Determination of Antioxidant Capacity and Antimicrobial Activity of Selected *Salvia* Species

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ABSTRACT

Aromatic plant species of genus *Salvia* are important medicinal plants, highly recommended due to a range of therapeutic properties: antirheumatic, antiseptic, antispasmodic, antimicrobial, carminative, antidiabetic. The present study attempts to compare the antioxidant and the antibacterial activity of the methanol extracts obtained by maceration, from *Salvia sclarea*, *Salvia lavandulifolia, Salvia officinalis Purpurascens*, *Salvia officinalis Tricolor* and *Salvia officinalis Icterina*. The antioxidant capacity was evaluated by the DPPH (1,1-diphenyl-2-picrylhydrazyl) radical method. Furthermore, the antibacterial effect of the extract was tested against five bacterial strains using the disk-diffusion method. *Salvia officinalis Tricolor* extract possesses the strongest antioxidant capacity. Moreover, all extracts showed antibacterial activity against Gram-positive *bacteria* tested (*Bacillus cereus* and *Staphylococcus aureus*) and against one Gram-negative bacteria (*Pseudomonas aeruginosa*). *Salmonella Typhimurium* was found to be resistant to *Salvia sclarea* extract and *Escherichia coli* to the extract from *Salvia officinalis Purpurascens*.

Keywords*: antioxidant capacity, antimicrobial activity, Salvia species*.

INTRODUCTION

In recent years, there has been growing concern regarding undesirable side effects of synthetic antimicrobial drugs/chemicals used for food preservation or in medicine. This necessitates the searching for new classes of safe and more effective antimicrobial agents with different acting mechanisms. A number of plants containing secondary metabolites could possess some of these ideal preservative characteristics mainly due to their antioxidant, antimicrobial and other biological potentials (Mehrsorosh *et al*., 2014).

There are more than 1 340 plants with defined antimicrobial compounds, and over

30 000 components have been isolated from phenol group-containing plant-oil compounds and used in the food industry. Edible, medicinal and herbal plants and spices such as oregano, rosemary, thyme, sage, basil, turmeric, ginger, garlic, nutmeg, clove, mace, savory and fennel, have been successfully used alone or in combination with other preservation methods. They exert direct or indirect effects to extend food-stuff shelf life or as antimicrobial agent against a variety of Gram-positive and Gram-negative bacteria (Tajkarimi *et al*., 2010).

The plant secondary metabolites spans an extremely large and diverse range of chemical compounds derived from plants. Therefore, they could have important implications for the development and implementation of therapeutic antimicrobial strategies and have a potential to be used in the food industry as a preservative (Balouiri *et al*., 2014).

The genus *Salvia*, the largest genus of *Lamiaceae* family, is widely distributed in various regions, including temperate and warmer zones. *Salvia* species have been used since ancient times for

different ailments ranging from aches to epilepsy, and mainly to treat colds, bronchitis, tuberculosis, hemorrhage and menstrual disorders. Based on their well-characterized antioxidant, aromatic and antimicrobial properties, it is not surprising that members of this genus have been used in the cosmetic industry, popular medicine, as food flavouring and preservation agents (Yousefzadi *et al*., 2007).

Species of *Salvia* also have a long-standing reputation as cognition-enhancing agents. Systematic and mechanistic studies of the effects of *Salvia* extracts have revealed multiple activities potentially relevant to brain function, aging and the preventative and symptomatic treatment of mild cognitive impairment and even Alzheimer's disease (Gomar *et al*., 2014). These plants are rich in volatiles such as mono-and sesquiterpenoids in their essential oil and non-volatile terpenoids especially di- and triterpenoids and synthesize polyphenols, including flavonoids and caffeic acid derivatives (Firuzi *et al*., 2013).

Cultivars of *S. officinalis* are quite variable in size, leaf and flower color and foliage pattern with many variegated leaf types. Modern cultivars include leaves with purple, rose, cream, and yellow in different combinations. Sage leaf contains tannic acid, oleic acid, ursonic acid, ursolic acid, cornsole, cornsolic acid, fumaric acid, chlorogenic acid, caffeic acid, niacin, nicotinamide, flavones, flavonoid glycosides and estrogenic substances (Amirmohammad et al., 2014).

Salvia officinalis Tricolor (a cultivar with white, yellow and green variegated leaves), *Salvia officinalis Icterina* (a cultivar with yellow-green variegated leaves) and *Salvia officinalis Purpurascens* (a purple-leafed cultivar) are cultivars of the common sage, grown primarily for their ornamental qualities, but these plants are also used for medicinal purposes.

Salvia sclarea L. is an important plant native to the Northern Mediterranean region and is largely cultivated in Europe. It is also known as "clary sage" and grown for its essential oil that has been traditionally used in food, pharmaceutical products, and also in aromatherapy. The essential oils or extracts of the aerial part of the S. *sclarea* plant have a broad spectrum of effects: analgesic, antiinflammatory, antioxidant, antifungal and antibacterial (Kuzma *et al*., 2009). Several reports on the evaluation of antioxidant and antimicrobial activities of clary sage have suggested it to be an excellent source of antioxidants*. S. typhimurium* was the most resistant microorganism while *Bacillus cereus, Bacillus brevis* and *Aeromonas hydrophila* were the most sensitive microorganism to the all extract examined (Tulukcu *et al*., 2009).

Salvia lavandulifolia is an annual herb originated in Iberian Peninsula, and it is widely distributed within East Spain, South France and North Africa. In the Mediterranean area, the aerial parts of *S. lavandulifolia*, which are rich in essential oils, are used as herbal remedy having sedative, analgesic, antioxidant and antiseptic properties (Porres-Martinez *et al*., 2014).

Additionally, the essential oil of Spanish sage is used in the pharmaceutical as well as in the perfume industry (Oelschlägel *et al*., 2012). Pharmacological studies of the plant have demonstrated its reputation as aromatic plant to enhance memory and anti-dementia drug by the inhibition of cholinesterase enzyme (Porres-Martinez *et al*., 2013).

Despite the well-known potentials of these plants and increased interest of their exploitation in food and pharmaceutical industry, to our knowledge, almost all studies available in the literature are restricted to *Salvia officinalis* (common sage). In this context, the aim of this work was to assess the antioxidant capacity o*f S. officinalis Tricolor, S. officinalis Icterina, S. officinalis Purpurascens, S. sclarea, S. lavandulifolia* extracts and the antimicrobial activity against a variety of microorganisms.

MATERIALS AND METHODS

The plant materials (aerial parts) were purchased from a greenhouse (Brasov, Romania) on June 2015. The leaves were dried immediately in a shady and well-aired place for two weeks. Afterwards, they were packed in paper bags and kept in a dark 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).

Plant extracts

The methanolic plant extracts were prepared using the method of Mureşan *et al*., 2012. Briefly, 1g of powdered material was extracted with 10 ml of methanol. The extract was separated and the residual tissue was re-extracted until the

extraction solvent 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 redissolved in 7 ml of methanol and stored in a freezer at -20°C until analyzed.

DPPH Radical Scavenging Assay

Antioxidant capacity (DPPH Free Radical Scavenging Activity) of the methanolic extracts was assessed on the basis of the radical scavenging effect of the stable DPPH free radical, using a method reported by Odriozola-Serrano *et al.*, 2008. A volume of 10 µl of the methanolic extract were mixed with 90 µl distilled water and added to 3.9 ml freshly prepared methanolic DPPH solution 0.025% (w/v). After 30 minutes incubation in darkness, the absorbance of each sample was measured at 515nm against a blank of methanol. The antioxidant capacity was calculated as follows:

% Radical scavenging activity (RSA) = $(A_0\text{-}A_1/A_0)$ 100,

where A_0 was the absorbance of DPPH free radical solution in methanol and A_1 the absorbance of the sample.

The analyses were run in triplicate and the results are expressed as average values with the standard error mean (SEM).

Bacterial Strains

The methanolic extracts were tested for antibacterial activity against the following bacterial strains: *Bacillus cereus* (ATCC 11778), *Staphylococcus aureus* (ATCC 6538P), *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25922) and *Salmonella typhimurium* (ATCC 14028). Each strain was grown into a test tube containing 10 ml sterile nutrient broth (Oxoid Ltd., Basingstoke, Hampshire, England) at 37°C for 24 h (except *B. cereus*: at 30°C for 24 h). The purity of the inoculum was confirmed by plating on appropriate selective media. A loopful of inoculum was transferred by streaking onto a selective medium: (i) MYP agar supplemented with Egg Yolk Emulsion and Polymyxin B (Oxoid Ltd., Basingstoke, Hampshire, England) for *B. cereus*, (ii) Baird-Parker agar base supplemented with Egg Yolk Tellurite emulsion (Oxoid Ltd., Basingstoke, Hampshire, England) for *S. aureus*, (iii) Pseudomonas-agar P, base (Merck KGaA, Darmstadt, Germany) for *P. aeruginosa*, (iv) TBX agar (Oxoid Ltd., Basingstoke, Hampshire, England) for *E. coli*, and (v) XLD agar (Oxoid Ltd., Basingstoke, Hampshire, England) for *S.* Typhimurium. Plates were incubated for 24 h at 30°C (*B. cereus*) or 37°C (*S. aureus*, *P. aeruginosa*, *E. coli*, and *S. typhimurium*). Bacterial morphology was confirmed by optical microscopy. Several colonies were collected with a sterile inoculating loop, transferred into sterile saline solution, and adjusted to the desired concentration using the McFarland nephelometer standards.

Antibacterial Assay

Antibacterial activity was determined using the disk diffusion test method according to the Clinical and Laboratory Standards Institute guidelines.

The inoculum was prepared to contain 10^8 CFU/ml by adjusting the suspension to match the McFarland No-0.5 turbidity standard according to National Committee for Clinical Laboratory Standards and dilutions were made that corresponding to a population of 1.5×10^5 CFU/ml. Then, 100 *µl* of each suspension bacteria were inoculated on Mueller-Hinton agar medium (Oxoid, Basingstoke, UK) plate's entire surface. The dried plant extracts (1g) were dissolved in methanol to a final volume of 7 ml. Extracts were pipetted (40 *µ*l) onto sterile paper discs (6 mm diameter) and placed onto the surface of inoculated agar plates. Gentamicin (0.04 $mg*ml^{-1}$ in saline solution) was used as a positive control and methanol was used as a negative control. Plates were incubated for 24 h at 30°C (*B. cereus*) or 37°C (*S. aureus, P. aeruginosa, E. coli,* and *S. Typhimurium*). A digital calliper was used to measure the inhibition zone diameter (in millimetres). All experiments were done in duplicates.

RESULTS AND DISCUSSION

DPPH is a stable free radical that is often used to evaluate the antioxidant activity of natural compounds in an easy, rapid and sensitive way (Al-Qudah *et al*., 2014).

By analyzing the antioxidant capacity of the different *Salvia* species it was demonstrated that all extracts studied had the capacity to scavenge DPPH free radicals (Table 1).

The results for the antioxidant activity that were expressed as percentage of decrease in the

absorbance value of each sample compared with the absorbance of DPPH reference solution, ranged between 79.48 and 92.07%. The best scavenging activity against DPPH radical was caused by *Salvia officinalis Tricolor* (92.07%) followed by *Salvia officinalis Icterina* (90.99%). These extracts showed DPPH radical scavenging effect over 90%. The lowest content in antioxidant compounds was recorded by *Salvia sclarea* (79.48%).

Disk diffusion methods are extensively used to investigate the antibacterial activity of natural antimicrobial substance and plant extracts. These assays are based on the use of disks as reservoirs containing the solution of substances to be examined.

The results obtained in the antibacterial assay are shown in Table 2. Methanol (control) had no inhibitory effects on the five microorganisms tested. As it can be seen in Table 2, all extracts showed varying degrees of antibacterial activity against of the Gram-positive and Gram-negative bacteria tested. *Salvia officinalis Tricolor* extract was the most active on the tested strains, presenting the largest zones of growth inhibition among the sensible strains and also possessing the maximum zone of inhibition. Hovewer, the extract from *Salvia sclarea* showed no antimicrobial activity against *Salmonella typhimurium* while *Escherichia coli* was found to be resistant to extract from *Salvia officinalis Purpurascens*. The plants showed antibacterial activity with zone of inhibition ranged from 8.93 mm to 16.48 mm. The maximum zone of inhibition was against Gram positive bacteria *Staphylococcus aureus* (16.48 mm) and the minimum zone of inhibition against Gram negative bacteria *Pseudomonas aeruginosa* (8.93 mm).

CONCLUSION

According to the results obtained, the aromatic plants studied have a high antioxidant capacity and may be considered as a good source of natural antioxidants. Almost all the screened *Salvia* species showed antibacterial potential, but with significant differences concerning the level of bacterial growth inhibition. *Salvia officinalis Tricolor* had the greatest antioxidant capacity and demonstrated the strongest antimicrobial efficacy

Tab.1. The antioxidant capacity of *Salvia officinalis Tricolor, Salvia officinalis Icterina*, *Salvia officinalis Purpurascens*, *Salvia lavandulifolia* and *Salvia sclarea* samples

Species	DPPH%
Salvia officinalis Tricolor	92.07±1.09
Salvia officinalis Icterina	90.99±0.42
Salvia officinalis Purpurascens	89.83±0.91
Salvia lavandulifolia	83.75±0.56
Salvia sclarea	79.48±1.21

Tab.2. Antibacterial activity of extracts from different *Salvia* species (zone of inhibition including the diameter of the paper disc, mm) by agar diffusion testing

Species	*E. coli* ATCC 25922	*S. aureus* ATCC 6538P	*S typhimurium* ATCC 14028	*P. aeruginosa* ATCC 27853	*B. cereus* ATCC 11778
S. Sclarea	9.55±0.05	10.20±0.47	-	8.93±0.11	12.32±0.18
S. off. Purpurascens	-	11.58±0.13	8.94±0.10	9.50±0.08	12.28±0.06
S. off. Tricolor	10.76±0.15	16.48±0.04	8.96±0.09	9.53±0.25	12.01±0.16
S.off. Icterina	9.74±0.16	9.79±0.05	8.94±0.03	9.03±0.02	11.57±0.16
S. lavandulifolia	13.13±0.13	9.40±0.21	8.76±0.03	9.38±0.23	12.71±0.15
Gentamicin	29.42±0.13	31.29±0.07	27.55±0.29	26.23±0.08	26.68±0.11
Methanol	-	-	-	-	-

Each value is the mean ± SD of two independent measurements
- No antimicrobial activity

against all bacterial strains. These data suggest the potential application of some herbal preparations alone or in combination with antibiotics for the treatment and prevention of pathologies associated with multi-resistant bacteria.

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