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DETERMINATION OF ANTIOXIDANT ACTIVIY *Salvia sclarea* L. AND *Rosmarinus officinalis* L. (LAMIACEAE) SPECIES FROM ESKİŞEHİR, TURKEY

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ABSTRACT

Salvia sclarea L. and *Rosmarinus officinalis* L. are an important medicinal and aromatic plants of Lamiaceae. The plant materials were collected from Eskişehir and the collected samples were macerated with 70% MeOH. In this study, antioxidant activity of plant extracts was evaluated using 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity, Trolox equivalent antioxidant capacity (TEAC assay) and β-carotene bleaching method. The *in vitro* antioxidant results, DPPH radical scavenging assay and β-carotene bleaching method, it is seen that *R. officinalis* extract shows a more effective antioxidant activity compared to *S. sclarea* extract, and in the TEAC assay, *S. sclarea* extract shows a more effective antioxidant activity than *R. officinalis* extract.

Keywords: *Salvia sclarea* L., *Rosmarinus officinalis* L., Lamiaceae, Antioxidant activity

1. INTRODUCTION

Salvia L. and *Rosmarinus* L. are an important species of the Lamiaceae family. *Salvia* is comprises of approximately 1000 species and represented in Turkey by nearly 99 species with 58 endemic species and *R. officinalis* type is represented with one taxon in Turkey [1-4].

Salvia species are rich in flavonoids, essential oil and rosmarinic acid [5]. Some species of *Salvia* are used to treat of stomach aches, cold and throat ache [6]. *S. sclarea* is a biennial or perennial plant and it is called as 'Ayıkulağı, Misk adaçayı, Tüylü adaçayı' in Turkey. The pale blue or lilac colored flowers and branches contain tannin, resin and essential oil. The leaves and flowering branches of the plant are used antiperspirant, stomach problems and sedative [7]. It is also used in alcoholic beverages, cosmetics, and perfumery [5]. Rosemary is called with different names such as 'Kuşdili, Hasalbal, Akpüren' in Turkey. *R. officinalis* is a significant species due to its phenolic components and essential oils [7, 8]. Rosemary was used for food and medical treatment in ancient Greece and Romans. It is used many fields such as cosmetics, perfumery, aromatherapy, pharmacy and food today [9].

Some studies have shown that the bioactive components of plants are used to prevent many diseases. The reason for this is that the polyphenols have antioxidant activity. Many polyphenolic components derived from plants exhibit a stronger antioxidant capacity than vitamins E and C from *in vitro* experiments. In addition to, *in vitro* experiments are thought to contribute to *in vivo* experiments [10]. It has also been reported that sage and rosemary extracts and essential oils can be used as a potential source of antioxidants [11]. The aim of this study is to evaluate the antioxidant activity of *S. sclarea* and *R. officinalis* collected from Eskişehir.

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2. MATERIALS AND METHODS

2.1. Plant Materials

In this study, *S. sclarea* L. and *R. officinalis* were collected during to flowering stage in 2016, Sarıcakaya (Eskişehir/Turkey). The specimens have been stored in Anadolu University, Faculty of Pharmacy Herbarium (ESSE). ESSE numbers are *S. sclarea* ESSE NO: 14701, *R. officinalis* ESSE NO: 14718.

Figure 1. *Salvia sclarea* L. (Photo by Kucuk S.)

Medicinal herbs dried and macerated with 70% MeOH. The extracts were obtained by evaporation and lyophilization.

2.2. Antioxidant Activity

2.2.1. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity

In this analysis free radical scavenging activity was calculated using the 1,1-Diphenyl-2-picrylhydrazyl (DPPH) assay. Serial dilutions were prepared with half the concentrations of the previous one, resulting in stock solutions (4 mg/mL). DPPH (the same amounts) were put in to the diluted solutions and the UV absorbance at 517 nm was measured after 30 minutes. The experiment, extract, and positive standard control were made in triplicate for gallic acid. The averages of the absorbances were recorded for per concentration. The percentage of prevention was measured by Equation 1 [12].

$$\text{Percentage Inhibition} = \left[\frac{(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}})}{\text{Abs}_{\text{control}}}\right] x100 \quad \text{Equation 1}$$

2.2.2. Trolox Equivalent Antioxidant Capacity (TEAC assay)

The measurements the ability of a components to swell the 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical contrasted to the antioxidant activity of Trolox, a water-soluble form of vitamin E used as a standard. The blue-green ABTS was reacted in dark medium with 7 mM ABTS, 2.5

mM sodium persulfate ($Na_2S_2O_8$) for 12-16 hours at room temperature conditions. The ABTS radical was diluted with C_2H_5OH at 734 nm until a final absorbance of 0.7-0.8. and added to 990 µl of 10 µl of the ABTS solution of the sample and the absorbance was measured for 1 min after the Trolox was added for 40 mins after the extract was added. Trolox (2.5 mM) stock solution was prepared in C_2H_5OH. Absorbance values were calculated in a UV/spectrophotometer [13].

2.2.3. Determination of Inhibition of β-Carotene/Linoleic acid Cooxidation

Antioxidant activity of the *S. sclarea* and *R. officinalis* extracts was evaluated according to β-carotene bleaching method [14, 15]. Briefly, 1 mL of β -carotene (0.2 mg/mL dissolved in chloroform) was added to a flask containing linoleic acid (40 mg) and Tween 20 (400 mg). Chloroform was evaporated under a stream of nitrogen. Distilled water (50 mL) was added and shaken vigorously. A control was prepared without sample or standards with same procedure. Blanks of control and sample were also prepared without β-carotene. Their absorbance were measured on a spectrophotometer at 470 nm. The samples were then subjected to thermal autoxidation by keeping them in a constant temperature water bath at 50°C for 105 minutes. The rate of bleaching of β-carotenewas monitored by taking the absorbance at 15 min intervals. Antioxidative activity (AA) was calculated according to Equation 2:

$$AA\% = [1-(Ab_{0sample}-Abs_{105sample})/(Ab_{0control}-Abs_{105control})] \times 100 \quad \text{Equation 2}$$

3. RESULTS AND DISCUSION

According to the antioxidant activity of *S. sclarea* and *R. officinalis* showed similar DPPH radical scavenging inhibitory activity with positive control gallic acid at the concentration 0.1 mg/mL. While gallic acid showed 69.5% inhibition, *S. sclarea* and *R. officinalis* extracts showed 67.7% and 70.9 % inhibition, respectively (Table 1).

Table 1. Neutralization of DPPH by *Salvia sclarea* and *Rosmarinus officinalis* Extract and Gallic Acid (as a Positive Control) in the DPPH Assay

Source	Inhibition (%)
Salvia sclarea	67.7
Rosmarinus officinalis	70.9
Gallic acid	69.5

β-carotene bleaching method conclusions were obtained as 16.7% and 27.0% for *S. sclarea* and *R. officinalis* extract, respectively while gallic acid standard was indicated: 67.3%. All the *Salvia* and *Rosmarinus* extracts indicated the resemblance TEAC assay with the scores of 0.5 mM TEAC and 0.21 mM TEAC in order of, while gallic acid showed 2.4 mM TEAC.

According to these results, DPPH radical scavenging activity results were found to be parallel to β-carotene bleaching method results. In the mentioned assays, the *R. officinalis* extract gave a more effective result than *S. sclarea* extract. However, in the TEAC assay, *S. sclarea* extract was more effective than *R. officinalis* extract.

Extracts of some Lamiaceae species, including *Salvia viridis* L., *Salvia multicaulis* Vahl species, have been reported to have significant DPPH radical scavenging activity [16]. In addition the antioxidant effect of *S. sclarea* species collected from Eskişehir locality has been showed by using Fe induced linoleic acid system, Rancimat method and DPPH [17]. However, in our study, the antioxidant effect of clary sage was also studied with β-carotene bleaching method and TEAC assay.

In the study of Tavassoli and Djomeh, methanol extracts of *R. officinalis* leaves were studied with the DPPH radical scavenging method and as a result, rosemary extract was showed antioxidant activity [18]. Nedamani et al. reported that rosemary extract showed a higher antioxidant activity than oak extract and a synthetic antioxidant BHT [19]. Bozin et al. reported that *Salvia officinalis* essential oil is more effective than *R. officinalis* essential oil by DPPH method. It has also been shown that both essential oils are more effective than BHT [20]. In our study, *R. officinalis* species showed a more effective score than *S. sclarea* species by DPPH method.

4. CONCLUSION

Antioxidants are compounds that delay or stop oxidizing chain reactions [15]. Determining the antioxidant properties of plants will contribute to the development of new drugs [16]. In this study, antioxidant activity of *S. sclarea* and *R. officinalis* species belonging to Lamiaceae family was investigated. It is thought that the studies on these two plant species, which are widely used by the public, will contribute to the popular antioxidant issue. Since both extracts act as standard control gallic acid at concentrations of 0.1mg/mL, the use of these plant extracts as food preservatives instead of synthetic antioxidants can be explored in more detail.

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