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Antioxidant Capacity of Catnip (*Nepeta cataria*)

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Antioxidants are emerging as prophylactic and therapeutic agents for various diseases. Antioxidants are important in protection against hypertension, diabetes, cardiovascular diseases and cancer. However, little is known about the antioxidant property of the extract from *Nepeta cataria*, a medicinally useful traditional medicinal herb. Here the antioxidant capacity of ethanolic extracts prepared from *Nepeta cataria* was evaluated. The present results demonstrate that *Nepeta cataria* extracts examined in this article exhibit antioxidant activity. This activity is correlated with the total phenolic compounds content in the extract which implicates that the *Nepeta cataria* extract may serve as potential natural source of antioxidants.

Key Words: Antioxidant activity, Catnip, *Nepeta cataria*.

INTRODUCTION

Catnip (*Nepeta cataria*) is a perennial herb belonging to the mint family, Labiatae[1,2]. Its cousins include basil, oregano and spearmint. All these plants produce essential oils that contain flavourful and aromatic terpenoids such as limonene, menthol and spearmint. It is also known as catnep, catmint, catrup, catwort, nip, nep and field balm[3,4]. The entire plant is harvested when in flower, which occurs from June to September[4,5]. It has a mint-like taste and odour and is strongly scented. Oil isolated from catnip by steam distillation is a repellent against insects, in particular mosquitoes, cockroaches and termites. Research suggests that in a test tube, distilled nepetalactone the active ingredient in catnip, repels mosquitoes ten times more effectively than commercial mosquito repellents. Additionally, catnip and catnip-laced products designed for use with domesticated cats are available to consumers. Catnip mixed together with chamomile tea can be used to lighten the colour of hair. Catnip and catmints are mainly known for the behavioural effects they have on cats, particularly domestic cats[3-6]. Compounds in catnip alter the behaviour of wild and domestic cats, other mammals and insects[6]. The main constituent that attracts cats is the *trans*, *cis* isomer of the unsaturated lactone, nepetalactone. epetalactone constitutes 70-99 % of the essential oil of the catnip plant. It is metabolized and excreted in the urine[7].

Catnip has been prepared and used by people for many years. It was originally used as a tea, juice, tincture, infusion and poultice and has been smoked and chewed.

It fell out of favour with the development of more effective drugs. More recently, it has been used by people for its hallucinogenic effects. It has a soothing effect and has been used to treat nervous headaches, hysteria and insanity. The root portion of the plant has the opposite effect. One reference stated, "*if the root be chewed it will make the quietest person fierce and quarrelsome*". It seems that catnip was used both as a mild stimulant and for its quieting effect on the nervous system. Folk medicine also suggests many other beneficial uses of catnip. The herb has been described as a remedy for colic, minor aches and pains in the gums and teeth and indigestion, to name just a few examples[6,7].

No detailed studies on the antioxidant capacity of catnip have previously been reported. The aim of this study is to investigate the antioxidant capacity of catnip.

EXPERIMENTAL

All chemicals used were analytical grade and obtained from either Sigma-Aldrich or Merck.

Plant materials and extraction procedures: Catnip (*Nepeta cataria*) was obtained from local market in Malatya province in Turkey. For ethanolic extraction, 25 g of catnip ground into a fine powder in a mill and were mixed 5 times with 100 mL of ethanol. Extraction continued until the extraction solvents became colourless (total solvent volume is 400 mL). For obtained crude extracts were filtered on Whatman No. 1 paper and the filtrate was collected, then ethanol was removed by a rotary evaporator at 50 ºC. This crude ethanolic extract of catnip (EEC) was used for antioxidant activity tests.

Antioxidant activity

Total antioxidant activity determination by ferric thiocyanate method (FTC): The antioxidant activity of ethanolic extract of catnip (EEC) and standards were determined according to the ferric thiocyanate method[8]. For preparation of stock solutions, 10 mg of EEC was dissolved in 10 mL of ethanol. Then, the solution of EEC (50 µg/mL) or standard samples (50 µg/mL) 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). The mixed solution (5 mL) was incubated at 37 ºC in a glass flask. During incubation the linoleic acid oxidation, peroxides are formed, which oxidize Fe^{2+} to Fe^{3+}. The latter ions form a complex with thiocyanate and this complex has a maximum absorbance at 500 nm. Therefore, high absorbance indicates high linoleic acid emulsion oxidation. The solutions without added extract or standard were used as control sample. All the data on total antioxidant activity are the average of triplicate analyses. The percentage inhibition of lipid peroxidation in linoleic acid emulsion was calculated by following equation.

$$\text{Inhibition of lipid peroxidation}\,(\%) = 100 - \left(\frac{A_{\text{Sample}}}{A_{\text{Control}}} \times 100\right)$$

Herein $A_{Control}$ is the absorbance of the control reaction and A_{Sample} is the absorbance in the presence of the sample (EEC) or standard compounds[9].

Reduction power (FRAP): The samples prepared for ferric cyanate method were used for this and the other assays. The reducing power of extract was determined by the method of Oyaizu[10]. Different concentrations of EEC (20-200 µg/mL) were mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferric cyanide [$K_3Fe(CN)_6$] (2.5 mL, 1 %). The mixture was incubated at 50 ºC for 20 min. 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 (Jasco V-530 UV/VIS spectrophotometer). Increased absorbance of the reaction mixture indicates increased reducing power.

Radical scavenging activity

2,2-Diphenyl-1-picryl-hydrazil (DPPH) free radical scavenging activity: The free-radical scavenging capacity of EEC was evaluated with the DPPH$^{\bullet}$ stable radical following the methodology described by Blois[11]. This method is described extensively elsewhere[12]. Wherein the bleaching rate of a stable free radical, DPPH$^{\bullet}$ is monitored at a characteristic wavelength in the presence of the sample. Briefly, 0.1 mM solution of DPPH$^{\bullet}$ in ethanol was prepared and 1 mL of this solution was added 3 mL of EEC solution in water at different concentrations (25-75 µg/mL). Thirty minutes later, the absorbance was measured at 517 nm. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity.

The capability to scavenge the DPPH$^{\bullet}$ radical was calculated using the following equation:

$$\text{DPPH}^{\bullet}\text{ scavenging effect (\%)} = \left(\frac{A_{Control} - A_{Sample}}{A_{Control}} \right) \times 100$$

wherein $A_{Control}$ is the absorbance of the control reaction and A_{Sample} is the absorbance in the presence of EEC[13].

Superoxide anion radical scavenging activity: Measurement of superoxide anion scavenging activity of EEC was based on the method described by Beacuhamp and Fridovich[14]. All solutions were 0.05 M in phosphate buffer (pH 7.8). The photo-induced reactions were performed in an aluminium foil-lined box with fluorescent lamps. The distance between reactant and lamp was adjusted until the intensity of illumination reached 4000 lx. The total volume of reactant was 5 mL and the concentrations of riboflavin, methionine and nitro blue tetrazolium (NBT) were 3×10^{-6}, 1×10^{-2} and 1×10^{-4} M, respectively. The reaction was performed at room temperature for 25 min. During this period riboflavin generated O^{2-} which reduced NBT to form formazan. The absorbance was read at 560 nm. Ethanolic extract of catnip, BHT or α-tocopherol as a standard were added to the reaction mixture, in which of O^{2-} was scavenged, thereby inhibiting the NBT reduction. The unilluminated reaction mixture was used as a blank. Decreased absorbance of the reaction mixture indicates increased

superoxide anion scavenging activity. The percentage inhibition of superoxide anion generation was calculated using the following formula:

$$\text{Scavenging (\%)} = \left(\frac{A_{\text{Control}} - A_{\text{Sample}}}{A_{\text{Control}}} \right) \times 100$$

where $A_{Control}$ is the absorbance of the control and A_{Sample} is the absorbance of EEC or standards[15].

Statistical analysis: All data on total antioxidant activity are the average of duplicate analyses. The other analyses were performed in triplicate. The data were recorded as mean ± standard deviation and analyzed by SPSS (version 9.0 for Windows 98, SPSS Inc.). One-way 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 significant.

RESULTS AND DISCUSSION

Antioxidant capacity is widely used as a parameter to characterize food, synthetic compounds or medicinal plants and their bioactive components. In this study, the antioxidant activity of EEC, BHA, BHT and α-tocopherol has been evaluated in a series of *in vitro* antioxidant assay.

Total antioxidant activity determination in linoleic acid emulsion system by ferric thiocyanate method: Total antioxidant activity of EEC and the reference compounds such BHA, BHT and α-tocopherol were determined by the ferric thiocyanate method. Ethanolic extract of catnip and standards compounds exhibited effective antioxidant activity. At the 50 mg/mL concentration, EEC, BHA and α-tocopherol on lipid peroxidation of linoleic acid emulsion are shown in Fig. 1. The percentage inhibition of α-tocopherol, EEC and BHA in linoleic acid system was 66.5, 61.1 and 51.5 %, respectively at the same concentration.

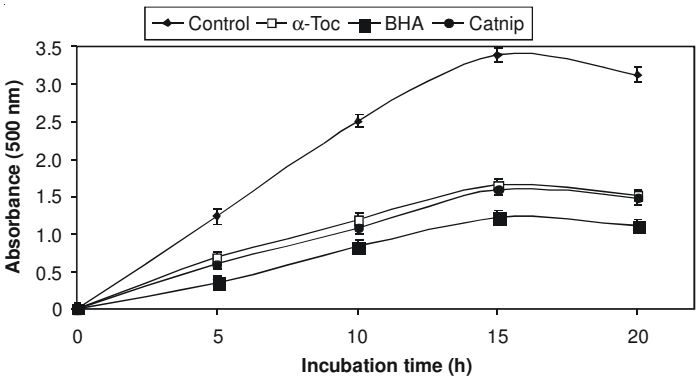

Fig. 1. Total antioxidant activities of different concentrations (5-20 mg/mL) of catnip and standard antioxidant compounds such as BHA, BHT and α-tocopherol at the same concentrations (α-Toc: α-tocopherol, BHA: Butylated hydroxyanisole, BHT: Butylated hydroxytoluene)

Reduction power by Fe^{3+}-Fe^{2+} transformation: The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. The presence of reductants such as antioxidants substances in the antioxidant samples causes the reduction of the Fe^{3+}/ferricyanide complex to the ferrous form. Therefore, the Fe^{2+} can be monitored by measuring the formation of Perl's Prussian blue[16] at 700 nm. Fig. 2 depicts the reducing power of EEC and standards (BHT, BHA and α-tocopherol) using the potassium ferricyanide reduction method. Like the antioxidant activity, the reducing power of EEC, BHT, BHA and α-tocopherol increased with increasing concentration. Reducing power of EEC and standard compounds exhibited the following order: BHA >BHT > α-tocopherol > EEC.

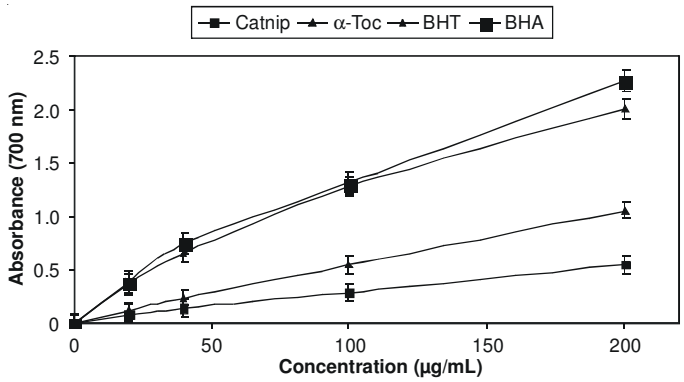

Fig. 2. Total reductive potential of different concentrations (15-200 mg/mL) of catnip and reference antioxidants; BHA, BHT and α-tocopherol determined by FRAP method (α-Toc: α-tocopherol, BHA: Butylated hydroxyanisole, BHT: Butylated hydroxytoluene)

Radical scavenging activity: Antioxidant properties, especially radical scavenging activities, are very important due to the deleterious role of free radicals in foods and in biological systems. Excessive formation of free radicals accelerates the oxidation of lipids in foods and decreases food quality and consumer acceptance. In this study, antioxidant activities of EEC and standard antioxidants were determined using a DPPH and superoxide scavenging method.

Free radical (DPPH) scavenging activity: DPPH has been widely used to evaluate the free radical scavenging effectiveness of various antioxidant substances in food systems[10,17]. DPPH free radical scavenging is an accepted mechanism by which antioxidants act in inhibiting lipid oxidation. The method has been used extensively to predict antioxidant activities because of the relatively short time required for analysis. The method is based on the reduction of alcoholic DPPH solution in the presence of a hydrogen-donating antioxidant due to the formation of the non-radical form DPPH-H by the reaction[9]. Fig. 3 illustrates a significant decrease ($p < 0.05$) in the concentration of DPPH radical due to the scavenging ability of EEC, α-tocopherol and BHT were used as references radical scavengers. The scaven-

ging effect of EEC and standards on the DPPH radical decreased in order: α-tocopherol > EEC > BHT. Free radical scavenging activity of these samples also increased with increasing concentration.

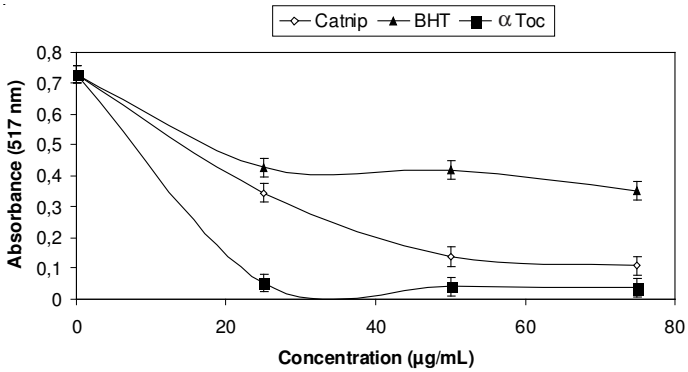

Fig. 3. DPPH free radical scavenging activity of different concentrations (25-75 mg/mL) of catnip and reference antioxidants; BHT and α-tocopherol (α-Toc: α-tocopherol, BHT: Butylated hydroxytoluene; DPPH•: 2,2-diphenyl-1-picryl-hydrazyl free radical)

Superoxide anion radical scavenging activity: Superoxide anion, which is a reduced form of molecular oxygen, has been implicated in the initiating oxidation reactions associated with aging[18]. It has also been implicated in several pathophysiological processes, due to its transformation into more reactive species such as hydroxyl radical that initiate lipid peroxidation. Superoxide has also been observed to directly initiate lipid peroxidation[19]. It has also been reported that antioxidant properties of some flavonoids are effective mainly *via* scavenging of superoxide anion radical[12,20]. Superoxide anion plays an important role in formation of other reactive oxygen species such as hydrogen peroxide, hydroxyl radical and singlet oxygen, which induce oxidative damage in lipids, proteins and DNA[10]. The decrease of absorbance at 560 nm with antioxidants indicates the consumption of superoxide anion in the reaction mixture[12,15]. Fig. 4 shows the percentage inhibition of superoxide radical generation by 4-16 mg/mL concentration of EEC, BHA and α-tocopherol. The inhibition of superoxide radical generation results of EEC and standard were found similar statistically ($p > 0.05$). As can see in Fig. 4, the percentage inhibition of superoxide generation by 4 mg/mL concentration of ethanolic extract of catnip, α-tocopherol and BHA were found as 14.09, 19.17 and 26.78 %, respectively. Superoxide radical scavenging activity of those samples showed the following order: EEC < α-tocopherol < BHA.

Conclusion

Catnip has been used by people for many years. It was traditionally used as a tea, juice, tincture, infusion and poultice and even it has been smoked and chewed. Antioxidant properties of plant extracts should be evaluated in a variety of model

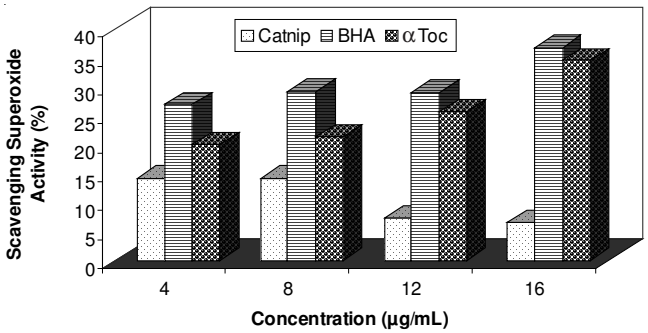

Fig. 4. Comparison of superoxide anion radical scavenging activity of 16 mg/mL concentration of catnip BHA and α-tocopherol (α-Toc: α-tocopherol, BHA: Butylated hydroxyanisole)

systems using several different indices because the effectiveness of such antioxidant materials. Obtained results in this study clearly showed that EEC of catnip has antioxidant activity against various antioxidant systems *in vitro*, moreover, this extract can be used as easily accessible source of natural antioxidants and as a possible food supplement or in pharmaceutical applications. It can also be used in stabilizing food against oxidative deterioration.

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