CHEMICAL COMPOSITION AND ANTIOXIDANT AND ANTIMICROBIAL ACTIVITY OF *HELICHRYSUM ITALICUM* (ROTH) G. DON. ESSENTIAL OIL FROM BOSNIA AND HERZEGOVINA

ORIGINAL SCIENTIFIC PAPER

Jasna Džananović Jaganjac[1]✉, Zahida Ademović[2], Broza Šarić Kundalić[1], Emir Horozić[2]

DOI: 10.5281/zenodo.5520600

RECEIVED 2021-07-05 **ACCEPTED** 2021-08-27

[1] Faculty of Pharmacy, University of Tuzla, Tuzla, Bosnia and Herzegovina
[2] University of Tuzla, Faculty of Technology, Urfeta Vejzagića 8, 75000 Tuzla, Bosnia and Herzegovina
✉ jasna.dzananovic@gmail.com

ABSTRACT:
In this study, the chemical profiles, antioxidant and antibacterial activity of *Helivhrysum italicum* essential oils from three plantation fields in Herzegovina were analysed. GC/MS analysis showed that all samples were rich in sesquiterpenes (45.19%-50.07%) and monoterpenes (21.15%-23.21%), followed by oxygenated monoterpenes (9.92%-14.03%). Diketones in the essential oil were detected in quantities ranging 5.72% to 6.67%. The main components in essential oils were γ-curcumene, α-pinene, β-selinene and neril-acetate. All tested essential oils exhibited relatively weak DPPH·-scavenging capacity. The antimicrobial activity of the essential oil was assayed by using the disk diffusion method. *E. coli* was most resistant against all three tested *H. italicum* essential oils, while moderate inhibitory activity against *S. aureus* and *C. albicans* was detected. The *L. monocytogenes* was the most sensitive where all three tested samples showed inhibitory activity.

KEYWORDS: *Helichrysum italicum*, essential oils, chemical profile, antioxidant activity, antibacterial activity

INTRODUCTION

Genus *Helichrysum* is a medicinal aromatic plant that belongs to the family *Asteraceae* and includes over 600 species distributed mainly in the Mediterranean area, at sea level up to 1700 m and growing preferably on sandy or loamy soils [1]. *Helichrysum italicum* (Roth.) G. Don. subsp. *italicum* is one of the most common species

The essential oil that is found mainly in the green parts of the plants is widely used in folk medicine as a source of choleretic, diuretic and expectorant material [2,3]. Also, it is known that the essential oil and extracts of *H. italicum* show antioxidant, antibacterial, anti-inflammatory, antialergic and antiviral properties [4-6].

The application of essential oil of immortelle depends on chemical composition of essential oil, which is affected by the plant genotype, the geographic origin and climatic conditions [7]. Three different chemotypes for the essential oils of *H. italicum* ssp. *Italicum* are reported: (I) a genotype rich in nerol and its esters; (II) a genotype with a dominance of α- and β-selinene; (III) a genotype with high amounts of γ-curcumene [8]. It was found that main components of *H. italic* essential oils from the Mediterranean part of Croatia are α-pinene, neryl acetate, α-cedrene, nerol, α-curcumene, γ-curcumene and geranyl acetate [9].

H. italicum has only recently been cultivated in Bosnia and Herzegovina (B&H) as a crop for industrial processing and for production of essential oil [10]. Previous *H. italicum* essential oil studies from the area of Bosnia and Herzegovina showed the differences in the chemical composition depend on the growing stage and the part of plants [11,12]. It was shown that the essential oils collected from wild growing *H. italicum* in B&H contained mainly monoterpenes and sesquiterpenes, followed by diketones, non-terpene esters and non-terpene ketones, which indicated that the essential oils from B&H are similar to those from Croatia and southeast Italy in their chemical composition [12].

The aim of this study was to determine the chemical composition and antioxidant and antimicrobial activity of *H. italicum* essential oils cultivated at three different locations in Bosnia and Herzegovina.

MATERIALS AND METHODS

PLANT MATERIAL

H. italicum plants were collected in June 2019 from three plantation fields in Herzegovina region at three locations in municipalities Stolac (Sample 1), Grude (Sample 2) and Posusje (Sample 3). Samples of collected plant material were identified and stored at Faculty of Pharmacy, University of Tuzla. The dried aerial parts of the plants, cut into small pieces, were water-distilled in semi-industrial distillation equipment. The essential oil was stored in a sealed vial at 4°C until the analysis.

STANDARDS AND REAGENTS

The reagents and chemicals used for the analysis were analytically pure. 2,2-diphenyl-1-picrylhydrazyl (DPPH), $FeSO_4x7H_2O$, $FeCl_3{\times}6H_2O$, HCl and methanol were obtained from Sigma-Aldrich (Germany) and 2,4,6-Tris (2-pyridyl)-s-triazine (TPTZ) was obtained from Himedia (India). FRAP reagent was prepared using the acetate buffer + TPTZ reagent + $FeCl_3$ x $6H_2O$ in a ratio of 10: 1: 1.

MICROBIAL STRAINS

The antimicrobial activity of essential oils was evaluated using the laboratory control strains: *Staphylococcus aureus* ATCC 25923, *Listeria monocytogenes* ATCC 19118, *Escherichia coli* ATCC 25922 and *Candida albicans.* Positive controls were Kanamycin for *S. aureus* and *L. monocytogenes* and Chloramphenicol for *E. coli.* Nystatin was positive control for *C. albicans.*

ANALYSIS

GC-MS method

The gas chromatograph-mass spectrometer GC-MS analysis was performed on a Shimadzu GC/MS QP 2010 Ultra GC with GC FID 2010 Plus-flame ionization detector. Operating conditions were as follows: column HP-5MS (30 m, internal diameter 0.32 mm, coating thickness 0.25 μm). Helium was carrier gas with a flow rate of 3 mL/min. Injector temperature was 250°C, injection volume 1 μL in split mode (1:50). The percentage share of individual components in each sample was calculated over the surface of the GC peak, and the percentages of components were calculated as mean values from duplicate samples of essential oil.

Antioxidant activity by DPPH

Samples were dissolved in 100 ml of methanol. A series of dilutions of different concentrations were made by adding different volumes of the essential oil stock solution and filling with methanol to 2 ml. Then, 500 μl of DPPH reagent solution was added to the solution. The mixture was incubated for 30 min in the dark at room temperature. The absorbance is measured against the blank (methanol) at 517 nm. The results are expressed as the IC_{50} value (mg/ml) or the concentration of extract that caused the 50% neutralization of DPPH radicals. Vitamin C was used as the comparator of the results of antioxidant activity.

Antimicrobial activity of the essential oils

The antibacterial activity of the essential oils was tested using the agar diffusion method according to the American Clinical Laboratory Standards Institute (CLSI) [13]. Mueller-Hinton agar (15 ml), sterilized in a flask and cooled up to 45–50 °C, was distributed to the sterilized Petri dishes. The sterile swab was used to transfer the bacterial suspension and inoculate the bacteria on the surface of Mueller-Hinton agar. The wells with a diameter of 10 mm were cut with the sterile stainless steel borer down to the plastic into Mueller-Hinton agar plates and then filled with a volume of 20, 50, and 100 μl of the extracts samples. The Petri dishes were incubated at 37 °C for 24 h. All experiments were performed in triplicate for each tested sample of essential oils and each microbial strain and the results were recorded as the mean. Data were analysed unidirectional using ANOVA analysis. The antimicrobial activity was measured on the basis of the diameter of the growth inhibition as follows: (<10 mm) – no antimicrobial activity; (10-15 mm) – weak antimicrobial activity; (16-20 mm) – moderate antimicrobial activity; (>20 mm) – strong antimicrobial activity [14]. Kanamycin, chloramphenicol, and nystatin (10.0 mg/mL) were used as references.

Statistical analysis

All the analyses were performed in triplicate. Statistical differences between samples were tested using ANOVA.

RESULTS AND DISCUSSION

THE CHEMICAL COMPOSITION

The chemical composition of the tested *H. italicum* essential oils characterized by GC/MS is presented in Table 1. A total of 45 compounds with a percentage share above 0.10% were identified, representing 92.65-93.52 % of the whole oil composition of the essential oils.

Table 1. The identified components and the percentage share in the sample. Components with a proportion less than 0.10% are not shown

Number	Component	RI	Sample 1 (%)	Sample 2 (%)	Sample 3 (%)
1	3- hexanone	914			0.11
2	α-pinene	937	17.83	15.96	17.98
3	Fenchen	941	0.33	0.26	0.38
4	Camphene	953	0.11	0.10	0.14
5	β-pinene	981	0.39	0.46	<0.10%
6	α-terpinene	1021	0.15	0.23	0.17
7	β-mircene	1024	<0.10%	0.11	<0.10%
8	Hexenil-acetat	1027	<0.10%	0.11	<0.10%
9	p-cimene	1029	0.13	0.12	0.19
10	Limonene	1035	2.23	3.26	3.17
11	1.8-cineole	1038	0.31	0.29	0.34
12	Izobutil angelat	1048	0.17	0.38	0.28
13	γ-terpinene	1061	0.38	0.47	0.47
14	α-terpinolene	1090	0.15	0.18	0.17
15	2-nanonene	1091	0.56	1.17	1.04
16	Methyl-butyrate	1097	0.18	0.21	0.16
17	Mt 170, Dione 2.4 dim	1155	0.60	1.23	0.85
18	Terpinene 4-ol	1172	0.18	0.26	0.28
19	3.4 octadion	1185	0.25	0.40	0.33
20	α-terpineole	1194	0.22	0.31	0.32
21	Nerol	1225	0.57	0.50	1.18
22	3.5-heptadione	1283	<0.10%	0.10	0.10
23	Neryl-acetate	1357	7.91	7.55	10.68
24	α-ylangene	1368	0.31	0.33	0.28
25	α-copaene	1375	2.49	2.71	2.19
26	Italicenes	1406	3.62	3.63	2.71
27	Bergamoten cis-α	1417	1.17	1.04	0.80
28	β-caryophillen	1427	5.44	5.06	4.96
29	Bergamoten trans-α	1436	1.07	0.96	0.79
30	Mt 210, italdione	1447	2.85	2.85	2.39
31	Neryl-propionate	1452	1.31	1.23	1.74
32	α-humulente	1461	0.19	0.40	0.22
33	γ-seline	1473	0.37	0.44	0.29
34	γ-curcumen	1480	17.72	20.62	14.39
35	Ar-curcumen	1485	3.89	2.92	3.57
36	β-seline	1488	9.92	8.51	10.24
37	α-seline	1497	5.13	4.10	5.73
38	Mt 224, dione	1512	1.66	1.50	1.34
39	γ-cardinene	1519	<0.10%	0.35	0.36
40	δ-cadinene	1526	1.16	1.24	1.13
41	Mt 238, dione	1533	0.66	0.55	0.68
42	Cariophilene-oxide	1581	0.52	0.44	0.50
43	Guaiol	1593	0.18	0.33	0.18
44	Naphtalene-methanol	1622	0.23	0.65	0.29
45	α-eudesmol	1637	0.11	<0.10%	0.13
TOTAL			**92.65**	**93.52**	**93.25**

Figure 1 shows distribution of terpene fractions and diketones in the tested essential oils samples. All three samples of essential oils were rich in sesquiterpenes (50.07% Sample 1, 49.27% Sample 2, and 45.19% Sample 3, respectively), while their oxygenated derivatives were presented in fewer quantities (from 0.81 up to 0.83%). Monoterpenes were the second most represented components with shares in the total composition of essential oil of 21.77% for Sample 1, 21.15% for Sample 2, and 23.21% for Sample 3. Oxygenated monoterpenes were also detected in percentages of 9.92% for Sample 1, 9.76% for Sample 2 and 14.03% for Sample 3. The proportion of diketones in the essential oil was 6.09% in Sample 1, 6.67% in Sample 2, and 5.72% in Sample 3. Comparing the composition of the tested essential oils with the results of studies conducted on samples of wild immortelle collected in Herzegovina, similar concentration of hydrocarbon monoterpenes and a lower concentration of sesquiterpenes were found, while the content of oxygenated monoterpenes and sesquiterpenes were also very low [11].

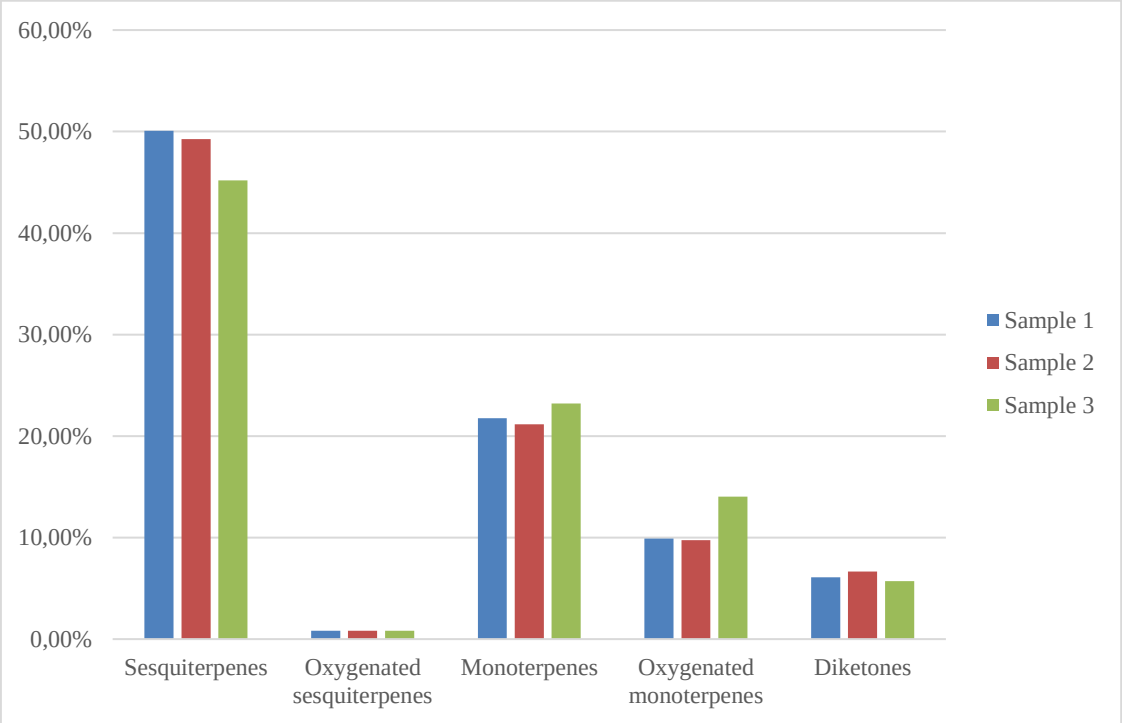

Figure 1. Distribution of terpene fractions and diketones in the tested essential oils

Regarding the results of GC-MS analysis of three *H. italicum* samples from Stolac, Grude and Posušje, it can be noticed that there were differences in the ratios of the most common active components within the tested oil, as well as differences between the analyzed samples. Also, the results showed that none of the samples stood out with the dominant content of active components.

Although all three oils have α-pinene (15.96-17.98%) and γ-curcumen (14.39-20.62%) as the most abundant components, Sample 1 contains equal proportions of these two components, Sample 2 contains more γ-curcumin and Sample 3 contains more α-pinene. The characteristic of essential oils from B&H and Croatia is α-pinene as the main component. Another important characteristic of the tested essential oils is the presence of 4,6,9-trimethyldec-8-ene-3,5-dione (2.39 to 2.85%) in the composition, which makes it different from other chemotypes. This component is also found in the main composition of wild immortelle from Herzegovina [11-12]. Also, the γ-selinen component was identified as a characteristic component that was not detected in other essential oils described in the literature. The content of this component in the analyzed essential oils is still slightly lower (0.29 to 0.37%) than the content determined in wild immortelle from Herzegovina (3.1%) [11]. According to the proportions of individual components in essential oils, the analyzed essential oils can be characterized as chemotype α-pinene, neryl acetate, γ-curcumen and β-seline. This chemical composition is very similar to the classification of *H. italicum* essential oils according to geographical origin proposed recently [6]. Here the authors classify essential oils obtained from *H. italicum* along the Adriatic coast as essential

oils where the main components are γ-curcumen (15–29%) or α-pinene (25–30%) as well as neryl acetate (4–14%). It can be concluded that the tested essential oils were chemically similar to the essential oils from Dalmatia.

ANTIOXIDANT ACTIVITY

The antioxidant activity of *H. italicum* extracts was determined using the DPPH method. The obtained results were compared with the activity of the natural antioxidant vitamin C (ascorbic acid). The highest antioxidant potential was registered by the Sample 2 essential oil, which an IC_{50} value of 5.76 mg/ml, while Samples 1 and 3 showed a slightly lower antioxidant capacity, with IC_{50} values of 6.48 and 8.74 mg/ml, respectively. Vitamin C is one of the strongest antioxidants and was used as a reference sample (IC_{50} 0.005 mg/ml) and it was used to compare the antioxidant activity of the tested samples of *H. italicum* essential oils. All tested essential oils exhibited relatively weak DPPH scavenging capacity. The DPPH values of the tested essential oils are similar to the DPPH values obtained for *H. italicum* from Montenegro [15].

ANTIMICROBIAL ACTIVITY

E. coli is a common pathogenic bacterium present in urinary tract infections and *S. aureus* is the cause of pneumonia and several infections in the intestines or urinary tract. Statistically significant results were determined by t-test at a statistically significant difference of $p < 0.05$. The effect of different volumes of three samples of essential oils on four microbial strains was analysed.

Table 2. Antimicrobial effects of *H. italicum*

Microbial strain	Volume applyed (µl)	Inhibition [mm]		
		1	2	3
E. coli	50	-	-	-
	100			
L. monocytogenes	20	10 (+)	10 (+)	12 (+)
	50	15 (+)	20 (++)	15 (+)
	100	Not tested		
S. aureus	50	-	-	-
	100	-	15 (+)	12 (+)
C. albicans	50	-	-	11 (+)
	100	-	17 (++)	11 (+)

None of the tested samples of *H. italicum* essential oils showed antimicrobial activity on *E. coli* and the zone of inhibition of all tested samples was <3 mm. Sample 1 showed activity on the strain *L. monocytogenes*, but did not show activity on strains *E. coli*, *S. aureus* and *C. albicans* with any tested volume, while Samples 2 and 3 showed activity on the microbial strain *L. monocytogenes* and *S. aureus*, as well as *C. albicans* when tested with 100 µl of essential oil.

Table 3. Analysis of antibacterial activity by t-test

Microbial strain	1-2	1-3	2-3
L. monocytogenes	0.000008	1.000000	0.000008
S. aureus	-	-	0.000562
C. albicans	-	-	0.000003

The results of the t-test show that the antimicrobial activity of essential oils on the tested strain of *L. monocytogenes* differed statistically significantly between samples 1-2 (p = 0.000008), as well as between samples 2-3 (p = 0.000008). Also, for *S. aureus* and *C. albicans*, a statistically significant difference in activity was found between Samples 2 and 3, while Sample 1 showed no antimicrobial activity on these two strains.

All studies indicated that *H. italicum* oils from the Mediterranean region have significant biological potential, but also significantly differ from the area and ecological conditions.

CONCLUSION

GC/MS results showed that the tested *H. italicum* essential oils collected from three plantation fields in Herzegovina region were rich in sesquiterpenes and monoterpenes and the rest consisted of diketones, oxygenated- terpenes and -sesquiterpenes. The main components detected were γ-curcumene, α-pinene, β-selinene and neril-acetate. The tested oils showed similarities in chemical composition with essential oils from Croatia.

Antibacterial activity of the essential oils were tested against microorganisms *E. coli*, *L. monocytogenes*, *S. aureus* and *C. albicans*. The *E. coli* was the most resistant and none of the tested essential oils showed inhibitory activity, while the *L. monocytogenes* strain was the most sensitive where all three tested samples showed inhibitory activity. *S. aureus* and *C. albicans* showed moderate sensitivity.

The tested essential oils contained flavoring substances, such as neryl acetate, pinenes, limonene, curcumenes and could be applied in food industry and as perfume supplement. Since the biological activity is correlated with the chemical composition, further research is needed on other biological activities.

REFERENCES

[1] Bjelic, V., Vodič za uzgoj ljekovitog i aromatičnog bilja u Bosni i Hercegovini. Udruženje "GEA" - Centar za istraživanje i studije, Banja Luka, 2012

[2] Mastelic, J., Politeo, O., Jerkovic, I., Radosevic, N., 2005. Composition and antimicrobial activity of *Helichrysum italicum* essential oil and its terpene and terpenoid fractions, Chem. Nat. Comp. 41 (2005) 35–40.

[3] Chinou, I. B., Roussi, V., Perdetzolou, D., Loukis, A.,. Chemical and biological studies on two *Helichrysum* species of Greek origin. Planta Med. 62 (1996) 339–377

[4] Viegas, D. A., Palmeira-De-Oliveira, A., Salgueiro, L., Martinez-De-Oliveira, J., Palmeira-De-Oliveira, R., *Helichrysum italicum*: From traditional use to scientific data. J. Ethnopharmacol. 151 (2014) 54-65.

[5] Mollova, S., Fidan, H., Antonova, D., Bozhilov, D., Stanev, S., Kostova, I., Stoyanova, A., Chemical composition and antimicrobial and antioxidant activity of *Helichrysum italicuam* (Roth) G. Don subspecies essential oils. Turk J Agric For. 44 (2020) 371-378

[6] Nincevic, T., Grdisa, M., Satovic, Z., Jug-Dujakovic, M.., *Helichrysum italicum* (Roth) G. Don: Taxonomy, biological activity, biochemical and genetic diversity. Ind Crops & Prod. 138 (2019) 1-10.

[7] Paolini, J., Desjobert, J.M., Costa, J., Bernardini, A.F., Castellini, C. B., Cioni, P.L., Flamini, G., Morelli, I., Composition of essential oils of *Helichrysum italicum* (Roth) G. Don subsp. *Italicum* from Tuscan archipelago islands. Flavour Fragr. J. 21 (2006) 805–808.

[8] Morone-Fortunato, I., Montemurro, C., Ruta, C., Perrini, R., Sabetta, W., Blanco, A., Lorusso, E., Avato, P., Essential oils, genetic relationships and *in vitro* establishment of *Helichrysum italicum* (Roth) G. Don ssp. *italicum* from wild Mediterranean germplasm. Industrial Crops and Products 32 (2010) 639-649.

[9] Mastelic, J., Politeo, O., Jerkovic, I., Radosevic, N., Composition and antimicrobial activity of Helichrysum italicum essential oil and its terpene and terpenoid fractions. Chem. Nat. Compd. 41 (2005) 35–40.

[10] Cagalj, M., Grgic, I., Zrakic Susac, M., Ivankovic, M., Economic evaluation (efficiency) of investment in organically grown immortelle [*Helichrysum italicum* ssp. *italicum*] in Bosnia and Herzegovina. The first report from Mediterranean Journal of Central European Agriculture, 20 (2019) 524-541.

[11] Talic, S., Odak, I., Martinovic Bevanda, A., Crnjac, N., Pastar, M. *Helichrysum italicum* (Roth.) G. Don subsp. *italicum* from Herzegovina: Volatile Composition, Variations during Seasons, Acetylcholinesterase Inhibition and Antioxidant Activity. Croat Chem Acta. 92 (2019) 69-77.

[12] Talic, S., Odak, I., Lukic, T., Brkljaca, M., Martinovic Bevanda, A., Lasic, A., Chemodiversity of Helichrysum italicum (Roth) G. Don subsp. Italicum essential oils from Bosnia and Herzegovina. Fresenius Environmental Bulletin 30 (2021) 2492-2502.

[13] CLSI Performance Standards for Antimicrobial Susceptibility Testing; 20th Informational Supplement. Wayne PA: Clinical and Laboratory Standards Institute document 2010, M100-S20, 30.

[14] Pirvu, L., Hlevca, C., Nicu, I., Bubueanu, C., Comparative analytical, antioxidant and antimicrobial activity studies on a series of vegetal extracts prepared from eight plant species growing in Romania. J. Planar Chromat. 275 (2014) 346-356.

[15] Kladar, N. V., Anackov, G. T., Rat, M. M., Srdjenovic, B. U., Grujic, N. N., Sefer, E. I., Bozin, B. N., Biochemical characterization of *Helichrysum italicum* (Roth) G.Don subsp. italicum (Asteraceae) from Montenegro: phytochemical screening, chemotaxonomy, and antioxidant properties. Chem. Biodiversity. 12 (2015) 419-431.