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Sustainable Extraction and Use of Natural Bioactive Compounds from the Waste Management Process of *Castanea* spp. Bud-Derivatives: The FINNOVER Project

Dario Donno [1,2,*], Federica Turrini [3], Raffaella Boggia [3], Maddalena Guido [4], Giovanni Gamba [1,2], Maria Gabriella Mellano [1,2], Isidoro Riondato [1] and Gabriele L. Beccaro [1,2]

[1] Department of Agriculture, Forestry and Food Science—DISAFA, University of Torino, 10095 Grugliasco (TO), Italy; giovanni.gamba@unito.it (G.G.); gabriella.mellano@unito.it (M.G.M.); isidoro.riondato@unito.it (I.R.); gabriele.beccaro@unito.it (G.L.B.)

[2] Chestnut R&D Center, 12013 Chiusa Pesio, Italy

[3] Department of Pharmacy—DIFAR, University of Genoa, 16148 Genova, Italy; turrini@difar.unige.it (F.T.); boggia@difar.unige.it (R.B.)

[4] Azienda Agricola Geal Pharma, 10060 Bricherasio, Italy; info@gealpharma.it

* Correspondence: dario.donno@unito.it; Tel.: +39-011-670-8751

SUPPLEMENTARY MATERIALS

Solvents, Chemicals, and Standards

Maceration and extraction solvents (ethanol and glycerol), analytical HPLC grade solvents (acetonitrile, methanol, and formic acid), reagents for HPLC buffer (potassium dihydrogen phosphate and phosphoric acid) were purchased from Fluka Biochemika (Buchs, Switzerland) and Sigma–Aldrich (St Louis, MO, USA). Cetyltrimethylammonium bromide (cetrimide) was purchased from Extrasynthése (Genay, France), while 1,2-phenylenediamine dihydrochloride (OPDA) was purchased from Sigma–Aldrich.

All polyphenolic standards (caffeic acid, chlorogenic acid, coumaric acid, ferulic acid, hyperoside, isoquercitrin, quercetin, quercitrin, rutin, ellagic acid, gallic acid, catechin, epicatechin, castalagin, vescalagin) were purchased from Sigma–Aldrich, while organic acids (citric acid, malic acid, oxalic acid, quinic acid, succinic acid, and tartaric acid) were purchased from Fluka Biochemika. Ascorbic acid and dehydroascorbic acid were purchased from Extrasynthése. Milli-Q ultrapure water was produced by Sartorius Stedim Biotech mod. Arium (Sartorius, Göettingen, Germany).

Stock solutions of cinnamic acids and flavonols with a concentration of 1.0 mg mL^{-1} were prepared in methanol. From these solutions, four calibration standards (1000 ppm, 50 ppm, 250 ppm, 125 ppm) were prepared by dilution with methanol; stock solutions of benzoic acids, tannins, and catechins with a concentration of 1.0 mg mL^{-1} were prepared in a solution of 95% methanol and 5% water. From these solutions, four calibration standards were prepared by dilution with 50% methanol-water. Stock solutions of organic acids with a concentration of 1.0 mg mL^{-1} were prepared in ultrapure water. From these solutions, four calibration standards were prepared by dilution with water. Finally, stock solutions of ascorbic and dehydroascorbic acids with a concentration of 1.0 mg mL^{-1} were prepared in methanol. From these solutions, four calibration standards were prepared by dilution with methanol.

Suppl. Table 1. Chromatographic conditions of the used HPLC methods.

Method	Classes of Interest	Mobile Phase[1]	Wavelength (nm)
A	cinnamic acids, flavonols	A: 10 mM KH_2PO_4/H_3PO_4, pH = 2.8; B: CH_3CN	330
B	benzoic acids, catechins, tannins	A: $H_2O/CH_3OH/HCOOH$ (5:95:0.1 v/v/v), pH = 2.5; B: $CH_3OH/HCOOH$ (100:0.1 v/v)	280
C	organic acids	A: 10 mM KH_2PO_4/H_3PO_4, pH = 2.8; B: CH_3CN	214
D	vitamin C	A: 5 mM $C_{16}H_{33}N(CH_3)_3Br$/50 mM KH_2PO_4, pH = 2.5; B: CH_3OH	261; 348

[1]Elution conditions

A. gradient analysis: 5%B to 21%B in 17 min + 21%B in 3 min (2 min conditioning time); flow: 1.5 mL min^{-1}

B. gradient analysis: 3%B to 85%B in 22 min + 85%B in 1 min (2 min conditioning time); flow: 0.6 mL min^{-1}

C. gradient analysis: 5%B to 14%B in 10 min + 14%B in 3 min (2 min conditioning time); flow: 0.6 mL min^{-1}

D. isocratic analysis: ratio of phase A and B: 95:5 in 10 min (5 min conditioning time); flow: 0.9 mL min^{-1}

Stationary phase: KINETEX – C18 column (4.6 × 150 mm, 5 μm)

Suppl. Table 2. HPLC-fingerprint of the chestnut bud-preparations and corresponding PUAE extracts obtained by from the processing waste.

Class	Molecule	Traditional bud-preparation *Mean value±SD* (mg/100 g FW)	PUAE extract *Mean value±SD* (mg/100 g FW)
Cinnamic acids	*caffeic acid*	1.61±0.02[a]	1.49±0.02[b]
	chlorogenic acid	14.23±0.29[a]	11.10±0.06[b]
	coumaric acid	n.d.	n.d.
	ferulic acid	4.34±0.22[a]	1.93±0.30[b]
Flavonols	*hyperoside*	3.03±0.10[a]	1.19±0.07[b]
	isoquercitrin	n.d.	n.d.
	quercetin	30.64±0.23[a]	15.39±0.22[b]
	quercitrin	29.01±0.82[a]	1.24±0.46[b]
	rutin	1.54±0.12[a]	0.29±0.14[b]
Benzoic acids	*ellagic acid*	48.95±0.13[a]	5.87±0.10[b]
	gallic acid	94.71±0.24[a]	2.36±0.11[b]
Catechins	*catechin*	1.28±0.32[a]	0.39±0.12[b]
	epicatechin	31.02±0.17[a]	4.14±0.10[b]
Tannins	*castalagin*	327.61±1.64[a]	23.37±0.81[b]
	vescalagin	153.61±0.40[a]	6.90±0.46[b]
Organic acids	*citric acid*	185.97±0.49[a]	9.19±0.45[b]
	malic acid	97.21±0.25[a]	5.01±0.02[b]
	oxalic acid	58.34±0.25[a]	18.97±0.06[b]
	quinic acid	53.47±0.40[a]	9.98±0.18[b]
	succinic acid	31.84±0.68[a]	2.78±0.14[b]
	tartaric acid	89.69±0.15[a]	27.14±0.15[b]
Vitamin C	*ascorbic acid*	15.79±0.12[a]	10.49±0.04[b]
	dehydroascorbic acid	2.29±0.14[a]	1.22±0.13[b]

Different letters represent significant statistical differences in accordance with the Student's t-test ($p < 0.05$; $N = 3$).