



Article

# Essential Oil Yield, Composition, and Antioxidant Activity in Two Umbel Maturity Stages of Wild Carrot (*Daucus carota* L. ssp. *carota*) from Montenegro

Jelena Stanojević <sup>1</sup>, Zoran S. Ilić <sup>2</sup>,\*, Ljiljana Stanojević <sup>1</sup>, Lidija Milenković <sup>2</sup>, Renata Kovač <sup>3</sup>, Dragana Lalević <sup>2</sup>, Ljubomir Šunić <sup>2</sup>, Aleksandra Milenković <sup>1</sup> and Dragan Cvetković <sup>1</sup>

- <sup>1</sup> Faculty of Technology, University of Niš, BulevarOslobođenja 124, 16000 Leskovac, Serbia
- Faculty of Agriculture, University of Priština in Kosovska Mitrovica, Kopaonička bb, 38219 Lešak, Serbia
- <sup>3</sup> Institute of Food Technology, University of Novi Sad, 21000 Novi Sad, Serbia
- \* Correspondence: zorans.ilic@pr.ac.rs; Tel.: +381-638-014-966

Abstract: The purpose of this study was to determine essential oil yield, composition, and antioxidant activity during two different maturation stages of umbels with seeds(I stage: premature-waxy; and II stage: mature-fully ripening) of wild carrot (Daucus carrota var. carota) from the Montenegrin coast. A higher yield of carrot essential oil (CEO) was determined in mature, fully ripening umbels (1.96 mL/100 g p.m) than in premature umbels at the waxy stage (mL/100 g p.m). Thirty-three components were identified in premature umbels, with  $\beta$ -bisabolene (32.3%), 11- $\alpha$ -(H)himachal-4-en-1- $\beta$ -ol (27.9%), elemicin (10.1%), and  $\alpha$ -longipipene (7.7%) being the main components. They were followed by  $\alpha$ -pinene (3.7%), (E)-asarone (3.4%), (E)-anethole (3.2%), and  $\beta$ -himachalene (2.0%). Thirty-two components were identified in CEO from mature umbels, with  $\beta$ -bisabolene (41.0%),  $11-\alpha$ -(H)-himachal-4-en-1- $\beta$ -ol(21.1%), elemicin (14.8%), and  $\alpha$ -longipipene (5.7%) being the most abundant. These components were followed by (E)-asarone (3.9%),  $cis-\alpha$ -bisabolene (2.4%), and  $\beta$ -himachalene (2.0%). The CEO isolated from mature umbelshowed better antioxidant activity (EC<sub>50</sub> value of 31.80 mg/mL) in comparison to the CEO isolated from premature umbels (EC<sub>50</sub> value of 49.18 mg/mL) during the incubation time of 60 min. The degree of DPPH radical neutralization increased as the incubation time increased from 20 to 60 min. Therefore, our findings recommend that wild carrot could be harvested in the fully ripening stage when the umbel improves CEO yield and antioxidant activity, without the risk of seed shedding from the umbel and seed losses.

**Keywords:** wild carrot; umbel; stage of maturation; essential oils; yield; composition; antioxidant activity



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# 1. Introduction

Wild carrot, *Daucus carota* L. ssp. *carota*, originates from the umbelliferae family with a high range of distribution and wide polymorphism [1,2]. It is a weed species that is undesirable in agricultural production, and grows in spontaneous flora. Its essential oils can be used for various medicinal purposes in the food technology, pharmaceutical, cosmetic, and sanitary industries (toiletry items, soaps, detergents), etc. Wild carrot essential oil (CEO) is used as a flavoring agent in beverages, bread and rolls, meat and meat products, fish, etc. For cystitis and prostatitis, CEO is used as an antiseptic in folk medicine in many European countries [3]. CEO is known as a treatment to strengthen the immune system and enhance liver function, and is also used against gastric ulcers, diabetes, and muscle and back pain, [4]. CEO extracted from different plant parts and development stages of wild carrot (flowering stage) has good antimicrobial activities and has been characterized with diuretic properties [5,6]. Traditionally, CEO is use to treat urinary calculus, cystitis, gout, and lithuria [5,7,8]. CEOalso has anti-tumor, anti-cancer properties, and antioxidant activity against a wide range of malignancies and diseases [9]. Many the medicinal properties are

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claimed for Iran's wild *D. carota*, characterized with nephroprotective, wound healing, and aphrodisiac activity [10]. Essential oils from the seeds of wild and cultivated carrot from Serbia are characterized by different content and components.

Sabinene and  $\alpha$ -pinene are the main components of CEO from wild carrot seeds. Carotol, the main component in cultivated carrot, has a pleasant spicy aroma and taste; thus, it has wide use as a favorable additional flavor in the cosmetics industry [11]. CEO from Italy, Lithuania, and Poland also contain  $\alpha$ -pinene and sabinene as predominated compounds [12–14]. In contrast, the main constituents of CEO from China and Egypt are carotol and daucol [15,16]. Sabinene (40.9%) and  $\alpha$ -pinene (30.1%) are the dominant compounds of wild CEO from northeast Serbia [11]. In the case of wild carrot from southeast Serbia, essential oil studies showed that geranyl-acetate (55.1%) and cubebol (10.5%) were the most abundant compounds [17]. Wild carrots, as an important source of biodiversity, represent a significant gene pool in the breeding program and creation of new varieties tolerant to different forms of abiotic stresses, resistant to diseases and pests, and male-sterile with better nutritional value. These wild species can be an important factor in the selection and process of improving vegetable quality and production [18].

The present paper aimed to compare the yield, chemical composition, and antioxidant activity of the essential oil isolated from the wild carrot (*Daucus carota* L. ssp. *carota*) umbels with seeds in two maturity stages (premature–waxy stage and mature–fully ripening) grown in Montenegro.

#### 2. Material and Methods

#### 2.1. Plant Material

Wild carrot (*Daucus carota* ssp. *carota*) is widely distributed in the coastal areaaround the Adriatic Sea, where it grows autochthonously. The plant material (inflorescences with seeds of different maturity) was collected in Herceg Novi (42°27′26.0928″ N and 18°31′53.31″ E) on the Montenegrin coast at the end of the growing season—September 10. After harvest, the plant material was dried in a drafty place in the shade and stored inpaper bags at room temperature until the analysis.

#### 2.2. ClevengerHydrodistillation

Disintegrated and homogenized plant material was used for essential oil isolation through Clevenger-type hydrodistillation with a hydromodulus (ratio of plant material: water) of 1:10 m/V for 120 min as described by Ilić et al. [19] and Milenković et al. [20].

# 2.3. Gas Chromatography/Mass Spectrometry (GC/MS) and Gas Chromatography/Flame Ionization Detection (GC/FID) Analysis

The details of the gas chromatography/mass spectrometry (GC/MS) and gas chromatography/flame ionization analyses are given in Ilić et al. [19].

## 2.4. DPPH Assay

The ability of the essential oil to scavenge free DPPH radicals was determined using the DPPH assay. Absorption was measured at 517 nm after 20, 40, and 60 min incubation with the radicals. All other relevant details of the assay used are given in Stanojević et al. [21,22].

### 2.5. Antimicrobial Activity

Microorganisms and substrates. Seven microorganisms were selected to determine the antimicrobial activity of the essential oil: (six bacterial strains) *Escherihia coli, Pseudomonas aeruginosa, Proteus vulgaris, Bacillus subtilis, Staphylococcus aureus,* and *Listeria monocytogenes,* and (fungal strain) *Candida albicans.* Microorganisms are from the collection of the Microbiology Laboratory, Faculty of Technology, Leskovac.

Disc-diffusion method. The agar disc-diffusion method explained by Milenkovic et al. was used for testing the antimicrobial activity [23]. The inoculation,

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incubation, and measurement of the inhibition zone diameter were performed by methods as described byllić et al. [24]. Every measurement was taken after three replications, and average values were calculated.

#### 2.6. Statistical Methods

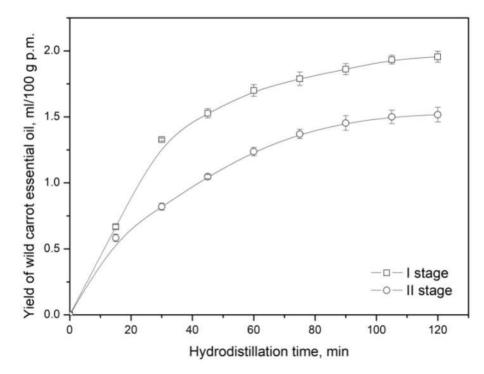
The difference between means of wild carrot essential oil yield was calculated with a T-test, while for other comparisons, ANOVA was used; in the case of wild carrot yield, one-way ANOVA was used, while for  $EC_{50}$ , factorial ANOVA was used. TIBCO Software Inc. (Palo Alto, CA, USA) (2020) Data Science Workbench, version 14. (http://tibco.com, accessed on 1 December 2020.), was used to perform all statistical calculations.

#### 3. Results and Discussion

#### 3.1. Essential Oil Content

Like all plants in the family *Umbelliferae*, the wild carrot is characterized by the successive flowering and ripening of inflorescences. Therefore, part of the seed can be shed and be lost if the harvest is too late. In contrast, if the harvest begins too early, the quality and quantity of the seeds will decrease. Due to no shattering risk in the wild carrot, the harvest should be carried out when the plants reach full maturity on the first umbel; the plants should be left to dry in a compartment, and then shaking should be carried out to release the seeds, or harvesting time could be delayed until the maturation of the secondary umbel. The yield of CEO depends on many factors, such as plant origin, variety, plant part, developmental stage, time of harvest, extraction methods, hydro-distillation time, etc. All parts of the plant contain the essential oil, but in different concentrations and compositions [15].

The essential oil yield was higher in mature  $(1.96 \, \text{mL}/100 \, \text{g p.m})$  than in premature umbels  $(1.52 \, \text{mL}/100 \, \text{g p.m})$ . The maximum yield and quality of wild carrot umbels were obtained when the primary umbel was fully mature. The stage of umbel maturity has a significant influence on wild carrot essential oil content (Figure 1).



**Figure 1.** The EO yield (ml/100 g p.m) of wild carrot seeds in two maturity stages (I-II) obtained after 2 h of hydrodistillation.

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Content of CEO from wild plants ranged between 0.5 and 0.8% (mL/100 g). Differences in CEO content between wild (1.67 mL/100 g)and cultivated (0.55 mL/100 g) carrot plants was significant [11]. The results of our research are in agreement with research from Poland, where the CEO obtained from mature umbels (1.06% mL/100 g)was higher than in flowering umbels (0.65 mL/100 g) [4].

The internal characteristics of the inflorescence, fruits, and seeds of the same plant in terms of schizocarpium (fruit) size, shape, mass, surface and anatomical features, vascular structure, and shape of exocarp cells [25] can affect the content of essential oils, but nothing else, and external environmental conditions of too high a temperature during flowering or heavy rain during ripening can the reduce content and composition of the essential oil in wild carrot seeds.

## 3.2. Essential Oil Composition

The chromatogram (GC/FID) of wild carrot essential oil from mature–fully ripening umbels is present in Figure 2.

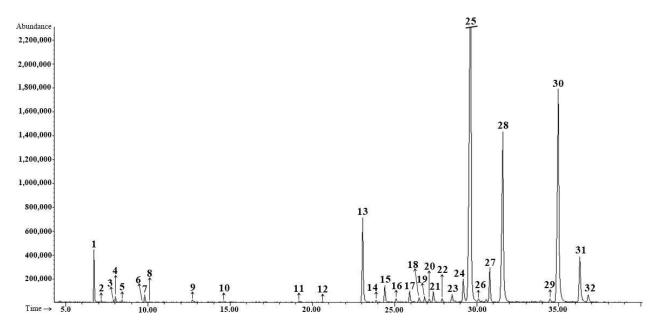


Figure 2. Chromatogram (GC/FID) of wild carrot essential oil from fully ripening umbels.

Variations in the chemical compositions of essential oils in response to different growth and development stages of the wild carrot are minor. The chemical composition of the essential oil obtained from premature and mature umbels is given in Table 1.

Thirty-three components were identified, mainly sesquiterpene hydrocarbons (48.2%), oxygenated sesquiterpenes (28.6%), phenylpropanoids (17.0%), monoterpene hydrocarbons (5.3%), and oxygenated monoterpenes (0.7%), representing 99.8% of the total essential oil composition from the premature–waxy wild carrot umbel (Table 1).  $\beta$ -bisabolene (32.3%), 11- $\alpha$ -(H)-himachal-4-en-1- $\beta$ -ol(27.9%), elemicin (10.1%), and $\alpha$ -longipipene (7.7%) were found as the most abundant components (structures given in Figure 3), followed by  $\alpha$ -pinene (3.7%), (*E*)-asarone (3.4%), (*E*)-anethole (3.2%), and  $\beta$ -himachalene (2.0%).

Thirty-two components were identified, mainly sesquiterpene hydrocarbons (54.7%), oxygenated sesquiterpenes (22.1%), phenylpropanoids (18.7%), monoterpene hydrocarbons (2.8%), and oxygenated monoterpenes (1.5%), representing 99.8% of the total essential oil composition from mature–fully ripening wild carrot umbels (Table 1).  $\beta$ -Bisabolene (41.0%), 11- $\alpha$ -(H)-himachal-4-en-1- $\beta$ -ol(21.1%), elemicin (14.8%), and  $\alpha$ -longipipene (5.7%) were the main components. These components were followed by (*E*)-asarone (3.9%), *cis*- $\alpha$ -bisabolene (2.4%), and  $\beta$ -himachalene (2.0%) (Table 1).  $\beta$ -sesquiphellandrene(0.2%) was present only in fully ripening umbels.

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 $\textbf{Table 1.} \ Chemical \ composition \ of \ EO \ isolated \ from \ wild \ carrot \ umbels \ at \ different \ maturity \ stages.$ 

No.	$t_{ m ret}.$ min	Compound	RI <sup>exp</sup>		Method of Identification	Content %		
				RI <sup>lit</sup>		Premature– Waxy Stage	Mature– Fully Ripening	
1.	6.73	α-Pinene	924	932	RI, MS	$3.7 \pm 0.09$	$2.0\pm0.04$	
2.	7.17	Camphene	940	946	RI, MS	0.1±	tr	
3.	7.92	Sabinene	965	969	RI, MS	tr	tr	
4.	8.03	β-Pinene	968	974	RI, MS, Co-I	0.4±	0.3±	
5.	8.43	Myrcene	981	988	RI, MS	0.3±	$0.1 \pm 0.01$	
6.	9.69	p-Cymene	1019	1020	RI, MS	tr	tr	
7.	9.80	Limonene	1022	1024	RI, MS, Co-I	$0.8 \pm 0.01$	0.4±	
8.	10.08	(Z)-β-Ocimene	1029	1032	RI, MS	tr	tr	
9.	12.07	Fenchone	1081	1083	RI, MS	tr	tr	
10	12.70	Linalool	1098	1095	RI, MS, Co-I	0.4±	tr	
11.	13.66	α-Campholenal	1121	1122	RI, MS	tr	-	
12.	14.61	trans-Verbenol	1143	1140	RI, MS	tr	tr	
13.	16.78	Methyl chavicol	1195	1195	RI, MS	0.3±	-	
14.	20.62	(E)-Anethole	1283	1282	RI, MS	$3.2 \pm 0.01$	tr	
15.	23.05	α-Longipipene	1341	1350	RI, MS	$7.7 \pm 0.01$	$5.7 \pm 0.01$	
16.	23.89	Longicyclene	1361	1371	RI, MS	tr	tr	
17.	24.40	Geranyl acetate	1372	1379	RI, MS	tr	$1.1\pm0.01$	
18.	25.10	β-Longipipene	1390	1400	RI, MS	0.3±	0.3±	
19.	25.92	(E)-Caryophyllene	1409	1417	RI, MS, Co-I	0.6±	0.8±	
20.	26.51	α-trans-Bergamotene	1423	1432	RI, MS	$0.3 \pm 0.01$	0.3±	
21.	26.85	Neryl acetone	1431	1434	RI, MS	$0.3 \pm 0.01$	$0.4\pm$	
22.	27.12	α-Himachalene	1439	1449	RI, MS	$0.3 \pm 0.02$	0.2	
23.	27.36	(Z)-β-Farnesene	1444	1440	RI, MS	$1.0 \pm 0.02$	$0.9 \pm 0.01$	
24.	27.91	Sesquisabinene	1457	1457	RI, MS	0.4±	0.3±	
25.	28.50	9-epi-(E)- Caryophyllene	1472	1464	RI, MS	$0.8 \pm 0.02$	$0.6 \pm 0.01$	
26.	29.18	β-Himachalene	1498	1500	RI, MS	$2.5 \pm 0.02$	$2.0 \pm 0.02$	
27.	29.62	β-Bisabolene	1500	1505	RI, MS	$32.3 \pm 0.01$	$41.0 \pm 0.06$	
28.	30.12	β-Sesquiphellandrene	1512	1521	RI, MS	-	$0.2 \pm 0.02$	
29.	30.80	cis-α-Bisabolene	1529	1529	RI, MS	$2.0 \pm 0.01$	$2.4\pm0.02$	
30.	31.57	Elemicin	1549	1555	RI, MS	$10.1 \pm 0.03$	$14.8 \pm 0.04$	
31.	34.48	1,10-di-epi-Cubenol	1625	1618	RI, MS	0.4±	0.3±	
32.	35.00	11-α-(H)-himachal-4- en-1-β-ol	1639	1638	RI, MS	$27.9 \pm 0.12$	$21.1\pm0.07$	
33.	36.28	(E)-Asarone	1672	1675	RI, MS	$3.4 \pm 0.02$	3.9±	
34.	36.80	Eudesm-7(11)-en-4-ol	1690	1700	RI, MS	0.3±	0.7±	

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No.	$t_{ m ret}.$ min	Compound				Content %			
			RI <sup>exp</sup>	RI <sup>lit</sup>	Method of Identification	Premature– Waxy Stage	Mature– Fully Ripening		
					Total identified	$99.8 \pm 0.41$	$99.8 \pm 0.32$		
	Grouped components (%)								
	Monote	erpene hydrocarbons (	1–8)		$5.3 \pm 0.10$	$2.8 \pm 0.05$			
	Oxyger	nated monoterpenes (9	-12, 17, 21)		$0.7 \pm 0.01$	1.5			
	Sesquit	erpene hydrocarbons	$48.2\pm0.12$	$54.7 \pm 0.14$					
	Oxygenated sesquiterpenes (30, 31, 33)					$28.6 \pm 0.12$	$22.1\pm0.04$		
	Phenyl	propanoids (13, 14, 29,	$17.0 \pm 0.06$	$18.7 \pm 0.09$					

tret: retention time; RIlit: retention indices from the literature (Adams, 2009) [26]; RIexp: experimentally determined retention indices using a homologous series of n-alkanes (C8–C20) on the HP-5MS column. MS: constituent identified using mass-spectra comparison; RI: constituent identified using retention index matching; Co-I: constituent identity confirmed using GC co-injection of an authentic sample; tr: trace amount (<0.05%).

Figure 3. Structures of the most abundant components in wild carrot umbels.

Very similar results, which are in agreement with our research on wild carrots from Montenegro, are also found in other Mediterranean regions and countries, such as Italy (Sardinia) and Portugal [6]. Dominant compounds in the CEO from northern Tunisia's Mediterranean basin were also characterized, with  $\beta$ -bisabolene as the main component [27].

It seems that CEO constituents are conditioned by ontogenetic development, origin, and geographical distribution. Essential oils obtained from flowering and mature umbels of wild plants show that  $\beta$ -bisabolene and 11- $\alpha$ -(H)-himachal-4-en-1- $\beta$ -ol predominate in the Mediterranean, while geranyl acetate and  $\alpha$ -pinene predominate on the Atlantic coast [6].

Although geographically close to Montenegro, we find a completely different composition of EO in wild carrots from Serbia, a neighboring continental country. Wild *Daucus carotagrowing* spontaneously in central Serbia presented in the study by Sokovic et al. [28] contained 48 compounds in CEO from ripe umbels, with sabinene as the main component, and 60 components in CEO from unripe umbels, with  $\alpha$ -muurolene as the main constituent. Similarly, in wild carrot seeds from north Serbia, CEO analysis shows that sabinene (40.9%) and  $\alpha$ -pinene (30.1%) are the main components. It is a specific variety of wild carrots containing sabinene and  $\alpha$ -pinene as the most abundant components and Horticulturae 2023, 9, 328 7 of 12

characteristic for continental areas of the Balkan region [11]. In carrot from Uzbekistan, the dominant compound of CEO was  $\beta$ -bisabolene (80.5%) [29].

The monoterpenes  $\alpha$ -pinene and sabinene dominated the CEOs of *D. gingidium* ssp. *gingidium* and *D. carota* ssp. *carota* (from Italy, Lithuania, and Poland) [13,14,30]. The major constituent of essential oils from *Daucus carota* subspecies *drepanensis* growing wildly inTunisia was (*E*)-methyl isoeugenol (58.7%), and for subsp. *hispidus*, the main component was  $\alpha$ -cadinol (13.6%), [31].

The main constituent of wild carrot essential oil from Poland [4] was sabinene (40.5%). Furthermore, (E)-methyl isoeugenol and  $\beta$ -bisabolene were identified as the main components of D. carota ssp. maximus essential oil, and reflect the unique composition of the essential oils of this Lebanese variety [32]. Myristicin is the main constituent of EO from Daucus sahariensisMurb. and is the chemical marker of this Saharan species [33]. As oxygenated monoterpenes, (E)-anethol and estragole are present as the main essential oil components of the D. reboudii growing in Bejaïa, Algeria [34]. Myrcene,  $\alpha$ -pinene, and sabinene are the most common components, but their amounts vary depending on plant species and variety, plant part, and ontogenetical stage [5,14].

Our study had sesquiterpene hydrocarbons and oxygenated sesquiterpene as the dominant groups. The results of the current study might be due to the synergistic activity of the phenylpropanoid group and the above-mentioned groups present in the oil.

# 3.3. Antioxidant Activity

Ripeness

Incubation Ripeness x incubation

The essential oil isolated from the mature–fully ripening umbels showed better antioxidant activity than the CEO from premature–waxy umbels during all incubation times studied (Table 2). The results obtained from ANOVA showed a significant effect of ripeness stage and incubation time; however, there is no interaction between these two factors. The degree of DPPH radical neutralization increased as the incubation time increased from 20 to 60 min (Figure 4). The CEO isolated from mature umbels showed the highest and, at the same time, better antioxidant activity (EC $_{50}$  value of 31.8- mg/mL) in comparison to the CEO isolated from premature umbels (EC $_{50}$  value of 49.2 mg/mL) during the incubation time of 60 min (Table 2).

F(*-1-0*1	EC <sub>50</sub> , mg/mL					
Essential Oil	20 min Incubation 40 min Incubation		60 min Incubation			
Wild carrot (I stage) Mature–Fully ripening stage	$47.7 \pm 0.18\mathrm{b}$	$33.8\pm0.20$ а	$31.8 \pm 0.25$ a			
Wild carrot (II stage) Premature–waxy stage	$74.5 \pm 0.33 \mathrm{c}$	$54.01 \pm 0.42\mathrm{b}$	$49.18\pm0.26\mathrm{b}$			

Table 2. EC<sub>50</sub>values of essential oil from the wild carrot seeds at different maturity stages.

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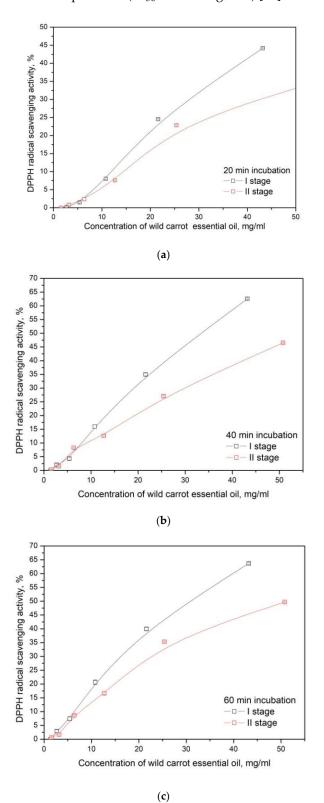
Lebanon wild carrot EO extract from the dried mature umbels exhibited antioxidant activity in all the assays (DPPH, FIC, and FRAP assays). The IC $_{50}$  value for the DPPH assay was determined to be 2.1 mg/mL. Nevertheless, it was a weaker antioxidant in comparison to Trolox (IC $_{50}$  value of 0.18 mg/mL) [35]. CEO from Lebanon wild plantsis characterized by significant antioxidant properties (DPPH activity, IC $_{50}$  = 0.29 mg/mL), [36].

The CEO from Algerian carrot leaves and seeds were found to be effective antioxidants in several in vitro assays. The carrot leaf extract provided the highest radical-scavenging activity, with lower  $IC_{50}$  values (83 mg/mL) than seeds (136 mg/mL). Theantioxidant activity of the CEO was lower than that of the synthetic antioxidant ascorbic acid ( $IC_{50}$ 

a-c: Numbers in column marked with same letter are not significantly different (at 0.05 level). \*\*: Significance of ripening stage (significance at 0.01).

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value of  $3.73 \mu g/mL$ ) [37]. The tested EOs from Moroccan *D. carota* demonstrated a low antioxidant potential (IC<sub>50</sub> = 73.31 mg/mL) [38].



**Figure 4.** The capacity of DPPH radical neutralization by the wild carrot umbels' essential oil after (a) 20, (b) 40, and (c) 60 min of incubation time.

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The essential oils isolated in this study showed weak antioxidant activity in comparison to the synthetic antioxidant BHT (EC<sub>50</sub> value of 0.021 mg/cm<sup>3</sup> after 20 min incubation with DPPH radical) [21]. They could, however, be considered as sources of  $\beta$ -bisabolene, 11- $\alpha$ -(H)-himachal-4-en-1- $\beta$ -ol, and elemicin.

Yeo et al. [39] demonstrated that i.p. injection of  $\beta$ -bisabolene can successfully target tumor cells in vivo. There is no information in the literature about the activity of the oxygencontaining sesquiterpene 11- $\alpha$ -(H)-himachal-4-ene-1- $\beta$ -ol. According to Maxia et al. [6], an oil type with high levels of  $\beta$ -bisabolene (17.6–51.0%) and 11- $\alpha$ -(H)-himachal-4-en-1- $\beta$ -ol (9.0–21.6%) had remarkable antifungal activity, implying that it could be useful for therapeutic purposes. On the other hand, according to the study by Al-Qahtani et al. [40], elemicin exhibits strong antioxidant activity by scavenging 100% of the DPPH radical at a 300 µg/mL concentration. The low antioxidant activity of the CEO studied in this paper could be due to interactions between different components that reduce its antioxidant potential.

# 3.4. Antibacterial Activity

CEO indicated antimicrobial activity against Escherichia coli, Staphylococcus aureus, Bacillus subtilis, and Candida albicans, but had no influence on Pseudomonas aeruginosa, Proteus vulgaris, and Listeria monocytogenes. CEO from fully ripe umbelsreduced the zone of inhibition of *Escherichia coli*, *Bacilus subtilis*, and *Candida albicans* better, but hada lesser effect on *S. aureus* than CEO from the premature—waxy stage. Efficacies increased with the ontogenesisripening stage. Despite the umbel stage of maturity, CEO did not havean effect on the inhibition zone of *P. aeruginosa*, *P. vulgaris*, and *L. monocytogenes* (Table 3).

		D 1		0. 1.1		T	
	Escherichia coli	Pseudo- monasae- ruginosa	Proteus vulgaris	Staphylo- coccus aureus	Bacillus subtilis	Listeria monocyto- genes	Candida albicans
Carrot umbel premature–waxy;I stage	20.3 <sup>a</sup>	0	0	11.7 <sup>a</sup>	17.7 <sup>d</sup>	0	11.7 <sup>a</sup>
Carrot umbel mature-full ripening;II stage	19.3 <sup>a</sup>	0	0	13.7 <sup>b</sup>	14.3 bc	0	11.3 <sup>a</sup>
	**			**	**		**

Table 3. Antimicrobial activity (inhibition zone, mm) of essential oils from wild carrot from Montenegro.

a–d: Numbers in column marked with same letter are not significantly different (at 0.05 level) \*\*: Significance of ripening stage (significance at 0.01).

EOs from carrot umbels in different stages of maturity did not show a significant difference on the microbial inhibition zone.

The antimicrobial activity of the essential oil of wild carrots from Serbia declines from ripe fruit to flowers, and is characterized with good antimicrobial potential [28]. In one study, CEO was efficaces for both Gram-positive and Gram-negative bacteria [4], but in other studies, it wasonly effective against Gram-positive bacteria [41,42].

Invasive weeds are a major threat to biological diversity, and can be difficult and costly to control. Currently, there isagreat potential for utilizing CEO as alternatives and supplements to conventional antimicrobial additives in the food industry (against *Escherichia coli, Bacilus subtilis, Staphylococcus aureus*, and *Candida albicans*), as antioxidant in the cosmetic industry, as or biopesticides against insects such as different mosquito species. As natural products become more and more in-demand, the use of wild carrots, in addition to the economic benefits, enriches agriculture and natural ecosystems, and it is necessary to sustainably manage this aggressive weed, as well as create products with added value [43]. An interdisciplinary approach through the preservation of biodiversity, along with the rational management of biological invasions through the economic exploitation of wild carrots should enable the use of CEO in the food and cosmetic industries.

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#### 4. Conclusions

Compensation for invasive weed control costs would be highly desirable solution, such as the development of new value-added products from the harvest of wild carrots. According to the results obtained, wild carrot could be harvested in the fully ripening stage, when umbels improve CEO yield and antioxidant activity without the risk of seed shedding from the umbels and seed losses. The main components of CEO were  $\beta$ -bisabolene, 11- $\alpha$ -(H)-himachal-4-en-1- $\beta$ -ol, and elemicin, regardless of umbels' ripening stage. CEO have shownsignificant activity inhibiting *Staphylococcus aureus* and *Candida albicans*. In addition to the current use of CEO as biopesticides, in food processing, and in the pharmacologic and cosmetic industries, future research could go in the direction of using CEO for medical purposes as an antitumor and antidiabetes agent in the treatment of humans.

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