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Chemical composition and antimicrobial and antioxidant activity of *Helichrysum italicum* (Roth) G.Don subspecies essential oils

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Abstract: The chemical composition and the antimicrobial and antioxidant activity of essential oils from two *Helichrysum italicum* (Roth) G.Don subspecies grown in Bulgaria were investigated. As a result, 95 compounds with concentrations above 0.05% were detected by GC/MS and 46 of them, mainly mono- and sesquiterpenes, representing 79.81% and 85.51% of the total content of the samples, were identified. The main constituents of the essential oil from *Helichrysum italicum* subsp. *microphyllum* (plant origin from Bosnia) were monoterpene α-pinene (20.84%) and sesquiterpene γ-curcumene (16.53%), followed by β-selinene (5.59%), *ar*-curcumene (4.39%), *trans*-caryophyllene (4.35%), β-diketone italidione I (4.32%), α-selinene (4.28%), and neryl acetate (3.81%). The sesquiterpene hydrocarbons were the dominant groups of chemical constituents in the essential oil, followed by the oxygenated aliphatic hydrocarbons. The main constituents in the *H. italicum* essential oil (plant origin from France) were neryl acetate (33.87%), γ-curcumene (8.84%), rosifoliol (5.46%), geranyl propionate (4.98%), *αr*-curcumene (4.31%), italidione I (3.56%), α-eudesmol (3.19%), and limonene (3.02%). The main class of chemical compounds was the oxygenated monoterpenes, followed by sesquiterpene hydrocarbons. *H. italicum* essential oil from France showed more pronounced antimicrobial activity against the Gram-positive bacteria *Staphylococcus aureus* and *Bacillus subtilis*, and the fungus *Aspergillus brasiliensis*, as well as stronger antioxidant potential.

Keywords: *Helichrysum italicum* (Roth) G.Don, essential oils, gas chromatography-mass spectrometry, chemical profile, antibacterial, antioxidant activity

1. Introduction

The genus *Helichrysum* (Asteraceae) is widespread throughout the world. Its species are naturally occurring mainly in the Mediterranean area. The essential oil that is found mainly in the green parts of the plants has wide usage in folk medicine and other areas of life (Mastelic et al., 2005).

Helichrysum italicum (Roth) G.Don, also known as immortelle or everlasting, is revered for its antiinflammatory and anticancer properties, and has been used in traditional medicine as a source of choleretic, diuretic, and expectorant material (Chinou et al., 1996).

The chemical profile of species distributed in different parts of the European countries has been studied by a number of authors (Chinou et al., 1996; Kladar et al., 2005; Mastelic et al., 2005; Cristofari et al., 2012). The main factors, affecting chemical composition of the essential oil, are the possible diversity of the plant genotype, the geographic origin and climatic conditions.

The main constituents found in the essential oil of *H. italicum* subsp. *microphyllum*, growing mainly in France, were reported in previous studies as neryl acetate (28.9%), rosifoliol (20.2%), neryl propionate (11.4%), γ-curcumene (11.4%–18.2%), linalool (14.9%), and nerol (10.7%) (Satta et al., 1999); neryl acetate (16.9%–21.4%), dihydro-occidentalil (7.6%–12.7%), nerol (5.4%–7.3%), and neryl propionate (4.6%–5.6%) (Marongiu et al., 2003); neryl acetate (33.3%) and γ-curcumene (8.6%) (Paolini et al., 2006); neryl acetate (17.6%–56.1%), eudesmen-5-en-11-ol (3.7%–23.5%), and nerol (3.7%–14.4%) (Usai et al., 2010); and isopropyl tetradecanoate (12.10%), α-pinene (12.02%), hexadecanoic acid (9.96%), (E)-caryophyllene (9.22%), ledol (9.11%), palustrol (5.55%), α-humulene

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(4.28%), caryophyllene (3.75%), and α-copaene (3.72%) (Bouchaala et al., 2016).

The composition of *H. italicum* subsp. *italicum* essential oil from plants grown in Italy has been observed as follows: neryl acetate (31%), γ-curcumene (10.7%), and neryl propanoate (5.1%) (Cristofari et al., 2012); nonyl acetate (10.5%), carvacrol (9.8%), and nonyl propanoate (6.8%); and *ar*-curcumene (6.4%) (Morone-Fortunato et al., 2010). Leonardi et al. (2013) also analyzed the composition of twenty-one essential oil samples isolated from *H. italicum* harvested in seven locations of Elba Island in Tuscany, Italy, that were characterized by different soil types, during three different periods (January, May, and October 2010). According to their findings, the oils were observed to contain a significant proportion of oxygenated monoterpenes (38.6%–62.7%), although other components in those oils such as monoterpene and sesquiterpene hydrocarbons were only observed in minor quantities of 2.3%–41.9% and 5.1%–20.1% of the identified constituents, respectively. The main oxygenated derivatives were reported to be nerol (2.8%–12.8%) and its ester derivative neryl acetate (5.6%–45.9%). *H. italicum*, unspecified subspecies, is largely distributed in western Balkan countries, and its essential oils are dominated by α-pinene (10.2%–29.9%), neryl acetate (4.1%–13.5%), and lower levels of γ-curcumene (0%–22%) (Blažević et al., 1995; Mastelic et al., 2005).

Helichrysum picardii, cultivated in Portugal, is dominated by α-pinene (53.5%) and γ-curcumene (27.4%) (Costa et al., 2015).

It has been shown that *Helichrysum* plants were used in pharmaceuticals and medicine because of their proven antiinflammatory (Sala et al., 2003), antioxidant (Rosa et al., 2007), antimicrobial, antiviral (Nostro et al., 2003), and anti-HIV (Appendino et al., 2007) properties. *H. arenarium* L. grows in Bulgaria, mainly in dry, rocky, or sandy areas, such as coastal parts of the Black Sea, the Danubian Plain (central part of the country), northeastern Bulgaria, and some parts of southeastern Bulgaria. Data are available on the use of this perennial herb in Bulgarian folk medicine (Petkov, 1982).

Despite being one of the most wide-ranging species, *H. italicum* has only recently been cultivated in Bulgaria as a crop for industrial processing and obtaining essential oil by importing plant seeds from different European countries. Planting material from Bosnia (*H. italicum*, unspecified subspecies) has been imported for cultivation and the plants have been harvested in the experimental field of the Institute of Rose, Essential and Medical Plants, Kazanlak. In the vicinity of the town immortelle plants originating from France have been also cultivated (*H. italicum* subsp. *microphyllum*). The town of Kazanlak is located in the Rose Valley, which is characterized by a transitional continental climate, with long-lasting colds and droughts. The soil in this region is low in humus and calcium, making this plant suitable for cultivating in this region.

The objective of this study was to investigate the chemical composition and properties of essential oils from two immortelle (*H. italicum*) subspecies with their potential for cultivation in Bulgaria and the applicability of the essential oil in cosmetics, perfumery, and pharmacy.

2. Materials and methods

2.1. Plant material

The immortelle planting materials, originating from France and Bosnia, were cultivated and harvested in a phase of mass flowering in the area of Kazanlak in 2017. The moisture of the plants (71.2–72.3 ± 0.07%) was determined by drying up to constant weight at 105 °C (State Pharmacopoeia of the USSR, 1990). The immortelle was cultivated on cinnamon forest soil with heavy mechanical composition, acidic pH 5.6, and poor nutrients (poor in nitrogen and humus, medium content of phosphorus, well stocked with potassium). Plant fertilization was performed with phosphorus as P_2O_5 (10 kg) before planting and nitrogen as NO_3 (8 kg) by the first soil tillage in spring. The planting scheme was 0.9 m between rows and 0.3 m between plants, or 3700 plants per acre.

2.2. Essential oils

The essential oil was isolated from 100 g of flowers by hydrodistillation for 3 h in a laboratory glass apparatus of the British Pharmacopoeia, modified by Balinova and Diakov (1974). The oil obtained was dried over anhydrous sodium sulfate and stored in tightly closed dark vials at 4 °C until analysis. The essential oil yields are represented on an absolute dry weight basis. In order to facilitate the discussion below, the essential oil isolated from *H. italicum* subsp. *microphyllum* (plant originating from France) is called immortelle essential oil originating from France, as is also the case for the plant originating from Bosnia.

2.3. Analysis

2.3.1. Gas chromatography-mass spectrometry (GC/MS)

The GC/MS analysis was performed on a HP 6890 GC System Plus gas chromatograph coupled to a HP 5973 MSD mass selective detector (Hewlett Packard, Palo Alto, CA, USA). An ultrainert nonpolar DB-5ms UI capillary column (J&W Scientific, Folsom, CA, USA) with 60 m column length, 0.25 mm i.d., and 0.25 μm film thickness was used. The oven temperature was programmed from 60 °C to 100 °C at a rate of 4 °C /min, from 100 °C to 155 °C at a rate of 2.5 °C /min, and from 155 °C to 220 °C at a rate of 4 °C/min (held for 10 min at the final temperature). Helium (99.999%) was used as a carrier gas at a constant flow rate of 0.8 mL/min. The split ratio was 1:125, the inlet temperature was set to 260 °C, and the transfer line

temperature was 280 °C. The mass selective detector was operated in electron impact ionization (EI) mode at 70-eV electron energy, the ion source temperature was set to 230 °C, and the quadrupole temperature was 150 °C. The mass scan range was 30–450 m/z. Instrument control and data collection were carried out using MSD Productivity ChemStation (E.02.02 SP2, Agilent Technologies).

2.3.2. Identification and quantitative analysis

The identification of the compounds was performed using commercial mass spectral libraries (NIST 05, Wiley 7th Mass Spectra Register) and retention times (linear retention indices, LIRs). In cases of lacking the corresponding reference data, the structures were proposed based on their general fragmentation and/or using reference literature mass spectra. For the quantitation, the normalization method (relative percentage, based on the total ion current (TIC) area) was used. Taking into account that TIC depends on the structural characteristics of the individual compounds, the GC/MS data are considered as semiquantitative.

2.4. Antimicrobial activity of the essential oils

The antibacterial activity of immortelle (*H. italicum*) essential oils was tested against test microorganisms provided by the National Bank for Industrial Microorganisms and Cell Cultures in Sofia, Bulgaria: Gram-positive bacteria *Staphylococcus aureus* ATCC 6538 and *Bacillus subtilis* ATCC 6633, Gram-negative bacteria *Escherichia coli* ATCC 8739 and *Pseudomonas aeruginosa* ATCC 9027, yeasts *Saccharomyces cerevisiae* ATCC 9763 and *Candida albicans* ATCC 10231, and fungal strains: *Aspergillus brasiliensis* ATCC 16404 and *Fusarium moniliforme* (clinical isolate).

The antimicrobial activity was determined by the agar well diffusion method with a well size of 8 mm. The growth media were tryptic soy agar (Merck) for the tested bacterial strains and Sabouraud dextrose agar (Merck) for the yeast and fungi. The media were inoculated with a 24-h suspension of the bacterial species with a density of approximately 10^7 cfu/mL (turbidity: 0.5 McFarland standard). Media melted and cooled to 50 °C were inoculated with the tested microorganisms and then equally dispensed into petri dishes. Next, a hole with a diameter of 8 mm was punched aseptically with a sterile cork borer, and 50 µL of the antimicrobial agent was introduced into the well. After that, the agar plates were incubated at 37 °C or 28 °C for 24 h or 72 h according to the microbial species. After cultivation, the distinct zone of the growth inhibition around the wells was measured using a digital caliper. The diameter of the zones, including the diameter of the well, was recorded in mm; for instance, at up to 15 mm the microbial culture was poorly sensitive, from 15 to 25 mm it was considered sensitive, and over 25 mm it was considered as very sensitive. The tests were performed in parallel with solvent controls (Tajkarimi et al., 2010).

2.5. Antioxidant activity of essential oils

2.5.1. ABTS assay

The Trolox equivalent antioxidant capacity (TEAC) was determined by using the colorimetric method reported by Re et al. (1999). For this assay, 2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) cation radical (ABTS+) solution was prepared by dissolving 7 mM ABTS in 2.45 mM $K_2S_2O_8$. This mixture was shaken for 12–16 h at ambient temperature in the dark until obtaining a stable oxidative state. For the study of the oils, the ABTS+ stock solution was diluted with methanol until absorbance became 0.70 ± 0.02 at 734 nm. Sample analysis was performed as follows: 2 mL of ABTS solution and 20 µL of sample or standard were mixed. The absorbance of the sample was measured at 734 nm with a spectrophotometer (Camspec M508, UK) after samples were incubated at 25 °C for 7 min. The calibration curve was plotted using 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox) as a standard. The results were expressed as µmol Trolox equivalents per g of dry weight (µM TE/g DW).

2.5.2. DPPH assay

Antioxidant activity was measured according to the procedure of Brand-Williams et al. (1995). In the test tubes, 100 µL of essential oil or Trolox and 2.9 mL of 0.12 mM DPPH (2,2-diphenyl-1-picrylhydrazyl) reagent, prepared with 4.8 mg of DPPH dissolved in 100 mL of CH_3OH), were mixed. The mixtures were shaken and then incubated for 30 min at room temperature (25 ± 1 °C). The absorbance was recorded at 517 nm with a spectrophotometer (Camspec M508, UK). To quantify the antioxidant activity, a standard Trolox curve (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was used with concentrations from 0.045 to 1.5 mmol Trolox. The results were expressed as µM TE/g dry weight.

IC_{50} (mg/mL) is defined as the concentration of essential oil that causes 50% loss of color. The mean IC_{50} was calculated on the basis of three repetitions and by means of interpolation of graphical dependence of concentration and degree of inhibition of the ABTS and DPPH radical.

2.6. Statistical analysis

All the analyses were performed in triplicate. Statistical differences between samples were tested using ANOVA. Data were expressed as mean ± SD. The level of significance was set at $P < 0.05$.

3. Results and discussion

In this study, the essential oil yield was 0.14 ± 0.00% for the samples from Bosnia and 0.10 ± 0.00% for the immortelle

originating from France. The essential oil yields are higher than those of Kladar et al. (2005), who reported that the average yield of essential oil of the samples was 0.03%. The essential oils have a light yellow color and a characteristic odor.

3.1. Chemical composition

As a result of the GC/MS analysis, 95 compounds with concentrations higher than 0.05% were detected in the immortelle essential oils and 46 of them, mainly mono- and sesquiterpenes, were identified.

The chemical profile of the immortelle essential oils, determined by means of GC/MS (TIC), is shown in Table 1.

The analyzed *H. italicum* essential oil samples, as seen in Figure 1, show similar qualitative content, though with significant quantitative differences, and appear to be representative of two different immortelle chemical phenotypes: the *α*-pinene chemotype (oil from Bosnia) and neryl acetate chemotype (oil from France).

3.1.1. Essential oil from Bosnia

In the framework of the current study, the immortelle essential oil originating from Bosnia was identified with 45 constituents representing 79.81% of the total content. The essential oil was dominated by monoterpene *α*-pinene (20.84%) together with sesquiterpene *γ*-curcumene (16.53%), followed by *β*-selinene (5.59%), *ar*-curcumene (4.39%), *trans*-caryophyllene (4.35%), *β*-diketone italidione I (4.32%), *α*-selinene (4.28%), and neryl acetate (3.81%). According to our results, the sesquiterpene hydrocarbons were the dominant groups of chemical constituents in the essential oil, followed by the oxygenated aliphatic hydrocarbons. The italidiones (a group of isomeric *β*-diketones), found only in *H. italicum* (Morone-Fortunato et al., 2010; Bouchaala et al., 2016), deserve special attention due to their biological activity. The content of italidiones in the analyzed essential oil was found to be 7.39%. The italidiones contribute to the specific fragrance of *H. italicum* and its remarkable antihematoma properties (Bouchaala et al., 2016).

The content of essential components does not differ from that of *H. italicum* subsp. *microphyllum* growing in the western part of Europe (Satta et al., 1999; Marongiu et al., 2003; Paolini et al., 2006; Usai et al., 2010). The difference in the quantities of the identified components can be attributed to the different soil and geographic features in the studied regions.

The differences measured in the quantitative contents of the essential oils were most probably caused by the different environmental conditions (soil, climate, etc.) of the western Balkan Peninsula and Bulgaria.

3.1.2. Essential oil from France

The immortelle essential oil originating from Bosnia was characterized to contain 44 compounds representing 85.51% of the total immortelle essential oil. The main constituents in the immortelle essential oil from France were neryl acetate (33.8%), *γ*-curcumene (8.8%), rosifoliol (5.46%), geranyl propionate (4.98%), *αr*-curcumene (4.31%), italidione I (3.56%), *α*-eudesmol (3.19%), and limonene (3.02%). The oxygenated monoterpenes were the main class of chemical compounds, followed by sesquiterpene hydrocarbons. The total content of italidiones was 6.13%.

The analyzed samples in this study showed a similar chemical profile with identical qualitative composition and only some minor quantitative differences for the unspecified *H. italicum* subspecies originating from western Balkan countries as observed in the literature (Blažević et al., 1995; Mastelic et al., 2005).

The results obtained in this study were not in agreement with the findings of Djihane et al. (2016), who determined that the most oxygenated sesquiterpene compounds were *a*-cedrene (13.61%), *a*-curcumene (11.41%), geranyl acetate (10.05%), limonene (6.07%), nerol (5.04%), neryl acetate (4.91%), and *a*-pinene (3.78%).

3.2. Antimicrobial activity

The results of the antimicrobial assays are presented in Table 2. The immortelle essential oil originating from Bosnia showed inhibitory activity only against *Aspergillus brasiliensis* (Figure 2). All other test microorganisms were resilient against the samples. The essential oil originating from France showed low inhibitory activity against Gram-positive bacteria *Bacillus subtilis* and *Staphylococcus aureus* and possessed a higher zone of inhibition (18.00 mm) against *Aspergillus brasiliensis*. Our results are in agreement with results reported by Mesic et al. (2019) that immortelle essential oil inhibited only Gram-positive bacteria and possessed antifungal effects. The established difference in antimicrobial activity of the two tested essential oils is explained by the different contents of oxygen derivatives that have higher antimicrobial action than hydrocarbons (Baser and Buchbauer, 2010). The results of this study are lower than the findings of Djihane et al. (2016), who reported that *H. italicum* inhibited the growth of *Staphylococcus aureus* ATCC 6538 (27 ± 0.1 mm) and *Bacillus cereus* ATCC 10876 (19 ± 0.2 mm), but not the growth of *E. coli* ATCC 25922, *K. pneumoniae* ATCC 4352, and *L. monocytogenes* ATCC 15313. Our results for antimicrobial activity are consistent with the results of Oji and Shafaghat, who reported a moderate effect on *S. aureus* (12.4 ± 0.15 mm) and *B. subtilis* (14.5 ± 0.12 mm).

3.3. Antioxidant activity

The results of the antioxidant activity are presented in Table 3. The data obtained by both methods showed that the immortelle essential oil originating from France is characterized by higher antioxidant activity compared to

Table 1. Chemical profile of the immortelle essential oils, as determined by GC/MS (TIC)*.

No.	Compounds	LRI, determined on DB-5	Rel.%, as determined by GC/MS (TIC)	
			Plant originating from Bosnia	Plant originating from France
1.	Ethanol	668	0.11 ± 0.01	n.d.
2.	2-Methyl-2-heptene	798	0.06 ± 0.00	0.08 ± 0.00
3.	Nonane	900	0.02 ± 0.00	trace
4.	*α*-Pinene	937	20.84 ± 0.5	1.89 ± 0.02
5.	*α*-Fenchene	941	0.23 ± 0.02	0.35 ± 0.01
6.	Camphene	953	0.11 ± 0.01	0.13 ± 0.00
7.	*β*-Pinene	981	0.41 ± 0.00	0.48 ± 0.01
8.	*α*-Terpinene	1021	0.13 ± 0.00	trace
9.	*p*-Cymene	1029	0.21 ± 0.01	0.26 ± 0.00
10.	Limonene	1035	2.02 ± 0.03	3.02 ± 0.03
11.	1,8-Cineole	1038	0.17 ± 0.02	0.14 ± 0.00
12.	i-Butyl angelate (2-Methyl-2-butenoic acid, i-butyl ester)	1048	0.16 ± 0.02	0.20 ± 0.02
13.	*γ*-Terpinene	1062	0.32 ± 0.00	0.21 ± 0.00
14.	2-Nonanone	1091	n.d.	0.19 ± 0.02
15.	*α*-Terpinolene	1092	0.15 ± 0.02	0.18 ± 0.02
16.	Linalool	1102	0.33 ± 0.01	1.58 ± 0.01
17.	Nonanal	1106	0.07 ± 0.03	n.d.
18.	iso-Pentyl angelate	1150	0.44 ± 0.00	0.51 ± 0.00
19.	Nerol oxide	1155	trace	0.12 ± 0.00
20.	*n*-Pentyl angelate	1163	trace	trace
21.	Unknown *β*-diketone	1185	0.12 ± 0.01	0.41 ± 0.01
22.	4-Terpineol	1190	0.07 ± 0.01	0.08 ± 0.00
23.	*α*-Terpineol	1204	0.10 ± 0.02	0.15 ± 0.00
24.	Decanal	1210	0.22 ± 0.01	trace
25.	Nerol	1228	0.15 ± 0.01	1.16 ± 0.01
26.	n-Hexyl angelate	1283	0.27 ± 0.00	trace
27.	Undecanone	1294	trace	0.06 ± 0.00
28.	Tridecane	1300	0.26 ± 0.03	0.09 ± 0.00
29.	Neryl acetate	1359	3.81 ± 0.02	33.87 ± 0.30
30.	*α*-Copaene+Unknown**	1386	1.47 ± 0.04	0.11 ± 0.00
31.	Curcumene (isomer)	1388	0.60 ± 0.03	0.79 ± 0.02
32.	*cis*-*α*-Bergamotene	1420	0.48 ± 0.00	0.44 ± 0.00
33.	*trans*-*β*-Caryophyllene	1433	4.35 ± 0.10	0.34 ± 0.00
34.	Italidione I (m/z 210)	1437	4.32 ± 0.05	3.56 ± 0.09
35.	Geranyl propionate	1448	0.66 ± 0.01	4.98 ± 0.05
36.	*α*-Humulene+*β*-Farnesene**	1468	0.75 ± 0.05	0.15 ± 0.00
37.	Italidione II (m/z 224)+*α*-Farnesene**	1481	3.07 ± 0.04	2.57 ± 0.03
38.	*γ*-Curcumene	1487	16.53 ± 0.12	8.84 ± 0.09
39.	*αr*-Curcumene	1489	4.39 ± 0.03	4.31 ± 0.03
40.	*β*-Selinene	1504	5.59 ± 0.01	0.32 ± 0.00

Table 1. (Continued).

41.	α-Selinene	1508	4.28 ± 0.01	0.60 ± 0.01
42.	*d*-Cadinene	1527	0.99 ± 0.02	trace
43.	Guaiol	1597	0.36 ± 0.01	1.93 ± 0.01
44.	Rosifoliol	1628	1.27 ± 0.02	5.46 ± 0.05
45.	β-Eudesmol	1648	0.48 ± 0.01	1.62 ± 0.01
46.	α-Eudesmol	1673	0.42 ± 0.01	3.19 ± 0.03
Total, %			79.81	85.51
Monoterpene hydrocarbons, %			24.06	3.82
Oxygenated monoterpenes, %			5.12	42.08
Sesquiterpene hydrocarbons, %			39.43	19.9
Oxygenated sesquiterpenes, %			3.53	12.12
Oxygenated aliphatic hydrocarbons, %			8.67	7.51

*Total ion current (TIC) depends on the characteristics of the compound and therefore GC/MS data are considered as semiquantitative.
**Co-eluting components on DB-5ms column; trace < 0.01%; n.d. - not detected.

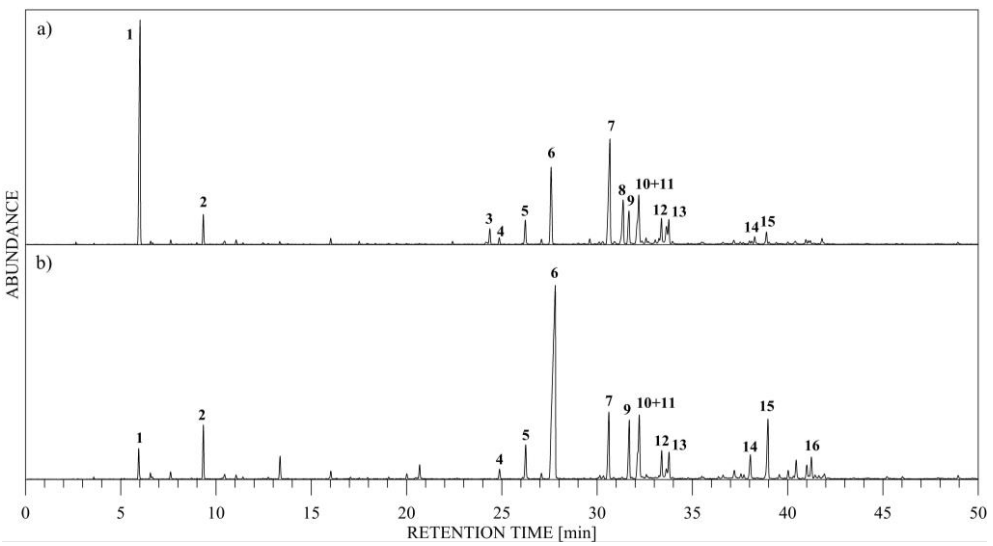

Figure 1. GC/MS (TIC) chromatogram of *H. italicum* samples: a) plants originating from France; b) plants originating from Bosnia. 1- α-Pinene, 2- limonene, 3- unknown, 4- linalool, 5- nerol, 6- neryl acetate, 7- γ-curcumene, 8- β-selinene, 9- italidione I, 10- *ar*-curcumene, 11- α-selinene, 12- italidione II, 13- guaiol, 14- rosifoliol, 15- β-eudesmol, 16-α-eudesmol.

that originating from Bosnia due to the difference in their chemical composition. In general, the results of this study are in line with the results from the literature (Sala et al., 2003; Rosa et al., 2007). Our results are not consistent with the findings of Ebrahimzadeh and Tavassoli (2015) for *Helichrysum pseudoplicatum* extract, as they reported that the total amount of phenolic compounds was determined as gallic acid equivalents of 22.7 ± 3.1 mg/g and the IC_{50} for DPPH radical-scavenging activity was 438.9 ± 15.6 µg/mL.

3.4. Conclusions

Subspecies of *Helichrysum italicum* originating from France and Bosnia were considered as potential material for cultivation and essential oil production. The immortelle essential oil originating from France was found to be rich in oxygenated monoterpenes (42.08%), while the samples derived from the plants originating from Bosnia are rich in sesquiterpene hydrocarbons (39.43%). Considering the influence of the chemical composition on the antioxidant

Table 2. Antimicrobial activity of immortelle essential oils.

Test microorganisms	Inhibition zone, mm	
	Plant originating from Bosnia	Plant originating from France
Staphylococcus aureus ATCC 6538	8.00 ± 0.00	11.30 ± 0.10
Bacillus subtilis ATCC 6633	8.00 ± 0.00	9.20 ± 0.08
Escherichia coli ATCC 8739	8.00 ± 0.00	8.00 ± 0.00
Pseudomonas aeruginosa ATCC 9027	8.00 ± 0.00	8.00 ± 0.00
Saccharomyces cerevisiae ATCC 9763	8.00 ± 0.00	8.00 ± 0.00
Candida albicans ATCC 10231	8.00 ± 0.00	8.00 ± 0.00
Aspergillus brasiliensis ATCC 16404	17.41 ± 0.16	18.00 ± 0.16
Fusarium moniliforme (clinical isolate)	8.00 ± 0.00	8.00 ± 0.00

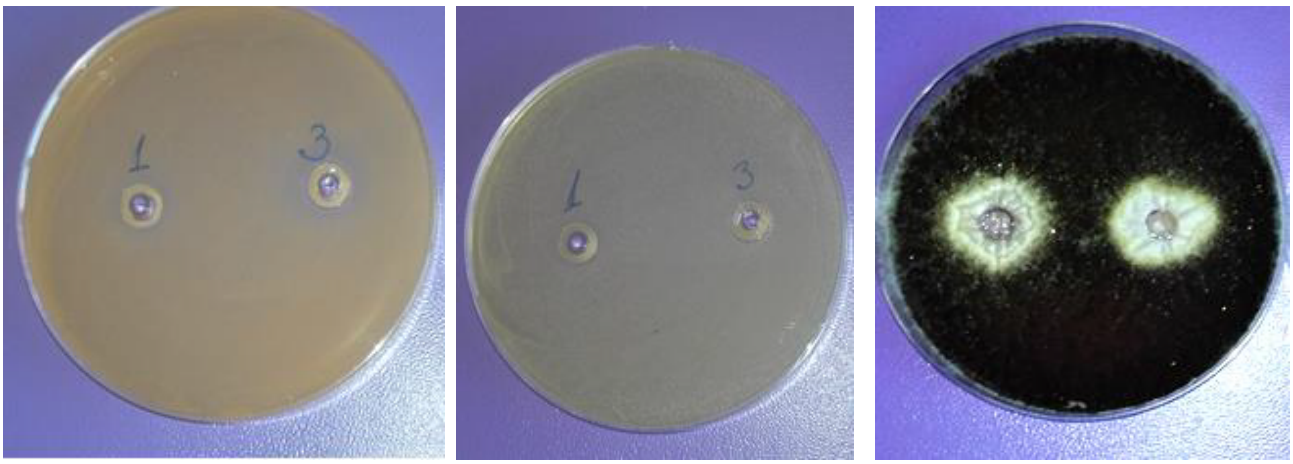

Figure 2. Antimicrobial activity against a) *Staphylococcus aureus*, b) *Bacillus subtilis*, and c) *Aspergillus brasiliensis*; 1 - originating from Bosnia, 3 - originating from France.

Table 3. Antioxidant activity of immortelle essential oils.

H. italicum	ABTS		DPPH	
Samples	μM TE/g DW	IC_{50}, mg/mL	μM TE/g DW	IC_{50}, mg/mL
Plant originating from Bosnia	35.64 ± 0.32	3.03 ± 0.03	0.16 ± 0.00	261.40 ± 1.91
Plant originating from France	52.42 ± 0.50	1.97 ± 0.01	0.52 ± 0.00	82.64 ± 0.80

and antimicrobial potential, it can be concluded that the *H. italicum* essential oil from France exhibits higher antimicrobial activity against Gram-positive bacteria and fungi, as well as higher phenolic content. Therefore, it can be considered as a valuable source of natural antioxidants in various pharmaceutical and cosmetic formulations.

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