SOUTHERN BRAZILIAN JOURNAL OF CHEMISTRY

STUDY ON THE DPPH FREE RADICAL-SCAVENGING ACTIVITY OF SALVIA NEMOROSA L. AT TWO GROWTH STAGES

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Received 12 April 2020; received in revised form 22 May 2020; accepted 22 June 2020

ABSTRACT

This study was designed to examine the DPPH free radical-scavenging activity, in different concentrations (0.025, 0.05, 0.07, 0.1, 0.2, 0.04 and 0.6) of methanolic extracts of Salvia nemorosa L. collected from the northwest of Iran (Zonouz and Ardabil regions) at two-stage of growth (vegetative stage leaves, flowering stage leaves, and flowers). The result showed that the mean of inhibition percentage in the Zonouz region increased in various concentrations and between flowers, vegetative stage leaves, and flowering stage leaves, compared with the plants of the Ardabil region. In each of the regions of Zonouz and Ardabil, the highest amount of DPPH inhibition was observed in the vegetative stage leaves in concentrations of methanolic extracts of Salvia nemorosa L. was observed that from each of the collected region, methanolic extracts from sage plants were dose-dependent and acted very effective and useful and the best antioxidant activity was in the high concentration of extracts, So in Zonouz and Ardabil regions, the content of inhibition of DPPH increased significantly, by increasing the concentration of 0.025 mg/ml to 0.6 mg/ml and in Zonouz region the content of inhibition of DPPH similarly increased in 0.2, 0.4 and 0.6 mg/ml concentrations. In the Ardabil region, the most content of inhibition of DPPH was seen in 0.4 mg/ml and 0.6 mg/ml concentrations, but in this region, the content of inhibition of DPPH in 0.2 mg/ml concentration there was only in vegetative stage leaves and flowering stage leaves.

Keywords: Salvia nemorosa, Lamiaceae, Growth stage, Antioxidant activity, 2,2-diphenyl-1-picrylhydrazyl (DPPH).

1. INTRODUCTION

It is known that 50,000-75,000 plant species are used in traditional and modern medicine worldwide (Schippmann et al., 2002). Medical and aromatic herbs have had essential components of healthcare throughout human history (Schippmann et al. 2002). From the past until now, by use of plants for the cure of different disease types in worldwide was usual because they contain components of therapeutic value. This was the basic formation of medicinal plants (Raja et al., 2010). Recently, there has been a general opinion that synthetic materials that are commonly used in the food and drug industry cause many diseases, such as cancer. This has led to an increasing global demand for natural and organic forms of medication. As a result of this demand, healing herbs and medicines of herbal

origins are commonly used worldwide as a part of traditional culture worldwide, and prefer alternative medicine methods at least once a year, and consume herbal drugs and healing herbs (Schipmann 2006).

Medical and aromatic herbs are used in such domains as cosmetics, medicine, dye, herbal tea, nutritional supplements, liquor, pesticide and fungicide, essential oil products, perfumes, flavoring liquids, and cleaning products. Previously, local usage of these herbs was common as part of the traditional culture, but today, these herbs have become an important source of national and international trade (Schipmann 2006).

The genus Salvia L. one of the largest genera in the Lamiaceae family (subfamily Nepetoideae), comprises over 900 species throughout the Old and New World (Hedge 1992).

The Lamiaceae (Labiate) is one of the most diverse and widespread plant families in terms of ethnomedicine, and its medicinal value is based on the concentration of the volatile oil (Sarac and Ugur 2007). Also, the Lamiaceae plant family is one of the largest families among the dicotyledons, many species belonging to the family being highly aromatic, due to the presence of external glandular structures that produce volatile oil (Giuliani and Bini 2008). Salvia nemorosa L., commonly known as wood sage, is growing in central Europe and Western Asia (Skała and Wysokińska 2004). In addition, S. nemorosa exhibits a considerable antioxidant, antibacterial, and enzyme inhibitory activities. Studies have showed that S. nemorosa is a rich source of bioactive metabolites (flavonoids and phenolic compounds) and could be used for preparing novel functional foods, cosmetics, and pharmaceutical ingredients (Bahadori et al., 2017).

Observing the considerable amount of phytochemicals of *S. nemorosa* with attempted to design a study to reveal the quantity of its antioxidant activity as well as the effect two different regions in northwest of Iran.

2. MATERIALS AND METHODS

2.1. Plant material

Salvia nemorosa L. plants were harvested at different growth stages (vegetative and flowering stages) from the northwest of Iran including Zonouz gardens in East Azarbaijan (longitude: 38.26° E', latitude: 45.46° N and altitude:1550 m) and 85 km from Khalkhal to Ardabil (Abbasabad village) in Ardabil province (longitude: 38.13° E', latitude: 48.19° N and altitude:1335 m). Plants were harvested during the vegetative stage from May 12th to May 26th, 2017, and were harvested during the flowering stage from May 19th to June 5th, 2017.

2.2. Extraction

The flowers and leaves of sage plants were dried at room temperature, and in shade conditions, then each was powdered, and they to extract were soaked in absolute methanol (50 ml), and extraction was carried out by shaking at room temperature for 72h. They were centrifuged for 20 minutes in 1000 rpm (Sarrou *et al.*, 2016).

2.3. DPPH radical scavenging

The ability of plant extracts to scavenge DPPH free radicals was determined according to the method described by Bondet *et al.*, (1997). The

sage extracts at different concentrations (0.025, 0.05, 0.07,0.1,0.2,0.4,0.6 mg/ml) were mixed with 2 ml of methanol solution of DPPH. The disappearance of DPPH was read against a blank at 517 nm. Free radical scavenging capacity was calculated by Equation 1:

% scavenging =
$$\left(100 - \frac{(Abc sample)}{(Abc DPPH)}\right) \times 100\%$$
 (Eq. 1)

Abs sample = Abs measured - Abs control (i.e., the absorbance of the sample tested without DPPH) (Barros *et al.*, 2011).

2.4. Statistical analysis

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

3. RESULTS AND DISCUSSION:

According to Table 1, there was no considerable difference in the content of inhibition of DPPH by regions (Zonouz and Ardabil) between flowers, vegetative stage leaves, and flowering stage leaves (Table1). But significant difference (p<0.01) was observed in each separate region between various concentrations (0.025, 0.05, 0.07, 0.1, 0.2, 0.4 and 0.6 mg/ml). In each of the regions of Zonouz and Ardabil, the highest amount of DPPH inhibition was observed in the vegetative stage leaves in comparison with flowering stage leaves and flowers. So, in two regions, from vegetative stage leaves to flowers, an insignificant decrease was observed (Figure 1).

The mean inhibition percentage in the Zonouz region showed an increase in various concentrations and in flowers, vegetative stage leaves, and flowering stage leaves, in comparison with the plants of the Ardabil region (Table 1). In this two regions, the content of inhibition of DPPH increased significantly, by increasing the concentration of 0.025 mg/ml to 0.6 mg/ml and in Zonouz region the content of inhibition of DPPH similarly increased in 0.2, 0.4 and 0.6 mg/ml concentrations (Figure 1)

Similar to our results, in the study of antioxidant activities of *Salvia officinalis* L. extracts was observed that DPPH inhibition in methanolic, ethanolic and n-hexane extracts was dosedependent and by increasing the concentration from 0 to 500 μ g/ml increased while, inhibition percentage in the highest concentrations (500 to 1000 μ g/ml) didn't show the significant change (Et-Touys *et al.*, 2016).

The more ever-present research showed that in each of regions of Zonouz and Ardabil, the highest amount of DPPH inhibition was observed in the vegetative stage leaves in comparison with flowering stage leaves and flowers. Similarly, in the study of Moghaddam *et al.*, (2018) on total phenolic content and antioxidant activity of *Fumaria vaillantii* L. extract at different growth stages was seen that highest accumulation amount of phenolic compounds was at the early growth stages, but the lowest was in the flowering stage.

In the Ardabil region, the most content of inhibition of DPPH was seen in 0.4 mg/ml and 0.6 mg/ml concentrations, but in this region, the content of inhibition of DPPH in 0.2 mg/ml concentration there was only in vegetative stage leaves and flowering stage leaves. Also, the content of inhibition of DPPH in flowers of the Zonouz region increased in comparison with flowers of the Ardabil region. In Zonouz and Ardabil regions, the content of inhibition of DPPH in higher concentrations (0.2, 0.4 and 0.6 mg/ml) significantly increased in compared with 0.25, 0.05, 0.07 and 0.1 mg/ml concentrations. In this regions, content of inhibition of DPPH in 0.07 and 0.1mmg/ml concentrations significantly decreased in compared with 0.4 and 0.6 mg/ml but significantly increased in compared with 0.025 and 0.05 mg/ml concentrations (Figure 1).

The influence of age and seasonal variation in the concentration of phenolic compounds are reported in previous researches by Munné-Bosch et al. (2000); Sellami et al. (2009) and Uddin et al., (2012). 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. (Wang and Lin 2000; Del Bano et al., 2003). Also, it seems that the existence of phenolic compounds during the earlier stages suggests a transport phenomenon toward the young organs. It was reported that some phenolic compounds disappeared from the vascular system by growing the plants. Also, endogenous biosynthesis and transportation in plants are two phenomena that can influence the distribution of phenolic compounds. It appears that a close relationship among different processes of biosynthesis, degradation, and transport are involved in the distribution of polyphenols in the plants (Del Bano et al. 2003). Furthermore, the type of solvent, the

degree of polymerization of phenolics, the interaction of phenolics with other food constituents, and the formation of the insoluble complex are also important (Gálvez *et al.*, 2005; Česonienė *et al.*, 2012).

Recently, phenolic compounds have received considerable attention because of their physiological functions, including antioxidant and free radical-scavenging abilities that are affected by the quality and nutritional value (Govindarajan et al., 2007). Therefore, antioxidant activity in plants 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. Due to the previous reports, the phenolic compounds are associated with redox properties, which allow them to act as reducing agents, hydrogen donators, and singlet oxygen quenchers as well as their metal-chelating abilities (Viuda-Martos et al., 2010). It is well-established that sage phenolic compounds (rosmarinic acid, carnosic acid, salvianolic acid, and its derivatives carnosol, rosmanol, epirosmanol, rosmadial) are highly effective free radical scavengers and antioxidants. It is showed that good antioxidant properties of S. officinalis by-products are a result of the high content of polyphenolics. Significance and role of significant phenolic compounds showing correlations with antioxidant tests are undoubted. Nevertheless, it should be taken into consideration that the total antioxidant capacity of sage extracts is the result of the potential cumulative or synergistic effects of the diversity of major and minor phenolic components (Lu and Foo 2001; Johnson and Jorge 2005).

Observed differences in this study such as the content of DPPH inhibition in higher concentrations, the mean of DPPH inhibition and differences at different growth stages between Zonouz and Ardabil regions probably due to variation in the amounts of phenolic compounds and environmental factors such as longitude, latitude, altitude, harvest season, plants age, growth stages, soil, temperature changes and annual humidity and rainfall in these regions. Similarly, Tavassoli and Djomeh (2011) stated that variation in the amounts of phenolic compounds could be attributed to several reasons such as plant species (genetic), parts of the plants and extrinsic factors such as environmental conditions (e.g., climate, height, soil characteristic, temperature, humidity 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.

4. CONCLUSIONS:

According to the present study, it can be concluded that observed differences in the content of antioxidant activities in higher concentrations, the increase of mean of DPPH inhibition in Zonouz region and the decrease of radical inhibition in flowers of Ardabil region probably due to differences in climatic conditions, the type of solvent, the degree of polymerization of phenolics, in two regions Zonouz and Ardabil that are affected antioxidant activities and phenolic contents in sage plants. Moreover, an increase in the number of antioxidant activities at the vegetative growth stage in these two regions shows that this stage is the best stage of the harvest to obtain the highest amount of natural antioxidants of salvia nemorosa for using in various industries, including food industries.

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Table 1. Variance Analysis to compare DPPH inhibition in different concentrations in Zonouz and Ardabil regions

Regions	Stages	Mean squer
Ardebil	Vegetative leaves	63.871 ^{ns}
	Flowering leaves	57.109 ^{ns}
	Flowers	37.799 ^{ns}
Zenouz	Vegetative leaves	72.997 ^{ns}
	Flowering leaves	65.541 ^{ns}
	Flowers	59.049 ^{ns}
		1.0/ =

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



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

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