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# Phenolic compounds profile of water and ethanol extracts of *Euphorbia hirta* L. leaves showing antioxidant and antifungal properties

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1 **Abstract**

2 The bioactive chemical constituents of water and ethanol extracts of *Euphorbia hirta* L. leaves have
3 been identified and quantified using an un-targeted mass spectrometric approach. The study allowed
4 the tentative identification of 123 individual phenolic compounds and 18 non-phenolic
5 phytochemicals, most of them described in *Euphorbia hirta* L. leaves for the first time.
6 Gallotannins, hydroxybenzoic and hydroxycinnamic acids were the most abundant phenolic classes
7 in *Euphorbia hirta* L. leaves, representing together the 71.5% (26.3%, 25.2% and 20%,
8 respectively) of the total amount of identified phenolics. The main phenolic compounds detected
9 were tri-*O*-galloyl-glucose isomers, feruloyl-coniferin, tetra-*O*-galloyl-glucose isomers, di-*O*-
10 galloyl-glucose isomers, ethyl-gallic acid, protocatechuic acid-*O*-pentoside-*O*-hexoside, 5-*O*-
11 caffeoyl-quinic acid *trans* isomer and digalloyl-quinic acid. Feruloyl-coniferin was found to be
12 approximately six times more concentrated in the ethanol extract with respect to the water extract.
13 The ethanol extract exhibited higher ABTS (1338.3 ± 85.3 and 802.3 ± 91.0 µmol ascorbic acid
14 equivalent/gram of dry extract, respectively) and superoxide anion (2014.6 ± 78.6 and 1528.0 ±
15 111.7 µmol ascorbic acid equivalent/gram of dry extract, respectively) scavenging abilities than the
16 water extract. Additionally, the ethanol extract also showed a remarkable anti-fungal effect against
17 *Fusarium oxysporum* f. sp. *vasinfectum*, *Alternaria solani* and *Rhizoctonia solani.* This study
18 provides new information about *Euphorbia hirta* L., offering reasons to promote this plant species
19 as rich source of phenolics and an excellent source of antifungal molecules that might have a
20 prospective use in controlling fungal diseases of vegetable crops.

21 ***Keywords:*** Bio-fungicides; phytochemicals; mass spectrometry; phytopathogenic mycetes; tomato.

22 **1. Introduction**

23 *Euphorbia hirta* L. (*E. hirta*) is a plant species commonly found in all tropical countries worldwide,
24 including Cameroon. *E. hirta* belongs to the spurge family of *Euphorbiaceae*. Although it can be
25 seen lying down sometimes, it is usually upright, slender-stemmed, spreading up to 80 cm tall (Abu
26 et al., 2011).

27 *E. hirta* is a very popular medicinal herb and has been used since ancient times as decoction or
28 infusion in traditional remedies to treat gastro-intestinal diseases and disorders (*e.g*. intestinal
29 parasites, diarrhoea and peptic ulcer), skin problems and asthma (Huang et al., 2012). More
30 recently, extracts from *E. hirta* have shown a broad range of biological properties, including
31 antimicrobial, antifungal, anti-inflammatory, antioxidant, anticancer and antidiabetic activities
32 (Almosnid et al., 2018; Kumar et al., 2010; Li et al., 2015). Several phytochemicals have been
33 already extracted and identified from *E. hirta* leaves, such as terpenoids, coumarins, lignans and
34 phenolic compounds (Kumar et al., 2010; Li et al., 2015; Yi et al., 2012). The latter components,
35 widely known for their antioxidant and biological activities, have been rarely investigated. In this
36 context, previous phytochemical studies showed that the leaves from *E. hirta* were characterized by
37 the presence of flavonols (quercetin and myricetin derivatives, and kaempferol), hydroxybenzoic
38 acids (gallic and protocatechuic acids), tannins (gallotannins and euphorbins), flavones (luteolin)
39 and lignans (pinocembrin, pinoresinol derivatives and syringaresinol derivatives) (Kumar et al.,
40 2010; Li et al., 2015; Yi et al., 2012). However, a comprehensive identification and quantification
41 of the phenolic profile of *E. hirta* leaves is still lacking.

42 Phytopathogenic fungi are the causative agents of several important diseases of cultivated plants,
43 responsible of enormous crop losses in agriculture (Eloff et al., 2017). In this context, the
44 application of chemical fungicides is the most widespread pest management strategy to prevent
45 yield and quality losses. Quite frequently, the development of resistance traits among the pathogens
46 is the result of massive and improper use of these chemicals (Lucas et al., 2015). Moreover, some of

47 these fungicides may seriously affect human health due to the environmental pollution and the
48 presence of residues frequently detected in fruits and vegetables.
49 Currently, the research on alternative natural products with potential use in pest management
50 strategies is very active (Bocquet et al., 2018; Eloff et al., 2017; Wu et al., 2018). To this purpose,
51 phenolic-rich plant extracts were shown to display antifungal activity against different pathogenic
52 fungi, including *Fusarium oxysporum*, *Rhizoctonia solani*, *Phytophthora nicotianae, Alternaria*
53 *alternata* and *Aspergillus* species (Eloff et al., 2017; de Rodríguez et al., 2015; Wu et al., 2018). In
54 addition, purified phenolics demonstrated a direct antifungal action as well, such as ferulic acid
55 against *Botrytis cinerea* or alkylresorcinols against different *Fusarium* spp. (Patzke et al., 2017;
56 Patzke and Schieber, 2018).
57 The aim of the present study was to identify and quantify the phenolic profile of two different
58 extracts (water and ethanol extracts) of *E. hirta* leaves by using an un-targeted mass spectrometry
59 approach. The two different extracts were also characterized for their antioxidant properties and
60 their ability to inhibit the growth of some plant pathogenic fungi affecting tomato.

61 **2. Materials and methods**

62 *2.1. Chemicals and reagents*

63 Phenolic standards (quercetin, quercetin-3-*O*-rutinoside, quercetin-3-*O*-glucoside, kaempferol,
64 epicatechin, ellagic acid, gallic acid, protocatechuic acid, caffeic acid, *p*-coumaric acid, and ferulic
65 acid) and reagents for analytical determination were purchased from Sigma-Aldrich (Milan, Italy).
66 Deionized water was obtained from a Milli-Q System (Bedford, MA, USA). The mass spectrometry
67 reagents and solvents for phenolic compounds extraction were obtained from BioRad (Hercules,
68 CA, USA).

69

70 *2.2 Fungal strains*

71 Three pathogenic fungi affecting tomato were used: *Fusarium oxysporum* f. sp. *vasinfectum* Snyder
72 et Hansen, strain FUSITS04 (from Cameroon), *Alternaria solani* Sorauer, strain ASU4 (from
73 Cameroon) and *Rhizoctonia solani* Kuhn, strain RsG1 (isolated in Italy and kindly provided by
74 Paola Nipoti, University of Bologna). The fungi were maintained on 3.9% (m/v) potato dextrose
75 agar (PDA) medium at 27°C until their use.

76

77 *2.3. Plant material*

78 A tropical plant species, *Euphorbia hirta* L. (family: *Euphorbiaceae*), native and widely spread in
79 Cameroon was selected for the production of the extracts. Plants were collected from a local area
80 (Central Region, Yaoundé-Mbankomo, Cameroon) and identified by Tadjouteu Fulbert by
81 comparison with the botanical collection of A. J. M. Leeuwenberg, number 10480 and registered at
82 the Cameroon National Herbarium in Yaoundé under the number 48982/HNC. Whole plants,
83 including roots, were harvested just before the flowering stage (**Figure S1**).

84

85 *2.4. Preparation of crude extracts*

86 Water and ethanol plant extracts were obtained as described in Nguefack et al. (2013). After manual
87 harvesting, the whole plants were shade-dried at a temperature of 35°C for 15 days. Dry leaves
88 were detached from plants and then milled into powder using a GRAIN MILL MAGNUM 4V
89 (motor power: 1 HP 750 Watt, 13,000 to 15,000 rpm). Aliquots of 100 g of plant powder were first
90 defatted by mixing with 600 mL of hexane on a rotary shaker at 120 rpm for 24 h at room
91 temperature. After filtration with a fine cloth, the plant residue was spread on an aluminium foil
92 under a sterile hood, allowing the complete hexane evaporation. Lipid-free dry powder was then
93 used for the extraction. Two solvents were simultaneously used in two different extraction
94 procedures: distilled water and 70% ethanol/water solution. For both extractions, the defatted plant
95 material was soaked and stirred in 600 mL of distilled water or, alternatively, 70% ethanol for 24 h
96 at room temperature, followed by filtration through Whatman N°1 filter paper. The resulting
97 filtrates were then centrifuged at 5,200×*g* for 10 min and the supernatants were evaporated into a
98 ventilate oven at 50°C overnight to obtain dried pellets. Dried pellets were named water extract and
99 ethanol extract. The average yields were 8.20% for water extract and 5.60% for ethanol extract.
100
101 *2.5. LC-ESI-IT-MS/MS analysis of phenolic compounds*
102 For LC-MS/MS analysis, 20 mg of powders from water and ethanol extracts of *E. hirta* leaves were
103 re-suspended in 1 mL of the respective solvents (water and 70% ethanol, respectively). The extracts
104 were then analysed on a HPLC Agilent 1200 Series system equipped with a C18 column (HxSil
105 C18 Reversed phase, 250×4.6 mm, 5 μm particle size, Hamilton Company, Reno, Nevada, USA) as
106 reported in Mena et al. (2016). The mobile phase consisted of (A) $H_2O$/formic acid (99:1, *v*/*v*) and
107 (B) acetonitrile/formic acid (99:1, *v*/*v*). The gradient started at 1% B for 1 min then linearly ramped
108 up to 40% B in 13 min. The mobile phase composition was raised up to 99% B in 13 min and
109 maintained for 2 min in order to wash the column before returning to the initial condition. The flow
110 rate was set at 1 mL/min. The samples were injected in the amount of 40 μL. After passing through

111 the column, the eluate was split and 0.4 mL/min were directed to an Agilent 6300 ion trap mass
112 spectrometer. Two MS experiments were performed, one in ESI negative ion mode and one using
113 positive ESI ionization, under the same chromatographic conditions. Identification of phenolic
114 compounds in all samples was carried out using full scan, data-dependent $MS^2$ scanning from *m/z*
115 100 to 800.
116 Ellagitannins were quantified as ellagic acid equivalents whereas gallotannins as gallic acid
117 equivalents. Flavonols were quantified as quercetin-3-glucoside or kaempferol equivalents. Flavan-
118 3-ols were quantified as catechin equivalent. Hydroxybenzoic acids were quantified as gallic acid or
119 protocatechuic acid equivalents whereas hydroxycinnamic acids as caffeic acid or coumaric acid or
120 ferulic acid equivalents. Isocoumarins were instead quantified as gallic acid equivalents.
121 ESI-IT-MS/MS parameters, limits of detection (LOD) and limits of quantification (LOQ) for the
122 different standards were the same as reported in Martini et al. (2017).
123 Quantitative results were expressed as mg of compounds per g of dry extract.
124
125 *2.6 Antioxidant activity analysis*
126 The antioxidant properties of water and ethanol fractions obtained from *E. hirta* leaves were
127 analysed by using four different assays. The samples were obtained by dissolving 20 mg of powders
128 from water and ethanol extracts in 1 mL of the respective solvents (water and 70% ethanol,
129 respectively). The radical scavenging ability was tested by using the ABTS assay according to Re et
130 al. (1999). For the determination of the reducing ability, a protocol based on the ferric
131 reducing/antioxidant power (FRAP) assay was used (Benzie and Strain, 1999). The capacity to
132 scavenge superoxide anion radicals was evaluated according to the methods reported by Martini et
133 al. (2017). The results were expressed as μmol of ascorbic acid equivalent per gram of dry extract.
134 The $Fe^{2+}$-chelation ability was instead evaluated by the ferrozine assay (Karama and Pegg, 2009).

135 Re-dissolved water and ethanol extracts of *E. hirta* leaves were diluted 20-fold in the respective
136 solvents and tested for the chelating ability. Results were expressed as percentage of bound $Fe^{2+}$.
137
138 *2.7 Antifungal activity*
139 The agar plate dilution method, as described by Rios et al. (1988), was performed to investigate the
140 direct antifungal activity of the extracts. Five increasing dilutions of the plant extracts were used to
141 obtain the following final concentrations: 1.25, 2.50, 5, 10, and 20 mg of dry extract/mL. PDA
142 plates without any addition of plant extracts were used as a negative control.
143 A 5 mm mycelial disk of each phytopathogenic fungus was placed on the centre of an agar plate and
144 then incubated at 26°C. After 7 days, in order to assess the fungal growth, the two perpendicular
145 diameters of the fungal mycelium were measured. Growth inhibition was calculated comparing
146 fungal growth on pre-treated PDA plates with the growth on PDA without any addition of plant
147 extracts. Growth inhibition percentage (% I) was calculated according to the formula developed by
148 Pandey et al., (1982):
149 $\% \text{ I} = [(MGC - MG)/MGC] \times 100$
150 where, MGC = mycelium growth diameter in the control PDA plate, *MG* = mycelium growth
151 diameter in the pre-treated PDA plate.
152 The concentration of plant extracts required to inhibit by 50% the fungal growth ($IC_{50}$) was
153 determined by plotting the growth inhibition percentage as a function of final plant extract
154 concentration (base-10 logarithm). $IC_{50}$ values were expressed as mg of extract/mL.
155
156 *2.8. Statistics*
157 Mass spectrometry and antioxidant activity data are displayed as mean ± SD for three replicates for
158 each prepared sample. Antifungal activity data are reported as mean ± SD for five replicates.
159 Univariate analysis of variance (ANOVA) with Tukey's post-hoc test was applied using GraphPad

160 prism 6.0 (GraphPad Software, San Diego, CA, U.S.A.) when multiple comparisons were
161 performed. The differences were considered significant with $P < 0.05$. $IC_{50}$ for antifungal activity
162 was calculated by using non-linear regression analysis (GraphPad prism 6.0; GraphPad Software,
163 San Diego, CA, U.S.A.).

164 **3. Results and discussion**

165 *3.1. Identification of the major phytochemicals in water and ethanol extracts of Euphorbia hirta*
166 *leaves*

167 In this study, the water and ethanol extracts of *E. hirta* leaves were analysed for their phytochemical
168 profile. The phytochemical composition of the extracts was investigated using an un-targeted
169 method through LC-ESI-IT-MS/MS experiments. Representative base peak chromatograms (BPCs)
170 are shown in **Figure 1**. This approach allowed the tentative identification of 123 individual phenolic
171 compounds, 7 organic acids, 4 terpenes, 3 amino acids, 1 dipeptide, 1 alkaloid, 1 anthraquinone and
172 1 norisoprenoid.

173 Ten compounds were identified by comparison with reference standards, while the remaining 131
174 compounds were tentatively identified based on the interpretation of their fragmentation patterns
175 obtained from mass spectra ($MS^2$ experiments) and by comparison with the literature. The mass
176 spectrum data along with peak assignments and retention time for the identified phytochemicals are
177 described in **Tables 1** and **2**.

178

179 *3.1.1. Ellagic acid derivatives and ellagitannins*

180 A total of 21 ellagic acid derivatives and ellagitannins were identified in the *E. hirta* extracts.
181 Compound **41.1** presented a negative charged molecular ion at *m/z* 285 ($[M\text{-}H]^{-1}$) and the same
182 fragmentation pattern as ellagic acid with a base peak at *m/z* 257 and secondary peaks at *m/z* 229
183 and *m/z* 185 (Calani et al., 2013). However, its *m/z* value was 16 Da lower than that of ellagic acid
184 and was tentatively identified as deoxyellagic acid. Compound **31.5** was identified as ellagic acid
185 by comparison of the retention time and mass spectral data with the authentic standard. Compounds
186 **28.1** and **30.4** showed an *m/z* ion at 519 ($[M\text{-}H]^{-1}$) and the typical fragmentation pattern of ellagic
187 acid. The loss of 218 Da is typical of a pentoside-malonyl group (loss of −86 Da and −132 Da
188 corresponding to malonyl and pentoside moieties, respectively) and these compounds were,

189 therefore, identified as isomers of ellagic acid malonyl-pentoside. Compound **32.5** gave a
190 pseudomolecular ion at *m/z* 601 ($[M-H]^{-1}$) and a fragmentation pattern consistent with gallagic acid
191 (Mena et al., 2012). Ellagitannins are characterized by the presence of a hexahydroxydiphenoyl
192 (HHDP) moiety, which results in the typical appearance of fragment ions at *m/z* 301 and *m/z* 275 in
193 the $MS^2$ spectra. Compounds **2.2**, **3.1** and **5.1** yielded the same pseudomolecular ion at *m/z* 481 and,
194 on the basis of the fragmentation spectra, they were identified as HHDP-hexoside isomers (Calani
195 et al., 2013). Three signals (compounds **6.7**, **11.1** and **20.1**) at *m/z* 633 were observed and identified
196 as galloyl-HHDP-hexoside (corilagin) isomers (Sousa et al., 2016). Additionally, two signals at *m/z*
197 631 (compounds **17.2** and **18.1**) showed the same fragmentation pattern of galloyl-HHDP-hexoside.
198 These compounds were identified as dehydro-corilagin isomers. Based on previously published
199 fragmentation spectra (Mena et al., 2012; Calani et al., 2013), compounds **17.1** and **22.2** were
200 identified as pedunculagin II and pedunculagin I, respectively, whereas compounds **21.2** and **27.2**
201 were identified as granatin B isomers. Finally, four signals (compounds **6.3**, **7.1**, **9.1** and **31.4**)
202 displayed the typical fragmentation pattern of ellagitannins, but it was not possible to assign them
203 an exact structure.
204
205 *3.1.2. Gallotannins*
206 In this study, 11 gallotannins were detected in the *E. hirta* leaves extracts. Gallotannins are
207 polyphenolic compounds with a sugar core linked to at least two gallic acid moieties. The $MS^2$
208 spectra of gallotannins usually gave typical fragment ions at *m/z* 331, 313 and 169, which
209 correspond to the moiety of galloyl-hexoside, galloyl-hexoside –$H_2O$, and gallic acid, respectively
210 (Gu et al., 2013). The typical losses included gallic acid moieties (152 or 170 Da) and sugar units
211 (162 Da) (Hukkanen et al., 2007). According to the proposed fragmentation pathway,
212 compounds **10.3** and **14.5** were identified as di-*O*-galloyl-glucose isomers (Gu et al., 2013).
213 Compounds **16.1**, **19.2**, **23.2** and compounds **23.4** and **27.3** were identified as isomers of tri- and

214 tetra-*O*-galloyl-glucose, respectively. The product ion at *m/z* 483 corresponding to the deprotonated
215 di-*O*-galloyl-glucose molecule and originating from successive loss of galloyl groups can be
216 observed in the $MS^2$ spectra of both tri-*O*-galloyl-glucose and tetra-*O*-galloyl-glucose. The
217 fragmentation of tetra-*O*-galloyl-glucose isomers also generated a signal at *m/z* 635 corresponding
218 to the deprotonated tri-*O*-galloyl-glucose molecule. Finally, compound **30.1** was assigned to penta-
219 *O*-galloyl-glucose. Compound **24.3** had a pseudomolecular ion at *m/z* 467 and produced at $MS^2$ *m/z*
220 315 and 169 corresponding to the loss of galloyl group (-152 Da) and galloyl group plus
221 deoxyhexose (-152 and -146 Da). Therefore, this compound was tentatively identified as di-*O*-
222 galloyl-rhamnose. Finally, two signals (compounds **6.4** and **33.1**) displayed the typical
223 fragmentation pattern of gallotannins but it was not possible to assign an exact structure to these
224 molecules.
225
226 *3.1.3. Flavonols*
227 Among the 34 flavonol derivatives (**Table 1**) detected, 16 compounds were identified as quercetin-
228 derivatives and 13 as kaempferol-derivatives. Quercetin-derivatives can be easily identified by the
229 presence of the typical fragment ions in the $MS^2$ spectra at *m/z* 301, 271, 179 and 151 derived from
230 the fragmentation of the quercetin aglycone (Fabre et al., 2001). Compound **40.1** was identified as
231 quercetin aglycone by comparison with the authentic standard. Compounds **32.3** and **33.3** presented
232 an identical pseudomolecular ion $[M-H]^-$ at *m/z* 433, releasing a fragment ion at *m/z* 301 (loss of a
233 pentose group), which might be coherent with quercetin-*O*-pentoside isomers. The appearance of
234 the signal at *m/z* 271 ($Y_0$-2H-CO) characteristic of 3-*O*-glycosyl flavonols in the $MS^2$ spectra of the
235 compound **32.3** pointed as the existence of a 3-*O*-pentoside binding site and the compound was
236 therefore identified as quercetin-3-*O*-pentoside (Ablajan et al., 2006; Martini et al., 2018).
237 Compound **33.3,** instead, showed the presence of a signal at *m/z* 273 ($Y_0$-CO), which is
238 characteristic of 7-*O*-glycosyl flavonols, and the compound was therefore identified as quercetin-7-

239 *O*-pentoside (Ablajan et al., 2006; Martini et al., 2018). Compound **33.4** showed a pseudomolecular
240 ion at *m/z* 477 and was identified as quercetin-3-*O*-rhamnoside due to the presence of the signals at
241 *m/z* 301 (quercetin aglycone originating from the loss of the rhamnosyl moiety) and 271.
242 Compound **23.3** had the same negative molecular ion (*m/z* 463) as compound **31.1**, which was
243 identified as quercetin-3-*O*-glucoside by comparison with the authentic standard. The analysis of
244 $MS^2$ spectra revealed the loss of 162 Da (hexose group) to produce an *m/z* 301 (quercetin) daughter
245 ion. Basing on the elution order, this compound was tentatively identified as quercetin-3-*O*-
246 galactoside (Del Rio et al., 2004). Compound **31.6** presented a pseudomolecular ion $[M-H]^-$ at *m/z*
247 477 releasing a fragment ion at *m/z* 301 (loss of a glucuronide group), which might be coherent with
248 quercetin-3-*O*-glucunoride (Dall'Asta et al., 2012). Compounds **32.4** and **35.1** showed the same
249 negative molecular ion (*m/z* 505), which gave product ions in the $MS^2$ spectra at *m/z* 463 (loss of
250 acetyl group) and 301 (loss of hexose group). The presence of the peak at *m/z* 271 allowed us the
251 identification of the peaks as quercetin-3-*O*-acetyl-hexoside isomers (Ablajan et al., 2006;
252 Cuyckens and Claeys, 2004). Compounds **33.5**, **35.5** and **37.3** exhibited identical negative
253 molecular ion (*m/z* 585) and peaks at *m/z* 433 (loss of a galloyl group) and 301 (loss of a pentoside
254 group) in the $MS^2$ spectra. The presence of the peak at *m/z* 273 allowed us the identification of the
255 peaks as quercetin-7-*O*-galloyl-pentoside isomers (Ablajan et al., 2006; Cuyckens and Claeys,
256 2004). Quercetin-3-*O*-rutinoside (compound **29.1**; *m/z* 609) was identified by comparison of
257 retention time and fragmentation spectra with the authentic standard. Compounds **31.7** and **37.2**
258 showed the same pseudomolecular ion at *m/z* 615, which gave product ions in the $MS^2$ spectra at
259 *m/z* 463 and 301, thus indicating a successive loss of a galloyl group (-152 Da) and a hexosyl
260 moiety (-162 Da). Due to the presence of a peak at *m/z* 271, these compounds were tentatively
261 identified as quercetin-3-*O*-galloyl-hexoside isomers (Ablajan et al., 2006; Cuyckens and Claeys,
262 2004). Compound **14.6** (*m/z* 625) presented peaks at *m/z* 463 (loss of a hexose group), 301 (loss of a
263 second hexose group), 300, 273 and 271 in the fragmentation spectra. The presence of the peak at

264 *m/z* 463 ($Y_1$) is indicative that the two hexosyl groups are attached in different position. The
265 observed peaks at m/z 273 and 271 indicated that one sugar is linked to the -OH group in position 3
266 and the other one to the -OH group in position 7 of the aglycone (Ferreres et al., 2004; Li and
267 Claeys, 1994). This peak was assigned to quercetin-3-*O*-hexoside-7-*O*-hexoside. Compounds **14.2**,
268 **24.4**, **25.3**, **31.2**, **32.2**, **33.2**, **33.6**, **35.3**, **35.4**, **36.1**, **36.2**, **37.1**, **38.1**, and **39.1** were characterized for
269 the presence in the $MS^2$ spectra of an intense signal at *m/z* 285, which is diagnostic of the
270 kaempferol aglycone (Fabre et al., 2001). Based on the same rules, as reported above for quercetin,
271 these compounds were assigned to kaempferol-derivatives, as depicted in **Table 1**. Similarly,
272 compounds **27.4** and **30.2** were characterized for the presence of the diagnostic peaks of the
273 myricetin aglycone (*m/z* 317 and 179) in the $MS^2$ spectra and identified as myricetin-derivatives as
274 reported in **Table 1** (Calani et al., 2013). Finally, two isorhamnetin-derivatives (compounds **11.3**
275 and **32.1**) were identified in the *E. hirta* leaves extracts (**Table 1**) (Mena et al., 2016).
276
277 *3.1.4. Flavan-3-ols, flavones, dihydroflavonols and isocoumarins*
278 Five flavan-3-ols were identified in the *E. hirta* leaves extracts (**Table 1**). Epicatechin (compound
279 **20.2**; *m/z* 289) was identified by comparison of retention time and fragmentation spectra with the
280 authentic standard. Three type-B procyanidin dimers ((epi)catechin-(epi)catechin) were identified at
281 *m/z* 577 (compounds **14.3**, **17.3** and **20.3**). The fragmentation pattern reported in **Table 1** is
282 consistent with previously reported data (Gu et al., 2003). Compound **27.6** showed a
283 pseudomolecular ion at *m/z* 597 and $MS^2$ fragments at *m/z* 435 and 315. The fragment at m/z 435
284 revealed the loss of *O*-linked hexoside group whereas the subsequent loss of 120 Da (fragment at
285 *m/z* 315) is characteristic of a *C*-linked hexoside group. Fragmentation did not generate the
286 aglycone, but it can be obtained through the calculation $[M-H]^-$-162-120-42 (Waridel et al., 2001).
287 The compound was tentatively identified as (epi)afzelechin-*O*-hexoside-*C*-hexoside.

288 Five flavones were identified in the *E. hirta* leaves extracts (**Table 1**). Compound **42.1** was
289 assigned to the aglycone chrysin based on previously published fragmentation pathway (Fabre et
290 al., 2001). Compounds **22.1**, **24.1**, **25.5** and **30.3** were instead identified as apigenin-derivatives.
291 Compound **22.1** presented a pseudomolecular ion at *m/z* 415 with a single peak in the $MS^2$ spectra
292 at *m/z* 269 originating from the loss of a rhamnosyl moiety and corresponding to the aglycone of
293 apigenin (Fabre et al., 2001). Compound **30.3** (*m/z* 431) was identified as apigenin-6-*C*-glucoside
294 due to the presence of the peak at *m/z* 341 (-90 Da) and 311 (-120 Da) diagnostic for a *C*-linked
295 hexoside group, and at *m/z* 413 (-18 Da) diagnostic of a 6-*C*-glycosidic bond (Waridel et al., 2001).
296 Compound **25.5** showed a negative molecular ion at *m/z* 563 and fragment ions at *m/z* 473, 443 and
297 413 resulting from the loss of 90, 120 and 150 Da, respectively, indicating the linkage of hexoside
298 to the *C*-position of aglycone (Ferreres et al., 2007). The presence of the fragment at m/z 545 (-18
299 Da) is diagnostic of a 6-*C*-hexoside bond. The fragments at *m/z* 383 and 353 are instead indicative
300 of the presence of an 8-*C*-linked pentoside moiety (Waridel et al., 2001). The compound was
301 identified as apigenin-6-*C*-hexoside-8-*C*-pentoside. Compound **24.1** (*m/z* 593) generated in the $MS^2$
302 spectra fragment at *m/z* 473 (loss of 120 Da), 431 (loss of 162 Da from the parent ion) and 311 (loss
303 of 120 Da from the ion at *m/z* 431). This compound was therefore identified as apigenin-8-*C*-
304 hexoside-4'-*O*-hexoside. Three dihydroflavonols were identified in the mass spectrum. Compound
305 **24.2** showed a pseudomolecular ion at *m/z* 449 and was identified as dihydrokaempferol-7-*O*-
306 hexoside due to the presence of the signals at *m/z* 287 (dihydrokaempferol aglycone originating
307 from the loss of the hexoside moiety) and 259 (characteristic of 7-*O*-glycosyl linkage). Compounds
308 **16.2** and **19.3** showed the same negative molecular ion (*m/z* 465) and a fragmentation pattern
309 typical of taxifolin-hexoside (Martini et al., 2017). Finally, 5 isocoumarins (brevifolin-derivatives),
310 corresponding to compounds **3.2**, **7.2**, **17.4**, **21.1** and **23.1**, were found in the extract (**Table 1**).
311 They were characterized for the presence of peaks in the $MS^2$ spectra corresponding to the
312 brevifolin aglycone (*m/z* 247) and brevifolin-carboxylic acid (*m/z* 291) (Lantzouraki et al., 2015).

313

314 *3.1.5. Hydroxycinnamic acids*

315 Compounds **1.1**, **25.1** and **34.1** were easily identified by comparison with authentic standards. On

316 the other hand, compounds **2.1**, **6.4**, **11.4**, **12.1**, **19.1** and **23.5** (*m/z* 353) were identified as

317 caffeoylquinic acids (CQAs) using the hierarchical keys previously developed by Clifford et al.

318 (2003) and the order of elution (Martini et al., 2017). Indeed, two isomers of 5-*O*-coumaroylquinic

319 acid (compounds **10.1** and **25.2**; *m/z* 337) and one of 5-*O*-feruloylquinic acid (compound **27.5**; *m/z*

320 367) were identified using the same hierarchical keys as reported above (Clifford et al., 2003;

321 Martini et al., 2017). Compound **34.2** showed a negative molecular ion at *m/z* 309 and a product ion

322 at *m/z* 193 (ferulic acid aglycone) due to the loss of a malic acid residue (-116 Da). Compound **14.1**

323 (*m/z* 341) was identified as caffeic acid-*O*-hexoside due to the presence of the peaks at *m/z* 179 (loss

324 of hexose residue) and 135 which are characteristic of caffeic acid (Martini et al., 2017). Compound

325 **10.4** (m/z 515) showed a fragmentation pattern typical of 3,5-*O*-dicaffeoylquinic acid (Clifford et

326 al., 2005). Compound **5.3** showed a pseudomolecular ion at *m/z* 517 and fragment in the $MS^2$

327 spectra at *m/z* 337 (loss of 180 Da associated with a coniferyl alcohol moiety) and 193 (loss of

328 hexose). This compound was identified as feruloyl-coniferin (Mena et al., 2012). Finally,

329 compounds **14.4** and **19.4** (*m/z* 529) were assigned to feruloyl-caffeoylquinic acid (Clifford et al.,

330 2006).

331

332 *3.1.6. Hydroxybenzoic acids*

333 Compounds **6.1**, **13.1** and **26.1** were easily identified by comparison with authentic standards.

334 Compounds **4.2**, **5.2**, **6.2** and **8.1** with a parent ion $[M-H]^-$ at *m/z* 331 revealed a daughter ion

335 $[M-H-162]^-$ at *m/z* 169 upon fragmentation, indicating the loss of a hexosyl moiety. They were

336 identified as galloyl-*O*-hexoside isomers (Erşan et al., 2016). The parent ion $[M-H]^-$ at *m/z* 493 of

337 compound **5.4** formed daughter ions $[M-H-162]^-$ at *m/z* 313 and $[M-H-162-162]^-$ at *m/z* 169 and

338 was tentatively identified as a galloyl-di-*O*-hexoside. Compounds **4.1**, **5.5** and **6.6** (*m/z* 343) were
339 identified as galloyl-quinic acid isomers due to the presence in the $MS^2$ spectra of peak at 191
340 (quinic acid moiety generated by the loss of a galloyl moiety) and 169 (galloyl moiety generated by
341 the loss of a quinic acid moiety) (Erşan et al., 2016). Compounds **15.1** and **21.3** were tentatively
342 identified as di- and tri-galloylquinic acids due to sequential losses of galloyl moieties (152 Da)
343 from their parent ions at *m/z* 495 and 647, respectively, and the formation of a final product ion at
344 *m/z* 191 (quinic acid moiety) (Erşan et al., 2016). Compound **27.1** presented a pseudomolecular ion
345 at *m/z* 505, which generated the daughter ions at *m/z* 343 (galloylquinic acid moiety; due to the loss
346 of a hexose group) and 313 (gallic acid hexose moiety; due to the loss of a quinic acid moiety). This
347 compound was identified as galloylquinic acid-*O*-hexoside. Compounds **108** and **119**, exhibited
348 parent ions $[M–H]^-$ at *m/z* 321 and 473. Their fragmentations resulted in product ions at *m/z* 169
349 and 125 characteristic of gallic acid. Thus, these compounds were tentatively identified as di- and
350 tri-gallic acids, due to sequential loss of galloyl moieties, yielding product ions specific for gallic
351 acid. Three additional compounds (**106**, **109** and **117**) showed the presence in the $MS^2$ spectra of
352 the typical product ions of gallic acid (*m/z* 169 and 125). Compound **106** (*m/z* 197) was
353 characterized for a loss of 48 Da generated by ethylic group. This compound was tentatively
354 identified as ethyl-gallic acid. Compound **109** exhibited a pseudomolecular ion $[M–H]^-$ at *m/z* 325
355 and was characterized by the loss of 156 Da, yielding a daughter ion at m/z 169. This compound
356 was identified as galloyl-shikimic acid (Erşan et al., 2016). Compound **117** (*m/z* 437) generated
357 after fragmentation a peak at *m/z* 169 and was identified as galloyl-salicilin (Itoh et al., 2000).
358 Compounds **107** and **118** showed negative parent ions at *m/z* 315 and 447, respectively, and their
359 fragmentations resulted in product ions at *m/z* 153 and 109 characteristic of protocatechuic acid. For
360 the compound **107** the signal at m/z 153 resulted from the loss of a hexose group and was identified
361 as protocatechuic acid-*O*-hexoside (Martini et al., 2017). Compound **118** presented in the $MS^2$
362 spectra a fragment at 315 (protocatechuic acid-hexoside group) arising from the loss of a pentose

363 group (-132 Da). This compound was tentatively identified as protocatechuic acid-*O*-hexoside-*O*-

364 pentoside.

365

366 *3.1.7. Other phytochemicals*

367 Seven organic acids (compounds **a.1**, **a.2**, **b.1**, **c.1**, **d.1**, **f.1** and **p.4**, **Table 2**) were easily identified

368 in the *E. hirta* extracts due to the characteristic fragmentation patterns that resulted in the loss of

369 $H_2O$ (-18 Da) and/or $CO_2$ (-44 Da) (Brent et al., 2014). Two *ent*-kaurene diterpenoids, albopilosin H

370 (*m/z* 331; compound **i.1**) and ponicidin (*m/z* 361; compound **l.1**) were identified according to the

371 fragmentation scheme proposed by Zhou et al. (2008). Based on the same scheme an *ent*-6,7-*seco*-

372 diterpenoids, isojaponins A (*m/z* 377; compound **l.2**), was identified in the extract (Zhou et al.,

373 2009). An additional diterpenoid, gibberellin CA29 (*m/z* 347; compound **e.1**), was identified basing

374 on the fragmentation spectrum reported by Urbanová et al. (2013). Two additional signals in the

375 negative mass spectra were assigned to crysophanol-8'-O-(6'-O-galloyl)-glucose (*m/z* 567;

376 compound **h.1**) and roseoside (*m/z* 385; compound **g.1**) (Cádiz-Gurrea et al., 2013; Ye et al., 2007).

377 In the positive MS spectra, 5 additional signals were identified. Three of them belonged to the

378 aromatic amino acids phenylalanine (*m/z* 166; compound **p.1**), tyrosine (*m/z* 182; compound **p.2**)

379 and tryptophan (*m/z* 205; compound **p.3**). Compound **p.5** was instead identified as the dipeptide

380 glutamic acid-tyrosine *(m/z* 311). Finally, the last signal (*m/z* 466; compound **p.6**) was assigned to

381 the alkaloid ternatoside C (Zhang et al., 2007).

382

383 *3.2. Quantitative profile of phenolic compounds in the Euphorbia hirta leaves*

384 **Tables 3-6** and **Figure 2** provide information about the amount of the 123 tentatively identified

385 phenolic compounds in the water and ethanol extracts of *E. hirta* leaves.

386 Water extract of *E. hirta* leaves contained more phenolic compounds than the ethanol extract,

387 163.62 ± 0.61 mg/g of extract vs 49.61 ± 0.39 mg/g of extract ($P < 0.05$), respectively. Water extract

388 was particularly rich in gallotannins and hydroxybenzoic acids (representing the 31.4% and 26.5%
389 of total phenolic compounds, respectively) (**Tables 3** and **6** and **Figure 2A**), whereas the ethanol
390 extract was rich in hydroxycinnamic acids and isocoumarin (representing the 45% and 16.7% of
391 total phenolic compounds, respectively) (**Tables 5** and **6** and **Figure 2B**). In the ethanol extract,
392 feruloyl-coniferin represented alone the 31.7% of total phenolic compounds and the 70.3% of total
393 hydroxycinnamic acids (**Table 6**).
394 **Figure 3** details the structure of the most important phenolic compounds identified in the *E. hirta*
395 leaves.
396
397 *3.3. Antioxidant activity analysis*
398 To fully characterize the antioxidant properties of the two extracts, the ability to scavenge
399 physiologically relevant radicals (superoxide anions), the organic nitro-radical ABTS and the
400 reducing power were evaluated. In addition, the $Fe^{2+}$-chelating ability of the two extracts was
401 assessed. The ethanol extract of *E. hirta* leaves was more effective, with respect to the
402 corresponding water extract, in scavenging ABTS ($P< 0.05$) and superoxide anion radicals ($P<$
403 $0.05$), despite the lower phenolic content measured by LC-MS analysis (**Figure 4**). Furthermore, the
404 ethanol extracts also showed higher reducing power with respect to the water extract ($P< 0.05$).
405 These results may be due the presence of non-phenolic antioxidant compounds or of unidentified
406 phenolic compounds in the ethanol extract. Alternatively, phenolic compounds present in the
407 ethanol extract may have a better antioxidant potential than those in the water extract. On the other
408 hand, the water extract exhibited better chelating ability towards $Fe^{2+}$ than the ethanol extract ($P<$
409 $0.05$).
410
411 *3.4. Antifungal activity analysis*

412 The *in vitro* antifungal activity of *E. hirta* leaves extracts was assayed, in order to check their
413 activity in inhibiting fungal growth. The assessment of any antifungal activity is pivotal for the
414 development and implementation of a suitable technology for the production of novel bio-
415 fungicides based on the exploitation of a possible antifungal activity of such extracts.
416 The extracts of *E. hirta* leaves displayed higher effectiveness in reducing the myceliar growth of
417 three pathogenic fungi to tomato, *R. solani*, *F. oxysporum* f. sp. *vasinfectum*, and *A. solani*, in a
418 concentration-dependent manner (**Table 7**). The ethanol extract was more effective in inhibiting
419 fungal growth than the water extract ($P< 0.05$; **Figure S2**). These results are in agreement with
420 those of other authors reporting that the *in vitro* antifungal and antimicrobial activities of some
421 ethanol extracts had higher efficacy than the aqueous extracts (Eloff, 1998; Kotze and Eloff, 2002;
422 Dakole et al., 2016).
423 Several plant extracts have been tested for their antifungal activity against the three pathogenic
424 fungi analysed in this study. Methanolic extracts of leaves from *Pulicaria incisa*, *Rhanterium*
425 *epapposum* and *Horwoodia dicksoniae* showed higher antifungal activity against *F. oxysporum* than
426 the *E. hirta* leaves extracts (Mohamed et al., 2017). However, *E. hirta* ethanol extract was as
427 effective as *Citrullus colocynthis* and *Gypsophila capillaris* leaves methanolic extracts (Mohamed
428 et al., 2017). Indeed, *E. hirta* ethanol extract displayed higher antifungal activity against *F.*
429 *oxysporum* and *A. solani* than *Vitis vinifera*, *Punica granatum* and *Ficus carica* leaves methanolic
430 extracts (El-Khateeb et al., 2013). The aqueous extracts of *Polystichum squarrosum, Adiantum*
431 *venustum Parthenium hysterophorus, Urtica dioeca* and *Cannabis sativa* leaves exhibited
432 antifungal activity against *R. solani*, *F. oxysporum* and *A. solani* with a lower effectiveness respect
433 to *E. hirta* ethanol and water extracts (Tapwall et al., 2011). Rongai et al. (2015) investigated the
434 antifungal properties of aqueous extracts from twenty plants against *F. oxysporum*. Among them,
435 extracts of *Rivina humulis, Brassica carinata*, *Brunfelsia calyicina, Salvia guaranitica* and *Punica*

436 *granatum* showed the best antifungal activity. Nevertheless, they were less effective than the *E.*
437 *hirta* water extract tested in this study.
438 The linearly increasing efficacy related to the concentration is a clear indication of the presence of
439 antifungal molecules in both extracts. Despite the higher amount of phenolic compounds identified
440 in water extract, a more pronounced antifungal activity was obtained using the ethanol extract ($P<$
441 $0.05$). This might be related to the very high content in isocoumarins and hydroxycinnamic acids of
442 the latter. Isocoumarins and hydroxycinnamic acids are well known phenolics, able to confer and/or
443 induce a non-specific resistance to several phytopathogens, when they affect their host plants. Such
444 plant-derived molecules belong to a group of antimicrobial substances called phytoalexins (Ingham,
445 1972) and are secondary metabolites produced in plants, especially as a result of biotic stresses
446 (Hammerschmidt, 1999).
447 Some of the phenolic compounds detected in the ethanol extract in higher amount, with respect to
448 the water extract, are described to possess a marked antifungal activity. For instance, ferulic and
449 coumaric acids showed a remarkable *in vitro* inhibiting effect on the growths of *F. oxysporum* and
450 *R. solani* (El Modafar and El Boustani, 2001; Hayashi, 1997). These compounds were also found in
451 higher concentration in date palm cultivars resistant to *F. oxysporum* infection, when compared to
452 the susceptible cultivars (El Modafar and El Boustani, 2001). Gallic acid showed antifungal activity
453 against *F. oxysporum* and *A. solani* (Alves Breda et al., 2016; Wu et al., 2009). Feruloyl-coniferin,
454 which represented alone more than 30% of the phenolic compounds in *E. hirta* leaves ethanol
455 extract (**Table 6**), seems to be particularly interesting. It is an ester between a molecule of coniferin
456 and a ferulic acid moiety (**Figure 3**). As reported above, ferulic acid was a potent inhibitor of fungal
457 growth, whereas coniferin was able to inhibit *in vitro* the growth of the pathogenic fungus
458 *Verticillium longisporum* (König et al., 2014). Indeed, mutant *Arabidopsis thaliana* plant lines
459 producing a high amount of coniferin were particularly resistant to *Verticillium longisporum*
460 infection (König et al., 2014). Induction of ferulic and coumaric acids synthesis is a common plant

461 defence mechanism to fungal infections. Panina et al. (2007) reported protection of tomato from *F.*
462 *oxysporum* as a consequence of ferulic and coumaric acids synthesis induced by the biocontrol non-
463 pathogenic fungus *F. oxysporum* CS-20 strain. Similarly, the biocontrol fungus *Pythium*
464 *oligandrum* elicited the accumulation of ferulic acid, protecting wheat from *Fusarium germinatum*
465 (Takenaka et al., 2003). Increased ferulic and coumaric acids level has been also associated to
466 tomato resistance to pathogens in resistant cultivars (Gayoso et al., 2010). The exact antifungal
467 mechanism of phenolic compounds is not yet fully elucidated, but may involve direct fungilytic
468 activity by disrupting cell membrane as well as inhibition of mycelial growth or the activation of
469 specific signalling pathways (Hayashi, 1997; Martins et al., 2015; Shalaby et al., 2016).
470
471 **4. Conclusion**
472 From this study, it emerges that *E. hirta* L. might be a potential and very rich source of phenolic
473 classes, such as gallotannins, hydroxybenzoic and hydroxycinnamic acids, and bioactive
474 components especially tri-*O*-galloyl-glucose isomers, feruloyl-coniferin, tetra-*O*-galloyl-glucose
475 isomers, di-*O*-galloyl-glucose isomers, ethyl-gallic acid, protocatechuic acid-*O*-pentoside-*O*-
476 hexoside, 5-*O*-caffeoyl-quinic acid *trans* isomer and digalloyl-quinic acid. The development and
477 implementation of new fungicides from these phenolics or, alternatively, the use of purified extracts
478 from *E. hirta*, may provide a new approach to control fungal diseases in tropical areas where, often,
479 sustainability of chemical control measures are not met. Additionally, since *E. hirta* is a very
480 common weed, the use of its extracts may provide an additional income to rural areas.

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## Figure captions

**Fig. 1. Representative negative ion mode base peak chromatograms (BPCs) of water (A) and ethanol (B) extracts from *Euphorbia hirta* leaves.** The shown BPCs are representative of three independent experiments**.**

**Fig. 2. Occurrence of phenolic classes in *Euphorbia hirta* extracts.** Global percentage of flavan-3-ols, flavonols, di-hydro-flavonols, hydroxybenzoic and hydroxycinnamic acids, gallotannins, ellagitannins, flavones and isocoumarins in water (A) and ethanol (B) extracts of *Euphorbia hirta* leaves. The total amounts of phenolic compounds quantified with mass spectrometry is also shown.

**Fig. 3. Structures of some newly identified *Euphorbia hirta* leaves phenolic compounds.** Examples of the phenolic structures present in highest concentration in the *Euphorbia hirta* leaves. (A) $R^1$: -H, coumaric acid; $-CH_3$, ferulic acid; (B) $R^1$: -OH, caffeoyl-quinic acid; $-CH_3$, feruloyl-quinic acid; (C) feruloyl-coniferin; (D) quercetin-7-*O*-pentoside; (E) apigenin-6-*C*-hexoside; (F) kaempferol-3-*O*-hexoside; (G) $R^1$: -OH, gallic acid; $-CH_2CH_3$, ethyl-gallic acid; (H) di-galloyl-quinic acid; (I) tri-galloyl-quinic acid; (J) gallotannins ($R^1$-$R^5$ may be –OH or –gallic acid); (K) brevifolin-carboxylic acid; (L) protocatechuic acid-*O*-pentoside-*O*-hexoside ($R^1$ and $R^2$ may identified a pentoside or hexoside moiety).

**Fig. 4. Antioxidant properties of water (black columns) and ethanol (grey columns) extracts from *Euphorbia hirta* leaves.** Antioxidant capacity (expressed as μmol ascorbic acid/g of powder) measured by three different assays (left *y*-axys). SOA: superoxide anion scavenging activity. The right *y*-axys detailed the $Fe^{2+}$-chelating ability of the two extracts expressed as percentage of bound $Fe^{2+}$. Each sample was run in triplicate and results are reported as mean values ± SD. Values with different letter within the same assay are significantly different ($P < 0.05$).

**Table 1**. Mass spectra data for phenolic compounds identified in water and ethanol extracts from *Euphorbia hirta* leaves.

| Peak | | Compound | [M-H]$^-$ (*m/z*) | MS$^2$ ion fragments (*m/z*) | Water extract | Ethanol extract |
|---|---|---|---|---|---|---|
| 1 | 1.1 | Coumaric acid | 163 | 119, 101 | - | + |
| 2 | 2.1 | 4-*O*-caffeoyl-quinic acid *cis* | 353 | 173, 191 | + | - |
| | 2.2 | HHDP-hexoside isomer | 481 | 301, 275 | + | - |
| 3 | 3.1 | HHDP-hexoside isomer | 481 | 301, 275 | + | - |
| | 3.2 | Dihydro-hydroxy-brevifolin-dicarboxylic acid | 353 | 291, 247, 203, 335 | - | + |
| 4 | 4.1 | Galloyl-quinic acid isomer | 343 | 191, 169, 125 | + | + |
| | 4.2 | Galloyl-glucose isomer | 331 | 169, 125, 271, 211 | + | + |
| 5 | 5.1 | HHDP-hexoside isomer | 481 | 301, 275 | + | - |
| | 5.2 | Galloyl-glucose isomer | 331 | 169, 125, 271, 211 | + | - |
| | 5.3 | Feruloyl-coniferin | 517 | 337, 193, 175, 217 | + | + |
| | 5.4 | Galloyl-di-*O*-hexoside | 493 | 331, 313, 271, 169, 211 | + | - |
| | 5.5 | Galloyl-quinic acid isomer | 343 | 191, 169, 125 | + | - |
| 6 | 6.1 | Gallic acid | 169 | 125 | + | + |
| | 6.2 | Galloyl-glucose isomer | 331 | 169, 125, 271, 211 | + | - |
| | 6.3 | Ellagitannin | 847 | 481, 301 | + | - |
| | 6.4 | Gallotannin | 465 | 271, 169, 313, 301 | + | + |
| | 6.5 | 4-*O*-caffeoyl-quinic acid *trans* | 353 | 173, 191 | + | - |
| | 6.6 | Galloyl-quinic acid isomer | 343 | 191, 169, 125 | + | - |
| | 6.7 | Galloyl-HHDP-hexoside isomer (corilagin) | 633 | 301, 481, 275 | + | - |
| 7 | 7.1 | Ellagitannin | 681 | 481, 301, 663, 619 | - | + |
| | 7.2 | Brevifolin-dicarboxylic acid-hexoside isomer | 497 | 335, 291, 247, 203 | - | + |
| 8 | 8.1 | Galloyl-glucose isomer | 331 | 169, 125, 271, 211 | + | - |
| 9 | 9.1 | Ellagitannin | 681 | 481, 301, 663, 619 | - | + |
| 10 | 10.1 | 5-*O*-coumaroyl-quinic acid trans | 337 | 191, 173, 233, 337 | + | + |
| | 10.2 | Galloyl-shikimic acid | 325 | 169, 125 | + | - |
| | 10.3 | Di-*O*-galloyl-glucose isomer | 483 | 271, 193, 211, 169, 313, 331 | + | - |
| | 10.4 | 3,5-*O*-dicaffeoyl-quinic acid | 515 | 191, 353, 179, 173 | + | - |
| | 10.5 | Protocatechuic acid-*O*-hexoside | 315 | 153, 109 | + | - |

| | | | | | | |
|---|---|---|---|---|---|---|
| 11 | 11.1 | Galloyl-HHDP-hexoside isomer (corilagin) | 633 | 301, 481, 275 | + | + |
| | 11.2 | Protocatechuic acid-*O*-pentoside-*O*-hexoside | 447 | 315, 153 | + | - |
| | 11.3 | Isorhamnetin-3-*O*-pentoside | 447 | 315, 300, 301 | + | - |
| | 11.4 | 3-*O*-caffeoyl-quinic acid cis | 353 | 191, 179, 135 | + | + |
| 12 | 12.1 | 3-*O*-caffeoyl-quinic acid trans | 353 | 191, 179, 135 | + | - |
| 13 | 13.1 | Protocatechuic acid | 153 | 109 | - | + |
| 14 | 14.1 | Caffeic acid-*O*-hexoside | 341 | 179, 135 | + | - |
| | 14.2 | Kaempferol-3-*O*-hexoside isomer | 447 | 285, 255 | + | - |
| | 14.3 | Procyanidin dimer B-type isomer | 577 | 407, 289, 245, 425 | + | - |
| | 14.4 | Feruloyl-caffeoyl-quinic acid isomer | 529 | 353, 173 | + | - |
| | 14.5 | Di-*O*-galloyl-glucose isomer | 483 | 271, 193, 211, 169, 313, 331 | + | - |
| | 14.6 | Quercetin-3-*O*-hexoside-7-*O*-hexoside | 625 | 463, 301, 273, 271 | + | - |
| 15 | 15.1 | Digalloyl-quinic acid | 495 | 343, 191, 169 | + | - |
| 16 | 16.1 | Tri-*O*-galloyl-glucose isomer | 635 | 271, 193, 211, 483, 169, 313, 331 | + | - |
| | 16.2 | Taxifolin-3-*O*-hexoside isomer | 465 | 303, 285, 241 | + | - |
| 17 | 17.1 | bis-HHDP-hexoside (pedunculagin I) | 783 | 301, 275 | - | + |
| | 17.2 | Dehydro-galloyl-HHDP-hexoside isomer | 631 | 451, 301, 275 | + | + |
| | 17.3 | Procyanidin dimer B-type isomer | 577 | 407, 289, 245, 425 | + | - |
| | 17.4 | Brevifolin-carboxylic acid-hexoside isomer | 453 | 291, 247 | + | + |
| | 17.5 | Digallic acid | 321 | 277, 169, 125 | - | + |
| 18 | 18.1 | Dehydro-galloyl-HHDP-hexoside isomer | 631 | 451, 301, 275 | + | + |
| 19 | 19.1 | 5-*O*-caffeoyl-quinic acid trans | 353 | 191 | + | - |
| | 19.2 | Tri-*O*-galloyl-glucose isomer | 635 | 271, 193, 211, 483, 169, 313, 331 | + | - |
| | 19.3 | Taxifolin-3-*O*-hexoside isomer | 465 | 303, 285, 241 | + | - |
| | 19.4 | Feruloyl-caffeoyl-quinic acid isomer | 529 | 353, 173 | + | - |
| 20 | 20.1 | Galloyl-HHDP-hexoside isomer (corilagin) | 633 | 301, 481, 275 | + | + |
| | 20.2 | Epicatechin | 289 | 245, 205, 179, 125 | + | + |
| | 20.3 | Procyanidin dimer B-type isomer | 577 | 407, 289, 245, 425 | + | - |
| 21 | 21.1 | Brevifolin-carboxylic acid | 291 | 247 | + | + |

| | | | | | | |
|---|---|---|---|---|---|---|
| | 21.2 | Granatin B isomer | 951 | 933, 301 | + | - |
| | 21.3 | Tri-galloyl-quinic acid | 647 | 495, 343 | - | + |
| 22 | 22.1 | Apigenin-7-*O*-rhamnoside | 415 | 269 | + | - |
| | 22.2 | Di-galloyl-HHDP-hexoside (pedunculagin II) | 785 | 481, 301 | + | - |
| 23 | 23.1 | Brevifolin-carboxylic acid-galloyl-hexoside | 605 | 453, 291, 247 | + | + |
| | 23.2 | Tri-*O*-galloyl-glucose isomer | 635 | 271, 193, 211, 483, 169, 313, 331 | + | - |
| | 23.3 | Quercetin-3-*O*-galactoside | 463 | 301, 179, 271, 151 | + | - |
| | 23.4 | Tetra-*O*-galloyl-glucose isomer | 787 | 635, 617, 483, 301 | + | - |
| | 23.5 | 5-*O*-caffeoyl-quinic acid cis | 353 | 191 | + | - |
| 24 | 24.1 | Apigenin-8-*C*-hexoside-4'-*O*-hexoside | 593 | 473, 431, 311, 301, 179, 271, 151 | + | + |
| | 24.2 | Dihydro-kaempferol-7-*O*-hexoside | 449 | 287, 269, 259 | + | - |
| | 24.3 | Di-*O*-galloyl-rhamnose | 467 | 423, 315, 169 | + | - |
| | 24.4 | Kaempferol-7-*O*-hexoside-3-*O*-rutinoside | 755 | 593, 375, 285, 255 | + | - |
| 25 | 25.1 | Caffeic acid | 179 | 135 | + | + |
| | 25.2 | 5-*O*-coumaroyl-quinic acid cis | 337 | 191, 173, 233, 337 | + | - |
| | 25.3 | Kaempferol-3-*O*-hexoside isomer | 447 | 285, 255 | + | - |
| | 25.4 | Galloyl-salicin | 437 | 313, 169, 125 | + | - |
| | 25.5 | Apigenin-6-*C*-hexoside-8-*C*-pentoside | 563 | 545, 473, 443, 413, 383, 353, 303 | + | + |
| 26 | 26.1 | Dihydroxy-benzoic acid | 153 | 109 | - | + |
| 27 | 27.1 | Galloyl-quinic acid-*O*-hexoside | 505 | 343, 313, 169 | + | - |
| | 27.2 | Granatin B isomer | 951 | 933, 301 | + | - |
| | 27.3 | Tetra-*O*-galloyl-glucose isomer | 787 | 635, 617, 483, 301 | + | - |
| | 27.4 | Myricetin-3-*O*-hexoside | 479 | 433, 316, 287, 179 | + | - |
| | 27.5 | 5-*O*-feruloyl-quinic acid | 367 | 191, 173 | + | - |
| | 27.6 | (Epi)afzelechin-*C*-hexoside-*O*-hexoside | 597 | 435, 315 | + | - |
| 28 | 28.1 | Ellagic acid-malonyl-pentoside isomer | 519 | 301, 501, 484, 413, 319, 275, 229, 199 | - | + |
| 29 | 29.1 | Quercetin-3-*O*-rutinoside | 609 | 301, 271, 179, 151 | + | - |
| 30 | 30.1 | Penta-*O*-galloyl-glucose | 939 | 785, 769, 617 | + | - |
| | 30.2 | Myricetin-3-*O*-pentoside | 449 | 316, 317, 287, 179 | + | - |

| | | | | | | |
|---|---|---|---|---|---|---|
| | 30.3 | Apigenin-6-*C*-hexoside | 431 | 341, 311, 283, 413 | + | + |
| | 30.4 | Ellagic acid-malonyl-pentoside isomer | 519 | 301, 501, 484, 413, 319, 275, 229, 199 | - | + |
| 31 | 31.1 | Quercetin-3-*O*-glucoside | 463 | 301, 179, 271, 151 | + | + |
| | 31.2 | Kaempferol-3-*O*-hexoside isomer | 447 | 285, 255 | + | - |
| | 31.3 | Ethyl-gallic acid | 197 | 169, 125 | + | + |
| | 31.4 | Ellagitannin | 765 | 301, 463, 626, 229 | + | - |
| | 31.5 | Ellagic acid | 301 | 271, 229 | + | + |
| | 31.6 | Quercetin-3-*O*-glucuronide | 477 | 301, 179, 271, 151 | + | + |
| | 31.7 | Quercetin-3-*O*-galloyl-hexoside isomer | 615 | 463, 301, 271 | + | - |
| 32 | 32.1 | Isorhamnetin-3-*O*-rutinoside | 623 | 315, 300, 301, 179 | + | - |
| | 32.2 | Kaempferol-3-*O*-rutinoside | 593 | 285, 255 | + | - |
| | 32.3 | Quercetin-3-*O*-pentoside | 433 | 301, 271, 179, 151, 300 | + | - |
| | 32.4 | Quercetin-3-*O*-acetyl-hexoside isomer | 505 | 300, 301, 463, 271, 179, 151 | + | - |
| | 32.5 | Gallagic acid | 601 | 313, 287, 211, 169 | + | - |
| 33 | 33.1 | Gallotannin | 659 | 465, 313, 489 | + | - |
| | 33.2 | Kaempferol-3-*O*-hexoside isomer | 447 | 285, 255 | + | - |
| | 33.3 | Quercetin-7-*O*-pentoside | 433 | 301, 273, 179, 151, 300 | + | - |
| | 33.4 | Quercetin-3-*O*-rhamnoside | 447 | 301, 179, 151, 271 | + | - |
| | 33.5 | Quercetin-7-*O*-galloyl-pentoside isomer | 585 | 433, 301, 273, 179, 255 | + | - |
| | 33.6 | Kaempferol-3-*O*-glucuronide | 461 | 285, 255 | + | - |
| 34 | 34.1 | Ferulic acid | 193 | 178, 149, 134 | + | + |
| | 34.2 | Feruloyl-malic acid | 309 | 193 | + | - |
| 35 | 35.1 | Quercetin-3-*O*-acetyl-hexoside isomer | 505 | 300, 301, 463, 271, 179, 151 | + | - |
| | 35.2 | Trigallic-acid | 473 | 271, 211, 169 | + | - |
| | 35.3 | Kaempferol-3-*O*-pentoside isomer | 417 | 285, 284, 255 | + | - |
| | 35.4 | Kaempferol-3-*O*-acetyl-hexoside | 489 | 327, 285, 255 | + | - |
| | 35.5 | Quercetin-7-*O*-galloyl-pentoside isomer | 585 | 433, 301, 273, 179, 255 | + | - |
| 36 | 36.1 | Kaempferol-3-*O*-pentoside isomer | 417 | 285, 284, 255 | + | - |
| | 36.2 | Kaempferol-3-*O*-rhamnoside | 431 | 285, 255 | + | - |
| 37 | 37.1 | Kaempferol-7-*O*-galloyl-pentoside isomer | 569 | 285, 257, 417 | + | - |

| | | | | | | |
|---|---|---|---|---|---|---|
| | 37.2 | Quercetin-3-*O*-galloyl-hexoside isomer | 615 | 463, 301, 271 | + | - |
| | 37.3 | Quercetin-7-*O*-galloyl-pentoside isomer | 585 | 433, 301, 273, 179, 255 | + | - |
| 38 | 38.1 | Kaempferol-7-*O*-galloyl-pentoside isomer | 569 | 285, 257, 417 | + | - |
| 39 | 39.1 | Kaempferol-7-*O*-galloyl-pentoside isomer | 569 | 285, 257, 417 | + | - |
| 40 | 40.1 | Quercetin | 301 | 151, 179 | + | - |
| 41 | 41.1 | Deoxyellagic acid | 285 | 257, 229, 185 | + | - |
| 42 | 42.1 | Chrysin | 253 | 209 | + | - |

HHDP: 2,3-(S)-hexahydroxydiphenoyl

**Table 2**. Mass spectra data for non-phenolic phytochemicals identified in water and ethanol extracts from *Euphorbia hirta* leaves.

| Peak | Compound | | $[M-H]^-$ *(m/z)* | $MS^2$ ion fragments *(m/z)* | Sample[a] | Class |
|---|---|---|---|---|---|---|
| a | a.1 | Hydroxycitric acid | 207 | 163, 119, 101 | EE | Organic acid |
| | a.2 | Quinic acid | 191 | 111 | WE and EE | Organic acid |
| b | b.1 | Shikimic acid | 173 | 155, 111 | EE | Organic acid |
| c | c.1 | Citric acid | 191 | 173, 111 | WE and EE | Organic acid |
| d | d.1 | Malic acid | 133 | 115 | EE | Organic acid |
| e | e.1 | Gibberelin CA29 | 347 | 303, 259, 163, 150 | EE | Terpene |
| f | f.1 | Chelidonic acid | 183 | 139 | EE | Organic acid |
| g | g.1 | Roseoside | 385 | 223, 153 | WE | Norisoprenoid |
| h | h.1 | Chrysophanol-8'-O-(6'-O-galloyl)-glucose | 567 | 331, 313, 271, 211, 169 | WE | Anthraquinone |
| i | i.1 | Albopilosin H | 331 | 313, 295, 255, 241 | EE | Terpene |
| L | l.1 | Ponicidin | 361 | 343, 325, 315, 271, 253, 235 | EE | Terpene |
| | l.2 | Isojaponins A | 377 | 359, 341, 315, 297 | EE | Terpene |
| p | p.1 | Phenylalanine[b] | 166 | 120 | WE and EE | Amino acid |
| | p.2 | Tyrosine[b] | 182 | 165, 136 | WE and EE | Amino acid |
| | p.3 | Tryptophan[b] | 205 | 188, 159, 144 | EE | Amino acid |
| | p.4 | Gluconic acid[b] | 235 | 118 | WE and EE | Organic acid |
| | p.5 | Glutamic acid-tyrosine[b] | 311 | 182, 165, 136 | WE and EE | Dipeptide |
| | p.6 | Ternatoside C[b] | 466 | 304, 258, 190 | EE | Alkaloid |

[a]WE means that the compound was found in the aqueous extract whereas EE in the ethanol extract
[b]Indicates $[M+H]^+$ rather than $[M-H]^-$

**Table 3**. Quantitative data for tannins (ellagitannins and gallotannins) identified in water and ethanol extracts from *Euphorbia hirta* leaves. Values are expressed as mg/g of dry extract and represent means ± standard deviation of triplicate determination (n.d. means not detected).

| | Compound | Water extract (mg/g) | Ethanol extract (mg/g) |
|---|---|---|---|
| **Ellagitannins[a]** | | | |
| 41.1 | Deoxyellagic acid | 0.80 ± 0.01 | n.d. |
| 31.5 | Ellagic acid | 1.40 ± 0.03 | 0.40 ± 0.01 |
| 2.2 | HHDP-hexoside isomer | 0.13 ± 0.01 | n.d. |
| 3.1 | HHDP-hexoside isomer | 0.21 ± 0.01 | 0.42 ± 0.01 |
| 5.1 | HHDP-hexoside isomer | 0.25 ± 0.01 | n.d. |
| 28.1 | Ellagic acid-malonyl-pentoside isomer | n.d. | 0.59 ± 0.02 |
| 30.4 | Ellagic acid-malonyl-pentoside isomer | n.d. | 0.54 ± 0.01 |
| 32.5 | Gallagic acid | 0.64 ± 0.03 | n.d. |
| 17.2 | Dehydro-galloyl-HHDP-hexoside isomer | 0.25 ± 0.01 | 0.24 ± 0.01 |
| 18.1 | Dehydro-galloyl-HHDP-hexoside isomer | 0.28 ± 0.01 | 0.17 ± 0.01 |
| 6.7 | Galloyl-HHDP-hexoside (corilagin) isomer | 0.14 ± 0.02 | n.d. |
| 11.1 | Galloyl-HHDP-hexoside (corilagin) isomer | 1.22 ± 0.09 | 0.26 ± 0.01 |
| 20.1 | Galloyl-HHDP-hexoside (corilagin) isomer | 1.95 ± 0.01 | 0.29 ± 0.02 |
| 6.3 | Ellagitannin | n.d. | 0.19± 0.01 |
| 7.1 | Ellagitannin | n.d. | 0.26 ± 0.01 |
| 9.1 | Ellagitannin | 0.27 ± 0.01 | n.d. |
| 17.1 | bis-HHDP-hexoside (pedunculagin I) | n.d. | 0.35 ± 0.02 |
| 22.2 | Di-galloyl-HHDP-hexoside (pedunculagin II) | 0.23 ± 0.01 | n.d. |
| 31.4 | Ellagitannin | 0.01 ± 0.00 | n.d. |
| 21.2 | Granatin B isomer | 0.88 ± 0.01 | n.d. |
| 27.2 | Granatin B isomer | 0.68 ± 0.01 | n.d. |
| | **Total ellagitannins** | **9.32 ± 0.10 (5.7%)** | **3.52 ± 0.03 (7.1%)** |

**Gallotannins**[b]

| | | | |
|---|---|---|---|
| 6.4 | Gallotannin | 0.57 ± 0.04 | 0.45 ± 0.01 |
| 24.3 | Di-*O*-galloyl-rhamnose | 3.23 ± 0.02 | n.d. |
| 10.3 | Di-*O*-galloyl-glucose isomer | 4.10 ± 0.08 | n.d. |
| 14.5 | Di-*O*-galloyl-glucose isomer | 6.76 ± 0.11 | n.d. |
| 16.1 | Tri-*O*-galloyl-glucose isomer | 3.17 ± 0.04 | n.d. |
| 19.2 | Tri-*O*-galloyl-glucose isomer | 5.57 ± 0.03 | n.d. |
| 23.2 | Tri-*O*-galloyl-glucose isomer | 10.11 ± 0.34 | n.d. |
| 33.1 | Gallotannin | 0.85 ± 0.01 | n.d. |
| 23.4 | Tetra-*O*-galloyl-glucose isomer | 0.95 ± 0.09 | n.d. |
| 27.3 | Tetra-*O*-galloyl-glucose isomer | 14.35 ± 0.06 | n.d. |
| 30.1 | Penta-*O*-galloyl-glucose | 1.64 ± 0.01 | n.d. |
| | **Total gallotannins** | **51.30 ± 0.39 (31.4%)** | **0.45 ± 0.01 (0.9%)** |

[a]Quantified as ellagic acid equivalent
[b]Quantified as gallic acid equivalent
Water and ethanol extracts were prepared by dissolving 20 mg of powder obtained from the extraction procedures in 1 mL of the respective solvent.
HHDP: 2,3-(S)-hexahydroxydiphenoyl

**Table 4**. Quantitative data for flavonols identified in water and ethanol extracts from *Euphorbia hirta* leaves. Values are expressed as mg/g of dry extract and represent means ± standard deviation of triplicate determination (n.d. means not detected).

| Compound | | Water extract (mg/g) | Ethanol extract (mg/g) |
|---|---|---|---|
| **Flavonols[a]** | | | |
| 40.1 | Quercetin | 0.78 ± 0.01 | n.d. |
| 32.3 | Quercetin-3-*O*-pentoside | 1.57 ± 0.21 | n.d. |
| 33.3 | Quercetin-7-*O*-pentoside | 3.77 ± 0.20 | n.d. |
| 33.4 | Quercetin-3-*O*-rhamnoside | 0.83 ± 0.03 | n.d. |
| 23.3 | Quercetin-3-*O*-galactoside | 0.20 ± 0.01 | n.d. |
| 31.1 | Quercetin-3-*O*-glucoside | 1.96 ± 0.01 | 0.07 ± 0.00 |
| 31.6 | Quercetin-3-*O*-glucuronide | 0.48 ± 0.01 | 0.11 ± 0.00 |
| 32.4 | Quercetin-3-*O*-acetyl-hexoside isomer | 0.53 ± 0.01 | n.d. |
| 35.1 | Quercetin-3-*O*-acetyl-hexoside isomer | 0.09 ± 0.00 | n.d. |
| 33.5 | Quercetin-7-*O*-galloyl-pentoside isomer | 0.33 ± 0.02 | n.d. |
| 35.5 | Quercetin-7-*O*-galloyl-pentoside isomer | 0.39 ± 0.01 | n.d. |
| 37.3 | Quercetin-7-*O*-galloyl-pentoside isomer | 1.05 ± 0.02 | n.d. |
| 29.1 | Quercetin-3-*O*-rutinoside | 0.31 ± 0.01 | n.d. |
| 31.7 | Quercetin-3-*O*-galloyl-hexoside isomer | 0.07 ± 0.00 | n.d. |
| 37.2 | Quercetin-3-*O*-galloyl-hexoside isomer | 0.16 ± 0.01 | n.d. |
| 14.6 | Quercetin-3-*O*-hexoside-7-*O*-hexoside | 0.28 ± 0.01 | n.d. |
| 35.3 | Kaempferol-3-*O*-pentoside isomer | 1.03 ± 0.04 | n.d. |
| 36.1 | Kaempferol-3-*O*-pentoside isomer | 3.00 ± 0.10 | n.d. |
| 36.2 | Kaempferol-3-*O*-rhamnoside | 0.15 ± 0.02 | n.d. |
| 14.2 | Kaempferol-3-*O*-hexoside isomer | 0.15 ± 0.01 | n.d. |
| 25.3 | Kaempferol-3-*O*-hexoside isomer | 0.14 ± 0.01 | n.d. |

| | | | |
|---|---|---|---|
| 31.2 | Kaempferol-3-*O*-hexoside isomer | 0.12 ± 0.01 | n.d. |
| 33.2 | Kaempferol-3-*O*-hexoside isomer | 0.14 ± 0.01 | n.d. |
| 33.6 | Kaempferol-3-*O*-glucuronide | 0.17 ± 0.01 | 0.07 ± 0.00 |
| 35.4 | Kaempferol-3-*O*-acetyl-hexoside | 0.19 ± 0.01 | n.d. |
| 37.1 | Kaempferol-7-*O*-galloyl-pentoside isomer | 0.13 ± 0.01 | n.d. |
| 38.1 | Kaempferol-7-*O*-galloyl-pentoside isomer | 0.23 ± 0.01 | n.d. |
| 39.1 | Kaempferol-7-*O*-galloyl-pentoside isomer | 0.47 ± 0.02 | n.d. |
| 32.2 | Kaempferol-3-*O*-rutinoside | 0.59 ± 0.01 | n.d. |
| 24.4 | Kaempferol-7-*O*-hexoside-3-*O*-rutinoside | 0.12 ± 0.00 | n.d. |
| 30.2 | Myricetin-3-*O*-pentoside | 0.31 ± 0.01 | n.d. |
| 27.4 | Myricetin-3-*O*-hexoside | 0.16 ± 0.01 | n.d. |
| 11.3 | Isorhamnetin-3-*O*-pentoside | 0.72 ± 0.02 | n.d. |
| 32.1 | Isorhamnetin-3-*O*-rutinoside | 0.16 ± 0.01 | n.d. |
| | **Total flavonols** | **20.78 ± 0.31 (12.7%)** | **0.26 ± 0.00 (0.5%)** |

[a]Quantified as quercetin-3 glucoside equivalent with the exception of the kaempferol-derivative which were quantified as kaempferol equivalent

Water and ethanol extracts were prepared by dissolving 20 mg of powder obtained from the extraction procedures in 1 mL of the respective solvent.

**Table 5**. Quantitative data for flavan-3-ols, flavones, dihydroflavonols and isocoumarins identified in water and ethanol extracts from *Euphorbia hirta* leaves. Values are expressed as mg/g of dry extract and represent means ± standard deviation of triplicate determination (n.d. means not detected).

| | Compound | Water extract (mg/g) | Ethanol extract (mg/g) |
|---|---|---|---|
| **Flavan-3-ols[a]** | | | |
| 20.2 | Epicatechin | 0.39 ± 0.01 | 0.08 ± 0.01 |
| 14.3 | Procyanidin dimer B-type isomer | 0.10 ± 0.00 | n.d. |
| 17.3 | Procyanidin dimer B-type isomer | 0.80 ± 0.01 | n.d. |
| 20.3 | Procyanidin dimer B-type isomer | 0.23 ± 0.01 | n.d. |
| 27.6 | (Epi)afzelechin-*C*-hexoside-*O*-hexoside | 0.46 ± 0.02 | n.d. |
| | **Total flavan-3-ols** | **1.97 ± 0.02 (1.2%)** | **0.08 ± 0.01 (0.2%)** |
| **Flavones[b]** | | | |
| 42.1 | Chrysin | 0.16 ± 0.01 | 0.26 ± 0.01 |
| 22.1 | Apigenin-7-*O*-rhamnoside | 0.96 ± 0.01 | n.d. |
| 30.3 | Apigenin-6-*C*-hexoside | 2.18 ± 0.11 | 0.32 ± 0.01 |
| 25.5 | Apigenin-6-*C*-hexoside-8-*C*-pentoside | 0.33 ± 0.01 | 0.18 ± 0.01 |
| 24.1 | Apigenin-8-*C*-hexoside-4'-*O*-hexoside | 0.13 ± 0.01 | 0.12 ± 0.01 |
| | **Total flavones** | **3.76 ± 0.11 (2.3%)** | **0.89 ± 0.01 (1.8%)** |
| **Dihydroflavonols[b]** | | | |
| 24.2 | Dihydro-kaempferol-7-*O*-hexoside | 0.04 ± 0.00 | n.d. |
| 16.2 | Taxifolin-3-*O*-hexoside isomer | 0.17 ± 0.01 | n.d. |
| 19.3 | Taxifolin-3-*O*-hexoside isomer | 0.09 ± 0.00 | n.d. |
| | **Total dihydroflavonols** | **0.30 ± 0.01 (0.2%)** | **n.d.** |
| **Isocoumarins[c]** | | | |
| 21.1 | Brevifolin-carboxylic acid | 5.25 ± 0.13 | 3.61 ± 0.05 |

| | | | |
|---|---|---|---|
| 3.2 | Dihydro-hydroxy-brevifolin-dicarboxylic acid | n.d. | 4.86 ± 0.05 |
| 17.4 | Brevifolin-carboxylic acid-hexoside | 2.04 ± 0.10 | 2.73 ± 0.06 |
| 7.2 | Brevifolin-dicarboxylic acid-hexoside | n.d. | 2.09 ± 0.02 |
| 23.1 | Brevifolin-carboxylic acid-galloyl-hexoside | 2.27 ± 0.04 | 0.51 ± 0.01 |
| | **Total isocoumarins** | **9.56 ± 0.17 (5.8%)** | **13.81 ± 0.09 (27.8%)** |

[a]Quantified as catechin equivalent

[b]Quantified as quercetin-3-glucoside equivalent

[c]Quantified as gallic acid equivalent

Water and ethanol extracts were prepared by dissolving 20 mg of powder obtained from the extraction procedures in 1 mL of the respective solvent.

**Table 6**. Quantitative data for phenolic acids (hydroxycinnamic and hydroxybenzoic acids) identified in water and ethanol extracts from *Euphorbia hirta* leaves. Values are expressed as mg/g of dry extract and represent means ± standard deviation of triplicate determination (n.d. means not detected).

| | Compound | Water extract (mg/g) | Ethanol extract (mg/g) |
|---|---|---|---|
| **Hydroxycinnamic acids[a]** | | | |
| 1.1 | Coumaric acid | n.d. | 2.58 ± 0.07 |
| 25.1 | Caffeic acid | 0.26 ± 0.01 | 0.16 ± 0.01 |
| 34.1 | Ferulic acid | 1.97 ± 0.02 | 2.55 ± 0.06 |
| 34.2 | Feruloyl-malic acid | 0.52 ± 0.03 | n.d. |
| 10.1 | 5-*O*-coumaroyl-quinic acid *trans* | 1.28 ± 0.04 | 1.05 ± 0.01 |
| 25.2 | 5-*O*-coumaroyl-quinic acid *cis* | 1.25 ± 0.01 | n.d. |
| 14.1 | Caffeic acid-*O*-hexoside | 0.17 ± 0.01 | n.d. |
| 2.1 | 4-*O*-caffeoyl-quinic acid *cis* | 0.15 ± 0.01 | n.d. |
| 6.5 | 4-*O*-caffeoyl-quinic acid *trans* | 1.29 ± 0.01 | n.d. |
| 11.4 | 3-*O*-caffeoyl-quinic acid *cis* | 0.22 ± 0.01 | 0.29 ± 0.01 |
| 12.1 | 3-*O*-caffeoyl-quinic acid *trans* | 1.33 ± 0.01 | n.d. |
| 19.1 | 5-*O*-caffeoyl-quinic acid *trans* | 7.46 ± 0.06 | n.d. |
| 23.5 | 5-*O*-caffeoyl-quinic acid *cis* | 0.65 ± 0.05 | n.d. |
| 27.5 | 5-*O*-feruloyl-quinic acid | 2.70 ± 0.03 | n.d. |
| 10.4 | 3,5-*O*-dicaffeoyl-quinic acid | 0.50 ± 0.01 | n.d. |
| 5.3 | Feruloyl-coniferin | 3.09 ± 0.08 | 15.71 ± 0.24 |
| 14.4 | Feruloyl-caffeoyl-quinic acid isomer | 0.24 ± 0.01 | n.d. |
| 19.4 | Feruloyl-caffeoyl-quinic acid isomer | 0.19 ± 0.01 | n.d. |
| | **Total hydroxycinnamic acids** | **23.26 ± 0.12 (14.2%)** | **22.34 ± 0.26 (45.0%)** |
| **Hydroxybenzoic acids[b]** | | | |
| 13.1 | Protocatechuic acid | n.d. | 0.49 ± 0.01 |
| 26.1 | Dihydroxy-benzoic acid | n.d. | 0.21 ± 0.01 |
| 6.1 | Gallic acid | 1.25 ± 0.04 | 6.07 ± 0.28 |
| 31.3 | Ethyl-gallic acid | 8.96 ± 0.14 | 0.43 ± 0.01 |
| 10.5 | Protocatechuic acid-*O*-hexoside | 3.34 ± 0.02 | n.d. |

| | | | |
|---|---|---|---|
| 17.5 | Digallic acid | n.d. | 0.33 ± 0.01 |
| 10.2 | Galloyl-shikimic acid | 1.29 ± 0.02 | n.d. |
| 4.2 | Galloyl-glucose isomer | 0.30 ± 0.01 | 0.26 ± 0.01 |
| 5.2 | Galloyl-glucose isomer | 0.86 ± 0.02 | n.d. |
| 6.2 | Galloyl-glucose isomer | 0.71 ± 0.01 | n.d. |
| 8.1 | Galloyl-glucose isomer | 0.68 ± 0.01 | n.d. |
| 4.1 | Galloyl-quinic acid isomer | 1.32 ± 0.01 | 0.48 ± 0.01 |
| 5.5 | Galloyl-quinic acid isomer | 0.84 ± 0.01 | n.d. |
| 6.6 | Galloyl-quinic acid isomer | 0.08 ± 0.00 | n.d. |
| 25.4 | Galloyl-salicin | 1.04 ± 0.01 | n.d. |
| 11.2 | Protocatechuic acid-*O*-pentoside-*O*-hexoside | 8.03 ± 0.13 | n.d. |
| 35.2 | Trigallic-acid | 0.58 ± 0.01 | n.d. |
| 5.4 | Galloyl-di-*O*-hexoside | 0.65 ± 0.05 | n.d. |
| 15.1 | Digalloyl-quinic acid | 7.11 ± 0.03 | n.d. |
| 27.1 | Galloyl-quinic acid-*O*-hexoside | 0.49 ± 0.05 | n.d. |
| 21.3 | Trigalloyl-quinic acid | 5.87 ± 0.09 | n.d. |
| | **Total hydroxybenzoic acids** | **43.37 ± 0.23 (26.5%)** | **8.27 ± 0.28 (16.7%)** |

[a]Quantified as caffeic acid equivalent (caffeic acid derivative) or coumaric acid equivalent (coumaric acid derivative) or ferulic acid equivalent (ferulic acid derivative)

[b]Quantified as gallic acid equivalent (gallic acid derivative) or protocatechuic acid equivalent (protocatechuic acid derivative)

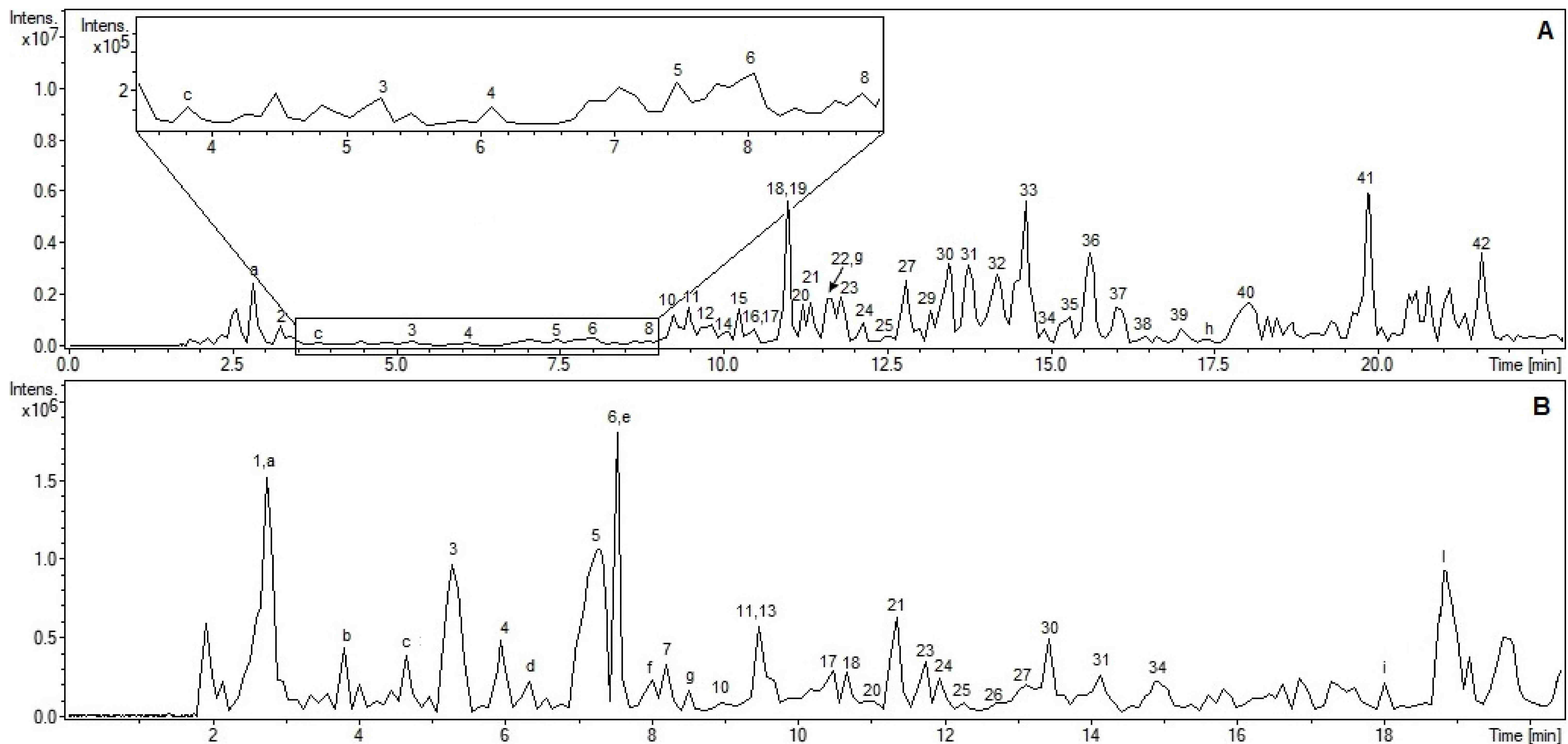
Water and ethanol extracts were prepared by dissolving 20 mg of powder obtained from the extraction procedures in 1 mL of the respective solvent.

**Table 7**. Mycelium growth inhibition of *Fusarium oxysporum* f. sp. *vasinfectum*, *Alternaria solani*, and *Rhizoctonia solani* as observed on potato dextrose agar medium added with the ethanol or water extracts of *Euphorbia hirta* leaves.

| **Extract** | ***Alternaria solani*** | ***Rhizoctonia solani*** | ***Fusarium oxysporum vasinfectum*** |
|---|---|---|---|
| | | **$IC_{50}$ (mg of dry extract/mL)** | |
| EE | $3.23 \pm 0.73^{a}$ | $3.66 \pm 0.11^{a}$ | $2.93 \pm 0.14^{a}$ |
| WE | $6.87 \pm 0.19^{b}$ | $32.14 \pm 0.59^{b}$ | $12.38 \pm 0.21^{b}$ |

Data are the average ± SD of five replications. Data in the same column followed by the different letters are significantly different ($p<0.05$).

WE means water extract of *Euphorbia hirta* leaves whereas EE ethanol extract of *Euphorbia hirta* leaves.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0.6
0.4
0.2
0.0
0.0
2.5
5.0
7.5
10.0
12.5
15.0
17.5
20.0
Time [min]
Intens.
x10^5
2
c
3
4
5
6
8
4
5
6
7
8
a
2
c
3
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27
29
30
31
32
33
34
35
36
37
38
39
h
40
41
42
B
Intens.
x10^6
1.5
1.0
0.5
0.0
2
4
6
8
10
12
14
16
18
Time [min]
1,a
b
c
3
4
d
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6,e
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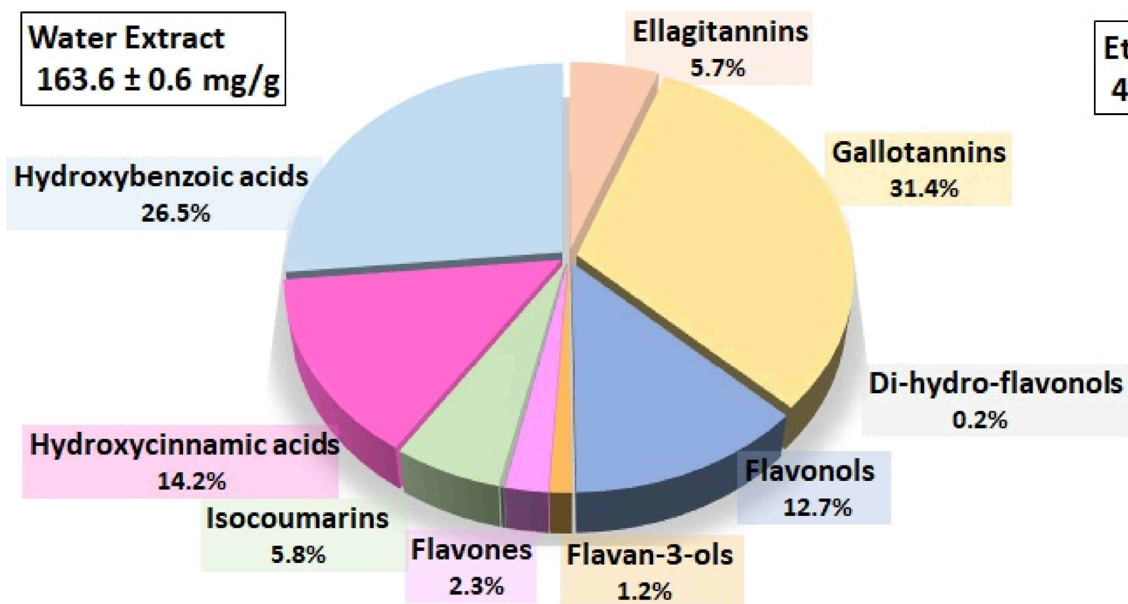
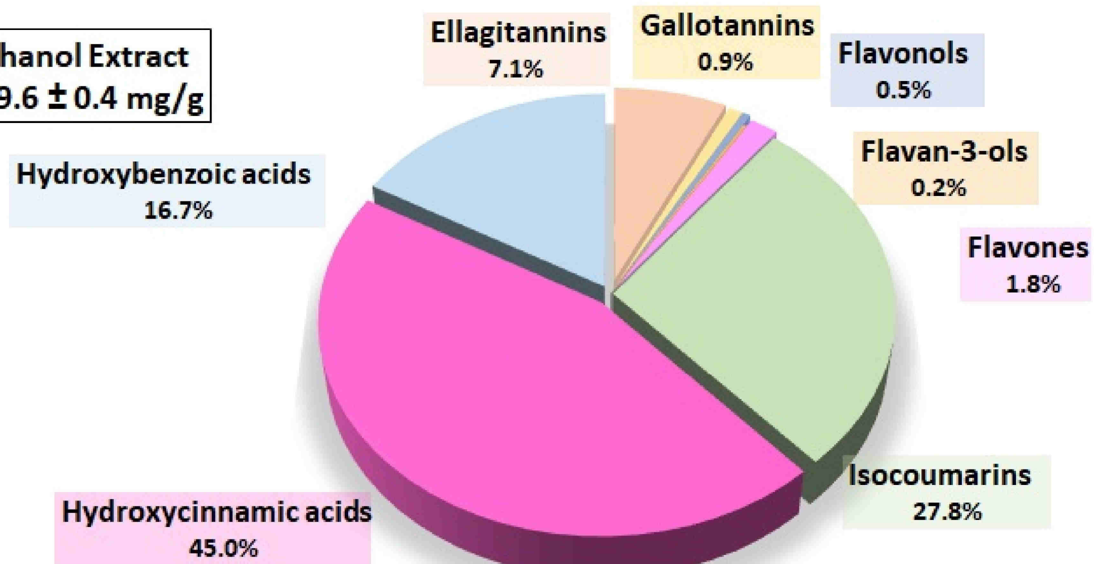
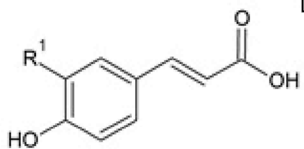


Water Extract
163.6 ± 0.6 mg/g
Ellagitannins 5.7%
Gallotannins 31.4%
Di-hydro-flavonols 0.2%
Flavonols 12.7%
Flavan-3-ols 1.2%
Flavones 2.3%
Isocoumarins 5.8%
Hydroxycinnamic acids 14.2%
Hydroxybenzoic acids 26.5%

Ethanol Extract
49.6 ± 0.4 mg/g
Ellagitannins 7.1%
Gallotannins 0.9%
Flavonols 0.5%
Flavan-3-ols 0.2%
Flavones 1.8%
Isocoumarins 27.8%
Hydroxycinnamic acids 45.0%
Hydroxybenzoic acids 16.7%

**A**



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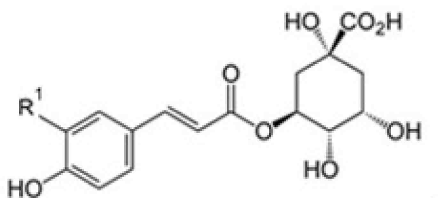

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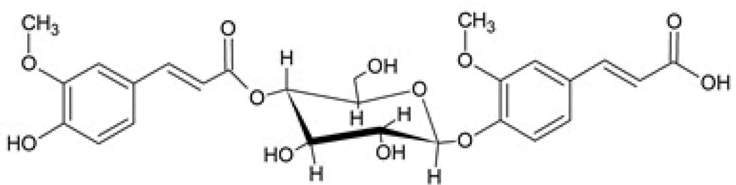

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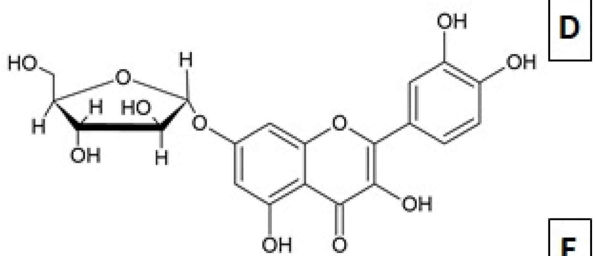

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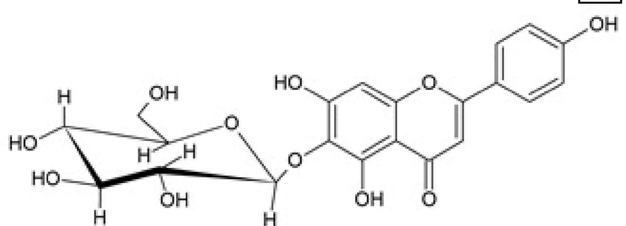

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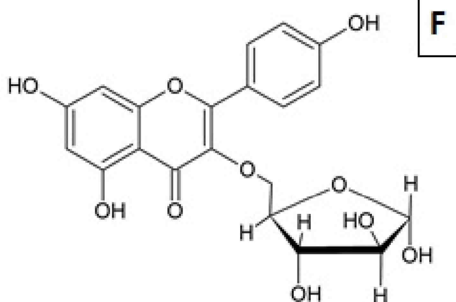

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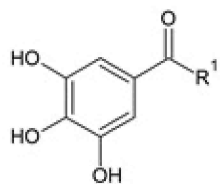

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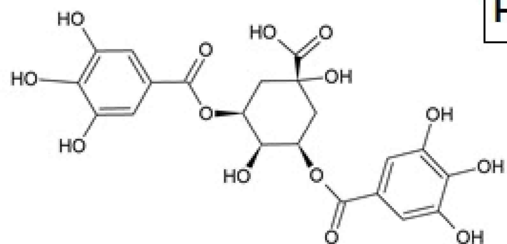

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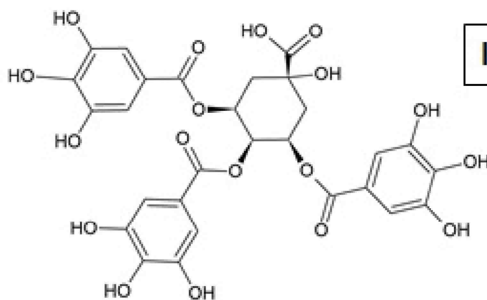

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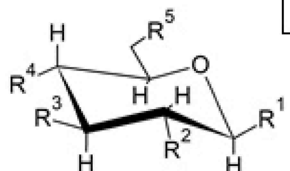

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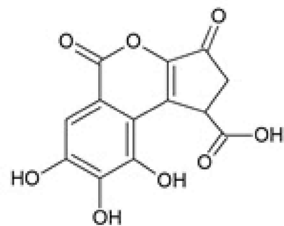

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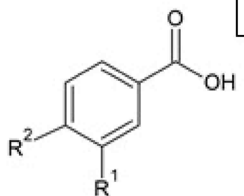

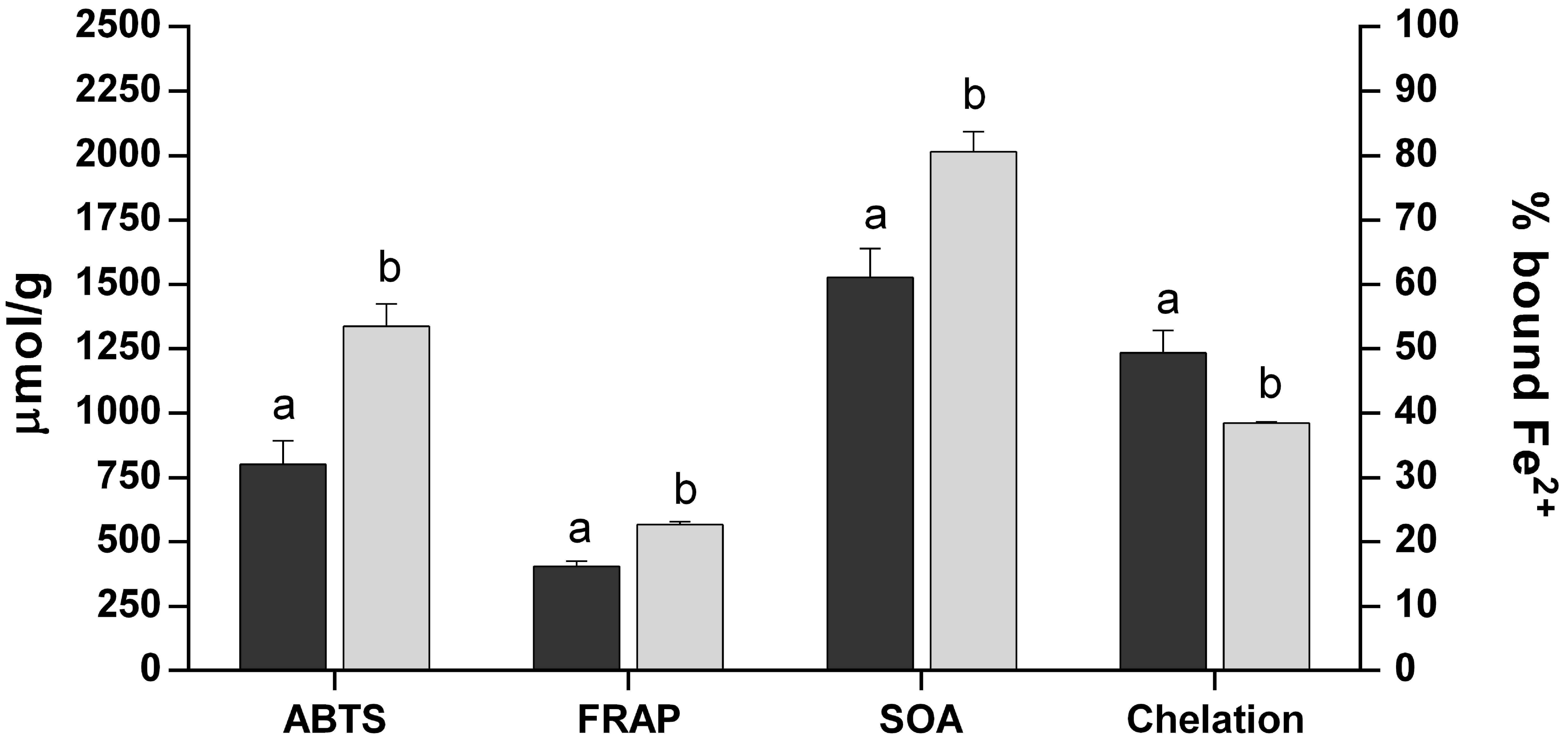

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1000
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b
a
b
a
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a
b
ABTS
FRAP
SOA
Chelation
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90
80
70
60
50
40
30
20
10
0
% bound $Fe^{2+}$