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Essential Oil Yield, Composition, Antioxidant and Microbial Activity of Wild Fennel (*Foeniculum vulgare* Mill.) from Monte Negro Coast

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Abstract: The aim of this work was to compare the chemical composition and antioxidant activity of the essential oils from two plant parts (leaves and stems) of fennel, wild-grown in the Montenegro seaside. The chemical composition of the isolated essential oils was determined by gas chromatography with mass spectrometry (GC/MS) and flame ionization detection (GC/FID). The yield of the fennel essential oils (FEOs) from leaves (0.83%) was four times higher than that from the fennel stems (0.21%). Forty-six compounds were identified from leaves' FEOs and were mainly aromatic compounds (68.5%), monoterpenes (17.8%), and others, where the most abundant compounds were (*E*)-anethole (51.4%) and methyl chavicol (9.3%). Forty-seven compounds were identified in the FEOs from stems, which were mainly aromatic compounds (69.7%), oxygen-containing monoterpenes (14.9%), where the most abundant compounds were also (*E*)-anethole (55.7%) and methyl chavicol (7.8%). The FEOs from stems showed higher antioxidant activity, with an EC_{50} value of 2.58 mg/mL, than in the fennel leaves, which had an EC_{50} value of 6.91 mg/mL. The FEOs show superior antimicrobial activity against *Candida albicans* (45.3 mm) and *Bacillus subtilis* (24.0 mm). Isolated essential oils could be used as a safer alternative to synthetic additives in the food industry.

Keywords: *Foeniculum vulgare* Mill.; plant part; essential oils; composition; antioxidant activity; microbial activity

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1. Introduction

Foeniculum vulgare Mill. (family Apiaceae), known as fennel, grows wild as an autochthonous aromatic herb in the wide area around the Mediterranean basin. As an essential oil-producing crop, it is wide spread in many countries of the world, including Asia, Europe and the United States [1]. It belongs to the subspecies, Capillaceum (Gil.), with several varieties, of which the important ones are: *var. vulgare* (Mill.) Thell. (bitter), *var. dulce* (Mill.) Thell. (sweet) and *var. azoricum* (Mill.) Thell. (Florence) [2]. All overground parts of the plant are aromatic and edible. It is native and used fresh or dry for the preparation of cooked meals, salads, tea, beverages or drinks [3]. Essential oils of fennel are used as aromatic flavoring agents in the food industry and as an ingredient in cosmetics and pharmaceutical products, such as in folk medicine, where it is used for female ailments, such as an antipyretic, antirheumatic and detoxifier [4]. Cultivated fennel is a vegetable plant with a tall stem and a thickened leaf base, which forms the edible part with the shortened stem. The thickened white base of the leaf is used for salads or stews, and after blanching it, it can be frozen and used during the winter and can also be marinated and pickled together with cucumber. Because of its antioxidant, antibacterial and antifungal properties, essential oil from fennel is used as an additive in bread, fish, salads, soups and cheese [5]. It is carminative and commonly used in the perfume industries [6], as well as in the manufacturing of soaps, cosmetics and cough drops [7].

The essential oils present in fennel (FEOs) are characterized by a number of pharmacological activities, such as hepatoprotective, antidiabetic [4], antispasmodic, cardiovascular, hypolipidemic [8], gastroprotective, antithrombotic [8] and anticancer properties [9]. In many studies, FEOs have been confirmed to have insecticidal and repellent activities, primarily because of their trans-anethole and fenchone presence [10].

FEOs' biological activities are determined by their chemical constituents that are conditioned by the plant genotype [11] and environmental conditions but are also influenced by other factors, such as the plant part, maturity stage, anatomical and physiological characteristics of plants [12], harvest time [13], etc.

Fenchone, estragole and anethole, as main constituents, strongly contribute to the flavor of FEOs. A bitter note originates from the estragole, while anethole has a sweet anise-like note [14]. The participation of individual components depends on the plant's origin and region. Due to differences in the content of individual components of essential oils, plants from southern Europe produce sweeter tastes, whereas plants from central and northern Europe produce a more bitter tastes [15]. The main components of the fennel essential oil isolated from the dried aerial parts were trans-anethole, α-pinene and limonene, whereas methyl chavicol was dominant in the EOs from the seeds [16]. The EOs components depend on the plant's development stage. Thus, the pre-fruiting fennel plants produce EOs that are particularly rich in *o*-cymene. Depending on the applied techniques of extraction (HSS or HS-SPME), the dominant aroma compounds of fennel were limonene and (*E*)-anethole [17]. Pinenes, fenchone, estragole, myrcene and camphene are the main components in cultivated fennel, while limonene is predominant in wild plants [18].

There is a limitation and lack of research on the content and composition of EOs depending on the part of the fennel plant. That is why our research aims to study the yield, phytochemical profile and antioxidant and microbial activity of the EOs from two plant parts (stalk and leaves) of indigenous fennel grown on the Monte Negro coast.

2. Material and Methods

2.1. Plant Material

Plant samples (leaves and stems) were collected from wild fennel (*Foeniculum vulgare* Mill.) in Monte Negro near the sea coast of Herceg Novi (with the coordinates of 42°27′26.0928″ N and 18°31′53.31″ E) on the tenth day of August 2021 and were identified by Prof. Dr. Danijela Prodanovic of the University of Priština in Kosovska Mitrovica. A voucher specimen has been deposited at the Herbarium of the Department of Biology in the Faculty of Natural Sciences and Mathematics. After drying in the shade, the plant material was stored in paper bags at room temperature until the moment of analysis, when it was chopped and ground in a porcelain mortar until particles of homogeneous size were obtained.

2.2. Clevenger-Hydrodistillation

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

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

The details of the gas chromatography/mass spectrometry (GC/MS) and gas chromatography/flame ionization analyses are given in Ilić et al. [20].

2.4. Antioxidant Activity (DPPH Assay)

The ability of the essential oil to scavenge free DPPH radicals was determined using the DPPH assay. The essential oil was dissolved in the ethanol, and a series of different concentrations were prepared. An ethanol solution of DPPH radical (1 cm^3, 300 µmol solution (3×10^{-4} mol/L)) was added to 2.5 mL of the prepared essential oil solutions. Absorption was measured at 517 nm after 40 min of incubation with radicals. Absorption at

517 nm was also determined for the ethanolic solution of DPPH radical as well, which was diluted in the aforementioned ratio (1 mL of the DPPH radical of the given concentration with 2.5 mL ethanol added). Ethanol was used as a blank. All other relevant details of the assay used are given in Stanojević et al. [21].

2.5. Antimicrobial Activity

Microorganisms and substrates. Nine microorganisms were selected to determine the antimicrobial activity of the essential oil: (eight bacterial strains) *Escherihia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Proteus vulgaris* (ATCC 8427), *Bacillus subtilis* (ATCC 6633), *Bacillus cereus* in house soj, *Staphylococcus aureus* (ATCC 25923), *Klebsiella pneumoniae* (ATCC 700603) and *Listeria monocytogenes* (ATCC 15313), and (one fungal strain) *Candida albicans* (ATCC 2091). The microorganisms are from the collection of the Microbiology Laboratory, Faculty of Technology, Leskovac.

Disc-diffusion method. The agar disc-diffusion method was used for testing the antimicrobial activity of the extract obtained [22]. The media were sterilized for 15 min in an autoclave at 121 °C under 110 kPa. The bacterial and fungal suspensions were prepared by applying the direct colony method. The colonies were taken directly from the plate and suspended in 5 mL of sterile 0.85% saline. The turbidity of the initial suspension was adjusted by comparing it with 0.5 McFarland's turbidity. After adjusting to the turbidity of the standard, the bacterium suspension contained about 10^8 colony-forming units (CFU)/mL, and the suspension of fungus contained 10^6 CFU/mL. Ten-fold dilutions of the initial suspension were additionally prepared into sterile 0.85% saline.

The bacterial cells were grown to the nutrient agar plates (Torlak, Belgrade, Serbia) and the fungal suspension to the Sabouraud maltose agar plates (Torlak, Belgrade, Serbia).

For screening, sterilized filter paper disks (9 mm dia., Schleicher & Schuell) were placed on the surface of inoculated media and impregnated with 10 µL of the essential oil. The incubation time of 24 h for bacteria and 48 h for yeasts (a type of fungus) represents a standard method for the determination of antimicrobial activity [23].

After incubation, the inhibition zone diameters were measured and expressed in mm. The presence of the inhibition zone indicates the activity of the tested samples against bacteria or fungi. The positive controls were cefalexin and nystatin antibiotics for bacteria and fungi, respectively [23].

All experiments were carried out in three replications, and the results represent the mean value ± standard deviation.

3. Results and Discussion

3.1. Essential Oil Content

While fennel (*Foeniculum vulgare* Miller) is a wild plant in the maritime region of Monte Negro and is used as a culinary crop, there has been no evaluation of the EOs accumulation and distribution in the different tissues of the plants. The relative content of the EOs isolated from the two plant parts of fennel, obtained after distillation time (2 h), are listed in Table 1.

Table 1. Yields of essential oil from the stems and leaves of wild-grown fennel obtained after 120 min of hydrodistillation (hydromodule 1:10 *m*/*v*).

Fennel (*Foeniculum vulgare* Mill.)	Essential Oil Yield, mL/100 g p.m. *
Stems	0.21 ± 0.0091
Leaves	0.83 ± 0.0183

* p.m.—plant material (dry).

The yields of the fennel essential oils (FEOs) from the stems and leaves obtained after 120 min of hydrodistillation were 0.21 mL/100 g and 0.83 mL/100 g of plant material, respectively.

The results from the same geographical region in the study of Božovic et al. [11] showed that ripe inflorescence (seed) from wild fennel had a higher essential oil content (FEOs) from Nikšić and Podgorica (those two were quite similar, 2.92% and 2.9%, respectively), whereas the fennel seeds from Kotor gave 2.33% of FEOs. One of the reasons for the lower content of EO in our research is that we did not have the fruit as a plant part because the collection was done in August when the plants were still blooming; thus, the fruit (florets) and seeds had not yet formed. The seeds are known to contain more oil than the other plant parts.

Ravid et al. [24] found different FEO contents depending on the developmental stage and plant organs. In the early fruiting stage, ripe umbels contained the highest EO yield (3%, *v*/*w*). According to Miraldi [25], the age of the fruit, as well as inadequate storage conditions, may negatively affect FEO yields.

The differences in the FEO contents between regions might be explained by the varied environmental conditions, diversification of fennel population, plant part (leaves, stem or fruit/seed), phenological stage, and the time and method of harvest, etc. The relative yield percentages, calculated per weight of the dried plant material (leaves and stems) for the cumulative yields over the entire extraction time, are shown in Figure 1.

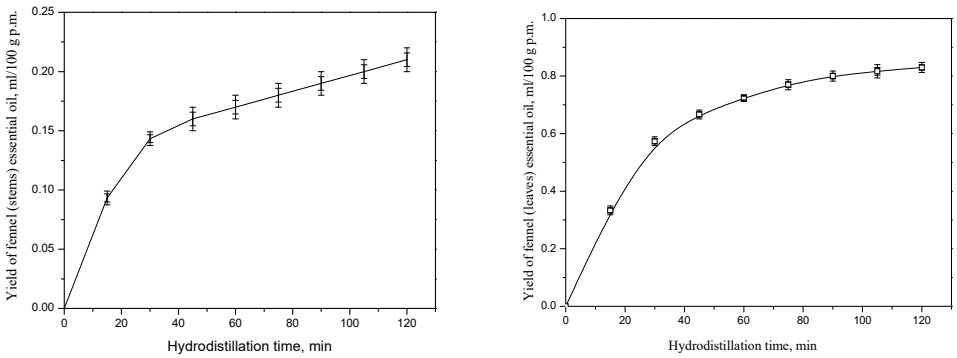


Figure 1. The dependence of the yield of wild fennel stems and leaves essential oil from the hydrodistillation time.

The content of FEOs also depends on the method and duration of the extraction time. The maximum amount of FEOs was obtained after 2 h, whereas the seeds needed at least 6 h for the extraction [12]. The distillation process from the literature cited is limited to 3 h or 4 h [26]. Following different studies, an extraction procedure of 2 h duration was applied to the fennel material in the present study.

3.2. Essential Oil Composition

Forty-eight compounds were identified from fennel stem essential oil, which were mainly aromatic compounds (69.7%), oxygen-containing monoterpenes (14.9%), monoterpenes (6.3%) and others, where the most abundant compounds were also (*E*)-anethole and methyl chavicol. Gas chromatography—flame ionization detector (GC-FID) chromatogram of fennel essential oil from stem are presented in Figure 2.

In addition to the most common components from fennel stem (E-anethole and methyl chavicol), the following components are somewhat less present: *p*-cymene (3.9%), cis-thujone (3.7%), ledol (3%), α-phellandrene epoxide (2.9%), camphor (2.6%), exo-fenchyl acetate (2.4%), and β-phellandrene (2.2%). The components of essential oils in fennel leaves that are represented in the range of 1% to 2% are fenchone (2%), α-phellandrene (1.7%) and methyl o-anisate (1.4%) (Table 2).

Forty-six compounds were identified in this research from fennel leaves essential oil, and were mainly aromatic compounds (68.5%), monoterpenes (17.8%), oxygen-containing monoterpenes (11.8%) and others, where the most abundant compounds were (*E*)-anethole (51.4%) and methyl chavicol (9.3%), Figure 3. Gas chromatography—flame ionization detector (GC-FID) chromatogram of fennel essential oil from leaves are presented in Figure 4.

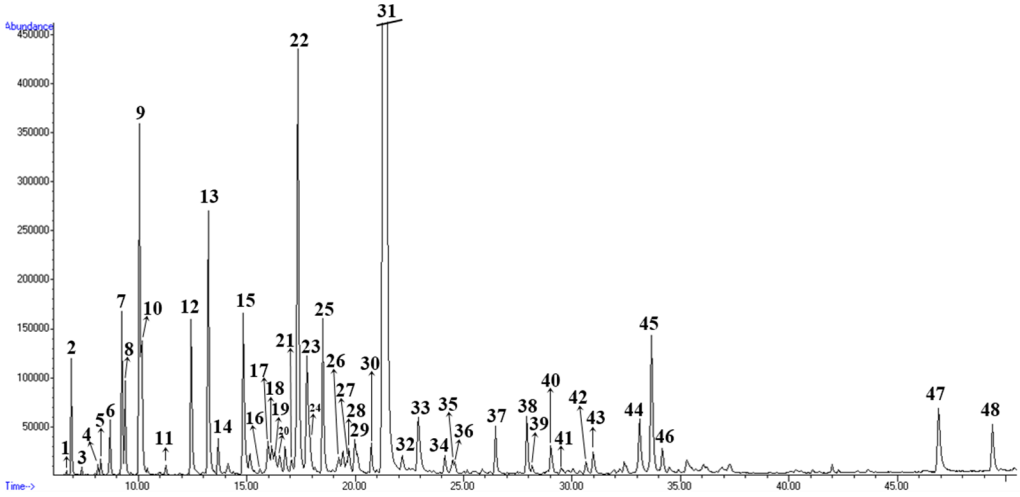


Figure 2. GC/FID chromatogram of essential oil isolated from wild fennel stem.

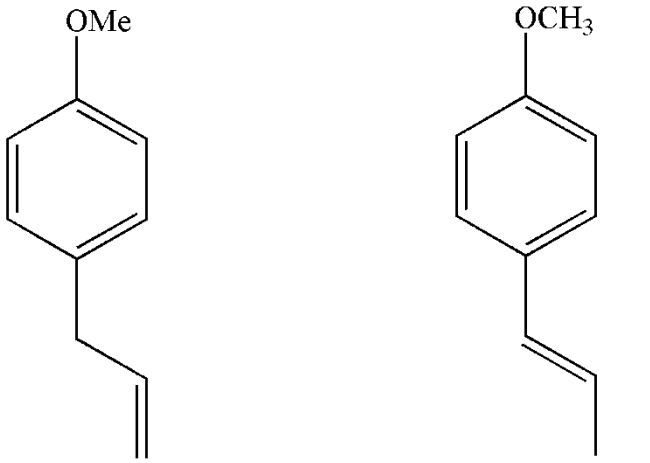
Table 2. Chemical composition of essential oil isolated from wild fennel stem.

N°	$t_{ret.}$, min	Compound	RI^{exp}	RI^{lit}	Method of Identification	*c* %
1.	6.70	α-Thujene	914	924	RI, MS	tr
2.	6.92	α-Pinene	922	932	RI, MS	0.9
3.	7.40	Camphene	937	946	RI, MS, Co-I	tr
4.	8.15	Sabinene	962	969	RI, MS	tr
5.	8.28	β-Pinene	967	974	RI, MS, Co-I	tr
6.	8.70	Myrcene	980	988	RI, MS	0.5
7.	9.26	α-Phellandrene	998	1002	RI, MS	1.7
8.	9.40	δ-3-Carene	1003	1008	RI, MS	1.0
9.	10.06	*p*-Cymene	1020	1020	RI, MS	3.9
10.	10.19	β-Phellandrene	1024	1025	RI, MS	2.2
11.	11.28	γ-Terpinene	1052	1054	RI, MS, Co-I	tr
12.	12.45	Fenchone	1083	1083	RI, MS	2.0
13.	13.24	cis-Thujone	1104	1101	RI, MS	3.7
14.	13.68	trans-Thujone	1114	1112	RI, MS	0.5
15.	14.85	Camphor	1142	1141	RI, MS, Co-I	2.6
16.	15.63	Pinocarvone	1161	1160	RI, MS	tr
17.	16.01	δ-Terpineol	1169	1162	RI, MS	0.4
18.	16.15	Borneol	1173	1165	RI, MS, Co-I	tr
19.	16.29	*p*-Mentha-1,5-dien-8-ol	1176	1166	RI, MS	tr
20.	16.52	Terpinen-4-ol	1182	1174	RI, MS, Co-I	tr
21.	17.06	*p*-Cymen-9-ol	1195	1204	RI, MS	tr
22.	17.36	Methyl chavicol	1202	1195	RI, MS	7.8
23.	17.79	α-Phellandrene epoxide	1200	-	MS	2.9
24.	17.91	endo-Fenchyl acetate	1215	1218	RI, MS	tr
25.	18.52	exo-Fenchyl acetate	1230	1229	RI, MS	2.4
26.	19.26	Carvone	1247	1239	RI, MS	tr
27.	19.61	cis-Piperitone epoxide	1256	1250	RI, MS	tr
28.	19.71	trans-Piperitone epoxide	1258	1252	RI, MS	tr
29.	19.97	*p*-Anisaldehyde	1265	1270	RI, MS	0.9
30.	20.74	Isobornyl acetate	1283	1283	RI, MS	0.4
31.	21.40	(E)-Anethole	1292	1282	RI, MS	55.7
32.	22.19	6-hydroxy-Carvotanacetone	1317	1309	RI, MS	tr
33.	22.89	Methyl o-anisate	1334	1334	RI, MS	1.4

Table 2. *Cont.*

N°	$t_{ret.}$, min	Compound	RI^{exp}	RI^{lit}	Method of Identification	*c* %
34.	24.15	(2E)-Undecenal	1364	1357	RI, MS	tr
35.	24.50	Piperitenone oxide	1373	1366	RI, MS	tr
36.	24.61	α-Copaene	1375	1374	RI, MS	tr
37.	26.49	(E)-Caryophyllene	1421	1417	RI, MS	0.7
38.	27.92	α-Humulene	1457	1452	RI, MS	0.9
39.	28.17	(E)-β-Farnesene	1464	1454	RI, MS	tr
40.	29.03	Germacrene D	1485	1484	RI, MS	0.5
41.	29.51	Phenyl ethyl 3-methylbutanoate	1497	1490	RI, MS	tr
42.	30.66	δ-Cadinene	1527	1522	RI, MS	tr
43.	30.98	Myristicin	1535	1525	RI, MS	tr
44.	33.13	Caryophyllene oxide	1592	1582	RI, MS	1.3
45.	33.67	Ledol	1606	1602	RI, MS	3.0
46.	34.17	Humulene epoxide II	1618	1608	RI, MS	tr
47.	46.90	Hexadecanoic acid	1969	1959	RI, MS	1.7
48.	49.36	13-epi-Manool	2069	2059	RI, MS	1.0
					Total identified	100.0
		Grouped components (%)				
		Monoterpene hydrocarbons (1–8, 10, 11)				6.3
		Oxygen-containing monoterpenes (12–20, 23–28, 30, 32, 35)				14.9
		Sesquiterpene hydrocarbons (36–40, 42)				2.1
		Oxygenated sesquiterpenes (44–46)				4.8
		Diterpenes (47)				1.0
		Aromatic compounds (9, 21, 22 *, 29, 31 *, 33, 41, 43)				69.7
		* Phenolics (22, 31)				* 63.5
		Others (34, 47)				1.7

RI^{exp}—retention indices, RI^{lit}—mass spectra with those of authentic standard as well as with those from Willey 6, NIST2011 and RTLPEST3 libraries and, wherever possible, by co-injection with an authentic standard (Co-I). C_x—concentration of the analytes in the sample, * 63.5% of phenolic components from the total 69.7% of aromatic compounds.



Methyl chavicol (E)-Anethole

Figure 3. Structures of the most abundant components in wild fennel essential oil.

Furthermore, *p*-Cymene (6.5%), α-phellandrene (5.9%), β-phellandrene (4.9%), and fenchone (3.2%) are components that are somewhat less represented. The components of essential oils in fennel leaves that are present in the range of 1% to 2% are α-thujene (1.9%), trans-piperitone epoxide (1.8%), δ-3-carene (1.7%), hexadecanoic acid (1.7%), γ-terpinene (1.4%), myrcene (1.3%), methyl o-anisate (1.3%), α-phellandrene epoxide (1.2%) and cis-

piperitone epoxide (1.2%). The content of all other components is below 1%, with some of them only in traces (Table 3).

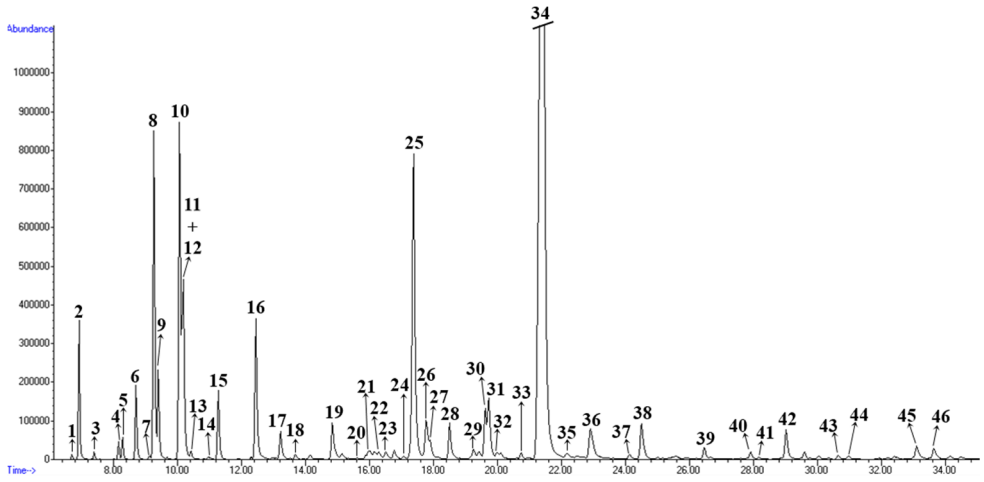


Figure 4. GC/FID chromatogram of essential oil isolated from wild fennel leaves.

The FEOs composition in plants collected from varied regions has different quantitative and qualitative properties. Based on the relative amounts of E-anethole, methyl chavicol and α-phellandrene found in the essential oils isolated from the aerial parts in the present study, we can include these chemotypes in group 1, as defined by Piccaglia and Marotti [27]. Regarding the FEOs samples analyzed in this study, anethole was present in each part of the plant as the most present component. Anethole was the main constituent (47–80.2%) in the FEOs, followed by fenchone (9.83%) and methyl chavicol (4.46%) [28].

Bozovic et al. [11] determined and recognized 18 chemical components in fennel seed from three locations in Monte Negro. The phenylpropanoids, anethole and estragole, and the monoterpenoids, α-terpineol and fenchone, are predominant constituents from the wild fennel collected from three localities in Montenegro. The FEOs from Podgorica were characterized as anethol-rich chemotypes, while the samples from Nikšic and Kotor α-terpineol and fenchone were predominant.

The differences between the previous literature reports [11] and these studies are the result of various factors that influence the chemical composition of FEO, such as the differences in plant tissue, seasonal variation and altitude.

The contents of EO in certain parts of the fennel plant are different. Thus, the main components of the EOs of the aerial part of wild fennel from Portugal are trans-anethole, α-pinene and limonene, whereas methyl chavicol was a predominant component of the EOs from seed [16]. The FEOs of the fruit from different locations in Iran varied from 2.7% to 4% [29]. Trans-anethole, estragole and fenchone were found to be the main components of FEOs in sweet and bitter fennel [29].

Anethol (ANE) and its isomer EST (also known as methyl chavicol) are very common ingredients of FEOs, and are usually present in fruits and flowers, as reported in numerous papers [30,31]. These phenylpropanoids contribute to the odor and flavor of many plants. ANE is responsible for the sweet, distinct, anise-like flavor characterizing fennel fruits [32].

The differences in the content of individual plant parts and their components of FEOs during the growing season were also observed. Thus, the FEOs from the leaves collected from April to June were found to contain limonene, fenchone, α-phellandrene and methyl chavicol as the major constituents. The FEOs from the stems gathered in September were characterized by a high content of α-phellandrene, (E)-anethole, α-pinene, limonene and p-cymene [33].

Table 3. Chemical composition of essential oils isolated from wild fennel leaves.

N°	$t_{ret.}$, min	Compound	RI^{exp}	RI^{lit}	Method of Identification	*c*, %
1.	6.70	α-Thujene	914	924	RI, MS	tr
2.	6.92	α-Pinene	922	932	RI, MS	1.9
3.	7.40	Camphene	937	946	RI, MS, Co-I	tr
4.	8.15	Sabinene	962	969	RI, MS	0.3
5.	8.28	β-Pinene	967	974	RI, MS, Co-I	0.4
6.	8.70	Myrcene	980	988	RI, MS	1.3
7.	9.12	3-Octanol	994	988	RI, MS	tr
8.	9.26	α-Phellandrene	998	1002	RI, MS	5.9
9.	9.40	δ-3-Carene	1003	1008	RI, MS	1.7
10.	10.06	*p*-Cymene	1020	1020	RI, MS	6.5
11.	10.14	Limonene	1022	1024	RI, MS, Co-I	tr
12.	10.19	β-Phellandrene	1024	1025	RI, MS	4.9
13.	10.42	(Z)-β-Ocimene	1030	1032	RI, MS	tr
14.	10.96	Benzene acetaldehyde	1044	1036	RI, MS	tr
15.	11.28	γ-Terpinene	1052	1054	RI, MS, Co-I	1.4
16.	12.45	Fenchone	1083	1083	RI, MS	3.2
17.	13.24	cis-Thujone	1104	1101	RI, MS	0.7
18.	13.68	trans-Thujone	1114	1112	RI, MS	tr
19.	14.85	Camphor	1142	1141	RI, MS, Co-I	1.0
20.	15.63	Pinocarvone	1161	1160	RI, MS	tr
21.	16.01	δ-Terpineol	1169	1162	RI, MS	tr
22.	16.29	*p*-Mentha-1,5-dien-8-ol	1176	1166	RI, MS	tr
23.	16.52	Terpinen-4-ol	1182	1174	RI, MS, Co-I	tr
24.	17.06	*p*-Cymen-9-ol	1195	1204	RI, MS	tr
25.	17.36	Methyl chavicol	1202	1195	RI, MS	9.3
26.	17.79	α-Phellandrene epoxide	1200	-	MS	1.2
27.	17.91	endo-Fenchyl acetate	1215	1218	RI, MS	0.5
28.	18.52	exo-Fenchyl acetate	1230	1229	RI, MS	1.0
29.	19.26	Carvone	1247	1239	RI, MS	tr
30.	19.61	cis-Piperitone epoxide	1256	1250	RI, MS	1.2
31.	19.71	trans-Piperitone epoxide	1258	1252	RI, MS	1.8
32.	19.97	p-Anisaldehyde	1265	1270	RI, MS	tr
33.	20.74	Isobornyl acetate	1283	1283	RI, MS	tr
34.	21.40	(E)-Anethole	1292	1282	RI, MS	51.4
35.	22.19	6-hydroxy-Carvotanacetone	1317	1309	RI, MS	tr
36.	22.89	Methyl o-anisate	1334	1334	RI, MS	1.3
37.	24.15	(2E)-Undecenal	1364	1357	RI, MS	tr
38.	24.50	Piperitenone oxide	1373	1366	RI, MS	1.2
39.	26.49	(E)-Caryophyllene	1421	1417	RI, MS	0.3
40.	27.92	α-Humulene	1457	1452	RI, MS	tr
41.	28.17	(E)-β-Farnesene	1464	1454	RI, MS	tr
42.	29.03	Germacrene D	1485	1484	RI, MS	0.9
43.	30.66	δ-Cadinene	1527	1522	RI, MS	tr
44.	30.98	Myristicin	1535	1525	RI, MS	tr
45.	33.13	Caryophyllene oxide	1592	1582	RI, MS	0.4
46.	33.67	Ledol	1606	1602	RI, MS	0.4
					Total identified	100.0
		Grouped components (%)				
		Monoterpene hydrocarbons (1–6, 8, 9, 11–13, 15)				17.8
		Oxygen-containing monoterpenes (16–23, 26–31, 33, 35, 38)				11.8
		Sesquiterpene hydrocarbons (39–43)				1.2
		Oxygenated sesquiterpenes (45, 46)				0.8
		Aromatic compounds (10, 14, 24, 25, 32, 34, 36, 44)				68.5
		* Phenolics (25, 34)				* 60.7
		Others (7, 37)				tr

RI^{exp}—retention indices; RI^{lit}—mass spectra with those of authentic standard as well as with those from Willey 6, NIST2011 and RTLPEST3 libraries and, wherever possible, by co-injection with an authentic standard (Co-I). C_x—concentration of the analytes in the sample. * 60.7% of phenolic components from the total 68.5% of aromatic compounds.

3.3. Antioxidant Activity

We chose the DPPH-radical scavenging capacity assay as the most frequently applied method. The antioxidant activity was measured as the free radical (DPPH), hydroxyl radical, and superoxide anion scavenging activities. Sánchez-Moreno et al. [34] classified the kinetic behavior of an antioxidant compound as rapid (30 min), based on the time required to reach a steady state at the concentration corresponding to a 50% decrease of the

initial DPPH concentration (EC_{50}). The FEOs evaluated in the present work should thus be classified as slow in terms of kinetic behavior.

When compared with the activity after 40 min of incubation with a radical, the activity decreases from the leaves (6.91 mg/mL) to the stem (2.58 mg/mL) (lower EC_{50} value represents better antioxidant activity), Table 4.

Table 4. EC_{50} values of essential oil from the wild fennel stems and leaves.

Essential Oil	EC_{50}, mg/mL	
	Without Incubation	40 min Incubation
Fennel stems	/	2.58 ± 0.101
Fennel leaves	/	6.91 ± 0.014

The antioxidative activities in stems were higher, which is probably related to the phenolics content (63.5%) in comparison with the phenolics content in leaves (60.7%).

Efficient concentration—EC_{50} values of oil from fennel stems and leaves during 40 min incubation were presented in Figure 5. With regard to the OH scavenging activity, large differences were observed among the different samples of fennel collected in different regions. The aerial parts of the fennel from the Ternisite and the fruits from Bologna were found to be the most active, with EC_{50} values of 10.5 and 28.5 µg/mL, respectively [35]. The EC_{50} values of the wild and cultivated fennel seeds from Italy are very different, with the wild fennel seed having an EC_{50} value of 31 µg/mL and the seed from the cultivated fennel being 83 µg/mL [36]. Mata et al. [37] also reported that the EC_{50} values for the ethanol extracts from Portugal were higher than for the synthetic antioxidants. The differences in antioxidant activity in the mentioned studies can be explained by the differences in origin, genotypes, agro-ecological conditions and plant parts.

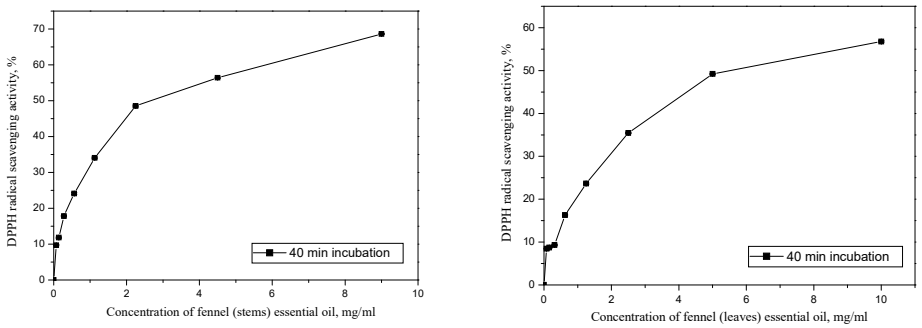


Figure 5. Antioxidant activity of wild fennel stems and leaves essential oil.

The total amount of volatile compounds is the highest in the fruits, less in the flowers, and significantly lowest in the leaves. A constant increase in estragole was observed during plant development [5].

Fenchone was the main constituent found in Algerian wild fennel essential oils seeds, while fennel umbel mainly contained α-pinene and carvacrol. The EC_{50} values were 9.96 mg/mL for the FEOs and 0.45 mg/mL for the BHT as a positive control [38]. The Egyptian fennel seed extract included higher radical scavenging activity (6.34 mg/g) than the Chinese fennel seed extract (EC_{50} = 7.17 mg/g) [39]. The Pakistan fennel seed extract had satisfied the scavenging activity (EC_{50} = 23.61 µg/mL) [40]. Wild fennel were found to exhibit a radical scavenging activity higher than that of edible fennel [35].

The main compounds found in both the wild and cultivated fennel were estragole, anethole and fenchone. Estragole was the main compound in cultivated fennel [41].

3.4. Antimicrobial Activity

Fennel essential oils are rich in trans-anethole and other compounds and are effective against *Candida albicans* ATCC 2091 (45.3 mm) and *Bacillus subtilis* ATCC 6633 (24 mm), Table 5.

Table 5. Antimicrobial activity of fennel essential oils (FEOs).

	Inhibition Zone (mm)								
	Escherihia coli	*Pseudomonas aeruginosa*	*Proteus vulgaris*	*Bacillus subtilis*	*Bacillus cereus*	*Staphylococcus aureus*	*Klebsiella pneumoniae*	*Listeria monocytogenes*	*Candida albicans*
Average	0	0	0	24.0	0	0	0	0	45.3
Stan dev				1.0					1.527
Cefalexin	n.t.	n.t.	n.t.	48.0	n.t.	n.t.	n.t.	n.t.	n.t.
Nystatin	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	17.0

These results are in agreement with the study declaring that monoterpenes hydrocarbon plus their oxygenated derivatives, which are the principal chemicals of essential oil, display potent antimicrobial activity. Due to the inhibition of the growth of pathogenic spoilage microorganisms, the extension of the shelf-life of food products is achieved. The effectiveness of EO is attributed to the presence of natural phenolic compounds, and they are good substitutes for synthetic preservatives and various additives. The use of fennel EOs in food preservation, is an alternative to synthetic preservatives and chemical additives in meat, cheese, vegetables and fruits, [42]. The antimicrobial activity of FEOs has encouraged researchers to investigate and use them with nanoparticles in the food industry as safe and non-chemical methods.

4. Conclusions

The variation in plant parts is important in the chemical responsible for the essential oil yield, composition and antioxidant activity. The essential oil yield of wild fennel from Herceg Novi was higher in the leaves than in the stems. Unlike the essential oil content, antioxidative activities in stems were higher, which is related to the phenolics content (63.5%) and is primarily due to the participation of (E)-Anethole and methyl chavicol in comparison with the phenolics content in leaves (60.7%). Aromatic compounds are the principal components of FEOs, which have an inhibiting capacity for the growth of *Bacillus subtilis* and *Candida albicans*. Future studies should be expanded to include other plant parts, such as florets and fruit/seed. Then, it will be much clearer to understand which plant parts and compounds are responsible for the specified effects. Fennel essential oils are a potential source of natural antioxidants, as a possible alternative to synthetic antioxidants in food products and can prevent their oxidative deterioration. Thus, there are many areas of research related to this plant that needs to be further explored to fully recognize the beneficial effects of fennel plants.

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