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# 1 Condensed tannins in extracts from European medicinal plants and herbal products

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9

## 10 ABSTRACT

11 Medicinal plant materials are not usually analysed for condensed tannins (CT). Thirty

12 commercially available European medicinal plants and herbal products were screened for CT

13 and fourteen CT samples were analysed in detail. This is also the first comprehensive CT

14 analysis of pine buds, walnut leaves and heather flowers and great water dock roots.

15 Acetone/water extracts contained between 3.2 and 25.9 g CT/100 g of extract, had CT with mean

16 degrees of polymerisation of 2.9 to 13.3, procyanidin/prodelphinidin ratios of 1.6/98.4 to 100/0

17 and *cis/trans* flavan-3-ol ratios of 17.7/82.3 to 97.3/2.7. The majority of samples contained

18 procyanidins, four contained A-type linkages (blackthorn flowers, heather flowers, bilberry

19 leaves and cowberry leaves) and one sample also had galloylated procyanidins (great water dock

20 roots).

21

22 *Keywords:* Proanthocyanidins, Flavan-3-ols, Molar response factors, Thiolytic, Mean degree of

23 polymerization

24

25

26

## 27 **1. Introduction**

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

44

## 45 **2. Materials and methods**

### 46 *2.1. Reagents*

47 Hydrochloric acid (37%, AR grade), butan-1-ol, acetic acid glacial (AR grade), acetone (AR  
48 grade), acetonitrile (HPLC grade), dichloromethane (LR grade), hexane (GLC, pesticide residue  
49 grade) and methanol (HPLC grade) were obtained from ThermoFisher Scientific  
50 (Loughborough, UK); benzyl mercaptan (99%), catechin hydrate ( $\geq 98\%$ ), epicatechin (90%),  
51 epigallocatechin (95%), galocatechin ( $\geq 97\%$ ), catechin gallate ( $\geq 98\%$ ), epicatechin gallate  
52 ( $\geq 98\%$ ), epigallocatechin gallate ( $\geq 95\%$ ), galocatechin gallate ( $\geq 98\%$ ), quercetin ( $\geq 99\%$ ),

53 isoquercitrin ( $\geq 90\%$ ), rutin hydrate ( $\geq 95\%$ ), naringin ( $\geq 95\%$ ), ( $\pm$ )-eriodictyol ( $\geq 90\%$ ) from  
54 Sigma-Aldrich (Poole, UK); naringenin (97%) from Alfa Aesar (Lancashire, UK); ( $\pm$ )-taxifolin  
55 (98%) from Apin Chemicals (Abingdon, UK); procyanidin A2 (PC A2,  $\geq 99\%$ ) and naringenin-  
56 7-*O*-glucoside ( $\geq 99\%$ ) from Extrasynthese (Genay Cedex, France); afzelechin (96-98%) from  
57 Plantech UK (Reading, UK) and Sephadex LH-20 from GE Healthcare (Little Chalfont, UK).

58

## 59 2.2. Plant materials

60 *Pruni spinosae flos*, *Callunae vulgaris flos*, *Crataegi inflorescentia*, *Tiliae inflorescentia*,  
61 *Betulae folium*, *Myrtilli folium*, *Vitis idaeae folium*, *Ribis nigri folium*, *Salicis cortex*, *Lupuli flos*,  
62 *Hydrolapathi radix* (from Poland) and *Pini gemmae* (typically from Ukraine) were obtained  
63 from Flos (Mokrsko, Poland); *Juglandis folium* (collected in June to August 2012, Poland) from  
64 Kawon (Gostyń, Poland); white clover flowers (*Trifolium repens* L., collected in April 2012,  
65 Poland) from Zioła z Kurpi (Jednorozec, Poland); (see Table 1). Details of other samples are in  
66 Table A1 and Appendix A.1.1. Plant materials were purchased in 2012/2013 and ground to pass  
67 a 1 mm sieve (impeller SM1 cutting mill, Retsch, Haan, Germany). Leaves and stalks were  
68 removed from blackthorn flowers and only flowers were used.

69

## 70 2.3. Screening plant material for CT with HCl/butan-1-ol

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

72

## 73 2.4. Preparation of plant extracts

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

75

## 76 2.5. CT derivatisation with benzyl mercaptan, HPLC and LC-MS analysis

77 CT in extracts were derivatised with benzyl mercaptan in triplicate [11]. Samples were analysed  
78 within 48 h by HPLC using gradient 1 [13]. However, heather flowers, bilberry and cowberry

79 leaf extracts were analysed with gradient 2 (solvent A: 1% acetic acid/Milli-Q H<sub>2</sub>O; solvent B:  
80 acetonitrile) as follows: 0-52 min, 0-36% B; 52-60 min, 36-50% B; 60-65 min, 50-100% B; 65-  
81 73 min, 100-0% B; 73-80 min, 0% B). Flavan-3-ols and their benzyl mercaptan (-BM) adducts  
82 were identified [14] and quantified [11] using peak areas at 280 nm and molar response factors  
83 relative to taxifolin: 0.30 for catechin and epicatechin; 0.06 for gallocatechin and  
84 epigallocatechin; 0.26 for catechin-BM and epicatechin-BM; 0.06 for gallocatechin-BM and  
85 epigallocatechin-BM [14-16]; 0.55 for PC A2, PC A-type trimers and their corresponding -BM  
86 adducts [17]; and 1.01 for epicatechin gallate and epicatechin gallate-BM [18] (Appendix A.1.4,  
87 A.1.5 and Table A.3). LC-MS was used to confirm the identity of terminal and extension units;  
88 MS spectra were recorded in the negative and positive ionisation scan mode and UV spectra at  
89 280 nm [13] (Table A.3 provides information on [M-H]<sup>-</sup> ions of each detected compound).

90

#### 91 2.6. *Quantification of free flavan-3-ols*

92 Extracts were also assayed for free flavan-3-ol monomers and their 3-*O*-galloylated derivatives  
93 as these interfere with the calculation of CT concentration and composition [19]. Extracts (4 mg)  
94 were dissolved in a mixture of methanol (2.05 ml), H<sub>2</sub>O (2.5 ml) and the internal standard  
95 (taxifolin, 0.5 ml; 0.05 mg/ml in methanol), vortexed and centrifuged (5 min, 3000 rpm) prior to  
96 RP-HPLC or LC-MS analysis. Samples were analysed in duplicate within 24 h.

97

#### 98 2.7. *Calculation of CT composition*

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

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

$$108 \quad \text{mDP}_{\text{(CT with B-type and A-type linkage)}} = \frac{\sum(\text{B-type TU}) + \sum(\text{B-type EU}) + \sum(n \times \text{A-type TU}) + \sum(n \times \text{A-type EU})}{\sum(\text{B-type TU}) + (\text{A-type TU})}$$

109 Equation 1

110 where: TU – terminal unit; EU – extension unit; n = 2 or 3 and is the degree of polymerisation of  
 111 terminal or extension units. The percentage of A-type linkages was calculated according to  
 112 Equation 2 [23] and takes A-type trimers into account:

$$113 \quad \%A - \text{type linkage} = \frac{\sum(\text{A-type TU}) + \sum(\text{A-type EU})}{\sum(\text{A-type TU}) + \sum(\text{B-type EU}) + \sum(n \times \text{A-type EU})}$$

114 Equation 2

115

### 116 **3. Results and discussion**

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

123

#### 124 *3.1. Analysis of CT*

125 Initial screening with HCl/butan-1-ol revealed the presence of CT in 20 of the 30 plant materials  
 126 (Table A.2). Samples with >3 g CT/100 g extract were selected for further analysis (see  
 127 Appendix A.3).

128

##### 129 *3.1.1. Discussion of response factors for quantifying terminal and extension units in CT*

130 Response factors relative to taxifolin at 280 nm are widely used for CT analysis after  
131 depolymerisation with benzyl mercaptan [14-16, 24]. However, the literature contains several  
132 different response factors for flavan-3-ol terminal and extension units [15, 16] and these can be  
133 affected to some extent by analysis conditions [25]. Some authors have also quantified extension  
134 units with response factor ratios against the epicatechin-BM adduct [26] by using the molar  
135 response factors reported previously [27]. Others have reported that catechin and epicatechin had  
136 the same molar response factors as their corresponding -BM adducts [28] or had relatively  
137 similar factors as in the case of epigallocatechin and its -BM adduct [27]. The relative molar  
138 responses of flavan-3-ols against taxifolin were, therefore, checked and found to be close to  
139 previous reports [14, 15, 24] (see Materials and Methods 2.5).

140 Although mass responses of B-type dimers and trimers relative to epicatechin were similar, i.e.  
141 0.96 and 1.04 [29], no information exists on the relative mass or molar response factors of A-  
142 type dimers and trimers against taxifolin. Two reports stated that the molar absorptivity of the  
143 epicatechin dimer (PC A2) was not equal to two times the molar absorptivity of epicatechin at  
144 280 nm [30, 31]. Indeed, we found a mass response factor of 0.29 and a molar response factor of  
145 0.55 for the PC A2 dimer against taxifolin, and used the molar response in this study. This is in  
146 line with other work, where the relative molar response was twice that of the corrected relative  
147 mass response of PC A2 against epicatechin [30]. Finally, the same relative molar response  
148 factors were used for the A-type dimer- and trimer-BM adducts in line with a previous report on  
149 A-type dimers and their BM adducts [23].

150

### 151 *3.1.3. Characterisation of B-type CT*

152 Overall, CT contents ranged from 3.2 to 20.2 g CT/100 g extract, mDP-values from 4.2 to 13.3,  
153 PC/PD ratios from 1.6/98.4 to 100/0 and *cis/trans* flavan-3-ol ratios from 17.7/82.3 to 97.3/2.7  
154 (Table 2). Interestingly, most samples contained only B-type PC, i.e. extracts from hawthorn  
155 flowers, hop strobiles, *Tilia* flowers, willow bark and walnut leaves. Only the extract from great



156 water dock roots had CT with galloylated flavan-3-ol subunits. White clover flowers had the  
157 highest percentage of PD (98.4%). *Cis*-flavan-3-ols accounted for >90% of the CT subunits in  
158 extracts from *Tilia* flowers, great water dock roots and hawthorn flowers; and blackcurrant  
159 leaves had the highest percentage of *trans*-flavan-3-ols (82.3%).  
160 This is the first report of the CT flavan-3-ol composition from pine buds and walnut leaf  
161 extracts: pine bud CT had an mDP of 7.8, a PC/PD ratio of 61.5/38.5 and a *cis/trans* flavan-3-ol  
162 ratio of 70.7/29.3 (Table 2); walnut leaf CT had an mDP of 5.1, consisted only of PC with a  
163 *cis/trans* flavan-3-ol ratio of 62.6/37.4. In the following paragraphs, we compare our results  
164 from medicinal and herbal products with literature data.  
165 White clover flowers contained CT with the highest mDP-value (13.3), the highest PD  
166 percentage (98.4%) and a moderate *cis/trans* ratio (61.1/38.9; Table 2). This closely resembles  
167 previous results where PD percentage was 99%, *cis/trans* ratio of 66/34; however, the mDP of  
168 4.4 was much lower [13]. Epigallocatechin was the main extension unit and galocatechin was  
169 the only terminal unit (Table 3). The blackcurrant leaf extract also contained CT that consisted  
170 mostly of PD (95.3%), had an mDP of 6.0 and a *cis/trans* ratio of 17.7/82.3 (Table 2). Whilst the  
171 mDP-value and PD percentage were similar to previous report (5.4 and 94.2, respectively), the  
172 *cis/trans* ratio differed noticeably (9.1/90.9) [13]. The birch leaf extract had mixed CT with, a  
173 PC/PD ratio of 58.9/41.1, an mDP of 4.2 and a *cis/trans* ratio of 62.9/37.1. These CT contained  
174 catechin as the main terminal unit and epicatechin as the main extension unit.  
175 The hawthorn flower extract had only PC with an mDP of 4.8 and a *cis/trans* ratio of 97.3/2.7  
176 (Table 2). Epicatechin was found in extension units and both catechin and epicatechin in  
177 terminal units (Table 3). Willow bark extract is one of the most studied medicinal plant  
178 preparations due to its anti-inflammatory and pain relieving effects [10]. In agreement with  
179 others, these CT consisted of pure PC [32] and with a low mDP-value (4.6). Catechin was  
180 mainly in the terminal position [32] and the *cis/trans* ratio was 68.7/31.3. Hop strobiles had pure  
181 PC in agreement with the literature [32], although LC-MS analysis detected traces of

182 gallo catechin and epigallocatechin in extension units (Table 3). These CT had an mDP of 5.1  
183 and a *cis/trans* ratio of 64.0/36.0 (Table 2). The *Tilia* flower extract also had pure PC with an  
184 mDP of 5.8, which concurs with a report that described PC oligomers up to pentamers [33].  
185 These CT had a very high proportion of *cis*-flavan-3-ols (90.6%).  
186 Of particular interest was the great water dock root (*Rumex hydrolapathum*) extract as it had the  
187 highest CT content (25.9 g/100 g extract), a high percentage of *cis*-flavan-3-ols (94.7%) and PC  
188 that contained 52% of the flavan-3-ols as gallate esters (Table 2). The chromatographic profile  
189 (Fig. A.2-A) was similar to CT from *R. obtusifolius* leaves [24]. *R. hydrolapathum* roots and *R.*  
190 *obtusifolius* leaves contained only PC with similar mDP-values (6.2 vs. 4.3) [24]. However, the  
191 extent of galloylation was much higher in *R. hydrolapathum* roots (52 vs. 8%) [24].

192

### 193 3.1.4. Characterisation of A-type CT in extracts

194 A-type linkages were only found amongst PC from blackthorn flowers, cowberry leaves,  
195 bilberry leaves, heather flowers and accounted for 25.3, 17.1, 7.3, and 6.7% of the CT,  
196 respectively (Table 2; Fig. A.2-B, C, D and E). A-type linkages are usually released by thiolysis  
197 as A-type dimers from terminal units or as A-type dimer-BM adducts from extension units [19,  
198 28]. Although one study also reported the release of an A-type trimer from extension units [34].  
199 The PC from blackthorn flower extract had a particularly low mDP (2.9) and a moderate  
200 *cis/trans* ratio (67.0/33.0), but had the highest percentage of A-type linkages (25.3%) which  
201 were present in terminal units (Table 2 and 3). Cowberry leaf extract had PC with an mDP of  
202 6.5, a *cis/trans* ratio of 68.5/31.5 and 17.1% of the flavan-3-ols had A-type linkages (Table 2).  
203 The presence of pure PC agrees with previous report [35]. A-type dimers occurred in terminal  
204 units and both A-type dimers were released from extension units (Table 3); somewhat unusually,  
205 A-type trimers also came from extension units. Bilberry leaf extract had PC with an mDP of 6.6,  
206 a *cis/trans* ratio of 92.2/7.8 and a low percentage of A-type linkages (7.3%, Table 2). A-type  
207 trimers were detected as terminal units and both A-type dimers and trimers as extension units.

208 However, no gallicocatechin and epigallocatechin were detected in our sample and this suggests  
209 that the percentages of PD can vary in bilberry leaves [35].  
210 The flavan-3-ol composition of CT from heather flower extract is reported here for the first time.  
211 These PC had an mDP of 7.2 and a *cis/trans* ratio of 87.5/12.5; and 6.7% of flavan-3-ols were  
212 present in A-type linkages. A-type trimers were detected as terminal units and A-type dimers as  
213 extension units.

214

### 215 3.2. Other flavonoids

216 Whilst this work focussed on CT we also provide information on a few flavonoids (rutin,  
217 quercetin and quercetin-hexoside) in the Appendix (Table A.3 and Fig. A.1 and A.2). Of  
218 particular interest here is an unusual observation in the thiolysed willow bark sample. This  
219 solution contained a major peak at 44.6 min that yielded an  $[M-H]^-$  ion at  $m/z$  of 271 (Fig. A.1-I)  
220 and a UV/VIS spectrum and retention time that matched authentic naringenin. However, this  
221 compound was not present in the original extract, which had contained two distinctive peaks at  
222 32.4 and 32.9 min that gave rise to  $[M-H]^-$  ions at  $m/z$  433,  $[M+H]^+$  ions at  $m/z$  435 and cleavage  
223 products of  $m/z$  273 (Table A.3). Both peaks were reduced to two minor peaks after thiolysis  
224 (Fig. A.1-J), which probably suggests that the naringenin peak was generated during thiolysis.  
225 However, authentic naringenin-7-*O*-glucoside (with a retention time of 34.5 min) was not  
226 cleaved during thiolysis. Given that naringenin, naringenin-7-*O*-glucoside and ( $\pm$ )-naringenin-5-*O*-  
227 glucoside (as two peaks) were previously detected in willow bark [10], we propose that,  
228 surprisingly, ( $\pm$ )-naringenin-5-*O*-glucoside was degraded under the relatively mild conditions of  
229 this thiolysis reaction.

230

## 231 4. Conclusions

232 Condensed tannins were characterised in acetone/water extracts from fourteen widely used  
233 medicinal plants and herbal products. Ten samples had pure procyanidins, two had almost pure  
234 prodelphinidins, and another two had CT as mixtures of procyanidins and prodelphinidins. Four  
235 extracts also contained A-type procyanidins and one extract had CT with 52% of the flavan-3-ol  
236 units as galloylated derivatives. To our knowledge, this is the first detailed analysis of CT in  
237 extracts from pine buds, walnut leaves, great water dock roots and heather flowers. Tannins  
238 occur in >80 dicotyledonous plant families [36]; however, information on tannin composition is  
239 generally hard to find, as their analysis is not trivial [31]. In contrast to food databases [37],  
240 European medicinal plants are not generally screened for CT contents or compositions [9]  
241 despite the fact that information on CT in medicinal plants would present opportunities for  
242 studying their health effects and could add useful information to a medicinal plant database.  
243 Such a database could provide a valuable tool for research into CT bioactivities or on their  
244 additive or synergistic effects with other compounds. In addition, it could contribute to the  
245 standardisation and quality control of herbal products.

246

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252

#### 253 **Appendix A. Supplementary data**

254

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**Table 1** Traditional uses of medicinal plant and herbal product samples investigated in this study.

English vernacular name and part used	Latin name of herbal substance	Botanical name(s)	Examples of pharmacological activities/indications (in single form for traditional uses)	Manufacturers' directions of use in form of infusion or decoction
Bilberry leaves (red)	<i>Myrtilli folium</i> <sup>b*, c</sup>	<i>Vaccinium myrtillus</i> L. <sup>b, d</sup>	-	Relaxing and soothing (bath) <sup>g</sup>
Birch leaves	<i>Betulae folium</i> <sup>b, c</sup>	<i>Betula pendula</i> Roth and/or <i>Betula pubescens</i> Ehrh. <sup>a, b</sup> ( <i>Betula</i> spp. <sup>d</sup> )	Rheumatic complains, urinary illness (irrigation) [1]	Mild urinary tract infections <sup>e</sup>
Blackcurrant leaves	<i>Ribis nigri folium</i> <sup>b, c</sup>	<i>Ribes nigrum</i> L. <sup>a, b</sup>	Diuretic, inflammatory disorders such as rheumatic conditions, spasmodic cough, colic, diarrhoea and topically to aid wounds [3]	Mild anti-rheumatic <sup>e</sup>
Blackthorn flowers	<i>Pruni spinosae flos</i> <sup>c</sup>	<i>Prunus spinosa</i> L.	-	General health benefits <sup>f</sup>
Cowberry leaves	<i>Vitis idaeae folium</i> <sup>c</sup>	<i>Vaccinium vitis-idaea</i> L.	-	Diuretic for mild urinary tract inflammation, renal calculus <sup>e</sup>
Great water dock roots	<i>Hydrolapathi radix</i> <sup>c</sup>	<i>Rumex hydrolapathum</i> Huds.	-	Relaxing and soothing (bath) <sup>g</sup>
Hawthorn flowers	<i>Crataegi inflorescentia</i> <sup>c</sup>	<i>Crataegus laevigata</i> Poir. <sup>a</sup> (leaves with flowers of <i>Crataegus</i> spp. <sup>d</sup> )	Reduction of cardiac performance (leaves with flowers – more recent use) [3]	Fatigue, initial mild cardiac failure where medication is not required <sup>e</sup>
Heather flowers	<i>Callunae vulgaris flos</i> <sup>c</sup>	<i>Calluna vulgaris</i> (L.) Hull. (leaves with flowers from inflorescence) <sup>d</sup>	Cystitis, urinary infections, rheumatism (leaves with flowers from inflorescence) [1]	Dietary supplement <sup>f</sup>
Hop strobiles	<i>Lupuli flos</i> <sup>b, c</sup>	<i>Humulus lupulus</i> L. <sup>a, b, d</sup>	Sedative, hypnotic, bactericidal (topically) [38] insomnia, excitability [1] neuralgia, priapism, mucous colitis, crural ulcers, restlessness (due to nervous tension headache and/or indigestion) [3]	Insomnia, nervous tension, calming <sup>e</sup>
Lime tree ( <i>Tilia</i> ) flowers	<i>Tiliae inflorescentia</i> <sup>c</sup> ( <i>Tiliae flos</i> <sup>b</sup> )	<i>Tilia cordata</i> Miller, <i>Tilia platyphyllos</i> Scop., <i>Tilia x vulgaris</i> Heyne or their mixtures <sup>a</sup> ( <i>Tilia</i> spp. <sup>d</sup> )	Sedative, antihypertensive [38], migraine, feverish cold [1], hysteria, arteriosclerotic hypertension, arterial pressure (due to arteriosclerosis and nervous tension) [3]	Feverish cold, diaphoretic <sup>e</sup>

Pine buds	<i>Pini gemmae</i> <sup>c</sup>	<i>Pinus</i> spp.	-	Illness of upper respiratory tract, mucolytic agent <sup>e</sup>
Walnut leaves	<i>Juglandis folium</i> <sup>b, c</sup>	<i>Juglans regia</i> L. <sup>b, d</sup>	Mild inflammation of skin, excessive perspiration of hand/feet (external use) [1]	Mild inflammation of skin, excessive perspiration of hand/feet (external) <sup>e</sup>
White clover flowers	<i>Trifolii albi flos</i>	<i>Trifolium repens</i> L.	-	Relaxing and soothing (bath) <sup>g</sup>
Willow bark	<i>Salicis cortex</i> <sup>b, c</sup>	<i>Salix</i> spp. (various including <i>S. purpurea</i> L.; <i>S. daphnoides</i> Vill.; <i>S. fragilis</i> L.) <sup>a, b</sup>	Anti-inflammatory, anti-rheumatic [38] muscular and arthrodiar rheumatism with pain and inflammation, gouty and rheumatoid arthritis, systemic connective tissue disorders with inflammation, influenza, respiratory catarrh, ankylosing spondylitis [3]	Feverish illness, mild anti-rheumatic <sup>e</sup>

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Note: <sup>a</sup> – [9], <sup>b</sup> – [39], <sup>b\*</sup> – no final opinion [39], <sup>c</sup> – as described by manufacturer, <sup>d</sup> – [1]; sold by manufacturer as either: <sup>e</sup> – medicinal product, <sup>f</sup> – dietary supplement or <sup>g</sup> – herbal product.

**Table 2** Condensed tannin (CT) contents, mean degree of polymerisation (mDP), procyanidin/prodelphinidin (PC/PD) and *cis*-/*trans*-flavan-3-ol ratios, percentages of galloylation and A-type linkages in aqueous acetone extracts of medicinal plants or herbal products.

Extract derived from	CT (g/100 g extract)	mDP	PC / PD	<i>cis</i> / <i>trans</i>	% galloylation
Great water dock roots	25.9 ± 0.7	6.2 ± 0.1	100.0 / 0.0 ± 0.1	94.7 / 5.3 ± 0.1	52.0 ± 0.1
					% A-type bond
Cowberry leaves	16.6 ± 0.4	6.5 ± 0.0	100.0 / 0.0 ± 0.0	68.5 / 31.5 ± 0.1	17.1 ± 0.0
Heather flowers	19.3 ± 0.4	7.2 ± 0.1	100.0 / 0.0 ± 0.1	87.5 / 12.5 ± 0.1	6.7 ± 0.1
Bilberry leaves	12.2 ± 0.5	6.6 ± 0.0	100.0 / 0.0 ± 0.0	92.2 / 7.8 ± 0.0	7.3 ± 0.0
Blackthorn flowers	4.0 ± 0.2	2.9 ± 0.1	100.0 / 0.0 ± 0.1	67.0 / 33.0 ± 0.2	25.3 ± 0.1
Hawthorn flowers	11.5 ± 0.4	4.8 ± 0.0	100.0 / 0.0 ± 0.0	97.3 / 2.7 ± 0.0	
Hop strobiles <sup>a</sup>	3.2 ± 0.1	5.1 ± 0.1	100.0 / 0.0 ± 0.1	64.0 / 36.0 ± 0.1	
<i>Tilia</i> flowers	19.5 ± 0.4	5.8 ± 0.1	100.0 / 0.0 ± 0.0	90.6 / 9.4 ± 0.0	
White clover flowers <sup>a</sup>	13.1 <sup>b</sup> ± 0.6	13.3 ± 0.1	1.6 / 98.4 ± 0.1	61.1 / 38.9 ± 0.1	
Pine buds	4.8 ± 0.3	7.8 ± 0.1	61.5 / 38.5 ± 0.0	70.7 / 29.3 ± 0.0	
Birch leaves <sup>a</sup>	4.8 ± 0.1	4.2 ± 0.1	58.9 / 41.1 ± 0.0	62.9 / 37.1 ± 0.0	
Blackcurrant leaves <sup>a</sup>	20.2 <sup>b</sup> ± 0.7	6.0 ± 0.1	4.7 / 95.3 ± 0.0	17.7 / 82.3 ± 0.0	
Willow bark	14.6 ± 0.7	4.6 ± 0.1	100.0 / 0.0 ± 0.1	68.7 / 31.3 ± 0.1	
Walnut leaves	5.9 ± 0.3	5.1 ± 0.1	100.0 / 0.0 ± 0.1	62.6 / 37.4 ± 0.1	

Note: <sup>a</sup> – no free flavan-3-ols detected, <sup>b</sup> – previously reported [11].

**Table 3** Composition of condensed tannins in terms of flavan-3-ols in terminal and extension units (as molar percentages).

Extract derived from	Flavan-3-ols (%)													
	GC	EGC	C	EC	GC -BM	EGC -BM	C -BM	EC -BM	ECg	ECg -BM				
Great water dock roots	0.0	0.0	5.3	2.4	0.0	0.0	0.0	40.3	8.5	43.5				
±	0.0	0.0	0.1	0.1	0.0	0.0	0.0	0.1	0.1	0.1				
									PC A-type trimer I	PC A-type trimer II	PC A-type dimer I	PC A-type dimer II	PC A-type trimer -BM	PC A-type dimer -BM
Cowberry leaves	0.0	0.0	9.0	2.6	0.0	0.0	16.8	53.7	0.0	1.5	5.8	0.4	5.8	4.4
±	0.0	0.0	0.1	0.1	0.0	0.0	0.2	0.1	0.0	0.1	0.0	0.1	0.0	0.1
Heather flowers	0.0	0.0	8.8	4.2	0.0	*	2.9	78.0	1.9	0.5	0.0	0.0	0.0	3.7
±	0.0	0.0	0.1	0.1	0.0	0.0	0.1	0.1	0.1	0.1	0.0	0.0	0.0	0.1
Bilberry leaves	0.0	0.0	2.1	13.3	0.0	0.0	5.3	72.7	0.0	1.3	0.0	0.0	2.5	2.9
±	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0
Blackthorn flowers	0.0	0.0	18.6	3.6	0.0	0.0	7.9	50.2	0.0	0.0	15.9	3.9	0.0	0.0
±	0.0	0.0	0.2	0.2	0.0	0.0	0.1	0.2	0.0	0.0	0.1	0.3	0.0	0.0
Hawthorn flowers	0.0	0.0	2.7	18.0	0.0	0.0	*	79.3						
±	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0						
Hop strobiles <sup>a</sup>	0.0	0.0	16.6	3.0	*	*	19.4	61.0						
±	0.0	0.0	0.1	0.1	0.0	0.0	0.1	0.1						
<i>Tilia</i> flowers	0.0	0.0	4.2	13.1	0.0	0.0	5.2	77.5						
±	0.0	0.0	0.1	0.1	0.0	0.0	0.1	0.0						
White clover flowers <sup>a</sup>	7.5	*	0.0	0.0	30.8	60.1	0.0	1.0						
±	0.1	0.0	0.0	0.0	0.1	0.1	0.0	0.1						
Pine buds	*	0.0	12.8	0.0	*	38.5	16.5	32.3						
±	0.0	0.0	0.1	0.0	0.0	0.0	0.1	0.0						

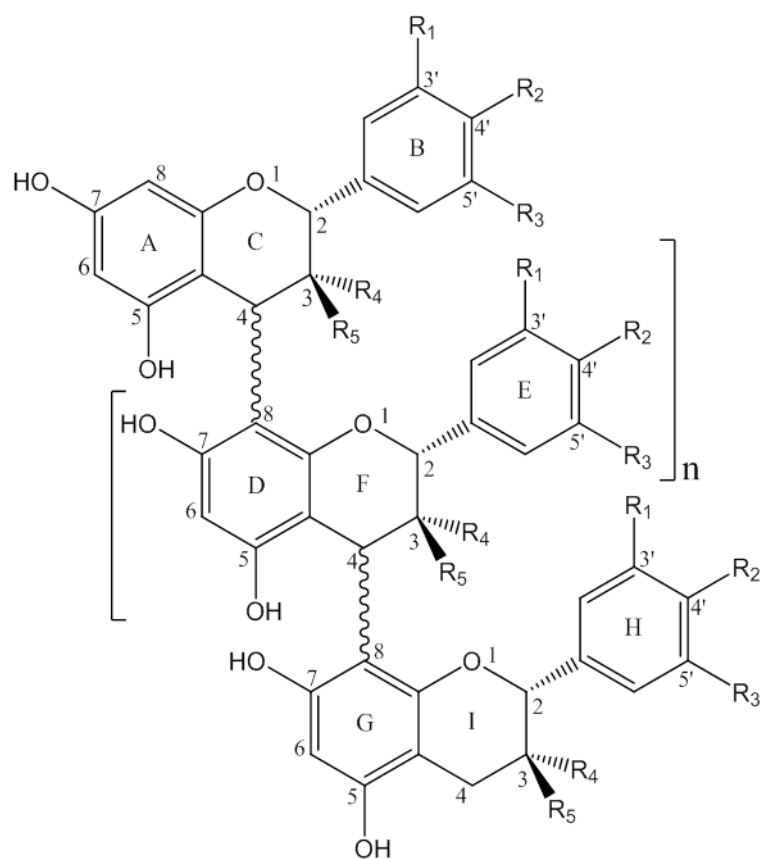
Birch leaves <sup>a</sup>	9.0	0.0	14.9	0.0	6.5	25.6	6.7	37.2
±	0.1	0.0	0.1	0.0	0.2	0.0	0.1	0.0
Blackcurrant leaves <sup>a</sup>	12.7	2.2	1.6	0.0	66.2	14.2	1.9	1.2
±	0.1	0.1	0.1	0.0	0.1	0.1	0.0	0.1
Willow bark	0.0	0.0	21.7	*	0.0	0.0	9.6	68.7
±	0.0	0.0	0.1	0.0	0.0	0.0	0.2	0.1
Walnut leaves	0.0	0.0	19.7	0.0	0.0	0.0	17.7	62.6
±	0.0	0.0	0.1	0.0	0.0	0.0	0.1	0.1

Note: <sup>a</sup> – no free flavan-3-ols detected; \* – trace amounts detected; GC – gallocatechin, EGC – epigallocatechin, C – catechin, EC – epicatechin, GC-BM – gallocatechin benzyl mercaptan adduct, EGC-BM – epigallocatechin benzyl mercaptan adduct, C-BM – catechin benzyl mercaptan adduct, EC-BM – epicatechin benzyl mercaptan adduct, ECg – epicatechin gallate, ECg-BM – epicatechin gallate benzyl mercaptan adduct, PC – procyanidins.

## **Figure Caption**

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

**Figure 1**



Flavan-3-ol subunits	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	R <sub>4</sub>	R <sub>5</sub>
Catechin	OH	OH	H	H	OH
Epicatechin	OH	OH	H	OH	H
Gallocatechin	OH	OH	OH	H	OH
Epigallocatechin	OH	OH	OH	OH	H



## **Appendix A.**

## **A.1. Material and methods**

### *A.1.1 Other plant materials*

Plant material of *Robiniae flos*, *Plantaginis maioris folium*, *Morus alba* L. *folium*, *Salviae folium*, *Menthae piperitae folium*, *Crataegi fructus*, *Pruni spinosae fructus*, *Sambuci fructus*, *Urticae radix*, *Frangulae cortex* *Calami rhizoma* (country of origin: Poland) and *Myrtilli fructus* (typical country of origin: Poland and Albania) was obtained from Flos (Mokrsko, Poland; GMP and GLP standard compliant); *Juniperi fructus* (collected in November to December 2012, Poland), *Aroniae fructus* (collected in August to September 2012, Poland) from Kawon (Gostyń, Poland; ISO 9001:2000 and HACCP compliant); *Linum usitatissimum* L. (golden linseeds variety, collected in July to August 2011 and/or 2012 from various European countries, de-fatted and milled in Poland) and *Chamomillae anthodium* (collected in June to July 2011 and/or 2012, Poland) from Herbapol (Lublin, Poland; GMP, BRC Global Standard Food and ECO compliant). Roots and fruits were ground first to pass a 5 mm and then a 1 mm sieve. Plant materials were stored in the dark at room temperature.

### *A.1.2 Screening of plant materials for CT with HCl/butan-1-ol*

Plant materials were first screened for CT presence with HCl/butan-1-ol in duplicate [12]. HCl/butan-1-ol (5 ml, 5:95 v/v) was added to the plant material (50 mg) in a 10 ml test tube and heated at 95 °C for 60 min. Corresponding blanks were kept at room temperature to check for the presence of flavan-4-ols or flavan-3,4-diols [40]. Plant materials that gave dark red colour were used for preparing CT extracts (see Table A.2).

### *A.1.3 Preparation of plant extracts*

Acetone/water was used to prepare the CT extracts [11]. Plant material (20 g) was extracted once with 70% acetone/H<sub>2</sub>O (250 ml) by stirring for 60 min and filtered under vacuum.

Chlorophyll and lipids were removed with dichloromethane (125 ml). Solvents were removed on a rotary evaporator at 35 °C and the aqueous phase was centrifuged for 3 min at 4500 rpm (Jouan CR3i Multifunction Centrifuge, Thermo Electron Corporation, Basingstoke, UK). Extracts were freeze-dried and stored at -20 °C (see Table A.2 for extract yields). Deionised water was purified in an Option 3 water purifier (ELGA Process Water, Marlow, UK).

#### *A.1.4 Standards*

Standards were injected in methanol for HPLC analysis: (+)-catechin hydrate, (-)-epicatechin, (-)-gallocatechin and (-)-epigallocatechin and (±)-taxifolin at 0.017 mg/ml; (-)-catechin gallate, (-)-epicatechin gallate, (-)-epigallocatechin gallate, (-)-gallocatechin gallate, PC A2 (+)-afzelechin and (±)-taxifolin at 0.013 mg/ml; (±)-eriodictyol, isoquercitrin, naringenin, naringin, naringenin-7-*O*-glucoside, quercetin and rutin at 0.1 mg/ml. Flavonoid standards were injected in methanol/water (80:20 v/v) for LC-MS analysis. Ultrapure water was purified in a Milli-Q Plus system (Millipore, Watford, UK).

Catechin hydrate, epicatechin, gallocatechin and epigallocatechin; catechin gallate, epicatechin gallate, epigallocatechin gallate, gallocatechin gallate, PC A2 and taxifolin were injected at above concentrations for molar response factor evaluation against taxifolin individually or in mixtures. Corresponding -BM adducts of these compounds were assumed to have the same molar response factor. PC A-type trimers and their -BM adducts were assumed to have the same molar response factor as PC A2. See Materials and Methods 2.5 and Result and Discussion 3.1.1.

#### *A.1.5. CT quantification*

Due to interfering peaks, quantification was done with taxifolin as an external standard [11], which was prepared as thiolysis blank. Taxifolin was dissolved in the 'thiolysis' reagent,

which was identical to the thiolysis solution used for samples, but it did not contain the sample and benzyl mercaptan was replaced by methanol.

## A.2 Calculations of CT composition

The following equations were used for the calculation of:

a) mDP-values of B-type CT and galloylated B-type CT [14, 20]:

$$\text{mDP} = \frac{\sum \text{TU (mol)} + \sum \text{EU (mol)}}{\sum \text{TU (mol)}}$$

Equation A.2.1

where: TU – terminal flavan-3-ol units; EU – extension flavan-3-ol units;

b) molar percentages of galloylated flavan-3-ols [20] (i.e. % galloylation):

$$\% \text{ galloylation} = \frac{\sum \text{galloylated TU} + \sum \text{galloylated EU}}{\sum \text{all types TU} + \sum \text{all types EU}} \times 100$$

Equation A.2.2

c) procyanidin/prodelphinidin (PC/PD) ratios [14]:

$$\text{PC / PD} = \frac{(\sum \text{C units} + \sum \text{EC units}) \times 100}{\sum \text{all units}} / \frac{(\sum \text{GC units} + \sum \text{EGC units}) \times 100}{\sum \text{all units}}$$

Equation A.2.3

where: C – catechin, EC – epicatechin, GC – gallocatechin, EGC – epigallocatechin, all units – TU + EU;

d) *cis/trans* flavan-3-ol ratios [14], where A-type units were not included:

$$\text{cis / trans} = \frac{(\sum \text{EC units} + \sum \text{EGC units}) \times 100}{\sum \text{all units}} / \frac{(\sum \text{C units} + \sum \text{GC units}) \times 100}{\sum \text{all units}}$$

Equation A.2.4.

### **A.3. Analysis of CT**

Extract yields ranged from 9 to 70 g/100 g plant dry weight (Table A.2); however, fruit sample extracts had particularly low contents (<3 g CT/100 g extract, data not shown).

No free flavan-3-ols were detected in hawthorn and white clover flowers, hop strobiles, birch and blackcurrant leaves (CT data without correction for free flavan-3-ol monomers are reported for comparison purposes and resulted in minor differences; Table 2 and 3 vs. Table A.5 and A.6).

**Table A.1** List of other medicinal plants and herbal products screened for condensed tannins.

Example of English vernacular name and part of plant used	Latin name of herbal substance	Botanical name of plant(s)
Bilberry fruits	<i>Myrtilli fructus</i> <sup>b, c</sup>	<i>Vaccinium myrtillus</i> L. <sup>a, b, d</sup>
Black locust flowers	<i>Robiniae flos</i> <sup>c</sup>	<i>Robinia pseudoacacia</i> L.
Blackthorn fruits	<i>Pruni spinosae fructus</i> <sup>c</sup>	<i>Prunus spinosa</i> L.
Broadleaf plantain leaves	<i>Plantaginis maioris folium</i> <sup>c</sup>	<i>Plantago maior</i> L.
Calamus rhizome	<i>Calami rhizoma</i> <sup>c</sup>	<i>Acorus calamus</i> L.
Chamomile flowers	<i>Chamomillae anthodium</i> <sup>c</sup>	<i>Matricaria chamomilla</i> L.
Chokeberries fruits	<i>Aroniae fructus</i> <sup>c</sup>	<i>Aronia</i> Medik.
Elderberry fruits	<i>Sambuci fructus</i> <sup>b*, c</sup>	<i>Sambucus nigra</i> L. <sup>b*</sup>
Frangula bark	<i>Frangulae cortex</i> <sup>b, c</sup>	<i>Rhamnus frangula</i> L. <sup>a, b, d</sup> ( <i>Frangula alnus</i> Miller) <sup>a</sup>
Hawthorn fruits	<i>Crataegi fructus</i> <sup>c</sup>	<i>Crataegus monogyna</i> Jacq. (Lindm.); <i>Crataegus laevigata</i> (Poir.) D.C. or other European <sup>a</sup> <i>Crataegus</i> spp. <sup>a, d</sup>
Juniper fruits/berry	<i>Juniperi fructus</i> <sup>c</sup> ( <i>Juniperi pseudo-fructus</i> <sup>b</sup> )	<i>Juniperus communis</i> L. <sup>a, b</sup>
Linseed seeds	<i>Lini semen</i> <sup>b</sup>	<i>Linum usitatissimum</i> L. <sup>a, b, d</sup>
Nettle roots	<i>Urticae radix</i> <sup>b, c</sup>	<i>Urtica dioica</i> L. <sup>d</sup> ; <i>Urtica urens</i> L. <sup>b</sup>
Peppermint leaves	<i>Menthae piperitae folium</i> <sup>b, c</sup>	<i>Mentha x piperita</i> L. <sup>a, b, d</sup>
Sage leaves	<i>Salviae folium</i> <sup>c</sup> ( <i>Salviae officinalis folium</i> <sup>b</sup> )	<i>Salvia officinalis</i> L. <sup>a, b, d</sup>
White mulberry leaves	<i>Morus alba</i> L. <i>folium</i> <sup>c</sup>	<i>Morus alba</i> L.

Note: <sup>a</sup> – [9], <sup>b</sup> – [39], <sup>b\*</sup> – no final opinion [39], <sup>c</sup> – as described by manufacturer, <sup>d</sup> – [1].

**Table A.2** Results of HCl/butanol screening of plant materials and yields (g extract/100 g dry weight of plant) of extracts.

Plant material	HCl/butanol		Yield (%)	Plant material	HCl/butanol		Yield (%)
	sample	control			sample	control	
Great water dock roots	+	-	26.5	Blackthorn fruits	+	-	29.0
Blackthorn flowers	+	-	24.5	Bilberry fruits	+	+	63.0
Hawthorn flowers	+	-	23.5	Heather flowers	+	+	9.0
Willow bark	+	-	20.5	Chokeberry fruits	+	+	46.0
Hop strobiles	+	-	18.0	Hawthorn fruits	+/-	-	65.0
<i>Tilia</i> flowers	+	-	21.5	Juniper fruits/berry	-	-	
White clover flowers	+	-	17.0	Black locust flowers	-	-	
Pine buds	+	-	15.0	Frangula bark	-	-	
Walnut leaves	+	-	19.0	Broadleaf plantain leaves	-	-	
Bilberry leaves	+	-	9.5	Linseed seeds	-	-	
Birch leaves	+	-	24.0	Sage leaves	-	-	
Blackcurrant leaves	+	-	17.5	Chamomile flowers	-	-	
Cowberry leaves	+	-	36.0	Peppermint leaves	-	-	
Elderberry fruits	+	-	44.0	White mulberry leaves	-	-	
Calamus rhizome	+	-	70.0	Nettle roots	-	-	

Legend: + positive (colour change to red), - negative (no colour change to red).

**Table A.3.** Typical HPLC retention times and  $m/z$  values of flavan-3-ols and selected flavonoids.

No	Compound	Retention times (min)		$m/z$	
		gradient 1	gradient 2	Molecular ion [M-H] <sup>-</sup>	Typical other fragments
1	Galocatechin	20.9	19.4	305	340
2	Epigallocatechin	24.5	25.2	305	340
3	Catechin	26.2	26.8	289	325
4	Epicatechin	28.6	31.1	289	325
5	Taxifolin	34.2	38.8	303	
6	3,4- <i>trans</i> -galocatechin-BM	40.6	50.7	427	303
7	3,4- <i>cis</i> -galocatechin-BM	40.9	51.8	427	303
8	3,4- <i>trans</i> -epigallocatechin-BM	41.3	53.8	427	303
9	3,4- <i>trans</i> -catechin-BM	43.4	57.6	411	447, 287
10	3,4- <i>cis</i> -catechin-BM	43.9	58.2	411	447, 287
11	3,4- <i>trans</i> -epicatechin-BM	44.4	58.8	411	447, 287
12	Benzyl mercaptan and unidentified compounds	48.5	47.8	-	-
13	Epicatechin gallate	33.2	39.6	441	477
14	Epicatechin-BM gallate	43.2 and 45.3	61.5 and 62.8	563	599
15	PC A-type trimer	28.2 and 31.5	29.1 and 32.9	863	497, 325, 289, 141
16	PC A-type dimer	31.7 and 33.2	37.5 and 40.1	575	615, 633, 594, 319, 275, 141
17	PC A-type trimer-BM	42.2	56.1	986	862, 510, 430, 301, 141, 113
18	PC A-type dimer-BM	45.3	63.1	697	733, 573, 437, 141, 113
a	Rutin	31.7	38.5	609	321
b	Quercetin-hexoside <sup>a, b</sup>	32.9	40.1	463	
c	Quercetin <sup>a</sup>	39.6	51.9	301	
d	Possibly naringenin- <i>O</i> -hexoside <sup>c</sup>	32.3 and 32.9	38.4 and 39.4	433	593



e Naringenin

44.9

60.3

271

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Note: -BM – benzyl mercaptan adduct, PC – procyanidins; previously reported: <sup>a</sup> – quercetin-3-*O*-glucoside, quercetin-3-*O*- $\beta$ -galactoside and quercetin were detected in bilberry leaves [35]; <sup>b</sup> – quercetin-3-*O*-glucoside in *Tilia* flowers [33]; <sup>c</sup> – in line with [10].

**Table A.4** Data of B-type condensed tannins and galloylated procyanidins in extracts that were not corrected for free flavan-3-ols.\*

Extract derived from	CT (g/100 g)	mDP	PC / PD		<i>cis</i> / <i>trans</i>		% galloylation
Great water dock roots	27.3 ± 0.7	4.9 ± 0.1	100.0 / 0.0	± 0.1	92.1 / 7.9	± 0.1	51.5 ± 0.1
							<hr/> % A-type bond <hr/>
Cowberry leaves	17.9 ± 0.4	4.5 ± 0.0	100.0 / 0.0	± 0.0	60.7 / 39.3	± 0.1	17.1 ± 0.0
Heather flowers	19.5 ± 0.4	6.8 ± 0.1	100.0 / 0.0	± 0.1	86.6 / 13.4	± 0.1	6.7 ± 0.1
Bilberry leaves	12.4 ± 0.5	6.1 ± 0.0	100.0 / 0.0	± 0.0	92.3 / 7.7	± 0.0	7.3 ± 0.0
Blackthorn flowers	4.3 ± 0.2	2.5 ± 0.1	100.0 / 0.0	± 0.1	62.9 / 37.1	± 0.1	25.3 ± 0.1
Hawthorn flowers	11.9 ± 0.4	4.2 ± 0.0	100.0 / 0.0	± 0.0	97.4 / 2.6	± 0.0	
<i>Tilia</i> flowers	21.0 <sup>a</sup> ± 0.4	5.1 ± 0.0	100.0 / 0.0	± 0.0	90.8 / 9.2	± 0.0	
Pine buds	4.8 ± 0.3	7.0 ± 0.1	62.2 / 37.9	± 0.0	69.6 / 30.4	± 0.0	
Willow bark	15.4 ± 0.7	3.8 ± 0.1	100.0 / 0.0	± 0.1	64.8 / 35.2	± 0.1	
Walnut leaves	6.2 ± 0.3	4.2 ± 0.1	100.0 / 0.0	± 0.1	59.5 / 40.5	± 0.1	

Note: \* – for comparison purposes only, CT – condensed tannin, mDP – mean degree of polymerisation, PC/PD – procyanidin/prodelphinidin ratios, *cis/trans* – *cis/trans*-flavan-3-ol ratios, <sup>a</sup> – previously reported [11].

**Table A.5** Composition of flavan-3-ol in extracts as molar percentages that were not corrected for free flavan-3-ols. <sup>a</sup>

Extract derived from	Flavan-3-ols (%)													
	GC	EGC	C	EC	GC -BM	EGC -BM	C -BM	EC -BM	ECg	ECg -BM				
Great water dock roots	0.0	0.0	7.9	2.3	0.0	0.0	0.0	38.3	10.2	41.3				
±	0.0	0.0	0.1	0.1	0.0	0.0	0.0	0.1	0.1	0.1				
									PC A-type trimer I	PC A-type trimer II	PC A-type dimer I	PC A-type dimer II	PC A-type trimer -BM	PC A-type dimer -BM
Cowberry leaves	0.0	0.0	17.7	2.3	0.0	0.0	15.2	48.6	0.0	1.4	5.3	0.4	5.2	3.9
±	0.0	0.0	0.0	0.1	0.0	0.0	0.2	0.1	0.0	0.0	0.0	0.1	0.0	0.1
Heather flowers	0.0	0.0	9.7	4.2	0.0	*	2.9	77.2	1.9	0.4	0.0	0.0	0.0	3.6
±	0.0	0.0	0.1	0.1	0.0	0.0	0.1	0.1	0.1	0.1	0.0	0.0	0.0	0.1
Bilberry leaves	0.0	0.0	2.0	14.8	0.0	0.0	5.2	71.4	1.3	0.0	0.0	0.0	2.4	2.9
±	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0
Blackthorn flowers	0.0	0.0	23.4	6.6	0.0	0.0	7.1	45.1	0.0	0.0	14.3	3.5	0.0	0.0
±	0.0	0.0	0.1	0.1	0.0	0.0	0.1	0.1	0.0	0.0	0.1	0.1	0.0	0.0
Hawthorn flowers	0.0	0.0	2.6	21.0	0.0	0.0	*	76.4						
±	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0						
<i>Tilia</i> flowers	0.0	0.0	4.1	15.6	0.0	0.0	5.1	75.2						
±	0.0	0.0	0.1	0.1	0.0	0.0	0.1	0.0						
Pine buds	*	0.0	14.2	0.0	*	37.8	16.2	31.7						
±	0.0	0.0	0.1	0.0	0.0	0.0	0.1	0.0						
Willow bark	0.0	0.0	26.1	*	0.0	0.0	9.1	64.8						
±	0.0	0.0	0.1	0.0	0.0	0.0	0.2	0.1						
Walnut leaves	0.0	0.0	23.7	0.0	0.0	0.0	16.8	59.5						
±	0.0	0.0	0.1	0.0	0.0	0.0	0.1	0.1						

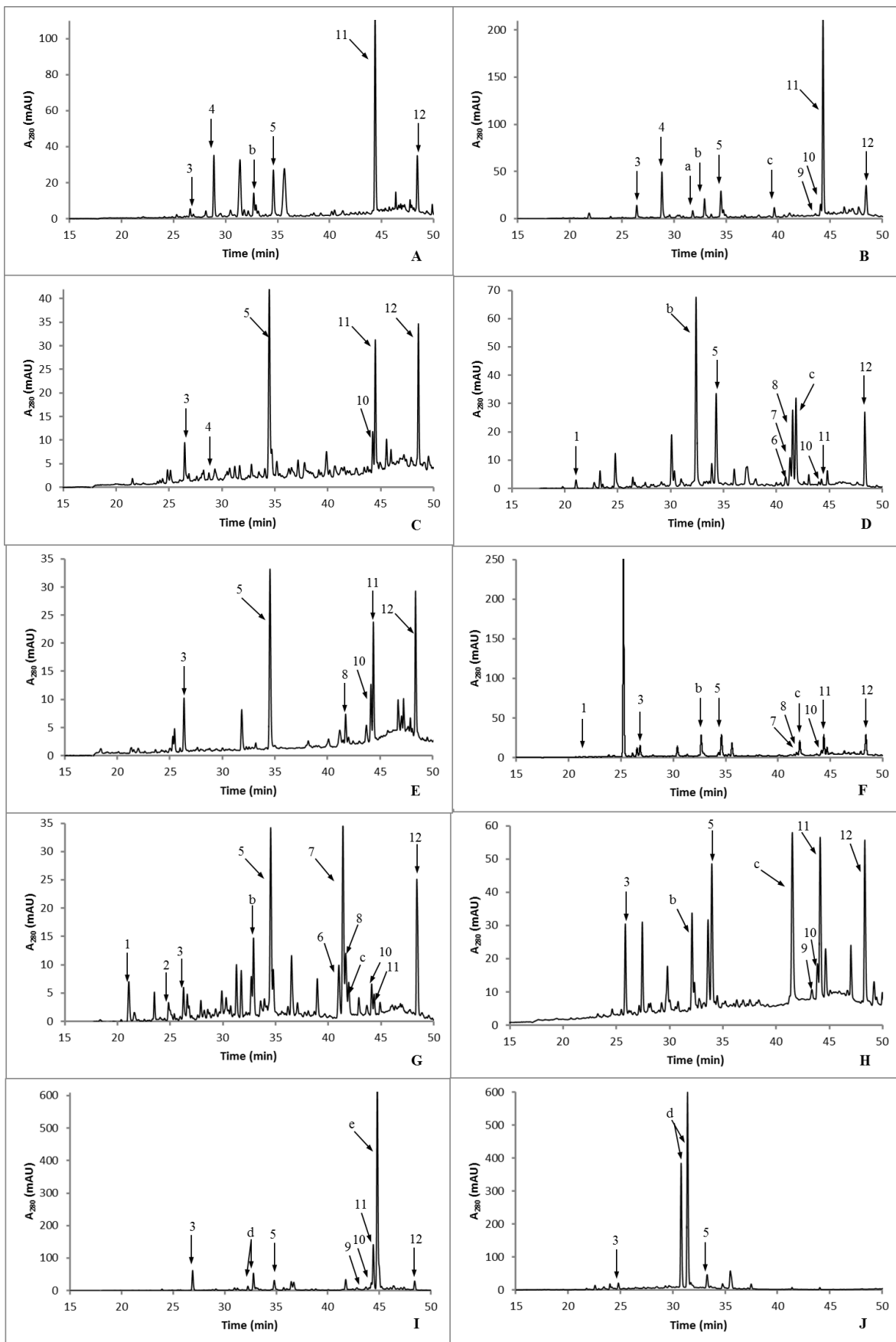
Note: <sup>a</sup> – for comparison purposes only, \* – trace amounts detected; GC – gallocatechin, EGC – epigallocatechin, C – catechin, EC – epicatechin, GC-BM – gallocatechin benzyl mercaptan adduct, EGC-BM – epigallocatechin benzyl mercaptan adduct, C-BM – catechin benzyl mercaptan adduct, EC-BM – epicatechin benzyl mercaptan adduct, ECg – epicatechin gallate, ECg-BM – epicatechin gallate benzyl mercaptan adduct, PC – procyanidins.

## Figure Captions

**Fig. A.1.** Examples of RP-HPLC chromatograms after thiolysis of extracts: A – hawthorn flowers, B – *Tilia* flowers, C – hop strobiles, D – white clover flowers, E – pine buds, F – birch leaves, G – blackcurrant leaves, H – walnut leaves, I – willow bark, J – willow bark (not thiolysed, shown for comparison); where: 1 – galliccatechin, 2 – epigallocatechin, 3 – catechin, 4 – epicatechin, 5 – taxifolin (internal standard), 6 – galliccatechin-BM (*trans*), 7 – galliccatechin-BM (*cis*), 8 – epigallocatechin-BM, 9 – catechin-BM (*trans*), 10 – catechin-BM (*cis*), 11 – epicatechin-BM, 12 – benzyl mercaptan and unidentified compounds; a – rutin, b – quercetin-*O*-hexoside, c – quercetin, d – possibly naringenin-*O*-hexoside.

**Fig. A.2.** Examples of RP-HPLC chromatograms after thiolysis of extracts: A – great water dock roots, B – cowberry leaves, C – heather flowers, D – bilberry leaves, E – blackthorn flowers; where: 1 – galliccatechin, 2 – epigallocatechin, 3 – catechin, 4 – epicatechin, 5 – taxifolin (internal standard), 6 – galliccatechin-BM (*trans*), 7 – galliccatechin-BM (*cis*), 8 – epigallocatechin-BM, 9 – catechin-BM (*trans*), 10 – catechin-BM (*cis*), 11 – epicatechin-BM, 12 – benzyl mercaptan and unidentified compounds, 13 – epicatechin gallate, 14 – epicatechin-BM gallate, 15 – procyanidin A-type trimer, 16 – procyanidin A-type dimer, 17 – procyanidin A-type trimer-BM, 18 – procyanidin A-type dimer-BM; a – rutin, b – quercetin-*O*-hexoside, c – quercetin.

Figure A.1



**Figure A.2**

