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 (λ_{max}) were recorded as wavelengths (nm) and log ε in methanol solution. Infrared spectra were recorded by using FTS165 FT-IR spectrophotometer. Major bands (λ_{max}) were recorded in wavenumber (cm^{-1}). ^{1}H and ^{13}C NMR spectra were performed on a Brüker AVANCE 300 MHz NMR or Varian UNITY INOVA 500 MHz NMR, Prince of Songkla University and Brüker AM 400 MHz at Kunming Institute of Botany, Chinese Academy of Sciences, China. Spectra were recorded in $CDCl_3$, Acetone-d_6, CD_3OD or DMSO-d_6 solution and were recorded as δ value in ppm down field from TMS (internal standard δ 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_2Cl_2, CH_2Cl_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_2Cl_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 °, c = 0.02 in MeOH (lit. –132.9 ° in 1% $CHCl_3$, -143 ° 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_2CO 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_2Cl_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_2Cl_2 and Me_2CO. 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_2Cl_2 and CH_2Cl_2-Me_2CO. 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_2Cl_2-MeOH (8:2) to give yellow needles **GD3** (5.3 mg).

UV (MeOH) λ_{max} nm (log ε): 323.0 (3.46), 257.2 (4.61), 215.8 (3.91)

FT-IR (neat) ν (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_2CO. The soluble fraction (130.3 mg) was chromatographed on CC using CH_2Cl_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) λ_{max} nm (log ε): 301.4 (3.48), 258.9 (3.97), 230.9 (3.79), 211.9 (3.91)

FT-IR (neat) ν (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) λ_{max} nm (log ε): 359.4 (4.06), 319.7 (4.29), 292.6 (4.12), 259.0 (4.50), 242.0 (4.51)

FT-IR (neat) ν (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) λ_{max} nm (log ε): 355 (3.90), 317.1 (4.25), 260.1 (4.32), 243.0 (4.44)

FT-IR (neat) ν (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_2Cl_2 and CH_2Cl_2. The major component was obtained and recrystallized from CH_2Cl_2-hexane. The yellow solid **GD7** was collected (35.9 mg).

Melting point: 197-199 °C (lit. 196-197 °C)

UV (MeOH) λ_{max} nm (log ε): 361.4 (4.06), 320.5 (4.30), 258.3 (4.47), 241.8 (4.50)

FT-IR (neat) ν (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

Faction B1D (0.236 g) was chromatographed on CC and eluted with CH_2Cl_2 and CH_2Cl_2-Me_2CO and crystallized from CH_2Cl_2 to give **GD8** as a yellow solid (11.3 mg).

Melting point: 228-230 °C

UV (MeOH) λ_{max} (nm) (log ε): 307.9 (3.18), 263.8 (3.48)

FT-IR (neat) ν (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_2Cl_2 and CH_2Cl_2-MeOH. The major fraction was rechromatographed and eluted with CH_2Cl_2-MeOH to give a yellow solid **GD9** (6.5 mg).

Melting point: 200-202 °C

UV (MeOH) λ_{max} (nm) (log ε): 333.6 (4.25), 271.7 (4.12), 238.0 (3.01), 215.8 (3.65)

FT-IR (neat) ν (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_2Cl_2 and CH_2Cl_2-MeOH. Repurified the major fraction by CC using CH_2Cl_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_2Cl_2, CH_2Cl_2-Me_2CO and Me_2CO 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_2Cl_2 and CH_2Cl_2-Me_2CO gave five combined fractions (B2A.1-B2A.5).

Fraction B2A.1 was further purified by CC using CH_2Cl_2 and CH_2Cl_2-Me_2CO as eluents to give a pale yellow solid **GD11** (8.7 mg).

Melting point: 65-66 °C

UV (MeOH) λ_{max} (nm) (log ε): 283.0 (4.46), 224.8 (4.40)

FT-IR (neat) ν (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_2Cl_2 (2:1) to give a yellow solid **GD12** (40.9 mg).

Melting point: 122-125 °C

UV (MeOH) λ_{max} (nm) (log ε): 268.2 (4.30), 212.1 (4.44)

FT-IR (neat) ν (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_2Cl_2 and CH_2Cl_2-Me_2CO 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_2Cl_2-Me_2CO to give **GD14** as a yellow solid (10.2 mg).

GD13

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

UV (MeOH) λ_{max} (nm) (log ε): 367.0 (3.83), 319.1 (4.20), 275.0 (4.07), 258.4 (4.37), 239.8 (4.32)

FT-IR (neat) ν (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**

GD14

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

UV (MeOH) λ_{max} (nm) (log ε): 359.7 (3.77), 317.8 (4.24), 260.9 (4.32), 242.7 (4.44)

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

^{1}H NMR (300 MHz) ($CDCl_3$): see **Table 38**

Isolation of GD15 and GD16

Fraction B2C showed two major spots on TLC. They were separated by CC and eluted with CH_2Cl_2. The major component, **GD15**, was obtained as yellow needles (45.4 mg). The minor component, **GD16**, was obtained and recrystallized from CH_2Cl_2-MeOH. The yellow solid **GD16** were collected (2.0 mg).

GD15

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

UV (MeOH) λ_{max} (nm) (log ε): 342.2 (3.51), 267.3 (4.67), 215.1 (4.60)

FT-IR (neat) ν (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) λ_{max} (nm) (log ε): 328.4 (1.72), 263.4 (3.05)

FT-IR (neat) ν (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_2Cl_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) λ_{max} (nm) (log ε): 392.9 (3.64), 324.6 (4.22), 262.5 (4.32), 245.5 (4.32)

FT-IR (neat) ν (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_2CO; the white solid formed was collected and further crystallized form Me_2CO-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**

^{13}C NMR (125 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_2Cl_2-Me_2CO. 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_2Cl_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) λ_{max} (nm) (log ε): 335.6 (4.14), 269.5 (4.17), 212.6 (4.47)

FT-IR (KBr) ν (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) λ_{max} (nm) (log ε): 350.1 (2.99), 291.6 (4.19), 274.3 (4.19), 223.8 (4.50)

FT-IR (KBr) ν (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_2Cl_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 C1**, 7.98 g) and aqueous fraction. The aqueous fraction was further extracted with n-butanol to give butanolic extract (**Crude C2**, 25.27 g) as shown in **Figure 7**.

Purification of Crude C1

Crude C1 (7.98 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 Me_2CO-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_2Cl_2 and CH_2Cl_2-Me_2CO as an 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_2Cl_2 and CH_2Cl_2-Me_2CO to give a yellow solid **GD21** (4.3 mg).

GD21

Melting point: 119-120 °C

UV (MeOH) λ_{max} (nm) (log ε): 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) ν (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_2Cl_2 and CH_2Cl_2-Me_2CO as eluents to give four fractions. The third fraction was further purified by PLC using CH_2Cl_2-Me_2CO (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_2Cl_2-Me_2CO (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) λ_{max} (nm) (log ε): 351.0 (4.00), 316.1 (4.46), 256.7 (4.52), 243.2 (4.62)

FT-IR (neat) ν (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_2Cl_2, CH_2Cl_2-Me_2CO, Me_2CO and finally with Me_2CO-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_2Cl_2 and CH_2Cl_2-Me_2CO as an eluents to give four fractions. The third fraction was further purified by CC and eluted with CH_2Cl_2-Me_2CO 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_2Cl_2 and CH_2Cl_2-Me_2CO as eluents to give seven fractions (C2C.1-C2C.7). The fractions C2C.2-C2C.5 were

further purified by CC using CH_2Cl_2-Me_2CO as an eluent to give a yellow solid **GD25** (225.8 mg).

Melting point: 200-203 °C

^{1}H NMR (500 MHz) (Acetone-d_6): see **Table 51**

^{13}C NMR (125 MHz) (Acetone-d_6): see **Table 51**

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_2Cl_2, CH_2Cl_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_2Cl_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_2Cl_2 and CH_2Cl_2 to give five fractions (D2.1-D2.5).

Fraction D2.1 was subjected to CC and eluted with 20% CH_2Cl_2-hexane to give yellow needles **RD1** (28.3 mg).

UV (MeOH) λ_{max} (nm) (log ε): 329.6 (2.61), 289.4 (3.98), 241.5 (3.58), 218.4 (3.53)

FT-IR (neat) ν (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_2Cl_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_2Cl_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_2Cl_2-Me_2CO to give a yellow solid. Repurified the solid by CC and eluted with CH_2Cl_2 to give a yellow solid **GD1** (80.2 mg).

Isolation of GD14

Fraction D6 was chromatographed on CC and eluted with CH_2Cl_2 and CH_2Cl_2-Me_2CO 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_2Cl_2 and CH_2Cl_2-Me_2CO 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_2Cl_2-Me_2CO to give a yellow solid **RD3** (12.9 mg).

Melting point: 210-212 °C

UV (MeOH) λ_{max} (nm) (log ε): 355.3 (5.82), 307.5 (6.13), 254.5 (6.36), 212.2 (4.01)

FT-IR (neat) ν (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_3OD): see **Table 54**

^{13}C NMR (125 MHz) ($CDCl_3$ + CD_3OD): see **Table 54**

Fraction D8.5 was crystallized from CH_2Cl_2 to give a white solid **RD4** (3.3 mg).

Melting point: 282-285 °C

^{1}H NMR (500 MHz) ($CDCl_3$ + DMSO-d_6): see **Table 55**

^{13}C NMR (125 MHz) ($CDCl_3$ + DMSO-d_6): see **Table 55**

Isolation of GD10, RD3 and RD5

Fraction D9 was further purified by CC and eluted with CH_2Cl_2-MeOH to give three fractions. The first fraction was further purified by CC and eluted with CH_2Cl_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_2Cl_2 to give a light brown crystals **RD5** (16.4 mg).

RD5

Melting point: 240-242 °C

$[\alpha]_D^{29}$: - 72.0 ° (c = 1.2 x 10^{-4} g/cm^3 in MeOH)

UV (MeOH) λ_{max} (nm) (log ε): 280.6 (2.92), 228.5 (3.75), 212.1 (4.12)

FT-IR (neat) ν (cm^{-1}): 3358 (O-H stretching), 1609, 1517 (C=C stretching)

HREIMS *m/z* 290.0771 $[M\text{-}O_2]^+$ (calcd for $C_{15}H_{14}O_6$, 290.0790)

EIMS *m/z* (% relative intensity): ($[M\text{-}O_2]^+$ 290, 21), 207 (29), 152 (39), 150 (87), 139 (92), 124 (100), 123 (66)

^{1}H NMR (300 MHz) ($CDCl_3$ + CD_3OD): see **Table 56**

^{13}C NMR (75 MHz) ($CDCl_3$ + CD_3OD): see **Table 56**

Isolation of RD6

Fraction D10 was further purified by dissolving in CH_2Cl_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_2Cl_2-MeOH. A yellow solid **RD6** (46.8 mg) was collected.

Melting point: 249-250 °C (lit. 246-247 °C)

UV (MeOH) λ_{max} (nm) (log ε): 351.5 (4.16), 266.2 (4.20), 245.1 (4.04)

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

^{1}H NMR (500 MHz) (DMSO-d_6): see **Table 57**

^{13}C NMR (125 MHz) (DMSO-d_6): see **Table 57**

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_2Cl_2 and CH_2Cl_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_2Cl_2 and CH_2Cl_2-Me_2CO 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) λ_{max} (nm) (log ε): 382.5 (2.89), 323.0 (3.39), 262.2 (3.50), 244.3 (3.49), 220.2 (3.20)

FT-IR (neat) ν (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 CH_2Cl_2 and CH_2Cl_2-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)

^{1}H NMR (300 MHz) ($CDCl_3$ + CD_3OD): see **Table 59**

^{13}C NMR (75 MHz) ($CDCl_3$ + CD_3OD): see **Table 59**

Isolation of GD20 and GD21

Fraction E3 (0.779 g) was chromatographed on CC and eluted with CH_2Cl_2 and CH_2Cl_2-MeOH, the major component, **GD20**, was obtained and recrystallized from CH_2Cl_2-MeOH (2:8) to give a yellow solid **GD20** (35.2 mg). The filtrate was concentrated and purified by CC using CH_2Cl_2 and CH_2Cl_2-MeOH to give three fractions. The second fraction was further purified by PLC, using CH_2Cl_2-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 CH_2Cl_2 and CH_2Cl_2-MeOH to give six fractions (E4.1-E4.6). Fraction E4.3 (175.0 mg) was crystallized from hexane-C_6H_6 (1:1) to give a yellow solid. The solid containing three components, was further purified by CC over silica gel and eluted with hexane-CH_2Cl_2 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)

^{1}H NMR (300 MHz) ($CDCl_3$): see **Table 60**

^{13}C NMR (125 MHz) ($CDCl_3$): see **Table 60**

RD10

Melting point: 167-168 °C (lit. 165.5 °C)

^{1}H NMR (300 MHz) ($CDCl_3$): see **Table 61**

^{13}C NMR (75 MHz) ($CDCl_3$): see **Table 61**

RD11

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

^{1}H NMR (300 MHz) ($CDCl_3$): see **Table 62**

^{13}C NMR (125 MHz) ($CDCl_3$): see **Table 62**

Fraction E4.4 (148.0 mg) was subjected to CC and eluted with hexane-CH_2Cl_2, CH_2Cl_2 and CH_2Cl_2-Me_2CO 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_2Cl_2, CH_2Cl_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_2CO. A few drops of CH_2Cl_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_2CO to form a solid. A yellow solid **RD13** (45.6 mg) was collected.

Melting point: 168-170 °C

UV (MeOH) λ_{max} (nm) (log ε): 334.8 (3.71), 268.3 (3.67), 213.2 (3.79)

FT-IR (neat) ν (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_2Cl_2. The solid formed was collected and further crystallized from CH_2Cl_2-MeOH to give a yellow solid **RD15** (114.2 mg).

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

UV (MeOH) λ_{max} (nm) (log ε): 345.4 (3.99), 316.9 (3.87), 265.1 (4.06), 244.8 (3.73)

FT-IR (neat) ν (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_2Cl_2 and CH_2Cl_2-MeOH to give ten fractions (E6.1-E6.10). Fraction E6.5 (43.5 mg) was chromatographed on CC and eluted with CH_2Cl_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)

$[\alpha]_D^{29}$: - 72.0 °, c = 0.038 in MeOH (lit. - 63.0 °)

UV (MeOH) λ_{max} (nm) (log ε): 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_2Cl_2 and CH_2Cl_2-Me_2CO. 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_2Cl_2 and CH_2Cl_2-Me_2CO to give a yellow solid **RD17** (11.6 mg).

Melting point: 170-172 °C

UV (MeOH) λ_{max} (nm) (log ε): 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)

^1H 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 G1**, 148.28 g and **Crude G2**, 38.43 g) and the methanolic extracts (**Crude M**, 126.65 g) were obtained.

A portion of **Crude G1** (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 G2** (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_2Cl_2, CH_2Cl_2-Me_2CO, Me_2CO, Me_2CO-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_2Cl_2 and CH_2Cl_2-Me_2CO 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_2O-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) λ_{max} (nm) (log ε): 376.9 (3.37), 309.3 (3.78), 273.8 (3.87), 241.4 (3.91)

FT-IR (neat) ν (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) λ_{max} (nm) (log ε): 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) ν (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) λ_{max} (nm) (log ε): 368.8 (4.15), 255.1 (4.13), 209.1 (4.10)

FT-IR (neat) ν (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 ° (c = 0.03 in MeOH)

UV (MeOH) λ_{max} (nm) (log ε): 356.8 (4.29), 266.2 (4.32), 256.5 (4.36)

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

EIMS *m/z* (% relative intensity): ($[M\text{-gal}]^+$ 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) λ_{max} (nm) (log ε): 335.0 (3.76), 287.7 (4.47), 224.1 (4.55)

FT-IR (neat) ν (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_2O-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_3OD): see **Table 75**

^{13}C NMR (100 MHz) (CD_3OD): see **Table 75**

FD8

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

UV (MeOH) λ_{max} (nm) (log ε): 346.3 (5.23), 290.2 (5.34), 274.8 (5.31), 255.4 (5.17), 216.7 (5.50)

FT-IR (neat) ν (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_3OD): see **Table 76**

^{13}C NMR (100 MHz) (CD_3OD): 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_2Cl_2, CH_2Cl_2-Me_2CO, Me_2CO and Me_2CO-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_2Cl_2 and CH_2Cl_2-Me_2CO 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) ν (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)

^{1}H NMR (300 MHz) ($CDCl_3$): see **Table 77**

^{13}C NMR (125 MHz) ($CDCl_3$): see **Table 77**

Isolation of GD7

Fraction H2 was further purified by CC and eluted with CH_2Cl_2, CH_2Cl_2-Me_2CO and Me_2CO to give a pale yellow solid **GD7** (21.5 mg).

Isolation of FD10

Fraction H3 was further purified by CC and eluted with CH_2Cl_2, CH_2Cl_2-Me_2CO and Me_2CO to give a pale yellow solid **FD10** (38.2 mg).

Melting point: 215-216 °C (lit. 216-218 °C)

^{1}H NMR (300 MHz) (DMSO-d_6): see **Table 78**

^{13}C NMR (125 MHz) (DMSO-d_6): see **Table 78**

Isolation of FD11

Fraction H4 was further purified by CC and eluted with CH_2Cl_2, CH_2Cl_2-Me_2CO and Me_2CO to give **FD11** as a yellow solid (2.37 g).

Melting point: 139-140 °C (lit. 135 °C)

$[\alpha]_D^{29}$: + 135.5 ° (c = 0.03 in MeOH)

^{1}H NMR (500 MHz) ($CDCl_3$): 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_2Cl_2, CH_2Cl_2-Me_2CO, Me_2CO and Me_2CO-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_2Cl_2 and CH_2Cl_2-Me_2CO 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_2Cl_2 and CH_2Cl_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_2Cl_2, CH_2Cl_2-Me_2CO, Me_2CO and Me_2CO-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_2Cl_2 and CH_2Cl_2-Me_2CO to give five fractions (J1.1-J1.5). Fraction J1.3 was rechromatographed on CC and eluted with hexane-CH_2Cl_2 (7:3) to give **FD12** (10.2 mg) and **FD13** (5.5 mg) as yellow solids.

FD12

Melting point: 128-130 °C

$[\alpha]_D^{29}$: + 96.0 °, c = 0.03 in MeOH (lit. +101 °, 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 CH_2Cl_2 and CH_2Cl_2-MeOH to give four fractions. The third fraction was further purified by CC and eluted with CH_2Cl_2-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 CH_2Cl_2-MeOH to give a yellow solid **FD14** (14.3 mg).

Melting point: 185-187 °C

UV (MeOH) λ_{max} (nm) (log ε): 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 $C_{28}H_{32}O_6$, 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) ($CDCl_3$ + CD_3OD): see **Table 82**

^{13}C NMR (125 MHz) ($CDCl_3$ + CD_3OD): see **Table 82**

Isolation of GD8 and GD14

Fraction J4 was rechromatographed on CC and eluted with CH_2Cl_2. Two major components, **GD8** and **GD14**, were obtained and recrystallized from hexane-CH_2Cl_2. 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_2Cl_2 and CH_2Cl_2-MeOH to give three fractions. The second fraction was rechromatographed on a silica gel column using CH_2Cl_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_2Cl_2 and CH_2Cl_2-MeOH. **FD15** and **RD6** were obtained and recrystallized from CH_2Cl_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_2Cl_2, CH_2Cl_2-Me_2CO, Me_2CO, Me_2CO-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_2Cl_2 (1:1). A yellow solid **FD15** was obtained (5.6 mg).

Melting point: 154-156 °C

UV (MeOH) λ_{max} (nm) (log ε): 358.1 (3.77), 317.9 (4.25), 256.2 (4.28), 241.7 (4.35), 211.7 (4.08)

FT-IR (neat) ν (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_2Cl_2-Me_2CO (1:1). A yellow solid **FD16** was obtained (23.8 mg).

Melting point: 213-215 °C

UV (MeOH) λ_{max} (nm) (log ε): 331.2 (4.44), 322.6 (4.43), 265.8 (4.53), 244.8 (4.54), 208.8 (4.38)

FT-IR (neat) ν (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_2Cl_2-Me_2CO (1:1). A yellow solid **FD17** was obtained (5.2 mg).

Melting point: 227-229 °C

UV (MeOH) λ_{max} (nm) (log ε): 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) ν (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_2Cl_2, CH_2Cl_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_2Cl_2 and CH_2Cl_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_2Cl_2-MeOH to give a yellow solid. The solid was recrystallized from CH_2Cl_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_2Cl_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_2Cl_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_2Cl_2-MeOH to give a yellow solid **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 **FD19** (35.3 mg).

Melting point: 281-283 °C

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

HRLCMS *m/z* 379.0428 $[M+K]^+$ (calcd for $C_{15}H_{16}O_9K$, 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 **Crude M** (37.11 g) was further fractionated by CC over silica gel. Gradient elution was conducted with CH_2Cl_2 and CH_2Cl_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 **GD20** and **FD1** (**Figure 21**).

Isolation of FD1

Fraction M4 was rechromatographed on CC and gradiently eluted with the CH_2Cl_2 and CH_2Cl_2-MeOH to give nine fractions (M4.1-M4.9). Fraction M4.6 was further purified by dissolving in MeOH and a few drops of CH_2Cl_2 was added to form a yellow solid **FD1** (5.6 mg). The filtrate was concentrated and chromatographed on CC and eluted with the CH_2Cl_2-MeOH (8:2) to give **FD1** (9.4 mg).

Isolation of GD20

Fraction M5 was dissolved in CH_2Cl_2. The solid formed was collected and further crystallized from CH_2Cl_2-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-CH_2Cl_2, CH_2Cl_2 and CH_2Cl_2-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 CH_2Cl_2 and CH_2Cl_2-MeOH to give four fractions. The second and the third fractions were further recrystallized from CH_2Cl_2-Me_2CO (8:2). A colourless solid **SD1** was collected (36.2 mg).

Fraction N5 was purified by CC and eluted with CH_2Cl_2 and CH_2Cl_2-MeOH to give five fractions. The second, the third and the forth fractions were recrystallized from CH_2Cl_2-Me_2CO (8:2). A colourless solid **SD1** (20.7 mg) was collected.

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

$[\alpha]_D^{29}$: - 10.6 °, c = 0.018 in MeOH (lit. –16.7 °, c = 0.70 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 CH_2Cl_2-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_2Cl_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_3OH) λ_{max} (nm) (log ε): 349.5 (4.58), 235.1 (4.46)

FT-IR (neat) ν (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_2Cl_2, CH_2Cl_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_2Cl_2 (1:1) and CH_2Cl_2 to give three fractions. The second fraction was purified by CC and eluted with hexane-CH_2Cl_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 CH_2Cl_2, CH_2Cl_2-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, CH_2Cl_2 and CH_2Cl_2-Me_2CO as the eluents to give eleven fractions (P1.1-P1.11). Fraction P1.2 (0.421 g) was rechromatographed, eluted with hexane-CH_2Cl_2 (1:1) and CH_2Cl_2 to give three fractions. The second fraction was further purified by CC using hexane-CH_2Cl_2 (1:1) to give a colourless solid **SD4** (142.2 mg).

Melting point: 89-90 °C

^{1}H NMR (300 MHz) ($CDCl_3$): see **Table 91**

^{13}C NMR (125 MHz) ($CDCl_3$): see **Table 91**

Fraction P1.6 (0.422 g) was fractionated by CC using hexane-CH_2Cl_2 (1:1), CH_2Cl_2 and CH_2Cl_2-Me_2CO as the eluents. A yellow solid **SD5** (2.4 mg) was obtained.

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

^{1}H NMR (500 MHz) ($CDCl_3$): see **Table 92**

Fraction P1.9 (0.37 g) was rechromatographed on CC and eluted with CH_2Cl_2 and CH_2Cl_2-Me_2CO to give four fractions (P1.9A-P1.9D). Fraction P1.9B was further

rechromatographed and eluted with CH_2Cl_2 and 2% Me_2CO in CH_2Cl_2. A yellow solid **RD12** (2.0 mg) was collected.

Isolation of GD20 and SD6

Fraction P2 (7.422 g) was further chromatographed on CC and eluted with CH_2Cl_2 and CH_2Cl_2-Me_2CO to give seven fractions. The third fraction was recrystallized from CH_2Cl_2-Me_2CO to give a colourless solid **SD6** (0.35 g). The fifth fraction was recrystallized from CH_2Cl_2-MeOH. A yellow solid **GD20** (9.40 g) was collected.

SD6

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

UV (MeOH) λ_{max} (nm) (log ε): 276.5 (3.63), 220.3 (3.91)

FT-IR (neat) ν (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 CH_2Cl_2-MeOH. The yellow solid **GD20** was obtained.

Isolation of SD7

Fraction P5 (3.751 g), containing a major component, was dissolved in CH_2Cl_2 and filtered. The solid was further crystallized from CH_2Cl_2-MeOH to give a yellow solid **SD7** (23.6 mg).

Melting point: 188-190 °C

UV (MeOH) λ_{max} (nm) (log ε): 318.4 (4.18), 244.9 (4.31), 213.2 (3.68)

FT-IR (neat) ν (cm^{-1}): 3397 (O-H stretching), 1612 (C=O stretching)

HRFABMS *m/z* 444.1788 $[M]^+$ (calcd for $C_{24}H_{28}O_8$, 444.1784)

FABMS *m/z* (% relative intensity): ($[M]^+$ 444, 2), 428 (100), 412 (15), 384 (20), 372 (17), 356 (15), 299 (13), 285 (7), 93 (6), 61 (7), 59 (10)

^{1}H NMR (300 MHz) ($CDCl_3$ + CD_3OD): see **Table 94**

^{13}C NMR (125 MHz) ($CDCl_3$ + CD_3OD): see **Table 94**

Isolation of SD8 and SD9

Fraction P6 (4.773 g) was chromatographed on CC and eluted with CH_2Cl_2 and CH_2Cl_2-MeOH to give ten fractions (P6.1-P6.10). Fraction P6.5 was chromatographed on CC and eluted with CH_2Cl_2 and CH_2Cl_2-MeOH to give a colourless solid **SD8** (59.7 mg).

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

UV (MeOH) λ_{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)

^{1}H NMR (500 MHz) (Acetone-d_6): see **Table 95**

^{13}C NMR (125 MHz) (Acetone-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

^{1}H NMR (300 MHz) (DMSO-d_6): see **Table 96**

^{13}C NMR (125 MHz) (DMSO-d_6): see **Table 96**

Isolation of SD10, SD11 and SD12

Fraction P7 (3.002 g) was chromatographed on CC and eluted with CH_2Cl_2 and CH_2Cl_2-MeOH to give nine fractions (P7.1-P7.9). Fraction P7.2 was chromatographed on CC and eluted with CH_2Cl_2 and CH_2Cl_2-MeOH. The third and fifth fractions were rechromatographed on CC and eluted with CH_2Cl_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 °, c = 0.018 in MeOH (lit. +59.5 °, c = 0.12 in MeOH)

UV (MeOH) λ_{max} (nm) (log ε): 282.6 (3.98), 223.4 (4.48)

FT-IR (neat) ν (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 °, c = 0.023 in MeOH (lit. –23 °, c = 0.40 in MeOH)

FT-IR (neat) ν (cm^{-1}): 3306 (O-H stretching), 1616, 1598 (C=C 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_2Cl_2 and CH_2Cl_2-MeOH to give five fractions. The third fraction was further purified by CC and eluted with CH_2Cl_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) ν (cm^{-1}): 3269 (O-H stretching), 1657, 1602 (C=O stretching)

^{1}H NMR (500 MHz) (Acetone-d_6): see **Table 99**

^{13}C NMR (125 MHz) (Acetone-d_6): see **Table 99**

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).

$$\% \text{ Inhibition} = \frac{(A_{control} - A_{sample})}{A_{control}} \times 100$$

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)

Sample	Average absorbances (517 nm)			% Inhibition ± S.D.
	0 min	30 min	60 min	30 min
Control	0.61	0.61	0.61	-
BHT	0.595	0.245	0.10	59.84 ± 0.02
green fruits				
DPPH + **Crude 1**	0.56	0.035	0.025	94.26 ± 0.05
DPPH + **Crude C**	0.60	0.46	0.385	24.59 ± 0.06
ripe fruits				
DPPH + **Crude 2**	0.505	0.075	0.055	87.70 ± 0.03
flowers				
DPPH + **Crude G1**	0.565	0.138	0.075	77.46 ± 0.06
DPPH + **Crude M**	0.545	0.085	0.07	86.07 ± 0.03
seeds				
DPPH + **Crude 3**	0.58	0.48	0.435	21.31 ± 0.08
DPPH + **Crude 4**	0.59	0.395	0.335	35.25 ± 0.06

Control = (0.05 mM DPPH)

Crude 1, **2**, **G1** = crude Me_2CO extract

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

Crude 3 = crude CH_2Cl_2 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)

Sample	Average absorbances (517 nm)			% Inhibition ± S.D.
	0 min	30 min	60 min	30 min
Control	0.61	0.61	0.61	-
Ascorbic acid	0.32	0.14	0.13	77.05 ± 0.01
BHT	0.45	0.35	0.35	42.62 ± 0.02
GD1	0.49	0.16	0.16	73.77 ± 0.03
GD3	0.51	0.50	0.50	18.03 ± 0.03
GD4	0.52	0.50	0.50	18.03 ± 0.01
GD5	0.53	0.52	0.52	14.75 ± 0.01
GD6	0.525	0.52	0.52	14.75 ± 0.02
GD7	0.52	0.51	0.50	16.39 ± 0.01
GD8	0.53	0.52	0.52	14.75 ± 0.03
GD9	0.53	0.475	0.47	22.13 ± 0.01
GD10	0.54	0.46	0.46	24.59 ± 0.02
GD11	0.51	0.49	0.46	19.67 ± 0.02
GD12	0.52	0.49	0.48	19.67 ± 0.01
GD13	0.53	0.52	0.52	14.75 ± 0.01
GD14	0.53	0.50	0.50	18.03 ± 0.01
GD15	0.525	0.515	0.51	15.57 ± 0.03
GD16	0.53	0.52	0.52	14.75 ± 0.02

Table 22 (Continued)

Sample	Average absorbances (517 nm)			% Inhibition ± S.D.
	0 min	30 min	60 min	30 min
GD17	0.53	0.53	0.52	13.11 ± 0.03
GD19	0.525	0.505	0.50	17.21 ± 0.02
GD20	0.49	0.30	0.29	50.81 ± 0.03
GD21	0.61	0.60	0.60	1.64 ± 0.02
GD22	0.58	0.54	0.52	11.48 ± 0.04
GD23	0.58	0.56	0.55	8.20 ± 0.03
GD25	0.60	0.59	0.59	3.28 ± 0.05
RD1	0.60	0.60	0.59	1.64 ± 0.02
RD3	0.58	0.55	0.54	9.84 ± 0.01
RD5	0.44	0.08	0.06	86.89 ± 0.03
RD6	0.53	0.52	0.52	14.75 ± 0.05
RD7	0.58	0.52	0.52	14.75 ± 0.04
RD8	0.54	0.50	0.50	18.03 ± 0.02
RD9	0.61	0.60	0.60	1.64 ± 0.02
RD10	0.61	0.56	0.54	8.20 ± 0.03
RD11	0.61	0.60	0.60	1.64 ± 0.02
RD12	0.56	0.54	0.52	11.48 ± 0.01
RD13	0.53	0.50	0.48	18.03 ± 0.02
RD14	0.52	0.19	0.18	68.85 ± 0.04
RD15	0.55	0.54	0.54	11.48 ± 0.02
RD16	0.43	0.11	0.10	81.97 ± 0.06
RD17	0.52	0.50	0.49	18.03 ± 0.02
FD1	0.58	0.58	0.57	4.92 ± 0.01
FD3	0.61	0.60	0.60	1.64 ± 0.01

Table 22 (Continued)

Sample	Average absorbances (517 nm)			% Inhibition ± S.D.
	0 min	30 min	60 min	30 min
FD4	0.61	0.56	0.55	8.20 ± 0.02
FD5	0.45	0.26	0.23	57.38 ± 0.05
FD6	0.50	0.39	0.35	36.07 ± 0.05
FD7	0.50	0.39	0.35	36.07 ± 0.04
FD8	0.47	0.27	0.26	55.74 ± 0.03
FD9	0.52	0.51	0.50	16.39 ± 0.01
FD10	0.51	0.41	0.37	32.79 ± 0.01
FD11	0.56	0.24	0.19	60.66 ± 0.05
FD12	0.44	0.25	0.23	59.02 ± 0.04
FD13	0.58	0.56	0.56	8.20 ± 0.01
FD14	0.52	0.52	0.51	14.75 ± 0.03
FD15	0.60	0.59	0.58	3.28 ± 0.01
FD16	0.61	0.60	0.60	1.64 ± 0.01
FD17	0.60	0.59	0.59	3.28± 0.01
FD18	0.60	0.60	0.59	1.64 ± 0.01
FD19	0.60	0.60	0.60	1.64 ± 0.01
SD1	0.57	0.56	0.56	8.20 ± 0.03
SD2	0.60	0.59	0.58	3.28 ± 0.01
SD3	0.60	0.60	0.60	1.64 ± 0.01
SD4	0.60	0.60	0.60	1.64 ± 0.01
SD5	0.58	0.57	0.57	6.56 ± 0.01
SD6	0.60	0.60	0.59	1.64 ± 0.01
SD7	0.59	0.57	0.57	6.56 ± 0.02
SD8	0.60	0.60	0.60	1.64 ± 0.01
SD9	0.60	0.60	0.60	1.64 ± 0.01

Table 22 (Continued)

Sample	Average absorbances (517 nm)			% Inhibition ± S.D.
	0 min	30 min	60 min	30 min
SD10	0.56	0.55	0.55	9.83 ± 0.03
SD11	0.58	0.58	0.57	4.92 ± 0.01
SD12	0.60	0.59	0.59	3.28 ± 0.01

Control = 0.05 mM DPPH

2.7.3 Evaluation of 50% inhibitory concentration (IC_{50})

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 (IC_{50}) 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

Sample		Final concentration (μM)									
		1.0	2.0	4.0	8.0	10.0	12.0	14.0	16.0	18.0	20.0
Control	A	0.61	0.61	0.61	0.61	0.60	0.61	0.61	0.61	0.61	0.61
Ascorbic acid	A	0.56	0.52	0.42	0.24	0.17	0.14	0.13	0.13	0.13	0.13
	% I	8.20	14.75	31.15	60.66	72.13	77.05	78.69	78.69	78.69	78.69
BHT	A	0.58	0.55	0.49	0.40	0.37	0.35	0.34	0.32	0.30	0.29
	% I	4.92	9.84	19.67	34.43	39.34	42.62	44.26	47.54	50.82	52.46
GD1	A	0.58	0.55	0.43	0.25	0.20	0.19	0.18	0.18	0.17	0.16
	% I	4.92	9.84	29.51	59.02	67.21	68.85	70.49	70.49	72.13	73.77
GD20	A	0.57	0.54	0.48	0.36	0.34	0.31	0.29	0.27	0.26	0.25
	% I	6.56	11.48	21.31	40.98	44.26	49.18	52.46	55.74	57.38	59.02
RD5	A	0.54	0.49	0.39	0.20	0.13	0.10	0.08	0.08	0.06	0.05
	% I	11.48	19.67	36.07	67.21	78.69	83.61	86.89	86.89	90.16	91.80
RD14	A	0.57	0.53	0.44	0.27	0.22	0.21	0.20	0.20	0.18	0.17
	% I	6.56	13.11	27.87	55.74	63.93	65.57	67.21	67.21	70.49	72.13
RD16	A	0.54	0.51	0.40	0.22	0.15	0.12	0.10	0.10	0.09	0.09
	% I	11.48	16.39	34.43	63.93	75.41	80.33	83.61	83.61	85.25	85.25
FD5	A	0.56	0.55	0.46	0.34	0.32	0.29	0.28	0.27	0.26	0.24
	% I	8.20	9.84	24.59	44.26	47.54	52.46	54.10	55.74	57.38	60.56
FD8	A	0.58	0.55	0.50	0.38	0.32	0.29	0.29	0.29	0.28	0.27
	% I	4.92	9.84	18.03	37.70	47.54	52.46	52.46	52.46	54.10	55.74
FD11	A	0.57	0.53	0.45	0.31	0.28	0.27	0.25	0.25	0.23	0.23
	% I	6.56	13.11	26.23	49.18	54.10	55.74	59.02	59.02	62.30	62.30
FD12	A	0.59	0.58	0.47	0.36	0.30	0.28	0.27	0.26	0.25	0.24
	% I	3.28	4.92	22.95	40.98	50.82	54.10	55.74	57.38	59.02	60.56

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