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A New Benzophenone from *Garcinia benthamiana*

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Abstract: A detailed phytochemical study on the stem bark of *Garcinia benthamiana* has afforded a new benzophenone, benthamianone (**1**), together with congestiflorone (**2**), a mixture of stigmasterol (**3**) and *γ*-sitosterol (**4**) as well as a mixture of phloroglucinol (**5**) and methyl palmitate (**6**). The structural elucidation of these compounds was performed through analysis of their spectroscopic data (NMR spectra and MS). This is the first report on the phytochemistry of *Garcinia benthamiana*.

Keywords: *Garcinia benthamiana*; Clusiaceae; benzophenone.

1. Introduction

Garcinia (Clusiaceae) species are mostly tropical green trees that are fairly widespread in tropical countries like Malaysia, Thailand and Brazil. They are traditionally used for numerous illnesses. *Garcinia oblongifolia* Champ is used to treat burns and inflammation[1] whereas *Garcinia cambogia* L. is used in cancer, ulcer and haemorrhoid treatments[2]. The medicinal properties of *Garcinia* species, such as antibacterial [3], antioxidant [4] and cytotoxic activities [5] are due to the high content of polyisoprenylated benzophenones and xanthones which possess these biological activities [6].

Garcinia benthamiana is locally known as Bacuri-pari or Bacuri of Nozzle. This plant was first found in the terra firme forest of the Amazon forest. This plant is a medium sized tree and can grow up to 20 metres in height. The tree trunk of this plant has a diameter of 15 to 40 cm. The fruits of Bacuri-pari are very sweet and refreshing. So far, there are no reports on this plant. Herein, we report the isolation and structural elucidation of a new benzophenone, benthamianone from *Garcinia benthamiana*, total phenolic content, DPPH free radical scavenging activity as well as the antibacterial activities of the extracts of *G. benthamiana*.

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2. Materials and Methods

2.1. General experimental procedures

UV spectra were recorded in $CHCl_3$ on a Shimadzu UV-160A UV-Visible Recording Spectrophotometer (Shimadzu Scientific Instruments, Kyoto, Japan). IR spectra were measured *via* the universal attenuated total reflection technique on a Perkin-Elmer 100 Series FT-IR spectrometer (Perkin Elmer, Waltham, MA, USA). The 1D (^1H, ^{13}C, and DEPT) and 2D (COSY, HMQC and HMBC) NMR spectra were recorded on a JNM-ECX500 500MHz NMR(Jeol, Tachikawa, Japan) in $CDCl_3$ (tetramethylsilane as the internal standard). EI-MS were measured using a Shimadzu GC-MS model QP2010 Plus spectrophotometer (Shimadzu Scientific Instruments, Kyoto, Japan).

2.2. Extraction and isolation

The stem bark of *G. benthamiana* (2.8 kg) was air-dried and ground into powder form. The powdered material was then macerated in hexane, chloroform ($CHCl_3$), ethyl acetate (EtOAc) subsequently for 72 hours (5 L × 3) at room temperature. All three hexane extracts were combined and concentrated in a rotary evaporator to give 14.74 g. The same procedure was repeated in $CHCl_3$ and EtOAc to obtain extracts weighing 19.00 g of $CHCl_3$ extract and 213.00 g of EtOAc extract. All the extracts were subjected to silica gel-packed vacuum column chromatography and eluted with a stepwise gradient system of hexane-$CHCl_3$, $CHCl_3$-EtOAc and EtOAc-MeOH. The hexane extract furnished 11 fractions from the vacuum column while EtOAc extract furnished nine fractions. The seventh fraction of the hexane extract was subjected to gravity column chromatography by eluting with hexane-$CHCl_3$ with increasing polarity to produce 14 fractions. The fourth and fifth fractions were combined and defatted with hexane. This produced a mixture of stigmasterol (**3**) and *γ*-sitosterol (**4**). Fraction thirteen was further purified using repeated column chromatography by eluting it with hexane-EtOAc (9:1) and hexane-$CHCl_3$ (3:7). This afforded 12.2mg of benthamianone (**1**) and 4mg of congestiflorone (**2**). Meanwhile, the vacuum column chromatographic separation of the EtOAc extract yielded four fractions. The second fraction was fractionated repeatedly by eluting with $CHCl_3$-EtOAc (3:2) and $CHCl_3$-MeOH (9:1). This produced a mixture of phloroglucinol (**5**) and methyl palmitate (**6**).

2.3. Spectral Data

Benthamianone (**1**): Yellow gum. UV (EtOH) λ_{max} (log ε): 214 (4.55) and 313 (4.18) nm; IR *ν* (cm^{-1}): 3262, 2925, 1721, 1609, 1449, 1274, 1127 and 700; for ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125MHz, $CDCl_3$) spectral data, see Table 1; EIMS *m/z* (rel. int.): 434 (18), 283 (20), 281 (20), 244 (23), 243 (100), 165 (60), 105 (27), 77 (14), 69 (34) and 55 (11); HR-ESI-MS *m/z* : 435.2181 $[M+H]^+$ (calculated for $C_{28}H_{35}O_4$, 435.2535) .

2.*4. Antibacterial Assay*

All the *G. benthamiana* extracts were assessed for *in vitro* antibacterial assay *via* disc diffusion (Kirby-Bauer) method, as described in literature [7]. Two bacteria were used in this assay, which included *Staphylococcus aureus* S 276 and *Bacillus subtilis* B 145. *Streptomycin* (10 mg/mL) was used as positive control. The bacteria were purchased from Institute for Medical Research, Malaysia.

2.5. DPPH Radical Scavenging Activity

The DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging assay was performed according to the method described by Yen and Hsieh [8] with some modifications. Various concentrations of extracts, ranging from 250 to 3.13 µg/mL were prepared by dissolving the extracts in EtOH. The assay were performed in 96 well plate by adding 40 µL of 0.6mM EtOH solution of DPPH radical to 60 µL of various concentration of tested extracts. The mixture was incubated in a dark room for 30 minutes before measuring the absorbance at 517 nm on a spectrophotometer. EtOH was used as blank in the

same plate. Ascorbic acid was used as reference antioxidant. The percentage of DPPH radical scavenging was calculated based on the formula below:

$$\%\ DPPH\ radical\ scavenging\ capacity = \frac{(OD_{blank} - OD_{sample})}{OD_{blank}} \times 100\%$$

Where OD_{blank} is the absorbance of EtOH with DPPH solution while OD_{sample} is the absorbance of sample with DPPH solution. The experiment was performed in triplicate and averaged.

2.6. Total Phenolic Content (TPC)

The TPC of *G. benthamiana* crude extracts was performed as described by Kahkonen et. al. [9] with minor modifications. Briefly, sample extracts (100 µL) were added to a mixture of 750 µL of Folin Ciocalteu's phenol reagent and 750 µL of $NaHCO_3$ (60g $NaHCO_3$/L distilled H_2O) in a 24 well plate. The plate was measured at 725 nm after being incubated in a dark room for 30 minutes. The calibration equation for gallic acid was $y = 0.002053x - 0.02435$ with $R^2 = 0.9993$. Hence, TPC was expressed in g gallic acid equivalents (GAE)/100g of crude extracts. The experiment was performed in triplicates and averaged.

3. Results and Discussion

3.1. Structure elucidation

Repeated column chromatography of the hexane extract has led to the isolation of congestiflorone (**2**) together with a new benzophenone, benthamianone (**1**). Compounds **3** to **6** were isolated from the chloroform ($CHCl_3$) and ethyl acetate (EtOAc) extracts. The structures of these compounds were elucidated by analysing their spectroscopic data and comparing with those in literature. (See Figure 1)

Compound **1** was isolated as a yellow gum. It has a molecular formula of $C_{28}H_{34}O_4$, which was deduced from the molecular ion peak at *m/z* 434 as shown in the EI-MS spectrum. The high resolution ESI mass spectrum (HR-ESI-MS) gave a positive molecular ion peak at *m/z* 435.2181 $[M+H]^+$ corresponding to the molecular formula $C_{28}H_{35}O_4$. The FTIR absorption suggested the presence of OH (3262 cm^{-1}), alkane CH stretching (2925 cm^{-1}), C=O of ketone group (1721 cm^{-1}), aromatic C=C (1609 and 1449 cm^{-1}), C-O of alcohol group (1274 and 1127 cm^{-1}) and alkene (700 cm^{-1}). From the UV-visible spectrum, the maximum absorption peaks at 214 and 313 nm were indicative of the presence of a benzophenone skeleton.

A singlet peak at δ_H 12.10 (s, 1H, 2-OH) observed in ^{1}H NMR showed the presence of a chelated OH group. The OH group was positioned at C-2 due to the ^2J and ^3J correlations of this hydroxy proton with C-2 (δ_C 163.7), C-1(δ_C 105.9) and C-3(δ_C 95.1), as seen in the HMBC spectrum (See Figure 2). The DEPT spectrum revealed C-1 and C-2 to be quaternary carbons while C-3 is a methine carbon. A sharp singlet proton signal which appeared at δ_H 6.00 (s, 1H, H-3) in the ^{1}H NMR spectrum has a ^1J connectivity with C-3 (δ_C 95.1) and ^2J and ^3J correlations with quaternary carbons C-2 (δ_C 163.7), C-4 (δ_C 156.7), C-1 (δ_C 105.9), C-5 (δ_C 100.1) and C-6 (δ_C 160.8) in the HMBC spectrum. Hence this sharp singlet was assigned to C-3.

The presence of a mono-substituted phenyl group (ring B) was confirmed through a series of aromatic proton signals seen in the ^{1}H NMR spectrum, which were a doublet at δ_H 7.48 (2H, J = 6.9 Hz, overlapped) and two sets of triplets at δ_H 7.35 (2H, J = 7.5 Hz, overlapped) and δ_H 7.42 (1H, J = 7.5 Hz). The DEPT and HMQC spectra showed that the overlapped methine carbons at δ_C 127.6 (C-2' and C-6') in the phenyl group were connected to the overlapped doublet protons at δ_H 7.48 (H-2' and H-6') and the methine carbons at δ_C 127.7 (C-3' and C-5', overlapped) with the triplet protons at δ_H 7.35 (H-3' and H-5'). The triplet proton at δ_H 7.42 (H-4') was connected to δ_C 130.4 (C-4') through ^1J correlation. In the HMBC spectrum, δ_H 7.35 (H-3' and H-5') has ^3J correlations with δ_C 142.7 (C-1'). The same correlation was also observed for δ_H 7.48 (H-2' and H-6') with δ_C 130.4 (C-4') and δ_C 200.2

(C-7) respectively. These correlations deduced that the phenyl group was connected to C-7 in this benzophenone skeleton.

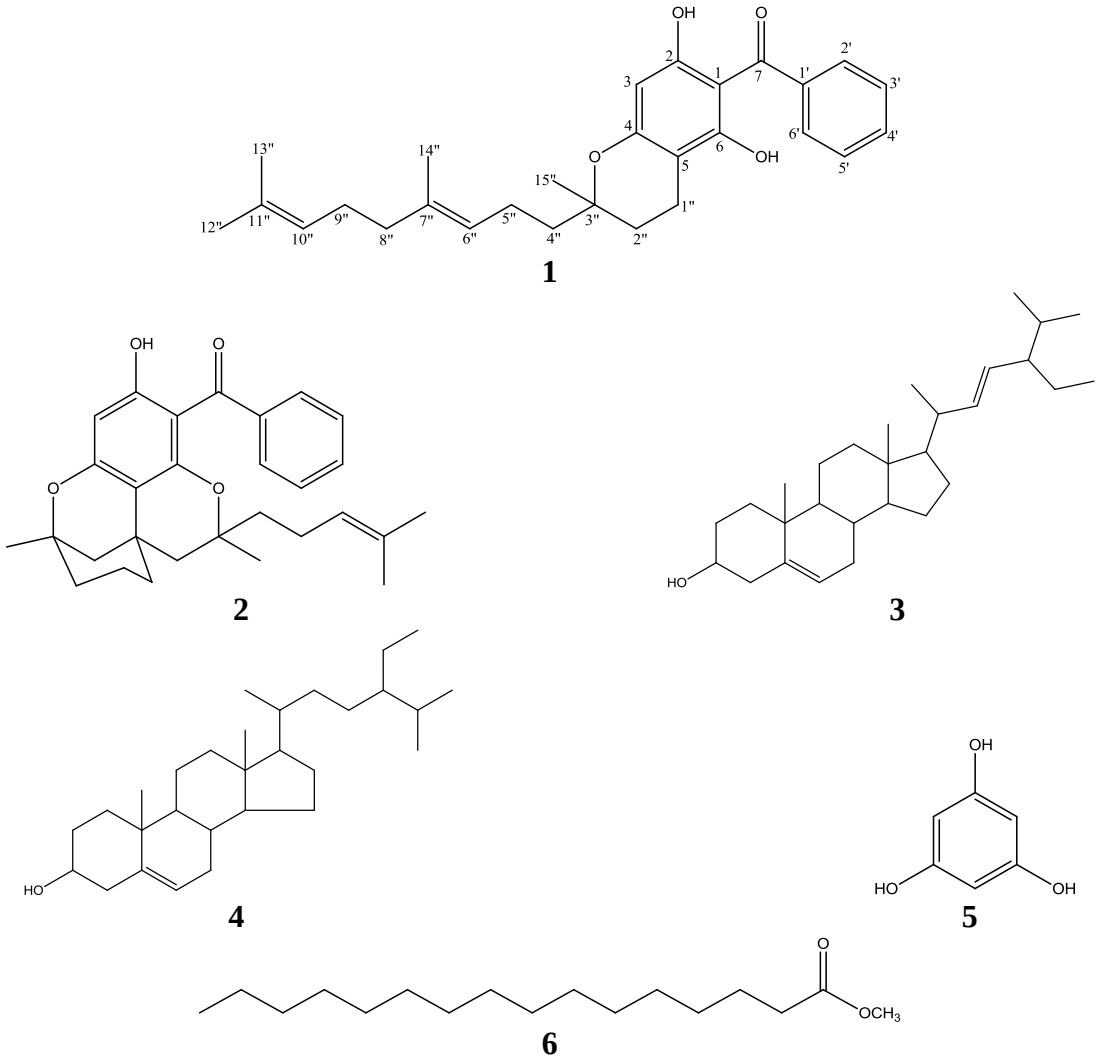

Figure 1. Structures of compounds **1**-**6**

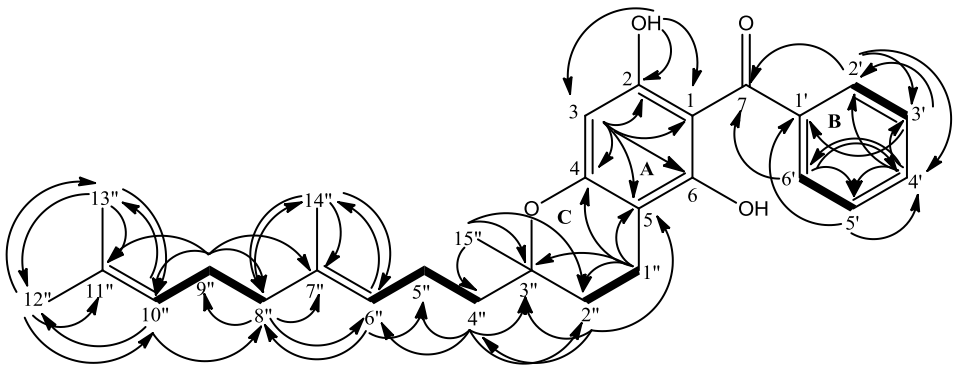

Figure 2. Arrows denote key HMBC cross-peaks and bold lines indicate COSY correlations for compound **1**

Other than that, the protons at δ_H 2.53 (m, 2H, H-1") and δ_H 1.62 (m, 2H, H-2") were seen to be coupled in the COSY spectrum. The proton resonance at δ_H 0.92 (s, 3H, H-15") indicated the presence of a 2-methyltetrahydro-2*H*-pyran (ring C). In the HMQC spectrum, δ_H 2.53 was found to be

directly bonded to δ_C 16.0 (C-1") while δ_H 1.62 was directly linked to δ_C 29.9 (C-2"). The DEPT spectrum suggested that C-1" and C-2" were methylene carbons. Besides, the short range ^2J correlations of δ_H 2.53 (m, 2H, H-1") with δ_C 1.62 (C-2") and δ_C 100.1 (C-5) as well as the long range ^3J correlations of this same proton (δ_H 2.53) with δ_C 77.7 (C-3") and δ_C 156.7 (C-4) demonstrated that the 2-methyltetrahydro-2*H*-pyran was fused to the benzophenone skeleton at C-4 and C-5. The methyl substituent of 2-methyltetrahydro-2*H*-pyran was located at C-3" due to the correlations of δ_H 0.92 with δ_C 29.9 (C-2"), δ_C 77.7 (C-3") and δ_C 39.8 (C-4").

Table 1. ^{1}H NMR and ^{13}C NMR spectroscopic data for compound **1** in $CDCl_3$.

Position	δ_H	δ_C
1		105.9
2		163.7
3	6.00 (*s*, 1H)	95.1
4		156.7
5		100.1
6		160.8
7		200.2
1'		142.7
2' & 6'	7.48 (*br d*, 2H, *J* = 6.9 Hz)	127.6
3' & 5'	7.35 (*t*, 2H, *J* = 7.5 Hz)	127.7
4'	7.42 (*t*, 1H, *J* = 7.5 Hz)	130.4
1"	2.53 (*m*, 2H)	16.0
2"	1.62 (*m*, 2H)	29.9
3"		77.7
4"	1.16 (*t*, 2H, *J* = 8.6 Hz)	39.8
5"	1.42 (*quin*, 2H, *J* = 7.5 Hz)	21.6
6"	4.85 (*t*, 1H, *J* = 6.9 Hz)	123.9
7"		135.2
8"	1.92 (*t*, 2H, *J* = 7.5 Hz)	39.7
9"	2.02 (*q*, 2H, *J* = 7.5 Hz)	26.7
10"	5.06 (*t*, 1H, *J* = 6.9 Hz)	124.3
11"		131.5
12"	1.68 (*s*, 3H)	25.8
13"	1.59 (*s*, 3H)	17.8
14"	1.48 (*s*, 3H)	16.0
15"	0.92 (*s*, 3H)	22.6
2-OH	12.10 (s, 1H)	

Further examination of the NMR spectra for benthamianone indicated the existence of 4,8-dimethylnona-3,7-dien-1-yl moiety. The proton resonance at δ_H 1.42 (quin, 2H, *J* = 7.5 Hz, H-5") was coupled to δ_H 1.16 (t, 2H, *J* = 8.6 Hz, H-4") and δ_H 4.82 (t, 1H, *J* = 6.9 Hz, H-6") while δ_H 2.02 (q, 2H, *J* = 7.5 Hz, H-9") was coupled with δ_H 1.92 (t, 2H, *J* = 7.5 Hz, H-8") and δ_H 5.06 (t, 1H, *J* = 6.9 Hz, H-10") respectively. These couplings were seen in the COSY spectrum. The HMBC spectrum exhibited the connections between δ_H 1.48 (s, 3H, H-14") with δ_C 123.9 (C-6"), δ_C 135.2 (C-7") and δ_C 39.7 (C-8"); δ_H 1.59 (s, 3H, H-13") with δ_C 124.3 (C-10"), δ_C 131.5 (C-11") and δ_C 25.8 (C-12"); as well as δ_H 1.68 (s, 3H, H-12") with δ_C 124.3 (C-10"), δ_C 131.5 (C-11") and δ_C 17.8 (C-13"). These connections were linked to each other through C-9" (δ_C 26.7). This was seen *via* the ^2J and ^3J correlations of δ_H 2.02 (H-9") with δ_C 39.7 (C-8"), δ_C 124.3 (C-10"), δ_C 135.2 (C-7") and δ_C 131.5 (C-11"). Further analysis of the HMBC spectrum concluded that the 4,8-dimethylnona-3,7-dien-1-yl moiety was attached to C-3" of the 2-methyltetrahydro-2*H*-pyran ring by cross peaks between δ_H 1.16 (H-4") with δ_C 21.6 (C-5"), δ_C 123.9 (C-6"), δ_C 77.7 (C-3") and δ_C 29.9 (C-2").

3.2. Antibacterial activity

The hexane, $CHCl_3$ and EtOAc extracts of *Garcinia benthamiana* were screened for antibacterial activities. The diameters of inhibition zones for the positive control, *streptomycin*, and extracts are listed in Table 2. It was deduced that all the extracts of *Garcinia benthamiana* possess weak inhibitory activities against *Staphylococcus aureus* and *Bacillus subtilis*.

Table 2. Antibacterial activities of hexane, $CHCl_3$ and EtOAc extracts of *G. benthamiana.*

	Diameter of the inhibition zone (mm)	
***G. benthamiana* Extracts**	***Staphylococcus aureus* (S 276)**	***Bacillus subtilis* (B 145)**
Hexane	7	7
$CHCl_3$	7	8
EtOAc	7	8
Streptomycin[a]	29	26

[a] Positive control

3.3. Total phenolic content and DPPH radical scavenging activity

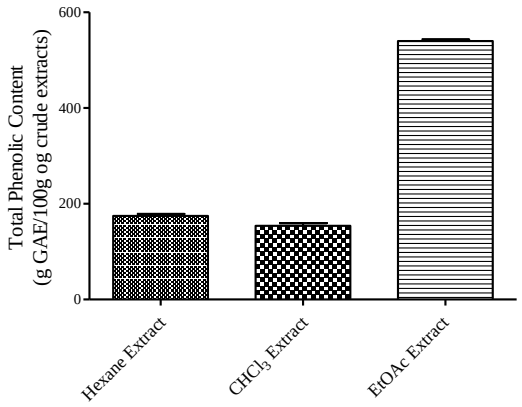

Figure 3. Total phenolic content (g GAE/100g of crude extracts) for hexane, $CHCl_3$ and EtOAc extracts of *Garcinia benthamiana*. Results are presented as means and SD of triplicate analysis.

All the crude extracts of *Garcinia benthamiana* were tested for total phenolic content (TPC) (See Figure 3) and DPPH radical scavenging activity (See Table 3). Ascorbic acid with an EC_{50} value of 19.28 µg/mL was used as a reference antioxidant in the DPPH radical scavenging assay. The correlation between TPC and DPPH radical scavenging activity [10] was determined in this experiment. The correlation is such that the higher the TPC, the lower the EC_{50} values. High EC_{50} values of 240.38 µg/mL and 225.09 µg/mL were obtained for the hexane and $CHCl_3$ extracts respectively in the DPPH radical scavenging activity tests. This implies that the hexane and $CHCl_3$ extracts possess weak scavenging activity. Moreover, these two extracts also gave low total phenolic content values. The EtOAc extract of *G. benthamiana* on the other hand, showed high total phenolic content and moderate antioxidant activity (EC_{50} = 60.78 µg/mL) compared to ascorbic acid.

Table 3. DPPH radical scavenging activity of hexane, $CHCl_3$, EtOAc extracts of *G. Benthamiana.*

***G. benthamiana* Extracts**	**EC_{50} [b] (µg/mL)**
Hexane	240.38 ± 8.38
$CHCl_3$	225.09 ± 5.01
EtOAc	60.78 ± 2.17
Ascorbic Acid [a]	19.28 ± 1.94

[a] Reference antioxidant

[b] EC_{50} values were determined through interpolation from linear regression analysis. All EC_{50} values were expressed as means ± standard deviation (n = 3).

4. Conclusions

One new benzophenone, benthamianone (**1)** along with congestiflorone (**2**), a mixture of stigmasterol (**3**) and γ-sitosterol (**4**) as well as a mixture of phloroglucinol (**5**) and methyl palmitate (**6**) were successfully isolated from the stem bark of *Garcinia benthamiana*. All the extracts (hexane, $CHCl_3$ and EtOAc extracts) of *G. benthamiana* showed weak antibacterial activities against *Staphylococcus aureus* and *Bacillus subtilis.* The EtOAc extracts of stem bark of this plant exhibited high level of TPC and moderate DPPH radical scavenging activity. However, the hexane and $CHCl_3$ extracts showed low TPC level and weak DPPH radical scavenging activity.

Acknowledgments

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