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CHEMICAL PROFILE OF *Nepeta cataria* L. var. *Citriodora* (Becker) ESSENTIAL OIL AND *in vitro* EVALUATION OF BIOLOGICAL ACTIVITIES

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ABSTRACT

Essential oil (EO) obtained by hydrodistillation in a Clevenger-type apparatus from aerial parts of *Nepeta cataria* L. var. *citriodora* (Becker), cultivated in Serbia was subjected to gas chromatography-mass spectroscopy (GC-MS) to determine the composition. Furthermore, *N. cataria* var. *citriodora* essential oil was tested to determine its antimicrobial, antioxidant, antihyperglycemic and anti-inflammatory activities *in vitro*. The antimicrobial activity was tested by broth microdilution method against 16 bacterial strains from American Type Culture Collection (ATCC). Four common tests for measuring *in vitro* antioxidant activity were used: 2, 2-diphenyl-1-picrylhydrazyl assay (DPPH), reducing power (RP), 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) and *β*-carotene bleaching assay (BCB). Antihyperglycemic activity was examined by using *α*-glucosidase inhibitory potential (AHgA), while anti-inflammatory activity (AIA) was determined by protein denaturation bioassay, using egg albumin. In total, 36 compounds were isolated and detected by GC-MS technique in *N. cataria* var. *citriodora* EO. The EO is mainly comprised of oxygenated monoterpenes (93.1%), and the main compounds were two monoterpenoid alcohols, nerol (38.5%) and geraniol (24.9%), followed by two aliphatic aldehyde, geranial (14.6%) and neral (11.0%). Antimicrobial activity of this EO shows growth inhibition of all tested bacteria strains, and exhibited good antioxidant, antihyperglycemic and anti-inflammatory activities. The EO obtained from *N. cataria* var. *citriodora* grown in Serbia shows valuable biological activity, indicating its potential for use as a supplement in everyday diet and as a natural preservative in food industry.

Key words: lemon catnip, antimicrobial activity, antioxidant activity, antihyperglycemic activity, anti-inflammatory activity

INTRODUCTION

There is increased interest in natural sources of bioactive compounds which can be widely used in everyday life: in food, perfume and cosmetic industries. Essential oils (EOs) occurred as safe, eco-friendly, cost-effective, renewable, and easily biodegradable compounds isolated from plants [Pandey et al. 2017].

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Due to their complex chemical composition, they possess a large spectrum of biological activities, such as antimicrobial, antioxidant, anti-inflammatory and immunostimulatory [Mucha and Witkowska 2021]. The global trend towards natural preservatives being used as alternatives to synthetic ones in food applications is supported by legislative actions and consumer concerns. In recent years, food industries have been using different EOs because of their ability to control the growth of pathogenic microorganisms and delay deterioration of food products [Prakash and Kiran 2016]. Furthermore, EOs have been considered to be natural antioxidants with high potential as additives in food to prevent degenerative diseases caused by oxidative stress [Abd Rashed et al. 2021].

Nepeta cataria L. (Lamiaceae), catnip or catmint, is a perennial herbaceous plant, which is characterized by nepetalactones in the EO. However, there are varieties of *N. cataria* which are characterized by being completely devoid of or producing little amounts of nepetalactones, and the main EO constituents are nerol, citronellal, neral and caryophyllene oxide. Because of these components, these varieties have a minty-lemony flavor, resemble true catnip, but are not attractive to cats. These varieties are called *N. cataria* L. var. *citriodora* (Becker) or lemon catnip [Gomes et al. 2020]. Because of its flavor, it is an appealing raw material in food industry and soft drinks, as well as vegetable and fruit canned food [Frolova et al. 2019]. Due to this, *N. cataria* var. *citriodora* is a valuable dietary supplement and a natural food additive. Furthermore, it is a good source for industrial production of citral (mixture of geranial and neral) widely used in the industry for improving organoleptic properties in pharmaceuticals, cosmetics, toothpastes, chewing gum and cigarettes [Pucci et al. 2020]. Investigations show that *N. cataria* var. *citriodora* can be used as a constituent of phytopharmaceutical preparations with a mild sedative, antispasmodic, antioxidant and anti-inflammatory activity [Modnicki et al. 2007, Ricci et al. 2010, Bernardi et al. 2011]. In addition, citral, main constituent of *N. cataria* var. *citriodora* EO, is known to possess antimicrobial, antioxidant, hypolipidemic and hepatoprotective effects, but it is also effective in cancer prevention and treatment [Onawunmi 1989, Uchida et al. 2017, Pucci et al. 2020]. Taking in account global demand for citral, *N. cataria* var. *citriodora* is occurring as new promising source of this compound, easy for commercial cultivation in temperate region [Aćimović et al. 2021].

The aim of this investigation was to determine the EO composition of *N. cataria* var. *citriodora* grown in Serbia and to evaluate its biological effects through antibacterial tests (against 16 bacterial strains that cause skin and food borne illness), antioxidant activity (DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ assays, reducing power and β-carotene bleaching assay), antihyperglycemic (AHgA) and anti-inflammatory (AIA) activity.

MATERIAL AND METHODS

Plant material. *Nepeta cataria* var. *citriodora* were cultivated at the Institute of Field and Vegetable Crops Novi Sad, at the Department for Vegetables and Alternative Crops Bački Petrovac (45°21'N; 19°35'E). The above ground flowering parts of plants were harvested at full flowering stage during July, 2019. The above ground parts were harvested manually, and the biomass was placed at room temperature until constant weight. Voucher specimens were confirmed and deposited at the Herbarium BUNS, the University of Novi Sad, Faculty of Sciences, Department of Biology and Ecology, under the acquisition number 2-1401.

Essential oil (EO) isolation. Air-dried aerial parts of *N. cataria* var. *citriodora* were submitted to hydrodistillation using the Clevenger apparatus. The obtained EO was separated from aqueous layer and dried over anhydrous sodium sulphate and stored in a dark glass vial at 4°C for further analysis. This process was performed in three replications, and average content of EO was 0.24%.

Gas chromatography-mass spectrometry analysis (GC-MS). The EO was analyzed using an HP 5890 gas chromatograph coupled to an HP 5973 MSD and fitted with a capillary column HP-5MS. The components were identified based on their linear retention index relative to C_8-C_{32n}-alkanes, comparison with data reported in the literature (Wiley and NIST databases). Quantification was done by external standard method using calibration curves generated by running GC analysis of representative authentic compounds.

Determination of antimicrobial activities. Antimicrobial activity of essential oil was tested against

16 bacterial strains from American Type Culture Collection (ATCC) by broth microdilution method according to Varga et al. [2019]. Tested bacterial strains with ATCC numbers are given in table 2. As a control, a gentamicin strip test was used for determination of minimal inhibitory concentration (MIC).

Determination of antioxidant activities. Four common tests for measuring *in vitro* antioxidant activity were used: DPPH• assay (DPPH) according to Gironés-Vilaplana et al. [2014], reducing power (RP) according to Oyaizu [1986], $ABTS^{\bullet+}$ method (ABTS) according to Mena et al. [2011] and *β*-carotene bleaching assay (BCB) according to Al-Saikhan et al. [1995]. The antioxidant activity was expressed as μmol of Trolox equivalents per 100 mL of essential oil (μmol TE 100 mL^{-1} EO).

***In vitro* antihyperglycemic activity assay.** Antihyperglycemic activity was examined by using *α*-glucosidase inhibitory potential (AHgA) according to Tumbas Šaponjac et al. [2014], using acarbose as control.

***In vitro* anti-inflammatory activity assay.** Anti-inflammatory activity (AIA) was determined by protein denaturation bioassay using egg albumin (from fresh hen's egg) according to Ullah et al. [2014], using diclofenac sodium as control.

RESULTS

There were 36 compounds detected in the *N. cataria* var. *citriodora* EO, among which 12 were not identified compounds (NI), comprising 2.1% of the total oil. However, EO is mainly comprised of oxygenated monoterpenes (93.1%). The main compounds were two monoterpenoid alcohols, nerol (38.5%) and geraniol (24.9%), followed by two aliphatic aldehyde, geranial (14.6%) and neral (11.0%), which mixture is called citral (in total 24.6%) – Table 1.

The *N. cataria* var. *citriodora* EO inhibited the growth of all Gram-positive bacteria at concentrations ranging from 3.55 μL mL^{-1} (*B. cereus, L. ivanovii, L. monocytogenes*, and *S. aureus*), to 14.20 μL mL^{-1} (*S. epidermidis*), while for Gram-negative bacteria *P. hauseri* and *S.* Enteritidis MIC value was 3.55 μL mL^{-1}, for *E. coli*, *K. aerogenes* and *S. typhimurium* MIC was 7.10 μL mL^{-1} (Table 2). Moreover, the *N. cataria* var. *citriodora* EO inhibited the growth of *P. aeruginosa* at a concentration of MIC/MBC 28.40 μL mL^{-1}.

In vitro antioxidant, α-glucosidase and anti-inflammatory activities of *N. cataria* var. *citriodora* are shown in Table 3. Employing the DPPH method, the *N. cataria* var. *citriodora* EO showed antioxidant activity of 86.33 μmol TE 100 mL^{-1} EO, reducing power of 16.28 μmol TE 100 g^{-1}, and scavenging capacity of ABTS radicals of 299.98 μmol TE 100 mL^{-1} EO. Furthermore, the activity obtained by β-carotene bleaching assay was found to be 118.63 μmol TE 100 mL^{-1} EO. In this study, *N. cataria* var. *citriodora* EO was less efficient in the antihyperglycemic assay (AhgA = 79.08%) than the positive control, acarbose. Based on the obtained results for *N. cataria* var. *citriodora* EO at a concentration of 250 mg mL^{-1}, it was capable of inhibiting the denaturation of the proteins for 51.36%. In this study, diclofenac sodium was used as a standard anti-inflammatory drug, which showed EC_{50} value of 1.14 mg mL^{-1}.

DISCUSSION

Nepeta cataria is characterized by chemical polymorphism [Baranauskiene et al. 2003]. There are several main chemotypes that were previously described. The one with nepetalactones (several steroisomers) as dominant compounds in the EO, which could be used as an insect repellent, for pharmaceutical preparations, as well as herbal tea and for culinary purposes, while another one containing citral (isomeric mixture of geranial and neral) i.e. var. *citriodora*, which is interesting in perfume industry due to the EO [Klimek and Modnicki 2005]. However, EO composition depends on many factors, such as the region, variety and cultivar, hybridization and mutation, climatic and stress conditions, harvest time and vegetation period (first or second cut), plant part and postharvest processing (fresh or dry), isolation technique and conditions, etc. [Aćimović et al. 2021].

Nepeta cataria EO showed antibacterial activity against different food-borne pathogens including *S. aureus*, *B. cereus*, *B. thuringiensis*, *L. monocytogenes*, *Yersinia enterococlitica*, *E. coli*, *Shigella* spp. and *Salmonella* spp. [Zomorodian et al. 2012, Vukovic et al. 2016]. However, the lower value of MICs (ranging from 0.125 to 2 μL mL^{-1}) are a result of different chemical compositions of the tested EOs in which the major compounds were detected nepetalactone iso-

Table 1. Chemical composition and molecular descriptors (MD) of compounds in *N. cataria* var. *citriodora* essential oil

No.	Compound	RI	%
1	sabinene	973	tr
2	*β*-pinene	977	0.2
3	6-methyl-5-hepten-2-one	987	0.3
4	myrcene	991	0.1
5	NI-1	1029	0.1
6	NI-2	1103	0.1
7	photocitral B	1107	0.2
8	*Z*-rose oxide	1113	0.1
9	*E*-rose oxide	1127	0.1
10	NI-3	1140	0.3
11	photocitral A	1151	0.5
12	citronellal	1153	2.7
13	*Z*-isocitral	1166	0.1
14	menthol	1174	tr
15	lavandulol	1175	0.2
16	rosefuran epoxide	1176	0.2
17	*E*-isocitral	1181	0.1
18	*α*-terpineol	1189	tr
19	NI-4	1208	0.2
20	citronellol	1225	0.1
21	nerol	1231	38.5
22	NI-5	1235	0.5
23	neral	1243	11.0
24	geraniol	1258	24.9
25	geranial	1273	14.6
26	NI-6	1275	0.1
27	NI-7	1283	0.2
28	NI-8	1337	0.2
29	NI-9	1362	0.1
30	*E*-caryophyllene	1421	0.7
31	*α*-humulene	1455	0.1
32	NI-10	1581	0.1
33	caryophyllene oxide	1583	0.6
34	NI-11	2051	0.1
35	NI-12	2063	0.2
36	phytol	2113	2.1
	monoterpene hydrocarbons		0.3
	oxygenated monoterpenes		93.1
	sesquiterpene hydrocarbons		0.8
	oxygenated sesquiterpenes		0.6
	oxigenated diterpenes		2.1
	other		0.5
	NI		2.1
	Total identified		99.5

RI – Retention Index from the NIST webbook database; other – aliphatic hydrocarbons, aliphatic aldehydes and alcohols, aliphatic acids, their esters and aldehydes, aromatic esters with aliphatic acids, alkyl-aromatic alcohols, or aryl esters of aromatic acids; NI – not identified compound.

Table 2. *In vitro* antibacterial activities of *N. cataria* var. *citriodora* essential oil (in μL mL^{-1}) and standard (gentamicin)

Antibacterial activity		EO		Gentamicin (MIC)
		MIC	MBC	
Gram-positive	*Bacillus cereus* (ATCC 11778)	3.55	3.55	0.19
	Bacillus spizizenii (ATCC 6633)	7.10	14.20	0.38
	Enterococcus faecalis (ATCC 29212)	7.10	7.10	8.00
	Listeria innocua (ATCC 33090)	7.10	14.20	0.50
	Listeria ivanovii (ATCC 19119)	3.55	7.10	0.50
	Listeria monocytogenes (ATCC 19111)	3.55	7.10	0.19
	Rhodococcus equi (ATCC 6939)	3.55	7.10	0.38
	Staphylococcus aureus (ATCC 25923)	3.55	3.55	0.38
	Staphylococcus epidermidis (ATCC 12228)	14.20	14.20	0.09
Gram-negative	*Escherichia coli* (ATCC 10536)	7.10	14.20	2.00
	Escherichia coli (ATCC 8739)	7.10	14.20	2.00
	Klebsiella aerogenes (ATCC 13048)	7.10	7.10	0.50
	Proteus hauseri (ATCC 13315)	3.55	7.10	1.00
	Pseudomonas aeruginosa (ATCC 27853)	28.40	28.40	1.00
	Salmonella enterica subsp. *enterica* serovar Enteritidis (ATCC 13076)	3.55	3.55	0.50
	Salmonella enterica subsp. *enterica* serovar Typhimurium (ATCC 14028)	7.10	7.10	0.50

MIC – minimal inhibitory concentration; MBC – minimal bactericidal concentration

Table 3. *In vitro* antioxidant, α-glucosidase and anti-inflammatory activities of *N. cataria* var. *citriodora* essential oil and standards

Bioactivity assay		EO	Standards
Antioxidant activity	DPPH	86.33 ±10.70	0.14 ±0.01[a]
	RP	16.28 ±0.47	0.12 ±0.02[a]
	ABTS	299.98 ±17.45	1.06 ±0.04[a]
	BCB	118.63 ±9.21	0.06 ±0.02[b]
Antihyperglycemic activity	AHgA	79.08 ±4.03	0.001 ±0.00[c]
Anti-inflammatory activity	AIA	51.36 ±8.36	1.14 ±0.03[d]

Mean value of three replicates (± standard deviation) for biological assays: DPPH – DPPH assay (μmol TE 100 g^{-1}); RP – reducing power (μmol TE 100 mL^{-1} EO); ABTS – $ABTS^{\bullet+}$ method (μmol TE 100 mL^{-1} EO): BCB – β-carotene bleaching assay (μmol TE 100 mL^{-1} EO); AHgA – antihyperglycemic activity (% of inhibition); AIA – anti-inflammatory activity (% of inhibition); IC_{50} values of used standard compounds in the bioactivity assays: [a] – Trolox; [b] – BHA, [c] – acarbose; [d] – Diclofenac sodium

mers [Zomorodian et al. 2012]. Slightly better antibacterial activity was observed for EOs with a smaller content of nepetalactones, and higher abundance of other compounds such as α and β-pinene, caryophyllene oxide, germacrene D and other compounds with known antibacterial potential [Vukovic et al. 2016]. Further, comprehensive report of antimicrobial activity of *N. cataria* EO against wider range of microorganisms (11 bacteria, 12 fungi and a yeast) indicated possibilities for application in food and pharmaceutical industry [Adiguzel et al. 2009]. Previous research, as well as this study, shoved that *N. cataria* EO is effective against bacteria *P. aeruginosa* which *cause* various *types of infections* [Kim et al. 2006]. Since *P. aeruginosa* represent one of the most resistant bacteria, this data will be valuable for new scientific studies in the future.

According to literature, the antioxidant activity of *N. cataria* has been studied by several authors and different methods. The results show that ethanol extract of *N. cataria* possesses scavenging DPPH radical in range between 0.449 and 0.491 mM TE g^{-1} depending on harvest time [Duda et al. 2015]. Furthermore, scavenging DPPH radical potential of *N. cataria* water extract was 220.2 μmol TE g^{-1} DW, while synthetic ABTS radical in water extract expressed activity of 292.0 μmol TE g^{-1} DW [Mihaylova et al. 2013]. Methanolic extract of *N. cataria* reduced stable DPPH radicals with an IC_{50} value of 171.98 μg ml^{-1}, while EO with dominant nepetalactones remained inactive [Adiguzel et al. 2009]. The fresh raw material of *N. cataria* from Poland was characterized with the ability to reduce free radicals (% DPPH) between 2.45 and 4.57% depending on crop age [Wieteska et al. 2018].

Experiments conducted *in vitro* using α-glucosidase activity tests showed that different extracts of *N. cataria* have significant beneficial effect on glycemic control, as well as in normalization of liver and pancreas function, so it may be applied for reducing complications against diabetes mellitus [Aly et al. 2010]. Furthermore, monoterpenes such as geraniol, nerol, citral, linalool, etc., have positive influence on glucose metabolism may have potential in the control of type 2 diabetes mellitus [Tan et al. 2016].

Investigations show that *N. cataria* EO in concentration of 0.0005 mL kg^{-1} in female rats expressed peripheric anti-inflammatory properties by reducing the induced edema after carrageenan injection [Ricci et al. 2010]. Furthermore, anti-inflammatory effect could be related to the free radical scavenging activity and depends on a synergic action of all the components [Miceli et al. 2005]. It is known that nerol, which can be found in many EOs, possesses potential anti-inflammatory and antinociceptive activities, supporting the application of this plants in treating various diseases associated with inflammation and pain [Bounihi et al. 2013].

CONCLUSIONS

The main compounds of *N. cataria* var. *citriodora* EO were nerol, geraniol, geranial and neral. EO of *N. cataria* var. *citriodora* shows significant antibacterial activity against all investigated bacterial strains, the most interesting being the antibacterial effect against *P. aeruginosa*, which is one of the most resistant bacteria. In view of the biological activity, examined essential oil might be considered a valuable natural constituent with antioxidant, antihyperglycemic, and anti-inflammatory properties, and potential application in food products.

CONFLICT OF INTEREST

The authors declare no conflict interest.

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