Application of the Nanofiltration Process for Concentration of Polyphenolic Compounds from *Geranium robertianum* and *Salvia officinalis* Extracts

G. Paun,[a,*] E. Neagu,[a] A. Tache,[a] G. L. Radu,[b] and V. Parvulescu[c]

[a]National Institute for Research-Development of Biological Sciences –
Centre of Bioanalysis, 296 Spl.Independentei,
PO Box 17-16, 060031, Bucharest 6, Romania

[b]Politehnica University of Bucharest, Faculty of Applied Chemistry
and Materials Science, 313 Spl.Independentei,
060042, Bucharest, Romania

[c]Institute of Physical Chemistry, Splaiul Independetei 202,
060021 Bucharest, Romania

The aim of this study was to prove the efficiency of the nanofiltration process for the concentration of polyphenolic compounds from *Geranium robertianum* and *Salvia officinalis* extracts and to evaluate the extract's antioxidant activity. A lab-scale cross-flow set-up using flat-sheet configuration membrane was employed for all experiments. Two nanofiltration membranes have been used: SelRO MPF-36 (Koch membrane) and an organic-inorganic membrane (polysulfone with SBA-15-NH$_2$). When the organic-inorganic membranes were used in the nanofiltration process, the obtained concentrated extracts proved to have higher polyphenol and flavonoid rejections, in both cases (*Geranium robertianum* and *Salvia officinalis*). The obtained values were over 88 % DPPH inhibition, for concentrated extracts, using the DPPH method. The concentrated extracts obtained after nanofiltration NF2 (organic-inorganic membrane) had the strongest scavenging activity for all extracts and almost completely inhibited DPPH absorption (92.9 % for *Geranium robertianum* concentrated extract and 90.1 % for *Salvia officinalis* concentrated extract). These features turn the studied, concentrated extracts into a good source for further medicinal applications.

Key words:

Geranium robertianum, *Salvia officinalis*, ultrafiltration, nanofiltration, organic-inorganic membrane

Introduction

In the last decade, there has been a great interest in the development of new extraction and separation techniques, especially in regards to the natural compounds with biological activity and potential benefits for human health.[1,2] Highlighting of the autochthonous, vegetal extracts obtained from *Geranium robertianum* and *Salvia officinalis* is justified by their use in traditional medicine for the treatment of human and animal diseases.

The *Geranium* genus phytochemistry is relatively well-known, the most studied classes of the active principles being tannins, volatile oils, flavonoids and polyphenols (hyperoside, ellagic acid, isoquercitrin, quercitrine, kaempferols, caftaric acid, ruthoside).[3,4] The phenol compounds, especially the flavonoids from *Geranium* spp. were re-

ported to exhibit antiviral, antitumor, hepatoprotective, anti-inflammatory, anticancer and immune stimulant effects.[5,6]

Salvia officinalis L. (sage) is a well-known medicinal plant, widely cultivated for its use in traditional medicine. Recently, the curing effects of sage have been assigned to the relatively large amounts of low molecular-mass compounds contained in the herb (phenolic acids, phenolic glycosides, diterpenoids, flavonoids). Many of these compounds possess a variety of biological activities, including the anti-oxidative, antitumoral and antiviral ones.[7,8]

The used traditional approaches to concentrate the bioactive compounds extracted from natural products include simple steam and vacuum distillation, generally at high temperature and high energy consumption. The former is inappropriate for heat-sensitive products. These methods may also result in low molecular mass compounds loss, as these could be removed within the solvent during

---
*Corresponding author: e-mail: gpaunroman@gmail.com;
tel./fax: 021-2200900

evaporation. The separation and concentration of biologically active compounds from the liquid media, through membrane techniques, offer a new approach in processing herb extracts. When applying membrane techniques, improved separation, purification and concentration of a certain compound is accomplished in a single phase at ambient temperature, without interference of other chemically reactive agents, as compared to traditional methods of separation and concentration of the biologically active compounds.

Although purification by microfiltration and concentration by ultrafiltration used in the industrial production of fruit and vegetable juices have been extensively investigated, in the past three decades only a few studies of these techniques' application in natural product purification and concentration have appeared in literature.[9–15]

The molecular mass cut-off (MMCO) of nanofiltration (NF) membrane (150–1000 Da) and pore size of 1 nm lie within the range between reverse osmosis (RO) and ultrafiltration (UF). NF appears to be a potential industrial scale method in these fields.

No literature is readily available on the performance of NF membranes, in separating and concentrating antioxidant compounds from *G. robertianum* and *S. officinalis*. The scope of this work includes evaluation of the nanofiltration processes' capability to concentrate the aqueous extracts from *Geranium robertianum* (Herb Robert) and *Salvia officinalis* (Sage) and testing a new organic-inorganic membrane. This is also a study of the antioxidative activity of the resulting retentates.

Experimental procedure

Preparation and concentration of extracts

The extracts were prepared by maceration, using cold, distilled water as solvent. The herbal was ground into powder using mill equipment; the contact time between the herbal and the solvent was 24 hours, with sporadic, mechanical stirring. The herbal's mass concentration in the solvent was 60 g L^{-1}.

The extracts were successively filtered through Whatman 1 (Medium-fast) filter paper and microfiltered through a 0.45 μm pore size membrane (Millipore), in order to remove any fine solid particles, which could initiate membrane fouling during ultra- and nanofiltration. Concentration experiments were also carried out on a two-stage membrane filtration set. First, the microfiltration extracts were treated by UF (membrane with cut-off 10,000 Da; Millipore), then, the UF permeate was fed and further treated by NF membrane. Each of the flat-sheet membranes used in the experiment had an effective area of 0.0028 m^2.

Membrane preparation

The membranes were prepared by the phase inversion method, using water as nonsolvent.[16] Polysulfone (PSF; M$_n$ 22000, Sigma-Aldrich Chemical Company, Inc.) was used as a membrane material and polyvinyl pyrrolidone K90 (PVP; Fluka) as an additive to make the membrane more porous. PSF and PVP powder were dissolved by stirring in N,N-dimethylformamid (DMF; Merck) to form a casting solution prepared from PSF 210 g L^{-1} and PVP 20 g L^{-1}. Organic-inorganic membranes were prepared by dispersion of SBA-15-NH$_2$ γ = 5 g L^{-1} into PSF solution γ = 210 g L^{-1} with PVP γ = 20 g L^{-1}. The polymer solution was then applied as a film with a "doctor blade " knife, followed by the polymer precipitation in the water coagulation bath. After precipitation, the membranes were kept in a water bath for 24 h and then washed with deionized water, before further experiments.

Molecular mass cut-offs of organic-inorganic membranes sample were measured using polyethylene glycols (PEG; Fluka) (MW 600–1500 Da) at a concentration of 10 mg L^{-1}. The concentrations of polyethylene glycol were measured by means of BaCl$_2$ (Roth, Germany) colorimetry.[17]

The surface and cross-section morphology were observed under SEM (EVO, Carl Zeiss Nano Technology Systems).

FTIR assay was performed using the FTIR-VAR technique with a beam incidence angle of 45°, on a Bruker TENSOR 27 instrument, using the OPUS software version 6.0. The samples were used without pre-treatment, as whole pieces fixed on a gold mirror and all the spectra were registered against a background of clean gold foil between 600 and 4000 cm^{-1}. The spectral resolution was 4 cm^{-1}, and the co-added scans 64, with an aperture of 6 nm.

Experimental set-up and procedure

The experiments were carried out in a KMS Laboratory Cell CF-1 type cross-flow lab-scale filtration unit. A schematic of the cross-flow equipment is presented in Fig. 1.

All experiments were run in a batch concentration mode, with the concentrate recycled to the feed tank.
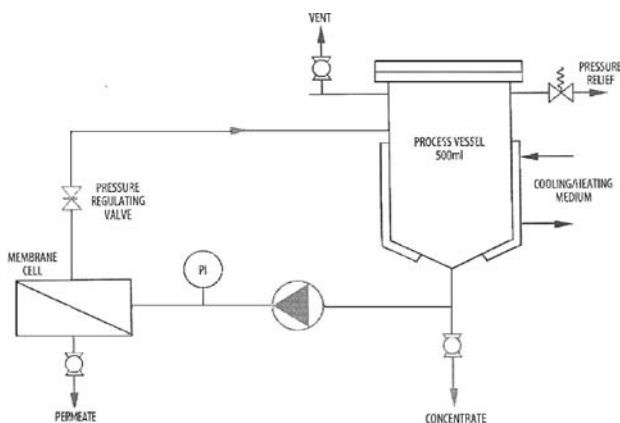
F i g . 1 – *KMS Laboratory Cell CF-1 experimental setup*

The effect of the nanofiltration process is usually measured by permeate flux and rejection rate. The flux is expressed in eq. (1)

$$J = \frac{V_p}{A \cdot t} \ (\text{L m}^{-2}\ \text{h}^{-1}) \qquad (1)$$

in which: V_p is the permeate volume (L), A is the effective membrane area (m^2), t is the time (h) necessary for the V liters of permeate to be collected. We counted the hours required for 100 mL of permeate to be collected and then calculated the flux.

The rejection R, was calculated using eq. (2) where γ_f and γ_p are the polyphenols/flavonoids concentrations in the feed and permeate:

$$R = (1 - \gamma_P/\gamma_f) \cdot 100 \ (\%) \qquad (2)$$

Several parameters were calculated in order to evaluate the performance of polyphenols/flavonoids recovery (in concentrate) for each tested membrane. The polyphenols/flavonoids percentage of recovery by mass balance to concentrate:

$$\% \text{ of recovery} = \left(\frac{\gamma_r \cdot V_r}{\gamma_f \cdot V_f} \right) \cdot 100 \qquad (3)$$

The polyphenols/flavonoids percentage of loss to permeate:

$$\% \text{ of loss} = \left(\frac{\gamma_p \cdot V_p}{\gamma_f \cdot V_f} \right) \cdot 100 \qquad (4)$$

where: γ_r is the polyphenols/flavonoids concentrations in the generated concentrate; V_p is the permeate volume obtained (L); V_f is the initial feeding volume (L); V_c is the concentrate volume obtained (L); the signification of γ_f and γ_p is the same as described in eq. 2.

Extract analysis

Phytochemical screening. Preliminary phytochemical screening was performed using the standard procedures.[18,19]

Total polyphenols and flavonoids assessment. The phenolic total content was determined by the Folin–Ciocalteu method.[20] Gallic acid (GAE) was used to calibrate the standard curve; total polyphenols contents were obtained from the regression equation of the calibration curve of gallic acid ($y = 0.0036x + 0.0203$, $R^2 = 0.9954$) and expressed as gallic acid (GAE) equivalent.

The total flavonoid content was determined according to the aluminium chloride colorimetric method with slight modifications.[21] Quercetin was used as standard, and results were expressed as microgram quercetin equivalents (QE) per mL. Total flavonoid contents were obtained from the regression equation of the calibration curve of quercetin ($y = 0.00989x + 0.01975$, $R^2 = 0.9977$).

Folin-Ciocalteu reagent and gallic acid were purchased from Sigma-Aldrich, whilst aluminium chloride and quercetin were purchased from Fluka.

HPLC analysis of extracts for phenolic acids and flavones was performed with a Shimadzu (Shimadzu Corp.) system equipped with a binary pump (LC-20Adsp), thermostat column with CTO-20AC and a diode-array detector (DAD: SPD-M20A). Spectral data for all peaks were recorded in the range 220–800 nm. Samples were injected at ambient temperature (20 °C) into a reverse-phase KROMASIL C$_{18}$ column, 4.6 x 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 solvents 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. The HPLC-grade solvents and the phenolic acids were purchased from Fluka (except gallic acid).

Antioxidant activity determination

The free radical scavenging activity of the feed (extracts), permeate and retentate was studied by DPPH method – based on the decrease of the 2,2-diphenyl-1-picrylhydrazyl (DPPH; Sigma-Aldrich) maximum absorbance at 519 nm in antioxidant presence.[22–24] DPPH$^•$ is a stable radical having a maximum absorbance at 519 nm. It can readily

undergo reduction by an antioxidant (AH), which runs as the following reaction:

$$DPPH\cdot + AH \rightarrow DPPH\text{-}H + A\cdot$$

The decreasing of the DPPH radical absorption by the action of antioxidants could be used for measuring the antioxidative activity.

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

$$\% \text{ inhibition} = [(A_0 - A_s)/A_0] \cdot 100 \qquad (5)$$

where: A_0 = blank absorbance; A_s = sample absorbance.

Statistical Analysis: The measurements were performed in triplicate and for statistical processing Excel 2007 was used, standard deviation (STDV) was < 10 %.

Results and discussion

The microfiltration (MF) process was meant to perform feed clarification and sterilization, while the ultra- (UF) and nanofiltration (NF) processes were meant for the concentration of bioactive compounds from *Geranium robertianum* and *Salvia officinalis* extracts.

Fig. 2 shows the morphology of the organic-inorganic membranes. SEM analysis of the cross-section of these organic-inorganic membranes provides information about the film thickness and the dispersion of the filler in the polymer matrix. The membranes obtained by coagulation in distilled water show denser morphology with smaller and fewer macrovoids in the sub-layer.

The retention performance of nanofiltration membranes is usually characterized by the molecular mass cut-off (MMCO), which is defined as the molecular mass of an uncharged solute with a rejection of 90 %.[25]

The estimated MMCO value was 1000 Da for organic-inorganic membrane, based on the experimental data.

The results of flux measurements of the NF membranes can be seen in Figs. 3 and 4. At the beginning of filtration, the permeate flux decreases sharply, as expected. Comparing the two NF membranes under the same pressure (10 bar), the Koch membrane (NF1) (43.3 L m^{-2} h^{-1} for Herb Robert extract, and respectively 47 L m^{-2} h^{-1} for sage extract) shows higher flux than organic-inorganic membrane (NF2) (19.4 L m^{-2} h^{-1} for Herb Robert extract and respectively 26.9 L m^{-2} h^{-1} for sage extract).

The loss of permeate flux in cross-flow ultra-filtration is caused by concentration polarization
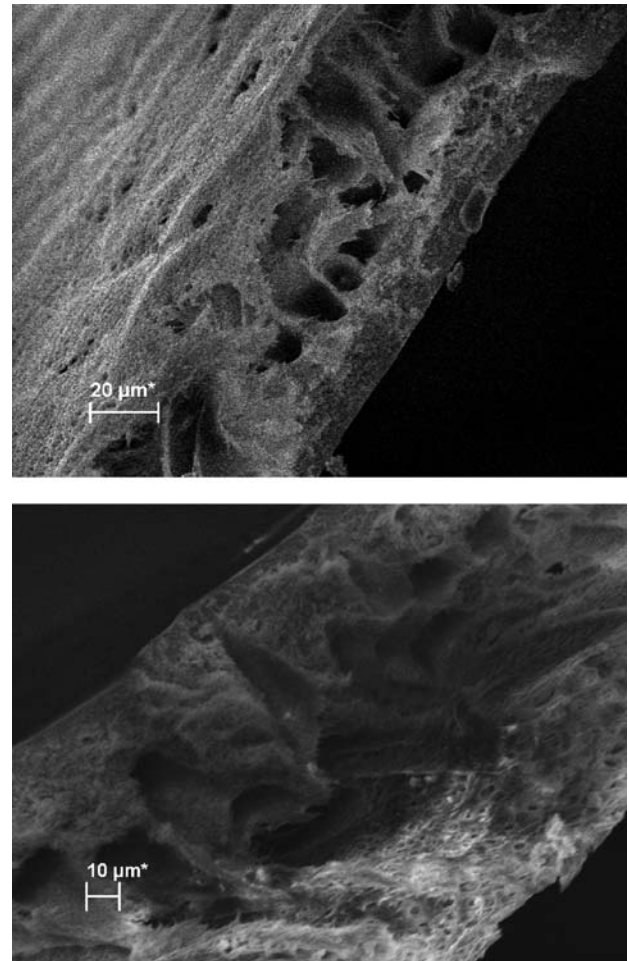
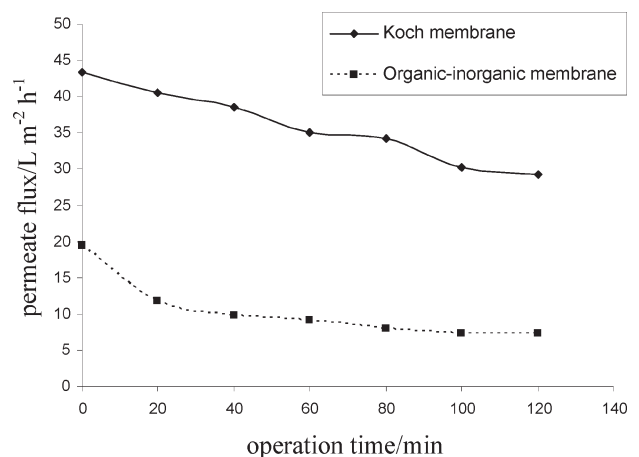F i g . 2 – *Morphology of nanofiltration organic-inorganic membranes from SEM*

F i g . 3 – *Comparison of G. robertianum extract flux values at different NF membranes vs. time*

and membrane fouling. The NF membrane fouling is related to the MMCO of UF membranes utilized at the previous step. We can reduce significantly the concentration polarization and the cake layer formed on the membrane using the UF membranes with MMCO 5,000 or 3,000.
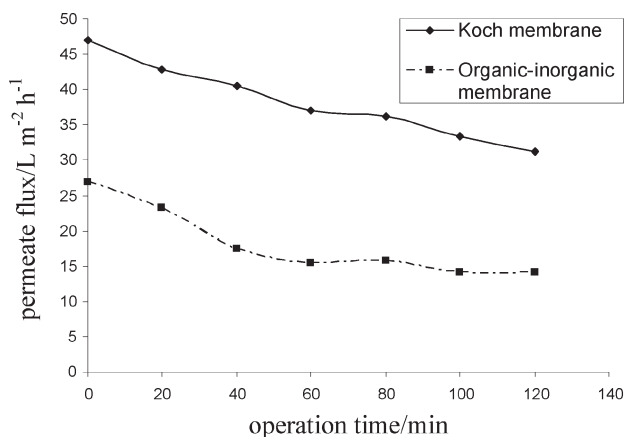
F i g . 4 – *Comparison of S. officinalis extract flux values at different NF membranes vs. time*

The phytochemical screening of the studied extracts (initial, after MF, UF and NF process) showed the presence of flavonoids, reducing sugars, terpenoids, saponins and aminoacids (Table 1). Other compounds like alkaloids and tannins were present in the extracts in trace amounts.

The data (Table 1) shows that all components from microfiltrate will gather as concentrated components in the nanofiltrate's retentate (NF1 and NF2), while in permeate they will remain in a very low concentration.

So far, as the phenolics represent one of the major groups of compounds acting as primary antioxidant or free radical terminator, it was reasonable to determine their total amount in the selected plant extracts. Total polyphenol and flavonoid contents were determined in permeate and retentate, after ultrafiltration process and also determined in retentate, after each nanofiltration process (Table 2).

T a b l e 1 – *Summary of phytochemical screening compounds*

Constituents	Tests	Geranium robertianum extract					Salvia officinalis extract				
		MF	UF		NF1	NF2	MF	UF		NF1	NF2
			P	R	R	R		P	R	R	R
Alkaloids	Dragendorff's	–	–	–	–	–	+	–	+	–	–
Tannins	Ferric chloride 0.1 %	++	+	++	++	++	+	±	+	+	+
Flavonoids	a) Alcalin (NaOH 10 %)	++	+	+	++	++	++	+	+	++	++
	b) Shinoda	++	+	+	++	++	++	+	+	++	++
Reducing sugars	Fehling	++	+	++	++	++	+	+	+	+	+
Polysaccharides	Molisch's	++	+	++	++	++	++	+	++	++	++
Terpenoides	Salkowski's	+	±	++	+	+	+	±	+	+	+
Saponins	Froth	+	+	+	+	+	+	+	+	+	+
Proteins	Xantoprotein's	+	–	++	–	–	++	±	++	++	++
Aminoacids	Ninhydrin 0.25 %	+	+	+	++	++	++	++	+	+	+

++ = Abundant; + = present; ± = poorly present; – = absent; P = permeate; R = retentate.

T a b l e 2 – *The results obtained on Geranium robertianum and Salvia officinalis extracts processed by ultrafiltration and nanofiltration*

Sample	Concentration of	Initial	Millipore membrane (UF)		Koch membrane (NF1)	Organic-inorganic membrane (NF2)
			Permeate	Retentate	Retentate	Retentate
Geranium robertianum extract	Polyphenols (mg GAE L^{-1})	1520±7.2	1270.3±10.3	1292.4±6.9	1449.6±8.6	1910.7±11.2
	Total flavonoids (mg QE L^{-1})	109.5±0.8	79.8±0.6	147.6±1.2	149.8±1.3	167.6±1.1
Salvia officinalis extract	Polyphenols (mg GAE L^{-1})	1106.6±8.6	815.14±5.1	999.2±7.2	1265.3±8.3	1343.7±8.7
	Total flavonoids (mg QE L^{-1})	130.6±1.1	85.3±0.5	160.5±1.4	162.7±1.5	193.1±1.7

The values are the means ± standard deviation (SD).

According to HPLC-DAD experiment, the main phenolic acids and flavonoids were found to be ferulic acid, caffeic acid, coumaric acid, rutin, quercetin, kaempferol in the *Geranium robertianum* extract and retentates and gallic acid, chlorogenic acid, coumaric acid, ferulic acid, rosmarinic acid in the *Salvia officinalis* extract and retentates, respectively. The highest concentrations of all phenolic acids in *Geranium robertianum* and *Salvia officinalis* retentates were obtained after nanofiltration with organic-inorganic membrane.

Table 3 shows the rejection of polyphenols and flavonoids, calculated with eq. (2). As can be seen from the results, the rejection ratios are higher for organic-inorganic membrane, but this is at the expense of lower flux (approximately 57 % for *S. officinalis* extract and 29 % for *G. robertianum* extract), as compared to Koch membrane. A similar result for biological compounds rejection values has been reported in literature.[26,27]

As shown in Table 4, for both NF membranes tested, recovery of polyphenols ranged between 45.6 % and 65.9 %. In the case of flavonoid compounds, the recovery was up to 90.5 %.

The nanofiltration technology is very efficient in accumulating flavonoid compounds in the final retentate, as seen by the high recovery yields (75.1 – 90.5 %) for both NF membranes tested.

The FTIR spectrum of the membrane before nanofiltration displays sharp peaks, typical for polysulfone membrane at 1018 cm^{-1} (stretching of aryl ether group), 1493, 1509 and 1686 cm^{-1} (aromatic ring stretch), 1157 and 1176 cm^{-1} (stretching of sulfonate group) and a sharp peak at 1259 cm^{-1} was due to N–H stretch (NH$_2$ group from SBA-15-NH$_2$), as can be seen from Fig. 5.

Only less intense peaks remain after nanofiltration, as a consequence of cake formation, covering the membrane surface. This layer acts as a barrier leading to lower flux and improved rejection.

The scavenging potential of concentrated extracts by nanofiltration was compared with known, standard antioxidants, such as Trolox (Fig. 6).

T a b l e   3 – *Rejection of total phenols and total flavonoids*[a]

Membrane type	Rejection, $R(\%)$			
	Total polyphenols		Total flavonoids	
	Geranium robertianum extract	*Salvia officinalis* extract	*Geranium robertianum* extract	*Salvia officinalis* extract
UF Millipore membrane (MMCO 10,000 Da)	16.4	26.3	27.1	34.4
NF Koch membrane (MMCO 1,000 Da)	70.4	70.1	77.3	80.4
NF Organic-inorganic membrane (MMCO 1,000 Da)	85.5	78.1	85.9	83.6

[a] Feed concentration: 1270.3±10.3 (mg GAE L^{-1}) for total phenols and 79.8±0.6 (mg QE L^{-1}) for flavonoids for Geranium robertianum extract; 815.14±5.1 (mg GAE L^{-1}) for total phenols and 85.3±0.7 (mg QE L^{-1}) for flavonoids for Sage extract (average values of three samples ± standard deviation).

T a b l e   4 – *Results of polyphenols recovery (%) by NF membranes*

V_r (mL)	γ_r (mg GAE L^{-1})	V_p (mL)	γ_p (mg GAE L^{-1})	% of loss	% of recovery
UF permeate *Geranium robertianum* extract generated by UF					
a) NF Koch membrane (NF1)					
100	1449.6	150	376.1	17.7	45.6
b) NF organic-inorganic membrane (NF2)					
100	1910.7	150	184.1	8.7	60.2
UF permeate *Salvia officinalis* extract generated by UF					
a) NF Koch membrane (NF1)					
100	1265.3	150	243.0	17.8	62.1
b) NF organic-inorganic membrane (NF2)					
100	1343.7	150	178.1	13.1	65.9

T a b l e 5 – *Results of flavonoids recovery (%) by NF membranes*

| V_r (mL)	γ_r (mg QE L^{-1})	V_p (mL)	γ_p (mg QE L^{-1})	% of loss	% of recovery
UF permeate *Geranium robertianum* extract generated by UF					
a) NF Koch membrane (NF1)					
100	149.8	150	15.6	13.8	75.1
b) NF organic-inorganic membrane (NF2)					
100	167.6	150	13.0	9.7	84
UF permeate *Salvia officinalis* extract generated by UF					
a) NF Koch membrane (NF1)					
100	162.7	150	16.7	11.7	76.3
b) NF organic-inorganic membrane (NF2)					
100	193.1	150	13.3	9.4	90.5

The values are the means
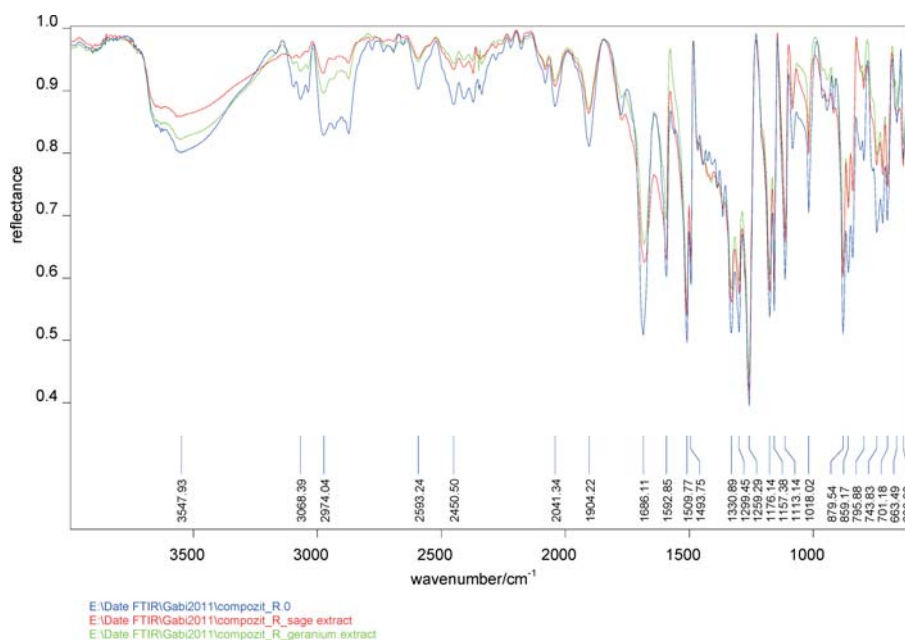
F i g . 5 – *FTIR-spectra of organic-inorganic membrane before and after nanofiltration*

The values obtained by the DPPH method were over 88 % DPPH inhibition for concentrated extracts. The concentrated extracts obtained after nanofiltration NF2 (organic-inorganic membrane) had the strongest scavenging activity for all extracts and almost completely inhibited DPPH absorption (92.9 % for *Geranium robertianum* concentrated extract and 90.1 % for *Salvia officinalis* concentrated extract).

The main role of the phenolic compounds as scavengers of free radicals is emphasized in our several reports.[10] Furthermore, the results are similar to other literature reports.[28–30] It can be observed that the content of polyphenols in the extracts correlates with their antiradical activity, proving that phenolic compounds are likely to contribute to the radical scavenging activity of these plant extracts. These results are in
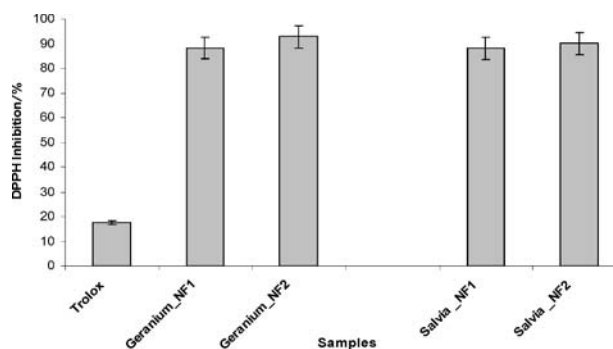

F i g . 6 – *Comparison of DPPH radical scavenging activity of the concentrated extracts and those of Trolox*

accordance to the obtained results for the higher rejection of polyphenols and flavonoids (Table 3).

For an almost complete retention of polyphenolic compounds, a membrane with MMCO <400 Da should be selected. Our aim was to test a new organic-inorganic nanofiltration membrane and to obtain concentrated extracts with antioxidant compounds, using membrane processes.

These results proved the ultra- and nanofiltration processes efficiency, in case of obtaining purified and concentrated extracts of *Geranium robertianum* and *Salvia officinalis*, with a very high antioxidative activity.

Conclusion

These results show that ultrafiltration and nanofiltration represent attractive, alternative processes for producing concentrated medicinal plant extracts, at low temperatures.

For both membranes, the rejection ratios for polyphenols and flavonoids were over 70 %. The rejection ratios are higher for organic-inorganic membrane, but this is at the expense of lower flux (approximately 57 % for *S. officinalis* extract and 29 % for *G. robertianum* extract), as compared to Koch membrane.

The results of the present study indicate that the concentrated extracts, obtained by nanofiltration through the organic-inorganic membrane, provide the highest antioxidant activity, therefore can serve as natural sources to develop free radical scavengers and antioxidant agents.

List of symbols

MMCO – molecular mass cut-off

MF   – microfiltration

UF   – ultrafiltration

NF   – nanofiltration

GAE – gallic acid

QE   – quercetin

J – permeate flux, L m^{-2} h^{-1}

V_p – volume of permeate, L

A – effective membrane area, m^2

t – time, h

R – rejection, %

γ_f – polyphenols/flavonoids concentrations in feed, mg L^{-1}

γ_p – polyphenols/flavonoids concentrations in permeate, mg L^{-1}

γ_r – polyphenols/flavonoids concentrations in concentrate, mg L^{-1}

V_f – volume of extract in the feeding, L

V_c – volume of concentrate, L

γ – mass concentration, g L^{-1}

References

1. *Naczak, M., Shahidi, F.*, J. Pharm. Biomed. Anal. **41** (2006) 1523.
2. *Cuvelier, M. E., Richard, H., Berset, C.*, J. Am. Oil Chem. Soc. **73** (5) (1996) 645.
3. *Fodorea, C. S., Vlase, L., Suciu, S., Tamas, M., Leucuta S. E.*, Rev. Med. Chir. Soc. Med. Nat. Iasi **109** (1) (2005) 174.
4. *Kobakhidze, K. B., Alaniya, M. D.*, Chem. of Nat. Comp. **40** (2004) 89.
5. *Shim, J. –U., Oh, P. –S., Lim, K. –T.*, J. Ethnopharmacol. **126** (2009) 90.
6. *Saija, A., Scalase, M., Lanza, M., Marzulla, D., Bonina, F., Castelli, F.*, Free Rad. Biol. and Med. **19** (4) (1995) 481.
7. *Lu, Y., Foo, L.*, Food Chemistry **75** (2001) 197.
8. *Lu, Y., Foo, L.*, Phytochemistry **59** (2002) 117.
9. *Vladisavljević, G. T., Vukosavljević, P., Bukvić, B.*, J. Food Eng. **60** (2003) 241.
10. *Paun Roman, G., Neagu, E., Radu, G. L.*, Rev. Chim.-Bucharest **61** (2010) 877.
11. *Sohrabi, M. R., Madaeni, S. S., Khosravi, M. , Ghaedi, A. M.*, Sep. Purif. Technol. **75** (2010) 121.
12. *Peshev, D., Peeva, L. G., Peev, G., Baptista, I. I. R., Boam, A. T.*, Chem. Eng. Res. Design **89** (2011) 318.
13. *Tzibranska, I., Seikova, I., Kochanov, R., Kancheva, V., Peev, G.*, Nanosci. Nanotechnol. **9** (2009) 196.
14. *Nawaz, H., Shi, J., Mittal G.S., Kakuda, Y.*, Sep. Purif. Technol. **48** (2006) 176.
15. *Qiao, X. L., Zhang, Z. J., Ping, Z. H.*, Sep. Purif. Technol. **56** (2007) 265.
16. *Paun Roman, G., Parvulescu, V., Radu, G.L., Su, B.-L.*, Rev. Chim.-Bucharest **58** (2007) 98.
17. *Sims, G. E. C., Snape, T. J.*, Anal. Biochem. **107** (1980) 60.
18. *Trease, G. E., Evans, W. C.*, Pharmacognosy, 13th edn. Bailliere Tindall, London, 1989.
19. *Harbone, J. B.*, Phytochemical Methods, London, Chapman and Hall Ltd, 1973.
20. *Singleton, V. L., Orthofer, R., Lamuela-Raventos, R.M.*, Meth. Enzymol. **299** (1999) 152.
21. *Lin, J. –Y, Tang, C. –Y*, Food Chem., **101** (2007) 140.
22. *Rice-Evans, C., Miller, N. J.*, Meth. Enzymol. **234** (1994) 279.
23. *Bondet, V., Brand-Williams, W., Berset, C.*, Lebensm. Wiss. U. Technol. **30** (1997) 609.
24. *Litescu, S., Radu, G. L.*, European Food Res. and Technol. Part A. **211** (2000) 218.
25. *Cooper A.R., Van Derveer, D.*, Sep. Sci. Technol. **14** (1979) 551.
26. *Mello, B. C. B. S., Petrus, J. C. C., Hubinger, M. D.*, J. Food Eng. **96** (2010) 533.
27. *Xu, L., Wang, S.*, Desalination **184** (2005) 305.
28. *Dorman, H. J. D., Kosar, M., Kahlos, K., Holm, Y., Hilturien, R.*, J. Agric. Food Chem. **51** (2003) 4563.
29. *Djeridane, A., Yousfi, M., Nadjemi, B., Vidal, N., Lesgards, J. F., Stocker, P.*, Eur. Food Res. Technol. **224** (2007) 801.
30. *Sokmen, M., Angelova, M., Krumova, E., Pashova, S., Ivancheva, S., Sokmen, A., Serkedjieva, J.*, Life Sciences **76** (2005) 2981.