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Guajaphenone C and D: two novel benzophenone glycosides from *Psidium guajava* (Linn.) leaves

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Abstract A guided fractionation and characterization of the methanol extract from the leaves of *Psidium guajava* L. (Myrtaceae) yielded two new benzophenone glycosides, Guajaphenone C (**I**) and Guajaphenone D (**II**) together with three known compounds, Guajaphenone A (**III**), Garcimangosone D (**IV)** and Guaijaverin (**V)**. Their structures were elucidated by analysis of spectroscopic data including 1D and 2D NMR and electrospray ionization mass spectrometry (ESI-MS). These data were also compared with those reported in the literature.

Keywords Benzophenone glycoside; Fractionation; *Psidium guajava;* Novel compound; Guajaphenone

1. Introduction

Plants owe their nutritional and therapeutic effectiveness to their metabolic products such as alkaloids, polyphenols, glycosides, etc. [1, 2]. This has led to the extensive exploration of various species of plants in order to establish an evidence-based report on their phytochemical composition and its relationship to human health in the treatment of various ailments or as natural blueprint for the development of newer drugs [3].

Psidium guajava L. (commonly called guava) is a medicinal plant used in Nigeria and several tropical countries to treat many health disorders. Different parts of the plant have been used for the treatment of various human and veterinary ailments in traditional medicine as antibacterial, hypoglycaemic, anti-inflammatory, analgesic, antipyretic, spasmolytic and CNS depressant agent [4, 5]. Many glycosidic and flavonoid derivatives have been isolated from various extracts of the leaf, stem and root parts of the plant [6, 7].

Recently, our team reported the isolation and structure elucidation of a new benzophenone glycoside (Guajaphenone A) and two previously reported flavonoid glycosides from the methanol leaf extract of this plant material [8, 9]. Our further investigations on the bioactive compounds from this plant species have yielded two new novel flavonoid derivatives, Guajaphenone C and Guajaphenone D. The structures of the new compounds were elucidated based on the detailed analysis of their spectroscopic data and comparison with literature.

2. Method

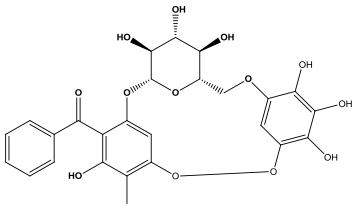
2.1 ***General experimental procedures*****:** 1D and 2D NMR spectra were recorded in DMSO-d6 or methanol-d4 on a Bruker DRX 500MHz 1H Larmor frequency using a 5mm QNP direct detection probe or AVANCE DMX 600 NMR spectrometers. HPLC/ESI-MS data were recorded on a Thermo-Finnigan LCQ-Deca mass spectrometer (Thermoquest, Germany) with an electrospray interface (ESI) coupled to an UV detector. HPLC-UV-DAD analysis

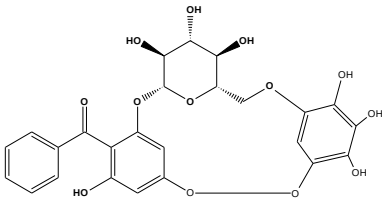
was carried out on a HP 1100 system equipped with a photodiode array detector (Agilent technologies, Palo Alto, CA) with a Eurospher C-18 column (5 mm, 125 X 4.6mm i.d; *Knauer*, Germany). The flow rate was at 400 µL/min and the absorbance detected at 254 nm with capillary temperature of 200 ^{0}C and drift voltage of 20eV. Analytical HPLC was performed with a Dionex P580A LPG pump equipped with a UV detector (UVD340S) using a Eurospher C-18 column (5mm, 125 X 4mm i.d; Knauer, Germany) while the semi-preparative HPLC was performed with Merck/Hitachi L-7100 pump coupled to a Merck/Hitachi UV detector (UV-L7400), photodiode array detector) using a Eurospher C-18 column (10mm, 300_8mm i.d; Knauer) . The detections were performed at 254 nm while a linear gradient of HPLC grade methanol and nanopure water were used for separation. Column chromatography was carried out using a Sephadex LH-20 column (3 X 60 cm) eluted with dichloromethane: MeOH (1:1) and the whole set up connected to a fraction collector (Retriever II, ISCO, Germany) and adjusted to a flow rate of 0.2 mL/min. TLC was performed on pre-coated TLC plates with Silica gel 60 F_{254} (layer thickness 0.2 mm, E. Merck, Darmstadt, Germany) with either CH_2Cl_2: MeOH (9:1) for semi-polar compounds or n-Hexane: EtOAc (8:2) for non-polar compounds as mobile phase. The compounds were detected by their UV absorption at 254 and 366 nm or by spraying the TLC plates with anisaldehyde reagent followed by heating at 110^{0}C. Measurements were done at the Institutes of Organic Chemistry and Pharmaceutical Biology/Biotechnology, Heinrich-Heine Universitat Düsseldorf, Germany.

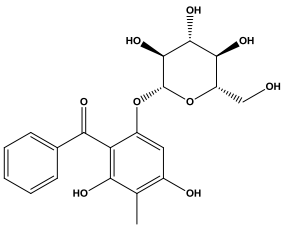
2.2 ***Plant material*****:** The leaves of the plant (*Psidium guajava L.*) were collected from the Botanical Reserved Area within the University of Port Harcourt, Nigeria between June and August. They were authenticated by the Plant Science & Biotechnology (PSB) Department of the University and the voucher specimen (UPHPCG0611) deposited in the Pharmacognosy herbarium of the Faculty of Pharmaceutical Sciences of the same institution. The leaves were prepared by drying under shade for 14 days. The dried leaves were pulverized and stored in air tight containers kept inside the refrigerator.

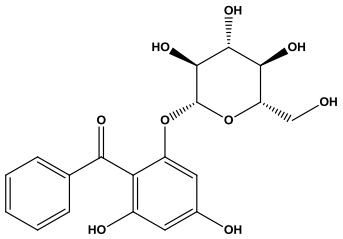
2.3 ***Extraction of plant materials and isolation of active constituents*****:** The pulverized air-dried leaves (600 g) were defatted with n-hexane and the dried marc (450 g) extracted with 5 L of 90 % methanol for 4 days at room temperature (25^{0}C) and the extract concentrated *in vacuo* with rotary evaporator. The dried methanol extract (35 g, 7.7 % w/w) was reconstituted in 20 mL of methanol, made up to 200 mL with distilled water, shaken for about 30 minutes and subjected to successive liquid-liquid extraction with chloroform (3 x 750 ml) and ethyl acetate (3 x 750 ml) to yield PsG-CF (6.0 g; 1.3% w/w), PsG-EF (10.7g; 2.4% w/w) and PsG-WF (8.6 g; 1.9% w/w) fractions respectively. A portion of PsG-EF (4.5 g) was separated on a Sephadex LH-20 column (3 X 60 cm) eluted with Dichloromethane: MeOH (1:1) to afford 10 pooled fractions PsG-EF1 to PsG-EF10. The fractions (PsG-EF1 to PsG-EF10) were subjected to analytical HPLC and the bioactive fraction, PsG-E4 was subjected to semi-preparative HPLC purification to isolate compounds **I** (3.2 mg), **II** (3.6 mg), **III** (3.5 mg), **IV** (3.0) mg) and **V** (4.5 mg).

3. Results and Discussion

I. 

II. 

III. 

IV. 

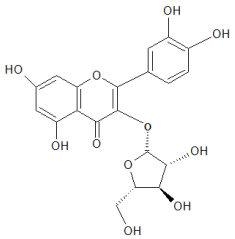
V. 

*Figure 1: Structure of Isolated Compounds: Guajaphenone C (**I**), Guajaphenone D (**II**), Guajaphenone A (**III**), Garcimangosone D (**IV**) and guaijaverin (**V**)*

The pulverized leaves of *Psidium guajava* L. leaves were initially defatted with n-hexane and then extracted with 90% methanol. The methanol extract was fractionated into chloroform and ethyl fractions by liquid-liquid partitioning. The ethyl acetate fraction (the active fraction) was further subjected to column chromatography on Sephadex LH-20 and finally purified using semi-preparative HPLC on reversed phase material to obtain two new phenolic compounds **I** & **II**, together with three known compounds guajaphenone A (**III**) Garcimangosone D (**IV)** and guaijaverin (**V)**.

3.1 Compound 1*:* Yield 3.2 mg; brownish semi-solid; UV (PDA): λ_{max} 262.0 and 362.0 nm;

^{1}H NMR (500 MHz, MeOD) δ = 7.50 (m, 2H), 7.41 (d, J=7.4, 1H), 7.27 (t, J=7.7, 2H), 7.10 (s, 1H), 6.15 (s, 1H), 4.76 (d, J=7.7, 1H), 4.55 (d, J=11.9, 1H), 4.34 (m, 1H), 3.50 (s, 2H), 2.50 (m, 1H), 2.02 (s, 3H).

^{1}H NMR (600 MHz, MeOD) δ = 7.50 (d, J=7.3, 2H), 7.41 (q, J=7.4, 1H), 7.27 (t, J=7.7, 2H), 7.10 (s, 1H), 6.15 (s, 1H), 4.77 (d, J=7.7, 2H), 4.55 (dd, J=1.9, 11.9, 2H), 4.35 (dd, J=4.6, 12.0, 1H), 3.51 (m, 1H), 3.32 (dd, 1H), 3. 28 (dd, 1H), 2.51 (dd, J=9.1, 17.8, 1H), 2.02 (s, 3H).

EI.MS m/z: 545.0 [M+1], 543.3 [M-1]

Compound **I** was isolated as brownish viscous semi-solid. It exhibited UV maxima at λ_{max} 262.0 and 362.0 nm. The molecular formula was deduced as $C_{26}H_{24}O_{13}$ based on the ESI-MS molecular ion peak at *m/z* 545.0 [M+H]+ in the positive mode and *m/z* 543.3 in the negative mode together with the analysis of ^{13}C NMR data. The ^{1}H and ^{13}C NMR data of **I** showed strong similarity with our previously reported benzophenone derivative, Guajaphenone A (**III**) [8, 9]. These included the presence of aromatic proton signals of the AA'BB'C system at δ_H 7.50 (2H, d, *J*= 7.3), 7.41 (1H, q, *J*= 7.4) and 7.27 (2H, t, *J*= 7.7) assigned to H-9/13, H-10/12 and H-11 respectively (Table 1). Compared to compounds **III** and **IV**, however, there was an aromatic singlet proton signal at δ_H 7.10 (1H, s) which neither 2D-COSY nor other heteronuclear correlations were able to assign as part of ring A or B of the compound. This suggested the presence of another aromatic nucleus (ring C) in compound **I**. Its ^{13}C-NMR spectrum confirmed this ring with signals between δ_C 115-125 ppm which were clearly absent in the spectra of Compounds **III** & **IV**. HMBC spectrum of the compound showed that this ring C proton is remotely linked to the signals at δ_C 108.6 (C-6").

Table 1: ^{1}H and ^{13}C-NMR data of compound I

Position	***δ_H***	***δ_C***	***HMBC***
1	-	105.0	
2	-	157.3	
3	-	115.5	
4	-	161.3	
5	6.15 s	93.6	1, 3, 4, 6
6	-	156.4	
7	-	177.7	
8	-	138.5	
9/13	7.50 d (7.3)	128.4	7, 11, 10, 12
10/12	7.27 t (7.7)	127.6	8, 9, 13
11	7.41 q (7.4)	134.2	9, 13
1'	4.77 d (7.7)	101.8	6
2'	2.51 dd	72.8	1', 3'
3'	3.28 dd	76.2	2', 4'
4'	3.32 dd	70.3	6'
5'	3.51 m	73.7	4'

6'	4.35 H_a (dd)	69.0	5'
	4.55 H_b (dd)		4'
1"	-	148.4	
2"	-	145.6	
3"	-	120.7	
4"	-	145.2	
5"	-	165.8	
6"	7.10 s	108.6	4, 1", 2", 4", 5"
3-CH_3	2.02 d (5.7)	8.0	2, 3, 4

NMR was measured at 600 MHz (^{1}H) and 150 MHz (^{13}C) (CD_3OD).

As in Compounds III and IV, compound I also showed proton signals from oxygenated carbon in the range of δ_H 2.51 to 4.55 ppm, which strongly suggested the presence of sugar moiety with an anomeric proton signal at δ_H 4.77 (d, *J*= 7.7). The analysis of the ^{1}H and ^{13}C NMR indicated the presence of a hexose-pyranose sugar with the trans diaxial orientation of all the sugar protons typical of the glucopyranose moiety. Also, the anomeric proton signal of the sugar observed as a doublet at δ_H 4.77 with diaxial coupling constant $J_{1',2'}$ = 7.7 Hz, confirmed a β-configuration and pyranose form of sugar unit [10, 11].

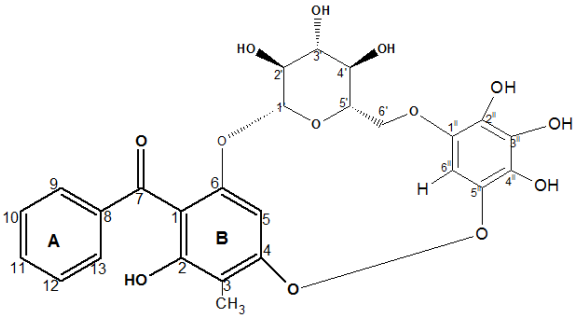


Figure 2: Numbering of Carbon Skeleton for Compound I

The proposed attachment of the sugar moiety at position 6 of the benzophenone skeleton was based on the observed correlation of the anomeric proton to C-6 (156.4 ppm) in HMBC. As observed in the ^{1}H-NMR spectrum of compound **III, I** also showed only one aromatic proton signal at δ_H 6.15 (1H, s) assigned to H-5 of ring B (Fig 2). The ^{1}H-NMR spectrum of **I** also showed an additional signal at the SP^3 hybridized C-H region (δ_H 2.02: 3H, s) integrating for three protons indicating the presence of a methyl carbon at C-3(CH_3). This suggested the presence of a methyl group at this position. A strong HMBC correlation of the methyl proton signals to C-3 supported the attachment of the methyl group at this position. Similarly, a HMBC correlation between the proton signal at δ_H 7.10 to C-4 (161.3 ppm), C-1" (148.4 ppm), C-2" (145.6 ppm), C-4" (145.2 ppm), and C-5" (165.8 ppm) suggested a link or bond between aromatic rings B and C. Compound **I** was thus elucidated with the name 2,4-dihydroxy-3-methyl-6-O-βD-glucopyranosylbenzophenone(4→5", 6'→1")benzene-2",3",4",5"-tetraol, for which we have proposed the trivial name *Guajaphenone C*. To the best of our knowledge, this compound has not been previously reported in any literature.

3.2 Compound II*:* Yield 3.6 mg; Brownish-yellow Semi-solid

^{1}H NMR (500 MHz, MeOD) δ = 7.61 (d, J=8.4, 2H), 7.44 (s, 1H), 7.31 (t, J=7.8, 2H), 7.09 (s, 1H), 6.16 (s, 1H), 6.05 (s, 1H), 4.50 (s, 1H), 4.33 (s, 1H), 3.58 (s, 1H), 2.77 (s, 1H).

UV λmax (MeOH) nm (Ɛ): 265; 294.

Compound **II** was isolated as brownish-yellow semi-solid with UV maxima at λ_{max} 265.0 and 294.0 nm and a molecular formula of $C_{25}H_{22}O_{13}$ which was deduced based on the ESI-MS molecular ion peak at 531.4 [$M+H^+$]. The ^{1}H and ^{13}C NMR data of **II** showed strong similarity with **I** in signals across the three aromatic rings and the sugar

moiety. As observed with compounds **III** & **IV**, the difference between the calculated positive ionization peak for **I** and **II** is -14, a value that is equivalent to the mass of a methylene group (-CH_2) and which could imply the loss of a methyl (-CH_3) group for a proton in this instance. Thus, the 1H-NMR spectrum of **II** showed signal for two different protons at δ_H 6.05 (1H, s) assigned to H-3 and δ_H 6.16 (1H, s) assigned to H-5 of ring B as against the lone signal δ_H 6.15 (1H, s) assigned to H-5 at the same aromatic region in compound **I** (Table 2).

Table 2: ^{1}H and ^{13}C-NMR data of compound II

Position	$\boldsymbol{\delta_H}$	$\boldsymbol{\delta_C}$	***HMBC***
1	-	105.0	
2	-	157.3	
3	6.05 s	115.5	
4	-	161.3	
5	6.16 s	93.6	1, 3, 4, 6
6	-	156.4	
7	-	177.7	
8	-	138.5	
9/13	7.61 d (8.4)	128.4	7, 11, 10, 12
10/12	7.31 t (7.8)	127.6	8, 9, 13
11	7.44 s	134.2	9, 13
1'	4.70 s	101.8	6
2'	2.77 s	72.8	1', 3'
3'	3.18 s	76.2	2', 4'
4'	3.42 s	70.3	6'
5'	3.58 s	73.7	4'
6'	4.33 dd H_a	69.0	5'
	4.50 dd H_b		4'
1"	-	148.4	
2"	-	145.6	
3"	-	120.7	
4"	-	145.2	
5"	-	165.8	
6"	7.09 s	108.6	4, 1", 2", 4", 5"

NMR was measured at 500 MHz (^{1}H) and 150 MHz (^{13}C) (CD_3OD).

Other analytical data were very similar to that of Compound **I** in all the regions. Compound **II** was thus elucidated as the demethylated analogue of **I** with the name 2,4-dihydroxy-6-O-βD-glucopyranosylbenzophenone (4→5", 6'→1") benzene-2",3",4",5"-tetraol. We have proposed the trivial name *Guajaphenone D* for this compound. To the best of our knowledge, this compound too has not been previously reported in any literature.

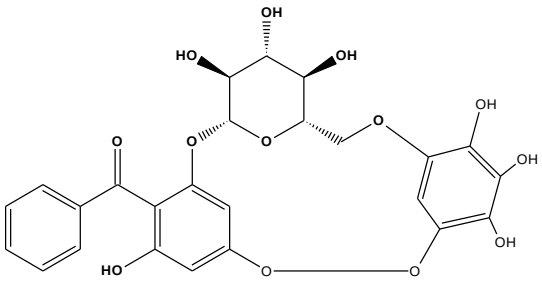


Figure 3: Structure of Compound II

Compound **III** was isolated as brownish-yellow semisolid. It exhibited UV maxima at λ_{max} 250.0 and 302.0 nm. The molecular formula was deduced as $C_{20}H_{22}O_9$ based on the ESI-MS molecular ion peak at *m/z* 406.8 [M+H]+ in the positive mode and 405.3 in the negative mode together with the analysis of ^{13}C NMR data. Analysis of the ^{1}H and ^{13}C NMR data of **III** showed that the compound is the previously reported benzophenone glycoside with the trivial name, guajaphenone A. The structure of the compound was elucidated by comparison of its 1D and 2D NMR data with those reported in the literature [8, 9].

Compound **IV** was isolated as yellowish semisolid with UV maxima at λ_{max} 254.0 and 296.0 nm. Its molecular formula was deduced as $C_{19}H_{20}O_9$ based on the ESI-MS molecular ion peak at 392.8 $[M+H]^+$. Analysis of the ^{1}H and ^{13}C NMR data of **IV** showed that it was the previously reported compound, Garcimangosone D. The structure of the compound was elucidated by comparison of its 1D and 2D NMR data with those reported in the literature [12-14].

Compound **V** was isolated as a brownish-yellow needle-like solid. It showed UV maxima at λ_{max} 257.0 and 354.0 nm. The molecular formula was deduced as $C_{20}H_{18}O_{11}$ based on the ESI-MS molecular ion peak at 435.0 $[M+H]^+$. Analysis of the ^{1}H and ^{13}C NMR data of 3 showed that the compound is the previously reported quercertin-3-O-α-L-arabinofuranoside with the trivial name, guaijaverin. The structure of the compound was elucidated by comparison of its 1D and 2D NMR data with those reported in the literature [11].

4. Conclusion

The further characterization of the methanol leaf extract of *Psidium guajava* yielded two new benzophenone glycosides Guajaphenone C (**I**) and Guajaphenone D (**II**) together with three known compounds guajaphenone A (**III**), garcimangosone D (**IV**) and guaijaverin **(V)**. The presence of these compounds may further explain the efficacy of extracts of *Psidium guajava* in the management of various disease states.

Acknowledgment

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