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Extraction and Determination of Cumin Essential Oil Compounds by GC-MS and Spectrophotometry and Evaluation of Antioxidant Potential under Different Growth Conditions

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Abstract
Evaluation of plant responses under different environmental conditions is a principal step towards a better understanding of their function and performance. In this investigation, cumin (*Cuminum cyminum*), which is known as an important medicinal plant, was examined under three different growing conditions including natural habitat, field and greenhouse conditions in order to clarify the effects of growing conditions on essential oil properties. Essential oil (EO) content was higher in natural habitat samples, but the composition of EO was varied along the three samples extracted from the above three conditions. Overall, 17 compounds were detected and the key component in all samples was thymol, with the highest amount of 18.03% in natural habitat samples. Cuminaldehyde, γ-terpinene, α-thujene and limonene were other substantial compounds of the EO. Some elements were not detected in all samples such as p-cymene which was not extracted from the EO of natural habitat sample and acetoxylinalool which was not observed in greenhouse sample analysis. Regards to phenol content, natural habitat samples showed the highest amount and the lowest value was obtained on field sample. Radical scavenging activity of EO was also higher in natural habitat samples and with respect to phenolic content analysis, it could be considered as a substantial advantage rather than the others. To sum up, results indicated some advantages of natural habitat samples, although field samples also showed superiority in some parameters.

Keywords:
Cuminum cyminum, GC-MS, Phenol, Spectrophotometry, Thymol

1.INTRODUCTION

Species with secondary metabolites that have desired aromatic qualities, therapeutic specificities, or provide a generous source material for the perfume and chemical industries have gained a great attention in recent years [1]. Due to their safety, usefulness, and accessibility, natural products are getting very popular and a wide range of studies are conducting related to their quantity and quality properties. Cumin (*Cuminum cyminum* L.), an important member of the Apiaceae family, used mainly in traditional and veterinary medicine and also as protective agent in food packaging, mainly to protect those foodstuffs that cannot be spiked or produced with additives, such as fresh products [1,2]. Cumin is generally adapted to dry climates and the main parts of growth period coincide with winter and spring rainfalls. Hence, the peak of water requirements of plant is provided and no further irrigation is usually needed. However, under severe dry periods supplementary irrigation is efficient. It is necessary to emphasis that due to the high probability of fungal diseases spreading, any extra water could reduce the yield in different grades.

The cultivation of medicinal species requires a good understanding of the effect of domestication on their biological activities associated with their chemical composition. In fact, it was reported that the lack of reproducibility of their bioactivity represented a major problem facing cultivation of medicinal and aromatic plants. Previous studies have demonstrated that domestication of some medicinal and aromatic species collected from the wild, can affect negatively or positively their chemical composition and consequently their biological properties [3-5]. This phenomenon has been attributed to the changes in the growth environment of the plants associated with their transplanting from a wild ecosystem to a cultivated

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field [6].

Domestication of medicinal plants is a multidimensional and complicated approach. One aspect which was evaluated is comparing conventional and organic cultivation systems. In peppermint, yield and essential oil production was affected by both the cultural system and the environmental conditions. The highest values of aboveground dry weight were recorded in the conventional system and the lowest in organic cultural system. In contrast, the highest oil content was found in organic system and the lowest in conventional system [7].

There are several researches reporting the essential oil chemical composition of cumin. The analysis of essential oil of *C. cyminum* revealed thymol as a major component, with its contribution to the oil 40.05 % [8]. In another research, Tunisian *C. cyminum* variety was investigated and twenty-one components were identified and *C. cyminum* contained cuminlaldehyde (39.48%), gamma-terpinene (15.21%), O-cymene (11.82%), beta-pinene (11.13%), 2-caren-10-al (7.93%), trans-carveol (4.49%) and myrtenal (3.5%) as a major component [9]. Results of a study on *Ocimum basilicum* indicated that different cultivation conditions affect essential oil content, even of the same variety. As such controlled greenhouse conditions favor or have no impact to the total production of essential oil in some of the varieties when comparing to the field conditions [10]. It is necessary to take into account that geographical location and the environmental characteristics of the habitat are affecting the chemical composition of the essential oil.

Considering the importance of cumin, this experiment is conducted in order to evaluate and compare plant quality under natural habitat conditions and also field and greenhouse cultivation.

2.EXPERIMENTAL

2.1.Natural habitat monitoring and sample collection

The experiment was conducted in three sections during 2018 to 2020. The first section was based on collecting of seeds from a well-known habitat in Joopar mountain, Kerman province, Iran with the height above mean sea level of 2950 m and 155 mm/year of mean rainfall. This region is traditionally considered as a natural habitat of cumin (*Cuminum cyminum*) and also *Bunium persicum* which also belongs to Appiaceae family and known as the most important medicinal plant of kerman province. The habitat was monitored three times during the growth season, began in November of 2018 and samples were collected in 12th of June, 2019. Seeds were transferred and stored under controlled conditions until the essential oil extraction and analysis.

2.2. Field experiment

The area of experimental field is located in Kerman province in South Eastern of Iran (29°30N 06°57E). The height above mean sea level is 1760 m and a mean rainfall of 144 mm/year. In late November of 2019, the same sampled seeds were planted in the experimental field. The distance of 10 cm in the rows and 50 cm between the rows was performed. The first irrigation was performed the day after the planting and repeated in 4 days to achieve the acceptable emergence. Next irrigations were applied in 12 days' intervals during the first 40 days (equivalent to 70% of soil field capacity) and 16 days' intervals until the harvest stage (equivalent to 60% of soil field capacity). Given the fact that cumin is a sensible plant to *Fusarium* wilt (*Fusarium oxysporum* f. sp. cumini) and it is known as a major constraint to production of cumin [11], irrigation level was reduced gradually in order to control the amount of water around plant root and stem. Based on the previous research of the authors and in order to provide the nutrient requirements of plant, vermicompost was incorporated with the soil (10 t/ha) prior to sowing. No chemical fertilizer, pesticide or herbicide was applied during the cultivation processes. In flowering stage, the field was exposed to the attack of *Fusarium oxysporum*, but it was not considerable and the damage estimation was about %10 to %15. Plants were harvested at 28th of May, 2020 and samples were randomly collected for further analysis.

2.3. Greenhouse experiment

The third section was a greenhouse experiment conducted in Kerman during 2019. The experiment designed under greenhouse controlled condition with 60-70% relative humidity and 25/15°C day/night temperatures. Plastic pots with 5 kg of soil capacity were used. In early December of 2019, randomly sampled seeds collected from the natural habitat (Joopar mountain) were sowed in the pots and irrigated. Further irrigations were applied in 8 days' intervals (equivalent to 60% of soil field capacity). Other cultivation operations were similar to the field experiment. Despite the sensitivity of plant and higher humidity and temperature, it was not attacked by *Fusarium* wilt in the greenhouse experiment. Plants were harvested at 4th of May, 2020 and samples were randomly collected for chemical analysis.

2.4. Plant material and extraction of EO

Collected samples from each section of experiment were air-dried at 25 °C and two samples (100 gr) from each experiment was hydrodistilled, using a Clevenger-type apparatus for 3 h. Extracted essential oils were dried with anhydrous sodium sulphate, weighed and stored at 4 °C until use.

2.5. Chemical analysis

2.5.1. Chemical composition of EOs analysis

Chemical constituents of EO samples were determined using GC-MS analyses. The GC-MS apparatus was a Varian GC-MS spectrometer consisting of a Varian Star 3400 GC equipped with a fused-silica column (DB-5, 30 m x 0.25 mm i.d., film thickness 0.25 µm; J and W Scientific Inc.), interfaced with a mass spectrometric detector (Varian Saturn 3). The components of essential oil were identified by using their retention indices (RI) obtained with reference to the n- alkane series (Sigma, UK) on the DB-5 column, mass spectra with those of authentic samples, composition of their mass spectra and fragmentation patterns reported in the literature, and computer matching with MS-data bank (Saturn version 4). Quantification of the relative amount of the individual components was performed according to the area percentage method [12].

2.5.2.Phenolic compounds

Total phenolic compound contents were determined using the Folin-Ciocalteau method [13]. The extract samples (0.5 ml) were mixed with Folin Ciocalteu reagent (5 ml, 1:10 diluted with distilled water) for 5 min and aqueous Na_2CO_3 (4 ml, 1 M) were then added. The mixture was stored for 15 min and phenols were determined according to the absorption at 765 nm. The standard curve was prepared by 0, 50, 100, 150, 200, and 250 mg.ml solutions of gallic acid in ethanol: water (50:50, v.v). Values are expressed in terms of gallic acid equivalent (mg.100g dry mass).

2.5.3.Radical scavenging activity assay

Bleaching of the purple colored ethanol solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH) was used to measure electron donation ability of *C. cyminum* essential oil. DPPH was employed as a reagent. Two ml of various concentrations of the samples (0.045-0.45 % w.v) in ethanol were added to 1 ml of a 2×10"4 M solution of DPPH. The decrease in absorbance at 517nm was determined by spectrophotometer after30 min for all samples. The absorbance of the ethanol solution DPPH radical without essential oil was measured as control [14].

3.RESULTS AND DISCUSSION

3.1.Essential oil content

It was indicated that the samples were varied in content of essential oil (EO) and the natural habitat collected samples had the highest percentage of EO (3.06%). Although the difference between field and greenhouse samples was not considerable, the field samples had the higher content of EO (2.26%) than the greenhouse samples (2.12%). A superior amount of EO in natural habitat collected samples is common in medicinal plants and it is generally accepted that grown seeds under wild and natural conditions contains more EO compared with the field and greenhouse conditions. As a matter of fact, it is an important setback in the domestication process of medicinal plants. In medicinal plants, EO percentage and composition is very different across the regions and habitats, but generally, the EO content of natural habitat collected samples was higher than the average percentage in other studies. The values for the EO content and its chemical constituents vary in samples from various locations. It was reported as 2.5% for Egyptian variety [15] and 1.45% for Iranian variety [16] and also 2.33% for Indian variety [17].

It has been shown that geographical and meteorological factors affect the content and composition of essential oil [18]. In this study, the harvest times were similar for all three experiments, so that the effect of the phonological cycle was eliminated. Moreover, the extraction and analytical methods were conducted in the same way. So the differences were mostly arising from environmental and geographical parameters. The effect of altitude on essential oil content of medicinal plants is controversial. In some reports, the lowest yield of essential oil was obtained at highest altitude, in *Artemisia roxburghiana* [19] and *Thymus praecox* [20] for instance. In contrary, investigation of wild populations of *Artemisia saharae* revealed that yields of essential oil were higher in populations of high altitudes [21].

3.2.Essential oil chemical composition

Based on the obtained data from GC MS analysis, 17 compounds were detected in the EO of three samples, with percentage variation. The key constituent in all samples was thymol, with the range of 15.25% in field samples and 18.03% in natural habitat samples. Cuminaldehyde was another substantial compound of the EO, but the variation of values was more extensive. It had the least amount in greenhouse sample (6.84%), while natural habitat samples percentage was 16.35%. Results also indicated γ-Terpinene as a main element of the EO. The important point was that the amount of γ-Terpinene in natural habitat collected sample was almost 35% less than the field sample. This difference was 20% compared with greenhouse sample. 1,8-Cineole was also a high value constituent with a similar trend. In fact, the concentration of the element in field sample was about four times more than the natural habitat collected sample. For α-Thujene, the percentage of field and greenhouse samples was resemble (5.12% and 5.04%), but it was substantially less in natural habitat collected samples (0.18%). There were also compounds that the concentration in natural habitat collected samples was noticeably higher than the other samples. For instance, limonene had 9.69% of this sample, while this value was 2.46% in greenhouse sample. The trend of variation of Linalool percentage was also considerable and all of the three samples showed a similar amount, to some extent. Some elements were not detected in all samples such as p-Cymene which was not extracted from the EO of natural habitat collected sample and Acetoxylinalool which was not observed in greenhouse sample analysis.

Thymol is known as one of the main constituents of cumin's essential oil and there are some reports which demonstrated thymol as the most important compound of cumin's EO [8]. It was found that thymol exert its biological functions by modulating the physiological and biochemical processes involved in ROS generation, with respect to its antioxidative properties [22]. Moreover, thymol facilitates the secretion of milk in lactating women [23].It necessary to consider that the difference between the concentrations of thymol in this experiment and the mentioned research is significant. Great diversity in the content of thymol in phenol-rich species associated with climatic factors is already shown [24].

Table 1. Essential oil chemical composition of cumin grown under natural habitat, greenhouse and field conditions

No.	Identified componed	RI	Field condition* (%)	Greenhouse condition* (%)	Wild samples* (%)
1	α-Pinene	931	9.24±0.17	8.49±0.53	10.79±0.09
2	a-Thujene	935	5.12±0.07	5.04±0.49	0.18±0.01
3	Sabinene	964	3.12±0.12	4.47±0.04	3.89±0.43
4	Camphene	973	2.25±0.38	2.78±0.08	3.09±0.33
5	β-Pinene	991	2.69±0.19	2.17±0.09	1.89±0.02
6	Myrcene	999	4.88±0.03	2.05±0.01	3.80±0.09
7	p-Cymene	1025	0.69±0.03	1.12±0.02	-
8	Limonene	1027	6.47±0.67	2.46±0.12	9.69±0.16
9	1,8-Cineole	1034	7.72±0.36	8.74±0.07	1.94±0.31
10	γ-Terpinene	1063	11.68±0.01	9.44±0.01	7.63±0.02
11	Terpinene- 4 - ol	1169	2.12±0.16	2.96±0.12	1.40±0.02
12	Acetoxylinalool	1191	0.93±0.08	-	2.09±0.11
13	Linalool	1195	7.83±0.09	7.49±0.63	7.11±0.05
14	a-Terpinolene	1199	0.29±0.03	1.58±0.17	0.96±0.06
15	Cuminaldehyde	1257	10.67±0.29	6.84±0.07	16.35±0.69
16	α-Terpinene-7-al	1296	1.34±0.11	4.44±0.11	1.70±0.02
17	Thymol	1299	16.01±0.53	15.25±0.13	18.03±0.43

*(Data are presented as mean ± standard deviation, n=3).

Cuminaldehyde (4 - isopropyl benzaldehyde) is known as the main constituent of the essential oil of *Cuminum cyminum* which has been used commercially in cosmetic industry. It has shown different roles including anti-platelet [25], antibacterial [26], antifungal [27] and anti-diabetic [28].

The literature review showed different portions of cuminaldehyde and in some of them it was almost one third of the essential oil (30.2%) [29]. Although the highest amount of the cuminaldehyde in this experiment was significantly lower than that, but the interesting point is the concentration of natural habitat collected sample was twice the greenhouse sample. This may be due to the environmental condition and especially irrigation and water availability. Comparing with the result of another study [30], cuminaldehyde percentage was significantly enhanced from 15.31% under control condition to 23.53% under moderate water deficit condition. Considering that the plants under natural habitat condition is mostly receive less moisture than the plants grow under greenhouse condition, so the results of these two experiments are comparable. It is also necessary to note that the concentration of some important constituents such as γ-Terpinene were lower in natural habitat collected sample. Thus, it cannot be concluded as a general principle that plants grow under natural conditions have higher concentrations of main essential oil constituents .In contrast of this experiment, when different populations of *Thymus migricus* were monitored in their natural habitats, the content of γ-terpinene correlated positively with the altitude [31].

Results of the comparison of wild and cultivated plants are not very consistent. For instance, the comparison of cultivated and wild Moroccan sage EOs showed a significant quantitative variation of some compounds. The oil obtained from wild plants showed a higher content of camphor (57.3%), while oil from cultivated plants was characterized by camphor (26.6%), camphene (22.0%) and α-pinene (20.6%) [4]. In contrary, when wild and cultivated *Lavandula mairei* were compared regards to the EOs chemical composition, it reveals very similar profiles for wild and cultivated plants, and the percentage of various constituents showed very low variations and carvacrol remained the main constituent with more than 76% in both cases [5]. The differences of major constituents of EO of cultivated and wild samples may be attributed to several factors including fertilization, since the wild populations did not receive any fertilization or irrigation. It was also implied by some researches that change of chemical composition in different regions is mostly due to the environmental factors that affect the biosynthesis pathway, in which this pathway begin by autooxidative conversion of γ-terpinene to p-cymene and then hydroxylation of p-cymene to thymol or carvacrol and this pathway over activated during flowering stage [32]. It was also proposed that a sudden change of environmental conditions can affect the essential oil compositions [33]. It is a very interesting point since the variation of climatic parameters in natural habitats is noticeable. The condition of cultivation is also frequently reported to influence the essential oil content and composition [34].

3.3.Phenol content

It was observed that phenolic content of the samples was affected by the growth conditions. The natural habitat collected samples showed the highest content of phenolic compounds and the lowest value was belonged to the field sample. Based on the achieved data, greenhouse sample had a higher phenolic content in comparison with the filed sample (Fig. 1). Although the condition in the greenhouse is much suitable for plant growth in general, it seems that in cumin it is dependent on a large group of parameters and greenhouse condition may not be necessarily more appropriate for *C. cyminum*. The values of total phenolic content are not genuine amounts of phenolic compounds and they derived from their chemical reducing capacity relative to gallic acid, but this measurement is generally able to introduce a valuable parameter in qualitative properties of the essential oil.

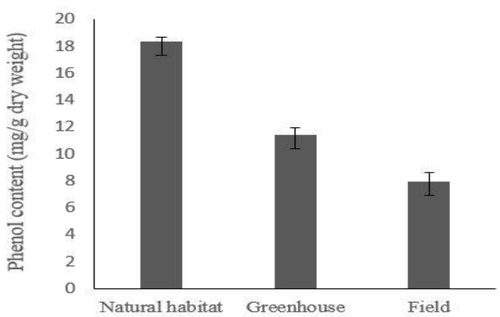

Fig 1. Phenol content of essential oil of samples grown under different conditions. (Data are presented as mean ± standard deviation, n=3).

Phenol compounds have gained a great attention as potential therapeutic agents against a wide range of ailments. The medicinal actions of phenolics is mostly ascribed to their antioxidant capacity, free radical scavenging, chelation of redox active metal ions, modulation of gene expression and interaction with the cell signaling pathways [35].

Phenolics conferring oxidative stress tole rance on plants. Use of plant materials rich in phenolic content in the food industry for their antioxidative advantages and health benefits is substantially elevating [36]. Although the values of total phenolic content are not genuine amounts of phenolic compounds and they derived from their chemical reducing capacity relative to gallic acid, but this measurement is generally able to introduce a valuable parameter in qualitative properties of the essential oil. It is also necessary to consider that the correlation between phenolic content and antioxidant capacity of the essential oil is very strong.

It is well established that phenolic compound biosynthesis is mostly enhanced under stress restrictions. It is known as a response to the oxidative stress generated by the formation of reactive oxygen species [37]. Elevation of phenolic content is suggested in a wide range of environmental stress, such as cumin when exposed to drought stress [38]. This enhancement was also reported under increased salinity level in mulberry [39]. Considering the harsh condition which plant should cope in natural habitats, the enhancement of phenolic compounds in these samples is seems to be reasonable.

3.4. Antioxidant activity

Results of the DPPH radical scavenging activity analysis demonstrated that natural habitat collected sample had a higher scavenging potential compared to the other two samples. Field sample also had the lowest scavenging potential (Fig. 2).This data also shows a strong correlation between the phenol content and antioxidant activity of the samples.

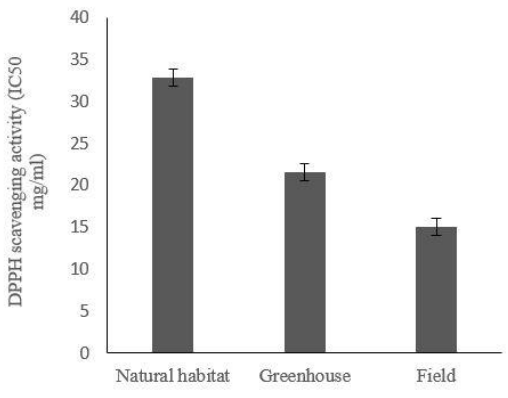

Fig 2. DPPH scavenging activity of essential oil of samples grown under different conditions. (Data are presented as mean ± standard deviation, n=3).

The DPPH oxidative assay is used worldwide in the quantification of radical-scavenging capacity. The capacity of biological reagents to scavenge the DPPH radical, can be ex pressed as its magnitude of antioxidation ability [40]. In *Nicotiana tabacum* L. leaf, highly significant positive correlation with altitude was observed and plants grown in higher altitude showed elevated DPPH radical scavenging activity. Other antioxidant activity indexes such as reducing power ability (RP) and ferric reducing antioxidant power (FRAP) showed entirely similar trends [41].

4. CONCLUSION

In this research, the differences between the cumin samples of natural habitat, greenhouse and field conditions were highlighted. Gathered data indicated that cultivation of plant under greenhouse and field conditions affects plant's quality in several aspects, although some of them were not significant. Generally, natural habitat samples showed some privileges regards to some components of EO and phenolic content and DPPH scavenging activity. Given the fact that the advantages of cultivation were also noteworthy, the interpretation of plant's preferred growing conditions should be conservative. Further long term and multidimensional research in this area is recommended.

CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest and they are responsible for the accuracy and integrity of the paper content.

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جداسازی و اندازه‌گیری ترکیبات اسانس زیره سبز به روش GC-MS و اسپکتروفتومتری و بررسی پتانسیل آنتی اکسیدانی آن تحت شرایط مختلف رشدی

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چکیده:

ارزیابی پاسخ های گیاه در شرایط رشدی متفاوت یکی از گام های اصلی به سمت درک بهتر کارکرد و عملکرد آنها محسوب می شود. با هدف روشن کردن اثر این شرایط متفاوت بر ویژگی های اسانس زیره سبز، این گیاه به عنوان یکی از مهمترین گیاهان دارویی ایران در این آزمایش در شرایط رشدی رویشگاه طبیعی، مزرعه و گلخانه مورد ارزیابی قرار گرفت. نتایج نشان دهنده درصد بالاتر اسانس در نمونه رویشگاه طبیعی بود و ترکیب شیمیایی اسانس در بین سه نمونه مورد بررسی متفاوت بود. در مجموع ۱۷ ترکیب مورد شناسایی قرار گرفت و تیمول به عنوان ترکیب اصلی در تمام نمونه های مورد بررسی جداسازی گردید و بیشترین مقدار آن (۱۸/۰۳ درصد) به نمونه رویشگاه طبیعی تعلق داشت. کومین آلدئید، گاما ترپنین، آلفا-توجن و لیمونن از دیگر اجزای کلیدی تشکیل دهنده اسانس بودند. برخی از ترکیبات در هر سه نمونه مورد بررسی شناسایی نشد. در خصوص ترکیبات فنولی، نمونه رویشگاه طبیعی بیشترین مقدار را به خود اختصاص داد و کمترین مقدار در نمونه مزرعه به دست آمد. بیشترین فعالیت مهارکنندگی رادیکال آزاد اسانس در نمونه رویشگاه طبیعی مشاهده شد و با توجه به بالاتر بودن محتوای ترکیبات فنولی در این نمونه، می‌تواند به عنوان یک مزیت بسیار مهم در مقایسه با دو نمونه دیگر محسوب گردد. در مجموع، نتایج نشان دهنده برتری نمونه رویشگاه طبیعی در مقایسه با سایر نمونه ها بود، هرچند که نمونه استخراجی از مزرعه نیز در خصوص برخی اجزاء، مزیت هایی را نشان داد.

کلمات کلیدی:

اسپکتروسکوپی جرمی، اسپکتروفتومتری، تیمول، زیره سبز، فنول، کروماتوگرافی گازی