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QUANTITATIVE ANALYSIS OF GALLIC ACID FROM *APIUM GRAVEOLENS*, *EQUISETUM ARVENSE* L. AND *PETROSELINUM CRISPUM* USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

The present paperwork refers to the quantitative analysis of gallic acid from the plant extracts belonging to the *Apium graveolens*, *Equisetum arvense* L. and *Petroselinum crispum*. The qualitative analysis was made using High Performance Liquid Chromatography (HPLC) and the researched species were: *Apium graveolens* – celery, *Equisetum arvense* L. – horsetail and *Petroselinum crispum* – parsley. From the experimental results one can notice that the highest content in gallic acid is found in the *Petroselinum crispum* extract (0.0179 mg/mL) while the lowest content is found in the *Equisetum arvense* L. extract (0.0037 mg/mL).

Keywords: antioxidants; gallic acid; HPLC; ethanol extracts.

INTRODUCTION

Gallic acid having the scientific name of 3,4,5 – trihydroxybenzoic acid, is found in free state in certain plants. It is highly spread in nature in the form of tannins from which can be obtained either by enzymatic hydrolysis with the help of certain moulds (which contain the specific enzyme - tannase), either by heating with diluted acids [1].

Physiologically gallic acid is considered to be a plant protection factor against bacterial infections (it precipitates the microorganisms), antioxidant (strong reducing agent), hydrogen transporter (participate in the cellular redox systems).

The studied material, *Apium graveolens*, *Equisetum arvense* L. and *Petroselinum crispum*, native species from Europe, Asia and North America respectively, contain gallic acid and have various pharmacological actions such as: anti-sweating, diuretic, de-chlorinating, haemostatic, re-mineralizing (*Equisetum arvense* L.); anti-anemia, anti-rachitic, anti-scorbutic, vasodilator, vermifuge (*Petroselinum crispum*); appetizer, anti-rheumatism, antiseptic, pulmonary and liver drain, blood regenerator (*Apium graveolens*) [2-4].

Prior to being analyzed, gallic acid has to be extracted from the vegetal material.

The most used extraction technique is maceration, which is used both for the obtaining of vegetal watery extracts and for the vegetal alcoholic extracts. The extraction time varies from 30-60 minutes for the water extraction up to 10 days for the alcoholic extraction.

High performance liquid chromatography (HPLC) is a technique that offers precision, is fast and has a high sensitivity [5].

Specialized literature does not offer information regarding the gallic acid content of the studied plants and, therefore, a study regarding chemical composition is necessary.

The aim of this paperwork is the quantitative determination of gallic acid from various plant parts of the species *Apium graveolens*, *Equisetum arvense* L. and *Petroselinum crispum* using high performance liquid chromatography.

MATERIALS AND METHODS

Reagents:

- Methanol and phosphoric acid (HPLC grade), Merck;
- Ethanol 96%, Merck;
- Gallic acid (99%), Roth.

Vegetal material:

- *Apium graveolens* – celery;
- *Equisetum arvense* L. – horsetail;
- *Petroselinum crispum* – parsley.

Preparation of the gallic acid standard solutions [6]:

The standard solutions of various concentrations were obtained using successive dilutions starting from a stock solution of gallic acid in methanol (0.15 mg/mL).

Preparation of the hydro alcoholic extracts.

Dried up and minced vegetal material was subjected to static extraction (maceration) with ethanol for 10 days at room temperature, stirring it 3 times a day, being kept in a dark place and with a ratio vegetal material: ethanol of 5:50 [7].

The extraction solutions were filtered through 4 layers of cloth, the residue washed with ethanol and the obtained solutions brought to 50 mL, thus obtaining the vegetal hydro-alcoholic extracts which were subjected to qualitative and quantitative analysis in order to determine gallic acid.

Instrumental and analysis protocols [6, 8].

An HPLC Varian Pro Star model 240 chromatograph was used, having a ternary pump, automated injection, thermostat set at room temperature and an UV-VIS detector (UV-VIS VARIAN MODEL 345).

Chromatographic separation was carried out on an Inertsil 5C8-3, 250 x 4,6 mm chromatographic column.

The mobile phase consisted of a mixture of methanol:phosphate buffer in a volumetric ratio of 45:55. Detection was at 215 nm.

The flow rate of the mobile phase was 1 mL/minute. The injected sample volume was 5 µL.

For the calibration curves we used a range of standards, the concentration of the standards being presented in Table I.

Table I. Gallic acid standard concentrations

Nr.	Standard	Gallic acid concentration [mg/mL]
1	C_1	0.15000
2	C_2	0.07500
3	C_3	0.03750
4	C_4	0.01875
5	C_5	0.01500
6	C_6	0.01000
7	C_7	0.00750

RESULTS

Identification of gallic acid was based on the retention times, at the specific wavelength from the UV-VIS domain for the analyzed standard.

The corresponding equation of the calibration curve for gallic acid was:

$$y = 1.0674 \cdot 10^7 \cdot x - 1.0401 \cdot 10^4$$

The correlation coefficient of the calibration curve was: $r^2 = 0.9998$.

The retention times and the concentrations in gallic acid for the studied hydro-alcoholic vegetal extracts are presented in Table II.

Table II. The retention times and the concentrations in gallic acid

Nr.	Vegetal extract	Retention time t_r, [min]	Content in gallic acid [mg/mL]
1	*Apium graveolens* – celery	4.543	0.0052
2	*Equisetum arvense* L. –horsetail	4.490	0.0037
3	*Petroselinum crispum* – parsley	4.522	0.0179

DISCUSSION

The highest amount of gallic acid that was extracted through maceration came from the *Petroselinum crispum* (parsley) while the lowest amount came from the *Equisetum arvense L.* extract (horsetail).

The content in gallic acid of the *Petroselinum crispum* extract (0.0179 mg/mL) is higher comparing to that of the vegetal extracts from the species *Alchemilla vulgaris* – Lady's mantle (0.0044 mg/mL) [6] and *Acorus calamus* – Sweet flag (0.0010 mg/mL) [6], obtained under the same extraction conditions.

CONCLUSION

High performance liquid chromatography proved to be a precise and selective method for the identification and quantitative determination of gallic acid from the studied vegetal extracts.

The obtained data allow the exact quantification of the gallic acid from the chemical composition of the studied plants.

REFERENCES

1. Beral E., Zapan M., "*Chimie organică*" (Organic chemistry), Ed. Tehnică, Bucureşti, 1973, p. 533.
2. Mocanu S., Răducanu D., "*Plante medicinale în terapeutică*" (Medicinal plants in therapeutics), Ed. Militară, Bucureşti, 1994.
3. Pârvu C., "*Universul plantelor. Mica Enciclopedie*" (Plant universe, Small Encyclopedia), Ed. Enciclopedică, Bucureşti, 2000.
4. Duță V., "*Tratamente naturiste*" (Homeopathic treatments), Editura Ştefan, Bucureşti, 2007, p. 84-305.
5. Cacig S., "*Posibilitatea utilizării extractelor vegetale ca ingredienți naturali pentru medicamente,*

cosmetice şi alimente" (The possibility of using vegetal extracts as natural ingredients for medicaments, cosmetics and foods), Ed. Politehnica, Timişoara, 2007, p.45.

6. Condrat D., Mosoarca C., Zamfir A.D. , Crişan F., Szabo M.R., Lupea A.X. , *"Qualitative and quantitative analysis of gallic acid in Alchemilla vulgaris, Allium ursinum, Acorus calamus and Solidago virga aurea by mass spectrometry with chip – electrospray ionization and high performance liquid chromatography"*, *Central European Journal of Chemistry*, Germania, Springer – Verlag GmbH, **8**(3) (2010) 530 - 535.
7. "*Farmacopeea Română*" (Romanian Pharmacopoeia), Ediția a X - a, Ed. Medicală, Bucureşti, 1994.
8. Condrat D., "*Contribuții la obținerea unor extracte din plante (phanerogame) cu efect antioxidant*" (Contributions to the obtaining of plant extracts (phanerograms) with antioxidant effects, Ed. Politehnica, Timişoara, 2010, p. 115-119.