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**SUPPLEMENTARY MATERIAL**

*Senna alata* leaves are a good source of propelargonidins

*Aina Ramsay*a, Irene Mueller-Harveya

*aChemistry and Biochemistry Laboratory, School of Agriculture, Policy and Development, University of Reading, 1 Earley Gate, P O Box 236, Reading RG6 6AT, U.K

*Corresponding author: aina.ramsay@hotmail.fr*

Proanthocyanidins (PA) in *Senna alata* leaves were investigated by thiolysis with benzyl mercaptan, LC-MS and NMR and consisted of rare, but almost pure propelargonidins with <6% procyanidins, had B-type linkages a mean degree of polymerisation of 3. Epiafzelechin was the major flavan-3-ol subunit (>94%) and epicatechin a minor constituent (6.4%) in residual PA and mainly detected as an extension unit.

**Keywords:** *Senna alata, Cassia alata, proanthocyanidins, propelargonidins, LC-MS, NMR, thiolysis*

1. **Experimental**
   
   **1.1 General**
   
   Acetone (analytical reagent grade), acetonitrile (HPLC grade), dichloromethane (HPLC grade) and hydrochloric acid (37%, analytical reagent grade), were purchased from ThermoFisher Scientific Ltd (Loughborough, U.K.); (+)-taxifolin (98%) and kaempferol (98%) from Apin Chemicals (Abingdon, U.K.); benzyl mercaptan (99%), epicatechin (EC) and quercetin (≥99% HPLC) from Sigma-Aldrich (Poole, U.K.). (+)-afzelechin (96-98%) from Plantech UK (Reading, UK). Sephadex™ LH-20 from GE Healthcare (Little Chalfont, UK). Deionised water was obtained from a Milli-Q System (Millipore, Watford, U.K.). Quercetin was dissolved in methanol/water (80/20 %) using six concentrations (n = 2) and peak areas were used for the calibration curve.

   **1.2 Samples**
   
   *Senna alata* leaves were harvested in December 2013 in private botanical garden in Trois-rivières, Guadeloupe, France. Any excess humidity was removed with kitchen paper, air-dried
for a few hours protected from direct light then immediately carefully packed and sent to the University of Reading, UK by airplane and arrived within 3 days. Upon arrival, leaves were freeze-dried and finely ground in an impeller SM1 cutting mill (Retsch, Haan, Germany) to pass a 1 mm sieve. The ground plant material was stored in the dark at room temperature.

1.3 Extraction and purification

1.3.1 Extractable proanthocyanidins
Finely ground leaves (10.2 g) were extracted for 1 h each with acetone/water (125 mL; 7:3; v/v). Acetone was removed under vacuum on a rotary evaporator; the remaining aqueous solution was centrifuged for 3 min at 4500 rpm (Jouan CR3i Multifunction Centrifuge, Thermo Electron Corporation, Basingstoke, UK) and freeze-dried to give the extract (extract = 1.5 g, yield = 15%). Acetone was allowed to evaporate from the plant residue in the fume cupboard overnight and protected from direct light before freeze-drying prior to analysis of unextractable proanthocyanidins.

1.3.2 Purification of proanthocyanidins
Dried extract (1.2 g) was dissolved in distilled water (500 mL) and passed through a Sephadex™ LH-20 resin (50 g), which had been conditioned with water. Distilled water was added to remove sugars and other impurities. The first fraction (F1-fraction) of proanthocyanidins was eluted with acetone/water (1 L; 3:7; v/v) and the second fraction (F2-fraction) with acetone/water (1 L; 1:1; v/v). Acetone was removed and the aqueous solutions were freeze-dried.

1.4 Analytical data

1.4.1 Thiolysis of extracted and fractionated proanthocyanidins
The freeze-dried extracts or fractions (8 mg) were placed into a screw cap glass tube with a stirring magnet, methanol (1.5 mL), methanol acidified with concentrated HCl (3.3%; 500 μL) and benzyl mercaptan (50 μL). The tubes were capped and placed into a water bath at 40°C for 1 h under vigorous stirring (ETS-D5, RCT basic, IKA® GmbH, Staufen, Germany). Then distilled water (2.5 mL) and internal standard, taxifolin in methanol (500 μL; 0.1 mg/mL), was added and thoroughly mixed. The mixture was transferred into a vial (0.8 mL; Chromacol 08-CPV; ThermoFisher Scientific Ltd), closed with a crimp top and analysed by liquid chromatography-mass spectrometry (LC-MS) within 12 h.
1.4.2 In situ thiolysis of proanthocyanidins

Freeze-dried leaves (200 mg) were reacted with the thiolysis reagent (2 mL methanol, 1 mL of 3.3% HCl in methanol, and 100 μL benzyl mercapta) as above. After the reaction, methanol (1 mL) was added to the mixture. The sample was mixed and centrifuged at 4000 rpm for 3 min (Centrifuge 5702, Eppendorf® GmbH, Hamburg, Germany) and supernatant (1 mL) was transferred into another empty and clean screw cap glass tube. Distilled water (9 mL) and internal standard, taxifolin in methanol (500 μL; 0.1 mg/mL), was added and thoroughly mixed. The mixture was transferred into a vial (0.8 mL; Chromacol 08-CPV; ThermoFisher Scientific Ltd), closed with a crimp top and analysed by liquid chromatography-mass spectrometry (LC-MS) within 12 h.

1.4.3 Liquid chromatography-mass spectrometry (LC-MS) analysis

LC-MS was used for confirmation of the identity of flavan-3-ols in terminal and extension units using instrument specifications and conditions as previously described (Williams et al. 2014). Flavan-3-ols and their benzyl mercaptan adducts (-BM adducts) were identified by their retention times and characteristic UV-VIS spectra between 220 and 595 nm (Gea et al. 2011). Peak areas of flavan-3-ols at 280 nm were integrated and quantified using molar response factors relative to taxifolin: 0.30 for epicatechin; 0.26 for epicatechin-BM; 0.15 for epiafzelechin was determined with an authentic standard and the same was assumed for epiafzelechin-BM. This provided information on the proanthocyanidin composition in terms of % terminal and % extension flavan-3-ol units; it also allowed calculation of the mean degree of polymerisation (mDP), % procyanidins (PC) and propelargonidins (PP), and % cis- and trans-flavan-3-ols within the proanthocyanidins (Gea et al. 2011). Other phenolics concentrations compounds were determined in quercetin equivalent using four different concentrations (n = 2) for calibration curve.

1.4.4 NMR analysis

The F2-fraction (10 mg) was dissolved in 500 μL DMSO-d6 and transferred to a 5 mm NMR tube. 1H-13C correlation 2D NMR (HSQC) spectra were recorded at 27 °C on a Bruker Avance III 500 (1H 500.13 MHz, 13C 125.76 MHz) instrument equipped with TopSpin 2.4 software and a 5-mm BBI 1H/13C gradient probe (Bruker, Coventry, U.K.). Spectral resonances were referenced to the residual signals of DMSO-d6 (2.49 ppm for 1H and 39.5 ppm for 13C spectra). For 1H-13C HSQC experiments, spectra were obtained using 128 scans
and 1024 scans for $^{13}$C experiments.

References


Table S1. Assignment of other phenolic compounds detected in *S. alata* leaves (mg/g of DW in quercetin equivalent, SD in parentheses; n = 3). Peak numbers refer to Figure S1.

<table>
<thead>
<tr>
<th>Peak number</th>
<th>Compounds</th>
<th>MW</th>
<th>t_R (min)</th>
<th>MS fragment m/z</th>
<th>Concentration (mg/g DW)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Aloe- emodin-O-glucoside</td>
<td>432</td>
<td>23.3</td>
<td>465.3 [M – H]^- Cl^-</td>
<td>1.8 (0.1)</td>
<td>Martin et al. 1998</td>
</tr>
<tr>
<td>2</td>
<td>Physcion-O-glucoside</td>
<td>446</td>
<td>26.5</td>
<td>479.3 [M – H]^- Cl^-</td>
<td>4.4 (0.1)</td>
<td>Smith et al. 1979</td>
</tr>
<tr>
<td>4</td>
<td>Kaempferol-O-gentiobioside</td>
<td>610</td>
<td>29.8</td>
<td>609.4 [M – H]^-</td>
<td>6.9 (0.2)</td>
<td>Moriyama et al. 2003</td>
</tr>
<tr>
<td>6</td>
<td>Kaempferol-O-glucoside</td>
<td>448</td>
<td>32.9</td>
<td>447.3 [M – H]^-</td>
<td>1.6 (0.1)</td>
<td>Hazni et al. 2008</td>
</tr>
<tr>
<td>8</td>
<td>Kaempferol</td>
<td>286</td>
<td>43.8</td>
<td>285.2 [M – H]^-</td>
<td>6.6 (0.2)</td>
<td>Rahaman et al. 2006</td>
</tr>
</tbody>
</table>
Figure S1: Epiafzelechin
Figure S2. HPLC chromatogram after in situ thiolysis of proanthocyanidins from S. alata leaves: 1, aloe-emodin-O-glucoside; 2, physcion-O-glucoside; 3, epiafzelechin; 4, kaempferol-O-gentiobioside; 5, internal standard (taxifolin); 6, kaempferol-O-glucoside; 7, epiafzelechin-BM; 8, Kaempferol. BM: benzyl mercaptan (see Table S1 for retention times and MS fragments).
Figure S3. HPLC chromatogram after thiolysis of proanthocyanidins in the F2-fraction: 3, epiafzelechin; 5, internal standard (taxifolin); 7, epiafzelechin-BM; 8, kaempferol; 9, epicatechin-BM. BM: benzyl mercaptan.
Figure S4. $^1$H – $^{13}$C HSQC NMR spectrum of F2-fraction PA from *S. alata* leaves (DMSO-$d_6$, 500 MHz).
Figure S5. $^{13}$C NMR spectrum of F2-fraction PA from *S. alata* leaves (DMSO-$d_6$, 125 MHz).