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Chemical Composition of Essential Oils from *Nepeta transcaucasica* Grossh. and *Nepeta cataria* L. Cultivated in Bulgaria and Their Antimicrobial and Antioxidant Activity

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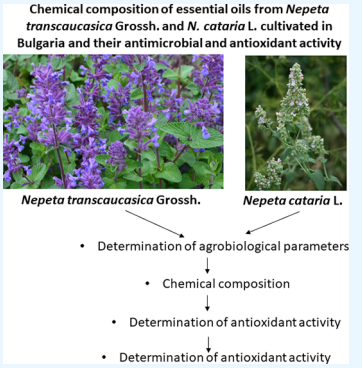
ABSTRACT: The genus *Nepeta*, belonging to the family Lamiaceae, includes about 300 species, most of which are used in folk medicine due to their pronounced biological properties. The aim of the present study was to evaluate the agrobiological characteristics of *Nepeta transcaucasica* (*N. transcaucasica*) Grossh. and *Nepeta cataria* (*N. cataria*) L., cultivated in Bulgaria, and obtain their essential oils and determine their antimicrobial and antioxidant activities. The agrobiological characteristics of the two species growing in Kazanlak were analyzed; therefore, high variability in the population of *N. transcaucasica* and comparative homogeneity in *N. cataria* was shown. The species *N. transcaucasica* contained 0.28% essential oil with main components *β*-citronellol (52.05%), eucalyptol (7.34%), *β*-citronellal (6.06%), germacrene D (5.45%), (*Z*)-*β*-ocimene (5.14%), and *β*-caryophyllene (3.06%). The species *N. cataria* consisted of 0.19% essential oil with main components *β*-citronellol (26.31%), geraniol (15.92%), neral (11.45%), nerol (9.56%), carvacrol (6.04%), and *β*-citronellal (5.35%). The antibacterial activity against Gram-positive bacteria *Listeria monocytogenes* and *Staphylococcus aureus* and Gram-negative bacteria *Escherichia coli* (*E. coli*) and *Salmonella enterica* subsp. *enterica* serovar Abony was determined. The essential oils showed antimicrobial activity only against *E. coli*. The diameters of the inhibition zones were found to be 26 mm for the species *N. transcaucasica* and 10 mm for the species *N. cataria*. The antioxidant activity of the two essential oils was also determined by four different methods, DPPH, ABTS, FRAP, and CUPRAC, with the highest values for the ABTS radical, for the species *N. transcaucasica* (48.72 *μ*M TE/mL), and the species *N. cataria* (310 *μ*M TE/mL).



INTRODUCTION

The genus *Nepeta* is one of the largest of the Lamiaceae family, with about 300 species distributed in temperate regions, mainly in central and southern Europe, the Middle East, central and south Asia, and some parts of Africa and North America. Most species of *Nepeta* are endemic, especially in Southwest Asia (Turkey and Iran). The plants of the genus are known for their medicinal properties, which is why they are mainly used in folk medicine to treat various diseases.[1−7]

The species *N. transcaucasica* Grossh. and *N. cataria* L. are most widely used for decorative and culinary purposes, as well as in folk medicine.[8−10]

The chemical composition of the essential oil of the species *N. cataria* has been the subject of several studies by authors from different countries. The oxygenated monoterpenes such as citronellal, neral, geranial, citronellol, nerol, and geraniol, some small amounts of other oxygenated monoterpenes, and sesquiterpenes such as *β*-caryophyllene and *α*-humulene have been identified as the main components in its composition[11−27] as and other volatile aromatic compounds.[20,28,29] The comparative analysis of these studies shows that the amount of the main components varies depending on the origin of the plant, the soil and climatic conditions, the individual stages of its development,[14] and the methods of drying the raw material before distillation.[15] The essential oil has proven antimicrobial[11,16,17,19−24] and antioxidant activity.[19,21−24] It also exhibits other biological properties,[8,17,18,21,22] which is why it finds a variety of applications.[12,30]

The *N. transcaucasica* essential oil has been the subject of less research. It was established that its main components were monoterpene hydrocarbons such as *α*-pinene, *β*-pinene, myrcene, limonene, and *γ*-terpinene[31] and their oxygenated derivatives such as citral, citronellol, and 1,8-cineole[20,32,33] as

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well as nepetalactones.[20,33,34] The essential oil has proven antimicrobial activity.[35,36]

In Bulgaria, species of the *Nepeta* genus are found mainly in the wild[37] and are used in folk medicine.[37−39]

For several years, two species of *N. transcaucasica* and *N. cataria*, which are currently not industrial raw materials for obtaining essential oil, have been grown in the experimental field of the Institute of Roses, Essential and Medical Plants, the city of Kazanlak, Bulgaria.

In this experimental field, systematic studies are carried out to establish the influence of soil and climatic conditions on the development of plants of both species, stem height, flower size, and flowering time, to select the most suitable samples for essential oil extraction.

The aim of the present study was to determine the chemical composition and the antimicrobial and antioxidant activities of essential oils from the species *N. transcaucasica* and *N. cataria*, grown under the conditions of the Kazanlak field, with possibilities for their application in food and cosmetic preparations.

MATERIALS AND METHODS

Plant Material. Populations of biennial plants of *N. transcaucasica* Grossh. and *N. cataria* L. were studied. Fresh stalks of the species *N. transcaucasica* and *N. cataria*, grown in the experimental field of Institute of Roses, Essential and Medical Plants, city of Kazanlak, Central Bulgaria (42.61°94′408″N 25.39°29′576″E, altitude of 407 m) were used. The experiments were conducted in 2019.

The soils in the area were leached cinnamon forest, developed on old diluvial deposits, structureless with good aeration and water permeability, with an acidic pH of 4.9, and poorly stocked with nitrogen 20.5 mg/1000 g, phosphorus 4.25 mg/100 g, potassium 21.75 mg/100 g, and a humus content of 1.8%.

Plants were grown under nonirrigated conditions, with planting patterns 0.7 m between rows and 0.3 m within the row.

Essential Oils. *Isolation of Essential Oils.* The raw material was collected during the mass flowering phase, with a moisture content between 60 and 65%, determined by drying at 105 °C to a constant mass.[40] It was processed by steam distillation in a laboratory copper distillation apparatus with a capacity of 5 L and a process duration of 2 h. The distillation type for obtaining essential oil at technological parameters was close to the production conditions in which the essential oils were mainly processed by steam distillation. The oil obtained was dried over anhydrous sulfate and stored in tightly closed dark vials at 4 °C until analysis.

Chemical Composition of Essential Oils. Prior to the GC−MS analysis, 100.0 μL of the essential oils was vacuum-dried in a centrifugal vacuum concentrator (CentriVap, Labconco, Kansas City, Missouri) at 40 °C. The dried residue was dissolved in a 100 μL solution of methoxyamine hydrochloride (20 mg/mL in pyridine) and heated at 70 °C for 1 h at constant shaking (300 min^{-1}) (Thermo Shaker TS-100, Analytik Jena AG, Germany). After cooling, 100 μL of *N,O*-bis(trimethylsilyl) trifluoroacetamide (BSTFA, silylation agent) was added, and the mixture was heated again while stirring (70 °C, 40 min, 300 min^{-1}). The injected sample volume was 1.0 μL.

GC−MS analysis was performed on an Agilent 7890A chromatograph interfaced with a 5975C mass-selective detector (Agilent Technologies, Inc., Santa Clara, USA). Separations were done on an HP-5ms column, 30 m × 0.25 mm (i.d.) coated with a 0.25 μm film of poly-(dimethylsiloxane) as the stationary phase. The instrumental parameters were as follows: the carrier gas was helium, maintained at a constant rate of 1.0 mL/min; an injector and transfer line temperature of 250 °C; an MS source temperature of 230 °C; the oven temperature program started from 100 °C (held for 2 min); then, the temperature was increased to 180 °C at a rate of 15 °C/min (held at 180 °C for 2 min) and then to 300 °C at a rate of 5 °C/min (held at 300 °C for 10 min); a total run time of 42 min; a split mode of 20:1; MS scans from 50 to 550 m/z. The components in the sample were identified based on their retention indices and by comparing their mass spectra with those in the NIST 08 spectra library.[41] The retention (Kovats) indices were calculated using a standard calibration mixture of *n*-alkanes (C_8−C_{40}) in *n*-hexane. The amounts of the identified compounds were expressed as a percentage of the total ion current (TIC), after normalization of the recorded peak areas.

Antimicrobial Activity of Essential Oils. The antimicrobial activity of essential oils and distillates was tested against pathogenic and opportunistic bacteria. The test cultures were derived from the National Bank for Industrial Microorganisms and Cell Cultures in Sofia, Bulgaria. Gram-positive bacteria used in our study were *Listeria monocytogenes* NCTC 11994 and *Staphylococcus aureus* (*S. aureus*) ATCC 25093, and Gram-negative bacteria were *Escherichia coli* (*E. coli*) ATCC 8739 and *Salmonella enterica* subsp. *enterica* serovar Abony NCTC 6017.

The medium was inoculated with a 24 h suspension of the bacterial species at a density of approximately 10^7 cfu/mL (turbidity: 0.5 McFarland standards). The antimicrobial activity was determined by a modification of the "agar diffusion" method by measuring the zones of inhibition of the growth of pathogens and fungi (mm) around sterile rings (Ø 6 mm) in which 0.05, 0.07, and 0.10 mL of essential oil were placed.[42]

Antioxidant Activity of Essential Oils. Chemicals and Reagents. Chromatographic-grade methanol and ethanol were used for HPLC analyses (VWR, Austria). Ammonium acetate, copper(II) chloride, glacial acetic acid, sodium acetate trihydrate, ferric chloride hexahydrate, quercetin, 6-hydroxy-2,5,7,8-tetramethychroman-2-carboxylic acid (Trolox), and reagents 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), neocuproine (2,9-dimethyl-1,10-phenanthroline), and 2,4,6-tripyridyl-*s*-triazine (TPTZ) were purchased from Sigma-Aldrich.

ABTS Assay. The Trolox equivalent antioxidant capacity (TEAC) was determined by using the colorimetric method reported by Re *et al.*[43] with slight modifications.[44]

DPPH Assay. The antioxidant activity was measured according to the procedure of Brand-Williams *et al.*[45]

Cupric Ion Reducing Antioxidant Capacity (CUPRAC) Assay. The cupric ion reducing antioxidant capacity (CUPRAC) method described by Apak *et al.* was performed.[46]

Ferric Reducing Antioxidant Power (FRAP) Assay. The ferric-reducing antioxidant power (FRAP) method described by Benzie and Strain was performed.[47]

Statistical Analysis. The analyses were made in triplicate. The data were expressed as the mean ± SD. Data were expressed as mean ± SD. The level of significance was set at $p < 0.05$. Statistical program SPSS 19.0 software was used for data analysis by one-way ANOVA followed by Tukey's *post hoc*

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Table 1. The Agrobiological Parameters of *N. transcaucasica*

		width		stems	inflorescences				
no.	height, cm	in the row, cm	row spacing, cm	diameter, mm	length, cm	width, cm	herb total, g	mass flowering	odor of the plant
1	80	125	125	5	20	3.5	1003	15 May	strong, rough
2	80	125	125	5	20	3	483	15 May	nice, fresh
3	58	50	75	4	20	3	230	15 June	foul smell, strong
4	80	75	80	5	20	3	750	15 May	pleasant
5	68	55	70	5	20	3	530	15 June	strong, pleasant
6	77	55	80	5	20	3	640	1 June	strong, pleasant
7	35	40	45	4	20	3	70	21 April	foul smell
8	90	110	130	5	25	3	998	15 June	fresh, pleasant
9	35	35	60	4	20	3	70	21 April	fresh, strong
10	78	110	120	5	18	3	1060	15 June	slightly foul smell
11	70	70	85	6	20	3	830	1 June	average
12	74	75	80	5	20	3	530	1 June	strong, pleasant
13	68	53	75	4	20	3	430	1 June	strong, pleasant
14	94	105	115	5	20	3	823	15 June	average
15	85	100	115	5	18	3.5	1173	15 June	strong, pleasant
16	65	65	95	5	15	2.5	153	15 June	rough
17	92	100	110	5	20	3	1540	22 June	strong, pleasant
18	85	95	106	5	25	4	1430	15 June	average
19	88	100	110	5	20	3	1200	15 June	slightly foul smell
20	80	95	108	5	20	3	830	15 June	average
				Mean value					
	74.1	81.9	95.5	4.9	20.1	3.1	738.7		
				Mean square deviation					
	13.2	2528	21.8	0.5	2.1	3.1	355.5		
				Coefficient of variation					
	17.0	27.8	21.6	9.6	10.3	9.9	36.4		

Table 2. The Agrobiological Parameters of *N. cataria*

			stems			herb				yield			
no.	height, cm	width, cm	above 6 mm, no.	5 mm, no.	under mm, no.	stems above 6 mm, g	stems, 5 mm, g	stems under 4 mm, g	total, g	stems above 6 mm, %	stems, 5 mm, %	stems under 4 mm, %	total, %
1	80	90	12	1	4	213	11	27	251	23.1	27.3	25.9	23.6
2	82	90	1	6	6	135	73	31	239	26.6	24.4	25.8	25.8
3	87	85	3	8	1	109	92	5	206	28.4	22.8	20.0	25.7
4	80	85	8	2	2	244	28	18	290	30.7	28.6	22.2	30.0
5	73	70	4	6	1	102	83	6	191	32.4	23.5	20.0	28.1
6	77	80	4	4	6	93	57	32	182	26.9	28.1	21.9	26.4
7	82	78	2	8	2	98	56	14	168	31.6	26.8	21.4	29.2
8	81	92	5	2	10	242	35	64	341	29.3	28.6	23.4	28.2
9	85	90	10	1	4	302	17	23	342	31.5	29.4	26.1	31.0
10	78	82	5	8	2	69	72	10	151	33.3	29.2	25.0	30.8
						Mean value							
	80.5	84.2	5.4	4.6	3.8	160.7	52.4	23	236.1	29.4	26.9	23.2	27.9
						Mean square deviation							
	3.8	6.3	3.5	2.2	3.0	78.1	21.9	19.5	67.3	2.9	2.2	2.27	2.3
						Coefficient of variation							
	4.7	7.5	46.1	35.2	52.5	39.4	33.1	55.8	26.5	9.7	8.3	9.7	8.2

test to evaluate differences between mean values of activities (SPSS, Inc., Chicago, IL, USA).

RESULTS

Plant Growth and Development. Agrobiological studies show that the soil−climatic conditions are favorable for the development of *Nepeta transcaucasica* and *Nepeta cataria* and form a significant and quality raw material.

The population of *N. transcaucasica* is highly heterogeneous according to the studied agrobiological parameters. Precocious plants numbered 7 and 9 stand out, which enter the mass flowering phase at the beginning of the third 10 days of April but form an insignificant above-ground mass and yield a fresh herb of only 70 g per plant. In the case of the late forms, entering the phase of mass flowering in the middle of June, the highest productive indicators are observed, reaching a fresh herb yield of 1540 g from one plant. The established significant

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Table 3. Chemical Composition of *N. transcaucasica* and *N. cataria* Essential Oils[a]

peak	RT, min	RI_{calc}	RI_{lit}	compounds	*N. transcaucasica*	*N. cataria*
1	9.45	933	932	α-pinene	0.24 ± 0.02[b]	0.12 ± 0.01[a]
2	10.76	969	969	sabinene	0.64 ± 0.05[b]	0.27 ± 0.02[a]
3	10.90	971	972	1-octen-3-one	0.04 ± 0.0	-*
4	11.00	975	974	β-pinene	0.94 ± 0.08[b]	0.59 ± 0.05[a]
5	11.18	978	979	3-octanone	0.33 ± 0.03[b]	0.20 ± 0.02[a]
6	11.33	989	988	myrcene	0.27 ± 0.02[b]	0.06 ± 0.0[a]
7	11.59	995	994	2-octanol	0.04 ± 0.0[a]	0.07 ± 0.0[a]
8	12.46	1020	1020	*p*-cymene	0.03 ± 0.0[a]	0.04 ± 0.0[a]
9	12.63	1023	1024	limonene	0.35 ± 0.03[b]	0.16 ± 0.01[a]
10	12.72	1026	1026	eucalyptol (1,8-cineole)	7.34 ± 0.70[b]	0.08 ± 0.0[a]
11	12.88	1031	1032	(*Z*)-β-ocimene	5.41 ± 0.50[b]	0.15 ± 0.01[a]
12	13.19	1045	1044	(*E*)-β-ocimene	0.83 ± 0.07[b]	0.27 ± 0.02[b]
13	13.37	1051	1051	bergamal	0.06 ± 0.0[a]	0.09 ± 0.0[a]
14	13.54	1055	1054	γ-terpinene	0.07 ± 0.0	-
15	13.92	1064	1065	(*Z*)-sabinene hydrate	0.05 ± 0.0	-
16	14.87	1095	1095	β-linalool	0.07 ± 0.0[a]	0.15 ± 0.01[a]
17	15.18	1107	1106	(*Z*)-rose oxide	1.30 ± 0.10[b]	0.44 ± 0.03[b]
18	15.67	1121	1122	(*E*)-rose oxide	0.63 ± 0.05[b]	0.10 ± 0.01[a]
19	15.73	1129	1128	allo-ocimene	0.26 ± 0.02[a]	0.14 ± 0.01[b]
20	16.02	1140	1140	(*E*)-verbenol	0.17 ± 0.01[a]	0.64 ± 0.05[b]
21	16.34	1144	1145	isopulegol	0.09 ± 0.0[a]	0.36 ± 0.03[b]
22	16.52	1148	1148	β-citronellal	6.06 ± 0.40[b]	5.35 ± 0.50[b]
23	16.66	1152	1152	3-(*Z*)-nonen-1-ol	0.18 ± 0.01[a]	1.54 ± 0.01[b]
24	17.01	1157	1157	2-(*E*)-nonen-1-al	0.12 ± 0.01[a]	0.46 ± 0.04[b]
25	17.29	1161	1160	(*Z*)-isocitral	0.26 ± 0.02[a]	0.89 ± 0.80[b]
26	17.76	1185	1186	α-terpineol	0.28 ± 0.02[a]	0.51 ± 0.05[b]
27	18.12	1193	1194	myrtenol		0.62 ± 0.06
28	18.99	1222	1223	β-citronellol	52.05 ± 5.00[c]	26.31 ± 2.50[b]
29	19.10	1126	1227	nerol	2.13 ± 0.20[b]	9.56 ± 0.90[c]
30	19.47	1234	1235	neral	2.42 ± 0.20[b]	11.45 ± 1.10[c]
31	19.91	1250	1249	geraniol	1.44 ± 0.10[b]	15.92 ± 1.40[c]
32	20.01	1265	1264	geranial	1.51 ± 0.10[b]	11.58 ± 1.10[c]
33	20.76	1299	1298	carvacrol	0.11 ± 0.01[a]	6.04 ± 0.50[b]
34	21.15	1313	1312	citronellic acid	0.25 ± 0.02[b]	0.12 ± 0.01[a]
35	22.09	1350	1350	citronellyl acetate	0.30 ± 0.03[b]	0.05 ± 0.0[a]
36	22.32	1349	1360	neryl acetate	0.05 ± 0.0[a]	0.08 ± 0.0[a]
37	22.40	1384	1385	4a-α,7α,7a-β-nepetalactone	0.08 ± 0.0[a]	0.06 ± 0.0[a]
38	22.83	1386	1387	β-bourbonene	0.06 ± 0.0	-
39	23.05	1388	1389	β-elemene	0.27 ± 0.02	-
40	23.21	1390	1391	4a-α,7β,7a-α-nepetalactone	1.62 ± 0.10[c]	0.33 ± 0.03[a]
41	24.01	1418	1417	β-caryophyllene	3.06 ± 0.30[a]	2.97 ± 0.22[a]
42	24.25	1431	1430	β-copaene	0.09 ± 0.0	-
43	24.81	1440	1440	(*Z*)-β-farnesene	0.27 ± 0.02[b]	0.14 ± 0.10[a]
44	24.91	1451	1452	α-caryophyllene	0.16 ± 0.01[a]	0.26 ± 0.02[a]
45	25.59	1485	1484	germacrene D	5.45 ± 0.50[b]	0.15 ± 0.01[a]
46	25.92	1500	1500	bicyclogermacrene	0.39 ± 0.30	-
47	26.21	1506	1505	β-bisabolene	0.11 ± 0.0	-
48	26.47	1514	1513	γ-cadinene	0.05 ± 0.0	-
49	26.60	1520	1521	β-sesquiphellandrene	0.08 ± 0.0	-
50	27.91	1576	1577	spathulenol	0.12 ± 0.0	-
51	28.05	1581	1582	caryophyllene oxide	1.54 ± 0.10[b]	1.33 ± 0.10[b]
52	29.00	1593	1592	viridiflorol	0.05 ± 0.0[a]	0.07 ± 0.0[a]
oxygenated aliphatics, %					0.71	2.28
monoterpene hydrocarbons, %					9.03	1.76
oxygenated monoterpenes, %					78.38	84.93
sesquiterpene hydrocarbons, %					10.02	3.53
oxygenated sesquiterpenes, %					1.71	1.40
phenylpropane hydrocarbons, %					0.03	0.04
oxygenated phenylpropanes, %					0.11	6.06

[a]The total ion current (TIC) depends on the characteristics of the compound, and therefore, GC/MS data are considered as semiquantitative. The asterisk (*) indicates not detected; different letters in the same row indicate significant differences ($p < 0.05$).

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Table 4. Inhibition Zones of *N. transcaucasica* and *N. cataria* Essential Oils, mm[a]

	concentration of essential oil, mL	*Escherichia coli*	*Salmonella enterica*	*Listeria monocytogenes*	*Staphylococcus aureus*
N. transcaucasica	0.10	26 ± 0.11[b]	-	-	-
	0.07	-[a]	-	-	-
	0.05	-	-	-	-
N. cataria	0.10	10 ± 0.12[a]	-	-	-
	0.07	9 ± 0.11[a]	-	-	-
	0.05	-	-	-	-

[a]The asterisk (*) indicates that no inhibition of microbial activity was observed; different letters in the same row indicate significant differences ($p < 0.05$).

Table 5. Antioxidant Activity of *N. transcaucasica* and *N. cataria* Essential Oils[a]

	ABTS		DPPH		CUPRAC	FRAP
essential oils	μM TE/mL	IC_{50}, mg/mL	μM TE/mL	IC_{50}, mg/mL	μM TE/mL	μM TE/mL
N. transcaucasica	48.72 ± 1.10[a]	57.48 ± 1.21[b]	5.97 ± 0.23[a]	265.60 ± 9.72[c]	8.82 ± 0.06[a]	3.68 ± 0.03[a]
N. cataria	310.66 ± 10.91[c]	6.37 ± 0.22[a]	101.50 ± 5.79[c]	8.17 ± 0.48[a]	113.55 ± 7.20[c]	132.33 ± 5.36[c]

[a]Different letters in the same row indicate significant differences ($p < 0.05$). The highest values are for the ABTS radical, from the species *N. transcaucasica* (48.72 μM TE/mL), and the species *N. cataria* (310 μM TE/mL). IC_{50} values were lower for *N. cataria* species, indicating that it had a higher antioxidant effect than *N. transcaucasica* oil.

differences in the organoleptic evaluation of fresh herbs are a prerequisite for selecting suitable forms with increased odor characteristics of the plant. The data are presented in Table 1.

The population of *N. cataria* is relatively uniform, with a typical citral odor of the drug without the off-flavor characteristic of specimens from the wild flora. The agro-biological characteristics of *Nepeta cataria* are presented in Table 2. A highly variable indicator is the number of stems of different thicknesses, forming a different structure in producing the fresh drug. The presence of plants with a more significant number of thinner stems determines the presence of more leaf mass. Therefore, it predetermines a higher essential oil content in the drug because the primary essential oil containers are found in the leaves. In this connection, differences are also observed in the drying rate, determined by the different thicknesses of the stems and their lignification.

Chemical Composition of Essential Oils. Essential oils are easily mobile light yellow liquids with a specific smell, which does not contradict the data from the literature.[12]

The chemical compositions of the oils are presented in Table 3. As seen, 51 components representing 99.72% of the total content were identified in the oil from *N. transcaucasica*. Thirteen of them were in concentrations over 1%, and the rest of the 38 constituents were in concentrations under 1%. The major constituents (about 3%) of the oil are as follows: β-citronellol (52.05%), eucalyptol (7.34%), β-citronellal (6.06%), germacrene D (5.45%), (*E*)-β-ocimene (5.14%), and β-caryophyllene (3.06%).

Forty-one compounds were identified in the composition of *N. cataria*, representing 99.72% of the total content identified in the oil. Ten of them were in a concentration over 1%, and the rest of the 30 constituents were in concentrations under 1%. The major constituents (about 3%) of the oil are as follows: β-citronellol (26.31%), geraniol (15.92%), neral (11.45%), nerol (9.56%), carvacrol (6.04%), and β-citronellal (5.35%).

Antimicrobial Activity. The diameters of the zones of inhibition compared to the tested test cultures are presented in Table 4. The data show that the oils are only active against the Gram-negative bacterium *E. coli*, with the other test cultures being resistant.

Antioxidant Activity. The antioxidant activity results are presented in Table 5.

DISCUSSION

The presence of plants with a more significant number of thinner branches determines the presence of more leaf mass that has not fallen; this, in turn, determines the content of essential oil in the drug because the leaves contain the primary containers of essential oil. In this regard, the drying rate depends on the thickness of the stems, which is determined by the different degrees of lignification.

The Nepeta plants can be harvested twice. The first mowing could be in the budding phase, the beginning of flowering when the plant has a significant leaf mass that has yet to fall from the high temperatures in July. The second mowing could be done in the fall, at the beginning of October, when the plants massively stop flowering, and a significant amount of essential oil accumulates in the inflorescences.

The soil−climatic conditions in the Kazanlak field are favorable for developing both species since they form a significant above-ground mass. The population of the species *N. transcaucasica* is highly heterogeneous according to the studied agrobiological parameters and allows the selection of suitable forms. The *N. cataria* population is relatively uniform with a typical citral smell of the plant without side, unpleasant notes, characteristic of specimens collected from natural habitats in Bulgaria.

These values approach the data described in the literature for the species *N. cataria* by Aćimović *et al.*[21] (0.24%) and Tiwari *et al.*[22] (0.1−03%). Still, they differ from the data of Baranauskienė *et al.*[20] (5.94%). For the species *N. transcaucasica*, yield data also differ from those published in the literature by Mishurova and Shikhiev[31] (0.94%) and Pelyakh *et al.*[32] (0.8−1.4%). The soil and climatic conditions can explain the established differences in the amount of essential oil in both species during the vegetation of the plants.

The chemical composition of the two investigated oils is similar, with the identified components differing only in quantity. The *N. transcaucasica* essential oil has a higher content of oxygenated monoterpenes eucalyptol and β-citronellol, of the monoterpene hydrocarbon β-ocimene, of

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the sesquiterpene hydrocarbon germacrene D, and nepetalactones. A higher amount of monoterpene alcohols nerol and geraniol, aldehydes neral and geranial, and phenol carvacrol was found in the oil of the *N. cataria* species. These differences can be explained by species characteristics, which were also found by other authors when studying the chemical composition of essential oils from different species of the genus Nepeta.[2,4,20,24,26]

The distribution of the compounds by groups (% of the composition) is presented in Table 3. The data show that oxygenated monoterpenes dominated (78.38 and 84.93%) the composition of the studied oils followed by sesquiterpene hydrocarbons (0.02 and 3.53%).

The comparative analysis of the chemical composition of *N. transcaucasica* essential oil shows that the amounts of the main components differ from the data in the literature. According to Baranauskienė *et al.*,[20] the main compounds were oxygenated monoterpenes such as citronellol (17.69%), geranial (9.05%), and geranyl acetate (8.20%). Mishurova and Shikhiev[31] determined that the main compounds were monoterpene hydrocarbons such as α-pinene (2.2%), β-pinene (11.8%), myrcene (0.5%), limonene (7.4%), and γ-terpinene (7.81%); by Pelyakh *et al.*,[32] the main compounds were oxygenated monoterpenes such as citral (8.1−24.6%) and citronellol (21.6−54.8%). Kilic[33] reported that the main compound was oxygenated monoterpene 1,8-cineole (14.4%). In the essential oil studied by us, the amounts of nepetalactones were deficient, in contrast to the data of Baranauskienė *et al.*[20] (14.34%), Kilic[33] (18.5%), Karakuş *et al.*[34] (93.75%), and İşcan *et al.*[35] (over 65%).

In the case of *N. cataria* essential oil, differences were also found with the data from the literature regarding the amounts of oxygenated monoterpenes such as β-citronellol, geraniol, neral, nerol, carvacrol, and β-citronellal. In the essential oil studied by us, the quantities of nepetalactones were deficient, in contrast to the data of Baranauskienė *et al.*[20] (over 80%), Tiwari *et al.*[22] (67.9−87.5%), and Azizian *et al.*[24] (81.3%). These differences can be explained mainly by how essential oils are obtained. In the steam distillation used in this study, only the primary oil is separated in the receiver of the apparatus, and the resulting distillates are discarded. In laboratory glassware, where the raw material is processed by water distillation, primary and secondary oils are separated in the receiver. The raw material boils with the distillation water, which can change the oil's chemical composition. The influence of various technological factors, such as temperature, pressure, and duration, on the chemical composition, including the content of nepetalactone, was investigated in the preparation of extracts with CO_2 and essential oil of the species *Nepeta persica* Boiss.[48]

Twenty-four known aromatic substances can cause allergic reactions, as described in the EU Directive (1223/2009).[49] It has been established that using various cosmetic preparations (emulsion creams, gels, lotions, shampoos, masks, etc.) containing some of the allergens listed in the Directive may cause various skin changes, the so-called allergic contact dermatitis on the face, hands, and scalp. Symptoms of allergic reactions are swelling, redness, rashes, severe itching, eczema, or increased skin sensitivity, expressed by a feeling of pain. These symptoms can appear hours or days after using the given cosmetic product, which is why specialists often experience difficulties accurately identifying the allergen. Allergic reactions can also include headache, sneezing, runny nose, watery eyes, etc.[50−57]

Six of the allergens specified in the EU Directive were found in the tested oils, and their quantity is as follows:

In the *N. transcaucasica* species are β-citronellol (52.05%), neral (2.42%), geranial (1.51%), geraniol (1.44%), limonene (0.35%), and β-linalool (0.07%).

In the *N. cataria* species are β-citronellol (26.31%), geraniol (15.92%), geranial (11.58%), neral (11.45%), limonene (0.16%), and β-linalool (0.15%).

The data show that the total amount of allergens is higher in *N. cataria* essential oil, which should be avoided or properly labeled when required by regulations about leave-on and rinse-off cosmetic products.

Despite the high content of oxygenated monoterpenes, which have proven biological properties,[58] the oils have weak antibacterial activity.

The essential oil of *N. cataria* species is known to have a strong inhibitory effect on the growth of three species of *Bacillus* bacteria—*Bacillus cereus*, *Bacillus subtilis*, and *Bacillus amyloliquefaciens*. It was found to have a low antimicrobial activity against *S. aureus*, and *E. coli* and *Pseudomonas aeruginosa* were resistant. This action is explained by the presence of nepetalactone in the oil.[17] It shows good antibacterial activity, especially on *Salmonella typhi*,[18] against the yeast *Candida albicans*[11] and other micro-organisms.[16,21−24,36]

The essential oil of the species *N. transcaucasica* has proven activity against *Candida* yeasts,[35] and extracts of the plant with polar solvents have antibacterial activity.[36]

Copper and iron are essential trace elements in many enzymes and physiological processes. However, in the free form, they have a toxic effect because Cu(II) catalyzes the oxidation of ascorbic acid, and reactive oxygen systems (ROS) such as superoxide radicals ($O_2^{\bullet-}$) and H_2O_2 are generated. In this catalytic process, Cu(II) and Fe(II) react with H_2O_2, and hydroxyl radicals ($^{\bullet}OH$) are generated via the Fenton reaction.[59]

Reactive oxygen species (ROS) are a result of cellular metabolism. Under physiological conditions, enzyme systems regulate ROS levels. They are known to damage vital molecules of biological importance such as phospholipids, proteins, and DNA. It has been shown that damage caused by them is associated with the development of a number of diseases (cancer, cardiovascular diseases, atherosclerosis, and Alzheimer's disease).[60] Even in a state of physiological health, the toxic effects of oxygen and its derivatives accumulating in the body lead to a reduction in life expectancy.[61]

In the present work, we investigated the antioxidant activity of the essential oil of two species of *Nepeta*. We used different methods to evaluate the ability to deactivate free radicals and the ability to reduce copper (Cu^{2+}) and iron (Fe^{3+}) ions.

Depending on the mechanism of the antioxidant reaction, the methods were divided into hydrogen atom transfer (HAT) and single electron transfer (SET) techniques. The HAT method is based on the antioxidant's ability to quench free radicals via a hydrogen donor. SET methods are characterized by the reductive ability of one electron transfer of the tested antioxidant to a radical species. FRAP and CUPRAC are single-electron transfer methods. ABTS and DPPH methods are considered as methods using both hydrogen and single-electron transfer.[62,63]

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There are data in the literature on the antioxidant activity of *Nepeta* species extracts[23] with different polar solvents, making it difficult to compare.

The antioxidant action of the oils is explained by the high content of oxygenated monoterpenes, which have a proven biological effect.[58] According to Tepe *et al.*,[64] the high antioxidant activity of *N. cataria* essential oil is due to the oxygenated monoterpene derivatives 1,8-cineole and linalool. In the studied oils, the first component is in greater quantity in the species *N. transcaucasica* (7.34%), while the species *N. cataria* is minimal (0.08%). The linalool content in both oils is in low concentrations, 0.07 and 0.15%, respectively. In a study by Azizian *et al.*,[24] it is stated that the species *N. cataria* has a high content of polyphenolic acids such as ferulic, chlorogenic, caffeic, and coumaric, as well as flavonoids, which determines antioxidant capacity by DPPH (77.26%) and FRAP (2.23 μmol Fe^{++} g^{-1}).

It is possible that the higher antioxidant activities of *N. cataria* compared to *N. transcaucasica* is due to the higher contents of *N. cataria* in the sum of oxygenated terpenoids (Table 3), such as oxygenated compounds, for example, verbenol, isopulegol, 3-(*Z*)-nonen-1-ol, α-terpineol, myrtenol, β-citronelol, nerol, geraniol, and carvacrol.

CONCLUSIONS

Comparative agrobiocharacteristics of two cultural species of the genus *Nepeta*, *N. transcaucasica* and *N. cataria*, cultivated in Bulgaria, were carried out. The soil and climatic conditions in the Kazanlak field are favorable for developing both species and determining the formation of significant and high-quality plant material. They differ in yield, chemical composition, and biological activity of essential oils. The species with higher antibacterial activity was *N. transcaucasica* essential oil, which can be included in food products. The *N. cataria* essential oil had more pronounced antioxidant activity. Still, it had a higher content of allergens in essential oil, which is an indicator that should be avoided in cosmetic products. The application of essential oils in food and cosmetic products could be a subject of our future research.

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S.M. and A.S. performed conceptualization; A.S., S.S., and H.F. conducted experiments; A.D., D.B., and S.M. performed formal analysis; R.U. and A.B. performed the methodologies; A.S., S.S., and H.F. wrote the original draft; S.E., A.A., and R.A.M reviewed and edited the manuscript.

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REFERENCES

(1) Klimek, B.; Modnicki, D. Terpenoids and sterols from *Nepeta cataria* L. var. citriodora (Lamiaceae). *Acta Pol. Pharm.* **2005**, *62*, 231−235.

(2) Formisano, C.; Rigano, D.; Senatore, F. Chemical constituents and biological activities of *Nepeta* species. *Chem. Biodiversity* **2011**, *8*, 1783−1818.

(3) Sharma, A.; Cannoo, D. Phytochemical composition of essential oils isolated from different species of genus *Nepeta* of Labiatae family: A review. *Pharmacophore* **2017**, *4*, 181−121.

(4) Asgarpanah, J.; Sarabian, S.; Ziarati, P. Essential oil of *Nepeta* genus (Lamiaceae) from Iran: A review. *J. Essent. Oil Res.* **2014**, *26*, 1−12.

(5) Salehi, B.; Valussi, M.; Jugran, A. K.; Martorell, M.; Ramírez-Alarcón, K.; Stojanovic-Radic, Z.; Antolak, H.; Kregiel, D.; Mileski, K.; Sharifi-Rad, M.; et al. *Nepeta* species: From farm to food applications and phytotherapy. *Trends Food Sci. Technol.* **2018**, *80*, 104−122.

(6) Suntar, I.; Nabavi, S.; Barreca, D.; Fischer, N.; Efferth, T. Pharmacological and chemical features of *Nepeta* L. genus: its importance as therapeutic agent. *Phytother Res.* **2018**, *32*, 185−198.

(7) Aćimović, M.; Stanković Jeremić, J.; Cvetković, M. Phyto-pharmacological aspects of *Nepeta nuda* L.: A systematic review. *Lek. Sirov.* **2020**, *40*, 75−83.

(8) Acimovic, M.; Zeremski, T.; Kiprovski, B.; Brdar-Jokanovic, M.; Popovic, V.; Koren, A.; Sikora, V. *Nepeta cataria*. Cultivation, chemical composition and biological activity. *J. Agron. Technol. Eng. Manag.* **2021**, *4*, 620−634. https://hdl.handle.net/21.15107/rcub_fiver_2344
(9) Reichert, W.; Park, C.; Juliani, R.; Simon, J. A new highly aromatic catnip *Nepeta cataria* L. cultivar rich in Z,E-nepetalactone. *Hort Sci.* **2016**, *51*, 588−591.
(10) Bol, S.; Caspers, J.; Buckingham, L.; Anderson-Shelton, G.; Ridgway, C.; Buffington, T.; Schulz, S.; Bunnik, E. M. Responsiveness of cats (Felidae) to silver vine (*Actinidia polygama*), Tatarian honeysuckle (*Lonicera tatarica*), valerian (*Valeriana officinalis*) and catnip (*Nepeta cataria*). *BMC Vet Res.* **2017**, *13*, 70.
(11) Zomorodian, K.; Saharkhiz, M. J.; Shariati, S.; Pakshir, K.; Rahimi, M.; Khashei, R. Chemical composition and antimicrobial activities of essential oil from *Nepeta cataria* L. against common causes of food-borne infections. *ISRN Pharm.* **2012**, No. 591953.
(12) Frolova, N.; Uktainets, A.; Korablova, O.; Voitsekhivskyi, V. Plants of *Nepeta cataria* var. *citriodora* Beck. and essential oils from them for food industry. *Slovak. J. Food Sci.* **2019**, *13*, 449−455.
(13) Chalchat, J.; Lamy, J. Chemical composition of the essential oil isolated from wild catnip *Nepeta cataria* L. cv. *citriodora* from the Drome region of France. *J. Essent. Oil Res.* **1997**, *9*, 527−532.
(14) Gomes, E.; Reichert, W.; Vasilatis, A.; Allen, K.; Wu, Q.; Simon, J. Essential oil yield and aromatic profile of lemon catnip and lemon-scented catnip selections at different harvesting times. *J. Med. Act Plants.* **2020**, *9*, 21−33.
(15) Mohammadizad, H.; Mehrafarin, A.; Badi, N. Qualitative and quantitative evaluation of essential oil of catnip (*Nepeta cataria* L.) under different drying conditions. *J. Med. Plants.* **2017**, *16*, 8−20.
(16) Hossan, M.; Jindal, H.; Maisha, S.; Raju, C.; Sekaran, S.; Nissapatorn, V.; Kaharudin, F.; Yi, L.; Khoo, T.; Rahmatullah, M.; Wiart, C. Antibacterial effects of 18 medicinal plants used by the Khyang tribe in Bangladesh. *Pharm. Biol.* **2018**, *56*, 201−208.
(17) Hee, K.; Jung, D.; Park, H. The composition of essential oil from *Nepeta cataria* and its effect on microorganism. *J. Ecol. Field Biol.* **2006**, *29*, 381−387.
(18) Ashrafi, B.; Ramak, P.; Ezatpour, B.; Talei, G. Biological activity and chemical composition of the essential oil of *Nepeta cataria* L. *J. Res. Pharm.* **2019**, *23*, 336−343.
(19) Adiguzel, A.; Ozer, H.; Sokmen, M.; Gulluce, M.; Sokmen, A.; Kilic, H.; Sahin, F.; Baris, O. Antimicrobial and antioxidant activity of the essential oil and methanol extract of *Nepeta cataria*. *Pol. J. Microbiol.* **2009**, *58*, 69−76.
(20) Baranauskienė, R.; Bendžiuvienė, V.; Ragažinskienė, O.; Venskutonis, P. Essential oil composition of five *Nepeta* species cultivated in Lithuania and evaluation of their bioactivities, toxicity and antioxidant potential of hydrodistillation residues. *Food Chem. Toxicol.* **2019**, *129*, 269−280.
(21) Aćimović, M.; Seregelj, V.; Simic, K.; Varga, A.; Pezo, L.; Vulic, J.; Cabarkapa, I. Chemical profile of *Nepeta cataria* L. var. *citriodora* (Becker) essential oil and *in vitro* evaluation of biological activities. *Acta Sci. Pol. Hortorum Cultus.* **2022**, *21*, 67−74.
(22) Tiwari, G.; Chaturvedi, T.; Gupta, A.; Lal, R.; Verma, R.; Srivastava, R.; Singh, P.; Gangwar, B.; Darokar, M.; Dhawan, S. Assessment of genetic diversity, micromorphology and antimicrobial activity in *Nepeta cataria* L. *Chem. Biodiversity* **2023**, 19.
(23) Nadeem, A.; Shahzad, H.; Ahmed, B.; Muntean, T.; Waseem, M.; Tabassum, A. Phytochemical profiling of antimicrobial and potential antioxidant plant: *Nepeta cataria*. *Front. Plant Sci.* **2022**, *13*, 18.
(24) Azizian, T.; Alirezalu, A.; Hassani, A.; Bahadori, S.; Sonboli, A. Phytochemical analysis of selected *Nepeta* species by HPLC-ESI-MS/MS and GC-MS methods and exploring their antioxidant and antifungal potentials. *J. Food Meas. Charact.* **2021**, *15*, 2417−2429.
(25) Malizia, R. A.; Molli, J. S.; Cardell, D. A.; Retamar, J. A. Volatile constituents of the essential oil of *Nepeta cataria* grown in Cordoba province (Argentinia). *J. Essent. Oil Res.* **1996**, *8*, 565−567.
(26) Hethelyi, E.; Szabo, L.; Korany, K. Volatile constituents of *Nepeta cataria* L., *N. glechoma* Benth. and *N. parviflora* M. Bieb. from Hungary. *Int. J. Hortic. Sci.* **2001**, *7*, 47−50.
(27) Morteza-Semnani, K.; Saeedi, M. Essential oils composition of *Nepeta cataria* L. and *Nepeta crassifolia* Boiss. and Buhse from Iran. *J. Essent. Oil-Bear. Plants.* **2004**, *7*, 120−124.
(28) Patel, H.; Gomes, E.; Yuan, B.; Lyu, W.; Wu, Q.; Simon, J. Investigation of volatile iridoid terpenes in *Nepeta cataria* L. (Catnip) Genotypes. *Molecules* **2022**, *27*, 19.
(29) Ganzera, M.; Crockett, S.; Tellez, M.; Khan, I. Determination of nepetalactone in *Nepeta cataria* by reversed phase high performance liquid chromatography. *Pharmazie* **2001**, *56*, 896−897.
(30) Sharma, A.; Cooper, R.; Bhardwaj, G.; Cannoo, D. S. The genus *Nepeta*: Traditional uses, phytochemicals and pharmacological properties. *J. Ethnopharmacol.* **2021**, *268*, 25.
(31) Mishurova, S. S.; Shikhiev, A. S. The monoterpene fraction of the essential oil of *Nepeta transcaucasica*. *Chem. Nat. Compd.* **1977**, *13*, 733−733.
(32) Pelyakh, Y.; Melnik, V.; Chobanu, V.; Unguryanu, I. Plants perspective with content from citrali in oil ethereal. *Studia Universitatis Moldaviae* **2016**, *96*, 29−33.
(33) Kilic, Ö. Essential oil and fatty acid composition of leaves of some Lamiaceae taxa from Turkey. *J. Essent. Oil Bear. Plants.* **2018**, *21*, 1706−1711.
(34) Karakuş, S.; Tiryaki, D.; Aydın, I.; Atıcı, Ö. Investigation of herbicidal effects of essential oil of *Nepeta transcaucasica* Grossh. on some cultivated and weed plants. *Doğu Fen Bilimleri Dergisi / J. Nat. Appl. Sci. East.* **2019**, *2*, 69−79.
(35) İşcan, G.; Köse, Y.; Demirci, B.; CAN BAŞER, K. H. Anticandidal activity of the essential oil of *Nepeta transcaucasica* Grossh. *Chem. Biodiversity* **2011**, *8*, 2144−2148.
(36) Öztürk, A. *In vitro* antibacterial activities of *Nepeta transcaucasica*. *Asian J. Chem.* **2009**, *21*, 6440−6444.
(37) Handjieva, N. V.; Popov, S. S.; Evstatieva, L. N. Constituents of essential oils from *Nepeta cataria* L. *N. grandiflora M.B. and N. nuda L. J. Essent. Oil Res* **1996**, *8*, 639−643.
(38) Kozuharova, E.; Benbassat, N.; Getov, I. Ethnobotanical records of not yet documented therapeutic effects of some popular Bulgarian medicinal plants. *Emir. J. Food Agric.* **2014**, *26*, 647−651.
(39) Petkov, V. *Contemporary phytotherapy. Medicine and physculture, Sofia, (Bulgarian language)*. 1982.
(40) *Association of Official Analytical Chemists (AOAC). Official Methods of Analysis*, 20th ed. AOAC International: Geithersburg, MD, 2016.
(41) Shen, V.; Siderius, D.; Krekelberg, W.; Hatch, H. (Eds.). *NIST Standard Reference Simulation Website, NIST Standard Reference Database 173*. National Institute of Standards and Technology: Gaithersburg MD, 20899. DOI: 10.18434/T4M88Q (accessed 10 January 2022).
(42) Zaika, L. Spices and herbs: their antimicrobial activity and its determination. *J. Food Saf.* **1988**, *9*, 97−118.
(43) Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.; Rice-Evans, C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biol. Med.* **1999**, *26*, 1231−1237.
(44) Bojilov, D.; Manolov, S.; Ivanov, I.; Mollova, S. Investigation of antioxidant activity of different extracts of *Helichrysum italicum* from Bulgaria. *J. Int. Sci. Publ.: Mater. Methods Technol.* **2019**, *13*, 241−249.
(45) Brand-Williams, W.; Cuvelier, M. E.; Berset, C. Use of a free radical method to evaluate antioxidant activity. *LWT - Food Sci. Technol.* **1995**, *28*, 25−30.
(46) Apak, R.; Güçlü, K.; Ozyürek, M.; Esin Karademir, S.; Erçağ, E. The cupric ion reducing antioxidant capacity and polyphenolic content of some herbal teas. *Int. J. Food Sci. Nutr.* **2006**, *57*, 292−304.
(47) Benzie, I.; Strain, J. The Ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power:" The FRAP assay. *Anal. Biochem.* **1996**, *239*, 70−76.
(48) Khajeh, M.; Yamini, Y.; Shariati, S. Comparison of essential oils compositions of *Nepeta persica* obtained by supercritical carbon

dioxide extraction and steam distillation methods. *Food Bioprod. Process.* **2010**, *88*, 227−232.
(49) The European Communities. *Labelling of ingredients in cosmetics Directive 76/768/EEC*; The European Communities Update February 2008. Available online: http://ec.europa.eu/consumers/sectors/cosmetics/files/doc/guide_labelling200802_en.pf.
(50) Hagvall, L.; Karlberg, A.-T.; Christensson, J. B. Finding the optimal patch test material and test concentration to detect contact allergy to geraniol. *Contact Dermatitis.* **2013**, *68*, 224−231.
(51) Ahlgren, C.; Axéll, T.; Möller, H.; Isaksson, M.; Liedholm, R.; Bruze, M. Contact allergies to potential allergens in patients with oral lichen lesions. *Clin. Oral Investig.* **2014**, *18*, 227−237.
(52) Bråred-Christensson, J.; Andersen, K.; Bruze, M.; Johansen, J.; Garcia-Bravo, B.; Gimenez-Arnau, A.; Goh, C.; Nixon, R.; White, I. Positive patch test reactions to oxidized limonene: Exposure and relevance. *Contact Dermatol.* **2014**, *71*, 264−272.
(53) Bråred-Christensson, J.; Hellsén, S.; Börje, A.; Karlberg, A.-T. Limonene hydroperoxide analogues show specific patch test reactions. *Contact Dermatitis.* **2014**, *70*, 291−299.
(54) Bråred-Christensson, J.; Hagvall, L.; Karlberg, A.-T. Fragrance allergens, overview with a focus on recent developments and understanding of abiotic and biotic activation. *Cosmetics* **2016**, *3*, 1−18.
(55) Deza, G.; García-Bravo, B.; Silvestre, J.; Pastor-Nieto, M.; González-Pérez, R.; Heras-Mendaza, F.; Mercader, P.; Fernández-Redondo, V.; Niklasson, B.; Giménez-Arnau, A.; GEIDAC. Contact sensitization to limonene and linalool hydroperoxides in Spain: A GEIDAC prospective study. *Contact Dermatol.* **2017**, *76*, 74−80.
(56) Sarkic, A.; Stappen, I. Essential oils and their single compounds in cosmetics - A critical review. *Cosmetics.* **2018**, *5*, 11.
(57) Dittmar, D.; Schuttelaar, M. L. A. Contact sensitization to hydroperoxides of limonene and linalool: results of consecutive patch testing and clinical relevance. *Contact Dermatitis.* **2019**, *80*, 101−109.
(58) Griffin, S. G.; Grant Wyllie, S.; Markham, J. L.; Leach, D. N. The role of structure and molecular properties of terpenoids in determining their antimicrobial activity. *Flavour Fragr. J.* **1999**, *14*, 322−332.
(59) Chalana, A.; Kumar Rai, R.; Karri, R.; Kumar Jha, K.; Kumar, B.; Roy, G. Interplay of the intermolecular and intramolecular interactions in stabilizing the thione-based copper(I) complexes and their significance in protecting the biomolecules against metal-mediated oxidative damage. *Polyhedron* **2022**, *215*, No. 115647.
(60) Heleno, S. A.; Martins, A.; Queiroz, M. J. R. P.; Ferreira, I. C. F. R. Bioactivity of phenolic acids: metabolites versus parent compounds: a review. *Food Chem.* **2015**, *173*, 501−513.
(61) Han, X.; Shen, T.; Lou, H. Dietary polyphenols and their biological significance. *Int. J. Mol. Sci.* **2007**, *8*, 950−988.
(62) Jimenez, A.; Selga, A.; Torres, J.; Julia, L. Reducing activity of polyphenols with stable radicals of the TTM series. Electron transfer versus H-abstraction reactions in flavan-3-ols. *Org. Lett.* **2004**, *6*, 4583−4586.
(63) Prior, R.; Wu, X.; Schaich, K. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *J. Agric. Food Chem.* **2005**, *53*, 4290−4302.
(64) Tepe, B.; Daferera, D.; Sihoglu-Tepe, A.; Polissiou, M.; Sokmen, A. Antioxidant activity of the essential oil and various extracts of *Nepeta flavida* Hub Mor. from Turkey. *Food Chem.* **2007**, *103*, 1358−1364.

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