Eucalyptone G, a new phloroglucinol derivative and other constituents from *Eucalyptus globulus* Labill

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Abstract
A new pholoroglucinol derivative named eucalyptone G (**4**) together with nine known compounds (**1-3, 5-10**) were isolated for the first time from the ethyl acetate fraction of the bark of small twigs of *Eucalyptus globulus* Labill. Their chemical structures were established on the basis of physical, chemical and spectroscopic methods 1D (^{1}H and ^{13}C) NMR and 2D (COSY, HMQC and HMBC) NMR in addition to mass spectrometry and comparison with literature data. Eucalyptone G displayed antibacterial activity against *Bacillus subtilis*, *Staphylococcus aureus* and *Escherichia coli*.

Keywords: *Eucalyptus globulus* Labill.; Myrtaceae; eucalyptone G, antibacterial activity

Introduction

Eucalyptus L'He`ritier (Myrtaceae) is one of the world's most important and most widely planted genera. In Australia, this genus is the second largest genus, after *Acacia*, and contains about 750 species.[1] Myrtaceous plants are known to be rich source of biologically active terpenoids and polyphenols, including flavonoids, phloroglucinol derivatives, and tannins[2]. Previous phytochemical studies on the *Eucalyptus globulus* Labill. reported the isolation of sesquiterpenes aromadendrene[1], acetogenin-sesquiterpenes, acetogenin monoterpenes[3-6], phloroglucinol sesquiterpenes[7], and *C*-methyl flavones[8]. The phloroglucinol compounds exhibit interesting biological activities[9]. *Eucalyptus globulus* Labill. (Myrtaceae) is used as a traditional remedy in many parts of the world for treatment of a wide variety of diseases including microbial infections[5]. In the present work we report the isolation and structure elucidation of one new phloroglucinol derivative named eucalyptone G, together with nine known compounds. The antibacterial activity of the new compound has been studied.

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Results and Discussion

The methanolic extract of the powdered bark of *Eucalyptus globulus* Labill. was concentrated to dryness and successively partitioned between *n*-hexane, ethyl acetate, *n*-butanol and water. The ethyl acetate fraction afforded one new phloroglucinol derivative named eucalyptone G (**4**) and nine known compounds (**1-3**, **5-10**).

Rhodomyrtone (**3**) appeared as orange-coloured spot on silica gel TLC plates when sprayed with anisaldehyde/H_2SO_4 reagent. The ESI-MS spectrum showed pseudomolecular ion peaks at *m/z* 443 (100) $[M+H]^+$ and 907 $[2M+Na]^+$. The UV spectrum exhibited three characteristic bands at λ_{max} (MeOH) 224.3, 263.1 and 301.3 nm. ^{1}H NMR spectrum revealed the presence of three downfield signals; two for OH groups at δ_H 13.04, 7.59 (each 1H, s), and one aromatic proton at δ_H 6.11 (1H, s, H-5), in addition to methine proton at δ_H 4.26 (1H, t, *J* = 5.4 Hz, H-9). Furthermore, the spectrum exhibited characteristic signals for isovaleryl group at δ_H 2.99 (2H, dd, *J* = 6.8, 15.5 Hz, H-2`), 2.28 (1H, m, H-3`), and 0.98 (6H, d, *J* = 6.6 Hz, two methyls at C-3`). On other hand, the ^{1}H NMR revealed the presence of isobutyl group at δ_H 1.48 (2H, m, H-1``) and 0.83, 0.87 (each 3H, d, *J* = 5.9 Hz, two methyls at C-2``), in addition to four singlet signals for four methyl groups at δ_H 1.37, 1.40, 1.42 and 1.54. ^{13}C and DEPT NMR spectra indicated the presence of eight methyls, two methylenes, four methines and twelve quaternary carbon atoms, three of the quaternary carbon atoms were assigned to three keto groups at δ_C 212.12 (C-3), 206.48 (C-1´) and 197.93 (C-1). The presence of isovaleryl and isobutyl groups was confirmed from ^{13}C signals at δ_C 206.48, 53.16, 25.10, 23.15 and 22.80 and at δ_C 45.83, 25.12, 23.48 and 22.74, respectively. COSY data allowed the unambiguous assignment of two spin systems. The first spin system showed correlation of the methylene at δ_H 2.99 (H_2-2´), methine at δ_H 2.28 (H-3´) and two geminal methyls at δ_H 0.98 (2 methyls at C-3´) corresponding to the isovaleryl group. The second consisted of a methine proton at δ_H 4.26 (H-9) correlated to a multiplet methylene signal at δ_H 1.48 (2H, m, H-1``), which further correlated with methine proton (overlapped with methyls) which gave a cross peak with two geminal methyl signals at $\delta_H$ 0.83 and 0.87, respectively. The assignment of protons and carbons was confirmed by HMQC data. The HMBC spectrum showed correlations of H-9 with C-1``, 2``, C-8a and 9a which established the connectivity of the isobutyl group at C-9. The position of the isovaleryl moiety at C-7 was confirmed by the HMBC correlation of H-2´ with C-7. The molecular weight, UV, and NMR data of compound (**3**) were very similar to those reported for rhoydomyrtone[10].

Eucalyptone G (**4**) was obtained as a yellow needle, physical characters, UV, ^{1}H, ^{13}C and DEPT NMR spectral data of **4** showed some similarities with those of **3**. IR spectrum showed absorption bands at γ_{max} (KBr) cm^{-1}; 3390 (OH), 1715 (C=O) and 1584 (C=C). The ESI-MS showed a pseudomolecular ion peak at *m/z* 677 $[M+H]^+$ (100%) which in conjunction with ^{1}H, ^{13}C and DEPT NMR spectral data suggested the molecular formula $C_{40}H_{52}O_9$. This was further confirmed by HRESI-MS. In comparison with **3**, compound **4** required 15 degrees of unsaturation instead of 10, which suggested the presence of an additional ring system. The significant differences in ^{1}H NMR between **3** and **4** were the appearance of new signals at δ_H

4.39 (1H, m), two methyls at δ_H 0.85 (6H, d, *J* = 6.0 Hz) indicating the presence of isobutyryl group, four methyl signals, as well as the disappearance of the aromatic proton H-5 in **4**. ^{13}C and DEPT NMR showed the presence of additional six methyls, one methine, seven quaternary carbon atoms in (**4**), three of them were assigned to three keto groups at δ_C 211.44 (C-3′′′), 210.01 (C-7′′′) and 197.47 (C-1′′′) in addition to the lack the doublet ^{13}C NMR signal at δ_C 94.76 (C-5). These data suggested that C-5 in (**4**) was blocked with a substituent. In COSY there is an additional spin system consisted of methine group and two methyl signals assigned to the isobutyryl moiety was observed. Comparison of the ^{1}H and ^{13}C NMR data with literature data[10] indicated the presence of 5-(isobutyryl)-2,2,6,6-tetramethyl-cyclohex-4-ene-1,3-dione moiety [substructure **A**] (Figure 2) and was further confirmed by the presence of fragment ion peak at *m*/*z* 441 $[M-C_{14}H_{19}O_3]^+$ in ESI-MS. The HMBC showed correlations of the 2 methyls at C-2′′′ with C-1′′′ and C-3′′′; 2 methyls at C-4′′′ with C-3′′′ and C-5′′′. The geminal dimethyls at δ_H 0.82 correlated with C-7′′′; H-8′′′ with C-7′′′ and C-5′′′ confirmed the attachment of the isobutyryl moiety at C-5′′′. The connectivity of the substructure **A** at C-5 was established by the disappearance of H-5 and downfield shift of C-5 to δ_C 104.81. To the best of our knowledge compound **4** is a new natural product and the name eucalyptone G was given to it. The known compounds were identified by analysis of the spectroscopic data (1D, 2D NMR and MS) and comparison of their data with those in the literature to be: stigmasterol (**1**)[11], ursolic acid (**2**)[12], rhodomyrtone (**3**)[10], 4′,5,7-trimethoxy kaemepferol (**5**)[13, 14], naringenin (**6**)[15], genistein (**7**)[13, 14], catechin (**8**)[15, 16], epicatechin (**9**)[15, 16] and octyl-β-D-glucopyranoside (**10**)[17]. This is the first report of compounds **1-3**, **5**, **7-10** in family Myrtaceae. Compound **6** was isolated for the first time from the plant.

Eucalyptone **G** was found to be active against the Gram-positive *Bacillus subtilis* and *Staphylococcus aureus* and caused an inhibition zone of 16 mm diameter after 24 h of incubation at 37 ^{0}C. Also, highly active against Gram-negative *E. coli* with an inhibition zone of 19 mm diameter.

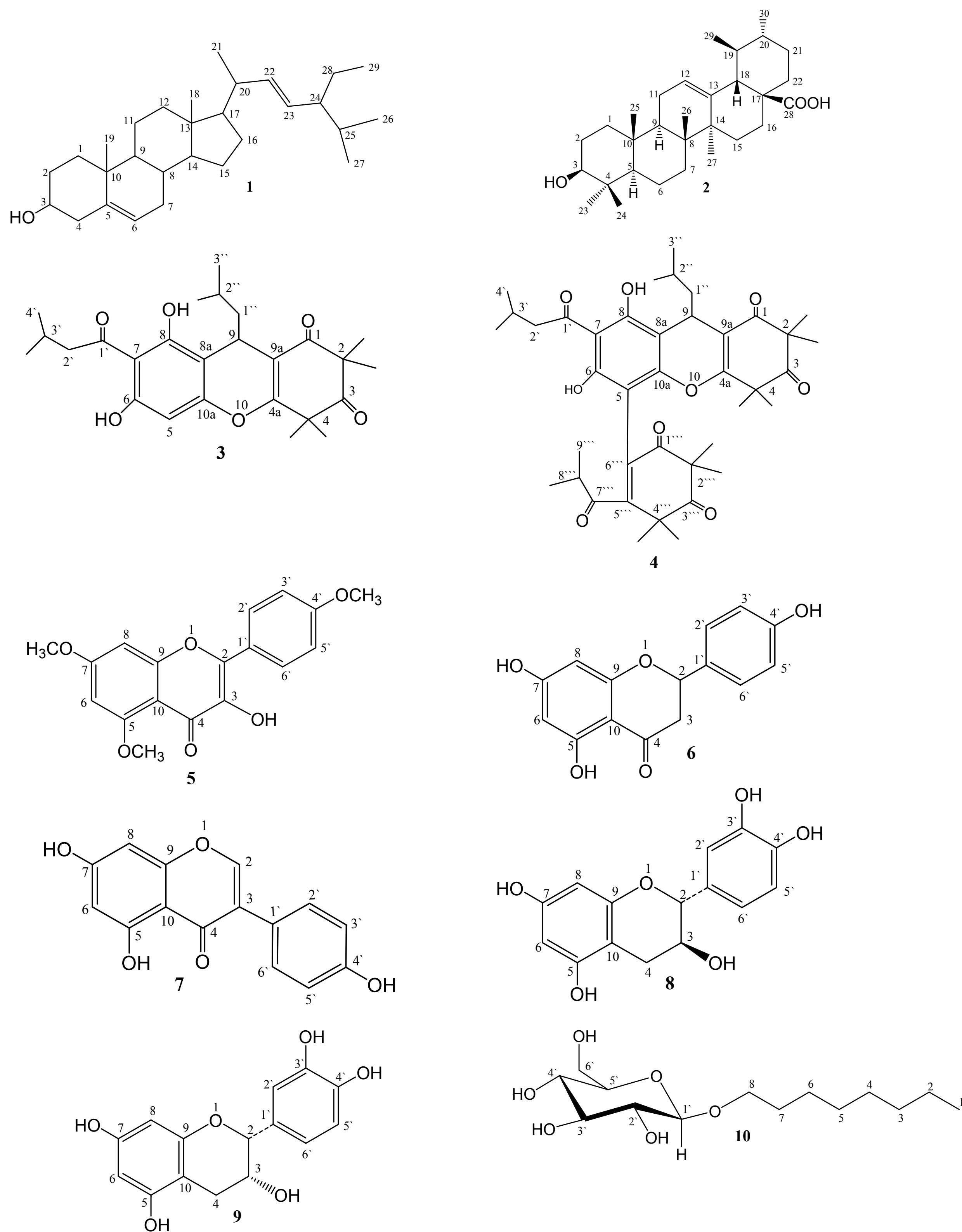

Figure 1. Chemical structures of the isolated compounds.

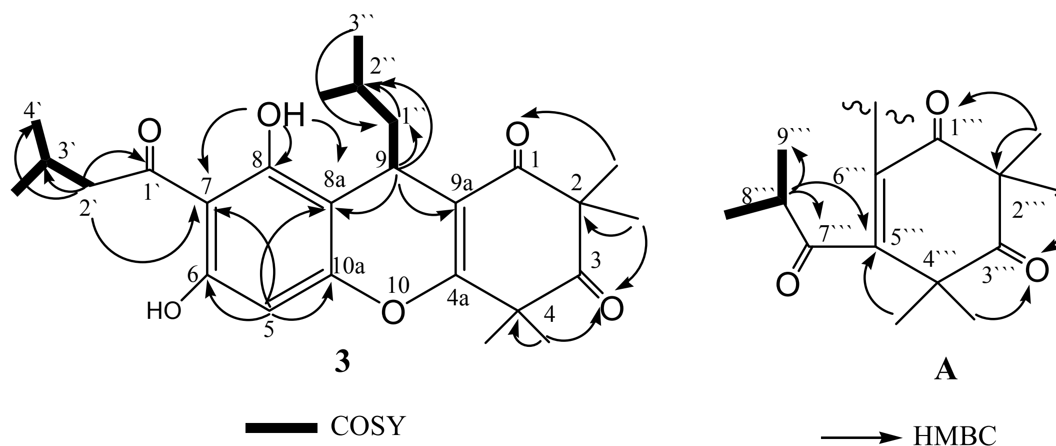

Figure 2. COSY and HMBC correlations of compound **3** and structure A.

Experimental Section

General Procedures. Melting points were determined in an Electrothermal 9100 Digital Melting Point (England). IR was measured on Schimadzu Infrared-400 spectrophotometer (Japan). The UV spectra were carried out in methanol (Merck) using a Perkin-Elmer Lambda 25 UV/VIS spectrophotometer. Electron impact mass spectra (EI-MS) were recorded on a Finnigan MAT TSQ 7000 mass spectrometer. Positive-ion electron spray ionization mass spectra (ESI-MS) were performed on a Thermofinnigan LCQ DECA mass spectrometer coupled to an Agilent 1100 HPLC system equipped with a photodiode-array detector. HRESI-MS was determined with a Micromass Qtof 2 mass spectrometer. ^{1}H and ^{13}C NMR spectra were measured on Bruker DRX 500 spectrometer (Bruker, Rheinstetten, Germany). 1D NMR spectral data were measured at temperature 23.7 ^{o}C in gradient sequences, while 2D NMR at 26.85 ^{o}C. High Performance Liquid Chromatography was performed on a HPLC system (Merck, Darmstadt, Germany) consisting of a Lachrom-Merck Hitachi L-7100 pump and an L-7400 UV detector using a C-18 column (250 × 8 mm i.d., prefilled with Eurospher 100, Knauer, Berlin, Germany) and UV detection at λ 280 nm. Vacuum liquid chromatography (VLC) was carried out on silica gel 60 (0.04-0.063 mm, 500 g, Merck). Column chromatographic separations were performed over silica gel 60 (0.04-0.063 mm, Merck) and Sephadex LH-20 (0.25-0.1 mm Merck). TLC analyses were carried out on pre-coated silica gel F_{254} aluminium sheets and RP-18 F_{254s} glass plates (Merck). Compounds were detected by UV absorption at λ 255 and 366 nm followed by spraying with anisaldehyde/H_2SO_4 reagent and heating at 110 ^{0}C for 1-2 min. The solvent systems used for TLC analyses were dichloromethane-methanol (97:3, solvent system I), dichloromethane-methanol (94:6, solvent system II) and dichloromethane-methanol (90:10, solvent system III). All solvents were distilled prior to use.

Extraction and isolation

The bark of small twigs of *Eucalyptus globulus* Labill. was collected in August 2004 from trees growing on the margins of the ponds and banks of the Nile-river, Mankabad, Assiut, Egypt. A voucher specimen has been deposited at the Herbarium of Pharmacognosy Department, Faculty of Pharmacy, Al-Azhar University, Assiut branch, Assiut, Egypt.

The air-dried powdered bark (900 g) was extracted with methanol (2.5 l × 4) at room temperature. The combined extract was concentrated under reduced pressure to afford a dark greenish brown residue (46.0 g). The latter was suspended in distilled water (200 ml) then partitioned between *n*-hexane (500 ml × 4), ethyl acetate (500 ml × 4) and *n*-butanol (500 ml × 3), successively. Each fraction was concentrated under reduced pressure to give *n*-hexane fraction (10.3 g), ethyl acetate fraction (7.6 g), *n*-butanol fraction (11 g) and aqueous (14.8 g) residue. The ethyl acetate fraction was subjected to VLC using *n*-hexane-ethyl acetate gradients, 6 fractions were obtained: E-1 to E-6. Fraction E-1 (1.1 g), fraction E-2 (0.89 g), fraction E-3 (1.3 g), fraction E-4 (0.95 g), fraction E-5 (1.4 g), and fraction E-6 (1.2 g). Fraction E-2 (0.89 g) was subjected to silica gel column chromatography (120 g × 50 × 3 cm) using dichloromethane-methanol 98:2 to afford compounds **1** (20 mg) and **2** (18 mg). Fraction E-3 (1.3 g) was subjected to VLC using dichloromethane-methanol gradients to obtain four subfractions E-3-A to E-3-D. Subfraction E-3-C (0.41 g) was subjected to silica gel column chromatography (90.0 g × 50 × 3 cm) using dichloromethane-methanol 98:2 to afford compounds **3** (22 mg) and **4** (9 mg). Fraction E-4 (0.95 g) was chromatographed over Sephadex LH-20 column (150 g × 100 × 5 cm) using methanol-dichloromethane 9:1 as an eluent to obtain three main subfractions E-4-A to E-4-C. Subfraction E-4-B (0.23 g) was subjected to silica gel column chromatography (90.0 g × 50 × 3 cm) using dichloromethane-methanol 92:8 to afford compounds **5** (14 mg), **6** (18 mg) and **7** (23 mg). About 100 mg of fraction E-5 was submitted to HPLC (HPLC gradient programm: 60:40 methanol/water at 0 and 5 min; 100:0 methanol/water at 38 and 45 min and a flow rate 5.0 ml/min) to yield compound **8** (19 mg) and **9** (24 mg). Silica gel column chromatography of fraction E-6 (1.2 g) using dichloromethane-methanol 88:12 gave compound **10** (26 mg).

Stigmasterol (1).[11] White needles; R_f = 0.86 (solvent system I); m. p. 169-170 ^oC, EI-MS *m/z* (rel. int.%): 412 $[M]^+$ (100). ^1H NMR (500 MHz, $CDCl_3$): δ_H 5.34 (1H, dd, *J* = 2.8, 5.1 Hz, H-6), 5.15 (1H, dd, *J* = 8.8, 15.1 Hz, H-22), 5.01 (1H, dd, *J* = 8.8, 15.1 Hz, H-23), 3.15 (1H, m, H-3), 1.02 (3H, s, CH_3-19), 1.00 (3H, d, *J* = 6.2 Hz, CH_3-21), 0.84 (3H, t, *J* = 6.3 Hz, CH_3-29), 0.79 (3H, d, *J* =7.5 Hz, CH_3-26), 0.78 (3H, d, *J* = 7.5 Hz, CH_3-27), 0.69 (3H, s, CH_3-18). ^{13}C NMR (125 MHz, $CDCl_3$): δ_C 140.71 (C-5, s), 138.29 (C-22, d), 129.24 (C-23, d), 121.69 (C-6, d), 71.79 (C-3, d), 56.83 (C-17, d), 55.92 (C-14, d), 51.21 (C-24, d), 50.12 (C-9, d), 42.23 (C-13, s), 40.47 (C-20, d), 39.65 (C-4, t), 37.22 (C-1, t), 36.48 (C-10, s), 31. 85 (C-8, 25, d), 31.59 (C-2, t), 31.50 (C-7, t), 28.89 (C-28, t), 25.38 (C-16, t), 24.34 (C-15, t), 21.19 (C-21, q), 21.07 (C-11, t), 21.05 (C-27, q), 19.37 (C-26, q), 18.96 (C-19, q), 12.23 (C-29, q), 12.02 (C-18, q).

Ursolic acid (2).[12] White amorphous powder; R_f = 0.71 (solvent system I); m. p. 292-293 ^oC, EI-MS *m/z* (rel. int.%): 456 $[M]^+$ (93). ^1H NMR (500 MHz, DMSO-d_6): δ_H 11.92 (1H, s, 28-CO<u>OH</u>), 5.11 (1H, dd, *J* = 3.8, 6.9 Hz, H-12), 4.28 (1H, d, *J* = 15.0 Hz, 3-OH), 2.98 (1H, m, H-

3), 2.10 (1H, d, *J* = 11.0 Hz, H-18), 1.02 (3H, s, CH_3-23), 0.90 (3H, d, *J* = 9.7 Hz, CH_3-29), 0.88 (3H, s, CH_3-27), 0.85 (3H, s, CH_3-26), 0.80 (3H, d, *J* = 6.6 Hz, CH_3-30), 0.73 (3H, s, CH_3-23), 0.66 (3H, s, CH_3-25). ^{13}C NMR (125 MHz, DMSO-d_6): δ_C 178.27 (C-28, s), 138.17 (C-13, s), 124.55 (C-12, d), 76.81 (C-3, d), 54.76 (C-5, d), 52.35 (C-18, d), 47.00 (C-17, s), 46.80 (C-9, d), 41.62 (C-8, 14, s), 38.48 (C-4, s), 38.42 (C-19, d), 38.37 (C-20, d), 38.21 (C-1, t), 36. 51 (C-22, t), 36.30 (C-10, s), 32.69 (C-7, t), 30.17 (C-21, t), 28.25 (C-23, q), 27.53 (C-15, t), 26.98 (C-2, t), 23.79 (C-16, t), 23.26 (C-27, q), 22.84 (C-11, t), 21.07 (C-30, q), 17.98 (C-6, t), 17.07 (C-26, q), 16.89 (C-29, q), 16.08 (C-25, q), 15.22 (C-24, q).

Rhodomyrtone (3).[10] Yellow needles; R_f = 0.78 (solvent system I); m. p. 186-187 °C, Positive-ion ESI-MS *m/z* (rel. int.%): 443 $[M+H]^+$ (100), in addition to significant fragment peak at *m/z* 385 $[M-C_4H_9]^+$. ^1H NMR (500 MHz, $CDCl_3$) see table 1, ^{13}C NMR (125 MHz, $CDCl_3$): see table 2.

Table 1. ^1H NMR data of compounds **3** and **4** ($CDCl_3$, 500 MHz)

3 [a]		**4** [a]	
Pos.[b]	δ_H (*J* Hz, m)	Pos.[b]	δ_H (*J* Hz, m)
2 × CH_3 at pos. 2	1.37, 1.40, each 3H, s	8 × CH_3 at pos. 2, 4, 2```, 4```	1.35-1.64, each 3H, s
2 × CH_3 at pos. 4	1.42, 1.54, each 3H, s		
5	6.11, 1H, s	5	-
9	4.26, 1H, t, *J* = 5.4 Hz	9	4.33, 1H, t, *J* = 5.4 Hz
2`	2.99, 2H, dd, *J* = 6.8, 15.5 Hz	2`	A 3.21, 1H, dd, *J* = 6.3, 17.3 Hz B 3.00, 1H, dd, *J* = 6.3, 17.3 Hz
3`	2.28, 1H, m	3`	2.38, 1H, m
2 × CH_3 at pos. 3`	0.98, 6H, d, *J* = 6.6 Hz	2 × CH_3 at pos. 3`	1.03, 6H, d, *J* = 6.3 Hz
1``	1.48, 2H, m	1``	overlapped with CH_3
2``	overlapped with CH_3	2``	overlapped with CH_3
2 × CH_3 at pos. 2``	0.83, 0.87, each 3H, d, *J* = 5.9 Hz	2 × CH_3 at pos. 2``	0.95, 0.89, each 3H, d, *J* = 5.9 Hz
		8```	4.39, 1H, m
		2 × CH_3 at pos. 8```	0.82, 6H, d, *J* = 6.0 Hz
6-OH	7.59, 1H, s	6-OH	-
8-OH	13.04, 1H, s	8-OH	13.48, 1H, s

[a] For clarity the same numbering system is used for **3** and **4**. [b] Position.

Table 2. ^{13}C NMR data of compounds **3** and **4** ($CDCl_3$, 125 MHz)

3 [a]			**4** [a]		
Pos.	δ_C (m)	HMBC	Pos.	δ_C (m)	HMBC
1	197.93 s		1	197.47 s	
2	56.05 s		2	56.28 s	
2 × CH_3 at pos. 2	24.56 q, 24.58 q	1, 2	4 × CH_3 at pos. 2, 2```	23.82 q, 24.19 q, 25.04 q, 25.56 q	1, 2, 1```, 2```
3	212.12 s		3	211.67 s	
4	47.15 s		4	47.25 s	
2 × CH_3 at pos. 4	24.71 q, 24.14 q	4, 4a	4 × CH_3 at pos. 4, 4```	24.36 q , 24.68 q, 24.87 q, 24.94 q	4, 4a, 4```, 5```
4a	167.14 s		4a	166.76 s	
5	94.76 d	6, 7, 8a, 10a	5	104.81 s	
6	158.15 s		6	152.35 s	
7	107.58 s		7	107.58 s	
8	162.61 s		8	160.61 s	
8a	106.49 s		8a	105.67 s	
9	25.15 d	8a, 9a, 1``, 2``	9	25.26 d	8a, 9a, 1``, 2``
9a	114.02 s		9a	114.27 s	
10a	155.61 s		10a	154.91 s	
1`	206.48 s		1`	204.58 s	
2`	53.16 t	1`, 3`, 7	2`	53.90 t	1`, 3`, 7
3`	25.10 d		3`	25.11 d	
2 × CH_3 at pos. 3`	23.15 q, 22.80 q	1`, 2`, 3`	2 × CH_3 at pos. 3`	23.27 q, 23.30 q	1`, 2`, 3`
1``	45.83 t	9, 2``	1``	45.47 t	9, 2``
2``	25.12 d		2``	25.26 d	
2 × CH_3 at pos. 2``	22.74 q, 23.48 q	1``, 2``	2 × CH_3 at pos. 2``	23.35 q, 23.78 q	1``, 2``
			1```	197.44 s	
			2```	56.03 s	
			3```	211.44 s	
			4```	47.28 s	
			5```	113.52 s	
			6```	111.74 s	
			7```	210.01 s	
			8```	39.21 d	5```, 7```, 2 × CH_3 at 8```
			2 × CH_3 at pos. 8```	22.82 q, 22.64 q	7```, 8```
			8-OH		7, 8, 8a

[a] For clarity the same numbering system is used for **3** and **4**.

Eucalyptone G (4). Yellow needles; R_f = 0.68 (solvent system I); m. p. 197-198 °C, Positive-ion ESI-MS *m/z* (rel. int.%): 677 $[M+H]^+$ (88); HRESI-MS *m/z* 677.3647 (calcd for $C_{40}H_{52}O_9$ 677.3611) in addition to a significant fragment ion peak at *m/z* 441 [M-236] .IR γ_{max} (KBr) cm^{-1}: 3390, 2975, 1715, 1709, 1620, 1584, 1460, 1323, 1120, 762. ^1H NMR (500 MHz, $CDCl_3$): see table 1, ^{13}C NMR (125 MHz, $CDCl_3$): see table 2.

**4`,5,7-Trimethoxykaempferol (5).**[13, 14] Yellow needles; $R_f$ = 0.54 (solvent system I); m. p. 152 °C, Positive-ion ESI-MS *m/z* (rel. int.%): 329 $[M+H]^+$ (100). $^1H$ NMR (500 MHz, $CDCl_3$): $\delta_H$ 8.19 (2H, dd, *J* = 1.9, 6.9 Hz, H-2`, 6`), 7.05 (2H, dd, *J* = 1.9, 6.9 Hz, H-3`, 5`), 6.57 (1H, d, *J* = 2.2 Hz, H-8), 6.36 (1H, d, *J* = 2.2 Hz, H-6), 3.88 (3H, s, 5-$OCH_3$), 3.91 (3H, s,7-$OCH_3$), 3.98 (3H, s, 4`-OCH_3). ^{13}C NMR (125 MHz, $CDCl_3$): δ_C 171.93 (C-4, s), 164.29 (C-7, s), 160.61 (C-5, s), 160.53 (C-4`, s), 158.85 (C-2, s), 142.25 (C-9, s), 137.43 (C-3, s), 128.86 (C-2`, 6`, d), 123.56 (C-1`, s), 113.99 (C-3`, 5`, d),106.24 (C-10, s), 95.64 (C-6, d), 92.39 (C-8, d), 56.39 (4`-OCH_3, q), 55.79 (7-OCH_3, q), 55.37 (5-OCH_3, q).

Naringenin (6).[15] Yellowish white amorphous powder; R_f = 0.86 (solvent system II); m. p. 257-258 °C, Positive-ion ESI-MS *m/z* (rel. int.%): 273 $[M+H]^+$ (100). ^1H NMR (500 MHz, DMSO-d_6): δ_H 12.14 (1H, s, 5-OH), 10.77 (1H, s, 7-OH), 9.57 (1H, s, 4`-OH), 7.30 (2H, d, *J* = 8.5 Hz, H-2`, 6`), 6.78 (2H, d, *J* = 8.5 Hz, H-3`, 5`), 5.86 (2H, br s, H-6, 8), 5.42 (1H, dd, *J* = 2.8, 12.6 Hz, H-2), 3.26 (1H, dd, *J* = 12.9, 17.0 Hz, H-$3_{ax}$), 2.67 (1H, dd, *J* = 2.8, 17.0 Hz, H-$3_{eq}$). $^{13}C$ NMR (125 MHz, DMSO-$d_6$): $\delta_C$ 196.40 (C-4, s), 166.62 (C-7, s), 163.47 (C-5, s), 162.93 (C-9, s), 157.71 (C-4`, s), 128.83 (C-1`, s), 128.34 (C-2`, 6`, d), 115.14 (C-3`, 5`, d), 101.75 (C-10, s), 95.76 (C-6, d), 94.94 (C-8, d), 78.41 (C-2, d), 41.95 (C-3, t).

Genistein (7).[13,14] Yellow amorphous powder; R_f = 0.77 (solvent system II); m. p. 303-304 °C, Positive-ion ESI-MS *m/z* (rel. int.%): 271 $[M+H]^+$ (100). ^1H NMR (500 MHz, DMSO-d_6): δ_H 12.94 (1H, s, 5-OH), 10.87 (1H, s, 7-OH), 9.58 (1H, s, 4`-OH), 8.31 (1H, s, H-2), 7.30 (2H, dd, *J* = 1.9, 6.6 Hz, H-2`, 6`), 6.78 (2H, dd, *J* = 1.9, 6.6 Hz, H-3`, 5`), 6.37 (1H, d, *J* = 2.2 Hz, H-8), 6.21 (1H, d, *J* = 2.2 Hz, H-6). $^{13}C$ NMR (125 MHz, DMSO-$d_6$): $\delta_C$ 180.20 (C-4, s), 164.25 (C-7, s), 161.98 (C-5, s), 157.57 (C-4`, s), 157.40 (C-9, s), 153.98 (C-2, d), 130.15 (C-2`, 6`, d), 122.25 (C-3, s), 121.18 (C-1`, s), 115.04 (C-3`, 5`, d), 104.45 (C-10, s), 98.95 (C-6, d), 93.64 (C-8, d).

Catechin (8).[15,16] Whitish powder; R_f = 0.69 (solvent system II); m. p. 244-245 °C, Positive-ion ESI-MS *m/z* (rel. int.%): 291 $[M+H]^+$ (100). ^1H NMR (500 MHz, DMSO-d_6): δ_H 9.17 (1H, s, 5-OH), 8.93 (1H, s, 7-OH), 8.86 (1H, s, 4`-OH), 8.81 (1H, s, 3`-OH), 6.70 (1H, d, *J* = 1.6 Hz, H-2`), 6.67 (1H, d, *J* = 8.2 Hz, H-6`), 6.57 (1H, dd, *J* = 1.6, 8.2 Hz, H-5`), 5.87 (1H, d, *J* = 2.2 Hz, H-8), 5.67 (1H, d, *J* = 2.2 Hz, H-6), 4.86 (1H, d, *J* = 5.86 Hz, H-2), 4.47 (1H, d, *J* = 7.6 Hz, 3-OH), 3.80 (1H, ddd, *J* = 5.3, 7.5, 12.9 Hz, H-3), 2.64 (1H, dd, *J* = 5.3, 16.1 Hz, H-4_{eq}), 2.33 (1H, dd, *J* = 7.9, 15.7 Hz, H-4_{ax}). ^{13}C NMR (125 MHz, DMSO-d_6): δ_C 156.45 (C-5, s), 156.17 (C-7, s), 155.35 (C-9, s), 144.83 (C-3`, 4`, s), 130.58 (C-1`, s), 118.34 (C-6`, d), 115.06 (C-5`, d), 114.15 (C-2`, d), 99.05 (C-10, s), 95.09 (C-6, d), 93.83 (C-8, d), 80.99 (C-2, d), 66.30 (C-3, d), 27.87 (C-4, t).

Epicatechin (9).[15,16] Yellowish residue; R_f = 0.69 (solvent system II); m. p. 241-242 °C, Positive-ion ESI-MS *m/z* (rel. int.%): 291 $[M+H]^+$ (100). ^1H NMR (500 MHz, DMSO-d_6): δ_H

9.17 (1H, s, 5-OH), 8.93 (1H, s, 7-OH), 8.85 (1H, s, 4`-OH), 8.75 (1H, s, 3`-OH), 6.96 (1H, d, *J* = 1.6 Hz, H-2`), 6.75 (1H, d, *J* = 8.2 Hz, H-6`), 6.71 (1H, dd, *J* = 1.6, 8.2 Hz, H-5`), 5.94 (1H, d, *J* = 2.2 Hz, H-8), 5.75 (1H, d, *J* = 2.2 Hz, H-6), 4.82 (1H, s, H-2), 4.69 (1H, d, *J* = 5.42 Hz, 3-OH), 4.07 (1H, d, *J* = 3.4 Hz, H-3), 2.64 (1H, dd, *J* = 4.1, 16.1 Hz, $H\text{-}4_{ax}$), 2.33 (1H, dd, *J* = 2.2, 15.4 Hz, $H\text{-}4_{eq}$). ^{13}C NMR (125 MHz, DMSO-d_6): δ_C 156.79 (C-5, s), 156.47 (C-7, s), 156.03 (C-9, s), 144.73 (C- 4`, s), 144.66 (C-3`, s), 130.90 (C-1`, s), 118.27 (C-6`, d), 115.12 (C-2`, d), 115.06 (C-5`, d), 98.79 (C-10, s), 95.36 (C-6, d), 94.38 (C-8, d), 78.31 (C-2, d), 65.19 (C-3, d), 28.47 (C-4, t).

Octyl–β-D-glucopyranoside (10).[17] White powder; R_f = 0.62 (solvent system III); m. p. 107 °C, FAB-MS *m/z* (rel. int.%): 315 $[M+Na]^+$ (100). ^1H NMR (500 MHz, DMSO-d_6): δ_H 4.92 (1H, s, 2`-OH), 4.89 (1H, s, 3` or 4`-OH), 4.86 (1H, s, 5`-OH), 4.08 (1H, d, *J* = 7.9 Hz, H-1`), 3.72, 3.41 (2H, m, H-6`), 3.72 (1H, m, H-8A), 3.40 (1H, m, H-8B), 3.10 (1H, m, H-5`), 3.06-3.03 (2H, m, H-3`, 4`), 2.90 (1H, m, H-2`), 1.49 (2H, quintet, *J* = 7.2 Hz, H-7), 1.29-1.24 (12 H, br s, -$(CH_2)_6$-), 0.84 (3H, d, *J* = 7.2 Hz, H_3-1). ^{13}C NMR (125 MHz, DMSO-d_6): δ_C 102.81 (C-1`, d), 76.79 (C-3`, d), 76.75 (C-5`, d), 73.41 (C-2`, d), 70.05 (C-4`, d), 68.52 (C-8, t), 61.06 (C-6`, t), 31.24 (C-7, t), 29.29 (C-6, t), 28.88 (C-5, t), 28.68 (C-4, t), 25.52 (C-3, t), 22.02 (C-2, t), 13.95 (C-1, q).

Agar plate diffusion assay.[18] Susceptibility disks (5 mm diameter) were impregnated with 5 μg of the isolated compound and then placed on agar plates inoculated with the test bacterium: *B. subtilis* 168 and *S. aureus* ATCC 25923 (for the Gram-positive bacteria) and *E. coli* ATCC 25922 (for Gram-negative bacteria). The plates were observed for zones of inhibition, after 24 h of incubation at 37 °C. In all cases, for the controls containing only the respective amount of solvent, no zones of inhibition were observed.

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