**SUPPLEMENTAL MATERIAL**

**CHEMICAL CONSTITUENTS AND ANTIOXIDANT ACTIVITY OF *Garcinia madruno* (Kunth) Hammel**

**Total phenolic content determination and antioxidant activity assays**

*Total phenolic content determination*

Total phenolic content was determined according to the Folin-Ciocalteau colorimetric method, using gallic acid as a standard phenolic compound. The extract solution (0.5 mL; 20 µg/mL) was mixed with 0.5 mL of the Folin-Ciocalteau reagent and 0.5 mL of 100 mg/mL Na2CO3, and the absorbance was measured at 760 nm after 1 h at room temperature. The mean ± standard deviation (SD) results of total phenolic contents of triplicate analyses were expressed as mg of gallic acid equivalent (GAE)/100g extract.

*DPPH free radical scavenging activity*

The stock solution was 20 mg/L of DPPH dissolved in methanol. The working solution was 990 μL of the stock solution with 10 μL of the sample (extract or pure compounds at different concentrations). For the blank, 10 μL of methanol was used instead of the sample, and a sample control was also made by mixing 10 μL of the sample with 990 μL of methanol. The change in color (from deep violet to light yellow) after a 30 min incubation period at room temperature was measured at 517 nm with a UV-visible spectrophotometer Jemway 6405. Results were expressed by the TEAC (Trolox equivalent antioxidant capacity) values as µmol of Trolox equivalent (TE)/100g of sample, determined from the calibration curve for Trolox. The experiment was performed in triplicate.

*ABTS radical cation scavenging activity*

ABTS radical cation was generated by oxidation of ABTS [2,2’-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)] with potassium persulfate. ABTS was dissolved in deionized water to 7.0 mM concentration and mixed with 2.45 mM potassium persulfate. The reaction mixture was left to stand at room temperature in the dark for 12 h before use. The ABTS solution was diluted with phosphate buffer solution (pH 7.4) to an absorbance of 0.700±0.050. Then, 950 µL of ABTS•+ solution was combined with 50 µL aliquots of extracts and pure compounds (diluted with ethanol), and the absorbance measured at 734 nm, exactly 30 min after mixing. Results were expressed by the TEAC (Trolox equivalent antioxidant capacity) values as µmol TE/100g of sample, determined from the calibration curve for Trolox. Appropriate solvent blanks were run in each assay. All determinations were carried out in triplicate.

*Ferric reducing power assay (FRAP)*

The FRAP reagent was prepared by mixing acetate buffer (pH 3.4) with TPTZ in HCl and FeCl3. The working solution was prepared with 900 µL of FRAP reagent, 50 µL of the sample, and 50 µL of ethanol. Absorbance readings were taken at 593 nm, exactly 30 min after mixing. Results were expressed by the AEAC (Ascorbic acid equivalent antioxidant capacity) values as mg of ascorbic acid equivalent (AAE)/100g of sample, determined from the calibration curve made with ascorbic acid. Appropriate solvent blanks were run in each assay. All determinations were performed in triplicate.

*Hydrophilic oxygen radical absorbance capacity assay (H-ORAC)*

The Hydrophilic ORAC method was carried out using Trolox as standard at controlled conditions of 37°C and pH 7.4. Peroxyl radicals were generated using [2,2’-azobis (2-amidinopropane) dihydrochloride] (AAPH). The stock solutions were 10.0 mM sodium fluorescein and 0.6 M AAPH dissolved in phosphate buffer solution (75 mM, pH 7.4). The working solution consisted of 21 µL of fluorescein, 3 µL of phosphate buffer solution, 30 µL of pure compound, and 50 µL of AAPH. Readings were carried out with an excitation wavelength of 493 nm (slit of 5 nm) and emission wavelength of 515 nm (slit of 13), using both an attenuator of 1% and without attenuator plate. ORAC values were calculated based on the net area under the curve (AUC), obtained by subtracting the AUC of the blank (phosphate buffer solution) from that of a sample (pure compound), and then compared to Trolox standard curve. The antioxidant capacity (ORAC) related to Trolox was estimated as:



Where *AUCsample* is the area under the curve of the pure compounds, *AUCblank* is the area under the curve for the blank, *AUCTrolox* is the area under the curve for Trolox, and *f* is the factor of dilution. Results were expressed in µmol TE/g of sample.

**Spectroscopic data of isolated compounds from *Garcinia madruno*.**

Glyceryl 1-stearate: IR (CHCl3) υmax (cm-1): 2918, 2852, 1734, 1469, 1178. 1H NMR (300 MHz, CDCl3): δ 4.27 (1H, dd, *J* = 11.7, 4.5, H-1a’), 4.23 (1H, dd, *J* = 11.7, 6.0, H-1b’), 3.97 (1H, qu, *J* = 4.5, H-2’), 3.76 (1H, dd, *J* = 11.4, 3.9, H-3a’), 3.66 (1H, dd, *J* = 11.4, 5.7, H-3b’), 2.40 (2H, t, *J* = 7.4, H-2), 1.67 (2H, qu, *J* = 7.4, H-3), 1.30 (28H, m), 0.92 (3H, t, *J* = 7.0, H-18). 13C NMR (75 MHz, CDCl3): δ 174.4 (C-1, C), 70.3 (C-2’, CH), 65.2 (C-1’, CH2), 63.4 (C-3’, CH2), 34.2 (C-2, CH2), 31.96 (C-16, CH2), 29.73-29.16 (12 CH2), 24.9 (C-3, CH2), 22.73 (C-17, CH2), 14.16 (C-18, CH3).

Garcinol: It was isolated as pale yellow needle crystals. UV (MeOH-H2O, 1:1) λmax (nm): 241, 270. IR (CHCl3) υmax (cm-1): 3373, 2949, 2835, 1730, 1654, 1032. 1H NMR (300 MHz, CDCl3): δ 7.03 (1H, dd, *J* = 8.0, 2.7, H-16), 7.02 (1H, d, *J* = 2.7, H-12), 6.64 (1H, d, *J* = 8.0, H-15), 5.14 (1H, m, H-25), 4.99 (1H, t, *J*= 7.0, H-18), 4.83 (1H, m, H-35), 4.44 (2H, d, *J* = 12.6, H-32a, H-32b), 2.81-1.49 (12H, m, allylic methylenes and methynes, H-6, H-29, H-34, H-7, H-24, H-17), 1.84 (3H, s), 1.80 (3H, s), 1.78 (3H, s), 1.74 (3H, s), 1.72 (3H, s), 1.64 (3H, s), 1.58 (2xCH3, s), 1.48-1.21 (6H, m, methyls, methylenes and methynes, H-30, H-22, H-23). 13C NMR (75 MHz, CDCl3): δ 209.6 (C-9, C=O, C), 199.3 (C-10, C=O, C), 195.5 (C-3, =C-OH, C), 194.3 (C-1, C=O, C), 150.3 (C-14, C), 148.5 (C-31, C), 144.2 (C-13, C), 135.7 (C-19, C), 133.4 (C-26, C), 132.4 (C-36, C), 128.1 (C-11, C), 124.5 (C-35, CH), 124.2 (C-16, CH), 123.0 (C-25, CH), 120.5 (C-18, CH), 116.9 (C-12, CH), 116.3 (C-3, C), 114.8 (C-15, CH), 113.1 (C-32, CH2), 70.2 (C-4, C), 58.3 (C-8, C), 50.1 (C-5, C), 47.2 (C-30, CH), 44.0 (C-6, CH), 43.1 (C-29, CH2), 36.6 (C-34, CH2), 33.0 (C-7, CH2), 29.3 (C-24, CH2), 27.4 (C-37, CH3), 26.8 (C-17, CH2), 26.5 (C-21, CH3), 26.3 (C-28, CH3), 26.2 (C-22, CH3), 26.1 (C-33, CH3), 23.1 (C-23, CH3), 18.6 (C-38, CH3), 18.3 (C-20, CH3), 18.0 (C-27, CH3).

Morelloflavone: It was isolated as yellow needle crystals. UV (MeOH-H2O, 1:1) λmax (nm): 249, 353. IR (CHCl3) υmax (cm-1): 3373, 2949, 2833, 1458, 1028, 670. 1H NMR (300 MHz, CDCl3): 13.12 (s, OH, OH-5), 12.31 (s, OH, OH-5’’), 7.31 (1H, d, *J* = 2.1, H-2’’’), 7.25 (1H, dd, *J* = 8.4, 2.1, H-6’’’), 7.10 (2H, d, *J* = 8.4, H-2’, H-6’), 6.86 (1H, d, *J* = 8.4, H-5’’’), 6.42 (2H, d, *J* = 8.4, H-3’, H-5’), 6.35 (1H, s, H-3’’), 6.25 (1H, s, H-6’’), 6.04 (1H, d, *J* = 2.1, H-6), 5.98 (1H, d, *J* = 2.1, H-8), 5.00 (1H, d, *J* = 12.0, H-2), 4.89 (1H, d, *J* = 12.0, H-3). 13C NMR (75 MHz, CDCl3): δ 198.2 (C-4, C), 182.4 (C-4’’, C), 170.8 (C-7, C), 166.8 (C-5, C), 164.3 (C-7’, C), 163.4 (C-8a, C), 163.0 (C-5’’, C), 161.1 (C-2’’, C), 157.4 (C-8a’’, C), 156.0 (C-4’, C), 149.5 (C-4’’’, C), 145.8 (C-3’’’, C), 129.2 (C-1’. C), 127.9 (C-2’, C-6’, CH), 122.1 (C-8’’, C), 119.3 (C-6’’’, CH), 115.4 (C-5’, CH), 114.2 (C-3’, C-5’, CH), 112.8 (C-2’’’, CH), 102.2 (C-4’’a, C), 101.9 (C-3’’, CH), 100.6 (C-4a, C), 98.5 (C-6’’, CH), 96.2 (C-8, CH), 95.2 (C-6, CH), 81.4 (C-2, CH), 49.5 (C-3, CH).

Volkensinflavone: It was isolated as yellow needle crystals. UV (MeOH-H2O, 1:1) λmax (nm): 288, 326. IR (CHCl3) υmax (cm-1): 3371, 2951, 2835, 1654, 1458, 1031. 1H NMR (300 MHz, CDCl3): 13.11 (s, OH, OH-5), 12.33 (s, OH, OH-5’’), 7.75 (2H, d, *J* = 8.0, H-2’’’, H-6’’’), 7.07 (2H, dd, *J* = 8.4, H-2’, H-6’), 6.93 (2H, d, *J* = 8.0, H-3’’’, H-5’’’), 6.41 (2H, d, *J* = 8.0, H-3’, H-5’), 6.26 (1H, s, H-6’’), 6.07 (1H, s, H-3’’), 6.03 (1H, d, *J* = 2.1, H-6), 5.97 (1H, d, *J* = 2.1, H-8), 5.72 (1H, d, *J* = 12.0, H-2), 5.03 (1H, d, *J* = 12.0, H-3). 13C NMR (75 MHz, CDCl3): δ 196.6 (C-4, C), 182.5 (C-4’’, C), 167.3 (C-7, C), 164.4 (C-2’’, C), 163.5 (C-5, C), 163.0 (C-8a, C), 161.9 (C-5’’, C), 161.1 (C-7’’, C), 157.4 (C-4’’, C), 156.0 (C-4’, C), 155.0 (C-8a’’, C), 129.2 (C-1’, C), 128.2 (C-2’’, C-6’’, CH), 127.8 (C-2’, C-6’, CH), 121.6 (C-1’’, C), 115.6 (C-3’’’, C-5’’’, CH), 114.2 (C-3’, C-5’, CH), 103.5 (C-4a’’, C), 102.7 (C-3’’, CH), 101.9 (C-8’’, C), 100.8 (C-4a, C), 96.1 (C-6’’, CH), 95.3 (C-8, CH), 95.1 (C-6, CH), 82.5 (C-2, CH), 49.6 (C-3, CH).