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Research Article

# Comparison of Chemical Compositions and Antioxidant Activities of Essential Oils from Ferula latisecta and Ferula gummosa Resins

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## PAPER INFO ABSTRACT

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#### **Keywords**:

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Ferula latisecta and Ferula gummosa are two members of the Apiaceae family native to Iran and have various ethnobotanical applications. This study aims to evaluate and compare the chemical compositions and antioxidant activities of the essential oils extracted from F. latisecta and F. gummosa gum resins. The essential oils were obtained using a Clevenger-type apparatus, and their chemical compositions were identified and quantified using GC-MS and GC-FID methods. Antioxidant activities of the various concentrations of the essential oils were measured using DPPH and ABTS radical scavenging methods. The results showed that 39 compounds were identified in the F. latisecta resin, with major components including  $\beta$ -pinene (30.7%),  $\alpha$ -pinene (25.1%),  $\alpha$ ferulene (6.4%), and  $\alpha$ -bisabolol (6.3%). On the other hand, 44 compounds were identified in the F. gummosa resin, with major components such as  $\beta$ -pinene (50.2%),  $\alpha$ -pinene (9.7%),  $\delta$ -3-carene (7.9%), and  $\beta$ -phellandrene (5.9%). The essential oil from *F. latisecta* demonstrated significant radical inhibitory activities against DPPH and ABTS (IC<sub>50</sub>= 16.86 and 6.99 mg/mL, respectively), which were more effective than that of F. gummosa (IC<sub>50</sub>= 26.72 and 13.97 mg/mL, respectively). These findings suggest that *F. latisecta* resin could be a promising source of bioactive phytochemicals for further research in the food and perfume industries.

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### 1. Introduction

Ferula is a genus of perennial herbs belonging to the Apiaceae family. This genus is distributed in Central and Southwest Asia, the Far East, North India, and the Mediterranean basin. The species in this genus are commonly used as spices and local medicines. These plants are also a rich source of aromatic gumresin (galbanum) used in folk medicine and the perfume industry [1,2].

The most abundant compounds identified in various parts of *Ferula* species are terpenoid hydrocarbons followed by sulfur-containing compounds [3]. The *Ferula* genus comprises about 185 species, with more than 30 species are found in the Iranian flora. Among these, 15 species, including *Ferula gummosa* Boiss. (Fig. 1); and *Ferula latisecta* Rech. f. et Aell (Fig. 2) are endemic.

*F. latisecta* has a strong characteristic odor and is traditionally used for treating infant colic [4]. Previous studies have reported cytotoxic, antimicrobial, and anti-dermatophyte activities of the extracts and essential oils from various parts of *F. latisecta* [5-7].

F. gummosa known as "Barijeh" in Iran, is famous for its gum resin. In Iranian traditional medicine, this resin is well known for treating various disorders including stomachache, cholera, diarrhea, epilepsy, inflammation, and pain. Numerous chemical compounds including terpenoids, sugars, and amino acids have also been reported from this species [8]. In the present study, our goal is to assess and compare the chemical composition of the essential oils extracted from F. latisecta and F. gummosa gum resins. Furthermore, we intend to evaluate the antioxidant activities of these essential oils against 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) free radicals.

In this article, for the first time, the phytochemical constituents and antioxidant effects of the gum resin of *F. latisecta* were determined. Comparing these results with those of *F. gummosa* can reveal the advantages of these plants over each other.



Fig. 1. F. gummosa



Fig. 2. F. latisecta

### 2. Experimental part

#### 2.1. Plant materials

The gum resins of *F. latisecta* (voucher no. 477) and *F. gummosa* (voucher no. 427) were collected from

the Hezar Masjed Mountains, in the Khorasan Razavi province of Iran by Dr. Mohammad Sadegh Amiri (Herbarium of the Payam-e-Noor University, Dargaz, Khorasan Razavi province, Iran).

## 2.2. Essential oils isolation

The essential oils from the resins (30 g) were extracted using a Clevenger-type hydrodistillation apparatus [9,10]. The obtained essential oils were dried over anhydrous sodium sulfate and stored at 4 °C.

### 2.3. GC-FID analysis

The chemical components of the essential oils were quantified using a gas chromatograph (GC, Varian CP-3800), equipped with a flame ionization detector (FID) and an HP-5MS capillary column (30 m  $\times$  0.32 mm; 0.25  $\mu$ m). The oven temperature program was set to start at 60°C and then increase to 250°C at a rate of 4°C/min. Nitrogen was used as the carrier gas with a flow rate of 1.0 mL/min. The injector and detector temperatures were maintained at 250°C. The injection volume was 1  $\mu$ L with a 1:100 split ratio. A mixture of normal alkanes (C<sub>6</sub>-C<sub>24</sub>) was injected under the same condition, to calculate retention indexes. The quantity of each component was determined using the peak area integration method [11, 12].

## 2.4. GC-MS analysis

The chemical components of the essential oils were identified using a gas chromatograph- mass spectrometer (GC-MS, Agilent 5977A) equipped with an HP-5MS capillary column (30 m  $\times$  0.32 mm; 0.25  $\mu$ m). The oven temperature program was set to start at 60°C and then increase to 250°C at a rate of 4°C/min. Helium was used as a carrier gas with a flow rate of 1.0 ml/min. The injector temperature was maintained at 250°C. The injection volume was 1  $\mu$ L with a 1:100 split ratio. The temperature and ionization voltage of the ion source were set at 200°C and 70 eV, respectively. A mixture of normal alkanes (C<sub>6</sub>-C<sub>24</sub>) was injected under the same

conditions to calculate retention indexes. The mass spectral data of each peak were compared with those provided in Wiley, Adams, and NIST mass spectral libraries [13,14].

## 2.5. DPPH radical scavenging assay

The antioxidant activities of the essential oils were assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging method [15, 16]. To do this, 50 µL of essential oil solution (160 µg/mL in methanol) was added to 200 µL of 100 mM DPPH solution in methanol. After a 30-minute incubation at room temperature in darkness, the decrease in absorbance (Abs) was measured at 517 nm. Butylated hydroxytoluene (BHT) was used as a positive control for this assay. The percentage of inhibition was determined using the following equation:

Inhibition (%) = 
$$\frac{Abs_{Control} - Abs_{Sample}}{Abs_{Control}} \times 100$$

All experiments were conducted at least in triplicate, and inhibition percentages were expressed as the mean  $\pm$  standard deviation. The inhibitory activities of various concentrations of the isolated compounds were assessed to determine the half-maximal inhibitory concentration (IC<sub>50</sub>).

## 2.6. ABTS radical scavenging assay

The antioxidant activities of the essential oils were measured using the ABTS (2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)) free radicals scavenging method [17]. To prepare the radical cationic solution, 5 ml of 7 mM ABTS reagent was mixed with 88  $\mu$ L of 140 mM potassium persulfate and left in darkness at room temperature for 14 hours. The solution was then diluted with ethanol until it reached an absorption of 0.7  $\pm$  0.02 at 734 nm. In a 96-well microplate, 150  $\mu$ L of the final ABTS solution was combined with 50  $\mu$ L of each essential oil (concentration ranging from 2.5 to 160  $\mu$ g/mL in methanol). After 6 minutes, the absorption changes were recorded at 734 nm. The results were reported as a percentage of inhibition using the

following equation:

$$Inhibition~(\%) = \frac{Abs_{Control} - Abs_{Sample}}{Abs_{Control}} \times 100$$

All experiments were conducted at least in triplicate, and the inhibition percentages were expressed as the mean  $\pm$  standard deviation. The inhibitory activities of various concentrations of the isolated compounds were evaluated to determine the half-maximal inhibitory concentration (IC<sub>50</sub>).

#### 3. Discussion and conclusion

### 3.1. Chemical compositions of the essential oils

The essential oils were extracted from the resins of F. latisecta and F. gummosa using a Clevenger apparatus, yielding  $7.7 \pm 0.2$  and  $21.7 \pm 0.8$  mL/g, respectively. The chemical compositions of the oils were then identified and quantified using GC-MS and GC-FID methods, respectively. The gas chromatograms of F. latisecta and F. gummosa resins are shown in Fig. 3 and Fig. 4, respectively. According to the results (Table 1), 44 compounds were identified in the F. gummosa resin, accounting for 97.7% of the essential oil. Additionally, 39 compounds were identified in the F. latisecta resin, representing 96.5% of its essential oil. Major components of the F. gummosa resin essential oil included  $\beta$ -pinene (50.2%),  $\alpha$ -pinene (9.7%),  $\delta$ -3carene (7.9%), and  $\beta$ -phellandrene (5.9%). In addition,  $\beta$ -pinene (30.7%),  $\alpha$ -pinene (25.1%),  $\alpha$ ferulene (6.4%), and  $\alpha$ -bisabolol (6.3%) were

determined as major components of the *F. latisecta* resin essential oil (Fig. 5).

As shown in Table 1, both essential oils were dominated by monoterpene hydrocarbons with 14 of the same compounds, including  $\alpha$ - and  $\beta$ -pinene,  $\beta$ -myrcene, (*E*)- and (*Z*)- $\beta$ -ocimene,  $\alpha$ -ferulene, shyobunon, and  $\alpha$ -bisabolol. On the other hand, *F. latisecta* essential oil contained 7.4% organosulfide compounds ((*E*)-sec-butyl propenyl disulfide, (*Z*)-sec-butyl propenyl disulfide, and, hexamethylene sulfide), while *F. gummosa* did not have any sulfurcontaining compounds (Fig. 6).

According to the results, the chemical constituents of the essential oils of F. latisecta and F. gummosa resins were dominated by mono- and sesquiterpenoids. 14 Compounds are the same in both samples of F. latisecta (73.84%) and F. gummosa (70.8%) essential oils, such as  $\alpha$ - and  $\beta$ -pinene,  $\beta$ -myrcene, (E)- and (Z)- $\beta$ -ocimene,  $\alpha$ -ferulene, shyobunon, and  $\alpha$ -bisabolol. On the other hand, F. latisecta essential oil has 7.4% organosulfide compounds, while F. gummosa has no sulfurcontaining compounds.

Previous studies have identified (Z)- and (E)-secbutyl propenyl disulfide in the essential oils from the leaves, fruits, and roots of F. latisecta [18-20]. Additionally, hexamethylene sulfide was previously detected in the essential oil of garlic [21].

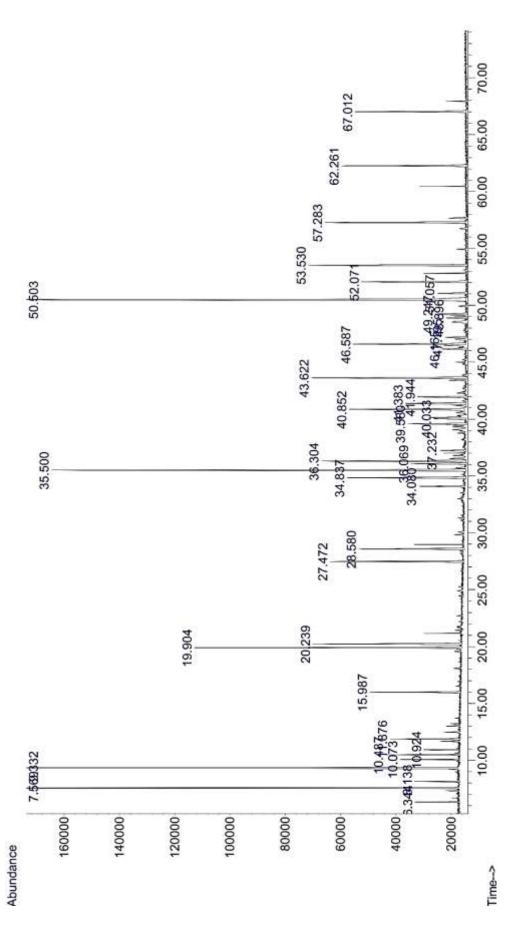


Fig. 3. GC chromatogram of the essential oil of F. latisecta resin

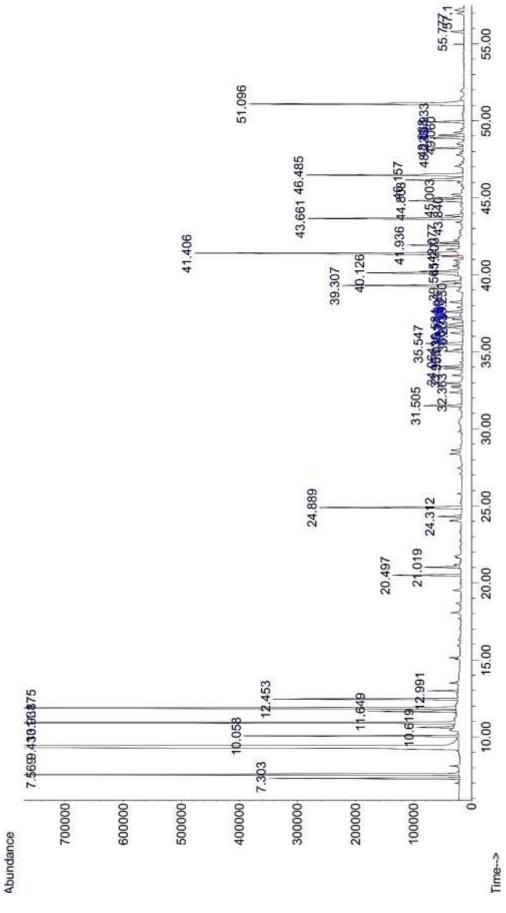


Fig. 4. GC chromatogram of the essential oil of F. gummosa resin

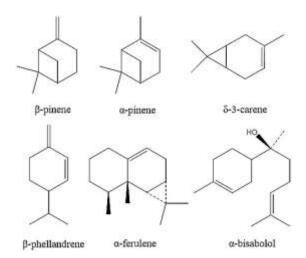


Fig. 5. Mian components of F. latisecta and F. gummosa resins

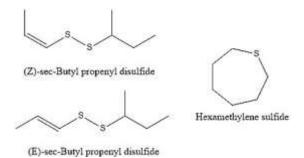


Fig. 6. Organosulfides of F. latisecta resin essential oil

**Table 1.** Chemical composition of the essential oils of F. gummosa and F. latisecta resins

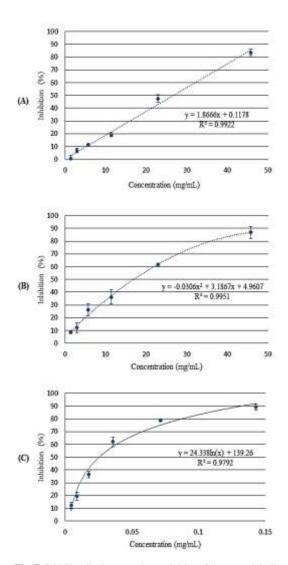
			Percentage (%)	
	RI*	Compound	F.	F.
			gummosa	latisecta
1	923	$\alpha$ -Thujene	1.2	-
2	933	$\alpha$ -Pinene	9.7	25.1
3	934	Camphene	-	0.9
4	982	$\beta$ -Pinene	50.2	30.7
5	991	$\beta$ -Myrcene	1.9	1.3
6	1002	Phellandrene	0.3	-
7	1011	$\delta$ -3-Carene	7.9	-
8	1021	<i>p</i> -Cymene	0.6	-
9	1024	o-Cymene	-	0.2
10	1029	Limonene	-	1.0
11	1029	$\beta$ -Phellandrene	5.9	-
12	1037	$(E)$ - $\beta$ -Ocimene	1.6	0.2
13	1045	$(Z)$ - $\beta$ -Ocimene	0.3	0.3
14	1153	(E)-Pinocarveol	-	0.3
15	1179	(3E,5Z)-1,3,5-		
		Undecatriene	0.7	-
16	1181	Pinocarvone	-	0.2
17	1188	(E)-sec-Butyl propenyl		
		disulfide	-	4.2
18	1188	(3 <i>Z</i> ,5 <i>E</i> )-1,3,5-		
		Undecatriene	0.3	-
19	1190	(3E,5E)-1,3,5-		
		Undecatriene	0.2	-
20	1194	(Z)-sec-Butyl propenyl		
		disulfide	-	2.3

			Percentage (%)	
	RI*	Compound	F. gummosa	F. latisecta
21	1216	Myrtenol	-	0.3
22	1236	3-Methoxy- <i>p</i> -cymene	0.2	-
23	1246	2-methoxy- <i>p</i> -Cymene	1.3	_
24	1291	Bornyl acetate	_	1.8
25	1293	(E)-Pinocarvyl acetate	0.1	0.4
26	1339	(E)-Methylgeranate	-	0.2
27	1342	α-Terpinyl acetate	0.4	_
28	1357	(+)-Cycloisosativene	0.1	-
29	1362	α-Cubebene	0.1	-
30	1366	$\alpha$ -Copaene	0.1	-
31	1375	$\beta$ -Bourbonene	0.1	-
32	1381	β-Cubebene	0.2	-
33	1383	$\beta$ -Elemene	0.3	0.8
34	1401	$\alpha$ -Cedrene	0.2	-
35	1409	(E)- $\beta$ -Caryophyllene	0.5	-
36	1425	γ-Elemene	0.4	-
37	1434	$\alpha$ -Ferulene	0.1	6.4
38	1432	Hexamethylene sulhide	-	0.9
39	1436	$\beta$ -Gurjunene	-	2.2
40	1443	$\alpha$ -Caryophyllene	0.2	-
41	1449	Dehydroaromadendrene	-	0.5
42	1450	$\beta$ -Farnesene	0.1	-
43	1461	4,11-Selinadiene	-	0.4
44	1473	(-)-Germacrene D	1.3	-
45	1476	$\beta$ - Selinene	0.2	0.7
46	1486	$\alpha$ -Selinene	1.2	-
47	1488	Bicyclogermacrene	0.2	-
48	1496	$\alpha$ -Gurjunene	-	1.2
49	1501	$\alpha$ -Amorphene	-	0.2
50	1504	$\beta$ -Bisabolene	-	0.2
51	1509	Shyobunon	2.8	0.8
52	1516	$(+)$ - $\delta$ -Cadinene	0.5	0.4
53	1520	Epizonarene	0.2	-
54	1549	Germacrene B	1.6	-
55	1565	Elemol	-	0.4
56	1567	Germacrene-4-ol	0.6	-
57	1571	epi-Ligulyl oxide	-	1.9
58	1592	α-Guaiol	0.6	0.4
59	1627	1-Hexadecyne	0.2	-
60	1640	$\beta$ -Selinenol	0.3	-
61	1643	α-Selinenol	0.3	0.4
62	1658	Bulnesol	0.3	-
63	1671	$\alpha$ -Cadinol	-	0.5
64	1684	$\alpha$ -Bisabolol	2.2	6.3
65	1751	1(5),3-		
		Aromadenedradiene	-	1.8
66	1808	(E,E)-Farnesyl acetate	-	0.2
67	1830	Isovalencenol	-	0.3
68	1919	2-Heptadecanone	-	0.2
69	1981	n-Hexadecoic acid	-	0.2
		Monoterpene		
		hydrocarbons	79.6	59.7
		Oxygenated		
		monoterpenes	2.0	3.2
		Sesquiterpene		
		hydrocarbons	7.6	14.8
		Oxygenated		
		sesquiterpenes	7.1	11.2
		Organosulfides	-	7.4
		Total	97.7	96.5

Previous studies have reported high values of organosulfides in the essential oils of leaves, roots, and fruits of F. latisecta, (48.0%, 60.8%, and 72.7%, respectively) [18]. In comparison, in the present study, it was found that organosulfide compounds made up 7.4% of the essential oil obtained from F. latisecta gum.

## 3.2. Antioxidant activities of the essential oils

The antioxidant activities of the various concentrations of essential oils were assessed by the ability to scavenge DPPH and ABTS free radicals, and the results were compared to those of BHT, a standard antioxidant agent. Curves displaying the percentage of inhibition versus the concentrations of essential oils were plotted (Fig. 7 and Fig. 8).



**Fig. 7.** DPPH radical scavenging activities of the essential oils of *F. gummosa* (A), *F. latisecta* (B) resins and BHT (C).

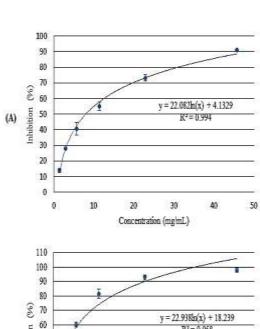
Based on the results of the DPPH and ABTS radical scavenging assays, the two essential oils showed significant and concentration-dependent activities.

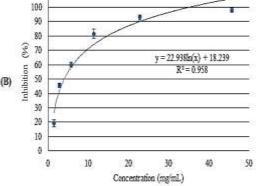
The IC<sub>50</sub> values were calculated using the equations of the curves and compared with that of BHT (Table 2).

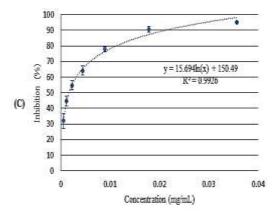
**Table 2.** Antioxidant activities of the essential oils of *F. gummosa* and *F. latisecta* resins against DPPH and ABTS

	DPPH*	ABTS*	
F. gummosa	26.72	13.97	
F. latisecta	16.86	6.99	
BHT	0.03	0.002	

<sup>\*</sup> IC50 (mg/mL)







**Fig. 8.** ABTS radical scavenging activities of the essential oils of *F. gummosa* (A), *F. latisecta* (B) resins and BHT (C).

 $IC_{50}$  indicates how much drug is needed to inhibit a biological process by half, providing a measure of the potency of an antagonist drug in pharmacological research. Therefore, a lower  $IC_{50}$  value means that the drug is potent at lower concentrations.

Based on the results, the essential oil of F. latisecta resin (IC<sub>50</sub>= 16.86 mg/mL) showed significant radical inhibitory activity, proving to be more effective than that of F. gummosa (IC<sub>50</sub>= 26.72 mg/mL) against DPPH radicals. In comparison, BHT had an IC<sub>50</sub> value of 0.03 mg/mL.

Usually, the antioxidant effects of synthetic compounds, such as BHT, are much higher than those of natural compounds, like essential oils. However, due to fewer complications, the use of natural compounds is preferred. Additionally, to better compare the results of various articles, the effects of compounds such as BHT are reported as a standard antioxidant [22].

The potency of the essential oil from F. latisectal resin (IC<sub>50</sub>= 6.99 mg/mL) was higher than that of F. gummosa (IC<sub>50</sub>= 13.97 mg/mL) against ABTS radical cation. In comparison, BHT exhibited an IC<sub>50</sub> value of 0.002 mg/mL.

Additionally, our observations confirm that the volatile oil from *F. latisecta* resin has high antioxidant activity and showed more activity than that of *F. gummosa*.

It appears that the higher antioxidant activities of F. *latisecta* essential oil can be attributed to two primary factors:

1. The presence of disulfide compounds in the essential oil of *F. latisecta* compared to *F. gummosa*. Some studies have proven the free radical inhibiting power of disulfide compounds. According to the mechanism of the reactions, when free radicals are added, disulfide bonds break and produce thiols and other derivatives (23).

2. Higher amounts of natural alcohols in F. latisecta gum essential oil. The total amount of natural alcohols in F. latisecta gum essential oil (8.9%) is more than twice that of F. gummosa (4.3%). Among these natural alcohols,  $\alpha$ -bisabolol has the highest amount in F. latisecta (6.3%) and F. gummosa (2.2%).

Previous studies have proved the high antioxidant activity of  $\alpha$ -bisabolol against free radicals, such as DPPH and ABTS (24). First and coworkers was reported IC<sub>50</sub> value of 43.88 mg/mL against DPPH free radicals (25).

 $\alpha$ -Bisabolol also increases the mRNA level of antioxidant proteins such as superoxide dismutase, catalase, and the keap1 gene product (26).

To the best of our knowledge, this is the first study to investigate the antioxidant activities of *F. latisecta* and *F. gummosa* gum resins. The results suggest that *F. latisecta* resin can be considered a source of bioactive phytochemicals for further studies in drug discovery and perfumery fields.

#### Acknowledgments

The authors would like to express their special gratitude to Dr. Mohammad Sadegh Amiri for identifying of the plant and collecting plant resins.

## Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this manuscript. Additionally, the authors have fully adhered to ethical standards, including avoiding plagiarism, obtaining informed consent, preventing misconduct, avoiding data fabrication or falsification, refraining from double publication or submission, and eliminating redundancy.

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