UCG UNIVERZITET CRNE GORE
MEDICINSKI FAKULTET
Broj 1801/1
Podgorica, 29. 11. 2024 god

Na osnovu člana 9 i stava 3 člana 31 a Pravila doktorskih studija (Bilten Univerziteta Crne Gore broj:513/20 i 561/22), a nakon razmatranja Drugog godišnjeg izvještaja mentora doc. dr Tanje Vojinović o napredovanju doktoranda mr ph spec. Đulije Hadžibeti broj:1801 od 05.11.2024.godine, Komisija za doktorske studije na sjednici održanoj dana 29.11.2024. godine je donijela

O D L U K U

1. Predlaže se Vijeću Medicinskog fakulteta da usvoji Drugi godišnji izvještaj mentora doc. dr Tanje Vojinović o napredovanju doktoranda mr ph spec. Đulije Hadžibeti broj:1801 od 05.11.2024. godine.

2. Predlog komisije i Izvještaj mentora sa objavljenim radom iz tačke jedan ove Odluke, dostavljaju se Vijeću Medicinskog fakulteta, na dalje izjašnjenje.

Obrazloženje

U skladu sa stavom 1 člana 31a Pravila doktorskih studija, mentor doc. dr Tanja Vojinović, blagovremeno je dostavila izvještaj o napredovanju doktoranda mr ph spec. Đulije Hadžibeti, broj: 1801 od 05.11.2024. godine.

Na osnovu stava 3 člana 31 a Pravila doktorskih studija, Komisija za doktorske studije na sjednici održanoj dana 29.11.2024. godine, nakon razmatranja navedenog izvještaja mentora, konstatovala da izvještaj sadrži sve elemente, da je pravilno popunjen i na osnovu njega se stiče konkretan uvid o ostvarenom napretku kandidatkinje. Mentor je visokim ocjenama okarakterisala dosadašnji istraživački rad doktorantkinje i dostavila objavljeni rad kao rezultat doktorskih istraživanja. Priloženi podaci u IM obrascu ukazuju na posvećenost kandidatkinje.

Na osnovu navedenog Komisija je odlučila kao u dispozitivu ove Odluke.

DOSTAVLJENO
-Vijeću Medicinskog fakulteta
-Studentskoj službi

KOMISIJA ZA DOKTORSKE STUDIJE
PREDSJEDNIK

Prof. dr Filip Vukmirović

GODIŠNJI IZVJEŠTAJ MENTORA O NAPREDOVANJU DOKTORANDA

Akademska godina za koju se podnosi izvještaj	2024/2025		
OPŠTI PODACI O DOKTORANDU			
Titula, ime, ime roditelja, prezime	Mr.ph.spec. Đulija Hadžibeti		
Fakultet	Medicinski fakultet		
Studijski program	Farmacija		
Broj indeksa	8/20		
MENTOR/MENTORI			
Prvi mentor	Doc.dr Tanja Vojinović	UCG Medicinski fakultet, Crna Gora	Farmaceutska tehnologija i kozmetologija
Drugi mentor	/	/	/

EVALUACIJA DOKTORANDA*	
Koliko ste zadovoljni kvalitetom održanih susreta sa doktorandom?	☐1 ☐2 ☐3 ☐4 ■5
(Ako je prethodni odgovor „1" ili „2" dati obrazloženje i prijedloge za poboljšanje)	
Da li je definisan plan rada sa doktorandom?	■DA ☐NE
Da li je doktorand ostvario napredak prema predviđenom planu rada?	■DA ☐NE
(Ako je prethodni odgovor „ne" dati obrazloženje i prijedloge za poboljšanje)	
Kvalitet napretka doktorandovog istraživačkog rada u periodu izmedju dva izvještaja je:	☐1 ☐2 ☐3 ☐4 ■5
(Ako je prethodni odgovor „1" ili „2" dati obrazloženje i prijedloge za poboljšanje)	
Dati ocjenu doktorandove spremnosti za konsultacije.	☐1 ☐2 ☐3 ☐4 ■5
Dati ocjenu planiranja i izvršavanja godišnjih istraživačkih aktivnosti i stručnog usavršavanja doktoranda.	☐1 ☐2 ☐3 ☐4 ■5
Dati ocjenu napretka u savladavanju metodologije naučno-istraživačkog rada.	☐1 ☐2 ☐3 ☐4 ■5
Dati ocjenu o aktivnostima sprovedenim na pisanju i objavljivanju naučnih radova	☐1 ☐2 ☐3 ☐4 ■5
Dati ocjenu doktorandovog generalnog odnosa prema studijama.	☐1 ☐2 ☐3 ☐4 ■5
Dati ocjenu ukupnog kvaliteta doktorandovog rada.	☐1 ☐2 ☐3 ☐4 ■5

*Ocjene su: 1 – nedovoljan, 2 – dovoljan, 3 – dobar, 4 – vrlo dobar, 5 – odličan

(Ako je prethodni odgovor „1" ili „2" dati obrazloženje i prijedloge za poboljšanje)	
SAGLASNOST ZA NASTAVAK STUDIJA	
Može li doktorand nastaviti studije?	■ Da ☐ Da, uz odredene uslove ☐ Ne
(Ako je prethodno dat odgovor pod „Da, uz odredjene uslove" ili „Ne" dati obrazloženje i prijedloge za poboljšanje)	
Napomene	
/	
IZJAVA MENTORA	
Doktorand je u fazi pisanja doktorske disertacije. Objavila je rad iz doktorske disertacije. pod nazivom: Chemical composition and antimicrobial activity of essential oil of Helichrysum italicum from Mediterranean coast. Hadžibeti Đ, Vojinović T, Nikolić M, Božić D.D, Antić Stanković J. Farmacia. 2024; (72):4: 1098-1104. https://doi.org/10.31925/farmacia.2024.5.12 SCIE u kategoriji Q2. Saglasna sam u svijetlu navedenog da doktorantkinja može nastaviti izradu svoje doktorske disertacije.	
U Podgorici, 05.11.2024.	Ime i prezime prvog mentora Ime i prezime drugog mentora MP

Prilog dokumenta sadrži:

- Gantogram aktivnosti (za prvi izvještaj mentora)
- Objavljevni rezultati rada na izradi doktorske disertacije (za drugi izvještaj mentora)

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ORIGINAL ARTICLE

CHEMICAL COMPOSITION AND ANTIMICROBIAL ACTIVITY OF ESSENTIAL OIL OF *HELICHRYSUM ITALICUM* FROM MEDITERRANEAN COAST

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Abstract

Gas chromatography-mass spectrometry has been used in the analysis of the chemical characteristics of the essential oil of *Helichrysum italicum*, cultivated in Montenegro, complete with its antimicrobial activity, the evaluation of which has been done against 7 microorganisms, involving Gram-positive and Gram-negative bacteria as well. The evaluation led to thirty-seven compounds being identified in the essential oil, with the following components: α-pinene (16.62%), γ-curcumene (14.06%), β-selinene (7.39%) and neryl acetate (6.04%). In addition, the importance of the following minor compounds found in the essential oil has been confirmed: β-caryophyllene (5.02%), bisabolane (4.28%), italicene (4.16%). The results of the antimicrobial activity tests have revealed that the essential oil of *Helichrysum italicum*, has rather strong antimicrobial activity, especially against *Enterococcus faecalis* (MIC is 0,008 mg/mL) and activity against *Staphylococcus epidermidis* (MIC is 0,016mg/mL) and *Staphylococcus aureus* (MIC is 0,062 mg/mL). The MIC value according to *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella abony* and *Pseudomonas aeruginosa* is greater than 4 mg/mL. Results have shown that the immortelle essential oil exhibited significant antibacterial activity against Gram-positive bacteria, and less against Gram-negative bacteria.

Rezumat

În cadrul studiului a fost determinată compoziția chimică a uleiului esențial de *Helichrysum italicum*, cultivat în Muntenegru, și evaluată activitatea sa antimicrobiană. În uleiul esențial au fost identificați 37 de compuși, dintre care: α-pinen (16,62%), γ-curcumen (14,06%), β-selinen (7,39%) și acetat de neril (6,04%). S-a demonstrat activitatea antimicrobiană a uleiului volatil de *Helichrysum italicum*, în special împotriva *Enterococcus faecalis* (CMI este 0,008 mg/mL), *Staphylococcus epidermidis* (CMI este 0,016mg/mL) și *Staphylococcus aureus* (CMI este 0,062 mg/mL). Rezultatele au arătat o activitate antibacteriană semnificativă a uleiului împotriva bacteriilor Gram-pozitive și mai puțin împotriva bacteriilor Gram-negative.

Keywords: *Helichrysum italicum*, imortelle essential oil, chemical compostion, antimicrobial activity

Introduction

Scientific research interest in pharmacy and medicine has recently focused on the study of natural resources (indigenous plants) and their potential application in pharmacotherapy. Medicinal plants have historically proven their value as a source of molecules with therapeutic potential, and today they continue to represent an important opportunity for the identification of new drugs. In recent decades, the pharmaceutical industry has mainly focused on synthetic compounds as a source of drug discovery. However, at the same time there is a downward trend in the number of new drugs coming to the market, thus influencing the renewed scientific interest in drug discovery from natural sources, despite the known challenges [1]. As regards traditional practice in medicine, the long-term use of plants and their significance when it comes to healing diseases among different nations, cultures and various ethnicity categories can not be neglected. According to the reports given by the WHO, 70 - 95% of the entire population on the planet is considered to be turning to the traditional medicine when it comes to addressing their health care needs [2]. First, it is necessary to mention *Helichrysum italicum* (immortelle), an ever green plant which possess flowers of an intense yellow colour, belonging to the family *Asteraceae*. The interpretation of its name is based on the following Greek terms: *helios* (which means "sun") and *chryos* (which means "gold"). The abovementioned plant is significant in terms of providing all kinds of companies with necessary pharmaceutical food and cosmetic raw materials. Concerning the genus *Helichrysum*, the existence of more than 600 separate species must be underlined, particularly when bearing in mind the fact that they can be found in every part of our planet. The following subspecies of the same plant are *serotinum*, *italicum* and *microphyllum*. A wide choice of 25 autochthonous species may be found scattered all

over the Mediterranean region. Among all of them *Helichrysum italicum* is considered to be the most dominant one [3].

It is characterised by bright yellow flowers that do not wither [4, 5]. Immortelle (*Helichrysum italicum*) grows on sunny rocky fields and slopes in Montenegro, in rock crevices, abandoned vineyards and along roadsides. When taking into consideration the fact that the plant itself can adjust to growing in a dry region without any water, complete with its ability to spread at the altitude of 2200 meters, one may not omit alkaline, infertile soil covered in sand that the plant is most likely to be found [6]. The tree is upright and overgrown with silky, dense hairs, up to 40 centimetres tall, and blooms from May to July. At the top of the tree, there are yellow flowers that are used to obtain oil. An outstanding number of more than a thousand species of *Helichrysum* Miller are reported.

However, the following species have been subjected to detailed examination: *Helichrysum italicum* [7], *Helichrysum stoechas* [8] and *Helichrysum arenarium* [9]. The Mediterranean region is where the first two given species are distributed the most [10]. On the other hand, the Adriatic region [11] and the Iberian Peninsula offer them a habitat in which they grow in particular. Moreover, the *Helichrysum arenarium* is specifically found in Central Europe. It has a wide range of biological activities, such as antimicrobial, anti-inflammatory and antioxidant properties, and its traditional use is related to respiratory, digestive and inflammatory conditions of the skin [13, 14].

Previous phytochemical investigations of Immortelle essential oil revealed the presence of sesquiterpenes such as γ-curcumene and β-selinene, as well as monoterpenes, with α-pinene and neryl acetate as major components [15].

Phytochemical studies so far indicate the presence of terpenes such as α-pinene, neryl-acetate, nerol, α-and γ-curcumin, as well as geranyl-acetate, in the essential oil of *H. italicum* [16].

Some of the most characteristic bioactive compounds found and researched in *H. italicum* are: caffeic acid, which shows antioxidant and anti-inflammatory effects; chlorogenic acid with antibacterial, antiviral and antioxidant properties; pinocembrin with anti-inflammatory effects; quercetin with antibacterial, anti-inflammatory and antioxidant effects; naringenin with antioxidant, antiviral and antibacterial action; gnaphalin with anti-inflammatory action; luteolin with antiviral action; tiliroside with anti-inflammatory and antioxidant action; arzanol with antiviral, antioxidant, anti-inflammatory and antibacterial action; ursolic acid with antioxidant, anti-inflammatory and antibacterial effect [17-19].

The chemical composition and antimicrobial activity of immortelle essential oil from the coast of Montenegro have not been tested yet.

The objectives of this study were to extract essential oils from *Helichrysum italicum*, identify and quantify the chemical constituents present in the essential oil, and evaluate its antimicrobial activity against selected strains of Gram-positive and Gram-negative bacteria. Oil from the area of the Montenegrin coast has not been sufficiently tested and there is not enough research that defines its specific content of bioactive substances.

Materials and Methods

Plant collection

Several factors significantly influence the quantity and quality of essential oil extracted from aromatic plants, including environmental and meteorological conditions. Furthermore, the age of plants and their collection season exert their own influence. To avoid this, immortelle flowers were collected manually from the same region, and their harvest was performed in the middle of the flowering stage. The collection of immortals was carried out in July at four different sites in the coastal area of Ulcinj (Montenegro).

Preparation of herb material

The identification of the plant material was performed by the agronomist Jusuf Katana. The drying of the plant material was carried out for 15 days at room temperature in the dark. After drying, the plant material was cut into 1 - 3 cm pieces, homogenised and stored in the dark until extraction.

Preparation of immortelle essential oil

Hydro distillation method is used to obtain the oil. The hydro distillation was performed in a Clevenger apparatus. Dried plant material (112 ± 35 g) was immersed in water within a distillation flask. The distillation lasted for 2.5 h. The essential oils were collected in n-pentane, which was also dried with anhydrous sodium sulphate and stored in the dark at 4°C until use.

Gas chromatography-mass spectrometry (GC-MS)

The characterisation of the composition of the essential oil is performed with a Shimadzu QP2020 gas chromatograph with an SSL injector, coupled with a triple-quadrupole mass spectrometer. The components are separated on a ZB-5MSplus column (30 m x 0.25 mm; 0.25 µm film thickness) with helium as carrier gas. Samples are released according to the SCAN method at a constant flow rate of 2.40 mL/min, recording the mass range m/z 50 - 500 (EI 70 eV). The essential oil analysis program lasts 58 minutes.

Microbial strains and media

The antimicrobial activity of *H. italicum* essential oil was tested on seven laboratory control strains of microorganisms (KWIK-STIK™, Microbiologics, Saint Cloud, MN, USA), three Gram-positive bacteria: *Staphylococcus aureus* subsp. *aureus* Rosenbach ATCC 6538, *Staphylococcus epidermidis* ATCC 12228 and *Enterococcus faecalis* ATCC 29212 and four Gram-

negative bacteria: *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* subsp. *pneumoniae* NCIMB 8267, *Salmonella enterica* subsp. *enterica* serovar Abony NCTC 6017 and *Pseudomonas aeruginosa* ATCC 27853. The microorganisms were reconstituted from the lyophilised pellet and then inoculated onto Tryptic Soy agar (TSA, Oxoid Ltd., Basingstoke and Hampshire, UK). The primary cultures were incubated for 24 h under aerobic conditions at 35 ± 1°C. The suspension of microorganisms used as inoculum was prepared from fresh cultures in sterile saline (bio-Mérieux, Marcy-l'Etoile, France) to a density of 0.5 according to the McFarland standard (McFarland Densimat densitometer, bioMérieux, Marcy-l'Etoile, France), corresponding to 1.5×10^8 CFU/mL of microorganisms. The Broth Microdilution Test for the determination of the minimum inhibitory concentration (MIC) of *H. italicum* essential oil was performed in accordance with the guidelines of the European Committee for Antimicrobial Susceptibility Testing (EUCAST 2020, 2023). A stock solution of *H. italicum* essential oil (HIEO) was prepared in Mueller-Hinton broth (MHB, Oxoid Ltd., Basingstoke, Hampshire, UK) after being dissolved in dimethyl sulfoxide (DMSO). Serial dilutions of *H. italicum* essential oil ranging from 0.004 - 4 mg/mL were prepared in MHB, and added in duplicate to flat-bottom, 96-well polystyrene microtiter plates (100 µL *per* well) (Nunc™ MicroWell™ 96-Well Microplates, Thermo Fisher Scientific, Waltham, MA, USA) and each concentration was inoculated with 5×10^5 CFU/mL of microorganisms. Growth controls (*i.e.*, only microorganisms in the medium) and negative controls (*i.e.*, only medium with samples) were included in the experiments. The microtiter plates were incubated for 24 hours under aerobic conditions at 35 ± 1°C, and the MIC was determined after addition of resazurin (7-hydroxy-3H-phenoxazin-3-one 10-oxide) (Sigma-Aldrich-Merck KGaA, Darmstadt, Germany). Resazurin is a redox indicator for the detection of cell growth and metabolism, and it is normally a blue and non-fluorescent dye when the cells are non-viable and it is reduced to a pink metabolite resorufin (7-hydroxy-3H-phenoxazin-3-one) when the microorganisms are viable. The MIC of the samples was determined as the lowest concentration of *H. italicum* essential oil that inhibits the growth of the microorganisms. Each test was repeated three times [20, 21].

Antimicrobial screening

The antimicrobial activity of the essential oil of *Helichrysum italicum* was evaluated *in vitro* using a broth microdilution assay against several laboratory microbial strains.

Antimicrobial resistance has been recognised by the WHO as one of the ten leading health problems at the global level. However, the dramatic worldwide rise of multidrug-resistant pathogens can not be counteracted by the current low development pace of new therapeutics, which favoured testing of the antimicrobial activity of herbal preparations.

Today, natural products are still one of the major sources of new drug molecules today [22-24].

As regards the essential oil of *Helichrysum italicum* and its antimicrobial effect, various kind of testing have been performed on seven different types of bacteria the significance of which has been confirmed in medicine. *Staphylococcus aureus* is an important Gram-positive pathogen that causes various infectious diseases (from skin and soft-tissue infections to bacteraemia, necrotising pneumonia, endocarditis and toxic shock syndrome) [25]. Since the second half of the XX century, around 80% of all *S. aureus* strains are resistant to penicillin. Methicillin-resistant *S. aureus* (MRSA) was discovered, two years after the introduction of methicillin in therapeutic protocols [26].

Staphylococcus epidermidis is a skin commensal and colonizes the axillae, head and nose. This bacterium can enter the human body and cause infections during surgical procedures. *S. epidermidis* is also the most common pathogen associated with infections after the use of peripheral or central intravenous catheters [27].

Enterococcus faecalis is a Gram-positive facultative anaerobe and it causes various hospital infections. The main reason why these microorganisms survive in the hospital environment is the high degree of resistance [28, 29].

Escherichia coli is a Gram-negative bacillus, and it is a part of commensal intestinal flora but can also be the cause of intestinal and extraintestinal illness in humans [30].

Klebsiella pneumoniae is a Gram-negative opportunistic pathogen that can cause urinary tract infections, bacteraemia, pneumonia and liver abscesses. In humans, *K. pneumoniae* takes place in the gastrointestinal tract and nasopharynx. Bacteria can enter the bloodstream or other tissues from the nasopharynx and cause an infection.

Today, *K. pneumoniae* is a major cause of medical infections in hospitals. Multiresistant strains of *K. pneumoniae* have become a serious problem in the treatment of infections caused by this bacterium [31, 32].

Salmonella species are widespread in the environment, and occur in cattle, pigs, poultry etc. Most cases of salmonellosis are caused by consumption of contaminated food [33].

Pseudomonas aeruginosa is a Gram-negative opportunistic pathogen and it is also one of the pathogens that cause hospital-acquired infections [34].

Results and Discussion

The chemical composition of the hydro distilled essential oil is shown in Table I. The presence of forty-four compounds is shown. The major components were α-pinene (16.62%) and γ-curcumene (14.06%), the

amount of several compounds in the essential oil was also significant: β-selinene (7.39%), neryl acetate (6.04%), β-caryophyllene (5.02%), α-selinene (4.92%). Djihane *et al.* [14] in their research presented the composition of essential oil of *H. italicum* from the North of Algeria which contains α-cedrene, α-curcumene and geranyl acetate and it differs from the composition of the essential oil that we have analysed from the Mediterranean coast in the area of Ulcinj.

The first task was to isolate essential oils from the plant immortelle (*Helicrysum italicum* subsp *italicum*), which was collected in Herzegovina, it was shown that its chemical composition mainly contained the compounds α-pinene (15.7%) and γ-curcumene (12.8%) [35]. Iso-italicene epoxide was prevailing in the essential oil, grown in Southern Italy [36]. The chemical composition varies in relation to the geographical origin, changed climatic condition, the vegetation cycle and whether fresh or dried plant material was used.

The chemical composition of the hydro distilled essential oil is shown in Table I. The presence of forty-four compounds is shown. The major components were α-pinene (16.62%) and γ-curcumene (14.06%). The amount of several compounds in the essential oil was also significant: β-selinene (7.39%), neryl acetate (6.04%), β-caryophyllene (5.02%), α-selinene (4.92%). Djihane *et al.* [14] presented the composition of essential oil of *H. italicum* from the North of Algeria which contained α-cedrene, α-curcumene and geranyl acetate and it differs from the composition of the essential oil that we have analysed from the Mediterranean coast in the area of Ulcinj. As regards immortelle (*Helicrysum italicum* subsp. *italicum*), which was grown in Herzegovina, it was isolated first, which eventually showed that the following compounds constituted its chemical composition: α-pinene (15.7%) and γ-curcumene (12.8%) [35]. The dominant constituent of the essential oil from Southern Italy was iso-italicene epoxide [36]. The chemical composition varies in relation to the geographical origin, changed climatic condition, the vegetation cycle and whether fresh or dried plant material was used.

Table I

Chemical composition of the essential oil of *H. italicum*

Compound	Percentage %
α-Pinene	16.62
α-Fenchene	0.34
Camphene	0.16
β-Pinene	0.45
Myrcene	0.08
α-Terpinene	0.19
p-Cimene	0.25
Limonen	2.69
Eucalyptol	0.53
Isobutyl angelate	0.43
γ-Terpinen	0.42
Terpinolen	0.18
Linalool	0.64
2-Methylbutyl 2-methylbutyrate	0.18
2-Methylbutyl angelate	1.25
Borneol	0.06
4-Terpineol	0.72
α-Terpineol	0.54
Decanal	0.04
Nerol	0.22
Neryl acetate	6.04
α-Ylangene	0.59
α-Copaene	2.99
Italicene	4.16
cis-α-Bergamotene	0.93
β-Caryophyllene	5.02
Bisabolane	4.28
Geranyl n-propionate	1.23
β-Farnesene	0.18
α-Humulene	0.43
α-Acoradiene	0.45
β-Acoradiene	0.46
γ-Curcumene	14.06
ar-Curcumene	2.97
β-Selinene	7.39
α-Selinene	4.92
γ-Cadinene	1.22
δ-Cadinene	0.13
Nerolidol (Z)	0.15
Guaiol	0.12
β-Eudesmol	0.4
α-Eudesmol	0.23
Geranyl hexanoate	0.2
Total	84.54

Antimicrobial effect of immortelle essential oil

The antimicrobial activity essay for *Helichrysum italicum* essential oil, as specified in the Table II.

Table II

Composition and codification of metronidazole gel formulations

Microorganism	MIC mg/mL
Staphylococcus aureus	0.053 ± 0.014
Staphylococcus epidermidis	0.021 ± 0.007
Enterococcus faecalis	0.012 ± 0.004
Escherichia coli	> 4
Klebsiella pneumoniae	> 4
Salmonella abony	> 4
Pseudomonas aeruginosa	> 4

MIC values ranged from 0.012 to 0.053 mg/mL. The essential oil showed the best activity against *E. faecalis* (MIC is 0.012 mg/mL) and activity against *S. epidermidis* (MIC is 0.021 mg/mL) and *S. aureus* (MIC is 0.053 mg/mL).

According to our findings, it can be stated that a more prominent antimicrobial effect of the essential oil of *H. italicum* is reported in case of gram-positive bacteria than when the same comparison is made including Gram-negative bacteria. The cell wall of a

Gram-negative bacterium is thinner but more complex than that of Gram-positive bacterium.

The Gram-negative bacteria have two membranes, the cytoplasmic cell membrane and the outer membrane. In addition, the Gram-negative bacteria tend to be more resistant to antimicrobial agents than Gram-positive bacteria [37].

After performing testing of the essential oil of *H. italicum* against different bacteria (including Gram-positive and Gram-negative species), demonstrated that essential oil acquired from endemic plants of Corsica was more useful against the Gram-positive bacterium *S. aureus* than against the Gram-negative strains [38], which is in accordance with our findings. Additionally, more pronounced antimicrobial activity of the essential oil of *Helichrysum italicum* from Croatia along with its terpenoid fraction was reported particularly against *Staphylococcus aureus* and *Candida albicans* [39]. Furthermore, the essential oil from *H. italicum* cultivated in the temperate climate of Central Europe (Warsaw, Poland) has a stronger bacteriostatic power against Gram-positive bacteria compared to Gram-negative, with *S. aureus* ATCC 25923 being the most sensitive among the tested [40]. Furthermore, the essential oil of *H. italicum* from France was reported to be more effective against the Gram-positive bacteria *Staphylococcus aureus* and *Bacillus subtilis* than the essential oil of *Helichrysum italicum* subsp. *microphyllum* (of plant origin from Bosnia) but essential oils did not have the same composition [41].

It is known that the quality of the essential oil is determined mainly by the composition. This parameter can vary significantly depending on genotypes, climatic conditions and nutrient supply during cultivation [42]. For example, the essential oil of *Helichrysum italicum* grown in Croatia, was made of the following constituents: α-pinene (10.2%), α-cedrene (9.6%), aromadendrene (4.4%), β-caryophyllene (4.2%) and limonene (3.8%), whereas the predominant oxygen-containing compounds were the following: neryl acetate (11.5%), 2-methylcyclohexyl pentanoate (8.3%), 2-methylcyclohexyl octanoate (4.8%) and geranyl acetate (4.7%) [43]. As regards the primary compounds of the essential oil from *Helichrysum italicum* subsp. *microphyllum* (plant origin from Bosnia) they were the following: monoterpene α-pinene (20.84%) and sesquiterpene γ-curcumene (16.53%), followed by β-selinene (5.59%), curcumene (4.39%), trans-caryophyllene (4.35%), β-diketone italidione I (4.32%), α-selinene (4.28%) and neryl acetate (3.81%). In addition, the *H. italicum* essential oil from France consisted of the following primary compounds): neryl acetate (33.87%), γ-curcumene (8.84%), rosifoliol (5.46%), geranyl propionate (4.98%), α-curcumene (4.31%), italidione I (3.56%), α-eudesmol (3.19%) and limonene (3.02%) [41].

This may apparently be that the antimicrobial effect of the essential oil originates from monoterpene or sesquiterpene compounds, as well as the synergistic effect of all bioactive components (neryl acetate and α-pinene) present in the essential oil of *H. italicum* [43]. What determines the antimicrobial activity of essential oils is primarily related to their chemical composition. On the other hand, the essential oils of different subspecies of *H. italicum* have different compositions. Geographical area, soil and climate also affect the chemical composition of the essential oil.

Conclusions

This study shows that the essential oil of Immortelle (*Helichrysum italicum*) from the Mediterranean Montenegro coast (Adriatic coast) has significant activity against three different microorganisms. The antimicrobial activity of *Helichrysum italicum* essential oil is due to the presence of a mixture of monoterpenes and oxygenated monoterpenes to the oxygenated monoterpenes). The identification of such compounds with a wide biological activity is critical for mankind as it helps in the search for chemical structures that should assist in designing new drugs as therapeutic agents against human pathogens. The abovementioned results verify the potential of *H. italicum* essential oil that may possibly be used in the food and pharmaceutical industries, which means that it can be applied as an alternative antimicrobial agent in natural medicine for the treatment of numerous infectious and skin diseases.

Conflict of interest

The authors declare no conflict of interest.

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