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# Phenolic Compounds and Antioxidant Activity of *Nepeta Nuda* Subsp. *Albiflora*

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**Abstract.** Phenolic content and antioxidant activity of *Nepeta nuda* subsp. *albiflora* Boiss. were reported in this study. The ethanol and water extracts of *Nepeta nuda* subsp. *albiflora* were prepared and used for biochemical analyses. Antioxidant capacities of the extracts were evaluated by three different *in vitro* bioanalytical methods including a reducing antioxidant method and two radical scavenging antioxidant methods. The water and ethanol extracts of the plant sample were found to have effective antioxidant potentials. Phenolic content of *Nepeta nuda* subsp. *albiflora* was determined by high performance liquid chromatography (HPLC). Rosmarinic acid (182.0±4.5 µg/g), apigenin (84.5±57.6 µg/g), and quercetin (44.5±62.9 µg/g) were identified as major compounds in the ethanol extract of the plant sample. This study has a potential scientific base for further studies about *Nepeta nuda* subsp. *albiflora* related to plant biochemistry and plant based pharmacological industry.

## Introduction

*Nepeta* L. (*Lamiaceae*) genus includes about 250 species in different parts of the world such as Asia, Europe, and North Africa. The plants of *Nepeta* L. (*Lamiaceae*) genus are mostly herbaceous perennials pleasantly aromatic herbs with erect or procumbent stems [1].

Phenolic compounds contain at least one hydroxyl group with an aromatic ring on their chemical structure. Antioxidant activities of phenolic compounds are mainly attributed to their redox properties which allow them to act as chelating metals, reducing agents, hydrogen donors, and quenchers of singlet oxygen [2].

Phenolic compounds and antioxidant activities of various aromatic plants have been evaluated extensively [3, 4]. Phenolic compounds are primary components and very important secondary metabolites of plants. They are widely composed by plants, fruits, and vegetables in response to microbial infections. Polyphenols gain remarkable attention due to their many useful properties on human health [5]. Medicinal herbs contain considerable phenolic acids that have a substantial role in decomposing peroxides and absorbing free radicals [6].

There are many studies on the determination of bioactive phytochemical constituents from different natural plant species [7-10]. However, the best of our knowledge there is no report about *Nepeta nuda* subsp. *albiflora* in the current literature. In this study, *Nepeta nuda* subsp. *albiflora* was used for determining its biochemical properties including polyphenol contents and antioxidant activities. For that aim, we analyzed the ferric ($Fe^{3+}$) ions reducing potentials, ABTS and DPPH radical scavenging activities of the water and ethanol extracts for evaluation of the antioxidant activity of the plant sample. These three methods have been used for the measurement of antioxidant profiles of foods, plants, and other materials by many researchers. Also, we identified the phenolic content of *Nepeta nuda* subsp. *albiflora* by HPLC technique.

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## Theory

Medicinal plants have been used for their healing effects with their natural constituents. They have some functions including biological activity related to their phenolic compounds. Determination of antioxidant potential and phenolic content of *Nepeta nuda* subsp. *albiflora* will contribute to the related studies on pharmacology and plant biochemistry.

## Materials and Methods

All experiments were done in Central Research Laboratories at Mus Alparslan University, Mus, Turkey.

### Chemicals

ABTS (2,2'-azino-bis 3-ethylbenzothiazoline-6-sulfonic acid), BHA (butylated hydroxyanisole), BHT (butylated hydroxytoluene), DPPH (1,1-diphenyl-2-picryl-hydrazyl), neocuproine (2,9-dimethyl-1,10-phenanthroline), TCA (trichloroacetic acid), α-tocopherol, trolox, and the standard phenolic compounds of HPLC (ascorbic acid, p-coumaric acid, gallic acid, myricetin, 4-hydroxybenzoic acid, 3,4-dihydroxybenzoic acid, abscisic acid, quercetin, apigenin, kaempferol, curcumin, catechol, vanillin, caffeic acid, cinnamic acid, rosmarinic acid and salicylic acid) were purchased from Sigma-Aldrich GmbH, Sternheim, Germany. The other chemicals and solutions were obtained from Mus Alparslan University Chemistry Research Laboratory.

### Identification and collection of the plant material

The plant sample, *Nepeta nuda* subsp. *albiflora*, was collected from Bingol, a Southeast city of Turkey, in July 2016 by Dr. Ömer Kılıç. The taxonomic description of the plant sample was made according to the "The Flora of Turkey and East Aegean Islands" [11]. The voucher specimen was deposited in the Bingol University, Department of Park and Garden Plant Herbarium.

### Preparation of water and ethanol extracts

The ethanol and water extractions of *Nepeta nuda* subsp. *albiflora* was carried out according to a previous study [12]. The leaves of *Nepeta nuda* subsp. *albiflora* were dried at room condition. For the preparation of the extracts, 20 g of air-dried leaves were powdered and mixed with 200 mL distilled water or ethanol (1/10:w/v), separately. The mixtures were homogenized by a magnetic mixer about 12 h, at room conditions. The homogeneous mixtures were filtered with filter papers. The filtrate sample from the water solvent was lyophilized in a lyophilizator (Labconco, Freezone 1L) at 5 mm Hg at -50 °C for preparing water extract. The ethanol filtrates were evaporated with a rotary evaporator (Heidolph 94200, Bioblock Scientific) for preparing the ethanol extract. The lyophilized and evaporated samples were stored at -30 °C until used.

### Determination of phenolic compounds by using HPLC analysis

The phenolic compounds of the plant sample were determined by using the HPLC instrument. For this aim, seventeen standard compounds were used. The standards were prepared at 10 mg/mL concentration and added into flasks. For preparing the stock solutions, primarily 1% acetic acid and acetonitrile were mixed (9:1 respectively) with methanol (1:1). The standard samples were used for the standard graphs. The solvent A was 1% acetic acid and the solvent B was 100% acetonitrile for gradient elution. The other parameters of HPLC were given in Table 1.

**Table 1**. The experimental conditions in HPLC analysis

| HPLC conditions | | Gradient elution | | |
|---|---|---|---|---|
| Model | Agilent Technologies 1260 Infinity II | Time (min) | A (%) | B (%) |
| Colon | ACE 5 C18 (250x4.6 mm id) | 0 | 90 | 10 |
| Colon Oven | G7130A | 25 | 60 | 40 |
| Detector | 1260 DAD WR | 39 | 40 | 60 |
| Pump | 1260 Quat Pump VL | 50 | 10 | 90 |
| Mobile Phase | A: 1% Acetic acid | 55 | 90 | 10 |
| | B: Acetonitrile | | | |
| Detection | 272, 280 and 310 nm | | | |
| Autosampler | 1260 Vialsampler | | | |
| Flow Rate | 1 mL/min | | | |
| Colon Temperature | 28 ºC | | | |
| Injection | 20 µL | | | |

### Determination of antioxidant activity

Reducing effects of the extracts were determined by ferric ions reducing antioxidant power (FRAP) method based on reduction of $Fe^{3+}$ to $Fe^{2+}$ according to the procedure as described previously [13]. Briefly, different concentrations of the water and ethanol extracts (10-50 µg/mL) in distilled water (0.75 mL) were mixed with phosphate buffer (1.25 mL 0.2 M, pH 6.6) and potassium ferricyanide solution (1.25 mL, 1%). The mixture was incubated at 50 ºC for 20 min and acidified with trichloroacetic acid (1.25 mL, 10%). Finally, $FeCl_3$ solution (0.5 mL, 0.1%) was transferred to the mixtures and absorbance was measured at 700 nm.

The DPPH free radical scavenging effects of the extracts were determined according to a previously described study [14]. DPPH free radical molecules show maximum absorbance at 517 nm. Thus, antioxidant molecules can reduce the absorbance. For this aim, DPPH radical solution in ethanol (0.5 mL, 0.1 mM) was transferred to the sample solution (1.5 mL) in ethanol (10-50 µg/mL) and incubated in dark for 30 min. Finally, the absorbance samples were recorded at 517 nm against blank samples lacking scavenging compounds. Analyses were achieved in triplicate.

ABTS cation radical scavenging method is based on reducing absorbance of ABTS radicals by existing antioxidant agents. First of all, ABTS radicals produced by treating ABTS with an oxidizing agent of potassium persulfate. The phosphate buffer (0.1 mM, pH 7.4) was used for dilution to the required absorbance (0.9±0.2) at 734 nm. Finally, 1 mL of ABTS radical solutions added to the 3 mL of the various concentrations (10-50 µg/mL) of the water and ethanol extract solutions. The absorbance was recorded at 734 nm [12].

### Statistical analysis

The experimental results were performed in triplicate. The data were assessed using the Microsoft Office Excel program. In our study values of $P<0.05$ were regarded as significant and descriptive statistics are presented as a mean ± standard deviation.

## Results and Discussion

### Phenolic compounds

Phenolic compounds in the plants have been related to some biological activities including antioxidant activity. Plant phenolic and flavonoid compounds do their antioxidant functions by scavenging reactive oxygen species and reducing radicals by donating protons [15, 16].

**Table 2.** HPLC parameters and amounts of standard compounds in *Nepeta nuda* subsp. *albiflora*)

| | **Standard compounds** | **RT** (min) | ***Nepeta nuda* water extract** | | ***Nepeta nuda* ethanol extract** | |
|---|---|---|---|---|---|---|
| | | | **Area** [mAU*s] | **Amount** (µg/mL) | **Area** [mAU*s] | **Amount** (µg/g) |
| 1 | Ascorbic acid | 3.77 | 8.2 | 0.5±0.7 | 11.8 | 0.6±1.0 |
| 2 | Gallic acid | 5.42 | | | | |
| 3 | 3,4-Dihydroxybenzoic acid | 7.95 | 221.9 | 0.4±0.6 | | |
| 4 | 4-Hydroxybenzoic acid | 11.28 | | | 83.2 | 2.1±0.4 |
| 5 | trans-p-Coumaric acid | 16.33 | 24.6 | 1.5±0.0 | 1122.7 | 18.5±0.4 |
| 6 | Myricetin | 22.29 | 35.0 | 2.8±0.6 | 187.5 | 9.3±0.4 |
| 7 | Abscisic acid | 26.74 | | | 212.1 | 17.5±7.2 |
| 8 | Quercetin | 27.63 | | | 1728.2 | 44.5±62.9 |
| 9 | Apigenin | 31.57 | | | 481.9 | 84.5±57.6 |
| 10 | Kaempferol | 34.10 | | | 126.8 | 5.7±8 |
| 11 | Curcumin | 43.03 | | | 58.4 | 17.1±0.5 |
| 12 | Catechol | 13.20 | 24.7 | 0.7±1.0 | 12.6 | 0.3±0.5 |
| 13 | Vanillin | 17.05 | 107.4 | | 52.7 | |
| 14 | Caffeic acid | 13.19 | 27.2 | | 30.9 | |
| 15 | Cinnamic acid | 18.60 | | | 530.3 | 4.5±3.3 |
| 16 | Rosmarinic acid | 21.53 | | | 14594.6 | 182.0±4.5 |
| 17 | Salicylic acid | 24.59 | | | 272.7 | 5.9±0.8 |

HPLC technique was used for the identification of the main organic compounds of *Nepeta nuda* subsp. *albiflora* by using of seventeen phenolic compounds as standards (Table 2). The results clearly showed the high amounts of both total phenolic and total flavonoid contents of *Nepeta nuda* subsp. *albiflora*. According to the HPLC experiments, rosmarinic acid (182.0±4.5 µg/g), apigenin (84.5±57.6 µg/g), and quercetin (44.5±62.9 µg/g) were identified as major compounds in the ethanol extract of *Nepeta nuda* subsp. *albiflora*. Also, the other detected compounds were ordered according to their concentrations as; trans-p-kumaric acid, abscisic acid, curcumin, mirisetin, salycylic acid, kaempferol, cinnamic acid and 4-hydroxybenzoic acid.

The phenolic content of water extract was determined to be very poor compared to the ethanol extract. The reason might be that the solubility of organic compounds in ethanol solvent is mostly better than in water. These various phenolic compounds might have affected the antioxidant activity of *Nepeta nuda* subsp. *albiflora*. The HPLC chromatograms of standard compounds in *Nepeta nuda* subsp. *albiflora* were given in Fig. 1.

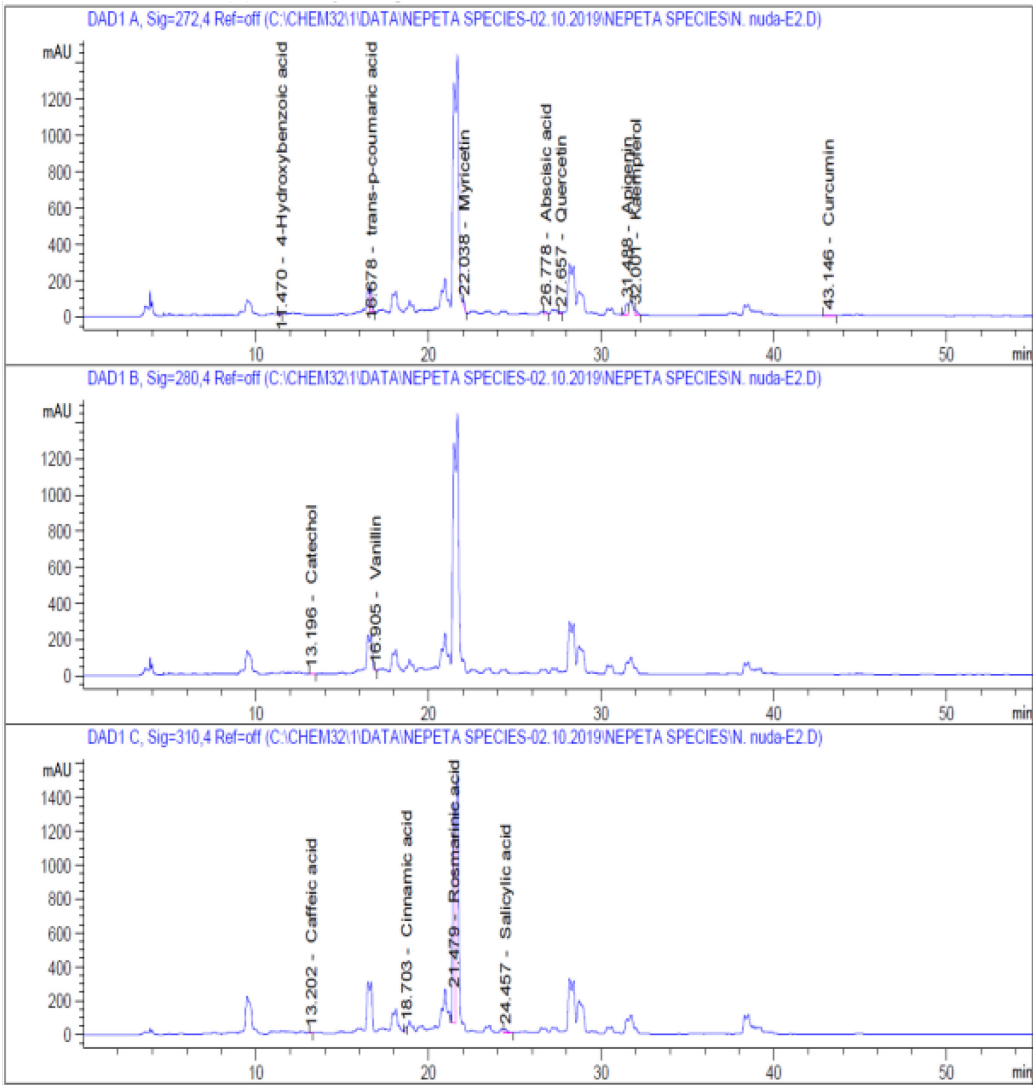


**Figure 1.** HPLC chromatograms of standard compounds of *Nepeta nuda* subsp. *albiflora*

**Antioxidant activity**

Antioxidant molecules or extracts can easily reduce and scavenge oxidant agents [17]. Antioxidant activity of *Nepeta nuda* subsp. *albiflora* was determined by analyzing radical scavenging and reducing the capacity of its water and ethanol extracts. The reducing potential of the plant sample was examined by using FRAP reducing ability method. Furthermore, ABTS and DPPH free radical scavenging assays were used for determining radical scavenging capacity. The results of the three methods were summarized in Table 3.

**Table 3.** Antioxidant activities of the extracts and standard compounds

| Antioxidants | $Fe^{3+}$-$Fe^{2+}$reducing | | ABTS | | DPPH | |
|---|---|---|---|---|---|---|
| | $\lambda_{700}$ | $r^2$ | Scavenging % | $IC_{50}$ | Scavenging % | $IC_{50}$ |
| **BHA** | 3.315±0.109 | 0.96 | 56.7±11.4 | 27.2±16.1 | 71.3±14.2 | 19.8±7.4 |
| **BHT** | 3.160±0.164 | 0.99 | 64.5±8.5 | 22.5±10.2 | 49.1±13.4 | 28.6±8.8 |
| **Ascorbic acid** | 1.633±0.334 | 0.91 | 71.5±3.0 | 20.9±11.0 | 40.5±13.2 | 34.8±9.1 |
| **Tocopherol** | 1.935±0.512 | 0.98 | 62.2±4.6 | 23.6±11.4 | 71.2±12.8 | 20.0±7.8 |
| **Trolox** | 2.028±0.303 | 0.96 | 63.5±10.4 | 23.2±10.5 | 69.9±13.8 | 20.3±7.6 |
| **Water extract** | 0.456±0.024 | 0.97 | 51.2±13.5 | 27.6±10.0 | 13.3±4.9 | 113±42 |
| **Ethanol extract** | 0.773±0.059 | 0.98 | 47.6±4.8 | 31.1±14.9 | 26.5±11.9 | 54.4±6.7 |

According to the FRAP method, the reducing potential of a sample can be determined by measuring the transformation of ferric ions ($Fe^{3+}$) to ferrous ions ($Fe^{2+}$) by single electron transfer of an antioxidant substance. The amount of $Fe^{2+}$ can be measured by measuring the absorbance of a

complex, which had a maximum absorbance at 700 nm. In this context, the ethanol extract and the water extract had potent reducing effects by using this method. As shown in Fig. 2, increasing absorbance indicates a high concentration of ferrous ions ($Fe^{2+}$) which means high reducing capacity.

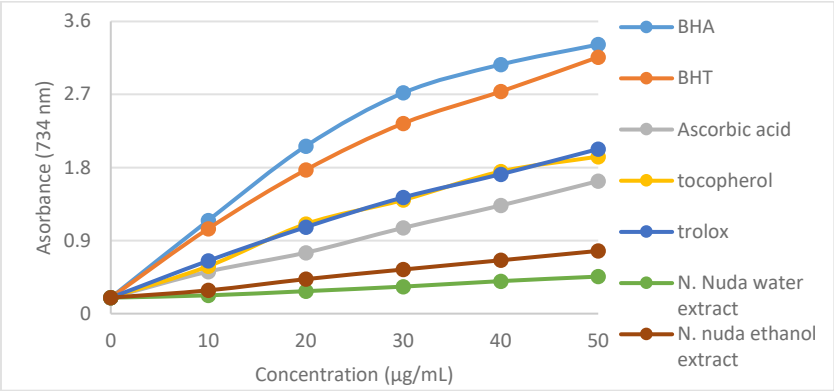


**Figure 2.** Reducing antioxidant activity of *Nepeta nuda* subsp. *albiflora* by using FRAP method

ABTS radical scavenging the extracts were given in Table 3 and Fig. 3. According to the results, the extracts and standards had decreasing absorbance with increasing concentration which means they scavenged more radicals. The water extract was demonstrated higher ABTS radicals scavenging activity than the ethanol extract.

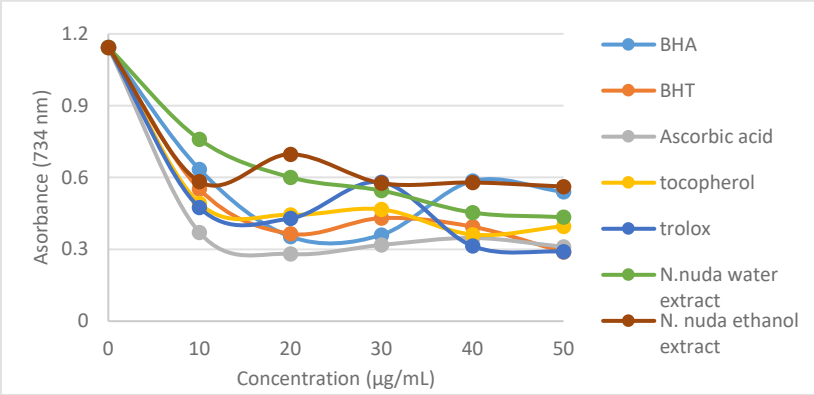


**Figure 3.** Radical scavenging antioxidant activity of *Nepeta nuda* subsp. *albiflora* by using ABTS method

Radical scavenging methods are common methods to determine the antioxidant capacities of plants or foods [18]. DPPH assay the most common spectrophotometric method for radical scavenging determination. Antioxidant substances can donate hydrogen and reduced DPPH radicals. The electron or hydrogen atom donation capacity of samples can be determined easily by this method. DPPH is a stable free radical and antioxidants reduce DPPH radical to diphenyl-picrylhydrazine by accepting electron or hydrogen radical. DPPH free radical has max absorbance at 517 nm, so decreasing absorbance at this wavelength indicates radical scavenging activity [19].

According to the data of the DPPH method, a remarkable correlation between radical scavenging potential and concentration was detected for standards (BHA, BHT, tocopherol, trolox, and ascorbic acid) and the plant extracts. The extracts and standards had decreasing absorbance with increasing concentration which means they scavenged more radicals. As a comparison, the ethanol extract demonstrated higher free radicals scavenging activity than the water extract. The effective free radical scavenging activities of extracts and standard antioxidants were shown in Fig. 4.

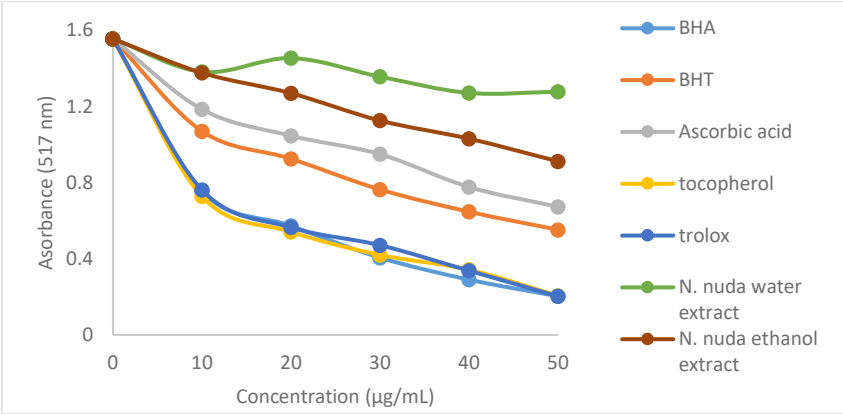


**Figure 4.** Radical scavenging antioxidant activity of *Nepeta nuda* subsp. *albiflora* by using DPPH method

Also, the $IC_{50}$ values of the extracts and standards for DPPH radical scavenging were given in Table 3. A lower $IC_{50}$ value means a higher DPPH free radical scavenging profile. So, the extracts were demonstrated effective free radical scavenging abilities, close to the level of standard compounds.

## Conclusions

This study provides important insights on phytochemistry and bioactivity of *Nepeta nuda* subsp. *albiflora* related to phenolic content and antioxidant capacity. According to the HPLC results, rosmarinic acid, apigenin, and quercetin were identified to be major phenolic and flavonoid compounds out of seventeen standard compounds. Also, the data of *in vitro* antioxidant methods showed that *Nepeta nuda* subsp. *albiflora* extracts were found to have effective antioxidant potentials. This report clarified the potential phenolic compounds with effective antioxidant activity of *Nepeta nuda* subsp. *albiflora*.

## Conflict of Interest

The authors declare no conflict of interest.

## Acknowledgment

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