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ORIGINAL PAPER

Tannins analysis from different medicinal plants extracts using MALDI-TOF and MEKC

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The matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF) and micellar electrokinetic chromatography (MEKC) methods were used to identify and quantify five tannins, (+)-catechin, (−)-epigallocatechin, (−)-epigallocatechin gallate, (−)-epicatechin gallate and (−)-epicatechin, from aqueous, ethanolic and acetonic extracts of *Calendula officinalis*, *Hypericum perforatum*, *Galium verum* and *Origanum vulgare*. The MALDI-TOF technique was used for screening tannins monomers and oligomers in plant extracts. The sandwich method and matrix 2,5-dihydroxybenzoic acid with a concentration of 10 mg mL^{-1} in acetonitrile/ultrapure water/trifluoroacetic acid (20 : 80 : 0.1, vol.) were used. The electrophoretic method developed for the separation and quantification of 5 catechins in 15 min exhibited good efficiency and precision, low limits of detection (0.0032–0.0153 μg mL^{-1}) and quantification (0.0096–0.0466 μg mL^{-1}). The correlation coefficients (R^2) exceeded 0.9986 and the recovery values ranged between 94.25 % and 102.50 %. The present work provides new information on some of the less studied compounds present in plants frequently used in traditional medicine.

Keywords: tannin, plant extract, MALDI-TOF, MEKC

Introduction

Tannins are natural compounds, generally water-soluble, with a molar mass between 300 Da and 20000 Da, and with a particular importance in traditional medicine and the leather industry. Depending on their chemical structures, tannins are divided into the following categories: gallotannins (galloyl units are bound to diverse polyol-, catechin- or triterpenoid units), ellagitannins (two units of galloyl are C—C coupled to each other), complex tannins (the catechin unit is bound glycosidically to a gallotannin or an ellagitannin unit) and condensed tannins (formed by the linkage of C-4 of one catechin with C-8 or C-6 of the next monomeric catechin). Due to their properties, tannins are used in many ways: as coagulants in rubber production, in the production of inks, textile dyes and cationic dyes, and as antioxidants in fruits, juices, wines and beer (Khanbabaee & van Ree, 2001). Different studies have demonstrated that tannins (especially epigallocatechin gallate) can inhibit carcinogenesis, or the growth of established cancers, in organs such as the stomach, liver, lung, mammary gland, colon and skin (Horie et al., 2005; Tabrez et al., 2013). Green tea polyphenols are known to influence the activities of tyrosine kinases receptors and signal transduction pathways, thereby altering the expression of genes involved in cell proliferation, angiogenesis and apoptosis (Khan & Mukhtar, 2008). In recent years, differ-

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ent methods have been developed to identify the tannins, especially catechins, in food products and green tea. These methods include thin-layer chromatography (TLC) (Li et al., 1996; Glavnik et al., 2009), liquid-chromatography (LC) with diode array detection (DAD) (Merken & Beecher, 2000), with fluorescence detection (FD) (Tsanova-Savova et al., 2005; Gürbüz et al., 2007), mass spectrometry detection (MS) (Flamini, 2003; Del Rio et al., 2004; Chang & Wu, 2011) and with electrochemical detection (ED) (Yang et al., 2000; Kolouchová-Hanzlíková et al., 2004). Due to their simplicity, selectivity and short migration time, capillary zone electrophoresis (CZE) and micellar electrokinetic chromatography (MEKC) are frequently used for the determination of various compounds from different plant extracts (Horie & Kohata, 2000; Bonoli et al., 2003; Weiss & Anderton, 2003). The MECK technique was first introduced by Terabe et al. (1984) and facilitates the simultaneous separation of both neutral and charged species and is more sensitive than simple CZE. Nuclear magnetic resonance (NMR) (Jáč et al., 2006) and Fourier transformnear infrared spectroscopy (FT-NIR) have also been used for the determination of catechins (Chen et al., 2009).

Matrix-assisted laser desorption ionisation (MALDI) is a "soft" ionisation technique which, combined with the unlimited mass range of time-of-flight mass spectrometry, represents an important tool for the analysis of larger (proteins, carbohydrates, peptide) and smaller molecules (mass 100–500 Da) (Menet et al., 2004; Navarrete et al., 2010; Olofson et al., 2014). Because the MALDI-TOF technique affords the simultaneous determination of masses in mixtures, it can also be used in the food industry (Reed et al., 2005). This technique is simple and affords rapid identification with a high sensitivity of the compounds of interest.

This work sought to identify and quantify the tannins from different extracts (aqueous, acetonic and ethanolics) prepared from four medicinal plants, namely Marigold (*Calendula officinalis*, *Asteraceae*), St John's wort (*Hypricum perforatum*, *Hypericaceae*), Yellow Bedstraw (*Galium verum*, *Rubiaceae*) and Oregano (*Origanum vulgare*, *Laminaceae*), using the MALDI-TOF and MEKC techniques. The extracts of these plants were previously studied in order to quantify the content of polyphenols and the correlated antioxidant activity (Danila et al., 2011). The present study reveals the advantages of using the MALDI-TOF technique to indicate the existence of tannins or other structures in plant extracts. The MEKC method thus developed and partially validated made possible the rapid identification (15 min) and sensitive quantification of 5 tannins: (+)-catechin, (−)-epigallocatechin, (−)-epigallocatechin gallate, (−)-epicatechin gallate and (−)-epicatechin in the different samples.

Experimental

(+)-Catechin (C, over 90 % purity) and (−)-epicatechin (EC, over 90 % purity) were purchased from Sigma–Aldrich (Germany). (−)-Epigallocatechin (EGC, over 90 % purity), (−)-epigallocatechin gallate (EGCG, over 97 % purity) and (−)-epicatechin gallate (ECG, over 98 % purity) were from Fluka (Germany). Ultrapure water, 0.1 M and 1 M sodium hydroxide solutions were supplied by Agilent Technologies (Germany). Potassium dihydrogen phosphate, sodium tetraborate and sodium dodecyl sulphate (SDS) used for capillary electrophoresis were from Sigma–Aldrich (Germany). Solvents (Merck, Germany), solutions and background electrolyte (BGE) were filtered on PVDF 0.2 μm membranes (Millipore, Bedford, MA, USA) and were degassed prior to each analysis. Stock standard solutions and plant extracts were stored at 4 ℃. Formic acid, the matrix and the calibration mixture used for MALDI-TOF, C104 (Bradykinin, Angiotensin II, Neurotensin, ACTH, Bovine Insulin chain B) were supplied by Bio-Laser (France).

Individual stock standard solutions were prepared by dissolving 1 mg of each compound in 1 mL of methanol. Working standard solutions were diluted in BGE and brought to ambient temperature prior to each analysis.

Sample preparation

From each plant, five different extracts were prepared using dried aerial parts of *C. officinalis* (Dacia Plant, L 81367 31), *H. perforatum* (Dacia Plant, L 81343 4), *G. verum* (S.C. Stef Mar S.R.L.) and *O. vulgare* (S.C. Stef Mar S.R.L.) obtained from a local pharmacy (Council of Europe, 2010) as follows: water extract at ambient temperature (1 g : 10 mL); 30 vol. %, 50 vol. % and 70 vol. % ethanol extracts (1 g : 10 mL); acetone extract [acetone : water, 80 : 20, vol.] (1 g : 30 mL); infusion at 95 ℃, (1 g : 10 mL). The aqueous and ethanol extracts were maintained at 40 ℃ for seven days, filtered through a Whatman filter paper and then stored at 40 ℃. Prior to each analysis, the extracts were filtered through 0.2 μm Millipore membrane filter. For MALDI-TOF analysis, the extracts were concentrated using a Concentrator plus Eppendorf and were then dissolved in acetone (9.6 mg mL^{-1}).

Analytical

The spectra were recorded on an Axima CFR-Plus MALDI-TOF instrument from Shimadzu-Kratos and processed using Launchpad software. The irradiation source was a nitrogen laser at 337 nm. The acceleration voltage was set at 20 V with a delay time of 100 ns and the measurements were performed in the positive-ion reflectron mode. A C104 (500–3500

Da) standard mix was used for external mass calibration. 10 mg mL^{-1} of 2,5-dihydroxybenzoic acid (DHB) in acetonitrile/ultrapure water/trifluoroacetic acid (20 : 80 : 0.1, vol.) was used as the matrix. The tannin samples were mixed with the matrix in equal amounts and then transferred onto the MALDI plate using the sandwich technique. The power laser was set at 188 μJ and the tolerance for recorded masses was ± 0.5 Da.

An Agilent capillary electrophoresis instrument equipped with a diode array detector was used for analysis, and data acquisition and processing were accomplished using ChemStation software. The compounds were separated using a fused-silica capillary with total length of 72 cm (effective length of 63.5 cm) and an internal diameter of 50 μm. The BGE consisting of 10 mM KH_2PO_4 and 8.3 mM sodium tetraborate buffer with 66.7 mM SDS, pH 7.0 (adjusted with 1 M hydrochloric acid) was used. Prior to each analysis, the capillary was rinsed with 1 M NaOH and 0.1 M NaOH for 10 min, followed by ultra-pure water (10 min) and buffer (20 min). The capillary was washed between runs with background electrolyte for 3 min. After 3 consecutive runs, the background electrolyte was changed. The samples were injected hydrodynamically for 12 sec (3500 Pa), the system was operated under the positive voltage (30 kV) and the cassette temperature was maintained constant at 30 °C. Electrochromatograms were recorded at 210 nm.

The results were evaluated using the statistical linear regression analysis MaxStat program (Version 3.60; $p < 0.0001$).

Results and discussion

Analysis of tannins by MALDI-TOF

The MALDI-TOF technique was used to analyse aqueous, ethanolic and acetonic extracts in order to establish the presence of tannins and to obtain more information on the structures and polymerisation degree of the constituents. Different types of matrix such as: α-cyano-4-hydroxycinnamic acid (α-CHCA), sinapinic acid (SA) and 2,5-dihydroxybenzoic acid (DHB) with NaCl (10 mg mL^{-1}) were tested and different concentrations (between 5–10 mg mL^{-1}) were used to find the optimal conditions of the laser desorption/ionisation processes. DHB was found to be the best matrix for analysis of the tannins from the samples.

The MALDI-TOF spectra of the plant extracts revealed, in particular, the presence of monomer constituents C/EC, EGC, EGCG and ECG with molecular mass of 290.3 Da, 306.3 Da, 458.3 Da and 442.3 Da, respectively. In all the extracts, the presence of fisetinidin was observed (which has the same structure as catechin but without the —OH group in the C5 position of the A-ring) and dimmer structure of fisetinidin. The

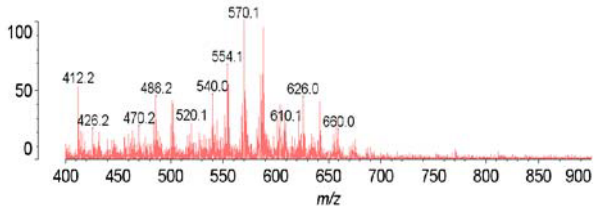

Fig. 1. MALDI-TOF spectrum in reflectron mode of 50 vol. % ethanol extracts of *H. perforatum* in the mass range of 400–900 Da.

detection of masses corresponding to a higher degree of polymerisation was achieved using the positive-ion reflectron mode with good resolution. The combination of masses of the oligomer peaks was calculated using the equation (Vázquez et al., 2013): $M + Na = 23 + 2 + 288.3A + 304.3B + 440.4C + 262(D/2) + 272.3F + 180S$; where: 23 is the mass of Na, 2 is the mass of two endpoint hydrogen atoms, A, B and C represent the number of mainly tannin monomer(s), F is the fisetinidin monomer, D is the number of dimmer structures of fisetinidin and S represents the number of sugar monomers found in the structure (generally rhamnose). Monomers and oligomers peaks were found in all the extracts in the mass range 1–3500 Da.

The spectra of the *C. officinalis* extracts presented the dominant peaks at $m/z = 290.3$ which indicated the presence of catechin/epicatechin structures. The spectra of these extracts also displayed small intensities corresponding to EGCG and ECG and, additionally, the presence of isorhamnetin-3-*O*-rutinoside ($m/z = 625.4$).

The positive-ion reflectron mode MALDI-TOF spectra of the *H. perforatum* extracts contained masses corresponding to the predominant structures of catechin/epicatechin, while poor signals were detected for EGC and ECG. The aqueous extracts spectra showed a high intensity at $m/z = 623.5$ corresponding to the structure of epicatechin gallate (442 Da) with a monosaccharide residue (180 Da) attached, as frequently appearing in aqueous extracts (Menet et al., 2004; Hoong et al., 2010; Navarette et al., 2010). It is desirable that the content of sugars should be as low as possible because their presence reduces the strength and water resistance of bonds in accordance with the amount added (Pizzi et al., 1983). In the extracts of *H. perforatum* (Fig. 1), naphtodianthrones such as hypericin ($m/z = 505.4$, $[M + H]^+$), pseudohypericin ($m/z = 521.1$, $[M + H]^+$), protopseudohypericin ($m/z = 523.1$, $[M + H]^+$) and phloglucinols like hyperforin ($m/z = 537.4$, $[M + H]^+$) are traceable. The MALDI-TOF analysis also exhibited a signal at $m/z = 610.1$ corresponding to the rutin structure.

The spectra of *G. verum* extracts presented peaks related to the masses of 497.9 Da, 775.9 Da, 1049.9 Da, 1324.3 Da and 1600 Da which indicate the presence of fisetinidin structures linked together. The part of the

spectrum with the low intensity peaks at 358.5 Da and 457.0 Da indicates the presence of pinoresinol and ursolic acid. For the *O. vulgare* extracts, the spectra showed intensity at $m/z = 716.2$ representing the theaflavin 3-gallate structure, found especially in the 70 vol. % ethanol extract.

Taking into account the results obtained by MALDI-TOF, it may be concluded that the acetonic extracts of all the plants studied in this paper showed higher intensities of compounds signals, a lower degree of polymerisation and a lower content of sugars.

Other compounds such as isorhamnetin (317.1 Da), galloyl (333.7 Da), di-epicatechin (428.1 Da) and disaccharide (343.4 Da) were identified by their molecular mass in these plant extracts. Isorhamnetin was detected in all the extracts and its presence confirms the results previously obtained by LC-APCI-MS (Matei et al., 2015). From these results, the MALDI-TOF technique may be deemed to be simple and fast (1.5 min), suitable for identifying and partially characterising the tannins and other compounds in plant extracts.

MEKC validation procedure

The MEKC method was applied to the quantitative determination of catechins identified using the MALDI-TOF technique. The method was derived from that described by Bonoli et al. (2003) with some modifications. The proposed method was validated for linearity, limits of detection (LOD) and quantification (LOQ), intra-day and inter-day precision and recovery. The linear detector response was in the concentration range of 2.0–40.0 μg mL^{-1}. Under these conditions, the LOD varied from 0.0032 μg mL^{-1} (EGCG) to 0.0153 μg mL^{-1} (C) and was calculated using the standard solution with lower concentration ($S/N = 3$). The LOQ was defined as the signal-to-noise ratio (S/N) of 10 and was between 0.0096 μg mL^{-1} (EGCG) and 0.0466 μg mL^{-1} (C) (Table 1).

Table 1. Values of analyses using external calibration curves ($n = 3$)

Compound	R^2	Linearity	LOD	LOQ
		μg mL^{-1}		
1	0.9990	2–40	0.0153	0.0466
2	0.9995	2–40	0.0075	0.0230
3	0.9986	2–40	0.0032	0.0096
4	0.9997	2–40	0.0132	0.0435
5	0.9994	2–40	0.0051	0.0152

The results obtained from the method validation are presented in Table 2. The intra-day ($n = 6$) and inter-day ($n = 6$) values were obtained after repeated injections of the standard solution at concentrations of 5 μg mL^{-1}, 10 μg mL^{-1} and 20 μg mL^{-1} for each analyte. The experimental results reveal the good precision of the method. The relative standard deviations were found to be between 0.03 % (EGC) and 1.10 % (EC) for the intra-day assay and between 0.16 % (ECG) and 1.20 % (EGC) for the inter-day assay.

Recovery values were determined by adding three different concentrations (4 μg mL^{-1}, 8 μg mL^{-1} and 12 μg mL^{-1}) of the standard catechins under investigation to the diluted sample solution of *G. verum*. The results were between 94.25 % for EGC and 102.50 % for EGCG (Table 3).

The method developed and validated in this study was compared with other data from the literature and the MEKC method described exhibited a higher accuracy than the other methods (HPLC, CZE and MEEK). Several methods for the quantitative analy-

Table 2. Intra- and inter-day precision data

Compound	Concentration	Intra-day	RSD	Inter-day	RSD
	μg mL^{-1}		%	μg mL^{-1}	%
1	5	5.070 ± 0.01	0.112	5.054 ± 0.037	0.323
	10	10.020 ± 0.020	0.254	10.054 ± 0.061	0.448
	20	20.030 ± 0.052	0.351	20.008 ± 0.062	0.501
2	5	4.999 ± 0.005	0.032	5.022 ± 0.059	0.385
	10	10.031 ± 0.015	0.178	9.979 ± 0.195	1.208
	20	20.028 ± 0.042	0.256	10.028 ± 0.095	0.932
3	5	5.040 ± 0.031	0.158	4.990 ± 0.027	0.352
	10	9.996 ± 0.062	0.452	10.076 ± 0.085	0.765
	20	20.107 ± 0.022	0.274	20.004 ± 0.059	0.421
4	5	5.042 ± 0.011	0.101	5.002 ± 0.035	0.168
	10	10.020 ± 0.125	0.921	10.084 ± 0.046	0.256
	20	20.090 ± 0.089	0.842	20.025 ± 0.063	0.482
5	5	5.034 ± 0.153	1.103	5.022 ± 0.043	0.249
	10	10.080 ± 0.036	0.194	10.060 ± 0.075	0.738
	20	20.060 ± 0.007	0.089	19.997 ± 0.024	0.262

Table 3. Recovery data for tannins compounds ($n = 3$)

Compound	Added	Found	Recovery
	μg mL^{-1}		%
1	4	3.89	97.25
	8	7.90	98.75
	12	12.19	101.58
2	4	3.96	99.00
	8	7.54	94.25
	12	11.57	96.42
3	4	4.03	100.75
	8	7.65	95.63
	12	12.30	102.50
4	4	3.78	94.50
	8	7.75	96.88
	12	11.36	94.66
5	4	3.91	97.75
	8	8.05	100.63
	12	12.13	101.08

Recovery = 100(Amount found/Amount added), sample was diluted 5 times.

sis of catechins in various types of tea and food are summarised in Table 4.

Quantification of tannins from plant extracts using MEKC method

The simultaneous determination of catechins was previously performed in different types of tea, especially in extracts of *Camellia sinensis* which is the plant most frequently studied due to its rich catechins content (Sang et al., 2011; Ananingsih et al., 2013). In the present study, the extracts from different commercial plants were analysed by the MEKC/DAD proposed method. The C, EGC, EGCG, ECG and EC found in plant extracts were identified by standard addition and by migration time. Fig. 2 shows an electrochromatogram of *G. verum* aqueous extract (diluted 5 times) (A) and an electrochromatogram of *G. verum* aqueous extract spiked with 8 μg mL^{-1} of each catechin. The 5 catechins are very well separated and the elution time is less than 15 minutes.

The tannin concentrations quantified by the proposed method showed some differences in the plant extracts analysed (Table 5).

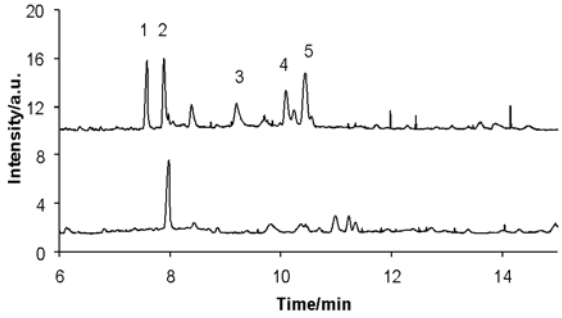

Fig. 2. Electrochromatograms of *G. verum* aqueous extract (λ = 210 nm): diluted 5 times (A); spiked with 8 μg mL^{-1} of: (+)-catechin (1); epigallocatechin (2); epigallocatechin gallate (3); epicatechin gallate (4); (−)-epicatechin (5).

The major component from the *C. officinalis* extracts is EGCG, ranging between 132.70 μg g^{-1} of dry mass (DW) in infusion and 370.43 μg g^{-1} of DW in 30 % ethanolic extract. The highest amount of EC was found in the 50 vol. % ethanol extract and the highest concentration of EGC (230.01 μg g^{-1} of DW) was detected in 70 vol. % ethanol extract. In comparison with the other plants, *C. officinalis* presented had lower concentrations of catechins. In the *H. perforatum* extracts, the highest amount of EGCG was identified in the 30 vol. % ethanol extract (1614.73 μg g^{-1} of DW) followed by those from the 70 vol. % ethanol extract (1388.74 μg g^{-1} of DW), the aqueous extract (1208.49 μg g^{-1} of DW), the acetone extract (544.24 μg g^{-1} of DW), the infusion (204.12 μg g^{-1} of DW) and the 50 vol. % ethanol extract (79.53 μg g^{-1} of DW). All the other compounds presented significant levels in different *H. perforatum* extracts (e.g. C with 387.89 μg g^{-1} of DW in the acetone extract, EGC with 949.51 μg g^{-1} of DW in the 70 vol. % ethanol extract and 862.80 μg g^{-1} of DW in the infusion, ECG with 749.67 μg g^{-1} of DW in the 50 vol. % ethanol extract and EC with 850.4 μg g^{-1} of DW in the infusion). In the *G. verum* extracts, only EGCG was found in noticeable concentrations in the 70 vol. % ethanol extract (1529.47 μg g^{-1} of DW) and C in the ace-

Table 4. Several methods applied to quantitative analysis of catechins

Sample extract	Number of analytes	Method	LOD/(μg mL^{-1})	Reference
Tea	11	HPLC	0.04–1.38	Tan et al. (2012)
Tea	9	HPLC	0.11–0.29	Rahim et al. (2014)
Tea	11	CZE	0.04–1.20	Arce et al. (1998)
Tea	7	MEEKC	0.30–6.61	Pomponio et al. (2003)
Vegetable and fruits	7	MEKC	0.618–1.93	López et al. (2011)
Tea	4	MEKC	1.00–2.50	Ye et al. (2014)
Medicinal plants	5	MEKC	0.003–0.015	Present study

Table 5. Catechins from plant extracts by MEKC (dry mass)

Plant	Extractant	C	EGC	EGCG	ECG	EC
		Content/(μg g^{-1})				
C. officinalis	Water	45.91	35.39	319.95	29.37	27.79
	Infusion	55.44	106.60	132.70	262.66	4.16
	Acetone	39.28	184.67	173.16	23.24	52.82
	30 vol. % Ethanol	12.08	93.93	370.43	130.34	22.74
	50 vol. % Ethanol	65.36	93.94	299.22	59.42	657.88
	70 vol. % Ethanol	30.61	230.01	253.07	543.01	5.98
H. perforatum	Water	203.06	34.98	1208.49	225.64	295.05
	Infusion	74.28	862.80	204.12	80.32	850.40
	Acetone	387.89	268.48	544.24	38.78	259.03
	30 vol. % Ethanol	38.90	53.74	1614.73	222.58	272.78
	50 vol. % Ethanol	108.20	782.30	79.53	749.67	62.02
	70 vol. % Ethanol	183.45	949.51	1388.74	47.10	5.94
G. verum	Water	519.81	58.41	330.57	20.40	133.44
	Infusion	238.80	42.12	10.93	61.80	0.15
	Acetone	1222.32	191.59	125.07	36.37	49.92
	30 vol. % Ethanol	23.76	66.25	64.50	52.36	24.44
	50 vol. % Ethanol	9.27	75.24	302.14	662.71	29.47
	70 vol. % Ethanol	20.50	34.95	1529.47	184.76	37.64
O. vulgare	Water	3.42	58.27	79.71	5.55	5.51
	Infusion	53.10	99.39	843.60	82.32	35.00
	Acetone	905.26	238.35	17947.66	2489.55	125.44
	30 vol. % Ethanol	531.55	158.17	1536.36	28.84	250.48
	50 vol. % Ethanol	129.77	1978.33	833.77	4.88	2032.31
	70 vol. % Ethanol	8012.33	2977.29	263.43	636.34	139.17

tone extract (1222.32 μg g^{-1} of DW). The quantitative results obtained for the *O. vulgare* extracts showed higher contents of EGCG in the infusion, acetone and 30 vol. % ethanol extracts (e.g. 17947.66 μg g^{-1} of DW in the acetone extract, being the highest quantity detected). All the other compounds were found in significant concentrations in various types of extracts, such as EC in the 50 vol. % ethanol extract (2032.31 μg g^{-1} of DW), C and EGC in the 70 vol. % ethanol extract (8012.33 μg g^{-1} and 2977.29 μg g^{-1} of DW, respectively) and ECG in the acetone extract (2489.55 μg g^{-1} of DW). The *O. vulgare* extracts exhibited the highest levels of tannins out of all the plants analysed.

The results of the current investigation reveal that the extraction environment played an important role in the process of catechins' extraction. To the best of our knowledge, no previous studies have been performed on tannins in the chosen medicinal plant materials that would render possible a comparison of the present results with data in the literature. The following plants were investigated in respect of volatile oil, phenolic acids, flavonoids and vitamins (Bilia et al., 2001; Mirza et al., 2004; Re et al., 2009; Lakić et al., 2010; De Falco et al., 2013; Quiroga et al., 2013). (+)-Catechin and (−)-epicatechin from ethanol extracts (30 vol. %, 50 vol. % and 70 vol. %) were identified among other polyphenolic compounds in our previous work (Matei et al., 2015) and the results accord with those obtained here.

With reference to the aqueous environment, which is more commonly used for consumption, it should be noted that EGCG was found to be plentiful in the aqueous extracts of *C. officinalis*, *H. perforatum* and *G. verum*, and in the *O. vulgare* infusion. (+)-Catechin was found in marked quantities in the aqueous extracts of *C. officinalis*, *H. perforatum* and *G. verum*, and EC in the *H. perforatum* (aqueous and infusion) and in the *G. verum* aqueous extract.

Major amounts of catechins were especially detected in the ethanolic extracts, namely 50 vol. % ethanolic extracts of *C. officinalis*, 70 vol. % ethanolic extracts of *H. perforatum*, *O. vulgare* and *G. verum*, but also in the acetone extracts of *O. vulgare* and *G. verum*.

The content of catechins found in the plant extracts analysed in the present work and used in traditional medicine is lower than the level of catechins reported in green tea extracts (the highest concentrations of catechins in the present samples was found in the acetonic extract of *O. vulgare*, approximately 21.6 mg g^{-1}, whereas a concentration of approximately 60 mg g^{-1} catechins was reported in a green tea infusion by Liu et al. (2014); however it may be noted that the presence of catechins in addition to other constituents renders the

consumption of these plants beneficial to human health.

Conclusions

The current study reports significant information provided by MALDI-TOF analysis on qualitative data recorded in a very short time (1.5 min). The MEKC method proved to be simple, fast and reliable, and showed excellent linearity, good precision and sensitivity for the analysis of catechins from different plant extracts. The quantitative data revealed that the ethanol extracts (especially 70 vol. %) had higher concentrations of catechins. To the best of our knowledge, no other studies have referred to the analysis of catechins in extracts of medicinal plants of *C. officinalis*, *H. perforatum*, *G. verum* and *O. vulgare*, using the MECK and MALDI-TOF techniques, or any other technique.

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