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Phenolic compounds from the fruit of *Garcinia dulcis*

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Abstract

Dulcinoside (**1**), dulcisisoflavone (**2**), dulcisxanthone A (**3**) and sphaerobioside acetate (**6**) together with 22 known compounds were isolated from the green fruit of *G. dulcis*. Dulcisflavan (**4**), dulcisxanthone B (**5**) and isonormangostin (**7**) together with 22 known compounds were isolated from the ripe fruit. Compounds **6** and **7** were synthetic known compounds. Their structures were determined by spectroscopic methods. The radical scavenging and antibacterial activities of some of the compounds were investigated.

Keywords: *Garcinia dulcis*; Guttiferae; Phenolic compounds; Xanthones; Isoflavones; Flavone C-glycoside; Radical scavenging; Antibacterial

1. Introduction

Garcinia dulcis Kurz. (Guttiferae) is known as an Asian medicinal plant used in folk medicines. In Thailand, its stem bark has been used as an antiinflammatory agent. The fruit juice has been used in traditional medicine as an expectorant. In Indonesia, the leaves and seeds have been used for the treatment of lymphatitis, parotitis and struma (Kasahara and Henmi, 1986). The use of this plant as a traditional medicine and the results from preliminary screening on biological activity of crude extracts from its fruit together with the knowledge that other members of the genus *Garcinia* are known to be a source of bioactive compounds (Likhitwitayawuid et al., 1998a; Mackeen et al., 2002; Mahabusarakam et al., 1986) led us to examine the fruit for substances active for radical scavenging and as antibacterial agents. We have isolated three new compounds and 22 known compounds from the green fruit. We also isolated two new compounds together with 22 known compounds from the ripe fruit. The structures of these compounds were elucidated on the basis of 1D and 2D NMR spectroscopic data which included ^{1}H, ^{13}C NMR, NOE, COSY, HMQC and HMBC experiments.

2. Results and discussion

Separation of an acetone extract of the green fruit of *G. dulcis* by solvent partitioning, chromatography and crystallisation generated three new compounds: named dulcinoside (**1**), dulcisisoflavone (**2**), dulcisxanthone A (**3**), and 22 known compounds: sphaerobioside acetate (**6**) (Roesler et al., 1965), camboginol (**8**) (Rama Rao et al., 1980), octadecanoic acid-2,3-dihydroxypropyl ester (**9**) (Chupin et al., 2001), derriscannoside A (**10**) (Dianpeng et al., 1999), 1,6-dihydroxy-3,7-dimethoxy-2-(3-methyl-2-butenyl)xanthone (**11**), cowanin (**12**) (Na Pattalung et al., 1994), cowaxanthone (**13**) (Likhitwitayawuid et al., 1998b), 1,7-dihydroxy-3-methoxy-2-(3-methyl-2-butenyl)xanthone (**14**) (Mahabusarakam et al., 1987), 1,5,8-trihydroxy-3-methoxy-2-(3-methyl-2-butenyl) xanthone (**15**) (Parveen and Khan, 1988),

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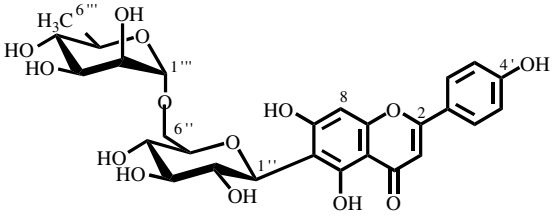
chandalone (**16**) (Falshaw et al., 1969), lupalbigenin (**17**) (Pistelli et al., 1996), BR-xanthone A (**18**) (Balasubramanian and Rajagopalan, 1988), mangostin (**19**) (Mahabusarakam et al., 1987), isolupalbigenin (**20**) (Tahara et al., 1994), 6,8,12-trihydroxy-7-(3-methyl-2-butenyl)-2-methyl-2-(4-methyl-3-pentenyl)pyrano(2′, 3′:7, 8)xanthone (**21**) (Mahabusarakam et al., 2005), 2-hydroxy-l, 2,3-propanetricarboxylic acid-1,3-dimethylester (**22**) (Pyo et al., 2002), vitexin (**23**) (Kartnig et al., 1991), morelloflavone (**24**) (Pelter et al., 1971), clusiaphenone B (**25**) (Delle Monache et al., 1991), mangostenol (**26**) (Suksamrarn et al., 2002), cratoxylone (**27**) and garcinone D (**28**) (Bennett et al., 1993). The ripe fruit of *G. dulcis* was extracted in the same manner as for the green fruit and on separation produced two new compounds: named dulcisflavan (**4**), dulcisxanthone B (**5**) and 22 known compounds: **8**, **9**, **15**, **18**, **19**, **24**, **26**, isonormangostin (**7**) (Mahabusarakam et al., 1987), l,6-dihydroxy-7-methoxy-8-(3,7-dimethyl-2,6-octadienyl)-2′,2′-dimethylpyrano[3,2-b]xanthen-9-one (**29**) (Mahabusarakam et al., 2005), tovophyllin A (**30**) (Bennett et al., 1993), betulinic acid (**31**) (Macías et al., 1994), kaemferol 3-*O*-β-glucopyranosyl-7-*O*-α-rhamnopyranoside (**32**) (Mahabusarakam et al., 2005), garcinone B (**33**) (Sen et al., 1982), l,3,6-trihydroxy-7-methoxy-2,5-bis(3-methyl-2-butenyl)xanthone (**34**) (Na Pattalung et al., 1994), l,6-dihydroxy-7-methoxy-8-(3-methyl-2-butenyl)-2′,2′-dimethylchromeno[5′,6′: 2,3]xanthone (**35**) (Sen et al., 1982), 8-desoxygartanin (**36**) (Govindachari et al., 1971), gartanin (**37**) (Govindachari et al., 1971), morusignin J (**38**) (Hano et al., 1993), apigenin (**39**) (Berghöfer and Hölzl, 1987), cambogin (**40**) (Rama Rao et al., 1980), kaemferol 3,7-di-*O*-α-rhamnopyranoside (**41**) (Mahabusarakam et al., 1987), and (−) epicatechin (**42**) (Sheehan et al., 1983).

Dulcinoside (**1**), 5,7,4′-trihydroxyflavone 6-C-[α-rhamnopyranosyl-(l → 6)]-β-glucopyranoside, was isolated as a yellow solid, m.p. 200–202 °C. A pseudomolecular ion in the HRFABMS at *m/z* 579.1742 $[M+H]^+$ was consistent for the molecular formula $C_{27}H_{30}C_{14}$. The ^{1}H NMR spectrum showed the characteristic resonances of an flavone proton at δ 6.63 (*s*, H-3), a hydrogen-bonded hydroxy proton at δ 13.31 (*s*, 5-OH) and an aromatic proton at δ 6.51 (*s*, H-8). Two doublet resonances ($J = 9.3$ Hz) at δ 7.83 (2H) and 6.90 (2H) were in agreement with the AA′BB′ type of aromatic proton H-2′, H-6′ and H-3′, H-5′. The presence of β-glucose moiety was suggested from the resonances at δ 4.58 (*d*, $J = 9.3$ Hz, H-1″), 3.42–3.40 (1H, *m*, H-5″) and 3.80 (*d*, $J = 9.3$ Hz, H-6″). A rhamnose moiety was identified from the resonances at δ 4.51 (*brs*, H-1‴), 4.10–4.02 (*m*, H-2″), 3.34 (*t*, $J = 9.3$ Hz, H-3‴), 3.14 (*t*, $J = 9.3$ Hz, H-4‴), 3.92–3.90 (*m*, H-5‴) and 1.06 (*d*, 7 = 6.0 Hz, H-6‴). The linkage of glucose unit to flavone nucleus was identified from the HMBC correlation (Table 1) of H-1″ to C-5, C-6 and C-7. The chemical shift values of C-1″ (δ 74.11 ppm) suggested that compound **1** is a C-glycoside. The (1 → 6) glycosidic bond, of rhamnose to glucose was characterised from the cross-peak of H-1‴ to C-6″ and the down field shift of C-6″ of glucose unit. The assignment of the linkage was confirmed by comparison with those of isovitexin-6″-*O*-glucoside (Lin et al., 1997).

Dulcisisoflavone (**2**), 7-hydroxy-2″,2″-dimethylchromano[5,6:6″,5″]-2‴,2‴-dimethylchromano[3′,4′:5‴,6‴] isoflavone, was isolated as a yellow solid and was assigned the molecular formula $C_{25}H_{26}O_5$ (*m/z* 406.1733) on the basis of HREIMS data analysis. The resonances of a characteristic isoflavone proton was shown at δ 7.67 (*s*, H-2) in the ^{1}H NMR spectrum. An ABX pattern from aromatic protons H-2′, H-5′ and H-6′ was present at δ 7.27 (*d*, $J = 1.7$ Hz), 6.73 (*dd*, $J = 8.5$ Hz) and 7.15 (*d*, $J = 8.5$ and 1.7 Hz), respectively whereas the isolated aromatic proton H-8 was at δ 6.42. The presence of two dimethylchroman rings was suggested from the resonances of four methyl groups at δ 1.33 (*s*, 2‴-Me_2), 1.39 (*s*, 2″-Me_2), four methylene groups at δ 1.80 (*t*, $J = 3.3$ Hz, H-3‴), 1.82 (*t*, $J = 3.3$ Hz, H-3″), 2.66 (*t*, $J = 6.6$ Hz, H-4″) and 2.80 (*t*, $J = 6.6$ Hz, H-4‴). The HMBC of H-4″ to C-5, C-6, C-7, C-2″, C-3″ and H-4‴ to C-2′, C-3′, C-4′, C-2‴, C-3‴ indicated that the dimethylchroman rings were fused to the isoflavone nucleus at C-5, C-6 and C-3′, C-4′, respectively. On the NOE experiment, the enhancement of the signal of H-2′ by irradiation at the resonance of H-4‴ confirmed the placement of the dimethylchroman rings. A carbonyl group was indicated from a carbon resonance at δ 177.50 ppm and a stretching band in the IR spectrum at 1652 cm^{-1}.

Dulcisxanthone A (**3**), l,3-dihydroxy-2,6-bis(3-methyl-2-butenyl)-2,2-dimethylchromeno(5‴,6‴:8,7)xanthone, was isolated as a yellow solid and was assigned the molecular formula $C_{28}H_{30}O_5$ on the basis of HREIMS data analysis. The ^{1}H NMR exhibited the resonances of a chelated hydroxy proton 1-OH (δ 13.70, *s*), H-4 (δ 6.26, *s*) and H-5 (δ 6.82, *s*). The presence of C-2 prenyl unit was indicated from the resonances at δ 3.57 (*d*, H-I′), 5.27 (*br t*, H-2′), 1.84 (*s*, H-4′) and 1.68 (*s*, H-5′). The signal of C-6 prenyl unit were shown at δ 3.46 (*d*, H-1″), 5.30 (*br t*, H-2″), 1.77 (*s*, H-4″) and 1.87 (*s*, H-5″). The HMBC correlations of H-1′ to C-l, C-2, C-3 and H-1″ to C-5, C-7 indicated that the two prenyl groups were at C-2 and C-6. The signals of two methyl groups (2‴-Me_2) and two vicinal olefinic protons (H-4‴ and H-3‴), associated with a chromene ring were present at δ 1.50 (6H, *s*), 7.99 (1H, *d*, $J = 10.2$ Hz) and 5.77 (*d*, $J = 10.2$ Hz), respectively. The down field chemical shift values of H-4‴ indicated that H-4‴ was near by C═O. The HMBC correlations (Table 2) of H-4‴ to C-2‴, C-8a and H-3‴ to C-8, C-2‴, C-4‴ indicated that the chromene ring was fused to the xanthone nucleus at C-7 and C-8.



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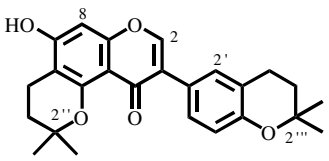

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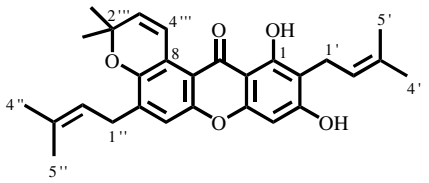

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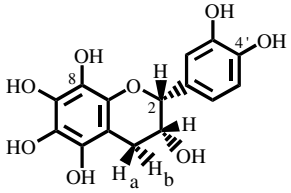

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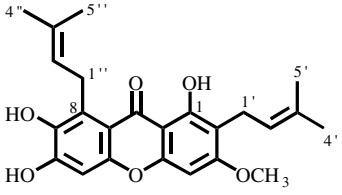

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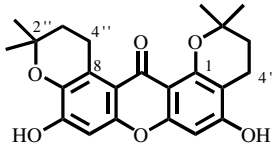

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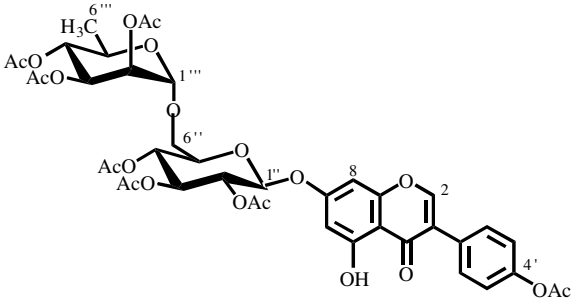

6

Dulcisflavan (**4**), 3,5,6,7,8,3′,4′-heptahydroxyflavan, was isolated as a light brown crystalline solid with m.p. 240–242 °C. The optical rotation was $[\alpha]_D^{29} - 72.0°$ ($c = 1.2 \times 10^{-2}$, MeOH). The molecular weight of 322 corresponded to $C_{15}H_{14}O_8$. The IR spectrum exhibited a strong absorption of O–H stretching at 3358 cm^{-1}. The ^1H NMR spectrum exhibited the signals of aromatic protons H-5′, H-6′ at δ 6.82 (2H) and H-2′ at δ 7.00. The resonances of methine protons H-2, H-3 were shown as singlets at δ 4.85 and 4.22 whereas the methylene protons H_a-4 and H_b-4 resonated as a doublet of doublet at δ 2.87 ($J = 15.0$ and 4.5 Hz) and 2.82 ($J = 15.0$ and 3.0 Hz). The enhancement of the resonances of H-3 and H_a-4 by irradiation at the resonance of H-2 in the NOE experiment indicated that these three protons were in the *cis*-configuration. The complete HMBC correlations (Table 3) confirmed the assigned structure. This compound is an C-3 epimer of elephantorrhizol (Moyo et al., 1999).

Dulcisxanthone B (**5**), 1,6,7-trihydroxy-3-methoxy-2,8-bis(3-methyl-2-butenyl)xanthone, was isolated as a yellow solid, m.p. 170–172 °C with molecular formula $C_{24}H_{26}O_6$. The ^1H NMR spectrum exhibited the singlet resonances of a hydrogen bonded hydroxy proton 1-OH at δ 13.35, H-4 at δ 6.26, H-5 at δ 6.75 and 3-OCH_3 at δ 3.83. The methoxy group was confirmed to be at C-3 by the NOE correlation of the methoxy resonance (3-OMe) to H-4. The presence of two prenyl groups was indicated

by two broad triplets at δ 5.16 (H-2′) and 5.24 (H-2″), two doublets at δ 3.28 (H-1′) and 4.27 (H-1″) and four singlets at δ 1.72 (H-5′ and H-4″), 1.60 (H-4′) and 1.82 (H-5″). The two prenyl groups were attached to C-2 and C-8 of the xanthone ring system on the basis of HMBC correlations (Table 3) from H-1′ to C-1 and C-3 and H-1″ to C-7 and C-8. The complete HMBC confirmed the structure.

Compounds **1**–**5** are new natural products. Compounds **6** and **7** have been previously reported as synthetic compounds (Roesler et al., 1965; Mahabusarakam et al., 1987) however this is the first report of these metabolites as natural products. Compounds **8**, **9**, **15**, **18**, **19**, **24**, **26** were isolated from both green and ripe fruit. Compound **8** was obtained in higher yield from the green-than from the ripe fruit. Compound **24** was previously isolated from the leaves of *G. dulcis* (Ansari et al., 1976). The isolation of other known compounds (**6**–**23**, **25**–**42**) from *G. dulcis* is reported for the first time.

The radical trapping activity and antibacterial activity of some of the compounds were evaluated. At the concentration of 10 μM (Table 4), compounds **4**, **8**, **24**, **40** and **42** were able to trap the DPPH radical with the % scavenging of 87, 74, 5.1, 69 and 82 which corresponded to IC_{50} of 5.90, 6.90, 13.00, 7.20 and 6.10 μM, respectively. For the antibacterial activity, compounds **8**, **13**, **17** and **19** were found to inhibit the growth of *Staphylococcus aureus*, both penicillin-sensitive strain ATCC 25923 and methicillin-resistant strain MRSA SK1 with MIC values ranging from 4 to 16 μg/mL, whereas the others showed either no or weak activities with MIC values greater than 16 μg/mL (Table 5).

In conclusion, dimethoxy citrate and nine types of the secondary metabolites: 22 xanthones, six isoflavones, three benzophenones, two flavans, three flavones, two flavonols, one biflavone, one glycerol derivative and one triterpene were isolated from the fruit of *G. dulcis*.

Table 1
HMBC correlation of compound **1**

H-position	**1**
3	C-2, C-4, C-4a, C-1′
8	C-4, C-4a, C-6, C-7, C-8a
2′, 6′	C-2, C-4′
3′, 5′	C-1′, C-4′
Glc-1″	C-5, C-6, C-7, C-3″, C-5″
2″	C-3″
3″	C-2″, C-4″
4″	C-3″, C-5″, C-6″
5″	C-6″
6″	C-4″, C-5″, C-1‴
Rha-1‴	C-6″, C-2‴, C-3‴, C-5‴
2‴	C-4‴
3‴	C-1‴, C-4‴
4‴	C-3‴, C-5‴
5‴	C-3‴, C-4‴
6‴	C-4‴, C-5‴
5-OH	C-4a, C-5, C-6

Table 2
HMBC correlation of compounds **2** and **3**

H-position	**2**	**3**
2	C-4, C-8a, C-1′	
4		C-2, C-3, C-4a, C-9a
5		C-6, C-7, C-8a, C-9, C-10a
8	C-4, C-4a, C-6, C7, C-8a	
1′		C-l, C-2, C-3
2′	C-4′, C-6′, C-4‴	C-2, C-4′, C-5′
4′		C-2′, C-3′, C-5′
5′	C-1′, C-3′, C-4′	C-2′, C-3′, C-4′
6′	C-2′, C-4′	
1″		C-5, C-7, C-2″, C-3″
2″		C-6, C-4″, C-5″
3″	C-6, C-2″, C-4″, 2″-Me	
4″	C-5, C-6, C-7, C-2″, C-3″	C-2″, C-3″, C-5″
5″		C-2″, C-3″, C-4″
3‴	C-3′, C-2‴, C-4‴, 2‴-Me	C-8, C-2‴, C-4‴
4‴	C-2′, C-3′, C-4′, C-2‴, C-3‴	C-8a, C-2‴
2″-Me	C-2″, C-3″	
2‴-Me	C-2‴, C-3‴	C-2‴, C-3‴
1-OH		C-l, C-2, C-9a
3-OH		C-2

Table 3
HMBC correlation of compounds **4** and **5**

H-position	**4**	**5**
2	C-3, C-4, C-1′, C-2′, C-6′	
3	C-4a, C-1′	
4	C-2, C-3, C-4a, C-5	C-2, C-3, C-4a, C-9a
5		C-6, C-7, C-8a, C-10a
1′		C-1, C-3, C-2′, C-3′
2′	C-2, C-1′, C-3′, C-4′, C-6′	C-2,C-1′, C-4′, C-5′
4′		C-2′, C-3′, C-5′
5′	C-1′, C-3′	C-2′, C-3′, C-4′
6′	C-2, C-1′, C-2′, C-4′	
1″		C-7, C-8, C-8a, C-2″, C-3″
2″		C-8, C-1″, C-4″, C-5″
3″		
4″		C-2″, C-3″, C-5″
5″		C-2″, C-3″, C-4″
1-OH		C-l, C-2, C-9a
3-OMe		C-3

Compounds **4**, **8**, **24**, **40** and **42** were effective scavengers of the DPPH radical. Compounds **13**, **8**, **17** and **19** showed moderate antibacterial activity.

3. Experimental

3.1. General method

Melting points were measured on a digital Electrothermal 9100 Melting Point Apparatus and are uncorrected. Infrared spectra were recorded on an FTS 165 FT-IR spectrometer. Ultraviolet absorption spectra were recorded using a UV-160A spectrometer (SHIMADZU). ^{1}H (500 MHz) and ^{13}C NMR (125 MHz) spectra were performed on a Varian UNITY INOVA 500

Table 4
Radical scavenging activity of compounds from the fruit of *G. dulcis* (10 μM)

Compounds	% scavenging of DPPH
1	22
2	15
3	2
4	87[a]
5	18
6	18
7	10
8	74[a]
10	18
11	15
12	15
13	16
14	15
15	25
16	20
17	20
18	15
19	18
20	16
21	13
23	17
24	51[a]
25	11
26	8
28	3
29	2
32	15
33	15
34	18
35	2
36	8
37	2
38	11
39	18
40	69[a]
41	11
42	82[a]
Ascorbic acid	77
BHT	43

[a] Compounds that gave a reduction greater than that produced by 10 μM BHT.

spectrometer in $CDCl_3$. The high resolution mass spectra were recorded on an MS25RFA spectrometer. Precoated TLC sheets (layer thickness 0.2 mm) and preparative TLC plates (layer thickness 1.25 mm) of silica gel 60 PF_{254} were used. Quick column and column chromatography (QCC and CC) were performed on silica gel 60H and silica gel 100 (Merck), respectively.

3.2. Plant material

The fruits of *G. dulcis* were collected from Songkhla province in the southern part of Thailand. The voucher specimen (Coll. No. 02, Herbarium No. 0012652) has been deposited at Prince of Songkla University Herbarium, Biology Department, Faculty of Science, Prince of Songkla University, Thailand.

Table 5
Antibacterial activity of compounds from the fruit of *G. dulcis*

Compounds	MIC (μg/mL)	
	S. aureus ATCC 25923	MRSA SK1
3	>128	>128
6	>128	>128
8	16	16
12	32	8
13	16	16
17	8	8
18	>128	>128
19	4	4
24	>128	>128
28	16	32
32	64	64
33	8	>128
34	16	32
40	128	64
41	>128	>128
Vancomycin	2	2

3.2.1. Extraction and isolation of compounds from the green fruit

The green fruits of *G. dulcis* (8 kg) were chopped and immersed in Me_2CO (5 days) then MeOH (3 days) to give, after evaporation, the Me_2CO extract (165.80 g) and the MeOH extract (154.63 g). The two layers obtained from the Me_2CO extract were separated. The upper layer was dried under reduced pressure to give a viscous liquid (fraction A, 84.85 g). The lower layer was further partitioned with BuOH. The residue from the BuOH layer was dissolved in CH_2Cl_2 followed by EtOAc to give a CH_2Cl_2 soluble-(fraction B1, 7.50 g), EtOAc soluble-(fraction B2, 37.53 g) and EtOAc insoluble fraction. The MeOH extract (48.31 g) was partitioned in EtOAc and H_2O to produce the EtOAc soluble-(fraction C1, 7.98 g) and an aqueous fraction. The aqueous fraction was further extracted with BuOH to give the BuOH extract (fraction C2, 25.27 g). Fraction A (84.85 g) was subjected to a QCC and eluted with CH_2Cl_2, CH_2Cl_2–Me_2CO, Me_2CO–MeOH and MeOH. The collected fractions were combined on the basis of TLC analysis to give fractions A1–A6. Chromatography of fraction A1 using CH_2Cl_2 as an eluent gave **8** (11.43 g). Fraction A3 was dissolved in $CHCl_3$, and a white solid that formed was further crystallized in Me_2CO to give **9** (1.51 g). Fraction A6 was further separated by CC and eluted with CH_2Cl_2 and CH_2Cl_2–Me_2CO to give **6** (5.3 mg) and **10** (11.5 mg). Fraction B1 was fractionated by CC using CH_2Cl_2–Me_2CO, Me_2CO–MeOH as eluents to give tractions B1A-B1L. Chromatography of fraction BIB on CC with hexane–CH_2Cl_2 and CH_2Cl_2 as eluents gave **11** (4.6 mg), **12** (40.6 mg) and **13** (35.9 mg). Fractions B1D, B1G and B1I were each fractionated on CC and eluted with CH_2Cl_2 to give **14** (11.3 mg), **1** (6.5 mg) and **15** (2.8 mg). Fraction B2 was fractionated by CC to give

fractions B2A–B2K. Fraction B2A and B2B were each further separated by CC, eluting with CH_2Cl_2 and CH_2Cl_2–Me_2CO to produce **16** (8.7 mg), **17** (40.9 mg) and **18** (30.2 mg), **19** (10.2 mg), respectively. Fraction B2C were separated by CC and eluted with CH_2Cl_2 followed by crystallization to give **20** (45.4 mg) and **2** (2.0 mg).

Fraction B2D was fractionated by CC using hexane–CH_2Cl_2 to give **21** (4.6 mg). Fraction B2H was dissolved in Me_2CO and then a white solid formed that was further crystallized in Me_2CO–MeOH (8:2) to give **22** (40.4 mg). Fraction B2I was fractionated by CC with CH_2Cl_2–Me_2CO to give **23** (8.7 mg) and **24** (20.6 mg). Fraction C1 (7.98 g) was separated by CC to give fractions C1A–C1G. Fractions C1B and C1C was further separated by CC using CH_2Cl_2 and CH_2Cl_2–Me_2CO as eluents to give **3** (4.3 mg), **14** (3.2 mg) and **25** (17.7 mg). A solid from fraction C1D was dissolved in CH_2Cl_2–Me_2CO (9:1) to give **26** (0.4 mg). Fraction C2 was subjected to CC to produce fractions C2A–C2E. Fractions C2B and C2C were further separated by CC, eluted with CH_2Cl_2–Me_2CO to give **27** (2.4 mg) and **28** (225.8 mg).

3.2.2. Extraction and isolation of compounds from the ripe fruit

The chopped ripe fruits (3 kg) were immersed in Me_2CO (5 days) at room temperature. Acetone was removed by evaporation to give a liquid extract that was further partitioned with hexane (30 mL) followed by EtOAc (30 mL). Solid were obtained from both the hexane-(fraction D, 7.86 g) and EtOAc soluble fraction (25.39 g). The solid from the EtOAc soluble fraction was further fractionated by dissolving in CH_2Cl_2 to give a CH_2Cl_2 soluble-(fraction E, 21.20 g) and insoluble-(fraction F, 40.90 g) fraction. Fraction D was subjected to CC and eluted with a gradient of CH_2Cl_2 and MeOH. The collected fractions were combined according to their TLC characteristics and evaporated to afford fractions D1–D11. Fractions D1, D2 and D3 were further fractionated by CC, eluted with CH_2Cl_2 and CH_2Cl_2–Me_2CO to give **18** (84.8 mg), **29** (28.3 mg), **30** (2.5 mg) and **26** (13.1 mg). Fractions D4, D5 and D7 were fractionated by CC using CH_2CI_2 and CH_2CI_2–Me_2CO as eluents to give **8** (80.2 mg). Fraction D6 generated **19** (9.7 mg) after separation by CC using CH_2Cl_2 and CH_2Cl_2–Me_2CO as eluents. Fraction D8 was further fractionated by CC, eluted with CH_2Cl_2–Me_2CO to give **7** (12.9 mg) and **31** (3.3 mg). Fraction D9 was subjected to CC using CH_2Cl_2–MeOH as eluent to give **4** (16.4 mg), **7** (25.2 mg) and **15** (5.8 mg). Fraction D10 was further crystallized in CH_2Cl_2–MeOH to give **32** (46.8 mg). Fraction E was fractionated by QCC to yield fractions E1–E6. Fractions E1 and E2 were fractionated by CC, eluted with CH_2Cl_2–Me_2CO to give **33** (20.3 mg) and **34** (85.1 mg), respectively. Fraction E3 was subjected to CC using CH_2Cl_2–MeOH to give **3** (13.6 mg) and **24** (35.2 mg). Fraction E4 was further purified by CC using CH_2Cl_2–MeOH as eluent to give **35** (22.4 mg), **36** (41.7 mg), **37** (31.2 mg) and **38** (35.3 mg). Fraction E5 was further purified by CC eluting with CH_2Cl_2–MeOH to give **9** (15.6 mg), **39** (45.6 mg) and **40** (288.8 mg). Fraction E6 was further crystallized in a mixture of CH_2Cl_2–MeOH (1:1) to give **41** (114.2 mg). The filtrate of fraction E6 was concentrated and fractionated by CC eluting with CH_2Cl_2–MeOH to give **24** (13.5 mg) and **42** (25.6 mg). Fraction F was fractionated by CC using CH_2Cl_2 and CH_2Cl_2–Me_2CO to give **6** (11.6 mg).

*3.2.2.1. Dulcinoside (**1**).* Yellow solid, m.p. 200–202 °C. HRFABMS *m/z* 579.1742 $[M+H]^+$ (calcd. for $C_{27}H_{31}O_{14}$, 579.1714). UV (CH_3OH) λ_{max} (nm) (log ε): 334 (4.25), 272 (4.12), 238 (3.01), 216 (3.65). IR (neat) ν (cm^{-1}): 3402, 1650. ^1H NMR (DMSO-d_6) (δ ppm): 13.31 (1H, *s*, 5-OH), 7.83 (2H, *d*, $J = 9.3$ Hz, H-2′, H-6′), 6.90 (2H, *d*, $J = 9.3$ Hz, H-3′, H-5′), 6.63 (1H, *s*, H-3), 6.51 (1H, *s*, H-8), 4.58 (1H, *d*, $J = 9.3$ Hz, H-1″), 4.51 (1H, *br s*, H-1‴), 4.10–4.02 (1H, *m*, H-2‴), 3.92–3.90 (1H, *m*, H-5‴), 3.80 (1H, *d*, $J = 9.3$ Hz, H-6″), 3.42–3.40 (2H, *m*, H-2″, H-5″), 3.34 (1H, *s*, $J = 9.3$ Hz, H-3‴), 3.24 (1H, *t*, $J = 9.3$ Hz, H-3″), 3.16 (1H, *t*, $J = 9.3$ Hz, H-4″), 3.14 (1H, *t*, $J = 9.3$ Hz, H-4‴), 1.06 (3H, *d*, $J = 6.0$ Hz, H-6‴). FABMS *m/z* (% rel. int): ($[M+H]^+$ 579, 25), 185 (70), 117 (100), 93 (98). ^{13}C NMR (DMSO-d_6) (δppm): 183.24 (C-4), 165.19 (C-2), 164.34 (C-7), 161.98 (C-5), 161.55 (C-4′), 157.65 (C-8a), 129.67 (C-2′, C-6′), 122.35 (C-1′), 117.28 (C-3′, C-5′), 109.32 (C-6), 104.55 (C-4a), 103.77 (C-3), 101.52 (C-1‴), 94.60 (C-8), 79.60 (C-3″), 74.11 (C-1″), 73.06 (C-5″), 71.56 (C-2‴), 71.47 (C-4″), 71.33 (C-4‴), 71.31 (C-2″), 70.71 (C-3‴), 69.40 (C-5‴), 68.54 (C-6″), 18.61 (C-6‴).

*3.2.2.2. Dulcisisoflavone (**2**).* Yellow solid, m.p. 178–180 °C. HREIMS *m/z* 406.1733 $[M]^+$ (calcd. for $C_{25}H_{26}O_5$, 406.1780). UV (CH_3OH) λ_{max} (nm) (log ε): 328 (1.72), 263 (3.05). IR (neat) ν (cm^{-1}): 3375, 1652. ^1H NMR ($CDCl_3$ + one drop DMSO-d_6) (δ ppm): 7.67 (1H, *s*, H-2), 7.27 (1H, *d*, $J = 1.7$ Hz, H-2′), 7.15 (1H, *dd*, $J = 8.5$, 1.7 Hz, H-6′), 6.73 (1H, *d*, $J = 8.5$ Hz, H-5′), 6.42 (1H, *s*, H-8), 2.80 (2H, *t*, $J = 6.6$ Hz, H-4‴), 2.66 (2H, *t*, $J = 6.6$ Hz, H-4″), 1.82 (2H, *t*, $J = 3.3$ Hz, H-3″), 1.80 (2H, *t*, $J = 3.3$ Hz, H-3‴), 1.39 (6H, *s*, 2″-Me_2), 1.33 (6H, *s*, 2‴-Me_2). EIMS *m/z* (% rel. int): ($[M]^+$ 406, 100), 385 (48), 351 (55), 295 (35), 57 (41). ^{13}C NMR ($CDCl_3$ + one drop DMSO-d_6) (δppm): 177.50 (C-4), 161.90 (C-7), 159.88 (C-8a), 157.00 (C-5), 154.90 (C-4′), 149.63 (C-2), 130.80 (C-2′), 128.20 (C-6′), 122.50 (C-1′), 120.50 (C-3′), 117.42 (C-3), 116.87 (C-5′), 107.38 (C-4a), 105.50 (C-6), 93.89 (C-8), 75.25 (C-2″), 74.27 (C-2‴), 32.82

(C-3‴), 31.40 (C-3″), 26.86 (2‴-Me_2), 26.56 (2″-Me_2), 22.47 (C-4‴), 17.12 (C-4″).

*3.2.2.3. Dulcisxanthone A (**3**).* Yellow solid, m.p. 119–120 °C. HREIMS *m/z* 446.1628 [M^+] (calcd. for $C_{28}H_{30}O_5$, 446.1629). UV (CH_3OH) λ_{max} (nm) (log ε): 329 (4.25), 301 (3.21), 288 (4.20), 265 (4.33), 247 (3.32), 208 (4.32). IR (KBr) *v* (cm^{-1}): 3402, 1622, 1579. ^1H NMR ($CDCl_3$)(δ ppm): 13.70 (1H, *s*, 1-OH), 7.99 (1H, *d*, *J* = 10.2 Hz, H-4‴), 6.82 (1H, *s*, H-5), 6.35 (1H, *s*, 3-OH), 6.26 (1H, *s*, H-4), 5.77 (1H, *d*, *J* = 10.2 Hz, H-3‴), 5.30 (1H, *br t*, *J* = 7.5 Hz, H-2″), 5.27 (1H, *br t*, *J* = 7.5 Hz, H-2′), 3.57 (2H, *d*, *J* = 7.2 Hz, H-1′), 3.46 (2H, *d*, *J* = 7.2 Hz, H-1″), 1.87 (3H, *s*, H-5″), 1.84 (3H, *s*, H-4′), 1.77 (3H, *s*, H-4″), 1.68 (3H, *s*, H-5′), 1.50 (6H, *s*, 2‴-Me_2). EIMS *m/z* (% rel. int): ([M]$^+$ 446, 21), 391 (55), 376 (100), 180 (40). ^{13}C NMR ($CDCl_3$) (δ ppm): 182.47 (C-9), 159.79 (C-3), 157.97 (C-1), 156.54 (C-4a), 153.07 (C-10a), 150.93 (C-7), 136.85 (C-6), 132.64 (C-3″), 132.34 (C-3‴), 131.33 (C-3′), 121.50 (C-2″), 121.07 (C-2′), 120.95 (C-4‴), 119.70 (C-8), 108.56 (C-8a), 104.39 (C-2), 103.89 (C-9a), 102.42 (C-5), 94.28 (C-4), 77.99 (C-2‴), 27.35 (2‴-Me_2), 25.86 (C-4″), 25.81 (C-4′), 22.58 (C-1″), 21.45 (C-1′), 17.98 (C-5′), 17.94 (C-5″).

*3.2.2.4. Dulcisflavan (**4**).* Light brown solid, m.p. 240–242 °C. $[\alpha]_D^{29}$: −72.0 °C ($c = 1.2 \times 10^2$, CH_3OH). HREIMS *m/z* 290.0771 [M–O_2]$^+$ (calcd. for $C_{15}H_{14}O_6$, 290.0790). UV (CH_3OH) λ_{max} (nm) (log ε): 281 (2.92), 229 (3.75), 212 (4.12). IR (neat) *v* (cm^{-1}): 3358, 1609, 1517. ^1H NMR ($CDCl_3$ + one drop CD_3OD) (δ ppm): 7.00 (1H, *s*, H-2′), 6.82 (2H, *br s*, H-5′, H-6′), 4.85 (1H, *s*, H-2), 4.22 (1H, *br s*, H-3), 2.87 (1H, *dd*, *J* = 15.0, 4.5 Hz, H_a-4), 2.82 (1H, *dd*, *J* = 15.0, 3.0 Hz, H_b-4). EIMS *m/z* (% rel. int): ([M–O_2]$^+$ 290, 21), 207 (29), 152 (39), 150 (87), 139 (92), 124 (100), 123 (66). ^{13}C NMR ($CDCl_3$ + one drop CD_3OD) (δ ppm): 156.43 (C-8), 155.90 (C-5), 155.78 (C-7), 144.52 (C-8a, C-4′), 144.39 (C-3′), 130.66 (C-6, C-1′), 118.20 (C-6′), 114.84 (C-5′), 113.92 (C-2′), 98.76 (C-4a), 78.47 (C-2), 66.15 (C-3), 27.89 (C-4).

*3.2.2.5. Dulcisxanthone B (**5**).* Yellow solid, m.p. 170–172 °C. HREIMS *m/z* 410.1731 [M^+] (calcd. for $C_{24}H_{26}O_6$, 410.1729). UV (CH_3OH) λ_{max} (nm) (log ε): 368 (4.04), 317 (4.25), 261 (4.49), 244 (4.50), 209 (4.25). IR (KBr) *v* (cm^1): 3406, 1642. ^1H NMR ($CDCl_3$) δ ppm): 13.35 (1H, *s*, 1-OH), 6.75 (1H, *s*, H-5), 6.26 (1H, *s*, H-4), 5.24 (1H, *br t*, *J* = 6.0 Hz, H-2″), 5.16 (1H, *br t*, *J* = 6.0 Hz, H-2′), 4.27 (2H, *d*, *J* = 6.3 Hz, H-1″), 3.83 (3H, *s*, 3-OCH_3), 3.28 (2H, *d*, *J* = 6.3 Hz, H-1′), 1.82 (3H, *s*, H-5″), 1.72 (6H, *s*, H-5′, H-4″), 1.60 (3H, *s*, H-4′). EIMS *m/z* (% rel. int): ([M + H]$^+$ 411, 70), 355 (100), 353 (45), 339 (27), 311 (33), 299 (37). ^{13}C NMR ($CDCl_3$) (δ ppm): 182.57 (C-9), 163.50 (C-3), 159.69 (C-1), 155.29 (C-4a), 153.50 (C-10a), 150.67 (C-6)*, 139.60 (C-7)*, 136.00 (C-3″), 132.00 (C-3′), 127.41 (C-8)*, 122.35 (C-2′), 121.52 (C-2″), 111.67 (C-8a), 111.37 (C-2), 101.13 (C-5, C-9a), 88.75 (C-4), 55.82 (3-OCH_3), 26.00 (C-1″), 25.85 (C-4″), 25.81 (C-4′), 21.35 (C-1′), 18.08 (C-5″), 17.78 (C-5′). * assignment may be interchangeable.

3.3. Radical scavenging activity

This was carried out at room temperature not 37 °C according to the previously reported procedure of Mahabusarakam et al. (2004).

3.4. Antibacterial activity

This was carried out according to the previously reported procedure of Mahabusarakam et al. (2004).

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