VERSITA

Cent. Eur. J. Chem. • 8(3) • 2010 • 530–535
DOI: 10.2478/s11532-010-0012-4

Central European Journal of **Chemistry**

Qualitative and quantitative analysis of gallic acid in *Alchemilla vulgaris*, *Allium ursinum*, *Acorus calamus* and *Solidago virga-aurea* by chip-electrospray ionization mass spectrometry and high performance liquid chromatography

Research Article

Dumitru Condrat[1], Cristina Mosoarca[2]*, Alina D. Zamfir[1,2], Florin Crişan[3], Maria R. Szabo[1], Alfa X. Lupea[4]

[1]*Aurel Vlaicu University, Department of Chemical and Biological Sciences, Arad 310330, Romania*
[2]*National Institute for Research and Development in Electrochemistry and Condensed Matter, Timisoara 300224, Romania*
[3]*County Lab for Pesticides Quality Control, Arad 310059, Romania*
[4]*Faculty of Industrial Chemistry and Environmental Engineering, Politehnica University, Timişoara 300006, Romania*

Received 07 October 2009; Accepted 16 December 2009

Abstract: This study presents the results obtained from qualitative and quantitative analysis of gallic acid from hydro-alcoholic extracts (methanol, ethanol) of plants from *Plantae* regnum. Plant qualitative analysis was performed using a novel mass spectrometric (MS) method based on fully automated chip-nanoelectrospray ionization (nanoESI) high capacity ion trap (HCT) while quantitative analysis was carried out by high performance liquid chromatography (HPLC). These methods were applied to *Alchemilla vulgaris* – common lady's-mantle (aerial part), *Allium ursinum* - bear's garlic (leaves), *Acorus calamus* - common sweet flag (roots), *Solidago virga-aurea* – goldenrod (aerial part). Obtained results indicated that methanol extracts (96%, 80%) have a gallic acid content ranging between 0.0011 - 0.0576 mg mL^{-1} extract while the ethanol extracts (96%, 60%) exhibit a gallic acid concentration that varies between 0.0010 -0.0182 mg mL^{-1} extract.

Keywords: *Gallic acid • HPLC • Chip-ESI MS • Hydro-alcoholic extracts • Plantae*

1. Introduction

Gallic acid (3,4,5-trihydroxybenzoic acid) has strong reducing character; in alkaline solution it becomes brown due to oxygen absorption. Its esters with glucose are components of tannins [1]. In nature, the free state is found in oak bark, gall fly donuts on oak leaves, and vegetal parts of some plants such as those from Plantae regnum.

From a physiological point of view, gallic acid is considered a protection factor for plants against bacterial infections (gallic acid exhibits antimicrobial activity), an antioxidant (strong reducing power), and a hydrogen carrier (participates in cellular redox systems) [2].

The gallic acid-containing native species, *Alchemilla vulgaris*, *Allium ursinum*, *Acorus calamus* and *Solidago virga-aurea*, originating from Central Europe and the Danube Delta, play different pharmacological roles, such as astringent, antidiarrheic, diuretic, depurative (*Alchemilla vulgaris*), intestinal antiseptic, bacteriostatic and bactericidal, tonic, anti-arthritic, cancer deterrent (*Allium ursinum*). Gallic acid is used as an effective treatment for gastric tonus problems, anorexia, bloating, gases, urinary diseases gout (*Acorus calamus*), haemostatic, skin repairer, sedative (*Solidago virga-aurea*) [3,4].

The analysis of gallic acid in plants involves the extraction of dried plant material and subsequent application of accurate and sensitive analytical methods

* *E-mail: mosoarca.c@gmail.com*

to perform the qualitative analysis in terms of composition and structure and the quantitative analysis of the native extract. Extraction can be achieved by either classical methods such as maceration and Soxhlet digestion, or by modern methods such as those using supercritical fluids, ultrasonication, microwaves etc. The qualitative and quantitative analysis can be performed by methods based on mass spectrometry (MS), absorption spectrophotometry in ultraviolet and visible (UV-VIS), nuclear magnetic resonance (NMR), liquid chromatography (LC), capillary electrophoresis (CE) *etc.*

Mass spectrometry is one of the most appropriate methods for the analysis of compounds in plant extracts [5], since it is able to provide essential information about the structure of intermediates produced by decomposition. For such analyses, mass spectrometry can be applied in parallel with NMR, UV-VIS or HPLC, although, for some specific and complex studies, concurrent application of all these techniques is necessary [6].

The advantages of modern mass spectrometry methods lay in the high speed, accuracy, reproducibility and sensitivity of the analysis, which enables the detection of intermediates with short lifetime and allows, consequently, a better understanding of the mechanisms and processes initiated by free radicals [7,8]. On the other hand, high performance liquid chromatography (HPLC) is nowadays a method of choice for identification and quantification of vegetal antioxidants from plant extracts, as it provides high separation efficiency and sensitivity. For instance, phenolic compounds such as (+)-catechin and (-)-epicatechin of apple juice were separated and quantified by HPLC with reverse phase, Nucleosil C18 column, Hypersil - ODS 4.6 mm × 250 mm, 5 µm using acidulated methanol-water as solvent and UV detection between 235 - 350 nm [9,10].

The aim of this work was to analyze the influence of the nature and composition of the extraction hydroalcoholic media (methanol, ethanol) on gallic acid extraction by maceration and ultrasonication of the dry plant material. Although similar studies of gallic acid expressed in oak species were reported earlier [11], in our investigation, qualitative analysis is for the first time performed by employing an advanced mass spectrometric system, which encompasses a fully automated robot (NanoMate) for sample delivery by chip-nanoelectrospray ionization (nanoESI) in direct coupling to a high capacity ion trap (HCT) mass spectrometer. Quantitative analysis was carried out by high-performance liquid chromatography (HPLC).

As compared to other methods of qualitative analysis of tannins, such as general methods for the phenolic groups [12] and protein precipitation [13], the modern chip-electrospray mass spectrometry technique enabled the detection of short-life intermediates, due to the high speed and sensitivity of the analysis.

2. Experimental Procedures

2.1. Materials

Methanol, phosphoric acid (HPLC grade), ethanol, were purchased from Merck (Darmstadt, Germany) and used without further purification. Gallic acid (99%) was obtained from Roth (Bucharest, Romania). Dried vegetal material: *Alchemilla vulgaris* – common lady's-mantle (aerial part); *Allium ursinum* - bear's garlic (leafs); *Acorus calamus* - common sweet flag (roots) and *Solidago virga-aurea* – goldenrod (aerial part) were obtained from Fares S.A. Orastie, Romania.

2.2. Samples

2.2.1. Preparation of gallic acid standard solution

1.5 mg gallic acid was transferred into a 10 mL calibrated flask and dissolved in methanol to obtain the stock solution. By successive dilution of the standard stock solution, samples of different concentrations (0.0075 - 0.15 mg mL^{-1}) were obtained. These solutions were used to map out the alibration curve (Fig. 1) for which a correlation coefficient of 0.999884 was determined.

2.2.2. Methods for obtaining extracts of Alchemilla vulgaris, Allium ursinum, Acorus calamus and Solidago virga – aurea.

2.2.2.1. Obtaining hydroalcoholic extracts by maceration.

Dried plant material, shredded prior to extraction, was subjected to extraction using different concentrations of alcohols, for ten days, in the dark, shaking three times a day at room temperature, having the molar ratio plant material: solvent = 5:50.The extraction solutions were filtered and the remaining residue was washed with

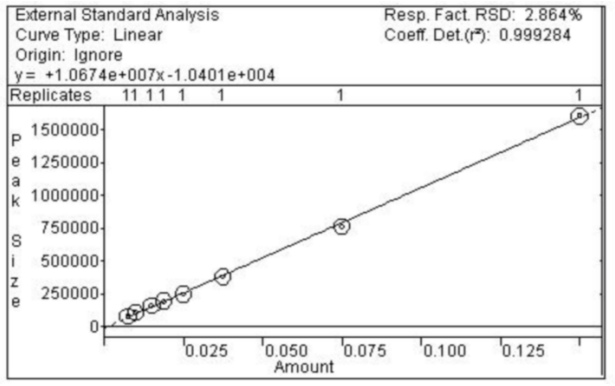

Figure 1. Calibration curve for gallic acid.

solvent (methanol, ethanol) an d diluted to 50 mL. Resulting hydroalcoholic extracts were subjected to qualitative and quantitative analysis for the determination of gallic acid content. In order to avoid degradation of substances which are light-unstable, the obtained tinctures were stored in a dark chamber.

2.2.2.2. Obtaining hydroethanolic extracts by ultrasound extraction.

Dried plant material, shredded previously, has undergone extraction with ethanol (96%, 60%) in an ultrasonic bath, with the molar ratio plant material: ethanol = 1:10, at a temperature of 20°C, for ten minutes. The extract solutions were filtered and the residue was washed with ethanol, the filtrate was diluted to a total volume of 10 mL. As a result, clear hydroalcoholic extracts of specific color were obtained.

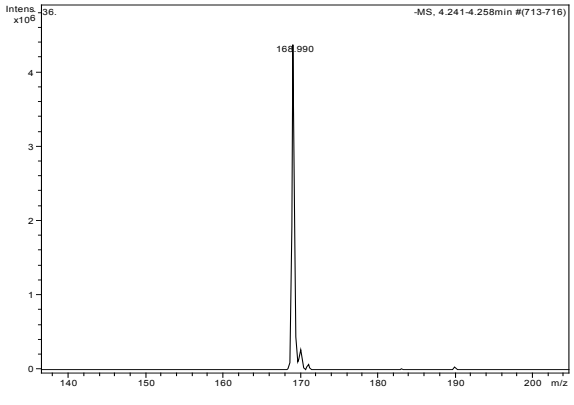

Figure 2. Fully automated chip (-) nanoESI HCT MS of standard gallic acid. Solvent: MeOH; ESI/MS parameters: ChipESI: -1.4 kV; capillary exit: -50 V. Back nitrogen pressure 0.30 p.s.i. Nitrogen nebullizer on MS at 50 p.s.i.

2.3. Mass Spectrometry

Mass spectrometry was conducted on a high capacity ion trap instrument (HCT MS) from Bruker Daltonics, Bremen, Germany, in laboratory coupled [14-16] with the NanoMate robot for automatic infusion of samples by chip-electrospray (NanoMate™) from Advion Biosciences, Norfolk, UK. HCT MS instrument was controlled and manipulated through the Esquire Control 6.1.512 software and experimental data were processed *via* Data Analysis 3.4.179. NanoMate robot was controlled through ChipSoft 7.1.1 software running under Windows XP. All mass spectra were recorded in the negative ion mode within (50-1000) *m/z* range, with a scan speed of 8000 m/z per second. The ESI process was initiated by applying -1.4 kV on the conductive pipette tip of the NanoMate and -50 V on the HCT counter-electrode. Nitrogen with a flow rate of 0.5 L min^{-1} was used for desolvation and as a nebulizer gas at 50 p.s.i. To enhance the desolvation of the generated ESI droplets, the source block temperature was maintained at 250°C [17].

Liquid chromatography was performed on a chromatograph type Varian Pro Star HPLC model 240, equipped with a ternary pump, automatic injector, thermostat set at room temperature and UV-Vis detector (UV-VIS VARIAN MODEL 345). The separation was performed on a chromatographic column, type Inertsil 5C8-3, 250x4.6 mm. The mobile phase consisted of a mixture of methanol:phosphate buffer in relative volumetric ratio of 45:55. The analytes were detected at 215 nm. The flow rate of the mobile phase was 1 mL min^{-1}. The injection volume was 5 µL.

3. Results and Discussions

3.1 Qualitative analysis of gallic acid in hydro-alcoholic extracts by chip-ESI HCT MS

(-)-Chip nanoESI ESI HCT mass spectra of gallic acid standard and the studied plant extracts, obtained under identical analysis conditions, are presented in Figs. 2-6.

The spectrum in Fig. 2 shows that in the negative ion mode, under the employed screening conditions, gallic acid is identifiable through its monodeprotonated [M-H]- ion at m/z 168.990. The spectra of *Alchemilla*

vulgaris, Allium ursinum, Acorus calamus and *Solidago virga-aurea* crude extracts depicted in Figs. 3-6 indicate the presence [M-H]- ion at m/z 168.990, which according to mass calculation, corresponds to gallic acid. The comparative standard-extract analysis unambiguously demonstrates the presence of gallic acid in the investigated extracts.

3.2 Quantitative analysis of gallic acid from the hydroalcoholic extracts

Quantitative determination of gallic acid, extracted under different experimental conditions of plant material was performed by the HPLC method. Correlation coefficient value corresponding to the calibration curve was $r^2>0.9980$ and the corresponding equation is given by:

$$y = 1{,}0674\times10^{7}x - 1{,}0401\times10^{4} \quad (1)$$

In Table 1 the values of the retention time (tr) and of gallic acid concentrations in methanol (96% and 80%) and ethanol (96%) extracts obtained by maceration of the plant material for 10 days at ambient temperature are presented.

From Table 1 it can be seen that the nature and the concentration of the extraction media influenced the quantity of the gallic acid extracted by maceration of plant material. Except for *Solidago virga-aurea* plant extract, in all cases increasing the concentration of methyl alcohol in the maceration system from 80% to 96% led to an increase in the amount of extracted gallic acid. This indicates that increasing the methanol concentration of the extraction media is an essential factor in obtaining better quality extracts.

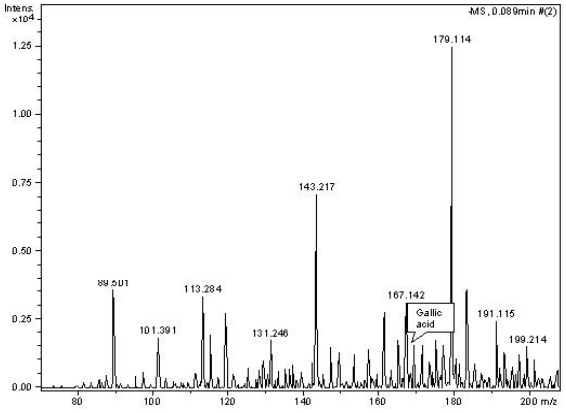

Figure 3. Fully automated chip (-) nanoESI HCT MS of Alchemilla vulgaris extract. Solvent: MeOH; ESI/MS parameters: ChipESI: -1.4 kV; capillary exit: -50 V. Back nitrogen pressure 0.30 p.s.i. Nitrogen nebullizer on MS at 50 p.s.i.

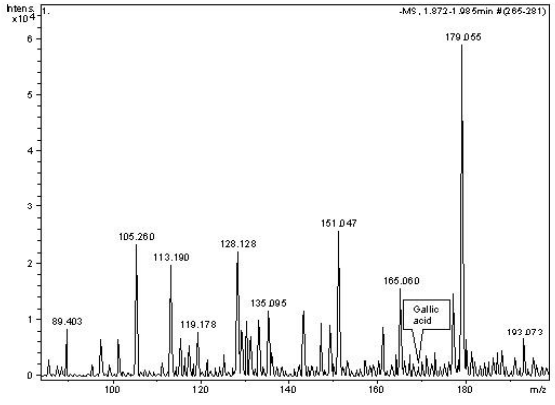

Figure 4. Fully automated chip (-) nanoESI HCT MS of Allium ursinum extract. Solvent: MeOH; ESI/MS parameters: ChipESI: -1.4 kV; capillary exit: -50 V. Back nitrogen pressure 0.30 p.s.i. Nitrogen nebullizer on MS at 50 p.s.i.

Table 1. Retention times and gallic acid content of the hydro-alcoholic extracts obtained by maceration.

Vegetal material	Hydroalcoholic extract	Retention time tr, [min]	Gallic acid content [mg mL^{-1}]
Alchemilla vulgaris (common lady's-mantle)	Methanol 96%,	4.327±0.04	0.0104
	Methanol 80%,	4.335±0.06	0.0092
	Ethanol 96%	4.327±0.04	0.0044
Allium ursinum (bear's garlic)	Methanol 96%,	4.479±0.09	0.0576
	Methanol 80%,	4.467±0.08	0.0165
	Ethanol 96%	4.415±0.05	0.0076
Acorus calamus (common sweet flag)	Methanol 96%,	4.290±0.04	0.0055
	Methanol 80%,	4.317±0.03	0.0020
	Ethanol 96%	4.276±0.05	0.0010
Solidago virga-aurea (goldenrod)	Methanol 96%,	4.502±0.06	traces
	Methanol 80%	4.500±0.06	0.0052
	Ethanol 96%	4.528±0.07	0.0045

Table 2. Retention times and gallic acid content of hydroethanolic systems (96%, 60%) obtained by alternative extraction with ultrasounds for 10 minutes, at 20°C

Vegetal material	Hydroethanolic extract concentration	Retention time tr, [min]	Gallic acid content [mg mL^{-1}]
Alchemilla vulgaris (common lady's-mantle)	96%	4.407±0.05	0.0020
	60%	4.407±0.05	0.0070
Allium ursinum (bear's garlic)	96%	4.455±0.06	0.0066
	60%	4.435±0.05	0.0743
Acorus calamus (common sweet flag)	96%	4.215±0.03	0.0015
	60%	4.294±0.04	0.0029
Solidago virga-aurea (goldenrod)	96%	4.477±0.06	0.0055
	60%	4.326±0.07	0.0186

At equal concentrations of alcohol in the extraction media, methanol has proven to be a better solvent for gallic acid than ethanol. A possible explanation is that gallic acid solubility decreases in th e following order: methanol > ethanol > water > ethyl acetate.

The largest amounts of gallic acid, which were extracted by maceration, regardless the nature and concentration of used alcohol were derived from the leaves of *Allium ursinum* (bear's garlic) and the lowest (0.0010 mg mL^{-1}) from the root of *Acorus calamus* (common sweet flag). In Table 2 the values of retention times and gallic acid content of the hydroethanolic systems (96%, 60%) obtained by alternative extraction with ultrasounds for 10 minutes at 20°C are given. mixture composition (the ethanol: water ratio) on gallic acid content in vegetable extracts was found. There ence of ultrasounds, an influence of the hydroalcoholic As in the maceration case, for the extraction in the presare also significant differences between 96% and 60% hydroethanolic extracts in terms of gallic acid quantity.

Gallic acid is better represented in 60% hydroethanolic extract (0.0743 mg mL^{-1} in - bear's garlic extract) than in the 96% hydroethanolic solution (0.0066 mg mL^{-1} in - bear's garlic extract). Bear's garlic extracts exhibited the highest content of gallic acid in all four types of extracts (0.0066 - 0.0743 mg mL^{-1} extract).

A possible explanation of these results is the extraction and chromatographic analysis conditions which may cause decarboxylation of the gallic acid. By comparing the results obtained from the perspective of the method used for extraction, a significant variation in gallic acid concentration can be noticed in the investigated extracts, which confirms the quality of ultrasound extraction. This can be rationalized by the fact that the ultrasonic field is intensifying the substance transfer in the solvent.

4. Conclusions

The present data indicate that mass spectrometry and high performance liquid chromatography can be applied successfully for the qualitative and quantitative investigation of gallic acid present in small amounts in the aerial part of *Solidago virga-aurea* and in significant amount in the leaves of *Allium ursinum* (0.0076 - 0.0743 mg mL^{-1} extract).

Chip-ESI HCT MS used for the analysis of studied extracts allowed a reliable and rapid identification of gallic acid as compared to the time and reagent consuming reactions of color and precipitation, which most often provide questionable results. Combined analysis of plant extracts by mass

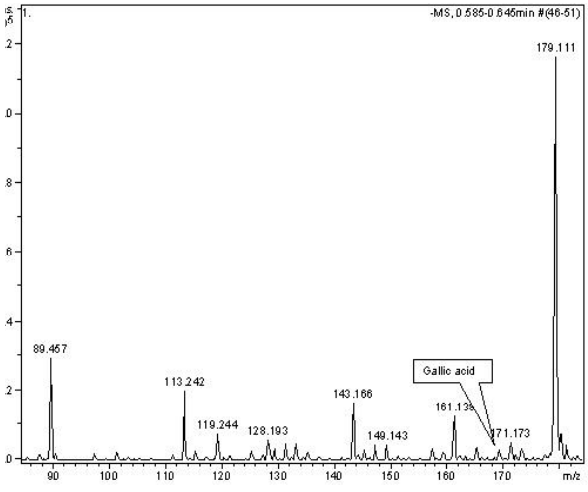

Figure 5. Fully automated chip (-) nanoESI HCT MS of *Acorus calamus* extract. Solvent: MeOH; ESI/MS parameters: ChipESI: -1.4 kV; capillary exit: -50 V. Back nitrogen pressure 0.30 p.s.i. Nitrogen nebullizer on MS at 50 p.s.i.

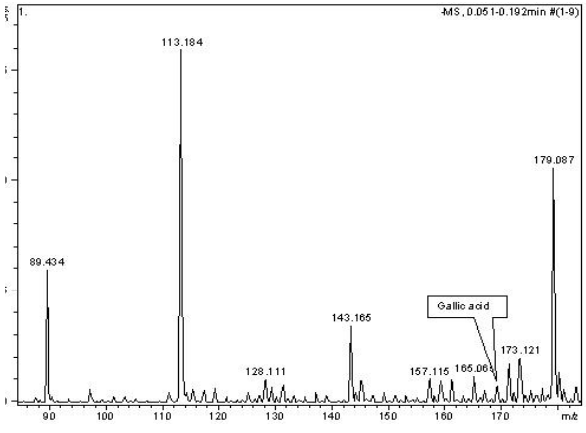

Figure 6. Fully automated chip (-) nanoESI HCT MS of *Solidago virga-aurea extract*. Solvent: MeOH; ESI/MS parameters: ChipESI: -1.4 kV; capillary exit: -50 V. Back nitrogen

spectrometry and high performance liquid chromatography provided not only the confirmation of gallic acid presence by screening mass spectra but also the determination of gallic acid concentration in herbal extracts.

Additionally, the results illustrate that the method of extraction assisted by ultrasounds is more effective and faster than the classical extraction technique, and from the biological point of view that the investigated plant extracts constitute a natural source of gallic acid.

References

[1] P. Buzzini et al., Mini Rev. Med. Chem. 8, 1179, (2008)
[2] J. Zhang, L. Li, S.H. Kim, A.E. Hagerman, J. Lü, Pharm. Res. 26, 2066, (2009)
[3] S.Z. Choi, S.U. Choi, S.Y. Bae, S. Pyo, K.R. Lee, Arch. Pharm. Res. 28, 49, (2005)
[4] H. Kim, T.H. Han, S.G. Lee, J. Ethnopharmacol. 122, 149, (2009)
[5] S. Moco, B. Schneider, J.Vervoort, J. Proteome Res. 8, 1694, (2009)
[6] N. Nakatani, J. Food Sci. 3447 (2002)
[7] N. Morita, M. Arisawa, Heterocycles 4, 373 (1976)
[8] A.D. Zamfir, J. Chromatogr. A 2, 1159, (2007)
[9] B. Suarez, J. Agric. Food. Chem. 42, 2732, (1994)
[10] J. Daigle, E.J. Conkerton, J. Chromatogr. 24, 262 (1982)
[11] P. Mämmelä, H. Savolainen, L. Lindroos, J. Kangas, T. Vartiainen, J. Chromatogr. A 891, 75, (2000)
[12] H.D. Graham, Food Chemistry 40, 801 (1992)
[13] A.E. Hagerman, M.E. Rice, N.T. Richard, J. Agric. Food. Chem., 46, 2590 (1998)
[14] R. Almeida et al., Anal. Biochem. 378, 43 (2008)
[15] T. Visnapuu, A.D. Zamfir, C. Mosoarca, M.D. Stanescu, T.Alamäe, Rapid Commun. Mass Spectrom. 23, 1337, (2009)
[16] A.D. Zamfir et al., Proteomics 9, 3435, (2009)
[17] A. Serb, C. Schiopu, C. Flangea, E. Sisu, A.D. Zamfir. J. Mass Spectrom. 44, 1434, (2009)