Polyphenols Analysis from Different Medicinal Plants Extracts Using Capillary Zone Electrophoresis (CZE)

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The electrophoretic method partially validated for separation and quantification of 16 polyphenols from aqueous and ethanolic extracts of Calendula officinalis, Hypericum perforatum, Galium verum and Origanum vulgare in 27 min showed good efficiency and precision, and low detection and quantification limits (between 0.06 - 1.38 μg mL^{-1} and 0.2 - 4.56 μg mL^{-1}, respectively). Correlation coefficients (r^2) exceed 0.994 and recovery values ranged between 86.66 and 101.54 %. Under these conditions the developed electrophoretic method would be suitable for the analysis of various extracts.

Keywords: polyphenols, plants extracts, CZE

Medicinal plants are promising and easily available sources of natural compounds with a wide range of biological activities and therefore attract the attention of researchers and ordinary people. Many medicinal plants are mainly used in home-type cure therapies, complementary medicine and modern medicine because of their supposed antioxidant, antimicrobial, anticancer, etc. properties. As a result, information on the antioxidant activity and phenolic compounds of medicinal plants is nowadays gaining increasing interest and the consumption of food rich in antioxidants is greater than ever. The biologic activities of medicinal plants are mainly endorsed by their antioxidant properties, since they can act as free-radical scavengers, electron or hydrogen donors and strong metal chelators, having neuroprotective properties and thus preventing the lipid peroxidation, DNA damage, etc. [1-5].

High-performance liquid chromatography (HPLC) was the most frequently method used for separation and quantification of polyphenolic compounds in different extracts with diode array, fluorometry and/or mass spectrometry detection. Capillary electrophoresis (CE) has proved to be a good alternative technique to HPLC due to its simplicity, good resolution, short analysis time and low consumption of chemicals and samples. For quantification of polyphenolic compounds from natural sources, the UV–Vis detection mode is satisfactory due to the fact that these compounds are biologically active in detectable quantities and not in trace amounts [6-8]. The major contribution of CE analysis compared to HPLC is the considerable low consumption of time, reagents and samples and a more simplified procedure.

The aim of this work was to describe specific polyphenols content from different extracts (aqueous and ethanolic) prepared from four medicinal plants, namely *Calendula officinalis*, *Hypericum perforatum*, *Galium verum* and *Origanum vulgare* using the CZE technique. The extracts of these plants were previously studied for antioxidant activity and some polyphenols composition using RP-HPLC and LC-MS methods [9, 10] and to quantify the tannins using micellar electro-kinetic chromatography (MEKC) [11]. In this study, ten polyphenolic acids (cinnamic acid, chlorogenic acid, sinapic acid, syringic acid, ferulic acid, coumaric acid, rosmarinic acid, salvianolic acid A, caffeic acid, gallic acid) and 6 flavonoids (rutin, naringenin, izoquercitrin, kaempferol, luteolin, quercetol) were quantified in 27 minutes from aqueous and ethanolic plant extracts using a reliable capillary zone electrophoretic method.

Experimental part

Materials and methods

Reagents and standard stock solution

Stock solutions (1 mg mL^{-1}) of caffeic acid (Sigma, C0625), quercetin (Sigma, Q4951), kaempferol (BioChemika, 60010), rutin (Sigma, R5143), luteolin (Fluka, 72511), ferulic acid (Aldrich, 128708), chlorogenic acid (Aldrich, C3878), gallic acid (Fluka, 48630), rosmarinic acid (Sigma, R4033), sinapic acid (Sigma, D7927), coumaric acid (Fluka, 28200), syringic acid (Fluka, 86230), naringenin (BioChemika, 71155), isoquercitrin (Roth, 7586.1), salvianolic acid A and cinnamic acid (Fluka, 96340) were prepared in methanol. Sodium tetraborate was purchased from Sigma (Germany) and sodium dodecyl sulfate (SDS) from Fluka (Switzerland). Ultrapure water and 0.1 and 1 N sodium hydroxide solutions were purchased from Agilent Technologies (Germany). Solvents (Merck, Germany) and solutions were filtered on 0.2μm membranes (Millipore, PTFE, Bedford, MA, USA) and degassed prior to use. Stock solutions for each standard were stored at +4° C. Working solutions were prepared daily by diluting the stock solutions in background electrolyte (BGE).

Sample preparation

Dried aerial parts of species *C. officinalis*, *H. perforatum*, *G. verum* and *O. vulgare* were bought from the local market. Five grams of each plant materials were added to 50 mL water and aqueous solutions of ethanol [30%, 50 and 70%, (w/v)]. The aqueous and ethanolic extracts were shaked daily for seven days at 4^{0}C in the dark. Samples were then filtered through Whatman filter paper and refrigerated at 4° C. Prior to each analysis the extracts were filtered through 0.2 μm membranes Millipore PTFE filter.

CZE equipment and method

An Agilent capillary electrophoresis instrument equipped with diode array detector was used for analysis; data acquisition and processing were done using ChemStation software. The compounds were separated using a fused-

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silica capillary column of 72 cm total length and 50μm internal diameter. The BGE consisting of 45 mM tetraborate buffer with 0.9 mM SDS (*p*H=9.35 adjusted with HCl 1 M) was used [12]. The capillary was rinsed between runs with BGE for 3 min. The sample was hydrodynamically injected for 12 s (35 mbar), the system was operated under positive voltage (30 kV) and the cassette temperature was maintained constant at 30°C. Electropherograms were recorded at 280 nm.

Statistical analysis

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

Results and discussions

Our electrophoretic method belongs to CZE category with direct UV detection. The anionic surfactant SDS improves the separation but was under critical concentration level for a micellar chromatography. The procedure that involves the use of tetraborate buffer at alkaline *p*H was based on prior experience described by Gatea et al. (2015) [13] with some modification regarding the polyphenolic compounds investigated in extracts (specific for plants).

Validation of the electrophoretic method

The main parameters used in the validation of the method are: the selectivity, linearity, precision, accuracy (recovery), limit of detection (LoD) and limit of quantification (LoQ). Table 1 presents the equations of regression lines which have good linearity in the range 2.5-60 μg mL^{-1} for caffeic acid, chlorogenic acid, gallic acid, salvianolic acid A, quercetol and kaempferol, 2.5 - 70μg mL^{-1} for luteolin, and 2.5-80μg mL^{-1}for the rest of the investigated compounds. LoD and LoQ used to assess sensitivity were estimated using a signal-to-noise ratio of 3 and 10, respectively.

Detection limits for the samples ranged between 0.06 μg mL^{-1} (cinnamic acid) and 1.38 μg mL^{-1} (rosmarinic acid). Linearity ranges used for compound quantification were acceptable, presenting correlation coefficients (r^2) between 0.994 and 0.999 for all 16 compounds taken into analysis. The method of standard additions was used for the identification of polyphenols, comparing their migration time with the migration times obtained for standard polyphenolic compounds (fig. 1)

Compound	t_R (min)	Linear regression equations	r^2	Linearity (μg mL^{-1})	LoD (μg mL^{-1})	LoD (μg mL^{-1})
Rutin	11.46±0.16	y = 0.582x + 1.652	0.998	2.5-80	0.48	1.59
Naringenin	11.77±0.13	y = 0.409x + 1.072	0.999	2.5-80	1.19	3.92
Izoquercitrin	12.48±0.11	y = 0.735x + 1.285	0.999	2.5-80	0.37	1.22
Cinnamic acid	13.12±0.14	y = 2.272x + 2.19	0.998	2.5-80	0.06	0.20
Chlorogenic acid	13.50±0.10	y = 0.912x + 1.260	0.999	2.5-60	0.44	1.45
Sinapic acid	13.81±0.10	y = 0.433x + 0.027	0.997	2.5-80	0.81	2.69
Syringic acid	15.19±0.09	y = 0.795x + 0.575	0.997	2.5-80	0.39	1.29
Ferulic acid	15.42±0.36	y = 1.326x + 1.467	0.998	2.5-80	0.16	0.53
Kaempferol	15.69±0.12	y = 3.12x + 0.666	0.998	2.5-60	0.15	0.50
Luteolin	16.56±0.14	y = 1.325x + 2.089	0.998	2.5-70	0.13	0.44
Coumaric acid	17.21±0.24	y = 2.267x + 1.647	0.998	2.5-80	0.17	0.56
Quercetol	18.27±0.24	y = 1.744x + 0.053	0.998	2.5-60	0.62	2.04
Rosmarinic acid	20.61±0.20	y = 0.659x + 2.439	0.998	2.5-80	1.38	4.56
Salvianolic acid A	22.24±0.33	y = 2.5281x + 1.8	0.994	2.5-60	0.22	0.74
Caffeic acid	23.99±0.52	y = 2.151x - 0.082	0.999	2.5-60	0.61	2.01
Gallic acid	26.24±0,68	y =0.644x + 0.415	0.999	2.5-60	0.60	1.97

Table 1
PERFORMANCE CHARACTERISTICS OF THE METHOD FOR POLYPHENOLIC COMPOUNDS SEPARATION

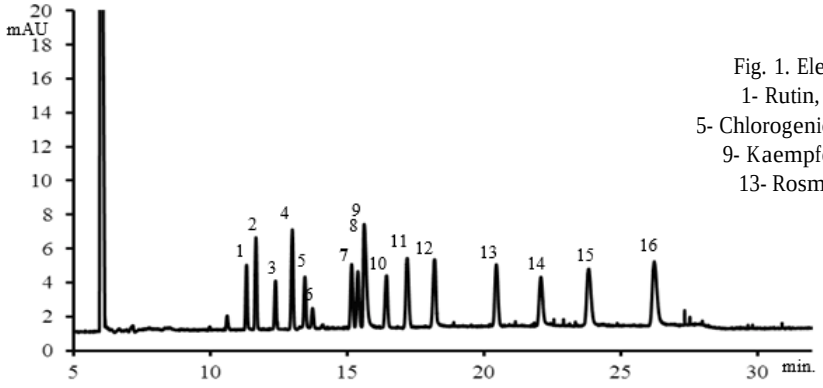

Fig. 1. Electropherogram of standard solutions (40μg mL^{-1}): 1- Rutin, 2- Naringenin, 3- Izoquercitrin, 4- Cinnamic acid, 5- Chlorogenic acid, 6- Sinapic acid, 7- Syringic acid, 8- Ferulic acid, 9- Kaempferol, 10- Luteolin, 11- Coumaric acid, 12- Quercetol, 13- Rosmarinic acid, 14-Salvianolic acid A, 15- Caffeic acid, 16- Gallic acid

Compound	Intra-assay Precision[a] (%, n = 5)	Inter-assay Precision[a] (%, n = 2x5)	Inter-assay Precision[b] (%, n = 2x5)	Inter-assay Precision[c] (%, n =2x5)
Rutin	1.02±0.09	2.42±0.36	4.34±0.44	4.52±0.30
Naringenin	2.85±0.30	4.66±0.38	3.45±0.83	2.99±1.37
Izoquercitrin	4.95±0.24	4.22±0.36	4.55±0.41	2.81±0.20
Cinnamic acid	4.57±0.40	3.52±0.26	3.22±0.58	2.35±0.66
Chlorogenic acid	4.09±0.35	4.76±0.26	4.80±0.78	3.41±0.90
Sinapic acid	4.06±0.35	4.40±0.42	5.07±0.54	3.54±0.52
Syringic acid	3.96±0.35	3.73±0.32	3.13±0.25	3.92±0.63
Ferulic acid	5.09±0.44	4.97±0.31	5.04±0.71	4.08±0.58
Kaempferol	3.12±0.28	4.87±0.39	2.77±0.93	2.45±0.32
Luteolin	3.58±0.32	4.55±0.51	3.82±0.31	3.21±0.22
Coumaric acid	4.92±0.42	4.15±0.25	2.13±0.52	2.91±0.98
Quercetol	5.00±0.44	4.80±0.43	3.35±0.27	4.08±0.24
Rosmarinic acid	4.32±0.38	4.71±0.44	3.81±0.54	3.97±0.32
Salvianolic acid A	5.12±0.46	4.38±0.41	4.36±0.36	4.11±0.29
Caffeic acid	4.90±0.44	4.30±0.45	4.07±0.50	4.96±0.75
Gallic acid	4.59±0.40	4.34±0.32	5.03±0.88	4.58±0.79

Table 2
PRECISION RESULTS OBTAINED FOR THE CZE SEPARATION METHOD

Standards concentrations: [a] 9 μg mL^{-1}; [b] 35 μg mL^{-1}; [c] 55 μg mL^{-1}; [*]mean values ± sd

Compound	Spiked concentration		
	12 μg mL^{-1}	24 μg mL^{-1}	36 μg mL^{-1}
	Recovery (%)* (mean values ±sd)		
Rutin	101.54 ± 3.75	98.45 ± 2.11	96.67.5 ± 1.67
Naringenin	96.71 ± 0.61	95.96 ± 5.63	95.02 ± 4.76
Izoquercitrin	92.54 ± 2.88	96.09 ± 3.61	97.54 ± 3.55
Cinnamic acid	99.39 ± 3.37	95.64 ± 3.54	99.77 ± 0.26
Chlorogenic acid	96.93 ± 4.27	98.92 ± 1.05	97.4 ± 3.14
Sinapic acid	96.02 ± 4.56	99.49 ± 3.20	98.39 ± 3.16
Syringic acid	96.84 ± 3.29	97 ± 3.47	93.75 ± 1.73
Ferulic acid	90.60 ± 2.99	86.88 ± 1.49	93.32 ± 4.16
Kaempferol	89.30 ± 3.64	94 ± 4.39	86.66 ± 1.93
Luteolin	94.81±0.96	97.45 ±3.05	93.52 ± 2.31
Coumaric acid	90.74 ± 4.35	91.54 ± 0.56	93.94 ± 2.71
Quercetol	94.27 ± 2.83	97.91 ± 2.83	98.27 ± 3.25
Rosmarinic acid	96.71 ± 4.80	96.07 ± 3.90	99.62 ± 5.28
Salvianolic acid A	97.30 ± 3.81	96.33 ± 0.90	93.48 ± 3.33
Caffeic acid	91.22 ± 3.21	92.87 ± 2.88	91.88 ± 4.11
Gallic acid	93.58 ± 5.03	94.05 ± 4.33	92.51 ± 3.41

Table 3
RECOVERY VALUES (%) OF POLYPHENOLS IN SAMPLE (*HYPERICUM PERFORATUM*)

**Recovery values expressed as [(average observed concentration) /(nominal concentration)] x100.*

The repeatability of the method was studied by repeated injections of the polyphenols mixtures (standards) 5 times in the same day (intra-day precision) whereas the reproducibility assimilated to inter-day precision was assessed by triplicate injections in 3 different days (table 2). The results are reported in terms of relative standard deviation (RSD). The RSD values for repeatability did not exceed 5.09 % for intra-day assays and 5.07 % for inter-day assays. Quantification limits maintained between 0.2μg mL^{-1} (cinnamic acid) and 4.56 μg mL^{-1} (rosmarinic acid).

In order to confirm the applicability of the proposed method for various types of polyphenolic extracts the recovery tests were performed for a 50% ethanolic sample of *Hypericum perforatum* (diluted 10 times) spiked with known concentrations of standard solutions (table 3). The recovery assays presented results between 86.66% and

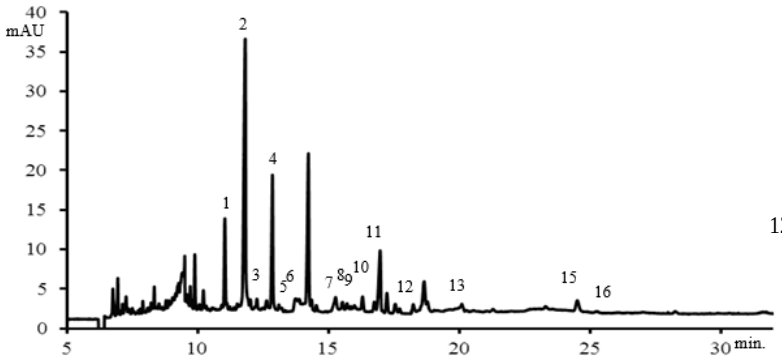

Fig. 2. Electropherogram of 70% ethanolic extract of *G. verum* (1- Rutin, 2- Naringenin, 3- Izoquercitrin, 4- Cinnamic acid, 5- Chlorogenic acid, 6- Sinapic acid, 7- Syringic acid, 8- Ferulic acid, 9- Kaempferol, 10- Luteolin, 11- Coumaric acid, 12- Quercetol, 13- Rosmarinic acid, 14-Salvianolic acid A, 15- Caffeic acid, 16- Gallic acid)

Table 4

COMPOSITION OF POLYPHENOLIC ACIDS AND FLAVONOIDS IN PLANTS EXTRACTS (mg g^{-1} DW)

Plants	Type of extract	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
C. officinalis	Aqueous	**2.62**	**2.05**	0.01	0.02	0.03	**1.51**	0.77	0.04	0.01	0	0.07	0.05	0	0	0.71	0.12
	Ethanolic 30%	**4.03**	**1.10**	0	0	0.22	**2.38**	0.69	0.41	0.09	0	0	0.05	0	0	0.48	0.40
	Ethanolic 50%	0.31	**2.33**	0.01	0	0.43	**0.96**	**1.71**	0	0.09	0.05	0.1	0.09	0	0.03	0.21	0.28
	Ethanolic 70%	**1.23**	**6.67**	**0.99**	0.22	0.17	**1.33**	0	0.52	0.36	0.06	0.02	0.08	0	0.01	0.23	0.17
H. perforatum	Aqueous	0.66	0.03	0.19	0.42	**1.57**	**1.13**	0	0	0.03	0	0.01	0.28	0	0	**0.81**	0
	Ethanolic 30%	0.01	0.20	0.73	0.50	**3.53**	**7.08**	0.09	0.01	0.23	0.46	0.32	**1.39**	0.29	0	**0.89**	0.52
	Ethanolic 50%	0.22	0.56	0.12	0.04	**0.98**	**2.16**	0.07	0	0.03	0.05	0	0.49	0	0	0.07	0
	Ethanolic 70%	0.18	**1.50**	0.33	**1.97**	0.37	**5.09**	0.27	0.14	0.21	**1.28**	0.13	0.12	**11.61**	0	0.15	0.55
G. verum	Aqueous	0.29	0.01	0.28	0	0.49	**4.19**	0	0.47	0.16	0	0	0.40	0	0	**1.35**	0.16
	Ethanolic 30%	0.01	0.10	0.46	0	**0.86**	0.61	0.29	**1.01**	0.06	0.03	**0.88**	0.31	0	0	**3.12**	0.74
	Ethanolic 50%	**5.16**	0.38	0.21	0	**1.87**	**9.14**	**1.70**	0.45	0	0.46	0	0.81	0	0	**1.08**	0.38
	Ethanolic 70%	**5.47**	**5.77**	0.25	**1.68**	**0.96**	**2.22**	**1.40**	0.34	0.15	0.41	**1.33**	0.28	0.20	0	0.37	0.26
O. vulgare	Aqueous	0.03	0	0.01	0.08	0.23	0.18	0.19	0.04	0	0.61	0.04	0.35	0.06	0.04	0.13	0.21
	Ethanolic 30%	0.62	0	**3.47**	0	0	0	0	0	0.11	**0.94**	0.35	0	**8.82**	0	**0.92**	**1.29**
	Ethanolic 50%	0.04	0	**4.48**	0	0	0	0	0.02	0.03	**1.13**	0.73	0.20	**19.33**	0.66	0.54	0
	Ethanolic 70%	**5.06**	0.52	**3.15**	0	0.32	0.28	0.29	0.09	0.26	**0.90**	0.57	0.14	**16.29**	0.28	0.59	**0.82**

1 - Rutin; 2- Naringenin; 3 - Izoquercitrin; 4 - Cinnamic acid; 5 - Chlorogenic acid; 6 - Sinapic acid; 7 - Syringic acid; 8 - Ferulic acid; 9 - Kaempferol; 10 - Luteolin; 11 - Coumaric acid; 12 - Quercetol; 13 - Rosmarinic acid; 14 - Salvianolic acid A; 15 - Caffeic acid; 16 - Gallic acid;

101.54%. Taking into consideration all validation parameters, the method is in accordance with validation requirements and is suitable for the analysis of plants extracts.

The obtained results recommend the method for polyphenolic compounds analysis from plants extracts or other extracts. Compared with other literature methods for quantification of polyphenols from plants extracts, the present method showed good accuracy; the global electropherograms displayed well separated peaks for all the identified phenolic compounds while being obtained faster than chromatographic procedures (fig. 1) [13-15].

Determination of polyphenolic content in samples

Different types of extracts (see samples preparation) from *C. officinalis*, *H. perforatum*, *G. verum* and *O. vulgare* were analysed through CZE. As an example, the electropherogram of *G. verum* (70% ethanolic extract) is presented in figure 2 and the content of polyphenolic compounds in the analysed samples is synoptically presented in table 4.

The results obtained in the present study (table 4) show that the concentrations of polyphenolic compounds differ considerably in the analyzed plants extracts. Generally, *C. officinalis* extracts presented lower concentrations of polyphenolic compounds than the other three plants studied in this work, in accordance with our previous studies [9, 10]. Chlorogenic and caffeic acids were quantified in all extracts and their concentrations ranged from 0.031 mg g^{-1} DW (aqueous extract) to 0.43 mg g^{-1} DW (50% ethanolic extract) and 0.21 mg g^{-1} DW (50% ethanolic extract) to 0.71 mg g^{-1} DW (aqueous extract), respectivelly. The largest amounts of rutin and naringenin were found in 30% ethanolic extract (4.03 mg g^{-1} DW) and 70% ethanolic extract (6.67 mg g^{-1} DW). In addition, naringenin was detected for the first time in all extracts of *C. officinalis.*

Regarding *H. perforatum* extracts, sinapic acid was the most significant polyphenol found in all types of extracts (the highest concentration was 7.08 mg g^{-1} DW in 30% ethanolic extract). These results are comparable with those reported by Dvorackova E. et al (2014) [16] from Czech Republic. The present study is the first to report the presence of naringenin with a content ranging between 0.03 mg g^{-1} DW (aqueous extract) and 1.50 mg g^{-1} DW (70% ethanolic extract). Other major compounds were chlorogenic acid (3.53 mg g^{-1} DW in 30% ethanolic), cinnamic acid (1.97 mg g^{-1} DW in 70% ethanolic), luteolin (1.28 mg g^{-1} DW in 70% ethanolic), quercetol (1.39 mg g^{-1} DW in 30%

ethanolic), rosmarinic acid (11.61 mg g^{-1} DW in 70% ethanolic) and caffeic acid (0.89 mg g^{-1} DW in 30% ethanolic).

According to our knowledge, this is the first report in which naringenin, sinapic acid and syringic acid were identified from aqueous and ethanolic extracts (30, 50 and 70%) of *G. verum* based on the described method. Sinapic acid was detected in high concentrations in all extracts, especially in 50% ethanolic extract (9.14 mg g^{-1} DW). Rutin (5.47 mg g^{-1} DW in 70% ethanolic), naringenin (5.77 mg g^{-1} DW in 70% ethanolic), chlorogenic acid (1.87 mg g^{-1} DW in 50% ethanolic), syringic acid (1.70 mg g^{-1} DW in 50% ethanolic), coumaric acid (1.33 mg g^{-1} DW in 70% ethanolic) and caffeic acid (3.12 mg g^{-1} DW in 30% ethanolic) were the main components quantified in *G. verum* extracts while gallic acid, ferulic acid and quercetol were found in noticeable amounts. The polyphenols profile obtained in this study is similar with a previously reported one by LC-MS analysis. [10]

O. vulgare presented, as expected, rosmarinic acid as predominant phenolic constituent in all ethanolic extracts (19.33 mg g^{-1} DW in 50% ethanolic was the highest one). This aspect is in accordance with previous studies about *Origanum* from Romania [9, 17] or from Lithuania, India and Greece [18-20]. Sinapic acid (not evidenced since now) and syringic acid were found only in aqueous and 70% ethanolic extracts while naringenin was detected only in 70% ethanolic extract. Other significant compounds identified in *O. vulgare* were gallic acid, rutin, luteolin, caffeic acid and isoquercitrin.

Although sinapic acid, syringic acid and naringenin are common phytochemicals in the human diet, they have not received as much interest from the scientific community as other hydroxycinnamic acids such as caffeic or ferulic acid. Sinapic acid showed antimicrobial [21-26], anti-inflammatory [27], anticancer [28], and anti-anxiety activities [29]; syringic acid was found to have antimicrobial [30], anticancer [31] and anti-diabetic activities [32] while naringenin possess antioxidant, anti-inflammatory, anti-proliferative and anti-mutagenic properties being a useful chemoprotective agent [33].

Conclusions

Aqueous and ethanolic extracts of *Calendula officinalis*, *Hypericum perforatum*, *Galium verum* and *Origanum vulgare* were evaluated for polyphenolic composition. Generally, these plants were studied mostly for content of essential oils and were less investigated for polyphenols, organic acids, vitamins or other chemicals composition (especially *G. verum*). In this study we used a simply, rapid and reliable capillary electrophoretic method for separation and quantification of 16 polyphenolic compounds usually found in plants extracts. Overall results of our studies indicated that aqueous extracts contain lower concentrations of polyphenols compared to ethanolic extracts. The results obtained are in accordance with our previous HPLC studies and, in addition, the presence of some compounds (*e.g.* naringenin in *Calendula* and *Hypericum,* naringenin, sinapic acid and syringic acid in *Galium*, sinapic acid in *Origanum*) was evidence for the first time in the investigated plants extracts.

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References

1. AFANASEV, I.B., DOROZHKO, A.I., BRODSKII, A.V., KOSTYUK, V.A., POTAPOVITCH, A.I., Biochem. Pharmacol., 38, 1989, p. 1763
2. BLOKHINA, O., VIROLAINEN, E., & FAGERTEDT, K.V., Ann. Bot., 91, 2003, p. 179
3. NAZARI, Q.A., KUME, T., TAKADA-TAKATORI Y., IZUMI, Y., AKAIKE, A., J. Pharmacol. Sci., 122, 2013, p. 109
4. TAVIANO, M.F., MARINO, A., TRAVATO, A., BELLINGHIERI, V., MELCHINI, A., DUGO, P., CACCIOLA, P., DONATO, P., MONDELLO, L., GUVENC, A., DE PASQUALE, R., MICELI, N., Food Chem. Toxicol., 58, 2013, p. 22
5. KORDAS, S., DEMIRTAS, I., OZEN, T., DEMIRCI, A.M., BEHCET, L., J. Sci. Food Agric., 95, 2015, p. 786
6. AMZOIU, E., ANOAICA, P.G., AVERIS, L.M.E., SIMIONESCU, A., BUBULICA, M.V., Rev. Chim. (Bucharest), **65**, no. 9, 2014, p. 1072
7. MARKHAM, K.R., MCGHIE, T.K., Phytochem. Anal., 7, 1996, p. 300
8. FUKUJI, T.S., TONIN, F.G., TAVARES, M.F.M., J. Pharm. Biomed. Anal., 51, 2010, p. 430
9. DANILA, A.O., GATEA, F., RADU, G.L., Chem. Nat. Compd., 47, 2011, p. 22
10. MATEI, A.O., GATEA, F., RADU, G.L., J. Chromatogr. Sci., 53, 2015, p. 1147
11. MATEI, A.O., GATEA, F., TEODOR E.D., RADU, G.L., Chem. Pap., (manuscript accepted)
12. GATEA, F., TEODOR, E.D., MATEI, A.O., BADEA, G.I., RADU, G.L., Food Anal. Methods, 8, 2015, p. 1197
13. POP, R.M., CSERNATONI, F., RANGA, F., FETEA, F., SOCACIU, Bull. UASVM Food Sci. Tech., 70, 2013, p. 99
14. MA, J., YANG, H., BASILE, M.J., KENNELLY, E.J., J. Agric. Food Chem., 52, 2004, p. 5873
15. VAHER, M., KOEL, M., J. Chromatogr. A, 990, 2003, p. 225
16. DVORACKOVA, E., SNOBLOVA, M., HRDLICKA, P., Food Res. J., 21, 2014, p. 1495
17. CIOANCA, O., MIRCEA, C., IANCU, C., GILLE, E., HANCIANU, M., SGEM 2013 Conference Proceedings, doi: 10.5593/Sgem2013/Bf6/S25.015
18. RADUSIENE, J., IVANAUSKAS, L., JANULIS, V., JAKSTAS, V., Biology, 54, 2008, p. 45
19. HITHAMANI, G., RAMALAKSHMI K., Int. J. Agric. Soil Sci. 1, 2013, p. 7
20. GREVSEN, K., FRETTE, X.C., CHRISTENSEN L.P., Eur. J. Hortic. Sci., 74, 2009, p. 193
21. NOWAK, H., KUJAVA, R., ZADERNOWSKI, R., ROCZNIAK, B., KOZLOWSKA, H., Eur. J. Lipid Sci. Technol., 94, 1992, p. 149
22. TESAKI, S., TANABE, S., ONO. H., FUKUSHI, E., KAWABATA, J., WATANABE, M., Biosci. Biotech. Biochem., 62, 1998, p. 998
23. BARBER, M.S., MCCONNELL, V.S., DECAUX, Phytochem., 54, 2000, p. 53
24. JOHNSSON, L., DUTTA, P.C., J. Chromatogr. A, 1064, 2005, p. 213
25. MADDOX, C.E., LAUR L.M., TITAN, L., Curr. Microbiol., 60, 2010, p. 53
26. ENGELS C., SCHIEBER, A., GANZLE, M.G., Eur. Food Res. Technol., 234, 2012, p. 535
27. YUN, K.J., KOH, D.J., KIM, S.H., PARK, S.J., RVU, J.H., KIM, D.G., LEE, J.Y., LEE, K.T., J. Agric. Food Chem., 56, 2008, p. 10265
28. HUDSON, E.A., DINH, P.A., KOKUBUN, T., SIMMONDS, M.S.J., GESCHER, A., Cancer Epidemiol. Biomarkers Prev., 9, 2000, p. 1163
29. YOON, B.H., JUNG, J.W., LEE, J.J., CHO, Y.W., JANG, C.G., JIN, C., OH, T.H., RVU, J.H., Life Sci., 81, 2007, p. 234
30. CHONG, Z.Z., SHANG, Y.C., MAIESE, K., Trends Cardiovasc. Med., 21, 2011, p. 151
31. ORABI, S.A., ABDELHAMIDET, M.T., J. Saudi Soc. Agric. Sci., 2014, p.1-10, doi:10.1016/j.jssas.2014.09.001
32. MUTHUKUMARAN, J., SRINIVASA, S., VENKATESAN, R.S., RAMACHANDRAN, V., MURUGANATHAN, U., J. Acute Disease, 2013, p. 304-309, doi: 10.1016/S2221-6189(13)60149-3
33. CAVIA-SAIZ, M., BUSTO, M.D., PILAR-IZQUIERDO, M.C., ORTEGA, N., PEREZ-MATEOS, M., MUNIZ, P., J. Sci. Food Agric., 90, 2010, p. 1238

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