agronomy

MDPI

Article

Helichrysum italicum (Roth) G. Don Essential Oil from Serbia: Chemical Composition, Classification and Biological Activity—May It Be a Suitable New Crop for Serbia?

Milica Aćimović [1,*], Jovana Ljujić [2], Jelena Vulić [3], Valtcho D. Zheljazkov [4], Lato Pezo [5], Ana Varga [6] and Vesna Tumbas Šaponjac [3]

1 Institute of Field and Vegetable Crops Novi Sad, Maksima Gorkog 30, 21000 Novi Sad, Serbia
2 Faculty of Chemistry, University of Belgrade, Studentski trg 16, 11000 Belgrade, Serbia; jovanalj@chem.bg.ac.rs
3 Faculty of Technology, University of Novi Sad, Bulevar cara Lazara 1, 21000 Novi Sad, Serbia; jvulic@uns.ac.rs (J.V.); vesnat@uns.ac.rs (V.T.Š.)
4 College of Agricultural Sciences, Oregon State University, Corvallis, OR 97331, USA; valtcho.jeliazkov@oregonstate.edu
5 Engineering Department, Institute of General and Physical Chemistry, University of Belgrade, Studentski trg 12/V, 11000 Belgrade, Serbia; latopezo@yahoo.co.uk
6 Institute of Food Technology, University of Novi Sad, Bulevar Lazara 1, 21000 Novi Sad, Serbia; ana.varga@fins.uns.ac.rs
* Correspondence: milica.acimovic@ifvcns.ns.ac.rs

Citation: Aćimović, M.; Ljujić, J.; Vulić, J.; Zheljazkov, V.D.; Pezo, L.; Varga, A.; Tumbas Šaponjac, V. *Helichrysum italicum* (Roth) G. Don Essential Oil from Serbia: Chemical Composition, Classification and Biological Activity—May It Be a Suitable New Crop for Serbia? *Agronomy* **2021**, *11*, 1282. https://doi.org/10.3390/agronomy11071282

Academic Editors: Alessandra Carrubba and Mauro Sarno

Received: 29 May 2021
Accepted: 21 June 2021
Published: 24 June 2021

Abstract: *H. italicum* essential oil (EO) is one of the most popular ingredients utilized by the cosmetic industry, and it is also used as natural antioxidant and as a value-added ingredient in food products. The chemical composition of the EO *H. italicum* cultivated in Serbia was analyzed by gas chromatography–mass spectrometry. The quantitative structure–retention relationship was used to predict the retention indices of the EO constituents acquired by GC-MS data, applying five molecular descriptors selected by factor analysis and a genetic algorithm. Also, antimicrobial activity, and biological activity by four common antioxidant tests (DPPH and ABTS assays, reducing power, and β-carotene bleaching test), and in vitro antihyperglycemic and anti-inflammatory capacities were evaluated. A total of 70 EO constituents were detected, of which 17 (8.5%) could not be identified. The *H. italicum* EO in this study belonged to γ-curcumene chemotype. The coefficients of determination reached the value of 0.964, demonstrating that this model could be used for prediction purposes. All applied tests showed that *H. italicum* EO possesses good biological activity and an interesting chemical composition. Therefore, the EO of *H. italicum* grown in Serbia has a potential to be used in food, cosmetic, and pharmaceutical products.

Keywords: immortelle; essential oil; chemotyping; antimicrobial; antioxidant; antihyperglycemic; anti-inflammatory

1. Introduction

The genus *HelichrysumMill.* includes over 300 species out of which 25 are distributed in Europe and the Mediterranean, while the others are distributed in Africa and Madagascar, Australia, and New Zealand [1–3], and also in temperate regions of Asia, including in India, Iran, and Turkey [4–6]. Mediterranean species belong to the Stoechadina section [7], and *H. italicum* (Roth) G. Don (syn. *H. angustifolium* D.C.) is the most widespread species, and it is found on alkaline, dry, sandy and poor soil [8] or even on coastal rocks where it identifies habitats protected by the European directive [9,10]. However, it is characterized by a high degree of anatomical and morphological polymorphism traits and a diversity of ecotypes [11,12]. Some botanists think that *H. italicum* is a complex species, which includes three species: *H. litoreum* Guss., *H. serotinum* Boiss. with two subspecies: ssp.

serotinum (Boiss.) P. Fourn. and ssp. *picardi* (Boiss. & Reut.) Galbany, L. Sáez, & Benedí, and *H. italicum* with four subspecies: ssp. *italicum*, ssp. *microphyllum* (Willd.) Nyman, ssp. *siculum* (Jord. & Fourr.) Galbany, L. Sáez, & Benedíand ssp. *tyrrheanicum* (Bacch., Brullo & Giusso) [8,13].

Today, immortelle or *H. italicum* essential oil (EO) is one of the most popular ingredients in cosmetic products, especially in skin care products, as it exhibits antiproliferative and tissue remodeling effects, thus helping with the wound healing process. Furthermore, as this EO stimulates blood circulation in the skin, it enables regeneration and has antiaging effects [14,15]. Essential oil composition depends on population, altitude, and climatic conditions [16], developmental stage (before flowering, full blossom, after flowering) [17,18], ecology and plant communities [19], plant part (leaves or flowers) [20], extraction method [20,21], etc.

H. arenarium is the only species in Serbian flora that grows spontaneously on sandy soil in dry grassy areas, mainly in the Vojvodina Province. Another species, *H. italicum*, is better known and cultivated to produce EO and extracts for the cosmetic industry. This plant is relatively new in agriculture as only wild populations were harvested in the past [8]. The growing interest in *H. italicum* can be attributed to the high price of its EO, especially in some countries in southern Europe, including the Balkans, Spain, and France [22]. Characteristic properties of EOs, as well as their quality and price, depend on their chemical composition [23]. Variability of the *H. italicum* EO composition is influenced by several factors including not only the plant growth stage and genotype, but also the geographic origin and environment [8]. Previous research showed that *H. italicum* EO exhibited antioxidant, antimicrobial, antiviral, anti-inflammatory, and antiproliferative activity [8].

The aim of this investigation was to characterize EO obtained by hydrodistillation from flowering parts of *H. italicum* introduced from Čapljina (Bosnia and Hercegovina) and grown in the experimental fields of the Institute of Field and Vegetable Crops in Novi Sad, Serbia. We conducted a systematic literature review of *H. italicum* EO composition using Science Direct Elsevier, SpringerLink, PubMed, Scopus, Scifnder, Web of Science, Wiley Online and Google Scholar, among others. The obtained data were used for cluster analysis and chemotyping of *H. italicum*. The main goal was to establish the quantitative structure–retention relationship (QSRR) model for anticipating the retention indices (RIs) of certain compounds in *H. italicum* EO obtained by GC-MS chromatography. Also, antimicrobial, antioxidant, in vitro antihyperglycemic, and anti-inflammatory activities of *H. italicum* EO were tested in this study.

2. Materials and Methods

2.1. Plant Material

H. italicum was grown in the Institute of Field and Vegetable Crops (IFVCNS) collection garden of medicinal and aromatic plants in Bački Petrovac (45.336508; 19.669867 geographic coordinates are given in decimal degrees (WGS-84)), confirmed by Milica Rat, PhD, and deposited at the Herbarium of the University of Novi Sad (BUNS), under the Vouch. No. 2-1375. Flowering tops of *H. italicum* were collected during full blossom stage (July 2019), and dried in a solar dryer at a temperature of 40° with air circulation.

2.2. EO Extraction and Analysis

After drying, the plant material was fragmented and the EO was extracted by hydro distillation in Clevenger apparatus, dried over anhydrous sodium sulfate, and stored in vials at 4–6 °C. The EO yield was 0.17% (*v*/*w*). The GC-MS analysis was carried out using an Agilent 7890A apparatus equipped with a 5975 C MSD, FID and a HP-5MS fused-silica capillary column. The EO constituents were identified based on their linear retention index relative to C8–C32 n-alkanes, compared with data reported in the literature (Adams4 and NIST11 databases). The relative percentage of the oil constituents was expressed as percentages by FID peak area normalization.

2.3. Phylogenetic Tree Diagram

The phylogenetic tree diagram was plotted using R software 4.0.2 (64-bit version). This diagram was calculated using R package "ape" (Analysis of Phylogentics and Evolution), applied as a graphical tool to represent the arrangements of similar EOs concentration (evaluated in the cluster analysis). The obtained experimental results were presented in the matrix, after which the hierarchical cluster analysis was performed. The distance matrix was determined using euclidean method, while the cluster analysis was performed using the "complete" method.

2.4. QSRR Analysis

The determination of molecular descriptors (MDs) was done using the PaDel-descriptor software [24,25]. The most relevant MDs for RIs prediction by factor analysis and genetic algorithm (GA) [26,27], using Heuristic Lab software. Statistical investigation of the data was investigated by the Statistica 10 software.

2.5. Artificial Neural Network (ANN)

Multilayer perceptron architecture (MLP) was used to build the artificial neural network model (ANN) for prediction of RIs for compounds found in *H. italicum* EO identified using GC-MS data [28]. Broyde–Fletcher–Goldfarb–Shanno (BFGS) algorithm was used to speedup the calculation of weight coefficients of the ANN [29]. More details regarding the descriptors could be studied in the Handbook of Molecular Descriptors [30]. The observed data were randomly separated to 70%, 15%, and 15% of data used for training, testing, and validations, respectively [31,32]. ANN calculations were executed with Statistica10.

2.6. Global Sensitivity Analysis

Yoon's global sensitivity method was used to calculate the relative impact of the selected MDs on RIs, according to weight coefficients of the developed ANN [33].

2.7. Biological Activity

In this study, all biological testswere conducted in vitro. In case of antimicrobial tests, it was repeated several times until the same value was obtained three times. Antioxidant, antihyperglycemic, and anti-inflammatory tests were conducted in three replications, and values are expressed as mean ± standard deviation (SD).

2.7.1. Antimicrobial Activity

The antimicrobial activity of the *H. italicum* EO was evaluated using 16 strains of American Type Culture Collection, using microtitre plate-based antibacterial assay incorporating resazurin as an indicator of cell growth. As a control, a Gentamicin strip test was used for determination of minimal inhibitory concentration (MIC) [34].

2.7.2. Antioxidant Activity

The potential biological effects of *H. italicum* EO were investigated using four in vitro antioxidant tests (DPPH$^{\bullet}$ and ABTS$^{\bullet}$+ assays, reducing power, and β-carotene bleaching antioxidant capacity). Antioxidant capacities of *H. italicum* EO were investigated by: DPPH$^{\bullet}$ assay (DPPH) according to Girones–Vilaplana et al. (2014) [35], reducing power (RP) as described in Oyaizu (1986) [36], 2,2′-azinobis-3-ethylbenzothiazoline-6-sulfonic acid radical cation (ABTS$^{\bullet}$+) method as described in Mena et al. (2011) [37], and β-carotene bleaching assay (BCB) as described by Al–Saikhan, et al. (1995) [38]. The antioxidant activities were expressed as μmol of Trolox equivalents per 100 g (μmoLTE/100 g).

2.7.3. In Vitro α-Glucosidase Inhibitory Potential

α-Glucosidase inhibitory potential was performed to examine in vitro antihyperglycemic activity (AHgA) of *H. italicum* EO using the method reported by Tumbas Šaponjac et al. (2014) [39], where each well contained 100 μL of 2 mmol L−14-nitrophenyl α-D-

glucopyranoside in 10 mmol/L potassium phosphate buffer (pH 7.0) and 20 µL of the samples at concentration 250 mg/mL, diluted in buffer. The reaction was started with the addition of 100 µL of the enzyme solution (56.66 mU/mL). The plates were incubated at 37 °C for 10 min. The absorbance of 4-nitrophenol released from 4-nitrophenyl α-D-glucopyranoside was measured at 405 nm.

2.7.4. Anti-Inflammatory Activity

The anti-inflammatory activity (AIA) was determined by protein denaturation bioassay using egg albumin (from fresh hen's egg), according to method adopted by Ullah et al. (2014) [40], where the absorbance was measured at 660 nm.

2.8. Statistical Analysis

The results represented are means ± standard deviation. Statistical study of the data was done using the Statistica 10 software. The cluster analysis (CA) was employed to investigate intra-and interpopulation variations and distinctions of EO constituents of *H. italicum* EO in samples gathered at various locations and/or introduced from literature reports, where the data were evaluated utilizing R software, 4.0.2 (64-bit version).

3. Results

The chemical composition of the *H. italicum* EO in this study was determined by GC-FID and GC-MS analyses, as shown in Table 1. A total of 70 compounds were detected, among which 17 were unidentified and accounted for 8.5%. The dominant compounds were: γ-curcumene (13.6%), β-selinene (12.2%), and α-pinene (11.8%), followed by β-caryophyllene (6.7%), ar-curcumene (5.0%), α-selinene (4.3%), and italicene (4.2%).

Table 1. Chemical composition of *H. italicum* essential oil.

No	Component	Cycle	RI[a]	$RI_{pred.}$	%
1	α-Pinene	Train.	926	935.054	11.8
2	α-Fenchene	Train.	942	1055.410	0.2
3	Camphene	Train.	944	1177.543	0.1
4	β-Pinene	Valid.	970	1415.606	0.3
5	α-Terpinene	Train.	1015	1373.881	0.2
6	*p*-Cymene	Test.	1021	1467.734	0.2
7	Limonene	Test.	1024	1571.383	2.6
8	1,8-Cineole	Train.	1027	928.460	0.3
9	Isobutyl angelate	Train.	1044	944.529	0.3
10	γ-Terpinene	Train.	1052	946.147	0.4
11	Terpinolene	Train.	1080	1033.521	0.2
12	Linalool	Train.	1091	1026.925	0.8
13	2-Methyl butyl-2-methyl butyrate	Valid.	1095	1045.050	0.3
14	Isoamyltiglate	Train.	1146	1026.019	1.8
15	Borneol	Train.	1159	1080.790	0.2
16	Menthol	Valid.	1166	1094.205	0.4
17	Terpinen-4-ol	Test.	1172	1147.990	0.2
18	5,6-Decanedione	Train.	1182	1155.678	0.2
19	α-Terpineol	Train.	1186	1183.886	0.2
20	Nerol	Train.	1225	1192.495	0.4
21	NI-1		1283		0.5
22	Menthyl acetate	Train.	1291	1224.095	0.2
23	Neryl acetate	Test.	1362	1287.671	5.5
24	NI-2		1364		0.4
25	α-Ylangene	Train.	1370	1393.100	0.4
26	α-Copaene	Train.	1374	1393.100	2.6
27	*iso*-Italicene	Train.	1398	1373.881	0.2

Table 1. *Cont.*

No	Component	Cycle	RI[a]	$RI_{pred.}$	%
28	Italicene	Test.	1402	1452.010	4.2
29	*cis*-α-Bergamotene	Valid.	1413	1481.175	1.3
30	β-Caryophyllene	Train.	1418	1440.820	6.7
31	*trans*-α-Bergamotene	Train.	1434	1461.915	1.3
32	Bisabolane	Train.	1441	1452.106	1.0
33	6,9-Guaiadiene	Train.	1442	1471.975	1.9
34	NI-3		1447		1.4
35	Nerylpropanoate	Train.	1452	1471.975	1.7
36	*trans*-β-Farnesene	Valid.	1456	1467.734	0.7
37	α-Acoradiene	Train.	1464	1453.653	0.6
38	β-Acoradiene	Train.	1466	1461.323	0.5
39	α-Selinene	Train.	1475	1492.297	1.8
40	γ-curcumene	Valid.	1480	1472.205	13.6
41	*ar*-Curcumene	Train.	1483	1503.786	5.0
42	β-Selinene	Train.	1486	1543.763	12.2
43	α-Selinene	Test.	1495	1627.550	4.3
44	α-Muurolene	Train.	1500	1608.356	0.4
45	NI-4		1507		0.5
46	β-Curcumene	Test	1511	1630.044	0.4
47	γ-Cadinene	Train.	1513	1630.137	0.5
48	δ-Cadinene	Train.	1523	1622.599	1.0
49	NI-5		1532		0.1
50	NI-6		1533		0.1
51	NI-7		1537		0.1
52	α-Calacorene	Train.	1542	1666.463	0.3
53	NI-8		1561		0.1
54	NI-9		1580		3.0
55	NI-10		1590		0.2
56	Guaiol	Train.	1594	937.385	0.3
57	NI-11		1600		0.5
58	Rosifoliol	Train.	1604	1145.600	0.7
59	NI-12		1610		0.3
60	NI-13		1645		0.3
61	1-*epi*-Cubenol	Train.	1629	1186.741	0.3
62	NI-14		1631		0.3
63	*epi*-α-Cadinol	Train.	1638	1500.806	0.2
64	β-Eudesmol	Train.	1647	1578.032	0.2
65	*neo*-Intermedeol		1652		0.6
66	*epi*-β-Bisabolol	Train.	1665	1585.105	0.2
67	NI-15		1693		0.1
68	Geranyl hexanoate	Valid.	1729	1965.463	0.1
69	NI-16		1777		0.2
70	NI-17		1801		0.2

Cycle—a cycle in artificial neural network model calculation; RI[a]: experimental Retention Index; RI_{pred}: Retention Index predicted.

With the intention to develop a QSRR model for prediction of RIs, PaDel-descriptor software was used. A large set of molecular descriptors (MDs) were calculated, and only the highly important descriptors were selected to build the forecasting RIs model. According to this initial examination, only around 250 descriptors remained for genetic algorithm (GA) calculation. The GA was applied to select among MDs for the highly influential variables for RIs anticipation. As an outcome, the five highly important molecular descriptors selected by GA were: three autocorrelation descriptors (AATS1m, AATSC4c, and MATS1c), Barysz matrix descriptor (VE3_Dzv), and Van der Waals volume descriptor (VABC).

Subsequently, the used MDs were appropriate to foresee the RIs of compounds in *H. italicum* by multivariate artificial neural network (ANN) model. Proposed descriptors

describe discrete aspects of the molecular bindings and were used to develop the QSRR models. The correlations between these molecular descriptors are presented in Table 2.

Table 2. Correlations between molecular descriptors for *H. italicum* essential oil.

	AATSC4c	MATS1c	VE3_Dzv	VABC
AATS1m	−0.1315 $p = 0.353$	−0.2060 $p = 0.143$	0.0211 $p = 0.882$	−0.1478 $p = 0.296$
AATSC4c		0.1945 $p = 0.167$	−0.0260 $p = 0.855$	0.1840 $p = 0.141$
MATS1			0.0165 $p = 0.908$	0.1076 $p = 0.448$
VE3_Dzv				0.0720 $p = 0.612$

AATS1m: average Broto–Moreau autocorrelation-lag 1/weighted by mass, AATSC4c: average centered Broto–Moreau autocorrelation-lag 4/weighted by charges, MATS1c: Moran autocorrelation-lag 1/weighted by charges, VE3_Dzv: logarithmic coefficient sum of the last eigenvector from Barysz matrix/weighted by van der Waals volumes, VABC: Van der Waals volume calculated using the method proposed in the Handbook of Molecular Descriptors [30].

With an idea to investigate the nonlinear relationship among RIs of compounds in and MDs selected by GA, ANN modelling tool was used to build a predictive model. The ANN model MLP 5-7-1 was constructed to predict the retention indices of compounds isolated from *H. italicum* EO. The coefficients of determination during the training cycle was 0.997, indicating that this model could be applied for prediction of RIs, having in mind the low prediction error and high r^2. The statistical results of the ANN model are shown in Table 3.

Table 3. ANN model summary (performance and errors), for training, testing, and validation cycles.

Net. Name	Performance			Error			Train. Algor.	Error Funct.	Hidden Activat.	Output Activat.
	Train.	Test.	Valid.	Train.	Test.	Valid.				
MLP 4-8-1	0.997	0.978	0.997	156.604	1175.567	6687.007	BFGS 117	SOS	Tanh	Identity

Performance term represent coefficients of determination, while error terms indicate a lack of data for the ANN model. ANN cycles: Train: training, Test: testing, Valid: validation, algor: algorithm, funct: function, activat: activation.

The predicted RIs are presented in Figure 1a, confirming the good fit of the constructed ANN by explaining the relationship among the predicted and experimental RIs values. The obtained results presented in Figure 1b show the good quality of the ANN model for anticipating the RIs of compounds in *H. italicum* EO obtained by GC-MS analysis. In this chapter, the impact of five most influential input variables on RIs, selected using genetic algorithm, was investigated. Based on the results presented on Figure 1b, MATS1c was the most influential MD for chemical compounds in *H. italicum* EO, with relative importance of +53.84%. The positive influence was observed for VE3_Dzv and AATSC4c descriptors, which expressed the relative importance of +27.63 and +10.17%. The negative influential MD for *H. italicum* EO was: VABC (with relative importance of −7.94%).

H. italicum showed low or no activity against tested bacteria. However, for all Gram negative bacteria (*E. coli*, *P. aeruginosa*, *Salmonella Typhimurium*, *S. enteritidis*, *K. aerogenes*, and *P. hauseri*) MIC and MBC values were higher than 454.50 µL/mL EO. For the Gram positive bacteria (*B. cereus*, *L. monocytogenes*, *R. equi,* and *S. epidermidis*) MIC and MBC values was 454.50 µL/mL, while for other (*B. spizizenii*, *E. faecalis*, *L. innocua*, *L.ivanovii,* and *S. aureus*) MIC and MBC values were higher than 454.50 µL/mL of EO (as illustrated in Table 4).

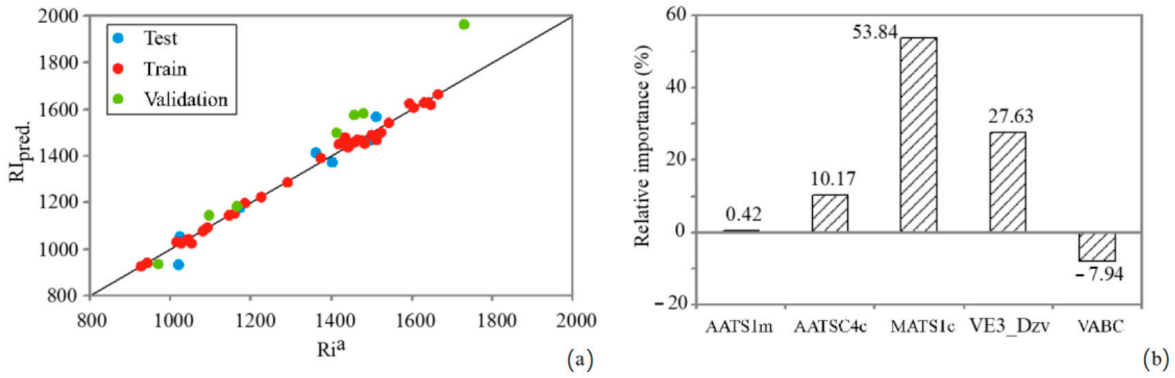

Figure 1. Retention indices of *H. italicum* EO composition from: experimentally obtained GC-MS data (RI^a) and predicted by the ANN ($RI_{pred.}$); (**a**) the relative importance of the molecular descriptors on RI, determined using Yoon interpretation method (**b**).

Table 4. In vitro antibacterial (µL/mL), antioxidant (µmoLTrolox equivalents (TE)/100 g), α-glucosidase (%), and anti-inflammatory (%) activities of *H. italicum* essential oil (µL/mL).

Antibacterial Activity			
Gram Negative	**MIC**	**MBC**	**Gentamicin (MIC)**
Escherichia coli (ATCC 8739)	>454.50	>454.50	2.00
Escherichia coli (ATCC 10536)	>454.50	>454.50	1.00
Pseudomonas aeruginosa (ATCC 27853)	>454.50	>454.50	1.00
Salmonella enteritidis (ATCC 13076)	>454.50	>454.50	0.50
Salmonella Typhimurium (ATCC 14028)	>454.50	>454.50	0.50
Klebsiellaaerogenes (ATCC 13048)	>454.50	>454.50	0.50
Proteus hauseri (ATCC 13315)	>454.50	>454.50	1.00
Gram Positive			
Bacillus cereus (ATCC 11778)	454.50	454.50	0.19
Bacillus spizizenii (ATCC 6633)	>454.50	>454.50	0.38
Enterococcus faecalis (ATCC 29212)	>454.50	>454.50	8.00
Listeria monocytogenes (ATCC 19111)	454.50	454.50	0.19
Listeria innocua (ATCC 33090)	>454.50	>454.50	0.50
Listeria ivanovii (ATCC 19119)	>454.50	>454.50	0.50
Rhodococcusequi (ATCC 6939)	454.50	454.50	0.38
Staphylococcus aureus (ATCC 25923)	>454.50	>454.50	0.38
Staphylococcusepidermidis (ATCC 12228)	454.50	454.50	0.094
Antioxidant Activity			
DPPH		254.66 ± 19.01	
RP		27.69 ± 0.40	
ABTS		734.24 ± 42.62	
BCB		96.58 ± 7.11	
α-Glucosidase Inhibitory Potential			
AHgA		66.02 ± 1.91	
Anti-Inflammatory Activity			
AIA		37.41 ± 0.42	

MIC: minimal inhibitory concentration; MBC: minimal bactericidal concentration. Data present mean value of three replicates ± SD.

The EO of *H. italicum* in DPPH method showed antioxidant activity of 254.66 µmol TE/100 g, reducing power of 27.69 µmol TE/100 g, scavenging capacity of ABTS radicals of 734.24 µmol TE/100 g, and BCB test 96.58 µmol TE/100 (as illustrated in Table 4). Furthermore, *H. italicum* EO at the concentration 250 mg/mL had strong inhibitory activity

on α-glucosidase (66.02%) and inhibited the denaturation of the proteins by 37.41% (as illustrated in Table 4).

4. Discussion

The essential oil content in *H. italicum* was reported to vary between 0.02% and 0.78%, depending on plant stage and location [11,16,17,41–53]. Systematic literature review of 104 *H. italicum* accessions with 17 most abundant compounds (in average presented with 1.0% or more) from EO is shown in Table 5.

According to cluster analysis (as illustrated in Figure 2), our sample belongs to γ-curcumene chemotype, as previously reported for Serbia [54] and Montenegro [55], but also found in Italy [45] and USA [49].

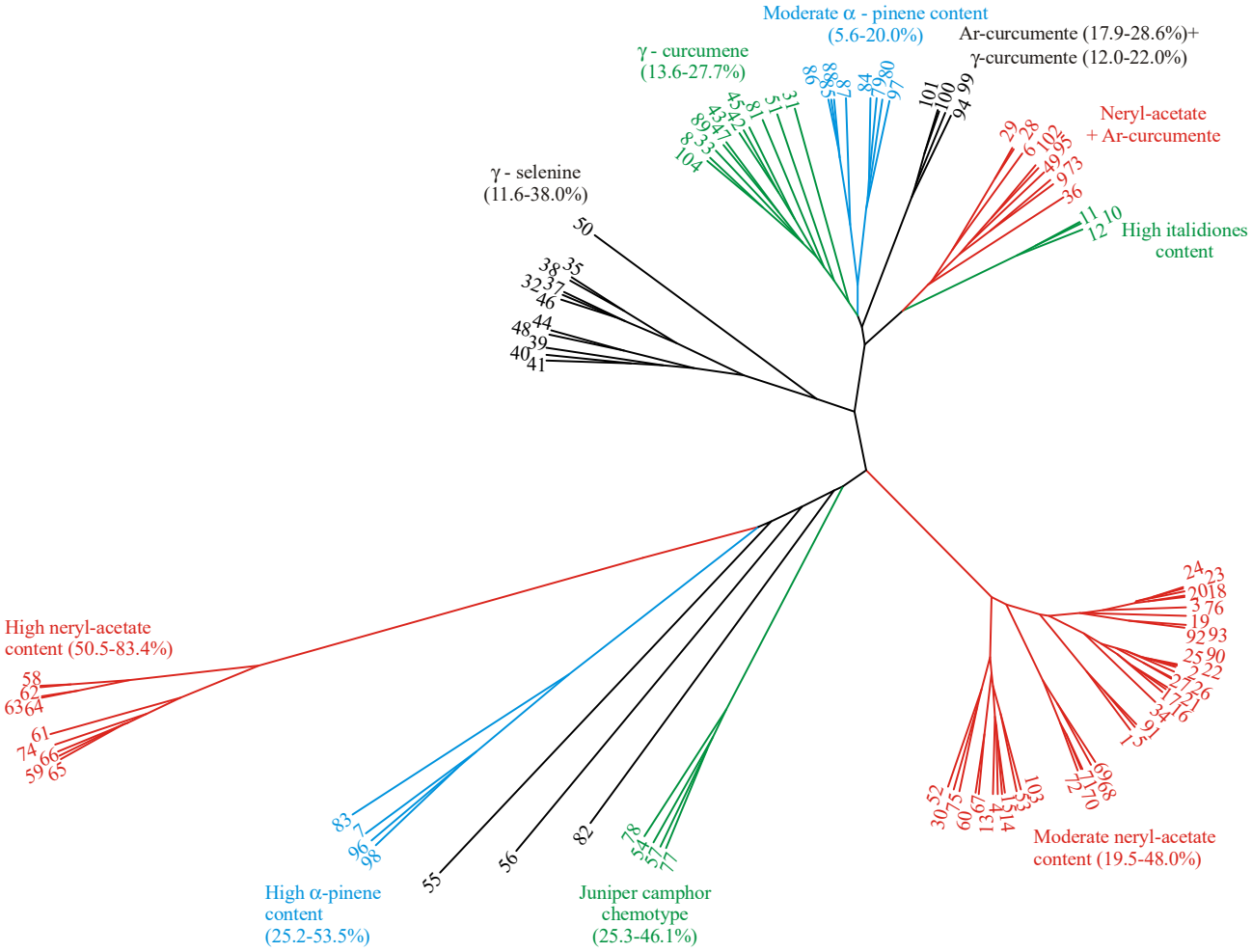

Figure 2. Unrooted phylogenetic tree of *H. italicum* samples based on concentration of EOs.

According to data from literature about *H. italicum* EO chemical composition (as illustrated in Table 1) and cluster analysis based on this data-unrooted phylogenetic tree (as illustrated in Figure 2), there are ten chemotypes of *H. italicum* depending on the main compounds in the EO: (1) high neryl-acetate chemotype (50.5–83.4%); (2) moderate neryl-acetate chemotype (19.5–48.0%); (3) neryl-acetate + ar-curcumene (3.9–20.3% and 0.8–14.5%, respectively); (4) ar-curcumene + γ-curcumene (17.9–28.6% and 12.0–22.0%, respectively); (5) γ-curcumene (13.6–27.7%); (6) high α-pinene chemotype (25.2–53.5%); (7) moderate α-pinene (5.6–20.0%); (8) juniper camphor (25.3–45.1%); (9) β-selinene (11.6–38.0%), and (10) italidiones chemotype.

Table 5. Chemical composition of different *H. italicum* accessions according to literature.

No.	Ref.	4,6,9-Trimethyldec-8-ene-3,5-dione	ar-Curcumene	Carvacrol	Eudesm-5-en-11-ol	Eudesmen-7-(11)-en-4-ol	Italicene	Limonene	Linalool	Nerol	Neryl Acetate	Neryl Propanoate	α-Pinene	α-Terpineol	β-Caryophyllene	β-Eudesmol	β-Selinene	γ-Curcumene
1	[42]	0.0	2.4	0.0	10.1	0.0	0.5	2.5	1.3	4.4	26.5	3.9	1.7	1.7	1.1	1.5	0.0	3.3
2	[42]	2.2	3.2	0.0	4.7	0.0	2.1	3.4	2.3	6.7	31.0	7.5	3.4	1.5	0.4	0.6	0.0	5.7
3	[42]	0.8	4.5	0.0	4.0	0.0	2.5	4.0	2.8	4.3	41.3	5.5	1.9	1.1	0.2	0.3	0.0	3.2
4	[42]	1.3	2.6	0.0	4.2	0.0	1.1	8.5	2.1	5.3	24.3	6.7	11.4	1.8	0.3	0.3	0.0	4.6
5	[42]	3.7	3.9	0.0	13.7	0.0	0.3	4.6	2.4	6.8	26.6	4.5	1.6	2.3	0.9	1.2	0.0	1.9
6	[42]	3.4	2.6	0.0	6.7	0.0	0.3	10.9	5.6	10.6	11.5	3.2	12.8	2.5	0.6	0.4	0.0	1.4
7	[42]	0.2	0.9	0.0	2.7	0.0	0.5	1.1	2.5	4.2	7.1	2.2	31.6	2.2	0.3	0.1	0.0	2.1
8	[54]	0.0	1.9	0.0	0.0	0.0	5.4	2.5	0.5	0.8	7.9	1.4	15.9	0.3	4.7	0.1	6.9	22.5
9	[43]	0.0	11.4	0.0	0.0	0.0	0.0	6.1	3.0	5.0	4.9	0.0	3.8	2.5	0.5	0.0	0.0	0.0
10	[44]	19.8	2.6	0.0	1.7	0.0	0.9	0.2	2.9	1.6	14.9	7.3	0.1	0.5	0.0	0.0	0.0	13.7
11	[44]	14.7	1.9	0.0	1.8	0.0	0.7	1.9	3.9	1.4	18.7	4.7	2.0	1.3	0.0	0.0	0.5	11.1
12	[44]	18.3	1.3	0.0	3.8	0.0	0.7	4.3	0.7	2.2	21.0	7.8	4.5	1.2	0.0	0.0	0.0	7.7
13	[44]	7.6	2.0	0.0	7.6	0.0	1.2	4.0	0.8	3.3	26.8	6.2	4.3	2.3	0.0	0.0	0.1	7.7
14	[44]	4.4	1.2	0.0	1.1	0.0	0.6	10.4	1.2	1.8	27.9	3.0	8.6	1.9	0.0	0.0	0.4	8.6
15	[44]	2.0	1.8	0.0	1.7	0.0	0.5	7.5	1.3	4.2	30.2	9.2	6.1	2.6	0.0	0.0	0.5	11.0
16	[44]	8.8	1.5	0.0	1.7	0.0	0.6	7.6	1.1	2.2	33.3	3.8	4.2	2.1	0.0	0.0	0.8	8.6
17	[44]	4.4	1.5	0.0	1.3	0.0	0.8	5.5	0.9	2.8	39.9	6.0	6.1	1.6	0.0	0.0	0.4	11.1
18	[44]	0.3	1.5	0.0	2.9	0.0	1.1	2.4	1.5	3.6	40.1	5.5	2.9	2.1	0.0	0.0	0.0	5.4
19	[44]	1.4	1.2	0.0	4.3	0.0	1.1	1.2	1.4	7.6	42.3	16.4	0.5	2.2	0.0	0.0	0.1	9.4
20	[44]	2.6	1.0	0.0	5.8	0.0	1.1	5.7	0.8	3.2	44.5	7.0	4.1	1.3	0.0	0.0	0.0	6.0
21	[56]	3.7	2.3	0.0	0.8	0.0	2.4	6.9	2.4	3.2	36.3	4.8	2.7	0.7	0.0	0.4	0.0	12.9
22	[56]	4.5	4.1	0.0	2.1	0.0	2.7	6.1	1.8	2.6	34.5	5.6	2.7	0.5	0.0	0.6	0.0	7.9
23	[56]	3.5	3.3	0.0	0.0	0.0	3.4	7.3	2.8	3.4	42.5	5.9	2.9	0.8	0.0	0.0	0.0	6.2
24	[56]	4.3	4.6	0.0	2.3	0.0	3.0	5.1	1.5	3.4	39.9	6.7	1.5	0.5	0.0	0.7	0.0	5.1
25	[56]	2.2	2.5	0.0	2.4	0.0	2.6	7.5	1.2	4.7	32.6	5.8	1.8	0.8	0.0	0.7	0.0	11.7
26	[56]	5.6	1.8	0.0	3.5	0.0	1.2	2.9	2.1	4.2	34.9	4.9	1.7	1.7	0.0	0.8	0.0	3.7
27	[56]	3.1	1.8	0.0	4.2	0.0	1.4	1.9	2.3	4.9	37.6	4.3	0.7	2.0	0.0	1.3	0.0	6.7
28	[56]	1.4	0.8	0.0	3.6	0.0	0.9	5.7	1.1	3.2	20.3	2.7	1.8	2.1	0.0	1.0	0.0	1.1
29	[56]	0.8	0.9	0.0	5.1	0.0	1.1	4.8	1.0	2.0	15.8	1.6	1.5	1.6	0.0	0.8	0.0	0.8
30	[45]	0.0	4.5	0.0	3.9	0.0	2.3	1.2	9.1	10.7	28.9	11.4	0.2	0.2	0.2	0.8	1.8	11.4
31	[45]	0.0	4.8	0.0	20.2	0.0	4.2	1.2	14.9	0.0	0.0	0.0	0.1	0.2	3.2	2.6	1.9	18.2
32	[57]	0.0	0.0	3.8	0.0	0.0	0.7	0.0	1.5	0.5	1.7	0.7	0.9	0.8	5.1	0.0	33.3	4.2
33	[57]	5.5	4.9	6.2	0.0	0.0	2.7	0.0	0.4	2.4	15.1	0.5	3.6	0.8	1.2	0.0	3.6	23.3
34	[57]	11.0	6.4	1.9	0.0	0.0	0.9	0.0	3.9	3.9	32.0	3.6	0.0	1.0	0.0	1.6	0.0	5.0
35	[57]	0.0	8.3	8.4	0.0	0.0	1.4	0.0	0.9	0.6	0.4	0.0	0.0	0.5	3.0	0.0	19.7	2.3
36	[57]	6.2	6.4	9.8	0.0	0.0	0.0	0.0	0.5	18.8	8.5	6.8	0.0	2.3	2.7	0.0	0.0	0.0
37	[57]	0.0	0.0	6.7	0.0	0.0	0.0	0.0	0.0	1.5	0.7	0.0	0.0	0.0	0.0	1.1	38.0	0.0
38	[57]	0.0	2.9	6.8	0.0	0.0	1.7	0.0	1.0	1.7	3.2	0.0	0.0	1.5	0.0	0.0	25.7	6.5
39	[57]	0.0	1.1	14.8	0.0	0.0	0.8	0.0	0.0	0.8	1.0	0.0	0.0	1.0	10.1	13.7	15.9	3.3
40	[57]	0.0	3.1	8.7	0.0	0.0	1.3	0.0	1.4	0.5	1.1	2.3	0.0	0.8	12.7	1.6	11.6	8.4
41	[57]	0.0	0.0	3.8	0.0	0.0	0.4	0.0	0.4	0.9	0.8	2.7	0.0	0.9	6.2	2.9	20.0	1.6
42	[57]	0.0	0.0	1.6	0.0	0.0	1.1	0.0	0.0	1.0	1.8	0.0	1.2	0.5	8.6	0.0	2.8	15.0
43	[57]	0.0	0.0	9.5	0.0	0.0	1.9	0.0	1.5	2.0	4.5	0.2	0.0	2.6	6.5	1.5	3.8	14.3
44	[57]	0.0	0.0	7.4	0.0	0.0	0.6	0.0	0.5	1.3	1.4	0.0	0.0	0.4	14.4	0.0	24.5	3.4
45	[57]	0.0	2.7	9.8	0.0	0.0	2.5	0.0	0.0	0.9	1.0	0.0	0.0	1.0	10.9	0.0	2.3	27.7
46	[57]	0.0	0.0	5.4	0.0	0.0	0.7	0.0	1.5	2.7	2.8	1.5	0.8	0.9	4.5	0.0	28.9	0.0
47	[57]	0.0	0.0	2.6	0.0	0.0	1.9	0.0	1.9	1.9	4.7	0.2	0.0	2.9	7.3	1.5	15.3	17.1
48	[57]	0.0	0.0	5.8	0.0	0.0	1.5	0.0	0.0	0.4	1.4	0.0	0.5	0.0	18.6	0.0	26.7	14.7
49	[57]	0.0	6.1	3.3	0.0	0.0	0.0	0.0	5.1	1.7	11.4	0.9	0.0	1.2	7.8	2.4	6.1	7.7
50	[57]	0.0	0.0	3.2	0.0	0.0	6.5	0.0	1.2	0.5	8.1	0.0	1.1	0.4	2.6	0.0	22.0	41.0
51	[57]	0.0	2.2	3.6	0.0	0.0	1.6	0.0	0.0	0.4	1.2	0.0	0.0	0.6	3.0	2.3	4.7	14.5
52	[46]	0.0	8.3	0.0	0.0	0.0	0.0	0.0	0.0	0.7	29.2	10.1	0.4	1.1	1.7	0.0	1.6	18.8
53	[58]	0.0	2.2	0.0	0.0	0.0	2.4	2.6	0.5	1.9	20.4	3.2	4.3	1.0	1.9	0.9	2.9	14.1
54	[16]	0.0	0.0	0.0	0.0	25.3	1.0	15.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.9	0.0	4.4
55	[16]	0.0	0.6	0.0	0.0	0.0	1.8	0.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.7
56	[16]	0.0	0.0	0.0	0.0	4.4	0.0	2.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
57	[16]	0.0	2.1	0.0	0.0	32.1	0.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.5	0.0	3.3
58	[16]	0.0	1.0	0.0	0.0	2.8	1.1	3.5	1.7	0.0	73.7	3.7	0.0	0.0	0.0	0.7	0.0	1.1
59	[16]	0.0	0.7	0.0	0.0	5.0	0.4	6.6	0.0	0.0	59.9	1.1	0.0	0.0	0.0	3.2	0.0	1.6
60	[16]	0.0	1.7	0.0	0.0	8.3	0.6	4.2	0.2	0.0	33.1	7.2	0.0	0.0	0.0	2.2	0.0	3.6

Table 5. *Cont.*

No.	Ref.	4,6,9-Trimethyldec-8-ene-3,5-dione	ar-Curcumene	Carvacrol	Eudesm-5-en-11-ol	Eudesmen-7-(11)-en-4-ol	Italicene	Limonene	Linalool	Nerol	Neryl Acetate	Neryl Propanoate	α-Pinene	α-Terpineol	β-Caryophyllene	β-Eudesmol	β-Selinene	γ-Curcumene
61	[16]	0.0	0.0	0.0	0.0	17.8	0.0	6.7	4.0	0.0	52.8	14.1	0.0	0.0	0.0	0.0	0.0	0.0
62	[16]	0.0	0.4	0.0	0.0	4.8	0.0	2.5	0.0	0.0	70.0	4.0	0.0	0.0	0.0	1.2	0.0	1.3
63	[16]	0.0	0.1	0.0	0.0	1.6	0.0	1.8	0.4	0.0	77.7	6.7	0.0	0.0	0.0	0.3	0.0	0.5
64	[16]	0.0	0.0	0.0	0.0	0.0	0.0	0.7	0.5	0.0	83.4	7.3	0.0	0.0	0.0	0.0	0.0	0.0
65	[16]	0.0	0.1	0.0	0.0	1.9	1.0	0.8	2.2	6.8	60.3	5.4	0.0	0.0	0.0	0.2	0.0	3.7
66	[16]	0.0	0.0	0.0	0.0	2.0	1.8	5.9	0.0	4.1	50.5	7.2	0.0	0.0	0.0	1.3	0.0	1.3
67	[16]	0.0	1.3	0.0	0.0	4.2	1.0	0.4	1.5	1.9	28.4	8.2	0.0	0.0	0.0	7.1	0.0	1.4
68	[16]	0.0	2.9	0.0	0.0	15.7	1.1	0.0	0.0	0.0	43.9	11.5	0.0	0.0	0.0	1.6	0.0	2.9
69	[16]	0.0	1.9	0.0	0.0	12.4	2.1	2.0	0.2	2.9	45.4	3.7	0.0	0.0	0.0	0.5	0.0	6.7
70	[16]	0.0	3.6	0.0	0.0	13.6	1.9	0.0	1.5	0.0	33.0	4.1	0.0	0.0	0.0	4.1	0.0	3.2
71	[16]	0.0	2.8	0.0	0.0	14.9	1.5	0.0	1.5	1.2	37.6	3.4	0.0	0.0	0.0	0.4	0.0	2.9
72	[16]	0.0	3.4	0.0	0.0	19.6	3.8	0.7	0.0	0.0	34.4	1.1	0.0	0.0	0.0	2.3	0.0	7.2
73	[16]	0.0	2.7	0.0	0.0	0.0	1.3	1.9	4.3	6.2	3.9	5.0	0.0	0.0	0.0	0.0	0.0	3.5
74	[16]	0.0	1.6	0.0	0.0	11.6	1.8	0.0	2.1	0.0	56.9	4.0	0.0	0.0	0.0	2.1	0.0	8.7
75	[16]	0.0	3.2	0.0	0.0	7.2	2.6	2.8	0.0	9.7	22.7	12.5	0.0	0.0	0.0	2.5	0.0	10.6
76	[16]	0.0	2.0	0.0	0.0	0.3	2.5	8.6	1.2	13.6	42.9	3.7	0.0	0.0	0.0	0.2	0.0	6.0
77	[16]	0.0	3.2	0.0	0.0	31.5	2.8	4.9	0.0	0.7	7.3	2.0	0.0	0.0	0.0	2.1	0.0	9.9
78	[16]	0.0	2.0	0.0	0.0	46.1	2.0	3.3	0.0	0.0	1.1	0.0	0.0	0.0	0.0	0.0	0.0	4.0
79	[47]	6.6	1.7	0.0	0.0	0.0	2.5	5.6	1.4	0.8	6.7	0.8	20.0	1.4	3.5	0.6	3.3	5.6
80	[48]	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0	12.0	0.0	9.2	0.0	0.7	0.0
81	[49]	0.0	1.1	0.0	0.0	0.0	1.8	1.1	1.0	4.5	0.0	6.5	3.4	0.0	1.9	0.4	0.0	16.0
82	[49]	0.0	20.8	0.0	0.0	0.0	1.1	10.7	17.3	14.6	0.0	1.8	0.5	0.0	0.0	0.3	0.5	0.0
83	[59]	0.0	2.8	0.0	0.0	0.0	1.2	0.5	0.1	0.0	0.0	0.0	53.5	0.4	0.0	0.0	0.0	27.4
84	[50]	0.0	2.3	0.0	0.0	0.0	0.0	4.0	0.0	1.1	10.4	0.7	12.8	2.0	2.0	3.6	2.0	0.0
85	[60]	0.0	0.0	0.0	5.7	0.0	0.0	1.9	0.9	2.3	17.1	0.0	5.6	2.8	4.9	3.1	0.0	18.1
86	[60]	0.0	0.0	0.0	5.0	0.0	0.0	2.9	1.9	2.1	20.6	0.0	8.8	2.7	4.8	5.6	0.0	12.5
87	[60]	0.0	0.0	0.0	1.9	0.0	0.0	4.5	1.1	0.6	12.0	0.0	19.5	0.4	3.1	5.1	0.0	25.8
88	[60]	0.0	0.0	0.0	8.1	0.0	0.0	1.7	0.5	0.2	18.0	0.0	7.0	0.3	3.3	3.1	0.0	13.0
89	[55]	0.0	10.4	0.0	0.0	0.0	0.0	0.7	0.1	0.0	3.9	0.0	0.7	0.2	5.4	0.0	11.3	14.1
90	[51]	4.6	3.5	0.0	2.3	0.0	3.0	4.6	2.7	4.0	31.0	5.1	1.8	0.5	0.0	1.3	0.0	10.7
91	[18]	0.0	0.0	0.0	14.7	0.0	0.0	3.9	1.9	8.7	29.0	2.9	0.1	5.2	0.5	3.2	0.0	1.1
92	[18]	0.0	0.0	0.0	5.2	0.0	0.0	1.1	3.4	7.4	48.0	6.9	0.0	1.8	0.1	2.2	0.0	0.2
93	[18]	0.0	0.0	0.0	11.2	0.0	0.0	1.6	3.9	5.7	41.8	4.2	0.0	1.0	0.2	3.7	0.0	0.6
94	[52]	0.0	28.6	0.1	0.0	0.0	1.7	0.4	0.4	1.4	6.2	0.0	4.2	10.1	0.2	0.0	0.0	12.0
95	[52]	0.0	14.5	0.4	0.0	0.0	0.0	5.7	2.0	2.2	11.8	0.0	2.3	0.8	3.3	0.0	0.0	9.9
96	[52]	0.0	1.0	0.4	0.0	0.0	1.3	6.2	2.6	1.0	4.1	0.0	29.9	1.1	1.8	0.0	0.0	9.6
97	[52]	0.0	10.2	0.0	0.0	0.0	1.3	4.1	1.8	0.7	13.0	0.0	10.1	1.0	10.8	0.0	0.0	4.9
98	[52]	0.0	9.3	0.0	0.0	0.0	9.3	2.8	0.4	2.4	13.5	0.0	25.2	0.9	0.4	0.0	0.0	0.0
99	[52]	0.0	25.1	0.1	0.0	0.0	2.6	0.2	1.4	1.4	5.9	0.0	0.4	0.2	1.1	0.0	0.0	22.0
100	[52]	0.0	23.3	0.0	0.0	0.0	1.3	0.1	0.1	3.8	7.5	0.0	0.1	0.4	1.0	0.0	0.0	12.0
101	[52]	0.0	17.9	0.1	0.0	0.0	1.2	0.2	3.3	2.0	6.7	0.0	3.7	0.1	1.1	0.0	0.0	12.2
102	[52]	0.0	7.8	0.1	0.0	0.0	1.4	0.6	1.0	2.0	12.0	0.0	0.4	0.0	0.0	0.0	0.0	15.4
103	[21]	0.0	3.7	0.0	0.0	0.0	0.0	1.3	2.3	6.7	19.5	5.1	0.0	0.0	0.0	0.0	0.0	4.0
104	TS *	0.0	5.0	0.0	0.0	0.0	4.2	2.6	0.8	0.4	5.5	1.7	11.8	0.2	6.7	0.2	12.2	13.6
Average		1.5	3.5	1.2	1.8	2.7	1.4	2.8	1.7	2.8	21.7	3.4	3.8	1.0	2.0	1.1	3.4	7.9

* TS: this study.

Samples with high content of neryl-acetate originate from Sardinia [16], while the ones with moderate neryl-acetate content have a wide range of distribution throughout Italy (Elba Island, Sardinia, and Tuscany) [16,18,21,42,44,57] and France (Corsica) [56,57], while one sample was from Montenegro [46] and anotherwas from Croatia [58]. Neryl acetate in combination with ar-curcumene is reported in Italy [16,42,57], Algeria [43], France [56], and Croatia [52]. Ar-curcumene in combination with γ-curcumene is reported in Croatia [52], while γ-curcumene chemotype is present in Serbia (commercial sample), Montenegro, Italy (Foggia, Palermo, and Sardinia), and USA [45,49,54,57].

Samples with high content of α-pinene are distributed in Italy [42], Portugal [59], and Croatia [52], while moderate α-pinene content in *H. italicum* EOs was recorded in Bulgaria, where it was introduced by the Corsican population [60], Croatia [52], Bosnia and Hercegovina [47], and Algeria [48]. This indicates that the chemical composition varies depending on the weather conditions and altitude [16].

Juniper camphor is reported as the main compound for other plant species (*Syzygium samarangense* (Blume) Merr. & L.M.Perry, *Artemisia argyi* Lév. & Vaniot, *Pulicaria somalensis* Hoffm.), as an EO compound responsible for antimicrobial, antioxidant, and phytotoxic properties [61]. However, *H. italicum* with juniper camphor as the dominant compound was reported in Sardinia [16]. Samples with the dominant β-selinene in the EO were also reported in Italy (Potenza, Taranto, Bari, Matera, Basilicata, and Siena) [57]. Furthermore, β-selinene is an important constituent for perfume industry, and thus, is commercially significant [62]. Furthermore, the *H. italicum* EO from Italy (Tuscany) could be divided into two main groups based on its chemical composition: (1) rich in β-diketones or italidiones (with 32.8–42.0%); (2) rich in neryl acetate (39.9–44.5%) that contained between 3.7 and 9.7% of β-diketones [44]. Neryl acetate is characterized by an orange blossom, rose, sweet, and fruity odor [63], while ar-curcumene are the prime contributors to the characteristic 'ginger' attribute [64]. However, γ-curcumene, as a valuable antioxidant compound, is very unstable and is easily transformed into italicene and isoitalicene, and further into α-curcumene when exposed to light [65]. Chemotypes containing these compounds as the dominant ones in the EO could be used in fragrance and perfume industry. They can also be used as natural antioxidants in preventing deterioration of foodstuff, beverage products and pharmaceuticals [46]. Italidiones act as anti-inflammatory agents and protect the skin against pollution and UV radiation [66]. Compounds such as α-pinene showed inhibitory activity on both collagenase and elastase enzymes associated with skin aging process, and thus, it is a valuable raw material in the cosmetic industry [53].

However, some samples, such as the two from Sardinia [16] with cis-β-guaiene (58.2%) or trans-nerolidol (55.6%) as the dominant compounds, did not fit into any of the above mentioned chemotypes. This was also the case with the one sample from USA with neryl acetone as the dominant compound (38.6%) [49]. This could be a consequence of the environmental conditions or crossing with other species or hybrids.

Antimicrobial agents from natural origin in the recent years are extensive studies. However, it depends on strong influence of the regional origin on chemical composition investigated plant species and microbial strain [67]. Antimicrobial activity of *H. italicum* EO from Algeria with α-cedrene, ar-curcumene, and geranyl acetate as dominant compounds assayed by disk diffusion method inhibited growth *S. aureus*, *M. luteus*, *E. cereus*, *B. cereus*, *S. epidermidis*, *B. subtilis*, *P. aeruginosa*, *E. faecalis*, and *P. mirabilis*, but did not affect *E. coli*, *K. pneumonia*, and *L. monocitogenes*. In addition, yeasts (*C. albicans* and *S. cervisae*) as well as fungi (*F. solani*, *A. niger*, *A. alternata* and *A. rabiei*) were also inhibited by *H. italicum* EO [66].

Antioxidants are mainly used to delay free radical accumulation and strengthen the oxidative stability [68]. Consumption of natural antioxidants from plant sources is a wise option for the prevention of oxidative stress-related disorders, aswith many chronic, neurodegenerative, and cardiovascular diseases [69]. The antioxidant properties of natural products, like EOs, are related to the nature of the bioactive compounds and sometimes to synergistic effects between them. There are several methodologies widely used to determine antioxidant capacity and, in this work, the free radical scavenging capacity

of *H. italicum* was determined using DPPH assay, RP, ABTS•+, and BCB assay. DPPH radical scavenging assay determines the ability of samples to donate an electron and scavenge DPPH radicals. RP of phytochemicals is associated with antioxidant capacity, since it is related to their ability to transfer electrons. One of the most frequently used organic radicals for the evaluation of antioxidant efficiency of pure substances and complex systems are stable synthetic ABTS•+. The BCB assay determines lipid peroxidation in oil-in-water emulsions; it measures the loss of the yellow color of β-carotene due to its reaction with formed linoleic acid radicals caused by oxidation, but this process could be minimized by the presence of antioxidants. EOs contain antioxidants which act in a hydrophobic environment, inhibiting lipid peroxidation and scavenge lipid peroxyl radicals, thus preventing the propagation of free radical-mediated chain reactions [70].

In the study by Kladar et al. (2015) [46], EO of *H. italicum* subsp. *italicum* exhibited IC50 value of 1.37 mg/mL to inhibit DPPH radicals, which was weak in comparison with that ofthe ethanol extract of the same plant. Furthermore, the activity obtained by β-carotene bleaching assay was found to be 96.58 μmoLTE/100 g. Poli et al. (2003) [71] performed investigation where DPPH test was performed on *H. italicum* dried flower heads from plants grown in different areas of north-east Italy under different growing conditions. The DPPH assay showed that all extracts in this study showed minimal antioxidant activity, even at the lowest test concentration of 5 mg/mL.

Also, the β-carotene bleaching test was applied in the same study, with respect to the variation in antioxidant activity after 28 and 56 h, and exhibited that the relative antioxidant activities values for the samples of dried flower heads from wild *H. italicum*, grown in experimental open fields, and commercial drug were quite similar at the first step (28 h). A comparison with the reference compound (BHA) showed that the antioxidant activity of these Helichrysum extracts was around 19% lower than that of BHA at the same concentration. However, dried flower heads from *H. italicum* produced in the nursery, exhibited lower activity than the other samples (43% lower than BHA at the same concentration). After 56 h, only the sample grown in experimental open fields maintained relatively constant relative antioxidant activities values, with a 6% drop from the first step (28 h).

α-Glucosidase, an enzyme located in the small intestine tract, plays a role in the final step of carbohydrate digestion, the breakdown of starch and disaccharides to glucose [72]. The enzyme α-glucosidase results in increased blood glucose levels. Inhibition of this enzyme regulates the liberation of D-glucose from complex carbohydrates [73].

Protein denaturation occurs when proteins lose their tertiary and secondary structure after the addition of external stress or compound, such as concentrated inorganic salt, an organic solvent, or heat, and consequently, proteins lose their biological function. Denaturation of protein tissues is one of the most causes of inflammation [74]. Djihane et al. (2017) [43] published a study where diclofenac sodium was found to be less effective when compared with that of *H. italicum* EO. The anti-inflammatory effect could be caused due to the synergistic effect rather than single constituent in *H. italicum* essential oil.

H. italicum was used in traditional medicine of countries where it grows spontaneous in the treatment wide range disorders, among them: allergies, liver, gallbladder and urinary disorders, infections, colds, cough, skin diseases, burns, snake bites, inflammation, sciatica and hernias, sleeplessness, and hysteria [75]. However, scientific data approve antioxidant [46,58,76–79], anti-inflammatory [76], and antimicrobial [58,78] activities of different types of *H. italicum* extracts and EOs.

5. Conclusions

The EO of flowering aerial parts of *H. italicum* introduced from Bosnia and Hercegovina and grown in the Serbia showed an interesting chemical composition. The results from this study showed that the selected five molecular descriptors were adequate in predicting the retention indices of the observed chemical constituents in *H. italicum* EO. The coefficients of determination for training cycle were 0.964 for compounds found in

H. italicum EO. Furthermore, the results suggest that this EO has a potential to be used as flavoring agent, natural antioxidant, and as a value-added bioactive ingredient in food and pharmaceutical products. Considering the demonstrated influence of the chemical composition of *H. italicum* EO on its biological properties, plant chemotypes may significantly influence the applications of this EO.

Author Contributions: Conceptualization, M.A. and V.D.Z.; methodology, J.L. and J.V.; software, L.P.; validation, V.T.Š.; formal analysis, A.V. and J.V.; investigation, J.L. and A.V.; resources, M.A.; data curation, L.P.; writing—original draft preparation, M.A.; writing—review and editing, J.V.; visualization, L.P.; supervision, V.D.Z.; project administration, V.T.Š.; funding acquisition, V.D.Z. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: The authors gratefully acknowledge Zoltan Kurunci for their administrative and technical support.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Baser, K.H.C.; Demirci, B.; Kirimer, N. Compositions of the Essential Oils of FourHelichrysumSpecies from Madagascar. *J. Essent. Oil Res.* **2002**, *14*, 53–55. [CrossRef]
2. Schmidt-Lebuhn, A.N.; Bruhl, J.J.; Telford, I.R.; Wilson, P.G. Phylogenetic relationships of Coronidium, Xerochrysum and several neglected Australian species of "Helichrysum" (Asteraceae:Gnaphalieae). *TAXON* **2015**, *64*, 96–109. [CrossRef]
3. Smissen, R.D.; Breitwieser, I.; Ward, J.M. Genetic diversity in the New Zealand endemic species Helichrysum lanceolatum (Asteraceae: Gnaphalieae). *N. Z. J. Bot.* **2006**, *44*, 237–247. [CrossRef]
4. Galbany-Casals, M.; Garcia-Jacas, N.; Sáez, L.; Benedí, C.; Susanna, A. Phylogeny, Biogeography, and Character Evolution in Mediterranean, Asiatic, and Macaronesian Helichrysum (Asteraceae, Gnaphalieae) Inferred from Nuclear Phylogenetic Analyses. *Int. J. Plant Sci.* **2009**, *170*, 365–380. [CrossRef]
5. Şenol, S.G.; Secmen, O.; Ozturk, B.; Galbany-Casals, M. *Helichrysum unicapitatum* (Asteraceae), a New Species from Turkey. *Ann. Bot. Fenn.* **2011**, *48*, 145–154. [CrossRef]
6. Azizi, N.; Sheidai, M.; Mozaffarian, V.; Nourmohammadi, Z. Karyotype and genome size analyses in species of Helichrysum (Asteraceae). *Acta Bot. Bras.* **2014**, *28*, 367–375. [CrossRef]
7. Aghababyan, M.; Greuter, W.; Mazzola, P.; Raimondo, F.M. Typification of Sicilian Helichrysum (Compositae) revisited. *TAXON* **2007**, *56*, 1285–1288. [CrossRef]
8. Ninčević, T.; Grdiša, M.; Šatović, Z.; Jug-Dujaković, M. *Helichrysum italicum* (Roth) G. Don: Taxonomy, biological activity, biochemical and genetic diversity. *Ind. Crop. Prod.* **2019**, *138*, 111487. [CrossRef]
9. Perrino, E.V.; Tomaselli, V.; Costa, R.; Pavone, P. Conservation status of habitats (Directive 92/43 EEC) of coastal and low hill belts in a Mediterranean biodiversity hot spot (Gargano—Italy). *Plant Biosyst. Int. J. Deal. Asp. Plant Biol.* **2013**, *147*, 1006–1028. [CrossRef]
10. European Commission DG Environment. *Interpretation Manual of European Union Habitats (Version EUR27)*; European Commission DG Environment: Brussels, Belgium, 2007.
11. Mancini, E.; de Martino, L.; Marandino, A.; Scognamiglio, M.R.; de Feo, V. Chemical Composition and Possible in Vitro Phytotoxic Activity of Helichrsyum italicum (Roth) Don ssp. italicum. *Molecules* **2011**, *16*, 7725. [CrossRef]
12. Melito, S.; Sias, A.; Petretto, G.L.; Chessa, M.; Pintore, G.A.M.; Porceddu, A. Genetic and Metabolite Diversity of Sardinian Populations of Helichrysum italicum. *PLoS ONE* **2013**, *8*, e79043. [CrossRef] [PubMed]
13. Brunetti, G.; Ruta, C.; Traversa, A.; D'Ambruoso, G.; Tarraf, W.; De Mastro, F.; De Mastro, G.; Cocozza, C. Remediation of a heavy metals contaminated soil using mycorrhized and non-mycorrhized *Helichrysum italicum* (Roth) Don. *Land Degrad. Dev.* **2018**, *29*, 91–104. [CrossRef]
14. Han, X.; Beaumont, C.; Stevens, N. Chemical composition analysis and in vitro biological activities of ten essential oils in human skin cells. *Biochim. Open* **2017**, *5*, 1–7. [CrossRef] [PubMed]
15. Sarkic, A.; Stappen, I. Essential Oils and Their Single Compounds in Cosmetics—A Critical Review. *Cosmetics* **2018**, *5*, 11. [CrossRef]
16. Melito, S.; Petretto, G.; Podani, J.; Foddai, M.; Maldini, M.; Chessa, M.; Pintore, G. Altitude and climate influence Helichrysum italicum subsp. microphyllum essential oils composition. *Ind. Crop. Prod.* **2016**, *80*, 242–250. [CrossRef]

17. Roussis, V.; Tsoukatou, M.; Petrakis, P.V.; Chinou, I.; Skoula, M.; Harborne, J.B. Volatile constituents of four Helichrysum species growing in Greece. *Biochem. Syst. Ecol.* **2000**, *28*, 163–175. [CrossRef]
18. Perrino, E.; Valerio, F.; Gannouchi, A.; Trani, A.; Mezzapesa, G. Ecological and Plant Community Implication on Essential Oils Composition in Useful Wild Officinal Species: A Pilot Case Study in Apulia (Italy). *Plants* **2021**, *10*, 574. [CrossRef] [PubMed]
19. Usai, M.; Foddai, M.; Bernardini, A.F.; Muselli, A.; Costa, J.; Marchetti, M. Chemical Composition and Variation of the Essential Oil of Wild SardinianHelichrysum ItalicumG. Don subsp. Microphyllum (Willd.) Nym from Vegetative Period to Post-blooming. *J. Essent. Oil Res.* **2010**, *22*, 373–380. [CrossRef]
20. Reidel, R.V.B.; Cioni, P.L.; Majo, L.; Pistelli, L. Evolution of Volatile Emission in Rhus coriaria Organs During Different Stages of Growth and Evaluation of the Essential Oil Composition. *Chem. Biodivers.* **2017**, *14*, e1700270. [CrossRef] [PubMed]
21. Marongiu, B.; Piras, A.; Desogus, E.; Porcedda, S.; Ballero, M. Analysis of the Volatile Concentrate of the Leaves and Flowers ofHelichrysum italicum(Roth) Don ssp.microphyllum (Willd.) Nyman (Asteraceae) by Supercritical Fluid Extraction and Their Essential Oils. *J. Essent. Oil Res.* **2003**, *15*, 120–126. [CrossRef]
22. Andreani, S.; Uehara, A.; Blagojević, P.; Radulović, N.; Muselli, A.; Baldovini, N. Key odorants of industrially-produced Helichrysum italicum subsp. italicum essential oil. *Ind. Crop. Prod.* **2019**, *132*, 275–282. [CrossRef]
23. Dobrnjac, M.; Hodžić, A.; Dobrnjac, S.; Dobrnjac, D. The new solution of the substance flow system in the steam distillation process of essential oil. Intern. *J. Aer. Innov.* **2017**, *5*, 153–155.
24. Yap, C.W. PaDEL-descriptor: An open source software to calculate molecular descriptors and fingerprints. *J. Comput. Chem.* **2010**, *32*, 1466–1474. [CrossRef]
25. Nekoei, M.; Mohammadhosseini, M. Chemical Compositions of the Essential Oils from the Aerial Parts ofAchillea wilhelmsi-iUsing Traditional Hydrodistillation, Microwave Assisted Hydro- distillation and Solvent-Free Microwave Extraction Methods: Comparison with the Volatile Compounds Obtained by Headspace Solid-Phase Microextraction. *J. Essent. Oil Bear. Plants* **2016**, *19*, 59–75. [CrossRef]
26. Goldberg, D.E. *Genetic Algorithms in Search, Optimization, and Machine Learning*; Addison-Wesley Professional: Longman, MA, USA, 1989; pp. 55–60.
27. Gramatica, P. Principles of QSAR models validation: Internal and external. *QSAR Comb. Sci.* **2007**, *26*, 694–701. [CrossRef]
28. Hu, X.; Weng, Q. Estimating impervious surfaces from medium spatial resolution imagery using the self-organizing map and multi-layer perceptron neural networks. *Remote. Sens. Environ.* **2009**, *113*, 2089–2102. [CrossRef]
29. Acimovic, M.; Pezo, L.; Jeremic, J.S.; Cvetkovic, M.; Rat, M.; Cabarkapa, I.; Tesevic, V. QSRR Model for Predicting Retention Indices of Geraniol Chemotype of Thymus serpyllum Essential Oil. *J. Essent. Oil Bear. Plants* **2020**, *23*, 464–473. [CrossRef]
30. Todeschini, R.; Consonni, V. Molecular Descriptors for Chemoinformatics. *Drug Sel.* **2009**, *41*, 1–962.
31. Aćimović, M.; Pezo, L.; Tešević, V.; Čabarkapa, I.; Todosijević, M. QSRR Model for predicting retention indices of Satureja kitaibelii Wierzb. ex Heuff. essential oil composition. *Ind. Crop. Prod.* **2020**, *154*, 112752. [CrossRef]
32. Xu, S.-G.; Zhao, Y.-J.; Liao, J.-H.; Yang, X.-B. Understanding the stable boron clusters: A bond model and first-principles calculations based on high-throughput screening. *J. Chem. Phys.* **2015**, *142*, 214307. [CrossRef]
33. Yoon, P.; Yoon, H.; Kim, Y.; Kim, G.-B. A Comparative Study on Forecasting Groundwater Level Fluctuations of National Groundwater Monitoring Networks using TFNM, ANN, and ANFIS. *J. Soil Groundw. Environ.* **2014**, *19*, 123–133. [CrossRef]
34. Varga, A.; Kocic-Tanackov, S.; Cabarkapa, I.; Acimovic, M.; Tomicic, Z. Chemical composition and antibacterial activity of spice essential oils against Escherichia coli and Salmonella Typhimurium. *J. Food Saf. Food Qual.* **2019**, *70*, 157–194. [CrossRef]
35. Girones-Vilaplana, A.; Mena, P.; Moreno, D.A.; Garcia-Viguera, C. Evaluation of sensorial, phytochemical and biological properties of new isotonic beverages enriched with lemon and berries during shelf life. *J. Sci. Food Agric.* **2013**, *94*, 1090–1100. [CrossRef] [PubMed]
36. Oyaizu, M. Studies on products of browning reaction. Antioxidative activities of products of browning reaction prepared from glucosamine. *Jpn. J. Nutr. Diet.* **1986**, *44*, 307–315. [CrossRef]
37. Mena, P.; García-Viguera, C.; Navarro-Rico, J.; Moreno, D.; Bartual, J.; Saura, D.; Martí, N. Phytochemical characterisation for industrial use of pomegranate (*Punica granatum* L.) cultivars grown in Spain. *J. Sci. Food Agric.* **2011**, *91*, 1893–1906. [CrossRef] [PubMed]
38. Al-Saikhan, M.S.; Howard, L.R.; Miller, J.C. Antioxidant Activity and Total Phenolics in Different Genotypes of Potato (*Solanum tuberosum*, L.). *J. Food Sci.* **1995**, *60*, 341–343. [CrossRef]
39. Šaponjac, V.T.; Gironés-Vilaplana, A.; Djilas, S.; Mena, P.; Ćetković, G.; Moreno, D.; Čanadanović-Brunet, J.; Vulić, J.; Stajčić, S.; Krunic, M. Anthocyanin profiles and biological properties of caneberry (Rubusspp.) press residues. *J. Sci. Food Agric.* **2014**, *94*, 2393–2400. [CrossRef]
40. Ullah, H.A.; Zaman, S.; Juhara, F.; Akter, L.; Tareq, S.M.; Masum, E.H.; Bhattacharjee, R. Evaluation of antinociceptive, in-vivo & in-vitro anti-inflammatory activity of ethanolic extract of Curcuma zedoaria rhizome. *BMC Complement. Altern. Med.* **2014**, *14*, 1–12. [CrossRef]
41. Zeljkovic, S.C.; Šolić, M.E.; Maksimović, M. Volatiles ofHelichrysum italicum(Roth) G. Don from Croatia. *Nat. Prod. Res.* **2015**, *29*, 1874–1877. [CrossRef]
42. Leonardi, M.; Ambryszewska, K.E.; Melai, B.; Flamini, G.; Cioni, P.L.; Parri, F.; Pistelli, L. Essential-Oil Composition ofHelichrysum italicum(Roth) G. Don ssp. Italicum from Elba Island (Tuscany, Italy). *Chem. Biodivers.* **2013**, *10*, 343–355. [CrossRef]

43. Djihane, B.; Wafa, N.; Elkhamssa, S.; Pedro, D.H.J.; Maria, A.E.; Mihoub, Z.M. Chemical constituents of Helichrysum italicum (Roth) G. Don essential oil and their antimicrobial activity against Gram-positive and Gram-negative bacteria, filamentous fungi and Candida albicans. *Saudi Pharm. J.* **2017**, *25*, 780–787. [CrossRef]
44. Paolini, J.; Desjobert, J.-M.; Costa, J.; Bernardini, A.-F.; Castellini, C.B.; Cioni, P.-L.; Flamini, G.; Morelli, I. Composition of essential oils ofHelichrysum italicum (Roth) G. Don fil subsp. Italicum from Tuscan archipelago islands. *Flavour Fragr. J.* **2006**, *21*, 805–808. [CrossRef]
45. Satta, M.; Tuberoso, C.; Angioni, A.; Pirisi, F.M.; Cabras, P. Analysis of the Essential Oil ofHelichrysum italicum G. Don ssp.Microphyllum (Willd) Nym. *J. Essent. Oil Res.* **1999**, *11*, 711–715. [CrossRef]
46. Kladar, N.V.; Anačkov, G.T.; Rat, M.; Srđenović, B.U.; Grujić, N.N.; Šefer, E.I.; Bozin, B. Biochemical Characterization ofHelichrysum italicum(Roth) G. Don subsp. Italicum (Asteraceae) from Montenegro: Phytochemical Screening, Chemotaxonomy, and Antioxidant Properties. *Chem. Biodivers.* **2015**, *12*, 419–431. [CrossRef]
47. Talić, S.; Odak, I.; Bevanda, A.M.; Crnjac, N.; Paštar, M. Helichrysum italicum (Roth) G. Don subsp. Italicum from Herzegovina. *Croat. Chem. Acta* **2019**, *92*, 69–77. [CrossRef]
48. Bouchaala, M.; Ramdani, M.; Chalard, P.; Figueredo, G.; Lograda, T. Chemical Composition, Antibacterial Activity and Chromosome Number of Helichrysum italicum from Algeria. *Int. J. Pharm. Phytoch. Res.* **2016**, *8*, 1675–1683.
49. Tucker, A.O.; Maciarello, M.J.; Charles, D.J.; Simon, J.E. Volatile Leaf Oil of the Curry PlantHelichrysum italicum(Roth) G. Don subsp. italicum and Dwarf Curry Plant subsp.microphyllum (Willd.) Nyman in the North American Herb Trade. *J. Essent. Oil Res.* **1997**, *9*, 583–585. [CrossRef]
50. Mastelić, J.; Politeo, O.; Jerković, I. Contribution to the Analysis of the Essential Oil of Helichrysum italicum (Roth) G. Don. Determination of Ester Bonded Acids and Phenols. *Molecules* **2008**, *13*, 795–803. [CrossRef]
51. Cristofari, G.; Znini, M.; Majidi, L.; Costa, J.; Hammouti, B.; Paolini, J. Helichrysum italicum subsp. italicum essential oil as environmentally friendly inhibitor on the corrosion of mil steel in hydrochlorid acid. *Int. J. Electrochem. Sci.* **2012**, *7*, 9024–9041.
52. Blazevic, N.; Petricic, J.; Stanic, G.; Males, Z. Variations in yields and composition of immortelle (*Helichrysum italicum*, Roth Guss.) essential oil from different locations and vegetation periods along Adriatic coast. *Acta Pharm.* **1995**, *45*, 517–522.
53. Fraternale, D.; Flamini, G.; Ascrizzi, R. In Vitro Anticollagenase and Antielastase Activities of Essential Oil of Helichrysum italicum subsp. italicum (Roth) G. Don. *J. Med. Food* **2019**, *22*, 1041–1046. [CrossRef]
54. Stupar, M.; Ljaljevic-Grbic, M.; Dzamic, A.; Unkovic, N.; Ristic, M.; Vukojevic, J. Antifungal activity of Helichrysum italicum (Roth) G. Don (Asteraceae) essential oil against fungi isolated from cultural heritage objects. *Arch. Biol. Sci.* **2014**, *66*, 1539–1545. [CrossRef]
55. Tzanova, M.; Grozeva, N.; Gerdzhikova, M.; Atanasov, V.; Terzieva, S.; Prodanova, R. Biochemical composition of essential oil of Corsican Helichrysum italicum (Roth) G. Don, introduced and cultivated in South Bulgaria. *Bulg. J. Agric. Sci.* **2018**, *24*, 1071–1077.
56. Bianchini, A.; Tomi, P.; Costa, J.; Bernardini, A.F. Composition of Helichrysum italicum (Roth) G. Don fil. subsp. italicum essential oils from Corsica (France). *Flavour Fragr. J.* **2001**, *16*, 30–34. [CrossRef]
57. 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. *Ind. Crop. Prod.* **2010**, *32*, 639–649. [CrossRef]
58. Dzamic, A.M.; Mileski, K.S.; Ciric, A.; Ristic, M.S.; Sokovic, M.; Marin, P.D. Essential Oil Composition, Antioxidant and Antimicrobial Properties of Essential Oil and Deodorized Extracts of Helichrysum italicum (Roth) G. Don. *J. Essent. Oil Bear. Plants* **2019**, *22*, 493–503. [CrossRef]
59. Costa, P.; Loureiro, J.M.; Teixeira, M.A.; Rodrigues, A.E. Extraction of aromatic volatiles by hydrodistillation and supercritical fluid extraction with CO_2 from Helichrysum italicum subsp. picardii growing in Portugal. *Ind. Crop. Prod.* **2015**, *77*, 680–683. [CrossRef]
60. Oliva, A.; Garzoli, S.; Sabatino, M.; Tadić, V.; Costantini, S.; Ragno, R.; Božović, M. Chemical composition and antimicrobial activity of essential oil of *Helichrysum italicum* (Roth) G. Don fil. (Asteraceae) from Montenegro. *Nat. Prod. Res.* **2020**, *34*, 445–448. [CrossRef] [PubMed]
61. Assaeed, A.; ElShamy, A.; El Gendy, A.E.-N.; Dar, B.; Al-Rowaily, S.; Abd-Elgawad, A. Sesquiterpenes-Rich Essential Oil from Above Ground Parts of Pulicaria somalensis Exhibited Antioxidant Activity and Allelopathic Effect on Weeds. *Agronomy* **2020**, *10*, 399. [CrossRef]
62. Singh, A.K.; Chanotiya, C.S.; Yadav, A.; Kalra, A. Volatiles of Callicarpa macrophylla: A Rich Source of Selinene Isomers. *Nat. Prod. Commun.* **2010**, *5*, 269–272. [CrossRef] [PubMed]
63. Soh, Z.; Saito, M.; Kurita, Y.; Takiguchi, N.; Ohtake, H.; Tsuji, T. A Comparison between the Human Sense of Smell and Neural Activity in the Olfactory Bulb of Rats. *Chem. Senses* **2013**, *39*, 91–105. [CrossRef]
64. Bednarczyk, A.A.; Kramer, A. Identification and Evaluation of the Flavor-Significant Components of Ginger Essential Oil. *Chem. Senses* **1975**, *1*, 377–386. [CrossRef]
65. Odak, I.; Lukic, T.; Talic, S. Impact of Storage Conditions on Alteration of Juniper and Immortelle Essential Oils. *J. Essent. Oil Bear. Plants* **2018**, *21*, 614–622. [CrossRef]
66. Combes, C.; Legrix, M.; Rouquet, V.; Rivoire, S.; Grasset, S.; Cenizo, V.; Moga, A.; Portes, P. 166 Helichrysum italicum essential oil prevents skin lipids peroxidation caused by pollution and UV. *J. Investig. Dermatol.* **2017**, *137*, S221. [CrossRef]

67. Riabov, P.A.; Micić, D.; Božović, R.B.; Jovanović, D.V.; Tomić, A.; Šovljanski, O.; Filip, S.; Tosti, T.; Ostojić, S.; Blagojević, S.; et al. The chemical, biological and thermal characteristics and gastronomical perspectives of Laurus nobilis essential oil from different geographical origin. *Ind. Crop. Prod.* **2020**, *151*, 112498. [CrossRef]
68. Halliwell, B.; Murcia, M.A.; Chirico, S.; Aruoma, O.I. Free radicals and antioxidants in food andin vivo: What they do and how they work. *Crit. Rev. Food Sci. Nutr.* **1995**, *35*, 7–20. [CrossRef] [PubMed]
69. Lee, H.P.; Zhu, X.; Casadesus, G.; Castellani, R.J.; Nunomura, A.; Smith, M.; Lee, H.-G.; Perry, G. Antioxidant approaches for the treatment of Alzheimer's disease. *Expert Rev. Neurother.* **2010**, *10*, 1201–1208. [CrossRef] [PubMed]
70. Fiedor, J.; Burda, K. Potential Role of Carotenoids as Antioxidants in Human Health and Disease. *Nutrients* **2014**, *6*, 466–488. [CrossRef]
71. Poli, G.; Leonarduzzi, G.M.; Biasi, F.; Chiarpotto, E. Oxidative Stress and Cell Signalling. *Curr. Med. Chem.* **2004**, *11*, 1163–1182. [CrossRef]
72. Čakar, U.; Grozdanić, N.; Pejin, B.; Vasić, V.; Čakar, M.; Petrović, A.; Djordjević, B. Impact of vinification procedure on fruit wine inhibitory activity against α-glucosidase. *Food Biosci.* **2018**, *25*, 1–7. [CrossRef]
73. Kumar, S.R.S.; Rao, K.V.B. Efficacy of Alpha Glucosidase Inhibitor from Marine Actino bacterium in the Control of Post-prandial Hyperglycaemia in Streptozotocin (STZ) Induced Diabetic Male Albino Wister Rats. *Iran J. Pharm. Res.* **2018**, *17*, 202–214.
74. Opie, E.L. On the Relation of Necrosis and Inflammation to Denaturation of Proteins. *J. Exp. Med.* **1962**, *115*, 597–608. [CrossRef]
75. 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.* **2014**, *151*, 54–65. [CrossRef] [PubMed]
76. Sala, A.; Recio, M.D.C.; Giner, R.M.; Máñez, S.; Tournier, H.; Schinella, G.; Ríos, J.-L. Anti-inflammatory and antioxidant properties of Helichrysum italicum. *J. Pharm. Pharmacol.* **2010**, *54*, 365–371. [CrossRef] [PubMed]
77. Kramberger, K.; Pražnikar, Z.J.; Arbeiter, A.B.; Petelin, A.; Bandelj, D.; Kenig, S. A Comparative Study of the Antioxidative Effects of *Helichrysum italicum* and *Helichrysum arenarium* Infusions. *Antioxidants* **2021**, *10*, 380. [CrossRef] [PubMed]
78. Mollova, S.; Fidan, H.; Antonova, D.; Bozhilov, D.; Stanev, S.; Kostova, I.; Stoyanova, A. Chemical composition and antimicrobial and antioxidant activity of Helichrysumitalicum (Roth) G.Don subspecies essential oils. *Turk. J. Agric. For.* **2020**, *44*, 371–378. [CrossRef]
79. Poli, F.; Muzzoli, M.; Sacchetti, G.; Tassinato, G.; Lazzarin, R.; Bruni, A. Antioxidant Activity of Supercritical CO_2 Extracts of Helichrysum italicum. *Pharm. Biol.* **2003**, *41*, 379–383. [CrossRef]