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INTRODUCTION

Garcinia is a major genus of tropical trees or shrubs belonging to the family Guttiferae. About twenty-six *Garcinia* species can be found growing in Thailand; some of them are well-known edible or medicinal plants e.g. *Garcinia mangostana, G. cowa* and *G. cambogia*. *Garcinia schomburgkiana* Pierre (Thai name: Ma-dan) is a small- to medium-size tree grown for its sour-tasting fruits. Its leaves have been used medicinally as an expectorant, laxative and blood tonic. Hexane extract of its stems was cytotoxic against both human breast cancer (BC) and carcinoma of the nasopharynx (KB) cell lines. The same extract, and dichloromethane extract of this plant part, also exhibited antimalarial activity against *Plasmodium falciparum* (K1, multi-drug resistant strain) [1]. Previous phytochemical studies of its stems have reported the presence of xanthone, benzophenones, quinones, flavonoids, phenolic aldehyde and steroids [1-3]. In this study, we report the isolation and identification of three xanthones and three biphenyls from the wood of *G. schomburgkiana*. These compounds were also evaluated for their cytotoxicity against five human cancer cell lines.

MATERIALS AND METHODS

The stems of *G. schomburgkiana* were collected in Nonthaburi, Thailand, in January 2009. Column chromatography was performed using silica gel 60 (Merck) and Sephadex LH-20 (Pharmacia). Thin layer chromatography was done on aluminium sheets precoated with silica gel 60 F_{254} (Merck). ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra were obtained on a Bruker Avance spectrometer. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra were obtained on a Varian Unity INOVA-500 spectrometer.

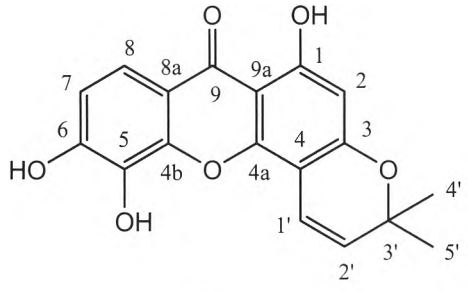
Oven-dried, powdered *G. schomburgkiana* stems (5.5 kg) were macerated with MeOH (3 × 15 L). The pooled extract was dried under reduced pressure, then redissolved in 70% MeOH and partitioned with dichloromethane (CH_2Cl_2) and ethyl acetate (EtOAc), respectively, to give CH_2Cl_2 (66.12 g), EtOAc (134.15 g) and aqueous (84.69 g) extracts after solvent evaporation. The CH_2Cl_2 extract (40 g) was separated on a silica gel column eluted with gradients of *n*-hexane-CH_2Cl_2 (1:0 → 0:1) and CH_2Cl_2-MeOH (1:0 → 1:2) into eight fractions (C-1 – C-8). Recrystallization of fraction C-6 in CH_2Cl_2-MeOH (1:1) yielded compound **1** (12.9 mg). Fraction C-4 was chromatographed on a Sephadex LH-20 column washed down with MeOH to give seven subfractions (C4-1 – C4-7). Silica gel chromatography of subfraction C4-1, eluted with *n*-hexane-EtOAc (4:1), afforded compound **2** (13.5 mg). Further purification of subfraction C4-6 on a silica gel column, employing *n*-hexane-EtOAc (2:1) as the eluent, gave five subfractions (C46-1 – C46-5). Recrystallization of subfraction C46-2 in EtOAc-acetone (1:1) afforded compound **3** (4.7 mg), whereas centrifugal chromatography of subfraction C46-5 on a chromatotron machine (model 8924), using a silica gel 60 F_{254} plate (0.25 mm thickness) eluted with *n*-hexane-EtOAc (2:1) (8 ml/min), gave compound **4** (3.4 mg). Gel filtration chromatography of fraction C-5, using Sephadex LH-20 eluted with CH_2Cl_2-MeOH (1:1), gave seven subfractions (C5-1 – C5-7). Purification of subfraction C5-6 on a silica gel column with *n*-hexane-EtOAc (3:1) as the eluent yielded compound **5** (3.2 mg), whereas separation of fraction C-7 on two successive Sephadex LH-20 columns, washed down with MeOH and CH_2Cl_2-MeOH (1:1), respectively, afforded compound **6** (3.4 mg).

The isolated compounds were evaluated for their *in vitro* cytotoxicity against human colon carcinoma (SW620), lung carcinoma (CHAGO), hepatocarcinoma (HepG2), breast carcinoma (BT474) and gastric carcinoma (KATO-III) cell lines using the tetrazolium dye (MTT) method [4]. IC_{50} values of the tested compounds which caused the percentage of cell survival to be lower than 50% were calculated and compared with doxorubicin as the positive control.

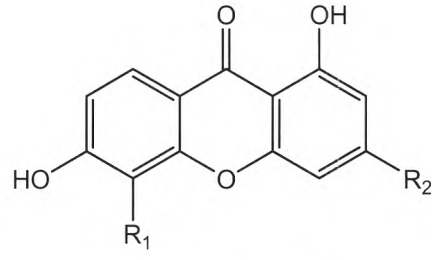
RESULTS AND DISCUSSION

Identification of compounds isolated from *G. schomburgkiana* stems was performed using 1- and 2-dimensional ^{1}H and ^{13}C NMR techniques and comparison with literature values. Compounds **1**, **2** and **6** were shown to be the xanthones isojacareubin [5], buchanaxanthone [6] and 1,3,5,6-tetrahydroxyxanthone [7-8], respectively, while compounds **3-5** were characterized as the biphenyls aucuparin [9], nigrolineabiphenyl B [10] and garcibiphenyl C [11], respectively. Their chemical structures are shown in **Figure 1** and their ^{1}H and ^{13}C NMR data are presented in **Tables 1** and **2**.

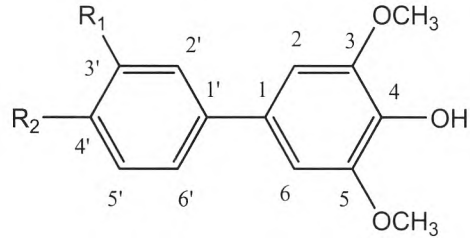
Although some of these natural compounds have been reported as constituents of certain members of the family Guttiferae and related families, all of them have been reported herein as constituents of *G. schomburgkiana* for the first time. Cytotoxicity against five human cancer cell lines, shown as percentages of cell survival, is presented in **Table 3**, and IC_{50} values of the active compounds **1**, **4** and **6** are shown in **Table 4**. the xanthone isojacareubin (**1**) was strongly cytotoxic against all cancer cell lines tested, and especially against the colon carcinoma (SW620) cells. The biphenyl nigrolineabiphenyl B (**4**) was specifically cytotoxic against the same cell line.



1



2: $R_1 = OCH_3$, $R_2 = H$
6: $R_1 = OH$, $R_2 = OH$



3: $R_1 = H$, $R_2 = H$
4: $R_1 = H$, $R_2 = OH$
5: $R_1 = OCH_3$, $R_2 = OH$

Figure 1 Xanthones and biphenyls isolated from the wood of *Garcinia schomburgkiana*

Table 1 ^1H and ^{13}C NMR data of xanthones from *G. schomburgkiana* wood (*J* in Hz, δ in ppm)

Position	1*		2**		6***	
	^1H	^{13}C	^1H	^{13}C	^1H	^{13}C
1	-	162.3	-	162.8	-	163.8
2	6.18 (1H, s)	98.4	6.76 (1H, dd, *J* 8.3, 0.9)	111.2	6.20 (1H, br s)	97.9
3	-	159.7	7.67 (1H, t, *J* 8.3)	137.5	-	165.5
4	-	100.9	7.05 (1H, dd, *J* 8.3, 0.9)	107.8	6.44 (1H, br s)	94.0
4a	-	151.3	-	157.0	-	157.8
4b	-	146.0	-	151.9	-	144.3
5	-	132.6	-	135.6	-	132.8
6	-	152.7	-	157.8	-	151.6
7	6.93 (1H, d, *J* 8.6)	113.4	7.04 (1H, d, *J* 8.8)	114.8	6.96 (1H, d, *J* 8.7)	112.8
8	7.51 (1H, d, *J* 8.6)	116.1	7.86 (1H, d, *J* 8.8)	122.2	7.58 (1H, d, *J* 8.7)	116.3
8a	-	112.8	-	115.0		111.8
9	-	180.0	-	182.3		180.2
9a	-	102.3	-	108.9		102.2
1′	7.06 (1H, d, *J* 10.0)	115.0	-	-		-
2′	5.77 (1H, d, *J* 10.0)	127.4	-	-		-
3′	-	78.2	-	-		-
4′	1.43 (3H, s)	27.9	-	-		-
5′	1.43 (3H, s)	27.9	-	-		-
1-OH	13.28 (1H, br s)	-	12.84 (1H, s)	-		-
5-OCH_3	-	-	4.01 (3H, s)	61.7		-

* 500 and 125 MHz, in DMSO-d_6 ** 500 and 125 MHz, in acetone-d_6
*** 300 and 75 MHz, in acetone-d_6

Table 2 ^{1}H and ^{13}C NMR data of biphenyls from *G. schomburgkiana* wood (*J* in Hz, δ in ppm)

Position	3*		4**		5***	
	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
1	-	132.3	-	133.1	-	132.0
2	6.90 (1H, s)	105.3	6.71 (1H, s)	104.0	6.81 (1H, s)	104.3
3	-	149.2	-	147.2	-	148.3
4	-	136.8	-	134.2	-	135.3
5	-	149.2	-	147.2	-	148.3
6	6.90 (1H, s)	105.3	6.71 (1H, s)	104.0	6.81 (1H, s)	104.3
1′	-	142.1	-	134.2	-	132.8
2′	7.61 (1H, d, *J* 7.8)	127.3	6.98 (1H, d, *J* 2.0)	109.7	7.43 (1H, d, *J* 8.6)	127.6
3′	7.39 (1H, t, *J* 7.8)	129.4	-	146.7	6.86 (1H, d, *J* 8.6)	115.6
4′	7.27 (1H, t, *J* 7.8)	127.3	-	145.0	-	156.8
5′	7.39 (1H, t, *J* 7.8)	129.4	6.95 (1H, d, *J* 8.1)	114.6	6.86 (1H, d, *J* 8.6)	115.6
6′	7.61 (1H, d, *J* 7.8)	127.3	7.02 (1H, dd, *J* 8.1, 2.0)	120.0	7.43 (1H, d, *J* 8.6)	127.6
3-OCH_3	3.89 (3H, s)	56.7	3.93 (3H, s)	56.4	3.87 (3H, s)	56.0
4-OH	7.75 (1H, br s)	-	-	-	-	-
5-OCH_3	3.89 (3H, s)	56.7	3.93 (3H, s)	56.4	3.87 (3H, s)	56.0
3′-OCH_3	-	-	3.95 (3H, s)	56.1	-	-

* 500 and 125 MHz, in acetone-d_6 ** 500 and 125 MHz, in $CDCl_3$
*** 300 and 75 MHz, in acetone-d_6

Table 3 Survival percentages of five human cancer cell lines when tested against compounds **1-6**

Compound	SW620	BT474	KATO-III	HepG2	Chago
1	10.0	31.0	19.5	27.5	9.5
2	57.0	48.5	76.5	69.0	64.5
3	73.0	57.0	74.5	78.0	84.0
4	25.5	78.5	72.5	86.0	69.0
5	80.5	92.0	97.0	96.5	84.0
6	29.0	63.5	46.0	66.0	32.5

Table 4 IC_{50} values of isojacareubin (**1**), nigrolineabiphenyl B (**4**) and 1,3,5,6-tetrahydroxyxanthone (**6**) against five human cancer cell lines

Compound	IC_{50} (μg/ml)				
	SW620	BT474	KATO-III	HepG2	Chago
Isojacareubin (**1**)	< 0.001	0.90	1.05	0.42	0.57
Nigrolineabiphenyl B (**4**)	0.1	> 10	> 10	> 10	> 10
1,3,5,6-Tetrahydroxyxanthone (**6**)	8.23	9.52	10	> 10	10
Doxorubicin	0.09	0.65	0.86	0.08	0.63

CONCLUSION

Three xanthones (isojacareubin, buchanaxanthone and 1,3,5,6-tetrahydroxyxanthone) and three biphenyls (aucuparin, nigrolineabiphenyl B and garcibiphenyl C) were isolated from the dichloromethane extract of the wood of *Garcinia schomburgkiana*. This is the first report of their occurrence in this *Garcinia* species. Isojacareubin was strongly cytotoxic against all human cancer cell lines tested, while nigrolineabiphenyl B and 1,3,5,6-tetrahydroxyxanthone also displayed interesting activity.

ACKNOWLEDGMENT

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REFERENCES

1. Koysomboon S. 2006. Isolation and biological activities of the chemical constituents from *Derris indica* and *Garcinia schomburgkiana* and seasonal variation in the major metabolites of the brown alga *Cystophora moniliformis*. PhD Dissertation, Program in Chemistry, Walailak University.

2. Häfner A, Frahm AW. 1993. Biflavanoids from the heartwood of *Garcinia schomburgkiana* and their structural elucidation as atropisomers. Planta Med. 59: A604.
3. Fun HK, Koysomboon S, Chantrapromma K, Chantrapromma S. 2006. A cocrystal of clusiacitran A, clusiacitran B, fluorinated clusiacitran A and fluorinated clusiacitran B (0.45:0.45:0.05:0.05). Acta Cryst. E E62: o3228-o3230.
4. Twentyman PR, Luscombe M. 1987. A study of some variables in a tetrazolium dye (MTT) based assay for cell growth and chemosensitivity. Br. J. Cancer 56: 279-285.
5. Wu QL, Wang SP, Du LJ, Yang JS, Xiao PG. 1998. Xanthones from *Hypericum japonicum* and *H. henryi*. Phytochemistry 49: 1395-1402.
6. Zhang Z, ElSohly HN, Jacob MR, Pasco DS, Walker LA, Clark AM. 2002. Natural products inhibiting *Candida albicans* secreted aspartic proteases from *Tovomita krukovii*. Planta Med. 68: 49-54.
7. Ishiguro K, Yamamoto R, Oku H. 1999. Patulosides A and B, novel xanthone glycosides from cell suspension cultures of *Hypericum patulum*. J. Nat. Prod. 62: 906-908.
8. Jiang DJ, Hu GY, Jiang JL, Xiang HL, Deng HW, Li YJ. 2003. Relationship between protective effect of xanthone on endothelial cells and endogenous nitric oxide synthase inhibitors. Bioorg. Med. Chem. 11: 5171-5177.
9. Kokubun T, Harborne JB, Eagles J, Waterman PG. 1995. Antifungal biphenyl compounds are the phytoalexins of the sapwood of *Saubus aucuparia*. Phytochemistry 40: 57-59.
10. Rukachaisirikul V, Tadpetch K, Watthanaphanit A, Saengsanae N, Phongpaichit S. 2005. Benzopyran, biphenyl, and tetraoxygenated xanthone derivatives from the twigs of *Garcinia nigrolineata*. J. Nat. Prod. 68: 1218-1221.
11. Chen JJ, Peng CF, Huang HY, Chen IS. 2006. Benzopyrans, biphenyls and xanthones from the root of *Garcinia linii* and their activity against *Mycobacterium tuberculosis*. Planta Med. 72: 473-477.