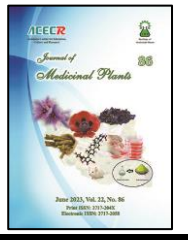
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Research Article

Phytochemical evaluation and antioxidant effects of the essential oil and distillates of *Nepeta crispa* Willd.

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ABSTRACT

Background: *Nepeta crispa* Willd. (Lamiaceae) is a medicinal plant endemic to Iran with numerous ethnobotanical uses. **Objective:** The current study focuses on the quality control of *N. crispa* growing in Hamadan province. **Methods:** Hydro-distilled essential oil from aerial parts of *N. crispa* was obtained using the Clevenger apparatus. Four samples *of N. crispa* distillate were purchased from various locations. Methanol extracts from aerial parts of this plant were obtained by the maceration method. Micromorphological characteristics, antioxidant properties, and chemical constituents of *N. crispa* essential oil and distillates were evaluated. Determination of the total phenols and flavonoid contents of the extract was performed. **Results:** GC/MS analysis revealed that the major components of *N.crispa* essential oil were 1,8-cineole (70.93 %), nepetalactones (5.29 %), and *β*-pinene (5.5 %). The volatile constituents of one of the distillates resemble the essential oil of *N. crispa*. The extract from aerial parts of *N. crispa* consistently exhibited more anti-oxidative power than its essential oil with an IC_{50} of 2.792 ± 0.385 mg/ml. The results from assays for total phenols and flavonoids revealed that this plant possesses phenolic and flavonoid content of 69.24 ± 3.34 µg GAEs/mg and 12.01 ± 0.26 µg QEs/mg of extract, respectively. Covering, glandular trichomes, diacytic stomata, and spiral vessels were seen in microscopic studies. **Conclusion:** Our results suggest that *N. crispa* possess considerable anti-oxidative potential with relatively high phenolic and flavonoid content in which 1,8-cineole is the major component. Due to the rarity of this plant, there are fraudulent specimens among its commercial distillates.

1. Introduction

The genus *Nepeta* (Nepetoideae, Lamiaceae) consists of about 300 species. Up to now, 79 endemic species of this genus have been discovered in Iran [1]. *Nepeta crispa* Willd. is an endemic, rare, threatened, and aromatic plant,

Abbreviations: QE, Quercetin Equivalent; GAE, Gallic Acid Equivalent; ABTS, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid; ABTS/PP, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid/ Potassium Persulfate; GC-MS, Gas Chromatography- Mass Spectrometry

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which grows specifically in the west of Iran, especially at the heights of Alvand Mountain, located in the northwest of Hamadan [2].

Due to the pungent and energetic scent of *N. crispa*, it goes by the name of "Moffarrah" among the locals which roughly translates to "what animates and brightens". The locals have been using this plant through the years in local homeopathy and herbal remedies for a variety of purposes such as anti-depressant, antiseptic, expectorant, anti-asthmatic, and pyrolytic uses [3].

Previous studies relate the effects of *Nepeta* genus to its essential oil and flavonoids. On the other hand, some studies are presenting the effects of environmental factors such as moisture, temperature, and soil on *Nepeta* essential oil both in terms of amount and composition [4]. There are some previous studies on the chemical compositions of *N. crispa* essential oil. Sonboli et al. reported the presence of 23 compounds with 1,8-cineole (47.9 %) and 4a*α*,7*α*,7a*β*-nepetalactone (20.3 %) as major components [3]. Mojab et al. discovered 12 components with 1,8-cineole (71 %) as the major constituent in the essential oil of *N. crispa* [4]. Abdoli et al. revealed that 1,8-cineole and nepetalactone are the main constituents of *N. crispa* essential oil in vegetative and flowering stages of growth [5]. Daryasari et al. used two methods of hydro-distillation (HD) and microwave-assisted hydro-distillation (MAHD) for the isolation of *N. crispa* essential oil. In their study, although 1,8-cineole and nepetalactone are the main components of *N. crispa* essential oil obtained by MAHD, nepetalactone was not observed in the HD method [6].

Recent studies have demonstrated that *Nepeta* genus in general possesses a rather high antioxidant potential [7]. There are some important studies on the antioxidant activity of *N. crispa* from different locations such as Lorestan province [8, 9].

Given the fact that *N. crispa* mostly grows in the hillsides of Alvand Mountain, located in the Hamadan region, there is not much research with a focus on this particular species. Moreover, due to the low availability and probably wrong identification of the genus and species, other plants might be used instead of *N. crispa* in its products and distillates in the market.

Considering, the current study aims to evaluate the chemical compositions of *N. crispa* essential oil and volatile constituents of four distillate samples purchased from the market. Furthermore, the antioxidant activity of *N. crispa* essential oil and extract was investigated by the ABTS method. Also, the total phenolic and flavonoid contents of *N. crispa* extract, as well as the microscopical features of its leaf were determined.

2. Materials and Methods

2.1. Preparation of the plant material

Aerial parts of *Nepeta crispa* Willd. (*N. crispa*) were collected in June of 2021 from the hillsides of Alvand Mountain, Hamadan province, Iran. Then they were dried and stored at room temperature. The plant material was authenticated and deposited a voucher specimen (Herbarium number: 72) in the Department of Pharmacognosy, School of Pharmacy, Hamadan University of Medical Sciences, Hamadan, Iran.

2.2. Preparation of the extract

The extract of *N. crispa* was prepared by a method used by Ahmadimoghadam et al. [10]. In order to obtain the extract, 100 g of the dried aerial parts of *N. crispa* was crushed, milled, and then soaked in 3 liters of methanol for 3 days at room temperature. The solvent was separated using a rotary vacuum evaporator (RE). The remains were concentrated and isolated in a separate container. This course of action was repeated 3 times to achieve the full amount of

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plant extract. The resulting solid masses were stored at 4 °C in a dark environment until use.

2.3. Preparation of the essential oil

The essential oil of *N. crispa* was prepared by hydro-distillation via a Clevenger-type apparatus [11]. For this purpose, 100 g of *N. crispa* dried parts were placed in a 2-liter balloon by adding one and a half liters of purified water. Then the system was heated for 4 hours. The essential oil was afterward isolated and stored in sealed dark glass vials at 4°C until use.

2.4. Isolation of the volatile components from distillates

Four samples of *N. crispa* distillates were purchased from different locations in downtown Hamadan. 500 ml of each distillate was deposited into a Clevenger-type apparatus. After 3 hours of heating at 90 °C, the non-polar fraction of the distillate was collected using a Pasteur pipette and analyzed by GC/MS.

2.5. ABTS assay

The radical scavenging activity of the methanol extract and the essential oil of *N. crispa* was measured using the method described by Gursoy et al. with minor modifications [12]. In order to perform this test, the $ABTS^{\bullet+}$ radical was prepared by mixing 7 mM ABTS solution in water and 2.45 mM potassium persulfate and allowing this solution to sit in the darkness for 18 hours at room temperature. Then, this solution was diluted to achieve the absorbance of 0.7 ± 0.05 at 734 nm. Afterward, 25 µl of $ABTS^{\bullet+}$ solution was added to 750 µl of the extract and the essential oil of *N. crispa* at various concentrations (8000 to 62.5 µg/ml). The mixtures were stored in the dark for 30 minutes. Then the absorbance was read at 734 nm against a blank sample that contained methanol instead of extract or essential oil of *N. crispa*. The scavenging capacity of $ABTS^{\bullet+}$ radicals was calculated using the following formula:

$$ABTS^{\bullet+} \text{ scavenging capacity \%} = [(Abs_{Control} - Abs_{Sample}) / Abs_{Control}] \times 100.$$

$Abs_{Control}$ is the absorbance of the control reaction (which contained all reagents except for the test compound) and Abs_{Sample} is the absorbance of the test compound. Gallic acid, ascorbic acid, and BHT were utilized as standards. Extract and essential oil concentrations providing 50% inhibition (IC_{50}) were obtained from the graph plotting of inhibition percentage against their concentration.

2.6. Determination of the total phenolic content

Using the procedures described by Nickavar et al., the total phenolic content of the methanol extract of *N. crispa* was determined [13]. In this assay, the Folin-Ciocalteu reagent and gallic acid were employed as standard. In order to perform this test, the Folin-Ciocalteu reagent was diluted at a ratio of 1:10. Then 150 µl of different concentrations of the extract (8000 to 62.5 µg/ml) was added to 750 µl of Folin-Ciocalteu solution in separate microtubes. After 10 minutes of incubation at room temperature, 600 µl of Na_2CO_3 (2 %) solution was added to each test sample. Then, all samples were allowed to sit at room temperature. At last, the absorbance was measured at 765 nm. A standard curve was also generated using various concentrations (1000-7.81 µg/ml) of gallic acid in ethanol. The concentrations of phenolic compounds were determined using the equation derived from the standard gallic acid graph.

2.7. Determination of the total flavonoid content

This assay was performed according to a procedure described by Arvouet-Grand et al. with a few modifications [14]. Briefly, 0.5 ml of

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20 mg/ml $AlCl_3$ was added to 0.5 ml of various concentrations of the methanol extracts (8000 to 62.5 µg/ml). After 30 minutes of incubation at room temperature, absorption values were measured at 415 nm against a blank sample which contained 1 ml of the extract solution and 1 ml of methanol without $AlCl_3$. The standard curve was also generated using various concentrations (1000-7.81 µg/ml) of quercetin in water. Concentrations of flavonoid components were determined using the equation derived from the standard quercetin graph.

2.8. GC/MS Analysis

The essential oil of *N. crispa* was analyzed via a Thermoquest Finnigan Trace gas chromatograph/mass spectrometer (GC/MS) fitted with a fused methyl silicon DB-5 column (30 m*0.25mm*0.25µm film thickness) [15]. Helium was utilized as carrier gas at a flow rate of 1.1 ml/min. The column temperature was maintained at 60 °C for two consecutive minutes, increased to 250 °C at a rate of 5 °C/min increase, and ultimately kept at 245 °C for 2 minutes. The injector temperature was 250 °C and the split ratio was adjusted at 1/100. The injection volume was 0.2 µL. The mass spectrometer condition was as follows: ionized potential 70 eV and source temperature 200 °C. Constituents were identified by comparison of their Retention Index in relation to C5-C24 n-alkanes by comparison of the RI, provided in the literature and by comparison of the mass spectra with those recorded by the MAINLIB and Willey. Furthermore, four samples of *N. crispa* distillates were also analyzed using the same technique.

2.9. Microscopical characteristics

Pharmacognostic studies of *N. crispa* leaf were carried out by examining its microscopical characteristics. Some of the leaves and stem tissue powder of *N. crispa* were separated and boiled in potassium hydroxide solution (20 %) in a beaker on the heater for 1 minute and washed with distilled water three times. After bleaching the powders with sodium hypochlorite, the powders were sequentially rinsed with distilled water. The prepared sample was placed on a microscope glass slide. Photomicrographs were taken using a Labomed microscope attached to a digital camera. Photomicrographs of sections were taken at different magnifications depending on the microscopic details.

3. Results

3.1. Chemical composition of the essential oil of Nepeta crispa and distillate sample A4

The total yield of the essential oil from aerial parts of *N. crispa* and distillate sample A4, which showed closest resemblance to essential oil chemical composition, were 0.85 % and 0.42 %, respectively. Table 1 shows the chemical compositions of the essential oil from aerial parts of *N. crispa* and sample A4 and their related chromatograms are brought in Fig. 1 and Fig. 2. In this study, 21 separate chemical constituents from the *N. crispa* essential oil were identified in GC/MS analysis. 1,8-cineole (70.93 %) was the major component of the essential oil from this plant, followed by *β*-pinene (5.5 %), linalool formate (4.92 %), *α*-pinene (2.22 %), and nepetalactones (for a total of 5.29 %). Secondary substituents of this essential oil were linalool (1.15 %), (*Z*)-*α*-Bisabolene (0.96 %), and cymene (0.65).

The result of chemical composition of the *Nepeta crispa* distillates are demonstrated in Table. 2 and the chromatogram for each sample (A1, A2 and A3) is in accordance with Fig. 3, Fig. 4 and Fig. 5, respectively.

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3.2. Microscopical characteristics

Microscopic features of *N. crispa* leaf were assessed. Fig. 6 represents the results microscopic inspection of *N. crispa*

3.2.1. Epidermal cells and stomata type

The epidermis is made up of polygonal axially elongated cells, closely fitted by a mixture of wavy and straight walls.

It is covered by a thin layer of sinuous cuticle walls and both epidermis of the leaf consisted diacytic-type stomata (amphistomatic). The stomatal study of the leaf showed that stomata are exclusively diacytic type. At the polar area of the guard cells, the stoma has two subsidiary cells. The guard cell's common wall will be at an angle to the common wall of the subsidiary cells.

Table 1. Chemical compositions of the essential oil from aerial parts of *Nepeta crispa* and distillate sample A4

No	RI	Compound	Area GC/MS %	
			Essential oil	Sample A4
1	928	*α*-Thujene	0.21	0.27
2	937	*α*-Pinene	2.22	2.53
3	978	Sabinene	0.86	1.11
4	986	*β*-Pinene	5.5	5.62
5	994	*β*-Myrcene	0.34	0.46
6	998	Dehydro-1,8-cineole	0.26	0.55
7	1006	Isobutyl 2-methylbutyrate	0.24	0.13
8	1024	*α*-Terpinene	0.14	
9	1034	*p*-Cymene	0.65	0.72
10	1038	Limonone	0.44	
11	1043	1,8-Cineole	70.93	62.64
12	1064	*γ*-Terpinene	0.56	0.24
13	1080	Terpinolene	0.85	1.58
14	1109	Linalool	1.15	1.41
	1113	*trans*-Sabinene hydrate		0.56
15	1184	*α*-Terpineol	2.1	2.92
16	1193	4-Terpineol	2.17	2.11
17	1210	Linalool formate	4.92	7.28
18	1377	4a*α*,7*α*,7a*α*-Nepetalactone	2.84	1.13
19	1413	4a*α*,7*β*,7a*α*-Nepetalactone	2.45	7.78
20	1453	(*E*)-*β*-Farnesene	0.21	0.24
21	1497	(*Z*)-*α*-Bisabolene	0.96	0.55

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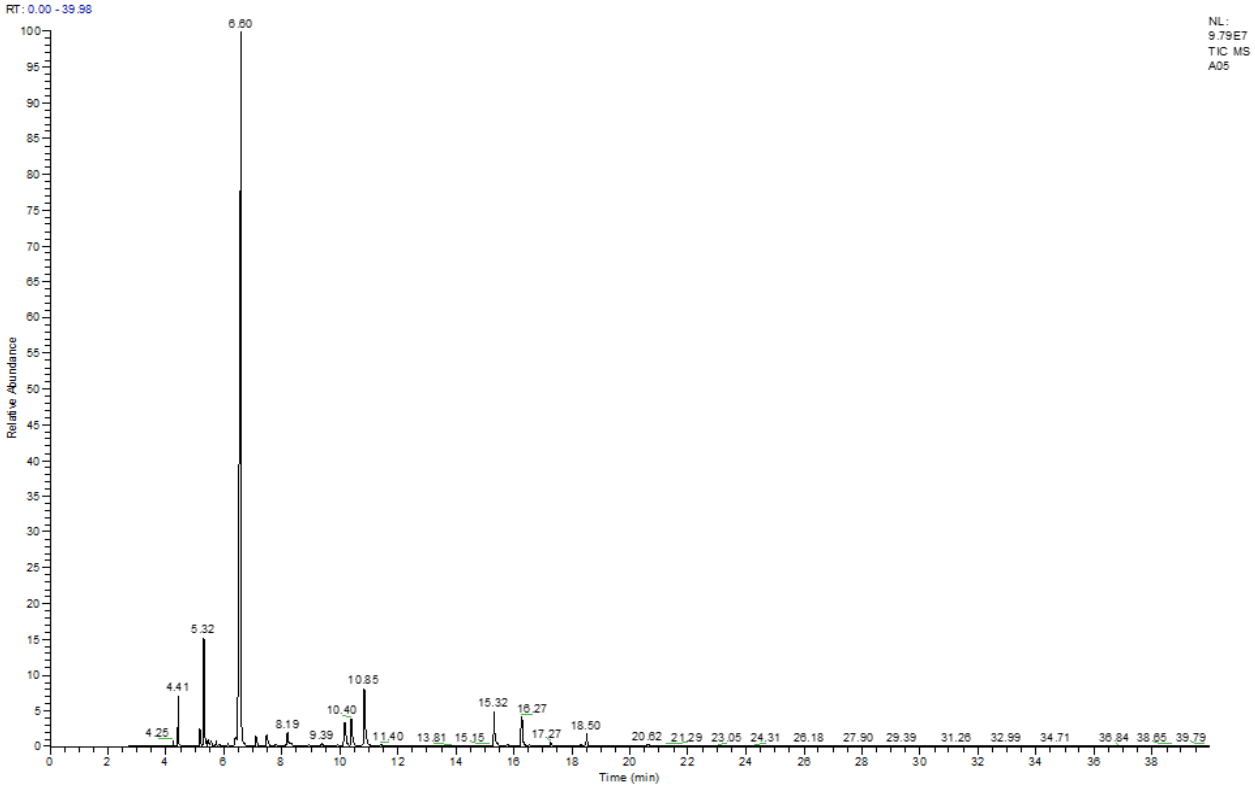


Fig. 1. GC/MS chromatogram of the essential oil from aerial parts of *Nepeta crispa* Willd.

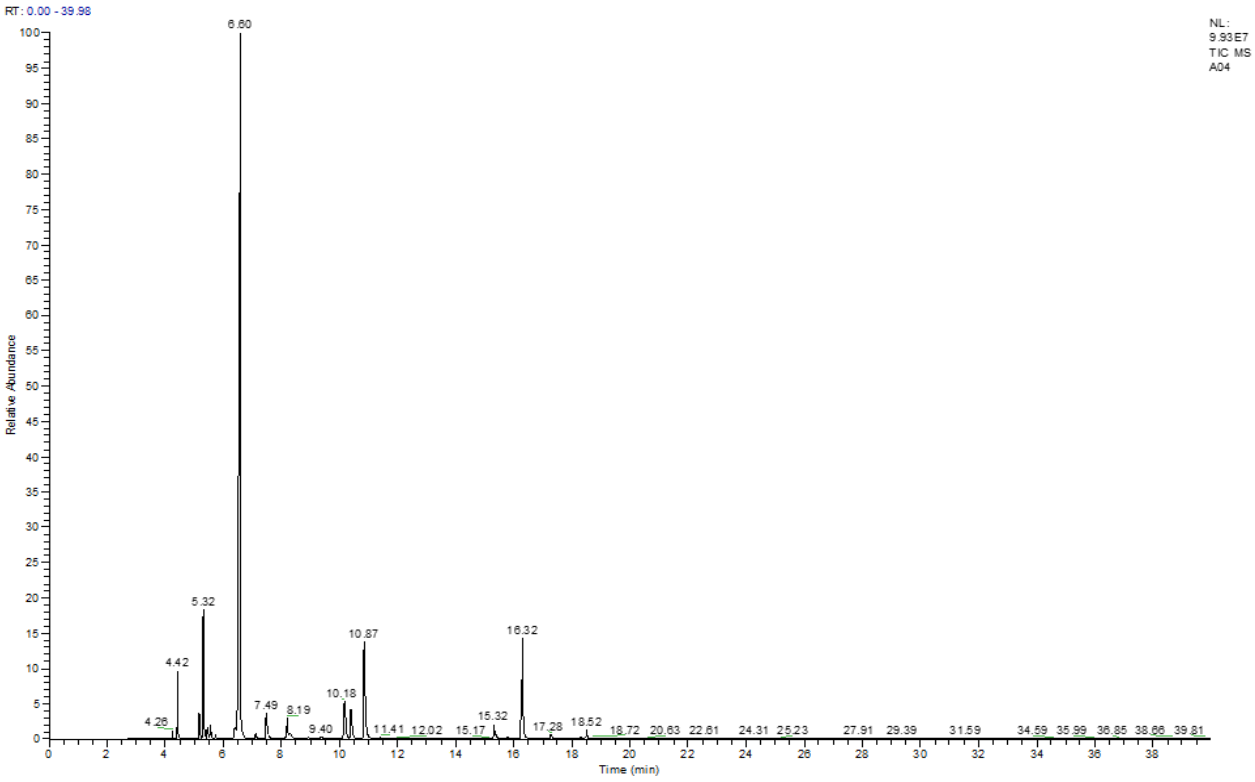


Fig. 2. GC/MS chromatogram of distillate sample A4

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Table 2. Chemical compositions of the essential oil of distillate samples A1, A2, A3

No	RI	Compound	Area GC/MS%		
			A1	A2	A3
1	902	2,5-Diethyltetrahydrofuran	0.04		
2	937	*α*-Pinene	0.03		
3	985	*β*-Pinene	0.03		
4	994	2-Octen-1-ol		0.48	
5	1006	3-Octanol	0.19		0.14
6	1035	Limonone	0.12		
7	1039	1,8-Cineole	0.39	38.22	16.64
8	1079	*cis*-Linalool oxide		0.17	
9	1096	*trans*-Linalool oxide		0.16	
10	1100	*cis*-Thujone		0.44	
11	1110	Linalool		2.64	
12	1137	*cis*-Sabinene hydrate		0.42	
13	1156	*trans*-Sabinene hydrate		0.44	
14	1159	*trans*-Pinocarveol			0.4
15	1162	Isopulegol	0.46	0.2	0.39
16	1169	Menthone	7.22	3.16	3.21
17	1178	*iso*-Menthone	4.14	0.5	2.04
18	1189	*α*-Terpineol		3.49	
19	1185	neo-Menthol	5.13		0.49
20	1198	4-Terpineol		12.25	
21	1205	Menthol	77.32		
22	1212	*trans*-Dihydro carvone			0.43
23	1214	Linalool formate		6.01	
24	1253	Pulegone	0.39	25.09	3.09
25	1260	Carvone	0.76		3.87
26	1269	Piperitone	1.14		0.23
27	1278	*neo*-Menthyl acetate	0.06		
28	1296	Menthyl acetate	1.7		0.18
29	1312	*iso*-Menthyl acetate	0.04		
30	1315	Carvacrol			2.29
31	1354	Piperitenone		0.31	
32	1389	*β*-Bourbonene	0.04		
33	1426	*E*-Caryophyllene	0.25		
34	1488	Germacrene D	0.07		
35	1587	Spathulenol		0.06	0.2
36	1600	Caryophyllene oxide		0.15	0.14
37	1633	Dill apiole	0.47		

3.2.2. Epidermal trichomes

Three types of indumentum are found in the leaf of *N. crispa*, which is composed of two main types of glandular: peltate and capitate, and non-glandular: branched and unbranched trichomes.

Glandular types of epidermal trichomes are abundant on the lamina, which are aroma-secreting glands. The glandular trichomes are two types; some of the glands are smaller in size and are capitate type; others are larger and peltate type.

Peltate glands feature a circular, horizontal secretary body plate and a two-celled stalk.

The epidermal cell that gives rise to the gland's stalk is encircled by several radiating rosette-shaped cells. Capitate glands are smaller and shorter. The gland has large spherical basal epidermal cells; these are two rectangular stalk cells, arranged one above the other. On the top of the stalk is a two-celled, spherical secretary body. Also, spiral xylem vessels in the leaf of *N. crispa* are observed.

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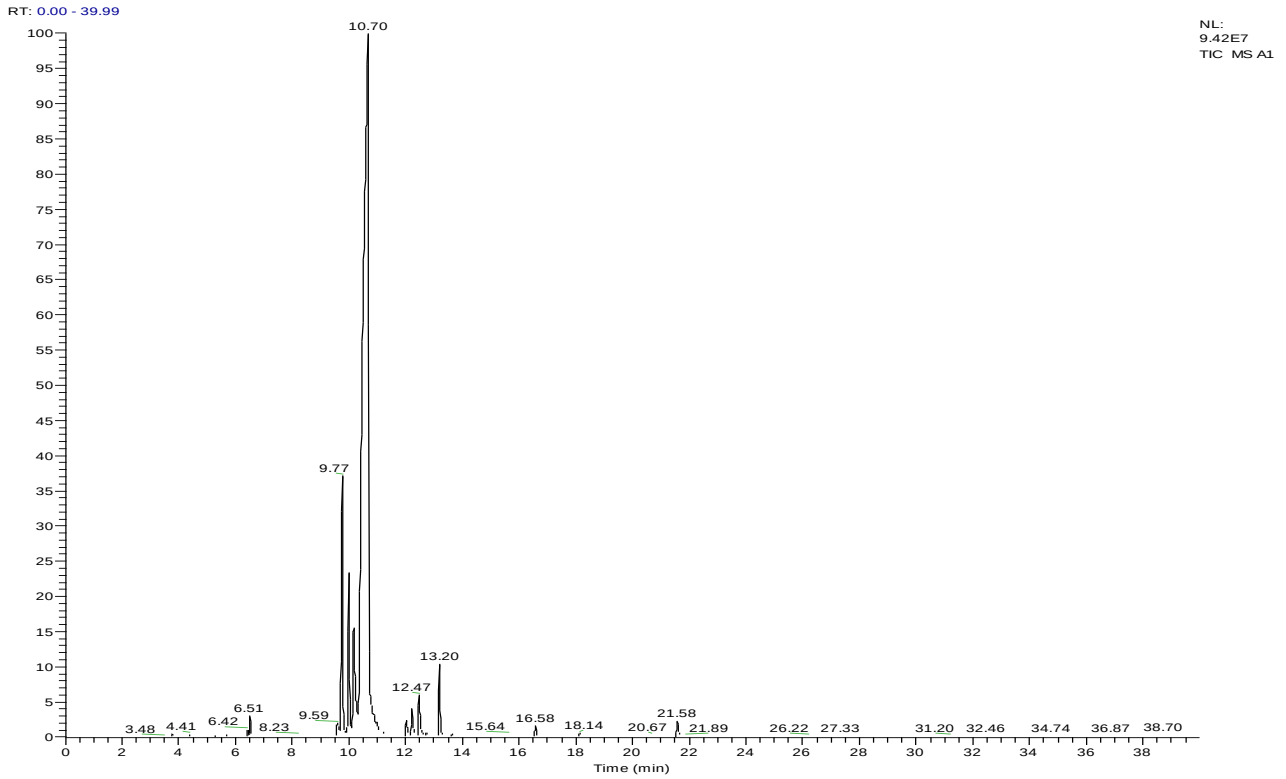


Fig. 3. GC/MS chromatogram of distillate sample A1

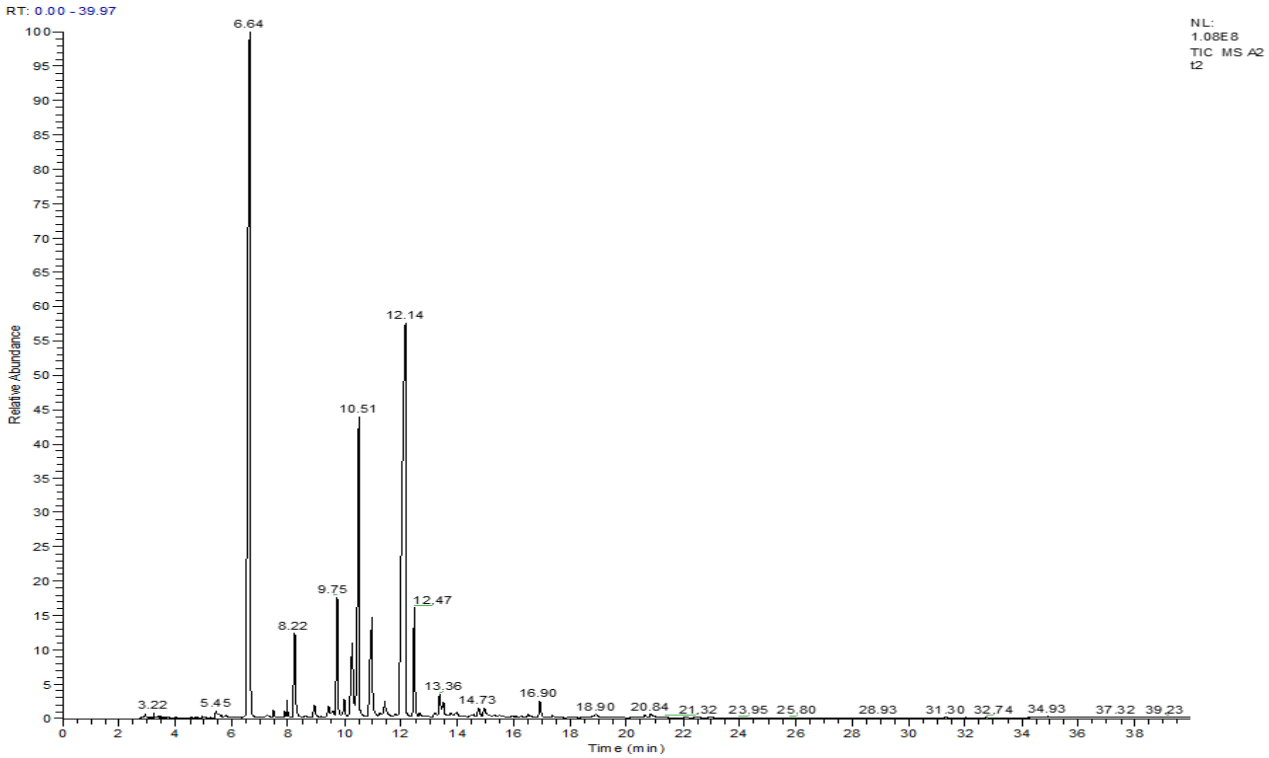


Fig. 4. GC/MS chromatogram of distillate sample A2

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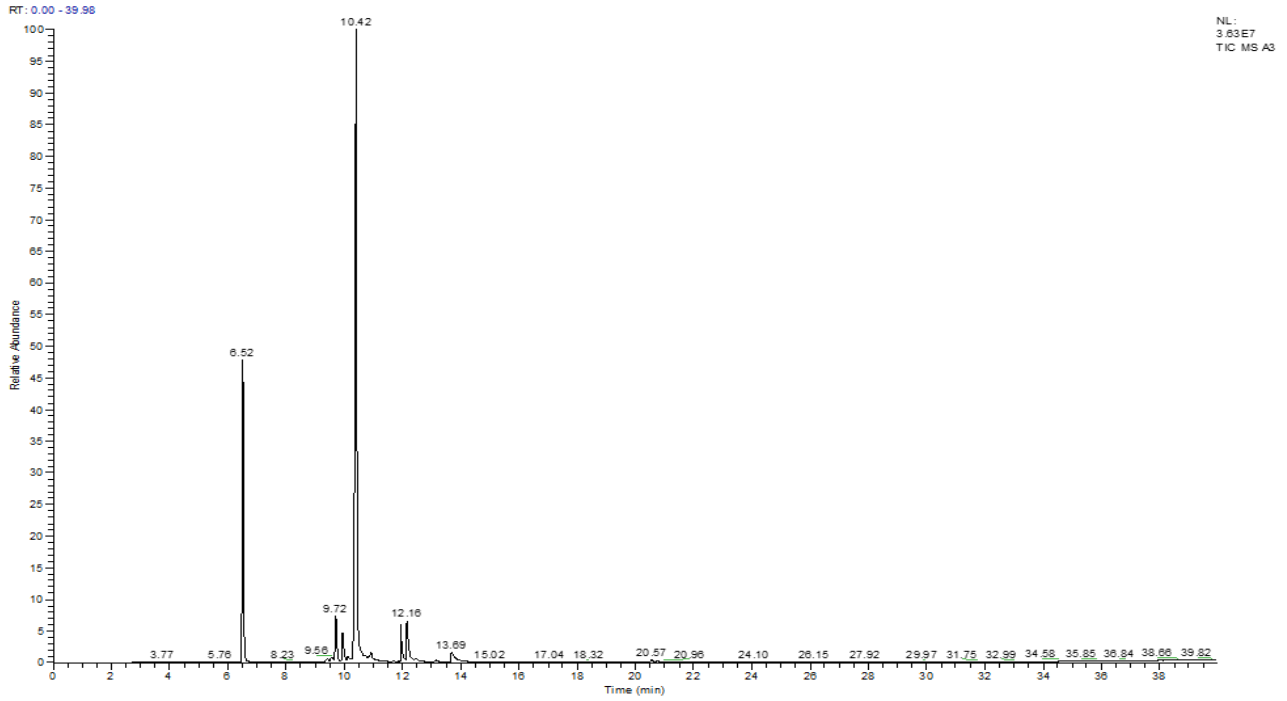


Fig. 5. GC/MS chromatogram of distillate sample A3

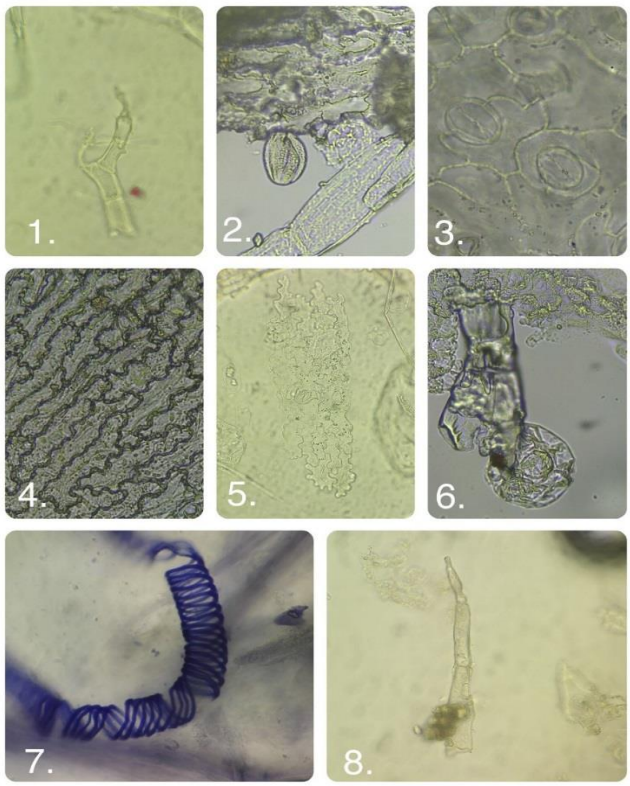


Fig. 6. Microscopical features of *N. crispa* (1. branched covering trichome 2. capitate glandular trichome 3. diacytic stomata 4. epidermis elongated cells 5. epidermis with cuticle 6. peltate glandular trichome 7. spiral vessel 8. unbranched covering trichome)

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3.3. Radical-scavenging activity in ABTS assay

The amount of antioxidant solution required to reduce the early ABTS concentration by 50 % (IC_{50}) is a commonly used criterion to assess antioxidant capacity [16]. A lower IC_{50} value corresponds to more antioxidant activity.

Table 3. shows the IC_{50} values for the extract and the essential oil of *N. crispa*. Our data demonstrated that a significant increase in extract concentrations will eventually result in the elevation of radical-scavenging activities. As can be seen from Table 3, the scavenging activity of the methanol extract was much more intense than the essential oil ($P < 0.05$) but compared to gallic acid (IC_{50} = 0.033 µg/ml) and BHT (IC_{50} = 0.12 µg/ml) as positive controls, was not significant. Surprisingly, ascorbic acid demonstrated very poor activity by the means of this assay, and the results were decided to be excluded from the conclusion.

Table 3. Scavenging effect (%) on $ABTS^{\bullet+}$ radicals

Sample	ABTS (IC_{50} mg/ml)
Methanol extract	2.79 ± 0.385
Essential oil	5.21 ± 0.861
Gallic acid	0.03 ± 0.002
BHT	0.12 ± 0.007

* Results are reported as Mean ± SD of three parallel measurements.

3.4. The total phenolic and flavonoid contents

Standard curves for gallic acid and quercetin are presented in Fig. 7 and Fig. 8 Using the equations derived from these standard graphs, the amounts of the total phenol and the flavonoid existent in the methanol extract from *N. crispa* were obtained. In this regard, *N. crispa* was found to have a phenolic content equal to 69.24 ± 3.34 µg GAEs/mg extract. In the case of the total flavonoid content, *N. crispa* possessed a capacity of 12.01 ± 0.26 µg QEs/mg extract.

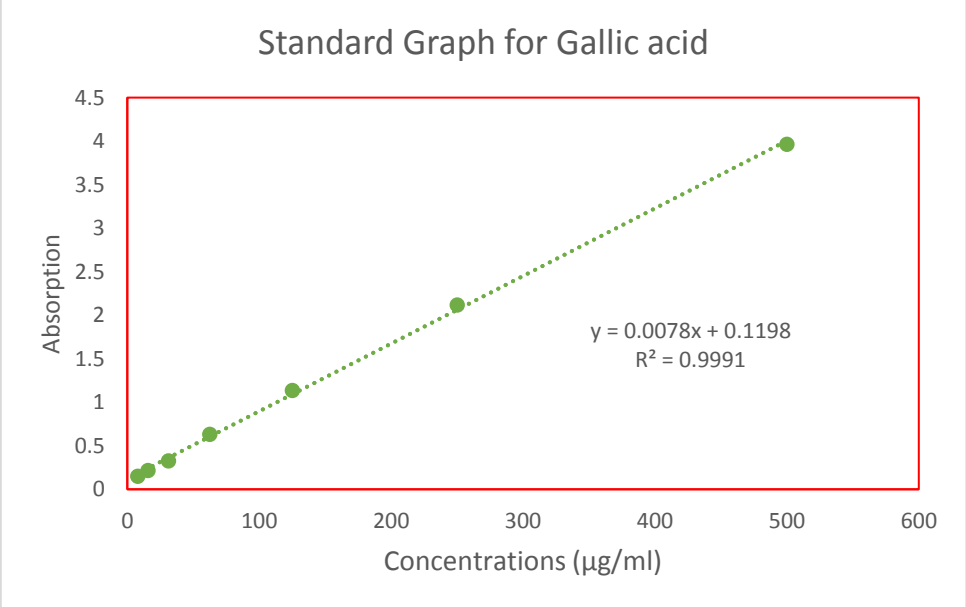


Fig. 7. Standard graph for gallic acid in assay for determination of the total phenolic content

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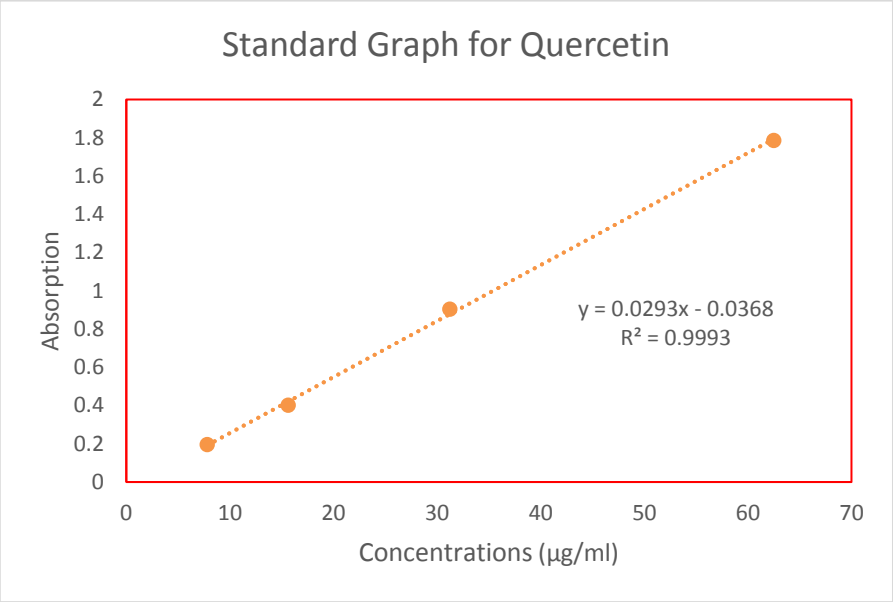


Fig. 8. Standard graph for quercetin in assay for determination of the total flavonoid content

4. Discussion

Our results from GC/MS analysis revealed that 1,8-cineole is the major component in the essential oil of *N. crispa* (70.93 %) and sample A4 (66.27 %). The contents of nepetalactones in volatile constituents of *N. crispa* and sample A4 were 5.29 % and 8.91 %, respectively. Linalool formate, *β*-pinene, *α*-pinene, *α*-terpineol, and 4-terpineol were other compounds with an amount of more than 2 % in both essential oils with different contents. The resting components were present below 2 %. Although two compounds (*α*-terpinene and limonene) were detected in the plant specimen, only trans-sabinene hydrate was present in sample A4.

According to the study conducted by Asgarpanah et al. two chemotypes of essential oils were obtained from the genus *Nepeta* in Iran. The first one was the nepetalactone chemotype and the second was 1,8-cineole or linalool chemotype [17]. Considering the results of the present study, the essential oils obtained from the plant specimen and sample A4 belong to the second chemotype as they contain about 70 % of 1,8-cineole.

The compositions of the essential oils will be the same if the plants grow in the same soil, the same climate, and are cultivated in the same season and time of the day, and the essential oil is isolated from the same plant part(s) with the same methods [17].

On the other hand, Jamzad et al. investigated the effect of altitude on the essential oil contents of *Nepeta binaludensis*. They revealed that 1,8-cineole and nepetalactones are positively and negatively correlated with altitude, respectively [18].

A study done by Sonboli et al. evaluated the chemical compositions of the essential oil from *N. crispa* that was collected from Alvand Mountain in July of 2003. These authors reported that the amounts of 1,8-cineole and nepetalactones were 47.9 % and 20.3 %, respectively [3].

A study by Abdoli et al. in 2016 focused on the analysis of chemical compositions of the essential oil from *N. crispa* in the flowering and vegetative stages [5]. The results demonstrated that the chemical components of the essential oil are different in contents and presence of certain

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constituents. At the flowering stage, a total of 31 constituents were detected as 1,8-cineole (44.25 %), 4a*α*,7*α*,7a*α*-nepetalactone (24.72 %), *α*-terpineol (6.3 %), and *δ*-terpineol (2.99 %) were the highest amounts. On the other hand, through the vegetative stage, 32 components were identified which 1,8-cineole (43.8 %), *α*-terpineol (11.03 %), 4aα,7α,7aα-nepetalactone (10.36 %), *δ*-terpineol (4.95 %), *α*-bisabolene (4.55 %), 4a*α*,7*β*,7a*α*-nepetalactone (3.67 %) and 4-terpineol (3.65 %) were the dominant components.

Mojab et al. (2009) obtained the essential oil from *N. crispa* contains 1,8-cineole (71 %), *β*-pinene (5 %), *α*-terpineol (4.1 %), *δ*-terpineol (2.8 %), and 4-terpineol (2.3 %). Other components are less than 2 % in their study. Interestingly, nepetalactones are not present even in trace amounts [4].

Daryasari et al. studied the effect of the isolation method (hydro-distillation and microwave-assisted hydro-distillation) on the essential oil of *N. crispa* surprisingly, the results are diverse [6].

Considering the results of these studies, it is possible to conclude that the composition of the compounds in wild herbs is subjected to climate conditions, altitude, annual rain volume, and other factors. Also, the domestication of some plants can have negative effects on them, both in terms of the amount of essential oil and the compounds in them.

N. crispa is a rare and endangered plant whose sole habitat are the hillsides of Alvand Mountain. It is sensible to witness this plant being sold at a high price for commercial use. Therefore, its distillates are expensive and prone to fraud and swindling. For instance, out of 4 specimens of *N. crispa* distillates that were purchased for the sake of this study, only one sample was from *N. crispa* essential oil. Two samples had menthol and another contained cineol and pulegone as the major components.

In Hamadan province, in the west of Iran, 10 species of the genus *Nepeta*, grow wild [19]. As a consequence, identification of this species is so critical.

It seems that since the plants belonging to the *Mentha* genus are more available and easy to be acquired than *N. crispa*, they are utilized in the production of commercial distillates. Thus, a surveillance program must be established to monitor and evaluate the authenticity of herbal commercial distillates.

Previously, antioxidant properties of *N. crispa* were investigated by DPPH, FRAP, *β*-carotene/linoleic acid, bleaching, TBARS, peroxide value, and iron chelating assays in studies conducted By Motaghed et al. [20] and Ranjbaran et al. [21].

According to the Scopus citation indexes, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$) radical cation-based assays along with 2,2-diphenyl-1-picrylhydrazyl (DPPH)-based and ferric-reducing antioxidant power (FRAP) are three popular accepted methods for measuring antioxidant effects throughout the world. ABTS/PP is the most popular method among different ABTS methods [22].

The results of the ABTS test from the current assay correlate with the ones from DPPH, done by Motaghed et al. In both tests, the essential oil of *N. crispa* was far less powerful than the extract. Moreover, none of them was comparable to the positive controls [20]. Further research into the similarities of the action mechanisms, which are involved in these tests, could be fruitful in explaining the cause of this matter.

The methanol extract and the essential oil of *N. crispa* had IC_{50} values of 93.2 ± 6.11 and 757 ± 29.48 µg/ml in the DDPH assay [20], and IC_{50} values of 2.79 ± 0.385 and 5.21 ± 0.861 µg/ml

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in the ABTS method, respectively. It seems that the ABTS method is superior to the DPPH assay. Following this, Floegel et al. investigated numerous edible materials and concluded that ABTS is the better method for measuring antioxidant properties in comparison to DPPH [23].

Due to the rarity of this particular species, the ABTS assay had never been performed on *N. crispa*. Tundis et al. studied methanol extracts of *N. crassifolia* and *N. binaludensis* by the ABTS method and revealed IC_{50} values of 13.4 ± 0.5 and 58.1 ± 0.9 µg/ml, respectively [24].

Flavonoids, phenolic acids, and tannins among the phenolic compounds are thought to contribute significantly to the antioxidant capacity of plants. Additionally, these antioxidants have a variety of biological properties, including anti-atherosclerotic and anti-inflammatory properties. Their antioxidant activity may be connected to these actions [25]. Consequently, this led to an evaluation of the overall phenolic and flavonoid content of *N. crispa* extract in this paper.

In this paper, the total phenolic content of the plant extract was measured using the Folin-Ciocalteu method. It was established that 69.23 ± 3.34 µg gallic acid is equivalent per milligram of extract. The total flavonoid content was determined by employing an aluminum chloride reagent and a standard graph for quercetin. The results showed that the extract contains 12.01 ± 0.26 µg of quercetin equivalent per milligram of extract.

In a study, Badrhadad et al. measured the antioxidant activity of methanol and hydroalcoholic extracts from cultivated *N. crispa* [8]. Their results revealed that the total phenolic content for methanol and hydroalcoholic extracts were 5.69 ± 0.19 and 4.85 ± 0.24 µg GAEs/mg extract, respectively. They also reported that the total flavonoid content for these extracts was 3.8 ± 0.07 and 3.77 ± 0.09 µg QEs/mg extract, respectively. Comparing these results with the data derived from this research, we realize that *N. crispa* collected from the mountains of Hamadan province possess far more phenolic and flavonoid content than the domestic species used by Badrhadad et al.

Another study by Tepe et al. focused on the determination of the total phenolic content of hexane, dichloromethane, and methanol fractions of extracts from *N. flavida* [26]. The highest phenolic content was remarked in polar fraction of methanol extract (200.16 ± 4.15 µg GAEs/mg extract) and non-polar fraction of methanol extract (188.61 ± 2.81 µg GAEs/mg extract). The lowest amounts of phenolic content were found in the hexane extract of the plant (11.9 ± 0.07 µg GAEs/mg extract). There was also an excellent positive correlation between the antioxidant activity and the total phenolic content, reported by the authors. Therefore, the high antioxidant capacity of polar fractions of the extract relates to their large amounts of phenolic compounds. Another research using a similar method showed that the total phenolic content for *N. italica*, *N. cicilia*, and *N. caesarea* is 21.4 ± 1.58, 24.8 ± 2.41, and 17.3 ± 0.97 µg GAEs/mg extract, respectively [27]. When compared to the results from *N. crispa*, it is deducted that all of these *Nepeta* species possess fewer phenolic compounds than *N. crispa*.

The total flavonoid content of *N. pogonosperma* was evaluated by Khalighi-Sigaroodi et al. These authors stated that 246.01 ± 9.25 µg of rutin are equivalents per milligram of the plant extract. Due to the different techniques used in this research, we could not perform an unmediated equivalence between their results and ours. Nevertheless, their work provides context for future studies [28].

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A similar study investigated the total flavonoid content of *N. binaludensis* and *N. crassifolia* [24]. They possessed 1.09 ± 0.07 and 0.38 ± 0.02 µg QEs/mg extract, respectively. Therefore, we observed that the flavonoid content of *N. crispa* is higher than both of these species.

Another study by Soleimani et al. evaluated the anti-oxidative potential of *Echinophora platyloba* and *Falcaria vulgaris* [18]. They utilized DPPH, FRAP, *β*-carotene/linoleic acid assay, and Iron-chelating test to determine the extent of their protective effects on oxidative stress. In this study, gallic acid and ascorbic acid demonstrated opposed anti-oxidative activities. Gallic acid was far more potent than ascorbic acid through the DPPH assay. Furthermore, the antioxidant activity of ascorbic acid was not good in the current research. As a result, it was excluded from the chart of reported results.

Due to the importance of studies on endemic plants and the lack of exclusive studies on them, the micromorphological properties of *N. crispa* leaf were investigated in this study. Microscopical studies revealed that the presence of glandular trichomes of peltate and capitate types, covering trichomes of unbranched and branched types, diacytic stomata in both epidermis and epidermis with polygonal axially elongated cells covered by waxy sinous cuticle walls.

A study conducted by Kahkeshani et al. on *N. menthoides* revealed capitate glandular trichomes and three types of covering trichomes (unicellular, multicellular branched, and unbranched), and paracytic stomata [29].

Amirmohammadian et al. (2021) detected only peltate glandular trichomes in micromorphological studies on *N.binaludensis* and *N. cataria* [30].

5. Conclusion

This study revealed that the extract from *Nepeta crispa* Willd. contains several components with high radical-scavenging profiles, as determined by the ABTS test and assay for total phenols and flavonoids. In this work, the methanol extract from aerial parts of *N. crispa* was relatively effective in confronting oxidative stress with impressive potency. On the other hand, the essential oil of *N. crispa* did not show significant antioxidant power regarding the ABTS assay. Our data justified as *N. crispa* constituents are not antioxidant agents but rather antimicrobial and antifungal.

Considering the chemical compositions of essential oil and distillates of this plant, we concluded that 1,8-cineole and nepetalactones are the principal compounds in the essential oil. Therefore, their footprints could be noticed in the distillates as well. Although three out of four distillates were not originated from *N. crispa*, the remaining sample exhibited a similar pattern in the chemical composition. It is suggested that a system must be in place to monitor the herbal distillates regularly to ensure the authenticity of plant distillates for commercial use.

Author Contribution

S.M. designed the experiments and supervised the work. S.M., M.B., and M.S. carried out the experimental bench work and analyzed and interpreted the data. All of the authors approved the final version of the manuscript and confirmed for submission.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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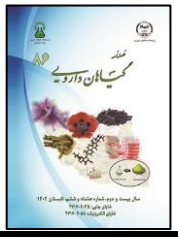
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مقاله تحقیقاتی

بررسی اجزای اسانس و اثرات آنتی‌اکسیدانی اسانس و عرق گیاه مفراح

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چکیده

مقدمه: گیاه مفراح یا پونه‌سای مواج عضو خانواده نعناعیان و گیاه دارویی بومی ایران همراه با خواص درمانی فراوان می‌باشد. **هدف:** این مطالعه به بررسی فارماکوگنوستیکی گیاه مفراح رویشی در همدان می‌پردازد. **روش بررسی:** اسانس گیاه از راه تقطیر آبی با استفاده از دستگاه کلونجر به دست آمد. چهار نمونه از عرق این گیاه از نقاط مختلف شهر همدان خریداری شد. عصاره‌ی متانولی اندام‌های هوایی نیز به طریق خیساندن استخراج شد. بررسی‌های خرده‌نگاری برگ، در کنار آنالیز ترکیبات اسانس و نمونه‌های عرق انجام شد. خواص آنتی‌اکسیدانی اسانس و عصاره نیز با تست ABTS سنجیده و محتوای فنلی و فلاونوئیدی عصاره نیز اندازه‌گیری شدند. **نتایج:** آنالیز کروماتوگرافی گازی متصل به طیف سنجی جرمی نشان داد، ۸،۱- سینئول، نپتالاکتون و بتا- پینن اجزا اصلی اسانس بودند. اجزای فرار یکی از نمونه‌های عرق به اسانس گیاه شباهت داشت. عصاره‌ی گیاه اثرات آنتی‌اکسیدانی قوی‌تری نسبت به اسانس آن نشان داد (IC_{50} برابر با ۲/۷۹ ± ۰/۳۸۵ میلی‌گرم در میلی‌لیتر). محتوای فنلی و فلاونوئیدی عصاره به ترتیب برابر با ۶۹/۲۴ ± ۳/۳۴ میکروگرم گالیک اسید در میلی‌گرم عصاره و ۱۲/۰۱ ± ۰/۲۶ میکروگرم کوئرستین در میلی‌گرم عصاره بودند. تریکوم‌های پوششی و ترشحی علاوه بر روزنه از نوع دیاسیتیک و آوند مارپیچی در مطالعات میکروسکوپی دیده شد. **نتیجه‌گیری:** نتایج این تحقیق نشان دادند که عصاره‌ی متانولی گیاه مفراح دارای پتانسیل بالای آنتی‌اکسیدانی و محتوای بالا فنلی و فلاونوئیدی می‌باشد. ۸،۱- سینئول جزء اصلی اسانس بوده و با توجه به کمیاب بودن نمونه گیاهی، در بین نمونه‌های عرق تقلباتی وجود دارد.

مخففها: QE، معادل کوئرستین؛ GAE، معادل گالیک اسید؛ ABTS، ۲،۲'-آزینو بیس (۳- اتیل‌بنزوتیازولین-۶- سولفونیک اسید)؛ ABTS/PP، ۲،۲'-آزینو بیس (۳- اتیل‌بنزوتیازولین-۶- سولفونیک اسید)/ پتاسیم پرسولفات؛ GC-MS، کروماتوگرافی گازی متصل به طیف‌سنج جرمی

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