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# **Enhancing the potential exploitation of food waste: Extraction, purification, and characterization of renewable specialty chemicals from blackcurrants (*Ribes nigrum* L.)**

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**Supplementary information.**

## MATERIALS AND METHODS

### Materials

Blackcurrant waste was obtained from GlaxoSmithKline (GSK) and more recently from A. R. House Ltd. UK, after the raw fruit grown in the UK was pressed in production of blackcurrant cordial (*Ribena*). The crude waste is referred to as pomace, which comprises the fruit skins (*ca.* 50 wt. %), seeds (*ca.* 45 wt. %) and extraneous matter (*e.g.* berry stalks, *ca.* 5 wt. %). GSK separated the seeds from this pomace (the seeds are a valuable source of  $\gamma$ -linolenic acid) and removed unwanted stalks; the material received was dried blackcurrant skins. Amberlite XAD-7HP was obtained from Rohm & Haas, Staines, UK. General purpose chemicals were obtained from Sigma-Aldrich. Delphinidin-3-*O*-glucoside (dp3glc) was purchased from Polyphenol AS, Sandnes, Norway.

### Extraction and semi-purification of the polyphenols

Dried blackcurrant fruit epicarp (dried skins, 30 g) were immersed in 600 mL water acidified with 0.01% *v/v* conc. HCl and stirred gently by magnetic follower at room temperature for 2 hours. The plant material was filtered off and the resulting aqueous extract (filtrate) was loaded onto an Amberlite XAD-7HP resin (60 g) until the eluent was almost colourless. Resin then washed with acidified water (0.01% *v/v* conc. HCl, 1L) before eluting the polyphenols with acidified ethanol (0.01% *v/v* conc. HCl). The collected ethanol fractions were combined and concentrated under vacuum on a rotary evaporator, and then subjected to high vacuum to remove trace solvent, yielding a dark violet amorphous solid (660 mg, yield 2.2%), which could be powdered by grinding.

<sup>1</sup>H NMR (**S2**) and HPLC (**Figure 1**) analyses confirmed the presence of four anthocyanins and other polyphenols in the extract. The dried blackcurrant extract (500 mg) was then dissolved in acidified water (50 mL, 0.1% *v/v* conc. HCl) and partitioned against isopropylacetate (1 × 70 mL) and ethylacetate (3 × 50 mL) in sequential manner. The organic layers were dried under reduced pressure to give isopropylacetate (yellow amorphous solid, 68.5 mg) and ethyl acetate extracts (yellow amorphous solid, 33 mg), whereas aqueous layer was freeze-dried to afford 398.5 mg (red amorphous solid) extract.

### HPLC

The extracts were analyzed with HPLC at every stage of the extraction and purification. The analytical HPLC system (Agilent 1290 infinity series) was equipped with diode-array detector (DAD), binary pump system connected with online degasser and Zorbax Eclipse XDB C18, 150 x 4.6 mm, 5  $\mu$ m. The flow rate was 1ml/min and the injection volume was 10  $\mu$ l. The chromatograms were recorded by scanning the absorption at 190-600 nm. The anthocyanins were monitored at 520 nm, flavonoids at 350 and hydroxycinnamates at 325 nm. For aqueous extract (anthocyanin analysis), the binary solvent system consisted of solvent A: water (0.5% TFA) and solvent B: acetonitrile (0.5%

TFA). The elution profile consisted of linear gradient from 5% B to 20% B in the first 20 min, then linear increase to 100% B at 20-23 min followed by isocratic elution (100% B) at 23-24 minutes, and then linear decrease to 5% B at 24-25 min followed by 5% B isocratic elution at 25-30 minutes. For ethylacetate and isopropylacetate extracts: the binary solvent system consisted of solvent A: water (0.1% TFA) and solvent B: acetonitrile (0.1% TFA). The elution profile consisted of linear gradient from 5% B to 20% B in the first 30 min, then linear increase to 100% B at 30-33 min followed by isocratic elution (100% B) at 33-34 minutes, and then linear decrease to 5% B at 34-35 min followed by 5% B isocratic elution at 35-40 minutes.

### **Preparative HPLC.**

The aqueous extract after liquid-liquid partitioning experiments was dried and 20 mg was re-dissolved in H<sub>2</sub>O/EtOH (9:1, 2 ml, acidified with 0.1% v/v HCl). It was then purified on semi-preparative HPLC to give anthocyanins **2-5**. The HPLC system (Agilent 1200 infinity series) was equipped with diode-array detector (DAD), binary pump system connected with online degasser. For anthocyanins: the extract was loaded on to a XBridge<sup>TM</sup> Prep C18, 10 × 50, 5 μm in 300 μl injections and eluted using gradient solvent system. The binary solvent system consisted of solvent A: water (0.5% TFA) and solvent B: acetonitrile (0.5% TFA). The elution profile consisted of linear gradient from 5% B to 20% B in the first 30 min, then linear increase to 100% B at 30-33 min followed by isocratic elution (100% B) at 33-34 minutes, and then linear decrease to 5% B at 34-35 min followed by 5% B isocratic elution at 35-40 minutes. The flow rate was 5 ml/min and five peaks were collected at 520 nm to give Dp3rut **4** (4.5 mg), Cy3rut **5** (4.1 mg), Dp3glu **2** (1.6 mg) and Cy3glu **3** (0.8mg) and polymeric anthocyanins (4.5 mg).

For flavonoids and hydroxycinnamates, the isopropylacetate extract (15 mg) and ethylacetate extract (10 mg) were both dissolved in methanol (2 ml) and purified on a semi-preparative column. The peaks were monitored at 325 for isopropyl acetate and 350 for ethylacetate extracts. The extracts were loaded on to XBridge<sup>TM</sup> Prep C18, 10 × 50, 5 μm in 300 μl injections and eluted at the flow rate of 5 ml/min using binary solvent system. The binary solvent system consisted of solvent A: water (0.1 % formic acid) and solvent B: acetonitrile (0.1% formic acid). The elution profile consisted of linear gradient from 5% B to 20% B in the first 30 min, then linear increase to 100% B at 30-33 min followed by isocratic elution (100% B) at 33-34 minutes, and then linear decrease to 5% B at 34-35 min followed by 5% B isocratic elution at 35-40 minutes. Caffeic acid **10** (3.3 mg) , p-coumaric acid **11** (5.5 mg), myricetin **12** (2.7 mg) and quercetin **13** (3.5 mg) were purified from the isopropylacetate extract whereas glucosides of myricetin **6** (4.7 mg) and quercetin **7** (3.0 mg) alongside nigrumin-p-coumarate **13** (1.5 mg) and nigrumin ferulate **14** (0.7 mg) were isolated from the ethylacetate extract (10 mg). Myricetin-3-β-rutinoside **8** (0.8 mg) and quercetin-3-β-rutinoside **9** (0.8 mg) were isolated from the aqueous extract (20 mg, monitored at 350 nm) using this method as well. The isolated

compounds were characterised using NMR, IR, UV/Vis spectroscopy and accurate mass spectrometry (vide infra).

### **HPLC quantification method (q-HPLC)**

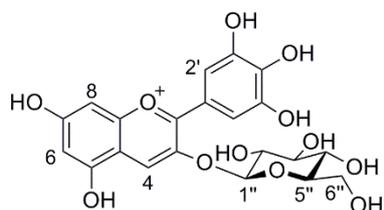
The anthocyanins in the post-SPE blackcurrant extract were quantified using calibration graphs for delphinidin-3-O-glucoside (dp3glc) from samples purified in this work and obtained commercially, using Agilent Chem Software. Delphinidin-3-O-glucoside (Dp3glc) was purchased from Polyphenol AS. The isolated as well as commercial samples of Dp3glc were dissolved in buffer pH 1.0 to give 1 mg/mL stock solutions and then several dilutions were prepared. UV/Vis absorption spectra were recorded on-line during HPLC analysis using a photodiode array detector and the external calibration graphs were obtained. Using these calibration graphs and Agilent Chem Software the absolute amount of delphinidin-3-O-glucoside and the relative amounts of rest of the anthocyanins were calculated. The relative ratios of the anthocyanins given by HPLC chromatograms and  $^1\text{H}$  NMR were in good agreement, therefore the difference in molar absorption coefficients was not taken into account. The amounts of neutral polyphenols is based on their isolated yield. The amounts of individual polyphenols were reflected in the relative peak area of each compound in  $^1\text{H}$  NMR of the post-SPE extract (S2).

### **NMR and Other methods**

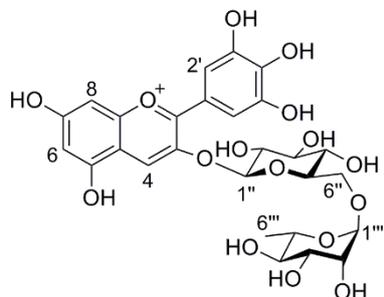
High resolution mass spectra (HRMS) were recorded on a Dionex Ultimate 3000 spectrometer using electrospray ionization (ESI). All masses quoted are correct to four decimal places. Agilent Carry Series UV/Vis spectrophotometer was used for uv/vis measurements. Infrared (IR) spectra were recorded using a Perkin Elmer Spectrum One FT-IR spectrophotometer or Bruker Alpha Platinum AR FTIR. Vibrational frequencies are reported in wavenumbers ( $\text{cm}^{-1}$ ).

The NMR experiments were done at 500 and 125 MHz for  $^1\text{H}$  and  $^{13}\text{C}$  respectively on Bruker DRX 500 spectrometer equipped with a multinuclear inverse probe for one-dimensional  $^1\text{H}$  and two-dimensional heteronuclear single quantum coherence ( $^1\text{H}$ - $^{13}\text{C}$  HSQC), heteronuclear multiple bond correlation ( $^1\text{H}$ - $^{13}\text{C}$  HMBC), and double quantum filtered correlation ( $^1\text{H}$ - $^1\text{H}$  COSY). The samples were either dissolved in  $\text{CD}_3\text{OD}$  or  $\text{CD}_3\text{OD}$ - $\text{CF}_3\text{COOD}$  (95:5) depending on nature of the compound. Chemical shifts ( $\delta$ ) are quoted in ppm downfield of tetramethylsilane or residual solvent peaks (3.31 and 49.0 ppm for  $\text{CD}_3\text{OD}$  in  $^1\text{H}$  and  $^{13}\text{C}$  respectively; 110 and 160 ppm for  $\text{CF}_3\text{COOD}$ ). The coupling constants (J) are quoted in Hz.

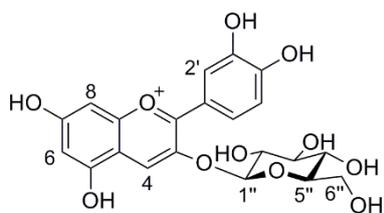
## Compound characterisation



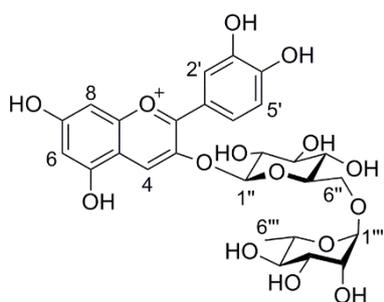
**Delphinidin-3-O-glucopyranoside 2 (Dp3gle):** It was isolated from the aqueous layer (purple solid, 1.6 mg, 7.7%). UV (buffer pH 1.0):  $\lambda_{\max}$  nm (log  $\epsilon$ ) 515 (3.85).  $^1\text{H}$  NMR (500 MHz,  $\text{CD}_3\text{OD}/\text{CF}_3\text{COOD}$  95:5):  $\delta$  8.98 (1H, s, H-4), 7.79 (2H, s, H-2', 6'), 6.88 (1H, d,  $J = 1.5$  Hz, H-8) and 6.66 (1H, d,  $J = 1.5$  Hz, H-6), 5.32 (1H, d,  $J = 7.5$  Hz, H-1''), 3.93 (1H, dd,  $J = 12.3, 2.1$  Hz, H-6a''), 3.73 (1H, dd,  $J = 12.3, 6.0$  Hz, H-6b''), 3.72 (1H, dd,  $J = 9.0, 7.5$  Hz, H-2''), 3.56 (1H, t,  $J = 9.1$  Hz, H-3''), 3.54 (1H, ddd,  $J = 9.0, 6.0, 2.1$  Hz, H-5'') and 3.47 (1H, dd,  $J = 9.0, 9.1$  Hz, H-4'').  $^{13}\text{C}$  NMR (125 MHz,  $\text{CD}_3\text{OD}/\text{CF}_3\text{COOD}$  95:5):  $\delta$  173.5 (C-7), 160.2 (C-2), 159.1 (C-5), 158.6 (C-9), 148.8 (C-4'), 147.6 (C-3', 5'), 145.9 (C-3), 136.3 (C-4), 117.6 (C-1'), 115.8 (C-10), 112.6 (C-2', 6'), 103.7 (C-1''), 103.3 (C-6), 95.0 (C-8), 78.8 (C-5''), 78.1 (C-3''), 74.8 (C-2''), 71.1 (C-4'') and 62.3 (C-6''). HRMS ( $\text{ESI}^+$ ) calculated for formula  $\text{C}_{21}\text{H}_{22}\text{O}_{12}$  [ $\text{MH}^+$ ] 466.1027, found 466.1037. IR (neat):  $\nu_{\max}$  3550-2440, 1680, 1540, 1505, 1310  $\text{cm}^{-1}$ .



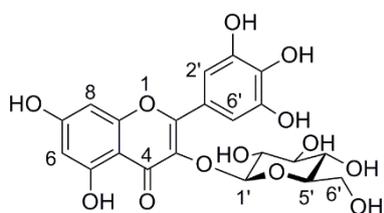
**Delphinidin-3-O-rutinoside 4 (Dp3rut):** It was isolated as purple solid from the aqueous layer (4.5 mg, 22.6%). UV (buffer pH 1.0):  $\lambda_{\max}$  nm (log  $\epsilon$ ) 520 (3.89).  $^1\text{H}$  NMR (500 MHz,  $\text{CD}_3\text{OD}/\text{CF}_3\text{COOD}$  95:5):  $\delta$  8.90 (1H, s, H-4), 7.78 (2H, s, H-2', 6'), 6.88 (1H, d,  $J = 2.0$  Hz, H-8) and 6.68 (1H, d,  $J = 2.0$  Hz, H-6), 5.30 (1H, d,  $J = 7.5$  Hz, H-1''), 4.65 (1H, d,  $J = 1.5$  Hz, H-1'''), 4.06 (1H, dd,  $J = 11.3, 1.8$  Hz, H-6a''), 3.80 (1H, dd,  $J = 3.5, 1.5$  Hz, H-2'''), 3.73 (1H, ddd,  $J = 9.0, 7.2, 1.8$  Hz, H-5''), 3.71 (1H, dd,  $J = 9.0, 7.5$  Hz, H-2''), 3.63 (1H, dd,  $J = 9.5, 3.5$  Hz, H-3'''), 3.59 (1H, dd,  $J = 11.3, 7.2$  Hz, H-6b''), 3.57 (1H, m, H-5'''), 3.55 (1H, t,  $J = 9.0$  Hz, H-3''), 3.43 (1H, t,  $J = 9.0$  Hz, H-4''), 3.33 (1H, t,  $J = 9.0$  Hz, H-4''') and 1.15 (3H, d,  $J = 6.0$  Hz, H-6''').  $^{13}\text{C}$  NMR (125 MHz,  $\text{CD}_3\text{OD}/\text{CF}_3\text{COOD}$  95:5):  $\delta$  170.3 (C-7), 164.1 (C-2), 159.0 (C-5), 157.6 (C-9), 147.6 (C-3', 5'), 145.8 (C-4'), 144.9 (C-3), 135.4 (C-4), 120.0 (C-1'), 113.1 (C-10), 112.7 (C-2', 6'), 103.4 (C-6), 103.3 (C-1''), 102.2 (C-1'''), 95.1 (C-8), 78.0 (C-5''), 77.5 (C-3''), 74.7 (C-2''), 73.9 (C-4'''), 72.5 (C-3'''), 71.9 (C-2'''), 71.2 (C-4''), 69.8 (C-5'''), 67.8 (C-6'') and 17.9 (C-6'''). HRMS ( $\text{ESI}^+$ ) calculated for formula  $\text{C}_{27}\text{H}_{32}\text{O}_{16}$  612.1681, found 612.1681. IR (neat):  $\nu_{\max}$  3390, 2975, 1650, 1510, 1490  $\text{cm}^{-1}$ .



**Cyanidin-3-O-glucopyranoside 3 (Cy3gle):** It was isolated from the aqueous layer (purple solid, 0.8 mg, 4%). UV (buffer pH 1.0):  $\lambda_{\max}$  nm (log  $\epsilon$ ) 510 (3.79).  $^1\text{H NMR}$  (500 MHz,  $\text{CD}_3\text{OD}/\text{CF}_3\text{COOD}$  95:5):  $\delta$  9.04 (1H, s, H-4), 8.27 (1H, dd,  $J = 8.5, 2.5$  Hz, H-6'), 8.06 (1H, d,  $J = 2.5$  Hz, H-2'), 7.02 (1H, d,  $J = 8.5$  Hz, H-5'), 6.91 (1H, d,  $J = 2.0$  Hz, H-8) and 6.69 (1H, d,  $J = 2.0$  Hz, H-6), 5.31 (1H, d,  $J = 8.0$  Hz, H-1''), 3.91 (1H, dd,  $J = 12.0, 2.0$  Hz, H-6a''), 3.72 (1H, dd,  $J = 12.0, 5.9$  Hz, H-6b''), 3.71 (1H, dd,  $J = 9.0, 7.0$  Hz, H-2''), 3.56 (1H, t,  $J = 9.0$  Hz, H-3''), 3.55 (1H, m, H-5''), and 3.44 (1H, t,  $J = 9.1$  Hz, H-4''). HRMS ( $\text{ESI}^+$ ) calculated for formula  $\text{C}_{21}\text{H}_{21}\text{O}_{11}$  449.1027, found 449.1037.

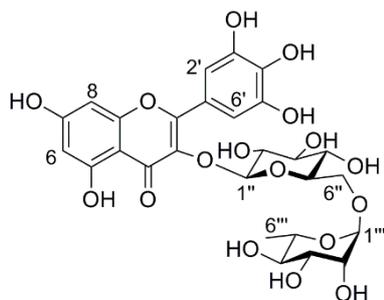


**Cyanidin-3-O-rutinoside 5 (Cy3rut):** It was isolated from the aqueous layer (purple solid, 4.1 mg, 20.4%). UV (buffer pH 1.0):  $\lambda_{\max}$  nm (log  $\epsilon$ ) 510 (3.87).  $^1\text{H NMR}$  (500 MHz,  $\text{CD}_3\text{OD}/\text{CF}_3\text{COOD}$  95:5):  $\delta$  8.95 (1H, s, H-4), 8.27 (1H, dd,  $J = 8.5, 2.5$  Hz, H-6'), 8.05 (1H, d,  $J = 2.5$  Hz, H-2'), 7.04 (1H, d,  $J = 8.5$  Hz, H-5'), 6.91 (1H, d,  $J = 1.5$  Hz, H-8) and 6.69 (1H, d,  $J = 1.5$  Hz, H-6), 5.29 (1H, d,  $J = 7.5$  Hz, H-1''), 4.65 (1H, d,  $J = 1.5$  Hz, H-1'''), 4.06 (1H, dd,  $J = 11.1, 1.5$  Hz, H-6a''), 3.80 (1H, dd,  $J = 3.5, 1.5$  Hz, H-2'''), 3.72 (1H, ddd,  $J = 9.0, 7.0, 1.5$  Hz, H-5''), 3.67 (1H, dd,  $J = 9.0, 7.5$  Hz, H-2''), 3.63 (1H, dd,  $J = 9.3, 3.0$  Hz, H-3'''), 3.59 (1H, dd,  $J = 11.1, 7.0$  Hz, H-6b''), 3.54 (1H, t,  $J = 9.0$  Hz, H-3''), 3.42 (1H, t,  $J = 9.0$  Hz, H-4''), 3.33 (1H, m, H-4''') and 1.13 (3H, d,  $J = 6.0$  Hz, H-6''').  $^{13}\text{C NMR}$  (125 MHz,  $\text{CD}_3\text{OD}/\text{CF}_3\text{COOD}$  95:5):  $\delta$  70.5 (C-7), 164.2 (C-2), 164.1 (C-5), 159.1 (C-9), 155.9 (C-4'), 147.5 (C-3'), 145.7 (C-3), 136.2 (C-4), 128.4 (C-6'), 120.3 (C-1'), 118.4 (C-2'), 117.5 (C-5'), 111.8 (C-10), 103.5 (C-6), 103.4 (C-1''), 102.2 (C-1'''), 95.2 (C-8), 78.0 (C-3''), 77.5 (C-5''), 74.7 (C-2''), 73.9 (C-4''), 72.5 (C-3'''), 71.9 (C-2'''), 71.2 (C-4'''), 69.8 (C-5'''), 67.4 (C-6'') and 17.9 (C-6'''). HRMS ( $\text{ESI}^+$ ) calculated for formula  $\text{C}_{27}\text{H}_{32}\text{O}_{15}$  596.1671, found 595.1711. IR (neat):  $\nu_{\max}$  3370, 2955, 1651, 1510, 1490  $\text{cm}^{-1}$ .

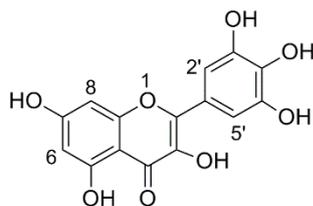


**Myricetin-3-O-glucopyranoside 6 (My3gle):** It was isolated from the ethyl acetate layer (pale yellow solid, 4.7 mg, 3.1%). UV (EtOH):  $\lambda_{\max}$  nm (log  $\epsilon$ ) 365 (4.23).  $^1\text{H NMR}$  (500 MHz,  $\text{CD}_3\text{OD}$ ):  $\delta$  7.31 (2H, s, H-2', 6'), 6.40 (1H, d,  $J = 2.0$  Hz, H-8) and 6.22 (1H, d,  $J = 2.0$  Hz, H-6), 5.23 (1H, d,  $J = 8.0$  Hz, H-1''), 3.73 (1H, dd,  $J = 12.0, 2.3$  Hz, H-6a''), 3.62 (1H, dd,  $J = 12.0, 5.0$  Hz, H-6b''), 3.51 (1H, dd,  $J = 8.9, 8.0$  Hz, H-2''), 3.44 (1H, t,  $J = 8.9$  Hz, H-3''), 3.39 (1H, t,  $J = 9.3$  Hz, H-4''), 3.24 (1H, ddd,  $J = 9.3, 5.0, 2.3$  Hz, H-5'').  $^{13}\text{C NMR}$  (125 MHz,  $\text{CD}_3\text{OD}$ ):  $\delta$  177.1 (C-4), 163.0 (C-7), 161.4 (C-5), 157.8 (C-2), 157.5 (C-

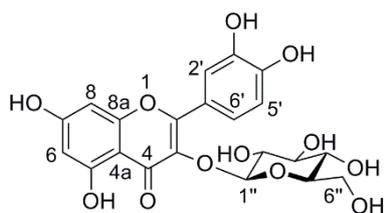
10), 142.8 (C-3' and C-5'), 137.5 (C-4'), 133.8 (C-3), 119.2 (C-1'), 108.6 (C-2' and C-6'), 102.8 (C-10), 103.1 (C-1''), 100.0 (C-6), 93.3 (C-8), 77.1 (C-5''), 76.8 (C-3''), 73.8 (C-2''), 69.7 (C-4''), 61.1 (C-6). HRMS (ESI<sup>+</sup>) calculated for formula C<sub>21</sub>H<sub>21</sub>O<sub>13</sub> [M+H] 481.0962, found 481.0974. IR (neat):  $\nu_{\max}$  3381, 1679, 1610 cm<sup>-1</sup>.



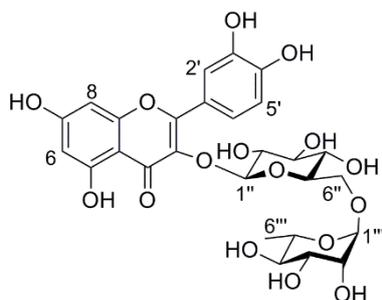
**Myricetin-3-O-rutinoside 8 (My3rut):** It was isolated from the aqueous layer (pale yellow amorphous solid, 0.8 mg, 3.1%). <sup>1</sup>H NMR (500 MHz, CD<sub>3</sub>OD):  $\delta$  7.30 (2H, s, H-2', 6'), 6.41 (1H, d, J = 2.5 Hz, H-8) and 6.22 (1H, d, J = 2.5 Hz), 5.08 (1H, d, J = 8.0 Hz, H-1''), 4.53 (1H, d, J = 1.3 Hz, H1'''), 3.80 (1H, dd, J = 11.5, 1.5 Hz, H6a''), 3.63 (1H, dd, J = 3.5, 1.5 Hz, H-2'''), 3.50 (1H, dd, J = 9.5, 3.5 Hz, H-3'''), 3.45 & 3.43 (under solvent peak), 3.42 (1H, dd, J = 11.5, 5.0 Hz, H-6b''), 3.40 (1H, t, J = 9.0 Hz, H-3) and 1.12 (3H, d, J = 6.0 Hz, H-6'''). HRMS (ESI<sup>+</sup>) calculated for formula C<sub>27</sub>H<sub>31</sub>O<sub>17</sub> [M+H] 627.1526, found 627.1548.



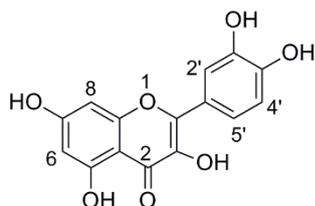
**Myricetin 12 (My):** It was isolated from the isopropyl acetate layer (pale yellow solid, 2.7 mg, 2.5%). UV (EtOH):  $\lambda_{\max}$  nm (log  $\epsilon$ ) 350 (4.33). <sup>1</sup>H NMR (500 MHz, CD<sub>3</sub>OD):  $\delta$  7.35 (2H, s, H-2' and 6'), 6.39 (1H, d, J = 2.2 Hz, H-8) and 6.19 (1H, d, J = 2.2 Hz, H-6). HRMS (ESI<sup>+</sup>) calculated for formula C<sub>15</sub>H<sub>9</sub>O<sub>8</sub> [M-H] 317.04, found 317.0304.



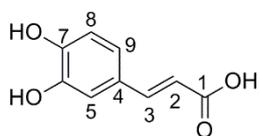
**Quercetin-3-O-glucopyranoside 7 (Qu3glc):** It was isolated from the ethyl acetate layer (pale yellow amorphous solid, 3 mg, 2%). UV (EtOH):  $\lambda_{\max}$  nm (log  $\epsilon$ ) 345 (4.24). <sup>1</sup>H NMR (500 MHz, CD<sub>3</sub>OD):  $\delta$  7.72 (1H, d, J = 2.0 Hz, H-2'), 7.59 (1H, dd, J = 8.5, 2.0 Hz, H-6'), 6.88 (1H, d, J = 8.5 Hz, H-5'), 6.41 (1H, d, J = 2.0 Hz, H-8), 6.22 (1H, d, J = 2.0 Hz, H-6), 5.24 (1H, d, J = 8.0 Hz, H-1''), 3.71 (1H, dd, J = 12.0, 2.5 Hz, H-6a''), 3.56 (1H, dd, J = 12.0, 5.2 Hz, H-6b''), 3.49 (1H, dd, J = 9.1, 8.0 Hz, H-2''), 3.43 (1H, t, J = 9.1 Hz, H-3''), 3.35 (1H, t, J = 9.5 Hz, H-4''), 3.22 (1H, m, H-5). <sup>13</sup>C NMR (125 MHz, CD<sub>3</sub>OD):  $\delta$  179.1 (C-4), 165.9 (C-7), 163.4 (C-5), 159.7 (C-9), 158.6 (C-2), 149.7 (C-3'), 145.9 (C-4'), 135.6 (C-3), 123.3 (C-1'), 123.2 (C-6'), 117.5 (C-2'), 116.0 (C-5'), 105.6 (C-10), 104.3 (C-1''), 99.9 (C-6), 94.7 (C-8), 78.4 (C-5''), 78.1 (C-3''), 75.7 (C-2''), 71.2 (C-4''), 65.6 (C-6''). HRMS (ESI<sup>+</sup>) calculated for formula C<sub>21</sub>H<sub>22</sub>O<sub>12</sub> [M+2] 466.1019, found 466.1092. IR (neat):  $\nu_{\max}$  3358, 3001, 1638 cm<sup>-1</sup>.



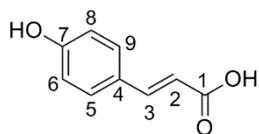
**Quercetin-3-O-rutinoside 9 (Qu3rut):** It was isolated from the aqueous layer (pale yellow amorphous solid, 0.8 mg, 3.2%). UV (EtOH):  $\lambda_{\max}$  nm (log  $\epsilon$ ) 360 (4.29).  $^1\text{H NMR}$  (500 MHz,  $\text{CD}_3\text{OD}$ ):  $\delta$  7.68 (1H, d,  $J = 2.2$  Hz, H-2'), 7.64 (1H, dd,  $J = 8.5, 2.2$  Hz, H-6'), 6.89 (1H, d,  $J = 8.5$  Hz, H-5'), 6.43 (1H, d,  $J = 2.0$  Hz, H-8), 6.23 (1H, d,  $J = 2.0$  Hz, H-6), 5.11 (1H, d,  $J = 8.0$  Hz, H-1''), 4.53 (1H, d,  $J = 1.5$  Hz, H-1'''), 3.80 (1H, dd,  $J = 11.0, 1.5$  Hz, H-6a''), 3.63 (1H, dd,  $J = 3.5, 1.5$  Hz, H-2'''), 3.54 (1H, dd,  $J = 9.5, 3.5$  Hz, H-3'''), 3.46 (1H, dd,  $J = 9.5, 8.0$  Hz, H-2'''), 3.43 (1H, m, H-5'''), 3.41 (1H, t,  $J = 9.5$  Hz, H-3'''), 3.39 (1H, dd,  $J = 11.0, 5.5$  Hz, H-6b''), 3.32 (under solvent peak, H-5''), 3.28 (1H, t,  $J = 9.5$  Hz, H-4'''), 3.26 (1H, d,  $J = 9.5$  Hz, H-4'') and 1.13 (3H, d,  $J = 6.5$  Hz, H-6'''). HRMS (ESI<sup>+</sup>) calculated for formula  $\text{C}_{27}\text{H}_{31}\text{O}_{16}$  [M+H] 611.1432, found 611.1464. IR (neat):  $\nu_{\max}$  3310, 2980, 1670, 1610  $\text{cm}^{-1}$ .



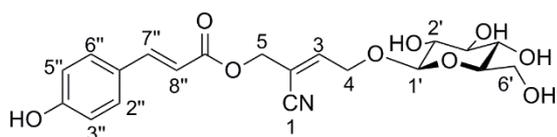
**Quercetin 13 (Qu):** It was isolated from the isopropyl acetate layer (pale yellow solid, 3.5 mg, 3.2%). UV (EtOH):  $\lambda_{\max}$  nm (log  $\epsilon$ ) 375 (4.32).  $^1\text{H NMR}$  (500 MHz,  $\text{CD}_3\text{OD}$ ):  $\delta$  7.74 (1H, d,  $J = 2.0$  Hz, H-2'), 7.63 (1H, dd,  $J = 8.5, 2.0$  Hz, H-6'), 6.89 (1H, d,  $J = 8.5$  Hz, H-5'), 6.39 (1H, d,  $J = 2.5$  Hz, H-8), 6.19 (1H, d,  $J = 2.5$  Hz, H-6).  $^{13}\text{C NMR}$  (125 MHz,  $\text{CD}_3\text{OD}$ ):  $\delta$  177.4 (C-4), 165.6 (C-7), 162.5 (C-5), 158.3 (C-8a), 148.8 (C-4'), 148.1 (C-2), 146.2 (C-3'), 137.2 (C-3), 124.2 (C-1'), 121.7 (C-6'), 116.2 (C-5'), 116.0 (C-2'), 104.5 (C-10), 99.3 (C-6), 94.4 (C-8). HRMS (ESI<sup>+</sup>) calculated for formula  $\text{C}_{15}\text{H}_9\text{O}_7$  [M-H] 301.0353, found 301.0354. IR (neat):  $\nu_{\max}$  3480, 3010, 1690, 1610, 1505  $\text{cm}^{-1}$ . The assignments were confirmed with a standard sample.



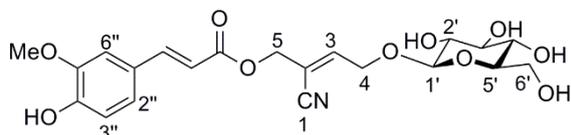
**Caffeic acid 10 (CA):** It was isolated from the isopropyl acetate layer (off white solid, 3.3 mg, 3%). UV (EtOH):  $\lambda_{\max}$  nm (log  $\epsilon$ ) 330 (4.34).  $^1\text{H NMR}$  (500 MHz,  $\text{CD}_3\text{OD}/\text{CF}_3\text{COOD}$  95:5):  $\delta$  7.54 (1H, d,  $J = 16.0$  Hz, H-3), 7.04 (1H, d,  $J = 2.0$  Hz, H-5), 6.94 (1H, dd,  $J = 8.0$  and 2.0 Hz, H-9), 6.78 (1H, d,  $J = 8.0$  Hz, H-8) and 6.22 (1H, d,  $J = 16.0$  Hz, H-2).  $^{13}\text{C NMR}$  (125 MHz,  $\text{CD}_3\text{OD}/\text{CF}_3\text{COOD}$  95:5):  $\delta$  171.0 (C-1), 149.5 (C-7), 147.1 (C-3), 146.8 (C-6), 127.8 (C-4), 122.9 (C-9), 116.5 (C-8), 115.5 (C-2), 115.1 (C-5). HRMS (ESI<sup>+</sup>) calculated for formula  $\text{C}_9\text{H}_7\text{O}_4$  [M-H] 179.0402, found 179.0360. IR (neat):  $\nu_{\max}$  3476, 3330, 1670, 1620, 1460  $\text{cm}^{-1}$ .



**p- Coumaric Acid 11 (p-CA):** It was isolated from the isopropyl acetate layer (pale yellow solid, 5.5 mg, 5%). UV (EtOH):  $\lambda_{\max}$  nm (log  $\epsilon$ ) 310 (4.39).  $^1\text{H}$  NMR (500 MHz,  $\text{CD}_3\text{OD}$ ):  $\delta$  7.60 (1H, d,  $J = 16.0$  Hz, H-3), 7.45 (2H, d,  $J = 8.5$  Hz, H-5,9), 6.81 (2H, d,  $J = 8.5$  Hz, H-6,8), 6.28 (1H, d,  $J = 16$  Hz, H-2);  $^{13}\text{C}$  NMR (125 MHz,  $\text{CD}_3\text{OD}$ ):  $\delta$  171.02 (C-1), 161.15 (C-7), 146.62 (C-3), 131.07 (C-5,9), 127.26 (C-4), 116.81 (C-6,8), 115.65 (C-2); HRMS (ESI-) calculated for formula  $\text{C}_9\text{H}_7\text{O}_3$  163.0503, found 163.0406. IR (neat):  $\nu_{\max}$  3336, 3026, 1667, 1626, 1446  $\text{cm}^{-1}$ .



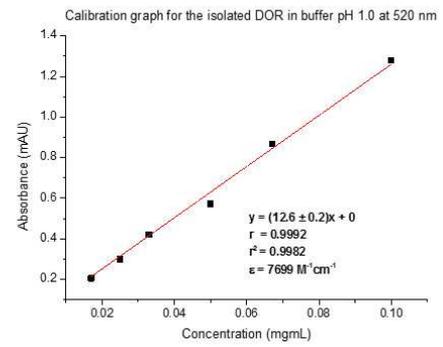
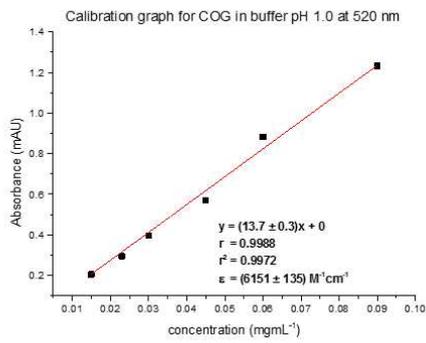
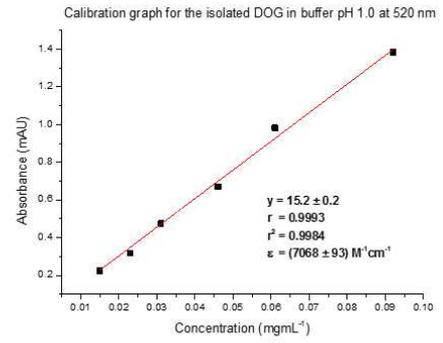
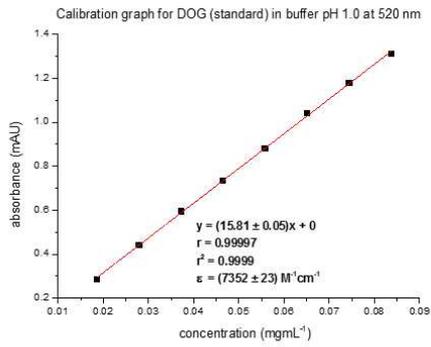
**Nigrumin-p-coumarate 14 (NCA):** It was isolated from the ethyl acetate layer (yellow glassy solid, 1.5 mg, 1%). UV (MeOH):  $\lambda_{\max}$  310, 211 nm.  $^1\text{H}$  NMR (500 MHz,  $\text{CD}_3\text{OD}$ ):  $\delta$  7.68 (1H, d,  $J = 16.0$  Hz, H-7''), 7.49 (2H, d,  $J = 9$  Hz, H-6'' and H-8''), 6.86 (1H, t,  $J = 6.5$  Hz, H-3), 6.82 (2H, d,  $J = 9.0$  Hz, H-3'' and H-5''), 6.37 (1H,  $J = 16.0$  Hz, H-8''), 4.82 (1H, s, H-5), 4.66 (1H, dd,  $J = 14.5, 5.9$  Hz, H-4b), 4.54 (1H, dd,  $J = 14.5, 6.5$  Hz, H-4a), 4.36 (1H, d,  $J = 8.0$  Hz, H-1'), 3.88 (1H, dd,  $J = 12.0, 1.0$  Hz, H-6b'), 3.69 (1H, dd,  $J = 12.0, 5.0$  Hz, 6a'), 3.21 (2H, t,  $J = 8.3$  Hz, H-2' and H-5'). HRMS (ESI) calculated for formula  $\text{C}_{20}\text{H}_{22}\text{NO}_9$  [M-H] 420.1706, found 420.1706. IR (neat):  $\nu_{\max}$  3360, 2950, 2850, 2030, 1662, 1480  $\text{cm}^{-1}$ . The  $^1\text{H}$  NMR assignments were confirmed with the literature.<sup>1,2</sup>



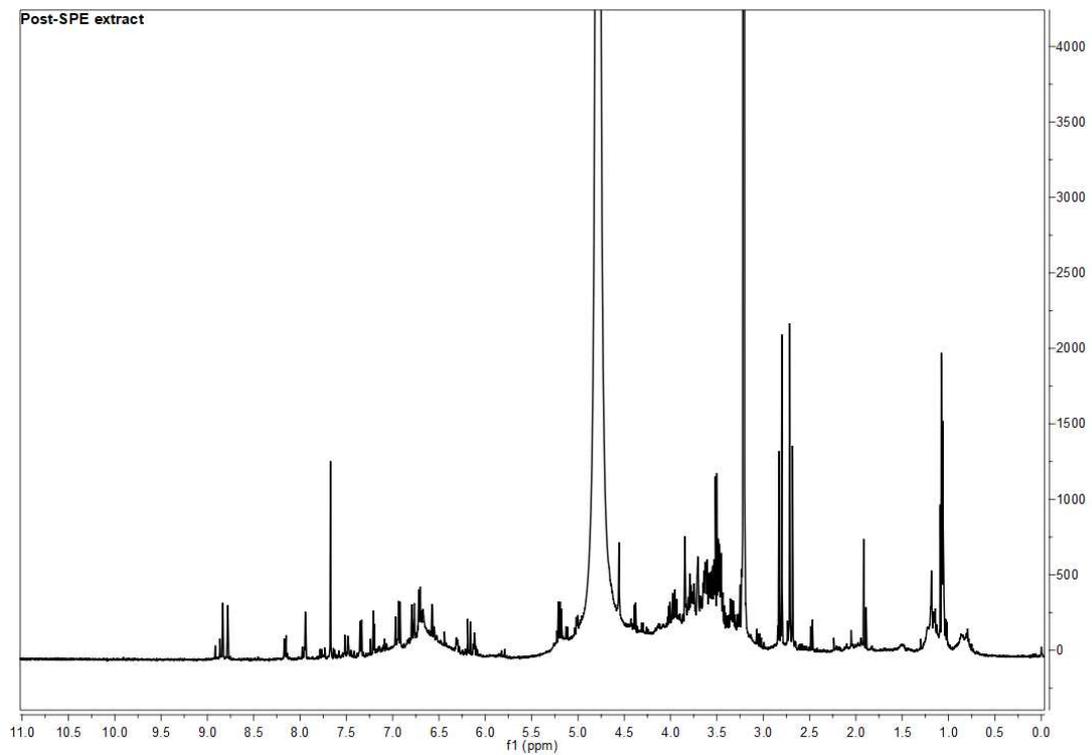
**Nigrumin ferulate 15 (NF):** It was isolated from the ethyl acetate layer (yellow glassy solid, 0.7 mg, 0.5%). UV (MeOH):  $\lambda_{\max}$  324, 210 nm.  $^1\text{H}$  NMR (500 MHz,  $\text{CD}_3\text{OD}$ ):  $\delta$  7.68 (1H, d,  $J = 16.0$  Hz, H-7''), 7.22 (1H, d,  $J = 2.0$  Hz, H-6''), 7.11 (1H, dd,  $J = 8.5, 2.0$  Hz, H-2''), 6.86 (1H, t,  $J = 6.5$  Hz, H-3), 6.82 (1H, d,  $J = 8.5$  Hz, H-3''), 6.37 (1H,  $J = 16.0$  Hz, H-8''), 4.82 (1H, s, H-5), 4.66 (1H, dd,  $J = 14.5, 5.9$  Hz, H-4b), 4.54 (1H, dd,  $J = 14.5, 6.5$  Hz, H-4a), 4.36 (1H, d,  $J = 8.0$  Hz, H-1'), 3.88 (1H, dd,  $J = 12.0, 1.0$  Hz, H-6b'), 3.69 (1H, dd,  $J = 12.0, 5.0$  Hz, 6a'), 3.21 (2H, t,  $J = 8.3$  Hz, H-2' and H-5'). HRMS (ESI) calculated for formula  $\text{C}_{21}\text{H}_{24}\text{NO}_{10}$  [M-H] 450.1406, found 450.1389. IR (neat):  $\nu_{\max}$  3360, 2950, 2840, 2190, 1662, 1450  $\text{cm}^{-1}$ . The  $^1\text{H}$  NMR assignments were confirmed with the literature.<sup>1,2</sup>

**Polymeric anthcyanins:** It was isolated from the aqueous layer (dull purple amorphous solid, 4.5 mg, 18%, isolated yield). UV (MeOH/12.1 M HCl, 99.9:0.1):  $\lambda_{\max}$  252, 277, 352, 520 nm.  $^1\text{H}$  NMR data indicated towards polymeric species with broad aromatic (6.00-7.80 ppm) and sugar region (3.52-4.51 ppm).

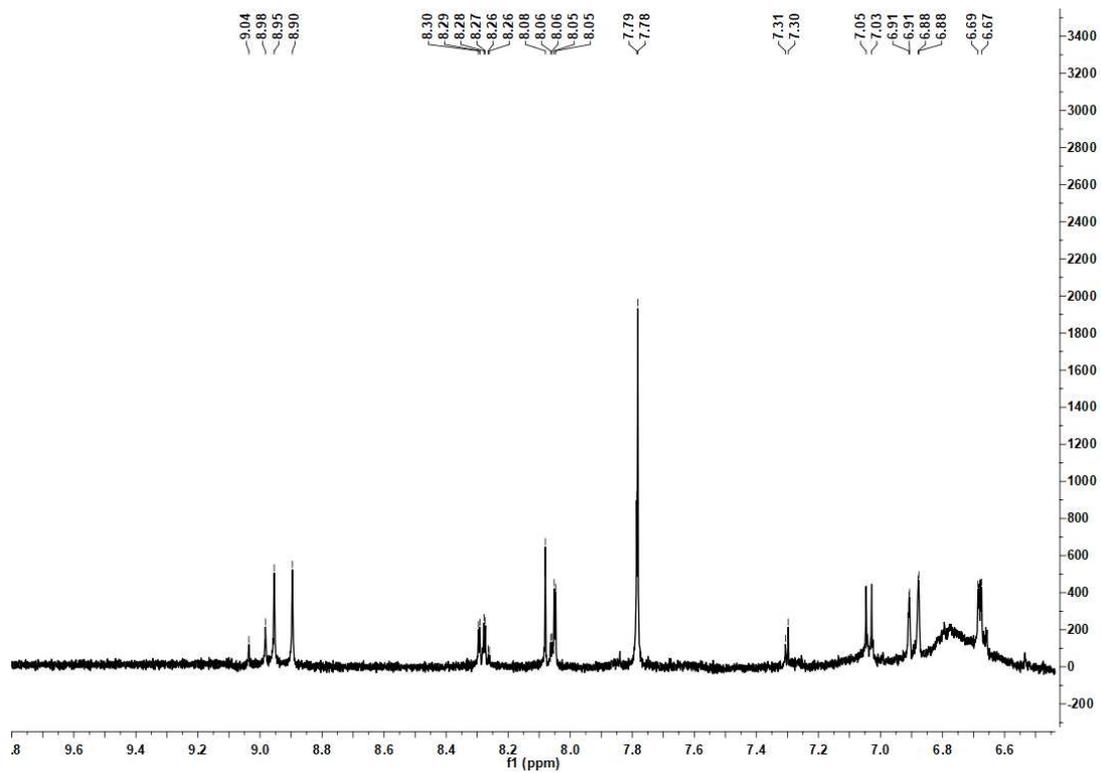
1. Lu, Y.; Foo, L.Y.; Wong, H. Nigrumin-5-p-coumarate and nigrumin-5-ferulate, two unusual nitrile-containing metabolites from black currant (*Ribes nigrum*) seed. *Phytochem.* **2002**, *59*, 465-468.
2. Mäkilä, L.; Laaksonen, O.; Alanne, A.-L.; Kortensniemi, M.; Kallio, H.; Yang, B. Stability of hydroxycinnamic acid derivatives, flavonol glycosides, and anthocyanins in black currant juice. *J. Agric. Food Chem.* **2016**, *64*, 4584-4598.



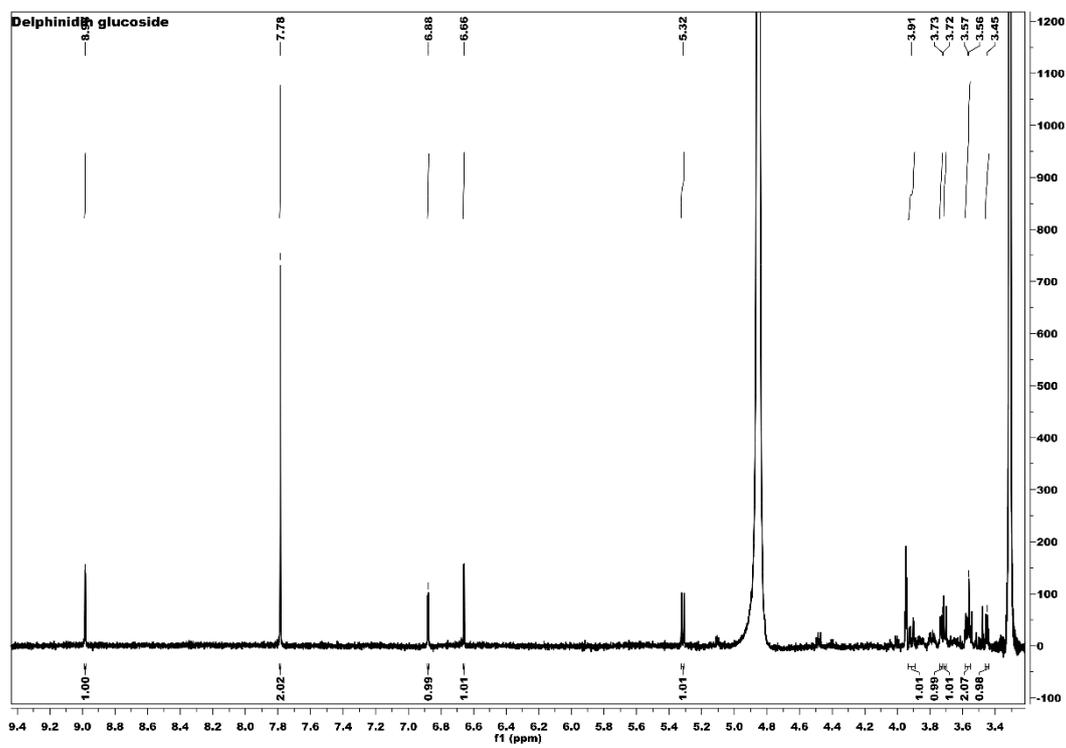
**S1. Calibration graphs for the extinction coefficient calculations (buffer pH 1.0).**



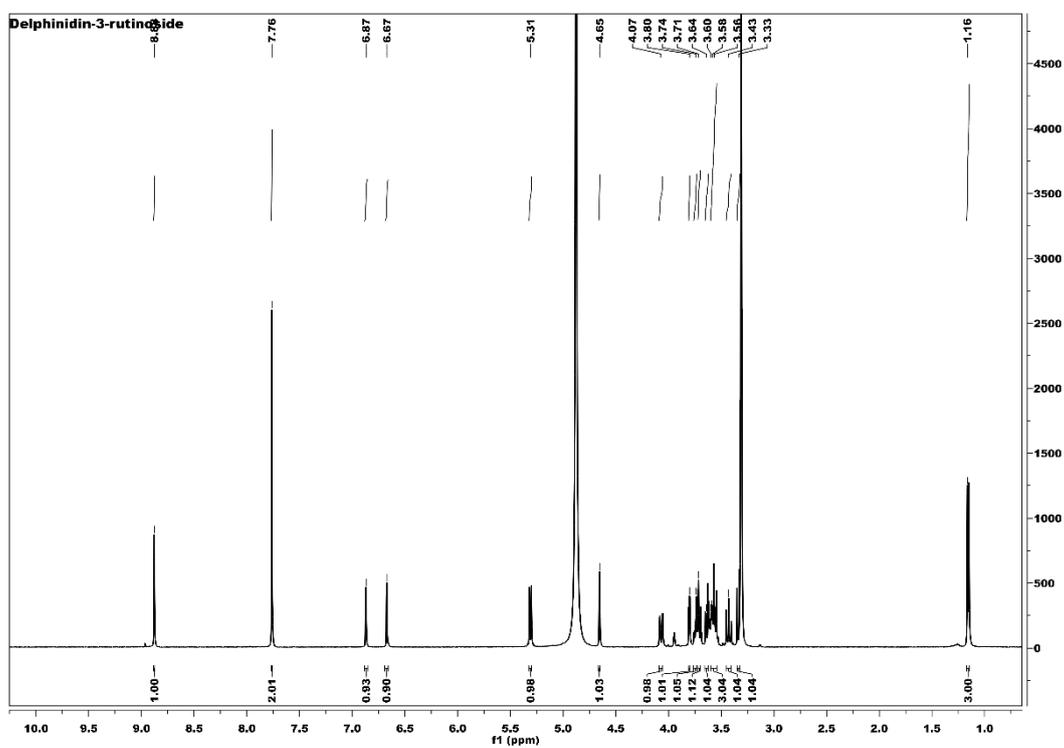
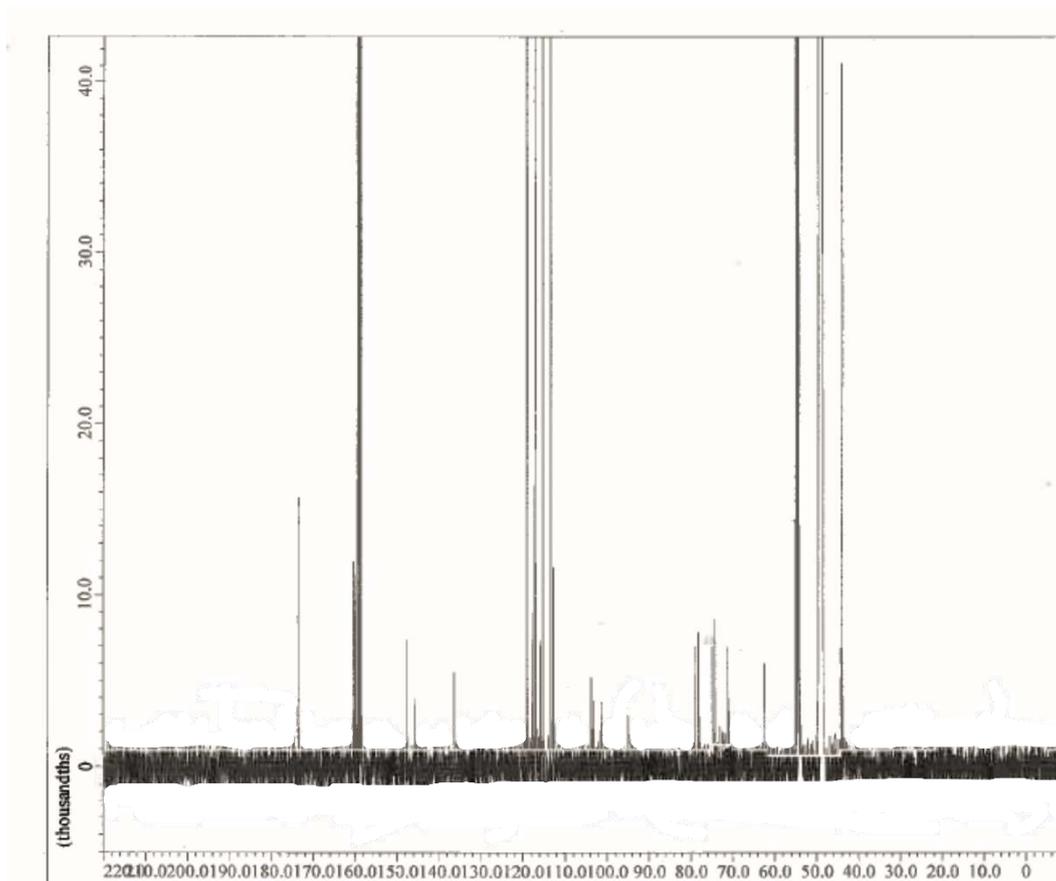
**S2. <sup>1</sup>H NMR of post-SPE blackcurrant extract (CD<sub>3</sub>OD/CF<sub>3</sub>COOD 95:5)**

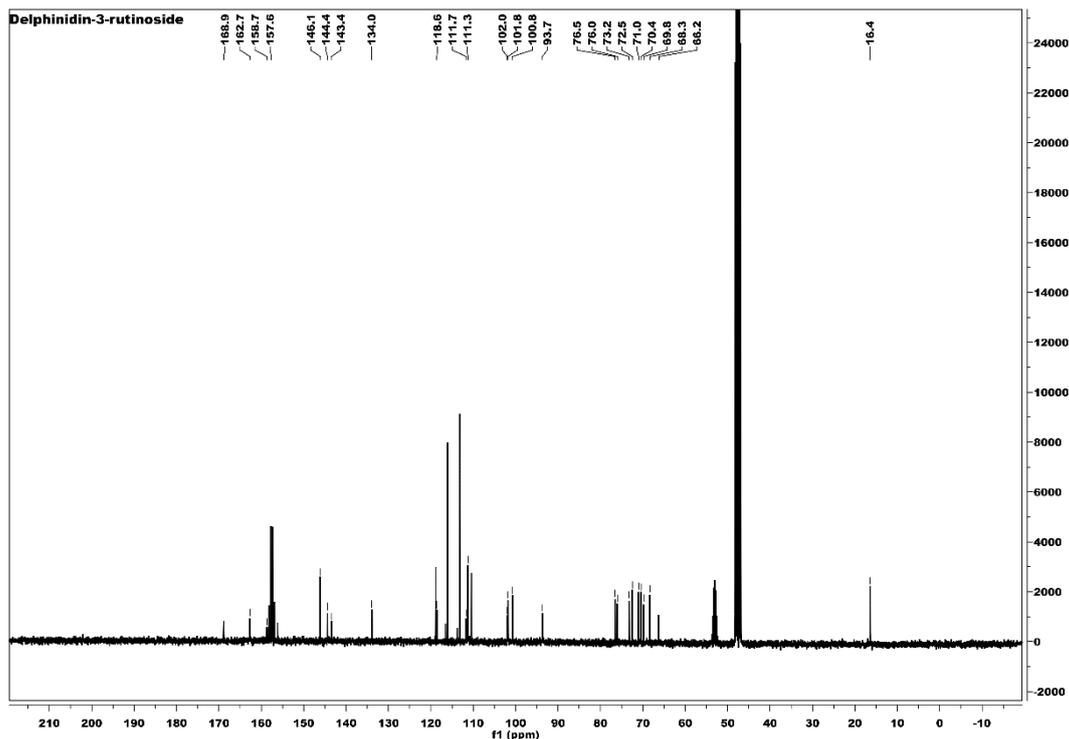


S3.  $^1\text{H}$  NMR of (post-liquid liquid extractions) blackcurrant extract ( $\text{CD}_3\text{OD}/\text{CF}_3\text{COOD}$  95:5)

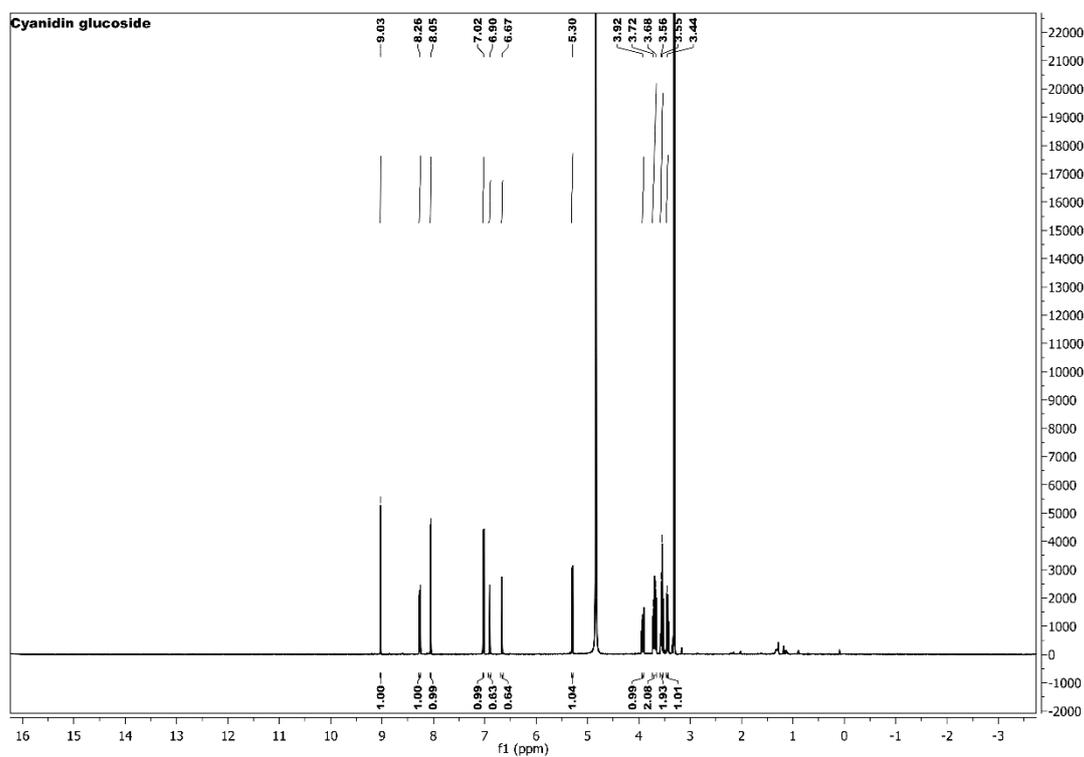


S4.  $^1\text{H}$  NMR spectrum for delphinidin-3-*O*-glucoside 2.

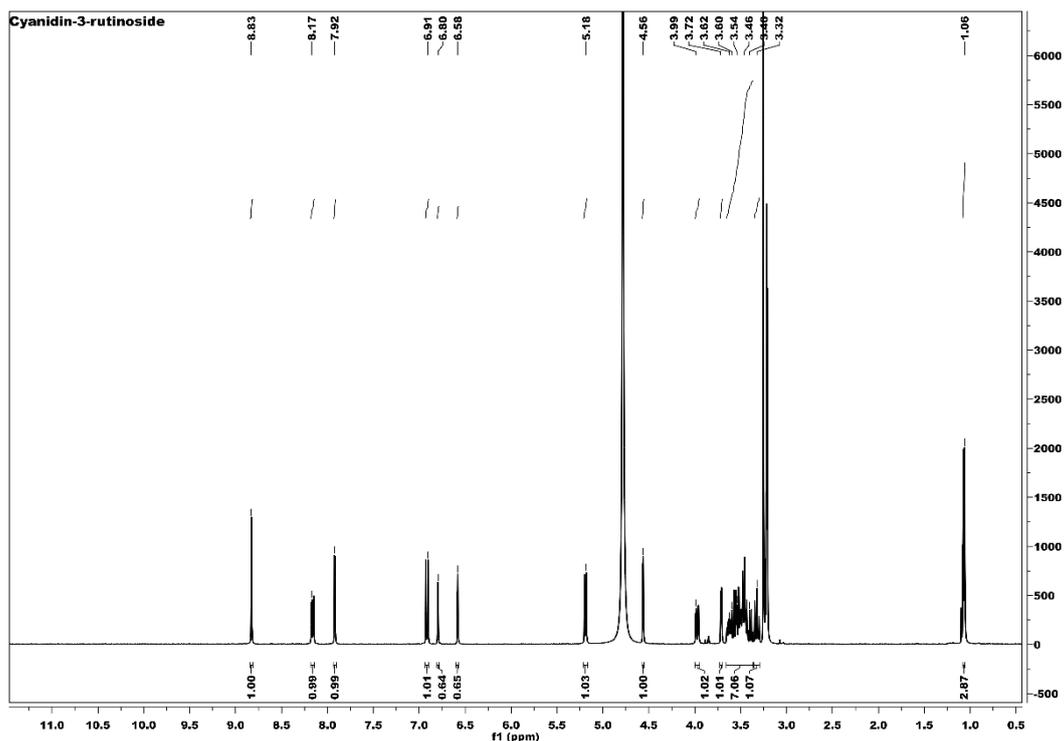




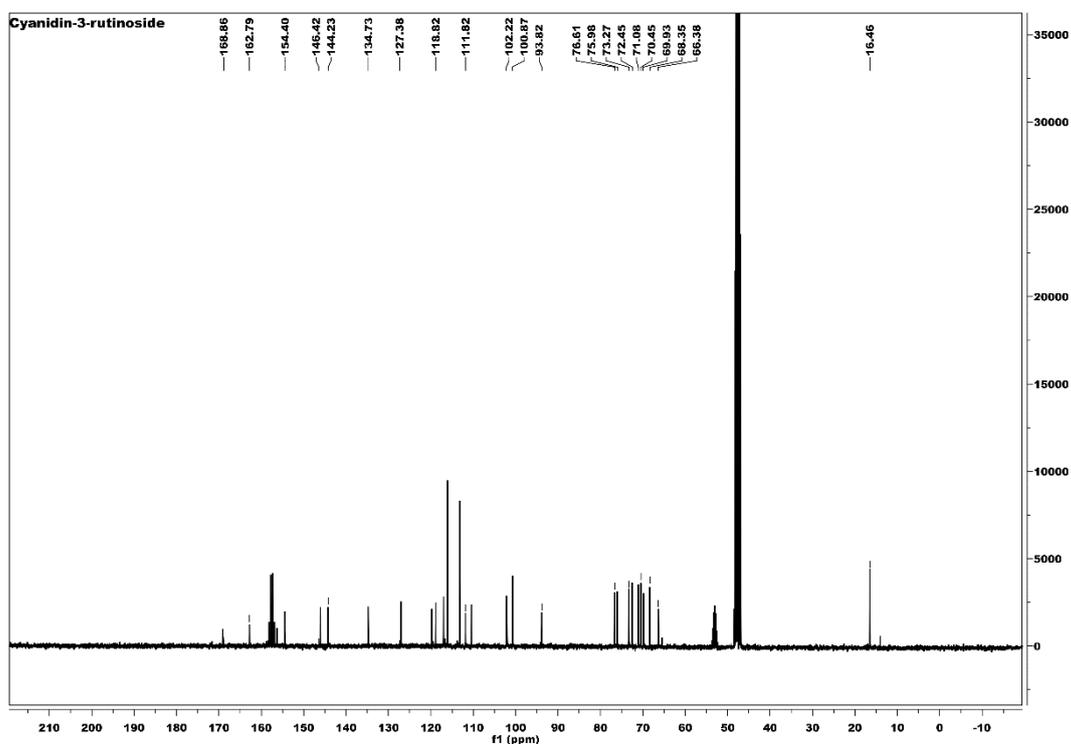
S7.  $^{13}\text{C}$  NMR spectrum for delphinidin-3-*O*-rutinoside **4**.



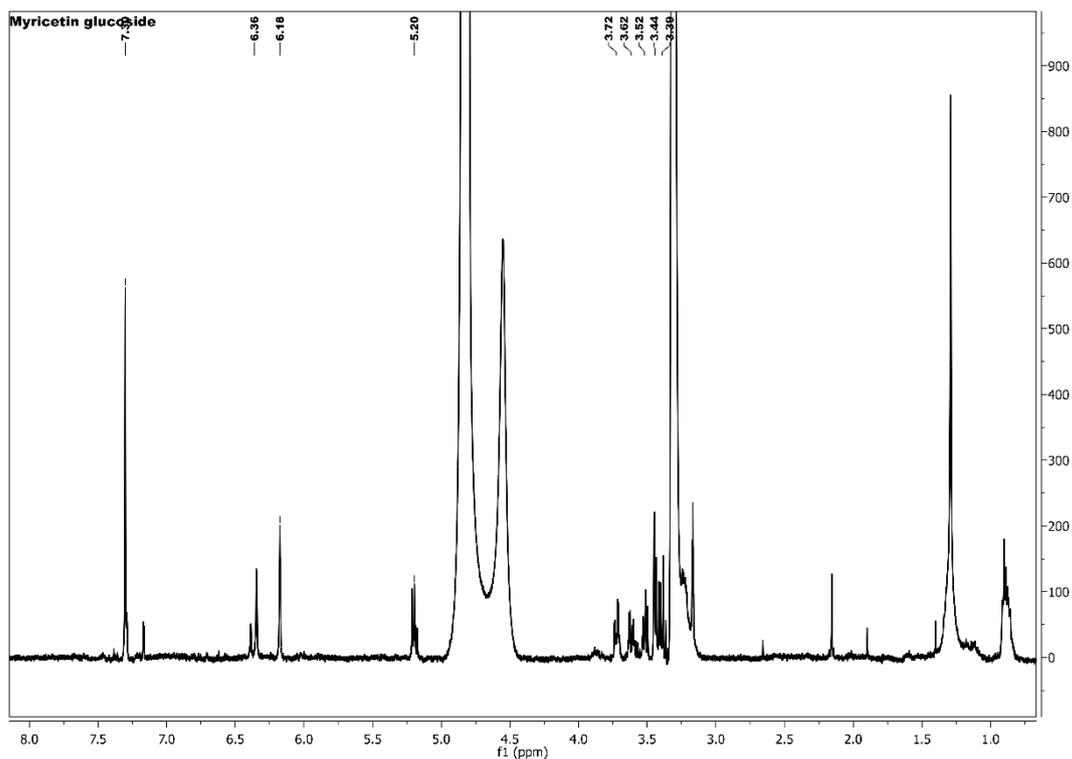
S8.  $^1\text{H}$  NMR spectrum for cyanidin-3-*O*-glucoside **3**.



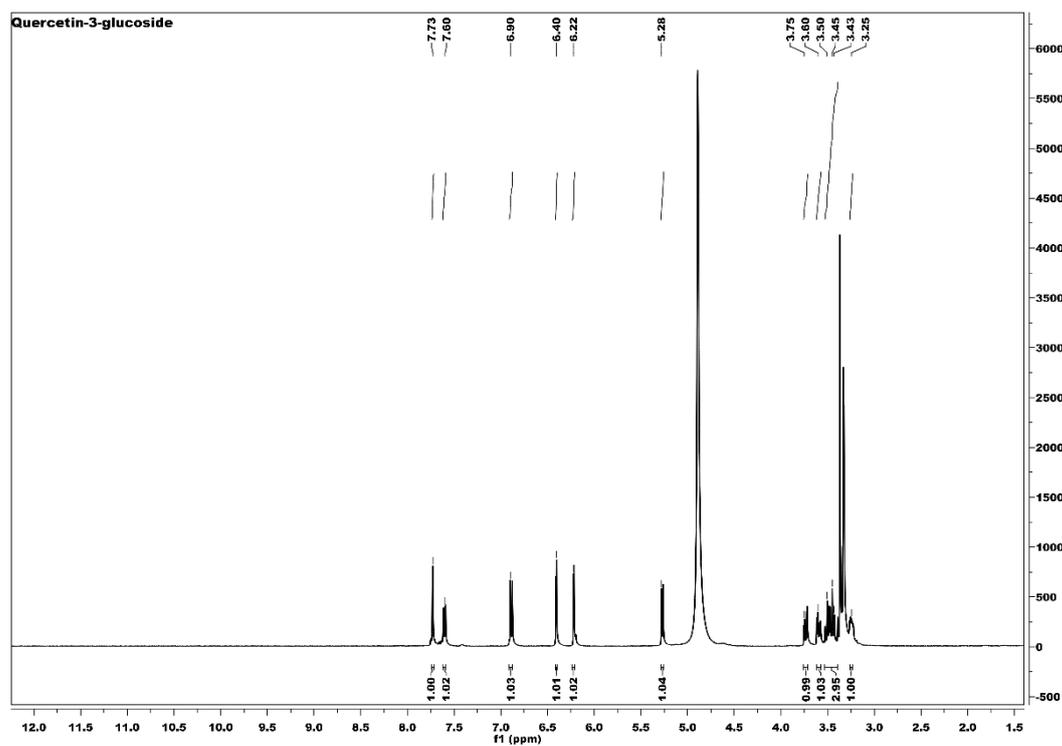
S9.  $^1\text{H}$  NMR spectrum for cyanidin-3-*O*-rutinoside 5.



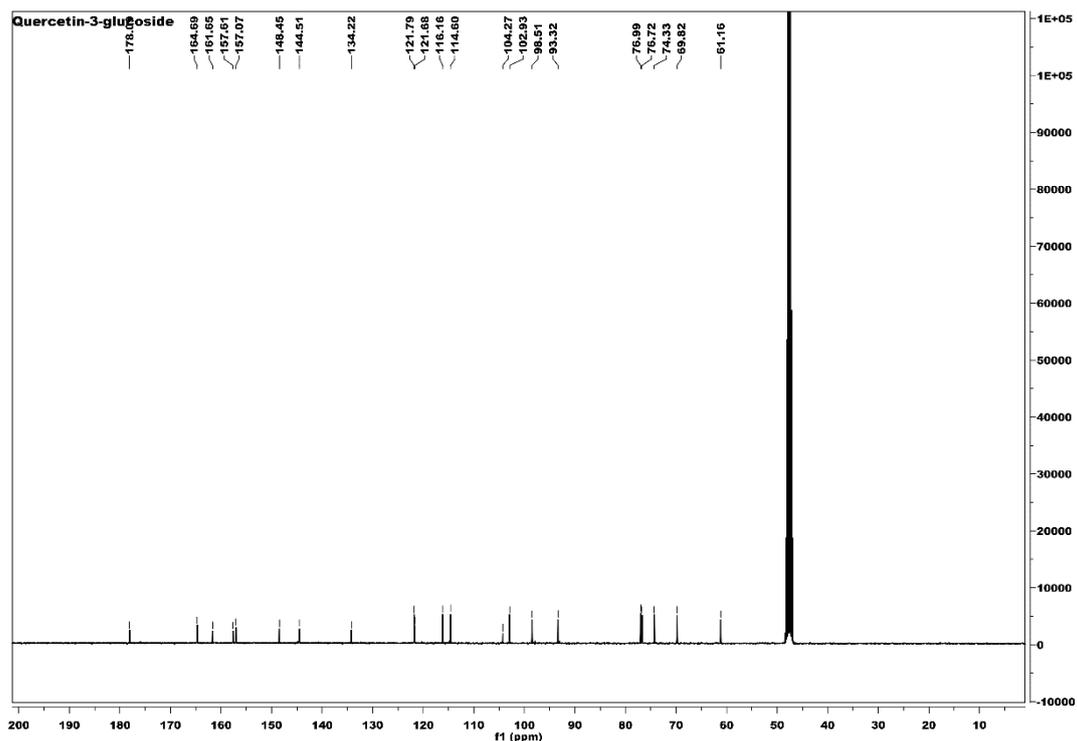
S10.  $^{13}\text{C}$  NMR spectrum for cyanidin-3-*O*-rutinoside 5.



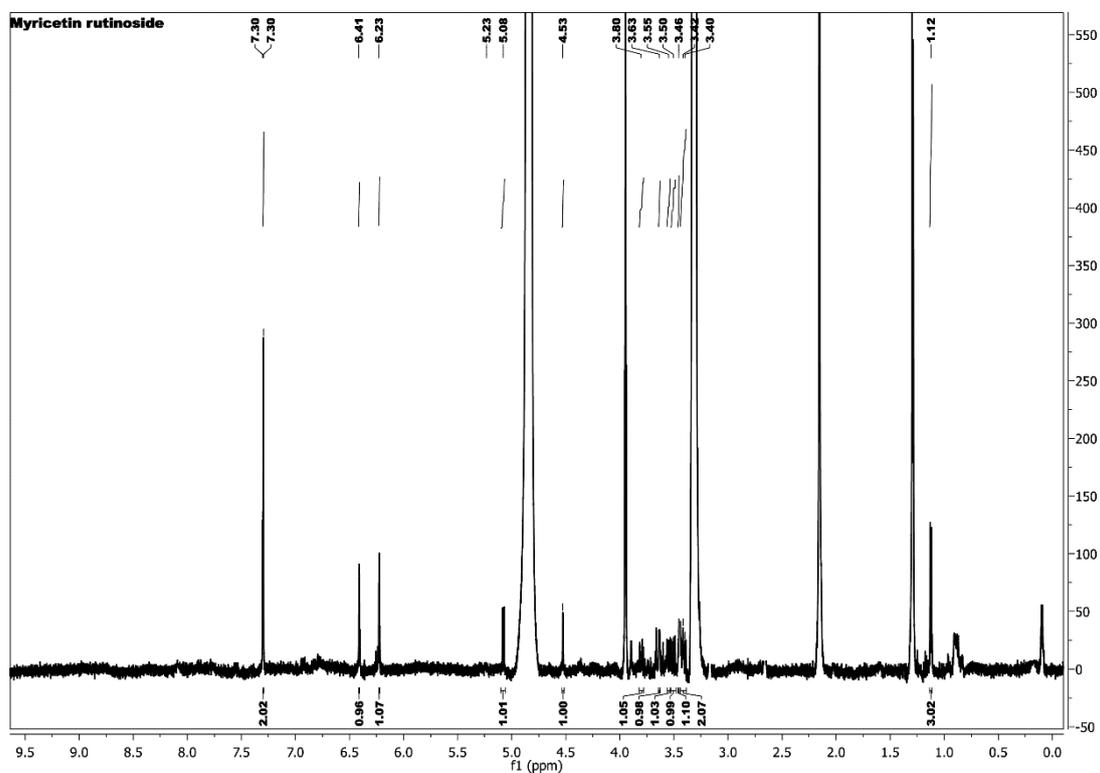
**S11.**  $^1\text{H}$  NMR spectrum for myricetin-3-*O*-glucoside **6** (peaks at 0.80 and 1.30 ppm are due to grease contaminants present in weak sample; residual  $\text{CHD}_2\text{OD}$  at 3.31ppm,  $\text{CD}_3\text{OH}$  at 4.60ppm and water at 4.87ppm).



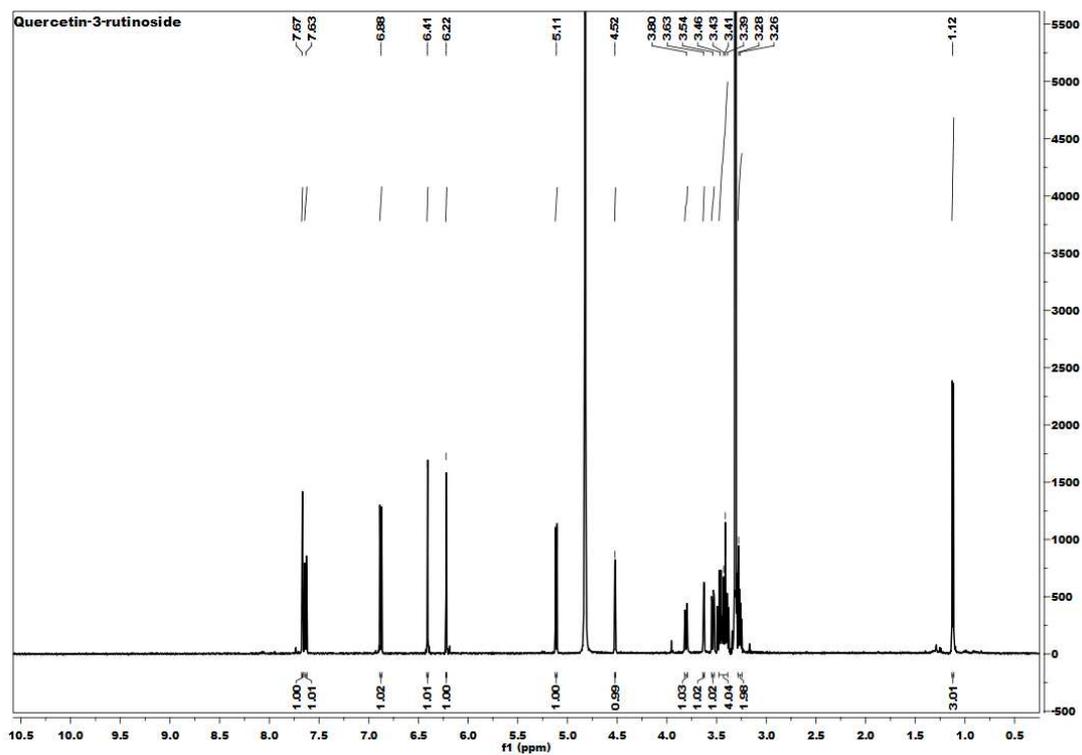
**S12.**  $^1\text{H}$  NMR spectrum for quercetin-3-*O*-glucoside **7**.



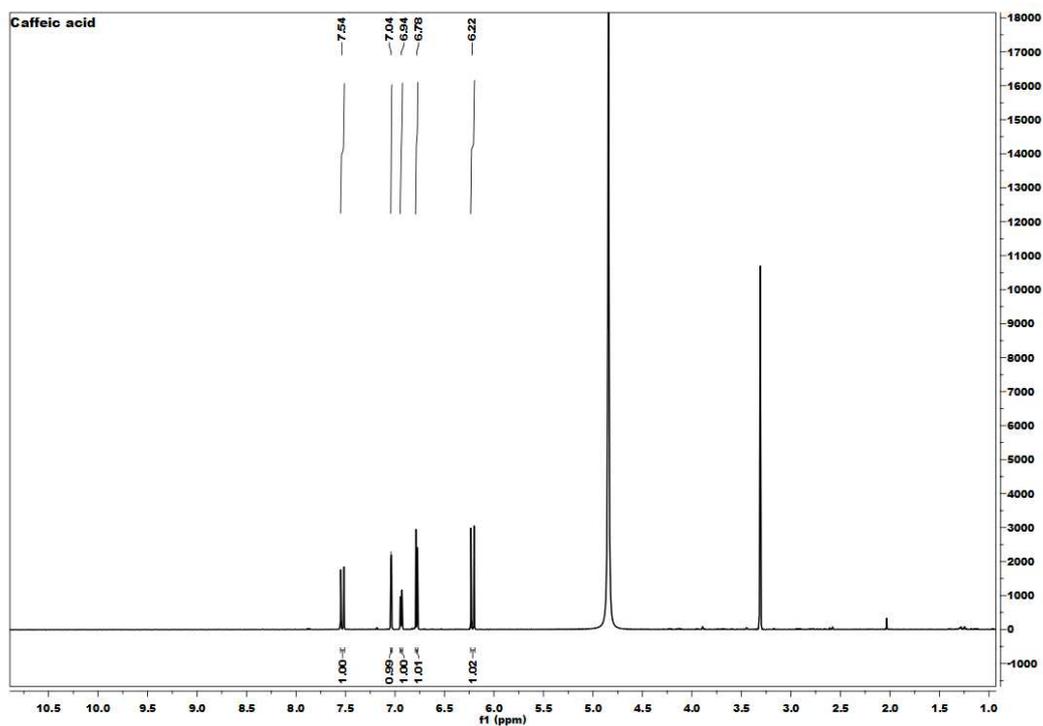
**S13.**  $^{13}\text{C}$  NMR spectrum for quercetin-3-*O*-glucoside **7**.



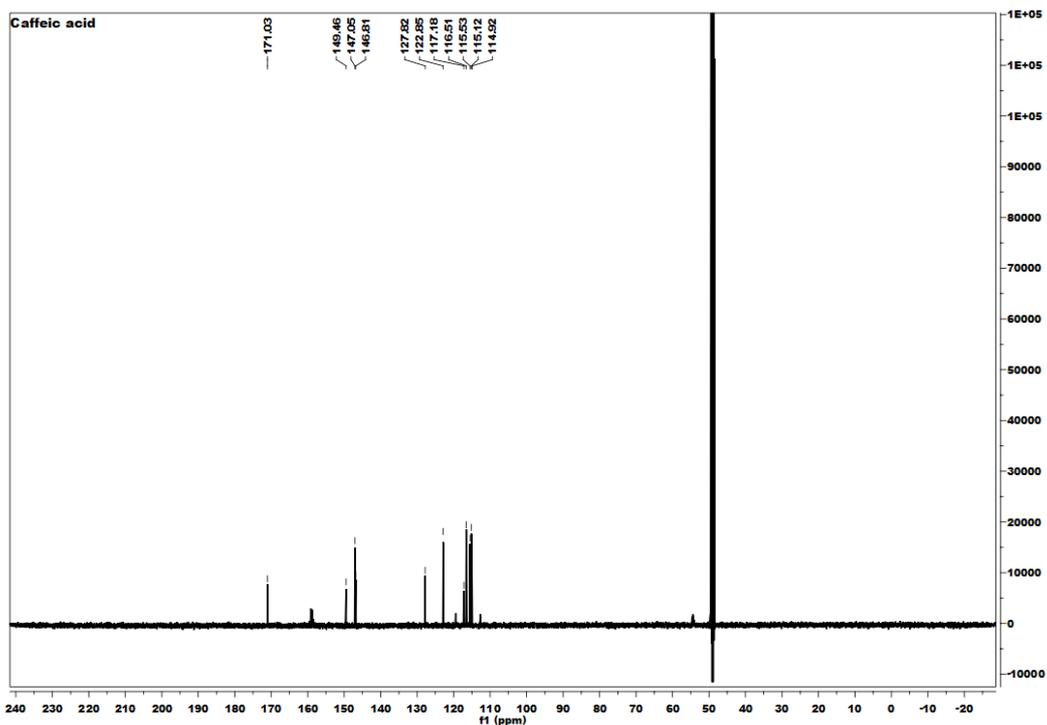
**S14.**  $^1\text{H}$  NMR spectrum for myricetin-3-*O*-rutinoside **8** (residual solvent peaks are present due to particularly weak sample; 1.30ppm (grease), 2.15ppm (acetone),  $\text{CHCD}_2\text{OD}$  at 3.31ppm and water at 4.87 ppm).



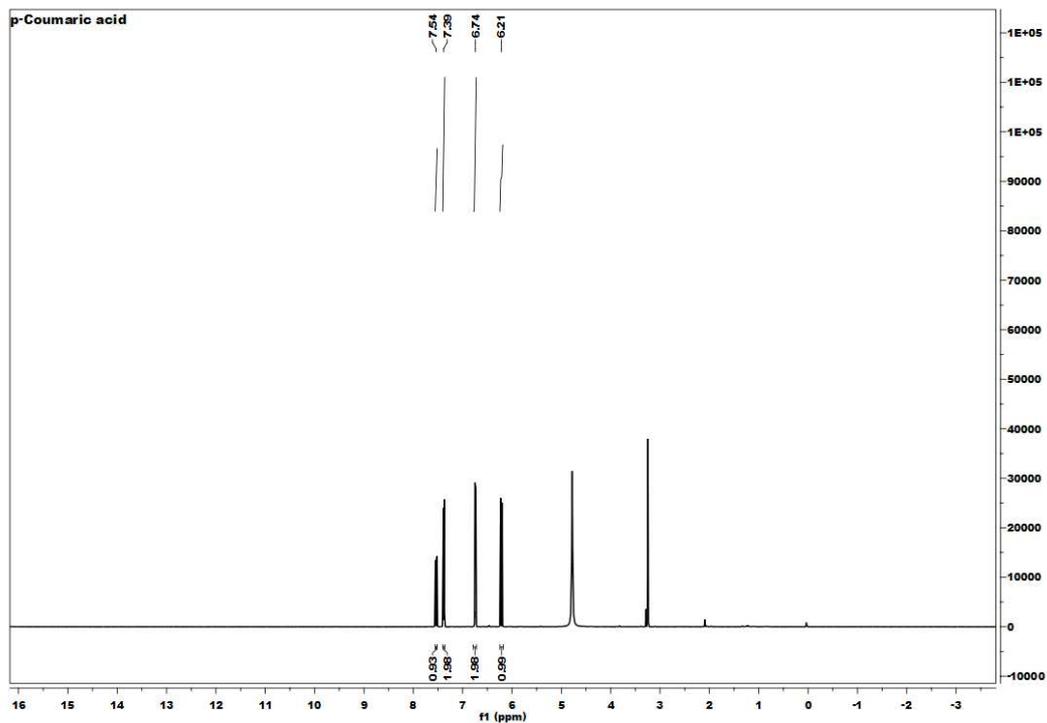
**S15.**  $^1\text{H}$  NMR spectrum for quercetin-3-*O*-rutinoside **9**.



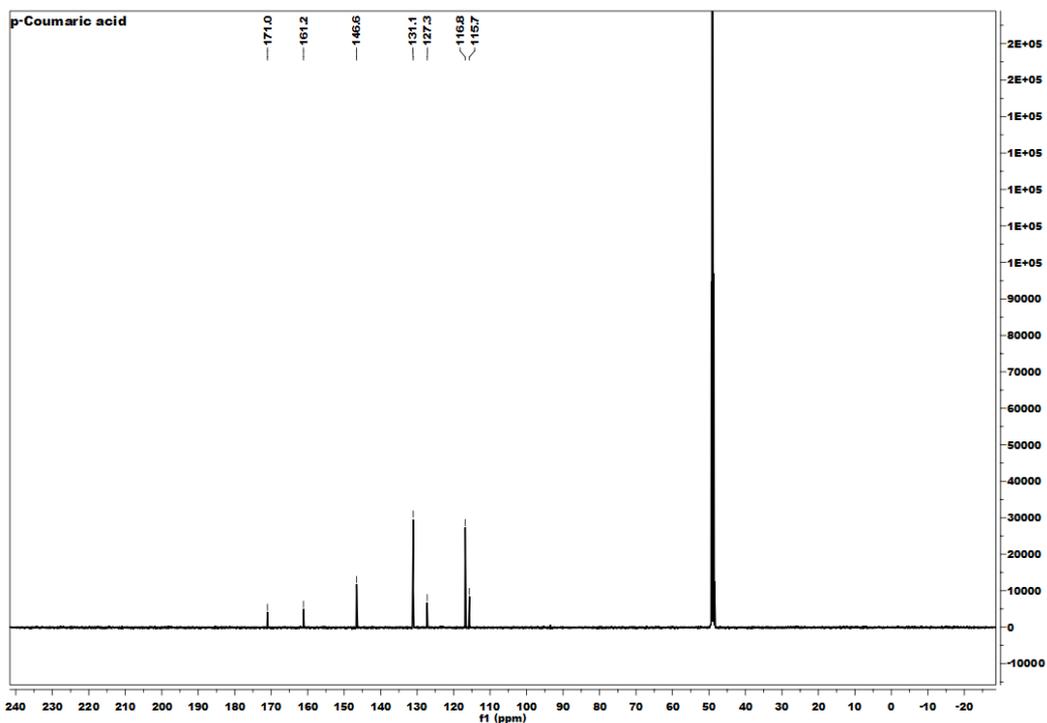
**S16.**  $^1\text{H}$  NMR spectrum for caffeic acid **10**.



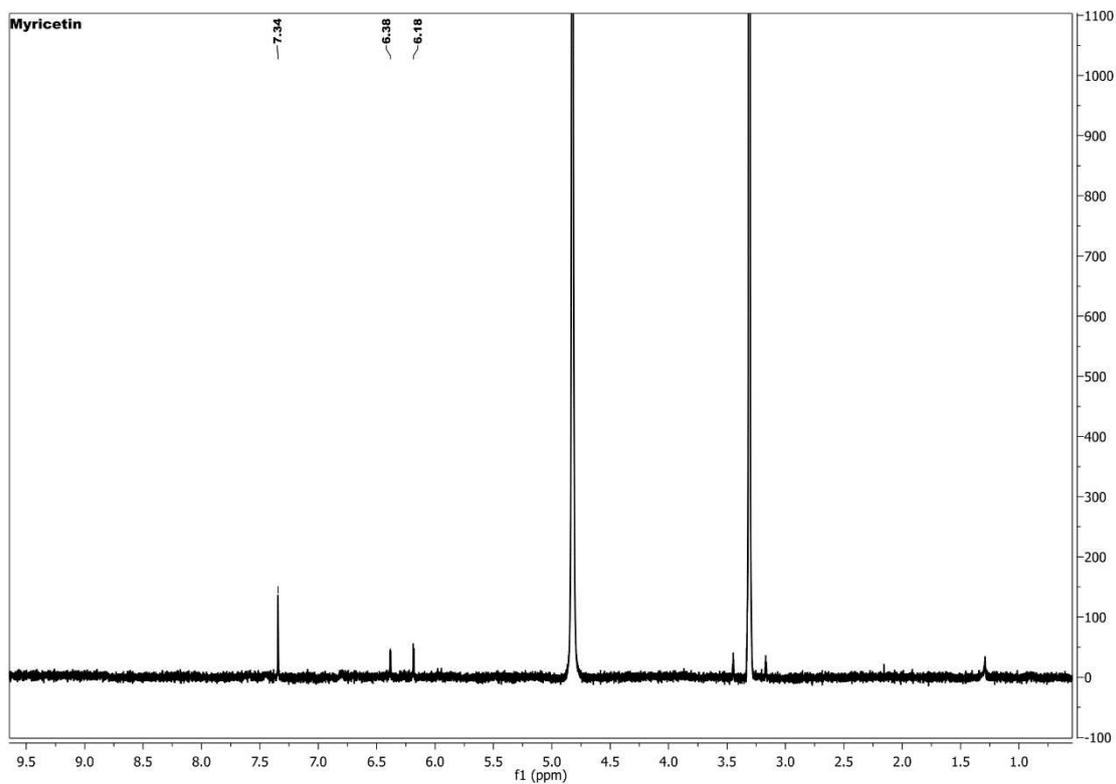
S17.  $^{13}\text{C}$  NMR spectrum for caffeic acid **10**.



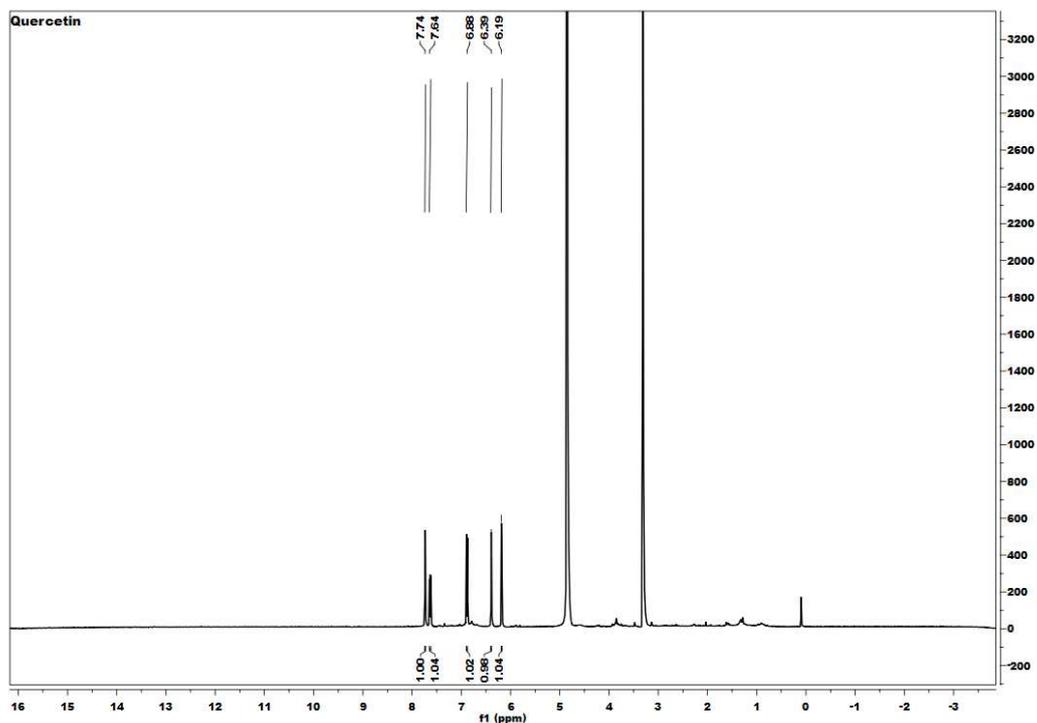
S18.  $^1\text{H}$  NMR spectrum for *p*-coumaric acid **11**.



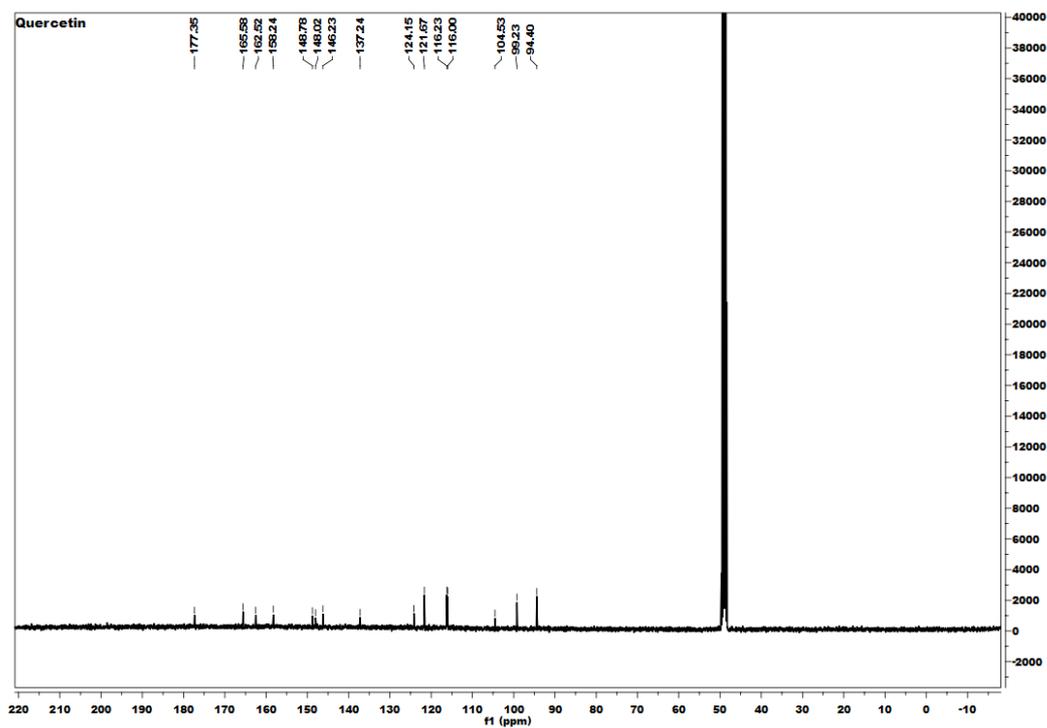
S19. <sup>13</sup>C NMR spectrum for *p*-coumaric acid **11**.



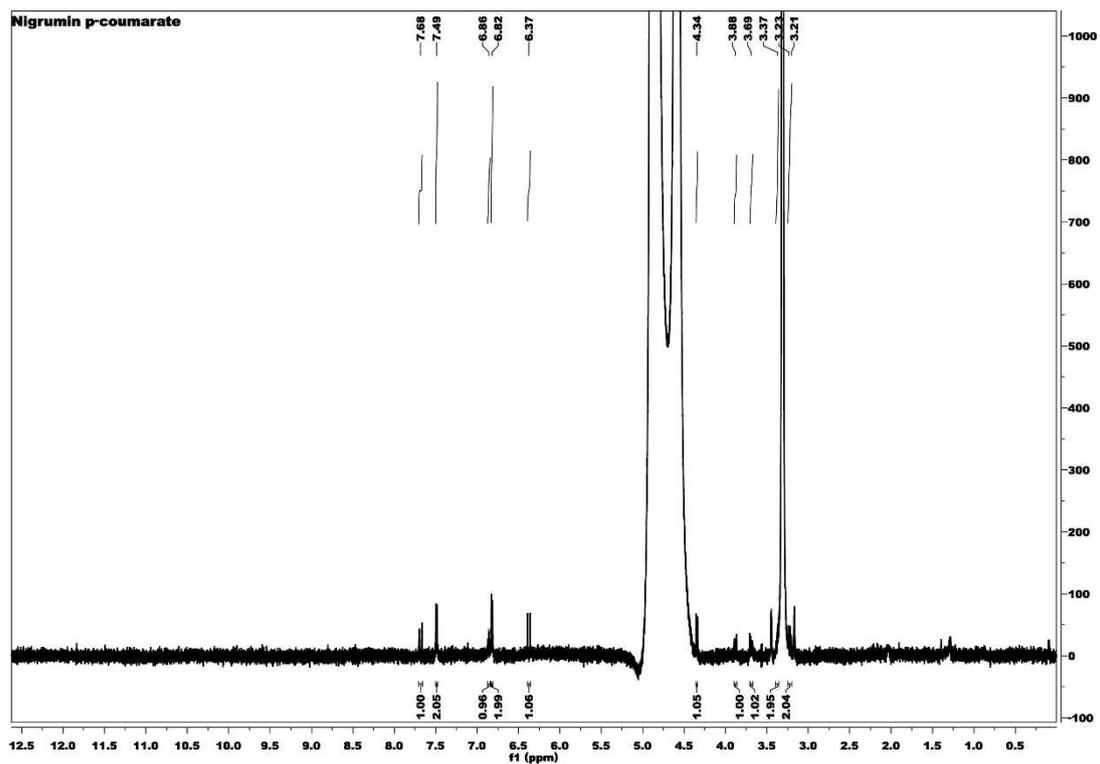
S20. <sup>1</sup>H NMR spectrum for myricetin **12**.



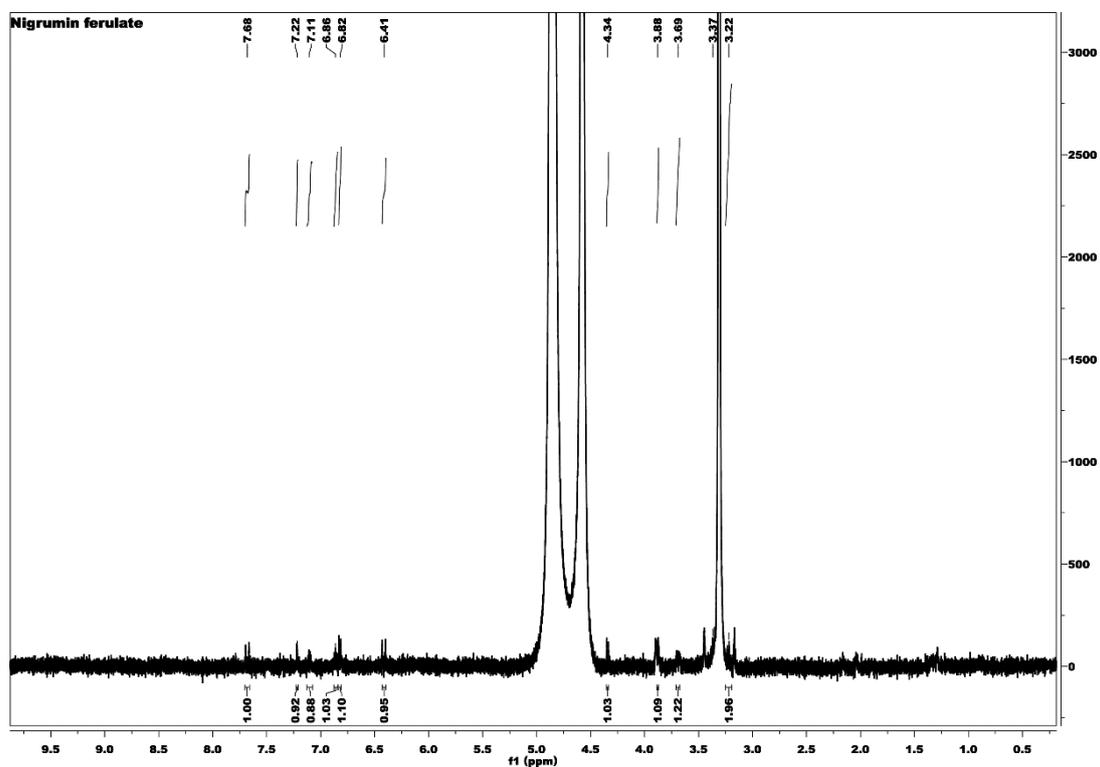
S21.  $^1\text{H}$  NMR spectrum for quercetin **13**.



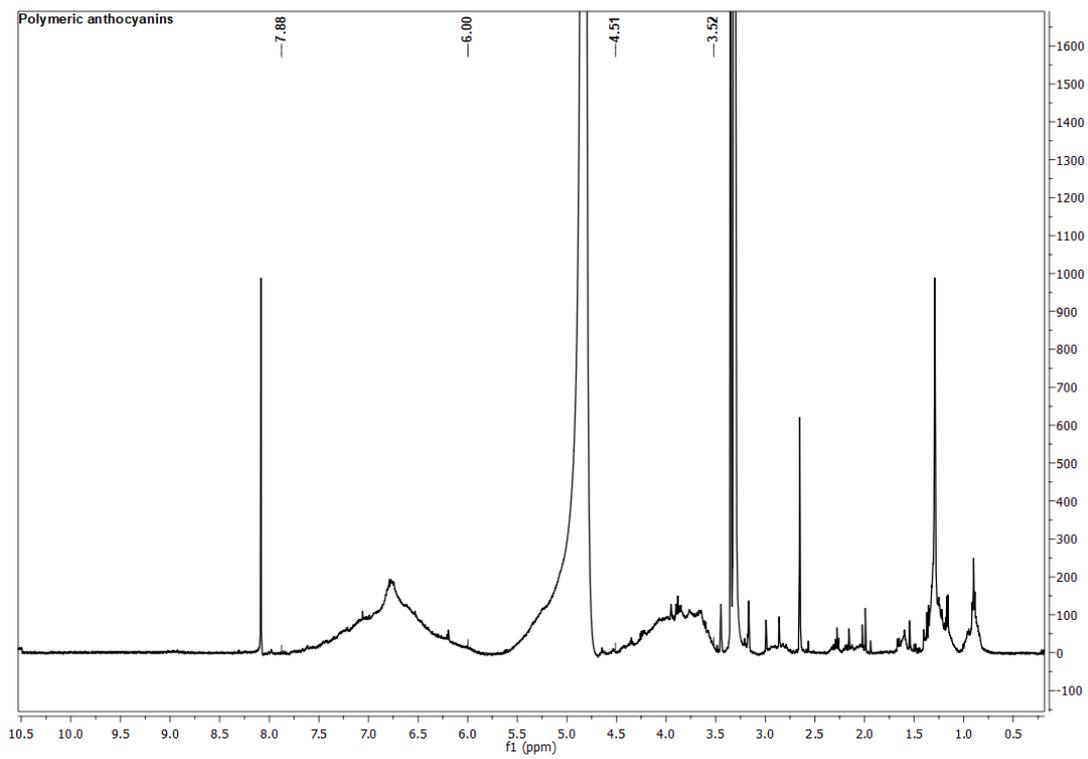
S22.  $^{13}\text{C}$  NMR spectrum for quercetin **13**.



S23. <sup>1</sup>H NMR spectrum for nigrumin-*p*-coumarate 14.



S24. <sup>1</sup>H NMR spectrum for nigrumin ferulate 15.



S25.  $^1\text{H}$  NMR spectrum for polymeric anthocyanins.