

# Phytochemical Composition, Antiradical and Anticholinesterase Potentials of *Centaurea alba* and *Centaurea jacea* Volatile Oils

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**Abstract:** This paper reports on the phytochemical composition as well as antiradical and anticholinesterase potential of volatile oils isolated from *Centaurea alba* and *Centaurea jacea*, from Croatia. The volatile components, obtained by hydrodistillation, were determined by GC and GC-MS analyses. A total of 18 compounds were identified in *C. alba* volatile oil with hexadecanoic acid, germacrene D and tetradecanoic acid as main compounds. A total of 29 compounds were identified in *C. jacea* volatile oil, with *epi*-bicyclosesquiphellandrene, aromadendrene and hexadecanoic acid as a major compounds. The tested volatile oils showed low DPPH inhibition potential as well as low to moderate antiAChE potential and low antiBuChE potential.

**Keywords:** *Centaurea alba* L., *Centaurea jacea* L., volatile oil, GC-MS, DPPH, AChE, BuChE

## INTRODUCTION

THE genus *Centaurea* L. is one of the richest genera in the Asteraceae family. According to today's concept, genus contains about 250 species which have the main spreading area in the Mediterranean region.<sup>[1,2]</sup>

The genus *Centaurea* had been extensively used in folk medicine for hundreds of years, especially in eastern Mediterranean ethnopharmacology.<sup>[3–5]</sup> It is confirmed that numerous *Centaurea* species have biological potential, as antimicrobial, antifungal, antiviral, anti*Helicobacter pylori*, antiulcerogenic, antioxidant, antiinflammatory and cytotoxic.<sup>[3,6–13]</sup>

Plants are good source of volatile components that are important in biological and ecological terms. These compounds could be good antioxidants or cholinesterase inhibitors. Antioxidants are compounds that delay autoxidation by inhibiting formation of free radicals or by interrupting propagation of the free radicals. Free radicals alter the structure and function of substances in the body and in high concentrations they can damage proteins, lipids and DNA. The brain is particularly susceptible to oxidative damage. There is substantial evidence that oxidative damage to

the brain is an early event in Alzheimer's disease (AD). Many biochemical mechanisms have been proposed to explain the neuropathology of AD but cholinergic hypothesis is the most accepted theory nowadays. It postulates that at least some of the cognitive decline experienced by patients with AD results from a deficiency of acetylcholine, or cholinergic neurotransmission.<sup>[14]</sup> Neutralizing free radicals and inhibition of acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) has become an important and persuasive therapeutic strategy against AD.<sup>[15]</sup>

As a part of our research on the phytochemical composition and biological potential of Croatian *Centaurea* sp.<sup>[16–18]</sup> we report here the results of phytochemical composition as well as antiradical and anticholinesterase potential of *Centaurea alba* L. and *Centaurea jacea* L. volatile oils from Croatia.

According to our knowledge, this is the first report about chemical composition of *Centaurea alba* L. and the second report about chemical composition of *Centaurea jacea* L. volatile oil, but the first one for the plant from Croatia. This is also the first record about biological potential of these oils.

## EXPERIMENTAL

### Plant Materials

Plant materials were collected during flowering period in a full blooming phase near Split, Croatia (*C. alba* L. was collected in Klis, while *C. jacea* L. was collected in Blaca, Kozjak). Plant material were and taxonomically identified by a botanist dr. Mirko Ruščić, associate professor, Department of Biology, Faculty of Science, Split, Croatia. Voucher specimens of plant materials have been deposited in herbarium at the Department of Biochemistry, Faculty of Chemistry and Technology, Split, Croatia and assigned as follows: 10-2016-CA and 3-2016-CJ.

### Materials

Analytical grade reagents and solvents: 2,2-diphenyl-1-picrylhydrazyl (DPPH) and Ascorbic acid were purchased from Sigma-Aldrich (St. Louis, SAD); Acetylcholinesterase (AChE, from *Electrophorus electricus* – electric eel, type V-S), Butyrylcholinesterase (BuChE, from equine serum), Acetylthiocholine iodide (ATChI), Butyrylthiocholine iodide (BTChI), 5,5-dithiobis (2-nitrobenzoic acid) (DTNB, Ellman's reagent) and Eserine were purchased from Sigma-Aldrich GmbH (Steinheim, Germany); Ethanol was purchased from Kemika, Croatia; Double deionised water was also used.

### Volatile Oils Isolation

The air dried aerial parts of plants were subjected to hydrodistillation using a Clevenger-type apparatus<sup>[19]</sup> for 3h. The obtained volatile oils were stored in a sealed vial, under  $-20\text{ }^{\circ}\text{C}$  until use.

### Gas Chromatography (GC) and Gas Chromatography–Mass Spectrometry (GC–MS) Analyses

GC and GC-MS analyses of isolated volatile oils were performed using Varian Inc. Gas Chromatograph, model 3900, Lake Forest, CA, SA, equipped with flame ionization detector and mass detector, model 2100T, with nonpolar capillary column VF-5MS (30 m  $\times$  0.25 mm *i.d.*; coating thickness 0.25  $\mu\text{m}$ ). Temperature program for VF-5MS column was: 60  $^{\circ}\text{C}$  isothermal for 3 min, then increased to 246  $^{\circ}\text{C}$  at a rate of 3  $^{\circ}\text{C min}^{-1}$  and held isothermal for 25 min. Carrier gas was helium at flow rate 1 mL  $\text{min}^{-1}$ , injector temperature was 250 $^{\circ}\text{C}$ , injected volume 1  $\mu\text{L}$ ; split ratio of 1 : 20; FID detector temperature was 300  $^{\circ}\text{C}$ . Mass spectrometer ionization voltage was 70 eV, mass scan range: 40–350 mass units and ion source temperature was 200  $^{\circ}\text{C}$ . Identification of volatile oils chemical composition was based on comparison of compound mass spectra with databases (Wiley 7 library - Wiley, New York, NY, USA; Adams 2007) and comparison of their retention indices (relative to series

of *n*-alkanes  $\text{C}_8\text{--C}_{40}$ ), with internal database retention indices and literature retention indices using NIST2002 (National Institute of Standards and Technology, Gaithersburg, MD, USA).<sup>[20,21]</sup> The internal database of compounds was created during previous analyses from authentic compounds obtained commercially and from more than thousand volatile oils obtained during our previous studies. The percentages of components were calculated as mean values from the GC and GC-MS peak areas.

### Antiradical Potential of Tested Extracts

Antiradical potential of volatile oils isolated from *C. alba* and *C. jacea* was performed by DPPH (2,2-diphenyl-1-picrylhydrazyl radical scavenging method) method of Brand-Williams et al.<sup>[22]</sup>. The DPPH radical scavenging activities of the tested extracts were calculated according to formula: % inhibition =  $[(A_0 - A_{\text{sample}}) / A_0] \times 100$ , where  $A_0$  was absorbance of the DPPH ethanol solution measured at the beginning and  $A_{\text{sample}}$  was absorbance of the sample measured after 60 min. The results were expressed as percentage inhibition of DPPH or  $\text{IC}_{50}$  (amount of antioxidant required for 50 % decrease in initial DPPH concentration). Ascorbic acid (vitamin C) was used as positive control. The experiment was performed in triplicate and the results were expressed as mean  $\pm$  standard deviations.

### Anticholinesterase Potential of Volatile Oils

Anti-acetylcholinesterase (antiAChE) and anti-butyrylcholinesterase (antiBuChE) potential of volatile oils isolated from *C. alba* and *C. jacea* were carried out by a slightly modified Ellman method.<sup>[23]</sup> A run consisted of 180  $\mu\text{L}$  of phosphate buffer (0.1 M, pH 8), 10  $\mu\text{L}$  of DTNB (at a final concentration of 0.3 mM prepared in 0.1 M phosphate buffer pH 7 with 0.12 M sodium bicarbonate added for stability), 10  $\mu\text{L}$  of sample solution (dissolved in 80 % EtOH), and 10  $\mu\text{L}$  of AChE/BChE solution (with final concentration 0.03 U  $\text{mL}^{-1}$ ). Reactants were mixed in a 96-well plate wells and reaction was initiated by adding 10  $\mu\text{L}$  of acetylthiocholine iodide / butyrylthiocholine iodide (ATChI / BTChI), to reach a final concentration of 0.5 mM). As a negative control, 80 % EtOH was used instead of sample solution. Non-enzymatic hydrolysis was also monitored by measurement of two blank runs for each run. All spectrophotometric measurements were performed at 405 nm and at room temperature for 6 min periods. The results are expressed as percentage inhibition of enzyme activity. The experiment was performed in triplicate and the results were expressed as mean  $\pm$  standard deviations.

## RESULTS AND DISCUSSION

The volatile oils of two *Centaurea* species were analyzed by GC and GC-MS. According to my knowledge, this is the first

report about chemical composition of *Centaurea alba* and the second report about chemical composition of *Centaurea jacea* volatile oil, but the first one for the plant from Croatia.

A total of 18 compounds were identified in *Centaurea alba* volatile oil, representing 98.5 % of the total volatile oil composition, Table 1. This oil was dominated by nonterpene compounds (61.7 %), especially by nonterpene acids (31.7 %) with hexadecanoic acid (19.1 %) and tetradecanoic acid (11.7 %) as main compounds. Nonterpene hydrocarbons (10.8 %) and aldehydes (9.9 %) were also presented in remarkable quantity. Among them pentadecanal (9.6 %) was presented as the most common. Terpene compounds

were presented with sesquiterpenes and sesquiterpenoids in quantity of 36.8 %. The main terpene compounds were germacrene D (14.3 %), hexahydrofarnesyl acetone (9.3 %) and ledol (5.8 %). Other terpene and nonterpene compounds were identified in quantity lower than 4 %.

A total of 29 compounds were identified in *Centaurea jacea* volatile oil, representing 91.1 % of the total volatile oil composition, Table 1. Sesquiterpene compounds (65.9 %) were the most abundant compounds of tested oil with *epi*-bicyclosesquiphellandrene (18.7 %) and aromadendrene (12.5 %) as major ones.  $\beta$ -Elemene (8.0 %) and caryophyllene oxide (6.6 %) was also identified in remarkable quantity. Nonterpene compounds were presented in

**Table 1.** Volatile oil composition of *Centaurea alba* and *Centaurea jacea* from Croatia.

Compound	KI	Identification	<i>C. alba</i>	<i>C. jacea</i>	Compound	KI	Identification	<i>C. alba</i>	<i>C. jacea</i>
Hexanal	805	KI, MS	0.3	0.8	caryophyllene oxide	1580	KI, MS	1.9	6.6
Octanal	1006	KI, MS	–	0.4	humulene epoxyde II	1608	KI, MS	–	1.2
2,4–heptadienal*	1013	KI, MS	–	1.2	$\alpha$ –cadinol	1653	KI, MS	–	2.1
benzene acetaldehyde	1049	KI, MS	–	1.4	pentadecanal	1712	KI, MS	9.6	–
nonanal	1102	KI, MS	–	0.9	$\alpha$ –cyperone	1750	KI, MS	3.8	–
$\beta$ –cyclocitral	1214	KI, MS	–	0.3	tetradecanoic acid	1787	KI, MS	11.7	–
pulegone	1244	KI, MS	–	0.3	hexahydrofarnesyl acetone	1845	KI, MS	9.3	–
anethole	1283	KI, MS	–	0.2	pentadecanoic acid	1882	KI, MS	0.9	–
bicycloelemene	1336	KI, MS	–	4.4	hexadecanoic acid	1977	KI, MS	19.1	8.8
$\delta$ –elemene	1340	KI, MS	–	0.4	heneicosane	2100	KI, MS	1.1	–
$\alpha$ –copaene	1376	KI, MS	3.5	1.7	docosane	2200	KI, MS	1.3	–
$\beta$ –patchoulene	1381	KI, MS	–	1.0	tricosane	2300	KI, MS	2.9	–
$\beta$ –cubebene	1390	KI, MS	–	1.9	heptacosane	2700	KI, MS	5.5	–
$\beta$ –elemene	1392	KI, MS	–	8.0	<b>Terpene compounds</b>			<b>36.8</b>	<b>77.4</b>
$\alpha$ –calarene	1428	KI, MS	–	4.8	<i>Monoterpenes</i>			–	–
aromadendrene	1427	KI, MS	–	12.5	<i>Monoterpenoids</i>			–	0.6
cis– $\beta$ –farnesene	1442	KI, MS	–	1.4	<i>Sesquiterpenes</i>			25.3	65.9
calarene	1445	KI, MS	–	1.6	<i>Sesquiterpenoids</i>			11.5	10.9
alloaromadendrene	1458	KI, MS	–	2.1	Diterpenes			–	–
$\gamma$ –patchoulene	1473	KI, MS	3.0	2.3	<b>Phenylpropane compounds</b>			–	<b>0.2</b>
germacrene D	1480	KI, MS	14.3	1.0	<b>Nonterpene compounds</b>			<b>61.7</b>	<b>13.5</b>
<i>epi</i> –bicyclosesquiphellandrene	1488	KI, MS	–	18.7	Aldehydes			9.9	4.7
valencene	1491	KI, MS	–	2.9	Acids			31.7	8.8
$\alpha$ –selinene	1498	KI, MS	3.0	–	Esters			9.3	–
$\gamma$ –cadinene	1519	KI, MS	1.5	1.2	Hydrocarbons			10.8	–
Ledol	1561	KI, MS	5.8	–	<b>TOTAL</b>			<b>98.5</b>	<b>91.1</b>
spathulenol	1564	KI, MS	–	1.0					

KI = Kovat's index determined on a VF-5MS column using the homologous series of *n*-alkanes (C<sub>6</sub>–C<sub>40</sub>); – = not identified.

lower quantity (13.5 %) with hexadecanoic acid (8.8 %) as main compound. Other compounds were identified in quantity lower than 5 %. The chemical composition of *C. jacea* essential oil was previously made from Milošević et al.<sup>[24]</sup> Results of this study showed sesquiterpenoid compounds, among which caryophyllene oxide (23.5 %) and spathulenol (8.9%) were the major ones, as well as fatty acids, with 9-octadecanoic acid (8.9 %) and hexadecanoic acid (6.6 %) as main components.

A literature search showed that *Centaurea* species were poor in monoterpene and monoterpenoid compounds and rich in fatty acids as well as sesquiterpene and sesquiterpenoid compounds. Among fatty acids, hexadecanoic acid was the most abundant compounds, while among sesquiterpene and sesquiterpenoid compounds germacrene D,  $\beta$ -caryophyllene, caryophyllene oxide, spathulenol and  $\beta$ -eudesmol were the most abundant compounds.<sup>[24–34]</sup> To date, several *Centaurea* volatile oils chemical analysis for plants from Croatia were performed. Hexadecanoic acid, germacrene D,  $\beta$ -caryophyllene and caryophyllene oxide were also the most abundant compounds. Hydrocarbon compounds, especially heptacosane, were also identified in significant quantity.<sup>[16–18,35–38]</sup>

Antiradical and anticholinesterase potential of *C. alba* and *C. jacea* volatile oils were also tested. Antiradical potential was tested by DPPH method,<sup>[22]</sup> while anti AChE and antiBuChE potential was tested by Ellman method,<sup>[23]</sup> Table 2. DPPH method is widely used for antiradical detection, while Ellman method is widely used for the detection of cholinesterase inhibitors. Both of used methods are sensitive, good repetitive and requires a little sample material. Results showed low DPPH inhibition potential for both of tested volatile oils ( $5.6 \pm 0.3$  % for *C. alba* and  $4.9 \pm 0.2$  % for *C. jacea*) in tested concentration of  $1 \text{ mg mL}^{-1}$  ( $0.048 \text{ mg mL}^{-1}$  in reaction system). For comparison, vitamin C as reference compound, showed  $95.3 \pm 1.7$  % inhibition of DPPH, for the same tested concentration. Results for tested cholinesterase inhibition potential showed low to moderate AChE potential

**Table 2.** Antiradical and anticholinesterase potential of *Centaurea alba* and *Centaurea jacea* volatile oils.

	DPPH inh. / %	AChE inh. / %	BuChE inh. / %
<i>C. alba</i> VO <sup>(a)</sup>	$5.6 \pm 0.3$	$28.3 \pm 2.8$	$5.4 \pm 0.9$
<i>C. jacea</i> VO <sup>(a)</sup>	$4.9 \pm 0.2$	$11.7 \pm 0.9$	$2.6 \pm 0.7$
vitamin C <sup>(a)</sup>	$95.3 \pm 1.7$	–	–
eserine <sup>(b)</sup>	–	$92.9 \pm 3.4$	$77.9 \pm 1.9$

VO = volatile oil.

<sup>(a)</sup> tested concentration was  $1 \text{ mg mL}^{-1}$  ( $0.048 \text{ mg mL}^{-1}$  for DPPH and  $0.045 \text{ mg mL}^{-1}$  for AChE / BuChE in reaction system).

<sup>(b)</sup> tested concentration was  $0.1 \text{ mg mL}^{-1}$  ( $4.8 \mu\text{g mL}^{-1}$  for DPPH and  $4.5 \mu\text{g mL}^{-1}$  for AChE / BuChE in reaction system).

of tested oils ( $28.3 \pm 2.8$  % for *C. alba* and  $11.7 \pm 0.9$  % for *C. jacea*) and low BuChE inhibition potential of tested oils ( $5.4 \pm 0.9$  % for *C. alba* and  $2.6 \pm 0.7$  % for *C. jacea*) for tested concentration of  $1 \text{ mg mL}^{-1}$  ( $0.045 \text{ mg mL}^{-1}$  in reaction system). For comparison, eserine as referent compound showed  $92.2 \pm 3.4$  % inhibition of AChE and  $77.9 \pm 1.9$  % inhibition of BuChE for tested concentration of  $0.1 \text{ mg mL}^{-1}$ .

Low DPPH inhibition potential could be connected to composition of these oils which does not contain components attributed to antiradical potential, like phenolic or monoterpenoid compounds.<sup>[39]</sup> Low to moderate anticholinesterase inhibition potential of tested volatile oils also could be connected to volatile oils composition or synergism among volatile oil constituents. Among volatile compounds identified as components of tested oils, pulegone, anethole,  $\alpha$ -copaene, aromadendrene, ledol and caryophyllene oxide were tested on AChE, while anethole,  $\alpha$ -copaene, ledol and caryophyllene oxide were tested on BuChE inhibition.<sup>[40]</sup> Among them, anethole and pulegone showed good AChE inhibition potential, while only anethole showed good BuChE potential.

## CONCLUSION

The volatile oils of *Centaurea alba* and *Centaurea jacea* from Croatia were subjected to phytochemical composition as well as antiradical and anticholinesterase potentials. *C. alba* volatile oil was dominated by nonterpene compounds, especially by nonterpene acids, with hexadecanoic acid and tetradecanoic acid as main compound. Nonterpene hydrocarbons and aldehydes were also presented in remarkable quantity with pentadecanal as most abundant. Terpene compounds were presented with sesquiterpenes and sesquiterpenoids. The main terpene compounds were germacrene D, hexahydrofarnesyl acetone and ledol. *C. jacea* volatile oil was dominated by sesquiterpene compounds, with *epi*-bicyclosesquiphellandrene and aromadendrene as major compounds.  $\beta$ -Elemene and caryophyllene oxide were also identified in remarkable quantity. Nonterpene compounds were presented in lower quantity, with hexadecanoic acid as main compound. Antiradical and anticholinesterase potential of *C. alba* and *C. jacea* volatile oils showed low DPPH inhibition potential for both of tested volatile oils as well as low to moderate AChE and low BuChE inhibition potential of tested oils.

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**Author Contribution Statement.** OP designed experiments, performed chemical analyses and wrote manuscript; AV performed biological assays; IC collected plant materials and assisted during the experiments.

## REFERENCES

- [1] A. Susanna, N. Garcia-Jacas in *The families and genera of vascular plants.*, Vol. 8, (Ed. K. Kubitzki), Springer Verlag, Berlin, Germany, **2007**, pp. 123–146.
- [2] A. Susanna, N. Garcia-Jacas in *Systematics, evolution, and biogeography of Compositae* (Eds. V. A. Funk, A. Susanna, T. F. Stuessy, R. J. Bayer), International Association for Plant Taxonomy, Vienna, Austria, **2009**, pp. 293–313.
- [3] E. Yeşilada, E. Sezik, G. Honda, Y. Takaishi, Y. Takeda, T. Tanaka, *J. Ethnopharmacol.* **1999**, *64*, 195–210. [https://doi.org/10.1016/S0378-8741\(98\)00133-0](https://doi.org/10.1016/S0378-8741(98)00133-0)
- [4] R. Arif, E. Küpeli, F. Ergun, *Gazi Üniv. Fen Bil. Dergisi*, **2004**, *17*, 149–164.
- [5] T. Baytop, in *Turkey, Past and Present*, 2<sup>nd</sup> Ed., Nobel Publishers, Istanbul, Turkey, **1999**.
- [6] A. Khammar, S. Djeddi, *Eur. J. Sci. Res.* **2012**, *84*, 398–416.
- [7] J. F. Severino, K. Stich, G. Soja, *Environ. Pollut.* **2007**, *146*, 707–714. <https://doi.org/10.1016/j.envpol.2006.04.006>
- [8] C. Karamenderes, S. Khan, B. L. Tekwani, M. R. Jacob, I. A. Khan, *Pharm. Biol.* **2006**, *44*, 534–539. <https://doi.org/10.1080/13880200600883080>
- [9] M. Shoeb, S. M. MacManus, M. Jaspars, J. Trevidu, L. Nahar, P. Kong-Thoo-Lin, S. D. Sarker, *Tetrahedron* **2006**, *62*, 11172–11177. <https://doi.org/10.1016/j.tet.2006.09.020>
- [10] C. Koukoulitsa, G. D. Geromichalos, H. Skaltsa, *J. Comput.-Aided Mol. Des.* **2005**, *19*, 617–623. <https://doi.org/10.1007/s10822-005-9018-y>
- [11] G. Stamatis, P. Kyriazopoulos, S. Golegou, A. Basayiannis, S. Skaltsas, H. Skaltsa, *J. Ethnopharmacol.* **2003**, *88*, 175–179. [https://doi.org/10.1016/S0378-8741\(03\)00217-4](https://doi.org/10.1016/S0378-8741(03)00217-4)
- [12] N. Garbacki, V. Gloaguen, J. Damas, P. Bodart, M. Tits, L. Angenat, *J. Ethnopharmacol.* **1999**, *68*, 235–241. [https://doi.org/10.1016/S0378-8741\(99\)00112-9](https://doi.org/10.1016/S0378-8741(99)00112-9)
- [13] G. Rusak, M. Krajacic, N. Plese, *Antiviral Res.* **1997**, *36*, 125–129. [https://doi.org/10.1016/S0166-3542\(97\)00045-4](https://doi.org/10.1016/S0166-3542(97)00045-4)
- [14] L. A. Craig, N. S. Hong, R. J. McDonal, *Neurosci. Biobehav. R.* **2011**, *35*, 1397–1409. <https://doi.org/10.1016/j.neubiorev.2011.03.001>
- [15] C. Costagli, A. Galli, *Biochem Pharmacol.* **1998**, *55* (10), 1733–1737. [https://doi.org/10.1016/S0006-2952\(97\)00676-X](https://doi.org/10.1016/S0006-2952(97)00676-X)
- [16] I. Carev, A. Maravic, M. Bektasevic, M. Ruscic, S. Siljak-Yakovlev, O. Politeo, *Croat. Chem. Acta* **2018**, *91*, 11–18. <https://doi.org/10.5562/cca3205>
- [17] I. Carev, M. Ruscic, M. Skocibusic, A. Maravić, S. Siljak-Yakovlev, O. Politeo, *Chem. Biodivers.* **2016**, *14*, e1600213. <https://doi.org/10.1002/cbdv.201600213>
- [18] O. Politeo, M. Skocibusic, I. Carev, F. Burcul, I. Jerkovic, M. Sarolic, M. Milos, *Nat. Prod. Comm.* **2012**, *7*, 1087–1090. <https://doi.org/10.1177%2F1934578X1200700831>
- [19] J. F. Clevenger, *J. Am. Pharm. Assoc.* **1928**, *17*, 346.
- [20] R. P. Adams, *Identification of essential oil components by gas chromatography / mass spectrometry*, 4<sup>th</sup> Ed, Allured Publ Corp Carol Stream, IL, USA., **2005**.
- [21] P. J. Linstrom, W. G. Mallard, *NIST Chemistry WebBook*, NIST Standard Reference Database Number 69. **2014**.
- [22] W. Brand-Williams, M. E. Cuvelier, C. Berset, *Food Sci. Tech.* **1995**, *28*, 25–30. [https://doi.org/10.1016/S0023-6438\(95\)80008-5](https://doi.org/10.1016/S0023-6438(95)80008-5)
- [23] G. L. Ellman, K. D. Courtney, V. Andres, R. M. Featherstone, *Biochem. Pharmacol.* **1961**, *7*, 88–90. [https://doi.org/10.1016/0006-2952\(61\)90145-9](https://doi.org/10.1016/0006-2952(61)90145-9)
- [24] T. Milosevic, C. Argyropoulou, S. Solujic, D. Murat-Spahic, *Nat. Prod. Comm.* **2010**, *5*, 1663–1668. <https://doi.org/10.1177%2F1934578X1000501030>
- [25] J. Novakovic, N. Rajcevic, S. Milanovici, P. D. Marin, P. Janackovic, *Chem. Biodivers.* **2016**, *13*, 1221–1229. <https://doi.org/10.1002/cbdv.201600029>
- [26] O. Kilic, E. Bagci, *J. Essent. Oil Bear. Pl.* **2016**, *19*, 185–193. <https://doi.org/10.1080/0972060X.2014.885315>
- [27] A. Maggio, L. Riccobono, S. Bancheva, M. Bruno, F. Senatore, *Nat. Prod. Commun.* **2014**, *9*, 1373–1376. <https://doi.org/10.1177%2F1934578X1400900938>
- [28] S. B. Erel, B. Demirci, S. Demir, C. Karaalp, H. H. C. Beser, *J. Ess. Oil. Res.* **2013**, *25*, 79–84. <https://doi.org/10.1080/10412905.2012.755476>
- [29] G. Zengin, A. Aktumsek, G. O. Guler, Y. S. Cakmak, Y. Kan, *Nat. Prod. Res.* **2012**, *26*, 1–10. <https://doi.org/10.1080/14786419.2010.527839>
- [30] C. Formisano, D. Rigano, F. Senatore, S. Bancheva, M. Bruno, A. Maggio, S. Rosselli, *Nat. Prod. Commun.* **2011**, *6*, 1339–1342. <https://doi.org/10.1177%2F1934578X1100600930>
- [31] F. Senatore, N. A. Arnold, M. Bruno, *Nat. Prod. Res.* **2005**, *19*, 749–754. <https://doi.org/10.1080/14786410412331302136>
- [32] A. Esmaeilli, A. Rustaiyan, M. Nadimi, *J. Ess. Oil. Res.* **2005**, *17*, 539–541. <https://doi.org/10.1080/10412905.2005.9698988>
- [33] H. Dural, Y. Bagci, K. Ertugrul, H. Demirelma, G. Flamini, P. L. Cioni, L. Morelli, *Biochem. Syst. Ecol.* **2003**, *31*, 1417–1425. [https://doi.org/10.1016/S0305-1978\(03\)00128-5](https://doi.org/10.1016/S0305-1978(03)00128-5)

- [34] F. Senatore, D. Rigano, R. de Fusco, M. Bruno, *Flavour Frag. J.* **2003**, *18*, 248–251.
- [35] M. Bruno, A. Milia, G. Catinella, S. Bancheva, *Nat. Prod. Commun.* **2018**, *13*, 1179.  
<https://doi.org/10.1177%2F1934578X1801300920>
- [36] L. Riccobono, Maggio, M. Bruno, S. Bancheva, O. Santucci, F. Senatore, *Plant Biosist.* **2017**, *51* (6), 1035–1044.  
<https://doi.org/10.1080/11263504.2016.1219419>
- [37] C. Formisano, F. Senatore, S. Bancheva, M. Bruno, S. Roselli, *Croat. Chem. Acta* **2010**, *83*, 403–408.
- [38] C. Formisano, F. Senatore, S. Bancheva, M. Bruno, A. Maggio, S. Roselli, *Nat. Prod. Commun.* **2010**, *5*, 1649–1654.  
<https://doi.org/10.1177%2F1934578X1000501027>
- [39] F. Bakkali, S. Averbeck, D. Averbeck, M. Idaomar, *Food Chem. Toxicol.* **2008**, *46*, 446–475.  
<https://doi.org/10.1016/j.fct.2007.09.106>
- [40] F. Burčul, I. Blažević, M. Radan, O. Politeo, *Curr. Med. Chem.* **2019** (Ahead of Print).  
<https://doi.org/10.2174/0929867325666180330092607>