PHARMACOGNOSTIC ANALYSIS AND ANALYSIS OF THE PHENOLIC COMPOUNDS OF THE AERIAL PARTS OF THE SPECIES *CERASTIUM BULGARICUM* UECHTR. SIN. *CERASTIUM GRACILE* DUFOUR.

Popescu Antoanela[1]
Suciu Felicia[2]
Lupu Carmen[3]
Stoicescu Iuliana[4]
Roșca Adrian Cosmin[5]
[1, 2, 3, 4, 5] Faculty of Pharmacy, Ovidius University of Constanta, Romania

ABSTRACT

The main objective of the paper was the pharmacognostic analysis of the microscopic and chemical species of the aerial part of the species *Cerastium bulgaricum* Uechtr. sin. *Cerastium gracile* Dufour, to establish the chemical composition and especially to identify active principles that scientifically substantiate the traditional use of the plant product.

The microscopic analyses of the vegetative organs (stem and leaf) the species *Cerastium bulgaricum* Uechtr led to the conclusion that its histo-anatomical structure is specific to *Caryophyllaceae*.

Following the global chemical analysis, active principles known in the literature for the antioxidant potential were identified. Following the preliminary quantitative determinations (drying loss, determination of soluble substances) results comparable to those in the literature on the content of volatile substances and soluble substances were obtained.

The separation, identification and quantification of poliphenols compounds were made through high performance of liquid chromatography (HPLC), standardized method according to USP30-NF25 Monograph.

Keywords: *Cerastium bulgaricum Uechtr, Cerastium gracile Dufour, Caryophyllaceae, phenol compound, HPLC*

INTRODUCTION

The aim of our study was the determination of microscopic characteristics, chemical composition, volatil oil and poliphenols compounds, of the aerial part of *Cerastium bulgaricum* Uechtr from România.

The species of the genus *Cerastium* (keras = horn, about its bent horn-shaped fruit) are herbaceous, annual or perennial, fragile, with heights between 5 and 60 cm, distributed in Romania in dry or wet places, on sandy or salty soils, on rocks and screes, close to walls or roadsides, from plain to the alpine zone [1], [2], [3].

No data regarding the therapeutic uses of the species of the genus *Cerastium* were encountered in the specialized medical literature, but in certain areas of the Apuseni Mountains the locals use some species of this genus empirically, as a decoction to stop the hemorrhage of various etiologies and as alcoholic extract to treat rheumatism [1].

Anne Catherine Emmerich (a Roman Catholic Augustinian Canoness Regular of Windesheim, mystic), using herbs for epilepsy. "She prayed many times to be told of a cure for them, and at last was able to describe a certain little flower known to her which she had seen St. Luke pick and use to cure epilepsy" [4].

MATERIALS AND METHOD

Cerastium bulgaricum Uechtr were collected from Celic Dere monastery, Tulcea in northern Dobrogea, Iunie 2019.

For the preliminary pharmacognostic determinations, the pharmacognostic analysis was used as a working tool (microscopic examination, qualitative chemical analysis, determination of drying loss).

The qualitative chemical analysis is based on the successful extraction of the plant product used, with solvents of different polarities and the identification by reactions characteristic of each group of active principles.

The reagents used in identifying the active principles are reagents for analysis from various domestic and imported companies.

Determination of drying loss is a preliminary quantitative pharmacognostic method that represents the degree of humidity of plant products, which must be within certain limits, to ensure the preservation of plant products.

The working method involves the following technique.

The weighing vials with the vegetable products previously brought to a constant mass, together with the sample taken, are kept in the oven at 105^0 C for 3-4 hours, unless otherwise provided, cooled in a desiccator and weighed. Continue drying for 1 hour, followed by cooling in the desiccator and weighing until the samples reach a constant mass. A KERN ABJ analytical balance was used to weigh the samples.

The volatile oil content of the vegetal products was detarminated with Clevenger method.

The separation, identification and quantification of poliphenols compounds were made through high performance of liquid chromatography (HPLC), standardized method according to USP30-NF25 Monograph [5].

- *Apparatus*: HPLC Agilent 1200, with quaternary pump, DAD, thermostat, degassing system, autosampler.
- *Performance conditions*: chromatographic column type C18, 250 mm × 4.6 mm; 5 µm (Zorbax XDB or equivalent); mobile phase: solution A - 0.1% phosphoric acid, solution B - acetonitrile, eluted in the

gradient (Table I); temperature: 35ºC; flow rate: 1.5 mL/min; detection: UV 310 nm; injection volume: 20 µl; analysis time: 22 minutes.

Table 1. *Work gradient of HPLC analysis*

Time, min.	**Solution A, mL %**	**Solution B, mL %**
0-13	90	10
13	7	22
13	78	22
14	60	40
17	60	40
17,5	90	10
22	90	10

The test sample is a methanolic extract obtained from the aerial part of the species *Cerastium bulgaricum* Uechtr and was prepared as follows: 10 g of vegetable product were extracted with 100 mL of 70% methanol. The extracted solution obtained was filtered and the 100 mL volumetric flask was filled with 70% methanol.

The obtained extractive solution was filtered and made up to the bottom with a 100 mL volumetric flask with 70% methanol.

Reference substances (solutions in 70% methanol): Standard (methanolic solutions 70%) used were: E – resveratrol = 37 mg/mL, Z – resveratrol = 0,22 mg/mL.(Z – resveratrol was obtained from E – resveratrol exposed 12 hours at 254 nm, UV), caffeic acid = 0,36 mg/mL, chlorogenic acid = 0,37 mg/mL, cinnamic acid = 0,58 mg/mL, vanillin = 0,42 mg/mL, gallic acid = 0,39 mg/mL, ferulic acid = 0,48 mg/mL, 3-methylgallic acid = 0,34 mg/mL, ellagic acid = 0,43 mg/mL, p-coumaric acid = 0,51 mg/mL (Table II, Figure 1).

Table 2. *The Retention time of standards (*standard deviation for six injections)*

Nr. Crt.	**Compound**	**Retention time** ± SD
1.	*E* - resveratrol	14,467 ± 0,017
2.	*Z* – resveratrol	15,751 ± 0,058
3.	Caffeic acid	4,598 ± 0,036
4.	Chlorogenic acid	3,501 ± 0,015
5.	Cinnamic acid	15,867 ± 0,007
6.	Vanilin	6,919 ± 0,051
7.	Gallic acid	0,990 ± 0,025
8.	Ferulic acid	8,565± 0,058
9.	Ellagic acid	15,303± 0,027
10.	p-Coumaric acid	7,187± 0,019
11.	3-Methylgallic acid	2,606± 0,008

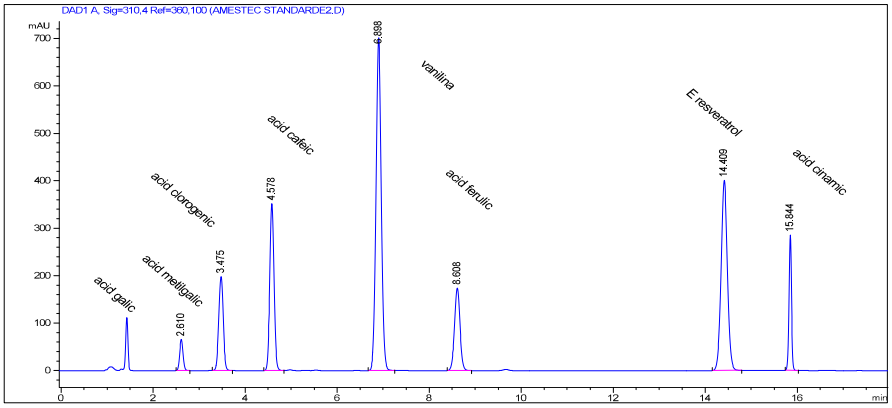

Fig. 1. *HPLC chromatogram of standards*

Statistical analysis

Statistical analyses were performed by analysis of variance (ANOVA soft SPSS 10). Data were presented as mean ± standard deviation (SD).

RESULTS AND DISCUSSIONS

The analysis of the transversal sections through the vegetative organs (stem and leaf) (Figure 2, Figure 3) the species *Cerastium bulgaricum* Uechtr led to the conclusion that its histo-anatomical structure is specific to *Caryophyllaceae* [6], [7], [8].

The microscopic analyses of the powder showed the presence of upper and lower epidermis of leaf, fragments of leaf lamina, the vessels of the stem, tectorial and secretory hairs and dyacitic stomata (figure 4).

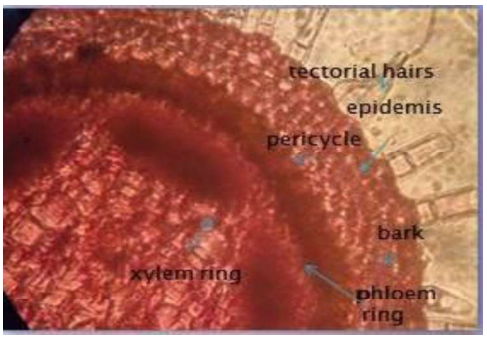

Fig. 2. *Transversal section through the stem10 x*

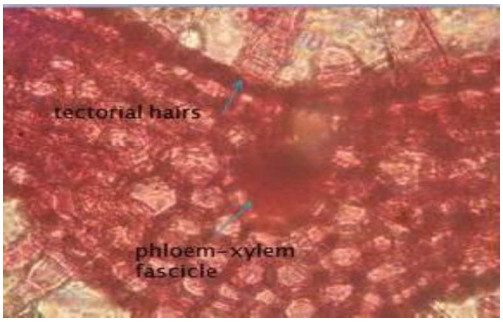

Fig. 3. *Transversal leaf section through the leaf 40 x*

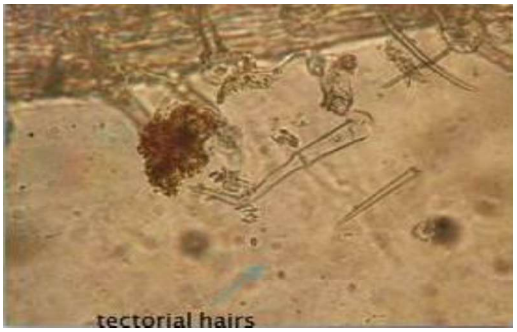

Fig. 4. *The microscopic analyses of the powder 10 x*

The vegetal product contains 2,0412% - 2,4202 g% water soluble substances.

The volatile oil content of the vegetal product determined by the Clevenger method was 0,08% mL.

The qualitative chemical analysis showed the presence of sterols, flavonoids and coumarins aglycons (in etheric solutions), flavonoids, coumarins, tannins, monosaccharides and other reducing compounds.

Using HPLC, we have determinated chlorogenic acid, caffeic acid, gallic acid, ellagic acid, cinnamic acid and p coumaric acid (figures 5, 6, 7), table III.

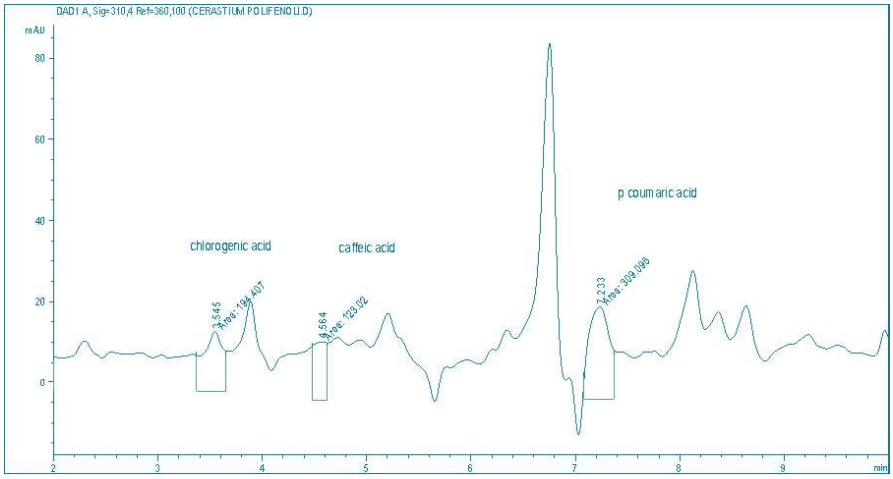

Fig. 5. *HPLC chromatogram*

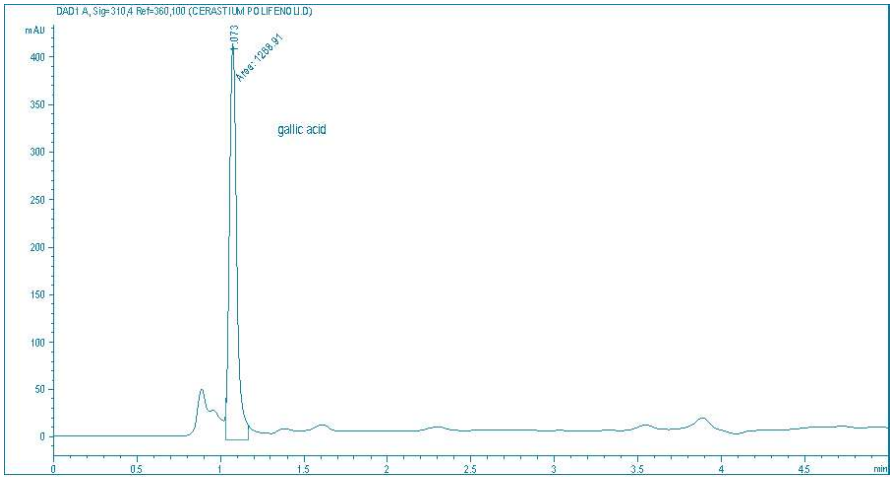

Fig. 6. *HPLC chromatogram*

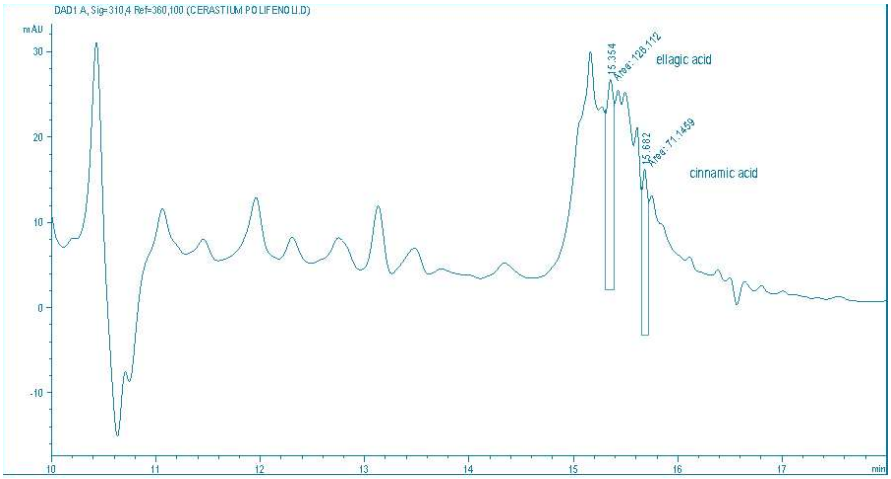

Fig. 7. *HPLC chromatogram*

Table 3. *Polyphenolic compounds determined by HPLC analysis*

Sample	Acid clorogenic mg%	acid cafeic mg%	Acid galic mg%	Acid elagic mg%	Acid cinamic mg%	Acid p cumaric mg%
Cerastium bulgaricum herba	4,086	10,986	1176,676	597,683	36,962	25,110

The presence of the free gallic acid is remarked. Verifying it with the general chemical analysis results we are sure that the intensity of the reaction for the identification of the tannins is given by its presence.

Science literature point out antiviral effects of phenol compounds (caffeic and gallic acids), antibacterial effect (caffeic, chlorogenic and gallic acids), antimycotic effect (caffeic and gallic acids), low level lipids effect (chlorogenic acid) [8].

CONCLUSIONS

The microscopic analyses of the species *Cerastium bulgaricum* Uechtr led to the conclusion that its histo-anatomical structure is specific to *Caryophyllaceae*.

The qualitative chemical analysis, as well as the determination of the volatile oil identified and detected active principles used in phytotherapy.

Cerastium bulgaricum herba contain phenolcarboxilic acids (chlorogenic, caffeic, p coumaric, ellagic, cinnamic and gallic). We measured some of them: chlorogenic acid (4,086 mg %), caffeic acid (10,986 mg %), ellagic acid (597,683 mg %) cinnamic acid (36,962 mg %) p coumaric acid (25,110 mg %) and gallic acid (1176,676 mg %).

All the detected compounds are recognised for antioxidant and anti-infective properties [8].

Cerastium bulgaricum Uechtr can be a future candidate for obtaining selective herbal extracts, rich in phenolic compounds with antioxidant activity [8].

The results obtained from HPLC analysis of polyphenolic compounds extracted from from the aerial part of the species in the study justify research orientation towards the assessment of antioxidant capacity.

REFERENCES

[1] Arcuş, M., Lilios, G., Doroftei, E., & Doicescu, D., THE HISTO-ANATOMOCAL ANALYSIS OF THE SPECIES CERASTIUM TOMENTOSUM L.(CARYOPHYLLACEAE). Annals of the Romanian Society for Cell Biology, 2012, 17,1.

[2] Dubois, M. A., Zoll, A., Bouillant, M. L., & Delaveau, P., Di-C-Glycosylflavones du Cerastium arvense ssp. arvense nouvelles pour les Caryophyllaceae. Planta medica, 46(09), 1982, 56-57.

[3] Couladis, M., & Tzakou, O., Volatile constituents of Cerastium candidissimum Corr. from Greece. Journal of Essential Oil Research, 12(6), 2000, 691-692.

[4] Emmerich A. C., The Life of The Blessed Virgin Mary, Translated by Sir Michael Palairet apud http://www.ecatholic2000.com/anne/lom.shtml.

[5] *** NF 25, United States Pharmacopeia and the National Formulary (USP 30/NF 25). Rockville (MD): The United States Pharmacopeial Convention, Inc.; 2007.p. 914.30/NF 25). Rockville (MD): The United States Pharmacopeial Convention, Inc.; 2007, 914.

[6] Dimitrov, D., & Vutov, V., BIODIVERSITY OF PLANTS AND NATURAL HABITATS IN THE VICINITIES OF ARAPYA BAY, ROPOTAMO NATURAL RESERVE, ALEPU BEACH, SINEMORETS AND SILISTAR CAPE (BULGARIAN SOUTHERN BLACK SEA COAST). Trakia Journal of Sciences, 17(4), 2019, 359.

[7] Adler, J. H., & Salt, T. A., Phytosterol structure and composition in the chemosystematics of the caryophyllales. In The Metabolism, Structure, and Function of Plant Lipids, Springer, Boston, MA, 1987, 119-121.

[8] Im LEE, J., KIM, Y. A., Chang-Suk, K. O. N. G., Jinhyeok, L. E. E., Haejin, K. I. M., & Youngwan, S. E. O., Inhibitory Effects of Crude Extract of the Arctic Terrestrial Plant Cerastium sp. on Growth of Human Cancer Cells, 한국생물공학회 학술대회, 2009, 154-154.