ANTIOXIDANT ACTIVITIES AND PHENOLIC COMPOSITION OF EXTRACTS FROM *NEPETA* PLANT SPECIES

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Abstract

The aim of this study was to assess antioxidant properties of four in Lithuanian growing *Nepeta* varieties, namely *N. cataria*, *N. cataria* var. *citriodora*, *N. transcaucasica* and *N. bulgaricum*. The plants were extracted using different polarity solvents, namely acetone, methanol, ethanol and water. The antioxidant properties were assessed by determining total content of phenolics, using free DPPH• radical scavenging assay, accelerated oil stability test and peroxide value measurement. Antioxidant activities of the analysed herb extracts were greatly dependent on the extraction solvent. Methanolic extract of *N. cataria* exhibited significantly higher antioxidant capacity comparing to other extracts. The sub-fractions isolated from the plant material were analysed by HPLC-MS and the concentration of some phenolic acids was determined. Rosmarinic acid was the major component in all extracts; luteolin, and caffeic acid were other identified constituents, however present in considerably lower amounts.

Key words: Antioxidant activity / DPPH• / Rosmarinic acid / polyphenolics/*Nepeta* spp.

Introduction

Catnips are perennial flowering plants, which have been used as aromatic herbs and for some other purposes. They belong to the genus *Nepeta,* a member of the Mint family (Lamiaceae), comprising about 250 species, which are native in Asia, North Africa and Europe; however it is most abundant in the Mediterranean region. Catnip leaves possess pleasant minty or lemony scent which depends on the plant species and variety. Some common species of *Nepeta* genus are *N. cataria* (field balm, true, catnip, and catmint), *N. cataria* var. *citriodora* (lemon catnip), *N. transcaucasica* and *N. bulgaricum.* The plant is also interesting because of its behavioral effects on cats; in addition its essential oil is an insect repellent (Kokdil et al., 1999). Some positive effects of plant preparations on human health were reported as well (Giamperi et al., 2009). Zenasni et al., (2008) indicated that the major component in *N. atlantica, N. tuberosa* and *N. cataria* oils was the stereoisomer 4a-α, 7-α, 7a-β-nepetalactone constituting up to 70% of oil, they also suggested that nepetalactone plays an important role in antibacterial activity against *E.coli* and *S. aureus* strains. Tittel et al., (1982) reported that the main constituents of *N. cataria* var. *citriodora* were citronellol (15.6%), elemol (11.9%), geraniol (9.5%), *β*-elemene (7.5%), *β*-caryophyllene oxide (4.5 %), α-cadinol (5.0%), nerol (3.7%), isopulegol (3.3%), cadinol (2.6%), citronellal (2.6%), hexahydrofarnesyl acetone (2.2%), linalool (1.8%), and neral (1.5%). Lee et al., (2010) determined that rosmarinic acid was one of the main antioxidant of *N. cataria.* Miceli et al., (2005) evaluated the anti-inflammatory activities of different species of *Nepeta* genus. In one of previous studies instrumental and sensory assessment of catnip essential oil was performed using different isolation techniques (Baranauskiene et al., 2003); however the data on antioxidant properties of various plant species and varieties are rather scarce.

The aim of this study was to evaluate antioxidative properties and composition of extracts isolated from four in Lithuania grown *Nepeta* varieties by different polarity solvents, such as methanol, acetone and water.

Materials and Methods

Materials. Leaves of *Nepeta cataria* (field balm, catnip or catmint)**,** *Nepeta cataria var. citriodora* (lemon catnip), *Nepeta transcaucasica* and *Nepeta bulgaricum* were collected from Kaunas Botanical Garden at Vytautas Magnus University, Lithuania. The plants were harvested during flowering stage, dried at room temperature and stored in glass containers in the dark. Before extraction dried material was ground in a laboratory mill (1095 Knifetec, Canada).

Extraction procedures. The extracts were isolated in a solid-liquid extractor (IKA Werke, Staufen, Germany) operating on the fluidized bed extraction principle. Extraction was completed in 3 hours; heating temperatures were 90 and 70 °C for methanol and acetone, respectively. The products are further referred as methanol (ME) and acetone (AE) extracts. Water extracts (WE) were prepared from the liquid herbal residues obtained after hydrodistilling the essential oil in a Clevenger-type apparatus. The solid residues were dried and extracted with acetone in a solid-liquid extractor to obtain deodorized acetone extract (DAE).
Evaluation of antioxidant properties. The oxidative stability of extracts was determined using an Oxipres apparatus (Mikrolab Aarhus, Denmark), which measures oxygen pressure changes in the vessel containing rapeseed oil with or without plant additives (Trojakova et al., 1999). The Shaal oven test was used to evaluate antioxidant activity by measuring weight gain during oil storage (Trojakova et al., 2001). Antioxidant power of Nepeta ssp. extracts in rapeseed oil were expressed as protection factors (PF). Radical scavenging capacity of extracts was measured using a method described by Brand-Williams et al., (1995). The assay was carried out by mixing 0.05 ml methanol solution of each extract with 2.0 ml of 6×10^{-5} M methanol DPPH• solution. The absorbance was recorded at 515 nm using a spectrophotometer (Spectronic Genesys 8, JAV).
Determination of total phenolic compounds (TPC). The amount of TPC in crude extracts was determined by Folin Ciocalteu method as described elsewhere (Taga et al., 1984). Gallic acid solutions in ethanol (0.045, 0.090, 0.135 and 0.180 mg ml^{-1}) were prepared for calibration curve. The concentration of TPC was expressed in gallic acid equivalents (GAE) in 1 g of extract.
Analysis of extract composition by HPLC. Composition of plant ME, AE, DAE and WE extracts was analysed by HPLC (Skalicka-Wozniak et al., 2008) with UV detection at 210–400 nm. Quantitative determination was performed at 254 nm by measuring two replicate samples. Rosmarinic acid, caffeic acid and luteolin were used as reference compounds for identification and quantitative assessment. Calibration curve was plotted by using rosmarinic acid, caffeic acid and luteolin reference compounds at four different concentrations, 0.8, 0.4, 0.2 and 0.1 mg in 10 ml). Quantification was performed by comparing the chromatographic peak areas of compounds present in extracts with those of external standards. The linear dependence was between peak area and concentration was determined for all reference compounds(R^2=0.9991).
Statistical data assessment. The measurements of radical scavenging capacity and the amount of TPC were performed in triplicate and the results were expressed as a mean ± standard deviation (SD). Other experiments were carried out in duplicate.

Results and Discussion

A screening of several *Nepeta* plants species was carried out in order to evaluate plant extracts for their antioxidative power using *in situ* assays, radical scavenging assay and determination of TPC. Three different solvents, acetone, methanol and water were used; their choice was based on polarity differences: dielectric constant (k) of water is 80.1; methanol 32.6 and acetone 20.7 (Demirel et al., 2010).
Two methods were selected to assess the influence of extracts on the PF of rapeseed oil oxidation at different storage conditions. In Oxipress apparatus the oil was heated at 110 °C and in general the effect of extracts on oil stability was insignificant; PF was almost equal for all analysed samples (Table 1). However, oil stability assay using weight gain measurement, (due to binding oxygen in the course of oxidation) performed at lower temperature (50 °C) revealed quite remarkable antioxidative effect of catnip extracts. In this case AEs were stronger antioxidants than other added to oil extracts; the highest antioxidative activity was found for *N. cataria* var. *citriodora* AE and DAE extracts, which increased oil PF up to two times.

Table 1

Comparison of Antioxidant Power (Expressed as Protection Factors) of 0.2% *Nepeta* ssp. Extracts in Rapeseed Oil

Additives	Oxipres method at 110 °C	Weight gain method at 50 °C
***N. cataria* var. *citriodora* (AE)**	1.3	2.0
***N. cataria* var. *citriodora* (ME)**	1.3	1.7
***N. cataria* var. *citriodora* (DAE)**	1.2	1.9
***N. cataria* var. *citriodora* (WE)**	1.2	1.1
***N. cataria* (AE)**	1.2	2.0
***N. cataria* (ME)**	1.3	1.2
***N. cataria* (DAE)**	1.3	1.7
***N. cataria* (WE)**	1.0	1.0
***N. bulgaricum* (AE)**	1.3	1.7
***N. bulgaricum* (ME)**	1.1	1.2
***N. bulgaricum* (DAE)**	1.0	1.1
***N. bulgaricum* (WE)**	1.2	1.0
***N. transcaucasica* (AE)**	1.2	1.9
***N. transcaucasica* (ME)**	1.2	1.1
***N. transcaucasica* (DAE)**	1.3	1.9
***N. transcaucasica* (WE)**	1.3	1.0

It is well known that antioxidative activity of plant extracts depends on plant species and extraction solvents. Radical scavenging capacity was assessed by using DPPH• assay, which is one of the most frequently, used methods for this purpose. The method is based on the ability of constituents present in plant extract to donate an electron to a free DPPH• radical; the reaction may be followed by the colour changes. The results of the antioxidant activity measurements are presented in Table 2. It was found that MEs were the most effective radical scavengers except for *N. cataria* var. c*itriodora*. Thus, *N. transcaucasica, N. cataria* and *N. bulgaricum* MEs inhibited approximatelly 80% in the reaction present DPPH• radicals, while *N. cataria* var. *citriodora* ME only 44%. Tepe et al., (2007) also reported that ME of *Nepeta flavida* possessed strong antioxidative effect. The radical scavenging percentage of other extracts was from 19 to 53%. The DAEs were the weakest radical scavengers for all *Nepeta* samples. Most likely, the majority of antioxidatively active constituents as polar compounds are dissolved in water during hydrodistillation; WEs obtained from the liquid hydrodistilation fraction were remarkably stronger antioxidants than DAEs. AEs isolated from the whole plant material were stronger radical scavengers than DAEs. It may be explained by two possible reasons: first, part of components dissolving in water during hydrodistillation at 100 °C may be also extracted by acetone from the whole material; second, AE may contain essential oil components which are removed during hydrodistillation and which may possess some antioxidant activity. Such activity was reported for *Nepeta* species essential oils (Giamperi et al., 2009; Saleh et al., 2010; Dapkevicius et al., 1998). It is interesting noting, that WEs were stronger radical scavengers than AEs, again except for *N. cataria* var. c*itriodora.*

Table 2

Radical Scavenging Capacity and Phenolic Compound Composition of *Nepeta* Extracts

Additives	DPPH• radical scavenging, %	Content (mg 100 g^{-1} extract)			Total phenolics mg GAE g^{-1} extract
		Luteo-lin	Rosmarinic acid	Caffeic acid	
***N. transcaucasica* AE**	33.0±2.4	tr.	60.6	tr.	8.9±2.1
***N. transcaucasica* DAE**	21.1±2.8	5.3	59.7	tr.	6.0±1.9
***N.transcaucasica* ME**	79.9±1.5	3.5	1082.8	tr.	12.7±0.9
***N.transcaucasica*WE**	45.3±0.9	3.7	184.9	300.2	8.6±1.3
***N. bulgaricum* AE**	44.8±1.2	3.5	387.3	tr.	15.2±3.1
***N. bulgaricum* DAE**	22.2±0.8	16.5	72.2	14.0	6.2±2.6
***N.bulgaricum* ME**	81.1±1.6	3.9	1404.7	17.2	17.4±1.5
***N.bulgaricum* WE**	52.6±1.1	6.9	587.3	284.2	10.8±0.7
***N. cataria* var. *citriodora* AE**	48.1±2.1	2.0	377.9	23.7	11.6±3.3
***N. cataria* var.*citriodora* DAE**	18.5± 1.8	7.2	42.2	tr.	–
***N. cataria* var. *citriodora* ME**	44.7±0.5	5.7	476.5	tr.	10.4±1.6
***N. cataria* var. *citriodora* WE**	29.4±0.6	3.9	166.7	186.3	2.1±1.1
***N. cataria* AE**	48.1±2.5	2.2	487.8	16.4	15.0±1.7
***N. cataria* DAE**	22.9±2.0	9.7	41.1	tr.	3.5±1.5
***N. cataria* ME**	80.9±1.4	4.4	1743.8	44.2	23.1±1.0
***N. cataria* WE**	52.7±0.9	7.8	193.3	302.3	11.0±1.4

tr. – trace amount

The content of TPC in the analysed extracts varied from 2.1 (WE of *N. cataria* var. c*itriodora*) to 23.1 mg GAE g^{-1} (ME of *N. cataria*). Methanol extracted the biggest amount of phenolic compounds from all studied *Nepeta* varieties, except for *N. cataria* var. c*itriodora*, when TPC was slightly higher in AE (Table 2). In general, the content of TPC was in correlation with radical scavenging capacity (R^2=0.7), however there were some exceptions. For instance, higher amount of TPC was found in *N. cataria* AE, than in its WE, while WE possessed higher radical scavenging capacity than AE. It may be explained by the differences in the composition and antioxidant capacity of individual constituents present in the extracts.

Antioxidative properties of *Nepeta* plants was mainly related to the presence of phenolic acids, particularly rosmarinic and caffeic acids (Lee et al., 2010). According to our results, rosmarinic acid was dominating in all extrtacts, except for WEs of *N. transcaucasica N. cataria* var. c*itriodora* and *N. cataria*, when the concentration of caffeic acid was higher than that of rosmarinic acid (Table 2). Flavonoid luteolin was identified in all extracts, however its concentration in most cases was lower comparing to phenolic acids.

Conclusions

1. It was shown that *Nepeta* plants analysed in this study contain the compounds possessing antioxidant activity.
2. In general, methanol extracts demonstrated superior antioxidant properties comparing to less polar, acetone extracts.
3. The extracts isolated from deodorized plant material possessed lower antioxidant power that the extracts isolated from the whole plants.

4. The major component in all extracts was strong antioxidant rosmarinic acid, while caffeic acid and luteolin were present in lower amounts.
5. The extracts of *Nepeta cataria* demonstrated higher radical scavenging capacity than other tested plant varieties. In general it may be concluded that extracts of *Nepeta* species possessed medium antioxidant power in rapeseed oil.

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