Full Length Research Paper

Determination of antimicrobial and antioxidant activities of Turkish endemic *Ajuga chamaepitys* (L.) Schreber subsp. *euphratica* P.H. Davis (Lamiaceae)

S. Turkoglu[1], I. Turkoglu[2]*, M. Kahyaoglu[3] and S. Celık[1]

[1]Department of Chemistry, Faculty of Science and Arts, Fırat University, Elazığ/Turkey.
[2]Department of Biology Education, Education Faculty, Fırat University, Elazığ/Turkey.
[3]Department of Primary Education, Faculty of Education, Siirt University, Siirt /Turkey.

In this study, the antioxidant and antimicrobial activities of methanol, water and chloroform extracts of *Ajuga chamaepitys* were investigated. The antioxidant properties of *A. chamaepitys* extracts were evaluated using different antioxidant tests, such as ABTS• radical scavenging capacity, DPPH• radical scavenging capacity, superoxide anion radical scavenging, hydrogen peroxide scavenging and metal chelating activities. These various antioxidant activities were compared with synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and α-tocopherol. The water extract of *A. chamaepitys* exhibited the higher antioxidant capacity than that of methanol and chloroform. In addition, total phenolic compounds in the extracts of *A. chamaepitys* were determined as pyrocatecol equivalents. Antimicrobial activity tests were carried out using disc diffusion methods with six bacteria strains and two yeast species. The obtained results indicate that *A. chamaepitys* is a potential source of natural antioxidant.

Key words: *Ajuga chamaepitys*, antimicrobial activity, antioxidant activity.

INTRODUCTION

Reactive oxygen species (ROS) are involved in an organism's vital activities including phagocytosis, regulation of cell proliferation, intracellular signalling, synthesis of biologically active compounds and ATP. With an insufficiency of the antioxidant protective system or under an intense influence of radical-initiating factors (ionizing radiation, ultraviolet radiation, xenobiotics, mineral dust), ROS are overproduced and oxidative stress develops. In vivo or in vitro, oxidative stress may cause various problems associated with degenerative aging diseases, such as arteriosclerosis and cancer (Halliwell et al., 1992). Free radicals play an important role in some pathogenesis of serious diseases, such as neurode-generative disorders, atherosclerosis, cataracts, diabetes and inflammation (Aruoma, 1998). Oxidation processes caused by reactive oxygen species are a major cause of

deterioration of various food products,that is, significant changes do occur in flavour, colour and texture and finally lead to loss of nutritive value or complete spoilage (Miliauskas et al., 2004, Jaitak et al., 2010).

In recent years, extracts of some plants have gained special interest as sources of natural antimicrobial and antioxidant agents. Since ancient times, spices and herbs have been used for different purposes, such as food, beverages, perfumery and drug (Draughon, 2004). They have been examined for their potential uses in alternative medicine for the treatment of many infectious diseases, because of their resistance to antibiotics (Essawi and Srour, 2000) and the preservation of foods from side effects of lipid peroxidation during storage and processing (Wang et al., 1998). The oxidation of lipids is the major reaction responsible for the deterioration in food quality affecting the colour, flavour, texture and nutritive value of the foods. Synthetic antioxidants have been commonly used in foods to prevent or retard lipid oxidation. However, use of synthetic antioxidants such as butylated hydroxy anisole (BHA) and butylated hydroxy

---
*Corresponding author. E-mail: isturkoglu@firat.edu.tr. Tel: +905323826626. Fax: +904242365064.

toluene (BHT) are commonly restricted by legislative rules because they are suspected to have some toxic effects and are possible carcinogens (Imaida et al., 1983, Madhavi and Salunkhe, 1996). Therefore, there is a great interest in finding new and safe antioxidants from natural sources (Gazzani et al., 1998).

Furthermore, multiple drug/chemical resistance in both human and plant pathogenic organisms have developed due to the indiscriminate use of commercial antimicrobial drugs/chemicals commonly used in the treatment of infectious diseases (Davis, 1994). This situation necessitated new antimicrobial substances from various sources such as medicinal plants (Clark, 1996).

The Labiatae is a widespread family of about 220 genera comprising almost 4000 species distributed throughout the world (Hedge, 1982).

The plants of genus *Ajuga* are evergreen, clump-forming rhizomatous annual or perennial herbaceous flowering species in the mint family, Lamiaceae, with most plants native to Europe, Asia and Africa, but also growing in Australia and North America (Coll and Tendrón, 2008).

Ajuga L. belongs to the Lamiaceae family and is represented in Turkey by 12 species and 22 taxa (Davis, 1975; Davis, 1988).

Ajuga species are used in folk medicine of different parts of the world for the treatment of rheumatism, gout, asthma, malaria, ulcers and diarrhea and have antibacterial, antitumor, antifeedant and vulnerary properties (Chen et al., 1996; Ben et al., 2000). *Ajuga* plants are used to treat diabetes,and hypertension (Eddouks et al., 2007). Several reported activities of Ajuga plants include anti-inflammatory (Marc et al., 2008) and antioxidant (Chenni et al., 2007). There are some reports on the phytochemical analysis of species belonging to *Ajuga* found in the literature but only a very small number of these species have so far been studied chemically for their essential oils. Some scientific studies on *Ajuga* species show the presence of many compounds belonging mainly to the groups of alkaloids, anthocyanins, tannins, withanolides, clerodane and neoclerodane diterpenoids, sterols, ionone, iridoid, phenethyl alcohol and phenylpropanoid glycosides (Chen et al., 1996; Ben et al., 2000; Akbay et al., 2003; Baser et al., 1999).

Furthermore, the antioxidant activity and radical scavenging capacity of *A. chamaepitys* has not previously been published. In this study, in vitro antioxidant, radical scavenging and antimicrobial properties of the methanol, water and chloroform extracts of *A. chamaepitys* growing in eastern part of Turkey have been investigated.

MATERIALS AND METHODS

Chemicals

Methanol and chloroform were purchased from E. Merck. Ferrous chloride, α-tocopherol, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), 3-(2-Pyridyl)-5, 6-bis (4-phenyl-sulfonic acid)-1,2,4-triazine (Ferrozine), nicotinamide adenine dinucleotide (NADH), butylatedhydroxyanisole (BHA) and butylated hydroxytoluene (BHT) were obtained from Sigma (Sigma-Aldrich GmbH, Sternheim, Germany). All other chemicals used in present study were of analytical grade and were obtained from Sigma (Sigma-Aldrich GmbH, Sternheim, Germany).

Plant material and extraction procedure

A. chamaepitys subsp. euphratica was collected from Kamışlık mountain, Elazığ region in Eastern Anatolia of Turkey when flowering (June 2009). The taxonomic identification of plant materials was determined by using flora of Turkey ((Davis, 1975). Fresh, plant material (20 g), was washed with tap water, dried and then chopped into small fragments, which was shade-dried and reduced to a coarse powder in a mortar and pestle. The powder material was extracted for three times with chloroform (99.0%), and methanol (99.8%) and followed by water in cycles of 48 h at room temperature. The filtrates of each extract were combined and concentrated in a rotavapor (IKA RV 05 basic) at reduced pressure below 40°C. Extracts were placed in a plastic bottle and then stored at -20°C until used. Then, all the extracts obtained were injected into blank antibiotic disks of 6 mm diameter (Oxoid) in amounts of 50 µl.

Microorganisms used

In vitro antimicrobial studies were carried out against six bacteria strains (Escherichia coli ATCC 25922, Staphylococcus aureus COWAN 1, Bacillus cereus FMC 3, Pseudomonas aeruginosa DSM 50071, Klebsillea pneumonia FMC 5, Enterobacter aerogenes CCM 2531), two yeast (Candida sp. and Saccharomyces cerevisiae), which were obtained from microbiology laboratory in Fırat University, Turkey.

ABTS• radical scavenging capacity

ABTS also forms a relatively stable free radical, which decolorizes in its non-radical form Shirwaikar et al. (2006). The spectrophotometric analysis of ABTS•+ radical scavenging capacity was determined according to the method of Re et al. (1999). ABTS•+ was produced by reacting 2 mM ABTS in H_2O with 2,45 mM potassium persulfate ($K_2S_2O_8$), stored for 12 h at room temperature in the dark. The ABTS•+ solution was diluted to give an absorbance of 0.750 ± 0.025 at 734 nm in 0,1 M sodium phosphate buffer (pH 7,4). Then, 1 mL of ABTS•+ solution was added to 3 mL of A. chamaepitys extracts in ethanol at 100 µg/mL concentrations. The absorbance was recorded for 30 min, after the mixing and percentage of radical scavenging were calculated for each concentration relative to a blank containing no scavenger. The extent of decolorization is calculated as percentage reduction of absorbance. The scavenging capability of test compounds was calculated by the following equation:

% Inhibition = [(Ao - A1)/Ao] x 100

where Ao is the absorbance of control and A1 is the absorbance in the presence of the sample of A. chamaepitys extracts or standards.

DPPH• radical scavenging capacity

The free radical scavenging capacity of A. Chamaepitys extracts was measured by 2,2-diphenyl-1-picryl-hydrazil (DPPH•) using the method of Shimada et al. (1992). Briefly, 0,1 mM solut ion of DPPH•

in ethanol was prepared and 1 mL of this solution was added to 3 mL of *A. chamaepitys* extracts solution in water at different concentrations (50,100 and 250 µg/ml). Absorbance at 517 nm was determined 30 min later against a blank solution containing the etanol. Lower absorbance of the reaction mixture indicates the higher free radical scavenging activity. When a hydrogen atom or electron was transferred to the odd electron in DPPH•, the absorbance at 517 nm is decreased proportionally to the increase of non-radical forms of DPPH (Gülçin et al., 2007). The capability to scavenge the DPPH• radical was calculated by the following equation:

DPPH• Scavenging Effect % = [(Ao - A1)/Ao] x 100

where Ao is the absorbance of control reaction and A1 is the absorbance in the presence of the sample of A. chamaepitys extracts.

Superoxide anion scavenging capacity

Measurement of superoxide anion scavenging capacity of *A. chamaepitys* extracts was based on the method described by Liu et al. (1997) with slight modification. One millilitre of nitroblue tetrazolium (NBT) solution (156 mmol/L NBT in 100 mmol/L phosphate buffer, pH 7.4), 1 mL NADH solution (468 mmol/L in 100 mmol/L phosphate buffer (pH 7.4), and 100 µL of sample solution of *A. chamaepitys* extracts in water were mixed. The reaction was started by adding 100 µL of phenazine methosulphate (PMS) solution (60 mmol/L PMS in 100 mmol/L phosphate buffer, pH 7.4) to the mixture. The reaction mixture was incubated at 25°C for 5 min, and the absorbance at 560 nm was measured against blank samples. Decreased absorbance of the reaction mixture shows increase in superoxide anion scavenging capacity. The percentage inhibition of superoxide anion generation was calculated by the following formula:

%Inhibition = [(Ao - A1)/Ao] x 100

where Ao is the absorbance of the control, and A1 is the absorbance of *A. chamaepitys* extracts or standards (Ye et al., 2000).

Hydrogen peroxide scavenging capacity

The ability of the *A. chamaepitys* extracts to scavenge hydrogen peroxide was determined according to the method of Ruch et al. (1989). A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). Hydrogen peroxide concentration was determined spectrophotometrically by measuring absorption with extinction coefficient for H_2O_2 of 81 $M^{-1}cm^{-1}$. Extracts (50, 100 and 250 µg/mL) in distilled water were added to a hydrogen peroxide solution (0.6 ml, 40 mM).

Absorbance of hydrogen peroxide at 230 nm was determined 10 min later against a blank solution containing the phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenging of both *A. chamaepitys* extracts and standard compounds was calculated by the following formula:

% Scavanged [H2O2] = [(Ao - A1)/Ao] x 100

where Ao is the absorbance of the control, and A1 is the absorbance in the presence of the sample of *A. chamaepitys* extracts or standards.

Metal chelating activity

The chelating of ferrous ions by the *A. chamaepitys* extracts and standards was estimated by the method of Dinis et al. (1994).

Briefly, extracts (50, 100 and 250 µg/mL) were added to a solution of 2 mM $FeCl_2$ (0.05 mL). The reaction was started by addition of 5 mM ferrozine (0.2 mL) and the mixture was shaken vigorously and left standing at room temperature for 10 min. Absorbance of the solution was then measured spectrophotometrically at 562 nm. All test and analyses were run in triplicate and averaged. The percentage of inhibition of ferrozine-Fe^{2+} complex formation was calculated by the following formula:

%Inhibition = [(Ao - A1)/Ao] x 100

where Ao is the absorbance of control, and A1 is the absorbance in the presence of the sample of A. chamaepitys extracts or standards. The control does not contain $FeCl_2$ and ferrozine, complex formation molecules.

Determination of total phenolic compounds

Total soluble phenolic compounds in the *A. chamaepitys* extracts were determined with Folin-Ciocalteu reagent according to the method of Slinkard et al. (1977) using pyrocatechol as a standard phenolic compound. Briefly, 1 mL of the A. chamaepitys extracts solution (contains 1000 µg extract) in a volumetric flask diluted with distilled water (46 ml). One milliliter of Folin–Ciocalteu reagent was added and the content of flask was mixed thoroughly. After 3 min, 3 mL of Na_2CO_3 (2%) was added and then was allowed to stand for 2 h with intermittent shaking. The absorbance was measured at 760 nm in a spectrophotometer. The total concentration of phenolic compounds in the A. chamaepitys extracts was determined as microgram of pyrocatechol equivalent by means of an equation obtained from standard pyrocatechol graph (Gulcin, 2002b).

Absorbance = 0.001 × total phenols [pyrocatechol equivalent (µg)] –0.0033

Antimicrobial activity

Antimicrobial tests were carried out by disc diffusion method using 100 µl of suspension containing 106 per/ml of bacteria and 104 per/ml yeast inoculated into Mueller Hinton Agar (Difco), and Malt Extract Agar (Difco). The discs (6 mm diameter) were impregnated with 50 µl placed on the inoculated agar. Petri dishes were placed at 4°C for 2 h. Then, the inoculated plates were incubated at 37 ± 0.1°C at 24 h for bacterial strains and also at 25 ± 0.1°C at 72 h for yeast. Antimicrobial activity was determined by measuring the zone of inhibition against test organisms.

RESULTS AND DISCUSSION

ABTS radical-scavenging capacity

All the tested compounds exhibited effective radical cation scavenging activity. The scavenging effect of *A. chamaepitys* and standards on ABTS•+ was decreased (Figure 1) by the following; BHA> BHT> α-tocopherol> water extract of *A. chamaepitys*> methanol extract of *A. chamaepitys*> chloroform extract of A. chamaepitys (99.9, 97.3, 96.9, 90.6, 77.2 and 52.4% respectively) at the concentration of 100 µg/mL (Table 1). No significant differences in ABTS•+ scavenging potential were found among *A. chamaepitys* water extract, BHA, BHT and α-tocopherol.
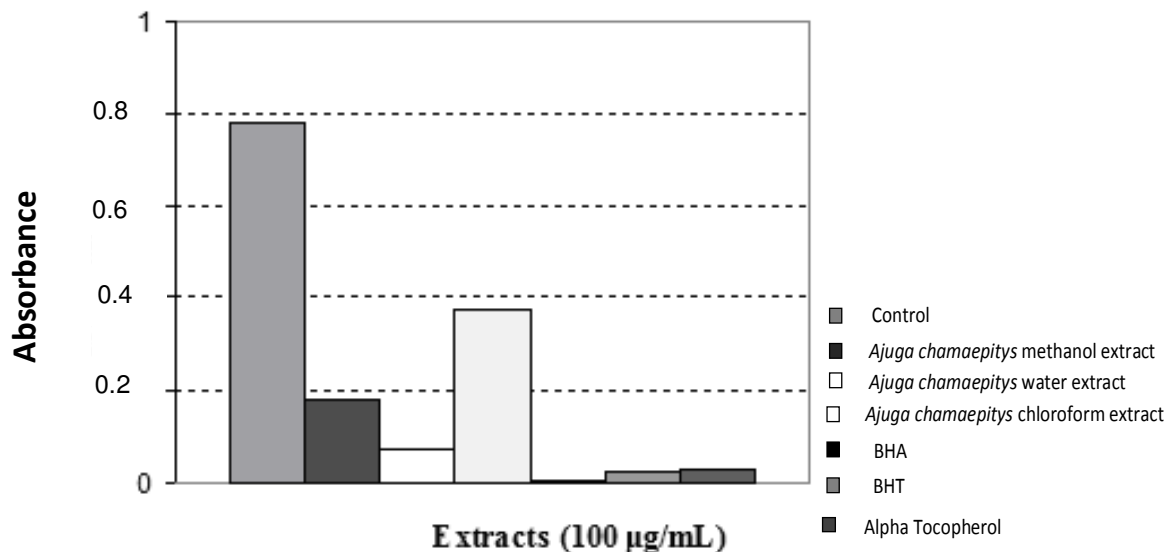
Figure 1. ABTS•+ radical-scavenging capacity of methanol, water and chloroform extracts of *A. chamaepitys*, BHA, BHT and α -tocopherol.

Table 1. (%) ABTS• radical-scavenging capacity of methanol, water and chloroform extracts of A. chamaepitys, BHA, BHT and α-tocopherol.

Extracts (100 µg/mL)	ABTS assay (%)
Ajuga chamaepitys methanol extract	77.2
Ajuga chamaepitys water extract	90.6
Ajuga chamaepitys chloroform extract	52.4
BHA	99.9
BHT	97.3
α-tocopherol	96.9

DPPH• radical scavenging capacity

DPPH• is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule (Soares et al., 1997). The reduction capability of DPPH• .radicals was determined by the decrease in its absorbance at 517 nm, which is induced by antioxidants. Hence, DPPH• is often used as a substrate to evaluate antioxidative activity of antioxidants (Duh et al., 1999). Figure 2 illustrates decrease in the concentration of DPPH• radical due to the scavenging ability of the extracts of A. chamaepitys. We used α-tocopherol as a standards. The scavenging effect of water and ethanol extracts of *A. chamaepitys* and standards on the DPPH• radical was decreased as: α-tocopherol> water extract of *A. chamaepitys*> methanol extract of *A. chamaepitys*> chloroform extract of *A. chamaepitys* (Figure 3). 100 µg of methanol, water and chloroform extracts of *A. chamaepitys* exhibited 77.8%, 79.6% and 62.6% DPPH• scavenging capacity, respectively. In the other hand, at

the same dose, α-tocopherol exhibited 95% DPPH• scavenging capacity. These results indicate that *A. chamaepitys* extracts have a noticeable effect on scavenging free radical.

Superoxide anion scavenging capacity

In the PMS/NADH-NBT system, superoxide anion derived from dissolved oxygen by PMS/NADH coupling reaction reduces NBT. The decrease in absorbance at 560 nm with antioxidants indicates the consumption of superoxide anion in the reaction mixture (Figure 4). Table 2 shows the % inhibition of superoxide radical generation of 100 µg/mL of methanol, water and chloroform extracts of *A. chamaepitys* and comparison with same doses of BHA, BHT and α-tocopherol. The extracts of *A. chamaepitys* have strong superoxide radical scavenging activity and exhibited higher superoxide radical scavenging activity than α-tocopherol. The percentage
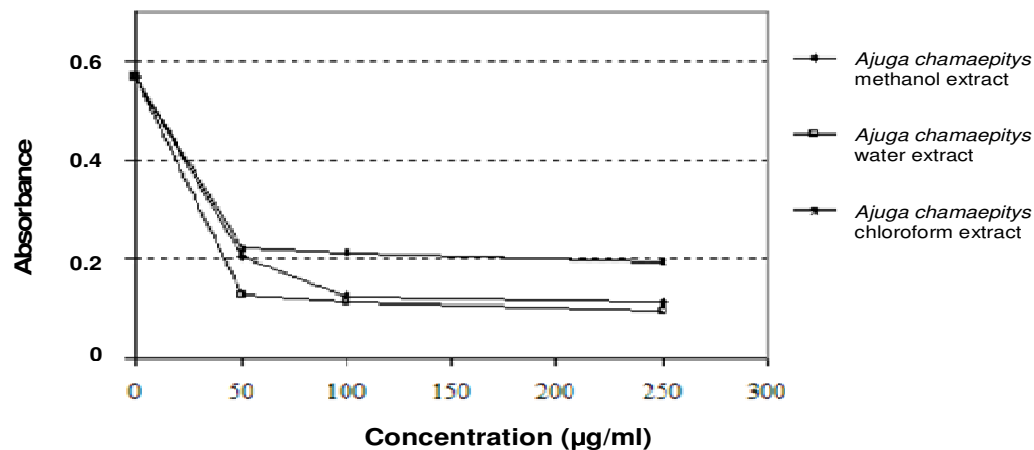
Figure 2. DPPH• radical scavenging capacity of methanol, water and chloroform extracts of *A. chamaepitys.*
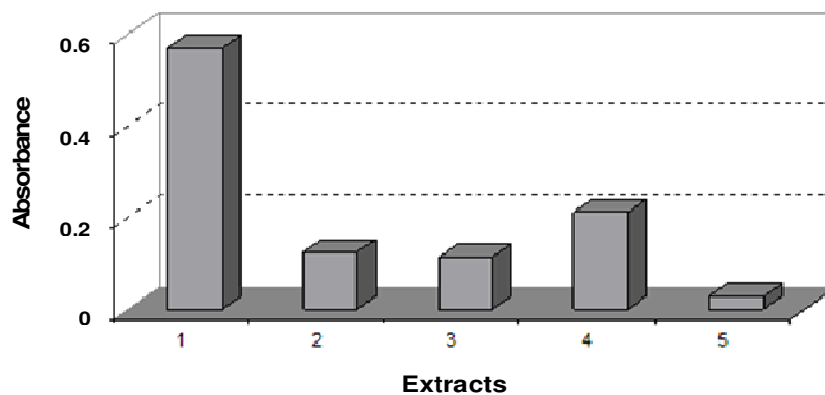

Figure 3. DPPH• radical scavenging capacity of methanol, water and chloroform extracts of A. chamaepitys (100 µg), α-tocopherol. (1. Control 2. Methanol extract of A. chamaepitys 3. Water extract of *A. chamaepitys* 4. Chloroform extract of A. chamaepitys 5. α-tocopherol).
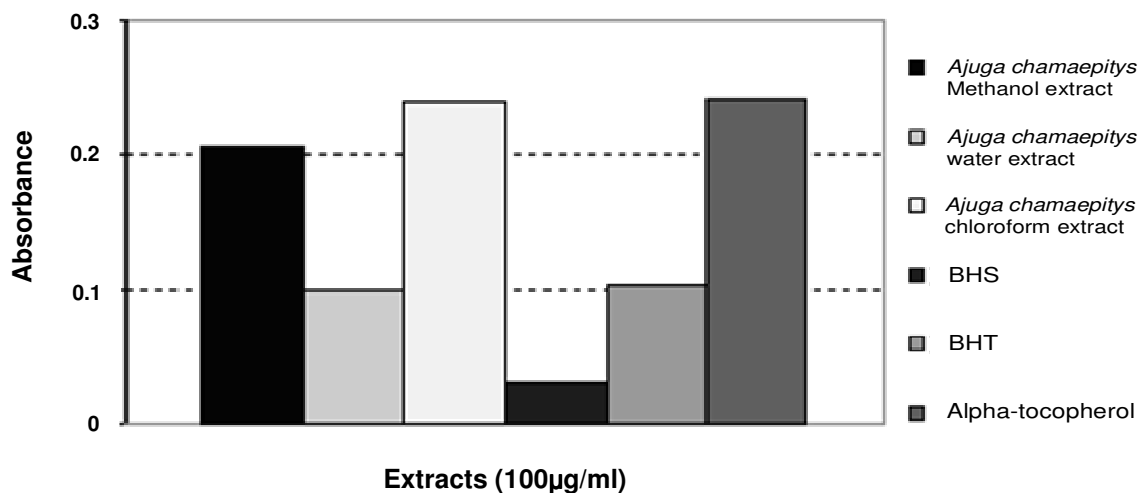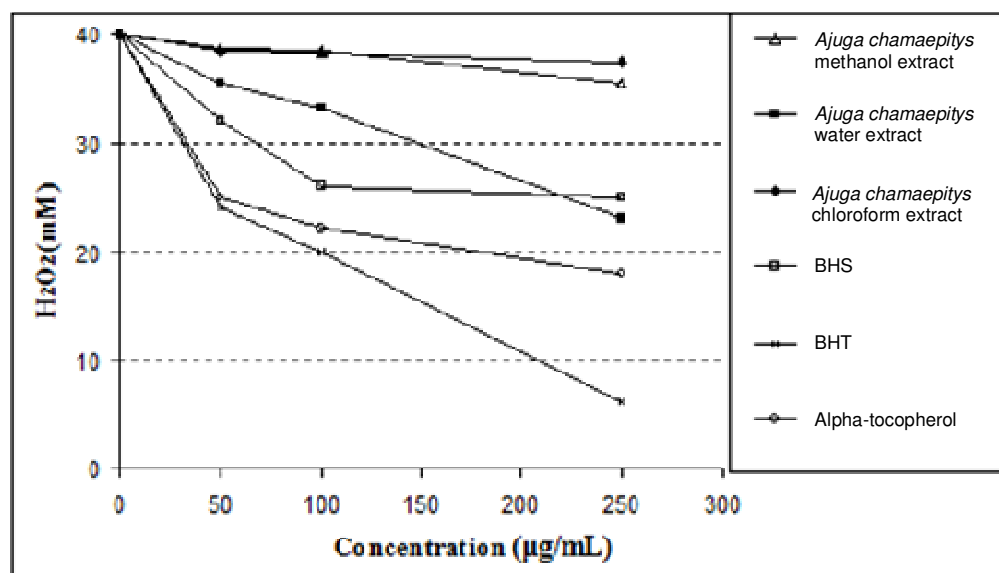

Figure 4. Superoxide anion radical scavenging activity of extracts of *A. chamaepitys*, BHA, BHT and α-tocopherol by the PMS–NADH–NBT method.

Table 2. % Superoxide anion scavenging activity of extracts of A. chamaepitys, BHA, BHT and α-tocopherol.

Extracts	%Superoxide anion scavenging activity (100 µg)
Ajuga chamaepitys methanol extract	60.9
Ajuga chamaepitys water extract	81.2
Ajuga chamaepitys chloroform extract	55.1
BHA	94.1
BHT	80.4
α-tocopherol	54.5

Figure 5. Hydrogen peroxide scavenging activities of methanol, water and chloroform extracts of *A. chamaepitys*, BHA, BHT and α-tocopherol.

inhibition of superoxide generation by 100 µg doses of BHA, BHT, methanol, water and chloroform extracts of A. chamaepitys was found as 94.1, 80.4, 60.9, 81.2 and 55.1% and greater than that of same doses of α -tocopherol (54.5%), respectively. Superoxide radical scavenging activity of those samples followed the order: BHA> water extract of *A. chamaepitys*> BHT> methanol extract of *A. chamaepitys*> chloroform extract of *A. chamaepitys* > α-tocopherol.

Hydrogen peroxide scavenging activity

The ability of extracts of *A. chamaepitys* to scavenge hydrogen peroxide was determined according to the method of Ruch et al. (1989). The scavenging ability of methanol, water and chloroform extracts of *A. chamaepitys* on hydrogen peroxide is shown Figure 5 and compared with BHA, BHT and α-tocopherol as standards. The extracts of *A. chamaepitys*   were

capableof scavenging hydrogen peroxide in an amount dependent manner. The hydrogen peroxide concentration of 250 µg/mL of methanol, water and chloroform extracts of A. chamaepitys were found to be 88.9, 57.8 and 93.8% respectively. In the other hand, at the same dose, BHA, BHT and α-tocopherol were found to be 37.5, 84.7 and 54.5%. These results show that the extracts of *A. chamaepitys* has the stronger hydrogen peroxide scavenging activity. Hydrogen peroxide itself is not very reactive, but it can sometimes be toxic to cell, because it may give rise to hydroxyl radical in the cells (Halliwell, 1991). Thus, the removing of H_2O_2 is very important for antioxidant defence in cell or food systems.

Metal chelating activity

The chelating of ferrous ions by the extracts of *A. chamaepitys* was estimated by the method of Dinis et al. (1994). Ferrozine can quantitatively form complex with
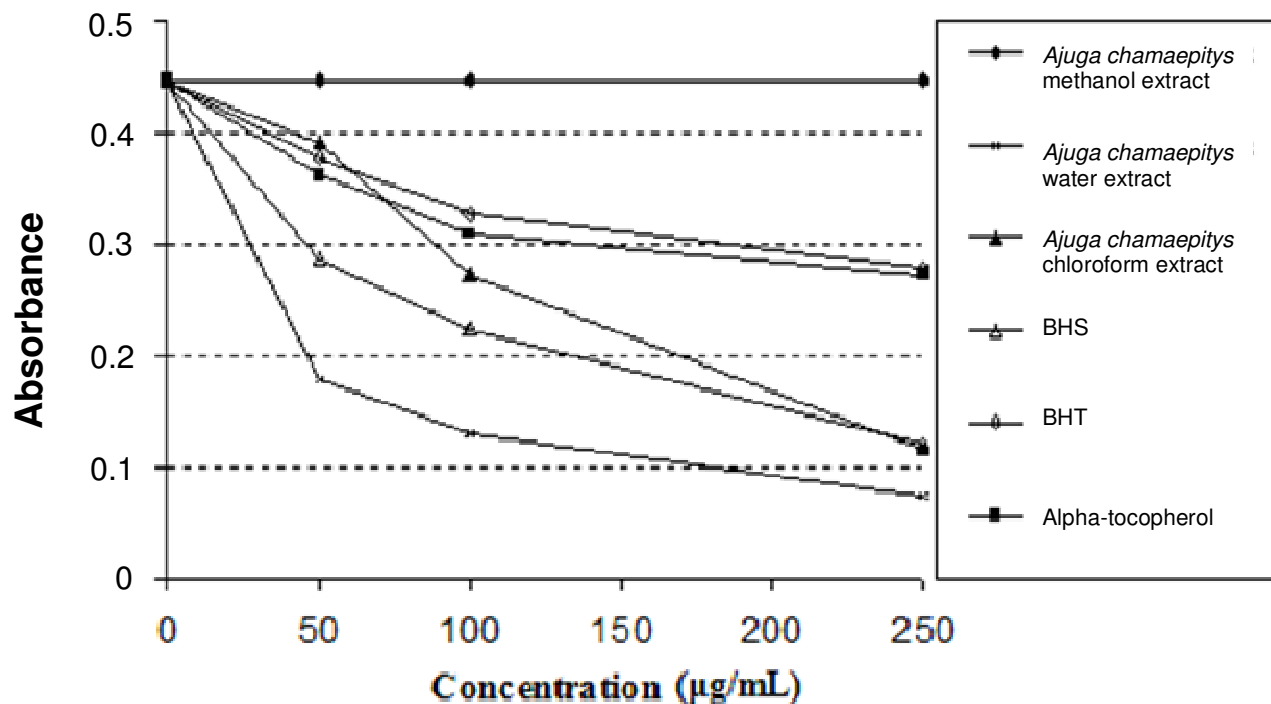
Figure 6. Metal chelating effect of different amount of methanol, water and chloroform extracts of *A. chamaepitys*, BHA, BHT and α-tocopherol.

Table 3. Result of the screening of A. chamaepitys methanol extract by means of the agar disc diffusion method. (Bacteria tested inhibisyon zone diameter (mm)).

Microorganisms	A.chamaepitys subsp. *euphratica*	Ampicillin* Nystatin**	Control
E. coli	13	12*	-
S. aureus	-	17*	-
B. cereus	-	17*	-
P. aeruginosa	15	11*	-
K. pneumonia	15	15*	-
E. aerogenes	12	11*	-
S. cerevisiae	-	20**	-
Candida sp.	-	18**	-

Control: (methanol); Ampicillin 20 µg; Nystatin 100 µg.

Fe^{2+}. In the presence of chelating agents, the complex formation is disrupted, resulting in a decrease in the red colour of the complex. Measurement of colour reduction therefore allows estimating the metal chelating activity of the coexisting chelator (Yamaguchi et al., 2000). In this assay, extracts of A. chamaepitys and standard compounds are interfered with the formation of ferrous and ferrozine complex, suggesting that they have chelating activity and are able to capture ferrous ion before ferrozine.

As shown in Figure 6, the formation of the Fe^{2+}–ferrozine complex is not complete in the presence of methanol, water and chloroform extracts of A. chamaepitys, indicating that extracts of A. chamaepitys chelate with the iron. The absorbance of Fe^{2+}–ferrozine complex was linearly decreased dose dependently (from 50 to 250 µg/ml). The percentages of metal scavenging capacity of 250 µg concentration of methanol, water and chloroform extracts of A. chamaepitys, BHA, BHT and α-tocopherol were found to be 2, 83.4, 74.3, 72.9, 38 and 39.2%, respectively.

Metal chelating capacity was significant, since it reducs

the concentration of the catalysing transition metal in lipid peroxidation (Duh et al., 1999). It was reported that chelating agents, which form S-bonds with a metal, are effective as secondary antioxidants, because they reduce the redox potential thereby stabilizing the oxidized form of the metal ion (Gordon, 1990). The data obtained from Figure 6 reveal that extracts of A. chamaepitys demonstrate a marked capacity for iron binding, suggesting that their action as peroxidation protector may be related to its iron binding capacity.

The total phenolic compounds

Phenols are very important plant constituents because of their scavenging ability due to their hydroxyl groups. According to the recent reports, a highly positive relationship between total phenols and antioxidant activity was found in many plant species (Gulcin et al., 2002b). 77, 27, 68 µg pyrocatechol equivalent of phenols was detected in 1 mg of methanol, water and chloroform extracts of A. chamaepitys.

The phenolic compounds may contribute directly to the antioxidative action (Duh et al., 1999). It is suggested that polyphenolic compounds may have inhibitory effects on mutagenesis and carcinogenesis in humans, when up to 1.0 g daily are ingested from a diet rich in fruits and vegetables (Liu et al., 1997). In addition, it was reported that phenolic compounds were associated with antioxidant activity and play an important role in stabilizing lipid peroxidation (Yen et al., 1993).

It is evaluated that the methanol, water and chloroform extracts of A. chamaepitys show strong antioxidant activity, ABTS• radical, DPPH• radical, superoxide anion scavenging, hydrogen peroxide scavenging and metal chelating activities when compared to standards such as BHA, BHT and α-tocopherol. The results of this study show that the methanol, water and chloroform extracts of A. chamaepitys can be used as easily accessible source of natural antioxidants and as a possible food supplement or in pharmaceutical industry and also in stabilising food against oxidative deterioration. However, the polyphenolic compounds or other components responsible for the antioxidant activity of methanol, water and chloroform extracts of A. chamaepitys are already unknown. Therefore, it is suggested that further work will be performed on the isolation and identification of the antioxidant components in extracts of A. chamaepitys.

Antimicrobial activity

The antimicrobial effect of A. chamaepitys subsp. euphratica extracts obtained by extraction techniques were tested against two gram-positive bacterial species (B. cereus, S. aureus), four gram-negative bacterial species (E. coli, E. aerogenes, P. aeruginosa, K. pneumonia), two yeast species (S. cerevisiae, Candida sp.). Methanol has no inhibitory effect on any of the test microorganisms in the control treatment. The results of these tests, as well as the effects of two control antibiotics, are presented in Table 3. The extracts obtained from A. chamaepitys subsp. euphratica show antimicrobial activity against to gram negative bacteria (E. coli 13 mm, P. aeruginosa 15 mm, K. pneumoniae 15 mm, E. aerogenes 12 mm) but has no antimicrobial activity against gram positive bacteria (B. cereus, S. aureus). Notably P. aeruginosa is more suspectible to the extract as compared to standard antibiotic (ampicillin). In addition, the extract of A. chamaepitys subsp. euphratica no antimicrobial activity against S. cerevisiae and Candida sp. yeast.

REFERENCES

Akbay P, Calis I, Heilmann J, Sticher O (2003). Ionone, iridoid and phenylethanoid glycosides from Ajuga salicifolia. Z. Naturforsch, 58: 177-180.

Aruoma OI (1998). Free radicals, oxidative stress, and antioxidants in human health and disease. J. Am. Oil. Chemists. Soc., 75: 199-212.

Baser KHC, Erdemgil Z, Ozek T, Demirci B (1999). Compositions of essential oils from two varieties of Ajuga chamaepitys subsp. chia from Turkey. J. Essent. Oil. Res., 11: 203-205.

Ben Jannet H, Harzallah-Skhiri F, Mighri Z, Simmonds MSJ, Blaney WM (2000). Responses of Spodoptera littoralis larvae to Tunisian plant extracts and to neo-clerodane diterpenoids isolated from Ajuga pseudoiva leaves. Fitoterapia, 71: 105-112.

Chen H, Tan RX, Liu ZL, Zhang Y, Yang L (1996). Antibacterial neoclerodane diterpenoids from Ajuga lupulina. J. Nat. Prod., 59: 668-670.

Chenni A, Yahia DA, Boukortt FO, Prost J, Lacaille-Dubois MA, Bouchenak M (2007). Effect of aqueous extract of Ajuga iva supplementation on plasma lipid profile and tissue antioxidant status in rats fed a high-cholesterol diet. J. Ethnopharmacol., 109: 207-213.

Clark AM (1996). Natural products as resource for new drugs. Pharm. Res., 13: 1133-1141.

Coll J, Tandrón YA (2008). Neo-clerodane diterpenoids from Ajuga: structural elucidation and biological activity. Phytochem. Rev., 7: 25-49.

Davis J (1994). Inactivation of antibiotics and the dissemination of resistance genes. Sci., 264: 375-382.

Davis PH, Mill RR, Tan K (Eds.) (1988). Flora Of Turkey And The East Aegean Islands. Edinburgh University Press, Edinburgh. Davis PH (Ed.) (1975). Flora Of Turkey And The East Aegean Islands. Vol. 7. Edinburgh University Press, Edinburgh.

Dinis TCP, Madeira VMC, Almeida LM (1994). Action of phenolic derivates (acetoaminophen, salycilate and 5-aminosalycilate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. Archives of Biochemistry and Biophysics, 315: 161–169.

Draughon FA (2004). Use of botanicals as biopreservatives in foods. Food. Technol., 58(2): 20-28.

Duh PD, Tu YY, Yen GC (1999). Antioxidant activity of water extract of Harng Jyur (Chrysanthemum morifolium Ramat). Lebensmittel-Wissenschaft und Technologie, 32: 269-277.

Eddouks M, Ouahidi ML, Farid O, Moufid A, Khalidi A, Lemhadri A (2007). The use of medicinal plants in the treatment of diabetes in Morocco. Phytotherapie, 5: 194-203.

Essawi T, Srour M. (2000). Screening of some Palestinian medicinal plants for antibacterial activity. J. Ethnopharmacol., 70: 343-349.

Gazzani G, Papetti A, Massolini G, Daglia M (1998). Antioxidative and pro-oxidant activity of water soluble components of some common diet vegetables and the effect of thermal treatment. Food. Chem., 6: 4118-4122.

Gordon MH (1990). The mechanism of antioxidant action in vitro. In Hudson BJF. (Ed.), Food antioxidants. London/ New York: Elsevier,

pp. 1-18.

Gülçin İ, Elmastas M, Aboul-Enein HY (2007). Determination of antioxidant and radical scavenging activity of basil (Ocimum basilicum) assayed by different methodologies. Phytotherapy. Res., 21: 354-361.

Gulcin I, Oktay M, Kufrevioglu OI, Aslan A (2002b). Determination of antioxidant activity of Lichen Cetraria islandica (L). Ach. J. Ethnopharmacol., 79(3): 325-329.

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

Halliwell B, Gutteridge JMC, Cross CE (1992). Free radicals, antioxidants and human disease: where are we now? J. Laboratory. Clin. Med, 119: 598-620.

Hedge IC (1982). Flora of Turkey and the East Aegean Islands. In P. H. Davis et al. (Eds.). Salvia L. Edinburgh: Edinburgh University Pres., 7: 400-461.

Imaida K, Fukushima S, Shirui T (1983). Promoting actions of butylated hydroxy anisole and butylated hydroxy toluene on 2-stage urinary bladder carcinogenesis and inhibition of c-glutamyl transpeptidase-positive foci development in the liver of rats. Carcinogenesis, 4: 895-899.

Jaitak V, Sharma K, Kalia K, Kumar N, Singh H.P., Kaul V.K. and Singh B (2010). Antioxidant activity of Potentilla fulgens: An alpine plant of western Himalaya. J. Food. Composıtıon. Anal 23(2): 142-147.

Liu F, Ooi VEC, Chang ST (1997). Free radical scavenging activity of mushroom polysaccharide extracts. Life. Sci., 60: 763-771.

Madhavi DL, Salunkhe DK (1996). Food antioxidants. In. Madhavi DL, Deshpande SS, Salunkhe DK (Eds.) New York: Dekker; pp. 267-359.

Marc EB, Nelly A, Annick D-D, Frederic D (2008). Plants used as remedies antirheumatic and antineuralgic in the traditional medicine of Lebanon. J. Ethnopharmacol. 120: 315-334.

Miliauskas G, Van Beek TA, Venskutonis PR, Linssen JPH, Waard PD, Sudholter EJR (2004). Antioxidant activity of P. Fruticosa. J. Sci. Food. Agric., 8: 1997-2009.

Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical. Biol. Med., 26: 1231-1237.

Ruch RJ, Cheng SJ, Klaunig JE (1989). Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea. Carcinogenesis, 10: 1003-1008.

Shimada K, Fujikawa K, Yahara K, Nakamura T (1992). Antioxidative properties of xanthin on autoxidation of soybean oil in cyclodextrin emulsion. J. Agric. Food. Chem., 40: 945-948.

Shirwaikar A, Shirwaikar A, Rajendran K, Punitha ISR (2006). In vitro antioxidant studies on the benzyl tetra tsoquinoline alkaloid berberine. Biol. Pharm. Bull., 29: 1906-1910.

Slinkard K, Singleton VL (1977). Total phenol analyses: automation and comparison with manual methods. Am. J. Enol. Viticult., 28: 49-55.

Soares JR, Dins TCP, Cunha AP, Ameida LM (1997). Antioxidant activity of some extracts of Thymus zygis. Free Radic. Res., 26: 469-478.

Wang MF, Li JG, Rangarajan M, Shao Y, LaVoie EJ, Huang TC, Ho CT (1998). Antioxidative phenolic compounds from sage (Salvia officinalis). J. Agric. Food. Chem., 46: 4869-4873.

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

Ye XY, Wang HX, Liu F, Ng TB (2000). Ribonuclease, cell-free translation-inhibitory and superoxide radical scavenging activities of the iron-binding protein lactoferrin from bovine milk. Int. J. Biochem. Cell. Biol., 32: 235-241.

Yen GC, Duh PD, Tsai CL (1993). Relationship between antioxidant activity and maturity of peanut hulls. J. Agric. Food. Chem., 41: 67-70.