Evaluation of the Antioxidant and Antimicrobial Activities of Clary Sage (*Salvia sclarea* L.)

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Abstract: The present work evaluates the antioxidant and antimicrobial activity of clary sage (CS) *Salvia sclarea* L. Antimicrobial, total antioxidant, DPPH radical scavenging, superoxide anion radical scavenging, hydrogen peroxide scavenging and metal chelating activities, reducing power, and total contents of phenolic compounds of dried herb samples extracted with chloroform and acetone were studied. The chloroform extract had stronger total antioxidant activity than the acetone extract and exhibited 93 and 68% inhibition of linoleic acid peroxidation, respectively. a-Tocopherol, quercetin, butylated hydroxyanisole and butylated hydroxytoluene were used as standard antioxidants. Antimicrobial activities of both CS extracts were examined by means of disk-diffusion methods with 11 microbial species (*Bacillus megaterium* NRS, *Proteus vulgaris* FMC 1, *Listeria monocytogenes* BRIE 1, *Bacillus cereus* FMC 19, *Staphylococcus aureus* FÜ, *Bacillus brevis* FMC 3, *Klebsiella pneumoniae* FMC 5, *Micrococcus luteus* LA 2971, *Pseudomonas aeruginosa* DSM 50071, *Escherichia coli* DM and *Mycobacterium smegmatis* CCM 2067) and 4 fungal species (*Penicillum frequentans*, *Fusarium equiseti*, *Aspergillus candidus* and *Byssochlamys fulves*). Both CS extracts were effective in inhibiting the growth of the organisms except for *Escherichia coli* DM. The antifungal activity of each of the above extracts is lower than the antimicrobial activity.

Key Words: Antioxidant activity, antimicrobial activity, clary sage, *Salvia sclarea*

Misk Adaçayının (*Salvia sclarea* L.) Antioksidan ve Antimikrobiyal Aktivitelerinin Değerlendirilmesi

Özet: Bu çalışma, misk adaçayının (*Salvia sclarea* L.) antioksidan ve antimikrobiyal aktivitelerini değerlendiren bir çalışmadır. Kurutulmuş bitki numunesinin kloroform ve aseton ekstrelerinin antimikrobiyal, total antioksidan, DPPH radikal giderme, süperoksit anyonu radikali giderme, hidrojen peroksit giderme, metal şelatlama ve indirgeme kuvveti aktiviteleri ile total fenolik bileşik içerikleri incelendi. Kloroform ekstresinin aseton ekstresinden daha fazla total antioksidan aktiviteye sahip olduğu gözlendi. Kloroform ekstresi linoleik asit emülsiyonunun peroksidasyonu % 93 inhibe ederken, aseton ekstresi linoleik asit emülsiyonunun peroksidasyonu % 68 inhibe inhibe ettiği gözlendi. a-Tokoferol, kuersetin, bütillenmiş hidroksi anisol (BHA) ve bütillenmiş hidroksi toluen (BHT) standart antioksidann olarak kullanıldı. Misk adaçayının her iki ekstresinin antimikrobiyal aktivitleri ise disk-difüzyon metoduna göre yapıldı. Bu amaçla *Bacillus megaterium* NRS, *Proteus vulgaris* FMC 1, *Listeria monocytogenes* BRIE 1, *Bacillus cereus* FMC 19, *Staphylococcus aureus* FÜ, *Bacillus brevis* FMC 3, *Klebsiella pneumoniae* FMC 5, *Micrococcus luteus* LA 2971, *Pseudomonas aeruginosa* DSM 50071, *Escherichia coli* DM ve *Mycobacterium smegmatis* CCM 2067 türler kullanıldı. *Penicillum frequentans*, *Fusarium equiseti*, *Aspergillus candidus* ve *Byssochlamys fulva* olmak üzere dört fungal tür kullanıldı. Her iki ekstrenin de *Escherichia coli* DM dışında kullanılan bütün mikroorganizmaların gelişimini durdurmada etkili oldukları belirlendi. Çalışmada kullanılan ekstrelerin fungusların gelişimlerini inhibisyon etkileri antimikrobiyal aktivitelere oranla çok daha düşük oldukları gözlendi.

Anahtar Sözcükler: Antioksidan aktivite, antimikrobiyal aktivite, misk adaçayı, *Salvia sclarea*

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Introduction

Salvia sclarea L. belongs to the family Labiatae. *S. sclarea*, also known as clary sage (CS), a plant native to southern Europe, is one of the most important aromatic plants cultivated worldwide as a source of essential oils and many other compounds derived from different parts of the plant. CS has shown diverse biological activities manifested by different components (mainly of essential oil) that allowed for many medicinal and pharmaceutical applications of the plant materials and/or extracts (Leung and Foster, 1996). Recent studies reported analgesic, anti-inflammatory (Moretti et al., 1997) and antimicrobial effects (Peana et al., 1999), virological evaluations (Hudaib, 2001), and genotoxic properties (Zani et al., 1991) of the plant essential oil fractions and their relation to the chemical composition of the oil. The effects of the plant extracts on skeletal and smooth muscles (Stanassova-Shopova 1970; Lis-Balchin and Harts, 1997) and the hematopoietic, immune and enzyme systems (Tkachuk and Shapoval, 1987) have also been reported. The chemical composition of the essential oil from CS was found to be almost exclusively determined by the geographical habitat 'source' of the plant growing wild or cultivated (Dzumayev et al., 1995, Torres et al., 1997; Souleles and Argyriadon, 1997, Peana et al., 1999) and few differences were observed from different plant parts (except for leaves) or different harvest and cultivation conditions (Dzumayev et al., 1995). In addition, the various bioactivities of the oil were found to correlate with its composition, particularly the major components (Moretti et al., 1997; Lis-Balchin et al., 1998). However, CS has bioactive compounds such as salvinolone, salvipisone, acetylsalvipisone, sclerapinone (Ulubelen et al., 1997) and sclareol (Ulubelen et al., 1994). In addition, the main constituent of *Salvia* species is salvianolic acid (Lu and Foo, 2001). CS is one of the most common salvia species. The aerial parts of this plant are used as antidiarrhea and tranquillizer drugs in Turkish folk medicine (Baytop, 1999). The present study aimed to assesses the antioxidant and antimicrobial activities of CS growing in Anatolia.

The oxygen consumption inherent in cell growth leads to the generation of a series of reactive oxygen species (ROS). These ROS are molecules such as superoxide anion radicals ($O_2^{.-}$) and hydroxyl radicals ($OH^{.}$). However, non-free radical species such as hydrogen peroxide (H_2O_2) and singled oxygen ($^1O_2$) are formed in vivo also. Both oxygen species play a positive role in energy production, phagocytosis, regulation of cell growth intercellular signalling, and synthesis of biologically important compounds. However, ROS may also be very damaging; they can attack the lipids of cell membranes and DNA. The oxidation induced by ROS can result in cell membrane disintegration, membrane protein damage and DNA mutation, which can further initiate or propagate the development of many diseases (Valentão et al., 2002; Gülçin et al., 2003c). As a result, they can easy initiate the peroxidation of the membrane lipids. ROS are continuously produced during normal physiologic events and are removed by antioxidant defense mechanisms (Büyükokuroğlu et al., 2001; Chang et al., 2001; Gülçin et al., 2002a; Gülçin et al., 2002b).

The major pathway of lipid peroxidation contains a self-catalytic free radical chain reaction. However, lipid peroxidation can be catalyzed by environmental factors, such as light, oxygen, free radicals and metal ions (Frankel, 1991). The discovery of the inhibition of lipid peroxidation by some phenolic compounds during the late 1940s has contributed to the application of synthetic antioxidants in the food industry (Sherwin, 1990). Lipid peroxidation is a major cause of food deterioration, leading to a loss of functional properties and nutritional value (Yen et al., 1999). Synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG) and tert-butylhydroquinone (TBHQ) have been dominant since their introduction. Because of this effect, they have been used as an antioxidant in foods for years. However, some physical properties of BHT and BHA such as their high volatility and instability at elevated temperatures, strict legislation on the use of synthetic food additives and consumer preferences have shifted the attention of manufacturers from synthetic to natural antioxidants (Porter, 1980). Consumers are increasingly avoiding foods prepared with preservatives of chemical origin, and natural alternatives are therefore needed to achieve sufficiently a long shelf life of foods and a high degree of safety with respect to food borne pathogenic microorganisms (Rauha et al., 2000, Oktay et al., 2003). Especially in the past few years, there has been increasing interest in finding natural antioxidants because they can protect the human body from free radicals and ROS related effects and retard the progress of many chronic diseases as well as retard lipid oxidative rancidity in foods (Pryor, 1991; Kinsella et al., 1993, Gülçin et al., 2003a).

Recent studies showed that a number of plants products include polyphenolic substances such as flavonoids and tannins. Those natural antioxidative substances usually have a phenolic moiety in their molecular structure. They have been found among flavonoids, tocopherols and catechines. Organic acids, carotenoids, protein hydrolysates and tannins can act as antioxidants or have synergistic effects when used together with phenolic antioxidants. Currently, materials that inhibit lipid oxidation can be obtained from plant materials, food waste, microorganisms, and animal cells (Dugan, 1980; Langseth, 1995).

The spoilage and poisoning of foods by microorganisms are problems that have not yet been brought under adequate control despite the range of robust preservation techniques available. In nature, there are a large number of different types of antimicrobial compounds that play an important role in the natural defense of all kinds of living organism.

Materials and Methods

Chemicals

1,1-Diphenyl-2-picryl-hydrazil (DPPH$\cdot$), 3-(2-Pyridyl)-5,6-bis (4-phenyl-sulfonic acid)-1,2,4-triazine (Ferrozine), ferrous chloride, polyoxyethylenesorbitan monolaurate (Tween-20), α-tocopherol, linoleic acid, nicotinamide adenine dinucleotide (NADH), pyrocatechol, Folin-Ciocalteu reagent and trichloracetic acid (TCA) were purchased from the Sigma Chemical Co. (St. Louis, USA). Ammonium thiocyanate was purchased from E. Merck (Darmstadt, Germany). All other chemicals were of analytical grade.

Plant materials and preparation of extracts

The plant material was collected from Kahramanmaraş in May and authenticated by Dr. Ahmet Ilçim, Department of Biology, Faculty of Science and Arts, Sütçü Imam University, Kahramanmaraş, Turkey (Herbarium number: I Varol 154 KSUH). CS was left on a bench to dry. The dried sample was chopped into small parts with a blender. For chloroform and acetone extracts, 20 g of dried powder of CS was extracted with 400 ml of solvent until extraction solvents became colorless; then extracts of each solvent were evaporated under reduced pressure and the final residues were used for the bioassays.

Microorganisms

Bacteria (*Bacillus megaterium*, *Proteus vulgaris*, *Listeria monocytogenes*,, *Bacillus cereus*, *Staphylococcus aureus*, *Bacillus brevis*, *Klebsiella pneumoniae*, *Micrococcus luteus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Mycobacterium smegmatis*) and fungi (*Penicillum frequentans*, *Fusarium equiseti*, *Aspergillus candidus* and *Byssochlamys fulva*) were obtained from the stock cultures of the Microbiology Laboratory, Department of Biology, Faculty of Science and Arts, Sütçü İmam University, Kahramanmaraş. The bacterial and fungal stock cultures were maintained on Muller Hinton Agar (Oxoid) slants, which were stored at 4 $^{\circ}$C. For antimicrobial evaluation 11 methicillin-resistant microorganisms, i.e. *Staphylococcus aureus,* were cultured in appropriate broths at 37 $^{\circ}$C overnight.

Antimicrobial activity determination

Agar cultures of the test microorganisms were prepared as described by Mackeen et al. (1997). For screening, 30 mg of extract was loaded onto each disks (ø, 6 mm) (Schleicher & Schül, Germany) and placed on the previously inoculated agar. The plates were incubated for 18 h at 30 $^{\circ}$C for bacterial species and 48 h at 30 $^{\circ}$C for fungal species. Clear inhibition zones around the disks indicated antimicrobial activity. Streptomycin (20 mg.disk^{-1}) was used as a positive control for bacteria.

Antioxidant activity determination

Total antioxidant activity of both CS extracts was determined according to the thiocyanate method described by Mitsuda et al. (1996). The inhibition of lipid peroxidation as a percentage was calculated by following equation:

% Inhibition = 100 - [(A$_1$ / A$_o$) x 100]

where A$_o$ was the absorbance of the control reaction and A$_1$ was the absorbance in the presence of the CS extract sample (Duh et al., 1999).

The reducing power of CS extracts was determined according to the method of Oyaizu (1986). Increased absorbance of the reaction mixture indicated increased reducing power. a-Tocopherol, quercetin and BHT, standard and commercial antioxidants, were used as reference standards.

The superoxide anion scavenging activity of both CS extracts and standards was measured based on the method described by Nishimiki et al. (1972). The PMS-NADH-NBT system was used in this assay.

The free radical scavenging activity of both CS extracts was measured by 1,1-diphenyl-2-picrylhydrazyl radicals using the method of Blois (1958).

The chelating of ferrous ions by the extracts of CS on ferrous ions was estimated by the method of Dinis (1994).

The ability of the CS extracts to scavenge hydrogen peroxide was determined according to the method of Ruch (1989).

Total soluble phenolics in both CS extracts were determined with Folin-Ciocalteu reagent according to the method of Slinkard and Singleton (1977) using pyrocatechol as a standard. The absorbance was measured at 760 nm in a spectrophotometer (8500 II, Bio-Crom Gmb, Zurich, Switzerland). The concentrations of total phenolic compounds in the CS extracts were determined as micrograms of pyrocatechol equivalent.

Statistical analysis

Experimental results were the mean ± S.D. of 3 parallel measurements. Analysis of variance was performed. Significant differences between means were determined by Duncan's multiple range tests. P values < 0.05 were regarded as significant and P values < 0.01 as very significant.

Results and Discussion

Disk diffusion methods are extensively used to investigate the antibacterial activity of natural antimicrobial substance and plant extracts. These assays are based on the use of disks as reservoirs containing the solution of substances to be examined. In the case of solutions with a low activity, however, a large concentration or volume is needed. The limited capacity of disks means that holes or cylinders are preferred (Bartner et al., 1994).

In this study, 11 different microbial and 4 different fungal species were used to screen the possible antimicrobial activities of CS extracts. Ten bacterial species and 4 fungal species were sensitive to the antimicrobial activity of extracts as shown in Table 1. All

Table 1. Antimicrobial activities of chloroform and acetone extracts of clary sage (30 μg. disk)$^{-1}$ and Streptomycin (20 mg.disk^{-1}).

Microorganisms	Clary sage extracts		Streptomycin
	Chloroform	Acetone	
Bacteria			
Bacillus megaterium	7 ± 1.00[*,b]	9 ± 0.00[*,b]	17 ± 2.00[*,a,b]
Proteus vulgaris	7 ± 1.00[*,b]	8 ± 2.00[*,b,c]	18 ± 3.00[*,a,b]
Listeria monocytogenes	-	-	19 ± 1.53[*,a]
Bacillus cereus	-	9 ± 1.00[*,b]	14 ± 0.57[*,d]
Staphylococceus aureus	7 ± 0.00[*,b]	-	17 ± 1.33[*,a,b,c]
Bacillus brevis	7 ± 1.00[*,b]	8 ± 1.33[*,b,c]	16 ± 1.00[*,a,b]
Klebsiella pneumoniae	-	7 ± 2,00[*,c]	15 ± 1,53[*,b,c]
Micrococcus luteus	8 ± 1.00[*a,b]	8 ± 1.00[*,b,c]	16 ± 0.00[*,a,b,c]
Pseudomonas aeruginosa	7 ± 1.53[*,b]	9 ± 1.53[*,b]	14 ± 2.00[*,c]
Mycobacterium smegmatis	9 ± 1.58[*,a]	7 ± 0.58[*,c]	15 ± 3.00[*,b,c]
Escherichia coli	-	-	-
Fungus			
Penicillum frequentans	-	-	ND
Fusarium equiseti	-	-	ND
Aspergillus candidus	7 ± 2.00[*,b]	-	ND
Byssochlamys fulva	9 ± 0.577[*,a]	22 ± 1.881[*,a]	ND

Values are the mean of 3 replicates. AE: Acetone extract (30 mg.disk^{-1}), CE: Chloroform extract (30 mg.disk^{-1}), Streptomycin (20 mg.disk^{-1}), ND: Not determined. *: Means in row with different superscripts differ significantly (P < 0.05)

of the extracts showed strong antibacterial activity against this bacterium (*Staphylococcus aureus*), but neither CS extract showed antibacterial activity against *Escherchia coli*, in that all of the extracts failed to inhibit the growth of *Escherchia coli* and *Listeria monocytogenes*.

In the present study, the antioxidant activities of acetone and chloroform extracts of CS were determined by thiocyanate methods. The amounts of peroxides formed in linoleic acid emulsion during incubation were determined spectrophotometrically by measuring absorbance at 500 nm. High absorbance was an indication of a high concentration of formed peroxides. As can be seen in Figure 1, antioxidant activity was shown by both extracts of CS assayed. Both extracts showed effective levels of inhibitory activity towards lipid peroxidation. The antioxidant activity of these extracts was higher than that of a-tocopherol. There was a significant difference between samples of CS extracts and the control ($P < 0.05$), in which there were no extract samples. Chloroform extract of CS showed stronger antioxidant activity than the acetone extract. There was a statistically significant difference between the antioxidant activities of the 2 solvent extracts, $P < 0.05$. This observation shows that antioxidant components present in the chloroform extract contributed to the increased activity over the acetone extract. The antioxidant activities of each extract increased with increasing amounts of extracts, but these values are not shown. The percentage of inhibition of 0.25 mg of chloroform and

acetone extract of CS on peroxidation in the linoleic acid system was 93 and 68%, respectively, and greater than that of the same doses of α-tocopherol (46%). The percentage of inhibition on peroxidation of chloroform and acetone extracts of CS and a-tocopherol followed the order: chloroform extract of *Salvia sclarea* > acetone extract of *Salvia sclarea* > a-tocopherol.

For the measurement of reductive ability, we investigated the Fe^{3+}-Fe^{2+} transformation in the presence of samples of the CS extracts and standard antioxidants, a-Tocopherol, quercetin and BHA, using the Oyaizu method (1986). It has been reported that reducing power was associated with antioxidant activity (Yen and Duh, 1993). As shown in Figure 2, chloroform and acetone extracts had statistically similar reductive capabilities. The differences between the both extracts and control were not statistically significant ($P > 0.05$). The reducing power of chloroform and acetone extracts of CS and standards followed the order: BHA > a-tocopherol > quercetin > chloroform extract of CS > acetone extract of CS.

In the PMS/NADH-NBT system, superoxide anion derived from dissolved oxygen by a PMS/NADH coupling reaction reduces NBT. The decrease of absorbance at 560 nm with antioxidants thus indicates the consumption of superoxide anion in the reaction mixture. Figure 3 shows the superoxide radical scavenging activity of 100 mg of both acetone and chloroform extracts of the CS and standards. Both extracts exhibited strong superoxide radical scavenging activity. The results were statistically
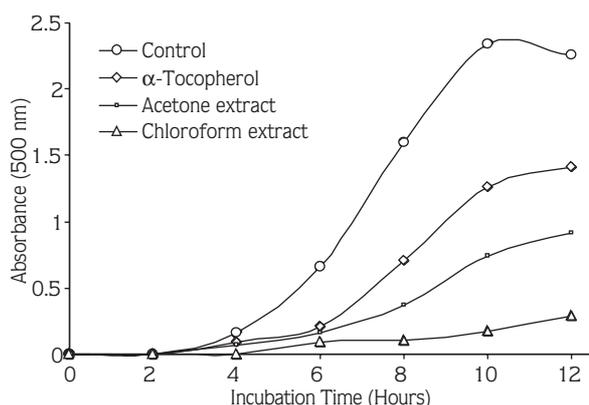

Figure 1. Determination of antioxidant activity of 250 µg of chloroform and acetone extracts of clary sage and α-tocopherol in the linoleic acid emulsion was determined by the thiocyanate method.
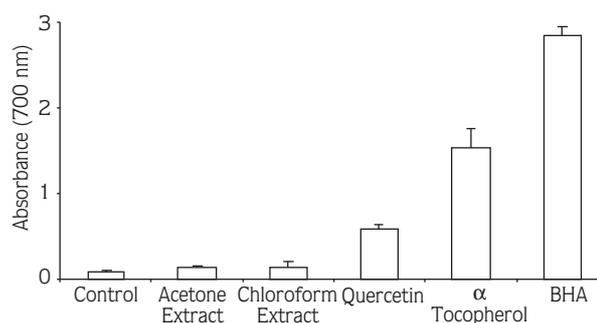

Figure 2. Comparison of reducing power of 100 µg of chloroform and acetone extracts of clary sage, quercetin, BHA and α-tocopherol by spectrophotometric detection of the Fe^{+3}-Fe^{+2} transformations (BHA: Butylated hydroxyanisole).

significant (P < 0.05). The superoxide anion radical scavenging activity of these samples of CS extracts followed the order: BHT > acetone extract of CS > BHA > chloroform extract of CS > quercetin.

DPPH· is a stable free radical in aqueous or ethanol solution and accepts an electron or hydrogen radical to become a stable diamagnetic molecule (Soares et al., 1997). In order to evaluate antioxidant potency through free radical scavenging with the test samples, the change in the optical density of DPPH radicals was monitored. Hence, DPPH· is usually used as a substrate to evaluate the antioxidative activity of antioxidants (Duh et al., 1999). Figure 4 illustrates the decrease in absorbance of DPPH radicals due to the scavenging ability of soluble solids in 100 mg of CS extracts and standards. Chloroform extracts of CS had stronger free radical scavenging activity than acetone extracts. The results were statistically significant when compared to the control values (P < 0.05). However, both extracts exhibited lower free radical scavenging activity than the standards. The free radical scavenging activity of both CS extracts and standard compounds followed the order: quercetin > BHT > BHA > chloroform extract of CS > acetone extract of CS.

Ferrozine can quantitatively form complexes with Fe^{2+}. In the presence of other chelating agents, the complex formation is disrupted with the result that the red color of the complex is decreased. Measurement of the rate of color reduction therefore allows estimation of the chelating activity of the coexisting chelator (Yamaguchi et al. 2000). In this assay both extracts of CS

and standard compounds interfered with the formation of ferrous and ferrozine complexes, suggesting that it has chelating activity and captures ferrous ions before ferrozine ions. The metal binding capacity of CS extracts and standard antioxidants was determined by assessing their ability to compete with ferrozine for the ferrous ions. As shown in Figure 5, the formation of ferrozine-Fe^{+2} complex is not complete in the presence of CS extracts and standards. The ability of chelating ferrous ions also increased with increasing amounts of CS extracts. It is indicated that CS extracts chelate the iron. The metal chelating effect of CS extracts is close to that of a-tocopherol and BHT, but lower than that of BHA and this difference was statistically significant (P < 0.05) when compared to the control. Furthermore, 250 mg of chloroform and acetone extracts of CS exhibited 31.6 and 37.7% iron binding capacity, respectively. On the other hand, the same concentration of a-tocopherol, BHT and BHA showed 43.0, 40.6 and 74.8% iron chelating activity, respectively. Those values obtained from Figure 5 demonstrated that the action of CS extracts, as a peroxidation protector, may be more related to its iron binding capacity.

H_2O_2 also forms OH· in the presence of metal ions and oxygen facilitates this reaction. Hence, metal chelating and H_2O_2 scavenging processes are important for living organisms (Gülçin et al., 2003b). The scavenging ability of CS extracts on hydrogen peroxide is shown in Figure 6 and compared with BHA, BHT and α-tocopherol as standards. CS extracts were capable of scavenging hydrogen peroxide in an amount–dependent manner.
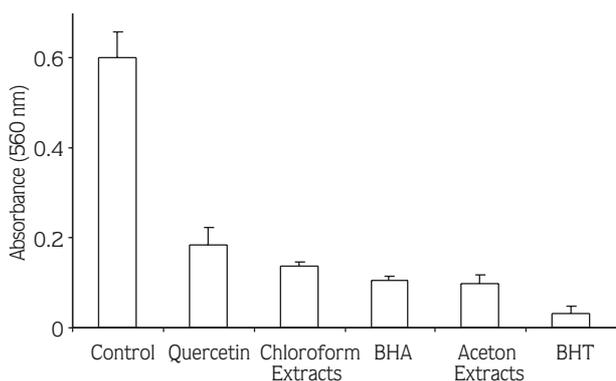

Figure 3. Superoxide anion radical scavenging activity of 100 mg of chloroform and acetone extracts of clary sage, quercetin, BHA and BHT by the PMS – NADH - NBT system (BHA: Butylated hydroxyanisole, BHT: Butylated hydroxytoluene).
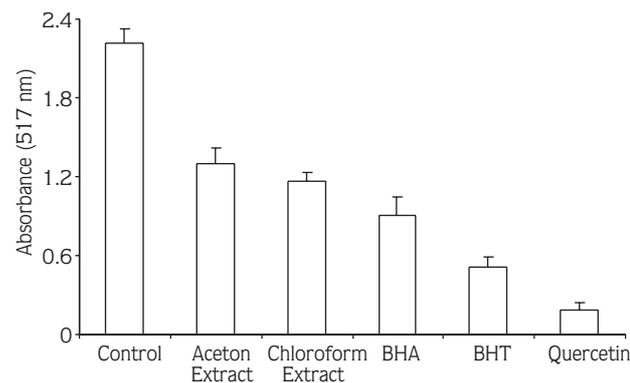

Figure 4. Free radical scavenging activity of 100 mg of chloroform and acetone extracts of clary sage, quercetin, BHA and BHT by 1,1-diphenyl-2-picrylhydrazyl radicals. (BHA: Butylated hydroxyanisole, BHT: Butylated hydroxytoluene).
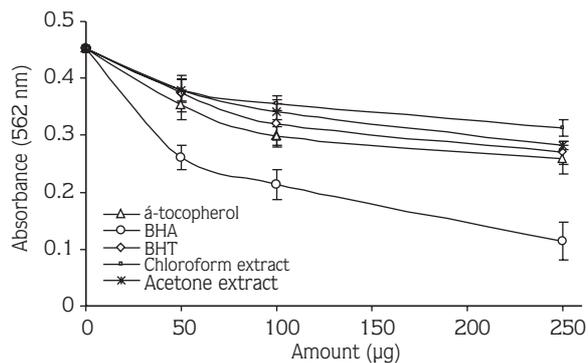
Figure 5. Metal chelating activity of different concentrations (50, 100 and 250 mg.ml^{-1}) of chloroform and acetone extracts of clary sage, α-tocopherol, BHA, and BHT on ferrous ions (BHA: Butylated hydroxyanisole, BHT: Butylated hydroxytoluene).
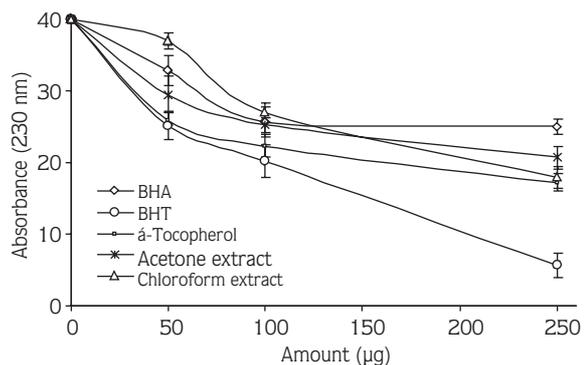

Figure 6. Hydrogen peroxide scavenging activity of different concentrations (50, 100 and 250 mg.ml^{-1}) of chloroform and acetone extracts of clary sage, α-Tocopherol, BHA, and BHT (BHA: Butylated hydroxyanisole, BHT: Butylated hydroxytoluene).

Acetone and chloroform extracts (250 mg) of CS exhibited 42.5 and 55.0% scavenging activity on hydrogen peroxide, respectively. On the other hand, at the same dose, BHA, BHT and a-tocopherol exhibited 37.3, 86.0 and 57.0% hydrogen peroxide scavenging activity. These results showed that CS extracts had stronger hydrogen peroxide scavenging activity than BHA. Those values are close to those of a-tocopherol but lower than those of BHT. There is a statistically significant correlation between these values and the control values ($P < 0.05$). Hydrogen peroxide itself is not very reactive, but it can sometimes be toxic to cells because it may give rise to hydroxyl radical in the cells (Halliwell, 1991; Gülçin et al., 2003b). Thus, the removal of H_2O_2 as well as O_2^- is very important for antioxidant defense in cell or food systems.

Phenolic antioxidants are potent free radical terminators (Shahidi and Wanasundara, 1992). Phenolic compounds, biologically active components, are the main agents that can donate hydrogen to free radicals and thus break the chain reaction of lipid oxidation at the first initiation step. This high potential of phenolic compounds to scavenge radicals may be explained by their phenolic hydroxyl groups (Sawa et al., 1999). The amount of total phenolic compounds was determined as the pyrocatechol equivalent using an equation obtained from a standard pyrocatechol graph. The equation is given below:

Absorbance = 0.001 x mg Pyrocatechol + 0.0033

The standard graph was prepared with 0.1 ml of pyrocatechol solution containing 0-400 mg of pyrocatechol in place of CS extracts. To determine the amount of total phenolic compounds in the extracts, the absorbance of a sample, which contains 1000 mg of dried extract, was measured at 760 nm using a spectrophotometer (8500 II, Bio-Crom Gmb, Zurich, Switzerland). The absorbance value was inserted in the above equation and the total amount of phenolic compound was calculated. The results of the present investigation indicated that the acetone extract of CS had higher total phenolic compounds than chloroform extract. As seen in Table 2, the total phenolic contents of acetone and chloroform extracts of CS are 35.24 to 28.91 mg of pyrocatechol equivalent. According to these results, there is no relationship between total phenols and antioxidant activity. In many cases the high antioxidant activity was not correlated with the phenol content; probably other factors played major roles as antioxidants (Velioglu et al., 1998).

In conclusion, both CS extracts have antioxidant and antimicrobial activities. Accordingly, this implies the inhibition of microbial pathogenesis, and cellular oxidation that is linked to pathological incidents such as heart disease, aging and cancer. However, the components responsible for the antioxidative and antimicrobial activities of these CS extracts are currently unclear. Therefore, it is suggested that further work be performed on the isolation and identification of the antioxidative and antimicrobial components of CS extracts.

Table 2. The percentage of inhibition of lipid peroxidation in linoleic acid emulsion and total phenolic compounds in 1 mg of chloroform and acetone extracts of clary sage.

	Total Phenolic Content (µg) *	Inhibition of Lipid Peroxidation (%)
Acetone Extract	35.24 ± 4.48[†,a]	68 ± 12.7*
Chloroform Extract	28.91 ± 3.12[†,b]	93 ± 10.8*

*Microgram equivalent of pyrocatechol. † :Means in row with different superscripts differ significantly (P < 0.05)

References

Bartner, A., K.P. Pfeiffer and H. Bartner, 1994. Applicability of diffusion methods required by the pharmacopoeias for testing antibacterial activity of natural compounds. Pharmazie. 49: 512-516.

Baytop, T. 1999. Therapy with medicinal plants (past and present). İstanbul, İstanbul University Publications, pp. 143.

Blois, M.S. 1958. Antioxidant determinations by the use of a stable free radical. Nature 26: 1199-1200.

Büyükokuroğlu, M.E., I. Gülçin, M. Oktay and O.İ. Küfrevioğlu, 2001. In vitro antioxidant activity of dantrolene sodium. Pharmacol. Res. 46: 491-494.

Chang, S.T., J.H. Wu, S.H. Wang, P.L. Kang, N.S. Yang and L.F. Shyur. 2001. Antioxidant activity of extracts from *Acacia confusa* bark and heartwood. J. Agric. Food Chem. 49: 3420-3424.

Dinis, T.C.P., V.M.C. Madeira and L.M. Almeida, 1994. Action of phenolic derivates (acetoaminophen, salycilate and 5-aminosalycilate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. Arch. Biochem. Biophys. 315: 161-169.

Dugan, L.R. 1980. Natural antioxidants. In: Autoxidation in food and Biological Systems, eds G., Simic and M., Karel, Cambridge, MA, USA, Pp. 263-282

Duh, P.D., Y.Y. Tu and G.C. Yen, 1999. Antioxidant activity of water extract of harng jyur (*Chrysanthemum morifolium* Ramat). Lebensm.-Wiss.u-Technol. 32: 269-277.

Dzumayev, K.K., I.A. Tsibulskaya, I.G. Zenkevich, K.G. Tkachenko and I.F. Satzyperova. 1995. Essential oils from *Salvia sclarea* L. produced from plants grown in southern Uzbekistan, J. Essent. Oil. Res. 7: 579–604.

Frankel, E.N. 1991. Recent advances in lipid oxidation. J. Sci. Food Agric. 54: 495-511.

Gülçin, I., M., Oktay, O.I. Küfrevioğlu and A. Aslan, 2002a. Determination of antioxidant activity of lichen *Cetraria islandica* (L) Ach. J. Ethnopharmacol. 79: 325-329.

Gülçin, I., M.E. Büyükokuroğlu, M. Oktay, and Ö.İ. Küfrevioğlu. 2002b. On the *in vitro* antioxidant properties of melatonin. J. Pineal Res. 33: 167-171.

Gülçin, I., M.E. Büyükokuroğlu, M. Oktay and Ö.İ. Küfrevioğlu, 2003a. Antioxidant and analgesic activities of turpentine of *Pinus nigra* Arn. Subsp. *pallsiana* (Lamb.) Holmboe. J. Ethnopharmacol. 86: 51–58.

Gülçin, I., M.E. Büyükokuroğlu, M. Oktay and Ö.İ. Küfrevioğlu, 2003b. Metal chelating and hydrogen peroxide scavenging effects of melatonin. J. Pineal Res. 34: 278-281.

Gülçin, İ., M. Oktay, E. Kireçci, and Ö.İ. Küfrevioğlu, 2003c. Screening of antioxidant and antimicrobial activities of anise (*Pimpinella Anisum* L.) seed extracts, Food Chem. 83: 371-382.

Halliwell, B. 1991. Reactive oxygen species in living systems: Source, biochemistry, and role in human disease. Am. J. Med. 91: 14-22.

Hudaib, M., M.G. Bellardi, C. Rubies-Autonell, J. Fiori and V. Cavrini. 2001. Chromatographic (GC-MS, HPLC) and virological evaluations of *Salvia sclarea* infected by BBWV-I. Ill Farmaco 56: 219-227.

Kinsella, J.E., E. Frankel, B. German and J. Kanner, 1993. Possible mechanisms for the protective role of antioxidant in wine and plant foods. Food Thechnol. 47: 85-89.

Langseth, L. 1995. Oxidant, antioxidants and disease prevention. JLSI Europe, Brussels, Belgium, pp 4-13.

Leung, A.Y. and S. Foster, 1996. Encyclopaedia of common natural ingredients used in food, drugs and cosmetics, 2nd ed. Wiley, New York, pp. 173–175.

Lis-Balchin, M., S.G. Deans and E. Eaglesham. 1988. Relationship between bioactivity and chemical composition of commercial essential oils, Flavour Fragrance J. 13: 98-104.

Lis-Balchin, M. and S. Harts. 1977. A preliminary study of the effect of essential oils on skeletal and smooth muscles in vitro. J. Ethnopharmacol. 58: 183-187.

Lu, Y. and L.Y. Foo. 2001. Salvianolic acid, a potent phenolic antioxidant from *Salvia officinalis*. Tetrahedron Let. 42: 8223-8225.

Mackeen, M.M., A.M. Ali, S.H. El-Sharkawy, M.Y. Manap, K.M. Salleh, N.H. Lajis and K. Kawazu. 1997. Antimicrobial and cytotoxic properties of some Malaysian traditional vegetables. Int. J. Pharmacog. 35: 237-243.

Mitsuda, H., K. Yuasumoto and K. Iwami, 1996. Antioxidation action of indole compounds during the autoxidation of linoleic acid. Eiyo to Shokuryo, 19: 210-214

Moretti, M.D.L., A.T. Peana and M. A. Satta, 1997. A study of anti-inflammatory and peripheral analgesic actions of *Salvia sclarea* oil and its main constituents, J. Essent. Oil Res. 9: 199-204.

Nishimiki, M., N.A. Rao and K. Yagi, 1972. The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. Biochem. Biophys. Res. Commun. 46: 849-853.

Oktay, M., İ. Gülçin and Ö.İ. Küfrevioğlu, 2003. Determination of *in vitro* antioxidant activity of fennel (*Foeniculum vulgare*) seed extracts, Lebens.-Wiss. u-Technol. 36: 263–271.

Oyaizu, M. 1986. Studies on product of browning reaction prepared from glucose amine. Jpn J. Nutr. 44: 307-315.

Peana, A.T., M.D.L. Moretti and C. Juliano, 1999. Chemical composition and antimicrobial action of the essential oils of *S. desoleana* and *S. sclarea*. Planta Med. 65: 752-754.

Porter, W.L. 1980. Recent trends in food applications of antioxidants. In: Autoxidation in Food and Biological Systems, (eds. G., Simic and M., Karel, Plenum Press, New York, USA, pp. 295-366.

Pryor, W.A. 1991. The antioxidant nutrients and disease prevention–What do we know and what do we need to find out? Am. J. Clin. Nutr. 53: 391-393.

Rauha, J.P., S. Remes, M. Heinonen, A. Hopia, M. Kahkonen, T. Kujala, K. Pihlaja, H. Vuorela and P. Vuorela, 2000. Antimicrobial effects of Finnish plant extracts containing flavonoids and other phenolic compounds. Int. J. Food Microbiol. 56: 3-12.

Ruch, R.J., S.J. Cheng and J.E. Klaunig, 1989. Prevention of cytotoxicity and inhibition of intrcellular communication by antioxidant catechins isolated from Chinese green tea. Carcinogen. 10: 1003-1008.

Sawa, T., M. Nakao, T. Akaike, K. Ono and H. Maeda, 1999. Alkylperoxyl radical scavenging activity of various flavonoids and other phenolic compounds: Implications for the antitumor promoter effect of vegetables. J. Agric. Food Chem. 47: 397-492.

Shahidi, F. and P.K.J.P.D. Wanasundara, 1992. Phenolic antioxidants. Crit. Rev. Food Sci. Nutrit. 32: 67-103.

Sherwin, E.R. 1990. Antioxidants. In: Food additives. (eds. A.L., Branen, P.M., Davidson and S., Salminen, Marcek Dekker Inc, New York, USA, pp. 139-193.

Slinkard, K. and V.L. Singleton, 1977. Total phenol analyses: Automation and comparison with manual methods. Am. J. Enol. Vitic. 28: 49-55.

Soares, J.R., T.C.P. Dinis, A.P. Cunha and L.M. Ameida, 1997. Antioxidant activity of some extracts of *Thymus zygis*. Free Rad. Res. 26: 469-478.

Souleles, C. and N. Argyriadon, 1997. Constituents of the essential oil of *Salvia sclarea* growing wild in Greece. Int. J. Pharmacogn. 35: 218-220.

Stanassova-Shopova, S. and S. Roussinov, 1970. Experimental studies on certain effects of the essential oil of *Salvia sclarea* L. on the central nervous system, Izv. Ins. Fiziol. (Sofia) 13: 89-95.

Torres, M.E., A. Velasco-Negueruela, M.J. Perez-Alonzo and M.G. Pinilla, 1997. Volatile constituents of two *Salvia* species grown wild in Spain. J. Essent. Oil Res. 9: 27-33.

Tkachuk, V.G. and V.V. Shapoval, 1987. Effect of *Salvia sclarea* essential oil on indices of the hematopoietic, immune and enzyme systems, Vrachebnoe Delo, 5: 83-84.

Ulubelen, A., U. Sönmez, and G. Topçu, 1997. Diterpenoid from the roots of *Salvia sclarea.* Phytochem. 44: 1297-1299.

Ulubelen, A., G. Topcu, C. Eriş, U. Sönmez, M. Kartal, S. Kurucu, and C. Bozok-Johansson, 1994. Terpenoids from *Salvia sclarea.* Phytochem. 36: 971-974.

Valentão, P., E. Fernandes, F. Carvalho, P.B. Andrade, R.M. Seabra and M.D. Bastos, Antioxidant activity of *Hypericum androsaemum* infusion: Scavenging activity against superoxide radical, hydroxyl radical and hypochlorous acid. Biol. Pharm. Bull. 25: 1320-1323.

Velioglu, Y.S., G. Mazza, L. Gao and B.D. Oomah, 1998. Antioxidant activity and total phenolics in selected fruits, vegetables and grain products. J. Agric. Food Chem. 46: 4113-4117.

Yamaguchi, F., T. Ariga, Y. Yoshimira and H. Nakazawa, 2000. Antioxidant and antiglycation of carcinol from *Garcinia indica* fruit rind. J. Agric. Food Chem. 48: 180-185.

Yen, G.C., H.Y. Chen and C.E. Lee, 1999. Measurement of antioxidative activity in metal ion-reduced lipid peroxidation systems. J. Sci. Food Agric. 79: 1213-1217.

Zani, F., G. Massimo, S. Benvenuti, A. Bianchi, A. Albasini, M. Melegari, G. Vampa, A. Bellotti and P. Mazza, 1991. Studies on the genotoxic properties of essential oils with Bacillus subtilis rec-assay and Salmonella/microsome reversion assay. Planta Medica 57: 237-241.