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ANTIOXIDANT CAPACITY OF SOME *SALVIA OFFICINALIS* CONCENTRATED EXTRACTS

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Were obtained new extracts from *Salvia officinalis* – 8%, 10% and 15 % (mass concentration) hydro-alcoholic extracts in 50 % ethanol – which have been processed using an original developed procedure of extract separation and concentration based on porous membranes (ultrafiltration). The extracts were characterized in terms of total polyphenols', flavones' content determination and antioxidant capacity assesement. The antioxidant capacity was assessed by two spectrophotometrical methods: 2,2-diphenyl-picrlyhydrazyl (DPPH) and 2,2'- azinobis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS). A proportionality between the polyphenols', flavones' concentrations and antioxidant capacity was observed, the highest antioxidant activity being found in case of extracts in 50% ethanol with 10% plant mass. The obtained results evidenced that the applied membranous (ultrafiltration) procedures resulted in some concentrated *Salvia officinalis* extracts having a high antioxidant capacity (89.89 percent of DPPH inhibition).

INTRODUCTION

Salvia officinalis L – herbaceous, perennial plant of *Fam. Lamiaceae* – is a semi-shrub cultivated as aromatic, condiment, medicinal, melliferous and ornamental plant, provided from South Europe (Mediterranean area), where it grows in the spontaneous flora. The genus includes more than 900 species spread within the whole world.[1,2] In Roumania 13 species of the *Salvia* genus vegetate, while one of them, *S. transilvanica* Schur – is endemic in Roumania.[1]

Salvia officinalis L. was long time known as remedy in the traditional medicine.

The ethanol tinctures, decoctions from aerial plan parts, as well as the salvia essential oils are used in treatment of wide range diseases; not only heart, nervous and circulator system' disturbances, respiratory, digestive diseases, but also metabolic and endocrine deregulations. Also, the salvia preparations have many other therapeutic effects. [3,4]

The essential oils of *Salvia officinalis* have a high antibacterial activity against *Stapphyloccocus aureus, Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis,* cytotoxic activity against Vero cells and antiviral activity against herpes simplex 1 virus and virus of vesicular stomatitis.[5,6] The ethanol extract of *Salvia officinalis* has antifungal properties against cells of *Saccharomyces cerevisiae.*[7]

The plant phytochemical investigations revealed the existence of numerous bioactive compounds – about 160 kinds of polyphenols, flavonoide, having various biological activities.[8] The polysaccharides extracted from *Salvia officinalis* exhibit immuno-modulator and antitussive properties.[9]

The *Lamiaceae* family includes a large number of plants, well known for their antioxidant properties. Among these, salvia has been widely used and most of its antioxidant components have been identified. It has been established that the antioxidant effects of salvia are mainly due to the polyphenolic compounds.[10-12] The major phenolic

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compounds identified in the extracts of salvia are rosmarinic acid, carnosic acid, salvianolic acid and its derivates – carnosol, rosmanol, epirosmanol, rosmadial and methyl carnosate.[13-15] Among these, the rosmanol is a major constituent of many *Salvia* species having strong antioxidant activities because these groups cause phenols to donate more easily the hydrogen atoms to activate free radicals, which interrupt the antioxidation chain reaction.[16]

The methanol extracts of eight *Salvia* species from Turkey – *S. aethiopis, S. candidissima, S. limbata., S. microstegia, S. nemorosa, S. pachystachys, S. verticillata, S. virgata* – exhibited different levels of antioxidant activity in all models studies.[17] The results from DPPH free radical-scavenging system revealed that *Salvia* had significant antioxidant and free radical-scavenging so these plants, notably *S. verticillata* and *S. virgata,* can be used as natural antioxidants. A positive linear correlation between total phenolic content and antioxidant activity of the extracts was observed.[17]

The high antioxidant capacity correlated with a increased content of polyphenols was reported, on methanolic extracts, at two others sage species: *Salvia officinalis* and *Salvia fruticosa.*[18,19]

Were realized studies on leaf and roots extracts of *Salvia przewalskii*, *Salvia miltiorrhiza, Salvia verticillata*.[20] The antioxidant capacity of the studied species is high, but differences between species and organs have been also revealed. *Salvia przewalskii* leaf extract was the strongest one in all tests, followed by *Salvia miltiorrhiza* root and *Salvia verticillata* leaf. Among the roots, the most active was *S. miltiorrhiza* extract, followed by *S. verticillata*. The antioxidant activity correlates to the total polyphenol and, depending on the assay, to the hydroxycinnamic acids content. The high content of tanshinones in both *S. miltiorrhiza* and *S. przewalskii* roots is unlikely to contribute to the antioxidant activity.[20]

Membrane processes are modern techniques used for simple and efficient separation, purification and concentration of bioactive compounds from plant extracts. They presents several advantages: low cost, separation, purification and concentration of a specific compound in one phase, at cold, without the intervention of chemical reagents, the absence of phase changes, preserving the quality of preparations, the possibility of coupling with other conventional separation processes. [21]

The aim of the work was to obtain new extracts of *Salvia officinalis* using an originally developed procedure of extract separation and concentration (through 4 type of membranes) and to characterize the obtained extracts in terms of content (flavones and total phenolic content equivalent) and antioxidant capacity.

MATERIALS AND METHODS

Reagents

ABTS (2,2'- azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt, potassium persulphate, Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), DPPH (2,2-diphenyl-1-picrylhydrazyl), Folin-Ciocalteu reagent, phenol, sulphuric acid, ethanol, sodium acetate, aluminum chloride, methanol were provided from Sigma-Aldrich Chemical Co. firm, while other used reagents were of highest analytical purity.

Equipments

The vegetal material – leaves of sage (provided from a particular farmer from Roumania) was ground to a fine powder by means of GRINDOMIX GM200 mill. The spectrophotometric measurements were realized using a Jasco V 530 spectrophotometer.

Extract Preparing

The leaves of *Salvia officinalis* were finely ground using the GRINDOMIX GM200 mill. The extracts of 8%, 10% and 15% mass concentration were obtained through grinding in 50% ethanol at room temperature, during 7 days, under gentle mixing. The 8% aqueous extracts were obtained at room temperature through maceration, during 24 h.

Concentration of extracts

Firstly, the extracts were filtered, and then concentrated on UF ultrafiltration Millipore membranes of regenerated cellulose, with 10,000 Da, 5,000 Da and 3,000 Da cut–ff and on polysulphona PSF. A KMS Laboratory Cell CF-1 installation, Koch Membrane – Germany was used for ultrafiltration and the concentration ratio was of 2:1.

Determination of flavones' content was realized by spectrophotometry, using the method described in "Farmacopeea Romana" Xth Edition, using sodium acetate 100 g/L and aluminum chloride 25 g/L.[22]

Determination of polyphenols' content was realized by spectrophotometry at 760 nm wavelength using the Folin–Ciocalteu reactive.[23] The polyphenols' concentration in sample was calculated based on an etalon curve of cafeic acid 10-100 μg/mL.

Assessment of antioxidant capacity

The Trolox Equivalent Antioxidant Capacity (TEAC) was determined by using two methods:

One method was based on the decrease of the **DPPH** (2,2-diphenyl-1-picrylhydrazyl) maximum absorbance at 519 nm in the antioxidant presence.[24,25]

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 was based on the decrease of the **ABTS** (2,2'- azinobis (3-ethylbenzothiazoline 6-sulfonic acid) diammonium salt maximum absorbance at 731 nm in the antioxidant presence.[26]

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

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

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 triplicates and for statistical processing a Program Microsoft Office Excel 2007 was used, the standard deviation (STDV) was <15.

RESULTS AND DISCUSSION

The hydrodynamic characteristics of the work membranes were evaluated by calculation of the distillated water and permeate flows through such membranes: Millipore 10.000 Da, 5.000 Da, 3.000 Da and PSF membrane. The experiments were carried out at pressure of 2 bars, the results being presented in Table 1.

Table 1

Flow of permeate through the membrane

	Flux, L/M^{2}h*			
Membrane type	**Millipore 10.000 Da**	**Millipore 5.000 Da**	**Millipore 3.000 Da**	**PSF**
AD	230.5	140.3	80.2	121.5
8% water extract	142.3	72.8	32.3	69.8
8% hydro-alcoholic extract in 50% alcohol	155.3	74.9	41.2	71.3

***** The permeate flow through membrane was calculated using the formula: $I = \frac{V \times 10^{-3} \times 3600}{28 \times 10^{-4} \times t(s)}$

It was concluded that the permeate flow decreased proportionally to the decrease of the diameter of the ultrafiltration membrane pores, in the succession: 10.000 Da →5.000 Da → PSF → 3.000 Da. The hydro alcoholic extracts had higher flows than water ones and the distillated water had flows almost double to hydro-alcoholic extracts.

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, the correlation factor R = 0.9962 (Table 2).

It was concluded that in initial salvia extracts, the *flavones'* content was proportional to the solvent concentration and plant mass, the maximum was recorded for 15% hydro-alcoholic extract - mass concentration, in 50% alcohol; The most proper membrane to increase the flavones' concentration in extract was Milli 5.000 Da membrane (the ratio between the concentrate and permeate flavones' contents was of 5.6), followed by Milli 10.000 Da and the last PSF.

Table 2

Flavones' content in *Salvia officinalis* hydro-alcoholic extracts

	Flavones mg/mL						
Type of membrane		**Millipore 10000 Da**		**Millipore 5000 Da**		**PSF**	
Sample	**initial**	**permeate**	**concentrate**	**permeate**	**concentrat**	**permeate**	**concentrat**
50% hydro-alcoholic 8% mass extract	36.43	20.81	57.93	27.34	65.25	29.33	37.09
50% hydro-alcoholic 10% mass extract	41.08	32.97	90.042	28.05	75.54	43.45	42.83
50% hydro-alcoholic 15% mass extract	***78.47***	***44.449***	***101.228***	***23.22***	***130.17***	***54.91***	***62.75***

Extracts' contents in total polyphenols

They were spectrophotometrically determined at λ =760 nm, with Folin–Ciocalteu reagent.[17] The concentration of polyphenols in samples was estimated using an etalon curve for cafeic acid within a concentration range of 10-100 μg/mL, the correlation factor R = 0.9918. The experiment results are presented in Table 3.

In initial salvia extracts the polyphenols' content proportionally increased as increased the concentration of mass plant and solvent, the highest values being obtained for the extract 15% – mass plant, in alcohol 50%.

Assessment of antioxidant capacity

The antioxidant activity of *Salvia officinalis* extracts was determined through two spectrophotometry methods (with DPPH and ABTS), the results are shown in Table 4 and Fig. 1.

Table 3

Total polyphenols' contents in *Salvia officinalis* hydro-alcoholic extracts

	Polyphenols μg/ mL						
Type of membrane		**Millipore 10000 Da**		**Millipore 5000 Da**		**PSF**	
	initial	**permeate**	**concentrate**	**permeate**	**concentrate**	**permeate**	**concentrate**
50% hydro-alcoholic 8% mass extract	356.06	311.91	356.5	358.37	***406.62***	336.9	390.11
50% hydro-alcoholic 10% mass extract	355.39	325.95	357.02	333.87	480.39	355	363.82
50% hydro-alcoholic 15% mass extract	***392.47***	354.38	357.97	360.61	372.30	352.247	377.07

Table 4

Antioxidant activity of analyzed *Salvia officinalis* hydro-alcoholic extracts

Sample			**$TEAC_{DPPH}$ (μmol Trolox/g)**	**$TEAC_{ABTS}$ (μmol Trolox/g)**
Acid ascorbic			21.97	419.17
Hydro-alcoholic extract				
50% EtOH 8% mass extract	initial		34.65	544.89
	UF1	permeate	33.30	495.07
		concentrate	53.16	534.94
	UF2	permeate	52.31	425.76
		concentrate	59.49	509.05
	UF3	permeate	25.29	464.16
		concentrate	31.21	545.44

Table 4 (continued)

50% EtOH 10% mass extract	initial		50.11	627.82
	UF1	permeate	35.68	515.99
		concentrate	33.93	656.99
	UF2	permeate	35.37	515.00
		concentrate	49.93	659.64
	UF3	permeate	25.77	543.21
		concentrate	46.28	626.49
50% EtOH 15% mass extract	initial		58.93	600.00
	UF1	permeate	45.70	564.27
		concentrate	76.91	745.05
	UF2	permeate	61.14	409.94
		concentrate	61.43	439.94
	UF3	permeate	56.02	672.33
		concentrate	***68.45***	***813.28***

where: UF1– membranes with cut-off of 10.000 Da of regenerated cellulose (Millipore);
UF2 – membranes with cut-off of 5.000 Da of regenerated cellulose (Millipore);
UF3 – polysulphone membranes; Perm – permeate; Conc – concentrate

It was concluded that – through both used methods – the antioxidant activity of the concentrated extracts was higher than that of permeates, and the highest antioxidant capacity had the hydro-alcoholic extracts in 50% ethanol of 15 % plant mass. In such extracts, the highest flavones and polyphenols amounts were also determined, especially in those concentrated through the ultrafiltration membranes with cut-off of 10.000 Da (UF1) and the polysulphone those (UF3).

Were calculated % DPPH inhibition for all types of extracts obtained, results varied between 59.67% and 89.89%. The highest inhibition percentage of DPPH, too: 89.25% – UF1 and 89.89% – UF3 in case of same type of hydro-alcoholic extract (in 50% ethanol with 15% plant mass) was calculated (Fig. 1).

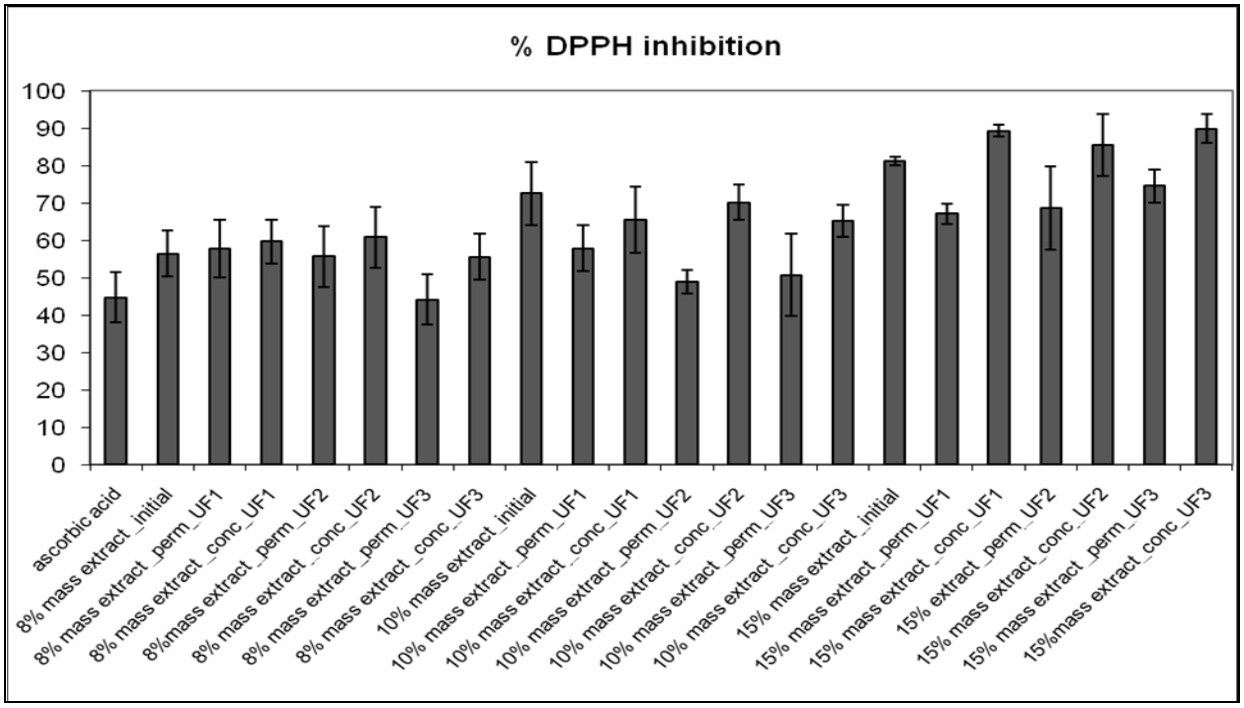

Fig. 1 – DPPH inhibition percent.

CONCLUSIONS

In the present study, 8%, 10% and 15% (mass concentration) hydro-alcoholic extracts in 50% ethanol of *Salvia officinalis* were realized through membranar – ultrafiltration procedures, by 3 types of PSF membranes and Millipore ultrafiltration membranes, of regenerated cellulose, with 10,000 Da and 5,000 Da cut-off. The concentration ratio was of 2:1.

In initial extracts, as well as in the permeates and concentrates, some biologic active principles were determined: flavones, total polyphenols. The content in flavones and polyphenols correlated with plant mass and with solvent concentration (the most proper in such case, was that of 50%). The highest amount of biologic active principles was found in extract 15% (mass concentration) in ethanol 50%.

The most adequate membrane to concentrate the biologic active principles was the Milli 5,000 Da membrane, followed by Milli 10,000Da, the last one being PSF.

The existence of a correlation between the polyphenols and flavones quantity and antioxidant activity was found. The highest antioxidant activity and the highest DPPH inhibition percent – above 89% – were determined in extracts with 50% ethanol and 15% (mass concentration) concentrated through membranes with cut-off of 10,000 Da (UF1) and polysulphone those (UF3). In such extract also the highest amount of flavones and total polyphenols was determined.

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