

2 EXPERIMENTAL

2.1 Instruments and chemicals

Melting points were recorded in celsius (°C) and are uncorrected. It was measured on a digital Electrothermal 9100 Melting Point Apparatus. Ultraviolet spectra (UV) were measured with Specord S100 spectrophotometer (Analytik Jena Ag). Principle bands ($\lambda_{\text{max}}$) were recorded as wavelengths (nm) and log $\varepsilon$ in methanol solution. Infrared spectra were recorded by using FTS165 FT-IR spectrophotometer. Major bands ($\lambda_{\text{max}}$) were recorded in wavenumber (cm$^{-1}$). $^1$H and $^{13}$C NMR spectra were performed on a Bruker AVANCE 300 MHz NMR or Varian UNITY INOVA 500 MHz NMR, Prince of Songkla University and Bruker AM 400 MHz at Kunming Institute of Botany, Chinese Academy of Sciences, China. Spectra were recorded in CDCl$_3$, Acetone-$d_6$, CD$_3$OD or DMSO-$d_6$ solution and were recorded as $\delta$ value in ppm down field from TMS (internal standard $\delta$ 0.00). Inverse-detected heteronuclear correlations were measured using HMQC and HMBC pulse sequences with a pulse field gradient. Optical rotation was measured in methanol solution with sodium D line (590 nm) on an AUTOPOL R II automatic polarimeter. High resolution mass spectra were recorded on an AEI-MS9 at University of Sydney, Australia, Thermofinnigan MAT 95 XL and Liquid Chromatograph-Mass spectrometer LCT, Micromass at Central Instrument Facilities, Prince of Songkla University. Pre-coated TLC aluminum sheets of silica gel 60 F$_{254}$ (20x20 cm, layer thickness 0.2 mm, Merck) were used for analytical purposes and the compounds were visualized under ultraviolet light or sprayed with anisaldehyde-sulfuric acid reagent. Preparative thin-layer chromatography was carried out on glass plates using silica gel 60 F$_{254}$ (20x20 cm,
layer thickness 1 mm, Merck), activated at 110 °C for 3 h were utilized in the case of preparative TLC. Bands were detected by exposure to short wavelength ultraviolet light. Quick column chromatography (QCC) was performed on silica gel 60H (Merck). Column chromatography was carried out using silica gel 100 (70-230 mesh ASTM, Merck), silica gel 60 (230-400 mesh ASTM, Merck), silica gel 60 RP-18 (40-63 µm, Merck) and sephadex LH-20 (Merck). Organic solvents for extraction and chromatography were distilled at their boiling point ranges prior to use except solvents for UV, IR and optical rotation which were analytical grade reagent. The analytical grade of absolute ethanol, 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical (Fluka), ascorbic acid (Fluka) and butylated hydroxy toluene (BHT, Fluka) were used for antioxidative activity testing and the absorptions of the test solutions were measured with a Spectronic 21 (MILTON ROY).

2.2 Plant material

Flowers and fruits of *G. dulcis* were collected on August 2002 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.
2.3 Chemical investigation of the green fruits

2.3.1 Extraction and isolation

The green fruits of *G. dulcis* (8 kg) were chopped and immersed at room temperature in acetone (5 days) and methanol (3 days), respectively, to give, after evaporation, the acetone extract (Crude 1, 158.50 g) and the methanolic extract (Crude C, 154.63 g). The two layers obtained from the acetone extract were separated. The upper layer was evaporated to dryness under reduced pressure to give a viscous liquid (Crude A, 84.85 g). The lower layer was further extracted with butanol to give butanolic extract (Crude B, 73.20 g). The process of extraction was shown in Figure 2.

2.3.2 Chemical investigation of Crude A

**Purification of Crude A**

Crude A (84.85 g) was subjected to QCC using silica gel as the stationary phase and gradiently eluted with CH$_2$Cl$_2$, CH$_2$Cl$_2$-MeOH and then with MeOH. On the basis of their TLC characteristic, the similar fractions (250 mL each) were combined to give fractions A1-A6 (Table 3). The selected fractions were further purified by CC and crystallization. Four pure compounds were obtained (Figure 3).

**Isolation of GD1**

Fraction A1 (12.240 g) appeared as a yellow solid with a major spot on TLC. It was further chromatographed on CC and eluted with CH$_2$Cl$_2$ to give a yellow solid GD1 (11.43 g).

Melting point: 125-128 °C (lit. 132 °C, 122 °C)

$[\alpha]_D^{29} - 128.5^\circ$, $c = 0.02$ in MeOH (lit. $-132.9^\circ$ in 1% CHCl$_3$, $-143^\circ$ in 1% CHCl$_3$)
EIMS m/z (% relative intensity): ([M]+ 602, 18), 466 (40), 465 (100), 355 (25), 341 (40), 231 (40), 137 (22), 110 (24), 69 (41)

$^1$H NMR (300 MHz) (CDCl$_3$): see Table 24

$^{13}$C NMR (75 MHz) (CDCl$_3$): see Table 24

**Isolation of GD2**

Fractions A3 and A5, obtained as a mixture of yellow viscous liquid and a white solid, were dissolved in CHCl$_3$. The white solid formed was collected and further crystallized in Me$_2$CO to give a white solid GD2 (1.51 g and 0.72 g).

Melting point: 63-65 °C

$^1$H NMR (500 MHz)(CDCl$_3$): see Table 25

$^{13}$C NMR (125 MHz) (CDCl$_3$): see Table 25

**Acetylation of GD2**

Compound GD2 (100 mg) was acetylated with acetic anhydride (2 mL) in pyridine (1 mL) at room temperature overnight. The reaction mixture was poured into ice water and then extracted with CH$_2$Cl$_2$. The lower layer was separated and washed with 10% hydrochloric acid and then water. The organic fraction was dried over anhydrous sodium sulfate and evaporated. The residue (145.0 mg) was further purified by CC and eluted with CH$_2$Cl$_2$ and Me$_2$CO. The acetylated product, GD2(A) was obtained as a colorless viscous liquid (135.0 mg).

$^1$H NMR (300 MHz) (CDCl$_3$): see Table 26

**Isolation of GD2, GD3 and GD4**

Fraction A6 (16.860 g) was dissolved in CHCl$_3$. Upon standing, a white solid formed was filtered to give GD2 (1.96 g). The filtrate was further chromatographed on
CC and gradiently eluted with CH$_2$Cl$_2$ and CH$_2$Cl$_2$-Me$_2$CO. The fractions containing similar components were combined to give twelve fractions (A6.1-A6.12).

Fraction A6.3 (0.09 g) was purified by CC and eluted with CH$_2$Cl$_2$-MeOH (8:2) to give yellow needles **GD3** (5.3 mg).

UV (MeOH) $\lambda_{max}$ nm (log $\varepsilon$): 323.0 (3.46), 257.2 (4.61), 215.8 (3.91)

FT-IR (neat) $\nu$ (cm$^{-1}$): 3410 (O-H stretching), 1646 (C=O stretching)

HREIMS $m/z$ 872.2377 [M]$^+$ (calcd for C$_{41}$H$_{44}$O$_{21}$, 872.2375)

EIMS $m/z$ (% relative intensity): ([M]$^+$ 872, 19), 871 (35), 829 (20), 641 (20), 628 (45), 562 (28), 561 (100)

$^1$H NMR (500 MHz) (CDCl$_3$): see Table 27

$^{13}$C NMR (125 MHz) (CDCl$_3$): see Table 27

Fraction A6.11 (1.89 g) was dissolved in Me$_2$CO. The soluble fraction (130.3 mg) was chromatographed on CC using CH$_2$Cl$_2$-MeOH (8:5) as an eluent. Repurified the major fraction by CC using the same eluent to give brown needles **GD4** (11.5 mg).

UV (MeOH) $\lambda_{max}$ nm (log $\varepsilon$): 301.4 (3.48), 258.9 (3.97), 230.9 (3.79), 211.9 (3.91)

FT-IR (neat) $\nu$ (cm$^{-1}$): 3402 (O-H stretching), 1642 (C=O stretching)

$^1$H NMR (500 MHz) (CDCl$_3$ + DMSO-$d_6$): see Table 28

$^{13}$C NMR (125 MHz) (CDCl$_3$ + DMSO-$d_6$): see Table 28

### 2.3.3 Chemical investigation of Crude B

**Extraction of Crude B**

**Crude B** (73.20 g) was separated into two fractions by dissolving in dichloromethane. The dichloromethane soluble- (**Crude B1**, 7.50 g) and insoluble fractions were obtained. The dichloromethane insoluble fraction was further dissolved
in ethyl acetate to give ethyl acetate soluble- (Crude B2, 37.53 g) and insoluble fractions as shown in Figure 4.

**Purification of Crude B1**

**Crude B1** (7.50 g) was fractionated by CC over silica gel. Elution was conducted with CH\_2Cl\_2, CH\_2Cl\_2-Me\_2CO, Me\_2CO and finally with MeOH. On the basis of their TLC characteristics, the similar fractions were combined to yield twelve fractions (B1A-B1L) as shown in Table 4. The selected fractions were further purified to give six compounds (Figure 5).

**Isolation of GD5, GD6 and GD7**

Fraction B1B (0.205 g) contained four major components. Three components were UV active. Rechromatographed of this fraction on CC and eluted with hexane-CH\_2Cl\_2 and CH\_2Cl\_2. The eluates containing similar components were combined into three fractions (B1B.1-B1B.3).

Fraction B1B.1 (6.0 mg) showed only one spot on TLC. Crystallization from hexane-CH\_2Cl\_2 gave a pale yellow solid GD5 (4.6 mg).

Melting point: 250-251 °C

UV (MeOH) \( \lambda_{\text{max}} \) nm (log \( \varepsilon \)): 359.4 (4.06), 319.7 (4.29), 292.6 (4.12), 259.0 (4.50), 242.0 (4.51)

FT-IR (neat) v (cm\(^{-1}\)): 3281 (O-H stretching), 1606 (C=O stretching)

\( ^1 \)H NMR (300 MHz) (CDCl\(_3\)): see Table 29

\( ^13 \)C NMR (75 MHz) (CDCl\(_3\)): see Table 29

Fraction B1B.2 (58.2 mg) which showed one spot on TLC was crystallized from hexane-CH\_2Cl\_2 to give a yellow solid GD6 (40.6 mg).
Melting point: 137-138 °C (lit. 135-137 °C)
UV (MeOH) \( \lambda_{\text{max}} \) nm (log \( \varepsilon \)): 355 (3.90), 317.1 (4.25), 260.1 (4.32), 243.0 (4.44)
FT-IR (neat) \( \nu \) (cm\(^{-1}\)): 3365 (O-H stretching), 1642 (C=O stretching)

\( ^1 \)H NMR (300 MHz) (CDCl\(_3\)): see Table 30

Fraction B1B.3 (0.050 g) was further chromatographed on CC and eluted with hexane-CH\(_2\)Cl\(_2\) and CH\(_2\)Cl\(_2\). The major component was obtained and recrystallized from CH\(_2\)Cl\(_2\)-hexane. The yellow solid GD7 was collected (35.9 mg).

Melting point: 197-199 °C (lit. 196-197 °C)
UV (MeOH) \( \lambda_{\text{max}} \) nm (log \( \varepsilon \)): 361.4 (4.06), 320.5 (4.30), 258.3 (4.47), 241.8 (4.50)
FT-IR (neat) \( \nu \) (cm\(^{-1}\)): 3369 (O-H stretching), 1646 (C=O stretching)
HREIMS \( m/z \) 410.1724 [M]\(^+\) (calcd for C\(_{24}\)H\(_{26}\)O\(_6\), 410.1729)
EIMS \( m/z \) (% relative intensity): ([M]\(^+\) 410, 5), 341 (18), 325 (25), 299 (31), 298 (47), 287 (66), 178 (68), 149 (69), 111 (30), 97 (46), 71 (67), 69 (83), 57 (100)

\( ^1 \)H NMR (300 MHz) (CDCl\(_3\)): see Table 31
\( ^{13} \)C NMR (75 MHz) (CDCl\(_3\)): see Table 31

**Isolation of GD8**

Fraction B1D (0.236 g) was chromatographed on CC and eluted with CH\(_2\)Cl\(_2\) and CH\(_2\)Cl\(_2\)-Me\(_2\)CO and crystallized from CH\(_2\)Cl\(_2\) to give GD8 as a yellow solid (11.3 mg).

Melting point: 228-230 °C
UV (MeOH) \( \lambda_{\text{max}} \) (nm) (log \( \varepsilon \)): 307.9 (3.18), 263.8 (3.48)
FT-IR (neat) \( \nu \) (cm\(^{-1}\)): 3387 (O-H stretching), 1650 (C=O stretching)

\( ^1 \)H NMR (300 MHz) (CDCl\(_3\)): see Table 32
\( ^{13} \)C NMR (75 MHz) (CDCl\(_3\)): see Table 32
Isolation of GD9

Fraction B1G (0.105 g) was chromatographed on CC, eluting with CH$_2$Cl$_2$ and CH$_2$Cl$_2$-MeOH. The major fraction was rechromatographed and eluted with CH$_2$Cl$_2$-MeOH to give a yellow solid GD9 (6.5 mg).

Melting point: 200-202 °C

UV (MeOH) $\lambda_{\text{max}}$ (nm) (log $\varepsilon$): 333.6 (4.25), 271.7 (4.12), 238.0 (3.01), 215.8 (3.65)

FT-IR (neat) v (cm$^{-1}$): 3402 (O-H stretching), 1650 (C=O stretching)

HRFABMS m/z 579.1742 [M+H]$^+$ (calcd for C$_{27}$H$_{31}$O$_{14}$, 579.1714)

FABMS m/z (% relative intensity): ([M+H]$^+$ 579, 25), 185 (70), 117 (100), 93 (98)

$^1$H NMR (500 MHz) (CHCl$_3$+ DMSO-$d_6$): see Table 33

$^{13}$C NMR (125 MHz) (CHCl$_3$+ DMSO-$d_6$): see Table 33

Isolation of GD10

Fraction B1I (0.362 g) was chromatographed on CC and eluted with CH$_2$Cl$_2$ and CH$_2$Cl$_2$-MeOH. Repurified the major fraction by CC using CH$_2$Cl$_2$ as an eluent to give a yellow solid GD10 (2.8 mg).

Melting point: 195-197 °C (lit. 193-195 °C)

$^1$H NMR (500 MHz) (CDCl$_3$): see Table 34

$^{13}$C NMR (125 MHz) (CDCl$_3$): see Table 34

Purification of Crude B2

Crude B2 (37.53 g) was fractionated by CC using CH$_2$Cl$_2$, CH$_2$Cl$_2$-Me$_2$CO and Me$_2$CO as eluents. The fractions containing similar components were combined into eleven fractions (B2A-B2K) (Table 5). The selected fractions were further purified to give ten compounds (GD11-GD20) as shown in Figure 6.
**Isolation of GD11 and GD12**

Fraction B2A was further separated on CC. Eluted with CH$_2$Cl$_2$ and CH$_2$Cl$_2$-Me$_2$CO gave five combined fractions (B2A.1-B2A.5).

Fraction B2A.1 was further purified by CC using CH$_2$Cl$_2$ and CH$_2$Cl$_2$-Me$_2$CO as eluents to give a pale yellow solid GD11 (8.7 mg).
Melting point: 65-66 °C

UV (MeOH) $\lambda_{\text{max}}$ (nm) (log $\varepsilon$): 283.0 (4.46), 224.8 (4.40)

FT-IR (neat) v (cm$^{-1}$): 3447 (O-H stretching), 1649 (C=O stretching)

$^1$H NMR (500 MHz) (CDCl$_3$): see Table 35

$^{13}$C NMR (125 MHz) (CDCl$_3$): see Table 35

Crystallization of fraction B2A.3 from hexane-CH$_2$Cl$_2$ (2:1) to give a yellow solid GD12 (40.9 mg).
Melting point: 122-125 °C

UV (MeOH) $\lambda_{\text{max}}$ (nm) (log $\varepsilon$): 268.2 (4.30), 212.1 (4.44)

FT-IR (neat) v (cm$^{-1}$): 3443 (O-H stretching), 1646 (C=O stretching)

$^1$H NMR (500 MHz) (CDCl$_3$): see Table 36

$^{13}$C NMR (125 MHz) (CDCl$_3$): see Table 36

**Isolation of GD13 and GD14**

Fraction B2B (0.315 g) was further subjected on CC and eluted with hexane, CH$_2$Cl$_2$ and CH$_2$Cl$_2$-Me$_2$CO to yield six combined fractions. The first fraction was rechromatographed on CC to give a yellow solid GD13 (30.2 mg). The second fraction was crystallized from CH$_2$Cl$_2$-Me$_2$CO to give GD14 as a yellow solid (10.2 mg).
**GD13**

Melting point: 182-183 °C (lit. 181-182 °C)

UV (MeOH) $\lambda_{\text{max}}$ (nm) (log $\varepsilon$): 367.0 (3.83), 319.1 (4.20), 275.0 (4.07), 258.4 (4.37), 239.8 (4.32)

FT-IR (neat) $\nu$ (cm$^{-1}$): 3387 (O-H stretching), 1646 (C=O stretching)

$^1$H NMR (500 MHz) (CDCl$_3$): see Table 37

$^{13}$C NMR (75 MHz) (CDCl$_3$): see Table 37

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**GD14**

Melting point: 182-183 °C (lit. 182-183 °C)

UV (MeOH) $\lambda_{\text{max}}$ (nm) (log $\varepsilon$): 359.7 (3.77), 317.8 (4.24), 260.9 (4.32), 242.7 (4.44)

FT-IR (neat) $\nu$ (cm$^{-1}$): 3418 (O-H stretching), 1645 (C=O stretching)

$^1$H NMR (300 MHz) (CDCl$_3$): see Table 38

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**Isolation of GD15 and GD16**

Fraction B2C showed two major spots on TLC. They were separated by CC and eluted with CH$_2$Cl$_2$. The major component, GD15, was obtained as yellow needles (45.4 mg). The minor component, GD16, was obtained and recrystallized from CH$_2$Cl$_2$-MeOH. The yellow solid GD16 were collected (2.0 mg).

**GD15**

Melting point: 164-166 °C (lit. 165-167 °C)

UV (MeOH) $\lambda_{\text{max}}$ (nm) (log $\varepsilon$): 342.2 (3.51), 267.3 (4.67), 215.1 (4.60)

FT-IR (neat) $\nu$ (cm$^{-1}$): 3380 (O-H stretching), 1642 (C=O stretching)

$^1$H NMR (300 MHz) (CDCl$_3$): see Table 39

$^{13}$C NMR (75 MHz) (CDCl$_3$): see Table 39
**GD16**

Melting point: 178-180 °C

UV (MeOH) $\lambda_{\text{max}}$ (nm) (log $\varepsilon$): 328.4 (1.72), 263.4 (3.05)

FT-IR (neat) $\nu$ (cm$^{-1}$): 3375 (O-H stretching), 1652 (C=O stretching)

HREIMS $m/z$ 406.1733 [M]$^+$ (calcd for C$_{25}$H$_{26}$O$_5$, 406.1780)

EIMS $m/z$ (% relative intensity): ([M]$^+$ 406, 100), 385 (48), 351 (55), 295 (35), 57 (41)

$^1$H NMR (500 MHz) (CDCl$_3$ + DMSO-$d_6$): see Table 40

$^{13}$C NMR (75 MHz) (CDCl$_3$ + DMSO-$d_6$): see Table 40

**Isolation of GD17**

Fraction B2D, containing a major component, was subjected to CC and eluted with hexane-CH$_2$Cl$_2$ to give six fractions. A yellow solid was obtained from the fifth fraction. Rechromatography of the solid by CC gave a yellow solid GD17 (4.6 mg).

Melting point: 93-95 °C

UV (MeOH) $\lambda_{\text{max}}$ (nm) (log $\varepsilon$): 392.9 (3.64), 324.6 (4.22), 262.5 (4.32), 245.5 (4.32)

FT-IR (neat) $\nu$ (cm$^{-1}$): 3454 (O-H stretching), 1665 (C=O stretching)

$^1$H NMR (500 MHz) (CDCl$_3$): see Table 41

$^{13}$C NMR (125 MHz) (CDCl$_3$): see Table 41

**Isolation of GD18**

Fraction B2H was dissolved in Me$_2$CO; the white solid formed was collected and further crystallized from Me$_2$CO-MeOH (8:2) to give a white solid GD18 (40.4 mg).

Melting point: 110-112 °C

$^1$H NMR (500 MHz) (CDCl$_3$ + DMSO-$d_6$): see Table 42
Isolation of GD19 and GD20

Fraction B2I was chromatographed on CC and gradiently eluted with CH$_2$Cl$_2$-Me$_2$CO. The eluates containing similar components were combined into nine fractions (B2I.1-B2I.9). Fraction B2I.6, a yellow solid, consisted of two components. The solid was chromatographed on CC over silica gel and eluted with CH$_2$Cl$_2$-MeOH and MeOH. The yellow solids **GD19** (8.7 mg) and **GD20** (20.6 mg) were collected.

**GD19**

Melting point: 258-259 °C (lit. 264-265 °C)

UV (MeOH) $\lambda_{max}$ (nm) (log $\varepsilon$): 335.6 (4.14), 269.5 (4.17), 212.6 (4.47)

FT-IR (KBr) $\nu$ (cm$^{-1}$): 3390 (O-H stretching), 1660 (C=O stretching)

$^1$H NMR (300 MHz) (CDCl$_3$ + DMSO-$d_6$): see **Table 43**

$^{13}$C NMR (75 MHz) (CDCl$_3$ + DMSO-$d_6$): see **Table 43**

**GD20**

Melting point: 305-307 °C (lit. 298-300 °C)

UV (MeOH) $\lambda_{max}$ (nm) (log $\varepsilon$): 350.1 (2.99), 291.6 (4.19), 274.3 (4.19), 223.8 (4.50)

FT-IR (KBr) $\nu$ (cm$^{-1}$): 3410 (O-H stretching), 1642 (C=O stretching)

EIMS $m/z$ (% relative intensity): ([M]$^+$ 556, 20), 430 (42), 404 (73), 268 (18), 149 (33), 126 (100), 69 (32)

$^1$H NMR (300 MHz) (DMSO-$d_6$) (380 K): see **Table 44**

$^1$H NMR (300 MHz) (DMSO-$d_6$) (295 K): see **Table 45**

$^{13}$C NMR (75 MHz) (DMSO-$d_6$) (380 K): see **Table 44**

$^{13}$C NMR (75 MHz) (DMSO-$d_6$) (295 K): see **Table 45**
Acetylation of GD20

Compound GD20 (100 mg) was acetylated with acetic anhydride in pyridine at room temperature overnight. The reaction mixture was worked up by pouring into ice water and then extracted with CH$_2$Cl$_2$. The lower layer was separated and washed with 10% hydrochloric acid then water. The organic fraction was dried over anhydrous sodium sulfate and evaporated. The acetyl derivative, GD20(A), was obtained as yellow needles (40.5 mg).

$^1$H NMR (500 MHz) (CDCl$_3$): see Table 46

$^{13}$C NMR (125 MHz) (CDCl$_3$): see Table 46

2.3.4 Chemical investigation of Crude C

Extraction of Crude C

A portion of Crude C (48.31 g) was dissolved in ethyl acetate-water (1:1) to afford the ethyl acetate soluble (Crude C$_1$, 7.98 g) and aqueous fraction. The aqueous fraction was further extracted with n-butanol to give butanolic extract (Crude C$_2$, 25.27 g) as shown in Figure 7.

Purification of Crude C$_1$

Crude C$_1$ (7.98 g) was fractionated by CC over silica gel. Elution was conducted with CH$_2$Cl$_2$, CH$_2$Cl$_2$-Me$_2$CO, Me$_2$CO and finally with Me$_2$CO-MeOH. On the basis of their TLC characteristics, similar fractions were combined to yield seven fractions (C1A-C1G) (Table 6). The selected fractions were further purified to give four compounds (GD8, GD21-GD23) as shown in Figure 8.
**Isolation of GD8 and GD21**

Fraction C1B was purified by CC using CH$_2$Cl$_2$ and CH$_2$Cl$_2$-Me$_2$CO as eluents to give five fractions. The yellow solid **GD8** (3.2 mg) was obtained from the first fraction. The third fraction was further purified by CC and eluted with CH$_2$Cl$_2$ and CH$_2$Cl$_2$-Me$_2$CO to give a yellow solid **GD21** (4.3 mg).

**GD21**

Melting point: 119-120 °C

UV (MeOH) $\lambda_{\text{max}}$ (nm) (log $\varepsilon$): 329.8 (4.25), 300.5 (3.21), 287.9 (4.20), 265.0 (4.33), 246.6 (3.32), 207.9 (4.32)

FT-IR (KBr) $\nu$ (cm$^{-1}$): 3402 (O-H stretching), 1622 (C=O stretching)

HREIMS $m/z$ 446.1628 [M]$^+$ (calcd for C$_{28}$H$_{30}$O$_5$, 446.1629)

EIMS $m/z$ (% relative intensity): ([M]$^+$ 446, 21), 391 (55), 376 (100), 180 (40)

$^1$H NMR (300 MHz) (CDCl$_3$): see Table 47

$^{13}$C NMR (125 MHz) (CDCl$_3$): see Table 47

**Isolation of GD22**

Fraction C1C was purified by CC using CH$_2$Cl$_2$ and CH$_2$Cl$_2$-Me$_2$CO as eluents to give four fractions. The third fraction was further purified by PLC using CH$_2$Cl$_2$-Me$_2$CO (8:2) as an eluent to yield **GD22** (17.7 mg) as yellow needles.

FABMS $m/z$ (% relative intensity): ([M]$^+$ 366, 7), 328 (33), 310 (21), 255 (24), 177 (25), 105 (100)

$^1$H NMR (300 MHz) (CDCl$_3$): see Table 48

$^{13}$C NMR (75 MHz) (CDCl$_3$): see Table 48
Isolation of GD23

Fraction C1D was further purified by dissolving in CH$_2$Cl$_2$-Me$_2$CO (9:1) to form a solid. A yellow solid GD23 (1.4 mg) was collected.

Melting point: 163-164 °C (lit. 157-159 °C)

UV (MeOH) \( \lambda_{\text{max}} \) (nm) (log \( \varepsilon \))): 351.0 (4.00), 316.1 (4.46), 256.7 (4.52), 243.2 (4.62)

FT-IR (neat) \( \nu \) (cm$^{-1}$): 3402 (O-H stretching), 1639 (C=O stretching)

$^1$H NMR (300 MHz) (CDCl$_3$): see Table 49

$^{13}$C NMR (75 MHz) (CDCl$_3$): see Table 49

Purification of Crude C2

Crude C2 (12.36 g) was separated by CC over silica gel and eluted with CH$_2$Cl$_2$, CH$_2$Cl$_2$-Me$_2$CO, Me$_2$CO and finally with Me$_2$CO-MeOH. The fractions were combined according to the characteristic on TLC and evaporated to yield five fractions (C2A-C2E) (Table 7). The selected fractions were further purified to give compounds GD24 and GD25 (Figure 9).

Isolation of GD24

Fraction C2B was purified by CC using CH$_2$Cl$_2$ and CH$_2$Cl$_2$-Me$_2$CO as an eluents to give four fractions. The third fraction was further purified by CC and eluted with CH$_2$Cl$_2$-Me$_2$CO to give yellow needles GD24 (2.4 mg).

$^1$H NMR (500 MHz) (Acetone-\( d_6 \)): see Table 50

$^{13}$C NMR (125 MHz) (Acetone-\( d_6 \)): see Table 50

Isolation of GD25

Fraction C2C was purified by CC using CH$_2$Cl$_2$ and CH$_2$Cl$_2$-Me$_2$CO as eluents to give seven fractions (C2C.1-C2C.7). The fractions C2C.2-C2C.5 were
2.4 Chemical investigation of the ripe fruits

2.4.1 Extraction and isolation

The chopped ripe fruits of *G. dulcis* (3,000 g) were immersed in acetone (5 day) at room temperature. Acetone was removed by evaporation to give a liquid extract (Crude 2, 60.50 g) which was further partitioned with hexane, ethyl acetate and then dichloromethane, respectively. Removal of solvent yielded the crude extracts of hexane soluble- (Crude D, 7.86 g), ethyl acetate insoluble- (20.11 g), dichloromethane soluble- (Crude E, 21.20 g) and dichloromethane insoluble- (Crude F, 40.90 g) fractions (Figure 10).

2.4.2 Chemical investigation of Crude D

Purification of Crude D

Crude D (7.86 g) was separated by CC over silica gel and eluted with CH$_2$Cl$_2$, CH$_2$Cl$_2$-MeOH and MeOH. The collected fractions were combined according to the characteristic on TLC and evaporated to give eleven fractions (D1-D11) (Table 8). The selected fractions were further purified to give compounds GD1, GD10, GD13, GD14, GD23 and RD1-RD6 (Figure 11).
**Isolation of GD13**

Fraction D1 was further chromatographed on CC and eluted with hexane-CH$_2$Cl$_2$ (1:1) to give a yellow solid **GD13** (84.8 mg).

**Isolation of RD1 and RD2**

Fraction D2 was further chromatographed on CC and gradiently eluted with hexane-CH$_2$Cl$_2$ and CH$_2$Cl$_2$ to give five fractions (D2.1-D2.5).

Fraction D2.1 was subjected to CC and eluted with 20% CH$_2$Cl$_2$-hexane to give yellow needles **RD1** (28.3 mg).

UV (MeOH) $\lambda_{max}$ (nm) (log $\varepsilon$): 329.6 (2.61), 289.4 (3.98), 241.5 (3.58), 218.4 (3.53)

FT-IR (neat) $\nu$ (cm$^{-1}$): 3410 (O-H stretching), 1646 (C=O stretching)

$^1$H NMR (300 MHz) (CDCl$_3$): see **Table 52**
$^{13}$C NMR (75 MHz) (CDCl$_3$): see **Table 52**

Fraction D2.4 was chromatographed on CC and eluted with CH$_2$Cl$_2$ to give a yellow solid **RD2** (2.5 mg).

Melting point: 212-215 °C (lit. 214-217 °C)

$^1$H NMR (300 MHz) (CDCl$_3$): see **Table 53**
$^{13}$C NMR (125 MHz) (CDCl$_3$): see **Table 53**

**Isolation of GD23**

Fraction D3 was further purified by CC and eluted with CH$_2$Cl$_2$. A yellow solid **GD23** (13.1 mg) was obtained.
**Isolation of GD1**

Fractions D4, D5 and D7 were chromatographed on CC and eluted with CH$_2$Cl$_2$-Me$_2$CO to give a yellow solid. Repurified the solid by CC and eluted with CH$_2$Cl$_2$ to give a yellow solid **GD1** (80.2 mg).

**Isolation of GD14**

Fraction D6 was chromatographed on CC and eluted with CH$_2$Cl$_2$ and CH$_2$Cl$_2$-Me$_2$CO to give a yellow solid **GD14** (9.7 mg).

**Isolation of RD3 and RD4**

Fraction D8 was chromatographed on CC over silica gel and using CH$_2$Cl$_2$ and CH$_2$Cl$_2$-Me$_2$CO as eluents. The fractions containing similar components were combined into five fractions (D8.1-D8.5).

Fraction D8.1 was further purified by CC and eluted with CH$_2$Cl$_2$-Me$_2$CO to give a yellow solid **RD3** (12.9 mg).

Melting point: 210-212 °C

UV (MeOH) $\lambda_{max}$ (nm) (log $\varepsilon$): 355.3 (5.82), 307.5 (6.13), 254.5 (6.36), 212.2 (4.01)

FT-IR (neat) $\nu$ (cm$^{-1}$): 3424 (O-H stretching), 1609 (C-O stretching)

HREIMS $m/z$ 396.1571 [M]$^+$ (calcd for C$_{23}$H$_{24}$O$_6$, 396.1573)

EIMS $m/z$ (% relative intensity): ([M]$^+$ 396, 65), 341 (100), 325 (46), 297 (56), 84 (70)

$^1$H NMR (300 MHz) (CDCl$_3$ + CD$_3$OD): see **Table 54**

$^{13}$C NMR (125 MHz) (CDCl$_3$ + CD$_3$OD): see **Table 54**

Fraction D8.5 was crystallized from CH$_2$Cl$_2$ to give a white solid **RD4** (3.3 mg).

Melting point: 282-285 °C
Isolation of GD10, RD3 and RD5

Fraction D9 was further purified by CC and eluted with CH$_2$Cl$_2$-MeOH to give three fractions. The first fraction was further purified by CC and eluted with CH$_2$Cl$_2$ to give the yellow solids GD10 (5.8 mg) and RD3 (25.2 mg). The second fraction (123.5 mg) was crystallized from CH$_2$Cl$_2$ to give a light brown crystals RD5 (16.4 mg).

RD5

Melting point: 240-242 °C
[$\alpha$]$_D^{20}$: -72.0 ° (c = 1.2 x $10^{-4}$ g/cm$^3$ in MeOH)
UV (MeOH) $\lambda_{max}$ (nm) (log $\varepsilon$): 280.6 (2.92), 228.5 (3.75), 212.1 (4.12)
FT-IR (neat) $\nu$ (cm$^{-1}$): 3358 (O-H stretching), 1609, 1517 (C=C stretching)
HREIMS $m/z$ 290.0771 [M-O$_2$]$^+$ (calcd for C$_{15}$H$_{14}$O$_6$, 290.0790)
EIMS $m/z$ (% relative intensity): ([M-O$_2$]$^+$ 290, 21), 207 (29), 152 (39), 150 (87), 139 (92), 124 (100), 123 (66)
$^1$H NMR (300 MHz) (CDCl$_3$ + CD$_3$OD): see Table 56
$^{13}$C NMR (75 MHz) (CDCl$_3$ + CD$_3$OD): see Table 56

Isolation of RD6

Fraction D10 was further purified by dissolving in CH$_2$Cl$_2$ and then a few drops of MeOH was added to form a yellow solid. The solid was filtered off and further crystallized from CH$_2$Cl$_2$-MeOH. A yellow solid RD6 (46.8 mg) was collected.

Melting point: 249-250 °C (lit. 246-247 °C)
UV (MeOH) $\lambda_{max}$ (nm) (log $\varepsilon$): 351.5 (4.16), 266.2 (4.20), 245.1 (4.04)
2.4.3 Chemical investigation of Crude E

Purification of Crude E

Crude E (21.20 g) was further fractionated by QCC and eluted with CH$_2$Cl$_2$ and CH$_2$Cl$_2$-MeOH. Fractions which showed the similar TLC chromatograms were combined and evaporated under reduced pressure to yield six fractions (E1-E6) (Table 9). The selected fractions were further purified to give thirteen compounds (Figure 12).

Isolation of RD7

Fraction E1 (0.258 g) was chromatographed on CC and eluted with CH$_2$Cl$_2$ and CH$_2$Cl$_2$-Me$_2$CO to give eight fractions (E1.1-E1.8). Fraction E1.3 was purified by CC to give a yellow solid. Further purification of the yellow solid by CC gave an orange solid RD7 (20.3 mg).

Melting point: 186-187 °C (lit. 185-187 °C)

UV (MeOH) $\lambda_{\text{max}}$ (nm) (log $\varepsilon$): 382.5 (2.89), 323.0 (3.39), 262.2 (3.50), 244.3 (3.49), 220.2 (3.20)

FT-IR (neat) $\nu$ (cm$^{-1}$): 3299 (O-H stretching), 1642 (C=O stretching)

$^1$H NMR (500 MHz) (CDCl$_3$): see Table 58

$^{13}$C NMR (125 MHz) (CDCl$_3$): see Table 58
**Isolation of RD8**

Fraction E2 (0.334 g) containing one major component was purified by CC and eluted with CH₂Cl₂ and CH₂Cl₂-MeOH to give three fractions. A bright yellow solid RD8 was obtained from the second fraction (85.1 mg).

Melting point: 223-224 °C (lit. 223-224 °C)

¹H NMR (300 MHz) (CDCl₃ + CD₃OD): see Table 59

¹³C NMR (75 MHz) (CDCl₃ + CD₃OD): see Table 59

**Isolation of GD20 and GD21**

Fraction E3 (0.779 g) was chromatographed on CC and eluted with CH₂Cl₂ and CH₂Cl₂-MeOH, the major component, GD20, was obtained and recrystallized from CH₂Cl₂-MeOH (2:8) to give a yellow solid GD20 (35.2 mg). The filtrate was concentrated and purified by CC using CH₂Cl₂ and CH₂Cl₂-MeOH to give three fractions. The second fraction was further purified by PLC, using CH₂Cl₂-MeOH (9.5:0.5) to give a yellow solid GD21 (13.6 mg).

**Isolation of RD9, RD10, RD11 and RD12**

Fraction E4 (0.370 g) was purified by CC and eluted with CH₂Cl₂ and CH₂Cl₂-MeOH to give six fractions (E4.1-E4.6). Fraction E4.3 (175.0 mg) was crystallized from hexane-C₆H₆ (1:1) to give a yellow solid. The solid containing three components, was further purified by CC over silica gel and eluted with hexane-CH₂Cl₂ to give six fractions (E4.3A-E4.3F). The yellow solids RD9 (22.4 mg), RD10 (41.7 mg) and RD11 (31.2 mg) were collected from the fraction E4.3B, E4.3D and E4.3F, respectively.

**RD9**

Melting point: 159-160 °C (lit. 156-157 °C)
RD10
Melting point: 167-168 °C (lit. 165.5 °C)
$^1$H NMR (300 MHz) (CDCl$_3$): see Table 60
$^{13}$C NMR (125 MHz) (CDCl$_3$): see Table 60

RD11
Melting point: 165-167 °C (lit. 167 °C)
$^1$H NMR (300 MHz) (CDCl$_3$): see Table 61
$^{13}$C NMR (75 MHz) (CDCl$_3$): see Table 61

Fraction E4.4 (148.0 mg) was subjected to CC and eluted with hexane-
CH$_2$Cl$_2$, CH$_2$Cl$_2$ and CH$_2$Cl$_2$-Me$_2$CO to give a yellow solid RD12 (35.3 mg).
Melting point: 183-185 °C (lit. 181-186 °C)
$^1$H NMR (500 MHz) (CDCl$_3$): see Table 63
$^{13}$C NMR (125 MHz) (CDCl$_3$): see Table 63

Isolation of GD2, RD13 and RD14

Fraction E5 (5.872 g) was subjected to CC and eluted with CH$_2$Cl$_2$, CH$_2$Cl$_2$-
MeOH and MeOH to give seven fractions (E5.1-E5.7). Fraction E5.2 (43.5 mg) was
dissolved in a small amount of Me$_2$CO. A few drops of CH$_2$Cl$_2$ was added to induce
precipitation. A white solid GD2 (15.6 mg) was obtained. Fraction E5.3 (74.2 mg) was
dissolved in Me$_2$CO to form a solid. A yellow solid RD13 (45.6 mg) was collected.
Melting point: 168-170 °C
UV (MeOH) \( \lambda_{\text{max}} \) (nm) (log \( \varepsilon \)): 334.8 (3.71), 268.3 (3.67), 213.2 (3.79)

FT-IR (neat) \( \nu \) (cm\(^{-1}\)): 3277 (O-H stretching), 1642 (C=O stretching)

\(^1\)H NMR (500 MHz) (CDCl\(_3\) + DMSO-\(d_6\)): see Table 64

\(^{13}\)C NMR (125 MHz) (CDCl\(_3\) + DMSO-\(d_6\)): see Table 64

Fraction E5.6 (0.352 g) was dissolved in a small amount of MeOH to induce a solid. A yellow solid RD14 (288.8 mg) was collected.

Melting point: 238-240 °C

\([\alpha]_D^{29}\) : -198.0 ° (c = 0.03 in MeOH)

HREIMS \( m/z \) 602.3642 [M]+ (calcd for C\(_{38}\)H\(_{50}\)O\(_6\), 602.3607)

EIMS \( m/z \) (% relative intensity): ([M]+ 602, 3), 464 (8), 341 (8), 122 (12), 105 (100), 77 (28), 69 (14)

\(^1\)H NMR (500 MHz) (DMSO-\(d_6\)): see Table 65

\(^{13}\)C NMR (125 MHz) (DMSO-\(d_6\)): see Table 65

**Isolation of RD15, GD20 and RD16**

Fraction E6 (0.342 g) was dissolved in CH\(_2\)Cl\(_2\). The solid formed was collected and further crystallized from CH\(_2\)Cl\(_2\)-MeOH to give a yellow solid RD15 (114.2 mg).

Melting point: 232-233 °C (lit. 229-230 °C)

UV (MeOH) \( \lambda_{\text{max}} \) (nm) (log \( \varepsilon \)): 345.4 (3.99), 316.9 (3.87), 265.1 (4.06), 244.8 (3.73)

FT-IR (neat) \( \nu \) (cm\(^{-1}\)): 3343 (O-H stretching), 1657 (C=O stretching)

\(^1\)H NMR (300 MHz) (DMSO-\(d_6\)): see Table 66

\(^{13}\)C NMR (75 MHz) (DMSO-\(d_6\)): see Table 66
The filtrate of the fraction E6 was concentrated, chromatographed on CC and eluted with CH$_2$Cl$_2$ and CH$_2$Cl$_2$-MeOH to give ten fractions (E6.1-E6.10). Fraction E6.5 (43.5 mg) was chromatographed on CC and eluted with CH$_2$Cl$_2$-MeOH. The second fraction which obtained was further purified by CC to give a yellow solid GD20 (13.5 mg). MeOH was added to fraction E6.8 (21.2 mg); a light brown crystals RD16 (25.6 mg) was collected.

**RD16**

Melting point: 235-236 °C (lit. 246 °C)  
[α]$_D$ $^{29}$: - 72.0 °, c = 0.038 in MeOH (lit. - 63.0 °)  
UV (MeOH) $\lambda_{max}$ (nm) (log $\varepsilon$): 280.8 (5.07), 229.7 (5.76), 212.3 (6.10)  
FT-IR (neat) ν (cm$^{-1}$): 3439 (O-H stretching)  
$^1$H NMR (300 MHz) (CDCl$_3$ + DMSO-$d_6$): see Table 67  
$^{13}$C NMR (125 MHz) (CDCl$_3$ + DMSO-$d_6$): see Table 67

### 2.4.4 Chemical investigation of Crude F

**Purification of Crude F**

Crude F (40.90 g) was further fractionated by CC and eluted with CH$_2$Cl$_2$ and CH$_2$Cl$_2$-Me$_2$CO. Fractions which showed the similar TLC chromatograms were combined and evaporated under reduced pressure to give six fractions (F1-F6) (Table 10). Fraction F2 was further purified to give RD17 (Figure 13).

**Isolation of RD17**

Fraction F2 (0.015 g) was chromatographed on CC and eluted with CH$_2$Cl$_2$ and CH$_2$Cl$_2$-Me$_2$CO to give a yellow solid RD17 (11.6 mg).  
Melting point: 170-172 °C  
UV (MeOH) $\lambda_{max}$ (nm) (log $\varepsilon$): 367.5 (4.04), 316.6 (4.25), 260.5 (4.49), 243.9 (4.50),
208.9 (4.25)
FT-IR (neat) ν (cm\(^{-1}\)): 3406 (O-H stretching), 1642 (C=O stretching)
HREIMS m/z 410.1731 [M]\(^+\) (calcd for C\(_{24}\)H\(_{26}\)O\(_6\), 410.1729)
EIMS m/z (% relative intensity): ([M]\(^+\) 410, 30), 355 (35), 339 (35), 311 (70), 268 (50),
240 (100), 212 (27), 61 (29)
\(^1\)H NMR (300 MHz) (CDCl\(_3\)): see Table 68
\(^{13}\)C NMR (75 MHz) (CDCl\(_3\)): see Table 68

2.5 Chemical investigation of the flowers

2.5.1 Extraction and isolation

The flowers of G. dulcis (1,200 g) were immersed at room temperature in dichloromethane (5 days), acetone (5 days and 7 days) and methanol (6 days), respectively. After evaporation, the dichloromethane- (81.45 g), the acetone- (Crude G\(_1\), 148.28 g and Crude G\(_2\), 38.43 g) and the methanolic extracts (Crude M, 126.65 g) were obtained.

A portion of Crude G\(_1\) (55.82 g) was separated into two fractions, by dissolving in dichloromethane, dichloromethane soluble- (Crude H, 15.28 g) and insoluble- (Crude I, 40.22 g) fractions were obtained. Crude G\(_2\) (38.43 g) was separated into two fractions, by dissolving in dichloromethane, the dichloromethane soluble- (Crude J, 8.74 g) and insoluble fractions were obtained. The dichloromethane insoluble fraction was further dissolved in ethyl acetate, the soluble- (Crude K, 8.52 g) and insoluble fractions were obtained. The ethyl acetate insoluble fraction was further dissolved in acetone, the soluble- (Crude L, 3.59 g) and insoluble portions (15.35 g) were obtained. The process of extraction was shown in Figure 14.
2.5.2 Chemical investigation of Crude G1

Purification of Crude G1

Crude G1 (92.06 g) was subjected to QCC using silica gel as the stationary phase and eluted with CH$_2$Cl$_2$, CH$_2$Cl$_2$-Me$_2$CO, Me$_2$CO, Me$_2$CO-MeOH and then with MeOH. On the basis of their TLC characteristic, the collected fractions (250 mL each) containing the same major components were combined, fractions G1.1-G1.17 were obtained (Table 11). The selected fractions were further purified to give nine pure compounds as shown in Figure 15.

Isolation of FD1

Fraction G1.5 (2.224 g) was chromatographed on CC and eluted with CH$_2$Cl$_2$ and CH$_2$Cl$_2$-Me$_2$CO to give eight fractions. The fifth fraction was purified by CC to give a yellow solid. Further purification of the solid by CC gave a yellow solid FD1 (29.1 mg).

Melting point: 247-250 °C (lit. ~250 °C)

$^1$H NMR (300 MHz) (DMSO-$d_6$) (390 K): see Table 69

$^{13}$C NMR (75 MHz) (DMSO-$d_6$) (390 K): see Table 69

Isolation of FD2, FD3, FD4, FD5 and FD6

Fraction G1.6 (7.731 g) was subjected to CC on sephadex LH-20 and eluted with a gradient of H$_2$O-MeOH to pure MeOH to give the yellow solids FD2 (3.0 mg), FD3 (9.0 mg), FD4 (62.0 mg), FD5 (27.0 mg) and FD6 (10.0 mg).

FD2

Melting point: 125-128 °C

UV (MeOH) $\lambda_{max}$ (nm) (log $\varepsilon$): 376.9 (3.37), 309.3 (3.78), 273.8 (3.87), 241.4 (3.91)

FT-IR (neat) $\nu$ (cm$^{-1}$): 3400 (O-H stretching), 1646 (C=O stretching)
HREIMS $m/z$ 332.0892 [M]$^+$ (calcd for C$_{17}$H$_{16}$O$_7$, 332.0896)

EIMS $m/z$ (% relative intensity): ([M]$^+$ 332, 100), 317 (98), 302 (21), 287 (15), 259 (17), 203 (10), 175 (13)

$^1$H NMR (300 MHz) (CDCl$_3$): see Table 70

$^{13}$C NMR (125 MHz) (CDCl$_3$): see Table 70

**FD3**

Melting point: 203-205 °C (lit. 196-201 °C)

UV (MeOH) $\lambda_{\text{max}}$ (nm) (log $\varepsilon$): 363.6 (3.47), 305.7 (4.10), 271.1 (4.05), 252.4 (4.37), 244.0 (4.40), 220.7 (4.02)

FT-IR (neat) $\nu$ (cm$^{-1}$): 3269 (O-H stretching), 1650 (C=O stretching)

EIMS $m/z$ (% relative intensity): ([M]$^+$ 302, 100), 287 (95), 259 (92), 216 (17), 122 (16)

$^1$H NMR (500 MHz) (CDCl$_3$): see Table 71

$^{13}$C NMR (125 MHz) (CDCl$_3$): see Table 71

**FD4**

Melting point: 215-216 °C

UV (MeOH) $\lambda_{\text{max}}$ (nm) (log $\varepsilon$): 368.8 (4.15), 255.1 (4.13), 209.1 (4.10)

FT-IR (neat) $\nu$ (cm$^{-1}$): 3417 (O-H stretching), 1657 (C=O stretching)

EIMS $m/z$ (% relative intensity): ([M]$^+$ 330, 100), 168 (30), 151 (70), 85 (30), 71 (43), 69 (44), 57 (66)

$^1$H NMR (500 MHz) (DMSO-$d_6$): see Table 72

$^{13}$C NMR (125 MHz) (DMSO-$d_6$): see Table 72
**FD5**

Melting point: 235-236 °C (lit. 238.5-239 °C)

$[\alpha]_D^{29}: - 26.0 ^\circ (c = 0.03 \text{ in MeOH})$

UV (MeOH) $\lambda_{\text{max}}$ (nm) (log $\varepsilon$): 356.8 (4.29), 266.2 (4.32), 256.5 (4.36)

FT-IR (neat) $\nu$ (cm$^{-1}$): 3446 (O-H stretching), 1598 (C=O stretching)

EIMS $m/z$ (% relative intensity): ([M-gal]$^+$ 302, 25), 84 (100), 66 (100)

$^1$H NMR (500 MHz) (DMSO-$d_6$): see Table 73

$^{13}$C NMR (125 MHz) (DMSO-$d_6$): see Table 73

**FD6**

Melting point: 270-271 °C (lit. 266-268 °C)

UV (MeOH) $\lambda_{\text{max}}$ (nm) (log $\varepsilon$): 335.0 (3.76), 287.7 (4.47), 224.1 (4.55)

FT-IR (neat) $\nu$ (cm$^{-1}$): 3417 (O-H stretching), 1635 (C=O stretching)

EIMS $m/z$ (% relative intensity): ([M]$^+$ 552, 10), 241 (17), 185 (17), 149 (66), 128 (25), 97 (32), 85 (39), 84 (55)

$^1$H NMR (500 MHz) (CDCl$_3$ + DMSO-$d_6$): see Table 74

$^{13}$C NMR (125 MHz) (CDCl$_3$ + DMSO-$d_6$): see Table 74

**Isolation of GD20**

Fraction G1.7 was crystallized from MeOH to give a yellow solid GD20 (1.52 g).

**Isolation of FD7 and FD8**

Fraction G1.13 was further separated on sephadex LH-20 and eluted with a gradient of H$_2$O-MeOH to pure MeOH to give yellow solids FD7 (148.0 mg) and FD8 (51.0 mg).
**FD7**

Melting point: 222-224 °C (lit. 219 °C)

EIMS m/z (% relative intensity): ([M-H]^+ 719, 100), 281 (25), 255 (28), 199 (13)

$^1$H NMR (400 MHz) (CD$_3$OD): see Table 75

$^{13}$C NMR (100 MHz) (CD$_3$OD): see Table 75

**FD8**

Melting point: 245-247 °C (lit. 242-243 °C)

UV (MeOH) $\lambda_{\text{max}}$ (nm) (log $\varepsilon$): 346.3 (5.23), 290.2 (5.34), 274.8 (5.31), 255.4 (5.17), 216.7 (5.50)

FT-IR (neat) $\nu$ (cm$^{-1}$): 3270 (O-H stretching), 1643 (C=O stretching)

EIMS m/z (% relative intensity): ([M-H]^+ 717, 100), 599 (35), 281 (14), 255 (15)

$^1$H NMR (400 MHz) (CD$_3$OD): see Table 76

$^{13}$C NMR (100 MHz) (CD$_3$OD): see Table 76

### 2.5.3 Chemical investigation of Crude H

**Purification of Crude H**

**Crude H** (15.28 g) was fractionated by CC using CH$_2$Cl$_2$, CH$_2$Cl$_2$-Me$_2$CO, Me$_2$CO and Me$_2$CO-MeOH as the eluents. The fractions containing similar components were combined to give six fractions (H1-H6) (Table 12). Fractions H1, H2, H3 and H4 were further separated on CC to give FD9, GD7, FD10 and FD11 (Figure 16).

**Isolation of FD9**

Fraction H1 was further purified by CC and eluted with CH$_2$Cl$_2$ and CH$_2$Cl$_2$-Me$_2$CO to give an orange solid FD9 (10.0 mg).
Melting point: 218-220 °C
UV (MeOH) λ_{max} (nm) (log ε): 335.4 (4.10), 301.0 (4.24), 289.1 (4.30), 221.0 (4.21), 205.6 (4.25)
FT-IR (neat) v (cm^{-1}): 3424 (O-H stretching), 1615 (C=O stretching)
HRFABMS m/z 460.1929 [M]^+ (calcd for C_{28}H_{28}O_{6}, 460.1886)
FABMS m/z (% relative intensity): ([M]^+ 460, 100), 444 (50), 61 (42)
^1H NMR (300 MHz) (CDCl₃): see Table 77
^13C NMR (125 MHz) (CDCl₃): see Table 77

**Isolation of GD7**
Fraction H2 was further purified by CC and eluted with CH₂Cl₂, CH₂Cl₂-Me₂CO and Me₂CO to give a pale yellow solid GD7 (21.5 mg).

**Isolation of FD10**
Fraction H3 was further purified by CC and eluted with CH₂Cl₂, CH₂Cl₂-Me₂CO and Me₂CO to give a pale yellow solid FD10 (38.2 mg).
Melting point: 215-216 °C (lit. 216-218 °C)
^1H NMR (300 MHz) (DMSO-d₆): see Table 78
^13C NMR (125 MHz) (DMSO-d₆): see Table 78

**Isolation of FD11**
Fraction H4 was further purified by CC and eluted with CH₂Cl₂, CH₂Cl₂-Me₂CO and Me₂CO to give FD11 as a yellow solid (2.37 g).
Melting point: 139-140 °C (lit. 135 °C)
[^1]H NMR (500 MHz) (CDCl₃): see Table 79
\(^{13}\)C NMR (125 MHz) (CDCl\(_3\)): see Table 79

### 2.5.4 Chemical investigation of Crude I

**Purification of Crude I**

Crude I (40.22 g) was subjected to QCC using silica gel as the stationary phase and eluted with CH\(_2\)Cl\(_2\), CH\(_2\)Cl\(_2\)-Me\(_2\)CO, Me\(_2\)CO and Me\(_2\)CO-MeOH. On the basis of their TLC characteristic, the collected fractions (200 mL each) which contained the same major components were combined to give eight fractions (I1-I8) (Table 13). The selected fractions were further purified to give GD13, GD20 and RD8 (Figure 17).

**Isolation of GD13**

Fraction I1 was chromatographed on CC over silica gel and using CH\(_2\)Cl\(_2\) and CH\(_2\)Cl\(_2\)-Me\(_2\)CO to give five combined fractions. The second fraction was further purified by CC to give a yellow solid GD13 (15.2 mg).

**Isolation of GD20**

Fractions I3, I5-I8 were crystallized from MeOH. The yellow solid GD20 (53.3 mg, 115.2 mg, 68.3 mg, 25.2 mg and 42.3 mg) was obtained.

**Isolation of RD8**

Fraction I4 was fractionated by CC using CH\(_2\)Cl\(_2\) and CH\(_2\)Cl\(_2\)-MeOH. The yellow solids RD8 (5.4 mg) and GD20 (28.5 mg) were obtained.
2.5.5 Chemical investigation of Crude J

Purification of Crude J

**Crude J** (8.74 g) was subjected to CC on silica gel, eluted with CH$_2$Cl$_2$, CH$_2$Cl$_2$-Me$_2$CO, Me$_2$CO and Me$_2$CO-MeOH. The fractions containing similar components were combined to give six fractions (J1-J6) (**Table 14**). The selected fractions were further purified to give GD8, GD14, GD20, RD6 and FD12-FD15 (**Figure 18**).

**Isolation of FD12 and FD13**

Fraction J1 was rechromatographed on CC and eluted with the CH$_2$Cl$_2$ and CH$_2$Cl$_2$-Me$_2$CO to give five fractions (J1.1-J1.5). Fraction J1.3 was rechromatographed on CC and eluted with hexane-CH$_2$Cl$_2$ (7:3) to give **FD12** (10.2 mg) and **FD13** (5.5 mg) as yellow solids.

**FD12**

Melting point: 128-130 °C

$[\alpha]_D^{20} + 96.0 ^\circ, c = 0.03$ in MeOH (lit. $+101 ^\circ, c = 0.5$ in CHCl$_3$)

$^1$H NMR (300 MHz) (CDCl$_3$): see **Table 80**

$^{13}$C NMR (75 MHz) (CDCl$_3$): see **Table 80**

**FD13**

Melting point: 256-257 °C (lit. 259-261 °C)

$^1$H NMR (500 MHz) (CDCl$_3$): see **Table 81**

$^{13}$C NMR (125 MHz) (CDCl$_3$): see **Table 81**
**Isolation of GD20**

Fraction J2 was chromatographed on CC and eluted with CH₂Cl₂ and CH₂Cl₂-MeOH to give four fractions. The third fraction was further purified by CC and eluted with CH₂Cl₂-MeOH (7:3) to give a yellow solid **GD20** (31.6 mg).

**Isolation of FD14**

Fraction J3 was rechromatographed on CC and gradiently eluted with CH₂Cl₂-MeOH to give a yellow solid **FD14** (14.3 mg).

Melting point: 185-187 °C

UV (MeOH) $\lambda_{max}$ (nm) (log $\varepsilon$): 369.1 (1.07), 322.7 (4.28), 258.4 (4.46), 242.9 (4.50), 207.4 (4.34)

FT-IR (neat) ν (cm⁻¹): 3450 (O-H stretching), 1711 (C=O stretching)

HREIMS $m/z$ 464.2199 [M]+ (calcd for C₂₈H₃₂O₆, 464.2199)

EIMS $m/z$ (% relative intensity): ([M]+ 464, 80), 421 (50), 409 (100), 339 (40), 297 (55), 285 (54), 69 (26)

$^1$H NMR (300 MHz) (CDCl₃ + CD₃OD): see Table 82

$^{13}$C NMR (125 MHz) (CDCl₃ + CD₃OD): see Table 82

**Isolation of GD8 and GD14**

Fraction J4 was rechromatographed on CC and eluted with CH₂Cl₂. Two major components, **GD8** and **GD14**, were obtained and recrystallized from hexane-CH₂Cl₂. The yellow solids **GD8** (20.2 mg) and **GD14** (31.7 mg) were collected.
Isolation of GD20

Fraction J5 was rechromatographed on CC and eluted with CH$_2$Cl$_2$ and CH$_2$Cl$_2$-MeOH to give three fractions. The second fraction was rechromatographed on a silica gel column using CH$_2$Cl$_2$ as an eluent to give a yellow solid GD20 (32.5 mg).

Isolation of FD15 and RD6

Fraction J6 was rechromatographed on CC and eluted with CH$_2$Cl$_2$ and CH$_2$Cl$_2$-MeOH. FD15 and RD6 were obtained and recrystallized from CH$_2$Cl$_2$-MeOH. The yellow solids FD15 (3.2 mg) and RD6 (12.3 mg) were collected.

2.5.6 Chemical investigation of Crude K

Purification of Crude K

Crude K (8.52 g) was rechromatographed over silica gel. Elution was conducted with CH$_2$Cl$_2$, CH$_2$Cl$_2$-Me$_2$CO, Me$_2$CO, Me$_2$CO-MeOH and finally with MeOH. On the basis of their TLC characteristic, the collected fractions with the same major components were combined to give twelve fractions (K1-K12) as shown in Table 15. The selected fractions were further purified to give FD15-FD17 (Figure 19).

Isolation of FD15

Fraction K2 was subjected to CC and eluted with hexane-CH$_2$Cl$_2$ (1:1). A yellow solid FD15 was obtained (5.6 mg).

Melting point: 154-156 °C

UV (MeOH) $\lambda_{\text{max}}$ (nm) (log $\varepsilon$): 358.1 (3.77), 317.9 (4.25), 256.2 (4.28), 241.7 (4.35), 211.7 (4.08)

FT-IR (neat) $\nu$ (cm$^{-1}$): 3446 (O-H stretching), 1650 (C=O stretching)
\(^1\)H NMR (500 MHz) (CDCl\(_3\)): see Table 83

\(^{13}\)C NMR (125 MHz) (CDCl\(_3\)): see Table 83

**Isolation of FD16**

Fraction K4 was rechromatographed on CC and eluted with CH\(_2\)Cl\(_2\)-Me\(_2\)CO (1:1). A yellow solid FD16 was obtained (23.8 mg).

Melting point: 213-215 °C

UV (MeOH) \(\lambda_{\text{max}}\) (nm) (log \(\varepsilon\)): 331.2 (4.44), 322.6 (4.43), 265.8 (4.53), 244.8 (4.54), 208.8 (4.38)

FT-IR (neat) \(\nu\) (cm\(^{-1}\)): 3479 (O-H stretching), 1587 (C=O stretching)

HREIMS \(m/z\) 408.1573 [M]\(^+\) (calcd for C\(_{24}\)H\(_{24}\)O\(_6\), 408.1573)

EIMS \(m/z\) (% relative intensity): ([M]\(^+\) 408, 60), 393 (55), 365 (70), 353 (100), 169 (15)

\(^1\)H NMR (500 MHz) (CDCl\(_3\) + DMSO-\(d_6\)): see Table 84

\(^{13}\)C NMR (125 MHz) (CDCl\(_3\) + DMSO-\(d_6\)): see Table 84

**Isolation of FD17**

Fraction K5 was rechromatographed on CC and eluted with CH\(_2\)Cl\(_2\)-Me\(_2\)CO (1:1). A yellow solid FD17 was obtained (5.2 mg).

Melting point: 227-229 °C

UV (MeOH) \(\lambda_{\text{max}}\) (nm) (log \(\varepsilon\)): 327.2 (3.51), 300.5 (3.62), 258.6 (4.25), 252.7 (4.24), 226.6 (3.96), 219.6 (3.93), 207.9 (3.88)

FT-IR (neat) \(\nu\) (cm\(^{-1}\)): 3343 (O-H stretching), 1657 (C=O stretching)

HREIMS \(m/z\) 206.0570 [M]\(^+\) (calcd for C\(_{11}\)H\(_{10}\)O\(_4\), 206.0579)

EIMS \(m/z\) (% relative intensity): ([M]\(^+\) 206, 100), 205 (95), 177 (11), 165 (8)

\(^1\)H NMR (500 MHz) (CDCl\(_3\)+ DMSO-\(d_6\)): see Table 85
$^{13}$C NMR (125 MHz) (CDCl$_3$+ DMSO-$d_6$): see Table 85

2.5.7 Chemical investigation of Crude L

Purification of Crude L

Crude L (3.59 g) was fractionated by CC using CH$_2$Cl$_2$, CH$_2$Cl$_2$-MeOH and MeOH as eluents. The fractions containing similar components were combined to give six fractions (L1-L6) (Table 16). The selected fractions were further purified to give GD20, RD7, RD12, FD1 and FD18 (Figure 20).

Isolation of RD7

Fraction L1 (0.035 g) was further separated by CC and eluted with CH$_2$Cl$_2$ and CH$_2$Cl$_2$-MeOH to give a yellow solid RD7 (2.6 mg).

Isolation of RD12

Fraction L3 (1.686 g) showed only one spot on TLC. It was subjected to CC, gradiently eluting with CH$_2$Cl$_2$-MeOH to give a yellow solid. The solid was recrystallized from CH$_2$Cl$_2$-MeOH (8:2) to give a yellow solid RD12 (2.3 mg).

Isolation of FD1

Fraction L4 (0.046 g) was chromatographed on CC and eluted with CH$_2$Cl$_2$-MeOH to give a yellow solid which was further purified by CC to give a yellow solid FD1 (5.2 mg).

Isolation of GD20

Fraction L5 (0.982 g) showed only one spot on TLC. Crystallization from CH$_2$Cl$_2$-MeOH (7:3) gave a yellow solid GD20 (2.8 mg).
**Isolation of FD18**

Fraction L6 (0.047 g) was chromatographed on CC and eluted with CH$_2$Cl$_2$-MeOH to give a yellow solid $\text{FD18}$ (15.2 mg).

Melting point: 179-180 °C (lit. 175-176 °C)

$^1$H NMR (500 MHz) (CDCl$_3$): see Table 86

$^{13}$C NMR (125 MHz) (CDCl$_3$): see Table 86

### 2.5.8 Chemical investigation of Crude M

**Isolation of FD19**

A solid was formed after removal of MeOH from the extract under reduced pressure. The solid was filtered and further washed with MeOH to give a white solid $\text{FD19}$ (35.3 mg).

Melting point: 281-283 °C

FT-IR (neat) $\nu$ (cm$^{-1}$): 3439 (O-H stretching), 1642 (C=O stretching)

HRLCMS $m/z$ 379.0428 [M+K]$^+$ (calcd for C$_{15}$H$_{16}$O$_9$K, 379.0431)

EIMS $m/z$ (% relative intensity): ([M-H]$^+$ 339, 95), 220 (20), 175 (30), 163 (80), 119 (100), 117 (15)

$^1$H NMR (300 MHz) (DMSO-$d_6$): see Table 87

$^{13}$C NMR (75 MHz) (DMSO-$d_6$): see Table 87

**Purification of Crude M**

A portion of $\text{Crude M}$ (37.11 g) was further fractionated by CC over silica gel. Gradient elution was conducted with CH$_2$Cl$_2$ and CH$_2$Cl$_2$-MeOH. Fractions with the similar TLC chromatograms were combined and evaporated under reduced pressure to yield twelve fractions (M1-M12) as shown in Table 17. The selected fractions were further purified to give $\text{GD20}$ and $\text{FD1}$ (Figure 21).
Isolation of FD1

Fraction M4 was rechromatographed on CC and gradiently eluted with the CH₂Cl₂ and CH₂Cl₂-MeOH to give nine fractions (M4.1-M4.9). Fraction M4.6 was further purified by dissolving in MeOH and a few drops of CH₂Cl₂ was added to form a yellow solid FD1 (5.6 mg). The filtrate was concentrated and chromatographed on CC and eluted with the CH₂Cl₂-MeOH (8:2) to give FD1 (9.4 mg).

Isolation of GD20

Fraction M5 was dissolved in CH₂Cl₂. The solid formed was collected and further crystallized from CH₂Cl₂-MeOH to give a yellow solid GD20 (15.2 mg).

2.6 Chemical investigation of the seeds

2.6.1 Extraction and isolation

The seeds of green fruits and ripe fruits of *G. dulcis* (900 g) were chopped and immersed in dichloromethane (3 days) and methanol (3 days) at room temperature, respectively. After evaporation, dichloromethane extract (Crude 3, 24.74 g) and methanolic extract (Crude 4, 51.19 g) were obtained. The dichloromethane extract was obtained as a viscous liquid with a white solid. The solid was then filtered off and washed with acetone to give a white solid GD2 (7.64 g) and an acetone soluble fraction (Crude N, 17.08 g). The methanolic extract (51.19 g) was dissolved in hexane to give, after removal of solvent, the crude extract of hexane soluble- (Crude O, 1.39 g) and insoluble- (Crude P, 48.19 g) fractions (Figure 22).
2.6.2 Chemical investigation of Crude N

Purification of Crude N

Crude N (17.08 g) was subjected to QCC using silica gel as the stationary phase and eluted with hexane, hexane-CH₂Cl₂, CH₂Cl₂ and CH₂Cl₂-MeOH. On the basis of their TLC characteristic, the collected fractions (250 mL each) which contained the same major components were combined to give fractions N1-N8 (Table 18). The selected fractions were further purified by CC and crystallized to yield compounds SD1 and SD2 (Figure 23).

Isolation of SD1

Fraction N4 was further subjected to CC and gradiently eluted with CH₂Cl₂ and CH₂Cl₂-MeOH to give four fractions. The second and the third fractions were further recrystallized from CH₂Cl₂-Me₂CO (8:2). A colourless solid SD1 was collected (36.2 mg).

Fraction N5 was purified by CC and eluted with CH₂Cl₂ and CH₂Cl₂-MeOH to give five fractions. The second, the third and the forth fractions were recrystallized from CH₂Cl₂-Me₂CO (8:2). A colourless solid SD1 (20.7 mg) was collected.

Melting point: 140-142 °C (lit. 153.5-154.5 °C)

\[\left[\alpha\right]_{D}^{29} = -10.6^\circ, c = 0.018 \text{ in MeOH (lit. } -16.7^\circ, c = 0.70 \text{ in MeOH)}\]

\(^1\)H NMR (500 MHz) (Acetone-\(d_6\)): see Table 88

\(^13\)C NMR (125 MHz) (Acetone-\(d_6\)): see Table 88

Isolation of SD2

Fraction N7 was chromatographed on CC and eluted with CH₂Cl₂-MeOH to give five fractions. The second fraction was purified by CC to give a yellow solid.
Further purification of the yellow solid by crystallization from CH$_2$Cl$_2$-MeOH (9:1) to give a yellow solid SD2 (25.2 mg).

Melting point: 218-220 °C (lit. 213-214.5 °C)

UV (CH$_3$OH) $\lambda_{\text{max}}$ (nm) (log $\varepsilon$): 349.5 (4.58), 235.1 (4.46)

FT-IR (neat) $\nu$ (cm$^{-1}$): 3284 (O-H stretching), 1620 (C=O stretching), 1602, 1580 (C=C stretching)

$^1$H NMR (500 MHz) (DMSO-$d_6$): see Table 89

$^{13}$C NMR (125 MHz) (DMSO-$d_6$): see Table 89

2.6.3 Chemical investigation of Crude O

Purification of Crude O

Crude O (1.39 g) was fractionated by CC and eluted with CH$_2$Cl$_2$, CH$_2$Cl$_2$-MeOH. Fractions which showed the similar TLC chromatograms were combined and evaporated under reduced pressure to give eleven fractions (O1-O11) (Table 19). Fractions O5 was further purified by CC to give SD3 (Figure 24).

Isolation of SD3

Fraction O5 (0.144 g) showed only one major spot on TLC. It was separated by CC and eluted hexane-CH$_2$Cl$_2$ (1:1) and CH$_2$Cl$_2$ to give three fractions. The second fraction was purified by CC and eluted with hexane-CH$_2$Cl$_2$ (1:1). The major component, SD3, was obtained as a yellow solid (11.4 mg).

Melting point: 78-80 °C

$^1$H NMR (300 MHz) (CDCl$_3$): see Table 90

$^{13}$C NMR (125 MHz) (CDCl$_3$): see Table 90
2.6.4 Chemical investigation of Crude P

Purification of Crude P

Crude P (48.19 g) was fractionated by QCC using silica gel as the stationary phase and eluted with CH₂Cl₂, CH₂Cl₂-MeOH and then with MeOH. On the basis of their TLC characteristic, the collected fractions (250 mL each) with the same major components were combined to give seven fractions (P1-P7) (Table 20). Each fraction was further purified by CC and/or crystallization to give eleven compounds (Figure 25).

Isolation of RD12, SD4 and SD5

Fraction P1 (5.667 g) was chromatographed on a silica gel column using hexane, CH₂Cl₂ and CH₂Cl₂-Me₂CO as the eluents to give eleven fractions (P1.1-P1.11). Fraction P1.2 (0.421 g) was rechromatographed, eluted with hexane-CH₂Cl₂ (1:1) and CH₂Cl₂ to give three fractions. The second fraction was further purified by CC using hexane-CH₂Cl₂ (1:1) to give a colourless solid SD4 (142.2 mg).

Melting point: 89-90 °C

¹H NMR (300 MHz) (CDCl₃): see Table 91

¹³C NMR (125 MHz) (CDCl₃): see Table 91

Fraction P1.6 (0.422 g) was fractionated by CC using hexane-CH₂Cl₂ (1:1), CH₂Cl₂ and CH₂Cl₂-Me₂CO as the eluents. A yellow solid SD5 (2.4 mg) was obtained.

Melting point: 228-230 °C (lit. 226-229 °C)

¹H NMR (500 MHz) (CDCl₃): see Table 92

Fraction P1.9 (0.37 g) was rechromatographed on CC and eluted with CH₂Cl₂ and CH₂Cl₂-Me₂CO to give four fractions (P1.9A-P1.9D). Fraction P1.9B was further
rechromatographed and eluted with $\text{CH}_2\text{Cl}_2$ and 2% $\text{Me}_2\text{CO}$ in $\text{CH}_2\text{Cl}_2$. A yellow solid \textbf{RD12} (2.0 mg) was collected.

**Isolation of GD20 and SD6**

Fraction P2 (7.422 g) was further chromatographed on CC and eluted with $\text{CH}_2\text{Cl}_2$ and $\text{CH}_2\text{Cl}_2$-$\text{Me}_2\text{CO}$ to give seven fractions. The third fraction was recrystallized from $\text{CH}_2\text{Cl}_2$-$\text{Me}_2\text{CO}$ to give a colourless solid \textbf{SD6} (0.35 g). The fifth fraction was recrystallized from $\text{CH}_2\text{Cl}_2$-$\text{MeOH}$. A yellow solid \textbf{GD20} (9.40 g) was collected.

\textbf{SD6}

Melting point: 128-130 °C (lit. 124 °C)

UV (MeOH) $\lambda_{\text{max}}$ (nm) (log $\varepsilon$): 276.5 (3.63), 220.3 (3.91)

FT-IR (neat) $\nu$ (cm$^{-1}$): 3446 (O-H stretching), 1646 (C=O stretching)

$^1$H NMR (500 MHz) (CDCl$_3$): see Table 93

$^{13}$C NMR (125 MHz) (CDCl$_3$): see Table 93

**Isolation of GD20**

Fractions P3 and P4 showed one major spot on TLC, crystallized from $\text{CH}_2\text{Cl}_2$-$\text{MeOH}$. The yellow solid \textbf{GD20} was obtained.

**Isolation of SD7**

Fraction P5 (3.751 g), containing a major component, was dissolved in $\text{CH}_2\text{Cl}_2$ and filtered. The solid was further crystallized from $\text{CH}_2\text{Cl}_2$-$\text{MeOH}$ to give a yellow solid \textbf{SD7} (23.6 mg).

Melting point: 188-190 °C

UV (MeOH) $\lambda_{\text{max}}$ (nm) (log $\varepsilon$): 318.4 (4.18), 244.9 (4.31), 213.2 (3.68)
Isolation of SD8 and SD9

Fraction P6 (4.773 g) was chromatographed on CC and eluted with CH₂Cl₂ and CH₂Cl₂-MeOH to give ten fractions (P6.1-P6.10). Fraction P6.5 was chromatographed on CC and eluted with CH₂Cl₂ and CH₂Cl₂-MeOH to give a colourless solid SD8 (59.7 mg).

Melting point: 132-133 °C (lit. 132-133 °C)

UV (MeOH) λ\text{max} (nm) (log ε): 278.3 (4.36), 224.2 (4.51)

FT-IR (neat) ν (cm⁻¹): 3247 (O-H stretching), 1653, 1606 (C=O stretching)

¹H NMR (500 MHz) (Acetone-\text{d}_6): see Table 95

¹³C NMR (125 MHz) (Acetone-\text{d}_6): see Table 95

Fraction P6.9 was dissolved in MeOH and filtered. A brown solid SD9 was obtained (10.6 mg). The filtrate was concentrated and MeOH was added. A light brown solid SD9 (5.0 mg) was formed and collected.

Melting point: 276-277 °C

¹H NMR (300 MHz) (DMSO-\text{d}_6): see Table 96

¹³C NMR (125 MHz) (DMSO-\text{d}_6): see Table 96
Isolation of SD10, SD11 and SD12

Fraction P7 (3.002 g) was chromatographed on CC and eluted with CH$_2$Cl$_2$ and CH$_2$Cl$_2$-MeOH to give nine fractions (P7.1-P7.9). Fraction P7.2 was chromatographed on CC and eluted with CH$_2$Cl$_2$ and CH$_2$Cl$_2$-MeOH. The third and fifth fractions were rechromatographed on CC and eluted with CH$_2$Cl$_2$-MeOH to give a pink solid SD10 (34.5 mg) and brown needles SD11 (12.8 mg), respectively.

SD10

Melting point: 150-152 °C (lit. 153-154 °C)

$[\alpha]_D^{29} : + 63.0 \degree$, $c = 0.018$ in MeOH (lit. +59.5 °, $c = 0.12$ in MeOH)

UV (MeOH) $\lambda_{max}$ (nm) (log $\varepsilon$): 282.6 (3.98), 223.4 (4.48)

FT-IR (neat) $\nu$ (cm$^{-1}$): 3373 (O-H stretching), 1510 (C=C stretching)

$^1$H NMR (500 MHz) (Acetone-$d_6$): see Table 97

$^{13}$C NMR (125 MHz) (Acetone-$d_6$): see Table 97

SD11

$[\alpha]_D^{29} : - 129.0 \degree$, $c = 0.023$ in MeOH (lit. –23 °, $c = 0.40$ in MeOH)

FT-IR (neat) $\nu$ (cm$^{-1}$): 3306 (O-H stretching), 1616, 1598 (C=O stretching)

$^1$H NMR (500 MHz) (Acetone-$d_6$): see Table 98

$^{13}$C NMR (125 MHz) (Acetone-$d_6$): see Table 98

Fraction P7.4 which contained one major component was subjected to CC and eluted with CH$_2$Cl$_2$ and CH$_2$Cl$_2$-MeOH to give five fractions. The third fraction was further purified by CC and eluted with CH$_2$Cl$_2$-MeOH (8:2). A pink solid SD12 (35.4 mg) was collected.

Melting point: 178-179 °C (lit. 157-158 °C)

FT-IR (neat) $\nu$ (cm$^{-1}$): 3269 (O-H stretching), 1657, 1602 (C=O stretching)
2.7 Estimation of the antioxidative activity

The potential antioxidative activities of the crude materials and pure compounds isolated from the flowers, fruits, and seeds of *G. dulcis* were assessed on the basis of the scavenging activity of the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical.

DPPH assay is one of the methods used for evaluation of antioxidative activity. The following assay procedure was modified from those described in previous report (Subhadhirasakul and Khumfang, 2000). The test solution in absolute ethanol (50 µL) was mixed with 0.05 mM DPPH solution in ethanol (3 mL). The absorbance (A) was then measured at 517 nm on spectrophotometer. BHT and ascorbic acid were used as a positive control. The measurements were performed at least in triplicate. The results expressed as % inhibition. The concentration of the sample at 50% inhibition (IC$_{50}$) was obtained by linear regression analysis of dose-response curve, which was plotted between % inhibition and concentration in µM (Subhadhirasakul and Khumfang, 2000).

\[
\text{% Inhibition} = \left( \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \right) \times 100
\]

2.7.1 Screening on the free radical scavenging activity of the crude extracts

The crude material was dissolved in absolute ethanol to prepare the solution with the concentration of 6.1 mg/mL. The solution of each sample (50 µL) was mixed
with 0.05 mM DPPH ethanolic solution (3 mL) in a cuvette to give the solution with the final concentration of 100 µg/mL. The trapping effect was assessed by measuring the absorbance change of the solution at 517 nm against 0.05 mM DPPH ethanolic solution after 30 and 60 min. Ascorbic acid and BHT were used as a positive control. The measurements were performed at least in triplicate. The degree of loss of color implied the activity. The results were shown in Table 21.

**Table 21** The average absorption and % inhibition of the crude extracts (100 µg/mL)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Average absorbances (517 nm)</th>
<th>% Inhibition ± S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 min</td>
<td>30 min</td>
</tr>
<tr>
<td>Control</td>
<td>0.61</td>
<td>0.61</td>
</tr>
<tr>
<td>BHT</td>
<td>0.595</td>
<td>0.245</td>
</tr>
<tr>
<td>green fruits</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DPPH + Crude 1</td>
<td>0.56</td>
<td>0.035</td>
</tr>
<tr>
<td>DPPH + Crude C</td>
<td>0.60</td>
<td>0.46</td>
</tr>
<tr>
<td>ripe fruits</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DPPH + Crude 2</td>
<td>0.505</td>
<td>0.075</td>
</tr>
<tr>
<td>flowers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DPPH + Crude G1</td>
<td>0.565</td>
<td>0.138</td>
</tr>
<tr>
<td>DPPH + Crude M</td>
<td>0.545</td>
<td>0.085</td>
</tr>
<tr>
<td>seeds</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DPPH + Crude 3</td>
<td>0.58</td>
<td>0.48</td>
</tr>
<tr>
<td>DPPH + Crude 4</td>
<td>0.59</td>
<td>0.395</td>
</tr>
</tbody>
</table>

Control = (0.05 mM DPPH)

**Crude 1, 2, G1** = crude Me₂CO extract

**Crude C, M, 4** = crude MeOH extract

**Crude 3** = crude CH₂Cl₂ extract
2.7.2 Screening on the free radical scavenging activity of pure compounds

The testing was performed as in 2.7.1 except the concentration of samples were made at 0.61 mM (final concentration 10 µM). The results were shown in Table 22.

Table 22 The average absorption and % inhibition of pure compounds (10 µM)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Average absorbances (517 nm)</th>
<th>% Inhibition ± S.D. 30 min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 min</td>
<td>30 min</td>
</tr>
<tr>
<td>Control</td>
<td>0.61</td>
<td>0.61</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>0.32</td>
<td>0.14</td>
</tr>
<tr>
<td>BHT</td>
<td>0.45</td>
<td>0.35</td>
</tr>
<tr>
<td>GD1</td>
<td>0.49</td>
<td>0.16</td>
</tr>
<tr>
<td>GD3</td>
<td>0.51</td>
<td>0.50</td>
</tr>
<tr>
<td>GD4</td>
<td>0.52</td>
<td>0.50</td>
</tr>
<tr>
<td>GD5</td>
<td>0.53</td>
<td>0.52</td>
</tr>
<tr>
<td>GD6</td>
<td>0.525</td>
<td>0.52</td>
</tr>
<tr>
<td>GD7</td>
<td>0.52</td>
<td>0.51</td>
</tr>
<tr>
<td>GD8</td>
<td>0.53</td>
<td>0.52</td>
</tr>
<tr>
<td>GD9</td>
<td>0.53</td>
<td>0.475</td>
</tr>
<tr>
<td>GD10</td>
<td>0.54</td>
<td>0.46</td>
</tr>
<tr>
<td>GD11</td>
<td>0.51</td>
<td>0.49</td>
</tr>
<tr>
<td>GD12</td>
<td>0.52</td>
<td>0.49</td>
</tr>
<tr>
<td>GD13</td>
<td>0.53</td>
<td>0.52</td>
</tr>
<tr>
<td>GD14</td>
<td>0.53</td>
<td>0.50</td>
</tr>
<tr>
<td>GD15</td>
<td>0.525</td>
<td>0.515</td>
</tr>
<tr>
<td>GD16</td>
<td>0.53</td>
<td>0.52</td>
</tr>
<tr>
<td>Sample</td>
<td>Average absorbances (517 nm)</td>
<td>% Inhibition ± S.D. 30 min</td>
</tr>
<tr>
<td>--------</td>
<td>-----------------------------</td>
<td>---------------------------</td>
</tr>
<tr>
<td></td>
<td>0 min</td>
<td>30 min</td>
</tr>
<tr>
<td>GD17</td>
<td>0.53</td>
<td>0.53</td>
</tr>
<tr>
<td>GD19</td>
<td>0.525</td>
<td>0.505</td>
</tr>
<tr>
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Table 22 (Continued)

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<th>Sample</th>
<th>Average absorbances (517 nm)</th>
<th>% Inhibition ± S.D.</th>
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</tr>
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<tr>
<td>SD3</td>
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<td>0.60</td>
</tr>
<tr>
<td>SD4</td>
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<td>0.60</td>
</tr>
<tr>
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<td>0.57</td>
</tr>
<tr>
<td>SD6</td>
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<td>SD7</td>
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<tr>
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</table>
Table 22 (Continued)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Average absorbances (517 nm)</th>
<th>% Inhibition ± S.D.</th>
</tr>
</thead>
<tbody>
<tr>
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<tr>
<td>SD10</td>
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<tr>
<td>SD11</td>
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<tr>
<td>SD12</td>
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<td>0.59</td>
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</tbody>
</table>

Control = 0.05 mM DPPH

2.7.3 Evaluation of 50% inhibitory concentration (IC₅₀)

Compounds GD1, GD20, RD5, RD14, RD16, FD5, FD8, FD11 and FD12 showed the strong activity, they were then selected for further study. The solution of DPPH (0.05 mM, 3 mL) and the sample were mixed to give the final concentration of 20.0, 18.0, 16.0, 14.0, 12.0, 10.0, 8.0, 4.0, 2.0 and 1.0 µM. The absorbances were measured at 517 nm for 30 minute. The results were expressed as % inhibition as shown in Table 23. The concentration that needed to decrease % inhibition of DPPH solution to 50 (IC₅₀) was obtained by linear regression analysis of dose-response curve.
Table 23 The average absorption and % inhibition of the samples solutions and standard antioxidants at various concentrations

<table>
<thead>
<tr>
<th>Sample</th>
<th>% I</th>
<th>Final concentration (µM)</th>
<th></th>
<th></th>
<th></th>
<th></th>
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<th></th>
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</thead>
<tbody>
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<td></td>
<td>1.0</td>
<td>2.0</td>
<td>4.0</td>
<td>8.0</td>
<td>10.0</td>
<td>12.0</td>
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<td>16.0</td>
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<tr>
<td>Ascorbic acid</td>
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<td>0.34</td>
<td>0.32</td>
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<tr>
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</tr>
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<td>0.25</td>
<td>0.20</td>
<td>0.19</td>
<td>0.18</td>
<td>0.18</td>
<td>0.17</td>
<td>0.16</td>
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<tr>
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<td>11.48</td>
<td>21.31</td>
<td>40.98</td>
<td>44.26</td>
<td>49.18</td>
<td>52.46</td>
<td>55.74</td>
<td>57.38</td>
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<td>57.38</td>
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Control = 0.05 mM DPPH; A = Average absorbance of samples at 517 nm for 30 min;
% I = % inhibition of samples at 517 nm for 30 min