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Condensed tannins in extracts from European medicinal plants and herbal products

Honorata M. Ropiak*, Aina Ramsay, Irene Mueller-Harvey*

Chemistry and Biochemistry Laboratory, School of Agriculture, Policy and Development, University of Reading, P O Box 236, Reading RG6 6AT, UK

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ABSTRACT

Medicinal plant materials are not usually analysed for condensed tannins (CT). Thirty commercially available European medicinal plants and herbal products were screened for CT and fourteen CT samples were analysed in detail. This is also the first comprehensive CT analysis of pine buds, walnut leaves, heather flowers and great water dock roots. Acetone/water extracts contained between 3.2 and 25.9 g CT/100 g of extract, had CT with mean degrees of polymerisation of 2.9 to 13.3, procyanidin/prodelphinidin ratios of 1.6/98.4 to 100/0 and *cis*/*trans* flavan-3-ol ratios of 17.7/82.3 to 97.3/2.7. The majority of samples contained procyanidins, four contained A-type linkages (blackthorn flowers, heather flowers, bilberry leaves and cowberry leaves) and one sample also had galloylated procyanidins (great water dock roots).

1. Introduction

Folk medicine in Europe uses plants against a wide range of illnesses [1,2], as food or dietary supplements and as herbal products [3]. The most popular oral intake is via herbal infusion, decoction or as ethanol extracts [2]. Several beneficial actions of medicinal plants have been attributed to tannins [4,5], and their traditional uses include treatments of diarrhoea, heavy metal poisoning [2] or mild peptic ulceration [5]. Condensed tannins (CT, Fig. 1) are also of interest for their antimicrobial, antiviral and antitumour effects; and for their health benefits in cases of cardiovascular and inflammatory issues and effects on innate immune responses [6–8]. However, commercially available medicinal plants are not usually analysed for CT and the European Pharmacopeia recommends that all tannins be quantified simply as pyrogallol equivalents [9]; but this provides no accurate information on CT contents or composition. Detailed information on these well-known antioxidants [6] in medicinal plants could prove useful for research into their bioactivities, whether on their own or in combination with other plant compounds [2,10] and may also contribute to the stability of active ingredients. Therefore, we first screened several medicinal plants and herbal products that are widely used in European folk medicine. A subset of extracts from materials with the highest CT contents was then analysed in detail for their flavan-3-ol compositions [11].

2. Materials and methods

2.1. Reagents

Hydrochloric acid (37%, AR grade), butan-1-ol, acetic acid glacial (AR grade), acetone (AR grade), acetonitrile (HPLC grade), dichloromethane (LR grade), hexane (GLC, pesticide residue grade) and methanol (HPLC grade) were obtained from ThermoFisher Scientific (Loughborough, UK); benzyl mercaptan (99%), catechin hydrate (≥98%), epicatechin (90%), epigallocatechin (95%), gallocatechin (≥97%), catechin gallate (≥98%), epicatechin gallate (≥98%), epigallocatechin gallate (≥95%), gallocatechin gallate (≥98%), quercetin (≥99%), isoquercitrin (≥90%), rutin hydrate (≥95%), naringin (≥95%), (±)-eriodictyol (≥90%) from Sigma–Aldrich (Poole, UK); naringenin (97%) from Alfa Aesar (Lancashire, UK); (±)-taxifolin (98%) from Apin Chemicals (Abingdon, UK); procyanidin A2 (PC A2, ≥99%) and naringenin-7-*O*-glucoside (≥99%) from Extrasynthese (Genay Cedex, France); afzelechin (96-98%) from Plantech UK (Reading, UK) and Sephadex LH-20 from GE Healthcare (Little Chalfont, UK).

2.2. Plant materials

Pruni spinosae flos, Callunae vulgaris flos, Crataegi inflorescentia, Tiliae inflorescentia, Betulae folium, Myrtilli folium, Vitis idaeae folium, Ribis nigri folium, Salicis cortex, Lupuli flos, Hydrolapathi radix (from Poland) and *Pini gemmae* (typically from Ukraine) were obtained from Flos (Mokrsko, Poland); *Juglandis folium* (collected in June to August 2012, Poland) from Kawon (Gostyń, Poland); white clover

* Corresponding authors.
E-mail addresses: h.m.ropiak@reading.ac.uk (H.M. Ropiak), i.mueller-harvey@reading.ac.uk (I. Mueller-Harvey).

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flowers (*Trifolium repens* L., collected in April 2012, Poland) from Zioła z Kurpi (Jednorożec, Poland); (see Table 1). Details of other samples are in Table A.1 and Appendix A.1.1. Plant materials were purchased in 2012/2013 and ground to pass a 1 mm sieve (impeller SM1 cutting mill, Retsch, Haan, Germany). Leaves and stalks were removed from blackthorn flowers and only flowers were used.

2.3. Screening plant material for CT with HCl/butan-1-ol

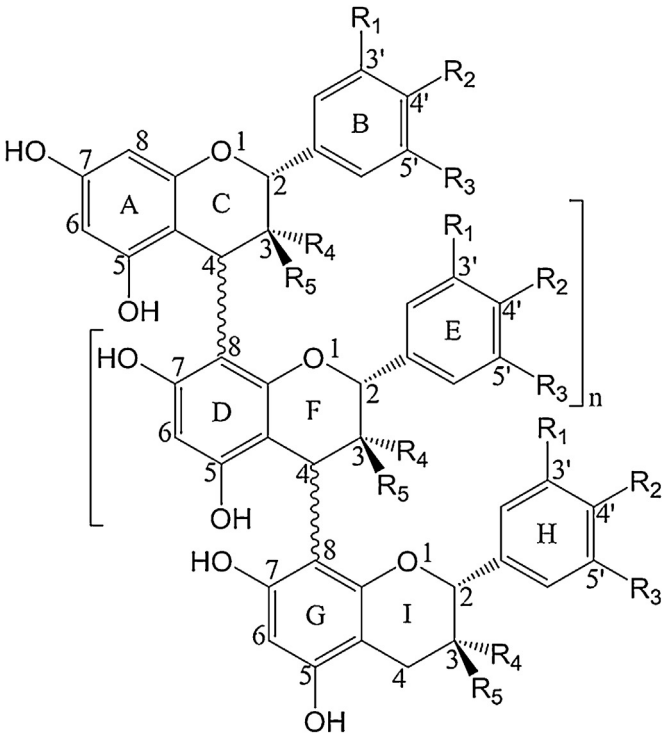
Plant materials were screened for CT presence with HCl/butan-1-ol [12] (see Appendix A.1.2).

2.4. Preparation of plant extracts

Acetone/water was used to prepare the CT extracts [11] (see Appendix A.1.3).

2.5. CT derivatisation with benzyl mercaptan, HPLC and LC–MS analysis

CT in extracts were derivatised with benzyl mercaptan in triplicate [11]. Samples were analysed within 48 h by HPLC using gradient 1 [13]. However, heather flowers, bilberry and cowberry leaf extracts were analysed with gradient 2 (solvent A: 1% acetic acid/Milli-Q H_2O; solvent B: acetonitrile) as follows: 0–52 min, 0–36% B; 52–60 min, 36–50% B; 60–65 min, 50–100% B; 65–73 min, 100-0% B; 73–80 min, 0% B). Flavan-3-ols and their benzyl mercaptan (-BM) adducts were identified [14] and quantified [11] using peak areas at 280 nm and molar response factors relative to taxifolin: 0.30 for catechin and epicatechin; 0.06 for gallocatechin and epigallocatechin; 0.26 for catechin-BM and epicatechin-BM; 0.06 for gallocatechin-BM and epigallocatechin-BM [14–16]; 0.55 for PC A2, PC A-type trimers and their corresponding -BM adducts [17]; and 1.01 for epicatechin gallate and epicatechin gallate-BM [18] (Appendix A.1.4, A.1.5 and Table A.3). LC–MS was used to confirm the identity of terminal and extension units; MS spectra were recorded in the negative and positive ionisation scan mode and UV spectra at 280 nm [13] (Table A.3 provides information on $[M-H]^-$ ions of each detected compound).



Flavan-3-ol subunits	R_1	R_2	R_3	R_4	R_5
Catechin	OH	OH	H	H	OH
Epicatechin	OH	OH	H	OH	H
Gallocatechin	OH	OH	OH	H	OH
Epigallocatechin	OH	OH	OH	OH	H

Fig. 1. Example of B-type condensed tannins.

$$\text{mDP (CT with B – type and A – type linkage)} = \frac{\Sigma(\text{B – type TU}) + \Sigma\ (\text{B – type EU}) + \Sigma\ (n \times \text{A – type TU}) + \Sigma\ (n \times \text{A – type EU})}{\Sigma(\text{B – type TU}) + (\text{A – type TU})} \quad (1)$$

2.6. Quantification of free flavan-3-ols

Extracts were also assayed for free flavan-3-ol monomers and their 3-*O*-galloylated derivatives as these interfere with the calculation of CT concentration and composition [19]. Extracts (4 mg) were dissolved in a mixture of methanol (2.05 ml), H_2O (2.5 ml) and the internal standard (taxifolin, 0.5 ml; 0.05 mg/ml in methanol), vortexed and centrifuged (5 min, 3000 rpm) prior to RP-HPLC or LC–MS analysis. Samples were analysed in duplicate within 24 h.

2.7. Calculation of CT composition

The mean degree of polymerisation (mDP)-value of B-type CT and galloylated B-type CT [14,20], molar percentages of galloylated flavan-3-ols [20], procyanidin/prodelphinidin (PC/PD) ratios and *cis*/*trans* flavan-3-ol ratios [14] were calculated as previously reported (see Appendix A.2 for equations to calculate CT composition); however, A-type units were not included in the calculations of *cis*/*trans* ratios. Flavan-3-ols in terminal and extension units [21] are reported as relative molar percentages.

The mDP-values of CT that had both B-type and A-type linkages were calculated according to Eq. (1), which is derived from a published formula for A-type dimers [7,22,23] and refers to molar ratios of terminal and extension flavan-3-ol units:

where TU—terminal unit; EU—extension unit; $n = 2$ or 3 and is the degree of polymerisation of terminal or extension units. The percentage of A-type linkages was calculated according to Eq. (2) [23] and takes A-type trimers into account:

$$\%\text{A – type linkage} = \frac{\Sigma(\text{A – type TU}) + \Sigma(\text{A – type EU})}{\Sigma(\text{A – type TU}) + \Sigma(\text{B – type EU}) + \Sigma(n \times \text{A – type EU})} \quad (2)$$

3. Results and discussion

Commercially available medicinal plants, and for that matter also other plants, are rarely analysed for CT contents or compositions, but these compounds are of interest as they have been implicated in numerous health effects [6–8]. Such information could be useful when searching for CT bioactivities or combination effects with other compounds that might be linked to their traditional uses. The main uses in traditional medicine of the samples investigated here are for treating minor urinary tract infections, feverish colds or mild rheumatism (Table 1).