1-1-2016

Antimalarial polyoxygenated and prenylated xanthones from the leaves and branches of Garcinia mckeaniana

Chiramet Auranwiwat
Chiang Mai University

Surat Laphookhieo
Mae Fah Luang University

Roonglawan Rattanajak
National Science and Technology Development Agency

Sumalee Kamchonwongpaisan
National Science and Technology Development Agency

Stephen G. Pyne
University of Wollongong, spyne@uow.edu.au

See next page for additional authors

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Abstract

The first phytochemical investigation of the leaf and branch extracts of Garcinia mckeaniana has led to the isolation and characterization of five new polyoxygenated and prenylated xanthones, mckeanianones A-E (1-5), and one new biflavone, mckeaniabiflavone (6), together with 13 known compounds (7-19). The diprenylated pyrano-xanthones (1-3) and the biflavone (6) were isolated from the leaves while the monoprenylated xanthones (4 and 5) were isolated from the branches. The structures of all isolated compounds were elucidated based on spectroscopic methods and were evaluated for their antimalarial activities against the Plasmodium falciparum strains, TM4 (drug sensitive strain) and K1 (a multidrug resistant strain), and cytotoxicity against a Vero cell line (African green monkey kidney epithelial cells). The pyrano-xanthones (1-3, 7 and 8), having two isoprene units, were generally the most active compounds with IC50 values in the range of 6.0±1.1 _ 8.5±1.2 and 3.6±1.7 _ 7.3±1.2 μM, respectively against the TM4 and K1 strains. Of these compounds 2, 3 and 8 were 2-5 times less cytotoxic against a Vero cell line (IC50 values of 12.6±0.9, 29.5±3.9 and 13.2±4.6 μM, respectively) in comparison with their antiplasmodial activities.

Disciplines

Medicine and Health Sciences | Social and Behavioral Sciences

Publication Details

Auranwiwat, C., Laphookhieo, S., Rattanajak, R., Kamchonwongpaisan, S., Pyne, S. G. & Ritthiwigrom, T. (2016). Antimalarial polyoxygenated and prenylated xanthones from the leaves and branches of Garcinia mckeaniana. Tetrahedron: the international journal for the rapid publication of full original research papers and critical reviews in organic chemistr, 72 (43), 6837-6842.

Authors

Chiramet Auranwiwat, Surat Laphookhieo, Roonglawan Rattanajak, Sumalee Kamchonwongpaisan, Stephen G. Pyne, and Thunwadee Ritthiwigrom

Antimalarial polyoxygenated and prenylated xanthones from the leaves and branches of *Garcinia mckeaniana*

Chiramet Auranwiwat [a], Surat Laphookhieo [b], Roonglawan Rattanajak [c], Sumalee Kamchonwongpaisan [c], Stephen G. Pyne [d], Thunwadee Ritthiwigrom [a,*]

[a] *Department of Chemistry, Faculty of Science, Chiang Mai University, Sutep, Muang, Chiang Mai, 50200, Thailand*

[b] *Natural Products Research Laboratory, School of Science, Mae Fah Luang University, Tasud, Muang, Chiang Rai, 57100, Thailand*

[c] *Medical Molecular Biology Research Unit, National Center for Genetic Engineering and Biotechnology, National Science and Technology Development Agency, Thailand Science Park, Pathumthani, 12120, Thailand*

[d] *School of Chemistry, University of Wollongong, Wollongong, New South Wales, 2522, Australia*

* Corresponding author. Tel. +66 53 943341-5; *e-mail address*: thunwadee.r@cmu.ac.th, othunwadee@gmail.com (T. Ritthiwigrom)

ABSTRACT

The first phytochemical investigation of the leaf and branch extracts of *Garcinia mckeaniana* has led to the isolation and characterization of five new polyoxygenated and prenylated xanthones, mckeanianones A-E (**1**-**5**), and one new biflavone, mckeaniabiflavone (**6**), together with thirteen known compounds (**7**-**19**). The diprenylated pyrano-xanthones (**1**-**3**) and the biflavone (**6**) were isolated from the leaves while the monoprenylated xanthones (**4** and **5**) were isolated from the branches. The structures of all isolated compounds were elucidated based on spectroscopic methods and were evaluated for their antimalarial activities against the *Plasmodium falciparum* strains, TM4 and K1 (a multidrug resistant strain), and cytotoxicity against a Vero cell line (African green monkey kidney cells). The pyrano-xanthones (**1**-**3**, **7** and **8**), having two isoprene units, were generally the most active

compounds with IC_{50} values in the range of 6.0±1.1 – 8.5±1.2 and 3.6±1.7 – 7.3±1.2 μM, respectively against the TM4 and K1 strains. Of these compounds **2**, **3** and **8** were 2-5 times less cytotoxic against a Vero cell line (IC_{50} values of 12.6±0.9, 29.5±3.9 and 13.2±4.6 μM, respectively) in comparison with their antiplasmodial activities.

Keywords: *Garcinia mckeniana*, Clausiaceae, pyrano-xanthone, antimalarial activity

1. Introduction

Garcinia plants (Clusiaceae) are well documented for producing a variety of secondary metabolites, especially xanthones,[1a-d] flavonoids,[1d] benzophenones[2] and terpenoids.[1a] These plants have been used in traditional medicines[1d,3a-c] and some of the compounds isolated from these plants have shown noteworthy pharmaceutical activities, including antimalarial,[4] antimicrobial,[5a-c] antioxidant[5c] and cytotoxic activities.[6] Thus *Garcinia* plants are an outstanding source of bioactive phytochemicals and therefore an important species to investigate for new bioactive compounds. In our continuous research for new bioactive metabolites from *Garcinia* plants,[5a-b,7a-b] we herein report our results from the first phytochemical investigation of the leaf and branch extracts from *Garcinia mckeaniana* Craib. This plant is locally named "Ma da" and is found in the North of Thailand. This study resulted in the isolation of six new natural products (**1-6**), together with thirteen known compounds (**7-19**). The biological activities of these compounds against the *Plasmodium falciparum* strains, TM4 and K1 (a multidrug resistant strain) and their cytotoxicities against a Vero cell line are reported.

2. Results and discussion

The concentrated acetone extract of the air dried leaves of *G. mckeaniana* was subjected to column chromatography and afforded three new xanthones, mckeanianones A-C (**1**-**3**), and one new biflavone, mckeaniabiflavone (**6**), together with three known compounds, bannaxanthones I (**7**)[8] and E (**8**),[6] and amentoflavone (**9**).[9] The crude acetone extract of the air dried branches of *G. mckeaniana* was separated by column chromatography resulting in the isolation of two new natural xanthones, mckeanianones D-E (**4**-**5**) along with ten known compounds, pancixanthones A and B (**10** and **17**),[10] neriifolone A (**11**),[11] 1,3,5-trihydroxy-2-(3-methylbut-2-enyl)-9H-xanthen-9-one (**12**),[12] 1,3,6-trihydroxy-7-methoxyxanthone (**13**),[13] 1,3,5-trihydroxyxanthone (**14**),[14] buchanaxanthone (**15**),[15] 5-*O*-methyl-2-deprenylrheediaxanthone B (**16**),[16] assiguxanthone A (**18**)[17] and 1,3,7-trihydroxy-2-(3-methylbut-2-enyl)-xanthone (**19**)[18] (Fig. 1). Full experimental information is provided in the Supplementary data.

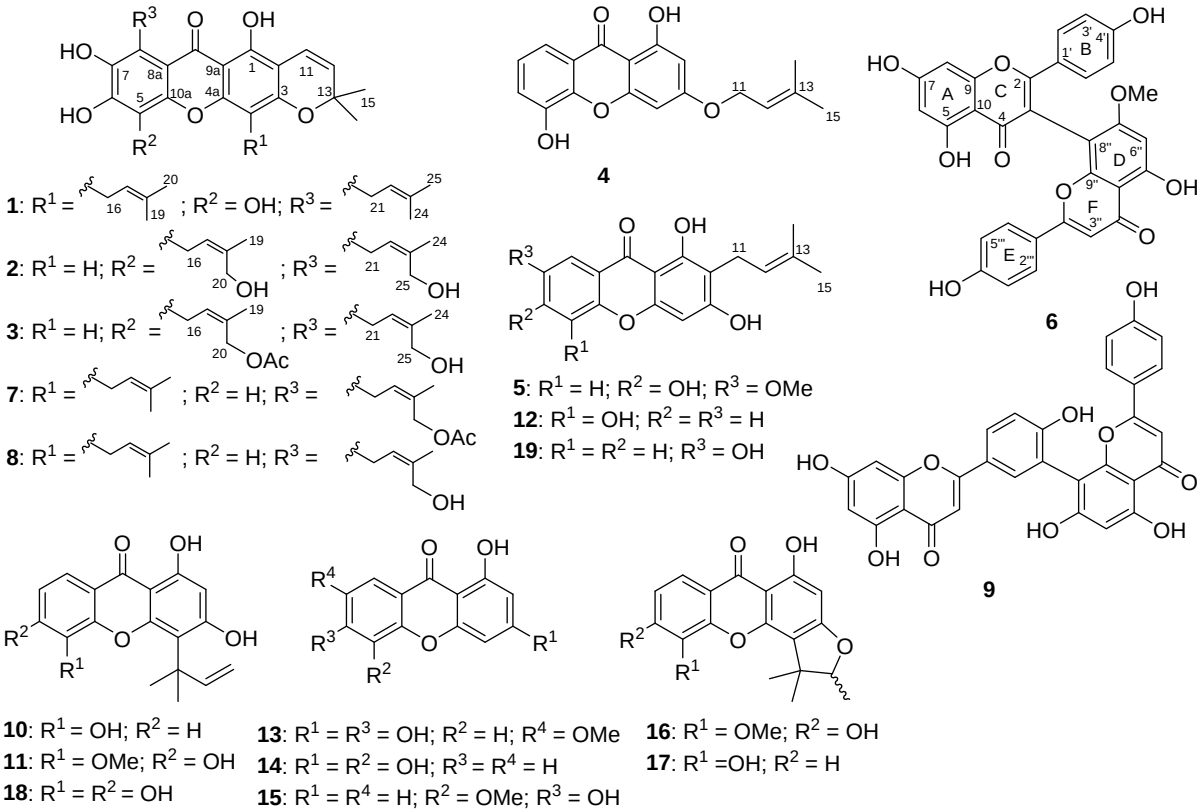

Fig. 1. Structures of compounds **1**-**19**

Mckeanianone A (**1**) was obtained as a yellow solid (mp 187-189 °C), whose molecular formula was determined to be $C_{28}H_{30}O_7$ from the positive ion HRESI-TOFMS [found *m/z* 479.2063 (calcd for 479.2070) $[M+H]^+$]. The UV spectrum displayed absorption bands at λ_{max} (MeOH) 217, 295, 337 and 384 nm, suggesting the presence of a xanthone chromophore.[19] The IR spectrum showed hydroxy and xanthone carbonyl functional group stretching bands at 3418 and 1649 cm^{-1}, respectively. The ^1H NMR spectrum (Table 1) displayed resonances for a chelated hydroxy proton at δ_H 13.97 (1H, s), a dimethylchromene unit [δ_H 6.69 (1H, d, *J* = 10.0 Hz, H-11), 5.69 (1H, d, *J* = 10.0 Hz, H-12) and 1.47 (6H, s, H-14, H-15)] and two sets of prenyl units [δ_H 5.31 (1H, brt, *J* = 6.2 Hz, H-22), 4.17 (2H, d, *J* = 6.2 Hz, H-21), 1.86 (3H, s, H-25) and 1.64 (3H, s, H-24); and 5.21 (1H, brt, *J* = 6.8, H-17), 3.42 (2H, d, *J* = 6.8 Hz, H-16), 1.83 (3H, s, H-20) and 1.63 (3H, s, H-19)]. The HMBC correlations between the 1-OH resonance (δ_H 13.97) and those of C-1 (δ_C 156.7) and C-9a (δ_C 104.1) confirmed that the chelated hydroxy group (δ_H 13.97) was attached at C-1. The dimethylchromene ring was located at C-2 (δ_C 104.7) and an ether linkage at C-3 (δ_C 157.7) on the basis of the HMBC correlations of the lowerfield olefinic proton resonance at δ_H 6.69 (corresponding to H-11) to C-2 and C-3. One of the prenyl units (δ_H 5.21, 3.42, 1.83 and 1.63) was assigned to position C-4 (δ_C 106.9), according to the HMBC correlations of the methylene protons, H-16 (δ_H 3.42), of this prenyl unit with C-3, C-4 and C-4a (δ_C 154.3). The other prenyl unit was located at C-8 (δ_C 129.0) as suggested from the HMBC correlations between the methylene protons, H-21 (δ_H 4.17) with the oxygenated carbons C-7 (δ_C 141.8), C-8 and C-8a (δ_C 111.8). The substituents at C-5 (δ_C 152.5) and C-6 (δ_C 152.6) were assigned as hydroxy groups on the basis of their chemical shifts. These correlations established mckeanianone A as 1,5,6,7-tetrahydroxy-6,6′-dimethyl-2*H*-pyrano(2′,3′:3,2)-4,8-di(3-methylbut-2-enyl)-xanthone (**1**).

Mckeanianone B (**2**) was obtained as a yellow viscous oil and the molecular formula, $C_{28}H_{30}O_8$, was determined by HRESI-TOFMS [found *m/z* 517.1839 (calcd for 517.1838) $[M+Na]^+$]. A careful analysis of its UV and IR spectra showed absorption bands similar to those of compound **1**. Furthermore, the ^{1}H NMR spectrum (Table 1) displayed resonances for a chelated hydroxy proton (δ_H 13.78, brs), a dimethylchromene ring [δ_H 6.69 (1H, d, *J* = 10.0 Hz, H-11), 5.54 (1H, d, *J* = 10.0 Hz, H-12) and 1.44 (6H, s, H-14, H-15)], one single aromatic proton (δ_H 6.25, H-4) and the two sets of oxygenated prenyl units [δ_H 5.40 (1H, t, *J* = 8.0 Hz, H-17), 4.30 (2H, s, H-20), 3.60 (2H, d, *J* = 8.0 Hz, H-16), 1.73 (3H, s, H-19) and 5.59 (1H, t, *J* = 7.8 Hz, H-22), 4.26 (2H, s, H-25), 4.13 (2H, d, *J* = 7.8 Hz, H-21) and 1.73 (3H, s, H-24)]. 2D-NMR Data ananlysis of compound **2** suggested the same pyrano-xanthone structure as compound **1** with the chelated hydroxy proton attached at C-1 (δ_C 157.7) and the dimethylchromene ring fused to C-2 (δ_C 104.3) and C-3 (δ_C 159.7). In addition, the aromatic proton resonating at δ_H 6.25 was assigned to H-4 due to its HMBC correlations with the ^{13}C NMR resonances for C-2, C-3, C-4a (δ_C 156.3) and C-9a (δ_C 103.8). The methylene proton resonances of the oxygenated prenyl, H-21 (δ_H 4.13), showed HMBC correlations to the oxygenated aromatic carbon C-7 (δ_C 139.1) and with C-8 (δ_C 124.2) and C-8a (δ_C 111.3). The methylene proton resonances for H-16 (δ_H 3.60), of the other oxygenated prenyl unit, showed HMBC cross peaks with the resonances of the aromatic carbons C-5 (δ_C 112.2), C-6 (δ_C 151.0) and C-10a (δ_C 149.7). These data established the location of these oxygenated prenyl units at C-8 and C-5, respectively. The configuration of the oxygenated prenyl units at their alkene moieties were assigned as *Z*-$\Delta^{17,18}$ and *Z*-$\Delta^{22,23}$ on the basis of the NOESY correlations that were observed between H-16 (δ_H 3.60) and H-20 (δ_H 4.30); H-17 (δ_H 5.40) and H-19 (δ_H 1.73); H-21 (δ_H 4.13) and H-25 (δ_H 4.26); and H-22 (δ_H 5.59) and H-24 (δ_H 1.73). Therefore, mckeanianone B was determined to be 1,6,7-trihydroxy-6,6′-dimethyl-2*H*-pyrano(2′,3′:3,2)-5,8-di(4-hydroxy-3-methylbut-2-enyl)-xanthone (**2**).

Table 1

NMR spectroscopic data (400 MHz, $CDCl_3$) for mckeanianones A-C (**1-3**)

Position	**1**			**2**			**3**		
	δ_H, (*J* in Hz)	δ_C (type)	HMBC	δ_H, (*J* in Hz)	δ_C (type)	HMBC	δ_H, (*J* in Hz)	δ_C (type)	HMBC
1		156.7 (C)			157.7 (C)			158.0 (C)	
2		104.7 (C)			104.3 (C)			104.5 (C)	
3		157.7 (C)			159.7 (C)			159.7 (C)	
4		106.9 (C)		6.25, s, 1H	94.1 (CH)	2, 3, 4a, 9, 9a	6.31, s, 1H	94.2 (CH)	2, 3, 4a, 9a
4a		154.3 (C)			156.3 (C)			156.3 (C)	
5		152.5[b] (C)			112.2 (C)			112.2 (C)	
6		152.6[b] (C)			151.0 (C)			151.3 (C)	
7		141.8 (C)			139.1 (C)			139.4 (C)	
8		129.0 (C)			124.2 (C)			124.0 (C)	
8a		111.8 (C)			111.3 (C)			111.3 (C)	
9		183.5 (C)			182.8 (C)			182.9 (C)	
9a		104.1 (C)			103.8 (C)			103.9 (C)	
10a		153.6 (C)			149.7 (C)			150.0 (C)	
11	6.69, d (10.0), 1H	116.5 (CH)	2, 3, 13, 14	6.69, d (10.0), 1H	115.8 (CH)	1, 2, 3, 13, 14, 15	6.73, d (10.0), 1H	115.9 (CH)	1, 2, 3, 13
12	5.69, d (10.0), 1H	128.0 (CH)	2, 13, 14	5.54, d (10.0), 1H	127.2 (CH)	2, 13, 14, 15	5.56, d (10.0), 1H	127.2 (CH)	2, 13, 14, 15
13		78.5 (C)			78.0 (C)			78.0 (C)	
14	1.47, s, 3H	28.4 (CH_3)	12, 13	1.44, s, 3H	28.4 (CH_3)	12, 13, 15	1.47, s, 3H	28.5 (CH_3)	12, 13, 15
15	1.47, s, 3H	30.6 (CH_3)	12, 13, 14	1.44, s, 3H	28.4 (CH_3)	12, 13, 14	1.47, s, 3H	28.5 (CH_3)	12, 13, 14
16	3.42, d (6.8), 2H	21.9 (CH_2)	3, 4, 4a, 17, 18	3.60, d (8.0), 2H	22.4 (CH_2)	5, 6, 10a	3.67, d (7.4), 2H	22.4 (CH_2)	5, 6, 10a, 17, 18
17	5.21, brt (6.8), 1H	123.3 (CH)	4, 16	5.40, t (8.0), 1H	125.4 (CH)	19, 20	5.52, t (7.4), 1H	127.5 (CH)	19, 20

18		131.4 (C)			134.7 (C)			130.8 (C)	
19	1.63, s, 3H	25.9 (CH_3)	17, 18, 20	1.73, s, 3H	22.6 (CH_3)	17, 18, 20	1.73, s, 3H	21.5 (CH_3)	17, 18, 20
20	1.83, s, 3H	18.1 (CH_3)	17, 18, 19	4.30, s, 2H	62.1 (CH_2)	17, 18, 19	4.87, s, 2H	63.6 (CH_2)	17, 18, 19, CO(Ac)
21	4.17, d (6.2), 2H	26.3 (CH_2)	7, 8, 8a, 22	4.13, d (7.8), 2H	26.1 (CH_2)	7, 8, 8a, 22, 23	4.21, d (8.0), 2H	26.5 (CH_2)	7, 8, 8a, 22, 23
22	5.31, brt (6.2), 1H	124.4 (CH)	8, 21	5.59, t (7.8), 1H	127.5 (CH)	8, 24, 25	5.73, t (8.0), 1H	128.5 (CH)	24, 25
23		131.5 (C)			133.7 (C)			132.9 (C)	
24	1.64, s, 3H	26.0 (CH_3)	22, 23, 25	1.73, s, 3H	22.9 (CH_3)	22, 23, 25	1.79, s, 3H	23.4 (CH_3)	22, 23, 25
25	1.86, s, 3H	18.3 (CH_3)	22, 23, 24	4.26, s, 2H	62.3 (CH_2)	22, 23, 24	4.37, s, 2H	63.2 (CH_2)	22, 23, 24
OH-1	13.97, s, 1H		1, 9a	13.78, brs, 1H			13.80, brs, 1H		
CO(Ac)								171.7 (C)	
CH_3(Ac)							2.12, s, 3H	21.2 (CH_3)	CO(Ac)

[a]Measured in acetone-d_6, [b]These assignments could be interchanged.

Mckeanianone C (**3**) was obtained as a yellow viscous oil, that gave a $[M+Na]^+$ ion at *m/z* 559.1945 in the HRESI-TOFMS which corresponded to the molecular formula $C_{30}H_{32}O_9$ (calcd for $C_{30}H_{32}O_9Na$, *m/z* 559.1944). The UV, IR, ^1H and ^{13}C NMR spectra indicated that compound **3** was closely related to compound **2**, except the IR spectrum showed an additional band at 1736 cm^{-1}, which indicated the presence of an ester carbonyl functional group. In conjunction with this, the ^1H NMR spectrum also contained an additional resonance for an acetoxy group at δ_H 2.12 (3H, s). The location of the acetoxy group at C-20 (δ_C 63.6) was based on the HMBC correlation between the resonance for H_2-20 (δ_H 4.87) and that for the acetoxy carbonyl (δ_C 171.7). This identified mckeanianone C as 1,6,7-trihydroxy-6,6′-dimethyl-2*H*-pyrano(2′,3′:3,2)-5-(4-acetoxy-3-methylbut-2-enyl)-8-(4-hydroxy-3-methylbut-2-enyl)-xanthone (**3**).

Mckeanianone D (**4**) was isolated as a yellow solid (mp 206-207 °C). The molecular formula $C_{18}H_{16}O_5$ was indicated from the mass ion peak at *m/z* 312.09995 $[M]^+$ in its HREIMS spectrum. The UV, IR and NMR spectra were similar to those of compound **14**,[14] except for the additional isoprene unit resonances [δ_H 5.51 (1H, t, *J* = 6.6 Hz, H-12), 4.72 (2H, d, *J* = 6.6 Hz, H-11), 1.80 (3H, s, H-15), 1.79 (3H, s, H-14)] in the ^1H and ^{13}C NMR spectra (Table 2). This isoprene unit was at C-3 (δ_C 167.2) based on the HMBC correlations between H-11 (δ_H 4.72) and the ^{13}C NMR resonances for C-3, C-12 (δ_C 120.0) and C-13 (δ_C 139.2). Therefore, mckeanianone D was determined to be 1,5-dihydroxy-3-*O*-(3-methylbut-2-enyl)-xanthone (**4**)

Mckeanianone E (**5**) was isolated as a yellow solid (decomposed >129 °C). Its HRESIMS displayed a positive molecular ion $[M+H]^+$ at *m/z* 343.1179, which was consistent with the molecular formula of $C_{19}H_{18}O_6$. The UV, IR and NMR spectra were similar to those of compound **13**,[13] with the only difference being an additional isoprene unit resonances [δ_H 5.30 (1H, t, *J* = 7.2 Hz, H-12), 3.37 (2H, d, *J* = 7.2 Hz, H-11), 1.80 (3H, s, H-15), 1.66 (3H, s,

H-14)] in the ^{1}H and ^{13}C NMR spectra (Table 2). This isoprene unit was at C-2 (δ_C 110.4) on the basis of the HMBC correlations between H-11 (δ_H 3.37) and the ^{13}C NMR resonances for C-1 (δ_C 161.2), C-2, C-12 (δ_C 123.4) and C-13 (δ_C 131.5). The structure of mckeanianone E was therefore confirmed to be 1,3,6-trihydroxy-7-methyoxy-2-(3-methylbut-2-enyl)-xanthone (**5**). This compound was synthesized earlier however its spectroscopic data was not reported.[20]

Table 2

NMR spectroscopic data (400 MHz in acetone-d_6) of mckeanianones D (**4**) and E (**5**)

Position	**4**			**5**		
	δ_H, (J in Hz)	δ_C (type)	HMBC	δ_H, (J in Hz)	δ_C[a] (type)	HMBC[a]
1		164.4 (C)			161.2 (C)	
2	6.35, d (2.2), 1H	98.4 (CH)	1, 3, 4, 9a		110.4 (C)	
3		167.2 (C)			163.2 (C)	
4	6.57, d (2.2), 1H	94.1 (CH)	2, 3, 4a, 9, 9a	6.49, s, 1H	93.9 (CH)	2, 3, 4a, 9, 9a
4a		158.5 (C)			156.6 (C)	
5		147.0 (C)		6.92, s, 1H	103.5 (CH)	7, 8a, 9, 10a
6	7.36, dd (7.9, 1.5), 1H	121.5 (CH)	8, 10a		155.0 (C)	
7	7.29, t (7.9), 1H	125.0 (CH)	5, 8a		146.6 (C)	
8	7.67, dd (7.9, 1.5), 1H	116.3 (CH)	6, 9, 10a	7.56, s, 1H	105.7 (CH)	6, 7, 9
8a		122.5 (C)			113.1 (C)	
9		182.0 (C)			180.5 (C)	
9a		104.2 (C)			103.2 (C)	
10a		146.1 (C)			153.3 (C)	
11	4.72, d (6.6), 2H	66.4 (CH_2)	3, 12, 13	3.37, d (7.2), 2H	22.0 (CH_2)	1, 2, 12, 13
12	5.51, t (6.6), 1H	120.0 (CH)	14, 15	5.30, t (7.2), 1H	123.4 (CH)	14, 15
13		139.2 (C)			131.5 (C)	
14	1.79, s, 3H	25.8 (CH_3)	12, 13, 15	1.66, s, 3H	25.9 (CH_3)	12, 13, 15
15	1.80, s, 3H	18.3 (CH_3)	12, 13, 14	1.80, s, 3H	17.9 (CH_3)	12, 13, 14
OH-1	12.91, brs, 1H			13.45, brs, 1H		
OMe-7				3.99, s, 3H	56.6 (CH_3)	7

[a] Recorded at 500 MHz

Mckeaniabiflavone (**6**) was obtained as a yellow solid (mp 237-239 °C). The molecular formula, $C_{31}H_{20}O_{10}$, was deduced from the HRESI-TOFMS which show a pseudomolecular ion at *m/z* 553.1155 (calcd 553.1135) $[M+H]^+$. The UV spectrum of compound **6** showed maximum absorption bands at λ_{max} (MeOH) 218, 269 and 331 nm while the IR spectrum showed absorption bands for a hydroxy group and a conjugated carbonyl group at 3444 and 1650 cm^{-1}, respectively. The ^{1}H NMR spectrum (Table 3) displayed resonances for two chelated hydroxy protons (δ_H 13.09 and 12.97, each 1H, brs), two sets of four-aromatic

protons for two *p*-disubstituted aromatic rings [δ_H 7.49 (2H, d, *J* = 8.4 Hz, H-2′, H-6′), 6.76 (2H, d, *J* = 8.4 Hz, H-3′, H-5′); and 7.74 (2H, d, *J* = 8.4 Hz, H-2‴, H-6‴), 6.97 (2H, d, *J* = 8.4 Hz, H-3‴, H-5‴)] which were assigned to the aromatic protons of rings B and E, respectively, four uncoupled (singlet) aromatic protons (δ_H 6.63, 6.57, 6.33 and 6.33, each 1H, H-3″, H-8, H-6 and H-6″, respectively) and one methoxy group (δ_H 3.82). The ^{13}C NMR and DEPT 135 spectra of **6** displayed only twenty-seven resonances for the total of thirty-one carbons; eighteen quaternary carbons (δ_C 183.3, 182.1, 165.2, 165.1, 164.7, 163.6, 163.5, 163.1, 163.0, 160.8, 158.9, 156.4, 124.8, 124.2, 111.2, 105.6, 104.7 and 100.5), twelve methine carbons [δ_C 130.9 (2C), 128.8 (2C), 116.1 (2C), 115.4 (2C), 104.5, 99.9, 99.8 and 94.7] and one methyl (δ_C 55.9) carbon. The chelated hydroxy proton at δ_H 12.97 showed HMBC correlations to C-5 (δ_C 163.1), C-6 (δ_C 99.8) and C-10 (δ_C 104.7). The aromatic proton resonating at δ_H 6.33 was assigned to H-6 due to its HMQC cross peak with C-6 as well as the HMBC correlations with C-7 (δ_C 165.2) and C-8 (δ_C 94.7). The aromatic proton H-6 correlated with H-8 (δ_H 6.57) in the ^{1}H-^{1}H COSY spectrum which correlated with C-4 (δ_C 182.1), C-6, C-7, C-9 (δ_C 158.9) and C-10 in the HMBC spectrum. The HMBC correlations between the aromatic protons H-2′ (δ_H 7.49, of the *p*-disubstituted aromatic ring) and C-2 (δ_C 165.1) established the attachment of this aromatic unit at C-2. The substituent at C-4′ (δ_C 160.8) of the *p*-disubstituted aromatic ring was assigned as a hydroxy group on the basis of its chemical shift. Consequently this data together with the chemical shifts of C-3 (δ_C 111.2), C-5 and C-7 suggested a 3-substitued-5,7-dihydroxy-(4-hydroxyphenyl) flavone moiety. In the other flavone unit, the chelated hydroxy proton (δ_H 13.09) was located at C-5″ (δ_C 163.0) due to its HMBC correlations to C-5″, C-6″ (δ_C 99.9) and C-10″ (δ_C 105.6). The olefinic proton at δ_H 6.63 (H-3″) showed cross peaks in the HMBC spectrum to C-2″ (δ_C 164.7), C-4″ (δ_C 183.3), C-10″ and C-1‴ (δ_C 124.2) of the *p*-disubstituted aromatic ring. In addition, C-4‴ of this aromatic ring was substituted by a hydroxy group due to its low field

chemical shift (δ_C 163.6). The aromatic proton resonating at δ_H 6.33 was located at C-6″ from the HMQC spectrum as well as the HMBC correlations between H-6″ and C-3, C-4″, C-5″, C-7″ (δ_C 163.5), C-8″ (δ_C 100.5) and C-10″. The HMBC correlations between H-6″ and C-3 indicated that the two flavone units were linked between C-3 and C-8″. This was further supported by a comparison of the NMR spectroscopic data of **6** with those of the related biflavones, biapigenin (the de-*O*-methyl derivative of **6**)[21] and its constitutional isomer, 4‴-*O*-methyl-biapigenin.[22] These three compounds had very similar ^{1}H and ^{13}C NMR spectroscopic data, except for the resonances associated with the presence or absence of the methoxy group. Thus compound mckeaniabiflavone was identified as 7″-methoxy-4′,4‴,5,5″,7-pentahydroxy-[3→8″]-biflavone (**6**).

Table 3

NMR spectroscopic data (400 MHz, acetone-d_6) for mckeaniabiflavone (**6**)

				6			
Position	δ_H, (*J* in Hz)	δ_C (type)	HMBC	Position	δ_H, (*J* in Hz)	δ_C (type)	HMBC
2		165.1 (C)		2″		164.7 (C)	
3		111.2 (C)		3″	6.63, s, 1H	104.5 (CH)	2″, 4″, 10″, 1‴
4		182.1 (C)		4″		183.3 (C)	
5		163.1 (C)		5″		163.0 (C)	
6	6.33, brs, 1H	99.8 (CH)	7, 8	6″	6.33, brs, 1H	99.9 (CH)	3, 4″, 5″, 7″, 8″, 10″
7		165.2 (C)		7″		163.5 (C)	
8	6.57, s, 1H	94.7 (CH)	4, 6, 7, 9, 10	8″		100.5 (C)	
9		158.9 (C)		9″		156.4 (C)	
10		104.7 (C)		10″		105.6 (C)	
1′		124.8 (C)		1‴		124.2 (C)	
2′	7.49, d (8.4), 1H	130.9 (CH)	2, 3′, 4′	2‴	7.74, d (8.4), 1H	128.8 (CH)	3‴, 4‴
3′	6.76, d (8.4), 1H	116.1 (CH)	2, 1′, 2′, 4′	3‴	6.97, d (8.4), 1H	115.4 (CH)	1‴, 4‴
4′		160.8 (C)		4‴		163.6 (C)	
5′	6.76, d (8.4), 1H	116.1 (CH)	2, 1′, 2′, 4′	5‴	6.97, d (8.4), 1H	115.4 (CH)	1‴, 4‴
6′	7.49, d (8.4), 1H	130.9 (CH)	2, 3′, 4′	6‴	7.74, d (8.4), 1H	128.8 (CH)	4‴, 5‴
OH-5	12.97, brs, 1H		5, 6, 10	OH-5″	13.09, brs, 1H		5″, 6″, 10″
				OMe-7″	3.82, s, 3H	55.9 (CH_3)	7″

All of the isolated compounds were tested for their antimalarial activities against the *Plasmodium falciparum* strains, TM4 and K1 (a multidrug resistant strain)[23] and their cytotoxic activity against a Vero cell line (African green monkey kidney cells)[24] (Table 4). The five pyrano-xanthones **1**-**3**, **7** and **8**, all isolated from the leaves and having two isoprene

substituents, were generally the most active compounds and showed significant activities against the TM4 and K1 strains with IC_{50} values in the range of 6.0±1.1 – 8.5±1.2 and 3.6±1.7 – 7.3±1.2 μM, respectively. Only compounds, **1** and **7**, were not tested against Vero cells (Table 4).

Table 4

Antimalarial and cytotoxic activities (IC_{50}, μM)

Compounds[a]	Antimalarial activity against *P. falciparum*		Cytotoxic activity
	TM4	K1	Vero cells
1	6.2±0.4	5.2±0.4	-[b]
2	6.7±0.6	6.4±0.5	12.6±0. 9
3	6.0±1.1	6.6±0.7	29.5±3.9
4	15.1.0±3.9	14.3±1.8	-[c]
5	27.7±3.4	25.7±2.3	-[c]
7	8.5±1.2	3.6±1.7	-[b]
8	8.3±0.9	7.3±1.2	13.2±4.6
10	13.7±4.5	19.8±2.0	36.5±8.0
11	22.1±0.7	19.5±1.0	-[c]
17	10.8±0.7	15.4±2.6	25.3±1.4
18	39.1±6.3	32.7±5.99	31.3±2.6
19	31.5±2.0	25.1±2.1	-[c]
Ellipticine[d]			0.4
Chloroquine[e]	0.03	0.3±0.04	
Cycloguanil[e]	0.04±0.01	3.2±0.8	
Pyrimethamine[e]	0.08±0.01	31.0±8.4	

[a] Compounds **6**, **9** and **12-16** were inactive in all assays. [b] Due to insolubility problems an accurate value could not be obtained. [c] Inactive at IC_{50} > 50 μM. [d] Positive control for cytotoxic assay. [e] Reference drugs for antiplasmodial activity.

Compounds **2**, **3** and **8** were 2-5 times less cytotoxic against Vero cells (IC_{50} values of 12.6±0.9, 29.5±3.9 and 13.2±4.6 μM, respectively) in comparison with their antiplasmodial activities. The xanthones, **4**, **5**, **10**, **11** and **17-19**, having one free or cyclized isoprene substituent had significantly weaker antiplasmodial activities and cytotoxicities. The xanthones **12** and **16**, the xanthones (**13-15**), having no isoprene substituent, and the biflavones, **6** and **9** were inactive in all assays. Other Garcinia species containing xanthones

have been documented to have antiplasmodial activities.[25a-c] Some of these xanthones have the 9-xanthone structure related to compounds **4**, **5**, **10**, **11** and **17-19** while three others (isolated from the stem bark of *G. vieillardii,* demethylcalabaxanthone, dombakina-xanthone and macluraxanthone) have the pyrano-xanthone stucture found in compounds **1-3**, **7** and **8** that were isolated here, but having one or two isoprene subsituents. These three compounds had antiplasmodial activities with IC_{50} values in the range of 0.9-1.9 μg/mL, however their cytotoxic activities were not reported.[25c]

3. Conclusions

Six new natural products and twelve known compounds was the first report that isolated from *G. mckeaniana*. The isolated compounds showed antimalarial activities against the *Plasmodium falciparum* strains, TM4 and K1, and cytotoxic activities against a Vero cell line. The isolated xanthone compounds, having a pyrano ring and two isoprene units, showed the highest antimalarial activities against the *P. falciparum* strains, and cytotoxic activities against a Vero cell line than those with only one isoprene unit. While the xanthones and biflavanoids, without an isoprene unit, were inactive in all assays.

4. Spectral data of new compounds

4.1 Mckeanianone A (1)

Yellow solid; mp 187-189 °C; R_f (20% acetone/hexanes) 0.29; UV (MeOH) λ_{max} (log ε) 217 (4.39), 295 (4.61), 337 (4.04), 384 (3.79) nm; IR (neat) ν_{max} 3418, 1649, 1610, 1463 cm^{-1}; δ_H (400 MHz, acetone-d_6) 13.97 (1 H, s, OH-1), 6.69 (1 H, d, *J* 10.0 Hz, H-11), 5.69 (1 H, d, *J* 10.0 Hz, H-12), 5.31 (1 H, br t, *J* 6.2 Hz, H-22), 5.21 (1 H, br t, *J* 6.8 Hz, H-17), 4.17 (2 H, d, *J* 6.2 Hz, H-21), 3.42 (2 H, d, *J* 6.8 Hz, H-16), 1.86 (3 H, s, H-25), 1.83 (3 H, s, H-20), 1.64 (3 H, s, H-24), 1.63 (3 H, s, H-19), 1.47 (6 H, s, H-14 and H-15); δ_C (100 MHz, acetone-d_6) 183.5, 157.7, 156.7, 154.3, 153.6, 152.6, 152.5, 141.8, 131.5, 131.4, 129.0, 128.0, 124.4,

123.3, 116.5, 111.8, 106.9, 104.7, 104.1, 78.5, 30.6, 28.4, 26.3, 26.0, 25.9, 21.9, 18.3, 18.1; HRESI-TOFMS: *m/z* 479.2063 $[M+H]^+$, calcd for 479.2070, $C_{28}H_{31}O_7$.

4.2 Mckeanianone B (2)

Yellow viscous oil; R_f (30% acetone/hexanes) 0.30; UV (MeOH) λ_{max} (log ε) 218 (4.49), 292 (4.57), 338 (4.18), 379 (3.96) nm; IR (neat) ν_{max} 3447, 1650, 1611, 1446, 1288 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 13.78 (1 H, s, OH-1), 6.69 (1 H, d, *J* 10.0 Hz, H-11), 6.25 (1 H, s, H-4), 5.59 (1 H, t, *J* 7.8 Hz, H-22), 5.54 (1 H, d, *J* 10.0 Hz, H-12), 5.40 (1 H, t, *J* 8.0 Hz, H-17), 4.30 (2 H, s, H-20), 4.26 (2 H, s, H-25), 4.13 (2 H, d, *J* 7.8 Hz, H-21), 3.60 (2 H, d, *J* 8.0 Hz, H-16), 1.73 (6 H, s, H-19 and H-24), 1.44 (6 H, s, H-14 and H-15); δ_C (100 MHz, $CDCl_3$) 182.8, 159.7, 157.7, 156.3, 151.0, 149.7, 139.1, 134.7, 133.7, 127.5, 127.2, 125.4, 124.2, 115.8, 112.2, 111.3, 104.3, 103.8, 94.1, 78.0, 62.3, 62.1, 28.4 (2C), 26.1, 22.9, 22.6, 22.4; HRESI-TOFMS: *m/z* 517.1839 $[M+Na]^+$, calcd for 517.1838, $C_{28}H_{30}O_8Na$.

4.3 Mckeanianone C (3)

Yellow viscous oil; R_f (20% acetone/hexanes) 0.31; UV (MeOH) λ_{max} (log ε) 217 (4.62), 292 (4.70), 338 (4.09), 382 (4.04) nm; IR (neat) ν_{max} 3418, 1736, 1650, 1597, 1448, 1244 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 13.80 (1 H, br s, OH-1), 6.73 (1 H, d, *J* 10.0 Hz, H-11), 6.31 (1 H, s, H-4), 5.73 (1 H, t, *J* 8.0 Hz, H-22), 5.56 (1 H, d, *J* 10.0 Hz, H-12), 5.52 (1 H, t, *J* 7.4 Hz, H-17), 4.87 (2 H, s, H-20), 4.37 (2 H, s, H-25), 4.21 (2 H, d, *J* 8.0 Hz, H-21), 3.67 (2 H, d, *J* 7.4 Hz, H-16), 2.12 (3 H, s, CH_3(Ac)), 1.79 (3 H, s, H-24), 1.73 (3 H, s, H-19), 1.47 (6 H, s, H-14 and H-15); δ_C (100 MHz, $CDCl_3$) 182.9, 171.7, 159.7, 158.0, 156.3, 151.3, 150.0, 139.4, 132.9, 130.8, 128.5, 127.5, 127.2, 124.0, 115.9, 112.2, 111.3, 104.5, 103.9, 94.2, 78.0, 63.6, 63.2, 28.5 (2C), 26.5, 23.4, 22.4, 21.5, 21.2; HRESI-TOFMS: *m/z* 559.1945 $[M+Na]^+$, calcd for 559.1944, $C_{30}H_{32}O_9Na$.

4.4 Mckeanianone D (4)

Yellow solid; mp 206-207 °C; R_f (20% EtOAc/hexanes) 0.35; UV (MeOH) λ_{max} (log ε) 252 (4.20), 312 (3.85), 369 (3.18) nm; IR (neat) ν_{max} 3385, 1653, 1569, 1456 cm^{-1}; δ_H (400 MHz, acetone-d_6) 12.97 (1 H, br s, OH-1), 7.67 (1 H, dd, *J* 7.9, 1.5 Hz, H-8), 7.36 (1 H, dd, *J* 7.9, 1.5 Hz, H-6), 7.29 (1 H, t, *J* 7.9 Hz, H-7), 6.57 (1 H, d, *J* 2.2 Hz, H-4), 6.35 (1 H, d, *J* 2.2 Hz, H-2), 5.51 (1 H, t, *J* 6.6 Hz, H-12), 4.72 (2 H, d, *J* 6.6 Hz, H-11), 1.80 (3 H, s, H-15), 1.79 (3 H, s, H-14); δ_C (100 MHz, acetone-d_6) 182.0, 167.2, 164.4, 158.5, 147.0, 146.1, 139.2, 125.0, 122.5, 121.5, 120.0, 116.3, 104.2, 98.4, 94.1, 66.4, 25.8, 18.3; HRMS (EI): *m/z* 312.09995 $[M]^+$ (calcd for 312.09977, $C_{18}H_{16}O_5$).

4.5 Mckeanianone E (5)

Yellow solid; mp decomposed >129 °C; R_f (30% EtOAc/hexanes) 0.28; UV (MeOH) λ_{max} (log ε) 258 (4.41), 320 (4.18), 366 (3.98) nm; IR (neat) ν_{max} 3269, 1647, 1559, 1482 cm^{-1}; δ_H (400 MHz, acetone-d_6) 13.45 (1 H, br s, OH-1), 7.56 (1 H, s, H-8), 6.92 (1 H, s, H-5), 6.49 (1 H, s, H-4), 5.30 (1 H, t, *J* 7.2 Hz, H-12), 3.99 (3 H, s, OMe-7), 3.37 (2 H, d, *J* 7.2 Hz, H-11), 1.80 (3 H, s, H-15), 1.66 (3 H, s, H-14); δ_C (125 MHz, acetone-d_6) 180.5, 163.2, 161.2, 156.6, 155.0, 153.3, 146.6, 131.5, 123.4, 113.1, 110.4, 105.7, 103.5, 103.2, 93.9, 56.6, 25.9, 22.0, 17.9; HRESI-TOFMS: *m/z* 343.1179 $[M+H]^+$ (calcd for 343.1182, $C_{19}H_{19}O_6$).

4.6 Mckeaniabiflavone (6)

Yellow solid; mp 237-239 °C; R_f (5% MeOH/CH_2Cl_2) 0.45; UV (MeOH) λ_{max} (log ε) 218 (4.57), 269 (4.51), 331 (4.30) nm; IR (neat) νmax 3444, 1650, 1608, 1508, 1178 cm^{-1}; δ_H (400 MHz, acetone-d_6) 13.09 (1 H, br s, OH-5″), 12.97 (1 H, br s, OH-5), 7.74 (2 H, d, *J* 8.4 Hz, H- 2‴ and H-6‴), 7.49 (2 H, d, *J* 8.4 Hz, H-2′ and H-6′), 6.97 (2 H, d, *J* 8.4 Hz, H-3‴ and H-5‴), 6.76 (2 H, d, *J* 8.4 Hz, H-3′ and H-5′), 6.63 (1 H, s, H-3″), 6.57 (1H, s, H-8), 6.33 (2 H, br s, H-6 and H-6″), 3.82 (3 H, s, OMe-7″); δ_C (100 MHz, acetone-d_6) 183.3, 182.1, 165.2, 165.1, 164.7, 163.6, 163.5, 163.1, 163.0, 160.8, 158.9, 156.4, 130.9 (2C), 128.8 (2C),

124.8, 124.2, 116.1 (2C), 115.4 (2C), 111.2, 105.6, 104.7, 104.5, 100.5, 99.9, 99.8, 94.7, 55.9; HRESI-TOFMS: *m/z* 553.1155 $[M+H]^+$, calcd for 553.1135, $C_{31}H_{21}O_{10}$.

Acknowledgements

This paper is dedicated to the memory of Mr. James F. Maxwell (Herbarium of Chiang Mai University). This work was supported by the Thailand Research Fund and Chiang Mai University (Grant No. TRG5880098). CA acknowledges the Science Achievement Scholarship of Thailand (SAST) for a scholarship. We thank the bioassay laboratory, BIOTEC, NSTDA, Thailand for KB and Vero cells and financial support to SK from NSTDA's Cluster Management Program.

Supplementary data

Experimental section, HRMS and 1D and 2D NMR spectroscopic data of compounds **1-6**. Supplementary data related to this article can be found at http://

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