Food Sci. Biotechnol. Vol. 18, No. 1, pp. 130 ~ 136 (2009)

Food Science and Biotechnology

© The Korean Society of Food Science and Technology

Protective Ability of Ethanol Extracts of *Hypericum scabroides* Robson & Poulter and *Hypericum triquetrifolium* Turra against Protein Oxidation and DNA Damage

Göksel Kızıl*, Murat Kızıl, and Bircan Çeken

Faculty of Science and Arts, Chemistry Department, University of Dicle, Diyarbakır 21280, Turkey

Abstract The present study was conducted to determine the protective ability of the ethanol extracts of *Hypericum scabroides* Robson & Poulter (HS) and *Hypericum triquetrifolium* Turra (HT) against the protein oxidation and DNA damage induced by Fenton system. The ability of HS and HT to prevent oxidative damage to bovine serum albumin (BSA) induced by Fe^{3+}/H_2O_2 and ascorbic acid was investigated. The ethanol extracts of HS and HT at different concentrations (50-1,000 μg/mL) efficiently prevented protein oxidation induced by hydroxy radical as assayed by protein oxidation markers including protein carbonyl formation (PCO) and polyacrylamide gel electrophoresis. The effect of ethanol extracts of HS and HT on DNA cleavage induced by UV-photholysis of H_2O_2 using pBluescript M13+ plasmid DNA were investigated. These extracts significantly inhibited DNA damage induced by reactive oxygen species (ROS). Therefore, HS and HT extracts may be useful in the food industry as effective synthetic antioxidants.

Keywords: protein oxidation, DNA damage, fenton chemistry, *Hypericum scabroides*, *Hypericum triquetrifolium*

Introduction

Oxygen derived reactive oxygen species (ROS) and their adverse effects are inevitable for aerobic organisms. Cellular constituents, lipids, proteins, and DNA are the crucial targets for ROS attact. Overexpression of ROS is being implicated in several degenerative diseases (1). Therefore intake of antioxidants from natural origin with less toxicity (compared to synthetic antioxidant) in relation to human health is useful.

A growing number of natural constituents of food, particularly fruits and vegetables, are regarded as possible antioxidants with a role in protecting the cell against free radical damage and chemicals, which can generate the oxidative forms (2). Several substances in plants express cytotoxic and genotoxic activities and show correlation with the incidence of tumors. Therefore, understanding of the health benefits and/or potential toxicity of these plants is important. Natural antioxidants constitute a broad range of compounds including phenolic compounds, flavonoids, and carotenoids. The antioxidant activity of several plant materials has recently been described (3). The search for newer natural antioxidants, especially of plant origin has ever since increased.

Plants of the genus *Hypericum* (Guttiferae) are widely used in herbal medicine for their important pharmacological properties and several studies have been published concerning antidepressant, antiviral, and wound healing (4). The antimicrobial activity of the essential oils or various extracts from several *Hypericum* species have also been reported (5-7). Nowadays, *Hypericum* species finds wide application for their antioxidant properties (8). Recent studies have demonstrated that, different species of *Hypericum* contains compounds such as flavonoids, xanthones, and phenolic and can be used as antioxidants (9).

The genus *Hypericum* which contains more than 400 species involved in relatively dry temperate zones of the world. In Turkey, the genus is represented 89 species of which 43 are endemic (10). These plants are used as sedatives and antiseptics in Turkish folk medicine under the names: *kantaron, peygamber çiçeği, kanotu, kuzukıran*, and *binbirdelik otu* (11). Various extracts of different *Hypericum* species have been examined for their antiinflammatory, antimicrobial, antioxidant, and hypolipidemic activity (7,12). In our previous study, the antioxidative potential of ethanol extracts of HS and HT were investigated using different antioxidant parameters such as 1,1-diphenyl-2-picrylhydrazyl (DPPH), metal chealating, reducing power, hydroxyl radical, total antioxidant activity, and lipid peroxidation inhibition assays. The amount of total phenolic compounds were previously also determined, and total phenolic content of 1 mg *H. triquetrifolium* ethanol extract was found to be equivalent to 267 μg gallic acid. The results obtained from our previous study indicate that ethanolic extracts of *Hypericum scabroides* Robson & Poulter (HS) and *Hypericum triquetrifolium* Turra (HT) are a potential source of natural antioxidants (13).

Oxygen-based reactants that escape detoxification by cellular antioxidant systems are responsible for roughly an estimated 10,000 DNA base modifications/cell/day. Thus many studies have focused on DNA damage as estimated by the levels of 8-hydroxy-deoxyguanosine after exposure to ROS (14). It has been showed that extracts from Indian medicinal plants have a significant effect on DNA cleavage induced by ultraviolet (UV)-photholysis of H_2O_2 (15).

Reactive species also damage proteins but, due to rapid turnover of proteins they are considered to contribute less

*Corresponding author: Tel: +90-412-2488550x3038; Fax: +90-412-2488039
E-mail: gokselk@dicle.edu.tr
Received June 4, 2008; Revised August 6, 2008;
Accepted August 11, 2008

prominently to total cellular damage. Recently, however the possibility that free radical damage to proteins might contribute to aging has also received attention and several groups have focused their research in this area. Thus, it has been shown that oxidatively modified proteins accumulate during aging and in some pathological conditions (16).

Exposure of proteins to ·OH, $O_2^{-\bullet}$ or both leads to gross structural modifications. These oxidatively modified proteins may undergo spontaneous protein fragmentation and cross-linking or exhibit a substantial increase in proteolysis. The principles of protein modification by ROS are well established as well as the characterized reaction products of protein interactions with ·OH and $O_2^{-\bullet}$. In addition to fragmentation, the oxidation of lysine, arginine, proline, and threonine residues may yield carbonyl derivatives. The presence of carbonyl groups has therefore been used as a marker of ROS-mediated protein oxidation (17). The end products of lipid peroxidation such as malondialdehyde (MDA) and 4-hydroxy-2-nonenal (HNE) as well as products from polyunsaturated fatty acids cause protein damage (18).

Natural antioxidants such as α-tocopherol and L-ascorbic acid are widely used, because they are seen as being safer and causing fewer adverse reactions, but their antioxidant activities are, however, lower than those of synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) (19). Currently, many kinds of synthetic antioxidants such as BHA, BHT, tertiary butyl hyroquinone (TBHQ), and propyl gallate have been used as materials or additives for oxidation suppressant in food, cosmetic and drug compositions. However, the use of these synthetic antioxidants for food or medicine components has been stricted by the toxicity and safety that can lead to problems of the potential health in human. Due to the reason, many researchers have tried to find the more effective oxidation inhibitors that may be used as antioxidants for food or medicine compositions without the side effects for the past several years. So many researchers have paid attention to many kinds of natural antioxidants that can be used without toxicity in human. Hence the need exists for safe, economic antioxidants with high activity from natural sources to replace these synthetic chemicals. The antioxidant compounds present in edible plants have recently been promoted as food additives because they display little or no toxic side effects (20).

There are some reports in the literature showing the protective effects of crude extracts of plants or their contituents against protein oxidation and DNA damage induced by ROS (21). So far, there is no report related to the protective ability of HS and HT extracts on protein and DNA damage. From the viewpoint of their high antioxidant potency, the consumption at high scale of many plants have been recommended (22), therefore, the evaluation of antioxidant activities of extracts and fractions is considered as an important step prior to the isolation of antioxidant phytochemicals they contain. Therefore, the aim of the present study was to test whether ethanol extract of HS and HT counteracts protein and DNA damage caused by ROS.

Materials and Methods

Collection of plant material Plants *Hypericum scabroides* Robson & Poulter (HS) and *Hypericum triquetrifolium* Turra (HT) were collected in Mardin and Şırnak in the area of south east of Turkey in June 2005, by Zuhal Toker. Voucher specimen have been deposited at the Herbarium of the Department of Biology, Faculty of Science and Arts, Dicle University (voucher no. DUF-2512-b and DUF-2512-a). They were identified by Professor A. Selçuk Ertekin from the same institution.

Preparation of crude extract Crude extracts were prepared as described before (13). Briefly, aerial parts (stems, leaves, and flowers) of HS (175 g) and HT (198 g) were dried for 10 days at room temperature. A total of 240 g of dried material was ground in an electric blender and then incubated into a glass flask with 2,000 mL ethanol (70%) for 3 days under magnetic stirrer. The crude ethanol extracts of HS (16 g) as brown colour and HT (23 g) as a purple colour were obtained and kept in dark glass bottles at 4°C until use.

Protein oxidation The effect of ethanol extract of both HS and HT on protein oxidation were carried out according to the slightly modified method of Wang *et al.* (23). Bovine serum albumin (BSA) was oxidized by a Fenton-type reaction. The reaction mixture (1.2 mL), containing sample extract (50-1,000 μg/mL), potassium phosphate buffer (20 mM, pH 7.4), BSA (4 mg/mL), $FeCl_3$ (50 μM), H_2O_2 (1 mM), and ascorbic acid (100 μM) were incubated for 30 min at 37°C. For determination of protein carbonyl content in the samples, 1 mL of 10 mM 2,4-dinitrophenyl-hydrazine (DNPH) in 2 M HCl was added to the reaction mixture. Samples were incubated for 30 min at room temperature. Then, 1 mL of cold TCA (10%, w/v) was added to the mixture and centrifuged at 3,000×g for 10 min. The protein pellet was washed 3 times with 2 mL of ethanol/ethyl acetate (1:1, v/v) and dissolved in 1 mL of guanidine hydrocholoride (6 M, pH 2.3). The absorbance of the sample was read at 370 nm. The data were expressed in terms of % inhibition, calculated from a control measurement of the reaction mixture without the test sample. BHT was used as a standard and the inhibition % of protein oxidation of the sample was calculated by the following equation.

$$\% \text{ Inhibition} = [(A_c - A_s)/A_c] \times 100$$

where A_c is the absorbance of the control, and A_s is the absorbance in the presence of samples of extracts or standard.

Sodium dodecyl sulfate-polyacrylamide gel electropheresis (SDS-PAGE) To investigate electrophoretic patterns of protective ability of ethanol extracts of HS and HT on proteins against H_2O_2/Fe^{3+}/ascorbic acid attact, BSA was used as a model protein. BSA (1 mg/mL) was dissolved in 20 mM potassium phosphate buffer (pH 7.4) and then 50 μM $FeCl_3$, 1 mM H_2O_2, 100 μM ascorbic acid were added in the reaction mixture and incubated in the presence or absence of BHT (50-1,000 μg/mL), ethanol extracts of HS (50-1,000 μg/mL), and ethanol extract of HT (50-1,000 μg/mL) in a final volume of 1.2 mL. After incubation for 3 hr at 37°C, the reaction mixture was analyzed by electrophoresis in 10% SDS polyacrylamide gel, using Laemmli's method

(24). Samples were mixed with equal volumes of sample buffer (Tris HCl pH 6.8, 2% SDS, 5% 2-mercaptoethanol, 10% sucrose, and 0.002% bromophenol blue). The mixture was then boiled for 5 min and 5 μL of each sample was loaded to the wells. The gel was run in a BioRad tank in running buffer (25 mM Tris pH 8.3, 190 mM glycine, and 0.1% SDS) at a maximum voltage and a constant current of 25 mAmp for a mini gel, using a BioRad 1,000/500 power supply. Gels were stained with 0.15% Coomassie brillant blue R-250 for 2 hr and then destained and digitally photographed.

Measurement of band intensity and graph representation To determine the amount of protein damage, band intensity was estimated using the Gel documentation system (Gel-Doc-XR; BioRad, Hercules, CA, USA). Bands on the gels were quantified discovery series Quantity One programme (version 4. 5. 2, BioRad). The density of each band was estimated and standardized with respect to the control group. Results show the average of 3 different measurements.

DNA oxidation DNA damage protective activities of ethanol extract of HS and HT were checked on pBluescript M13+ plasmid DNA. Plasmid DNA was isolated by Qiagene plasmid mini prep kit. Plasmid DNA was oxidized with H_2O_2+UV treatment in presence of different concentrations of HS or HT extracts and checked on 1% agarose according to Attaguile *et al.* (25) after modification. In brief, the experiments were performed in a volume of 10 in a microfuge tube containing 200 ng of plasmid DNA in phospate buffer (7.14 mmol phospate and 14.29 mmol NaCl), pH 7.4, H_2O_2 was added at a final concentration of 2.5 mmol/L with and without 1 μL of (100, 200, 300, and 400 μg/mL) ethanol extracts of HS and HT. The reactions were initiated by UV irradiation and continued for 5 min on the surface of a UV transilluminator (8,000 μW/cm) at 300 nm under room temperature. After irradiation the reaction mixture (10 μL) with gel loading dye was placed on 1% agarose gel for electrophoresis. Electrophoresis was performed at 40 V for 3 hr in the presence of ethidium bromide (10 mg/mL). Untreated pBluescript M13+ plasmid DNA was used as a control in each run of gel electrophoresis along with partial treatment i.e., only UV treatment and only H_2O_2. Percent inhibition of the DNA strand scission was calculated as follows (26):

$$\text{Inhibition } (\%)=I-[(S_{m+a}-S_c)/(S_m-S_c)]$$

Where S_{m+a} is % remaining supercoiled after treatment with mix plus agent, S_c is % remaining supercoiled in control untreated plasmid, and S_m is % remaining supercoiled with mix without agent.

Densitometric analysis of treated and control pBluescript M13+ plasmid DNA Gel was scanned on Gel documentation system (Gel-Doc-XR; BioRad). Bands on the gels were quantified discovery series Quantity One programme (version 4. 5. 2, BioRad).

Statistical analysis Data are presented as the mean± standard deviation (SD). Significant differences among the groups were determined by one-way analysis of variance (ANOVA) using SPSS 12.0 software package program. The results were considered significant if the value of p was less than 0.05. Differences were considered significant at $p<0.05$.

Results and Discussion

Protein oxidation The modification of proteins may proceed along several pathways either by direct oxidation of side chains or indirectly by reaction with products of lipid or carbohydrate oxidation or modification of the sugar moieties of glycoproteins. ROS may play an important role in many dysfunctions. They could potentially react with nucleic acids, lipids, and proteins, causing extensive oxidative damage. ROS leading to protein oxidation include radical species such as superoxide ($O_2^{-\bullet}$), hydroxyl (•OH), peroxyl ($RO_2^{\bullet}$), alkoxyl ($RO^{\bullet}$), hydroperoxyl ($HO_2^{\bullet}$), and nonradical species such as hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl), ozone (O_3), singlet oxygen ($^1O_2$), and peroxynitrite ($ONOO^-$) (27).

The oxidative protein damages, provoked by free radicals, have been also demonstrated to play a significant role in aging and several pathological events (17). Radical mediated damages to proteins might be initiated by electron leakage, metal-ion dependent reactions, and autoxidation of lipids and sugars. Major molecular mechanisms, leading to structural changes in proteins are free-radical mediated protein oxidation characterized by carbonyl formation (PCO). Indeed, measurement of PCO has been used as a sensitive assay for oxidative damages of proteins (28). The usage of protein carbonyl groups as biomarkers of oxidative stress has some advantages in comparison with the measurement of other oxidation products because of the relative early formation and the relative stability of carbonylated proteins. Most of the assays for detection of protein carbonyl groups involve derivatisation of the carbonyl group with 2,4-dinitrophenylhydrazine (DNPH), which leads to formation of a stable dinitrophenyl (DNP) hydrazone product. This then can be detected by various

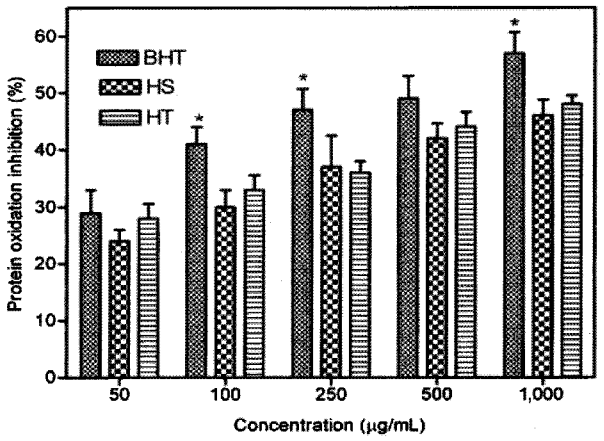
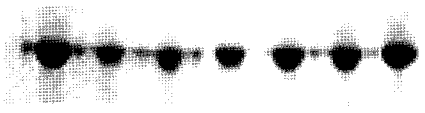


Fig. 1. Inhibitory effect of butylated hydroxytoluene (BHT), ethanol extract of *Hypericum scabroides* (HS), and ethanol extract of *Hypericum triquetrifolium* (HT) on protein (BSA) oxidation expressed as protein carbonyl inhibition induced Fenton system (Fe^{3+}/H_2O_2/ascorbic acid). Each value is expressed as mean±SD of 3 different experiments. All the values statistically significant than control ($p<0.05$). *BHT statistically significant than ethanol extract of HS.



+	+	+	+	+	+	+	BSA
-	+	+	+	+	+	+	Fe^{3+}/H_2O_2/Asc
-	-	50	100	250	500	1,000	BHT (μg)

Fig. 2A. Protection of BSA oxidative damage by BHT. The electrophoretic pattern of BSA. BSA was oxidised by Fenton system (Fe^{3+}/H_2O_2/ascorbic acid).

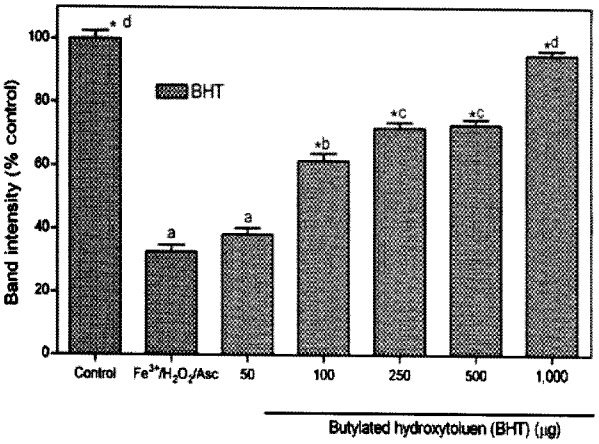


Fig. 2B. The densitometric analysis of the corresponding band intensity. Each bar represents the mean±SD of 3 different experiments. Means with different letters at a time differ significantly, $p<0.05$. *$p<0.05$ compared with Fe^{3+}/H_2O_2/ascorbic acid treated group.

+	+	+	+	+	+	+	BSA
-	+	+	+	+	+	+	Fe^{3+}/H_2O_2/Asc
-	-	50	100	250	500	1,000	HS (μg)

Fig. 3A. Protection of BSA oxidative damage by ethanol extract of *Hypericum scabroides* (HS). The electrophoretic pattern of BSA. BSA was oxidised by Fenton system (Fe^{3+}/H_2O_2/ascorbic acid).

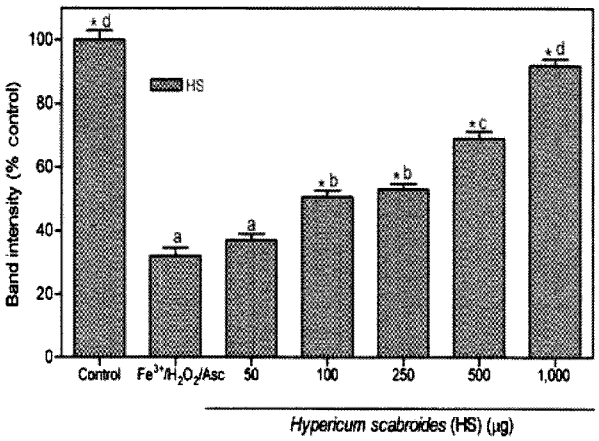


Fig. 3B. The densitometric analysis of the corresponding band intensity. Each bar represents the mean±SD of 3 different experiments. Means with different letters at a time differ significantly, $p<0.05$. *$p<0.05$ compared with Fe^{3+}/H_2O_2/ascorbic acid treated group.

means, such as spectrophotometric assay, enzyme-linked immunosorbent assay (ELISA), and 1-dimensional or 2-dimensional electrophoresis followed by Western blot immunoassay (29).

The effect of ethanol extracts of HS, HT, and BHT, on oxidative damage of BSA induced by ·OH is shown in Fig. 1. BHT, HS, and HT in the range of 50-1,000 μg/mL, showed concentration dependent reduction of albumin oxidation, induced by the H_2O_2/Fe^{3+}/ascorbic acid system, which resulted in formation of a carbonyl groups. The effect of BHT and ethanol extracts of HS and HT at 1,000 μg/mL, reached a plateau of 57± 3.7, 46±2.8, and 48±1.5% inhibition, respectively. BHT and ethanol extracts of HS and HT showed a significant inhibitory effect compared with the control ($p<0.05$). However, when BHT compared with ethanol extracts of HS and HT, the former had a significant inhibitory effect than latters at 100, 250, and 1,000 μg/mL concentrations. Furthermore, at 50 and 250 μg/mL, no significant difference was found between inhibitory effect of BHT and ethanol extracts of HS and HT. The inhibition of protein oxidation by HS and HT was the result of its scavenging effect on H_2O_2/Fe^{3+}/ascorbic acid generated reactive oxygen species.

Electrophoretic patterns of BSA after incubation with Fe^{3+}/H_2O_2/ascorbic acid system in the absence or presence of different concentrations of BHT and the corresponding densitometric analyses of the corresponding bands were presented in Fig. 2A and B. Three hr after incubation with Fe^{3+}/H_2O_2/ascorbic acid, the density of BSA band decreased to 32±4.5% of control. 1,000 μg/mL of BHT almost completely prevented the oxidative degradation of BSA. At that concentration BHT restored BSA band intensity to 95±2.5% of control levels.

The different concentrations of ethanol extracts of HS and HT also showed protective effect on BSA degradation induced by Fe^{3+}/H_2O_2/ascorbic acid system. The electrophoretic pattern of BSA and densitometric analysis of the corresponding bands are summarized in Fig. 3A and B and Fig. 4A and B. Ethanol extracts of HS (Fig. 3A and B) and HT (Fig. 4A and B), in the range of 50-1,000 μg/mL, showed a concentration-dependent inhibition of protein oxidation induced by Fe^{3+}/H_2O_2/ascorbic acid system. Densitometric analysis of each protein band showed quantified gel image and confirmed the protective effect of HS and HT on protein against ROS attact more clearly. One-thousand μg/mL HS and HT protected BSA very efficiently and restored the BSA band intensity to 92±3.5 and 95±3.0% of control levels, respectively.

DNA oxidation DNA damage caused by oxygen-derived species including free radicals is the most frequent type encountered by aerobic cells. When this type of damage occurs to DNA, it is called oxidative DNA damage and it can produce a multiplicity of modifications in DNA including base and sugar lesions, strand breaks, DNA-protein crosslinks and base-free sites. Many such lesions have been identified in cells and tissues at steady-state levels and upon exposure

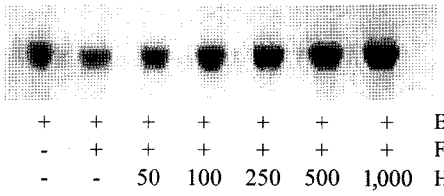


Fig. 4A. Protection of BSA oxidative damage by ethanol extract of *Hypericum triquetrifolium* (HT). The electrophoretic pattern of BSA. BSA was oxidised by Fenton system (Fe^{3+}/H_2O_2/ ascorbic acid).

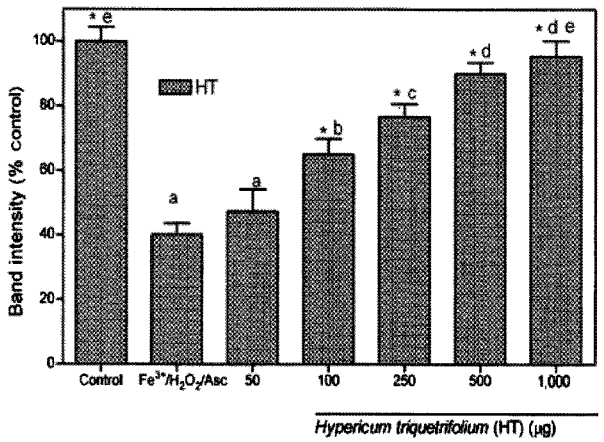


Fig. 4B. The densitometric analysis of the corresponding band intensity. Each bar represents the mean±SD of 3 different experiments. Means with different letters at a time differ significantly, $p<0.05$. *$p<0.05$ compared with Fe^{3+}/H_2O_2/ascorbic acid treated group

to free radical-generating systems. Data accumulated over many years clearly show that oxidative DNA damage plays an important role in a number of disease processes. Thus, oxidative DNA damage is implicated in carcinogenesis and neurodegenerative diseases such as Alzheimer's disease. There is also strong evidence for the role of this type of DNA damage in the aging process (30).

DNA damage protective activity of ethanol extracts of HS and HT were checked on pBluescript M13+ vector in *E. coli* XL-1 Blue strain. Plasmid DNA was isolated by Qiagene mini prep kit. Figure 5A and 6A show the quantified band intensity for the circular supercoiled DNA (form I), circular relaxed DNA (form II), and linear DNA (form III). Figure 5B and 6B show the electrophoretic pattern of DNA after UV-photolysis of H_2O_2 (2.5 mM) in the absence and presence of ethanol extracts of HS and HT (100, 200, 300, and 400 μg/mL), respectively. DNA derived from pBluescript M13+ DNA plasmid showed 2 bands on agarose gel electrophoresis (lane 1), the faster moving band corresponded to the native form of supercoiled circular DNA (scDNA) and the slower moving band was the circular rellaxed form (ocDNA). The UV irradiation of DNA in the presence of H_2O_2 (lane 2) resulted in the cleavage of scDNA to linear form (linDNA), indicating that OH radical generated from UV photolysis of H_2O_2 produced DNA strand scission. The addition of extracts (Fig. 5B and 6B, lane 6-9) to the reaction mixture suppressed the formation of linear DNA and induced a partial recovery of scDNA. The inhibition activities of the ethanol extracts of HS and HT on DNA damage were 95.60±3.0, 95.32±3.2, 97.14±3.4, 97.00±2.6, and 90.15± 3.06, 91.24±3.0, 95.94±3.5, and 95.61±2.6% at the concentrations of 100, 200, 300, and 400 μg/mL, respectively. And these inhibitory activities on DNA damage slightly increased with increment of the concentrations. In Fig. 5B and 6B, lane 5 represent the effects of ethanol extracts and UV on DNA without H_2O_2. The ethanol extracts of HS (200 μg) and HT (200 μg) have no significant effect on DNA. As shown in Fig. 5B and 6B (lane 6-9), ethanol extract from aerial parts of HS and HT showed a concentration-independent hydroxyl radical scavenging effect. In order to characterize the oxygen radicals leading to DNA cleavage, the effects of 3 free radical scavengers and inhibitors of DNA cleavage were studied. Figure 6D shows the electrophoretic pattern of DNA after UV-photolysis of H_2O_2 (2.5 mM) in the absence and presence

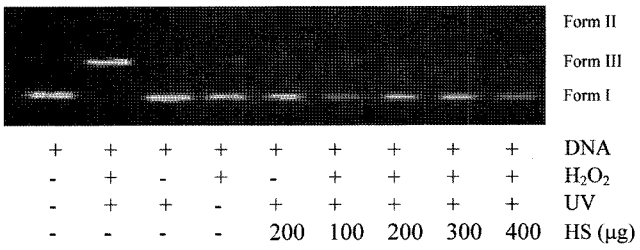


Fig. 5B. Electrophoretic pattern of pBluescript M13+ DNA after UV-photolysis of H_2O_2 in the presence or absence of ethanol extract of *Hypericum scabroides* (HS). Electrophoresis running buffer: TAE (40 mM Tris acetate, 1 mM EDTA, pH 8.2).

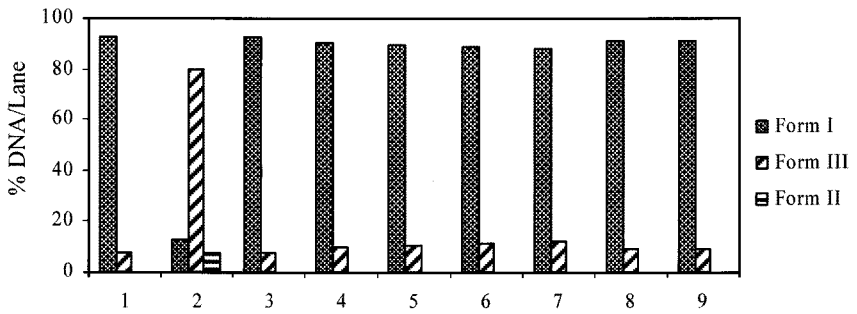


Fig. 5A. The quantified band intensity for the circular supercoiled DNA (Form I), circular relaxed DNA (Form II), and linear DNA (Form III) with Quantity One 4.5.2. version software.

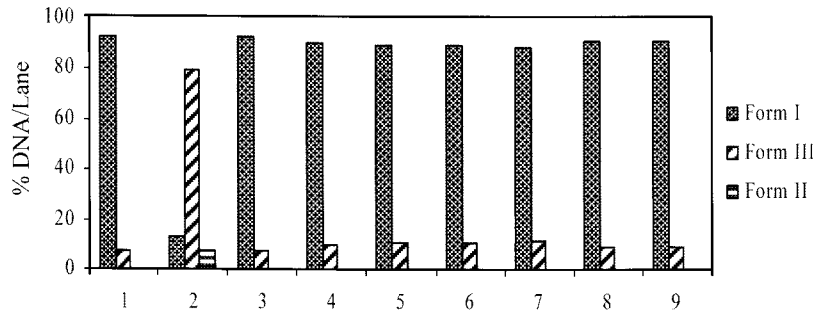


Fig. 6A. The quantified band intensity for the circular supercoiled DNA (Form I), circular relaxed DNA (Form II), and linear DNA (Form III) with Quantity One 4.5.2. version software.

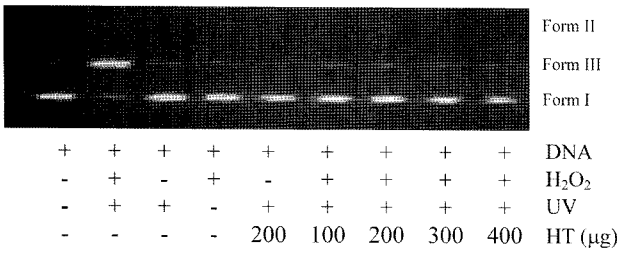


Fig. 6B. Electrophoretic pattern of pBluescript M13+ DNA after UV-photolysis of H_2O_2 in the presence or absence of ethanol extract of *Hypericum triquetrifolium* (HT). Electrophoresis running buffer: TAE (40 mM Tris acetate, 1 mM EDTA, pH 8.2).

of 50 mM dimethyl sulfoxide (DMSO), 10 mM thiourea, and 50 mM KI which are known as hydroxyl radical scavengers. The action of these extracts was comparable to 50 mM DMSO (lane 6), 10 mM thiourea (lane 7), and 50 mM KI (lane 8). DMSO, thiourea, and potassium iodide inhibited the DNA cleavage by 48.0±3.30, 78.5±1.55, and 77.30±1.13%, respectively. Ethanol extracts of HS and HT have more protecting activity than DMSO, thiourea, and KI on DNA cleavage.

Thus, the identification of natural products able to give protection against UV radiation-induced inflammatory responses and the generation of oxidative stress may have important human health implications. The DNA cleavage analysis demonstrated the strong antioxidant properties of HS and HT. In fact, both these extracts suppressed the formation of linear DNA, generated by exposure of plasmid DNA to OH radical generated by UV-photolysis/ H_2O_2, and induced a partial recovery of scDNA. DNA damage protecting activity of both extracts are corresponding to its antioxidant potential.

Results obtained herein on protective ability of ethanol extracts of HS and HT against the protein oxidation and DNA damage support the traditional medicinal use of these plants and provide grounds for further establishing its use as a functional food.

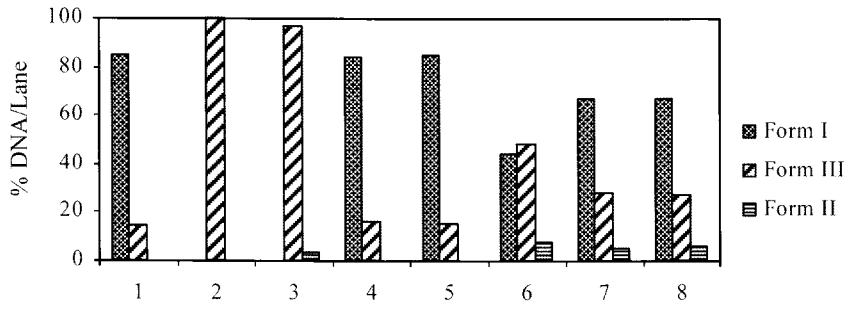


Fig. 6C. The quantified band intensity for the circular supercoiled DNA (Form I), circular relaxed DNA (Form II), and linear DNA (Form III) with Quantity One 4.5.2. version software.

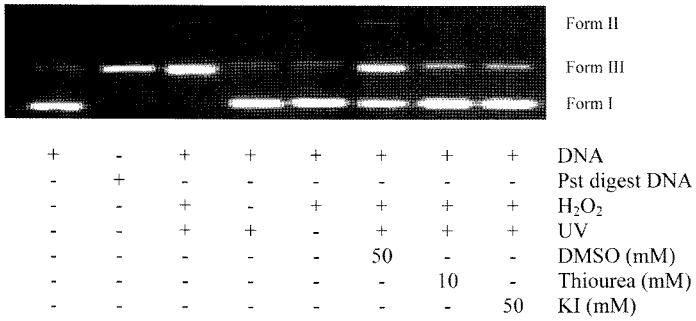


Fig. 6D. Electrophoretic pattern of pBluescript M13+ DNA after UV-photolysis of H_2O_2 in the presence or absence of DMSO, thiourea, and potassium iodide. Electrophoresis running buffer: TAE (40 mM Tris acetate, 1 mM EDTA, pH 8.2).

The present study suggest that, the ethanol extracts of HS and HT has antioxidative activity, is capable of supressing DNA cleavage and protein oxidation *in vitro*. The polyphenol plant extracts content seems to be responsible for the scavenging activity of the ROS, resulting in protection against protein oxidation and DNA damage induced by oxidative stress. Therefore may be beneficial in the preventation of ROS related diseases, such as cardiovascular, inflammatory, and cancer as well as materials or additives for oxidation suppresssant in food, cosmetic and drug compositions.

In conclusion, it is still not know how fruits, vegetables, and plants protect against cancer, but it seems increasingly unlikely that it is simply because they contain high concentrations of antioxidants. The effects of phytochemicals (which may or may not be antioxidants) on many other cellular functions, including cell-signalling, apoptosis, antioxidant enzymes, the phase I and II xenobiotics-metabolising enzymes, DNA repair, plus of course, the enormous potential for effects on gene expression that might have an impact on the carcinogenic process.

Acknowledgments

This study was supported by Dicle University Research Foundation (Project no: 04-FF-55 and 03-FF-63), Turkey.

References

1. Halliwell B, Gutteridge JMC. Free radicals in biology and medicine. Oxford University Press, Oxford, UK. pp. 1-25 (1997)
2. Collins BH, Horska A, Hotten PM, Riddoch C, Collins AR. Kiwifruit protects against oxidative DNA damage in human cells and *in vitro*. Nutr. Cancer 39: 148-153 (2001)
3. Bergman M, Varshavsky L, Gottlieb HE, Grossman S. The antioxidant activity of aqueous spinach extract: Chemical identification of active fractions. Phytochemistry 58: 143-152 (2001)
4. Bombardelli E, Morazzoni P. *Hypericum perforatum*. Fitoterapia 66: 43-68 (1995)
5. Kizil G, Toker Z, Özen HÇ, Aytekin Ç. The antimicrobial activity of essential oils of *Hypericum scabrum, Hypericum scabroides*, and *Hypericum triquetrifolium*. Phytother. Res. 18: 339-341 (2004)
6. Toker Z, Kizil G, Özen HÇ, Kizil M, Ertekin S. Compositions and antimicrobial activities of the essential oils of two *Hypericum* species from Turkey. Fitoterapia 77: 57-60 (2006)
7. Sokmen A, Jones MB, Ertürk M. Antimicrobial activity of extracts from the cell cultures of some Turkish medicinal plants. Phytother. Res. 13: 355-357 (1999)
8. Conforti F, Statti AG, Tundis R, Menichini F, Houghton P. Antioxidant activity of methanolic extract of *Hypericum triquetrifolium* Tura. Fitoterapia 73: 479-483 (2002)
9. Cakir A, Mavi A, Yıldırım A, Duru ME, Harmandar M, Kazaz C. Isolation and characterization of antioxidant phenolic compounds from the aerial parts of *Hypericum hyssopifolium* L. by activity-guided fractionation. J. Ethnopharmacol. 87: 73-83 (2003)
10. Davis PH. Flora of Turkey and the East Aegean Islands. Edinburgh University Press, Edinburgh, UK. p. 399 (1988)
11. Baytop T. Therapy with Medicinal Plants in Turkey. İstanbul University Press, İstanbul, Turkey. pp.166-167 (1999)
12. Hakimoglu F, Kizil G, Kanay Z, Kizil M, Isi H. The effect of ethanol extract of *Hypericum lysimachioides* on lipid profile in hypercholesterolomic rabbits and its *in vitro* antioxidant activity. Atherosclerosis 192: 113-122 (2007)
13. Kizil G, Kizil M, Yavuz M, Emen S, Hakimoglu F. Antioxidant activities of ethanol extracts of *Hypericum triquetrifolium* and *Hypericum scabroides*. Pharm. Biol. 46: 231-242 (2008)
14. Kuchino Y, Mori F, Kasai H, Inonue H, Iwai S, Miura K, Ohtsuka E, Nishimura S. Misreading of DNA templates containing 8-hydroxydeoxyguanosine at the modified base and at adjacent residues. Nature 327: 77-97 (1987)
15. Russo A, Izzo AA, Cardile V, Borrelli F, Vanella A. Indian medicinal plants as antiradicals and DNA cleavage protectors. Phytomedicine 8: 125-132 (2001)
16. Youngman LD, Park JY, Ames BN. Protein oxidation associated with aging is reduced by dietary restriction of protein or calories. P. Natl. Acad. Sci. USA 89: 9112-9116 (1992)
17. Stadtman ER, Levin RL. Protein oxidation. Ann. NY Acad. Sci. 899: 191-208 (2000)
18. Esterbauer H, Schaur RJ, Zollner H. Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde, and related aldehydes. Free Radical Bio. Med. 11: 81-128 (1991)
19. Barlow SM. Toxicology aspects of antioxidants used as food additives. Vol. 253, pp. 253-307. In: Food Antioxidants. Hudson BJF (ed). Elsevier, New York, NY, USA (1990)
20. Elzaawely AA, Xuan TD, Tawata S. Antioxidant and antibacterial activities of *Rumex japonicus* HOUTT. aerial parts. Biol. Pharm. Bull. 28: 2225-2230 (2005)
21. Ardestani A, Yazdanparast R. Inhibitory effects of ethyl acetate extract of *Teucrium polium* on *in vitro* protein glycoxidation. Food. Chem. Toxicol. 45: 2402-2411 (2007)
22. Kitts DD, Yuan YV, Wijewickreme AN, Hu C. Antioxidant properties of a North American gingseng extract, Mol. Cell. Biochem. 203: 1-10 (2000)
23. Wang BS, Lin SS, Hsiao WC, Fan JJ, Fuh LF, Duh PD. Protective effects of an aqueous extract of Welsh onion green leaves on oxidative damage of reactive oxygen and nitrogen species. Food. Chem. 98: 149-157 (2006)
24. Laemli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 27: 680-685 (1970)
25. Attaguile G, Russo A, Campisi F, Savoca F, Acquaviva R, Ragusa N, Vanella A. Antioxidant activity and protective effect on DNA cleavage of extracts from *Cistus incanus L.* and *Cistus monspeliensis L.* Cell Biol. Toxicol. 16: 83-90 (2000)
26. Fukuhara K, Miyata N. Resveratrol as a new type of DNA-cleaving agent. Bioorg. Med. Chem. Lett. 8: 3187-3192 (1998)
27. Dean RT, Fu S, Stocker R, Davies MJ. Biochemistry and pathology of radical-mediated protein oxidation. Biochem. J. 324: 1-18 (1997)
28. Reznick AZ, Packer L. Oxidative damage to proteins: Spectrophotometric method for carbonyl assay. Method Enzymol. 233: 357-363 (1994)
29. Dalle-Donne I, Rossi R, Giustarini D, Milzani A, Colombo R. Protein carbonyl groups as biomarkers of oxidative stress. Clin. Chim. Acta 329: 23-38 (2003)
30. Dizdaroglu M. Introduction to serial reviews on oxidative DNA damage and repair. Free Radical Bio. Med. 32: 677 (2002)