PHYTOCHEMICAL STUDY OF SOME ACTIVE PRINCIPLES WITH ANTIOXIDANT ACTION FROM THE *ROSMARINUS OFFICINALIS* AND *SALVIA OFFICINALIS* SPECIES

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Abstract: Many natural products have been reported to contain large amounts of antioxidants other than vitamin E, C, carotenoids (Javanmardi et al., 2003). These antioxidants play a role in delaying, intercepting, or preventing oxidative reaction (Vilioglu et al., 1998) catalysed by free radicals. This antioxidant activity may be mainly do to the present of phenolic components such as flavoinoids (Pietta,1998), phenolic acids and phenolic diterpenes (Shahidi et al., 1992). It is necessary to find out if medicinal plants could provide the antioxidant substances that may help to modulate oxidative stress who are associated with many pathological disorders.

This phytochemical study emphasized the existence of some compounds with antioxidant action flavon and poliphenol types in hydroalcoholic extracts of *Salvia officinalis* and *Rosmarinus ofiicinalis*.

INTRODUCTION

The term „antioxidant” is mainly used for a chemical compound consuming molecular oxygen.

Antioxidant agents are substances capable to protect the organism against destructions caused by free radicles.

They can be divided into: primary and secodary. Most of the antioxidants used are primary antioxidants. They are phenol compunds having various substitutes attached: phenol acids, flavonoids, antocianydins, lignans, tannins, coumarins. Secondary antioxidants include metallic complex agents, singlet oxygen and others (Larson R.A., 1997).

First details regarding the antioxidating activity of the medical plants appeared in the 50’s, when Chipault and Co. did a study on 72 medical plants, testing their antioxidant capacity (Chipault and Co,1956; Chipault and Co.,1952). Rosemary and sage proved to be the most efficient resources of antioxidants. These plants have been treated very carefuly lately. Various studies proved the efficiency of these plants and had as result different commercial applications (Braco and Co, 1981).

The active compounds from the sage and the rosemary, presenting antioxidant properties are the phenol acids, flavonoids, natural pigments (capsaicin and curcumin), and terpens (rosemanol, carnosol, carnosic acid, epirosemanol, isorosemanol) (Cuvelier and Co., 1994).

Chang *et al* studied the antioxidant properties of rosemary and sage extracts in a wider variety of solvents (hexane, benzene, ethyl ether, chloroform, dichlorethylene, dioxane and methanol) applied to lard stored at 60ºC in the dark. This time, peroxide value was determined and rosemary extracts in dichlorethylene, ethyl ether and methanol showed the lowest results. Wu *et al* confirmed the antioxidant efficiency of the rosemary (0,02%) methanolic extract in lard stored in the dark for 6, 14, 21, 28 and 36 days through peroxide value determination.

In this paper we proved the presence of the polyphenols and flavones, substances with antioxidant properties, in vegetal hydroalcoholic extractsobtained from the sage (*Salvia officinalis)*) and the rosemary (*Rosmarinus officinalis*).

MATERIAL AND METHODS

As raw materials, we used 2 species of medical plants, that is sage (*Salvia officinalis)* and rosemary (*Rosmarinus officinalis)*. The biological material comes from the spontaneous flora and was purchased in 2006 from SC A&C Network Ltd Brăila (rosemary) and from certified natural person in Tulcea (sage) and in that year they were also analysed.

From the two species of plants, we did more hydroalcoholic extracts obtained by processing the air part, varying the ethanolic concentration (40, 50 and 70%) as well as the report plant - solvent (1:7 and 1:10). The phytochemical analysis consisted of the assessment of the contents in polyphenols and flavones. For the polyphenols, the caffeic acid and the rosmarinic acid were dosed.

The determination of the contents in flavones was done using reactives: ethylic alcohol of 50^0; sodium acetate, sol.10%; aluminium chloride, sol. 2,5%; ruthenium (s.r.), sol. 0,1 g/l in ethylic alcohol of 50^0.

Determination completion

3-4ml for test are weighed in a 50 ml baloon and dilluted at the sign with ethylic alcohol of 50° (solution A). In a 25ml baloon we put 3ml of A solution, we add 5ml of sodium acetate solution and 3ml of aluminium chloride solution, stirring after each add. We dilute at the sign with ethylic alcohol of 50° and we stir.

Compensation solution: in a 25 ml balloon we put 3 ml of A solution, diluted up to the sign with ethylic alcohol of 50°.

After 15 minutes the absorbance of the analysed solution is measured at 430 nm, as compared to the compensation solution.

Determination of the contents in rosmarinic acid was done using the reactives: ethylic alcohol of 50^0; chloride acid of 0,5M; sodium hydroxid 1n; Arnow reactive: 10g sodium nitrite and 10g of sodium molybdate are dissolved in 100ml of distilled water.

Determination completion

1 -1-2 ml of fluid extract are weighed in a 50 ml balloon, then diluted up to the sign with ethylic alcohol of 50°(A solution).

2 -1ml of A solution is introduced in a 10 ml level test tube. We add 2 ml of chloride acid 0,5 M, 2 ml Arnow reactive, 2 ml of sodium hydroxid 1n, diluted with water up to 10ml and then we mix.

3 -Compensation solution: In another 10 ml level test tube we put: 1ml A solution, 2 ml chloride acid 0,5M, 2 ml sodium hydroxid 1n and diluted in water up to 10ml.

We immediately measure the absorbance of the analysed solution at 505 nm, as compared to the compensation solution.

RESULTS AND DISCUSSIONS

Quantitative phytochemical analysis for polyphenols and flavones at *Salvia officinalis*

From *Salvia officinalis,* air part, we did various types of hydroalcoholic extracts, using a plant: solvent report (ethylic alcohol) of 1:7 and 1:10 and three ethanolic concentrations: 40, 50 and 70%. The extracts were done both in hot and in cold (tinctures). The solutions were clear, redish brown colour, specific smell.

The ethanolic extracts obtained from the vegetal matter were analysed from a quantitative point of view for the assessment of the contents in polyphenols (expressed in caffeic and rosmarinic acids) and total flavones (expressed in rutozid). Details are found in tables 1 and 2.

Table no 1. Polyphenols and flavones content from hot extracts of *Salvia officinalis*

No.	Tests	Concentration EtOH	Polyphenols (g % s.u.)		Flavones rutozid, (g % s.u.)
			Caffeic acid	Rosmarinic acid	
1.	*Salvia officinalis* 1:7	40%	2,547	1,176	2,222
2.		50%	3,008	1,387	2,297
3.		70%	3,088	1,426	2,425
4.	*Salvia officinalis* 1:10	40%	1,902	0,878	0,488
5.		50%	1,936	0,893	0,512
6.		70%	2,087	0,963	0,681

Table no 2. Polyphenols and flavones content from cold extracts (tinctures) of *Salvia officinalis*

No.	Tests	Concentration EtOH	Polyphenols (g % s.u.)		Flavones rutozid, (g % s.u.)
			Caffeic acid	Rosmarinic acid	
1.	*Salvia officinalis* PLV (1:10)	40%	1,614	0,745	1,091
2.		50%	1,932	0,892	2,566
3.		70%	2,209	1,020	3,102
4.	*Salvia officinalis* PLV (1:7)	40%	2,001	0,924	2,047
5.		50%	2,320	1,071	2,829
6.		70%	2,777	1,283	3,111

The analysis of the results obtained in case of hot extracts (table 1), prove rather high quantities of poliphenols and flavones. There is a direct correlation between the concentration of the ethanolic extract and the quantity of the total polyphenols and flavones dosed in here.

Dosed flavones have values between 0,488 g% s.u. and 2,425 g% s.u. We can see that the lower percentage of flavones (0,488g% s.u.), belongs to the EtOH 40% and the plant: solvent report of 1:10, and the highest percentage in flavones (2,425g% s.u.), belongs to the EtOH 70% concentration and the plant: solvent report of 1:7.

Polyphenols dosed in hot extracts have values between 1,902 g% s.u. and 3,088 g% s.u., for the caffeic acid and between 0,878 g% s.u. and 1,426 g % s.u., for the rosmarinic acid. We see that the lowest percentage of polyphenols expressed in caffeic acid (1,902 g% s.u.) belongs to the 40% concentration and the plant: solvent report of 1:10, and the highest one (3,088 g% s.u.) to the 70% concentration and the plant: solvent report of 1:7. Also, the lowest percentage of polyphenols expressed in rosmarinic acid (0,878 g% s.u.) belongs to the 40% concentration and the plant: solvent report of 1:10, and the highest one (1,426 g% s.u.) to the 70% concentration and the plant: solvent report of 1:7.

Tinctures obtained from *Salvia officinalis*, have increased quantities of flavones and polyphenols, as compared to the extracts obtained in hot (table 2). This way, at the 70% concentration and the plant: solvent report of 1:7, the value for the polyphenols expressed in caffeic acid is of 2,777 g% s.u., and for the rosmarinic acid is of 1,283 g% s.u. At the same concentration (70%) for flavones we get a value of 3,111 g% s.u.

Phytochemical quantitative analysis at *Rosmarinus officinalis*

From *Rosmarinus officinalis* we did various types of vegetal extracts, using ethanolic concentrations of 40, 50 and 70% and a report between plant and solvent (ethylic alcohol) de 1:10. Extracts were done in hot and cold (tinctures – extraction time being of 8 days at room temperature). Solutions were clear, redish brown colour, specific smell.

The quantitative analyses were based on the polyphenol dosing, coffee and rosemary type and the flavones expressed in rutozid (tables 3 and 4).

Table no 3. Polyphenols and flavones content from hot extracts of *Rosmarinus officinalis*

No.	Tests	Concentration EtOH	Polyphenols (g % s.u.)		Flavones rutozid, (g % s.u.)
			Caffeic acid	Rosmarinic acid	
1.	*Rosmarinus officinalis* 1:10	40%	1,453	0,671	1,706
2.		50%	1,897	0,876	2,147
3.		70%	2,003	0,925	2,206

Table no 4. Polyphenols and flavones content from cold extracts (tinctures) of *Rosmarinus officinalis*

No.	Tests	Concentration EtOH	Polyphenols (g % s.u.)		Flavones rutozid, (g % s.u.)
			Caffeic acid	Rosmarinic acid	
1.	*Rosmarinus officinalis* 1:10	40%	2,599	1,200	1,304
2.		50%	2,859	1,320	1,429
3.		70%	3,227	1,490	1,700

For the ethanolic extracts obtained in cold, we see a quantitative variation of the polyphenols expressed in caffeic acid, from 1,453 g% s.u. (concentration EtOH 40%) to 2,003 g% s.u. (concentration EtOH 70%). To these extracts, the values of the rosmarinic acid contents are under 1 g% s.u., with the lowest value of 0,671 g% s.u. for the EtOH concentration of 40%, and of 0,925 g% s.u. for the EtOH concentration of 70%.

Flavones dosed in extracts have values between 1,706 g% s.u. (concentration EtOH 40%) up to 2,206 g% s.u. (concentration EtOH 70%). In the case of rosemary there is also a direct correlation between the concentration of the ethanolic solution and the quantity of active principles dosed in here; the highest quantities of polyphenols and flavones belong to the extract version 70%, the extraction output of this version being the highest one.

The tinctures obtained from R*osmarinus officinalis* have increased quantities of polyphenols expressed in caffeic and rosmarinic acids (Table 4), as compared to the extracts obtained in hot. For the caffeic acid, the values are between 2,599 g% s.u. and 3,277 g% s.u., and for the rosmarinic acid the values are between 1,200 g% s.u. and 1,490 g% s.u. The highest quantities of polyphenols belong to the concentration of 70%, the value for the polyphenols expressed in caffeic acid is of 3,227 g% s.u., and for the polyphenols expressed in rosmarinic acid of 1,490 g% s.u. A ligh decrease of the values for flavones at rosemary tinctures was observed, as compared to the extracts in hot, so that the minimum is of 1,304 g% s.u. and the maximum 1,700 g% s.u. At the same concentration (70%) for flavones, the highest value belongs to the extracts in hot (2,206 g% s.u.), as compared to only 1,700 g% s.u. at the tincture.

CONCLUSIONS

In case of hydroalcoholic sage extracts, the values for polyphenols and flavones range according to the ethanolic concentration and the report between solvent and vegetal matter. The minimum quantity of polyphenols espressed in caffeic acid (1,614g % s.u.) is also met in the cold extract of *Salvia officinalis* (40% ethanolic concentration and the plant: solvent report of 1:10), and the maximum (3,088 g% s.u.) is also met at the hot extract of sage (70% ethanolic concentration and the plant: solvent report of 1:7); the latter one is also characterized by the highest values for polyphenols expressed in rosmarinic acid (1,426 g% s.u.).

The maximum quantity of flavones (3,111 g% s.u.) is met in the cold sage extract (70% ethanolic concentration and the plant: solvent report of 1:7).

Tinctures obtained from *Rosmarinus officinalis* have higher quantities of polyphenols expressed in caffeic and rosmarinic acids, as compared to the hot extracts. The minimum quantity of polyphenols expressed in caffeic acid (1,453 g% s.u.) and in rosmarinic acid (0,671 g% s.u.), belong to the hot rosemary extract (ethanolic concentration of 40%), and the maximum quantity

of polyphenols expressed in caffeic acid (3,227 g% s.u.) and in rosmarinic acid (0,490 g%s.u.), belong to the cold rosemary extract (70% ethanolic concentration).

A slight decrease of the values was observed for the flavons, at the rosemary tinctures, as compared to the hot extracts, so that the minimum is of 1,304 g% s.u. in the hot extract (40% ethanolic concentration), and the maximum 2,206 g% s.u. in the cold extract (70% ethanolic concentration).

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