Marmara Pharmaceutical Journal 21/4: 889-897, 2017
DOI: 10.12991/mpj.2017.27

RESEARCH ARTICLE

# Biflavonoids from the leaves and stem bark of *Garcinia griffithii* and their biological activities

Wan Mohd Nuzul Hakimi Wan SALLEH, Nik Shazwani Afifah Nik SAZALI, Farediah AHMAD, Muhammad TAHER

**ABSTRACT**

The phytochemical and biological activity of the leaves and stem barks of *Garcinia griffithii* have been investigated. Extraction and chromatographic purification of the leaves and stem barks extracts have successfully afforded five biflavonoids namely amento-4"-methylether **(1)**, 3,8"-binaringenin **(2)**, morelloflavone **(3)**, 3,8"-binaringenin-7"-*O*-glucoside **(4)** and morelloflavone-7"-*O*-glucoside **(5)**, together with squalene **(6)**, canophyllol **(7)**, friedelin **(8)** and β-amyrin **(9)** which were characterized spectroscopically. All extracts and phytochemicals were tested for antioxidant, antityrosinase and antibacterial activities. The antioxidant assay on DPPH radical scavenging showed that the *n*-hexane extract of the stem barks had the highest radical scavenging activity with $IC_{50}$ value of 96.4 μg/mL, while compound **(3)** was found to be the strongest antioxidant compound with $IC_{50}$ value of 57.6 μg/mL. The methanol extract of the leaves showed the highest total phenolic content with 444.1 mg/g of gallic acid equivalent (GAE/L) and 423.1 mg/g of cathechin equivalent (CE/L). The extracts and all compounds were found to have weak anti-tyrosinase activity. The antimicrobial assays of all the extracts were carried out by minimum inhibition concentration (MIC) and minimum bactericidal concentration (MBC). The methanol extract of the leaves showed the most significant antimicrobial activity towards *E. faecalis* and *K. pneumoniae* with MIC and MBC value ranged between 225-450 μg/mL compared to the other extracts.

**Key words:** *Garcinia griffithii* ; antioxidant ; antityrosinase ; antibacterial

## 1. Introduction

Plants of Clusiaceae family have been the subject of a great effort in the isolation of secondary metabolites which characterize the family as an abundant source of phenolic compounds such as xanthones, benzophenones, flavonoids, antraquinones, anthrone derivatives and coumarins. Triterpenoids have been reported as the emerging group of secondary metabolites of this family which include those of oleananes, lupanes, friedelanes, lanostanes and friedolanostanes [1]. In addition, Bennett and Lee [2] also reported that xanthones or related benzophenones have been found in the entire major and several minor genera within the family. A review by Waterman and Hussain [3] on African *Garcinia* reported that the genus is a significant source of xanthones, benzophenones and biflavonoids.

The genus *Garcinia* is the biggest genus with the common village fruit-trees such as *G. atroviridis* (*asam gelugor*), *G. cowa* (*kandis*) and *G. prainiana* (*kechupu*). The trees are small to medium, rarely taller than 30 m; therefore the trees are almost completely confined to the interior of the forest,

Wan Mohd Nuzul Hakimi Wan Salleh
*Department of Chemistry, Faculty of Science and Mathematics, Universiti Pendidikan Sultan Idris (UPSI), 35900 Tanjong Malim, Perak, Malaysia*

Nik Shazwani Afifah Nik Sazali, Farediah Ahmad
*Department of Chemistry, Faculty of Science, Universiti Teknologi Malaysia (UTM), 81310 Johor Bahru, Johor, Malaysia*

Muhammad Taher
*Kulliyah of Pharmacy, International Islamic University Malaysia, Jalan Sultan Ahmad Shah, Bandar Indera Mahkota, 25200 Kuantan, Pahang, Malaysia*

**Corresponding Author:**
Wan Mohd Nuzul Hakimi Wan Salleh
*e-mail: wmnhakimi@fsmt.upsi.edu.my*

**Submitted / Gönderilme:** 10.05.2017 **Revised / Düzeltme:** 20.07.2017
**Accepted / Kabul:** 22.07.2017

**How to cite this article :** Salleh WMNHW, Sazali NSAN, Ahmad F, Taher M. Bioflavonoids from the leaves and stem bark of *Garcinia griffithii* and their biological activities. Marmara Pharm J 2017; 21 (4): 889-897

in shade. Many species of *Garcinia* have very similar leaves but differ in flower and fruit characters. The fruit hull of *G. mangostana* has found many uses in traditional medicine such as for healing skin infections and wounds in Thai folk medicine [4-5].

Species of *G. griffithii* (apple-*kandis* or *kandis gajah*) is a small to medium tree that may reach 23 m tall and conspicuous from the large leaves and fruits. Its latex is yellowish white and the inner bark with opaque yellow exudate. The sapwood and heartwood cannot be distinct and in dark red-brown colour. The leaves have very large blade with size from 15×7 to 28×16 cm, broady elliptic, strongly ribbed and pointed. The edges incurved and has base rounded. The young leaves are pink and the drying leaves are blackish green with thin-texture. The flowers mainly in short woody on the twigs behind the leaves and have four sepals and petals with yellow flushed red colours at the base. The fruits are characteristically globose, faintly ribbed, subsessile, fattened at the top, clustered on the branched, edible and turning brownish yellow with watery acid flesh like green apple. The stigma is usually sunken entirely, flat or slightly convex. The species is common in Peninsular Malaysia at the low land forest [4-6].

To date, there are three reports on the phytochemicals of *G. griffithii* collected from Singapore (2005) [7] and Indonesia (2006, 2009) [8-9]. However, the bioactivities of the plants are not studied thoroughly. Although the climate and ecology of Singapore and Indonesia are similar with Malaysia, this study is still being continued and is hoped to get variations of chemical constituents with interesting bioactivities. The data obtained will be utilized for future research on the chemical markers of *Garcinia* species from Malaysia. Herein, we report the biflavonoids and other constituents, together with their biological activities from the leaves and stem barks of *G. grafithii*. The structure of the isolated compounds was elucidated on the basis of spectral analysis and comparison with published data.

## 2. Results and Discussion

In continuation of our research on the medicinal plants from Malaysian flora, we have performed a phytochemical investigation on the stem bark of a Malaysian Guttiferae, *G. griffithii* which has led to the isolation of nine compounds (Figure 1), characterised as five biflavonoids namely amento-4"-methylether **(1)**, 3,8"-binaringenin **(2)**, morelloflavone **(3)**, 3,8"-binaringenin-7"-*O*-glucoside **(4)** and morelloflavone-7"-*O*-glucoside **(5)**, together with squalene **(6)**, canophyllol **(7)**, friedelin **(8)** and β-amyrin **(9)**. These metabolites were identified by analysing their spectroscopic data and comparing them with the literature data. Compounds **(3)** and **(4)** have been isolated previously from *G. travancoria* [10], while compound **(2)** from *G. atroviridis* [11]. Besides, compound **(5)** has been reported previously from *G. cowa* [12]. There are several reports on

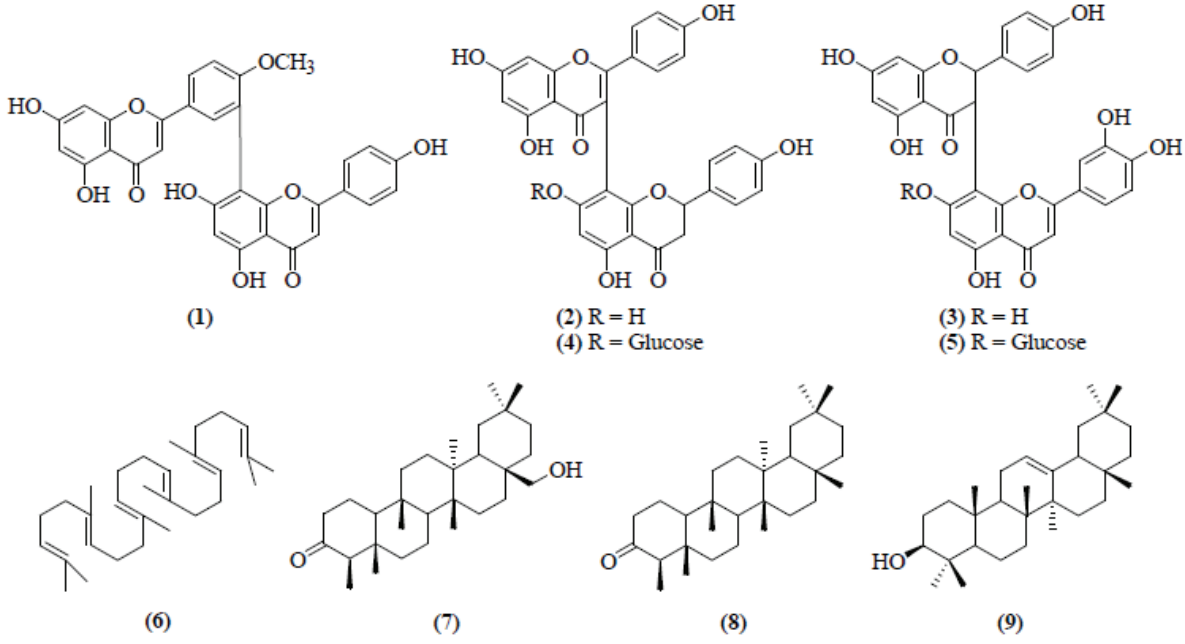


**Figure 1.** Chemical structures of compounds isolated from *G. griffithii*

biflavonoids isolated from the *Garcinia* species, such as *G. hombroniana* [13], *G. bakeriana* [14], *G. subelliptica* [15], *G. brasiliensis* [16], *G. livingstonei* [17], *G. gardneriana* [18], *G. kola* [19], *G. multiflora* [20], *G. thwaitesii* [21], *G. densivenia* [22], *G. terpnophylla* and *G. echinocarpa* [23].

The polyisoprenylated benzophenones cambogin or isoxanthochymol, and guttiferone I, as well as the xanthones 1,7-dihydroxyxanthone, 1,3,6,7- tetrahydroxyxanthone, 1,3,5,6-tetrahydroxyxanthone, 1,5-dihydroxy- 3,6-dimethoxy- 2,7-diprenylxanthone, 1,6-dihydroxyxanthone and a bixanthone, griffipavixanthone, have been isolated and identified from *G. griffithii* [8, 9]. The current phytochemical study provides different skeletons of metabolites as compared to the previous studies. Elfita et al. [21] have reported the significant antiplasmodial and antiprotozoal activity of 1,5-dihydroxy-3,6-dimethoxy-2,7-diprenylxanthone ($IC_{50}$ of 7.25 µM) and isoxanthochymol [$IC_{50}$ values of 2.65 µM against *Trypanosoma cruzi*, 1.91 µM (*Trypanosoma brucei*), 2.03 µM (*Leishmania infantum*), and 4.47 µM (*Plasmodium falciparum*)], respectively. On the other hand, griffipavixanthone has shown high *in vitro* cytotoxity against P388 (leukemia), LL/2 (cellosaurus) and Wehi 164 (murine fibrosarcoma) cell line with $ED_{50}$ values 3.4, 6.8 and 4.6 µg/mL, respectively [8].

The antioxidant activities of the extracts and isolated compounds were tested by DPPH radical scavenging and total penolic contents of the extracts were determined. The results are shown in Table 1. The total phenolic content was expressed as mg GAE per gram of extract using the standard equation of gallic acid ($y = 0.001x + 0.013$, $r^2 = 0.999$) and mg CE per gram using the standard equation of catechin ($y = 0.001x + 0.034$, $r^2 = 0.994$) as standard equations. The MeOH extract of leaves recorded the highest total phenolic content with 444.1 ± 6.6 mg/g of GAE/L and 423.1 ± 6.6 mg/g of CE/L. It has been proven from this study that the MeOH extract which contained biflavonoids contributed to the highest phenolic content. The presence of non-polar compounds in the *n*-hexane extract of the leaves yielded the lowest total phenolic content.

The antioxidant activities of *G. griffithii* extracts and isolated biflavonoids were assessed based on the DPPH radical scavenging activity. The *n*-hexane extract of stem barks was found to show the highest free radical scavenging activity ($IC_{50}$ of 96.4 ± 2.6 µg/mL) compared to other extracts. As for the test compound, morelloflavone **(3)** displayed the highest radical scavenging activity ($IC_{50}$ of 57.5 ± 0.5 µg/mL) followed by morelloflavone-7"-*O*-glucoside **(5)**, 3,8"-binaringenin **(2)**, 3,8"-binaringenin-7"-*O*-glucoside **(4)** and amento-4'-methylether **(1)**. Compound **(3)** showed the greatest radical scavenging activity due to the presence of the *ortho* 3"",4""-dihydroxyl groups at ring E, the *meta* 5",7"-dihydroxy moiety at ring D and the 2",3"-double bond with 4"-keto group at ring F as they are the determinants of scavenging free radical. Replacement of a free hydroxyl group at C-7"

**Table 1.** Antioxidant and antityrosinase activities of the extracts and biflavonoids from *G. griffithii*

| Samples | Total phenolic content (mg GAE/g) | DPPH radical scavenging $IC_{50}$ (µg/mL) | Tyrosinase inhibition (I%) at 0.1 mg/mL |
|---|---|---|---|
| **Extracts** | | | |
| **GGLH** | 30.0 ± 2.8 | 975.0 ± 2.5 | NA |
| **GGLD** | 104.90 ± 1.8 | 941.7 ± 1.1 | 7.3 ± 2.6 |
| **GGLM** | 444.10 ± 6.7 | 188.0 ± 2.2 | 15.0 ± 4.6 |
| **GGSH** | 223.50 ± 9.4 | 96.4 ± 2.7 | NA |
| **GGSE** | 393.20 ± 3.1 | 107.7 ± 1.5 | 5.3 ± 1.8 |
| **GGSM** | 220.10 ± 2.9 | 534.1 ± 1.8 | 20.0 ± 4.4 |
| **Compounds** | | | |
| **(1)** | ND | 1033 ± 1.7 | 2.8 ± 3.5 |
| **(2)** | ND | 112.4 ± 0.9 | 11.5 ± 0.9 |
| **(3)** | ND | 57.6 ± 0.5 | 34.6 ± 2.5 |
| **(4)** | ND | 134.0 ± 0.8 | 6.9 ± 2.6 |
| **(5)** | ND | 108.1 ± 0.9 | 7.6 ± 3.5 |
| Ascorbic acid | - | 17.4 ± 1.2 | - |
| Kojic acid | - | - | 90.2 ± 0.7 |

GGLH = *n*-Hexane leaves extract; GGLD = Dichloromethane leaves extract; GGLM = MeOH leaves extract; GGSH = *n*-Hexane stem bark extract; GGSE = EtOAc stem bark extract; GGSM = MeOH stem bark extract; GAE/L = total phenolic was expressed as mg of gallic acid equivalent; NA = No activity; ND = Not determined

with glucoside for compound **(5)** resulted in the decreasing of the scavenging activity. Lacked of 2,3-double bond for ring F of compound **(2)** and **(4)** further reduced the antioxidant activity. Compound **(1)** displayed the lowest antioxidant activity due to replacement of methoxyl group and a linkage at C-4' and C-3' of ring B [24].

Tyrosinase inhibitors are chemical agents capable of reducing enzymatic reactions, such as food browning and melanisation of human skin. Based on Table 1, all the extracts were found to be poorly active towards mushroom tyrosinase compared to kojic acid. The MeOH extract for both leaves and stem barks were the only extracts that showed tyrosinase inhibitor activity more than 10% because these extracts contained bioflavonoids. The *n*-hexane and EtOAc extracts were found inactive towards tyrosinase mushroom due to the absence of polar compounds. Among the naturally occurring components, flavanoids and terpenoids containing a-hydroxyketone group have been reported to be a good mushroom-tyrosinase inhibitors such as kojic acid [25]. Amento-4'-methyl ether **(1)** showed the lowest value of activity due to the existence of methoxyl group. In fact, glycosylation and methoxylation of the specific hydroxyl group on the aromatic ring would heavily affect the inhibitory activity [26]. Therefore, the glucosidic biflavonoid, morelloflavone-7"-*O*-glucoside **(5)** and 3,8"-binaringenin-7"-*O*-glucoside **(4)** showed weak activity compared to their corresponding aglycones, 3,8"-binaringenin **(2)** and morelloflavone **(3)**.

The minimum inhibitory concentrations (MIC) of the crude extracts against the microorganisms tested are shown in Table 2. The antimicrobial activity revealed that the crude extracts have moderate to strong and in a few cases very weak activity against the tested bacteria. The MeOH crude extract of the leaves (GGLM) exhibited strong inhibition against *E. faecalis* and *K. pneumonia*, both with MIC value 225 ppm. The EtOAc extract of the stem barks displayed (GGSE) strong activity against *E. coli* with MIC 225 ppm. The MeOH extract of the stem barks (GGSM) was found strongly active against *K. pneumonia* with MIC 225 ppm. The evaluation of MBC of the extracts showed that the MeOH extracts of leaves (GGLM) exhibited strong inhibition at concentration 400 ppm against *E. Faecalis* and at concentration 450 ppm against *B. subtilis* and *K. pneumoniae* except for *E. coli*. The *n*-hexane (GGSH) and EtOAc extracts (GGSE) from the stem barks were found as active inhibitors both with value 450 ppm against *B. subtilis*. GGSE which also found as a strong inhibitor at 450 ppm against *E. coli* while the MeOH extract (GGSM) showed strong inhibition against *K. pneumoniae*. Based on the MIC and MBC results, all the crude extracts from leaves and stem barks of *G. griffithii* gave positive results towards both Gram-positive and Gram-negative bacteria. The performance patterns showed that *G. griffithii* had broad spectrum of antibacterial activity and can be a source of bioactive substances. Out of the six extracts, the MeOH extracts from the leaves (GGLM) showed the highest activity against the tested organism. The decrease of antimicrobial activity of all the crude extracts against the tested bacteria as summarized as GGLM > GGSE > GGSH > GGSM > GGLD > GGLH.

## 3. Conclusion

In conclusion, chemical study of the leaves and stem barks of *G. griffithii* have successfully isolated nine compounds, which comprised of five biflavonoids and four terpenes. The crude MeOH extract of the leaves has highest value of phenolic compounds in the total phenolic content assay and these were proved by the isolation of biflavonoids. Since *G.*

**Table 2.** Antimicrobial activities of the extracts of *G. griffithii*

| Samples | Gram positive bacteria | | | | Gram negative bacteria | | | |
|---|---|---|---|---|---|---|---|---|
| | *Enterococcus faecalis* | | *Bacillus subtilis* | | *Escherichia coli* | | *Klebsiella pneumoniae* | |
| | MIC | MBC | MIC | MBC | MIC | MBC | MIC | MBC |
| **GGLH** | 450 | 900 | 900 | 1800 | 900 | 1800 | 900 | 1800 |
| **GGLD** | 450 | 900 | 900 | 900 | 450 | 900 | 900 | 1800 |
| **GGLM** | 225 | 400 | 450 | 450 | 450 | 900 | 225 | 450 |
| **GGSH** | 450 | 900 | 450 | 450 | 450 | 900 | 450 | 900 |
| **GGSE** | 450 | 900 | 450 | 450 | 225 | 450 | 450 | 900 |
| **GGSM** | 450 | 900 | 900 | 900 | 450 | 900 | 225 | 450 |
| **SS** | 14.1 | 14.1 | 14.1 | 28.1 | 14.1 | 14.1 | 14.1 | 28.1 |

GGLH = *n*-Hexane leaves extract; GGLD = Dichloromethane leaves extract; GGLM = MeOH leaves extract; GGSH = *n*-Hexane stem bark extract; GGSE = EtOAc stem bark extract; GGSM = MeOH stem bark extract; MIC = Minimum inhibitory concentration (µg/mL); MBC = Minimum bactericidal concentration (µg/mL); SS = Streptomycin sulfate

*griffithii* was found to have high concentration of biflavonoid compounds, future work on the other bioactivities such as cytotoxic, anti-diabetic and anti-inflammatory would permit SAR study on the compounds. Chemical reaction such as reduction, addition and oxidation can be carried out on the compounds to give useful derivatives for bioactivity studies.

## 4. Materials and Methods

### *4.1 General experimental procedures*

The mass spectra were obtained on a Finnigan-MAT-95 mass spectrometer from National University of Singapore. The UV spectra were recorded in methanol on a Shimadzu UV 1601PC spectrophotometer. The IR spectra were obtained on a Perkin-Elmer 1600 FTIR spectrophotometer. The 1D and 2D NMR spectra were recorded in deuterated chloroform on a Bruker Avance 400 MHz spectrometer, chemical shifts are reported in ppm on δ scale, and the coupling constants (*J*) are given in Hz. Vacuum liquid chromatography (VLC) was performed using Merck silica gel 230-400 mesh, while column chromatography (CC) was performed using Merck silica gel 70-230 mesh. Preparative thin layer chromatography (PTLC) was prepared using silica gel 60 PF254. Thin layer chromatography (TLC) aluminum sheets pre-coated with silica gel 60 F254 (0.2 mm thickness) was used to detect and monitor components presence in the crude samples or fractions. The TLC and PTLC spots were visualized under UV light (254 and 366 nm) followed by spraying with 5% $H_2SO_4$ and 1% vanillin in MeOH and heating at 120°C for 5 min. All solvents were AR grade.

### *4.2 Plant materials*

Samples of *G. griffithii* were collected from Sungai Pandan, Panching, Pahang on April 2010. The samples were identified by Dr. Shamsul Khamis and the plant specimens (MT23) were deposited at Herbarium, Kulliyah of Pharmacy, International Islamic University Malaysia, Pahang.

### *4.3 Extraction and isolation*

The dried and ground leaves (350 g) and stem barks (350 g) were extracted sequentially by Soxhlet extractor (stem bark: using *n*-hexane, EtOAc, MeOH; leaves: using *n*-hexane, DCM, MeOH) for 8 h each. Each solvent was concentrated and the extract was stored at 4°C for further use. The MeOH leaves extract (20 g) was fractionated by VLC eluted with $CHCl_3$:EtOAc:$Me_2CO$ of increasing polarity to give 14 fractions (GGLM1-14). Fraction GGLM5 (2.0 g) was further fractionated by CC eluted with $CHCl_3$:MeOH (9:1 and 8:2) to yield new fraction, GGLM5A-C which then re-chromatographed using the same eluent system to give **(1)** (25.3 mg), **(2)** (153.6 mg), and **(3)** (743.0 mg), respectively. Fraction GGLM7 was purified by CC with eluent $CHCl_3$:MeOH (5% increasing step gradient) to obtain three fractions GGLM7A-C (20.3 mg; 50.5 mg; and 136.2 mg). Fraction GGLM7C underwent purification by preparative TLC to give **(4)** (74.1 mg). Fraction GGLM8-9 was combined (497.9 mg) and further purified through preparative TLC to obtain two spots, A and B with eluent $CHCl_3$: MeOH (4:1). Spot B was washed using $Me_2CO$ to give **(5)** (65.9 mg). Fractionation and purification processes on the *n*-hexane leaves extract (20.0 g) have yielded 10 fractions (GGSH1-10). Repeated purification of fraction GGSH6 yielded friedelin **(8)** (151.9 mg), which was a major compound isolated from the *n*-hexane leaves extract. Repeated purifications of fraction GGSH9 followed by trituration with *n*-hexane gave β-amyrin **(9)** (73.9 mg). The MeOH extract of the stem barks (10.0 g) was fractionated using $CHCl_3$:MeOH with 10% step gradient of polarity to afford **(3)**. The EtOAc extract of the stem barks (5.0 g) was chromatographed using *n*-hexane:$CHCl_3$:MeOH with 10% step gradient to yield **(1)** and **(3)**. Fractionation and purification processes on the *n*-hexane stem bark extract (20.0 g) have yielded 11 fractions (GGSH1-11). Purification of the combined fractions GGSH1-3 (339.0 mg) gave squalene **(6)** (44.3 mg) and multiple purification of fraction GGSH5 yielded canophyllol **(7)** (35.0 mg).

Amentoflavone-4'-methyl ether **(1)** - Yellow amorphous powder (25.3 mg); m.p. 221-223°C; IR $\nu_{max}$ (film) $cm^{-1}$: 3304, 3019, 2843, 1645, 1602, 1501, 1239, 1168; $^1H$ NMR $\delta_H$ ($CDCl_3$): 3.83 (3H, s, $OCH_3$), 6.26 (1H, d, *J* = 2.0 Hz, H-6), 6.47 (1H, s, H-6"), 6.53 (1H, d, *J* = 2.0 Hz, H-8), 6.74 (1H, s, H-3"), 6,75 (1H, s, H-3), 6.96 (2H, d, *J* = 8.8 Hz, H-3'"/5'"), 7.27 (1H, d, *J* = 8.8 Hz, H-5'), 7.76 (2H, d, *J* = 8.8 Hz, H-2'"/6'"), 8.06 (1H, d, *J* = 8.8, 2.4 Hz, H-6'), 8.14 (1H, d, *J* = 2.4 Hz, H-2'), 13.03 (1H, s, 5-OH), 13.17 (1H, s, 5"-OH); $^{13}C$ NMR $\delta_C$ ($CDCl_3$): 55.0 ($OCH_3$), 93.9 (C-8), 98.9 (C-6), 99.0 (C-6"), 103.3 (C-3), 103.4 (C-3"), 103.6 (C-8"), 104.5 (C-10"), 104.7 (C-10), 114.4 (C-3'"/5'"), 116.7 (C-5'), 120.0 (C-3'), 122.5 (C-1'), 123.4 (C-1'"), 128.0 (C-6'), 128.3 (C-2'"/6'"), 131.7 (C-2'), 155.3 (C-9"), 158.0 (C-9), 159.5 (C-4'), 161.8 (C-5), 161.9 (C-5"), 162.5 (C-4'"), 162.7 (C-7), 163.9 (C-2"), 164.1 (C-7"), 164.2 (C-2), 182.2 (C-4"), 182.6 (C-4); CIMS *m/z* 553 [$M+H^+$, $C_{31}H_{21}O_{10}$] [27].

3,8"-Binaringenin **(2)** - Yellow amorphous powder (153.6 mg); m.p. 220-225°C; IR $\nu_{max}$ (film) $cm^{-1}$: 3191, 2894, 1595, 1509, 1447, 1158, 1076; $^1H$ NMR $\delta_H$ ($CDCl_3$): 2.61 (1H, dd,

*J* = 13.2, 3.2 Hz, H-3"a), 2.72 (1H, dd, *J* = 13.2, 12.0 Hz, H-3"b), 4.72 (1H, d, *J* = 12.0 Hz, H-3), 5.33 (1H, dd, *J* = 12.0, 3.0 Hz, H-2"), 5.85 (1H, m, H-2), 5.94 (1H, s, H-6"), 5.98 (2H, s, H-6/8), 6.73 (2H, m, H-3'/5'), 6.83 (2H, m, H-3'"/5'"), 6.92 (2H, m, H-2'"/6'"), 7.24 (2H, br s, H-2'/6'), 12.20 (1H, m, 5"-OH), 12.32 (1H, m, 5-OH); $^{13}$C NMR $\delta_C$ ($CDCl_3$): 43.3 (C-3"), 48.2 (C-3), 79.3 (C-2"), 82.0 (C-2), 94.9 (C-6"), 95.8 (C-8), 96.2 (C-6), 101.5 (C-10"), 102.0 (C-10/8"), 114.8 (C-3'"/5'"), 115.3 (C-3'/5'), 129.0 (C-2'/6'/2'"/6'"), 130.9 (C-1/1'"), 145.2 (C-4'"), 157.7 (C-4'), 163.4 (C-9"), 164.6 (C-5/5"/7"), 166.2 (C-7), 196.3 (C-4), 196.9 (C-4"); CIMS *m/z* 543 [M+H$^+$, $C_{30}H_{22}O_{10}$] [26].

Morelloflavone **(3)** - Pale yellow amorphous powder (743.0 mg); m.p. 275-278°C; IR $\nu_{max}$ (film) cm$^{-1}$: 3331, 2977, 1637, 1513, 1451, 1158, 1051; $^1$H NMR $\delta_H$ ($CDCl_3$): 5.00 (1H, d, *J* = 12.4 Hz, H-3), 5.88 (1H, d, *J* = 12.4 Hz, H-2), 6.04 (2H, br s, H-6/8), 6.32 (1H, s, H-6"), 6.48 (1H, s, H-3"), 6.54 (2H, d, *J* = 8.0 Hz, H-3'/5'), 7.03 (1H, d, *J* = 8.0 Hz, H-5'"), 7.25 (2H, d, *J* = 8.0 Hz, H-2'/6'), 7.52 (1H, br s, H-2'"), 7.54 (1H, br d, *J* = 8.0 Hz, H-6'"), 9.62 (1H, br s, 4'-OH), 12.34 (1H, s, 5-OH), 13.17 (1H, s, 5"-OH); $^{13}$C NMR $\delta_C$ ($CDCl_3$): 49.2 (C-3), 81.5 (C-2), 95.2 (C-8), 96.3 (C-6), 98.7 (C-6"), 100.8 (C-10"), 102.1 (C-10), 102.9 (C-3"), 104.0 (C-8"), 113.3 (C-2'"), 114.6 (C-3'/5'), 115.8 (C-5'"), 119.6 (C-6'"), 122.6 (C-1'"), 128.4 (C-2'/6'), 129.2 (C-1'), 145.5 (C-3'"), 149.3 (C-4'"), 155.9 (C-9"), 157.5 (C-4'), 161.7 (C-7"), 163.4 (C-2"), 164.0 (C-9), 164.7 (C-5/5"), 166.4 (C-7), 182.3 (C-4"), 196.5 (C-4); CIMS *m/z* 558 [M+H$^+$, $C_{30}H_{21}O_{11}$] [26].

3,8"-Binaringenin-7"-*O*-glucoside **(4)** - Yellow amorphous powder (74.1 mg); m.p. 215-220°C; IR $\nu_{max}$ (film) cm$^{-1}$: 3307, 2915, 1619, 1514, 1450, 1163, 1071; $^1$H NMR $\delta_H$ ($CDCl_3$): 2.64 (1H, m, H-3"a), 2.75 (1H, m, H-3"b), 3.30 – 4.00 (m, H-2''''/4''''/3''''/5''''/6''''), 4.75 (1H, d, *J* = 10.4 Hz, H-3),4.87 (1H, s, H-1''''), 5.44 (1H, d, *J* = 11.6 Hz, H-2²), 5.77 (1H, m, H-2), 5.84 (1H, s, H-6"), 5.95 (2H, m, H-6/8), 6.74 (2H, d, *J* = 8.4 Hz, H-3'/5'), 6.81 (2H, m, H-3'"/5'"), 6.90 (2H, m, H-2'"/6'"), 7.36 (2H, d, *J* = 8.4 Hz, H-2'/6'), 12.16 (1H, m, 5"-OH), 12.21 (1H, m, 5-OH); $^{13}$C NMR $\delta_C$ ($CDCl_3$): 43.7 (C-3"), 48.4 (C-3), 61.6 (C-6''''), 70.3 (C-4''''), 73.6 (C-2''''), 76.9 (C-3''''), 77.2 (C-5''''), 79.4 (C-2"), 82.3 (C-2), 94.9 (C-8/6"), 96.1 (C-6), 100.3 (C-1''''), 101.9 (C-10"), 103.4 (C-8"), 103.6 (C-10), 115.0 (C-3'/5'), 115.2 (C-3'"/5'"), 129.1 (C-2'"/6'"), 129.8 (C-2'/6'), 145.2 (C-4'"), 157.8 (C-4'), 163.5 (C-9"), 164.5 (C-5, 5"/7"), 166.4 (C-7), 197.0 (C-4), 197.3 (C-4"); CIMS *m/z* 705 [M+H$^+$, $C_{36}H_{32}O_{15}$] [26].

Morelloflavone-7"-*O*-glucoside **(5)** - Shiny yellow amorphous (65.9 mg); m.p. 239-241°C; IR $\nu_{max}$ (film) cm$^{-1}$: 3244, 2919, 1640, 1594, 1450, 1163, 1062; $^1$H NMR $\delta_H$ ($CDCl_3$): 3.43 (1H, m, H-2''''), 3.48 (1H, m, H-4''''), 3.58 (2H, m, H-3''''/5''''), 3.83 (2H, m, H-6''''), 5.05 (1H, d, *J* = 12.0 Hz, H-3), 5.31 (1H, d, *J* = 7.6 Hz, H-1''''), 5.94 (1H, m, H-2), 6.03 (2H, br d, *J* = 7.2 Hz, H-6/8), 6.12 (1H, s, H-6"), 6.53 (2H, m, H-3'/5'), 6.62 (1H, s, H-3"), 7.03 (1H, d, *J* = 7.6 Hz, H-5'"), 7.30 (2H, m, H-2'/6'), 7.52 (1H, br d, *J* = 7.6 Hz, H-2'"), 7.54 (1H, br d, *J* = 7.6 Hz, H-6'"), 13.00 (1H, s, 5-OH), 13.19 (1H, s, 5"-OH); $^{13}$C NMR $\delta_C$ ($CDCl_3$): 49.4 (C-3), 61.7 (C-6''''), 70.3 (C-4''''), 74.0 (C-2''''), 77.0 (C-3''''), 77.2 (C-5''''), 81.5 (C-2), 95.2 (C-8), 96.4 (C-6), 98.4 (C-6"), 100.3 (C-10"), 100.8 (C-1''''), 102.1 (C-10), 103.0 (C-3"), 103.7 (C-8"), 113.4 (C-2'"), 114.5 (C-3'/5'), 115.8 (C-5'"), 119.7 (C-6'"), 122.3 (C-1'"), 128.5 (C-2'/6'), 129.4 (C-1'), 145.7 (C-3'"), 149.6 (C-4'"), 155.3 (C-9"), 157.9 (C-4'), 161.4 (C-7"), 163.5 (C-2"), 164.7 (C-5/9/5"), 166.6 (C-7), 182.5 (C-4"), 196.0 (C-4); CIMS *m/z* 719 [M+H$^+$, $C_{36}H_{30}O_{16}$] [26].

Squalene **(6)** - Pale yellow liquid (44.3 mg); IR (film) $\upsilon_{max}$ cm$^{-1}$: 3097, 2855, 1669, 1448, 1381; spectral data were consistent with the literature [28].

Canophyllol **(7)** - White solid (35.0 mg); m.p 279-281°C; IR (film) $\upsilon_{max}$ cm$^{-1}$: 3540, 2854, 1706, 1460, 1377, 1157; spectral data were consistent with the literature [29].

Friedelin **(8)** - White needle (151.9 mg); m.p 261-264°C; IR (film) $\upsilon_{max}$ cm$^{-1}$: 2854, 1716, 1459, 1377; spectral data were consistent with the literature [30].

β-Amyrin **(9)** - White solid (73.9 mg); m.p 187-190°C; IR (film) $\upsilon_{max}$ cm$^{-1}$: 3345, 2854, 1643, 1459, 1377, 1261; spectral data were consistent with the literature [31].

### *4.4 Biological activities*

***Solvents and chemicals*** - ***Antioxidant:*** 1,1-diphenyl-2-picrylhydrazyl (DPPH), ascorbic acid, gallic acid, and butylated hydroxytoluene (BHT) were obtained from Sigma-Aldrich (Germany). Analytical grade methanol, ethanol and dimethylsulfoxide (DMSO) were purchased from Merck (Germany). ***Antityrosinase:*** Mushroom tyrosinase enzyme (EC1.14.18.1), kojic acid and L-dopa were purchased from Sigma-Aldrich (Germany). ***Antibacterial:*** Nutrient agar (NA), nutrient broth (NB), and streptomycin sulphate were purchased from Oxoid (Italy). The tested microorganisms were purchased from Mutiara Scientific (Malaysia).

### *4.5 Antioxidant activity*

#### *4.5.1 Total phenolic content (TPC)*

The TPC of the extracts was determined by Folin-Ciocalteau's assay [32]. In brief, a stock solution of was

diluted in methanol and was prepared in triplicate. A 0.05 mL aliquot of sample was pipetted into a test tube followed by 3.95 mL of distilled water, then 0.25 mL Folin-Ciocalteu's reagent was added, and the flask was thoroughly shaken. After 2 min, 0.75 mL of 20% $Na_2CO_3$ solution was added and the mixture was allowed to stand for 1 h with intermittent shaking. The absorbance measurements were recorded at 765 nm. The same procedure was repeated for standard gallic acid solutions with the concentrations from 25 to 500 µg/mL in triplicate. A calibration graph of standard gallic acid and (±)-catechin were constructed, respectively. Total phenolic content of the extract was expressed as mg Gallic acid equivalent (GAE) per gram of extract.

#### *4.5.2 DPPH Radical scavenging assay*

DPPH radical scavenging assay of all tested samples was carried out by the DPPH method with minor modification [33]. Stock solution of the extracts was diluted to final concentrations from 1000 to 7.81 µg/mL. Then, a total of 3.8 mL of 50 µM DPPH methanolic solution was added to 0.2 mL of each sample solution and allowed to react at room temperature for 30 min. The absorbance of the mixtures was measured at 517 nm. A control was prepared without sample or standard and measured immediately at 0 min. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity, and vice versa. Inhibitions of DPPH radical in percent (I%) were calculated as follow:

$$[\ I\% = (A_{blank} - A_{sample} / A_{blank}) \times 100\ ]$$

where $A_{blank}$ is the absorbance value of the control reaction (containing all reagents except the samples) and $A_{sample}$ is the absorbance values of the samples. The sample concentration that provides 50% inhibition ($IC_{50}$) was calculated by plotting inhibition percentages against concentration of the sample. All tests were carried out in triplicate and $IC_{50}$ values were reported as means ±SD of triplicate.

### *4.6 Antityrosinase activity*

Tyrosinase inhibition assay was performed according to previous study with minor modification [34]. The samples and kojic acid were dissolved in DMSO prepared as 0.1 mg/mL. The reaction was carried out using 96-well microplate and ELISA microplate reader (VersaMax Molecular Devices, USA) was used to measure the absorbance at 515 and 655 nm. 40 µL of samples were dissolved in DMSO with 80 µL of phosphate buffer (pH 6.8), 40 µL of tyrosinase enzyme (EC 1.14.18.1) and 40 µL of L-Dopa were placed in each well. Each sample was accompanied by a blank and had all the components except for L-Dopa. Kojic acid was used as references standard inhibitor for comparison. Inhibitions of tyrosinase in percent (I%) were calculated as follow:

$$[\ I\% = (A_{control} - A_{sample} / A_{control}) \times 100\ ]$$

where $A_{control}$ is the absorbance value of the control reaction and $A_{sample}$ is the absorbance values of the samples.

### *4.7 Antibacterial activity*

The test microorganisms, *Enterococcus faecalis* (ATCC19433), *Bacillus subtilis* (ATCC6633), *Escherichia coli* (ATCC10536) and *Klebsiella pneumonia* (ATCC13883) were used. The strains were grown on Nutrient broth (NB) for the bacteria. The minimal inhibitory concentration (MIC) was determined by broth micro dilution method using 96-well microplates [35-36]. The inocula of the microbial strains were prepared from 24 h broth cultures and McFarland standard turbidity of suspensions was adjusted to 0.5. Sample (1 mg) was dissolved in DMSO (1 mL) to get 1000 µg/mL stock solution. A number of wells were reserved in each plate for positive and negative controls. The mixture of samples and sterile broth (100 µL) were transferred to each well in order to obtain a twofold serial dilution of the stock samples (concentration of 1000, 500, 250, 125, 62.5, 31.3, 15.6 and 7.8 µg/mL). The inoculated bacteria (100 µL) were added to each well. The final volume in each well was 200 µL. Streptomycin sulfate was used as positive controls for bacterial. Plates were incubated at 37°C for 24 h. Microbial growth was indicated by the turbidity and the presence of pellet at the bottom of the well.

**Acknowledgements**

The authors would like to thank the Ministry of Higher Education (MOHE) Malaysia for the research grant and financial support under Grant University Project (GUP) QJ130000.7126.02H30 and Department of Chemistry, Faculty of Science, Universiti Teknologi Malaysia for the NMR facilities.

**Authorship contributions**

Concept – W.M.N.H.W.S., N.S.A.N.S., F.A.; Design – W.M.N.H.W.S., N.S.A.N.S.; F.A.; Supervision – F.A.; Resource – F.A.; Materials – F.A.; Data Collection and/or Processing – W.M.N.H.W.S., N.S.A.N.S.; Analysis and/or Interpretation – W.M.N.H.W.S., N.S.A.N.S., F.A.; Literature Search

– W.M.N.H.W.S., N.S.A.N.S.; Writing – W.M.N.H.W.S., N.S.A.N.S.; Critical Reviews – W.M.N.H.W.S., N.S.A.N.S., F.A.

## Conflict of interest

The authors declared no conflict of interest.

## References

1. Gautam B. Natural product: Chemistry, Biochemistry and Pharmocology. Alpha Science International Ltd. (Oxford, UK), 2009.
2. Bennett GJ, Lee HJ. Xanthones from Guttiferae. Phytochemistry 1989; 28: 967-998.
3. Waterman PG, Hussain RA. Systematic significance of xanthones, benzophenones and biflavonoids in *Garcinia*. Biochem Syst Ecol 1983; 11: 21-28.
4. Whitemore TC. Malayan Forest Records No. 26: Tree of Malaya: A Manual for Foresters. Longman (Kuala Lumpur), 1972.
5. Parveen M, Khan NU, Acchari B, Dutta PK. A triterpene from *Garcinia mangostana*. Phytochemistry 1991; 30: 361-2.
6. Corner EJH. Wayside Trees of Malaya, Government Printing Office, 1940.
7. Nilar LH, Nguyen D, Ganpathi V, Sim K-Y, Harrison LJ. Xanthones and benzophenones from *Garcinia griffthii* and *Garcinia mangostana*. Phytochemistry 2005; 66: 1718-23.
8. Xu YJ, Cao SG, Wu XH, Lai YH, Tan BHK, Pereira JT, Goh SH, Venkatraman G, Harrison LJ, Sim KY. Griffipavixanthone, a novel cytotoxic bixanthone from *Garcinia griffithii* and *G. parvifolia*. Tetrahedron Lett 1998; 39: 9103-6.
9. Elfita E, Muharni M, Madyawati L, Darwati D, Ari W, Supriyatna S, Husein HB, Dachriyanus D, Paul C, Louis M, Kenne F, Sandra A, Luc P. Antiplasmodial and other constituents from four Indonesian *Garcinia* spp. Phytochemistry 2009; 70: 907-12.
10. Anu Aravind AP, Asha KRT, Rameshkumar KB. Phytochemical analysis and antioxidant potential of the leaves of *Garcinia travancorica* Bedd. Nat Prod Res 2016; 30: 232-6.
11. Permanaa D, Abas F, Maulidiani, Shaari K, Stanslas J, Ali AM, Lajis N. Atrovirisidone B, a new prenylated depsidone with cytotoxic property from the roots of *Garcinia atroviridis*. Z Naturforsch 2005; 60: 523-6.
12. Panthong K, Hutadilok-Towatana N, Panthong A. Cowaxanthone F, a new tetraoxygenated xanthone, and other anti-inflammatory and antioxidant compounds from *Garcinia cowa*. Canadian J Chem 2009; 87: 1636-40.
13. Jamila N, Khairuddean M, Khan SN, Khan N. Complete NMR assignments of bioactive rotameric (3→8) biflavonoids from the bark of *Garcinia hombroniana*. Magn Reson Chem 2014; 52: 345-52.
14. Al-Shaghdari A, Bello Alarcón A, Cuesta-Rubio O. Determination of biflavonoids in different parts of *Garcinia bakeriana* (Urb) borhidi. Rev Cuba Farm 2014; 48: 307-15.
15. Ito T, Yokota R, Watarai T, Mori K, Oyama M, Nagasawa H, Matsuda H, Iinuma M. Isolation of six isoprenylated biflavonoids from the leaves of *Garcinia subelliptica*. Chem Pharm Bull 2013; 61: 551-8.
16. Gontijo VS, Judice WAS, Codonho B, Pereira IO, Assis DM, Januário JP, Caroselli EE, Juliano MA, De Carvalho Dosatti A, Marques MJ, Junior CV, Santos MHD. Leishmanicidal, antiproteolytic and antioxidant evaluation of natural biflavonoids isolated from *Garcinia brasiliensis* and their semisynthetic derivatives. Eur J Med Chem 2012; 58: 613-23.
17. Kaikabo AA, Eloff JN. Antibacterial activity of two biflavonoids from *Garcinia livingstonei* leaves against *Mycobacterium smegmatis*. J Ethnopharmacol 2011; 138: 253-5.
18. Castardo JC, Prudente AS, Ferreira J, Guimarães CL, Monache FD, Filho VC, Otuki MF, Cabrini DA. Anti-inflammatory effects of hydroalcoholic extract and two biflavonoids from *Garcinia gardneriana* leaves in mouse paw oedema. J Ethnopharmacol 2008; 118: 405-11.
19. Nworu CS, Akah PA, Esimone CO, Okoli CO, Okoye FBC. Immunomodulatory activities of kolaviron, a mixture of three related biflavonoids of *Garcinia kola* Heckel. Immunopharmacol Immunotoxicol 2008; 30: 317-32.
20. Lin YM, Anderson H, Flavin MT, Pai YHS, Mata-Greenwood E, Pengsuparp T, Pezzuto JM, Schinazi RF, Hughes SH, Chen FC. In vitro anti-HIV activity of biflavonoids isolated from *Rhus succedanea* and *Garcinia multiflora*. J Nat Prod 1997; 60: 884-8.
21. Gunatilaka AAL, Sriyani HTB, Sotheeswaran S, Waight ES. 2,5-dihydroxy-1,6-dimethoxyxanthone and biflavonoids of *Garcinia thwaitesii*. Phytochemistry 1983; 22: 233-5.
22. Waterman PG, Crichton EG. Xanthones and biflavonoids from *Garcinia densivenia* stem bark. Phytochemistry 1980; 19: 2723-6.
23. Bandaranayake WM, Selliah SS, Sultanbawa MUS, Davii OW. Biflavonoids and xanthones of *Garcinia terpnophylla* and *G. echinocarpa*. Phytochemistry 1975; 14:, 1878-80.
24. Rice-Evans CA, Miller NJ, Paganga G. Antioxidant properties of phenolic compounds. Trends Pl Sci Rev 1997; 2: 152-9.
25. Te-Sheng C. Review: An updated review of tyrosinase inhibitors. Int J Mol Sci 2009; 10: 2440-75.
26. Chen FC, Lin YM, Hung JC. Phenolic compounds from the heartwood of *Garcinia multiflora*. Phytochemistry 1975; 14: 300-3.
27. Ryu YB, Jeong HJ, Kim JH, Kim YM, Park JH, Kim D, Naguyen TTH, Park SJ, Chang JS, Park KH, Rho MC, Lee WS. Biflavonoids from *Torreya nucifera* displaying SARS-CoV 3CLpro inhibition. Bioorg Med Chem 2010; 18: 7940-7.
28. Ragasa CY, Levida RM, Don MJ, Shen CC. Cytotoxic isothiocyanates from *Moringa oleifera* Lam seeds. Philippine Sci Lett 2012; 5: 46-52.
29. Nozaki H, Suzuki H, Hirayama T, Kasai R, Wu RY, Lee KH. Antitumor triterpenes of *Maytenus diversifolia*. Phytochemistry 1986; 25: 479-85.
30. Hui WH, Ko PDS, Lee YK, Li MM, Arthur HR. Triterpenoids from the ten *Lithocarpus* species of Hong Kong. Phytochemistry 1975; 14: 1063-6.
31. Tahany MAAR, Hegazy AK, Sayed AM, Kabiel HF, El-Alfy T, El-Komy SM. Study on combined antimicrobial activity of some biologically active constituents from wild *Moringa*

*peregrine* Forssk. J Yeast Fungal Res 2010; 1: 15-24.

32. Salleh WMNHW, Ahmad F. Antioxidant and anti-inflammatory activities of essential oils of *Actinodaphne macrophylla* and *A. pruinosa* (Lauraceae). Nat Prod Commun.2016; 11: 853-5.
33. Salleh WMNHW, Ahmad F, Khong HY, Zulkifli RM. Chemical constituents and bioactivities from the leaves of *Beilschmiedia glabra* Kosterm (Lauraceae). Marmara Pharm J 2016: 20: 400-6.
34. Salleh WMNHW, Ahmad F, Khong HY, Zulkifli RM. Comparative biological activities of extracts from three Malaysian *Beilschmiedia* species. Marmara Pharm J 2016; 20: 224-31.
35. Zavala MA, Perez GS, Perez GRM. Antimicrobial screening of some medicinal plants. Phytother Res 1997; 11: 368-71.
36. Petrus EM, Tinakumari S, Chai LC, Ubong A, Tunung R, Elexson N, Chai LF, Son R. A study on the minimum inhibitory concentration and minimum bactericidal concentration of nano colloidal silver on food-borne pathogens. Int Food Res J 2011; 18: 55-66.