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EVALUATION OF ANTIOXIDANT CAPACITY OF *GERANIUM ROBERTIANUM* EXTRACTS

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In this study 6% and 10% *Geranium robertianum* aqueous extracts, as well as 10% (mass concentration) hydro-alcoholic extracts in 50% and 70% alcohol were obtained. They were purified and concentrated using procedures based on porous membranes. The extracts were characterized in terms of total polyphenols' content determination and antioxidant capacity assessment. Total polyphenols were, quantified using Folin–Ciocalteu method, while the antioxidant capacity was assessed by two spectrophotometrical methods: 2,2-diphenyl-picrlyhydrazyl (DPPH) and 2,2'- azinobis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS).The obtained results evidenced that the applied membranous (micro- and ultrafiltration) procedures resulted in some concentrated *G. robertianum* extracts having a high antioxidant capacity (92% percent of DPPH inhibition).

INTRODUCTION

Numerous aromatic and medicinal plants contain substances with antioxidant properties. In recent decades, natural antioxidants have attracted great attention from consumers over the world due to their lower toxicity than synthetic antioxidants.[1] A lot of researches performed on such plants lead to the development of some natural products containing antioxidants from various plants especially from those used in food, pharmaceutical and cosmetics industries.[2,3]

The oxidative stress caused by "reactive species of oxygen" (ROS) in living cells is associated to many pathological states, like coronary disease, cancer, cataract, macular degeneration and ageing. [4,5]

The oxidation reactions could be induced by so called ROS and could be initiated and accelerated by heavy metals and other reactive substances. ROS are continuously generated in living organisms within different metabolic paths or could be introduced into organisms from external sources through foods, drugs, or could be generated by environment pollution.[6] Nowadays, the use of the natural antioxidants as food, pharmaceutical or cosmetic additives to inactivate free radicals, focused the researchers' attention due to their advantage of being natural products and due to their exhibited highly efficiency as radical scavengers.[2]

Geranium robertianum (Geraniaceae) – is an annual or biennale herbaceous plant growing in moist places, solely in the shade of spruce fir, beech forests located at altitudes above 1500 m. The *Geranium* genus phytochemistry is relatively well known in present, the most studied classes of active principles being tannins, volatile oils, flavonoids and polyphenols (hyperoside, ellagic acid, isoquercitrin, quercitrine, kaempferols, caftaric acid, rutoside).[7,8]

Geranium species are used as antiasthmatic, antiallergic, antioxidant, antidiarrhoeic, antihepato-toxic, diuretic, tonic, haemostatic, stomachic and

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antidiabetic in folk medicine. [9] *Geranium pratense* ssp *finiticum* is used to treat stomach-ache. [10] *Geranium maculatum* is used to trat duodenals ulcers as well as in diarrhoe and hemorrhoids in Canada.[11] *Geranium sibiricum* Linne (GSL) has been used for trating diarrhea and intestinal inflammation in traditional Korean folk medicine. [12] The leaves of *Geranium thunbergii* have been used as an antidiarrhoeic in Japan [13] and *Geranium sanguineum* was reported to posess a potent effect against influentza virus.[14] In China *Geranium maximowiczii* has been used used to alleviate rheumatism.[15]

The crystalline tannin geraniin is claimed to be the main component in these plants. [16] In recent times, A-type proanthocyanidins, designated geranins A,B,C and D, wich showed significant antioxidant and antiprotozoal acrivity, were isolated from *Geranium niveum.* [17,18]

Recently studies of some plants growing in Central and Eastern Europe showed *Geranium macrorhizum* (DPPH absorbtion inhibition % - 92,3) and *Potentilla fruticosa* to have distinct radical scavenging properties and to be rather effective in artificial model tests[2].

Significant antioxidant, antitumoral, antiviral and antibiotic activities are frequently reported for many plants.[18-21]

Antioxidant activity of several *Geranium* species has been reported: *Geranium sanguineum*, [22] *Geranium bellum*, [23] *Geranium purpureum*, [24] *Geranium sibiricum* Linne (GSL) was reported to posess anti-oxidative and anti-proliferative activity in the Chang liver cells.[12]

Membrane separation processes may have an improved efficiency and reduced operating cost in comparison with the traditional concentration processes used in the pharmaceutical industry and for concentration of medicinal plant extracts.[25-28]

The aim of the work was to obtain new extracts of *Geranium robertianum* using an own developed procedure of extract separation and concentration, and to characterize the obtained extracts in terms of content (total phenolic content equivalent) and antioxidant capacity.

EXPERIMENTAL

Reagents

ABTS (2,2'- azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt solution $2.5x10^{-3}$ M in persulphate of potassium potassium persulfate, Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) $2.5x10^{-4}$ M in methanol, DPPH (2,2-diphenyl-1-picrylhydrazyl), Potassium persulphate $K_2S_2O_8$ solution $2.5x10^{-3}$ M in water, Folin-Ciocalteu reagent, methanol were purchased from the Sigma-Aldrich Chemical Co. All other used chemicals were of the highest purity grade available.

Equipments and Methods

The plant material was grounded to a fine powder , using the GRINDOMIX GM200 mill. Spectrometric measurements were performed using a Jasco V 530 spectrophotometer.

Obtaining the extracts and their processing

The leaves of *Geranium robertianum* were dried, homogenized and grounded to a fine powder; the extracts were prepared by maceration in aqueous: alcoholic (ethanol) mixtures 50% and, respectively 70%. The contact duration between the plant and the solvent was of 24 h for aqueous extracts and 7 days for hydro-alcoholic extracts, extracts were sporadic mechanically stirring, working temperature (20 ^{o}C). The plant's mass concentration in the solvent was of 6% and 10% - for aqueous extracts and 10% for hydro-alcoholic extracts.

The extracts were processed through microfiltration (MF), using Millipore membranes with 0.45 μm pores, to remove the coarse suspensions and impurities. Then, the resulted permeate was concentrated through the ultrafiltration (UF), using 2 types of membranes with cut-off of 10.000 Da: one of regenerated cellulose (Millipore)(UF1), and another of polysulphone (prepared in laboratory)(UF2). The concentration ratio (expressed as volume ratio between permeate and concentrate) was of 2:1. A KMS Laboratory Cell CF-1 installation purchased from Koch Membrane firm – Germany, was used for both MF and UF.

Assessment of total polyphenols - the Folin–Ciocalteu method was used[29]. The calculus of polyphenols' concentration used on an calibration curve of 0.1-1 g/L caffeic acid.

Assessment of antioxidant capacity - The Trolox Equivalent Antioxidant Capacity (TEAC) was determined by using of two methods:

One method based on the decrease of the **DPPH** maximum absorbance at 519 nm in the antioxidant presence. [30,31]
The antioxidant activity (radical scavenging activity) was calculated using the expression:

$$\% \text{ inhibition} = [(A_B - A_A)/A_B] \times 100$$

where: A_B = control absorbance; A_A = sample absorbance.

Another method based on the decrease of the **ABTS** maximum absorbance at 731 nm in the antioxidant presence. [32]

The results were obtained using the equation (1), the equivalent of the antioxidant capacity being expressed as Trolox equivalent:

$$TEAC_{sample} = C_{Trolox} \cdot f \cdot \frac{A_{sample} - A_{blank}}{A_{Trolox} - A_{blank}} \quad (1)$$

where: A_{blank} = control absorbance; A_{sample} = sample absorbance; A_{Trolox} = absorbance for Trolox in measurement cell; C_{Trolox}= concentration of Trolox in measurement cell; f-dilution factor

Statistical Analysis: The tests were realized in triplicate and for statistical processing was used a Program Microsoft Office Excel 2007, standard deviation (STDV) was <15.

RESULTS AND DISCUSSION

The total polyphenols' content, expressed in caffeic acid equivqlent, was spectrophotometrically assessed for all *Geranium robertianum* extracts. The results are given in Table 1.

Table 1

Total polyphenols' content in analyzed *Geranium robertianum* extracts

Sample	Total Polyphenols (mg/mL)
6% aqueous extract	1.96
10 % aqueous extract	3.19
10 % _ MF	3.15
10 % perm_UF$_1$	3.16
10% conc_UF1	4.22
10% perm_UF2	3.61
10% conc_UF2	4.68
50% hydro-alcoholic extract	4.21
70% hydro-alcoholic extract	3.38

where: MF – microfiltration; UF1 – membranes with cut-off of 10.000 Da of regenerated cellulose (Millipore); UF2 – polysulphone membrane; Perm – permeate; Conc – concentrate

Was revealed that the content in total poliphenols of *Geranium robertianum* extracts depends, as expected, on the polarity of extraction solvent used and with plant's dry mass level, consequently the polyphenols content increased with increasing of the amount of alcohol in solvent mixture and with the increasing of plant's mass. It was established that the appropriate solvent mixture is ethanol: water, 50%.

The lowest polyphenols' content was found in 6% aqueous extract, while the highest in 50% hydro-alcoholic extract. The obtained results also showed that the highest polyphenols' content was in UF1 concentrate (using the cellulose membrane - Millipore).

The antioxidant activity of *Geranium robertianum* extracts determined through the two spectrophotometry methods (with DPPH and ABTS), the results are shown in Table 2 and Figures 1 and 2.

Table 2

Antioxidant activity of analyzed extracts

Sample	$TEAC_{DPPH}$ (μmol Trolox/g)	$TEAC_{ABTS}$ (μmol Trolox/g)
6% aqueous extract	24,21	404,03
10 % aqueous extract	223,99	768,22
10 %_MF	220,20	772,10
10 % perm_UF$_1$	221,88	720,98
10% conc_UF1	418,02	1239,93
10% perm_UF2	206,15	1360,50
10% conc_UF2	464,40	2609,00
50% hydro-alcoholic	217,06	1509,27
70% hydro-alcoholic	242,75	782,30

where: MF – microfiltration; UF1 – membranes with cut-off of 10.000 Da of regenerated cellulose (Millipore); UF2 – polysulphone membrane; Perm – permeate; Conc – concentrate

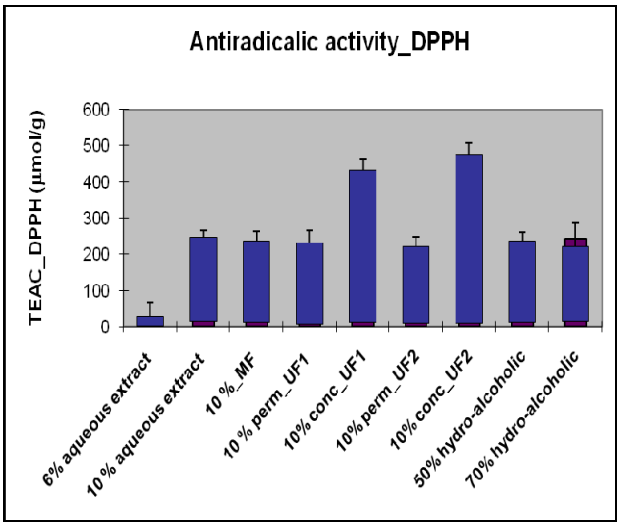

Fig. 1 – Antiradicalic activity_DPPH.

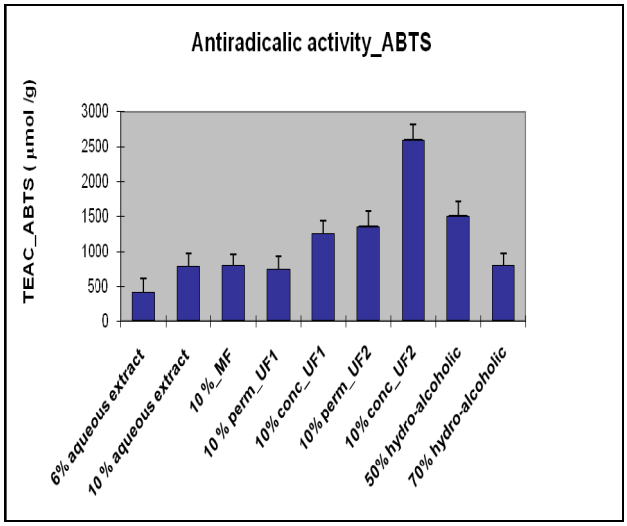

Fig. 2 – Antiradicalic activity_ABTS.

Comparing results of two radical scavenging tests good correlation between both tests and the determined content of total phenolics can be observed. The antiradical activity of extracts against ABTS radical cation lead to higher values with respect to tests performed against DPPH radical, the rationale of this behaviour consisting in the fact that polyphenols efficacy when addressed by ABTS is related to the total number of phenolic HO- from the structures of investigated polyphenols, while DPPH is more selective assay, due to the fact that only β-substituted polypohenols are able to scavange the DPPH radical .

A correlation between the extracts polyphenols' content and their antioxidant activity was revealed, namely: the lowest antioxidant activity was registered in case of 6 % aqueous extract which also had the lowest polyphenols' content, the highest antioxidant activity was determined in case of UF2 concentrate through both methods: DPPH and respectively ABTS and in concentrated extracts the antioxidant activity was higher than in permeates and in hydro-alcoholic extracts higher than in aqueous ones.

In addition, the inhibition extent of DPPH$^{\bullet}$ radicals was determined, and the obtained results are shown in Figure 3.

The obtained inhibition values varied from 77.6% for 6% aqueous *Geranium robertianum* extract till 95.3% in UF2 concentrated extract. The extracts concentrated by both UF types had a strong antioxidant activity, the DPPH inhibition percent being above 92% in both cases.

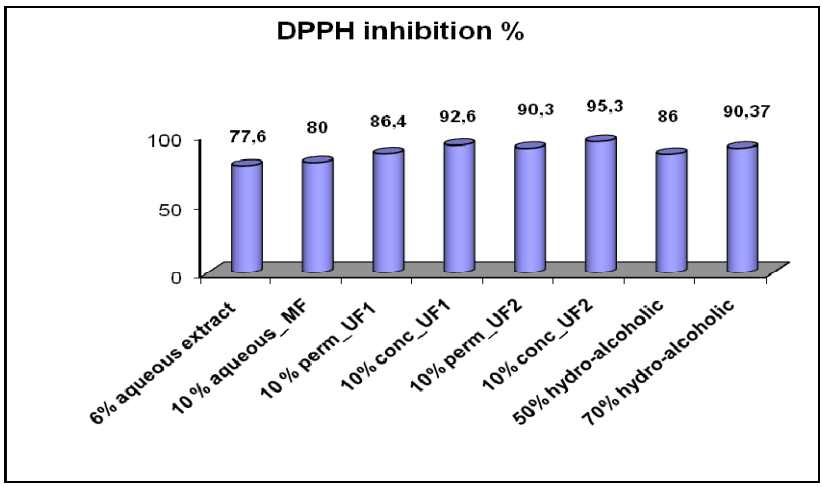

Fig. 3 – DPPH inhibition percent.

CONCLUSIONS

In this work new *Geranium robertianum* extracts were obtained and characterized from a antioxidant capacity point of view and to assesse the total polyphenols' content.

In this work 6% and 10% aqueous *Geranium robertianum* extracts and 10% (mass concentration) hydro alcoholic extracts in 50% and 70% alcohol were obtained. They were purified and concentrated through MF and UF membranare procedures.

The results as regards the extracts' antioxidant capacity obtained through the two (ABTS and DPPH) applied methods correlated and confirmed the correlation between the polyphenols' content and the antioxidant activity.

It was ascertained that is possible to obtain concentrated *Geranium robertianum* extracts having very high antioxidant activity (above 92% percent of DPPH inhibition) by use of the membranare processes to concentrate the biologic active principles (polyphenols).

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