SOUTHERN BRAZILIAN JOURNAL OF CHEMISTRY

COMPARATIVE STUDY ON ANTIOXIDANT ACTIVITY OF *SALVIA NEMOROSA* L. FROM TWO DIFFERENT LOCATIONS

GHAFIYEHSANJ, Elham[1]; DILMAGHANI, Kamaladdin*[2]; CHAPARZADE, Nader[3]; SAADATMAND, Sara[4];

1,4 Islamic Azad University, Science and Research Branch, Faculty of Science, Biology Department

2 Islamic Azad University, Marand Branch, Faculty of Science, Biology Department

[3]Azerbaijan Tarbiat-e-Moallem University, Faculty of Science, Department of Biology
** Correspondence author*
e-mail: dilmaghanikamal@gmail.com

Received 11 March 2020; received in revised form 30 May 2020; accepted 23 June 2020

ABSTRACT

In order to investigate the antioxidant activity of *Salvia nemorosa* L. collected from Ahar and Urmia regions in Iran at different growth stages, aerial parts of sage after collecting were dried, and for measurement, the ability of scavenge DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) radical in different concentrations (0.025, 0.05, 0.07, 0.1, 0.2, 0.04 and 0.6) of methanolic extracts were prepared. The result showed that the ability to scavenge DPPH radical and amount of inhibition percent of vegetative stage leaves, flowering stage leaves, and flowers increased with increasing concentrations of methanolic extracts from 0.25 to 0.6 mg/ml. In the region of Urmia, the highest amount of DPPH inhibition there was in vegetative stage leaves, and the lowest amount of DPPH inhibition was seen in flowers. In Ahar regions, unlike the Urmia region, the highest amount of DPPH inhibition there was in flowers, but the lowest of DPPH inhibition was seen in flowering stage leaves. Also, the content of inhibition of DPPH in Ahar and Urmia regions similarly increased between two phenological stages (vegetative stage leaves, flowering stage leaves, and flowers) in 0.4 mg/ml and 0.6 mg/ml concentrations.

Keywords: *Lamiaceae, Salvia nemorosa, Antioxidant activity, Methanolic extracts, 2,2-diphenyl-1-picrylhydrazyl (DPPH)*

1. INTRODUCTION

The enlarged lamiaceae contains about 236 genera and 6,900 to 7,200 species, and the most abundant genera are Salvia. The most common purpose of medical and aromatic herbs is *Salvia* spp (Mirjalili *et al*., 2006). The genus Salvia is represented in Iran by 58 species, 17 of which are endemic. *Salvia nemorosa* L., commonly known as wood sage, is growing in central Europe and Western Asia (Skala and Wysokinska, 2004). The members of the genus display a remarkable diversity in growth form, floral morphology, pollination biology, and secondary compounds. They are distributed all around the world (Walker *et al*., 2004). Many species of Salvia are used in traditional medicine throughout the world. In Iranian folk medicine, several Salvia species have been used as antiseptic for wounds, as a diuretic, stomach tonic, anti flatulent, and reconstitute, and for the treatment of eye disorders, diarrhea, dyspepsia, fever, rheumatism, excessive menstruation, common cold, coughing, pertussis, and sinusitis (Naghibi *et al*., 2005). There is a growing interest in medicinal plants as therapeutic agents against many illnesses such as Alzheimer's disease, diabetes mellitus, and oxidative damages (Zengin *et al*., 2014).

Antioxidants such as flavonoids, tannins, coumarins, xanthons, phenolics, lignans, and terpenoids are found in various plant products (such as fruits, leaves, seeds, and oils) (Jeong *et al*., 2004). DPPH is one of the most popular sensitive and frequently used methods for the determination of the antioxidant activity of plant extracts, which was usually explained with the presence of phenolic acids and flavonoids in them. (Fazal *et al*., 2011). The effect of antioxidants on DPPH radical scavenging is due to their hydrogen donating ability, which is accepted as an electron or hydrogen radical to become a stable diamagnetic molecule (Hadbaoui *et al*., 2010). Studies have showed that *S. nemorosa* is a rich source of bioactive metabolites (flavonoids).

Also, *S. nemorosa* exhibited considerable antioxidant, antibacterial, and inhibitory enzyme activities. In conclusion, *S. nemorosa* is a valuable source of natural products and could be used for preparing novel functional foods, cosmetics, and pharmaceutical ingredients. It was reported, phenolic compounds of *Salvia nemorosa* are gallic acid, protocatechuic acid, catechin, p-hydroxybenzoic acid, caffeic acid, epicatechin, vanillin, p-coumaric acid, ferulic acid, sinapic acid, benzoic acid, o-coumaric acid, rutin, hesperidin, rosmarinic acid, trans-cinnamic acid, quercetin, luteolin, kaempferol, apigenin (Bahadori *et al*., 2017).

Herein, we aim to evaluate the influence of the region on the antioxidant activity of *S. nemorosa* in the northwest of Iran.

2. MATERIALS AND METHODS

2.1. *Plant material*

Sage plants (*Salvia nemorosa* L.) were collected at two vegetative and flowering stages from two regions in the northwest of Iran including Ahar (75 km from Tabriz to Ahar) (longitude: 38.26◦E' , latitude: 47.04◦N and altitude:1391m) and in East Azarbaijan and Urmia, Qasemlu village (longitude: 37.65 ◦E', latitude: 47.05◦N and altitude:1328m) in western Azarbaijan. Plant collection times at the vegetative stage were May 12th to May 26th, 2017, and collection times at the flowering stage were May 19th to June 5th, 2017. After collecting the plants, they were dried at room temperature.

2.2. Extraction

After collecting plants at two growth stages (vegetative and flowering) from two regions (Ahar and Urmia) and after drying flowers and leaves in shade conditions, each was powdered, and they were soaked using 50 ml absolute methanol and were extracted for three days by a shaker. Extracts, then, were centrifuged for 20 minutes in 1000 rpm (Sarrou *et al*., 2016).

2.3. DPPH radical scavenging

The antioxidant activity of sage plants extracts in this study was evaluated using DPPH (2,2-diphenylpicrylhydrazyl) scavenging assay. The hydrogen atom or electron donation abilities of the corresponding extracts were measured from the bleaching of the purple-colored methanol solution of DPPH. Various concentrations (0.025, 0.05, 0.07,0.1,0.2,0.4,0.6 mg/ml) of the extracts in methanol were added to 2 ml of methanol solution of DPPH. The absorbance was read against a blank at 517 nm. Inhibition of free radical DPPH in percent (I%) was calculated with the following equation:

$I\% = (A_{blank} - A_{sample} / A_{blank}) \times 100$ (Sarrou *et al*. 2016).

2.4. Statistical analysis

Data on DPPH radical activity in this study were performed with SPSS statistical software. Multi-Way-ANOVA analysis of variance was used to compare variables in regions, stages, different concentrations, and their interactions. Duncan's test was used to show the least significant difference in the probability level of 5% (P-value ≤0.05).

3. RESULTS AND DISCUSSION:

The capability of sage plants in quenching the oxidant radicals have previously been reported (Et-Touys *et al*., 2016). According to table 1, there was no considerable difference in the content of inhibition of DPPH by regions (Ahar and Urmia) between flowers, vegetative stage leaves, and flowering stage leaves (Table1). In the region of Urmia, the highest amount of DPPH inhibition there was in vegetative stage leaves, and the lowest amount of inhibition was seen in flowers. In Ahar, unlike the Urmia region, the highest amount of DPPH inhibition there was in flowers, but the lowest of DPPH inhibition was seen in flowering stage leaves (Figure 1).

In the Ahar region, the most the content of inhibition was observed in flowers in 0.025, 0.05, 0.07, and 0.2 mg/ml concentrations, but there was no considerable difference between vegetative stage leaves and flowering stage leaves. Also, in the Ahar region in 0.1 mg/ml concentration, the most and the lowest content of inhibition of DPPH were in vegetative stage leaves, and flowering stage leaves. In the Urmia region, the most and the lowest the content of inhibition of DPPH by increasing the concentration of 0.025 mg/ml to 0.2 mg/ml was seen in vegetative stage leaves and flowers, respectively. So that, in this region from the vegetative stage to an insignificant flowering decrease was observed (Figure 1). Similar to our results, in the study of Et-Touys *et al*. (2016) on antioxidant activity of *Salvia officinalis* was seen that DPPH inhibition in methanolic, ethanolic and en-hexane extracts were dose-dependent and by increasing the concentration from 0 to 500 µg/ml increased, and the most of inhibition percentage in methanolic extracts was observed. While inhibition percentage by increasing the concentration from 500 to 1000µg/ml didn't show the significant change (Et-Touys *et al*. 2016).

Moreover, in this study was observed that the methanolic extracts of vegetative stage leaves of plants collected from Urmia region had higher antioxidant properties in comparison with methanolic extracts of flowers and flowering stage leaves of sage plants while the methanolic extracts of flowers in Ahar region plants showed the highest antioxidant activities (Figure 1). About the amount of phenolic compound in the growth of different stages of plants, several studies were done that the effect of plant growth stages shows on the amount of this compound. According to the results of previous studies, the total phenolic content of extracts from fenugreek (Omezzine and Haouala 2013) and *Asparagus racemosus* decreased from vegetative to fructification stages (Verma and Kasera 2007).

In the study of the effect of DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical trapping in different concentrations of methanolic extracts of *S. nemorosa* was observed that from each of the collected region, methanolic extracts prepared from plants samples in scavenger of DPPH radical dose-dependent acted very effective and useful and the best antioxidant activity was in the high concentration of extracts. So that, by increasing concentration from 0.025 to 0.6 mg/ml, the content of inhibition percentage showed an increase in each growth stage (flowers, vegetative stage leaves, flowering stage leaves). The content of the inhibition percentage showed the same increase in the highest concentrations (0.4 and 0.6mg/ml) in different phonologic stages (Figure 1).

Also, it has been shown that total polyphenolic content increased at the flowering stage in *Boerhavia diffusa*, *Sida cordifolia* (Verma and Kasera 2007) and *Crithmum maritimum* (Males *et al*., 2003), during floral budding in *Hypericum hyssopifolium*, *H. scabrum* and at full flowering in *H. pruinatum* (Ayan *et al*., 2007). In addition, in spinach leaves, higher levels of total phenolics and antioxidant capacity were reported at the mid-maturity stage in comparison with the immature stage (Pandjaitan *et al*., 2005). Furthermore, leaf methanolic extracts of *Aegle marmelos* (Siddique *et al*., 2010), *Myrtus communis* var. *italica* (Wannes *et al*., 2010), *Artemisia absinthium* (Riahi *et al*., 2013) and *Malva sylvestris* (Barros *et al*., 2011) showed the highest antioxidant capacity compared to other plant parts (Alimpić *et al*., 2014). It seems that a decrease in phenolic content with age is probably due to their dilution with growth or that may be because of great cellular division at the early growth stages and formation transport phenomenon toward the young organs (Wang and Lin 2000; Del Bano *et al*., 2003). Also, it was reported that the existence of by-products, especially during the flowering stage, maybe considered a significant source of natural antioxidants in sage plants. Antioxidant activity is related to phenolic compounds that play a crucial role in neutralizing free radicals as a result of the fact that phenolics have a hydroxyl group (Viuda-Martos *et al*., 2010).

According to previous studies, the major phenolic compounds identified in the extracts of sage plants are rosmarinicacid, carnosic acid, salvianolic acid, and its derivatives carnosol, rosmanol, epirosmanol and rosmadial (Lu and Foo 2001). Among these, rosmanol is a major constituent of many salvia species and possesses strong antioxidant capacity because these groups cause phenols to more easily donate hydrogen atoms to activate free radicals to interrupt the chain reaction of antioxidants (Weng and Wang 2000). Furthermore, natural antioxidant compounds have various mechanisms such as prevention of chain initiation and breaking by donating hydrogen atoms or electrons, decomposition of peroxides, and prevention of continued hydrogen abstraction (Fazal *et al*. 2011). In addition, the reductive potential measures the ability of a sample to act as an electron donor and, therefore, reacts with free radicals converting them to more stable products and thereby terminates radical chain reactions (Sarikurkcu *et al*., 2010).

Observed differences in antioxidant activities of sage plants in Ahar and Urmia regions in this study can be caused by the effect of the difference in environmental factors such as longitude, latitude, altitude, harvest season, plants age, growth stages, soil and climate conditions in these regions. For example, it has been reported that the concentration of phenolic compounds can be affected by age and seasonal variation (Uddin *et al*., 2012). Also, variation in the amounts of phenolic compounds can be attributed to several reasons. Many of intrinsic factors such as plant species (genetic), parts of the plants and extrinsic factors such as environmental conditions (e.g., climate, soil, irrigation, temperature range, exposure to diseases and pests), cultural practices, harvest season, drying methods, handling and storage factors can influence the phenolic content of plants during the plant growth cycles (Tavassoli and Djomeh 2011).

4. CONCLUSIONS:

On the basis of all the analyses, it could be concluded that the methanolic extracts of vegetative stage leaves in the Urmia region showed the highest amount of antioxidant activity, but in the Ahar region, the highest amount antioxidant activity in the methanolic extracts of flowers was observed. So, different stages of growth of Salvia nemorosa plants, harvest times, different in climatic condition, major habitat and direct relation between ecological and genetic factors have led to differences in the antioxidant activity of sage plants in Ahar and Urmia regions which, can be investigated as a source of plant antioxidants, with potential use in food, cosmetics, and pharmaceutical fields.

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Table 1. *Variance Analysis to compare DPPH inhibition in different concentrations in Ahar and Urmia regions*

Regions	Stages	Mean squer
Ahar	Vegetative leaves	57.732^{ns}
	Flowering leaves	53.847 ^{ns}
	Flowers	65.444 ^{ns}
Urmia	Vegetative leaves	71.448 ^{ns}
	Flowering leaves	68.702 ^{ns}
	Flowers	50.854 ^{ns}

Note: 'ns' indicate non-significant diffrence at %5.

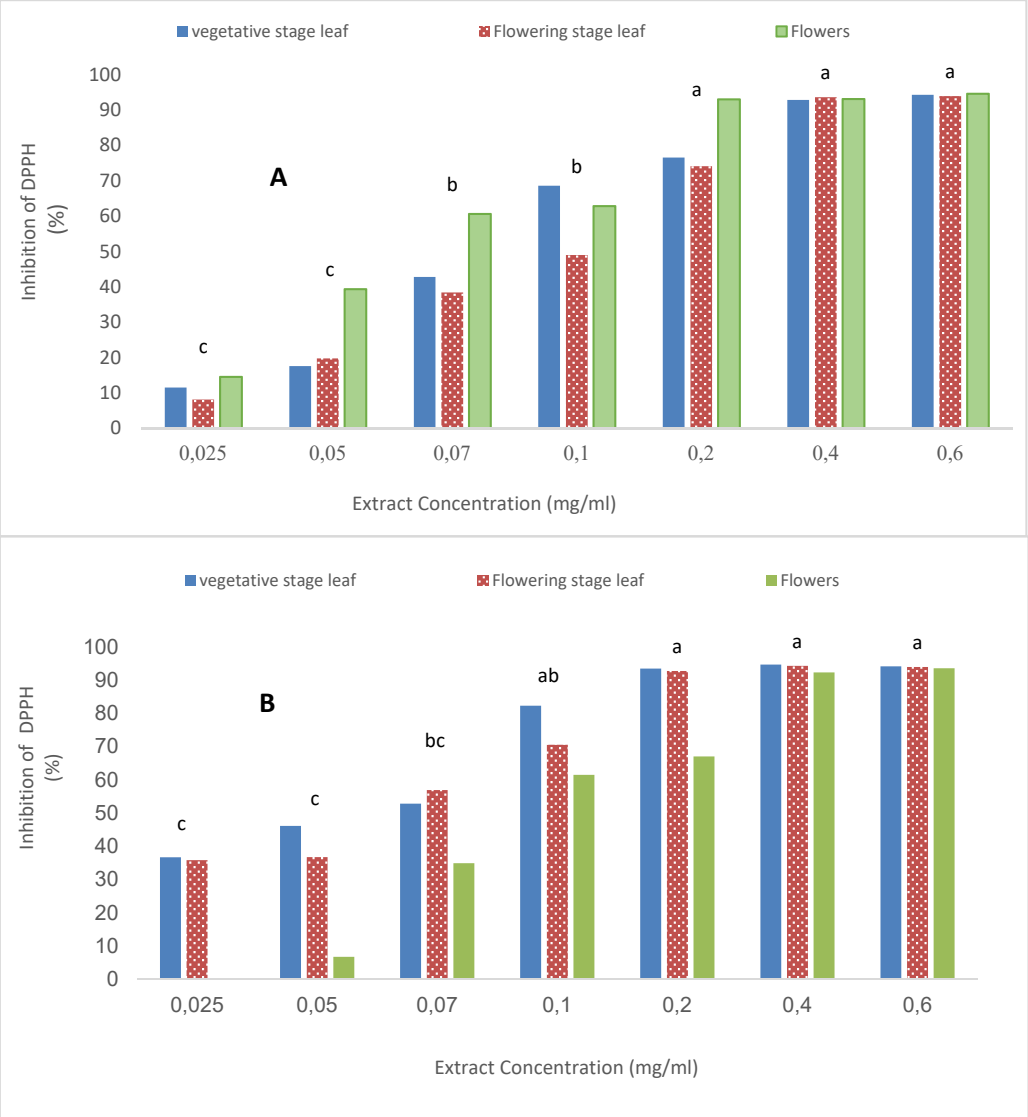

Figure 1. *The percentage of DPPH inhibition in different concentrations of Ahar (A) and Urmia (B) regions separately in different stages of growth*