In vitro Antioxidant Activities of Extracts from Some Nepeta Species

Sercan ÖZBEK YAZICI1, Ismail ÖZMEN2, Umut CELIKOGLU2, Hasan ÖZCELİK2, Hasan GENC3

1Health School, Mehmet Akif Ersoy University, Burdur, Turkey
2Faculty of Arts and Sciences, Süleyman Demirel University, Isparta, Turkey
3Faculty of Education, Mehmet Akif Ersoy University, Burdur, Turkey

Received: 01.11.2011 Accepted: 14.01.2012 Published: 15.02.2012

Abstract
In this study, the purpose is to investigate and compare the in vitro antioxidant activities of methanol extracts of Nepeta italica, Nepeta cilicia and Nepeta caesarea. The antioxidant capacities of 3 different species collected from different locations and extracted with methanol were assayed by 1, 1- diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging activity and cupric reducing antioxidant capacity (CUPRAC) assay. The total phenolic contents of N. italica, N. cilicia, N. caesarea extracts measured by Folin-Ciocalteu method were 24.8, 21.4 and 17.3 µg/mg (extract) as gallic acid equivalent whereas the antioxidant activities found by CUPRAC expressed as trolox equivalent antioxidant capacity and DPPH assay expressed as IC50; 114.5±1.4, 90.4±1.05 and 80.4±0.71 µmol TR/g and 25.5 ± 0.55 33.4 ± 1.25 and 39.1± 0.76 µg/ml, respectively.

The present results showed that the extracts exhibited antioxidant properties. Especially, the extract of N. italica has higher activity than others for all assays. It may be suggested that the extracts of Nepeta species possessed middle antioxidant power when compared to other plants belonging to the Lamiaceae family.

Key words: Nepeta species, Antioxidant activity, DPPH, Folin-Ciocalteu, CUPRAC assay

INTRODUCTION
Nepeta belongs to the Lamiaceae family of plant and is a genus of perennial or annual herbs which is found in Asia, Europe and North Africa. About 280 species of Nepeta are reported of which, 17 species are present in Turkey (Davis 1982). Nepeta species are widely used in folk medicine because of their antispasmodic, expectorant, diuretic, antiseptic, antitussive, antiasthmatic and febrifuge activities (Baser et al. 2000). Most of the studies on Nepeta species have been conducted to determine their essential oil composition and biological activities (Tepe et al. 2007). It has been found that some Nepeta species have insect repellant, anti-inflammatory (Miceli et al. 2005), anti-fungal, antimicrobial (Sonboli et al. 2004), analgesic (Aydın et al. 1998) and sedative activities (Rabbani et al. 2008). However, in vitro antioxidant activity of essential oil and some extracts of Nepeta species have also been demonstrated (Dapkevicius et al. 1998; Miceli et al. 2005; Tepe et al. 2007; Alim et al. 2009).

The medical properties of Nepeta species are usually attributed to their essential oils and flavonoids (Jamzad et al. 2003). It has been reported that the essential oils of Nepeta species contain nepetalactones, caryophyllene oxide, and 1,8-
cineole and/or linalool as the main components but different oil compositions have been detected in several *Nepeta* species (Tepe et al. 2007; Giamperi et al. 2009).

The essential oil of *N. italica* L. has been characterized by a high percentage of monoterpenes (95%), mainly 1,8-cineole; however, a major component of the essential oil of *N. cincerea Boiss* has been found as β-caryophyllene oxide (% 40.7), a sesquiterpene (Kokdil et al.1997). Yet in another study, limonene was major component and chemical differences between the plant contents have been explained with a difference of collecting area (Karaman and Comlekiciglu 2007).

*N. caesarea* Boiss. (*Lamiaceae*), an endemic species to Turkey, has folkloric uses in southern Anatolia and is used as an herbal tea to treat gastric disorders (Saracoglu et al. 1990). Baser and Ozek (1994) reported that 4α, 7α, 7α β-nepetalactone was found to be the major component in the *N. caesarea* oil. Moreover, *N. caesarea* was found to show significant analgesic activity, as well as marked sedation (Aydın et al. 1998).

The essential oil composition of *N. italica, N. cincerea* and *N. caesarea* has been reported previously (Baser and Ozek 1994; Kokdil et al. 1997; Kokdil et al. 1999), but we have found little information on their antioxidant activities. Therefore, in this study, the purpose is to investigate and compare the in vitro antioxidant activities of methanol extracts of the *Nepeta* species by three “in vitro” systems.

**MATERIAL AND METHODS**

Plant Materials

Wild growing *N. italica, N. caesarea* and *N. cincerea* was collected at the stage of full blooming, *N. italica* - Turkey, Ç3 Burdur to Aglasun 20 km, road sides 1200-1400m, 30.06.2009. *N. cincerea* Boiss: Turkey, C4 Akseki to Seydişehir, 10 km, limestone 1265m, 06.06.2009. *N. caesarea* Sütcüler (Isparta) Tot-Hacı Ilyas mountain, 1600-2000m, 27.8.2009. The voucher specimens were determined by Dr. Hasan Özcelik1 and Dr. Hasan Genc2 at the Department of Biology, Suleyman Demirel University and Mehmet Akif Ersoy University and have been deposited at the Herbarium of the Department of Biology, Suleyman Demirel University, Isparta-Turkey.

Preparation of Extract

The air ground plants were dried in the shade at room temperature and then pulverized. Extracts of plant materials were prepared by using solvent and the extraction protocol which is given below:

50 g of pulverized plant materials defatted with petrol in soxhlet apparatus. The solvent free plant materials were extracted with methanol by using soxhlet apparatus (6h for each solvent).

**Antioxidant Activity Determine**

**Assay for total phenolic contents**

Total phenolic constituents of the extracts of *Nepeta* were determined by literature methods involving Folin-Ciocalteu reagent and gallic acid as standard (Singleton et al. 1999). Extract solution (0.1 ml) containing 1000 µg of extract was taken in a volumetric flask; 46 ml of distilled water and 1 ml Folin-Ciocalteu reagent were added and the flask was thoroughly shaken. After 3 min., 3 ml of a solution of 2% Na2CO3 were added and the mixture was allowed to stand for 2 h with intermittent shaking. Absorbance was measured at 760 nm. The same procedure was repeated for all standard gallic acid solutions (0–1000 µg 0.1 ml-1) and a standard curve was obtained with the equation given below:

Absorbance = 0.0012 x Gallic acid (microgram) + 0.0003

**DPPH (2,2-diphenylpicrylhydrazyl) Assay**

The hydrogen atom-or-electron donation ability of the corresponding extracts and some pure compounds was measured from the bleeding of the purple colored methanol solution of DPPH•. This spectrophotometric assay uses the stable radical, DPPH•, as a reagent (Burits and Bucar 2000). Fifty microliters of various concentrations of the extracts in methanol were added to 3 ml of a 0.0024% methanol solution of DPPH•. After a 30 min incubation period at room temperature, the absorbance was read against a blank at 517 nm. Inhibition of free radical, DPPH•, in percent (I %) was calculated in following way:

I% = (Ablank – Asample/Ablank) X 100

Where Ablank is the absorbance of the control reaction (containing all reagents except the test compound), and Asample is the absorbance of the test compound. Extract concentration providing 50% inhibition (IC50) was calculated from the graph plotted of inhibition percentage against extract concentration.

**CUPRAC (Cupric Ion Reducing Antioxidant Capacity) Assay**

1 ml CuCl2 (1.0x10-2 M), 1 ml neocuproine alcoholic solution (7.5x/10-3 M) and 1 m NH4Ac buffer solution were added to a test tube, followed by mixing; (x) ml herbal of extract followed by (1.1-x) ml water were then added (total volume, 4.1 ml) and mixed well. Absorbance against a reagent blank was measured at 450 nm after 30 min. Since the molar absorptivity of trolox in the CUPRAC method is ε =1.66x104 l/mol/cm, and the calibration curve for trolox is a line passing through the origin, the trolox equivalent molar concentration of the herbal extract sample in the final solution may be found by dividing the observed absorbance by the ε for trolox. The trolox equivalent antioxidant capacity may be
three parallel measurements. The data were tested using SPSS (Version 15.0). Statistical analysis of the results was based on Mann–Whitney U-test and Pearson correlation analyses.

RESULTS

In this study, the extracts were individually assessed for their possible antioxidant activities by employing three complementary tests; assays for total phenolics, DPPH free radical-scavenging, and CUPRAC were given.

The phenolic contents of the extracts of *N. italic*, *N. cilia* and *N. caesarea* are reported in (Table 1). The amount of phenolic contents was 24.8, 21.4 and 17.3 µg of gallic acid equivalents (GAE)/mg extract in *N. italic*, *N. cilia* and *N. caesarea* respectively.

<table>
<thead>
<tr>
<th>Plant species</th>
<th>TPC*</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>N. italic</em></td>
<td>24.8</td>
</tr>
<tr>
<td><em>N. cilia</em></td>
<td>21.4</td>
</tr>
<tr>
<td><em>N. caesarea</em></td>
<td>17.3</td>
</tr>
</tbody>
</table>

* Values are mean (n=3). Expressed as µg gallic acid/mg of the extract.

DPPH Assay

The extracts of *N. italic*, *N. cilia* and *N. caesarea* exhibited a concentration dependent antiradical activity. by quenching DPPH radical with values IC$_{50}$ of 25.5 ± 0.55 33.4 ± 1.25 and 39.1± 0.76 µg/ml, respectively (Figure 1). The IC$_{50}$ of butylatedhydroxyanisole (BHA) as a standard antioxidant is higher than that of the extracts.

CUPRAC Assay:

CUPRAC values of antioxidant capacities of studied extracts reported in Figure 2 show that the extracts of *N. italic*, *N. cilia* and *N. caesarea* gave trolox equivalent of 114.5±1.4, 90.4±1.05 and 80.4±0.71 µmol TR/g, respectively.

**DISCUSSION**

Natural antioxidants mostly exist on plants which contain phenolic compounds. The phenolic content and composition of plants depend on genetic and environmental factors (Tepe et al. 2007; Koksal et al. 2011). There are many different antioxidant compounds in plants and it is very hard to determine each antioxidant component separately. Therefore, the antioxidant potential of extracts is evaluated by an approach with multiple assays. In this study, the three methods, Folin, DPPH radical-scavenging activity, CUPRAC, were studied to identify antioxidant capacity of the *Nepeta* species.

The extraction yield and the antioxidant activity of extracts are related to the solvent polarity. The highest yields are usually achieved with methanol, ethanol and their mixture with water (Franco et al. 2008; Adıguzel et al. 2009). Also, it was reported that *Nepeta* species extracted with methanol were usually higher phenolic content (Tepe et al. 2007; Kraujalis et al. 2011). The amounts of total phenolic in the extracts are shown in Figure 1. The amount of total phenolics was highest in the extract of *N. italic*. As far as our literature survey could ascertain, several studies have been carried out with the *Nepeta* species and their total phenolic content results support our findings (Adıguzel et al. 2009; Kraujalis et al. 2011). However, Tepe et al. (2007) and Safaei-Ghomi et al. (2011) found that the amounts of total phenolic of *N. flavida*, *N. laxiflora* and *N. sessilifolia* were much higher than the *Nepeta* species analyzed.

Radical scavenging capacity was assessed by using DPPH assay. The IC$_{50}$ values are expressed as µg/ml and represent the concentration of extracts that is required for 50% of free...
radicals inhibition. As shown in Figure 1, the extracts were able to reduce the stable radical. The extract of *N. italicca* had the strongest free radical-scavenging capacity and it was close to value of the standard antioxidant (BHA). We found only one study on antioxidant activity of extracts from *Nepeta* species in our study and the study was found that the extract of *N. italicca* was highly effective against DPPH radicals (Emre et al. 2011). Also, some researchers reported that extracts obtained from other *Nepeta* species possessed strong antioxidative activity in DPPH assay (Miceli et al. 2005; Tepe et al. 2007; Kraujalis et al. 2011).

In this study, CUPRAC assay was chosen to determine antioxidative capacity of the extracts. Because it has been reported that the results obtained from in vitro CUPRAC measurements might be more efficiently extended to the possible in vivo reactions of antioxidants (Gulcin et al. 2011). Cupracy iron reducing capacity of the extracts of *Nepeta* species were evaluated for the first time here and expressed as trolox equivalent antioxidant capacity (TEAC) (Figure 2). For this reason, there is no data on TEAC values of *Nepeta* species, the results were just compared to the data on TEAC values of different plant species. Apak et al. (2006) found that CUPRAC values of some herbal teas were quite high when compared to our findings. On the contrary, Gorinstein et al. (2010) found that CUPRAC values of exotic fruits were low when compared to our findings.

Test statistics indicate that the differences between total phenolic content and antioxidant activities of the extracts were not significant (p ≥ 0.05). However; the results of DPPH and CUPRAC assays are found to be correlated with the total phenolic content, r= -0.971, r= 0.947 respectively. Also, the correlation between the antioxidant activity assays is significant (r>0.983). There is a reverse correlation between DPPH assay values and other methods. Similar correlations between phenols and antioxidant activity were emphasized by researchers (Apak et al. 2006; Guclu et al. 2006; Liu et al. 2009; Ulusoy et al. 2010).

In conclusion, the present results are showed that the extracts exhibited antioxidative properties. Especially, the extract of *N. italicca* has higher activity than others for all assays. It may be suggested that the extracts of *Nepeta* species possessed middle antioxidant power when compare to other plants belonging to the *Lamiaceae* family. On the other hand, further detailed studies of essential oils and extracts of *Nepeta* species are required to determine which of their components are more responsible for its antioxidant effect and to clarify their biological properties.

REFERENCES


