SHORT REPORT

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Rec. Nat. Prod. 8:3 (2014) 312-316

records of natural products

Phytochemicals from the Bark of *Garcinia hombroniana* and Their Biological Activities

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(Received December 14, 2013; Revised February 14, 2014; Accepted March 12, 2014)

Abstract: The dichloromethane and ethyl acetate extracts from the bark of *Garcinia hombroniana* yielded lupeol (**1**), lupeol acetate (**2**), 3*β*-acetoxy- lup-12,20(29)-diene (**3**), *β*-sitosterol (**4**), 22-dehydroclerosterol (**5**), mangiferolic acid (**6**), ursolic acid (**7**), betulin (**8**), betulinic acid (**9**), 1,3,6-trihydroxy-7-methoxy-2,8-(3-methyl-2-butenyl)xanthone (**10**), leucodin (**11**), *p*-hydroxycinnamate (**12**), *p*-hydroxybenzoic acid (**13**) and stearic acid (**14**). Compounds **10**, **12** and **13** exhibited significant antioxidant activities in DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,2-azinobis-3-ethylbenzothiazoline-6-sulfonic acid) and FRAP (ferric ion reducing antioxidant power) assays. Antibacterial studies showed strong inhibitory effects for compound **11** while other compounds (**1**-**9** and **14**) were either inactive or moderately active for the bacterial strains investigated. This study presents the first report on the isolation and biological activities of the chemical constituents from the bark of *G. hombroniana*. The compounds **3**, **5**-**12** and **14** are reported for the first time from this source.

Keywords: *Garcinia hombroniana*; bark; triterpenoids; antioxidant activities; antibacterial activities.

1. Plant Material

Garcinia hombroniana Pierre is native to Peninsular Malaysia and can be found in the coastal regions, from the lowland forests near the sea to the lower mountain forests and the highlands [1]. In Malaysia, *G. hombroniana* is mostly cultivated for ornamental purposes but its fruit is also used for making juices and jellies [2]. The roots and leaves decoction is traditionally used to relieve itching and as a protective medicine after child birth [3, 4]. This paper describes the phytochemical screening of crude extracts, isolation and characterization of the pure chemical constituents, and evaluation of their antioxidant and antibacterial activities. The plant (*G. hombroniana*) was collected from Penang Botanical Garden, Penang, Malaysia. A voucher specimen PBGK12 has been deposited at the Herbarium of this garden.

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The article was published by Academy of Chemistry of Globe Publications
www.acgpubs.org/RNP © Published 05/01/2014 EISSN: 1307-6167

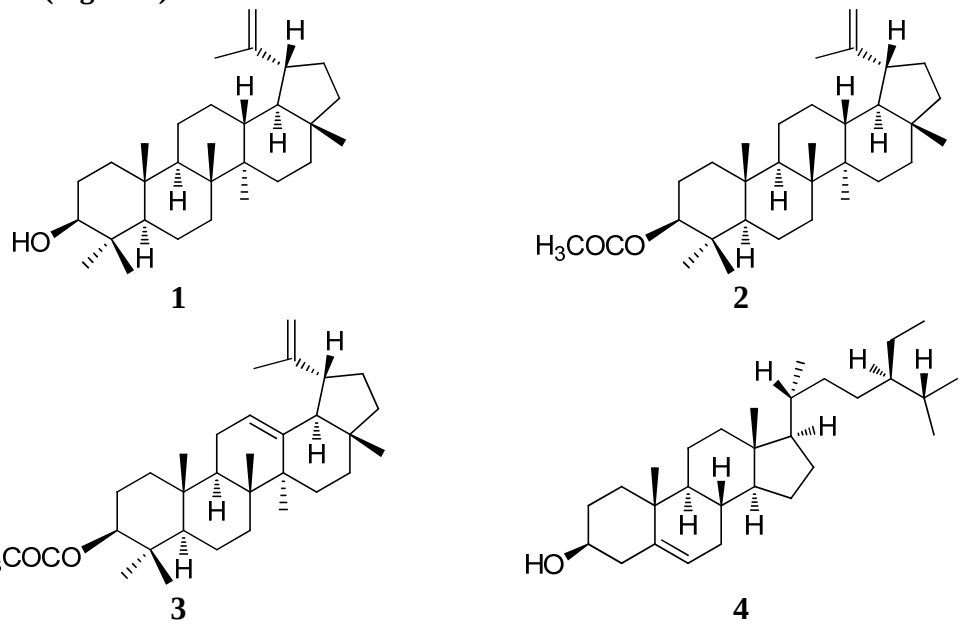
2. Previous Studies

Previous studies on the twigs, pericarp and leaves of *G. hombroniana* yielded triterpenoids, xanthones, benzophenones and flavonoids [5-7]. The pharmacological studies of some phenolic and triterpenoidal constituents isolated from the twigs, have shown copper-mediated LDL antioxidation, antiplatelet aggregation and antibacterial activities against MSSA (methicillin-susceptible *Staphylococcus aureus*) and MRSA (methicillin-resistive *Staphylococcus aureus*) [8].

3. Present Study

Air-dried 5.2 kg ground bark of *G. hombroniana* was successively extracted using Soxhlet extractor with *n*-hexane (*n*-C_6H_{14}), dichloromethane (DCM), chloroform ($CHCl_3$), ethyl acetate (EtOAc) and methanol (MeOH). The aqueous extract (aqueous) was obtained by the partition of methanol extract with distilled water in separatory funnel. Also a separate aqueous extract (aqueous-di) was obtained by the direct maceration of the plant material in hot distilled water for 24 hours. The different extracts were screened for the detection of various secondary metabolites such as sterols, terpenoids, phenolics, flavonoids and reducing sugars and the results were as presented in **Table S1** given in supporting information.

The dichloromethane and ethyl acetate extracts, upon isolation through repeated silica gel column chromatography (CC) yielded 14 known compounds belonging to different classes of phytochemicals. The dichloromethane extract gave triterpenoids as major constituents while the moderately polar fractions of ethyl acetate extract afforded some simple phenolic compounds along with triterpenoids, already isolated from dichloromethane extract. These constituents were identified as: lupeol (**1**) [9], lupeol acetate (**2**) [10], 3*β*-acetoxy-lup-12,20(29)-diene (**3**) [11], *β*-sitosterol (**4**) [12], 22-dehydroclerosterol (**5**) [13], mangiferolic acid (**6**) [14], ursolic acid (**7**) [15], betulin (**8**) [16], betulinic acid (**9**) [17], 1,3,6-trihydroxy-7-methoxy-2,8-(3-methyl-2-butenyl)xanthone (**10**) [18], leucodin (**11**) [19], *p*-hydroxycinnamate (**12**), *p*-hydroxybenzoic acid (**13**) and stearic acid (**14**) by comparison of their 1D and 2D NMR spectroscopic data and EI mass spectrometry with those reported in the literature **(Figure 1)**.



1 **2** **3** **4**

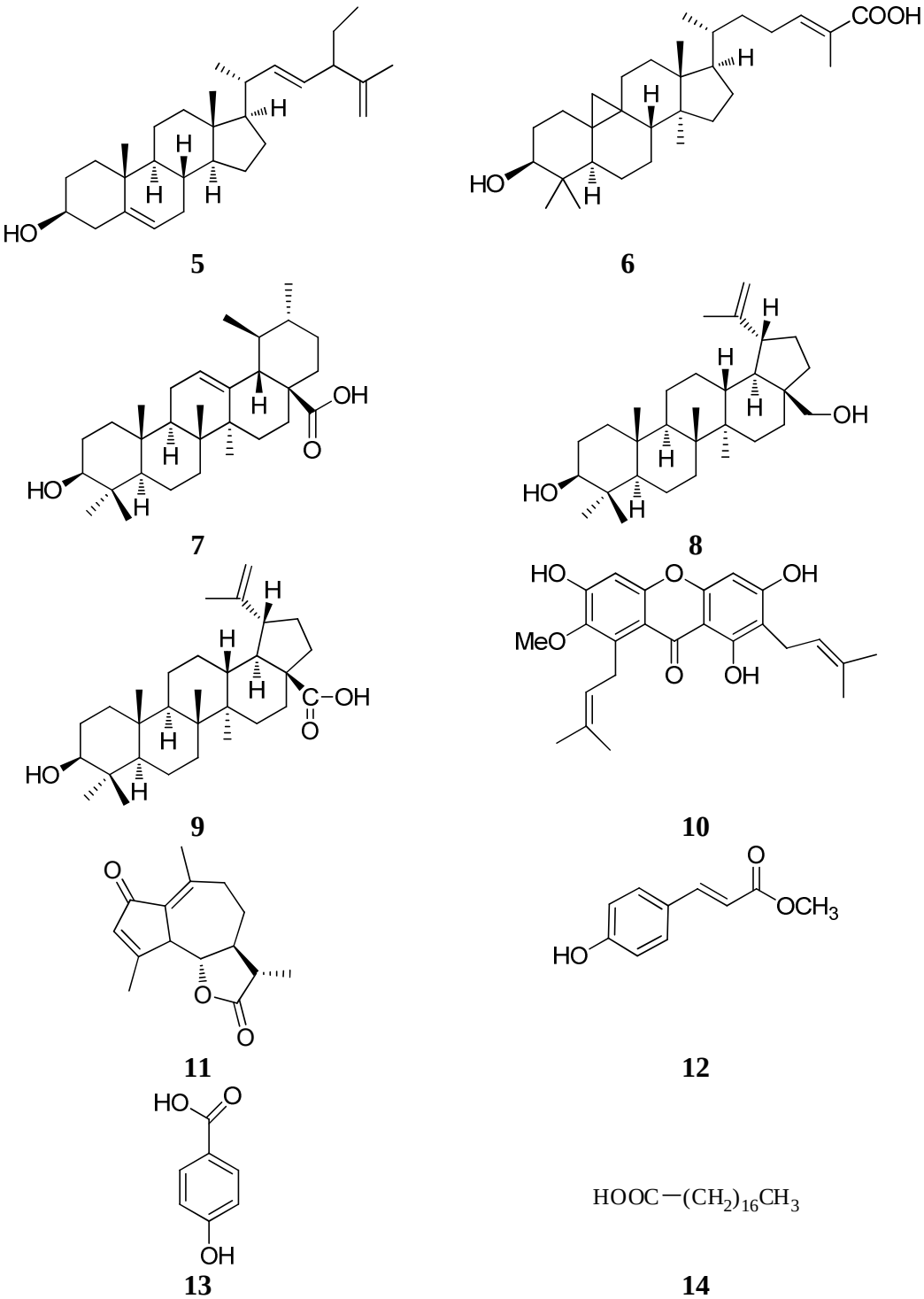


Figure 1. Chemical structures of compounds **1-14**

Antioxidant Activities: The results of antioxidant activities from all three assays indicated that only compounds **10**, **12** and **13** are capable of inhibiting DPPH and ABTS free radicals and also reducing the ferric ions. Compound **10** exhibited strong antioxidant activities (IC_{50} 2.43 µM in DDPH, 2.10 µM in ABTS and 478.5 µM TE in FRAP) followed by compounds **13** (IC_{50} 7.42 µM in DDPH, 6.99 µM in ABTS and 239.3 µM TE in FRAP) and **12 (**IC_{50} 4.98 µM in DDPH, 4.31 µM in ABTS and 285.2 µM TE in FRAP**)**. Compound **10**, in antioxidant activities was stronger even than the positive controls (trolox, gallic acid and ascorbic acid). In DPPH assay, compound **13** showed stronger activities than

the standard gallic acid while in ABTS assay it was slightly lower. The rest of the compounds (**1-9**, **11** and **14**) did not show any antioxidant activity. The detailed results of antioxidant activities are shown in **Table 1**.

Antibacterial Activities: In antibacterial assay, compound **11** was found to be the most active against Gram positive bacteria (*S. aureus* and *B. Subtilis*), with inhibition of MIC value of 31.25 µM, followed by compounds **7** (MIC 62.5 µM) and **10** (MIC 250.0 µM). Compound **7** also exhibited the strongest inhibition against *P. aeruginosa*; a Gram negative bacterium with the MIC value of 15.65 µM. The overall results of the disc diffusion and minimum inhibitory assays are shown in **Table 2**.
The present study reported the isolation of various chemical constituents identified by spectroscopic techniques for the first time from the bark of *G. hombroniana*. The investigated constituents were analyzed for antioxidant and antibacterial activities. This biochemical investigation study of the isolated compounds justifies the traditional uses of *G. hombroniana* against infections after child birth and itching problems.

Table 1. Antioxidant Activities of the Compounds Isolated from *G. hombroniana*

Compounds	DPPH assay (IC_{50} in µM)	ABTS assay (IC_{50} in µM)	FRAP assay µM TE
10	2.43±0.21[a]	2.10±0.40[a]	478.5±5.19[c]
12	7.42±0.54[d]	6.99±0.38[d]	239.3±3.14[a]
13	4.98±0.68[b]	4.31±0.31[c]	285.2±3.14[b]
Trolox	22.4±0.81[e]	11.7±0.19[e]	-
Gallic acid	6.76±0.05[c]	3.12±0.09[b]	-
Ascorbic acid	4.39±0.95[b]	4.06±0.09[c]	-

Results are mean values of 3 replicates ± SD
The mean difference in the antioxidant activities is statistically significant ($p<0.05$)

Table 2. Antibacterial Activities of the Compounds Isolated from *G. hombroniana*

Compounds	Disk Diffusion (mm)				Minimum inhibitory concentration (MIC) µM			
	S. aureus	*B. subtilis*	*P. aeruginosa*	*E. coli*	*S. aureus*	*B. subtilis*	*P. aeruginosa*	*E. coli*
7	19.5	18.0	20.0	8.5	62.5	62.5	15.65	>500
10	13.0	14.5	-	-	250	250	>500	>500
11	15.5	17.3	-	-	31.25	31.25	250	250
12	15.0	16.2	-	-	>500	>500	>2000	>2000
Vancomycin*	16.0	26.0	-	12.0	31.25	31.25	250	250
Gentamicin*	21.0	22.0	19.0	24.5	15.65	15.65	31.25	7.81

Results are mean values of triplicate assays
- = no inhibition observed (6 mm); * = positive control

Acknowledgments

Authors are thankful to the Universiti Sains Malaysia (USM) for providing research grants RU1001/PKIMIA/811129 and RU1001/PKIMIA/811050). One of the authors (Nargis Jamila) is also thankful to TWAS and USM for providing TWAS-USM PG fellowship. The authors also thank to Mr. Zahari Othman for NMR measurements.

Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/RNP

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