

# Antioxidant, Antityrosinase Activities and Composition of Essential Oils Obtained from Roots and Fruits of *Ferulago longistylis* Boiss.

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**ABSTRACT:** This study was aimed to evaluate the potential uses of *Ferulago longistylis* Boiss. essential oils in cosmetic applications. The air dried roots and fruits were subjected to water distillation by using a Clevenger apparatus for 3 h. Both essential oils were analyzed by gas chromatography (GC-FID) and gas chromatography-mass spectrometry (GC/MS) systems, simultaneously. The essential oil yields of the fruits and roots were calculated as 4% and 0.91%, respectively. The major compounds were identified as 2,3,6-trimethylbenzaldehyde (29.5%), *cis*-chrysanthenyl acetate (15.2%), (*Z*)- $\beta$ -ocimene (12.4%),  $\alpha$ -pinene (12.0%) and myrcene (11.4%) in the fruit essential oil while the root essential oil was characterized with high content of  $\alpha$ -pinene (90.5%). Both essential oils were evaluated for their potential antioxidant and antityrosinase activities. The both essential oils showed weak Trolox equivalent antioxidant capacity and DPPH radical scavenging activity. The fruit essential oil showed weak antityrosinase (%25 inhibition, 1 mg/mL) activity while the root essential oil was inactive. The results concluded that both essential oils were not promising in cosmetic applications.

**KEYWORDS:** *Ferulago longistylis*;  $\alpha$ -pinene; 2,3,6-trimethylbenzaldehyde; antioxidant, antityrosinase.

## 1. INTRODUCTION

Humanity has long used medicinal herbs for skin care. Medicinal and aromatic plants attracted considerable attention since they play a critical role in skin health and beauty. This has led to great interest in biologically active natural based products for skin whitening, skin antiaging, photoaging, skin cancers, skin inflammatory disorders, etc. Herbal products as cosmetics are used as personal care, and provide the nutrients necessary for healthy skin and bioactive compounds affect the skin's biological functions [1].

One of the targets in skin health/beauty is tyrosinase which plays efficient role in melanin biosynthesis. Tyrosinase plays an important role in melanin biosynthesis in melanocytes via the conversion of L-tyrosine to dopaquinone. In humans, melanin gives color to hair, eyes and skin. From ancient times, skin color has been considered important in the context of beauty, and also the overproduction of melanin causes hyperpigmentation, spots, freckles and melasma [2]. Most researchers are trying to discover new tyrosinase inhibitors that are more effective than kojic acid. In tyrosinase inhibitor studies, kojic acid used as positive control is a chemical product from various type of fungi such as *Aspergillus* species [3].

In today's life, the skin is often exposed to environmental events, including excessive UV exposure and increased air pollution, which usually causes oxidative stress. During these stages, high levels of reactive oxygen species (ROS) may damage the skin and cause skin diseases. This can lead to an imbalance between ROS and endogenous antioxidant systems and resulting in a severe decrease in antioxidant content and marked formation of active oxygen intermediates [4]. For the researches in skin health/beauty products, radical scavenging activity evaluation is still popular.

Among the secondary metabolites identified in medicinal and aromatic plants, essential oils have gained more interest for the cosmetic industry. One of the largest essential oil families is Apiaceae. Apiaceae

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encompasses 3780 species in 434 genera in the world. The Apiaceae plants have their characteristic flavours due to the presence of schizogenous ducts that contain essential oil, mucilage or resin and which are deposited in roots, leaves, stems and fruits [5]. The plants in this family have a wide variety of uses in the fields of food, beverages, and spices, pharmaceutical and cosmetic [6,7].

The genus *Ferulago* belongs to the tribe Peucedaneae of the Apiaceae. *Ferulago* species grow in area with a mild climate, mainly in Europe (except Northern), Southwest and Middle Asia, the Caucasus and North Africa. The greatest number of species is occurred in southern and western Turkey which is considered to be the main center of diversity in the genus [8]. In Turkey, 34 species are recorded and 18 species are endemic. *Ferulago* species are known as “Çakşırotu” in Turkey [9]. These species have been used for medicinal purposes for centuries. The decoction of the roots of *F. sylvatica* (Besser) Reichb. has been used for skin diseases. And also, *F. pachyloba* (Fenzl) Boiss. fruit and leaves (infusion) have been used as immunostimulant, *F. cassia* Boiss. seeds (decoction) have been used to stimulate milk secretion and treating eye diseases, *F. confusa* Velen. inflorescence (infusion) has been used for bronchitis [9]. Besides coumarin and its derivatives as major secondary metabolites in *Ferulago* species, also the volatile compounds in essential oils have been identified [8].

Among the endemic *Ferulago* species, *F. longistylis* was found to have limited studies on its phytochemicals and activities [10-13].

This present study was aimed to characterize of volatiles of *Ferulago longistylis* roots and fruits essential oils collected from Tunceli. GC-FID and GC/MS systems were employed to analyze and identify volatile compounds in both essential oils. The identification of the volatile compounds were accomplished using standard compounds or in house and commercial libraries. Also, the both essential oils were investigated for their antioxidant effects and antityrosinase effects by *in vitro*.

## 2. RESULTS

### 2.1. Chemical composition of the essential oils

The essential oil yields of the fruits and roots were calculated as 4% and 0.91% (*v/w*), respectively. GC and GC/MS analyzes resulted that totally thirty three volatile compounds were detected and identified.

Twenty-seven constituents were characterized as representing 99.6% of the fruit essential oil. 2,3,6-Trimethylbenzaldehyde (29.5%), 2-*cis*-chrysanthenyl acetate (15.2%), (*Z*)- $\beta$ -ocimene (12.4%),  $\alpha$ -pinene (12%), and myrcene (11.4%) were found to be the most abundant compounds.

Twenty-five compounds were detected, representing 99.3% of the root essential oil.  $\alpha$ -Pinene (90.5%) was a major compound in the root essential oil (Table 1).

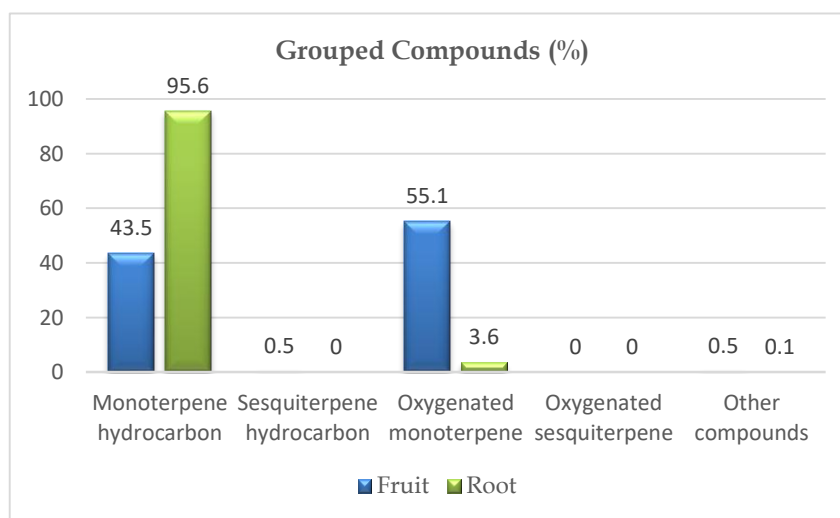
In the fruit essential oil, oxygenated monoterpenes (55.1%) and monoterpene hydrocarbons (43.5%) were the most abundant groups among the identified constituents. The percentage of sesquiterpene ( $\beta$ -caryophyllene (0.5%)) was found to be very low. On the other hand, in the root essential oil monoterpene hydrocarbons were dominant and oxygenated monoterpenes percentage was 3.6%, and no sesquiterpenes were detected (Figure 1).

A recent study on the essential oils obtained from *Ferulago longistylis* was published by Demirci et al., 2020. *F. longistylis* collected from Erzincan-Refahiye in 2016 its aerial parts, roots and fruits essential oils were characterized by the presence of  $\alpha$ -pinene (91.7%),  $\beta$ -pinene (2.0%) and myrcene (1.1%) in the root oil while 2,3,6-trimethyl benzaldehyde (26.5%),  $\alpha$ -pinene (14.9%), (*Z*)- $\beta$ -ocimene (14.1%), myrcene (7.5%), sabinene (7.3%) in the fruit oil [13]. Another study reported that 2,3,6-trimethylbenzaldehyde (29.4%),  $\alpha$ -pinene (16.7%), (*Z*)- $\beta$ -ocimene (15.9%), sabinene (6.2%), myrcene (5.7%) and bornyl acetate (4.4%) were detected in the fruit essential oil obtained from *F. longistylis* collected from Erzincan in 2006 [10]. Kilic et al (2010) published the results of four endemic *Ferulago* species collected from Turkey [11]. Among them, *F. longistylis* was collected from Erzincan in 2006. The essential oil hydrodistilled from the aerial parts of *F. longistylis* was characterized with 2,3,6-trimethylbenzaldehyde (32.7%) and bornyl acetate (12.6%).

**Table 1.** Volatile compounds in the essential oils of *Ferulago longistylis* fruits and roots

RRI <sup>a</sup>	RRI <sup>b</sup>	Compounds	Fruit (%)	Root %	IM
1032	1008-1039 <sup>c</sup>	<b><math>\alpha</math>-Pinene</b>	<b>12.0</b>	<b>90.5</b>	
1076	1043-1086 <sup>c</sup>	Camphene	0.3	0.4	t <sub>R</sub> , MS
1118	1085-1130 <sup>c</sup>	$\beta$ -Pinene	0.5	1.9	t <sub>R</sub> , MS
1132	1098-1140 <sup>c</sup>	Sabinene	3.6	0.4	t <sub>R</sub> , MS
1174	1140-1175 <sup>c</sup>	<b>Myrcene</b>	<b>11.4</b>	1.3	t <sub>R</sub> , MS
1188	1154-1195 <sup>c</sup>	$\alpha$ -Terpinene	0.1	-	t <sub>R</sub> , MS
1203	1178-1219 <sup>c</sup>	Limonene	0.7	0.5	t <sub>R</sub> , MS
1210	1188-1233 <sup>c</sup>	$\beta$ -Phellandrene	0.1	tr	t <sub>R</sub> , MS
1244	1244 <sup>d</sup> , 1243 <sup>e</sup>	Amyl furan	-	tr	t <sub>R</sub> , MS
1246	1211-1251 <sup>c</sup>	<b>(Z)-<math>\beta</math>-Ocimene</b>	<b>12.4</b>	0.2	MS
1255	1222-1266 <sup>c</sup>	$\gamma$ -Terpinene	1.6	0.2	t <sub>R</sub> , MS
1266	1232-1267 <sup>c</sup>	(E)- $\beta$ -Ocimene	0.4	-	t <sub>R</sub> , MS
1280	1246-1291 <sup>c</sup>	p-Cymene	0.4	0.2	t <sub>R</sub> , MS
1290	1261-1300 <sup>c</sup>	Terpinolene	-	tr	t <sub>R</sub> , MS
1294	1292 <sup>h</sup>	1,2,4-Trimethyl benzene	0.5	-	t <sub>R</sub> , MS
1583	1561 <sup>c</sup>	<b>cis-Chrysanthenyl acetate</b>	<b>15.2</b>	1.0	MS
1590	1549-1597 <sup>c</sup>	Bornyl acetate	0.8	0.8	t <sub>R</sub> , MS
1611	1564-1630 <sup>c</sup>	Terpinen-4-ol	0.6	0.1	t <sub>R</sub> , MS
1612	1569-1632 <sup>c</sup>	$\beta$ -Caryophyllene	0.5		t <sub>R</sub> , MS
1645	1645 <sup>g</sup>	cis-Verbenyl acetate	0.3	tr	t <sub>R</sub> , MS
1661	1613 <sup>e</sup>	Safranal	0.3	-	MS
1662	1661 <sup>g</sup>	trans-Pinocarvyl acetate	0.3	-	t <sub>R</sub> , MS
1664	1643-1671 <sup>c</sup>	trans-Pinocarveol	-	0.1	t <sub>R</sub> , MS
1668	1647-1668 <sup>c</sup>	cis-Verbenol	0.8	-	MS
1683	1665-1691 <sup>c</sup>	trans-Verbenol	3.2	0.2	MS
1747	1680 <sup>f</sup>	p-Mentha-1,5-dien-8-ol	1.3	tr	MS
1764	1751-1765 <sup>c</sup>	cis-Chrysanthenol	tr	0.1	MS
1779	1729-1779 <sup>c</sup>	(E,Z)-2,4-Decadienal	-	tr	MS
1827	1770-1834 <sup>c</sup>	(E,E)-2,4-Decadienal	-	0.1	MS
1908	1908 <sup>i</sup>	Thymoquinone	0.5	-	MS
1925	1925 <sup>k</sup>	2,3,4-Trimethylbenzaldehyde	2.3	0.1	MS
2019	2019 <sup>k</sup>	<b>2,3,6-Trimethylbenzaldehyde</b>	<b>29.5</b>	0.8	MS
2239	2239 <sup>d,e</sup>	Carvacrol	-	0.4	t <sub>R</sub> , MS
<b>TOTAL</b>			<b>99.6</b>	<b>99.3</b>	

RRI<sup>a</sup>; relative retention indices calculated against *n*-alkanes (C<sub>8</sub> to C<sub>25</sub>). RRI<sup>b</sup>; RRI from literature for polar column values c [14]; d [15]; e [16]; f [17]; g [18]; h [19]; i [20]; k [13]. %; calculated from the FID chromatograms. tr; trace amount (<0.1%). -; not detected. Identification method (IM): t<sub>R</sub>, identification based on the retention times (t<sub>R</sub>) of genuine compounds on the HP Innnowax column; MS, identified on the basis of computer matching of the mass spectra with those of the in-house Baser Library of Essential Oil Constituents, Adams, MassFinder and Wiley libraries and comparison with literature data.



**Figure 1.** Compound group distribution of the essential oils

In the literature, the essential oils of *Ferulago* species growing wild in Turkey were determined by different researchers (Table 2). Roots, fruits, aerial parts, flowers were investigated for their essential oil compositions. In general, the fruit essential oil yield is the highest depending on the collection site. The main distillation technique was water distillation. 2,3,6-Trimethylbenzaldehyde is the major compound in the essential oils of *F. asparagifolia* (fruit), *F. platycarpa* (aerial parts), and *F. longistylis* (fruits). The essential oils of *F. bracteata* (flowers), *F. galbanifera* (fruits), *F. isaurica* (fruits), *F. longistylis* (aerial parts and roots), and *F. sandrasica* (aerial parts and roots) were found to be rich in  $\alpha$ -pinene. Among them, the root essential oil obtained from *F. longistylis* collected from Erzincan was highly rich in  $\alpha$ -pinene with 91.7% of the oil. Our findings on the essential oil of *F. longistylis* are highly correlated with the published data by Demirci et al. [13].

**Table 2.** Main constituents of the essential oils of *Ferulago* species growing wild in Turkey

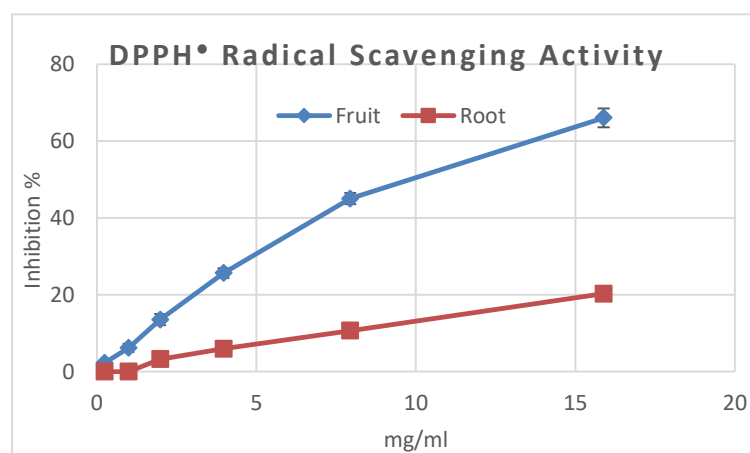
Plant	Collection place	Parts/Oil yield (%)	Main constituent of the essential oil (%)	Reference
<i>F. asparagifolia</i> Boiss.	Antalya	Fruits/6.9	2,3,6-Trimethylbenzaldehyde (38.9%) Myrcene (18.2%)	[21]
<i>F. asparagifolia</i>	Antalya	Fruits/7.0	2,3,6-Trimethylbenzaldehyde (38.9%) Myrcene (18.2%)	[22]
<i>F. blanchiana</i> Post.	Kayseri	Aerial parts/0.032	Bornyl acetate (11.7%) $\beta$ -Caryophyllene (10.2%)	[23]
<i>F. blanchiana</i>	Kayseri	Flowers/0.15	Sabinene (23.2%) Myrcene (17.5%)	[23]
<i>F. blanchiana</i>	Kayseri	Roots/0.032	(E)-2-Decenal (20.3%) Caryophyllene oxide (17.8%)	[23]
<i>F. bracteata</i> Boiss. & Hausskn.	Gaziantep	Aerial parts/0.59	7-Methoxy-6-(3-methyl-2-butenyl)-coumarin (86.7%)	[24]
<i>F. bracteata</i>	Gaziantep	Flowers/1.61	$\alpha$ -Pinene (12.1%) $\alpha$ -Phellandrene (22.8%)	[24]
<i>F. bracteata</i>	Gaziantep	Roots/0.11	Hexadecanoic acid (40.4%) (E)-2-Decenal (13.9%)	[24]
<i>F. cassia</i> Boiss.	Lakes Region	Fruits/5-7.8	Chrysanthenyl acetate (13.5%-24.5%) 2,3,6-Trimethylbenzaldehyde (5.9%-25.5%) L-Limonene (4.7%-27.4%) $\alpha$ -Pinene (7.6%-12.4%) $\beta$ -Myrcene (3.4%-10.4%)	[25]
<i>F. galbanifera</i> (Miller) W. Kosch	Eskişehir	Fruits/1.3	$\alpha$ -Pinene (31.8%) Sabinene (15.8%)	[21]
<i>F. humilis</i> Boiss.	Muğla	Fruits/3.9	(Z)- $\beta$ -Ocimene Limonene (17.3%)	[21]
<i>F. isaurica</i> Peşmen	Antalya	Aerial parts/0.08	Nonacosane (25.5%) Hexadecanoic acid (14.8%)	[11]
<i>F. isaurica</i>	Antalya	Fruits/12.0	$\alpha$ -Pinene (31.5%) Limonene (24.2%) Myrcene (17.0%)	[20]
<i>F. isaurica</i>	Antalya	Roots/0.7	Terpinolene (42.1%) Myrcene (27%)	[20]
<i>F. longistylis</i> Boiss.	Erzincan	Aerial parts/0.27	$\alpha$ -Pinene (18.7%) Bornyl acetate (11.8%) 2,3,6-Trimethylbenzaldehyde (9.3%)	[13]
<i>F. longistylis</i>	Erzincan	Roots/0.86	$\alpha$ -Pinene (91.7%)	[13]
<i>F. longistylis</i>	Erzincan	Fruits/6.1	2,3,6-Trimethylbenzaldehyde (26.5%) $\alpha$ -Pinene (14.9%) (Z)- $\beta$ -Ocimene (14.1%)	[13]
<i>F. longistylis</i>	Erzincan	Aerial parts/0.16	2,3,6-Trimethylbenzaldehyde (32.7%) Bornyl acetate (12.6%)	[11]
<i>F. longistylis</i>	Erzincan	Fruits/6.4	2,3,6-Trimethylbenzaldehyde (29%) $\alpha$ -Pinene (17%) (Z)- $\beta$ -Ocimene (16%)	[10]
<i>F. pachyloba</i> (Fenzl) Boiss.	Niğde	Aerial parts/1.5	(Z)- $\beta$ -Ocimene (25.7%) $\alpha$ -Pinene (9.8%)	[11]

**Table 2.** Main constituents of the essential oils of *Ferulago* species growing wild in Turkey (continuation)

Plant	Collection place	Parts/Oil yield (%)	Main constituent of the essential oil (%)	Reference
<i>F. pachyloba</i>	Niğde	Aerial parts/0.11	Sabinene (16.0%)	[24]
<i>F. pachyloba</i>	Niğde	Flowers/1.62	(Z)- $\beta$ -Ocimene (15.1%)	[24]
<i>F. pachyloba</i>	Niğde	Roots/0.063	Sabinene (25.8 %)	[24]
<i>F. pachyloba</i>	Niğde	Fruits/0.033	(Z)- $\beta$ -Ocimene (27.5%)	[24]
<i>F. platycarpa</i> Boiss. & Bal.	Nevşehir	Aerial parts/0.07	(E)-2-Decenal (14.3%)	[24]
<i>F. sandrasica</i> Peşmen & Quezel	Denizli	Leaves/0.62	Bicyclogermacrene (11.1%)	[24]
<i>F. sandrasica</i>	Denizli	Aerial parts/0.1	2,3,6-Trimethylbenzaldehyde (29.8%)	[11]
<i>F. sandrasica</i>	Denizli	Roots/0.88	<i>cis</i> -Chrysanthenyl acetate (24.2%)	[26]
<i>F. setifolia</i> C. Koch	Gümüşhane	Aerial parts/0.26	Ocimene (30.5%)	[26]
<i>F. syriaca</i> Boiss.	Hatay	Fruits/4.8	$\delta$ -3-Carene (27.4%)	[27]
<i>F. syriaca</i>	Hatay	Roots/1.1	$\alpha$ -Pinene (17.8%)	[27]
<i>F. thirkeana</i> (Boiss.) Boiss.	İstanbul	Fruits/4.1	$\alpha$ -Pinene (26.4%)	[27]
<i>F. trachycarpa</i> (Fenzl) Boiss.	Konya	Aerial parts/0.6	$\alpha$ -Pinene (27.9%)	[27]
<i>F. trachycarpa</i>	Antalya	Aerial parts/0.59	Limonene (26.1%)	[27]
<i>F. trachycarpa</i>	Antalya	Roots/0.033	$\delta$ -3-Carene (14.2%)	[28]
<i>F. trachycarpa</i>	Antalya	Fruits/0.11	2,4,5-Trimethylbenzaldehyde (77.8%)	[28]
<i>F. trachycarpa</i>	Karaman	Fruits/7.3	2,3,4-Trimethylbenzaldehyde (6.2%)	[20]
			Myrcene (15.3%)	[20]
			4,6-Guaiadiene (10.7%)	[20]
			Bornyl acetate (69.4%)	[20]
			Terpinolene (12.5%)	[29]
			Ferulagone (63.5%)	[29]
			(Z)- $\beta$ -Ocimene (34.1%)	[21]
			(Z)- $\beta$ -Ocimene (13.8%)	[24]
			Spathulenol (25.0%)	[24]
			(E)-2-Decenal (11.9%)	[24]
			Spathulenol (32.8%)	[24]
			Bicyclogermacrene (23.0%)	[24]
			(Z)- $\beta$ -Ocimene (30.7%)	[30]
			Myrcene (27.7%)	[30]

## 2.2. DPPH radical scavenging activity

DPPH radical scavenging activity of the both essential oils were tested *in vitro*. The results are given in Figure 2. The fruit essential oil showed better activity ( $IC_{50}$  value of  $8.6 \pm 0.1$  mg/mL) than the root essential oil. The effects were weak compared to positive controls (vitamin C,  $IC_{50}$  value of  $9.3 \pm 0.01$   $\mu$ g/mL, gallic acid,  $IC_{50}$  value of  $1.93 \pm 0.02$   $\mu$ g/mL).



**Figure 2.** DPPH• scavenging activity of the essential oils of *Ferulago longistylis*



### 2.3. Trolox equivalent antioxidant capacity (TEAC)

The total antioxidant capacity of the essential oils was determined by using a Trolox equivalent antioxidant capacity assay. The results were given in Table 3. The fruit essential oil presented better antioxidant capacity than the root essential oil at 10 mg/mL concentration. However, no effect was observed at 1 mg/mL concentration.

**Table 3.** Total antioxidant capacity of essential oils

Samples (10 mg/mL)	Trolox equivalent antioxidant capacity (mmol/L)
Fruit EO	0.026 ± 0.001
Root EO	0.022 ± 0.001

Data are given as mean ± SD ( $n = 3$ ).

### 2.4. Tyrosinase inhibition

The anti-tyrosinase activity of the essential oils at 1 mg/mL concentration was evaluated using L-DOPA, and the effects were compared with kojic acid. The results were given in Table 4. The fruits essential oil inhibit the tyrosinase weakly, and the root essential oil did not show any inhibition.

**Table 4.** Anti-tyrosinase activity of essential oils

Samples	Antityrosinase activity (Inhibition %)
Fruit EO	24.9 ± 0.7
Root EO	inactive
Kojic acid*	3.6 ± 0.01 (IC <sub>50</sub> , µg/mL)

Data are given as mean ± SD ( $n = 3$ ).

## 3. CONCLUSION

Our findings on the essential oils of *Ferulago longistylis* resulted that the fruit essential oil was found to be rich in oxygenated monoterpenes while the root essential oil was characterized with high monoterpene hydrocarbons. Both essential oils were poor in sesquiterpenes and their oxygenated derivatives. The root oil highly rich in  $\alpha$ -pinene exerted very low antioxidant activity and no activity was recorded for tyrosinase inhibition. The fruit essential oil characterized with 2,3,6-trimethylbenzaldehyde,  $\alpha$ -pinene, (Z)- $\beta$ -ocimene showed weak antioxidant and antityrosinase activity. Both essential oils were not found to be promising in cosmetic applications for antioxidant and antityrosinase purposes.

## 4. MATERIALS AND METHODS

### 4.1. Plant material

*Ferulago longistylis* was collected from Pülümür, Tunceli, Turkey, on July 05, 2021 (The plant material was collected by Mehmet Yavuz Paksoy and Ahmet Duran, the identification was done by Ahmet Duran (Retired Prof. Dr.). The samples were kept with number 10816 at Ahmet Duran's individual herbarium collection).

### 4.2. Chemicals, enzyme and solvents

L-DOPA (Sigma), kojic acid (Sigma-Aldrich), tyrosinase from mushroom (Sigma-Aldrich), 1,1-diphenyl-2-picrylhydrazyl (DPPH•) (Aldrich), 2,2'-azinobis-(3-ethylbenzothiazoline)-6-sulfonic acid (Sigma-Aldrich), Trolox ((S)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid, Sigma-Aldrich), gallic acid (Sigma), ascorbic acid (Sigma-Aldrich), sodium dihydrogen phosphate dihydrate (Merck), dipotassium hydrogen phosphate (Merck), sodium sulfate anhydrous (Sigma-Aldrich), potassium persulfate (Sigma-Aldrich), methanol (Merck), ethanol (Sigma-Aldrich), and *n*-hexane (Sigma-Aldrich) were purchased with high purity and at least of analytical grade.

#### 4.3. Isolation of essential oils

The air-dried roots (59 g) and fruits (120 g) of *F. longistylis* Boiss. were subjected to hydrodistillation, using a Clevenger-type apparatus for 3h. The obtained oils were kept at +4°C in the dark until the experiments.

#### 4.4. Essential oil analyzes (GC-FID and GC/MS) and identification of volatile compounds

The GC-FID and GC/MS analyzes were done according to Kurkcuoglu et al. [31]. The parameters for GC and GC/MS analyzes and also identification method of the volatiles were the same with the published data [31].

#### 4.5. *In vitro* activity methods

The potential tyrosinase inhibitory activity (L-DOPA as a substrate) and DPPH radical scavenging activity of the essential oils were determined using the method of Agalar and Temiz, 2021 [32]. Total antioxidant capacity (TEAC) of the essential oils was tested with the method described by Re et al. (1999) with using ABTS<sup>•+</sup> radical, and the results were expressed as Trolox equivalent (mmol/L Trolox) [33].

#### 4.6. Statistical analysis

All the experiments were carried out in triplicate, and data were expressed as means  $\pm$  standard deviation (SD). Statistical analysis were performed using Sigmaplot 14.0 software (Systat Software, Inc., San Jose, CA, USA). IC<sub>50</sub> values were calculated by regression analysis.

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