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Essential oil composition of five *Nepeta* species cultivated in Lithuania and evaluation of their bioactivities, toxicity and antioxidant potential of hydrodistillation residues

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ABSTRACT

Essential oils of *Nepeta cataria* var. *citriodora*, *N. transcaucasica*, *N. melissifolia*, *N. sibirica* and *N. nuda* were investigated. The yields of EO were from 0.78 (*N. nuda*) to 5.94 (*N. cataria*) mg/g plant dry weight (pdw). In total, 143 compounds were identified and quantified in *Nepeta* plant EOs by GC-MS/FID. 4aα,7α,7aβ-Nepetalactone (NL) was dominant constituent in *N. cataria* and *N. nuda* EO (50.16 and 55.72%, respectively) followed by 4aα,7α,7aα-NL (35.64 and 6.20%, respectively); other quantitatively important compounds were β-caryophyllene, caryophyllene oxide, some monoterpene alcohols and their aldehydes. *N. transcaucasica* EO was composed mainly of citronellol (17.69%), 4aα,7β,7aα-NL (14.34%), geranial (9.05%) and geranyl acetate (8.20%), whereas EOs of *N. melissifolia* and *N. sibirica* contained high percentages of 1,8-cineole (37.35 and 42.58%, respectively) and caryophyllene oxide (22.06 and 20.35%, respectively). In order to valorize EO distillation residues their antioxidant potential was evaluated by several *in vitro* assays: water extracts were considerably stronger radical scavengers than acetone extracts isolated from the solid EO distillation residue. The bioactivities and toxicological data of *Nepeta* spp. and their main EO components were assessed based on the most recently reported data.

1. Introduction

Essential oil (EO) bearing plants due to their aromatic, medicinal and various other beneficial properties have been in the focus of humankind since ancient times. And although in modern times a huge amount of scientific knowledge has been obtained in the field of chemistry and bioactivities of EO constituents, the interest in these important secondary plant metabolites has not decreased, both due to the enormous chemical and biological diversity of the Plant Kingdom and their benefits for food, pharmaceutical, cosmetic and other industries.

The genus *Nepeta* (Lamiaceae) comprising about 300 perennial species (commonly called catnips) is widespread in the central and southern Europe, the Near East, central and southern Asia, and some areas of Africa; it was also naturalized in North America (Formisano et al., 2011). Many *Nepeta* spp. are well known for their healing properties and have been widely used in folk medicine. Moreover, extensive studies of *Nepeta* plants during last decades, which have been reviewed in several articles (Formisano et al., 2011; Sharma and Cannoo, 2013; Asgarpanah et al., 2014; Salehi et al., 2018), enabled to characterize 193 compounds (until 2011) and revealed numerous bioactivities of their preparations, extracts and purified constituents (Fig. 1). *N. cataria* (catnip) is the most intensively studied *Nepeta* species; it has been used for ornamental and culinary purposes and as a folk-medicine remedy. Leaves, shoots and flowers of catnip have been used as flavourings in sauces, soups, stews, beverages, wine, liqueurs, herbal teas, especially for providing a strong mint odour notes.

The yields and the composition of *Nepeta* spp. EOs were reported in numerous articles and have been reviewed in the several above-mentioned articles. For instance, Formisano et al. (2011) summarized that extensive phytochemical investigations of 88 *Nepeta* species led to the isolation of 193 compounds with a variety of C-skeletons; however, the authors also concluded that there are still many *Nepeta* spp. that have received no or only little attention. It is also important noting that remarkable chemo diversity of the same species has been reported due to the differences in plant genotype, chemotype, cultivation and climatic

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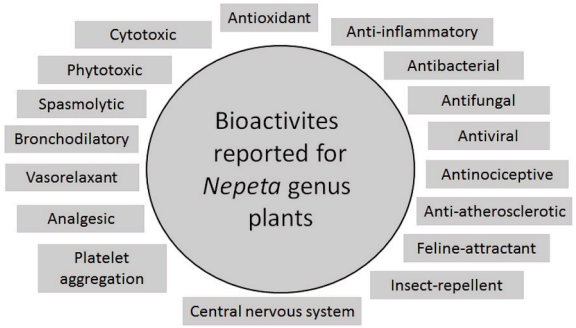

Fig. 1. Bioactivities of *Nepeta* genus plants essential oils and other preparations (adapted from Formisano et al., 2011).

peculiarities, harvesting time and other factors. Therefore, investigations of new, as well as known plant species from different geographic zones, may considerably expand the existing knowledge on the EO bearing plants.

Regarding EO composition *Nepeta* genus plants may be conditionally divided into 2 large groups (Sharma and Cannoo, 2013): first, with quite unique nepetalactones (further abbreviated as NL) as major constituents, and, second, with other type dominant compounds, which are found in numerous aromatic plants such as citral derivatives, 1,8-cineole, caryophyllene oxide, β-caryophyllene and others. Many bioactivities of *Nepeta* EOs and extracts (Fig. 1) are attributed to the presence of high concentrations of NLs (Alim et al., 2009; Asgarpanah et al., 2014; Gkinis et al., 2010; Gormez et al., 2013; Yazici et al., 2012; Mihaylova et al., 2013; Nestorović et al., 2010; Tsuruoka et al., 2012; Zomorodian et al., 2012; Ashrafi et al., 2019). Besides health benefits and other useful properties of *Nepeta* EOs, their toxicological safety is also an important issue.

Considering polymorphism of *Nepeta* genus plants as well as the variety of factors, which may influence EO composition, five cultivated in Lithuania *Nepeta* spp., namely *N. cataria* var. *citriodora*, *N. transcaucasica*, *N. melissifolia, N. sibirica* and *N. nuda* have been selected for the present study. Besides EO yields and composition, antioxidant potential of hydrodistillation residues was evaluated by the *in vitro* assays, while bioactivities and toxicological issues were surveyed based on the most recent and/or previously not reviewed reports. To the best of our knowledge, the composition of EO of *N. melissifolia* has not been reported previously.

2. Materials and methods

2.1. Plant material

N. cataria var. *citriodora* Balbis, *N. transcaucasica* Grosch., *N. melissifolia* L., *N. sibirica* L. and *N. nuda* L. were cultivated in Kaunas Botanical Garden at Vytautas Magnus University (Lithuania). The main geographical and climatic characteristics of the cultivation area are the following: 54° 52′ 14″ N of latitude, 23° 54′ 37″ E of longitude, elevation (altitude) 84 m; average temperature in July +18 °C; average precipitation 660 mm per year; period of vegetation ~180–200 days. The plants were collected at the beginning of flowering phase (butonization) in the period of 27 May – 19 July. Harvested herbs were dried at room temperature in a ventilated room in the absence of direct sunlight and stored in the dark glass containers. Before analysis, the leaves were separated from the stems and ground in an ultra-centrifugal mill ZM 200 (Retsch, Haan, Germany) using 0.5 mm hole size sieve.

2.2. Chemicals and solvents

Stable 2,2-diphenyl-1-picrylhydrazyl hydrate radical (DPPH$^{\bullet}$, 95%); gallic acid (GA); 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox 97%); anhydrous Na_2CO_3; 2,2'-azobis (2-amidinopropane) dihydrochloride (AAPH); 2M Folin–Ciocalteu's phenol reagent, 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS); fluorescein sodium salt; pentane, diethyl ether, acetone (GC grade), ethanol and HPLC grade methanol were purchased from Sigma-Aldrich Chemie (Steinheim, Germany). NaCl, Na_2SO_4, Na_2HPO_4, KCl, $K_2S_2O_8$ were from Merck (Darmstadt, Germany); KH_2PO_4 from Jansen Chimica (Beerse, Belgium); the mixture of C_7—C_{30} saturated *n*-alkanes was from Supelco Analytical (Bellefonte, PA, USA).

2.3. Isolation of essential oil and extraction of non-volatile compounds

Volatile compounds were isolated in a Likens-Nickerson simultaneous distillation-solvent extraction apparatus (further referred as L-N) from 40 g dried herb leaves placed in a 2 L round-bottom flask with ~1 L distilled water during 2.5 h. Evaporated volatile compounds were condensed and simultaneously extracted with 20 mL mixture of pentane:diethyl ether (1:1) in duplicate from each species. The L-N extracts were dried over Na_2SO_4, sealed and kept at −18 °C temperature for at least 24 h. The solvent was removed under a stream of nitrogen and pure EOs were kept at −18 °C before chromatographic analysis. Their yields were calculated on a dry weight basis (w/w).

The residues after distillation were separated into liquid and solid fractions by filtration. The liquid fractions were lyophilized in a freeze dryer Maxi Dry Lyo (Hetto-Holton AIS, Allerod, Denmark) resulting in water extracts (WE). The solid fractions were dried at 30 °C in a Dörrer dryer (Germany). Deodorized acetone extracts (DAE) were produced by extracting solid distillation residue with 150 mL acetone; all extractions were carried out in three steps at room temperature for 2 h; the solvent was removed in a Rotavapor R-114 (Büchi, Flavil, Switzerland) at 0.06 MPa and 40 °C, and finally dried under flow of nitrogen in 20 min. The yields were expressed in g of extract (edw) and plant dry weight (pdw).

2.4. Gas chromatography with flame ionization (GC–FID) and time-of-flight mass spectrometry (GC–TOFMS) detectors

2.4.1. GC–FID

For quantitative evaluation diluted in pentane (5 μL/mL) EOs were analysed on a Perkin Elmer Clarus 500 GC (Shelton, WA, USA) equipped with a FID and an Elite-5 (5% diphenyl, 95% dimethylpolysiloxane) fused silica capillary column, 30 m length, 0.25 mm id, 0.25 μm film thickness (Perkin Elmer, Shelton, WA, USA). The carrier gas was helium with an inlet pressure of 15 psi at 50 °C, which is equivalent to a 1.3 mL/min volumetric flow. Detector's temperature was 300 °C, oven temperature was programmed from 50 °C (2 min) to 280 °C (10 min) at the rate of 5 °C/min. One μL of EO solution was injected into a split/splitless injector heated at 260 °C in a split mode 1:10. The percentage of each EO component was determined by peak area normalization without using correction factors as the mean of quadruplicate GC runs.

2.4.2. GC–TOFMS

The qualitative composition of *Nepeta* spp. EOs was analysed on a GC × GC-TOFMS LECO Pegasus 4D system, consisting of an Agilent 7890A GC, Gerstel Multipurpose Sampler MPS (Gerstel GmbH, Mulheim an der Ruhr, Germany), high-speed TOFMS detector (LECO, St. Joseph, MI, USA) and four jet cryogenic modulator (Zoex, Houston, TX, USA). The column set consisted of a primary column BPX-5 (30 m, 0.25 mm id, 0.25 μm film thickness) (SGE Analytical Science, Australia) connected to a secondary column, BPX-50 (2.0 m, 0.10 mm id, 0.1 μm film thickness). The primary oven programming was 2 min at 50 °C then ramped to 280 °C at 5 °C/min (hold 10 min); the secondary oven programming was 2 min hold at 65 °C then ramped to 295 °C at 5 °C/min. The transfer line temperature was 250 °C. The GC injector port was kept at 280 °C with desorption time of 5 min. The TOFMS acquisition rate

was 10 spectra/s, the mass range used for identification was from 35 to 550 m/z units. Detector's voltage was set at 1550 V and ion source temperature of 250 °C. Data from the GC × GC-TOFMS system was collected by ChromaTOF software v.4.22 (LECO) after a solvent peak delay of 420 s, split ratio was 1:20; signal-to-noise threshold was set as 50, and the minimum accepted similarity was 750.

Volatile components were identified by comparing their Kováts Retention Indices (KI) relative to C_7—C_{30} *n*-alkanes, obtained on non-polar Elite-5 column with those provided in literature (Adams, 2009) and by comparing their mass spectra with the data provided by the NIST, Mainlib, Replib and Adams mass spectral libraries. Positive identification was assumed when good match of mass spectrum and KI was achieved.

2.5. Evaluation of antioxidant potential of extracts

2.5.1. Total phenolic content (TPC)

The Folin–Ciocalteu method with slight modifications was used (Slinkard and Singleton, 1977). A linear calibration curve was built by mixing 0.25 mL reference GA solutions in ethanol (0–0.35 mg/mL) with 1.25 mL of diluted with distilled water (1:10) Folin–Ciocalteu's phenol reagent and 1 mL of 7.5% Na_2CO_3 solution in distilled water. The absorption was recorded after 30 min at 765 nm on a Spectronic Genesys 8 spectrophotometer; calibration curve was y = 10.39x + 0.0793, R^2 = 0.9997. For the determination of TPC in the analysed samples, 0.25 mL of appropriate concentration of extracts in methanol (0.05% WE; 0.25% DAE) and distilled water as a blank were used. The TPC was expressed as mg GA equivalents per g (mg GAE/g edw) and recalculated to mg GAE/g pdw. Four replicate measurements were performed for each sample.

2.5.2. DPPH$^{\bullet}$ scavenging capacity (RSC) assay

The RSC of extracts against stable DPPH$^{\bullet}$ was determined spectrophotometrically by using a slightly modified method of Brand-Williams et al. (1995). The solution of DPPH$^{\bullet}$ in methanol (60×10^{-5} M) was prepared daily before measurements on a UV/Vis spectrophotometer (Spectronic Genesys 8, Rochester, USA) at 515 nm. *Nepeta* extracts at three different concentrations were dissolved in methanol, while not fully dissolved extracts were treated in the ultrasonic bath ASTRASON™, model 9H (Heat Systems Ultrasonics, NY, USA) and filtered. A 2 mL aliquot of DPPH$^{\bullet}$ solution was mixed with 50 μL of extract solution in a 1 cm path length quartz cuvette. The decreasing absorption was recorded during 40 min reaction time at 1 min intervals until the absorption curve reached the plateau. Simultaneously the absorption of a blank sample containing the same amount of methanol and DPPH$^{\bullet}$ solution was prepared and measured daily. The experiments were carried out in triplicate, the RSC was calculated as $I = [(A_B – A_X)/A_B] \times 100$, where I is DPPH$^{\bullet}$ inhibition (%); A_B is the absorbance of a blank sample (t = 0 min); A_X is the absorption of extract solution (t = 40 min). The results are expressed as an effective concentration EC_{50} (%), showing the amount of extract required to decrease the initial DPPH$^{\bullet}$ concentration in the reaction mixture by 50%.

2.5.3. ABTS$^{\bullet+}$ decolourisation assay

The RSC of extracts was also determined by ABTS$^{\bullet+}$ decolourisation assay (Re et al., 1999). Stock solution of ABTS (2 mM, pH = 7.4) was prepared by dissolving in 50 mL of phosphate buffered saline (PBS), containing 8.18 g NaCl, 0.27 g KH_2PO_4, 1.42 g Na_2HPO_4 and 0.15 g KCl in 1 L of ultra-pure water. ABTS$^{\bullet+}$ was produced by reacting 50 mL ABTS stock solution with 200 μL of 70 mM $K_2S_2O_8$ solution and allowing the mixture to stand in the dark at room temperature for 15–16 h. The radical was stable in this form for more than 2 days. For the assessment of extracts, the ABTS$^{\bullet+}$ solution was diluted with PBS to obtain the absorbance of 0.800 ± 0.030 at 734 nm. Three mL aliquot of ABTS$^{\bullet+}$ solution were mixed with 30 μL methanol solution of plant extract at three different concentrations in a 1 cm path length quartz microcuvette. The absorbance was read at ambient temperature after 1, 4, 6 and 10 min. PBS solution was used as a blank sample. All determinations were performed in triplicate. The percentage decrease of the absorbance at 734 nm was calculated as $I = [(A_B – A_X)/A_B] \times 100$, where I is the ABTS$^{\bullet+}$ inhibition (%); A_B is the absorption of blank sample (t = 0 min); A_X is the absorption of extract solution (t = 10 min).

2.5.4. Oxygen radical absorbance capacity (ORAC)

ORAC assay was performed as described by Prior et al. (2003) using fluorescein as a fluorescent probe and AAPH as a peroxyl radical generator. The reaction was carried out in 75 mM phosphate buffer (pH 7.4), stock solution of fluorescein were prepared according to Prior et al. (2003). The samples were prepared by dissolving plant extracts in methanol to obtain 0.0005% concentration and 25 μL of sample or trolox and 150 μL (14 μM) of fluorescein solutions were mixed in a 96-well transparent flat-bottom microplate, the mixture was incubated for 15 min at 37 °C followed by a rapid addition of 25 μL of AAPH solution (240 mM). The microplate was immediately placed in the FLUOstar Omega reader (BMGLABTECH, Ortenberg, Germany), automatically shaken prior to each reading and the fluorescence was recorded at every cycle (1 min × 1.1), in total 81 cycles. The 485-P excitation and 520-P emission filters were used. At least three independent measurements were performed for each sample. Raw data were exported from the Mars software to Excel 2003 (Microsoft, Roselle, IL) for further calculations. Antioxidant curves (fluorescence versus time) were normalized and then the area under the fluorescence decay curve (AUC) was calculated as follows: $AUC = (1 + f_1/f_0 + f_2/f_0 + f_3/f_0 + f_4/f_0 + \ldots + f_i/f_0) \times CT$, where f_0 is the initial fluorescence reading at 0 min and f_i is the fluorescence reading at time i; CT = cycle time in min. The final ORAC values were calculated by using a regression equation between the Trolox concentration and the net area under the curve (AUC). A series of trolox solutions in the concentration range of 3.12–125 μM were used for the calibration and the TEAC was expressed in μmol TE/g edw.

2.6. Statistical analysis

Mean values ± standard deviations of extracts yields and antioxidant capacity values (RSC, ORAC, TPC) were calculated from 3 to 4 replicate determinations, while the content of EO constituents was calculated from quadruplicate injections using MS Excel software. Statistical analysis of the obtained results was performed by one-way analysis of variance (ANOVA, vers.2.2), significant differences among the samples were evaluated by the Duncan's multiple-range test at the probability level ($P < 0.05$).

3. Results and discussion

3.1. Essential oil and extract yields

Product yields as well as the recovery of target fractions and/or constituents are very important indicators of process efficacy, particularly in the development of industrial biorefining technologies for the production of high value active functional substances from various crops. The strategy for developing the so-called 'zero-waste' processing technologies has been particularly emphasized for achieving sustainability in the exploitation of natural resources. So far as the yields of EO from many aromatic plants are lower than 1%, this study was extended for evaluating the possibilities to obtain other, non-volatile soluble fractions from the EO distillation residues. It is expected that application of such approach may significantly increase economic effectiveness of EO production. The yields of *Nepeta* spp. solid residual fraction after hydrodistillation, DAEs, WEs and EOs are provided in Table 1.

The yields of EO from the studied *Nepeta* spp. were rather low, from 0.08% (*N. nuda*) to 0.59% (w/w) (*N. cataria* var. *citriodora*). The latter

Table 1
The yields of solid phase, DAE and WE extracts and EOs of five *Nepeta* spp.

Nepeta species	Solid phase yield, mg/g pdw	DAE yield, mg/g spdw	DAE yield, mg/g pdw	WE yield, mg/g pdw	EO yield, mg/g pdw
NN	638.4 ± 5.8^{b}	42.75 ± 0.08^{d}	27.08 ± 0.00^{b}	276.5 ± 0.3^{bc}	0.78 ± 0.01^{a}
NT	617.1 ± 6.5^{ab}	38.58 ± 1.96^{a}	23.80 ± 0.95^{a}	279.9 ± 12.2^{bc}	1.75 ± 0.14^{a}
NS	648.5 ± 4.2^{bcd}	47.05 ± 0.32^{c}	30.51 ± 0.41^{c}	290.4 ± 12.2^{c}	1.32 ± 0.26^{a}
NC	684.4 ± 32.0^{d}	38.14 ± 1.73^{a}	26.08 ± 0.04^{b}	221.5 ± 5.7^{a}	5.94 ± 0.64^{b}
NM	587.6 ± 11.8^{a}	46.41 ± 0.09^{c}	27.28 ± 0.60^{b}	277.4 ± 23.9^{bc}	1.55 ± 0.30^{a}

Results are expressed as a mean ± standard deviation (n = 2,3); Values within columns followed by the same superscript letter (a-d) do not differ statistically at $P < 0.05$ (Duncan test). pdw – plant dry weight; spdw – plant solid phase dry weight. N – *N. nuda*; T – *N. transcaucasica*; S – *N. sibirica*; C— *N. cataria* var. *citriodora*; M – *N. melissifolia*.

Nepeta species was the most aromatic herb. Previously reported EO yields from *N. cataria* were in a similar range: 0.3, 0.5 and 0.9% from the plants harvested in Iran at vegetative, floral budding and full flowering phases, respectively (Zomorodian et al., 2012); 0.74% from the plants collected in Turkey (Adiguzel et al., 2009) and 1.02% from Moroccan plants (Zenasni et al., 2008). The yields of EO from *N. cataria* var. *citriodora* grown in Poland were from 0.45 to 0.80% (Klimek and Modnicki, 2005). However, previously reported EO yields from *N. nuda* were remarkably higher: 0.40% from former Yugoslavia (Chalchat et al., 1998), 0.97% from Greece (Gkinis et al., 2010), and even 2.1% (subsp. *Abiflora*) from Turkey (Alim et al., 2009). *N. sibirica* from Mongolia gave 0.16% EO (Tsuruoka et al., 2012), while the same species from Altai region in Russia yielded up to 1.0% (Letchamo et al., 2005).

The solid residues remaining after L-N procedure were separated from the liquid distillation 'soup' by filtration and dried; their yields were from 58.76% (*N. melissifolia*) to 68.44% (*N. cataria* var. *citriodora*) of the initial herb material subjected to hydrodistillation. The yields of WEs were from 22.1% (*N. cataria* var. *citriodora*) to 29.0% (*N. sibirica*), which indicates that *N. cataria* contained the lowest amount of the soluble in boiling water substances. The DAE yields obtained by extracting dried solid residues with acetone were from 3.8 (*N. cataria* var. *citriodora*) to 4.7% (*N. sibirica*), which is equivalent to 2.6 and 3.1% from the initial dry plant material (Table 1). Consequently, this simple fractionation from each species gave 4 different products, namely EO, WE, DAE and insoluble residue; the latter, most likely, consists mainly of polymeric cell wall carbohydrates, proteinaceous and other complex substances. To the best of our knowledge, this approach was applied to *Nepeta* spp. for the first time. Several *Nepeta* species were previously fractionated using acetone, methanol and water (Dienaitė et al., 2018): acetone extract yields from defatted with hexane *N. cataria* and *N. sibirica* were 3.01% and 4.73%, while extraction of the whole plant material with 80 °C water gave the yields from 26.55% (*N. nuda*) to 36.21% (*N. cataria*), which is higher than WE yields obtained after hydrodistillation in our study.

3.2. The composition of essential oils (EOs)

In general, the use of sensitive GC × GC-TOFMS system enabled to identify remarkably higher number of EO constituents in *Nepeta* plants with high level of reliability comparing with the previously reported data for the same species. Consequently, the results of this study extend the knowledge on the chemical diversity of *Nepeta* EOs. The detailed list of EO compounds, their percentage composition and odour descriptions are presented in Table 2. In total, 143 compounds were identified in the hydrodistilled from 5 *Nepeta* spp. EOs. The sums (in %) and the numbers of the identified constituents in *N. cataria* L. var. *citriodora*, *N. nuda*, *N. transcaucasica*, *N. melissifolia* and *N. sibirica* were 99.73/56, 98.27/90, 96.79/96, 93.81/88 and 95.02/79, respectively.

It is evident that *N. cataria* analysed in our study belongs to NL chemotype containing 4aα,7α,7aβ-NL (50.16%), 4aα,7α,7aα-NL (35.64%) and 4aα,7β,7aα-NL (1.80%), which represent 87.60% of the total EO volatiles. β-Caryophyllene (3.07%), caryophyllene oxide (1.95%) and citronellol (1.06%) were other three compounds exceeding 1%, while the content of nerol, geraniol, geranial, neral, carvacrol, (*Z*)-β-farnesene, α-humulene, *allo*-aromadendrene, δ-cadinene, spathulenol and phytol were between 0.1 and 1%. *N. cataria*, probably, is the most widely studied *Nepeta* species. Thus, compared to *N. cataria* investigated in our study, the EO of plants from Iran contained similar (Zomorodian et al., 2012) or remarkably lower (Asgarpanah et al., 2014) percentages of 4aα,7α,7aβ-NL (55–58 *vs* 28.8%) and 4aα,7β,7aα-NL (30–31.2 *vs* 11.9%). The plants studied by Asgarpanah et al. (2014) had high percentages of α-humulene (14.4%), 1,8-cineole (13.5–21%), α-pinene (10.3%), geranyl acetate (8.2%) and β-caryophyllene (5.7%). NLs were also major volatiles in the EO of *N. cataria* from Turkey, however their percentages were different: 4aα,7α,7aβ-NL constituted 70.4%, 4aα,7α,7aα-NL 6%, and 4aα,7β,7aα-NL 2.5%; other compounds found at > 1.2% were thymol, pulegone, piperitenone, piperitenone oxide and pinocarvone (Adiguzel et al., 2009). EO of *N. cataria* from Morocco (Zenasni et al., 2008) had even higher content of 4aα,7α,7aβ-NL (77.4%) followed by dihydronepetalactone (5.0%), α-terpinene (4.2%) and limonene (4.1%), while the same species from Argentina (Malizia et al., 1996) besides 4aα,7α,7aβ-NL (57.3%) were particularly rich in caryophyllene oxide (19.3%) and β-caryophyllene (8.1%). However, *N. cataria* var. *citriodora* grown in Poland (Klimek and Modnicki, 2005) was of completely different chemotype: its EO was composed of geraniol (23.49%), nerol (24.36%), citronellol (13.96%), a/b citrals (8.22/6.63%), caryophyllene oxide (2.48%) and β-caryophyllene (1.83%). Monoterpene alcohols (citronellol, geraniol) and especially their esters (geranyl acetate, citronellyl acetate) were the major volatile constituents of the previously analysed *N. cataria* cultivar from Lithuania (Baranauskienė et al., 2003).

4aα,7α,7aβ-NL (55.72%) and 4aα,7α,7aα-NL (6.20%) were also major EO constituents of *N. nuda*, followed by caryophyllene oxide (5.53%), nerol (4.79%), geranial (4.03%), neral (2.92%) and geraniol (2.64%). The same NL isomer was dominant in *N. nuda* from Greece (Gkinis et al., 2010), however its content in plant leaves was approx. 3-fold lower that in its verticillasters, 24.7 *vs* 75.7%. Instead, the leaves had higher percentages of 1,8-cineole (16.7 *vs* 3.3%) and caryophyllene oxide (16.3 *vs* 2%). Nepetalactones were recently reported as major constituents in EO of *N. nuda* subsp. *glandulifera*, composed of 70.94% of 4aβ,7α,7aβ and 3.51% of 4aα,7α,7aα-isomers (Sarikurkcu et al., 2018). 1,8-cineole chemotype of *N. nuda* from former Yugoslavia with 42.8–63.8% of this terpene epoxide was reported 20 years ago by Chalchat et al. (1998). EO of *N. nuda* from Turkey was composed of 4aα,7β,7aα-NL (18.10%), germacrene (15.68%), elemol (14.38%), β-caryophyllene (8.81%), spathulenol (6.14%) and cubenol (5.10%) as major volatile components (Bozari et al., 2013; Gormez et al., 2013); while NLs in *N. nuda* subsp. *abiflora* were not detected; the main EO constituents were β-caryophyllene (23.97%), isopulegone (12.60%), *Z*-sabinol (10.11%) and β-pinene (10.01%) (Alim et al., 2009).

EO of *N. transcaucasica* contained the highest percentage of 4aα,7β,7aα-NL isomer (14.34%), while the major constituent was citronellol (17.69%) followed by geranial (9.05%), geranyl acetate (8.20%), neral (6.28%), geraniol (5.97%), 1,8-cineole (5.61), and caryophyllene oxide (5.07%) (Table 2). İşcan et al. (2011) reported 27

Table 2
Chemical composition of *Nepeta* spp. essential oils, GC peak area percentage.

No[#]	Compound[A]	KI[B]	KI[C]	KI[D]	NC	NN	NT	NM	NS	Odour description[1]
1	Hexen-3-en-1-ol	865	859	1391	0.05 ± 0.01^{a}	0.09 ± 0.01^{a}	0.25 ± 0.04^{b}	0.41 ± 0.03^{c}	0.53 ± 0.04^{d}	green, moss, fresh
2	2-Heptanone	885	892	1182[E]	–	–	–	0.13 ± 0.01^{a}	0.19 ± 0.03^{b}	soap
3	Nonane	900	900	900	tr^{a}	–	tr^{b}	–	–	alkane
4	α-Thujene	930	930	1021	–	–	0.06 ± 0.01^{a}	0.13 ± 0.00^{a}	0.27 ± 0.08^{b}	wood, green, herb
5	α-Pinene	939	939	1032	tr^{a}	–	0.10 ± 0.04^{b}	0.11 ± 0.01^{b}	0.21 ± 0.01^{c}	pine, turpentine[1]; woody[2]
6	Camphene	962	954	1075	–	–	–	tr^{a}	–	camphor[1]; vanilla[2]
7	1-Octen-3-ol	985	979	1444[E]	tr^{a}	0.14 ± 0.01^{c}	0.66 ± 0.04^{d}	tr ^{ab}	0.07 ± 0.00^{b}	cheese, creamy, earthy, herbaceous, vegetable, meaty, fishy[2]
8	Thuja-2,4(10)-diene	970	960	1122[E]	–	–	–	0.07 ± 0.00^{b}	0.05 ± 0.00^{a}	
9	Benzaldehyde	970	960	1495	0.08 ± 0.02^{b}	–	0.05 ± 0.01^{a}	0.06 ± 0.00^{ab}	0.11 ± 0.04^{c}	almond, burnt sugar[1]; almond, cherry, sweet[2]
10	Sabinene	978	975	1123	–	–	0.05 ± 0.00^{a}	0.45 ± 0.02^{b}	0.50 ± 0.04^{c}	pepper, turpentine, wood[1]
11	β-Pinene	981	979	1116	0.05 ± 0.01^{a}	–	0.13 ± 0.07^{b}	0.70 ± 0.02^{c}	1.10 ± 0.04^{d}	pine, resin, turpentine[1]; woody[2]
12	3-Octanone	990	983	1255[E]	–	–	–	1.47 ± 0.03^{b}	1.35 ± 0.14^{b}	banana, berry, butter, cheese, musty, spicy, herbaceous, vegetable, earthy, green[2]
13	6-Methyl-5-hepten-2-one	992	985	1337[E]	tr^{a}	0.09 ± 0.01^{a}	0.93 ± 0.18^{b}	–	–	oily, herbaceous, green[2]
14	3-Octanol	999	991	1392[E]	–	–	0.07 ± 0.01^{a}	0.17 ± 0.01^{c}	0.13 ± 0.01^{b}	melon, musty, oily, herbaceous, nutty, earthy, minty, spicy, waxy, woody[2]
15	Decane	1000	1000	1000	0.08 ± 0.01^{b}	–	0.07 ± 0.01^{a}	–	–	alkane
16	*p*-Menta-1(7),8-diene	1007	1004		-	-	0.10 ± 0.03^{a}	–	–	
17	(*2E,4E*)-Heptadienal	1017	1007	1401	-	-	0.06 ± 0.00^{a}	0.16 ± 0.01^{b}	0.17 ± 0.01^{c}	nut, fat, fried[1]; cinnamon, hazelnut, fatty[2]
18	α-Terpinene	1024	1017	1178	-	-	0.13 ± 0.04^{a}	–	–	lemon[1]; berry, lemon, sweet, vegetable, woody, camphoraceous, medicinal, pepper[2]
19	*p*-Cymene	1031	1024	1261	tr^{a}	0.09 ± 0.01^{ab}	0.41 ± 0.16^{d}	0.17 ± 0.00^{bc}	0.20 ± 0.01^{c}	solvent, gasoline, citrus
20	Limonene	1035	1029	1178	tr^{a}	0.05 ± 0.00^{a}	0.29 ± 0.01^{c}	0.19 ± 0.01^{b}	0.75 ± 0.03^{d}	lemon, orange[1]; lemon, orange, citrus, sweet[2]
21	β-Phellandrene	1037	1029	1209	–	tr^{a}	–	–	–	mint, terpentine
22	*1,8*-Cineole	1039	1031	1213	0.10 ± 0.01^{a}	0.65 ± 0.03^{a}	5.61 ± 1.36^{b}	37.35 ± 0.62^{c}	42.58 ± 2.42^{d}	mint, sweet[1]; citrus, herbaceous, fruity, sweet, vanilla, woody, spicy, minty, pepper[2]
23	(*Z*)-β-Ocimene	1045	1037	1245	tr^{a}	0.10 ± 0.00^{c}	tr^{b}	–	–	citrus, herb, flower
24	Benzene acetaldehyde	1053	1042	1641[E]	0.05 ± 0.01^{a}	0.08 ± 0.00^{b}	0.20 ± 0.02^{c}	0.23 ± 0.01^{d}	0.29 ± 0.02^{e}	hawthorne, honey, sweet
25	(*E*)-β-Ocimene	1059	1050	1242	tr^{a}	0.11 ± 0.03^{b}	0.19 ± 0.04^{c}	–	0.09 ± 0.01^{b}	sweet, herb
26	Bergamal	1060	1056		–	0.05 ± 0.00^{a}	0.21 ± 0.01^{b}	–	–	
27	γ-Terpinene	1064	1059	1238	tr^{a}	0.06 ± 0.01^{c}	tr^{b}	0.11 ± 0.00^{e}	0.07 ± 0.00^{d}	gasoline, turpentine[1]; herbaceous, citrus[2]
28	(*Z*)-Sabinene hydrate	1075	1070	[1421]	–	0.08 ± 0.00^{a}	0.08 ± 0.00^{a}	0.35 ± 0.00^{b}	0.60 ± 0.12^{c}	balsamic
29	(*Z*)-Linaloloxide	1080	1072	1420	–	0.07 ± 0.01^{a}	0.34 ± 0.03^{b}	0.05 ± 0.00^{a}	–	flower
30	Camphenilone	1092	1082		–	–	–	0.10 ± 0.00^{a}	–	
31	(*E*)-Linaloloxide	1094	1086	1449	–	–	0.13 ± 0.02^{a}	–	–	flower
32	Terpinolene	1096	1088	1282[E]	–	tr^{a}	–	0.16 ± 0.00^{c}	0.05 ± 0.00^{b}	sweet-piney, plastic[2]
33	Linalool	1103	1096	1537	0.05 ± 0.01^{a}	0.29 ± 0.01^{c}	0.74 ± 0.10^{d}	0.21 ± 0.01^{b}	0.22 ± 0.02^{bc}	flower, lavender[1]; lemon, orange, floral, citrus, sweet[2]
34	(*E*)-Sabinene hydrate	1106	1098	[1458]	–	tr^{a}	0.14 ± 0.02^{c}	0.05 ± 0.00^{a}	0.08 ± 0.01^{b}	wood, balsamic
35	Nonanal	1110	1100	1385	–	0.05 ± 0.01^{a}	0.20 ± 0.02^{b}	–	0.07 ± 0.01^{a}	fat, citrus, green
36	α-Fenchocamphorone	1108	1105		–	–	–	0.07 ± 0.01^{a}	–	
37	(*E*)-Vertocitral C	1110	1106		tr^{a}	–	–	–	–	camphor
38	6-Methyl-3,5-heptadien-2-one	1112	1107		–	0.06 ± 0.01^{a}	0.24 ± 0.02^{b}	–	–	
39	(*Z*)-Rose oxide	1116	1108	1337	–	0.10 ± 0.01^{a}	0.65 ± 0.17^{b}	–	–	sweet, rose, green, flower
40	(*E*)-Thujone	1122	1114	1439[E]	–	–	–	0.08 ± 0.01^{a}	–	
41	(*E*)-*p*-Mentha-2,8-dien-1-ol	1128	1122	[1639]	–	–	–	–	0.06 ± 0.01^{a}	
42	Fotocitral A	1129	1125		–	–	tr^{a}	–	–	
43	(*E*)-Rose oxide	1131	1125	1373	–	–	0.26 ± 0.07^{c}	0.24 ± 0.01^{abc}	0.18 ± 0.04^{ab}	flower
44	α-Campholenal	1134	1126	1496[E]	-	tr^{a}	0.28 ± 0.02^{c}	0.18 ± 0.00^{b}	0.17 ± 0.04^{b}	
45	*(E)*-Pinocarveol	1144	1139	1632	-	0.23 ± 0.01^{b}	0.12 ± 0.02^{a}	2.16 ± 0.02^{d}	1.81 ± 0.12^{c}	flower
46	Nopinone	1146	1140	1573[E]	-	0.10 ± 0.00^{a}	0.22 ± 0.02^{b}	0.61 ± 0.00^{d}	0.53 ± 0.04^{c}	
47	(*Z*)-Verbenol	1148	1141	1660[E]	-	0.05 ± 0.00^{a}	0.13 ± 0.02^{b}	–	0.06 ± 0.01^{a}	
48	(*E*)-Sabinol	1151	1142	1717[E]	-	-	-	0.11 ± 0.00^{a}	–	
49	(*Z*)-β-Terpineol	1152	1144	1646	-	-	-	0.09 ± 0.00^{a}	–	must
50	(*E*)-Verbenol	1153	1144	1680[E]	-	-	0.08 ± 0.01^{a}	–	0.10 ± 0.00^{b}	
51	(*E*)-dihydro-α-Terpineol	1155	1147		-	0.12 ± 0.00^{a}	0.18 ± 0.01^{b}	–	–	
52	Isoamyl angelate	1155	1147		-	-	-	0.09 ± 0.00^{b}	0.06 ± 0.01^{a}	

(*continued on next page*)

Table 2 (*continued*)

No[#]	Compound[A]	KI[B]	KI[C]	KI[D]	NC	NN	NT	NM	NS	Odour description[1]
53	Citronellal	1159	1153	1488	0.10 ± 0.02[a]	0.95 ± 0.01[b]	2.47 ± 0.19[c]	–	0.10 ± 0.01[a]	fat[1]; cherry, lemon, green, rose, sweet[2]
54	Nerol oxide	1162	1158	1479	-	-	0.11 ± 0.02[a]	–	–	oil, flower
55	Sabina ketone	1167	1159	[1651]	-	-	-	0.33 ± 0.01[b]	0.24 ± 0.01[a]	
56	β-Pinene oxide	1170	1159		-	tr[a]	0.30 ± 0.01[b]	–	–	
57	Pinocarvone	1172	1164	1575[E]	-	0.12 ± 0.01[d]	tr[a]	0.86 ± 0.03[c]	0.87 ± 0.08[c]	
58	δ-Terpineol	1175	1166	1679[E]	-	tr[a]	0.09 ± 0.01[b]	0.39 ± 0.01[d]	0.15 ± 0.02[c]	
59	Rosefuran epoxide	1184	1177		-	0.10 ± 0.03[a]	0.16 ± 0.01[b]	–	–	
60	Terpinen-4-ol	1185	1177	1591	tr[a]	0.13 ± 0.02[b]	0.41 ± 0.01[c]	0.60 ± 0.01[e]	0.52 ± 0.04[d]	turpentine, nutmeg, must
61	Isogeranial	1186	1180	[1516]	-	–	0.13 ± 0.01[a]	–	–	green
62	*p*-methyl Acetophenone	1190	1182	1774[E]	-	–	–	–	0.13 ± 0.04[a]	hawthorne, fruity[2]
63	*p*-Cymen-8-ol	1192	1182	1848[E]	-	–	–	0.11 ± 0.01[a]	–	
64	Cryptone	1195	1185	1675[E]	–	0.44 ± 0.01[b]	0.41 ± 0.04[a]	–	–	
65	α-Terpineol	1197	1188	1688	tr[a]	0.13 ± 0.01[b]	0.18 ± 0.01[c]	0.39 ± 0.01[e]	0.23 ± 0.01[d]	oil, anise, mint[1]; lilac[2]
66	Isoamyl tiglate	1198	1192		–	–	–	tr[a]	–	fruity, wine-like[2]
67	Methyl salicylate	1202	1191	1745	0.06 ± 0.01[a]	–	–	–	–	peppermint[1]; spicy, minty, sweet[2]
68	Myrtenal:Myrtenol (~2:1)	1202	1195	1596	-	0.10 ± 0.01[a]	0.07 ± 0.01[a]	1.71 ± 0.01[b]	1.58 ± 0.15[b]	spice
69	Estragole	1205	1196	1655	-	0.06 ± 0.01[b]	-	tr[a]	-	licorice, anise
70	Verbenone	1215	1205	1721[E]	-	0.08 ± 0.01[a]	0.35 ± 0.00[b]	0.21 ± 0.01[ab]	1.20 ± 0.29[c]	
71	*iso*-Dihydrocarveol	1222	1214	[1562]		tr[a]	0.06 ± 0.00[b]	-	-	wood, spice
72	(*E*)-Carveol	1225	1216	1839	0.18 ± 0.02[a]	1.61 ± 0.03[d]	0.99 ± 0.06[c]	-	0.29 ± 0.05[b]	caraway, solvent
73	(*E*)-Sabinene hydrate acetate	1228	1221	[1610]	-	0.07 ± 0.00[a]	0.19 ± 0.06[c]	0.21 ± 0.01[b]	-	
74	Citronellol	1231	1225	1762	1.06 ± 0.21[b]	0.12 ± 0.01[a]	17.69 ± 0.53[c]	0.19 ± 0.01[a]	-	rose[1]; geranium, rose[2]
75	Nerol	1238	1229	1770	0.82 ± 0.15[c]	4.79 ± 0.03[d]	0.56 ± 0.10[b]	0.30 ± 0.01[a]	-	sweet
76	(*Z*)-Carveol	1240	1229	1846	-	-	-	0.17 ± 0.01[b]	0.11 ± 0.01[a]	fresh, spearmint, caraway
77	Neral	1250	1238	1667	0.40 ± 0.07[a]	2.92 ± 0.08[b]	6.28 ± 0.25[c]	0.21 ± 0.01[a]	-	lemon
78	Cumin aldehyde	1251	1241	1759	-	0.33 ± 0.02[c]	-	tr[a]	0.12 ± 0.01[b]	acid, sharp
79	Carvone	1254	1243	1720	0.08 ± 0.00[a]	0.18 ± 0.00[b]	0.10 ± 0.03[a]	0.24 ± 0.01[c]	0.68 ± 0.06[d]	caraway, mint, basil, fennel
80	3-Methyl pentyl angelate	1258	1252		-	-	-	0.23 ± 0.01[a]	0.34 ± 0.03[b]	
81	Geraniol	1262	1252	1847	0.41 ± 0.20[b]	2.64 ± 0.04[c]	5.97 ± 0.07[d]	0.16 ± 0.01[a]	0.05 ± 0.01[a]	rose, geranium
82	Geranial	1279	1267	1715	0.66 ± 0.10[b]	4.03 ± 0.09[c]	9.05 ± 0.08[d]	0.13 ± 0.01[a]	0.11 ± 0.01[a]	lemon, mint
83	Citronellyl formate	1284	1273	1615[E]	tr[a]	0.35 ± 0.01[b]	0.34 ± 0.04[b]	-	-	apricot, honey, peach, plum, rose[2]
84	Methyl dihydrocinnamate	1286	1279		-	tr[a]	0.15 ± 0.05[b]	-	-	
85	(*3Z*)-Hexenyl valerate	1287	1281		-	0.08 ± 0.01[b]	-	tr[a]	-	
86	Neryl formate	1284	1282	1674[E]	tr[a]	tr[b]	-	0.05 ± 0.00[c]	0.09 ± 0.01[d]	rose, medicinal herb, citrus, tropical fruit, citrus[2]
87	Hydroxy citronellal	1294	1288		-	-	0.11 ± 0.04[a]	-	-	
88	Thymol	1296	1290	2164[E]	-	-	-	0.05 ± 0.01[a]	–	woody, fruity, sweet, minty, earthy, spicy, smoky, wine-like, coffee[2]
89	*p*-Cymen-7-ol	1299	1290	2101[E]	-	-	0.06 ± 0.01[ab]	0.09 ± 0.01[b]	0.10 ± 0.01[ab]	weak citrus-like
90	Lavandulyl acetate	1300	1290	1602[E]	tr[a]	-	0.18 ± 0.00[c]	-	-	
91	Geranyl formate	1308	1298	1697[E]	tr[a]	0.16 ± 0.02[b]	-	-	-	green, fruity, rose, sweet[2]
92	Carvacrol	1312	1299	2211[E]	0.63 ± 0.10[b]	–	0.06 ± 0.01[a]	0.08 ± 0.03[a]	-	
93	Dihydroedulan II	1322	1316		-	-	-	0.11 ± 0.01[a]	0.35 ± 0.04[b]	
94	α-Terpinyl acetate	1358	1349	1700	-	0.12 ± 0.01[a]	-	-	-	wax
95	Citronellyl acetate	1360	1352	1607	tr[a]	0.14 ± 0.01[c]	0.21 ± 0.00[b]	0.21 ± 0.01[b]	-	rose, dust
96	4aα,7α,7aα-Nepetalactone	1374	1360	[1970]	35.64 ± 5.36[c]	6.20 ± 0.10[b]	2.76 ± 0.86[ab]	0.11 ± 0.01[a]	0.19 ± 0.01[a]	
97	Neryl acetate	1376	1361	1742	tr[a]	0.33 ± 0.01[d]	0.19 ± 0.08[c]	0.11 ± 0.01[b]	-	fruit
98	α-Copaene	1389	1376	1488	0.06 ± 0.01[a]	0.08 ± 0.01[b]	-	0.15 ± 0.01[d]	0.10 ± 0.01[c]	wood, spice
99	Geranyl acetate	1395	1381	1711	0.05 ± 0.01[a]	0.43 ± 0.01[c]	8.20 ± 0.21[d]	0.25 ± 0.03[b]	-	rose
100	β-Bourbonene	1398	1388	1585	-	0.38 ± 0.00[b]	0.60 ± 0.04[c]	0.25 ± 0.03[a]	0.70 ± 0.03[d]	herb
101	β-Cubebene	1400	1388	1546	-	0.19 ± 0.01[b]	0.06 ± 0.00[a]	-	-	citrus, fruit
102	4aα,7α,7aβ-Nepetalactone	1406	1387		50.16 ± 4.31[b]	55.72 ± 0.24[c]	0.21 ± 0.06[a]	0.97 ± 0.02[a]	0.57 ± 0.05[a]	
103	4aα,7β,7aα-Nepetalactone	1412	1392		1.80 ± 0.27[b]	0.05 ± 0.01[a]	14.34 ± 1.85[c]	0.41 ± 0.03[a]	0.17 ± 0.02[a]	
104	(*Z*)-Caryophyllene	1419	1408	1570	-	-	tr[a]	0.05 ± 0.00[b]	0.06 ± 0.01[b]	wood
105	(*E*)-Caryophyllene	1432	1419	1594	3.07 ± 0.09[e]	1.05 ± 0.01[b]	0.74 ± 0.06[a]	1.29 ± 0.03[c]	1.54 ± 0.12[d]	wood, spice
106	β-Ylangene	1434	1420	1577[E]	-	0.07 ± 0.00[a]	-	-	-	fruity[2]
107	β-Copaene	1440	1432	1580[E]	-	0.05 ± 0.00[a]	0.11 ± 0.02[d]	0.10 ± 0.01[bcd]	0.09 ± 0.01[b]	
108	γ-Elemene	1454	1436	1636	0.05 ± 0.01[b]	tr[a]	-	-	-	green, wood, oil
109	Aromadendrene	1459	1441	1600	0.05 ± 0.01[b]	0.10 ± 0.01[d]	tr[a]	0.05 ± 0.00[b]	0.09 ± 0.00[d]	wood
110	(*Z*)-β-Farnesene	1461	1442	1648	0.26 ± 0.02[c]	0.10 ± 0.01[b]	-	0.08 ± 0.01[a]	-	citrus, green
111	α-Humulene	1468	1454	1663	0.26 ± 0.01[c]	1.24 ± 0.03[d]	0.12 ± 0.01[ab]	0.11 ± 0.01[a]	0.14 ± 0.01[b]	wood
112	*allo*-Aromadendrene	1475	1460	1639	0.19 ± 0.06[a]	-	-	-	-	wood
113	γ-Gurjunene	1493	1477	1668[E]	-	0.05 ± 0.00[a]	-	-	-	
114	Germacrene D	1494	1481	1705	tr[a]	0.06 ± 0.00[a]	0.11 ± 0.02[a]	1.37 ± 0.02[b]	1.80 ± 0.21[c]	wood, spice
115	Citronellyl isobutanoate	1495	1483	1705	-	-	0.21 ± 0.08[a]	-	-	fruit, rose[2]
116	(*E*)-β-Ionone	1498	1488	1912	-	-	0.05 ± 0.01[a]	-	0.11 ± 0.01[b]	seaweed, violet, flower, raspberry
117	Bicyclogermacrene	1510	1500	1738	-	0.06 ± 0.01[a]	-	-	0.05 ± 0.00[a]	green, wood
118	Aciphyllene	1512	1501		-	-	-	0.09 ± 0.03[b]	tr[a]	

(*continued on next page*)

Table 2 (*continued*)

No[#]	Compound[A]	KI[B]	KI[C]	KI[D]	NC	NN	NT	NM	NS	Odour description[1]
119	Cuparene	1520	1504	1816[E]	-	0.07 ± 0.02[b]	tr[a]	-	-	
120	β-Bisabolene	1522	1505	1714	-	0.06 ± 0.00[a]	-	-	-	balsamic
121	β-Sesquiphellandrene	1527	1522	1782	-	tr[a]	-	-	0.06 ± 0.00[b]	wood
122	δ-Cadinene	1529	1523	1749	0.15 ± 0.03[ab]	0.61 ± 0.02[c]	0.14 ± 0.01[a]	0.17 ± 0.01[b]	0.14 ± 0.02[a]	thyme, medicine, wood
123	(*Z*)-Nerolidol	1538	1532	2010	tr[a]	-	-	-	-	wax[1]; apple, citrus, woody, green, rose[2]
124	α-Calacorene	1556	1545	[2207]	-	tr[a]	-	-	-	wood
125	Elemol	1569	1549	2089	-	0.35 ± 0.02[a]	-	2.53 ± 0.01[c]	2.30 ± 0.12[b]	green, wood
126	Germacrene B	1568	1561	1864	-	-	0.35 ± 0.01[a]	–	–	wood, earth, spice
127	Longicamphenylone	1579	1563		-	-	-	0.11 ± 0.00[a]	-	
128	1-nor-Bourbonanone	1583	1563		-	0.07 ± 0.00[ab]	0.05 ± 0.01[a]	0.22 ± 0.06[c]	0.10 ± 0.00[b]	green-woody, sharp
129	(*3Z*)-Hexenyl benzoate	1585	1566	2119[E]	tr[a]	-	-	-	-	woody, herbaceous, green[2]
130	Spathulenol	1595	1578	2129	0.20 ± 0.03[a]	0.96 ± 0.04[b]	1.34 ± 0.03[c]	3.04 ± 0.14[d]	0.88 ± 0.11[b]	herb, fruit
131	Caryophyllene oxide	1600	1583	1962	1.95 ± 0.10[a]	5.53 ± 0.07[b]	5.07 ± 0.06[b]	22.06 ± 0.09[d]	20.35 ± 1.49[c]	herbaceous, sweet, spice
132	Widdrol	1614	1599		-	tr[a]	0.05 ± 0.01[b]	-	0.10 ± 0.01[c]	
133	Humulene epoxide II	1628	1608	2047[E]	0.14 ± 0.02[a]	0.54 ± 0.02[b]	0.64 ± 0.01[c]	0.88 ± 0.06[d]	0.97 ± 0.07[e]	woody spicy
134	γ-Eudesmol	1642	1632	2185	-	tr[a]	-	-	0.12 ± 0.02[b]	wax, sweet
135	τ-Cadinol	1655	1640	2170[E]	-	0.06 ± 0.00[a]	0.07 ± 0.01[a]	0.19 ± 0.01[c]	0.13 ± 0.02[b]	
136	Cubenol	1660	1646	[1993]	-	0.08 ± 0.04[a]	-	-	0.16 ± 0.01[b]	spice, herb, green tea
137	β-Eudesmol	1671	1650	2246	-	0.15 ± 0.03[b]	-	-	0.06 ± 0.02[a]	wood, green
138	α-Cadinol	1674	1654	2191	-	0.37 ± 0.03[b]	tr[a]	0.88 ± 0.09[c]	1.22 ± 0.27[b]	herb, wood
139	14-hydroxy-(*Z*)-Caryophyllene	1685	1667		tr[a]	0.18 ± 0.05[ab]	0.35 ± 0.03[b]	1.76 ± 0.10[d]	1.06 ± 0.24[c]	
140	Mustakone	1693	1677		-	-	0.28 ± 0.01[a]	0.28 ± 0.07[b]	-	
141	α-Bisabolol	1703	1685	2235	tr[a]	0.20 ± 0.04[c]	0.08 ± 0.01[b]	0.65 ± 0.02[d]	-	spice, flower
142	β-Bisabolenol	1789	1789		-	-	0.06 ± 0.00[a]	-	-	
143	Phytol	2109	1943	2571	0.21 ± 0.1[a]	0.08 ± 0.01[a]	0.25 ± 0.01[a]	1.94 ± 0.10[a]	1.81 ± 0.52[b]	flower[1]; balsamic, floral[2]
	Total compounds identified/representing % of total oil				56/99.73	90/98.27	96/96.79	88/93.81	79/95.02	
	Grouped compounds (%)									
	Monoterpene hydrocarbons				0.24	0.48	1.53	2.12	3.29	
	Oxygenated monoterpenes				4.80	22.28	64.48	48.74	52.96	
	Nepetalactones				87.60	61.97	17.31	1.49	0.93	
	Sesquiterpene hydrocarbons				4.11	4.26	2.33	3.71	4.81	
	Oxygenated sesquiterpenes				2.39	8.55	8.03	32.60	27.45	
	Aldehydes and ketones				tr	0.20	1.43	1.76	1.78	
	Alcohols and esters				0.11	0.31	0.98	1.02	1.13	
	Alkanes				0.10		0.11			
	Aromatics				0.13	0.08	0.25	0.29	0.40	
	Norterpenoids					0.06	0.09	0.14	0.46	
	Diterpenes				0.21	0.08	0.25	1.94	1.81	

Compounds are listed in order of their elution from nonpolar *Elite-5* capillary column.
[A] Identified on the basis of GC–TOFMS spectra and calculated Kováts retention index of GC–FID response.
[B] Kováts retention indices calculated against C_7—C_{30} *n*-alkanes on nonpolar *Elite*-5 column.
[C] Kováts retention indices on nonpolar DB-5 column reported in literature (Adams, 2009).
[D] Kováts retention indices on polar carbowax 20 M column reported in literature (Flavornet, http://www.flavornet.org/flavornet.html).
[E] Kováts retention indices on polar carbowax 20 M column reported by Davies (1990).
[1]Odour description from http://www.flavornet.org. (and without number indications) [2] Odour description from SAFC®Flavors & Fragrances (www.safcglobal.com).
tr – trace (≤0.04%); RSD%, average coefficient of variance of individual compounds.
Values within rows followed by the same superscript letter (a-f) do not differ statistically at $P < 0.05$ (Duncan test).

volatiles in the EO of *N. transcaucasica* with high percentages of 4aα,7α,7aβ-NL (39.8%), 4aα,7α,7aα-NL (28.4%) and germacrene D (15.6%). To the best of our knowledge *N. transcaucasica* with so high content of citronellol/geraniol/nerol type terpenes has not been reported previously.

It is evident that *N. melissifolia* and *N. sibirica* do not depend to NL chemotype; the total contents of NLs were only 1.49% and 0.93%, respectively. The key components in both species were 1,8-cineole (37.35 and 42.58%) and caryophyllene oxide (22.06 and 20.35%), while the other compounds exceeding 1% were β-caryophyllene (1.29 and 1.54%) and elemol (2.53 and 2.30%). Spathulenol (3.04%) was found only in *N. melissifolia*, some differences in minor constituents were also observed (Table 2). Only two reports were found on *N. sibirica* EO: neoepinepetalactone (78.8%), germacrene D (9.4%) and β-ocimene (4.8%) were major constituents in the plants from Altai region in Russia (Letchamo et al., 2005), while exclusively 4aα,7α,7aα-NL was determined in the EO of Mongolian *N. sibirica* (Tsuruoka et al., 2012). To the best of our knowledge, the composition of EO from *N. melissifolia* was not reported previously.

3.3. Antioxidant potential of Nepeta extracts

In general, the antioxidant potential of plant extracts is mainly associated with the presence of phenolic compounds, which may scavenge free radicals *via* single electron (SET) or hydrogen atom (HAT) transfer reactions. Huang et al. (2005) recommended to use several

methods for a more comprehensive evaluation of antioxidant properties of biomaterials; therefore, TPC, DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ scavenging and ORAC assays were used for the *Nepeta* spp. extracts in our study. It should be noted that although the values obtained with Folin-Ciocalteu reagent are referred as a total phenolic content (TPC) the principle of this reaction is also based on SET. Numerous studies reported good correlation between the RSC and TPC values (Žugić et al., 2014).

The TPC values for WEs (6.5–10.3%, w/w) were considerably higher than for DAEs (2.2–3.6%, w/w) (Table 3). Moreover, considering remarkably higher yields of WEs than those of DAEs, the percentage distribution of recovered phenolics from the initial herbal material (pdw) in water and solid fractions were from 1.4% (*N. cataria* var. *citriodora*) to 3.0% (*N. sibirica*) and from 0.05% (*N. transcaucasica*) to 0.09% (*N.cataria* var. *citriodora*), respectively. Consequently, the main part of polar phenolics was dissolved in water during herb distillation. Sarikurkcu et al. (2019) recently reported similar TPC value (86.30 mg GAE/edw) for polar methanolic extract of *N. nuda* subsp. *glandulifera*. Acetone is an aprotic organic solvent extracting compounds of lower polarity, while most polyphenolic antioxidants are polar due to the presence hydroxyl groups and attached saccharides in their structures. The sum of TPC isolated with both solvents from the dried plants were from 15.40 (*N. cataria* var. *citriodora*) to 30.76 (*N. sibirica*) mg GAE/g pdw. The TPC values in *N. cataria* var. *citriodora* DAE and WE, 36.17 ± 0.69 and 65.28 ± 1.20 mg GAE/g edw, respectively, were in similar ranges compared to the previously reported values for *N. cataria* extracts, which, depending on extraction method and solvent, were from 29.8 to 49.3 mg GAE/g dw (Mihaylova et al., 2013). TPC content in methanolic extract of *N. melissifolia* leaves was 31.06 mg GAE/g dw (Proestos et al., 2013), which is quite similar to the TPC of DAE (24.80 mg GAE/g edw) determined in our study. Dienaitė et al. (2018) reported that TPC values for different *Nepeta* spp. varied in a wider range, from 62.44 to 669.0 mg GAE/g edw and from 2.27 to 241.2 mg GAE/g pdw; however, plant fractionation methodology in this study was different.

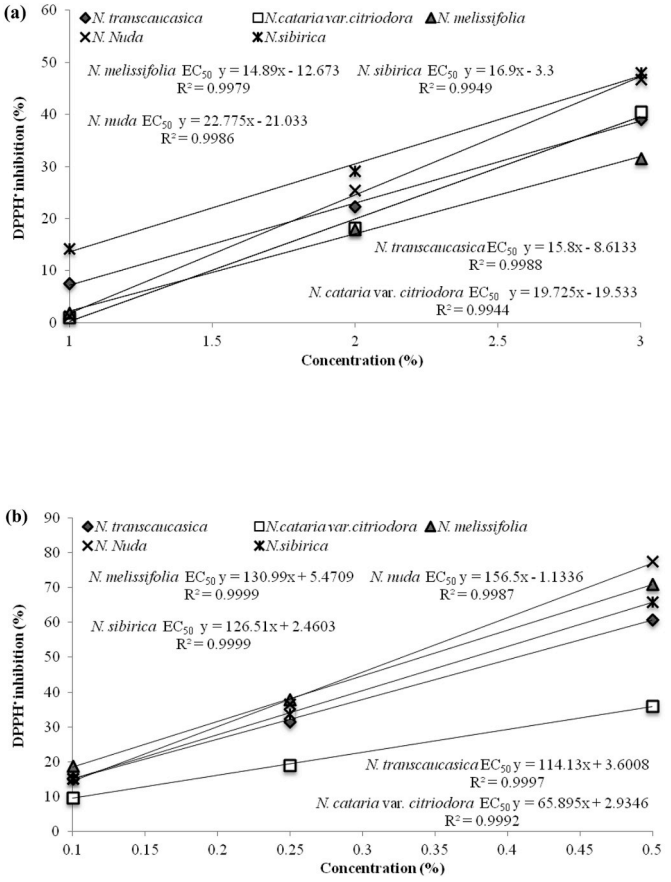

Fig. 2. Effects of different concentration water (WE) (a) and deodorized acetone (DAE) (b) extracts on DPPH$^{\bullet}$ scavenging capacity.

Table 3
The TPC content of *Nepeta* spp. extracts.

Nepeta species	TPC content					Total TPC, mg GAE/g pdw
	Water extract (WE)		Deodorised acetone extract (DAE)			
	mg GAE/g edw	mg GAE/g pdw	mg GAE/g edw	mg GAE/g spdw	mg GAE/g pdw	
NN	81.86 ± 1.44^{b}	22.64 ± 0.40^{b}	22.76 ± 0.23^{a}	0.97 ± 0.01^{c}	0.62 ± 0.01^{b}	23.25 ± 0.40^{b}
NT	85.38 ± 1.86^{c}	23.90 ± 0.52^{c}	22.30 ± 0.76^{a}	0.86 ± 0.03^{a}	0.53 ± 0.02^{a}	24.43 ± 0.52^{c}
NS	103.34 ± 1.53^{e}	30.01 ± 0.44^{d}	24.56 ± 1.07^{b}	1.16 ± 0.05^{b}	0.75 ± 0.03^{d}	30.76 ± 0.45^{e}
NC	65.28 ± 1.20^{a}	14.46 ± 0.27^{a}	36.17 ± 0.69^{c}	1.38 ± 0.03^{d}	0.94 ± 0.02^{e}	15.40 ± 0.26^{a}
NM	92.53 ± 2.14^{d}	25.67 ± 0.59^{d}	24.80 ± 1.24^{b}	1.15 ± 0.06^{b}	0.68 ± 0.03^{c}	26.34 ± 0.60^{d}

Results are expressed as a mean ± standard deviation (n = 4); Values within columns followed by the same superscript letter (a-e) do not differ statistically at $P < 0.05$ (Duncan test). edw – extract dry weight; pdw – plant dry weight. N – *N. nuda*; T – *N. transcaucasica*; S – *N. sibirica*; C– *N. cataria* var. *citriodora*; M – *N. melissifolia*.

The principles of the applied *in vitro* RSC methods are similar; however, DPPH$^{\bullet}$ scavenging assay is more applicable for hydrophilic compounds, while ABTS$^{\bullet+}$ decolouration assay is equally suitable both for lipophilic (better soluble in acetone) and hydrophilic (better soluble in water) antioxidants. DPPH$^{\bullet}$ scavenging is measured by the colour changes from purple to yellow due to the SET reaction between antioxidant molecules and radicals (Huang et al., 2005). The results obtained in our study clearly demonstrate that WEs of *Nepeta* plants were remarkably stronger antioxidants than DAEs, however all tested extracts reacted with the radical rather slowly, and after 40 min the percentage of scavenged radicals was dose-dependent: for instance, WE applied at 0.5% and DAE at 3% scavenged 36.04–77.6% and 31.6–48.0% of DPPH$^{\bullet}$. EC_{50} values representing the concentration reducing 50% of radicals were estimated from the curves in Figs. 2 and 3. Based on EC_{50} values WEs were ~6–10 times stronger antioxidants than DAEs, with EC_{50} = 0.33–0.71% *vs* 3.12–4.12% (Table 4). Comparing the studied plants, WEs of *N. nuda* and *N. melissifolia* were the strongest, while that of *N. cataria* var. *citriodora* the weakest radical scavengers. For comparison, previously reported DPPH$^{\bullet}$ scavenging EC_{50} for methanol extract of *N. melissifolia* was 5.1 μg/mL (Proestos et al., 2013), for *N.nuda* EO ~40 μg/mL (Gkinis et al., 2010) and for methanol extract of *N. nuda* 148.98 μg/mL (Žugić et al., 2014). RSC of various *Nepeta* extracts was also determined in trolox equivalents (TE). Dienaitė et al. (2018) measured DPPH$^{\bullet}$ scavenging capacity for various *Nepeta* spp. and reported that water extracts were the strongest (403–973 μM TE/g), followed by methanol (95–380 μM TE/g) and acetone extracts (32–94 μM TE/g). Mihaylova et al. (2013) for water and hydroethanolic extracts of *N. cataria* reported the values ranging from 220.0 to 335.5 μM TE/g dw; Sarikurkcu et al. (2019) for *N. nuda* subsp. *glandulifera* methanolic extracts, depending on the assay, from

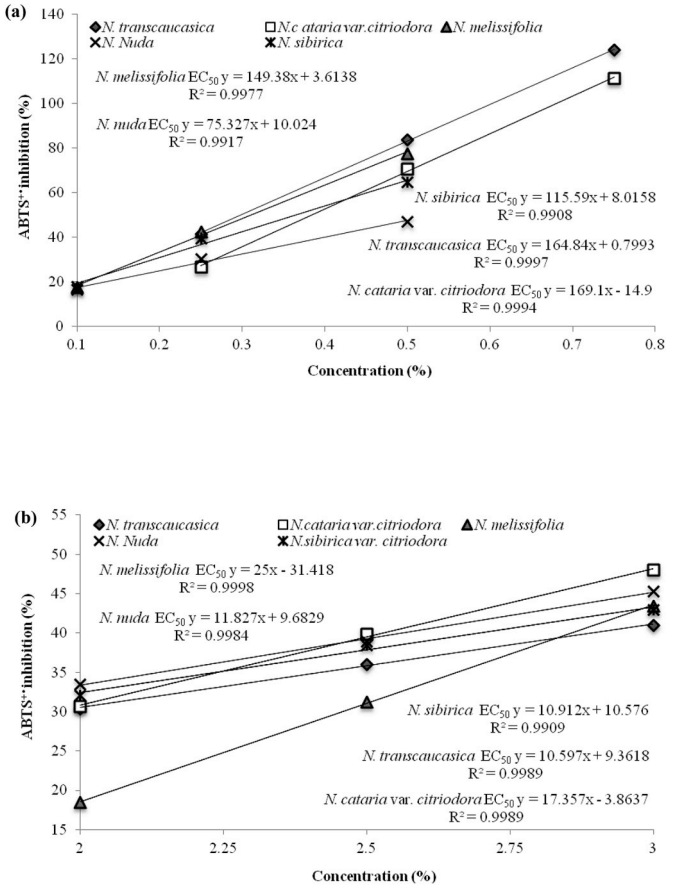

Fig. 3. Effects of different concentration water (WE) (a) and deodorized acetone (DAE) (b) extracts on $ABTS^{+\bullet}$ scavenging capacity.

Table 4
Radical scavenging and ORAC capacity of *Nepeta* spp. extracts.

Nepeta species	$DPPH^{\bullet}$ (EC_{50}),%	$ABTS^{+\bullet}$ (EC_{50}), %	ORAC, μmol TE/g edw
WN	0.33 ± 0.01^{a}	0.35 ± 0.01^{b}	1342 ± 1.3^{c}
WT	0.41 ± 0.01^{c}	0.31 ± 0.01^{a}	888 ± 0.44^{a}
WS	0.38 ± 0.01^{b}	0.33 ± 0.02^{ab}	1122 ± 1.9^{b}
WC	0.71 ± 0.01^{d}	0.39 ± 0.01^{c}	3399 ± 2.6^{d}
WM	0.34 ± 0.01^{a}	0.32 ± 0.02^{a}	4488 ± 1.5^{e}
AN	3.12 ± 0.05^{a}	3.58 ± 0.18^{b}	1476 ± 0.6^{d}
AT	3.71 ± 0.05^{d}	3.97 ± 0.13^{c}	1013 ± 2.1^{b}
AS	3.15 ± 0.10^{b}	3.36 ± 0.23^{ab}	1889 ± 1.3^{e}
AC	3.53 ± 0.10^{c}	3.04 ± 0.06^{a}	900 ± 4.1^{a}
AM	4.12 ± 0.20^{e}	3.32 ± 0.17^{ab}	1050 ± 1.4^{c}

Results are expressed as a mean ± standard deviation (n = 3,4); Values within columns (for one group of extract) followed by the same superscript letter (a-e) do not differ statistically at $P < 0.05$ (Duncan test). edw – extract dry weight. The extracts isolated with water and acetone are referred by the abbreviation composed of the first letter of the solvent and the first letters plant species, W and A, respectively. N – *N. nuda*; T – *N. transcaucasica*; S – *N. sibirica*; C– *N. cataria* var. *citriodora*; M – *N. melissifolia*.

94.6 (ABTS) to 242.94 (DPPH) mg TE/edw. It is interesting noting that antioxidant capacity of EO from the same species was many times lower in $DPPH^{\bullet}$ than in $ABTS^{\bullet+}$ scavenging assay (IC_{50} = 153.24 *vs* 2.72 mg/mL); most likely, it may be explained by the lipophilic character of EO (Sarikurkcu et al., 2018). However, Ashrafi et al. (2019) reported very low IC_{50} (80.62 μg/mL) in $DPPH^{\bullet}$ scavenging assay for *N. cataria* EO containing 62.54% nepetalactones.

In $ABTS^{\bullet+}$ scavenging assay blue radical cation is decolourized *via* reaction with antioxidant molecules. In general, the data obtained by this assay are similar to the $DPPH^{\bullet}$ scavenging results (Table 4). The EC_{50} values for WEs and DAEs were from 0.31% (*N. transcaucasica*) to 0.39% (*N. cataria* var. *citriodora*) and from 3.04% (*N. cataria* var. *citriodora*) to 3.97% (*N. transcaucasica*), respectively. Strong positive linear correlation was observed between TPC − $DPPH^{\bullet}$ (R_2 = 0.9011) and TPC − $ABTS^{\bullet+}$ (R^2 = 0.9175) values.

The ORAC assay is based on radical chain breaking by HAT and evaluates inhibition of oxidation induced by peroxyl radical; it is believed that this method is more relevant to the expected antioxidant activity of phenols in biological systems. ORAC values measured in our study for WEs and DAEs of different *Nepeta* spp. were 888–4488 and 900–1889 μmol TE/g edw, respectively (Table 4). WE of *N. melissifolia* and *N. cataria* var. *citriodora* demonstrated the highest ORAC, 4488 and 3399 μmol TE/g edw, which is equivalent to 1.12 and 0.85 g/g of trolox, respectively. Consequently, the extracts may be recognised as strong peroxyl radical scavengers. Only one study applied ORAC assay to *Nepeta* spp. extracts previously (Dienaitė et al., 2018); it reported that ORAC values were solvent and species dependent and varied from 331.4 to 2476 μmol TE/g edw and from 15.95 to 862.6 μmol TE/g pdw.

3.4. Bioactivities and toxicity of Nepeta spp. and their constituents

The phytochemical composition, bioactivities (Fig. 1) and applications of previously studied *Nepeta* species were comprehensively reviewed in several articles (Formisano et al., 2011; Sharma and Cannoo, 2013; Asgarpanah et al., 2014; Salehi et al., 2018). Therefore, this section will shortly discuss only the most recent results, which have not been covered in the above-mentioned review articles, and which are most relevant to the plants (and their EO constituents) investigated in the present study.

Several articles reported cytotoxic effects of various *Nepeta* spp. Rich in (*E,Z*)-NL *N. rtanjensis* EO from Serbia exhibited cytotoxic activity on HeLa, 1 < 562, A549, LS -174 and MDA-MB-231 cancer cell lines, whereas normal cell line (MRC-5) was the least sensitive; IC_{50} value for MRC-5 was not reached within the tested range of EO concentrations up to 0.1 μL/mL (Skorić et al., 2017). EO of *N. sintenisii* (NLs > 63%) also was remarkably more cytotoxic in the cancer cell lines (A2780, HeLa, LS180 and MCF-7) than in the normal HUVEC cell line: IC_{50} for HUVEC was 219 μg/mL, while for HeLa 20.37 μg/mL (Shakeri et al., 2016). *N. sibirica* EO consisting mainly of 4aα,7α,7aα-NL was cytotoxic to HL60 myeloma and Kato III stomach carcinoma cell lines (Tsuruoka et al., 2012). *N. menthoides* EO containing two NLs (~40%) showed a significant cytotoxic effect against T47D, HT-29 and Caco-2 cell lines (IC_{50} = 19.37, 30.7, and 32.24 μg/mL, respectively), and acetylcholinesterase inhibitory activity with IC_{50} = 64.87 μg/mL (Kahkeshani et al., 2014). Methanol extract of *N. nuda* subsp. *nuda* had antiviral activity against herpes simplex virus demonstrating the IC_{50} values of 320 μg/mL against HSV-1 and 510 μg/mL against HSV-2 (Todorov et al., 2015).

EO of *N. nuda* containing 46.0% of 1,8-cineole inhibited 5 tested bacterial strains (Miladinovic et al., 2015). In addition, the authors performed chemo-informatics survey, which confirmed that the antagonistic interactions between 1,8-cineole and standard antibiotics are due to a membrane potential/proton motive force dissipation. EO of *N. sintenisii* had antimicrobial effects against 11 tested bacteria and *Candida albicans*, while its *in vitro* antioxidant capacity was weak (Shakeri et al., 2016). Methanol extract of *N. transcaucasica* had antibacterial activity against 14 out of 50 bacteria, while some of the extracts had the lowest minimal inhibitory concentration (MIC) values (31.25 μg/mL) as compared with the standard drug, maxipime (Ozturk, 2009). MICs of *N. nuda* EO against twenty phytopathogenic bacteria were from 7.81 to 31.25 μL/disc (Gormez et al., 2013); *N. sibirica* EO consisting mainly of 4aα,7α,7aα-NL showed antimicrobial effects against *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans* and *Aspergillus fumigatus* (Tsuruoka et al., 2012); EO of *N. transcaucasica* with 4aα,7α,7aβ-NL (39%), 4aα,7α,7aα-NL (28%), and germacrene D (15%) effectively inhibited *Candida glabrata* and *C. tropicalis* yeasts, at MICs of 0.09 and

0.375 mg/mL, respectively (İşcan et al., 2011). Different polarity fractions of *N. nuda* (subsp. *Lydiae*) had different antioxidant capacity and phytochemical composition (Geçibesler et al., 2017). EO of *N. nuda* ssp. *nuda* inflorescence from Greece with 4aα,7α, 7aβ-NL (75.7%) exhibited pro-oxidant activity at the highest applied concentration (Gkinis et al., 2010).

EO of *N. meyeri* with 4aα,7α,7aβ-NL (83.4%) and 4aα,7α,7aα-NL (8.83%), as the major components, had strong inhibitory activity against *Bromus danthoniae* and *Lactuca serriola* weeds and *Brassica napus* and *Zea mays* crop plants by causing a change in random amplified polymorphic DNA profiles (Kekeç et al., 2013). EO of *N. nuda* with 4aα,7β,7aα-NL (18.10%), germacrene (15.68%) and elemol (14.38%) inhibited root and stem growth of *Zea mays* seedlings (Bozari et al., 2013). *N. nuda* subsp. *albiflora* with 4aα,7α,7α,β-NL (74.27%) revealed significant herbicidal actions by inhibiting the germination, radicle and plumule elongation (Bozok et al., 2017).

Zhu (2011) demonstrated the efficacy of commercial catnip oil with 90% (*Z,E*)-NL + (*E,Z*)-NL as repellent of *Stomoxys calcitrans* and other filth flies; whereas Oz et al. (2018) reported that the LC_{50} and LC_{90} values on the larvae of *Culex pipiens* L. induced by *N. cadmea* EO with caryophyllene oxide (22.96%), viridiflorol (12.23%), *cis*-calamenene (10.67%) and only 3.09% of (*Z-E*)-NL were 28.7 and 39.82 ppm, respectively. High insecticidal activity towards aphis species *L. erysimi* (LC_{50} = 2.18 and 2.73 mg/mL; LT_{50} = 15.24 and 17.18 h) demonstrated 7*R*-(*E,E*)-NL isolated from the EO of *N. elliptica*, which was comparable with synthetic pesticide, monocrotophos (Kumar et al., 2015).

Methanolic extracts of *N. sibirica* and *N. rtanjensis* containing (*Z,E*)-NL and (*E,Z*)-NL did not increase lethality of zebrafish embryos (Živković et al., 2018). *N. cataria* EO was reported among 5 most effective oils against *Aedes*, *Anopheles*, and *Culex* mosquitoes (Amer and Mehlhorn, 2006). EO vapours of *N. racemosa* caused the highest mortality at 2 μL/L air doses in red spider mite (*Tetranychus urticae*) and silverleaf whitefly (*Bemisia tabaci*) (Çalmaşur et al., 2006), while *N. racemosa* EO was the most effective against the adults of *Lasioderme serricorne* (Aslan et al., 2005). EO of *N. nuda* (ssp. *pubescens*) with low content of NLs (2.55%) was less active than *N. curviflora* (containing 5.57% of dihydro-NL) against the nematode *Panagrolaimus rigidus* (Musso et al., 2017). The acute oral LD_{50} of catnip oil was found to be 3160 mg/kg body weight (BW) and 2710 mg/kg BW in female and male rats, respectively, while the acute dermal LD_{50} was > 5000 mg/kg BW; the acute inhalation LD_{50} was > 10 000 mg/m^3 (Zhu et al., 2009).

Some *Nepeta* species accumulate high amounts of other than NLs volatile compounds, e.g. 1,8-cineole and caryophyllene oxide (*N. melissifolia*, *N. sibirica*); citronellol, geranial and geranyl acetate (*N. transcaucasica*). De Vincenzi et al. (2002) reviewed the toxicity of 1,8-cineole and proposed 0.1 mg/kg as a tolerable daily intake (TDI) dose, although toxic effects in male rats after gavage were observed at higher than 600 mg/kg doses, while in mice no effect was seen even at the levels up to 1200 mg/kg. Reported lethal doses for 1,8-cineole are quite high; oral LD_{50} (rat) = 2480 mg/kg; subcutaneous LD_{50} (mouse) = 1070 mg/kg; intraperitoneal TDLO (mouse) = 150 mg/kg (https://pubchem.ncbi.nlm.nih.gov/compound/Eucalyptol#section = Top). Most recent reviews on *Eucalyptus* leaf (Dhakad et al., 2018), and *Rosmarinus officinalis* (Borges et al., 2018) EOs, *i.e.* the plants containing high percentages of 1,8-cineole, did not reveal any unsafe toxicity of this compound to mammals at the realistic levels of uses. Moreover, such health benefits as anti-inflammatory, anti-depressive, antialgic, antioxidant, smooth muscle relaxant activities (Borges et al., 2018) as well as undeniable therapeutic action of 1,8-cineole in many drugs (Dhakad et al., 2018) were noted.

β-Caryophyllene and its oxide are found in numerous plants EOs. Caryophyllene oxide was reported to be an allergen of a moderate strength (Skold et al., 2006). *Artemisia campestris* ssp. *campestris* EOs with dominant caryophyllene oxide were toxic in brine shrimp (*Artemia* sp.) assay with LC_{50} values ranging to 20 μg/mL (Judzentiene et al., 2010). Caryophyllene oxide was devoid of genotoxic effects, inducing neither point mutations nor chromosomal damages (Di Sotto et al., 2013). On the other hand, caryophyllene oxide demonstrated anticancer activities by affecting the growth and proliferation of numerous cancer cells (Fidyt et al., 2016). The most recent study demonstrated a moderate antioxidant activity of β-caryophyllene and its oxide, with a 25% and 40% inhibition of the ROS-levels, respectively, increased by cigarette smoke (Di Giacomo et al., 2018). Consequently, in terms of 1,8-cineole and caryophyllenes *Nepeta* plants containing these phytochemicals as quantitatively important EO constituents may be regarded as safe at the expected levels of uses.

The toxicity of *dl*-, *l*- and (+)-(*R*)-citronellol, as fragrance materials, was comprehensively reviewed 10 years ago by Lapczynski et al. (2008a,b,c). The following acute toxicity values were reported for racemic mixture of isomers (LD_{50} in g/kg): oral (rats) 3.45; dermal (rabbits) 2.65; intramuscular (mice) 4.0 and subcutaneous (mice) 0.88. Two years later the RIFM expert panel published comprehensive safety assessment data on non-cyclic alcohols with unsaturated branched chain (Belsito et al., 2010) including the compounds abundant in *N. transcaucasica* EO. In general, toxicity studies on various *Nepeta* spp. and their important EO components have demonstrated that the herbs and their extracts should be quite safe at the expected levels of uses.

4. Conclusions

Comprehensive evaluation of essential oils of *Nepeta cataria* var. *citriodora*, *N. transcaucasica*, *N. melissifolia, N. sibirica* and *N. nuda* provided new information on chemical diversity of *Nepeta* genus. Although the yields of hydrodistilled EO were rather low, from 0.78 (*N. nuda*) to 5.94 (*N. cataria*) mg/g plant dry weight, in total, 143 compounds were identified and quantified in the *Nepeta* spp., which is remarkably higher number comparing to the previously reported data for these species. In Lithuania cultivated *N. cataria* and *N. nuda* may be assigned to the nepetalactone chemotypes; *N. transcaucasica* EO was composed mainly of citronellol, 4aα,7β,7aα-nepetalactone, geranial and geranyl acetate; whereas the EOs of *N. melissifolia* and *N. sibirica* are characterized by high percentage of 1,8-cineole and caryophyllene oxide. Evaluation of antioxidant potential of EO distillation residues revealed that water extract is a strong radical scavenger, particularly in oxygen radical absorbance capacity (ORAC) assay. Numerous beneficial bioactivities were reported for *Nepeta* spp. and their main EO components, while existing toxicological data provides reliable evidence that the investigated herbs, their EOs and extracts may be regarded as safe at the expected levels of their uses.

Author contributions

Petras Rimantas Venskutonis: conceptualization, writing-review and editing, supervision, project administration, funding acquisition; Renata Baranauskienė: methodology, software, investigation, writing-preliminary draft, Vilma Bendžiuvienė: methodology, software, investigation; Ona Ragažinskienė: conceptualization, writing-review and editing.

Conflicts of interest

The authors have declared no conflicts of interest.

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