000.2526.06H55 and the Faculty of Science, Universiti Teknologi Malaysia for the research facilities.

References

[1] Julian, K. C. M., Rachel, C., Penny, S., Rainer, F., Richard, M. Richard M. T. 2005. Plant Derived Pharmaceuticals–The Road Forward. *TRENDS in Plant Science*. 12: 1360–85.
[2] Sasidharan, N. 2004. *Biodiversity Documentation of Kerala. Part:6 Flowering Plants.* Kerala forest Research Institute. Peechi. Thrissur. Kerala. 336–57.
[3] Kochummen, K. M., Go, R. 2000. *Tree Flora of Sabah and Sarawak.* Ampang Press Sdn. Bhd: Kuala Lumpur. 3: 181.
[4] Whitmore, T. C. 1978. *Tree Flora of Malaya.* Longman Malaysia Sdn. Bhd: Kuala Lumpur. 3: 119–67.
[5] Ng, F. S. P. 1992. *Manual of Forest Fruits, Seeds and Seedlings*. Forest Research Institute Malaysia, Kepong: Kuala Lumpur. 2: 451–54.
[6] Nomura, T., Hano, Y., Aida, M. 1998. Isoprenoid-substitutes Flavanoids from *Artocarpus* Plants (Moraceae). *Heterocycles*. 47: 1179–205.
[7] Jamil, S., Sirat, H. M., Jantan, I., Aimi, N., Kitajima, M. A 2008. New Prenylated Dihydrochalcone from the Leaves of *Artocarpus lowii*. *J Nat Med*. 62: 321–24.
[8] Hakim, E. H., Fahriyati, A., Kau, M. S., Achmad, S. A., Makmur, L., Ghisalberti, E. L., *et al.* 1999. Artoindonesianins A and B, Two New

Prenylated Flavones from the Root of *Artocarpus champeden*. *J Nat Prod*. 62: 613–5.

[9] Arung, E. T., Yoshikawa, K., Shimizu, K., Kondo, R. 2010. 3-Prenyl Luteolin, A New Prenylated Flavone with Melanin Biosynthesis Inhibitory Activity from Wood of *Artocarpus heterophyllus. Fitoterapia*. 81: 640–3.

[10] Pedro, M., Ferreira, M. M., Cidade, H., Kijjoa, A., Rocha, E. B., Nascimento, M. S. J. 2005. Artelastin as a Cytotoxic Prenylated Flavone that Disturbs Microtubules and Interferes with DNA Replication in MCF-7 Human Breast Cancer Cells. *Life Science*. 77: 293–311.

[11] Kamkaen, N., Mulsri, N., Treesak, C. 2007. Screening of Some Tropical Vegetables for Anti-tyrosinase Activity. *Thai Pharmac. & Health Science Journal*. 2: 15–9.

[12] Likhitwitayawuid, K., Sritularak, B. 2001. A New Dimericstilbene with Tyrosinase Inhibitory Activity from *Artocarpus gomeizianus*. *J Nat Prod*. 64: 1457–9.

[13] Mbaveng, A. T., Ngameni, B., Kuete, V., Simo, I. K., Ambassa, P. Roy, R., *et al.* 2008. Antimicrobial Activity of the Crude Extracts and Five Flavonoids from the Twigs of *Dorstenia barteri*(moraceae). *J of Ethnopharmacology*.116: 483–9.

[14] Wang, Y. H., Hou, A. J., Lei, C., Chen, D. F., Sun, H. D., Zhao, Q. S., *et al.* 2004. New Isoprenylated Flavones, Artochamins A-E, and Cytotoxic Principle from *Artocarpus chama*. *J Nat Prod*. 67: 757–61.

[15] Zheng, Z. P., Chen, S., Wang, S., Wang, X. C., Cheng, K. W., Wu, J. J., *et al.* 2009. Chemical Components and Tyrosinase Inhibitors from the Twigs of *Artocarpus heterophyllus*. *J Agric Food Chem*. 57: 6649–55.

[16] Shimizu, K., Kondo, R., Sakai, K., Buabarn, S., Diokkunanant, U. A. 2000. Geranylated Chalcone with 5α-reductase Inhibitory Properties from *Artocarpus incisus*. *Phytochemistry*. 54: 737–9.

[17] Khatib, S., Nerya, O., Musa, R., Shmuel, M., Tamir, S., Vaya, J. 2005. Chalcones as Potent Tyrosinase Inhibitors: The Importance of a 2, 4-Substitutetd Resorcinol Moiety. *Bioorganic and Medicinal Chemistry*. 13: 433–41.

[18] Hashim, N., Rahmani, M., Cheng, L. E., Sukari, M. A., Yahayu, M., Amin, M. A., *et al.* 2012. Antioxidant, Antimicrobial and Tyrosinase Inhibitory Activities of Xanthones Isolated from *Artocarpus obtusus* F. M. Jarret. *Molecules*. 17: 6071–82.

[19] Shimizu, K., Kondo, R., Sakai, K. 1999. Inhibition of Tyrosinase by Flavonoids, Stilbenes and Related 4-Substituted Resorcinols: Structure-Activity Investigations. *PlantaMedica*. 66: 11–5.