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Essential oil composition, phenolic content and antioxidant activity in Romanian *Salvia officinalis* L.

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Abstract

Salvia (*Salvia officinalis*), a member of *Lamiaceae* family, is one of the most important herb known for its essential oil richness and extensive use in folk medicine. The aim of the present paper was to characterize the chemical composition of the essential oil of *S. officinalis* L. and to determine the antioxidant activity and total phenols from sage extract in an attempt to contribute to the use of *Salvia officinalis* as alternative product in medicine and natural antioxidant agent in foodstuff. Thirty two constituents were identified in *S. officinalis oil* that represented 99.98 % of the oil. The main components of the oil were α-thujone (28.27%), camphor (16.76%) and 1,8-cineole (10.01%). The total phenolic content and the antioxidant activity of plant extract were determined by Folin-Ciocalteu and by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assays, respectively.

Keywords: antioxidant capacity, essential oil, GC-MS, phenolic content, *Salvia officinalis*.

1. Introduction

Genus *Salvia* (*Lamiaceae*) comprises about 900 species, spread throughout the world, some of which are economically important since they are used as a raw material in medicine, food industry and perfumery. *Salvia officinalis* L., was described by C. Linnae in 1753, and the name of *Salvia* has the origin in the latin word ”salvare”, to be healthy, to heal, to save, while the word *officinalis* referrs to the medical use of the plant [27].

Sage (*Salvia officinalis* L.) enjoys the reputation of being a panacea because of its wide range of medicinal effects: it has been used as an antihydrotic, spasmolytic, antiseptic and in the treatment of mental and nervous conditions [7], anti-inflammatory [4], anticancer [24], antioxidant [23], antimicrobial [2], antiviral [16], antidiabetic [11], antimutagenic [28] and [9].

The Food and Drug Administration (FDA) includes sage on its list of substances considered generally recognized as safe (GRAS) for use as spices and other natural seasonings and flavorings, and on the list of GRAS essential oils, oleoresins (solvent-free), and natural extractives (including distillates) [9].

S. officinalis contains many biologically active substances like essential oils, phenolic acids, flavones, phenylpropanoid glycosides, tannins and others [1]. Sage produces substantial amounts of essential oils with aromatic properties, used by different industries including pharmaceutical, food and cosmetic, wound treatment, bathing, washing, skin, hair care. Essential oils are a mixture of natural volatile substances

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produced from different parts of sage plants as secondary metabolites [3]. They contain a mixture of terpenes (monoterpenes, sesquiterpenes, or even diterpenes), low molecular weight aliphatic hydrocarbons (linear, branched, saturated and unsaturated), acids, alcohols, aldehydes, acyclic esters or lactones and exceptionally nitrogen and sulphur compounds, cumarines, and homologous of phenylpropanes [20]. Reports on the essential oil composition of this specie have been published by several authors ([6, 8, 15, 24]. The researches indicate that the major compounds were 1,8-cineole, camphor and a wide variety of thujenes [21].

On the other hand, sage is very rich in phenolic compounds such as flavonoids, phenolic acids and phenolic diterpenes and possess high antioxidant activities [2]. The antioxidant properties of sage have been studied intensively, and are found to be related to the presence of rosmarinic acid and carnosic acid. In addition, salvianolic acid, which is a rosmarinic acid dimer isolated from the sage extract, showed a high antioxidant activity and is a very significant scavenger of free radicals [13].

The aim of the present paper was to characterize the chemical composition of the essential oil of indigenous *S. officinalis* L. and to determine the antioxidant activity and total phenols from sage extract in an attempt to contribute to the use of *Salvia officinalis* as alternative product in medicine and natural antioxidant agent in foodstuff.

2. Materials and methods

The plant material (aerial parts) was collected in July 2014 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. All the reagents used were purchased from Sigma-Aldrich or Merck (Darmstadt, Germany). The moisture content for the dry plant material was 9.59% .

Essential oil extraction: The essential oil was extracted by hydro-distillation as follows: 50g of grinded dried leaves and 750 ml distilled water were placed in the distillation flask. For homogenization, a few glass beads were also added. The distillation time was 3 hours since the distillation began. At the end of extraction the obtained essential oil was collected and measured. In order to remove any traces of water, in the storage vials, anhydrous sodium sulfate was added. Essential oil sample was stored in a refrigerator in tightly sealed vials until further analysis.

Preparation of Extract:The methanol extract was prepared using the method of Mureşan *et al*., 2012 [17] with some modifications. For sample extraction, 1g of powdered material was extracted with 10 ml of methanol in a ultrasonic bath for 10 min. The extract was separated and the residual tissue was re-extracted until the extraction solvents became colorless (the total solvent volume was between 100-200 ml). The filtrates were combined in a total extract, which was dried by vacuum rotary evaporator at 40°C. The dry residues were recovered in 7 ml of methanol and stored in a freezer at -20°C until analyzed.

Total phenolic content: The determination of the total phenolics content was performed using a modified Folin-Ciocalteu method, according to Singleton *et al.* 1999 [25]. A 0.1 mL of the methanolic extract obtained above were mixed with 6 mL of water and 0.5 mL of Folin–Ciocalteu reagent. After 4 minutes at room temperature, 1.5 mL of a sodium carbonate (Na_2CO_3) solution 7.5% was added and the samples were diluted to a final volume of 10 ml with distilled water.

After incubation for 120 min at room temperature, the absorbance was measured at 750 nm on a Shimadzu UV-1700 PharmaSpec spectrophotometer. A calibration curve was performed using different concentrations of standard gallic acid solutions (r^2 = 0.9997) and the concentration of TPC was expressed as mg GAE/g dried material.

Determination of 2,2-diphenylpicrylhydrazil radical scavenging capacity (DPPH)

Scavenging activities of the extracts on the stable free radical DPPH were assayed using the method adapted after Odriozola-Serrano *et al*. 2008 [18].

A volume of 10 µL of methanolic extracts was mixed with 90 µL distilled water and 3.9 mL methanolic DPPH solution. After 30 minutes incubation in darkness, the absorbance of each sample was measured at 515nm against a blank of methanol.

The percentage of inhibition of DPPH was calculated by measuring the absorbance of the sample and applying the following equation:

$$\% \text{ of inhibition} = [1 - (As/A_0)] \times 100$$

where *A*s is the absorbance of sample, and A_0 is the absorbance of the DPPH solution.

GC-MS analysis

The analysis of the essential oils was performed using ITEX/GC-MS technique on a Shimadzu GCMS QP-2010 model gas chromatograph – mass spectrometer (Shimadzu Scientific Instruments, Kyoto, Japan) equipped with a CombiPAL AOC-5000 autosampler. An aliquot of 1µL essential oil was introduced in a sealed vial and incubated for 10 min at 60 ºC. The volatile compounds accumulated in the headspace phase of the vial were separated on a ZB-5ms capillary column of 30m x 0.25mm i.d. and 0.25µm film thickness. The GC temperature program was: 40.0ºC (3 min) rose to 160.0ºC with 4ºC/min and to 240 ºC with 10 ºC/min and held for 5 min. The ion source temperature and interface temperature were set at 250ºC and the MS mode was EI. The carrier gas was helium 0.84ml/min and the split ratio of 1:100. The mass range scanned was 40-650m/z at a Scan speed of 3333u/s. Components were tentatively identified on the basis of their mass spectra using the NIST147 and NIST27 GC-MS libraries.

3. Results and discussion

Antioxidant activity

Phenolic compounds are commonly found in both edible and non edible plants, and they have been reported to have multiple biological effects, including antioxidant activity. The total phenolic content in *S.officinalis* was measured by Folin Ciocalteu reagent. According to the Table 1, *S. officinalis* has a high phenolic content (2711.786 mg GAE/100g DW) and antioxidant activity (81.56%) and can be used as a natural source of phenolics and antioxidants.

Essential oil composition

The volume of essential oil obtained by hydro-distillation calculated for 100g of dry matter was 2.43 ml. The volatile profile of the sage essential oil extracted by hydro-distillation is shown in the GC-MS chromatogram illustrated in figure 1.

The components of the oil, the concentration of each constituent (expressed as percent from total composition) and the retention time are summarized in Table 2. Thirty-two identified compounds reached 96.98% of the oil. The main components were α-thujone (28.27%), camphor (16.76%) and 1,8-cineole (10.01%) reaching together 55.04% of the oil. The identification of the separated compounds was made by mass spectra using the NIST147 and NIST27 GC-MS libraries.

Table 1. Total phenolic content and radical scavenging activity of *S. officinalis*

Plant Species	Total phenolic content (mg GAE/g)	DPPH%
S. officinalis	2711.786	81.56

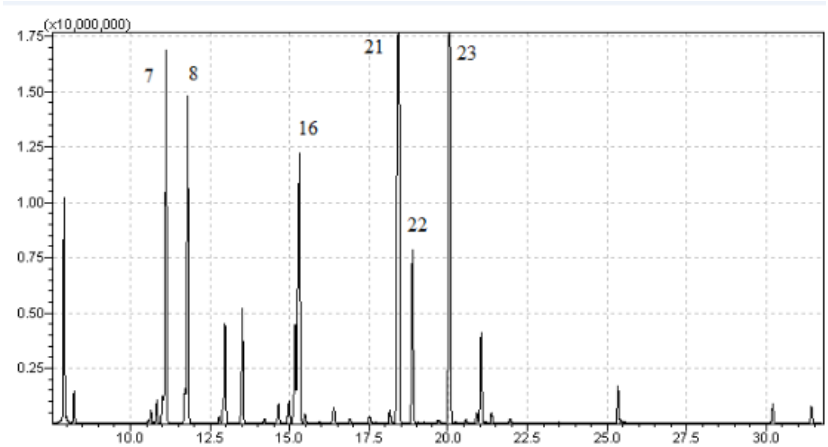

Figure 1. GC-MS chromatogram of sage essential oil. For peak identification see table 2

Table 2. The identified compounds from *S.officinalis* oil by GC-MS

No.	Compounds	Retention time (min)	Concentration %
1.	Cyclopropane, hexylidene	7.915	4.76
2.	2-Hexenal	7.981	0.03
3.	3-Undecyne	8.231	0.66
4.	β-pinene	10.569	0.08
5.	Tricyclene	10.643	0.27
6.	α-thujene	10.831	0.51
7.	α-pinene	11.118	9.35
8.	Camphene	11.801	8.51
9.	β-phellandrene	12.787	0.15
10.	β-pinene	12.973	2.58
11.	β-myrcene	13.52	2.84
12.	α-phellandrene	14.203	0.12
13.	4-carene	14.653	0.54
14.	m-cymene	14.982	0.42
15.	D-Limonene	15.172	2.93
16.	**1,8-cineole**	**15.307**	**10.01**
17.	β-trans-ocimene	15.486	0.27
18.	δ-terpinene	16.387	0.51
19.	4-carene	17.507	0.23
20.	β-linalool	18.135	0.33
21.	**α-thujone**	**18.416**	**28.27**
22.	β-thujone	18.854	4.91
23.	**Camphor**	**20.063**	**16.76**
24.	3-pinanone	20.577	0.04
25.	Isothujol	20.907	0.27
26.	Borneol	21.046	2.53
27.	1-Terpinen-4-ol	21.378	0.29
28.	α-terpineol	21.961	0.11
29.	Bornyl acetate	25.346	0.98
30.	β-Caryophyllene	30.194	0.27
31.	α-Caryophyllene	31.417	0.37
32.	Alloaromadendrene	35.483	0.08

Alpha-thujone is mentioned as a major component of sage essential oil in most literature reports and that is also the case of our essential oil. The chemical composition of *Salvia officinalis* L. essential oil has been investigated in various countries and the essential oils were divided into five groups according to the amount of the major constituents [8]:

1. camphor > α-thujone >1,8-cineole > β-thujone
2. camphor > α-thujone > β-thujone > 1,8-cineole
3. β-thujone > camphor > 1,8-cineole > α-thujone
4. 1,8-cineole > camphor > α-thujone > β-thujone

5. α-thujone > camphor > β-thujone > 1,8-cineole

The composition of the essential oil of *S. officinalis* of our study is very similar to that of essential oil from Spania [5], India [22], R. Macedonia [26] and Montenegro [10]. β-thujone was present in smaller quantities (4.91%) in our sample then the values reported in literature ([6, 12, 14]. Other Romanian researchers [19] analyzed the essential oil from 4 samples of *Salvia officinalis* leaves: fresh material and air-dried sample, harvested from plants cultivated in Cluj-Napoca and 2 commercial samples. They found major compound in all analyzed oils α-thujone (31.23-52.86 %), followed by camphor (8.33-22.49 %) and viridiflorol (8.06-12.39 %).

4. Conclusion

Thirty two constituents were identified in *S. officinalis* essential oil that represented 99.98% of the oil, α-thujone, camphor and 1,8-cineole being the major compounds. The methanolic extract investigated presented a strong antioxidant activity due to the contribution of phenolic compounds. In conclusion, the present research work suggested that this medicinal plant, widespread in Romania, has a strong antiradical activity and can be considered as a good source of natural antioxidants, useful for human health but also for other medicinal and commercial uses.

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Compliance with Ethics Requirements. Authors declare that they respect the journal's ethics requirements. Authors declare that they have no conflict of interest and all procedures involving human / or animal subjects (if exist) respect the specific regulation and standards.

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