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Effect of collection time on biological activity of Clary sage (*Salvia sclarea*)

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Summary

Salvia sclarea (Clary sage, Lamiaceae) is commonly known as 'misk ada cayi' in Turkey. It is widely cultivated for extractive purposes in France, Bulgaria and USA. This study was designed to examine the effect of seasonal variation on in vitro antimicrobial and antioxidant activities of methanolic extract of Clary sage. The results indicated that the extract of the plants collected in midday contained higher levels of phenolic content and revealed higher antioxidant capacities compared to the materials collected at other times of the day. No differences were found between the methanolic extract of the plants collected at the years of 2005 and 2006 for phenolic content and antioxidant activities. Antimicrobial activities of the extracts against thirteen microorganisms were also investigated. *Salmonella typhimurium* was the most resistant microorganism whereas *Aeromonas hydrophila*, *Bacillus brevis* and *Bacillus cereus* were the most sensitive microorganisms to the all extracts examined in this study.

Introduction

A variety of herbs and spices are widely utilized by the human beings in different medical and domestic purposes. These herbs have many potential therapeutic and clinical applications in the modern medicine, as numerous studies have disclosed that they contain bioactive components which revealed antioxidant activities (MERKEN et al., 2001; ZHENG and WANG, 2001; MILIAUSKAS et al., 2004). The use of plants, herbs as antioxidants in processed foods is becoming of increasing importance in the food industry as an alternative to synthetic antioxidants (PROESTOS et al., 2006). Synthetic food antioxidant, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) are widely used to prevent lipid peroxidation which is major cause for the deterioration of food containing fat during storage and processing. Nevertheless, these synthetic antioxidants could have toxicological effects on human health (BARLOW, 1990). For this reasons, research on natural products including plant extracts and essential oils are very important. Particularly, spice extracts have appeared on the market as natural antioxidants for food industry use in recent years. The antioxidant capacity of some of these compounds has been proved to be comparable to and sometimes higher than that of synthetic antioxidants (CUVELIER et al., 1990; POKORNY, 1991). Close attention is currently paid to plants and phytochemicals of potential use in risk reducing of oxygen stress related chronic diseases, such as cardiovascular disease and cancer (SALAH et al., 2006). Also, spice extracts and essential oils have gained great importance due to their antimicrobial properties (DEANS and SVOBODA, 1990; PANIZZI et al., 1993).

Salvia belongs to the Lamiaceae family and is represented of by 88 species and 93 taxa of which ca. 51% are endemic in the flora of Turkey (DAVIS, 1982; GUNER et al., 2000). *Salvia* species are used variously as herbal tea and in folk medicine for wound healing and in alleviating stomach, liver, and rheumatism pains and for treating the common cold in various parts of the world and Turkey (BAYRAK and AKGUL, 1987). Previous reports indicated that the species which belong to the genus *Salvia* had antimicrobial, antiviral and cytotoxic activities (SIVROPOULOU et al., 1997; SOKOVIC et al., 2002). Also many diterpenes, isolated from plants of several species of the genus *Salvia*, have been demonstrated to have interesting pharmacological properties, such as antioxidant, anti-inflammatory, analgesic, antipyretic, haemostatic, hypoglycemic and antitumor activities (FIORE et al., 2006).

S. sclarea (Clary sage) is commonly known as 'misk ada cayi' in Turkey. It is widely cultivated for extractive purposes in France, Bulgaria, USA and former USSR (LAWRANCE, 1992). The oil of flowering spikes and leaves are used as a sedative and for treatment of stomach-ache, constipation and to reduce sweating (BAYTOP, 1999). Antifungal activity of Clary sage has also been reported (DIKSHIT and HUSAIN, 1984). Among the major components of the oil, linalool is known for its antibacterial activity (DEANS and SVOBODA, 1988).

Chemical and biological diversity of aromatic and medicinal plants depending on such factors, as cultivation area, climatic conditions, vegetation phase, genetic modifications and others is an important impetus to study flora present in different growing sites, countries and geographical zones (MILIAUSKAS et al., 2004).

In this study, the purpose was to screen methanolic extract of Clary sage for free radical scavenging capacity, antioxidant and antimicrobial activities with a special focus on variations in harvesting periods.

Materials and Methods

The sampling of Clary sage

The materials *Salvia sclarea* L. (Clary sage) were collected from plants cultivated at the Experimental Horticulture area at the Cumra Agricultural Vocational School, Konya, Turkey. Clary sage belongs to the Lamiaceae Germaplasm Bank of this School. It produces high quality essential oils during the summer and can be cultivated from wild plant seeds collected by the School. Moreover, this is a wildly grown plant in Turkey and has not been cultivated in Turkey until we cultivated.

The tested Clary sage was cultivated in Cumra county of Konya province located in central Anatolia with a 300 mm rainfall and 1040 m altitude. The seeds were sown during March 2004, and then standard maintenance procedure was applied for the crop. They were harvested during the flowering stage during the June and July 2005-2006.

Aerial parts of Clary sage were collected at different time intervals over a period of two years. The sampling periods were in the mornings (7 - 8 am), middays (0 -1 pm) and evenings (7 - 8 pm) between June, 15 2005 - July, 15 2005 and at the same period of 2006.

Preparation of the methanolic extracts

Air dried plant (at room temperature) was ground to fine powder with a grinder. Then the powdered material (10 g) was extracted in a Soxhlet extractor with 100 mL methanol (MeOH) at 60 °C for 6 h. The extract was filtered and concentrated to dryness under reduced pressure at 40 °C with a rotary evaporator. After determining the yield, the extract was dissolved in MeOH for further study.

Determination of total phenolic content

The Folin-Ciocalteau colorimetric method was used to determine total phenolic content of methanolic extract (SINGLETON and ROSSI, 1965). Forty µL of each plant extract solution (1 mg/mL) was transferred into a test tube and then mixed thoroughly with 2.4 mL distilled water. This solution was then mixed with 200 µL folin-ciocalteau reagent. After 30 second, 600 µL sodium carbonate solution (20 % Na_2CO_3) and 760 µL distilled water were added and mixed. The absorbance of reaction mixture was measured at 765 nm against a methanol blank after incubation at room temperature for 2 h in dark. The total phenolic content was determined using a standard curve with gallic acid (0-1 mg/mL) as the standard. The mean of three readings was used and expressed as mg of gallic acid equivalents (GAE)/g extract.

Determination of antioxidant activity

The antioxidant activity of plant extract was evaluated by the phosphomolybdenum method according to the procedure of PRIETO et al. (1999). For this purpose, 0.4 mL of plant extract was mixed with 4 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The effective concentration of extract was 1000 µg/mL in the reaction mixture. The tubes were capped and incubated in an incubator at 95 °C for 90 min. After the samples were cooled to room temperature, absorbance of the mixture was measured at 695 nm. A typical blank solution contains 4 mL reagent solution and the appropriate volume of the same solvent was used for the extract. The antioxidant activity was determined using a standard curve with ascorbic acid (0-1 mg/mL) as the standard. The mean of three readings was expressed as mg of ascorbic acid equivalents (AAE) /g extract.

Free radical scavenging activity

The free radical scavenging activity of plant extract for the radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) was measured as described LEE et al. (1998) with some modifications. A series of extract concentration in methanol, i.e. 2000, 1000, 500, 250, 100 ppm were prepared, and 50 µL of each concentration of plant extract was added to 450 µL tris-HCl and 1000 µL 0.1 mM methanol solution of DPPH. Methanol was used as a control instead of the extract. After a 30 min incubation period at room temperature in the dark, the absorbance was read against the blank at 517 nm. IC_{50} (concentration causing 50% inhibition) values of each extract was determined graphically. The measurements were performed in triplicate and the results were averaged. Tests were carried out in triplicate.

Inhibition of free radical DPPH in percent (I%) was calculated in following equation:

I (%) = 100 x (1-Absorbance of sample/ Absorbance of control)

Determination of antimicrobial activity

The test organisms used in this study were as follows: *Aeromonas hydrophila* ATCC 7965, *Bacillus brevis* FMC 3, *Bacillus cereus* RSKK 863, *Escherichia coli* ATCC 25922, *E. coli* O157:H7 932, *Klebsiella pneumoniae* FMC 5, *Listeria monocytogenes* 1/2B, *Morganella morganii*, *Salmonella typhimurium* NRRLE 4463, *Staphylococcus aureus* ATCC 29213, *Yersinia enterocolitica* ATCC 1501, *Candida albicans* ATCC 1223, *Saccharomyces cerevisiae* BC 5461.

Test yeasts; *C. albicans* and *S. cerevisiae* were grown in malt extract at 25 °C for 18 h, and *Y. enterocolitica* was grown in nutrient broth above same temperature and time. The other microorganisms were grown in nutrient broth at 35 °C for 18 h. All test microorganisms in nutrient broth or malt extract broth were enumerated by using the serial dilution method. Their final cell concentrations were 10^6-10^7 cfu/mL. The agar diffusion method was used to detect antimicrobial activity (SAGDIC, 2003; SAGDIC et al., 2006). For this purpose, 250 µL of each microorganism was added into a flask containing 25 mL sterile nutrient agar or malt extract agar at 45 °C and poured into Petri dishes (9 cm diameter). Then the agars were allowed to solidify at 4 °C for 1 h. The equidistant holes were made in the agar using sterile cork borers (Ø = 4 mm). The extracts (50 µL) were prepared at 1%, 2.5%, 5% and 10% concentrations in absolute methanol and were applied to the holes using a pipettor and absolute methanol without herb extract was used as a control. *Y. enterocolitica, C. albicans* and *S. cerevisiae* was incubated at 25 °C for 14-24 h in the inverted position. The other microorganisms were incubated at 35 °C for 18-24 h. At the end of the period, all plates were examined for any zones of growth inhibition, and the diameters of these zones were measured in millimeters. All the tests were performed in duplicate and the results were presented as averages.

Statistical analyses

Data from the experiments were subjected to analysis of variance (ANOVA) using SPSS (2001) for Windows (ANONYMOUS, 2001). Percentage data were transformed using arcsine $\sqrt{x}$ before ANOVA. Means were separated at the 5% significance level by the least significant difference (LSD) test.

Results and discussion

The percent yield of each sample is presented Tab. 1. The extract yields of Clary sage collected at different periods of two years ranged from 19.62 to 26.08%. The highest yield was obtained from the plants collected in June 2005 at the evenings whereas the least yield was obtained from the plants collected in July 2006 at the mornings. Extract yield varied depending on the harvesting time and the year. Methanolic extract of the plant collected in June 2005 provided maximum yield.

The total phenolic contents of the extracts determined by Folin-Ciocalteu method were reported as gallic acid equivalents (Tab. 1). The differences among total phenolic contents of the extracts obtained from Clary sage collected at different times were statistically significant. The total phenolic contents of extracts ranged from 38.34 ± 1.01 to 97.84 ± 1.17 mg GAE/g dry extract. The highest extractable phenolic contents were observed in the extracts of plants collected in July 2005 in the morning and in June 2005 in the midday while the least content was also observed in the extract of the plants collected in July 2005 at the evening. Total phenolic contents of Clary sage tested have been reported here for the first time.

The antioxidant activities detected with the phosphomolybdenum reduction assay in Clary sage extract was shown as ascorbic acid equivalents in Tab. 1. Methanol extracts of all the samples had antioxidant activities in the range from 106.40 ± 0.48 to 217.11 ±

Tab. 1: Harvest year, collection time, yield, total phenolic content, antioxidant activity and IC_{50} of Clary sage methanolic extracts

Sample	Harvest Year	Collection Time	Yield, %	Phenolic content (mg GAE/g dry extract)	Antioxidant activity (mg AAE/g dry extract)	IC_{50} (µg/mL)
S1	15 June 2005	Morning	23.36	83.84 ± 4.65c	133.48 ± 0.26f	26.34i
S2	15 June 2005	Midday	24.33	97.84 ± 0.59a	143.94 ± 3.07d	23.49l
S3	15 June 2005	Evening	26.08	70.12 ± 0.59e	174.08 ± 1.39b	28.41f
S4	15 July 2005	Morning	20.53	97.84 ± 1.17a	106.40 ± 0.48h	24.74k
S5	15 July 2005	Midday	21.90	83.64 ± 1.55c	136.99 ± 3.75e	26.39h
S6	15 July 2005	Evening	19.74	38.34 ± 1.01h	143.78 ± 2.16d	27.40g
S7	15 June 2006	Morning	23.80	44.76 ± 2.11g	11.66 ± 3.12g	45.70a
S8	15 June 2006	Midday	20.62	61.33 ± 1.17f	144.85 ± 0.7d	30.17e
S9	15 June 2006	Evening	21.28	77.56 ± 0.59d	162.02 ± 0.95c	31.62c
S10	15 July 2006	Morning	19.62	77.56 ± 1.55d	139.51 ± 1.60e	30.95d
S11	15 July 2006	Midday	22.54	88.72 ± 0.59b	217.11 ± 3.39a	25.36j
S12	15 July 2006	Evening	21.16	77.13 ± 1.22e	143.40 ± 1.00d	36.77b

Total phenolic content is expressed as gallic acid equivalents (GAE; mg GAE/g dry extract). Antioxidant activity is expressed as ascorbic acid equivalents (AAE; mg AAE/g dry extract). Each value is presented as mean±SD (n=3). Means within each column with different letters (a-l) differ significantly ($p < 0.05$).

3.39 mg AAE/g dry extract. The differences among the antioxidant activities of the extracts obtained from Clary sage collected at different times were statistically significant. High antioxidant activity was observed in the plants collected during the midday and evening. Maximum antioxidant capacity was observed in the extract of plants collected in the midday of July 2006 whereas minimum antioxidant capacity was also observed in the extract of plants collected in the mornings in July 2005. Also, statistically no differences were found between the methanolic extract of plants collected at 2005 and 2006 for the phenolic content and antioxidant activity. The antioxidant activity of Clary sage measured by phosphomolybdenum method has been reported here for the first time. However, the antioxidant activity of many *Salvia* species was determined by the different methods. Lu and Foo (2001) studied the antioxidant activity of flavonoids and phenolic acids of the isolates from the sage (*S. officinalis*) using three different test methods.

The free radical scavenging potentials of the extracts from Clary sage plants collected at different time of two years were tested by DPPH method. All of the extracts exhibited the scavenging activity depending on extract concentration at various degrees. The differences among radical scavenging activities of the extracts were statistically significant ($p < 0.05$). The extract of the plant collected in the mornings in June 2006 showed minimum activity with an IC_{50} of 45.70 µg/mL whereas the extract of the plant collected during the midday in June 2005 showed maximum activity with an IC_{50} of 23.49 µg/mL at all the tested concentrations (Tab. 1).

Biological activities of different *Salvia* species were reported in a previous paper. Bozan et al. (2002) determined that the methanol extracts of Clary sage and other *Salvia* species exhibited antioxidant and free radical scavenging activity. Similar to our results, previous studies on the free radical scavenging activities of different *Salvia* species showed also that the *Salvia* species had strong free radical scavenging activity. In a previous paper, Tepe et al. (2006) determined that the methanol extracts of six *Salvia* species from Turkey showed free radical-scavenging activities between 20.7-49.7 µg/ml and that of Clary sage 23.4 µg/ml. Tepe et al. (2004) reported that essential oil and methanol extract of *Salvia cryptantha* and *S. multicaulis* have the capacity to scavenge free radicals. Also, same author and his colleagues determined that aqueous methanol extract of *S. tomentosa* and the methanol extracts of *S. verticillata* subsp. *verticillata* and *S. verticillata* subsp. *amasiaca* exhibited strong free radical scavenging activity (IC_{50} = 18.7 µg/mL) in DPPH assay (Tepe et al., 2005; Tepe et al., 2007). It is reported that extract of *S. miltiorrhiza* possessed high scavenging activities (Zhao, 2006). DPPH scavenging activity of the extract of three different *Salvia* species was also determined by Kamatou et al. (2005).

The antimicrobial activities of the studied Clary sage extract at a concentration of 10, 5, 2.5, and 1% are shown in Tab. 2. Methanol (control) had no inhibitory effects on the thirteen microorganisms tested. Statistical differences within the microorganisms were important in the agar diffusion assay. However, the differences among antimicrobial activities of the extracts obtained from Clary sage collected at different time were not statistically significant ($p > 0.05$). Antimicrobial activities of the extracts varied for the tested organisms. For example, *A. hydrophila, B. brevis* and *B. cereus* were the most sensitive microorganisms to the all extracts examined in this study. None of *S. sclarea* extracts inhibited the growth of *Escherichia coli* ATCC 25922, *M. morganii* and *Y. enterocolitica*, also the extracts had no inhibitory effects on two yeasts: *C. albicans* and *S. cerevisiae.*

Several studies have been conducted for the antimicrobial activities of essential oils and extracts from different *Salvia* species (Haznedaroglu et al., 2001; Tepe et al., 2004; Tepe et al., 2005; Delamare et al., 2007). In this study, the results supported the observations of some other researchers. But it is difficult to compare the results of these studies due to the differences in the *Salvia* species, different methods and/or different microorganisms used for the evaluation of antimicrobial activities. For example, Tepe et al. (2005) showed that non-polar extracts and subfractions of *S. tomentosa* showed moderate antimicrobial activities while polar extracts remained almost inactive. Again, antimicrobial potential of the methanolic extracts of *S. cryptantha* and *S. multicaulis* was also shown by Tepe et al. (2004) and Kabouche et al. (2005) noted that the acetone extract of the roots of *S. jaminiana*, remarkably inhibited the growth of *B. subtilis*, *S. aureus* ATCC 25923 and *Streptococcus* α-hemolitic. Kuzma et al. (2007) determined that the crude dichloromethane fractions from acetone extracts of *S. sclarea* roots as well as four pure abietane diterpenoids isolated from the hairy root cultures had antimicrobial activity against Gram (+) bacteria tested. Kamatou et al. (2005) showed the extract of three different *Salvia* species had antibacterial activity.

Tab. 2: Antimicrobial activities of methanolic extracts of Clary sage at different concentrations (inhibition zones, mm)

Samples		Microorganisms							
		A. hydrophila	*B. brevis*	*B. cereus*	*E. coli* O157:H7	*K. pneumoniae*	*L. monocytogenes*	*S. typhimurium*	*S. aureus*
S1	10%	15.5 ± 0.7	17.5 ± 0.7	18.5 ± 0.7	11.5 ± 0.7	11.5 ± 0.7	8.0 ± 0.0	-	7.0 ± 0.0
	5%	12.0 ± 2.8	15.5 ± 2.1	16.5 ± 0.7	7.5 ± 0.7	6.0 ± 0.0	6.0 ± 0.0	-	7.0 ± 0.0
	2.5%	9.0 ± 1.4	13.5 ± 2.1	13.5 ± 2.1	6.0 ± 0.0	-	-	-	6.0 ± 0.0
	1%	6.0 ± 0.0	10.0 ± 0.0	8.5 ± 0.7	-	-	-	-	-
S2	10%	24.5 ± 0. 7	24.5 ± 2.1	20.5 ± 0.7	11.0 ± 1.4	-	8.0 ± 0.0	8.0 ± 1.4	-
	5%	20.0 ± 0.0	22.5 ± 4.9	14.0 ± 4.2	8.5 ± 0.7	-	6.0 ± 0.0	-	6.0 ± 0.0
	2.5%	13.0 ± 0.0	19.5 ± 3.5	11.0 ± 1.4	7.0 ± 0.0	-	-	-	-
	1%	8.0 ± 0.0	15.5 ± 0.7	8.0 ± 0.0	-	-	-	-	-
S3	10%	14.0 ± 1.4	18.0 ± 4.2	19.0 ± 1.4	12.5 ± 2.1	12.5 ± 0.7	6.0 ± 0.0	-	6.5 ± 0.7
	5%	9.0 ± 0.0	18.5 ± 0.7	14.5 ± 0.7	8.0 ± 0.0	9.0 ± 1.4	-	-	6.0 ± 0.0
	2.5%	8.0 ± 0.0	16.0 ± 1.4	11.5 ± 2.1	6.0 ± 0.0	-	-	-	-
	1%	6.5 ± 0.7	11.0 ± 1.4	9.0 ± 0.0	-	-	-	-	-
S4	10%	24.5 ± 0.7	33.0 ± 0.0	23.0 ± 2.8	14.0 ± 1.4	8.0 ± 1.4	8.0 ± 0.0	-	6.0 ± 0.0
	5%	17.5 ± 0.7	28.0 ± 1.4	18.5 ± 2.1	8.0 ± 0.0	6.0 ± 0.0	-	-	-
	2.5%	12.0 ± 0.0	24.0 ± 2.8	11.5 ± 0.7	7.0 ± 0.0	-	-	-	-
	1%	9.5 ± 2.1	17.5 ± 2.1	8.5 ± 0.7	-	-	-	-	-
S5	10%	20.5 ± 0.7	26.0 ± 1.4	25.5 ± 0.7	20.5 ± 0.7	-	-	-	7.0 ± 0.0
	5%	16.5 ± 0.7	20.0 ± 1.4	22.0±2.8	8.5±0.7	-	-	-	6.0 ± 0.0
	2.5%	9.5 ± 0.7	17.0 ± 1.4	14.5±0.7	6.5±0.7	6.0±0.0	-	-	-
	1%	7.5 ± 0.7	12.5 ± 2.1	8.5 ± 0.7	-	-	-	-	-
S6	10%	20.0 ± 2.8	25.0 ± 1.4	23.5 ± 0.7	10.0 ± 1.4	-	8.0 ± 0.0	-	-
	5%	16.0 ± 1.4	21.0 ± 1.4	19.0 ± 0.0	7.0 ± 1.4	-	-	-	-
	2.5%	9.5 ± 2.1	13.0 ± 0.0	14.0 ± 1.4	-	-	-	-	-
	1%	6.0 ± 0.0	11.5 ± 0.7	8.5 ± 2.1	-	-	-	-	-
S7	10%	12.5 ± 0.7	24.5 ± 2.1	22.5 ± 0.7	8.0 ± 1.4	6.5 ± 0.7	-	7.0 ± 1.4	6.5 ± 0.7
	5%	12.0 ± 0.0	18.0 ± 2.8	18.5 ± 2.1	6.0 ± 0.0	6.0 ± 0.0	-	-	-
	2.5%	6.0 ± 0.0	12.0 ± 0.0	10.0 ± 2.8	-	-	-	-	-
	1%	5.5±0.0	10.5 ± 0.7	7.5 ± 2.1	-	-	-	-	-
S8	10%	19.0 ± 0.0	20.5 ± 0.7	15.0 ± 0.0	-	6.0±0.0	-	-	7.0 ± 0.0
	5%	12.5 ± 0.7	15.5 ± 0.7	14.0 ± 0.0	-	-	-	-	6.5 ± 0.7
	2.5%	12.0 ± 0.0	14.5 ± 0.7	11.5 ± 2.1	-	-	-	-	-
	1%	11.0 ± 0.0	13.0 ± 1.4	9.0 ± 0.0	-	-	-	-	-
S9	10%	20.0 ± 1.4	24.5 ± 0.7	20.0 ± 0.0	-	6.5 ± 0.7	-	-	6.5 ± 0.7
	5%	12.5 ± 2.1	20.0 ± 0.7	16.5 ± 0.7	-	-	-	-	-
	2.5%	11.0 ± 1.4	18.0 ± 0.7	13.0 ± 1.4	-	-	-	-	-
	1%	8.5 ± 0.7	12.0 ± 1.4	8.5 ± 2.1	-	-	-	-	-
S10	10%	24.5 ± 0.7	21.0 ± 1.4	21.0 ± 1.4	7.5 ± 0.7	6.5 ± 0.7	8.0 ± 0.0	-	7.0 ± 0.0
	5%	22.0 ± 2.8	18.5 ± 2.1	15.0 ± 1.4	-	-	6.0 ± 0.0	-	-
	2.5%	13.0 ± 1.4	15.0 ± 1.4	10.0 ± 0.0	-	-	-	-	-
	1%	10.0 ± 0.0	12.0 ± 0.0	8.0 ± 1.4	-	-	-	-	-
S11	10%	20.0 ± 0.0	18.5 ± 0.7	16.0 ± 1.4	17.5 ± 0.7	11.0 ± 0.0	13.5 ± 2.1	-	18.5 ± 2.1
	5%	18.5 ± 0.7	17.0 ± 1.4	14.0 ± 1.4	15.5 ± 0.7	6.0 ± 0.0	-	-	7.0 ± 0.0
	2.5%	15.0 ± 1.4	12.0 ± 1.4	11.5 ± 0.7	12.0 ± 0.0	-	-	-	-
	1%	6.0 ± 0.0	10.0 ± 0.0	8.5 ± 0.7	6.0 ± 0.0	-	-	-	-
S12	10%	20.5 ± 0.7	24.5 ± 0.7	16.5 ± 2.1	10.5 ± 2.1	11.5 ± 0.7	-	-	9.5 ± 0.7
	5%	12.0 ± 2.8	16.0 ± 0.0	13.5 ± 0.7	8.0 ± 0.0	6.5 ± 0.7	-	-	7.0 ± 0.0
	2.5%	10.0 ± 0.0	13.5 ± 0.7	10.5 ± 0.7	7.0 ± 0.0	-	-	-	-
	1%	7.5 ± 0.7	12.0 ± 0.0	8.0 ± 1.4	6.5 ± 0.7	-	-	-	-

-: no inhibition.

S: Samples were coded and determined above Tab. 1.

This is the first report that envisages the effect of seasonal variation on in vitro antimicrobial, antiradical and antioxidant activities of methanol extracts of Clary sage. No differences were found between the methanolic extracts of plants collected at 2005 and 2006 for phenolic contents and antioxidant activities. However, the higher phenolic content and antioxidant activity observed from Clary sage collected during the midday. Also, according to the findings of this study, antimicrobial, antiradical and antioxidant activities of Clary sage seems worthy of further consideration.

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