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Concentration of the bioactive principles in *Geranium robertianum* extracts through membranare procedures (ultrafiltration)

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Abstract

8%, 10% and 15% (mass concentration) hydro-alcoholic extracts of Geranium robertianum in 50%, 70% and 96% alcohol were obtained. They were further purified and concentrated through membranare procedures (ultrafiltration). 2 types of membranes were used: Millipore ultrafiltration membranes of regenerated cellulose with 10.000 Da cut–off and composite membrane: polysulphona–polyaniline - PSF/PANI. The contents of flavones, total polyphenols, proteins and reducing sugars in all extracts' types ranged between: 7.233 – 72.71 mg/mL –flavones, 271.17 – 437.07 µg/ mL – polyphenols, 1.262 – 2.40 µg/ mL –proteins and 153.10 – 661.60 µg/ mL –sugars. It was ascertained that such contents were proportional to plant mass and to the used solvent concentration, the maximum being obtained in extracts realized in 70% ethanol, with 10% plant mass. The increase of ethanol concentrations and plant mass above the mentioned values resulted in the decrease of the concentration of the studied compounds.

Keywords: *Geranium robertianum*, membranare procedures, ultrafiltration, Millipore membrane, PSF/PANI membrane, flavones, sugars, polyphenols.

Introduction

Since immemorial times humans used the plants to maintain their health due to plant prophylactic and therapeutic stock, being well known as an important source of biologic active compounds. In the last years the plants became a significant source to discover new medicaments /1/.

The *Geranium* genus (Geraniaceae fam.) comprises about 300 species used in popular medicine as antifebrile, purgative and antidiarrheic agents, as well as against renal pains /2-5/. The *Geranium* species are used as antiasthmatic, antiallergenic, antihepatotoxic, diuretic, tonic, haemostatic, antidiabetic agents in popular medicine /5/.

The chemical analysis of plants belonging to this genus demonstrated the existence of some significant amounts of hydrolysable tannins, phenol compounds and flavonoids /6,7/. The phenol compounds, especially the flavonoids are main components among the biologic active compounds of *Geranium* species. The flavonoids have an important role in plant biochemistry and action at physiologic level through the hepatoprotective, antithrombotic, antiinflammatory, antiviral, antiallergic, antiproliferative, anticancer and immune stimulant activities /8,9/.

Membrane separation processes have been extensively studied and developed for their application to medicinal plant extracts purification and separation /10-12/. In the current

paper, 8%, 10%, 15% (mass concentration) *Geranium robertianum* extracts in 50%, 70% and 96% alcohol were obtained, then concentrated through the membranare procedures (ultrafiltration) on 2 types of membranes: polysulphona–polyaniline - PSF/PANI, a composite membrane and Millipore membrane for ultrafiltration, of regenerated cellulose, with 10.000 Da cut-off.

Materials and methods

Extract Preparing

The leaves of *Geranium robertianum* were finely grinded using a GRINDOMIX GM200 mill. The extracts of 8%, 10% and 15% mass concentration were obtained in 50%, 70% and 96% ethanol at room temperature, during 7 days, under gentle mixing.

Concentration of extracts

Firstly, the extracts were filtered, and then concentrated on UF ultrafiltration Millipore membranes of regenerated cellulose with 10.000 Da cut–ff and on polysulphona–polyaniline - PSF/PANI, composite membranes obtained in the laboratory of Faculty of Applied Chemistry and Science of Materials (UPB). A KMS Laboratory Cell CF-1 installation was used for ultrafiltration (Koch Membrane – Germany) and the concentration ratio was of 2:1.

Determination of flavones' content was realized by spectrophotometry through the method described in "Farmacopeea Romana" Xth Edition, using sodium acetate 100 g/L and aluminum chloride 25 g/L /13/.

Determination of polyphenols' content was realized by spectrophotometry at 760 nm wavelength using the Folin–Ciocalteu reactive /14/. The concentration of polyphenols in sample was calculated based on an etalon curve of cafeic acid 10-100 µg/mL.

Determination of proteins' content was realized by spectrophotometry at 660 nm wavelength, by Lowry method /15/.

Determination of sugars content was realized by spectrophotometry at 490 nm wavelength with phenol – sulphuric acid - Dubois method /16/.

Results and discussions

Flavones' content in obtained extracts was determined by spectrophotometry at 430 nm wavelength, based on an etalon curve using rutozide on a concentration domain between 1-12 mg/mL (fig. 1, Table 1)

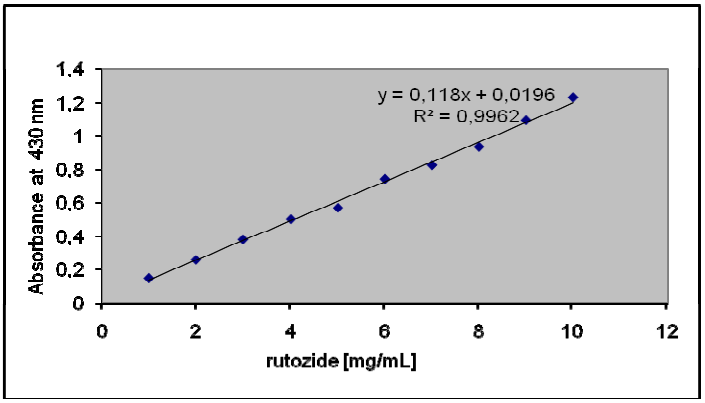

Figure 1. Etalon curve for the determination of flavones' content

Table 1. Flavones' content in *Geranium robertianum* extracts

Type of membrane			**Millipore 10000 Da**		**PSF/PANI**	
Sample		initial	permeate	concentrate	permeate	concentrate
50% hydro-alcoholic 8% mass extract	**d.w.%**	0.92	0.8	1.63	0.64	0.874
	flavones mg/ mL	10.004	7.233	12.88	10.81	11.28
50% hydro-alcoholic 10% mass extract	**d.w.%**	1.63	0.78	1.04	1.24	1.64
	flavones mg/ mL	17.56	12.31	21.17	11.72	21.76
70% hydro-alcoholic 10% mass extract	**d.w.%**	1.56	1.26	2.29	1.23	1.52
	flavones mg/ mL	29.20	15.19	72.71	19.12	32.57
50% hydro-alcoholic 15% mass extract	**d.w.%**	1.51	1.21	1.64	1.41	1.45
	flavones mg/ mL	17.99	15.41	48.97	18.21	21.38
70% hydro-alcoholic 15% mass extract	**d.w.%**	1.65	1.209	1.99	1.29	1.49
	flavones mg/ mL	47.23	14.42	42.98	17.55	20.28
96% hydro-alcoholic 15% mass extract	**d.w.%**	1.173	0.61	0.97	0.34	0.54
	flavones mg/ mL	16.54	12.53	17.89	15.29	18.18

It was concluded that:

The flavones amount was correlated to the plant mass and solvent concentration, the highest quantity of flavones was found in 10% (mass concentration) extract in 70% ethanol. The increase of alcohol concentration and plant mass above such values not resulted in flavones' amount increase, but to it decrease.

For concentration, the Millipore membrane was more efficient than the PSF/PANI membrane, the highest concentration of flavones was obtained on the concentrate of 10% (mass concentration) extract in 70 % ethanol.

Content of total polyphenols in extracts was determined by spectrophotometry at λ=760 nm, with Folin–Ciocalteu reactive. The polyphenols' concentration in sample was calculated using an etalon curve for caffeic acid of 10-100 µg/mL concentration (fig. 2); the results are shown in Table 2.

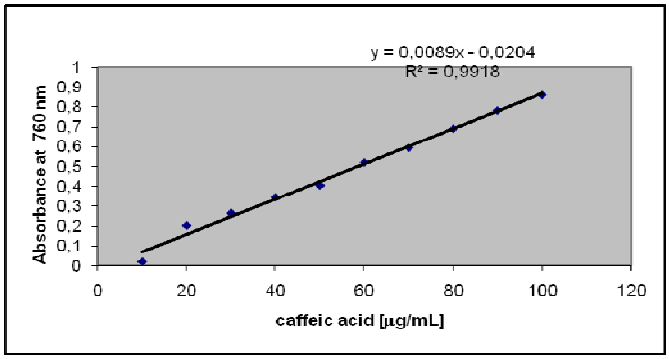

Figure 2: Etalon curve for the determination of total polyphenols

Table 2:. Polyphenols content in *Geranium robertianum* extracts

	Polyphenols µg/ mL				
Type of membrane		**Millipore 10000 Da**		**PSF/PANI**	
Sample	**initial**	**permeate**	**concentrate**	**permeate**	**concentrate**
50% hydro-alcoholic 8% mass extract	335.449	271.17	330.61	251.79	319.43
50% hydro-alcoholic 10% mass extract	364.32	350.11	363.48	326.40	363.93
70% hydro- alcoholic 10% mass extract	374.32	333.76	***437.97***	342	397.07
50% hydro-alcoholic 15% mass extract	319.94	304.88	315.84	327	337.52
70% hydro-alcoholic 15% mass extract	297.97	267.24	310.73	271.46	284.55
96% hydro- alcoholic 15% mass extract	292.86	219.71	273.87	250.06	264.45

In case of total polyphenols, similar concentrations were obtained for 8 and 10% extracts in 50% and 70% alcohol after which the content in polyphenols decreased, as the plant amount and alcohol concentration increased.

After concentration, small differences appeared between the concentrations found for permeates against the concentrates for both types of used membranes.

The highest amount of total polyphenols was found in concentrate of 10% (mass concentration) extract in 70% ethanol, ultrafiltrated through Millipore membrane. Same conclusion was also made in the case of flavones.

Content of proteins in extracts was assessed by spectrophotometry with Lowry method (I.F. Dumitru, 1988) at λ= 660 nm, using an etalon curve of bovine serum albumin (BSA) on 0-100 µg/mL concentration domain (figure 3); the results are shown in Table 3.

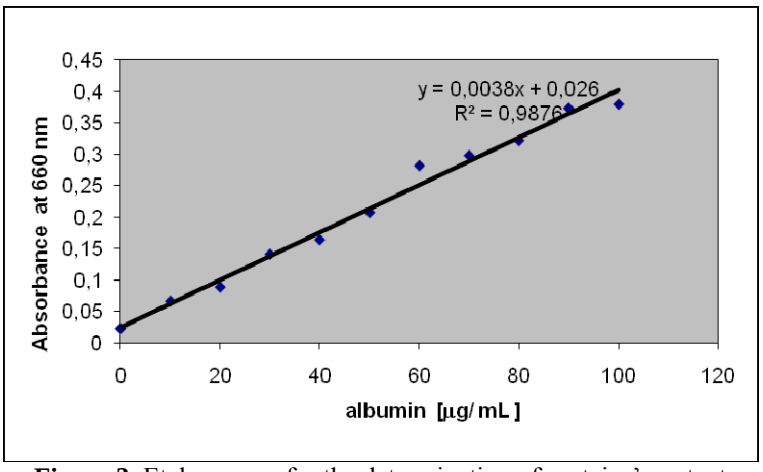

Figure 3. Etalon curve for the determination of proteins' content

Table 3. Proteins' content in *Geranium robertianum* extracts

	Proteins mg/ mL				
Type of membrane		**Millipore 10000 Da**		**PSF/PANI**	
Sample	**initial**	**permeate**	**concentrate**	**permeate**	**concentrate**
50% hydro-alcoholic 8% mass extract	1.262	1.787	1.931	1.757	1.937
50% hydro-alcoholic 10% mass extract	1.706	1.622	1.739	1.678	2.031
70% hydro-alcoholic 10% mass extract	2.242	1.867	2.40	1.851	1.917
50% hydro-alcoholic 15% mass extract	1.296	1.205	1.397	1.102	1.332
70% hydro-alcoholic 15% mass extract	1.180	1338.15	1.493	1.247	1.282
96% hydro- alcoholic 15% mass extract	1.117	0.936	1.234	1.110	1.150

It was observed that:

The content in proteins of extracts was proportional to the plant mass and solvent concentration, only till the level of 10% for plant mass and 70% solvent concentration, after which it decreased as the solvent concentration and plant mass increased. The highest protein content was found in 10% (mass concentration) extract in 70% ethanol, while the lowest in extract with highest mass concentration (15%) of plant and 96% solvent.

Both types of membranes gave similar results at concentration. The 50% hydro-alcoholic extract (10% plant mass) was best concentrated through the PSF/PANI membrane, while through the Millipore membrane the 70% hydro-alcoholic extract (10% plant mass).

Determination of sugars' content was realized by spectrophotometry at 490 nm with phenol – sulphuric acid - Dubois method. The results were obtained using an etalon curve realized with 50 mg% dilutions of glucose on a 5- 25 µg/mL concentration domain (figure 4). The results for the three variants of hydro-alcoholic extracts are shown in Table 4.

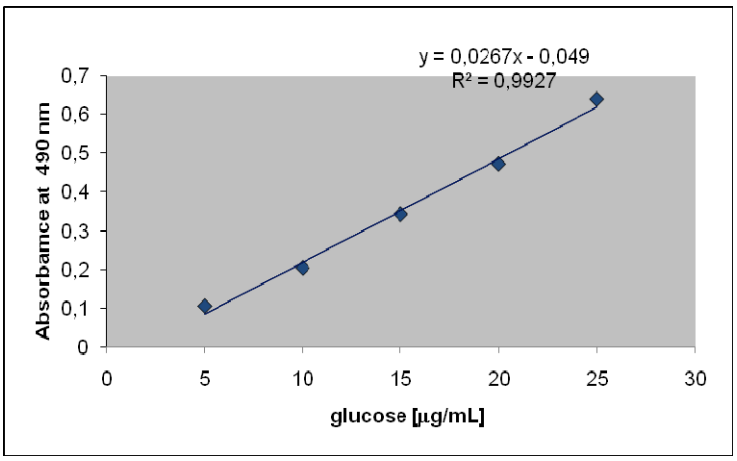

Figure 4. Etalon curve for the determination of glucose at λ= 490 nm

Table 4. Reducing sugars' content in *Geranium robertianum* extracts

	Reducing sugars µg/ mL				
Type of membrane		**Millipore 10000 Da**		**PSF/PANI**	
Sample	**initial**	**permeate**	**concentrate**	**permeate**	**concentrate**
50% hydro-alcoholic 8% mass extract	353.10	252.19	269.54	153.10	172.05
50% hydro-alcoholic 10% mass extract	386.06	*293.10*	*536.93*	250.80	383.62
70% hydro- alcoholic 10% mass extract	479.5	*363.41*	*661.60*	377.77	455.12
50% hydro-alcoholic 15% mass extract	267.7	156.9	256.7	186.9	297.01
70% hydro-alcoholic 15% mass extract	288.3	166.4	277.8	284.4	364.1
96% hydro- alcoholic 15% mass extract	257.2	181.3	196.1	142.2	162.02

In case of reducing sugars it was observed that:

The sugars amount in extracts increased with the increase of the plant mass till 10% level, its highest values being obtained in both 10% extracts realized in 70% and 50% alcohol;

The increase of plant quantity and solvent concentration above 10% plant mass and 70% alcohol not resulted in the increase of the sugars' amounts, but contrary, the smallest quantity of sugars was determined in most concentrated extracts (15% plant mass in 96% alcohol);

The Millipore Membrane with 10.000 Da cut-off was more efficient than PSF/PANI one, the concentration was maximum in case of the 10% hydro-alcoholic extracts.

Conclusions

In present study, hydro-alcoholic extracts of *Geranium robertianum* were realized: 8% (mass concentration) in 50% ethanol, 10% (mass concentration) in 50% and 70% ethanol and 15% (mass concentration) in 50%, 70% and 96% alcohol;

The extracts were concentrated by membranare procedure – ultrafiltration, through 2 types of membranes: PSF/PANI and ultrafiltration Millipore of regenerated cellulose, with 10.000 Da cut-off, the concentration ratio being 2:1;

The biologic active principles: flavones, polyphenols, proteins, sugars were assessed in the initial extracts and in permeates and concentrates;

The content in flavones, polyphenols, proteins, sugars was correlated to the plant mass and with the solvent concentration, the highest amount of biologic active principles being found in 10% (mass concentration) extract in ethanol 70%. The increase of plant concentration or solvent concentration above such value not resulted in increase of mentioned compounds, but, on contrary, lead to the diminution of their quantities;

The most efficient membrane for the concentration of the studied biologic active principles was the Millipore membrane of regenerated cellulose with 10.000 Da cut-off.

Acknowledgment

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