Antibacterial and Antioxidant Xanthones and Benzophenone from *Garcinia smeathmannii*

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Key words
- *Garcinia smeathmannii*
- Guttiferae
- xanthone
- benzophenone
- antibacterial
- antioxidant

Abstract

A new prenylated xanthone, 1,3,5,8-tetrahydroxy-2-(3-methybut-2-enyl)-4-(3,7-dimethyl-octa-2,6-dienyl) xanthone (**1**), and a new benzophenone (**2**), together with four known xanthone derivatives, cheffouxanthone (**3**), smeathxanthone A (**4**), smeathxanthone B (**5**), ananixanthone (**6**), and two pentacyclic triterpenes, *epi*-friedelinol (**7**) and friedelin (**8**), were isolated from the stem bark of *Garcinia smeathmannii*. The structures of the compounds were elucidated on the basis of 1D and 2D NMR experiments, and compound **2** was further characterized and confirmed by single X-ray analysis. Compounds **1, 2**, and **3** exhibited the most prominent antibacterial activity against gram-positive *Enterococcus faecalis* with minimal inhibitory concentration values of 8, 8, and 2 µg/mL, respectively, while compounds **1, 3, 4**, and **6** showed the capacity to scavenge free radicals.

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Introduction

Plants of the genus *Garcinia* (Guttiferae) have been extensively investigated from both phytochemical and biological points of view, and they are well known as a rich source of natural xanthones [1], biflavonoids [2], benzophenones [2] as well as triterpenoids [3].

Phenolic constituents from *Garcinia* species have been reported to display antibacterial [4–6], cytotoxic [7], prooxidant [8] as well as anticancer activities [9] and to inhibit α-glucosidase [10] and HIV [11]. *Garcinia smeathmannii* Oliver (syn. *Garcinia barteri*) is widely distributed in the lowland tropical rainforests of Western and Central Africa [12–14]. *G. smeathmanii* is used as an antidote for many poisons and for the treatment of ophthalmia [14]. Previously, xanthones, benzophenones, and triterpenes from the root and stem bark of *G. smeathmannii* were reported by our research group [15–17].

In the course of our continuing phytochemical investigation on *Garcinia* plants found in Cameroon, phytochemical studies of the stem bark of *G. smeathmannii* and their biological activities were carried out. We report herein the isolation and characterization of one new xanthone and one new benzophenone, together with four known xanthones and two pentacyclic triterpenes. We also report the antibacterial and antioxidant activities of the isolated compounds.

Results and Discussion

A phytochemical investigation of the methanol extract of *G. smeathmannii* led to the isolation of a new prenylated xanthone, 1,3,5,8-tetrahydroxy-2-(3-methybut-2-enyl)-4-(3,7-dimethyl-oct-2,6-dienyl) xanthone (**1**), and a new benzophenone (**2**), along with four known xanthone derivatives, cheffouxanthone (**3**) [15], smeathxanthone A (**4**) [16], smeathxanthone B (**5**) [16], and ananixanthone (**6**) [18], and two pentacyclic triterpenes, *epi*-friedelinol (**7**) [19] and friedelin (**8**) [17]. Compounds **1, 2, 3, 4**, and **6** were tested for their antibacterial and antioxidant activities. In addition, the known compounds **3, 4, 5**, and **8** were previously isolated from the root and stem bark of *G. smeathmannii* [15–17], while compound **6** was reported in *Garcinia paucinervis* [18]. Compound **7** is reported in *Garcinia* species for the first time.

Compound **1** was obtained as a yellow powder, and its molecular formula was assigned to be $C_{28}H_{32}O_6$ from its HR-EI-MS and NMR data (**Ta-**

Table 1 ^{1}H NMR (500 MHz) and ^{13}C-NMR (125 MHz) data of compound **1** in d_6-acetone.

No.	^{1}H (multi.; *J* in Hz)	^{13}C	HMBC
1	–	158.8	
2	–	111.6	
3	–	162.3	
4	–	107.7	
5	–	138.1	
6	7.31 (d; *J* = 8.8)	124.6	138.1; 145.0; 154.2
7	6.62 (d; *J* = 8.8)	109.9	108.3; 138.1; 154.2
8	–	154.2	
9	–	186.0	
4a	–	153.6	
8a	–	108.3	
9a	–	102.7	
10a	–	145.0	
1′	3.68 (d; *J* = 7.2)	22.2	107.7; 122.8; 136.7; 153.6; 162.3
2′	5.31 (t; *J* = 7.2)	122.8	16.4; 22.2; 40.4
3′	–	136.7	
4′	1.98 (t; *J* = 7.0)	40.4	16.4; 27.2; 122.8; 136.7
5′	2.05 (m)	27.2	40.4; 124.6; 131.7
6′	5.04 (d; *J* = 7.2)	124.9	17.6
7′	–	131.7	
8′	1.56 (s)	25.7	17.6; 124.9; 131.7
9′	1.51 (s)	17.6	25.7; 124.9; 131.7
10′	1.86 (s)	16.4	40.4; 122.8; 136.7
1′′	3.45 (d; *J* = 7.3)	22.0	111.6; 122.7; 132.7; 158.8; 162.3
2′′	5.23 (d; *J* = 7.3)	122.7	18.0; 25.8
3′′	–	132.7	
4′′	1.66 (s, 3H)	25.8	18.0; 122.7; 132.7
5′′	1.79 (s, 3H)	18.0	25.8; 122.7; 132.7
1-OH	12.34 (s)	–	102.7; 111.6; 158.8
3-OH	–	–	–
5-OH	–	–	–
8-OH	11.31 (s)	–	108.3; 109.9; 154.2

ble 1), indicating thirteen double bond equivalents. The IR spectrum displayed hydroxyl (3350 cm^{-1}), carbonyl (1720 cm^{-1}), and aromatic (1627, 1617, 1580 cm^{-1}) absorptions. The UV spectrum showed absorption characteristic for a tetrahydroxylated xanthone [20,21]. The ^{1}H and ^{13}C NMR data (**Table 1**) of compounds **1** and **3** were similar. The difference was observed at position 2 where the proton signal at δ_H 6.38 (s) in compound **3** disappeared in compound **1** and by the presence of new signals at δ_H 5.23 (t, *J* = 7.3 Hz), 3.45 (d, *J* = 7.3 Hz), 1.66 (s), and 1.79 (s), characteristic of a prenyl group. This was confirmed by ^{13}C NMR spectra of compound **1** with five more signals at δ_C 22.0, 122.7, 132.7, 25.8, and 18.0. In the HMBC spectrum, the signal of the benzylic proton δ_H 3.68 (H-1′) of the geranyl substituent showed cross-peaks with the carbon signals at δ_C 107.7 (C-4), 153.6 (C-4a), 162.3 (C-3), 136.7 (C-3′), and 122.8 (C-2′), demonstrating the attachment of the geranyl group to C-4. In addition, the proton of H-1′′ (δ_H 3.45) showed a correlation with C-1 (δ_C 158.8), C-2 (δ_C 111.6), and C-3 (δ_C 162.3), confirming that the prenyl group is linked to C-2. The EI mass spectrum of compound **1** showed a parent peak at *m/z* 464 and two fragment ions at *m/z* 123 (11%) and 69 (25%), suggesting the presence of one geranyl and one prenyl substituent. In addition, the base peak at *m/z* 285 (100%) was a result of the loss of two neutral fragments, C_4H_7 for a prenyl group and C_9H_{15} for a geranyl group, with such a loss being characteristic for compounds having a prenyl group adjacent to hydroxyl groups [22]. Thus, compound **1** was characterized as 1,3,5,8-tetrahydroxy-2-(3-methybut-2-enyl)-4-(3,7-dimethylocta-2,6-dienyl) xanthone.

Compound **2** was obtained as a yellow crystal, m. p. 87–88 °C, and its molecular formula was assigned to be $C_{16}H_{16}O_6$ from its HR-ESI-MS data. The UV and IR are characteristic for an oxygenated benzophenone [23]. The ^{1}H and ^{13}C NMR data of **2** (**Table 2**) were almost identical to those of 3′,6-dihydroxy-2,4,4′-trimethoxybenzophenone isolated from *Garcinia mangostana* by Nilar et al. [23]. Its structure was solved by X-ray crystallography (**Fig. 2**) as 2,3′-dihydroxy-2′,4,6-trimethoxybenzophenone (**Fig. 1**).

Results of the antibacterial activity of the compounds are shown in **Table 3**. Apart from compound **2**, all the tested compounds showed significant to moderate activity against at least three of the six bacterial strains used. Compounds **1, 2**, and **3** exhibited the most prominent antibacterial activity against gram-positive *Enterococcus faecalis* with MIC values of 8, 8, and 2 µg/mL, respectively. Although no clear-cut structure-activity relationship could be derived, some basic activity trends could be observed from the comparison of the chemical structures of the compounds and their different activities. Compound **2** was the least active one against all the bacterial strains used with minimal inhibitory concentration (MIC) values greater than 256 µg/mL, except against *Staphylococcus aureus* (MIC 128 µg/mL). Among the tested samples, compound **2** appears to be a unique non-prenylated compound. Therefore, the absence of the lipophilic prenyl group in the xanthonic scaffold is probably the reason for the loss of this activity for compound **2**. Many naturally occurring xanthones and their prenylated derivatives have been found to exhibit significant biological and pharmacological properties, such as antimicrobial and antitumor activities, and it can be inferred that the

No.	^{1}H (multi.; *J* in Hz)	^{13}C	HMBC
1	–	107.1	–
2	–	167.7	–
3	6.13 (d; *J* = 2.3)	94.3	91.7; 107.1; 167.7
4	–	168.1	–
5	5.99 (d; *J* = 2.3)	91.7	94.3; 107.1; 164.0; 168.1; 199.1
6	–	164.0	–
1′	–	138.6	–
2′	–	144.7	–
3′	–	150.4	–
4′	6.96 (dd; *J* = 7.1; 1.9)	117.9	118.4; 144.7
5′	6.96 (brt; *J* = 7.1)	124.4	118.4; 144.7; 150.4; 138.6
6′	6.66 (dd; *J* = 7.2; 1.9)	118.4	117.9; 138.6; 144.7; 150.4; 199.1
2′-OMe	3.66 (s)	61.4	143.9
4-OMe	3.87 (s)	56.0	168.1
6-OMe	3.44 (s)	56.1	164.0
2-OH	13.25 (s)	–	94.3; 107.1; 167.7
3′-OH	8.22 (s)	–	–
O=C	–	199.1	–

Table 2 ^{1}H-(500 MHz), HMBC, and ^{13}C-NMR (125 MHz) data for compound **2** in CD_3OCD_3.

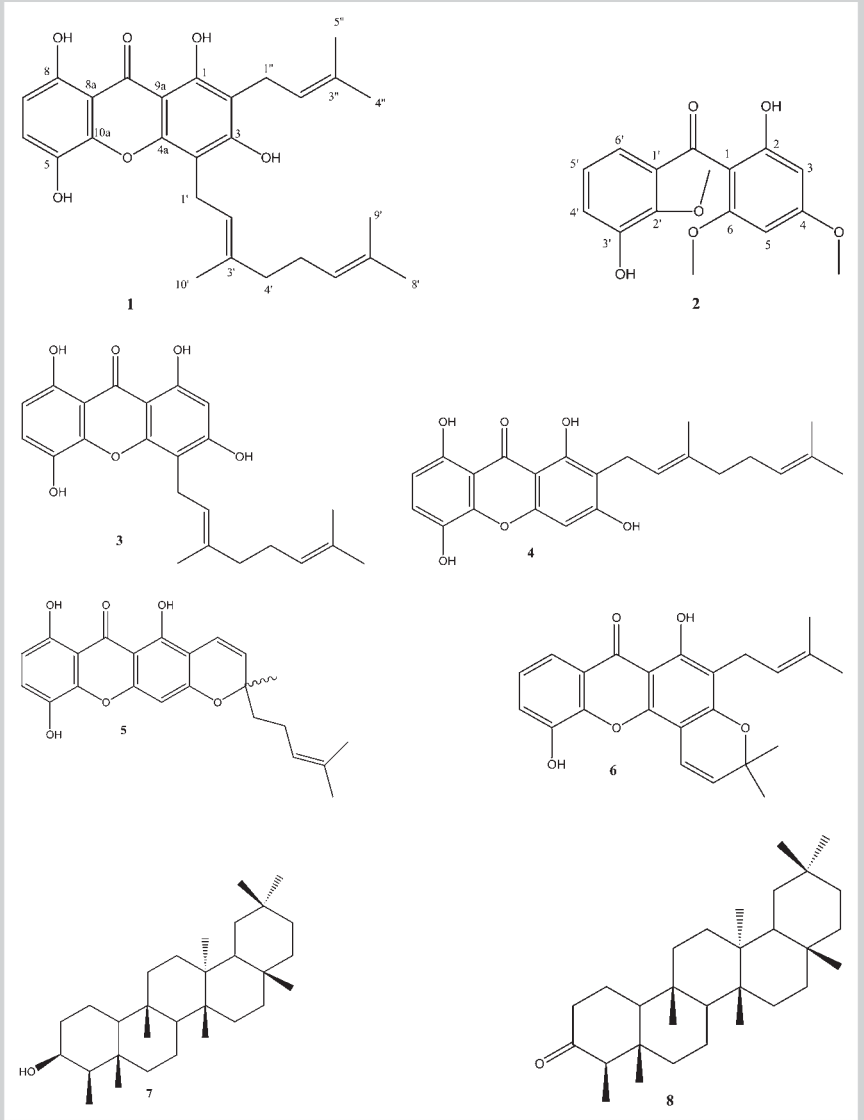

Fig. 1 Structures of the isolated compounds.

presence of prenyl groups can be associated with an improvement of potency and selectivity for some of these properties [24].

The antioxidant properties of compounds **1**–**4** and **6** were also studied, and the IC_{50} values are summarized in ◉ **Table 4**. All the

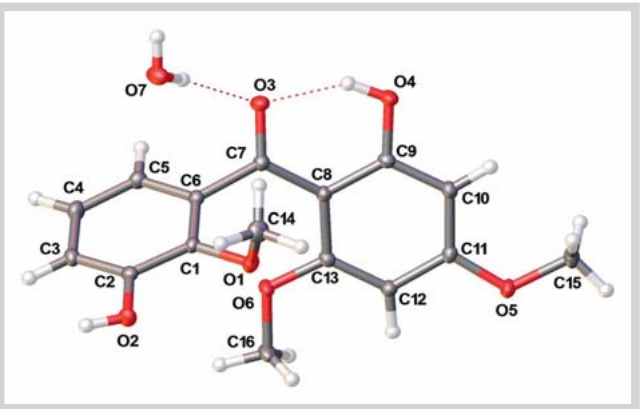

Fig. 2 ORTEP plot of **2**. (Color figure available online only.)

tested compounds (except compound **2**) showed the capacity to scavenge free radicals. IC_{50} values ranged from 1.90 to 34.50 µg/mL in DPPH and ABTS assays, while the $FeSO_4$/g equivalent varied from 46.17 to 1527.17 µmol $FeSO_4$/g in the FRAP method. Compounds **3** and **4** showed the most potent antioxidant capacity, while compound **2** showed the lowest activity with an IC_{50} value greater than 100 µg/mL. Considering that antioxidant activity is generally accepted to depend on the structure and substitution pattern of hydroxyl groups, we found a correlation between the radical scavenging activity and the number of free hydroxyl groups in the xanthone scaffold [25]. Some of the xanthones presented herein are potential antioxidants and antimicrobials or possibly derivatives with improved activity, which could be of interest for further development of the compounds taking into account the growing health problems related to oxidative stress and antimicrobial resistance.

Material and Methods

General

Melting points were determined on a Büchi-540 melting point apparatus. IR spectra were determined on a Nicolet 380 Fourier Transform IR spectrometer. UV spectra were determined on a Spectronic Unicam spectrophotometer. The ^1H, ^{13}C, and DEPT NMR spectra as well as two-dimensional experiments (COSY, NOESY, HMQC, and HMBC using pulsed field gradients) were recorded on a Bruker DRX 500 FT-NMR spectrometer, operating at 500 MHz (^1H) and 125 MHz (^{13}C) and a Bruker Avance 600 FT-NMR spectrometer, operating at 600 MHz (^1H) and 150 MHz (^{13}C) in $CDCl_3$, d_6-acetone, or d_6-DMSO with TMS as the internal standard.

EI mass spectra and accurate mass spectra were recorded with an LC linear ion trap instrument (Esquire 3000) using electrospray ionization in the negative or positive mode and a sector field mass spectrometer (Autospec X). Vacuum liquid chromatography (VLC) was carried out using Merck silica gel 60 F_{254} (230–400 mesh), column chromatography using Si Gel 60 (230–400 mesh, 70–230 mesh), and TLC analysis was performed on silica gel (Merck silica gel 60 with fluorescent indicator UV_{254}, 0.20 mm, 20 × 20 cm) with different mixtures of petrol ether, cyclohexane, dichloromethane, ethyl acetate, acetone and methanol as eluents; spots were visualized under UV lamps (254 nm and 365 nm) or by MeOH-H_2SO_4 reagent. Solvent evaporation was done using a rotavapor (laborota 4000; Heidolph).

Plant material

The stem bark of *G. smeathmannii* was collected from Cheffou-Baham, Western Province, Cameroon in April 2010 and was identified by Victor Nana of the Cameroon National Herbarium (CNH), Yaoundé, where a voucher specimen (35 169/HNC) has been deposited.

Table 3 Antibacterial activity of compounds **1**–**4** and **6** (MIC in µg/mL).

Compounds	Microorganisms					
	S. aureus	*E. faecalis*	*B. cereus*	*P. aeruginosa*	*E. coli*	*S. typhimurium*
1	64	8	256	16	32	64
2	128	–	–	–	–	–
3	64	8	32	128	64	–
4	32	16	64	256	32	256
6	32	2	64	64	128	256
Gentamicin	0.25	0.12	0.25	0.5	0.25	0.25

-: > 256 µg/mL

Compounds	DPPH, IC_{50} (µg/mL)	ABTS, IC_{50} (µg/mL)	FRAP [µg Fe (II)/g]
1	4.10 ± 1.64	> 100	1527.17 ± 21.46
2	> 100	> 100	46.80 ± 11.92
3	2.94 ± 0.73	1.90 ± 0.03	960.83 ± 31.58
4	3.16 ± 0.28	1.92 ± 0.06	996.93 ± 21.01
6	34.50 ± 2.23	5.63 ± 0.65	221.84 ± 25.43
Trolox	5.36 ± 0.10	3.71 ± 0.21	Nd
Ascorbic acid	2.80 ± 0.03	2.61 ± 0.08	Nd

Nd: not determined

Table 4 Antioxidant activity of compounds **1**–**4** and **6**.

Extraction and isolation

The air-dried, powdered stem bark of *G. smeathmannii* (2.5 kg) was extracted at room temperature for three days using distilled methanol (12 L). The crude methanol extract (207 g) obtained was partitioned with petroleum ether (88 g; 2.5 L), dichloromethane (20.4 g; 1.5 L), and ethyl acetate (32 g; 2 L).

The petroleum ether fraction (80 g) was subjected to flash column chromatography using silica gel (230–400 mesh; 800 g) eluted with petroleum ether, petroleum ether-EtOAc (9 : 1), petroleum ether-EtOAc (7.5 : 2.5), petroleum ether-EtOAc (1 : 1), EtOAc and EtOAc-MeOH (7.5 : 2.5) to give five main fractions labeled A (27 g), B (22 g), C (14 g), D (17 g), and E (6.4 g), respectively.

Fraction A (25 g) was then subjected to column chromatography (5 × 100 cm) on silica gel (600 g, 230–400 mesh) and eluted by a petroleum ether-EtOAc mixture of increasing polarity (20 : 1–3 : 1). A total of 90 fractions of ca. 300 mL each were collected, concentrated and combined on the basis of TLC to give five subfractions indexed A1 to A4. Subfraction A2 (2.3 g) was subjected to column chromatography (4 × 30 cm) on silica gel (30 g, 70–230 mesh) and eluted with petroleum ether-EtOAc (19 : 1) to give *epi*-friedelinol (**7**, 10 mg, ≥ 99% purity) and friedelin (**8**, 25 mg, ≥ 99% purity). The subfraction A3 (1.9 g) was further subjected to column chromatography (4 × 30 cm) on silica gel (25 g, 70–230 mesh) and eluted with petroleum ether-EtOAc (18 : 2) to yield compound **1** (8 mg, ≥ 98% purity). The subfraction A4 (19.2 g), after column chromatography (5 × 50 cm) on silica gel (350 g, 70–230 mesh), yielded a mixture of polyprenylated benzophenones.

Fraction B (20 g) was also subjected to column chromatography (5 × 100 cm) on silica gel (600 g, 230–400 mesh) and eluted with a petroleum ether-EtOAc mixture of increasing polarity (20 : 1–3 : 1). Fifty fractions of ca. 300 mL each were collected and regrouped into three subfractions, B1 to B3, on the basis of their TLC profile. Subfraction B1 (1.8 g) was subjected to column chromatography (4 × 30 cm) on silica gel (20.0 g, 70–230 mesh) and eluted with a mixture of petroleum ether-EtOAc (17 : 3) to give compound **5** (5.0 mg, ≥ 99% purity). Subfraction B2 (8.0 g) was subjected to column chromatography (4 × 30 cm) on silica gel (20 g, 70–230 mesh) and eluted with a mixture of petroleum ether-EtOAc (13 : 7) to yield compounds **6** (25.0 mg, ≥ 97% purity) and **3** (18.5 mg, ≥ 98% purity). Subfraction B3 (2.6 g) was also subjected to column chromatography (4 × 30 cm) on silica gel (30 g, 70–230 mesh) and eluted with a mixture of petroleum ether-EtOAc (12 : 8) to afford compounds **4** (15.0 mg, ≥ 98% purity) and **2** (22.5 mg, ≥ 98% purity).

1,3,5,8-Tetrahydroxy-2-(3-methybut-2-enyl)-4-(3,7-dimethylocta-2,6-dienyl)xanthone (**1**): yellow powder, m. p. 172–173 °C; UV (MeOH): λ_{max} (log ε) = 283 (4.6), 325 (4.2), 350 (4.3) 400 (3.8) nm; IR (KBr pellet): ν = 2919, 2851, 1627, 1617, 1580, 1485, 1463, 1314, 1217, 1176, 1099, 1007, 967, 830, 809, 719, 704, 618, 589 cm^{-1}; ^1H NMR and ^{13}C NMR (**Table 1**); HR-EI-MS: *m/z* = 464.21 920 $[M]^+$ (calcd. for $C_{28}H_{32}O_6$; 464.21 934).

2,3′-Dihydroxy-2′,4,6-trimethoxybenzophenone (**2**): yellow crystals, m. p. 87–88 °C; UV (MeOH) λ_{max} (log ε) = 220 (3.7), 230 (4.1), 304 (4.3) nm; IR (KBr pellet): ν = 3391, 2944, 2365, 2339, 1613, 1600, 1468, 1458, 1437, 1421, 1339, 1292, 1224, 1208, 1156,1116, 1070, 1054, 986, 861, 802, 758, 698, 641, 597, 527, 461 cm^{-1}; ^1H NMR and ^{13}C NMR (**Table 2**); HR-ESI-MS: *m/z* = 327.08 301[M + Na] $^+$ (calcd. for $C_{16}H_{16}O_6Na^+$; 327.08 391).

X-ray structure determination

A single crystal of **2** was selected under paratone oil and transferred on a SuperNova, single source at offset, Eos diffractometer. The crystal was kept at 100.0(1) K during data collection. Using Olex2 [26], the structure was solved with ShelXS-97 and refined with the ShelXL-97 [27] refinement package using least squares minimization. The sum formula is $C_{16}H_{16}O_6 \cdot H_2O$, M = 322.30 g/mol, triclinic, space group P$\bar{1}$ (no. 2), a = 7.2955(3) Å, b = 9.9851 (4) Å, c = 11.0641(4) Å, α = 109.954 (4)°, β = 94.051 (3)°, γ = 91.808 (3)°, V = 754.37(5) Å^3, Z = 2, μ(MoKα) = 0.112 mm^{-1}, *Dcalc* = 1.419 g/cm^3, 15 039 reflections measured (5.6° ≤ 2Θ ≤ 60.0°), 4403 unique (R_{int} = 0.0204), which were used in all calculations. The final R_1 was 0.0393 (I > 2σ(I)) and wR_2 was 0.1137 (all data). CCDC 1 016 089 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

Antibacterial activity

Compounds **1**–**4** and **6** were tested for their antimicrobial activity against six bacteria: three gram-positive bacteria (*Staphylococcus aureus* ATCC 29 213, *Enterococcus faecalis* ATCC 29 212, *Bacillus cereus* ATCC 14 579) and three gram-negative bacteria (*Escherichia coli* ATCC 25 922, *Pseudomonas aeruginosa* ATCC 27 853 and *Salmonella typhimurium* ATCC 14 028). This activity was evaluated by determining the MIC using a rapid broth microdilution technique with p-iodonitrotetrazolium violet (INT) as the growth indicator as described by Eloff [28]. Gentamicin (50 mg/mL, Virbac) was used as a reference antibiotic drug. The samples were serially diluted to provide a final concentration range of 256 to 2 μg/mL and 4 to 0.03 for gentamycin.

Antioxidant activity

DPPH and ABTS assays: The DPPH and ABTS radical scavenging activities were determined using the modifications of the 96-well microtiter plate method described by Brand-Williams et al. [29] and Re et al. [30], respectively. 2,5,7,8-Tetramethylchroman carboxylic acid (trolox; 97% purity) and L-ascorbic acid (99% purity; purchased from Sigma-Aldrich) were used as positive controls, methanol was used as a negative control, and samples without DPPH or $ABTS^{\bullet+}$ solution were used as blanks. Results are expressed as percentage reduction of the initial DPPH or $ABTS^{\bullet+}$ absorption in relation to the control. The IC_{50} values were calculated from the graph plotted as inhibition percentage against the concentration.

Ferric reducing antioxidant power (FRAP) assay: The FRAP assay was carried out according to the procedure of Benzie and Strain [31] with slight modifications. Briefly, the FRAP reagent was prepared from acetate buer (pH 3.6), 10 mM TPTZ solution in 40 mM HCl, and 20 mM iron (III) chloride solution in proportions of 10 : 1 : 1 (v/v), respectively. The FRAP reagent was prepared fresh daily and was warmed to 37 °C in a water bath prior to use. Fifty μL of sample were added to 1.5 mL of the FRAP reagent. The absorbance of the reaction mixture was then recorded at 593 nm after 4 min. The standard curve was constructed using $FeSO_4$ solution (0.1–2 mM), and the results are expressed as μg $FeSO_4$/g of the compound. All the measurements were taken in triplicate, and the mean values were calculated.

Supporting information

^1H-NMR, ^{13}C-NMR, HMQC, HMBC, mass, and HR-mass spectra of compounds **1** and **2** are available as Supporting Information.

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Conflict of Interest

The authors declare no conflict of interest.

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