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Essential Oil Composition, Antioxidant and Antifungal Activities of *Salvia sclarea* L. from Munzur Valley in Tunceli, Turkey

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Abstract

The essential oil composition and in vitro antioxidant and antifungal activity of the *Salvia sclarea* L. from Munzur Valley in Tunceli, Turkey were evaluated in this research. The *in vitro* antifungal activity of ethanol, hexane and aqueous extracts of *S. sclarea* against pathogen fungi *Epicoccum nigrum* and *Colletotrichum coccodes* were investigated. The essential oil of aerial parts of *S. sclarea* was obtained by hydrodistillation and was analysed by GC and GC-MS. Total antioxidant status was determined by using Rel assay diagnostics TAS assay kit (Lot.RL024) by Multiscan FC (Thermo). 33 compounds were identified representing the 85.0% of the total oil. The most abundant components (>5%) of the S. sclarea essential oils were caryophyllene oxide (24.1%), sclareol (11.5%), spathulenol (11.4%), 1H-naphtho (2,1,6) pyran (8.6%) and β-caryophyllene (5.1%). The best antifungal and antioxidant effect was seen in ethanolic *S. sclarea* extract. It can be said that *Salvia sclerae* could be used as natural antioxidant.

Key words: *Salvia sclarea*, Essential oil, Antioxidant activity, Antifungal activity.

Introduction

The human body produces reactive oxygen species (ROS), such as superoxide anion radical, hydroxyl radical and hydrogen peroxide, by many enzymatic systems through oxygen consumption (1). It has been reported that ROS largely contribute to cellular aging (2), mutagenesis (3) and several diseases.

The Lamiaceae family includes a large number of plants that are well known for their antioxidant properties. Among these, sage has been widely used and most of its antioxidant components have been identified (4,5,6). Salvia species are generally known for their multiple pharmacological effects including their antibacterial (7), antiviral (8) antioxidative (9), antidiabetic (10), cardiovascular, antitumor and anticancer (11). Also, some studies showed that a part of these activities depended on essential oil composition. Essential oils of plants are of growing interest both in the industry and scientific research because of their antibacterial, antifungal, and antioxidant properties and make them useful as natural additives in foods (12). Plants produce a great deal of secondary metabolites, many of them with antifungal activity. Well-known examples of these compounds include flavonoids, phenols and phenolic glycosides, unsaturated lactones, sulphur compounds, saponins, cyanogenic glycosides and glucosinolates (12).

Salvia is the largest and the most important genus of the family Lamiaceae. It is includes nearly 900 species spread throughout the world. This genus is represented in Turkey by 89 species and altogether 94 taxa, 45 of which are endemic in Turkey (13,14,15).

To the best of our knowledge, there are no available reports on essential oils composition antioxidants and antifungal activities of *S. sclarea* L. collected from Tunceli, Turkey. Therefore, the aim of the present work was carried out to study in vitro antioxidant and antifungal activities of *S. sclarea* and to evaluate the component of essential oils by GC–MS.

Materials and methods

Plant material

The specimens of *S. sclarea* was collected Munzur Valley in Tunceli (Eastern Anatolia, Turkey) in 2011 (Figure 1). Voucher specimens are kept at the Fırat University Herbarium (FUH), Elazig, Turkey.

Figure 1. *Salvia sclarea* L. from Munzur Valley in Tunceli.

Isolation of the essential oils

Air-dried aerial parts of the plant materials (100g) were subjected to hydrodistillation using a Clevenger-type apparatus for 3 h.

Gas chromatographic (GC) analysis

The essential oil was analysed using HP 6890 GC

equipped with FID detector and HP- 5 MS (30 m x 0.25 mm *i.d.,* film tickness 0.25 µm) capillary column was used. The column and analysis conditions were the same as in GC-MS expressed as below. The percentage composition of the essential oils was computed from GC-FID peak areas without correction factors.

Gas chromatography / mass spectrometry (GC-MS) analysis

The oils were analyzed by GC-MS, using a Hewlett Packard system. HP- Agilent 5973 N GC-MS system with 6890 GC in Plant Products and Biotechnology Res. Lab. (BUBAL) in Firat University. HP-5 MS column (30 m x 0.25 mm *i.d.,* film tickness 0.25 µm) was used with helium as the carrier gas. Injector temperature was 250 ^{o}C, split flow was 1 ml / min. The GC oven temperature was kept at 70^{o}C for 2 min. and programmed to 150 ^{o}C at a rate of 10 ^{o}C/min and then kept constant at 150 ^{o}C for 15 min to 240^{o}C at a rate of 5 ^{o}C / min. Alkanes were used as reference points in the calculation of relative retention indices (RRI). MS were taken at 70 eV and a mass range of 35-425. Component identification was carried out using spectrometric electronic libraries (WILEY, NIST).

Plant Extracts Preparations

Aqueous, hexanic, and ethanolic extracts were obtained from leaves of *S. sclarea.* Fresh plant material was washed with tap water, air dried and then chopped into small fragments, which was shade-dried and reduced to a coarse powder in a mortar and pestle. The aerial parts of the plant samples (2 g) were extracted with 20 ml ethanol, water and hexane. The organic solvents were evaporated to dryness under vacuum at low temperature using a rotary evaporator.

Total Antioxidant Status (TAS)

Total antioxidant status was determined by using Rel assay diagnostics TAS assay kit (Lot.RL024) by Multiscan FC (Thermo). Antioxidants in the sample reduce dark blue-green colored ABTS radical to colorless reduced ABTS form. The change of absorbance at 660 nm is related with total antioxidant levels of the sample. The assay is calibrated with a stable antioxidant Standard solution which is traditionally named as Trolox Equivalent that is a vitamine E analog (16).

Antifungal activity

In this study, pathogen fungus *Epicoccum nigrum* and *Colletotrichum coccodes* were used in the antifungal assay. This fungal strain was provided from Dicle University, Department of Biology. Antifungal assays were performed according to (17). The *in vitro* tests were carried out to measure the effects of *S. sclarea* extracts on radial growth of *E. nigrum* and *C. coccodes*. Sabouroud dextrose agar (SDA) medium was used in the study. To every 13 ml of sterile SDA medium in Petri dishes, 5 ml either aqueous, hexanic, or ethanolic extracts of each plant were added at a concentration of 2.5% (w/v). The solution in each Petri dish was gently swirled and allowed to solidify. The extract-amended medium in the Petri dishes were inoculated each alone at the centre with 5 mm inoculum-disc of each test fungus and incubated at 27 °C for 15 days. The medium with inoculum-disc but without any extract served as control. When the mycelium of fungus reached the edges of the control plate (without added extract) the antifungal index was calculated as follows:

Antifungal Index = DC - DT / DC x 100, where: DC = diameter of control, DT = diameter of test, each experiment was performed three times.

Statistical analysis

All values are presented as mean ± S.E. TAS and AFI values were compared by one-way analyses of variance (ANOVA) and post hoc multiple comparasions were done with Duncan test in SPSS/PC software program (version 12.0; SPSS Inc., Chicago, IL, USA) to determine the differences in extract types.

Results

Total antioxidant status of *S. sclarea* collected from Tunceli is shown in Table 1. The ethanolic extract of *S. sclarea* exhibited highest total antioxidant status among all samples evaluated in this study (4,40 mmolTrolox Equiv./L.)

Constituents of the essential oils of *S. sclarea* from Tunceli are shown in Table 1. In present study, the essential oil yields of S. sclarea L. was 0.1% v/w. In the case of S. sclarea, 33 compounds were identified representing the 85.0% of the total oil. The most abundant components (>5%) of the S. sclarea essential oils were caryophyllene oxide (24.1%), sclareol (11.5%), spathulenol (11.4%), 1H-naphtho (2,1,6) pyran (8.6%) and β-caryophyllene (5.1%) (Table 2).

In our study, the extracts produced different levels of antifungal activity against *E. nigrum and C. coccodes*. The result revealed that highly significant percent inhibition (65.2 %) of mycelial growth of *E. nigrum* was observed in SDA media amended with ethanolic leaf extract of *S. sclerae* and the lowest activity was observed

Table 1. Total antioxidant status of *S. sclarea* collected from Tunceli.

Plant Species	Part of plants	Solvents for extraction	TAS*
Salvia sclarea L.	Leaf	Ethanol	4.40±0.04^{a}
		Hexane	4.20±0.02^{ab}
		Water	4.11±0.01^{b}

***TAS:** Total Antioxidant Status; mmolTrolox Equiv./L, n: 3, Mean±SE. Means with different letter in the same column are significantly different at P< 0.05 according to Duncan's Multiple Range Test.

in hexane extract of *S. sclarae* (32.1 %) (Figure 2).

Table 2. Constituents of the essential oils of *S. sclarea* from Tunceli.

N°	Compound	RRI	% concentration
1	δ− 3 carene	1252	0.1
2	α− cubebene	1337	0.1
3	α− copaene	1360	4.0
4	α− bourbonene	1366	0.1
5	β− elemene	1369	1.5
6	β− caryophyllene	1393	5.1
7	α −caryophyllene	1418	0.3
8	Germacrene D	1435	1.3
9	Bicyclogermacrene	1445	0.5
10	Naphthalene	1456	1.0
11	1H-cycloprop(e)azulene	1480	1.0
12	1,5-epoxysalvial-4(14)-ene	1490	1.2
13	Spathulenol	1496	11.4
14	Caryophyllene oxide	1499	24.1
15	Veridoflorol	1502	0.5
16	Dihydroaromadendrene	1503	0.4
17	Salvial- 4(14)-en-1-one	1504	1.1
18	Cyclododecane	1512	0.5
19	Ledol	1514	1.2
20	Ylangene	1517	1.1
21	Benzoic acid	1524	0.3
22	1H-cyclopropa(a)naphthalene	1526	1.1
23	δ−selinene	1534	1.1
24	α−calacorene	1544	0.6
25	Caryophyllene-II	1547	1.1
26	12-Norcyercene-B	1558	1.0
27	Vulgarol A	1590	1.2
28	Valencene	1591	0.2
29	Aromadendrene oxide	1610	0.8
30	1H-Naphtho(2,1,6)pyran	1655	8.6
31	S-Estrone	1676	0.5
32	Sclareol	1859	11.5
33	Nonacosane	1942	0.5
	Total		**%85.0**

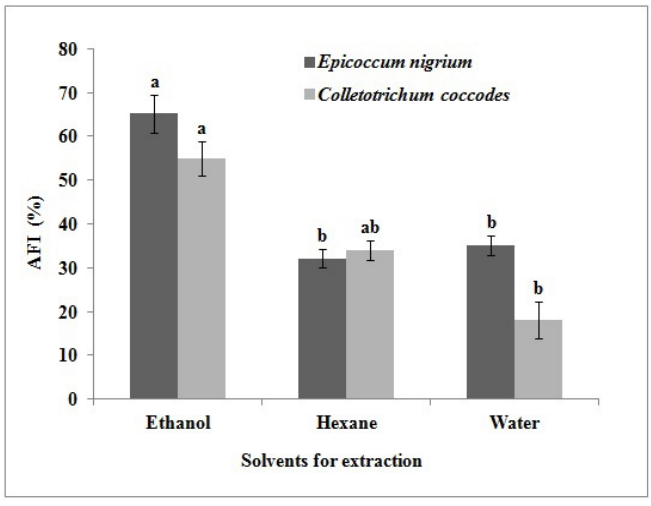

Figure 2. Antifungal activity of *S. sclarea* leaf extract on *E. nigrium* and *C. coccodes*, **AFI:** Antifungal Index, n: 3, Mean±SE. Means with different letter on the same colour bar are significantly different at P< 0.05 according to Duncan's Multiple Range Test.

Discussion

The role of free radicals is becoming increasingly recognized in the pathogenesis of many human diseases (18). All organisms are protected against free radical damage by oxidative enzymes or by chemicals such as atocopherol, ascorbic acid, carotenoids, polyphenols and glutathione (19). Thus, to increase antioxidant intake in human diet is one important way to minimize such oxidative damage (20). Natural antioxidants are considered to be useful agents for the prevention of diseases (21,22,23,24).

Many *Salvia* species having strong antioxidant activities because these groups cause phenols to donate more easily the hydrogen atoms to activate free radicals, which interrupt the antioxidation chain reaction (24). The methanol extracts of eight *Salvia* species from Turkey – *S. aethiopis, S. candidissima, S. limbata, S. microstegia, S. nemorosa, S. pachystachys, S. verticillata, S. virgata* – exhibited different levels of antioxidant activity in all models studies (25). Farhat et al. (26) found that the methanolic extracts of postdistilled *S. officinalis* might be valuable antioxidant natural sources and seemed to be applicable in both the health medicine and food industries. Yumrutas et al. (27) suggested that *Salvia euphratica var. euphratica* and *Salvia euphratica var. leiocalycina* could be used as natural antioxidant. In the present study, the ethanolic extract of *S. sclarea* exhibited highest total antioxidant status among all samples evaluated in this study (4,40 mmol-Trolox Equiv./L.) The results of antioxidant effects obtained in this study are similar to those reported in the above studies. *S. sclarea* could be used as natural antioxidant (Table 1). Many studies have shown that phenolic compounds in plant essential oils display antioxidant activity as a result of their capacity to scavenge free radicals (28,29) thus, we have studied esssential oil composition of *S.sclera.* Tenore et al. (30) investigated that the essential oil of aerial parts of *Salvia lanigera* Poir. (Lamiaceae) growing wild in Cyprus. A total of 67 compounds, representing 93.6% of the oil, were identified, and the major components were showed to be thymol (12.1%), hexadecanoic acid (6.0%), carvacrol and α-thujone (5.7%) . The essential oil from the aerial parts of *Salvia chrysophylla* Staph (Lamiaceae), endemic to Turkey, was investigated by using GC and GC-MS. Fifty-four of 55 components, represented 99.52% of the total oil, and was identified. The major components of the essential oil were found to be α-terpinenyl acetate (36.31%), β- caryophyllene (15.29%), linalool (8.12%) and β-elemene (4.26%) (31). The variation in the chemical composition of the essential oil of *Salvia officinalis*, growing in different habitats, was studied (26). GC-MS analysis revealed 57 compounds representing 94.68−96.80% of total oils. The major components were α-thujone (11.55−19.23%), viridiflorol (9.94−19.46%), 1,8-cineole (8.85−15.60%), camphor (5.08−15.06%), manool (5.52−13.06%), β-caryophyllene (2.63−9.24%), α-humulene (1.93−8.94%), and β-thujone (5.45−6.17%), showing significant differences between different collection sites. It was evaluated the in vitro antioxidant activities of n-hexane (Hex), dichloromethane (DCM), methanol (MeOH) and essential oils (EO) extracts obtained from *Salvia euphratica* var. *euphratica* and *Sal-*

via euphratica var. *leiocalycina*. The chemical compositions of essential oils of two varieties were analysed and their main components were determined as eucalyptol (18.4%) and trans-pinocarvyl acetate (24.9%), respectively (27). In our study, the essential oil yields of *S. sclarea* L. was 0.1% v/w. In the case of *S. sclarea*, 33 compounds were identified representing the 85.0% of the total oil. The most abundant components (>5%) of the S. sclarea essential oils were caryophyllene oxide (24.1%), sclareol (11.5%), spathulenol (11.4%), 1H-naphtho (2,1,6) pyran (8.6%) and $\beta-$caryophyllene (5.1%) (Table 2).

Many studies have shown that *Salvia* species have antifungal activities. Yildirim et al. (32) investigated that ethanol, methanol, hexane, and aqueous extracts of sage (*Salvia* sp) against pathogen fungi *E. nigrum*. The highest antifungal activity by hexane extract of sage (*Salvia* sp.) was found 10.6 mm against*, E. nigrium*. The ethanol extracts obtained from the leaves, rootstock and the combined formulation of endemic *Salvia tigrina* Hedge & Hub.-Mor. (Labiaceae) have been investigated for their antifungal activities (33). The minimum inhibitory concentration (MIC) ranged from 3.12 to 25 mg/mL. All the extracts exhibited a strong antifungal effect against the fungal cultures. The extracts exhibited greater antifungal effect against *C. albicans, C. neoformans* and *B. cinarea.* The findings provide support for the use of this plant in traditional medicine for fungal infections especially against candidiasis. The ethanol extract of *Salvia officinalis* has antifungal properties against cells of *Saccharomyces cerevisiae* (34). In another study, the antifungal activities of the essential oils of *Salvia lavandulifolia, S. officinalis* and *S. sclarea* against various *Candida* species were reported to be high (28). The essential oil of *S. multicaulis, S. kronenburgii* and *S. verticillata* were tested against *Candida albicans* and some bacteria and were found to be very effective especially against *Candida albicans* (35).

In our study, the extracts produced different levels of antifungal activity against *E. nigrum* and *C. coccodes*. The result revealed that highly significant percent inhibition (65.2 %) of mycelial growth of *E. nigrum* was observed in SDA media amended with ethanolic leaf extract of *S. sclerae* and the lowest activity was observed in hexane extract of *S. sclarae* (32.1 %) (Figure 2).

In this research, we showed that *S. sclarea* is an important medicinal plant in Tunceli, Tunrkey. This study focuses on essential oil composition, antioxidant and antifungal activities of *S. sclarea* in an attempt to contribute to use of these as alternative products and natural antioxidant and antifungal agent for food and medicinal uses. This findings candidate the plant as a good case for more in-depth studies and we wish our future research lead to the identification and structure elucidation of biologically active molecules present in its extract.

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