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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 Introduction

Senna alata (L.) Roxb grows in Central America, Africa and the Caribbean area. The leaves are traditionally used as decoctions for dermatological applications (skin rashes, shingles (*Herpes zoster*), eczema, mycosis) and against constipation (Hennebelle et al. 2009). *S. alata* has exhibited antibacterial, antifungal, anti-diabetic and anti-inflammatory activities (Hennebelle et al. 2009; Sagnia et al. 2014). Apart from a few reports on flavonoids and anthraquinones, there is little information on their proanthocyanidin (PA) composition (Abii & Onuha 2014, Hennebelle et al. 2009), which is being reported in full here for the first time.

2 Results and discussion

2.1 Characterisation and flavan-3-ol composition of PA in S. alata

2.1.1 Analysis by thiolytic degradation

S. alata leaves were subjected to thiolysis to determine PA content and composition. Terminal and extension flavan-3-ol units within PA were analysed by HPLC-ESI-MS (see Supplementary) and PA content and composition are described in Table 1. Figure S2 illustrates the HPLC chromatogram of the thiolysis reaction applied directly to *S. alata* leaves. Several other phenolic compounds were also detected and assigned according to the literature (Table S1). The PA content was 2.5 g/100 g of dry weight (DW) and the mean degree of polymerisation (mDP) of the PA was 3. These PA were almost pure propelargonidins that consisted of epiafzelechin (EAz) (Figure S1, Figure S2, Table 1). Epiafzelechin was assigned to peak 3 at 29.3 min and generated fragment ions at *m/z* 309.3 $[M - H]^- Cl^-$ and *m/z* 273.3 $[M - H]^-$. The epiafzelechin-BM adduct was assigned to peak 7 at 44.9 min and generated fragment ions at *m/z* 431.3 $[M - H]^- Cl^-$, *m/z* 395.3 $[M - H]^-$ and after loss of the benzyl mercaptan molecule (– 124 amu) at *m/z* 271.2. Extractable PA (ePA) were analysed in the aqueous acetone extract and unextractable PA (uPA) in the plant residue after extraction. More ePA than uPA

were found (1.7 vs 0.7 g/100 g DW), but residual uPA had a slightly larger mDP-value (4.4 vs 3.4). Extraction removes impurities and can concentrate PA in the extract and residue; this appeared to facilitate a more sensitive PA analysis. As a result, low proportions of epicatechin were detected as extension units in ePA and uPA, although this was not detected during direct PA analysis of whole leaves. Slightly more epicatechin was detected in extension units of uPA than ePA (6.4 vs 2.4 %) (fragment ions at *m/z* 447.3 $[M - H]^- Cl^-$, *m/z* 411.3 $[M - H]^-$ and after loss of the benzyl mercaptan molecule (– 124 amu) at *m/z* 287.3). Sephadex™ LH-20 was used to purify the PA from the extract to confirm this PA characterisation and yielded two fractions. The F1-fraction had 34 g PA/100 g fraction and F2-fraction had 50 g PA/100 g fraction. PA in the F1-fraction contained only EAz and no epicatechin, but PA in the F2-fraction were slightly larger (mDP 6.4 vs 2) and epicatechin was detected as an extension unit (Figure S3).

2.1.2 NMR analysis of F2-fraction

The F2-fraction was subjected to NMR analysis–and the $^{1}H - ^{13}C$ HSQC spectrum clearly shows the presence of PP (Figure S1, Figure S4): signals at 6.7 ppm and 7.3 ppm were assigned to H/C-3'/5' (PP) and to H/C-2'/6' (PP), respectively. The signal at 4.7 ppm was assigned to H/C-4 and at 6.00 ppm to H/C-6 and H/C-8; which confirms that *S. alata* PA contains B-type linkages between $C_4 \rightarrow C_8$ (Appeldoorn et al. 2009). The signal at 5.1 ppm was assigned to H/C-2; and signals at 3.8 and 4.2 ppm to H/C-3. More than one signal was detected for H/C-3 and could be due to the *cis/trans* configurations at the C-ring (Foo et al. 2000). The same results are also visible in the ^{13}C-spectrum (Figure S5). Signals at 112.8 ppm (C-3'/5'), 125.4 ppm (C-2'/6') and 157.1 ppm (C4') belong to PP subunits. C-4, C-6, C-10 and C-8 were identified at 35.3, 96.2, 103.2 and 106.6 ppm as well as C-1' at 130 ppm and C-5/7/9 at 154.8 ppm. Signals at 65.0 and 72.1 ppm were attributed to terminal and extension units of *cis* and *trans*-flavan-3-ols for C-3. The signal at 76.7 ppm corresponds to C-2 from an extension unit (*cis*). The predominance of this signal over the C-3 signals from extension unit (*cis* + *trans*) strongly suggests that the stereochemistry of the PP subunit is mainly *cis* and confirms the thiolysis results and the *epi*-configuration. Moreover, no signal corresponding to a *trans* extension unit for C-2 was detected.

It remains to be seen whether the presence of these PP can account for the fact that *S. alata* leaves are used for skin treatments in traditional medicine: PP have a low hydroxylation pattern (just 1 OH-group in the B-ring, Figure 1) compared to procyanidins (PC, 2 groups) or prodelphinidins (PD, 3 groups). Dobreva (2012) showed that PC had lower affinities and enthalpies during PA-protein binding than PD. Therefore, PP can be expected to bind less strongly to skin proteins than most other PA, but the implications of this hypothesis will need further research. To conclude, plants with pure PP are relatively rare (Falleh et al. 2011, Van

Huynh & Bevington 2014) and *S. alata* leaves are a useful source of PP for research into their biological activities compared to other PA.

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Table 1. Total, extractable, unextractable and purified fractions of proanthocyanidins (PA) from *S. alata* leaves in terms of PA content (g PA/100 g DW), mean degree of polymerisation (mDP), percentages (%) of propelargonidins (PP), procyanidins (PC), and *cis*-flavan-3-ols. The flavan-3-ol composition of PA in terminal and extension units is shown separately (percentages are relative molar percentages; SD in parentheses; n = 3).

						Terminal unit	Extension unit	
	PA	mDP	% PP	% PC	% *cis*	EAz	EC	EAz
Total PA	2.5 (0.1)	3.4 (0.1)	100 (0)	0 (0)	100 (0)	29.6 (0.4)	0.0 (0.0)	70.5 (0.1)
Extractable PA	1.7 (0.1)	3.1 (0.1)	97.7 (0.1)	2.4 (0.1)	100 (0)	24.6 (0.4)	2.3 (0.1)	73.1 (0.5)
Unextractable PA	0.7 (0.1)	4.4 (0.1)	93.7 (0.6)	6.4 (0.6)	100 (0)	22.6 (0.1)	6.4 (0.0)	71.1 (0.1)
F1-fraction	34.1 (1.1)	2.0 (0.1)	100 (0)	0 (0)	100 (0)	33.9 (0.1)	0.0 (0.0)	66.1 (0.2)
F2-fraction	50.4 (0.1)	6.4 (0.1)	98 (0)	2 (0)	100 (0)	13.5 (0.1)	1.9 (0.1)	84.7 (0.1)