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Research Article

Chemical Constituents from Stem Bark of *Garcinia prainiana* and Their Bioactivities

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ABSTRACT

Phytochemical investigations on the hexane and methanol extracts of the dried stem barks of *Garcinia prainiana* resulted in the isolation of two triterpenes; 3β-hydroxyeupha-5,22-diene (**1**), 3β-acetoxyeupha-5,22-diene (**2**) as well as five flavonoids, morelloflavone (**3**), *O*-methylfukugetin (**4**), volkensiflavone (**5**), amentoflavone (**6**) and 4‴-methoxyamentoflavone (**7**). Except for (**3**) and (**5**), the rest of the isolated compounds are reported here for the first time from this plant. The structures were established based on spectral analyses. The crudes and compounds were screened for DPPH radical scavenging assay and tyrosinase inhibition activity. The methanol extract and compound (**3**) were found to show high radical scavenging activity with IC_{50} 15.1 and 15.7 μg/mL, respectively. As for tyrosinase inhibition assay, compound (**3**) was found to exhibit the highest activity with IC_{50} 34.0 μg/mL.

Keywords: *Garcinia prainiana*, Guttiferae, Biflavanoids, Antibacterial, Antioxidant, Anti-tyrosinase.

INTRODUCTION

Plant kingdom serves as the most productive source of organic compounds, which many have been used for medicinal purposes. The secondary metabolites from plants with interesting biological activities have led to the drug development and help to maintain human health. Guttiferae family is distributed mainly in tropical regions and comprises about 40 genera and 1200 species[1]. Plants of the genus *Garcinia* (Guttiferae) are widely distributed in tropical Africa, Asia, New Caledonia, and Polynesia[2]. The genus is well known as the sources of variety oxygenated and prenylated polyphenol compounds. Continuous study of chemical constituents as well as biological activities by previous workers have identified many compounds such as xanthones[3-5], biflavanoids[6,7], benzophenones[7,8] and triterpenoids[9,10]. Some of these plants have been reported to exhibit cytotoxicity, antifungal and anti-inflammatory activities[11]. *Garcinia* also is well known as medicinal plants since it was used as expectorant, treatment of lymphatitis, parotitis and struma[11] by Thailand and Indonesia local folks. *Garcinia prainiana* is native to Malaysia and Thailand. This species is a small to medium-sized tree up to 18 m high with a narrow, dense and bushy crown. It produces white latex, locally known as cerapu, cerpu, mencupu and kecupu in Malaysia. Previous phytochemical investigations on twigs and leaves of *G. prainiana* has led to the isolation of friedelin, eupha-8, 24- diene 3-β -ol, stigmasterol, teraxerone, teraxerol[11], and prainianonide, (2*S*)-eriodictyol 7-O-*β*-D-glucuronide, naringenin 7-O-*β*-D-glucuronide, (–)-GB-1a, (+)-morelloflavone and amentoflavone[12]. However, to date, there is no report on the phytochemicals content from the stem bark of *G. prainiana.* Herein, we report the isolation of chemicals constituents from the stem bark of *G. prainiana* and their bioactivities including antioxidant, anti-tyrosinase and antibacterial.

MATERIALS AND METHODS

Plant material

The stem bark of this plant was collected from Agricultural Garden, Kuantan in November 2009. A voucher specimen (MT19) was deposited in the herbarium of Kulliyyah of Pharmacy, International Islamic University, Malaysia.

Isolation procedure

The dried and powdered stem bark of *G. prainiana* (350 g) was extracted by Soxhlet extractor for 18 hours with *n*-hexane (2.5 L) and methanol (2.5 L) successively at room temperature. Concentration of each solvent under reduced pressure afforded a crude hexane extract, GPSH (13 g) and crude methanol extract, GPSM (21 g) as a sticky brown liquid. The hexane extract (8 g) was submitted to vacuum liquid chromatography (VLC) on silica gel using a gradient of hexane-chloroform 90:10, 70:30, 50:50, 30:70, 10:90, chloroform, chloroform-ethyl acetate 90:10, 70:30, 50:50, 30:70 and ethyl acetate, to afford 11 fractions. Fraction 5 (90 mg) was subjected to a silica gel column chromatography using a gradient of hexane-chloroform (98:2, 96:4, 94:6, 90:10, 80:20, 50:50, 30:70) and chloroform, to give seven sub-fractions. Sub-fraction 5 (50 mg) showing two major components was submitted to purification by silica gel column eluted with a gradient

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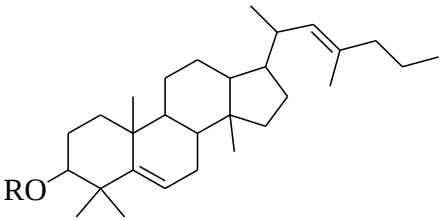

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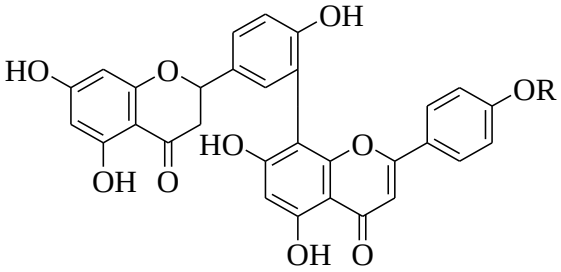
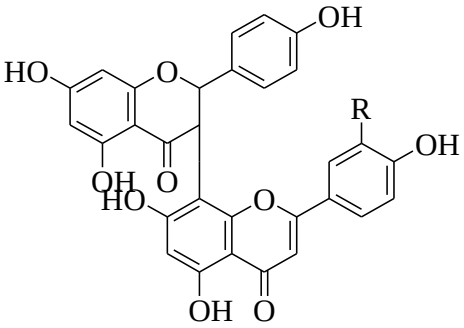
(2) R = Ac



(3) R = H
(4) R = CH_3



(5) R = OH
(6) R = OCH_3
(7) R = H

hexane-chloroform afforded triterpenes; 3β-hydroxyeupha-5,22-diene (**1**, 18 mg) and 3β-acetoxyeupha-5,22-diene (**2**, 20 mg). The methanol extract (10 g) was submitted to vacuum liquid chromatography (VLC) on silica gel using gradient of chloroform-ethyl acetate 80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80, 10:90 and ethyl acetate, furnishing 9 fractions. Fraction 7 (150 mg) was applied to silica gel chromatography column eluting with chloroform–methanol (99:1, 98:2, 97:3, 96:4, 95:5, 94:6, 93:7, 92:8, 91:9, 90:10) led to the isolation of biflavanoids characterized as morelloflavone (**3**, 21 mg), *O*-methylfukugetin[13] (**4**, 15 mg), volkensiflavone[14] (**5**, 13 mg), amentoflavone (**6**, 13 mg), 4'''-methoxyamentoflavone[15,16] (**7**, 10 mg). The identification of all compounds was achieved by UV, IR, MS, ^{1}H, ^{13}C NMR and 2D NMR methods. Their structures were also confirmed by comparing with the previously reported spectral data.

DPPH-radical scavenging assay

The free radical scavenging activity was evaluated using DPPH with modification by using flat bottom 96-well microplates[17]. Briefly, each sample of stock solution (1.0 mg/mL) was diluted with methanol to a final concentration of 1000, 500, 250, 125, 62.5, 31.3, 15.6 and 7.8 µg/mL. 50 µM DPPH methanolic solution (1 mg/50 mL) was prepared and 100 µL of this solution was mixed with 100 µL to produce the sample solution. The plates were shaken and allowed to stand at room temperature for 30 min in a dark room. The absorbance of the mixtures was measured at 517 nm using an ELISA Reader (EPOCH, Bio-Tek Instrumentation). A control was prepared without either sample or standard and measured

Anti-tyrosinase assay

Tyrosinase-inhibition activity of the crude extract of *G. prainiana* stem bark and isolated compounds were performed by using L-DOPA as a substrate with slight modification[18]. All extracts or compounds were first dissolved in DMSO at 1.0 mg/ml and then diluted to different concentration using DMSO. Each sample (40 µl) was diluted with 80 µl of 0.1 M sodium phosphate (pH 6.8) and 40 µl of L-DOPA solution (with 0.1 M sodium phosphate, pH 6.8). The samples was incubated for 10 minutes at 25 ºC. Then, 40 µl of mushroom tyrosinase solution (100 units) was added in the reaction and incubated for 20 minutes at 25 ºC. The dopachrome formation was measured using UV-Vis spectrophotometer at 475 nm. The percentage of tyrosinase-inhibition activity was calculated by using this formula: % Tyrosinase inhibition = [A- (B-C)]/A * 100 Where, A = absorbance of control treatment; without test sample, B = absorbance of test sample treatment; with tyrosinase, C = absorbance of test sample blank treatment; without tyrosinase. The extent of inhibition by the addition of samples is expressed as the percentage necessary for 50% inhibition (IC_{50}).

Antibacterial assay

Disc diffusion assay

The antibacterial activity of the extracts and compounds **4** and **5** were screened by the disc agar diffusion method[19]. The samples were diluted with DMSO. Streptomycin sulfate was used as the reference antibacterial compound; 10 µL of each of the samples and reference were used. A negative control experiment using the solvent, DMSO, was also included. The controls and samples were placed on the surface of agar plates at well-spaced intervals and

Table 1: DPPH-radical scavenging activity of tested samples.

	DPPH Scavenging Capacity	
Samples	S (%)[a]	SC_{50} (µg/mL)[b]
GPSH	20.1±0.6	480.2±0.30
GPSM	88.5±0.5	15.1±0.24
(3)	78.8±0.4	25.5±0.20
(4)	67.8±1.7	100.1±0.21
(5)	87.3±0.4	15.7±0.20
(6)	58.5±1.7	110.1±0.21
(7)	84.5±1.7	20.1±0.21
Vitamin C[c]	96.1±0.5	11.9±0.22

[a] Percent scavenging at 125 µg/mL as mean ± SD of triplicate experiments. [b] SC_{50} is defined as the concentration sufficient to obtain 50% of the maximum scavenging capacity. [c] Standard control

Table 2: Tyrosinase inhibition activity of tested samples.

	Inhibition activity	
Samples	Inhibition (%)[a]	IC_{50} (µg/mL)[b]
GPSH	69.9±0.7	48.2±0.20
GPSM	81.2±0.2	42.0±0.20
(3)	83.1±0.5	38.0±0.20
(4)	81.7±1.7	43.1±0.24
(5)	86.8±0.4	34.0±0.22
(6)	80.9±1.5	40.1±0.23
(7)	85.6±1.5	36.1±0.23
Kojic acid[c]	88.2±0.5	12.0±0.20

[a] Percent inhibition at 100 µg/mL as mean ± SD of triplicate experiments. [b] IC_{50} is defined as the concentration sufficient to obtain 50% of the inhibition activity. [c] Standard control

incubated at 37°C for 24 h. The activity was expressed in mm diameter of the inhibition zones including 6 mm disc diameter.

Minimum inhibitory concentration

The MIC was tested by the broth micro dilution method and performed in sterile round bottom 96-well microplates[20]. The samples (3.6 mg) were diluted in DMSO to give 1800 µg/mL stock solution. The stock solution samples (100 µL) were added to each microplate well in rows A and B. Sterile broth (100 µL) was added to each well from row B to H. Then, the mixture of samples and broth (100 µL) from row B was transferred to each well to obtain a twofold serial dilution of the stock samples (concentration of 1800, 900, 450, 225, 112.5, 56.25, 28.13 and 14.07µg/mL). An inoculum of bacteria (100 µL) was added to each well. A number of wells were reserved in each plate for the controls. Streptomycin sulfate was used as the positive control and the plates were incubated at 37°C for 24 h. Bacterial growth was identified by the presence of turbidity and a pellet at the bottom of the well.

RESULTS AND DISCUSSION

A total of seven compounds were isolated from the stem bark of *G. prainiana* through consecutive column chromatographic separations. On the basis of chemical and physiochemical properties as well as comparison of published data, the compounds were identified as 3β-hydroxyeupha-5,22-diene (**1**), 3β-acetoxyeupha-5,22-diene (**2**), morelloflavone (**3**), *O*-methylfukugetin (**4**), volkensiflavone (**5**), amentoflavone (**6**) and 4"'-methoxyamentoflavone (**7**). All compounds except (**3**) and (**5**) were reported for the first time from this species. All crude extracts and compounds were evaluated for their antioxidant activity by DPPH radical scavenging assay. The scavenging percentage (S) and scavenging concentration (SC) to obtain 50% of the maximum scavenging capacity of DPPH by the tested extracts and the most abundant compounds (**3-7**) are presented in Table 1. The reduction of DPPH radicals can be observed by its absorbance decrement at 517 nm and was visually noticeable as a discoloration from purple to yellow[21]. The methanol crude extract showed high ability to act as a radical scavenger with the highest SC_{50} value (50 µg/mL) compared to the hexane extract. Meanwhile, all biflavonoids exhibited antioxidant activity with the SC_{50} ranging from 110 – 15.7 µg/mL. Morelloflavone (**3**) exhibited the strongest antioxidant activity (SC_{50} 15.7 µg/mL) among all biflavonoids, comparable to the standard vitamin C (SC_{50} 11.9 µg/mL). The large number of phenolic groups contributed to its strong antioxidant activity and act as hydrogen donor to scavenge the DPPH

Table 3: Antibacterial activity of tested samples

Bacteria		GPSH	GPSM	1	2	3	4	5	6	7	SS
B. subtilis	DD	7.0±0.0	7.8±1.5	7.0±0.0	7.2±0.5	7.5±0.7	7.2±0.1	7.0±0.0	7.2±0.1	7.0±0.0	17.5±0.2
	MIC	900	450	900	900	900	900	900	900	900	14.05
S. aereus	DD	7.3±0.6	7.0±0.0	7.2±0.1	7.50±0.6	7.0±0.0	7.8±0.9	7.8±0.9	7.8±0.6	7.5±0.9	17.8±0.2
	MIC	900	900	900	900	900	900	900	900	900	14.05
E. coli	DD	7.5±0.5	7.5±0.4	7.5±0.4	7.2±0.4	7.7±0.9	7.8±0.5	7.8±0.5	7.8±0.2	7.8±0.5	17.6±0.2
	MIC	900	450	900	900	900	900	900	900	900	14.05
P. aeruginosa	DD	7.0±0.0	7.6±0.1	7.0±0.0	7.5±0.1	8.5±1.6	8.0±0.2	7.5±0.6	7.5±0.3	7.5±0.2	17.3±0.2
	MIC	900	450	900	900	900	900	900	900	900	14.05

[a]Data represent mean ±SD of four independent experiments.DD – Disc diffusion (zone of inhibition including the diameter of disc: 6 mm); MIC –Minimum inhibitory concentration (µg/mL); SS – streptomycin sulfate.

radical. For the tyrosinase inhibitory activity, each extracts and compounds were assayed employing L-DOPA as the substrate. The inhibition percentage and inhibition concentration to obtain 50% of the inhibition activity (IC_{50}) of tyrosinase by the tested extracts and compounds were presented in Table 2. The methanol extract showed potent anti-tyrosinase activity compared to the hexane extract. The biflavanoids (**3-7**) showed strong anti-tyrosinase inhibitory activity but lower than kojic acid. Morelloflavone (**3**) showed the highest inhibitory activity than other biflavanoids suggesting the presence of the catechol moiety in the ring B enhanced the tyrosinase inhibitory activity[22]. In addition, biflavanoids (**4**) and (**6**) showed weaker activity compared to other compounds. It could be deduced that the methoxylation of the hydroxyl group reduced the anti-tyrosinase activity[23]. The antibacterial activity (Table 3) of the extracts and compounds (**3-7**) were tested against Gram-positive (*Bacillus subtilis* and *Staphylococcus aureus*) and Gram-negative bacteria (*Escheria coli* and *Pseudomonas aeruginosa*). The activity was determined qualitatively using the disc diffusion method and quantitatively using minimum inhibitory concentration (MIC). The inhibition zones in the disc diffusion method were in the range of 6.5-10.5 mm. The MIC results indicated that the methanol crude extract weakly inhibited the growth of all the tested bacteria. The methanol crude extract gave the lowest MIC value (450 µg/mL) on the Gram- negative bacteria, *P. aeruginosa* and *E. coli* and Gram-positive bacteria, *B. subtilis*. This activity may be due to the presence of biflavanoids and related metabolites that have been implicated for their antibacterial activities[24]. From the presence study, it can be concluded that *G. prainiana* can serve as medicinal plants since the extract and the chemical constituents especially morelloflavone (**3**) shows significant antoxidant and anti-tyrosinase activity.

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REFERENCES

1. Santa-Cecília FV, Vilela FC, da Rocha CQ, Dias DF, Cavalcante GP, Freitas LS, dos Santos, MH, Giusti-Paiva A. Anti-inflammatory and antinociceptive effects of *Garcinia brasiliensis*. Journal of Ethnopharmacology. 2011; 133: 467–73.
2. Ampofo SA, Waterman PG. Xanthones from three *Garcinia* species. Phytochemistry. 1986; *25*(10): 2351-2355.
3. Rukachaisirikul V, Kaewnok W, Koysomboon S, Phongpaichit S, Taylor WC. Caged-Tetraprenylated Xanthones from *Garcinia scortechinii*. Tetrahedron. 2000; 56(43): 8539.
4. Rukachaisirikul V, Painuphong P, Sukpondma Y, Koysomboon S, Sawangchote P, Taylor WC. Xanthone and Sesquiterpene Derivatives from the Fruits of *Garcinia scortechinii.* Journal of Natural Product, 2003; 66(7): 933.
5. Rukachaisirikul V, Naklue W, Phongpaichit S, Towatana NH, Maneenoon K. Phloroglucinols, depsidones and xanthones from the twigs of *Garcinia parvifolia*. Tetrahedron, 2006;62(36): 8578.
6. Parveen M, Ilyas M, Mushfiq M, Busudan OA, Muhaisen HMH. A new biflavonoid from leaves of *Garcinia nervosa*. Natural Product Research. 2004; 18(3): 269.
7. Yang H, Figueroa M, To S, Baggett S, Jiang B, Basile MJ, Weinstein IB, Kennelly EJ. Benzophenones and biflavonoids from *Garcinia livingstonei* fruits. *Journal of Agricultural* Food Chemistry. 2010; 58(8): 4749.
8. Jantan I, Saputri, FC. Benzophenones and xanthones from *Garcinia cantleyana var. cantleyana* and their inhibitory activities on human low-density lipoprotein oxidation and platelet aggregation. Phytochemistry. 2012; 80: 58-63.
9. Rukachaisirikul V, Pailee P, Hiranrat A, Tuchinda P, Yoosook C, Kasisit J, Taylor WC, Reutrakul V. Anti-HIV-1 Protostane Triterpenes and Digeranylbenzophenone from Trunk Bark and Stems of *Garcinia speciosa*. Planta Medica. 2003; 69(12): 1141.
10. Rukachaisirikul V, Saelim S, Karnsomchoke P, Phongpaichit S. Friedolanostanes and lanostanes from the leaves of *Garcinia hombroniana*. Journal of Natural Product. 2005; 68(8): 1222.
11. Mawa S, Said IM. Chemical constituents of *Garcinia prainiana*. Sains Malaysiana. 2012; 41: 585–590.
12. Klaiklay S, Sukpondma Y., Rukachaisirikul V, Phongpaichit S. Flavanone glucuronides from the leaves of *Garcinia prainiana*. Phytochemistry. 2013; 85: 161.
13. Waterman PG, Crichton EG. Xanthones and biflavonoids from *Garcinia densivenia* stem bark. Phytochemistry. 1980; 19: 2733.
14. Hui Y, Mario F, Satoshi T, Scott B, Bei J, Margaret JB, Bernard W1, Edward JK. Benzhophenone and Biflavonoids from *Garcinia livingstonei*. Journal of Agricultural Food Chemistry, 2010; 58: 4749.
15. Kaikabo AA, Samuel BB, Eloff JN. Isolation and activity of two antibacterial Biflavonoids from leaf extracts of *Garcinia livingstonei* (Clusiaceae). Natural Product Communication. 2009; 4(10): 1363.
16. Kaikaboo AA, Eloff JN. Antibacterial activity of two biflavonoids from *Garcinia livingstonei* leaves against Mycobacterium smegmatis. Journal of Ethnopharmacology. 2011; 138: 253.
17. Hashim NM, Rahmani M, Ee GCL, Sukari MA, Yahayu M, Amin MAM, Ali AM, Go R. Antioxidant, antimicrobial and tyrosinase inhibitory activities of xanthones isolated from *Artocarpus obtusus* F.M. Jarrett. Molecules. 2012; 17: 6071-6082.
18. Kobo I, Kinst-Hori I, Chaudri SK, Kubo Y, Sanchez T, Ogura T. Flavanols from Heterotheca inuloide: tyrosinase inhibitory activity and structural criteria. Bioorganic Medicinal Chemistry. 2000; 8: 1749-1755.

19. Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolken RH. Manual of clinical microbiology. 1999; 7th ed. ASM, Washington, DC, USA.
20. Gulluce M, Sokmen M, Sahin M, Sokmen A, Adiguzel A, Ozel H. Biological activities of essential oil and methanolic extract of *Micromeria fruticosa* (L.) Druce ssp. *serpyllifolia* (Bieb) PH Davis plants from the eastern Anatolia region of Turkey. Journal of Science Food Agriculture. 2004; 84: 735-741.
21. Zhao Q, Zhao Y, Wang K. Antinociceptive and free radical scavenging activities of alkaloids isolated from *Lindera angustifoli*a Chen. Journal of Ethnopharmacology. 2006; 106: 408-413.
22. Khatib S, Nerya O, Musa R, Shmuel M, Tamir S, Vaya J. Chalcones as potent tyrosinase inhibitors: the importance of a 2,4-substituted resorcinol moiety. Biiorganic & Medicinal Chemistry. 2005; 13: 433-441.
23. Saewan N, Koysomboon K, Chantrapromm K. Anti-tyrosinase and anti-cancer activities of flavonoids from *Blumea balsamifera* DC. Journal of Medicinal Plant Research. 2011; 5: 1018-1025.
24. Syamsudin, Kumala S, Sutaryo B. Screening of some extracts from *Garcinia parvifolia* Miq. (Guttiferae) for antiplasmodial, antioxidant, cytotoxic and antibacterial activities. Asian Journal of Plant Sciences. 2007; 6: 972-976.