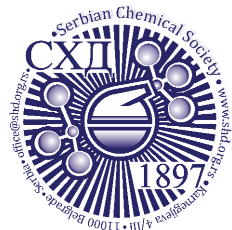

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Application of membrane processes for the concentration of *Symphytum officinale* and *Geranium robertianum* extracts to obtain compounds with high anti-oxidative activity

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Abstract: The paper reports the successful application of membranes processes to obtain good quality extracts with compounds of high antioxidative activity and therapeutic value. In this study, the phenolic compounds from two plant species used in Romanian ethno-medicine were investigated and their antioxi/-dant and cytotoxic activities evaluated. Three extracts prepared from *Geranium robertianum* and *Symphytum officinale* were concentrated by microfiltration and ultrafiltration. The levels of phenolic compounds and flavonoids were determined by UV–Vis spectroscopy and high-pressure liquid chromatography (HPLC). The free-radical scavenging activity of the concentrated extracts was determined by the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) method. The preliminary tests of cytotoxic activity for the concentrated extracts were performed on human epidermoid laryngeal carcinoma cell line (Hep-2p) and normal monkey kidney cells (RM). The results showed that all the concentrated extracts had a very low cytotoxicity against healthy cells, but a significant cytotoxic effect on Hep-2p tumor cells. The concentrated extracts had a high antioxidant activity (% DPPH inhibition > 80 %).

Keywords: antioxidant compounds; cytotoxicity; ultrafiltration; *Geranium robertianum*; *Symphytum officinale*; free-radical scavenger.

INTRODUCTION

The major therapeutical activity of phytochemicals is described in relation to their biologically active polyphenol components, such as flavonoids and phenolic acids, which possess significant antioxidant activity.[1–4] Antioxidant substances

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block the action of free radicals involved in the pathogenesis of many diseases, including atherosclerosis, ischemic heart disease, Alzheimer's disease, Parkinson's disease, cancer and the aging process.[5] With respect to this, in recent years, considerable attention has been paid to plants as sources of antioxidants.

In the present study, interest was focused on medicinal plants extracts obtained from *Symphytum officinale* and *Geranium robertianum* L. (Geraniaceae), both of them known for a long time as remedies in traditional medicine.[6–9] *S. officinale* L. – comfrey (Boraginaceae family) is used in the traditional medicine of Romania to treat different human and animal diseases, such as ulcerations in the gastrointestinal tract, lung congestion, and joint inflammation, and to promote wound healing.[6,7] *G. robertianum* (herb Robert) is used as an anti-inflammatory, haemostatic, anti-diabetic, antibacterial, immunomodulatory and anti-cancer remedy in popular medicine, although very little information is available on the constituents.[8,9]

Medicinal plants have been used for centuries in the cure of human diseases due to their content of components with therapeutic value.[10] Moreover, the wide use of plant extracts in the food, cosmetic and pharmaceutical industries suggests that a systematic study of medicinal plants is very important to identify the active compounds.[10]

Previous studies revealed that ultrafiltration could be used for the concentration of medicinal plant extracts.[4,11] Ultrafiltration processes offer more benefits over conventional technologies. Its application in the phyto-pharmaceutical industry raises particular interest, since membrane processes occur at mild temperatures, avoiding damages caused by thermal processes, thus maintaining the original characteristics of the processed products. Compared with other purification and concentration techniques, including evaporation and dialysis, ultrafiltration has the capability to process larger amounts at greater speeds and it is a very efficient process. Vacuum evaporation is a slower process and is feasible only with small volumes of samples.

The major aim of the present study was to apply various membrane techniques to obtain good quality extracts with high value antioxidant compounds and therapeutic value. The second aim was to determine the cytotoxic effect of *S. officinale* and *G. robertianum* extracts for application as herbal medicine in extract preparation.

In addition, in various research studies, the level of minerals present in medicinal plants were investigated, since these are essential for human nutrition.[12,13] Recently, the importance of the determination of toxic metals level in aqueous extracts and on the phyto-products made from medicinal plants,[14] has been emphasized not only for their benefits, but also for their safe use.

In this study, the metal ions present in the extracts, considering both essential and non-essential ions to the human body, were also determined. The analysis

was performed using flame atomic absorption spectroscopy. The levels of phenolic compounds and flavonoids were determined by UV–Vis spectroscopy and reversed phase-high pressure liquid chromatography (RP-HPLC). To the best of our knowledge, there are no experimental data reported in the literature on the content of microelements and the cytotoxic effect of *S. officinale* and *G. robertianum* extracts.

EXPERIMENTAL

Chemicals and equipments

All chemicals and solvents were purchased from Sigma Aldrich (Germany), Fluka (Switzerland) and Roth (Germany). Deionized water was used for all the performed analysis (Millipore, Bedford, MA).

Microfiltration membranes with 0.45 μm pores and ultrafiltration membranes from regenerated cellulose (MWCO 10000 Da and 1000 Da) were purchased from Millipore (SUA).

The medicinal plants (*S. officinale* and *G. robertianum*) were obtained from a provider specialized in medicinal and aromatic plants – Phytogentec srl (Romania).

Determinations of Ca, Mg, Mn, Zn, Fe, Ni and Pb were performed using a flame atomic absorption spectrometer – FAAS SOLAAR 969 AA (ATI Unicam). All of the absorbance measurements were realized in the area integration mode. Samples were prepared in triplicate and their signals were subtracted from their blanks.

The experiments are performed in a KMS Laboratory Cell CF-1 (Koch Membrane – Germany) with a type cross-flow lab-scale filtration unit.

The rejection, R, was calculated using Eq. (1):

$$R\ \% = 100\,(1 - c_p/c_f) \quad (1)$$

where c_f and c_p are the polyphenols/flavones concentrations in the feed and permeate, respectively.

Preparation and concentration of the extracts

Three extracts were prepared by maceration: *G. robertianum* aqueous extract using cold distilled water as the solvent and for *S. officinale* and *G. robertianum*, alcoholic extracts using 50 % aqueous ethanol (v/v) as the solvent. The dried root (comfrey) and dried leaves (herb Robert) were ground into powder using mill equipment (Grindomix G200), and then mixed with the selected solvent. The herbal mass concentration in the solvent was 6 % (w/v).

The extracts were then successively filtered through Whatman 1 (Medium-fast) filter paper, and microfiltered through 0.45 μm pore size membrane (Millipore), to remove any fine solid particles that could cause membrane fouling during the ultrafiltration (UF).

The concentration experiments were realized on a two-stage membrane filtration set. First, the microfiltration (MF) extracts are treated using a UF1 membrane (cut-off 10000 Da), then the permeate obtained from UF1 was introduced into the cross-flow circuits for UF2 membrane (cut-off 1000 Da) treatment. Each of the flat sheet membranes used in the experiment had an effective area of 0.0028 m^2.

The concentration ratio in ultrafiltration processes (expressed as permeate and concentrate volume ratio) was 2:1. All ultrafiltration experiments were performed at room temperature (*ca*. 23 °C).

Determination of phenolic compounds

The phenolic total content (TPC) was determined by the Folin–Ciocalteu method.[15] Gallic acid (GAE) was used to calibrate the standard curve; the total polyphenols content was obtained from the regression equation of the calibration curve of gallic acid ($y = 0.0036x + 0.0203$, $R^2 = 0.9954$) and is expressed as gallic acid (GAE) equivalent.

The characterization of the bioactive phenolic compounds was effected by UV–Vis spectroscopy and HPLC.

Determination the total flavonoid compounds (TFC)

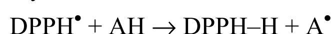
The total flavonoid content was determined according to the aluminum chloride colorimetric method with slight modifications.[16] Rutin was chosen as the standard in the concentration range: 0.005 to 0.1 mg mL^{-1} and the total flavonoid content is expressed as microgram rutin per mL. Total flavonoid content was obtained from the regression equation of the calibration curve of rutin ($y = 0.00989x + 0.01975$, $R^2 = 0.9977$).

HPLC analysis of the extracts for polyphenols and flavones

The analysis of extracts for polyphenols and flavones was performed with a Shimadzu HPLC system equipped with a binary pump (LC-20Adsp), a CTO-20AC column thermostat and a diode-array detector (DAD: SPD-M20A). The spectral data for all peaks were recorded in the range 220–800 nm. Samples were injected at ambient temperature (20 °C) onto a reverse-phase KROMASIL C_{18} column, 4.6 mm×150 mm, 5.1 µm. An auto injector was used to inject 15 µL of the test solution into the HPLC system. The binary mobile phase consisted of solvent A (water acidified with 1 % formic acid, pH 3.0) and B (acetonitrile acidified with 1 % formic acid, pH 3.0). The gradient elution started with 5 % B and changed to 50 % B in 50 min, then reached 5 % B in 5 min. The flow rate was 1.0 mL min^{-1}. The quantitative determinations were made by the calibration curves for caffeic acid, gallic acid, coumaric acid, ferulic acid, chlorogenic acid, rosmarinic acid, rutin, quercetin and kaempferol.

Determination of free radical scavenging activity

The free radical scavenging activities of the feed (extracts), permeate and retentate were studied by the DPPH method – based on the decrease in the maximum absorbance at 519 nm of the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) (Sigma–Aldrich) in the presence of an antioxidant.[17-19] DPPH· is a stable radical that can readily undergo reduction by an antioxidant (AH), which occurs by the reaction:



$$\mathrm{DPPH}^{\bullet} + \mathrm{AH} \rightarrow \mathrm{DPPH{-}H} + \mathrm{A}^{\bullet}$$

The decreasing of the DPPH radical absorption by the action of antioxidants could be used as a measure the antioxidative activity.

The antioxidant activity (radical scavenging activity) was calculated using the expression:

$$I\,\% = 100\,((A_0 - A_s)/A_0) \quad (3)$$

where A_0 is the blank absorbance and A_s the sample absorbance.

Cytotoxic activity analysis

The preliminary tests of the cytotoxic activity of the concentrated extracts were performed on the human epidermoid laryngeal carcinoma cell line (Hep-2p) and normal monkey kidney cells (RM).

The cellular cultures of human neoplasm origin (Hep-2p cells) were cultured in DMEM medium (Dulbeco's modified essential medium, Biochrom AG, Germany) supplemented with

10 % fetal bovine serum (Sigma, Germany), 100 μg mL^{-1} streptomycin (Biochrom, Germany), 100 IU mL^{-1} penicillin (Biochrom, Germany) and 50 μg mL^{-1} amphotericin B (Biochrom, Germany), at a density of 5×10^5 cells mL^{-1} flasks, in a humidified 5 % CO_2 atmosphere at 37 °C. When the cells reached confluence, they were detached from the flask with 0.25 % trypsin + 0.02 % ethylenediaminetetraacetic acid (EDTA, Biochrom, Germany) in normal medium and then centrifuged at 1800 rpm for 2 min. The cells were seeded at a density of 1×10^5 cells mL^{-1} in experimental tubes containing 2 mL DMEM medium. The medium of the 24 h cell cultures was replaced either by a normal one (control cultures) or by one containing the vegetal extracts (treated cultures), in a variable dose. After 24 and 48 h of *in vitro* treatment, the total cell number (cytometry), the dead cells/living cells (exclusion test with Trypan Blue) and the cell cultures development were estimated. The cytotoxic property of the studied biopreparations was calculated using the expression:[20]

$$\text{Cytotoxicity level} = 100(N_{tct} - N_{cat})/N_{tc} \quad (4)$$

where N_{tct} = total number of treated cells, N_{cat} = number of treated living cells and N_{tcc} = control cell total number.

Statistical analysis

The measurements were performed in triplicate and Excel 2007 was used for statistical processing, standard deviation (*SD*) was < 10 %.

RESULTS AND DISCUSSION

The microfiltration (MF) process is performed for feed clarification and sterilization, while the ultrafiltration (UF) processes were employed to concentrate the bioactive compounds in the extracts from *S. officinale* and *G. robertianum.*

As plant phenolics represent one of the major groups of compounds acting as primary antioxidants or free radical scavengers, it was important to determine their total amount in the selected plant extracts. The total contents of polyphenols (Fig. 1) and flavones (Fig. 2) were determined in the permeate and retentate after ultrafiltration of the extracts.

The obtained results for the concentration of the extracts by ultrafiltration (after UF2) ranged between 72–78 % for the polyphenols retention, while for flavonoids, the retention ranged between 46–61 %. The degree of retention of polyphenols was between 19–33 % when the extracts were processed using the UF1 membrane. The retention of polyphenols by the UF1 membrane (cut-off 10000 Daltons) was probably due to colloids, which were clustered together with the colloidal matter on the membrane.

The contents of the individual polyphenolic compounds (flavones and polyphenol carboxylic acids) in the extracts were determined by RP-HPLC, after processing by MF–UF. The obtained values are presented in Tables I and II.

Quantitative and qualitative analysis showed that pure water was not the best solvent for the extraction of phenolic compounds (*G. robertianum* aqueous extract compared with *G. robertianum* aqueous alcoholic extract). This is in concordance with previous studies, which reported that aqueous alcohol solvent is the best solvent for the extraction of phenolic compounds from plant materials.[21,22]

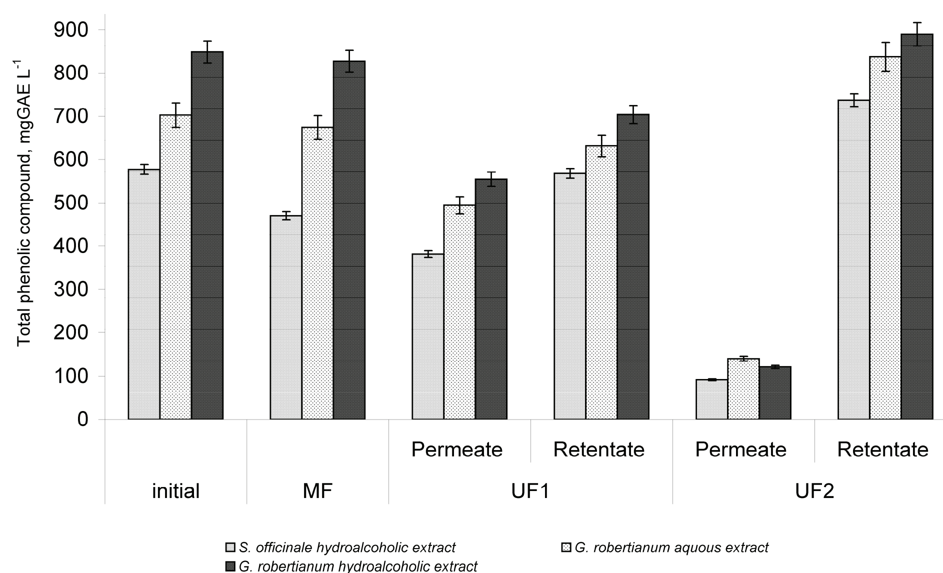


Fig. 1. Total phenolic compounds in *S. officinale* and *G. robertianum* extracts concentrated by ultrafiltration.

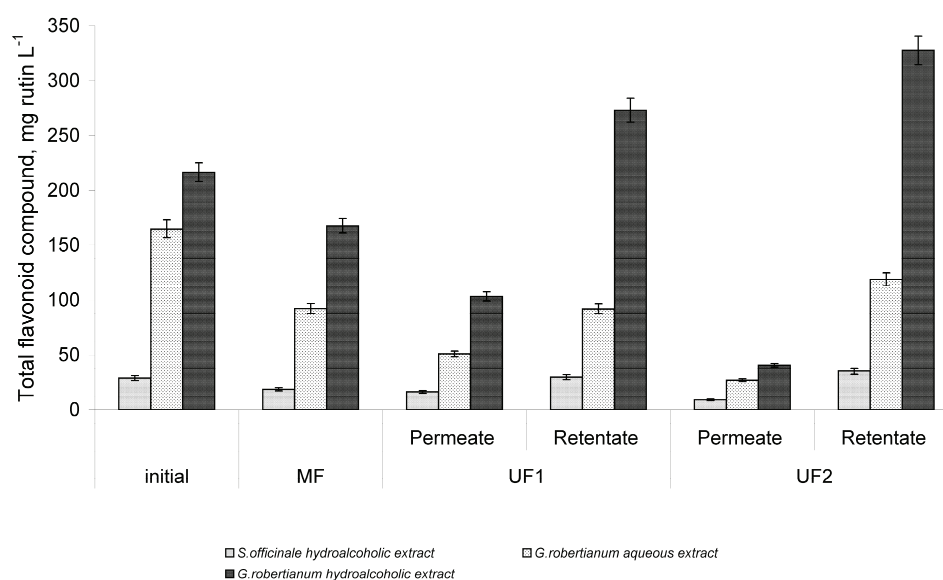


Fig. 2. Total flavonoid compounds in *S. officinale* and *G. robertianum* extracts concentrated by ultrafiltration.

The most important compound identified in the comfrey extract was rosmarinic acid (146.3 mg kg^{-1} in the microfiltrate and 198.66 mg kg^{-1} in the final concentrate extract) and a less important one was luteolin (1.11 mg kg^{-1} in the

microfiltrate and 1.23 mg kg^{-1} in the final concentrate extract). In the *G. robertianum* aqueous alcoholic extract, kaempferol (284.57 mg kg^{-1} in the microfiltrate and 402.83 mg kg^{-1} in the final concentrated extract) and quercetin (54.6 mg kg^{-1} in the microfiltrate and 74 mg kg^{-1} in the final concentrate extract) were the major compounds identified. *p*-Coumaric (9.22 mg kg^{-1} in the microfiltrate and 21.05 mg kg^{-1} in the final concentrate extract) followed by ferulic acid (11 mg kg^{-1} in the microfiltrate and 18.12 mg kg^{-1} in the final concentrate extract) were of minor importance.

TABLE I. Contents of phenolic acids (mg kg^{-1}) and flavonoids in *S. officinale* extracts

Sample	Chlorogenic acid	Caffeic acid	Ferulic acid	*p*-Coumaric acid	Rutin	Rosmarinic acid	Luteolin	Quercetin	Kaempferol	Apigenin
MF	3.23	13.35	3.51	8.51	7.86	146.30	1.11	1.50	1.49	1.81
UF1 permeate	1.58	7.43	1.99	2.43	2.23	41.05	0.96	0.42	1.28	1.63
UF1 retentate	2.25	9.97	4.12	5.30	4.89	137.37	1.04	1.48	1.43	1.72
UF2 retentate	4.11	12.58	8.51	11.51	10.45	198.66	1.23	1.75	1.53	1.78

TABLE II. Contents of phenolic acids (mg kg^{-1}) and flavonoids in *G. robertianum* extracts

Sample		Rutin	Caffeic acid	*p*-Coumaric acid	Ferulic acid	Quercetin	Kaempferol
G. robertianum aqueous extract							
MF		2.67	8.18	0.77	1.18	4.83	0.00
UF1	Retentate	10.72	10.97	0.52	3.63	0.00	0.00
	Permeate	1.75	7.85	0.43	1.23	4.83	0.00
UF2	Retentate	1.87	8.32	0.50	1.25	5.60	0.00
G. robertianum aqueous alcoholic extract							
MF		38.95	20.18	9.22	11.00	54.60	284.57
UF1	Retentate	42.78	25.28	22.80	12.58	66.90	375.30
	Permeate	11.73	13.97	6.80	4.27	40.20	188.27
UF2	Retentate	26.97	24.48	21.05	18.12	74.00	402.83

The polyphenol-rich extracts were obtained by combining the two retentate fractions.

The total phenolic contents in the *G. robertianum* and *S. officinale* concentrated extracts were proportional to their free-radical scavenging-linked antioxidant activities.

The results obtained by the DPPH method showed 90 % DPPH inhibition by the *G. robertianum* concentrated extracts (Fig. 3) and over 80 % DPPH inhibition by the comfrey concentrated extract (Fig. 4). The retentate from the aqueous alcoholic and aqueous *G. robertianum* extracts showed the highest antioxidant

activity. The results indicate that the antioxidant activity of the all concentrated extracts is higher than that of Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2--carboxylic acid).

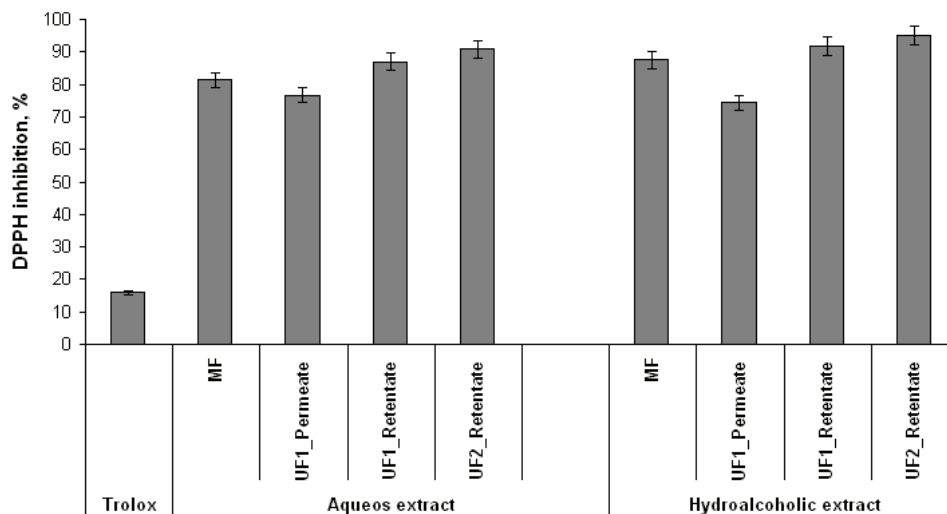


Fig. 3. Comparison of the DPPH radical scavenging activity of the concentrated *G. robertianum* extracts and that of Trolox.

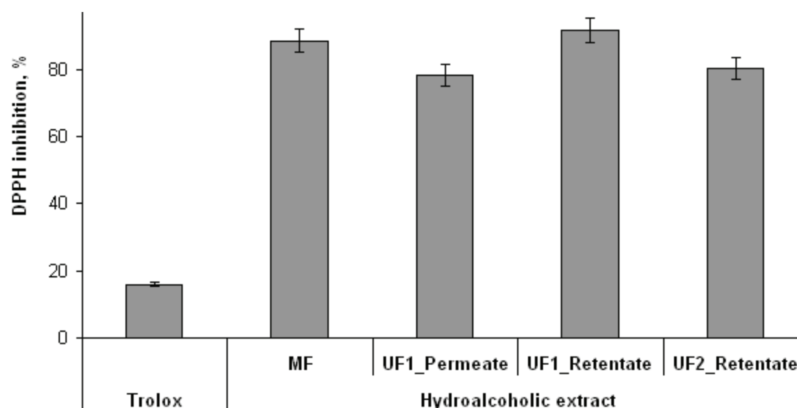


Fig. 4. Comparison of the DPPH radical scavenging activity of the concentrated *Symphytum officinale* extracts and that of Trolox.

A good correlation coefficient (Pearson's correlation coefficient $r \geq 0.98$) between TPC and DPPH scavenging activity was observed.

The strong correlation observed in this study between the total phenolics and the total antioxidant capacity, together with the lack of the cytotoxicity, indicates

the necessity for the use of comfrey and herb Robert, in order to increase health benefits.

There is a direct relationship between the quality of phytotherapeutic products and the bioprocessing of herbals based on the latest developed methods. The present research contributes to the field of medicinal and aromatic plant processing, as the ultrafiltration process showed good results in concentration and purification processes of the active principles with high antioxidant activity from *S. officinale* (comfrey) and *G. robertianum* (herb Robert).

The contents of microelements in the extracts, expressed as mg L^{-1}, are given in Table III. Some metals, such as Ca, Mg, Mn, Zn and Fe, are reported as essential for human health, whereas others, such as Pb and Ni, have been identified as toxic.

TABLE III. Contents of microelements in comfrey (*S. officinale*) and herb Robert (*G. robertianum*) extracts; DL = detection limit; MF = microfiltrate: UF = ultrafiltrate

Sample		Content of microelements, mg L^{-1}						
		Ca	Mg	Mn	Zn	Fe	Ni	Pb
S. officinale	MF	0.052±0.004	0.054±0.005	< DL	0.079±0.009	0	0.050±0.004	0.354±0.011
	Permeate UF1	0.052±0.005	0.053±0.004	< DL	0.072±0.008	0	0.041±0.003	0.298±0.02
	Permeate UF2	< DL	< DL	< DL	0.030±0.001	0	0.048±0.003	1.22±0.09
	Retentate UF2	0.051±0.004	0.052±0.003	< DL	0.082±0.007	0	0.023±0.001	0.196±0.02
G. robertianum aqueous extract	MF	0.935±0.08	10.4±0.3	0.893±0.07	0.071±0.006	3.2±0.1	0.051±0.003	< DL
	Permeate UF1	0.89±0.07	9.4±0.6	0.734±0.06	0.071±0.006	2.3±0.1	0.043±0.003	< DL
	Permeate UF2	0.705±0.06	8.82±0.4	0.236±0.01	0.050±0.004	0.7±0.05	0.042±0.003	< DL
	Retentate UF2	1.72±0.1	12.8±0.9	0.919±0.08	0.097±0.008	5.6±0.3	0.044±0.004	< DL
G. robertianum Aqueous alcoholic extract	MF	0.927±0.08	9.78±0.7	0.819±0.07	0.069±0.006	1.8±0.1	0.047±0.003	< DL
	Permeate UF1	0.896±0.07	9.12±0.6	0.729±0.06	0.064±0.006	1.3±0.09	0.041±0.003	< DL
	Permeate UF2	0.792±0.05	7.69±0.6	0.254±0.01	0.031±0.004	0.3±0.03	0.037±0.003	< DL
	Retentate UF2	1.34±0.09	11.7±0.9	1.273±0.09	0.11±0.009	2.9±0.2	0.046±0.004	< DL

The concentrations of Ca, Mg, and Fe in permeate of the *S. officinale* extract after the UF2 were below the detection limit of the instrument. Mn was an exception as its concentration was below the detection limit even in the microfil-

tration step. In the case of the *G. robertianum* extracts, only Pb was below the detection limit even in the microfiltration step.

The elemental studies of the plants showed that they contained large amounts of nutrients (Mg, Ca and Fe); WHO limits for these metals have not been established.

On comparing the two extracts, it could be observed that the concentrations of the toxic heavy metals (Pb and Ni) in the UF2 retentate were higher in the *S. officinale* extracts. The Pb concentration levels ranged from 0.196 to 0.354 mg L^{-1} and Ni concentration levels from 0.023 to 0.05 mg L^{-1} but they did not exceed the limits of 10 mg kg^{-1} Pb and 8 mg kg^{-1} Ni recommended for medicinal plants.[23] The concentrations of all the microelements determined in the studied medicinal plants were well below the critical limits.

Determination of the cytotoxic action of the studied extracts on the viability of Hep-2p tumor cell cultures and the normal RM cell cultures was based on the calculation of viability percentage of the treated cell cultures, in relation to the controls. The obtained results are presented in Tables IV–VI.

TABLE IV. The cytotoxic impact of *S. officinale* aqueous alcoholic extract processed by MF-UF on Hep-2p tumor cells and RM normal cells; ES = standard deviation; NS = no significant

Sample	Number of alive cells		Number of dead cells		Cytotoxicity, %
	$(X\pm ES)\times10^{-4}$	p	$(X\pm ES)\times10^{-4}$	p	
Hep-2p cells					
Control	94.60±5.34(5)	–	3.90±1.08(5)	–	4.0
Retentate UF2	32.85±1.97(5)	NS	5.25±2.34(5)	NS	13.8
70 % Ethanol	1.11±0.6(5)	<0.001	4.8±1.2(5)	<0.001	81.2
RM cells					
Control	39.90±3.10(5)	–	3.00±1.26(5)	–	6.9
Retentate UF2	39.30±2.83(5)	<0.001	2.50±0.97(5)	<0.001	6.0
70% Ethanol	0.18±0.2(5)	<0.001	1.11±0.4(5)	<0.001	86.0

The comparative analysis of the number of alive and dead cells from the Hep-2p cell cultures/normal RM cell cultures, control and treated for 48 h with extracts, at a dose of 1.5 mg mL^{-1}, emphasized the different behavior of these two types of cell cultures. Thus, the control cultures were characterized by a greater number of living cells than dead ones, while the treated cultures showed a greater number of dead cells than living ones.

It is highly important that all concentrated extracts, namely the ethanolic extract of *S. officinale* and *G. robertianum*, and the aqueous extract of *G. robertianum* showed very low cytotoxicity against healthy cells, but selective cytotoxicity against the Hep-2p tumor cells. The low cytotoxic potential of the aqueous extracts is of great significance for their traditional use in the treatment of various disorders, other than cancer.

TABLE V. The cytotoxic impact of *G. robertianum* aqueous extract processed by MF-UF on Hep-2p tumor cells and RM normal cells; MF = microfiltrate extract, ES = standard deviation; NS = no significant

Sample	Number of alive cells		Number of dead cells		Cytotoxicity, %
	$(X \pm ES) \times 10^{-3}$	p	$(X \pm ES) \times 10^{-3}$	p	
Hep-2p cells					
Control	314.89±9.98(5)	–	10.11±0.32(5)		3.1
MF	271.85±7.60(5)	<0.01	21.47±0.60(5)	<0.001	7.3
UF1 Permeate	320.44±9.04(5)	NS	2.77±0.08(5)	<0.001	0.9
UF1 Retentate	101.53±3.91(5)	<0.001	48.97±1.89(5)	<0.001	32.5
UF2 Retentate	319.58±7.18(5)	NS	19.20±0.07(5)	<0.001	5.7
RM cells					
Control	214.15±11.72(5)	–	6.87±0.38(5)	–	3.1
MF	176.41±9.40(5)	<0.05	7.17±0.38(5)	NS	3.9
UF1 Permeate	210.72±6.79(5)	NS	3.29±0.11(5)	<0.001	1.5
UF1 Retentate	124.98±5.26(5)	<0.001	12.72±0.54(5)	<0.001	9.2
UF2 Retentate	195.93±7.68(5)	NS	10.43±0.41(5)	<0.001	5.1

TABLE VI. The cytotoxic impact of *G. robertianum* aqueous alcoholic extract processed by MF-UF on Hep-2p tumors cells and RM normal cells; MF = microfiltrate extract, ES = standard deviation; NS = no significant

Sample	Number of alive cells		Number of dead cells		Cytotoxicity, %
	$(X \pm ES) \times 10^{-3}$	p	$(X \pm ES) \times 10^{-3}$	p	
Hep-2p cell					
Control	318.70±10.10(5)	–	6.30±0.20(5)	–	1.9
MF	251.60±7.63(5)	<0.001	71.70±2.17(5)	<0.001	22.2
HA	239.66±3.85(5)	<0.001	15.58±0.25(5)	<0.001	6.1
UF1 Permeate	247.61±3.61(5)	<0.001	19.77±0.29(5)	<0.001	7.4
UF1 Retentate	92.73±1,70(5)	<0.001	32.37±0.60(5)	<0.001	25.9
UF2 Retentate	167.66±2.73(5)	<0.001	41.49±0.67(5)	<0.001	19.8
RM cell					
Control	214.15±11.72 (5)	–	6.87±0.38 (5)	–	1.9
HA	157.42±7.24 (5)	<0.002	53.47±2.46 (5)	<0.001	25.4
MF	182.18±3.91 (5)	<0.02	15.96±0.34 (5)	<0.001	8.1
UF1 Permeate	195.55±3.92 (5)	NS	9.73±0.20 (5)	<0.001	4.7
UF1 Retentate	118.44±1.79 (5)	<0.001	34.06±0.51 (5)	<0.001	22.3
UF2 Retentate	185.30±3.40 (5)	<0.02	10.75±0.20 (5)	<0.001	5.5

The concentrated aqueous alcoholic extract from *G. robertianum* was the most cytotoxic on Hep-2 cell lines, 19.8 % cytotoxicity for the UF2 retentate and 25.9 % cytotoxicity for the UF1 retentate, while the cytotoxicity of the concentrated aqueous extracts from *G. robertianum* were 32.5 % for the UF1 retentate and 5.7 % for UF2 retentate.

Comparison of the cytotoxicity of *S. officinale* and *G. robertianum* may lead to the conclusion that this effect is mainly related to their phenolic compounds.

The cytotoxic effect of the comfrey extract concentrate may also be associated with the toxic metal level (Pb and Ni).

This information helps to establish modern complementary and alternative medicine treatment methods, which may offer efficient cures to large populations suffering from different diseases, including cancer.

CONCLUSIONS

This study showed that it is possible to obtain concentrated comfrey and herb Robert extracts with high antioxidant activity by membrane processes.

Moreover, the *Geranium* aqueous and aqueous alcoholic extracts, both purified and concentrated by membrane processes, and the concentrated *Symphytum* aqueous alcoholic extract diminished the viability of tumor cells. This proved that these extracts had a moderate cytotoxic potential.

The present study evidenced that the heavy metal and minerals contents of the selected medicinal plants were within safe limits. Thus, it was proven that the health of the human body is unlikely to be affected by the extracts of these medicinal plants containing such low contents of heavy metals.

It should be stressed that, hitherto, no reports on the microelements and cytotoxic effect of extracts of *S. officinale* and *G. robertianum* exist in the available literature.

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ИЗВОД

ПРИМЕНА МЕМБРАНСКОГ ПРОЦЕСА ЗА КОНЦЕНТРОВАЊЕ ЕКСТРАКАТА БИЉАКА *Symphytum officinale* И *Geranium robertianum* РАДИ ДОБИЈАЊА ЈЕДИЊЕЊА ВЕЛИКЕ АНТИОКСИДАТИВНЕ АКТИВНОСТИ

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У раду је описана примена мембранског процеса за добијање екстраката једињења велике антиоксидативне активности и могуће примене у терапији. Испитивана су фенолна једињења биљних врста које се користе у румунској етномедицини и утврђена је њихова антиоксидативна и цитотоксична активност. Екстракти добијени из *Geranium robertianum* и *Symphytum officinale* су концентровани микрофилтрацијом и ултрафилтрацијом. Концентрације фенолних једињења и флавоноида су одређене UV–Vis спектроскопијом и методом HPLC. Способност хватања слободних радикала концентрованих екстраката утврђена је методом DPPH. Прелиминарни тестови цитотоксичности изведени су на ћелијској линији епидермоидног карцинома (Hep-2p) и на нормалним буб-

режним ћелијама мајмуна. Резултати су показали да концентровани екстракти испољавају ниску цитотоксичност спрам здравих ћелија, док је цитотоксичност спрам Hep-2p туморских ћелија значајна. Концентровани екстракти су испољили и велику антиоксидативну активност (DPPH инхибиција је преко 80 %).

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