Evaluation of Antioxidant Activity and Phenolic Content in Different *Salvia officinalis* L. Extracts

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ABSTRACT

The use of medicinal plants to improve health is an ancient practice and in recent years it has been observed an increasing interest of scientific researchers for the study of plants with biological properties and active principles responsible for their therapeutic effects. *Salvia officinalis* L. is considered the queen of herbs and belongs to the *Lamiaceae* (*Labiatae*) family. Due to the increasing interest in plants health benefits, the aim of the present study was to characterize various extracts of Romanian sage regarding their content in compounds with antioxidant activity. Three different techniques and five solvents were used for extraction of bioactive compounds from *Salvia officinalis* L. The total phenolic content and the antioxidant activity of plant extract were determined by Folin-Ciocalteu method and respectively by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay. Methanolic extract exhibited the highest content in phenolic compound (1974.89 mg GAE/100g dw) as well ass the strongest antioxidant capacity (85.12%).

Keywords: *antioxidant capacity, extraction, phenolic content, Salvia officinalis.*

INTRODUCTION

Plenty of aromatic, medicinal and other herbs which contain biological active compounds are widely used in food and cosmetic industries, drug production, or as precursors in the synthesis of new products with specific bioactive properties. Spices and their alcoholic extracts belong to the most studied vegetal species because of their high antioxidant activity. In recent years, spice extracts have appeared on the market as antioxidants for food industry use (Pizzale *et al*., 2002).

Total phenolic content, antioxidant activity and antimicrobial activity of herbal extracts are of particular interest to food industry which is looking for plant extracts with significant antimicrobial activity to be used as alternatives to conventional food preservatives (Skotti *et al*., 2014). Regarding these beneficial effects and properties of plant, phenolics interest is considerably increasing in finding naturally occurring antioxidants from botanical sources, to be use in the food industry and in preventive medicine (to replace synthetic antioxidants that are being restricted due to their possible toxicity and carcinogenicity). (Bettaieb *et al*., 2011).

Sage (*Salvia officinalis* L.) is a common herbal plant widely cultivated in various parts of the world, but it is native to the Mediterranean region. It is an aromatic herb that is used extensively to add a distinctive aroma and flavor to food. Its

leaves contain essential oils and tannins that can help facilitate digestion with anticonvulsant, anti-fever, antiseptic and anti-diabetic effect. Modern evidence shows possible uses as an anti sweating, antibiotic, antifungal, astringent, antispasmodic, estrogenic, hypoglycemic and tonic agent. This plant is also used for the treatment of gout, chronic rheumatism, dizziness and headache. Investigations have been performed to use sage as a medication for Alzheimer's disease (Amirmohammadi *et al*., 2014). *Salvia officinalis* extract and rosmarinic acid can be considered as a potential therapeutic agents for treating diseases related to skin pigmentation. Rosmarinic acid is the major phenol compound of sage and relevant biological activities have been attributed to it (Oliveira *et al*., 2013). Recent findings also suggested that sage extract attenuates morphine-induced memory impairment (Gomar *et al*., 2014).

In the past few decades sage has been the subject of an intensive study for its phenolic content, especially due to strong antioxidant and antimicrobial properties of its extracts. Sage polyphenols comprise a great diversity of structures, ranging from rather simple molecules to complex polymers among which flavonoids and phenolic acids are usually distinguished (Dragovic-Uzelac *et al*., 2012). The leaves are reported to contain carnosolic acid, flavonoids including salvigenin, genkwanin, hispidulin, luteolin and its derivatives; phenolic acids including rosmarinic, caffeic, labiatic (Shamnas *et al*., 2014).

Extraction is an important step involved in the discovery of bioactive components from medicinal plants and different extraction methods have been used to extract polyphenolic compounds from plant materials (Murugan and Parimelazhagan, 2014).

Some extraction techniques, such as hydrodistillation, maceration, Soxhlet extraction, ultrasonic extraction, percolation, are widely used for obtaining extractable substances from different parts of a number of plants. Some specific applications require more sophisticated and costly extraction techniques using specialized equipment such as supercritical-fluid extraction (Balouiri *et al*., 2014).

For the extraction of polyphenols from plant material different solvent systems have been used, extraction yield being dependent on the solvent and the method of extraction. Water and aqueous mixtures of ethanol and methanol are commonly used in plant extraction. Roby *et al*., 2013 reported that methanol was the best solvent for extracting phenolic compounds, followed by ethanol, diethyl ether and hexane from thyme, sage and marjoram. In another work, aqueous ethanol was found to be a better solvent, for extracting sage polyphenols, than methanol. In addition, the contents of phenols and flavonoids as well as the antioxidant activity were greater in the extracts obtained by maceration than those obtained by sonication (Veličković et al., 2011).

In the present study several extraction techniques (maceration, hot extraction and sonication) and five extracting solvents of different polarity (S1- methanol, S2 - ethanol, S3 - aqueous methanol (80% v/v), S4 - diethyl ether and S5 – hexane) were used in order to assess their effiency in extracting the bioactive compounds from the leaves of *Salvia officinalis* L.

MATERIALS AND METHODS

Plant material

The plant material (aerial parts) was collected at the vegetative stage (23 March 2015) from the green-house of Phytotechny Department of UASVM. The leaves were air-dried, in a cool dark place. Then, they were packed in paper bags and kept in a dark, dry and cool place until analysis. Before use, dry leaves were crushed using a house blender.

Preparation of Extracts

Maceration

A ground dried sample of 0.5 g was weighted and then 15 ml of solvents with varying polarities (methanol, aqueous methanol, ethanol, diethyl ether and hexane) were added to the plant samples. Maceration was carried out at room temperature for 14 days, under continuous agitation. After filtration and washing the final volume was adjusted to 25 ml (Soran *et al*., 2009).

Sonication

Extraction was carried out in an Bandelin Sonorex ultrasonic bath (RK100H, Berlin, Germany). Flasks containing 0.5 g of air-dried and crushed plant material and 10 ml of solvent (S1-S5) were immersed in the ultrasonic bath. After 15 min of sonication the extract was separated and the sample was once again subjected to another 15 min of sonication with 10 ml solvent (S1-S5).

The sample was finally filtered and the residue washed. The extracts were reunited and then the final volume was adjusted to 25 ml (Soran *et al*., 2009).

Hot extraction

The method described by Danesi *et al.* 2014, with some modifications was used. An amount of 40 mg freeze-dried powder were extracted with 1ml of solvents (S1-S5) and homogenized. The homogenates were incubated for 20 minutes at 50°C in a Thermoblock TB2. After cooling at room temperature, the extract was centrifuged using a Hettich centrifuge (EBA20, Tuttlingen Germany) for 10 min and the supernatant was collected.

Total phenolic content

The total phenolic content was determined using the modified Folin-Ciocalteau assay (Socaci *et al*., 2013). Briefly, 0.1 ml of extract was mixed with 6 ml distilled water and 0.5 ml Folin-Ciocalteu reagent. After 4 min, 1.5 ml Na_2CO_3 solution (7.5%) was added and the sample was brought to a final volume of 10 ml with distilled water. The incubation was carried out at room temperature, in the dark, for 2 h and the absorbance was measured at 750 nm using a Shimadzu UV-VIS spectrophotometer (UV-1700 PharmaSpec, Shimadzu Scientific Instruments, Kyoto, Japan). Standard curve was prepared by using different concentrations of gallic acid. Total phenolic content was expressed as mg gallic acid/g dry weight (mg GAE/g DW). Analyses were performed in triplicate.

Antioxidant activity

The antioxidant capacity was assessed using the DPPH free radical scavenging assay according to Odriozola-Serrano *et al.*, 2008. DPPH is the most easy, simple and reasonably costly method and hence it may be the most commonly used method for the evaluation of the antioxidant activity of a sample (Alam *et al*., 2013).

A volume of 10 µl of sample was mixed with 90 µl of distilled water and 3.9 ml methanolic DPPH solution. After 30 minutes of incubation in darkness, the absorbance of each sample was measured at 515 nm against a blank of methanol using a Shimadzu UV-VIS spectrophotometer (UV-1700 PharmaSpec, Shimadzu Scientific Instruments, Kyoto, Japan). Negative control sample was prepared using 10 µl methanol, 90 µl distilled water and 3.9 ml DPPH solution. Positive control sample was prepared using 10 µl gallic acid aqueous solution (0.5 mg/ml), 90 µl distilled water and 3.9 ml DPPH solution in methanol and was used as standard antioxidant. The percentage of DPPH radical scavenging capacity of each plant extract was calculated using the Eq. (1):

$$\% \text{ RSA (Radical scavenging activity)} = \frac{Abs_{DPPH} - Abs_{sample}}{Abs_{DPPH}} \frac{Abs_{DPPH} - Abs_{sample}}{Abs_{DPPH}} * 100 \quad (1)$$

where:

Abs_{DPPH} is the absorbance of DPPH free radical solution in methanol;

Abs_{sample} is the absorbance of DPPH free radical solution mixed with sample/standard.

RESULTS AND DISCUSSION

Depending on the extraction method, the amount of total phenolics varied between 1930.58 - 1974.89 mg GAE/100 g for the methanolic extract, between 1919.78 - 1967.74 mg GAE/100 g for the 80% methanolic extracts, between 1891.01 - 1949.61 mg GAE/100 g for the ethanolic extracts, between 1399.71 - 1549.28 mg GAE/100 g for the diethyl ether extracts and between 1170.43 - 1306.52 mg GAE/100 g for the hexane extracts (Table 1). Results showed that methanol was the best solvent for extracting phenolic compounds and the maceration was the most effective extraction method. The lower polarity solvents, particularly diethyl ether and hexane showed much lower ability in extracting the phenolic compounds as compared to the polar solvents. Thus, the lowest total polyphenolic content was obtained for hexane extracts, 1306.52 mg GAE/100g for the extract obtained by maceration, 1249.72 mg GAE/100g for the extract obtained by sonication and 1170.43 mg GAE/100g for the extract obtained by hot extraction.

The ranking of antioxidant activity values of plant extracts was found to be the same with the ranking of values of the total phenolic content for the same plant extracts (Table 1). The methanolic extract obtained by maceration had the greatest antioxidant activity (85.12%) followed by those obtained by sonication (80%) and hot extraction (78.32%). Hexane extract obtained by hot

Tab.1. The content of total phenols and antioxidant activity of sage extracts

Solvent	Extraction methods	Total phenols (mg GAE/g)	DPPH (%)
Methanol	Maceration	1974.89±0.136	85.12±0.51
	Sonication	1942.76±0.116	80.03±0.29
	Hot extraction	1930.58±0.159	78.32±0.42
Aqueos methanol	Maceration	1967.74±0.142	80.68±0.40
	Sonication	1928.91±0.273	76.58±0.33
	Hot extraction	1919.78±0.372	72.82±0.19
Ethanol	Maceration	1949.61±0.248	78.43±0.25
	Sonication	1906.21±0.072	72.97±0.09
	Hot extraction	1891.01±0.372	70.83±0.18
Diethyl ether	Maceration	1549.28±0.216	67.94±0.26
	Sonication	1406.12±0.151	61.41±0.07
	Hot extraction	1399.71±0.051	60.04±0.14
Hexan	Maceration	1306.52±0.021	60.52±0.37
	Sonication	1249.72±0.101	56.38±0.29
	Hot extraction	1170.43±0.241	55.89±0.07

extraction exhibited the weakest activity (55.89%). The negative control registered a 2.94±0.10% RSA value and the positive control 87.38±0.47%. The higher antioxidant activity presented by the methanolic extract could be related to higher levels of specific phenolic compounds, such as caffeic acid, luteolin-7-O-glucoside, apigenin acetylglucoside and hispidulin. Furthermore, other molecules besides phenolic compounds are present in another extracts studied and might exert some antagonistic effects in the antioxidant activity (Martins *et al*., 2015).

Thus, it could be concluded from the results shown in the Table 1 that the higher polar solvents were more efficient in extracting phenolic compounds from sage than the less polar ones. Our results are in agreement with other studies which found that methanolic extracts of S. *officinalis* provide the most significant bioactivities, which are positively related to their phenolic composition (Balouiri *et al*., 2014; Roby *et al*., 2013).

Concerning the extraction method, all methanolic extracts obtained by maceration had a greater content of phenolic compound and a higher antioxidant activity than other extracts obtained by sonication and hot extraction. This can be explained by the use of high temperatures that may cause a loss of bioactive compounds and a degradation of a part of these compounds by interaction with highly reactive hydroxyl radicals formed during sonication (Quiroz-Reyes *et al*., 2013; Veličković *et al*., 2011).

CONCLUSION

Antioxidant activity as well as total phenols content of *Salvia officinalis* L. extracts obtained by different extraction method and solvents were studied. Methanolic extract obtained by maceration exhibited the highest extraction ability for phenolic compound and presented higher antioxidant capacity. This allowed us to conclude that maceration is an effective method and methanol was found to be a better solvent for the extraction of phenolic compounds from sage. Therefore, methanolic extracts of sage can be considered as a good source of natural antioxidants.

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