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Andjelka ŠĆEPANOVIĆ*, Slađana KRIVOKAPIĆ, Valentina ŠĆEPANOVIC, Vladimir ŽIVKOVIĆ and Svetlana PEROVIĆ [1]

CHEMICAL CONSTITUENTS AND BIOLOGICAL POTENTIAL OF ESSENTIAL OILS OF *HELICHRYSUM ITALICUM* (ROTH) G. DON FROM MONTENEGRO

SUMMARY

The chemical constituents of essential oils isolated from the *Helichrysum italicum* (Roth) G. Don by steam-distillation were analyzed by GC-MS. The oils were predominantly composed of sesquiterpene hydrocarbons with 52.35%, with γ-curcumene as major constituent (14,11%), β-selinene (11,31%) and α-curcumene (10,42%). The antimicrobial activity of the essential oils was evaluated against Gram-positive and Gram-negative bacteria and fungi. Results showed that the oils exhibited antibacterial activities and that *Staphylococcus aureus*, *Listeria monocytogenes* and *Bacillus subtilis* are very sensitive.

Keywords: *Helichrysum italicum*, GC-MS, antimicrobial activity

INTRODUCTION

The interest in herbal remedies has been significantly increased in the last few decades. In the worldwide, all the natural resources including medicinal plants, fungi and algae are screened for their biological activities (Enazi *et al.* 2018). Medicinal plants play an important role in the discovery and isolation of new drugs. The essential oils are known as a secondary plant metabolite which form part of naturopathic therapy, are widely known for their antimicrobial properties (Rapper *et al.* 2013; Hosseini *et al.* 2016). Various biological characteristics, such as digestive, anti-inflammatory, sedative, antioxidant, antimicrobial, antiviral, and also cytotoxic activities have been attributed to the essential oils (Carvalho *et al.* 2018).

Numerous members of the *Asteraceae* family are important crop species of cut flowers and ornamentals, as well as being medicinal and aromatic plants, many of which produce essential oils used in pharmaceutical industries (Abad *et al.* 2013). Helichrysum is one of the important genera for medical purposes. This genus is currently widely distributed in Africa, Madagascar, the Mediterranean basin, Macaronesia, central Asia and India (Juliano *et al.* 2018) includes more than a thousand taxa that have a higher occurrence in the Mediterranean areas of

[1]Andjelka Scepanovic* (corresponding author: andjelka.s@ucg.ac.me), Sladjana Krivokapic, Valentina Scepanovic, Svetlana Perovic, University of Montenegro, Faculty of Natural Science and Mathematics, Department of Biology, Dzordza Vasingtona bb, Podgorica, MONTENEGRO; Vladimir Zivkovic, Center for Ecotoxicological Research Podgorica, Boulevard Sarla de Gola 2, Podgorica, MONTENEGRO..

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Europe (Viegas *et al.* 2014; Facino *et al.* 1988). Almost 25 species are native of Mediterranean area and the most widespread species is *Helichrysum italicum* (Roth) G. Don (Guinoiseau *et al.* 2013). *H. italicum* is a typically Mediterranean plant. It is a small aromatic shrub with yellow flowers, up to 40–50 cm high, growing on dry cliffs and sandy soil. It is widespread along the East coast and on the islands of the Adriatic sea (Mastelic *et al*, 2005). The genus Helichrysum has an important source of secondary metabolites such as flavonoids, phytocannabinoids, triterpenoids, diterpenoids, steroids, organic acids, phloroglucinol and acetophenone derivatives (Guinoiseau *et al.* 2013).

The aim of this study was to determine the chemical composition and antimicrobial activity of *H. italicum* oils from Montenegro.

MATERIAL AND METHODS

Plant material of *Helichrysum italicum* was collected from several sites in the south of Montenegro; Isolation of the essential oils the dried plant samples was subjected to steam distillation for 3 hours. Samples oils were dried over anhydrous sodium sulphate and stored at low temperature before analysis. The analyses were performed on a gas chromatograph-mass spectrometer, GC-MS QP 2010 plus, Shimadzu, equipped with split-splitless injector and a ZB-5MS capillary column (30m × 0.25 mm; 0.25 μm film thickness). The chromatographic conditions were as mentioned in the preceding paragraph. Injector was heated at 260 °C, detector (MSD) was heated at 260 °C, while the column temperature was linearly programmed from 35 to 270 °C (5.0 °C min-1). The EI MS spectra (70 eV) were obtained in the scan mode in m/z range 50–500.

Antimicrobial activity

To assess the antimicrobial properties of essential oil of *Helichrysum italicum*, nine strains of pathogenic microorganisms were used in the study (Table 1). All microorganisms were derived from the culture collection of the Institute of the Health of Montenegro and Department of Biology, Faculty of Sciences and Mathematics, University of Montenegro. Bacteria were subcultured from nutrient agar slopes into nutrient broth and in Sabouraud dextrose broth for *Candida albicans*. The resulting bacterial broth was used as the inoculum in microbial analysis. Cell numbers of the inoculum were standardized at 10^5 cell/mL. Incubation lasted 18 h at 37°C for bacteria and 48 h at 26°C for *C. albicans.*

Essential oils dilutions were prepared directly in the Mueller-Hinton broth and tested in concentrations range from final concentrations of 71, 35, 14, 7, 3.5, 1.4 μl/ml.

The minimal inhibition concentration (MIC) values were determined for the microbial strains to the essential oils of *Helichrysum italicum.* The MIC is defined as the lowest concentration at which the microorganism does not demonstrate visible growth. Tests were carried out in duplicate

Table 1. Microbial strains and reference number ATCC

Ref. number	**Microorganisms**
ATCC 25922	*Escherichia coli*
ATCC 25923	*Staphylococcus aureus*
ATCC 6633	*Bacillus subtilis*
ATCC 13076	*Salmonella enteritidis*
ATCC 19111	*Listeria monocytogenes*
ATCC 25933	*Proteus mirabilis*
ATCC 27853	*Pseudomonas aeruginosa*
ATCC 19433	*Streptococcus faecalis*
ATCC 10231	*Candida albicans*

RESULTS AND DISCUSSION

Chemical analysis

The results of the chemical analyses of essential oils of. *H. italicum* investigated are presented in Table 2.

Table 2. Chemical composition (% of compound) of the essential oils from *H. italicum*

	Compound	**RT**	**RI**	**%**
1.	alpha.-pinene	11.49	939	0.72
2.	beta-myrcene	13.65	991	0.03
3.	para-cymene	14.85	1026	0.23
4.	limonene	15.02	1031	0.72
5.	1,8-cineol	15.10	1033	0.22
6.	gamma-terpinen	16.03	1062	0.10
7.	linalool	17.41	1098	0.11
8.	beta-thujone	17.61	1114	0.62
9.	camphor	18.91	1143	0.16
10.	borneol	19.71	1165	0.10
11.	alpha-terpineol	20.41	1189	0.16
12.	neryl acetate	25.00	1365	3.91
13.	alpha-copaene	25.57	1376	3.68
14.	beta-elemene	25.91	1391	0.20
15.	alpha-cedrene	26.37	1409	6.25
16.	alpha-bergamotene	26.52	1415	0.92
17.	beta-caryophyllen	26.74	1418	5.36
18.	gamma-curcumene	28.17	1480	14.11
19.	alpha-curcumene	28.24	1483	10.42
20.	beta-selinene	28.52	1485	11.31
21.	alpha-selinene	28.69	1494	6.07
22.	delta-cadinene	29.20	1524	1.40
23.	caryophyllene oxide	30.77	1581	0.52

The main components in *H. italicum* oils are γ-curcumene (14,11%), β-selinene (11,31%) and α-curcumene (10,42%). From Table 2 it can be seen that in the essential oils from *H. italicum* dominates sesquiterpene hydrocarbons with 52.35%. Other components that are present in the essential oil are: α-selinene (6.07%), neryl acetate (3,91%) and α-copaene (3,68%). The monoterpene 1,8-cineole is represented in essential oil with 0,22%. The twenty three compounds were identified, representing about 67.32% of the total oil. According to the literature, essential oil of *H. Italicum* has significant chemical and biological potential. However, the composition of essential oils varies considerably depending on the method of extraction, time of maturation, soil composition and climatic conditions. Research work Paolini *et al*, 2006 reported that in composition of essential oils of *H. italicum* dominated monoterpenes such as neryl acetate, neryl propanoate and α-pinene. According research Morone-Fortunato *et al.* 2010 in essential oils of *H. italicum* are a large proportion of sesquiterpenes. The samples from Italian essential oils of *H. italicum* contained mainly γ-curcumene, β-selinene and α-selinene (Morone-Fortunato *et al.* 2010).

Antimicrobial potential

The data of the antimicrobial activity assessed by dilution method showed that in general the antimicrobial activities of the tested essential oil were varied on the type of microorganism (Table 3).

Table 3. Minimal Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC) of *H. italicum* tested Essential Oils against microorganisms using dilution method

Microbial strains	**MIC (**μg/mL)	**MBC (**μg/mL)
Echerichia coli ATCC25922	n.d.	n.d.
Staphylococcus aureus ATCC25923	<1.4	1.4
Bacillus subtilis ATCC6633	1.4	14
Salmonella enteritidis ATCC13076	n.d.	n.d.
Listeria monocytogenes ATCC19111	<1.4	1.4
Proteus mirabilis ATCC25933	n.d.	n.d.
Pseudomonas aeruginosa ATCC27853	n.d.	n.d.
Streptococcus faecalis ATCC19433	7.1	14
Candida albicans ATCC10231	n.d.	n.d.

∗ n.d. not detected from tested concentrations

It was demonstrated that *Staphylococcus aureus* and *Listeria monocytogenes* (with an MIC of <1.4 μg /mL), and *Bacillus subtilis* (with an MIC of 1.4 μg /mL) were the most sensitive bacteria. Investigation according Nostro *et al* (2001) reported that essential oil of *H. italicum* to inhibit the growth of *Staphylococcus aureus* in a concentration dependent manner, with no

difference in sensitivity between methicillin-resistant *Staphylococcus aureus* and methicillin-sensitive *Staphylococcus aureus* strains.

The antimicrobial activity of the essential oil of *H. italicum* from Montenegrian origin has been evaluated for the first time

CONCLUSIONS

Our results revealed that γ-curcumene, β-selinene and α-curcumene are the major components of *H. italicum* essential oil from Montenegro. The oils possess rather a significant activity against microorganisms *S. aureus*, *L. monocytogenes* and *B. subtilis.* Investigations by other authors have indicated that *H. italicum* oils from the Mediterranean region has significant biological potential, but also significantly differs from the area and ecological conditions. Since these first reports of the biological activity of *H. italicum* from the area of Montenegro research should be continued and extended to biotesting.

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