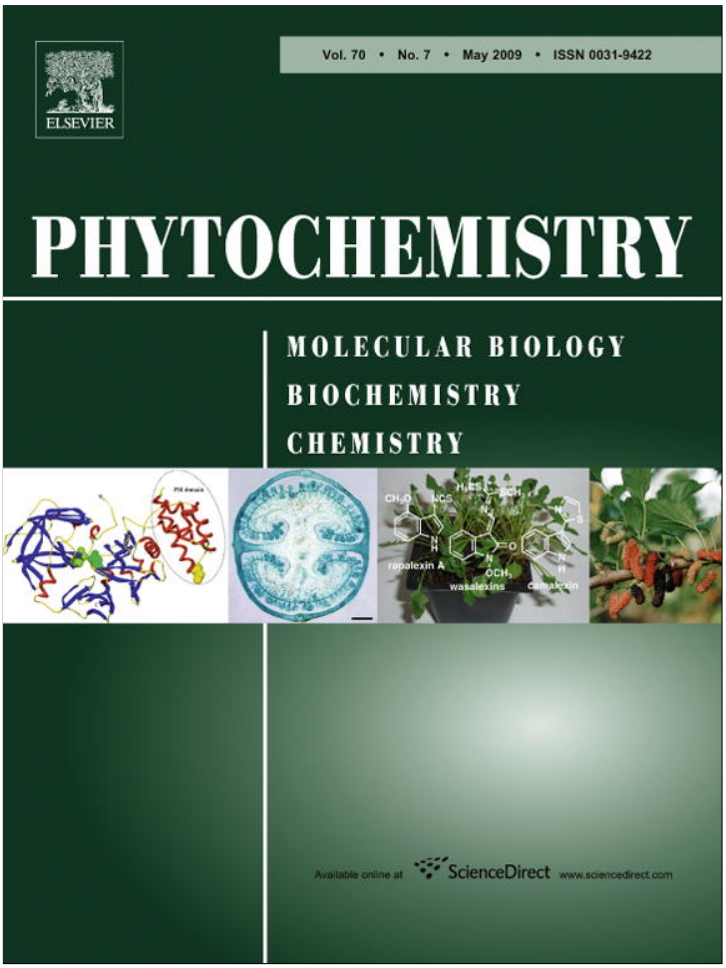

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Antiplasmodial and other constituents from four Indonesian *Garcinia* spp.

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Isoxanthochymol
Garcihombronane D
3β-Hydroxy-5-glutinen-28-oic acid
Antiprotozoal activity
Plasmodium falciparum

ABSTRACT

Phytochemical investigations of four *Garcinia* spp. from Indonesia, i.e. *Garcinia griffithii* T. Anderson, *Garcinia celebica* L., *Garcinia cornea* L. and *Garcinia cymosa* K. Schum (Clusiaceae), have resulted in the isolation of a xanthone, 1,5-dihydroxy-3,6-dimethoxy-2,7-diprenylxanthone, 1,7-dihydroxyxanthone, isoxanthochymol, β-sitosterol-3-*O*-β-D-glucoside and stigmasterol-3-*O*-β-D-glucoside from the stem bark of *G. griffithii*; friedelin and 3β-hydroxy-23-oxo-9,16-lanostadien-26-oic acid or garcihombronane D from leaves of *G. celebica*; 23-hydroxy-3-oxo-cycloart-24-en-26-oic acid and epicatechin from stem bark of *G. cornea*; (±)-morelloflavone, morelloflavone-7-*O*-β-D-glucoside or fukugiside, the triterpene 3β-hydroxy-5-glutinen-28-oic acid and canophyllol from stem bark of *G. cymosa*. The xanthone and garcihombronane D displayed a selective activity against *Plasmodium falciparum*; isoxanthochymol and the triterpene β-hydroxy-5-glutinen-28-oic acid a broad but non-selective antiprotozoal activity.

1. Introduction

Garcinia species are known to contain a wide variety of oxygenated and prenylated xanthones, as well as polyisoprenylated benzophenones such as the guttiferones (Nilar et al., 2005). Xanthones show a wide range of biological and pharmacological properties, e.g. antioxidant, antiinflammatory, antimicrobial and cytotoxic activities (Minami et al., 1994; Mbwambo et al., 2006; Chin et al., 2008). Guttiferones have been reported as anti-HIV, trypanocidal and cytotoxic agents (Gustafson et al., 1992; Williams et al., 2003; Merza et al., 2006; Vlietinck et al., 1998; Cos et al., 2008).

Garcinia griffithii (locally named "kandis gajah" in Indonesia) is a medium sized tree ocurring in South East Asia. The polyisoprenylated benzophenones cambogin or isoxanthochymol, and guttiferone I, as well as the xanthones 1,7-dihydroxyxanthone, 1,3,6,7-tetrahydroxyxanthone, 1,3,5,6-tetrahydroxyxanthone, and the bixanthone griffipavixanthone, have been isolated and identified from *G. griffithii* (Nilar et al., 2005; Xu et al., 1998). However, it should be noted that the name guttiferone I has also been applied for different compounds obtained from *Garcinia virgata* (Merza et al., 2006) and from *Garcinia humilis* (Herath et al., 2005). No phytochemical investigations have been carried out yet on *Garcinia celebica*, *Garcinia cornea* and *Garcinia cymosa*. In our continuing phytochemical investigation of *Garcinia* plants found in Indonesia, the isolation and structure elucidation from *G. griffithii*, *G. celebica*, *G. cornea* and *G. cymosa* of a new xanthone, a new triterpene, isoxanthochymol, some unusual triterpenes and some common compounds, as well as the antiparasitic activity of the new xanthone, the new triterpene and some other constituents are reported here.

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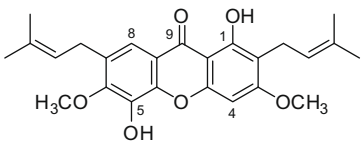
2. Results and discussion



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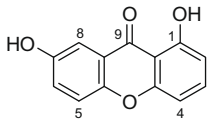

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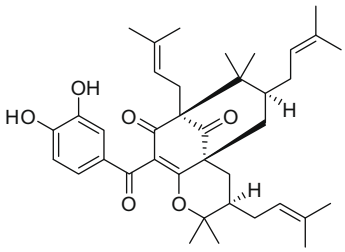

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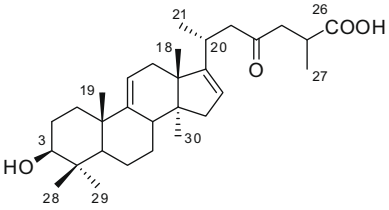

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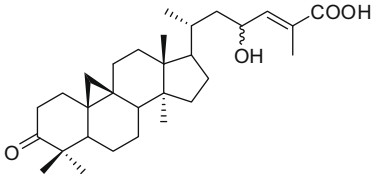

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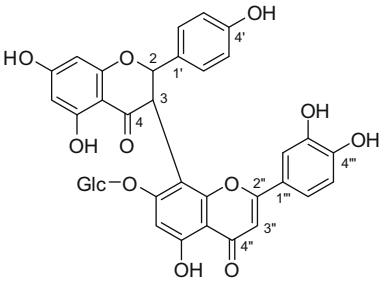

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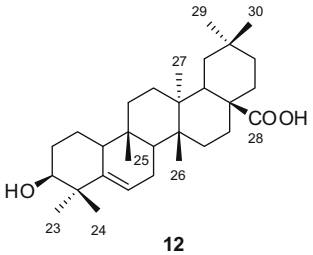

12

1,5-Dihydroxy-3,6-dimethoxy-2,7-diprenylxanthone (**1**), 1,6-dihydroxyxanthone (**2**), isoxanthochymol (cambogin) (**3**), β-sitosterol-3-*O*-β-D-glucoside (**4**), stigmasterol-3-*O*-β-D-glucoside (**5**), and friedelin (**6**) were obtained from *G. griffithii*. The ^{1}H NMR spectrum of compound **1** showed the presence a chelated hydroxyl at δ 12.95. The presence of two prenyl groups was evident from the four methyl groups at δ 1.67, 1.73 ppm, 1.75 and 1.78, four allylic methylene protons at δ 3.35 ppm (2H, *d*, *J* = 7.4 Hz) and 3.40 ppm (2H, *d*, *J* = 7.1 Hz), and two vinylic methine protons at δ 5.22 and 5.29. The spectrum also revealed the presence of two methoxyl groups at δ 3.91 and 3.96, two aromatic protons at δ 6.50 ppm (1H, *s*) and 7.61 ppm (1H, *s*), and an additional non-chelated hydroxyl group at δ 5.70 ppm. The ^{13}C NMR spectrum showed 25 carbon resonances assigned to a carbonyl group (δ 180.38), ten quaternary aromatic carbons and two aromatic CH groups (δ 89.80 and δ 116.55), two methoxyl groups (δ 55.98 and δ 61.12), and two groups of five resonances showing the typical pattern of a prenyl group. The ^{1}H and ^{13}C NMR data suggested a xanthone-like structure (Shahat et al., 2003). A literature search showed that the ^{1}H and ^{13}C NMR data were in complete agreement with a 1-hydroxyl, 2-prenyl, 3-methoxyl substitution pattern of one of the aromatic rings of the xanthone moiety, as in β-mangostin or cudraxanthone F (Likhitwitayawuid et al., 1998; Hano et al., 1990). This substitution pattern was confirmed by the HMBC spectrum. The methylene protons at δ 3.35 (H-1′) showed five correlations (^{2}J: C-2, C-2′; ^{3}J: C-1, C-3, C-3′), which required the placement of one of the 3-methylbut-2-enyl groups at C-2. The methoxyl proton at δ 3.91 showed a long-range correlation ^{3}J with C-3, confirming its position. The aromatic proton at δ 6.50 showed four correlations (^{2}J: C-3, C-4a; ^{3}J: C-2, C-9a), confirming C-4 as the unsubstituted position. The substitution pattern of the first aromatic ring being established, it was evident that one hydrogen, one hydroxyl, one methoxyl and one prenyl group were left as substituents for the second ring. No known compound that completely matched the NMR spectra was found. From a statistical point of view there were 24 possibilities. However, several structural proposals could readily be excluded for various reasons: the hydroxyl group could not be situated at C-8, since its chemical shift of δ 5.70 did not indicate a chelated hydro-

xyl group; the methoxyl resonance at δ 61.12 in ^{13}C NMR indicated a position between two other substituents and not next to an unsubstituted position; other proposals were identical to known compounds but showed different NMR spectra. The HMBC spectrum indicated that position C-8 had to be the unsubstituted one, as evident from the correlations between H-8 (*d* 7.61) and C-9 (carbonyl, δ 180.38, ^3J), and C-4b (δ 143.85, ^3J). The methylene protons at δ 3.40 (H-1″) showed five correlations (^2J: C-7, C-2″; ^3J: C-6, C-8, C-3″), which required the placement of the second prenyl group at C-7. The exchangeable proton of the hydroxyl functionality (δ 5.70) was correlated to C-4b (δ 143.85, ^3J) and C-6 (δ 149.82, ^3J), confirming its position at C-5, and the methoxyl proton at δ 3.96 was correlated with C-6 (δ 149.82, ^3J), indicating its position at C-6. Hence the structure of compound **1** could unequivocally be established as 1,5-dihydroxy-3,6-dimethoxy-2,7-diprenylxanthone, being a new compound, and all ^{1}H and ^{13}C NMR signals assigned. This was confirmed by ESIMS producing a pseudomolecular ion at *m/z* 423 ($[M-H]^-$) in the negative ion mode and high-resolution MS confirming the molecular formula as $C_{25}H_{28}O_6$. Until now this substitution pattern in one of the aromatic rings of a xanthone had only been observed in cudrafrutixanthone A from *Cudrania fruticosa* (Wang et al., 2004). The ^{1}H and ^{13}C NMR assignments for this part of the molecule were in good agreement for both compounds.

The ^{1}H NMR spectrum of compound **2** showed the presence of six aromatic protons; the coupling pattern was in agreement with one spin system consisting of three protons, and another spin system consisting of two protons that were *ortho*-coupled to each other, and one proton that was *meta*-coupled to one of these. The exchangeble protons were observed, one of them at δ 12.61 indicating a chelated hydroxyl group. Also the ^{13}C NMR spectrum showed the typical pattern of a xanthone with two hydroxyl substituents. Based on this information only two possibilities were left, i.e. 1,6-dihydroxy- or 1,7-dihydroxyxanthone. Indeed the NMR and other spectral data were in good agreement with those reported for 1,7-dihydroxyxanthone, not the 1,6-dihydroxy analogue; e.g. in 1,7-dihydroxyxanthone and in compound **2** C-8 was observed around δ 107, whereas this should be around δ 102 in 1,6-dihydroxyxanthone (Yang et al., 2001; Lin et al., 1993). 1,7-Dihydroxyxanthone was reported before from *G. griffithii* by Nilar et al. (2005).

The ^{1}H and ^{13}C NMR spectra of compound **3** showed the typical features of a guttiferone, i.e. the typical coupling pattern of a 1,3,4-trisubstituted benzophenone moiety and three prenyl groups in the ^{1}H NMR spectrum, and four carbonyls in the ^{13}C NMR spectrum. A quaternary ^{13}C NMR signal occurred at δ 86.34, and together with the fact that no tautomeric equilibrium was observed, in contrast to most of the guttiferones, this indicated a structure where this tautomerism was blocked by a ring closure between one of the hydroxyl groups and a prenyl group. Indeed the NMR and other spectral data were in agreement with those reported for isoxanthochymol or cambogin, reported before from this plant (Nilar et al., 2005; Gustafson et al., 1992). Compounds **4** and **5** were isolated as a mixture and were identified as β-sitosterol-3-*O*-β-D-glucoside and stigmasterol-3-*O*-β-D-glucoside (Voutquenne et al., 1999; Alam et al., 1996).

From *G. celebica*, two triterpenes were obtained. Compound **6** could readily be identified as the common 3-keto-triterpenoid friedelin (Klass et al., 1992). The ^{13}C NMR spectrum of compound **7** showed a non-conjugated carbonyl functionality at δ 211.12, two quaternary unsaturated carbons (δ 157.16 and δ 151.09), and two unsaturated CH-signals (δ 121.54 and δ 115.70), suggesting a triterpene with two double bonds. The HSQC spectrum showed that the latter two signals were correlated with a broad singlet at δ 5.27 and a doublet at δ 5.33 (J = 5.8 Hz), respectively. Presumably the carbonyl functionality could not be located at C-3, since the usual signal of 3-hydroxy-triterpenes at δ 79.61 in ^{13}C NMR and δ 3.14 (*dd*, J = 8.1 and 8.0 Hz) was present. In the ^{13}C NMR spectrum only 29 carbon signals were observed; nevertheless, from the long-range correlations observed in the HMBC spectrum the presence of an additional signal at δ 181.00 could be deduced. Careful analysis of the two-dimensional NMR spectra (COSY, HSQC and HMBC) allowed determination of the position of the carbonyl and carboxyl functionalities, and the two double bonds in a lanostadiene-like skeleton. The ^{1}H and ^{13}C NMR spectra were found to be in complete agreement with those reported for 3β-hydroxy-23-oxo-9,16-lanostadien-26-oic acid or garcihombronane D (**7**), reported before from *Garcinia hombroniana* (Rukachaisirikul et al., 2000, 2005). The ^{1}H and ^{13}C NMR assignments of compound **7** are added as Supplementary Data (Table S1). The absolute configuration at C-25 of the same compound isolated from *Garcinia speciosa* was established as *R* by X-ray diffraction (Vieira et al., 2004).

Compounds **8** and **9** were obtained from *G. cornea*. The ^{1}H and ^{13}C NMR spectrum of compound **8** indicated a triterpenoid structure; the presence of a non-conjugated carbonyl (δ 214.89), a carboxyl (δ 169.00), a double bond (CH-group at δ 146.00, and a quaternary carbon at δ 125.30), and a cyclopropyl moiety (quaternary carbons at δ 25.45 and 20.49; a CH_2-group at δ 28.79) were obvious, suggesting a cycloartenone-like structure (Baldé et al., 2001). No CH-OH functionality as expected for 3-hydroxylated triterpenes was observed, suggesting that the C-3 substituent was a ketone. By means of two-dimensional NMR spectra the structure could be resolved as 23-hydroxy-3-oxo-cycloart-24-en-26-oic acid or 23-hydroxy-mangiferonic acid, reported before from *Mangifera indica* as a mixture with the corresponding 3α,22-dihydroxy derivative (Anjaneyulu et al., 1999); the two compounds could only be resolved after acetylation. Hence this is the first report of the isolation of 23-hydroxy-3-oxo-cycloart-24-en-26-oic acid as a pure compound, and the first complete assignment of its ^{1}H and ^{13}C NMR spectra (Table 2). The absolute configuration at C-23 has not been determined. Compound **9** from *G. cornea* was identified as epicatechin (Davis et al., 1996).

Table 1
^{1}H (400 MHz) and ^{13}C (100 MHz) NMR assignments for 1,5-dihydroxy-3,6-dimethoxy-2,7-diprenylxanthone (**1**) in CD_3OD.

Carbon no.	^{13}C NMR δ (ppm)	^{1}H NMR δ (ppm), mult., J (Hz)
1	159.78	–
2	112.14	–
3	164.19	–
4	89.90	6.50
4a	155.93	–
4b	143.85	–
5	136.74	–
6	149.82	–
7	133.52	–
8	116.55	7.61
8a	117.13	–
9	180.38	–
9a	103.58	–
1′	21.37	3.35, *d*, 7.4
2′	122.04[a]	5.22, *m*[a]
3′	131.94[b]	–
4′	25.79	1.75, *d*, 1.0[c]
5′	17.88[c]	1.73, *br s*[b]
1”	28.45	3.40, *d*, 7.1
2”	121.75[a]	5.29, *m*[a]
3”	131.31[b]	–
4”	25.79	1.67, *d*, 1.0[c]
5”	17.78[c]	1.78, *br s*[b]
3-OMe	55.98	3.91, *s*
6-OMe	61.12	3.96, *s*
1-OH	–	12.95, *s*
5-OH	–	5.70, *s*

[a,b,c]Assignments bearing the same superscript may be interchanged within the same column.

Table 2
^{1}H (400 MHz) and ^{13}C (100 MHz) NMR assignments for 23-hydroxy-3-oxo-cycloart-24-en-26-oic acid (**8**) in DMSO-d_6.

Carbon no.	^{13}C NMR δ (ppm)	Characteristic ^{1}H NMR signals δ (ppm), mult., J (Hz)
1	32.54[a]	1.63, *m*/1.50, *m*[a]
2	37.00	2.67, *ddd*, 13.8, 13.8, 6.3 2.14, *m*
3	214.89	–
4	49.54	–
5	47.79	1.64, *m*
6	21.01	1.47, *m*/0.92, *m*
7	27.78	1.84, *m*/1.25, *m*
8	47.38	1.55, *m*
9	20.49	–
10	25.45	–
11	25.54[b]	1.31, *m*/1.07, *m*
12	32.65[a]	1.75, *m*/1.63, *m*[a]
13	45.03	–
14	48.43	–
15	35.16	1.27, *m*
16	25.98[b]	1.98, *m*/1.18, *m*
17	52.27	1.55, *m*
18	17.97[c]	0.97, *s*
19	28.79	0.71, *d*, 3.7 0.61, *d*, 3.7
20	31.75	1.67, *m*
21	18.10[c]	0.89, *d*, 6.2
22	42.74	1.55, *m*/0.88, *m*
23	64.40	4.33, *m*
24	146.00	6.54, *d*, 7.8
25	125.30	–
26	169.00	–
27	12.38	1.70, *s*
28	20.44	1.01, *s*
29	22.18	0.92, *s*
30	19.06	0.84, *s*

[a,b,c]Assignments bearing the same superscript may be interchanged within the same column.

Table 3
^{1}H (400 MHz) and ^{13}C (100 MHz) NMR assignments for fukugiside (**11**) in CD_3OD.

Carbon no.	^{13}C NMR δ (ppm)	characteristic ^{1}H NMR signals δ (ppm), mult., J (Hz)
2	82.84	5.74, *d*, 11.6
3	51.01	4.83[b]
4	197.64	
5	164.82	
6	96.51	5.98, *br s*
7	165.72	
8	97.70	5.93, *br s*
9	168.33	
10	103.32	
1′	130.39	
2′,6′	129.40	7.10, *d*, 8.1
3′,5′	115.46	6.35, *d*, 8.1
6′	158.46	
2″	166.10	
3″	103.58	6.38, *s*
4″	183.90	
5″	162.71	
6″	99.51	6.62, *s*
7″	161.64	
8″	104.13	
9″	156.65	
10″	106.47	
1‴	123.05	
2‴	114.38	7.31, *br s*
3‴	146.72	
4‴	151.13	
5‴	116.87	6.87, *d*, 8.3
6‴	120.84	7.26, *br d*, 8.3
1⁗	101.52	5.15, *d*, 7.5
2⁗	75.19	3.3–3.9, *m*
3⁗	78.32[a]	3.3–3.9, *m*
4⁗	71.10	3.3–3.9, *m*
5⁗	78.52[a]	3.3–3.9, *m*
6⁗	62.47	3.3–3.9, *m*

[a] Assignments bearing the same superscript may be interchanged within the same column.
[b] Masked by solvent.

Phytochemical investigation of *G. cymosa* resulted in the isolation and identification of compounds **10**–**13**. Compound **10** could readily be identified as the biflavonoid morelloflavone, based on its NMR and mass spectral data. Recently we have obtained (+)-morelloflavone from *Garcinia livingstonei*, with a specific optical rotation of +202 (Mbwambo et al., 2006). However, this sample showed an $[\alpha]_D^{25}$ of only +10 (*c* 0.25 g/100 ml, DMSO), indicating an almost racemic mixture. Indeed, (±)-morelloflavone has been reported as a constituent of many *Garcinia* spp. (Dictionary of Natural Products on DVD, 2007). Compound **11** showed the same pattern as **10** in ^{1}H and ^{13}C NMR, but additional signals for a glucosyl moiety were present. This was confirmed by mass spectrometry ($[M-H]^-$ at *m/z* 717). Detailed analysis of the 2D-NMR spectra (COSY, HSQC and HMBC) allowed assignment of the ^{13}C NMR signal at δ 161.64 to C-7 of the lower unit (De Bruyne et al., 1996, 1999). This carbon showed a long-range correlation with the anomeric proton at δ 5.15 in ^{1}H NMR, establishing unambiguously the position of the glucoside. Morelloflavone-7″-*O*-β-D-glucoside is known as fukugiside. The specific optical rotation of our sample ($[\alpha]_D^{25}$ + 165, *c* 1.07 g/100 ml, MeOH) was comparable to the reported value (155), further confirming its identity. Fukugiside has been reported before from *Garcinia xanthochymus*, *Garcinia spicata* and *Garcinia atroviridis* (Baggett et al., 2005; Konoshima and Ikeshiro, 1970; Permana et al., 2003). This is the first report of the ^{13}C NMR assignments of fukugiside (Table 3).

The ^{1}H and ^{13}C NMR spectra of compound **12** were in good agreement with those observed for 3β-hydroxy-glutin-5-ene reported in *Maytenus horrida* by Gonzalez et al. (1987). However, one methyl group was lacking but a carboxylic acid moeity appeared to be present. This was confirmed by MS ($[M-H]^-$ observed at *m/z* 455). The position of the carboxylic acid residue was determined by detailed analysis of the 2D-NMR spectra (COSY, HSQC and HMBC) and unambiguously assigned to C-28, based on the long-range correlation observed between C-28 at δ 184.75 and H-18 at δ 2.37. 3β-Hydroxy-5-glutinen-28-oic acid was a new compound. Complete ^{1}H and ^{13}C NMR assignments were based on 2D-NMR analysis (Table 4). Duplication of signals due to rotational isomerism was observed; only assignments for the major rotamer are given. Assignments of all ^{1}H NMR peaks of the glucosyl moiety was not possible due to signal overlap. Further spectral analysis allowed identification of compound **13** as the triterpene 28-hydroxy-friedelin or canophyllol (Ali et al., 1999).

Selected compounds were evaluated in an integrated antimicrobial and antiparasitic screening including *Trypanosoma cruzi*, *Trypanosoma brucei*, *Leishmania infantum*, *Plasmodium falciparum* (Ghana strain), *Staphylococcus aureus*, *Escherichia coli*, and evaluation of cytotoxicity against MRC-5 cells. The new xanthone 1,5-dihydroxy-3,6-dimethoxy-2,7-diprenylxanthone (**1**) showed selective activity against *P. falciparum* with an IC_{50} of 7.25 μM, all other IC_{50} values, including cytotoxicity, being >64 μM. In contrast, 1,7-dihydroxyxanthone (**2**) was completely inactive. Isoxanthochymol (**3**) exhibited a broad but non-selective antiprotozoal activity, with IC_{50} values of 2.65 μM against *T. cruzi*, 1.91 μM (*T. brucei*), 2.03 μM (*L. infantum*), and 4.47 μM (*P. falciparum*), but it was also cytotoxic (IC_{50} 7.46 μM). Similar to the xanthone (**1**), 3β-hydroxy-23-oxo-9,16-lanostadien-26-oic acid or garcihombronane D (**7**) showed a selective activity against *P. falciparum* (IC_{50} 7.71 μM). In contrast, 23-hydroxy-3-oxo-cycloart-24-en-26-oic acid (**8**) was completely inactive. The new triterpene from *G. cymosa* (**12**) displayed a broad but weak and non-specific antiprotozoal activity and cytotoxicity, with IC_{50} values of 10.64 μM against *T. cruzi*, 11.09 μM (*T. brucei*),

Table 4
^{1}H (400 MHz) and ^{13}C (100 MHz) NMR assignments for 3β-hydroxy-5-glutinen-28-oic acid (**12**) in CD_3OD.

Carbon no.	^{13}C NMR δ (ppm)	^{1}H NMR δ (ppm), mult., J (Hz)
1	18.30	1.44/1.52
2	27.77	1.67/1.82
3	76.31	3.45, *br s*
4	40.79	–
5	141.46	–
6	121.72	5.60
7	23.43	1.75/1.95
8	47.71	1.50
9	35.07	–
10	49.93	1.98
11	34.47	1.36/1.51
12	30.88	1.35/1.45
13	38.61	–
14	37.11	–
15	32.41	1.20
16	35.82	1.48
17	44.73	–
18	37.73	2.37, *dd*, 13.1, 3.8
19	34.84	1.14/1.29
20	28.45	–
21	32.75	1.46
22	29.23	1.65/2.29, *dd*, 14.7, 9.5
23	28.92	1.01, *s*
24	25.42	1.12, *s*
25	15.59	0.80, *s*
26	20.29	0.89, *s*
27	18.22	0.96, *s*
28	184.75	–
29	34.31	0.91, *s*
30	29.72	1.01, *s*

8.64 μM (*L. infantum*), 31.05 μM (*P. falciparum*), and 6.87 μM (MRC-5 cells). None of the selected compounds showed any antibacterial activity.

3. Experimental

3.1. General experimental procedures

Melting points were determined on a micromelting point apparatus. UV and IR spectra were measured on Cary Varian 100 Conc., Beckman DU-700 (UV), and Shimadzu FTIR 8400 spectrophotometers, respectively. The ^{1}H, ^{13}C and DEPT NMR spectra, as well as two-dimensional experiments (COSY, HSQC, HMBC using pulsed field gradients) were recorded on a Bruker DRX 400 spectrometer, operating at 400 MHz (^{1}H) and 100 MHz (^{13}C) in $CDCl_3$, CD_3OD or DMSO-d_6, using standard software packages and pulse sequences. Mass spectra were recorded with a LXQ linear ion trap instrument (Finnigan) using electrospray ionisation in the negative or positive mode. Positive ion mode accurate mass spectra were acquired using a Q-TOF II instrument (Waters, Manchester, UK). The MS was calibrated prior to use and the spectra were lock mass corrected using the know mass of the of a PEG-cluster ion (*m/z* 432.2809). Vacuum Liquid Chromatography (VLC) was carried out using Merck Si Gel 60 GF254 (230–400 mesh), column chromatography using Si Gel 60 (70–230 mesh), TLC analysis was performed on Si Gel plates (Merck kieselgel 60 GF254, 0.25 mm, 20 × 20 cm).

3.2. Plant material

Stem bark of *G. griffithii* was collected on April 2006 from the Sarasah Bonta, Lembah Arau, Kabupaten Lima Puluh Kota, West Sumatra. The plant was identified by the staff at the Andalas University Herbarium (ANDA), Padang and a voucher specimen has been deposited at the Herbarium.

Fresh leaves *of G. celebica*, stem bark of *G. cornea* and stem bark of *G. cymosa* were collected on April 2006 from the Kebun Raya Bogor, West Java. The plants were identified by the staff at the Herbarium Bogoriense, Bogor and voucher specimens have been deposited at the Herbarium. Stem bark of *G. cornea* and *G. cymosa* was roasted first, based on the traditional use (to sterilise the plant material in order to avoid microbial degradation).

3.3. Extraction and isolation

Powdered stem bark (1 kg) of *G. griffithii* was successively extracted with *n*-hexane, CH_2Cl_2 and MeOH, yielding 35.2 g *n*-hexane extract, 23.5 g CH_2Cl_2 extract, and 125.4 g MeOH extract. A portion (20 g) of the CH_2Cl_2 extract was fractionated by VLC eluted with *n*-hexane – CH_2Cl_2 mixtures of increasing polarity and CH_2Cl_2 – MeOH (9:1 and 8:2) to give six fractions A–F (2.6 g; 2.2 g; 1.7 g; 8.0 g; 4.0 g and 5.8 g respectively) (Supplementary Data, Scheme 1S). Fraction B was further fractionated by column chromatography eluted with *n*-hexane – EtOAc (9:1 and 2:8) to given six fractions B1–B6 (92 mg; 86 mg; 146 mg; 134 mg; 67 mg; and 125 mg, respectively). Fraction B4 was subjected to column chromatography eluted with *n*-hexane – CH_2Cl_2 (3:7) and after crystallisation, compound **1** (14 mg) was obtained. Fraction D was further fractionated by column chromatography eluted with *n*-hexane – EtOAc (3:7–1:9) and EtOAc–MeOH (9:1 and 8:2) to give five fractions D1–D5 (517 mg; 231 mg; 428 mg; 479 mg; and 312 mg, respectively). Fraction D1 was subjected to column cromatography eluted with *n*-hexane–EtOAc (3:7) and recrystalisation afforded compound **2** (10 mg). Fraction D4 was further fractionated by column chromatography eluted with *n*-hexane–EtOAc (3:7, 2:8 and 1:9) and EtOAc–MeOH (9:1) to given four fractions D4.1–D4.4 (64 mg; 31 mg; 40 mg; and 29 mg, respectively). Fraction D4.2 yielded compound **3** (5 mg) after recrystalisation. Compounds **4** and **5** (6 mg) were obtained as a mixture from the CH_2Cl_2 extract by eluting with CH_2Cl_2–EtOAc mixtures of increasing polarity.

Fresh leaves (1 kg) of *G. celebica* were extracted successively with *n*-hexane and EtOAc. The EtOAc extract was evaporated to dryness under reduced pressure, and subjected to VLC using *n*-hexane, *n*-hexane–EtOAc mixtures, EtOAc and MeOH (gradient of increasing polarity) as eluents to give 7 fractions (1–7). Fraction 1 was further separated by VLC using the same solvents to yield again 7 fractions (1.1–1.7). Subfractions 1.2 and 1.3 yielded compound **6** (26.3 mg). VLC of fraction 3 using the same solvents produced 5 subfractions (3.1–3.5), and further VLC under the same conditions 4 subfractions (3.3.1–3.3.4). Subfraction 3.3.1 yielded compound **7** (5.2 mg).

Roasted stem bark (amount 1 kg) of *G. cornea* was extracted successively with *n*-hexane and EtOAc. The EtOAc extract was evaporated to dryness under reduced pressure, and subjected to VLC (elution with solvent mixtures of *n*-hexane–EtOAc–MeOH of increasing polarity) yielding 10 fractions. Fraction 5 afforded compound **8** (15 mg). Fraction 3 was subjected to column chromatography (elution with *n*-hexane–EtOAc (7:3)) and produced 7 fractions. Fraction 3 yielded compound **9** (11 mg).

Roasted stem bark of *G. cymosa* (2 kg) was extracted successively by maceration with *n*-hexane, ethyl acetate and methanol for 5 days. Evaporation of each extract to dryness under reduced pressure, afforded the *n*-hexane (56 g), ethyl acetate (48 g) and methanol (30 g) extracts. The EtOAc extract was subjected to VLC on Si gel eluted with EtOAc, EtOAc: MeOH (1:1), and MeOH to yield 4 fractions. The third fraction was further separated by column chromatography on Si gel eluted with EtOAc: MeOH (1:1) to yield 5 fractions. From the first fraction compound **10** (12 mg) was obtained. Further VLC of the fourth fraction yielded compound **11** (15 mg). The crude *n*-hexane extract (30 g) was subjected to VLC on Si gel and eluted with a solvent mixture of increasing polarity

(*n*-hexane - EtOAc) to afford 5 fractions. Fraction 2 (5 g) was further purified by VLC on Si gel using a gradient (*n*-hexane–*n*-hexane: EtOAc 1:1, and EtOAc) to yield 5 subfractions. After column chromatography on Si gel using a gradient of *n*-hexane–EtOAc, the second subfraction (520 mg) yield compound **12** (9 mg). Further separation of fraction 4 (805 mg) by column chromatography on silica gel using a gradient system EtOAc–MeOH afforded 7 subfractions. After column chromatography on Si gel using a gradient system of EtOAc–MeOH, the sixth subfraction (82 mg) yielded compound **13** (6 mg).

*3.3.1. 1,5-Dihydroxy-3,6-dimethoxy-2,7-diprenylxanthone (**1**)*

Yellow needles; m.p. 208–210 °C. UV (MeOH) λ_{max} (nm): 260, 316; UV (MeOH + NaOH) λ_{max} (nm): 285, 336. IR (KBr) ν_{max} cm^{-1}: 3441 (OH), 1651 (C=O), 1601, 1570, 1466 (C=C aromatic), 1115 (C–O ether). ^1H and ^{13}C NMR: Table 1. ESIMS *m/z* 423 ($[M-H]^-$); HRESIMS *m/z* 425.1961 ($[M+H]^+$) (calc. for $C_{25}H_{29}O_6^+$, 425.1964).

*3.3.2. 3β-Hydroxy-5-glutinen-28-oic (**12**)*

White amorphous powder; $[\alpha]_D^{25}$ +54 (*c* 0.49 g/100 ml, $CHCl_3$); ^1H and ^{13}C NMR: Table 1. ESIMS *m/z* 455 ($[M-H]^-$).

3.4. Biological evaluation

Antimicrobial testing and evaluation of cytotoxicity was carried out as reported before (Cos et al., 2006).

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Appendix A. Supplementary material

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.phytochem.2009.04.024.

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