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Antioxidant, antimicrobial, antiulcer and analgesic activities of nettle (*Urtica dioica* L.)

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Abstract

In this study, water extract of nettle *(Urtica dioica* L.) (WEN) was studied for antioxidant, antimicrobial, antiulcer and analgesic properties. The antioxidant properties of WEN were evaluated using different antioxidant tests, including reducing power, free radical scavenging, superoxide anion radical scavenging, hydrogen peroxide scavenging, and metal chelating activities. WEN had powerful antioxidant activity. The 50, 100 and 250 μg amounts of WEN showed 39, 66 and 98% inhibition on peroxidation of linoleic acid emulsion, respectively, while 60 μg/ml of α-tocopherol, exhibited only 30% inhibition. Moreover, WEN had effective reducing power, free radical scavenging, superoxide anion radical scavenging, hydrogen peroxide scavenging, and metal chelating activities at the same concentrations. Those various antioxidant activities were compared to standard antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), quercetin, and α-tocopherol. In addition, total phenolic compounds in the WEN were determined as pyrocatechol equivalent. WEN also showed antimicrobial activity against nine microorganisms, antiulcer activity against ethanol-induced ulcerogenesis and analgesic effect on acetic acid-induced stretching.

Keywords: Antioxidant activity; Antimicrobial activity; Antiulcer activity; Analgesic activity; Nettle; *Urtica dioica* L.

1. Introduction

Lipid peroxidation is an important deteriorate reaction in food during storage and processing. It not only causes a loss in food quality but also is believed to be associated with some diseases such as carcinogenesis, mutagenesis, ageing, and arteriosclerosis (Yagi, 1987). The role of active oxygen and free radicals in tissue damage in such diseases, are becoming increasingly recognized (Halliwell and Gutteridge, 1985). Cancer, emphysema, cirrhosis, arteriosclerosis, and arthritis have all been correlated with oxidative damage. Active oxygen, either in the form of superoxide ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($OH^{\bullet}$), or singled oxygen ($^1O_2$), is a product of normal metabolism and attacks biological molecules, leading to cell or tissue injury. When the mechanism of antioxidant protection becomes unbalanced by exogenous factors such as smoking, ionising radiation, certain pollutants, organic solvents and pesticides and endogenous factors such as normal aerobic respiration, stimulated polymorphonuclear leukocytes and macrophages, and peroxisomes may occur, resulting in above-mentioned diseases and accelerating ageing (Büyükokuroğlu et al., 2001). However, antioxidant supplements or foods rich in antioxidants may be used to help the human body in reducing oxidative damage by free radicals and active oxygen (Halliwell and Gutteridge, 1984; Mau et al., 2001; Gülçin et al., 2002b). Recently, various phytochemicals and their effects on health, especially the suppression of active oxygen species by natural antioxidants from teas, spices and herbs, have been intensively studied (Ho et al., 1994). The most commonly used antioxidants at the present time are butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG), and *tert*-butylhydroquinone (TBHQ) (Sherwin, 1990). However, they are suspected of being responsible for liver damage and carcinogenesis in laboratory animals (Grice, 1986; Wichi, 1988). Therefore, the development and utilization of more effective antioxidants of natural origin are desired (Gülçin et al., 2002a; Oktay et al., 2003).

Aqueous infusions of Mediterranean herbs including *Urtica dioica*, exhibit antioxidant activity towards iron-promoted oxidation of phospholipids, linoleic acid, and deoxyribose (Matsingou et al., 2001). Also, the electrogen-

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erated bromine method was used for estimating the antioxidant capacity of plant materials such as *Urtica dioica* and plant-based medicinal preparations (Abdullin et al., 2002). It was reported that *Urtica dioica* prevent the damage of rat liver tissue structure (Lebedev et al., 2001).

Urtica dioica herbs are used to treat stomachache in Turkish folk medicine (Yeşilada et al., 2001). In addition, this herb is used to treat rheumatic pain and for colds and cough (Sezik et al., 1997) and is used against liver insufficiency (Yeşilada et al., 1993).

The aim of the present study was to investigate antioxidant activity by using different antioxidant tests including reducing power, free radical scavenging, superoxide anion radical scavenging, hydrogen peroxide scavenging, and metal chelating activities. An important goal of this research was to examine antimicrobial, antiulcer, and analgesic activity of WEN.

2. Materials and methods

2.1. Antioxidant activities

2.1.1. Chemicals

Ammonium thiocyanate was purchased from E. Merck. Ferrous chloride, polyoxyethylenesorbitan monolaurate (Tween-20), α-tocopherol, 1,1-diphenyl-2-picryl-hydrazyl (DPPH), 3-(2-pyridyl)-5,6-bis (4-phenyl-sulfonic acid)-1,2, 4-triazine (ferrozine), nicotinamide adenine dinucleotide (NADH), butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and trichloroacetic acid (TCA) were purchased from Sigma (Sigma-Aldrich GmbH, Sternheim, Germany).

2.1.2. Plant material and extraction

Nettle was collected in May, in Dumlu area in Erzurum, Turkey, and authenticated by Prof. Dr. İsmet Hasenekoğlu, Department of Biology Education, Kazım Karabekir Education Faculty, Atatürk University. Then, nettle was left on a bench to dry. The dried sample was chopped into small parts with a blender. For water extraction, 20 g dried aerial parts of nettle ground into a fine powder in a mill and was mixed with 400 ml boiling water by magnetic stirrer during fifteen minutes. Then the extract was filtered over Whatman No.1 paper. The filtrate was frozen and lyophilized in a lyophilizator at 5 μmHg pressure at −50 °C (Labconco, Freezone 1L). The extract of nettle was placed in a plastic bottle, and then stored at −20 °C until used.

2.1.3. Total antioxidant activity determination

The antioxidant activity of WEN was determined according to the thiocyanate method (Mitsuda et al., 1996). For stock solution; 20 mg lyophilized WEN was dissolved in 20 ml water. Then the solution, which contains different amount of stock WEN solution or standards samples (50, 100 and 250 μg) in 2.5 ml of potassium phosphate buffer (0.04 M, pH 7.0) was added to 2.5 ml of linoleic acid emulsion in potassium phosphate buffer (0.04 M, pH 7.0). Each solution was then incubated at 37 °C in a glass flask in the dark. At intervals during incubation, each solution was stirred for 3 min, 0.1 μl this incubation solution, 0.1 ml $FeCl_2$ and 0.1 ml thiocyanate were transferred to the test tube, which containing 4.7 ml ethanol. Then this solution incubated for 5 min. Finally, the peroxide value was determined by reading the absorbance at 500 nm in a spectrophotometer (8500 II, Bio-Crom Gmbh, Zurich, Switzerland). During the linoleic acid oxidation, peroxides formed and these compounds oxidize Fe^{2+} to Fe^{3+}. The latter Fe^{3+} ions form complex with SCN^-, which has a maximum absorbance at 500 nm. Therefore higher absorbance values indicate higher linoleic acid oxidation. The solutions without added WEN or standards were used as blank samples. Five millilitres linoleic acid emulsion is consisting of 17.5 μg Tween-20, 15.5 μl linoleic acid and 0.04 M potassium phosphate buffer (pH 7.0). On the other hand, 5 ml control composed of 2.5 ml linoleic acid emulsion and 2.5 ml potassium phosphate buffer (0.04 M, pH 7.0). All data about total antioxidant activity are the average of duplicate analyses. The inhibition of lipid peroxidation in percentage was calculated by the following equation:

$$\text{Percent inhibition} = \left[\frac{A_0 - A_1}{A_0}\right] \times 100$$

where A_0 was the absorbance of the control reaction and A_1 was the absorbance in the presence of the sample of WEN (Duh et al., 1999).

2.1.4. Reducing power

The reducing power of WEN was determined according to the method of Oyaizu (1986). The different doses of WEN (50, 100 and 250 μg) in 1 ml of distilled water were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide $[K_3Fe(CN)_6]$ (2.5 ml, 1%). The mixture was incubated at 50 °C for 20 min. A portion (2.5 ml) of TCA (10%) was added to the mixture, which was then centrifuged for 10 min at $1000 \times g$ (MSE Mistral 2000, UK, Serial No.: S693/02/444). The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1%), and the absorbance was measured at 700 nm in a spectrophotometer (8500 II, Bio-Crom GmbH, Zurich, Switzerland). Higher absorbance of the reaction mixture indicated greater reducing power.

2.1.5. Superoxide anion scavenging activity

Measurement of superoxide anion scavenging activity of WEN was based on the method described by Liu et al. (1997) with slight modifications (Gülçin et al., 2003c). Superoxide radicals are generated in phenazine methosulphate (PMS)–nicotinamide adenine dinucleotide (NADH) systems by oxidation of NADH and assayed by the reduction of nitroblue tetrazolium (NBT). In this experiments, the superoxide radicals were generated in 3 ml of Tris–HCl buffer

(16 mM, pH 8.0) containing 1 ml of NBT (50 μM) solution, 1 ml NADH (78 μM) solution and 1 ml sample solution of WEN (100 μg/ml) were mixed. The reaction was started by adding 1 ml of PMS solution (10 μM) to the mixture. The reaction mixture was incubated at 25 °C for 5 min, and the absorbance at 560 nm in a spectrophotometer (8500 II, Bio-Crom GmbH, Zurich, Switzerland) was measured against blank samples. L-Ascorbic acid was used as a control. Decrease in absorbance of the reaction mixture indicated increased superoxide anion scavenging activity. The percentage inhibition of superoxide anion generation was calculated using the following formula

$$\text{Percent inhibition} = \left[\frac{A_0 - A_1}{A_0}\right] \times 100$$

where A_0 was the absorbance of the control (L-Ascorbic acid), and A_1 was the absorbance of WEN or standards (Ye et al., 2000).

2.1.6. Free radical scavenging activity

The free radical scavenging activity of WEN was measured by 1,1-diphenyl-2-picryl-hydrazil ($DPPH^\bullet$) using the method of Shimada et al. (1992). Briefly, 0.1 mM solution of $DPPH^\bullet$ in ethanol was prepared. Then, 1 ml of this solution was added to 3 ml of WEN solution at different doses (50–250 μg). The mixture was shaken vigorously and allowed to stand at room temperature for 30 min. Then the absorbance was measured at 517 nm in a spectrophotometer (8500 II, Bio-Crom GmbH, Zurich, Switzerland). Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The $DPPH^\bullet$ concentration (mM) in the reaction medium was calculated from the following calibration curve, determined by linear regression (R^2: 0.9769):

$$\text{Absorbance} = 104.09 \times [DPPH^\bullet]$$

The DPPH radical concentration was calculated using the following equation:

$$DPPH^\bullet \text{ scavenging effect (\%)} = 100 - \left[\frac{A_0 - A_1}{A_0} \times 100\right]$$

where A_0 was the absorbance of the control reaction and A_1 was the absorbance in the presence of the sample of WEN (Oktay et al., 2003).

2.1.7. Metal chelating activity

The chelating of ferrous ions by the WEN and standards was estimated by the method of Dinis (Dinis et al., 1994). Briefly, extracts (50–250 μg) were added to a solution of 2 mM $FeCl_2$ (0.05 ml). The reaction was initiated by the addition of 5 mM ferrozine (0.2 ml) and the mixture was shaken vigorously and left standing at room temperature for ten minutes. After the mixture had reached equilibrium, the absorbance of the solution was then measured spectrophotometrically at 562 nm in a spectrophotometer (8500 II, Bio-Crom GmbH, Zurich, Switzerland). The percentage of inhibition of ferrozine-Fe^{2+} complex formation was given by the formula:

$$\text{Percent inhibition} = \left[\frac{A_0 - A_1}{A_0}\right] \times 100$$

where A_0 was the absorbance of the control, and A_1 was the absorbance in the presence of the sample of WEN and standards. The control contains $FeCl_2$ and ferrozine (Gülçin et al., 2003a).

2.1.8. Scavenging of hydrogen peroxide

The ability of the WEN 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 from absorption at 230 nm in a spectrophotometer (8500 II, Bio-Crom GmbH, Zurich, Switzerland). Extracts (50–250 μg) in distilled water were added to a hydrogen peroxide solution (0.6 ml, 40 mM). Absorbance of hydrogen peroxide at 230 nm was determined after ten minute against a blank solution containing in phosphate buffer without hydrogen peroxide. The percentage of scavenging of hydrogen peroxide of WEN and standard compounds was calculated using the following equation:

$$\text{Percent scavenged } [H_2O_2] = \left[\frac{A_0 - A_1}{A_0}\right] \times 100$$

where A_0 was the absorbance of the control, and A_1 was the absorbance in the presence of the sample of WEN and standards (Gülçin et al., 2003b).

2.1.9. Determination of total phenolic compounds

Total soluble phenolic compounds in the WEN were determined with Folin–Ciocalteu reagent according to the method of Slinkard (Slinkard and Singleton, 1977) using pyrocatechol as a standard phenolic compound. Briefly, 1 ml of the WEN 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 the 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 (8500 II, Bio-Crom GmbH, Zurich, Switzerland). The total concentration of phenolic compounds in the WEN determined as microgram of pyrocatechol equivalent by using an equation that was obtained from standard pyrocatechol graph (Gülçin et al., 2002b):

$$\begin{aligned}\text{Absorbance} &= 0.0053 \\ &\quad \times \text{total phenols [pyrocatechol equivalent } (\mu g)] \\ &\quad - 0.0059.\end{aligned}$$

2.2. Antimicrobial activities

2.2.1. Preparation of test microorganisms

For the purpose of antimicrobial evaluation ten microorganisms were used. *Pseudomonas aeruginosa* (ATCC 9027, Gram-negative), *Escherichia coli* (ATCC 9837, Gram-negative), *Proteus mirabilis* (Clinical isolate, Gram-negative), *Citrobacter koseri* (Clinical isolate, Gram-negative), *Enterobacter aerogenes* (Clinical isolate, Gram-negative), *Staphylococcus aureus* (ATCC 6538, Gram-positive), *Streptococcus pneumoniae* (ATCC 49619, Gram-positive), *Micrococcus luteus* (Clinical isolate, Gram-positive), *Staphylococcus epidermidis* (clinical isolate, Gram-positive), and *Candida albicans* (ATCC 10231) microorganism strains were employed for determination of antimicrobial activity. Clinical isolates of microorganisms were defined by Dr. Ekrem Kireçci, Department of Microbiology, Medical Faculty, Atatürk University, Erzurum.

Bacteria and yeast were obtained from the stock cultures of Microbiology Laboratory, Department of Microbiology, Medical Faculty, Atatürk University, Erzurum. The bacterial and yeast stock cultures were maintained on Muller Hinton Agar (Oxoid CM 337, Basingstoke, Hampshire, UK) slants, respectively, which were stored at 4 °C. These bacteria were maintained on Blood agar base (Oxoid CM55, Basingstoke, Hampshire, UK). The yeast was maintained on Sabouraud-dextrose agar (Oxoid CM41, Basingstoke, Hampshire, UK).

2.2.2. Antimicrobial activity determination

Agar cultures of the test microorganisms were prepared as described by Mackeen et al. (1997). Three to five similar colonies were selected and transferred with loop into 5 ml of Tryptone Soya broth (Oxoid CM 129, Basingstoke, Hampshire, UK). The broth cultures were incubated for 24 h at 37 °C. The WEN was dissolved in sterile water for the assay by magnetic stirrer. For screening, sterile, 6 mm diameter filter paper disc were impregnated with 250 μg of the WEN. Then the paper discs were placed onto Mueller Hinton agar (Oxoid CM337, Basingstoke, Hampshire, UK). The inoculum for each organism was prepared from broth cultures. The concentration of cultures was adjusted to 10^8 colony forming units (1×10^8 CFU/ml). The results were recorded by measuring the zones of growth inhibition surrounding the disc. Clear inhibition zones around the discs indicated the presence of antimicrobial activity. All data on antimicrobial activity are the average of triplicate analyses. Netilmicin (30 μg per disc), amoxicillin-clavulanic acid (20–10 μg per disc), ofloxacin (5 μg per disc, BBL™ Sensi disc™), and antifungal miconazole nitrate (40 μg per disc, DRG International) were used as reference standards, which as recommended by the National Committee for Clinical Laboratory Standards (NCCLS).

2.3. Antiulcer activities

Forty albino Sprague–Dawley male rats with a weight of 190–225 g were used for the experiment. The rats were fed with standard laboratory chow and water before the experiment. The laboratory was windowless with automatic temperature (22 ± 1 °C) and lighting controls (14 h light/10 h dark). Rats were divided into five equal groups ($n = 8$) and housed in cages. Twenty-four hours before the experiment, the rats were fasted and allowed access to water ad libitum.

Anti-ulcerogenic effect of WEN was investigated by using the ethanol-induced ulcer model (Büyükokuroğlu et al., 2002). On the day of the experiment, groups 1, 2 and 3 were injected with 10 mg/kg WEN, while group 4 was injected with 20 mg/kg famotidine and group 5 with saline solution. All of drugs were administered intraperitoneally in 0.5 ml vehicle. Following a 30-min-period, all the animals were given 1 ml of ethanol (70%) by oral gavages. One hour after the administration of ethanol, animals were sacrificed by decapitation. The stomach of each was removed and opened along the greater curvature and washed in physiological saline solution. For the measurement of the gross gastric mucosal lesions, freshly excised stomach was laid flat and the mucosal lesions were traced on clear acetate paper. Gross mucosal lesions were recognized as haemorrhage or linear breaks (erosions) with damage to the mucosal surface. The area of stomach and gross lesions were approximately calculated by planimetry using a simple magnifier. The results were translated to the term of "total ulcer area/total gastric area" and these were expressed as an ulcer index (%).

2.4. Writhing test

All experiments were performed on no-fasted male and female albino Swiss mice weighing 30–38 g, which were obtained from animal house in the Atatürk University, Medical Faculty. Animals were divided into five equal groups of 6 each. Animals were pretreated with 50, 100 and 200 mg/kg doses of WEN and 200 mg/kg dose of metamizol as reference drug. Control animals received an equal volume of 0.9% NaCl in distilled water. Drugs and saline were given 60 min before acetic acid injection.

Writhing test was determined according to the method of Zakaria et al. (2001). Writhing was induced by 10 mg/kg of intraperitoneally acetic acid (0.6%) injection. Ten millimetres after acetic acid injection, the mice were placed in a transparent box and the number of writhes was counted for period of 10 min. Writhing movement was accepted as contraction of the abdominal muscles accompanied by stretching of the hind limbs. Antinociceptive effect was expressed as the reduction of the number of writhing between control and pretreated mice.

$$\text{Percentage reduction of the number of writhing (\%)} = \left[\frac{A_0 - A_1}{A_0}\right] \times 100$$

where A_0 was the number of writhing of the control, and A_1 was the number of writhing of pretreatment with WEN (Gülçin et al., 2003d).

2.5. Statistical analysis

Experimental results concerning this study were mean ± S.D. of three parallel measurements. Analysis of variance was performed by ANOVA procedures. 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 very significant.

3. Results and discussion

3.1. Antioxidant capacity

The antioxidant activity of putative antioxidants have been attributed to various mechanisms, among which are prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity and radical scavenging (Diplock, 1997; Oktay et al., 2003). Numerous antioxidant methods and modifications have been proposed to evaluate antioxidant activity and to explain how antioxidants function. Of these, total antioxidant activity, reducing power, DPPH assay, metal chelating, active oxygen species such as H_2O_2, $O_2^{\bullet-}$ and $OH^{\bullet}$ quenching assays are most commonly used for the evaluation of antioxidant activities of extracts (Duh et al., 1999; Amarowicz et al., 2000; Chang et al., 2002).

Total antioxidant activity of WEN was determined by the thiocyanate method. WEN exhibited effective antioxidant activity at all doses. The effects of various amounts of WEN (from 50 to 250 μg) on peroxidation of linoleic acid emulsion are shown in Fig. 1. The antioxidant activity of WEN increased concentration dependently. WEN (50, 100 and 250 μg) showed higher antioxidant activities than that of 100 μg concentration of α-tocopherol. After incubation times the percentage inhibition of peroxidation in linoleic acid emulsion was 39, 66 and 98%, respectively, and greater than that of α-tocopherol (30%).

Fig. 2 shows the reductive capabilities of WEN compared to tocopherol. For the measurements of the reductive ability, we investigated the Fe^{3+}–Fe^{2+} transformation in the presence of WEN samples using the method of Oyaizu (1986). The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity (Meir et al., 1995). Like the antioxidant activity, the reducing power of WEN increased concentration dependently. All of the concentrations of WEN showed higher activities than the control in a statistically significant ($P < 0.05$) manner.

In the PMS–NADH–NBT system, superoxide anion derived from dissolved oxygen by PMS–NADH coupling reaction reduces NBT. The decrease of absorbance at 560 nm with antioxidants indicates the consumption of superoxide anion in the reaction mixture (Oktay et al., 2003). Fig. 3 shows the percentage inhibition of superoxide radical generation by 100 μg of WEN and comparison with same doses of BHA, BHT and α-tocopherol. The WEN exhibited higher superoxide radical scavenging activity than BHA, BHT and α-tocopherol ($P < 0.01$). The percentage inhibition of superoxide generation by 100 μg amount of WEN was found as 97% and greater than that of some doses of BHA, BHT, and tocopherol (95, 83 and 60%), respectively. Superoxide radical scavenging activity of those samples followed the order: WEN > BHA > BHT > α-tocopherol.

The effect of antioxidants on DPPH radical scavenging is thought to be due to their hydrogen donating ability. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The model of scavenging the stable DPPH radical is a widely used method to evaluate antioxidant activities in a relatively short time compare to other methods (Soares et al., 1997). The reduction capability on the DPPH radical is determined by the decrease in its absorbance at 517 nm induced by antioxidants. The maximum absorption of a stable DPPH rad-

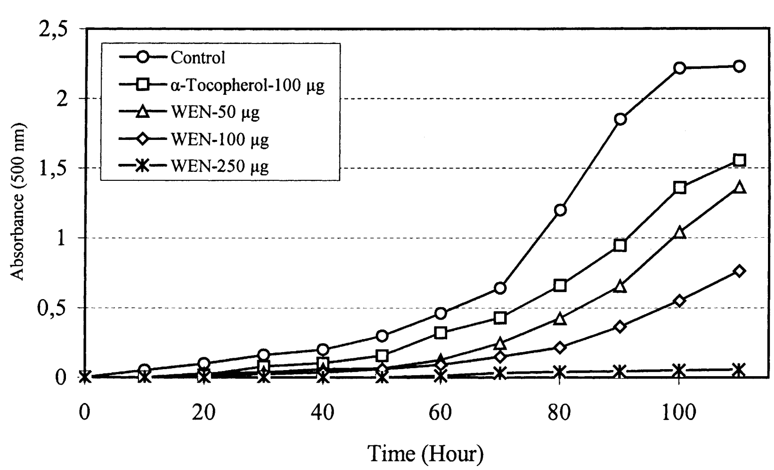

Fig. 1. Antioxidant activity of different doses of WEN and α-tocopherol in the linoleic acid emulsion was determined by the thiocyanate method. The indicated amounts of dried extract of WEN were presented in 5 ml of linoleic acid emulsion. The control was the linoleic acid emulsion without WEN extract. Results are average of duplicate experiments (WEN: water extract of nettle).

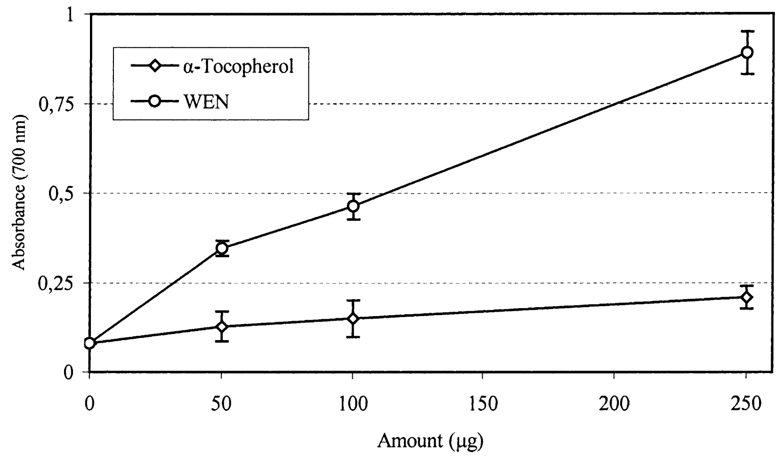

Fig. 2. Reducing power of WEN, and α-tocopherol by spectrophotometric detection of the Fe^{3+}–Fe^{2+} transformation. Control was test sample without extract or α-tocopherol. Higher absorbance indicates higher reducing power (WEN: water extract of nettle).

ical in ethanol is at 517 nm. The decrease in absorbance of DPPH radical caused by antioxidants is due to the reaction between antioxidant molecules and radical, which results in the scavenging of the radical by hydrogen donation. This is visualized as a discoloration from purple to yellow. Hence, DPPH is usually used as a substrate to evaluate antioxidant activity (Duh et al., 1999; Chang et al., 2002; Gülçin et al., 2003c). Fig. 4 illustrates a significant ($P < 0.01$) decrease in the concentration of DPPH radical due to the scavenging ability of the WEN and standards. WEN and BHA showed almost equal DPPH scavenging activity, however, significantly are lower than that of quercetin. The scavenging effect of WEN and standards on the DPPH radical decreased in the order of quercetin > WEN > BHA and were 93, 37 and 32% at the concentration of 60 μg/ml, respectively.

It was reported that oxidative stress, which occurs when free radical formation exceeds the body's ability to protect itself, forms the biological basis of chronic conditions such as arteriosclerosis (Fatimah et al., 1998). Based on the data obtained from this study, WEN exhibits free radical inhibitor or scavenger activity as well as a primary antioxidant that reacts with free radicals, which may limit free radical damage occurring in the human body.

The chelating of ferrous ions by WEN was estimated with the method of Dinis et al. (1994). Ferrozine can quantitatively form complexes with Fe^{2+}. In the presence of chelating agents, the complex formation is disrupted and eventually that the red colour of the complex fades. Measurement of colour reduction therefore allows estimation of the chelating activity of the co-existing chelator (Yamaguchi et al., 2000). In this assay WEN and standard antioxidant compound interfered with the formation of ferrous and ferrozine complex, suggesting that they have chelating activity and capture ferrous ion before ferrozine. Iron can stimulate lipid peroxidation by the Fenton reaction, and also accelerates peroxidation by decomposing lipid hydroperoxides into peroxyl and alkoxyl radicals that can themselves abstract hydrogen and perpetuate the chain reaction of lipid peroxidation (Chang et al., 2002; Halliwell, 1991).

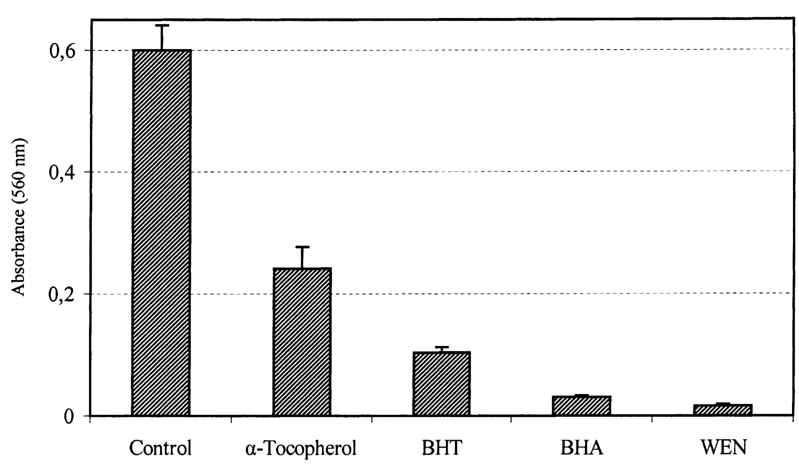

Fig. 3. Superoxide anion radical scavenging activity of 100 μg of WEN, BHA, BHT, and α-tocopherol by the PMS–NADH–NBT method (BHA: butylated hydroxyanisole, BHT: butylated hydroxytoluene, WEN: water extract of nettle).

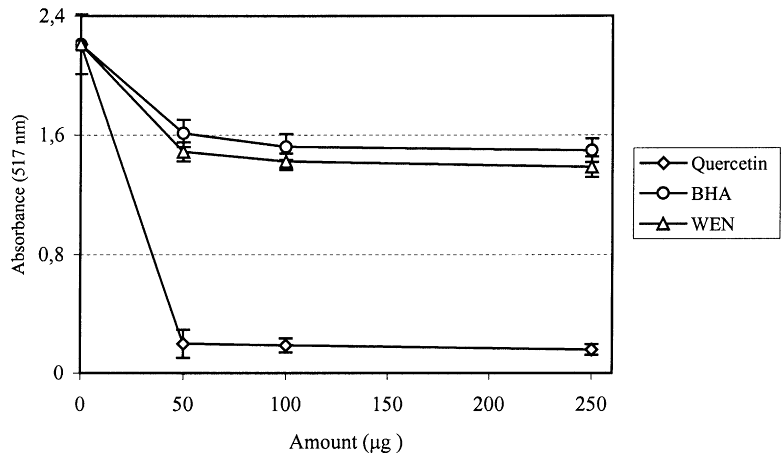

Fig. 4. Comparison of free radical scavenging activity of quercetin, BHA, and WEN on 1,1-diphenyl-2-picrylhydrazyl radical (BHA: butylated hydroxyanisole, WEN: water extract of nettle).

As shown in Fig. 5, the formation of the Fe^{2+}-ferrozine complex was not completed in the presence of WEN, indicating that WEN chelates the iron. The absorbance of Fe^{2+}-ferrozine complex was linearly decreased dose-dependently (from 50 to 250 μg). The difference between WEN and the control was statistically significant ($P < 0.01$). The percentages of metal chelating capacity of 250 μg concentration of WEN, α-tocopherol, BHA, and BHT were found as 92, 43, 66 and 41%, respectively. The metal scavenging effect of WEN and standards decreased in the order of WEN > BHA > α−tocopherol > BHT.

Metal chelating capacity is important since it reduced the concentration of the catalysing transition metal in lipid peroxidation (Duh et al., 1999). It was reported that chelating agents, which form 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 Fig. 5 revealed that WEN demonstrate a marked capacity for iron binding, suggesting that their action as peroxidation protector may be related to its iron binding capacity.

Scavenging of H_2O_2 by WEN may be attributed to their phenolics, which could donate electrons to H_2O_2, thus neutralizing it to water The H_2O_2 scavenging capacity of an extract may be attributed to the structural features of their active components, which determine their electron donating abilities (Wettasinghe and Shahidi, 2000).

The ability of WEN to scavenge H_2O_2 was determined according to the method of Ruch et al. (1989). The scavenging ability of WEN on H_2O_2 is shown in Fig. 6 and compared with BHA, BHT and α-tocopherol as standards. WEN was capable of scavenging H_2O_2 in a dose-dependent manner. Two-hundred and fifty micrograms of WEN exhibited 23% scavenging activity on H_2O_2. On the other hand, at the same concentration; BHA, BHT and α-tocopherol showed 38, 86 and 57% activity respectively. These results indicated that WEN posses effective H_2O_2 scavenging activity but lower than BHA, BHT and α-tocopherol. However, there was sta-

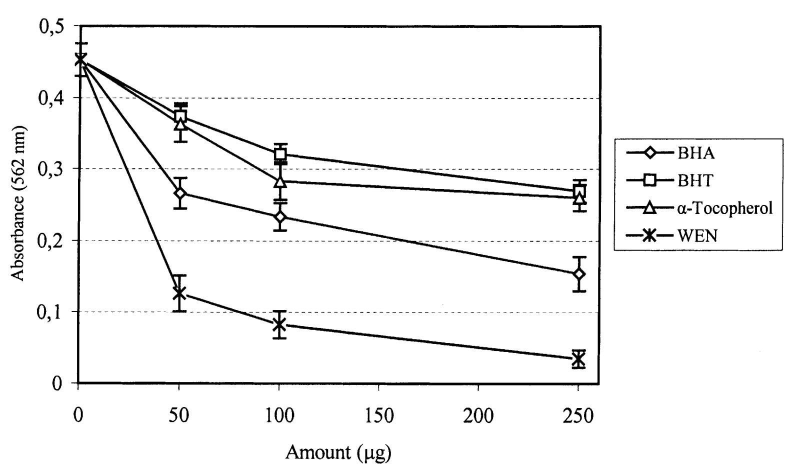

Fig. 5. Metal chelating effect of different amount of water extract of nettle, BHA, BHT, and α-tocopherol on ferrous ions (BHA: butylated hydroxyanisole, BHT: butylated hydroxytoluene, WEN: water extract of nettle).

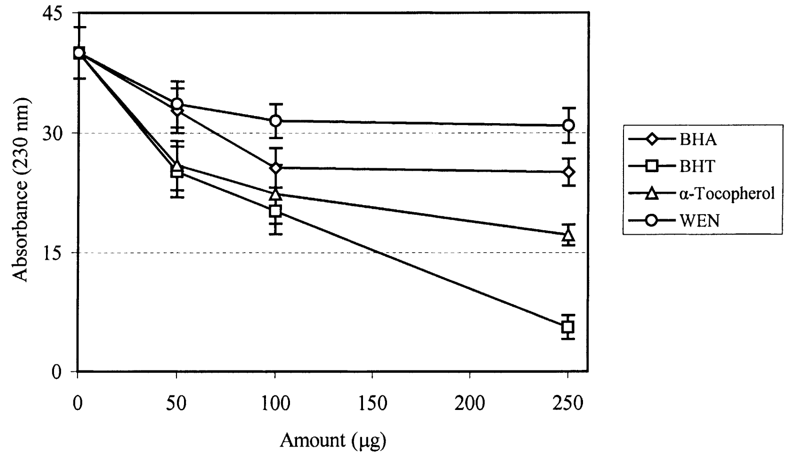

Fig. 6. Hydrogen peroxide scavenging activity of different amount of WEN, BHA, BHT, and α-tocopherol (BHA: butylated hydroxyanisole, BHT: butylated hydroxytoluene, WEN: water extract of nettle).

tistically a very significant correlation between those values and control ($P < 0.01$). The H_2O_2 scavenging effect of same dose (250 μg) of WEN and standards decreased in the order of BHT > α−tocopherol > BHA > WEN. Hydrogen peroxide itself is not very reactive, but it may be toxic to cell since it may give rise to hydroxyl radicals in cells (Halliwell, 1991).

Phenols are very important plant constituents because of their scavenging ability due to their hydroxyl groups (Hatano et al., 1989). According to the recent reports, a highly positive relationship between total phenols and antioxidant activity was found in many plant species (Vinson et al., 1998; Velioglu et al., 1998; Gülçin et al., 2002b; Oktay et al., 2003). 25.3 μg pyrocatechol equivalent of phenols was detected in 1 mg of WEN.

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 (Tanaka et al., 1998). 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).

3.2. Antimicrobial activity

In this study, nine different microbial and one yeast species were used to screen the possible antimicrobial activity of WEN. WEN exhibited antimicrobial activity against all tested microorganisms. Of the species used, *Staphylococcus aureus* is one of the most common Gram-positive bacteria causing food poisoning. Its source is not the food itself, but the humans who contaminate food after if has been processed (Rauha et al., 2000). Interestingly WEN showed antibacterial activity against this bacterium. As it is shown in Table 1, the generation of most bacterial and the yeast species was inhibited by WEN. *Escherichia coli*, belonging to the normal flora of humans, is a Gram-negative bacterium. However, an enterohemmoragic strain of *Escherichia coli* has caused serious cases of food poisoning and preservatives to eliminate its growth are needed. *Candida albicans* is the microbe responsible for most clinical yeast infections, e.g. in mouth infections. Miconazole nitrate (40 μg per disc), amoxicillin-clavulanic acid (20–10 μg per disc), ofloxacin (5 μg per disc), and netilmicin (30 μg per disc) were used as positive controls for bacteria and yeast.

3.3. Effects on acute gastric mucosal lesions induced by ethanol

Ulcer indices (UI) are shown in Table 2. Per-oral administration of 70% ethanol produced multiple mucosal lesions in the rat stomach. Pre-treatment with WEN and famotidine were found to inhibit the ethanol-induced gastric mucosal

Table 1
Antimicrobial activities of WEN (250 μg per disc), and miconazole nitrate, amoxicillin-clavulanic acid, ofloxacin, and netilmicin

Microorganisms	Diameter of zone of WEN (mm)	Antimicrobial agent (mm)			
		MN	ACA	O	N
Pseudomonas aeruginosa	ND	–	ND	ND	10
Escherichia coli	8	–	15	23	25
Proteus mirabilis	8	–	24	26	25
Citrobacter koseri	9	–	22	15	24
Staphylococcus aureus	8	–	15	12	27
Streptococcus pneumoniae	9	–	15	24	18
Enterobacter aerogenes	9	–	12	23	23
Micrococcus luteus	13	–	19	20	22
Staphylococcus epidermidis	11	–	24	21	25
Candida albicans	8	20	–	–	–

WEN: water extract of nettle; MN: miconazole nitrate (40 μg per disc); ACA: amoxicillin-clavulanic acid (20–10 μg per disc); O: ofloxacin (5 μg per disc); N: netilmicin (30 μg per disc); ND: not detected activity at this amount of WEN or standards.

Table 2
The effects of different doses of WEN and famotidine on the ethanol-induced gastric mucosal injury (WEN: water extract of nettle)

Groups	Ulcer index (%) (mean ± S.E.M.)	Percent decrease of gastric mucosal injury (%)
Control	6.75 ± 0.66	–
Famotidin 20 (mg/kg)	4.43 ± 0.25*	34.4
WEN 50 (mg/kg)	2.18 ± 0.16*	67.7
WEN (100 mg/kg)	2.63 ± 0.24*	61.1
WEN (200 mg/kg)	1.50 ± 0.60*	77.8

Results are means ± S.E.M. and data are evaluated by using one-way analysis of variance (Tukey test).
* $P < 0.01$, compared to control.

Table 3
Effects of different doses of WEN and metamizol on acetic acid-induced writhing in mice (WEN: water extract of nettle)

Groups	Writhing number (mean ± S.E.M.)	Percent decrease of acetic acid-induced writhing in mice (%)
Control	25.3 ± 2.3	–
Metamizol (200 mg/kg)	15.3 ± 2.6*	39.4
WEN (25 mg/kg)	7.5 ± 3.8*	62.1
WEN (50 mg/kg)	2.7 ± 2.9*	70.4
WEN (100 mg/kg)	9.6 ± 1.4*	89.2

Results are means ± S.E.M. and data are evaluated by using one-way analysis of variance (Tukey test).
* $P < 0.01$, compared to control, $n = 8$.

injury in rats. Preventive effects of 50, 100 and 200 mg/kg WEN were in a dose-dependent manner (percent inhibitions were 67.7, 61.1 and 77.8, respectively, compared to control) and there was a statistically significance between the effects of used WEN doses ($P < 0.005$). Famotidine also significantly inhibited the ethanol-induced gastric lesion (percent decrease was 34.4, compared to ethanol). There were significant differences between all concentrations of WEN effects and famotidine effect ($P < 0.001$).

3.4. Analgesic effect

Writhing numbers are shown in Table 3. Pretreatment with WEN and metamizol were found to inhibit the acetic acid-induced writhing in mice. Inhibitor effects of 50, 100 and 200 mg/kg WEN were in a dose-dependent manner and significant (percent decrease, compare to control: 62.1, 70.4 and 89.2%, respectively). As seen in Table 3, metamizol also inhibited the acetic acid-induced writhing significantly ($P < 0.01$) (decrease compare to control: 39.4%).

4. Conclusion

It is known in traditional therapy that *Urtica dioica* L. (Urticaceae) or nettle has a hypertensive effect (Garnier et al., 1961). Therewith, some other actions of this plant were reported such as anti-inflammatory and antirheumatic effects (Obertreis et al., 1996; Riehemann et al., 1999), acute diuretic, natriuretic and hypotensive effects (Tahri et al., 2000), cardiovascular effects (Testai et al., 2002), and stimulation of proliferation of human lymphocytes (Wagner et al., 1989). The effects of the nettle are also evoked in the therapy of the prostatic hyperplasia (Krzeski et al., 1993; Hiramo et al., 1994; Lichius and Muth, 1997), but this plant has no hypoglycemic action, as reported by Raman-Ramos et al. (1992) and Swanston-Flatt et al. (1989). Moreover, this plant has been used in the traditional therapy of hypertension (Ziyyat et al., 1997).

On the basis of the results of this study, it is clearly indicated that WEN has a powerful antioxidant activity against various oxidative systems in vitro; moreover, WEN can be used as accessible source of natural antioxidants and as a possible food supplement or in pharmaceutical industry. The various antioxidant mechanisms of WEN may be attributed to strong hydrogen donating ability, a metal chelating ability, and their effectiveness as scavengers of hydrogen peroxide, superoxide, and free radicals. Phenolic compounds appear to be responsible for the antioxidant activity of WEN. In addition, free radicals have been demonstrated to be a contributing factor in the tissue injury and modulation of the pain (Khalil et al., 1999; Van der Laan et al., 1997). Some studies have revealed that the antioxidants melatonin and β-carotene potentiate the antinociceptive responses (Penn, 1995; Pang et al., 2001). It was indicated that vitamin E has beneficial effects in improvement of rheumatic disease, intermittent claudication or angina pectoris due to its antioxidant activity (Rapola et al., 1996; Sangha and Stucki, 1998; Kleijnen and Mackerras, 2000). According to the above information, it is said that there is a relationship between antioxidant and analgesic activities. Analgesic activities may be related to antioxidant activity.

Finally, all concentrations of WEN possessed noticeable antimicrobial activity against Gram-positive and -negative bacteria when compared with standard and strong antimicrobial compounds such as miconazole nitrate, amoxicillin-clavulanic acid, ofloxacin, and netilmicin. At the same time WEN has effective antiulcer activity against ethanol-induced ulcerogenesis and analgesic effect on acetic acid-induced stretching and it can be used for therapy of ulcerogenesis and gastric mucosal injury.

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