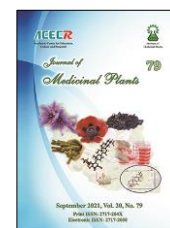




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Research Article

The differences between *Froriepia subpinnata* (Ledeb.) Baill. and *Pimpinella anisum* L. commonly named as anarijeh based on major components of the essential oil; a marker for resolve ambiguities

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ABSTRACT

Background: *Froriepia subpinnata* (Ledeb.) Baill. and *Pimpinella anisum* L. are two important medicinal plants belong to the Apiaceae family. Due to the similar Persian name in ethnobotanical studies, namely “anarijeh”, these two medicinal plants are mistakenly used instead of each other in the Iranian medicinal plants market and even in scientific reports. **Objective:** In this study, the correct morphological description of studied species were introduced and the chemical composition of their essential oils and their antioxidant activities were determined. **Methods:** The aerial parts of *F. subpinnata* and the seeds of *P. anisum* were crushed separately followed by hydro-distillation method for 3 h using a Clevenger apparatus to obtain essential oils (EOs) and their constituents were analyzed by GC/MS. Also, the potential of antioxidant inhibitory of essential oils were determined using DPPH and FRAP methods. **Results:** *p*-Cymen-8-ol (51.13 %), α -terpinolene (7.69 %) and limonene (6.83 %) were the major components of *F. subpinnata* EO while *trans*-anethole (85.65 %) and carvone (5.31 %) were the major components in *P. anisum* EO. The results of antioxidant activities in DPPH and FRAP assays at the concentration of 250 μ g/ml were 53.03 and 62.72 % for *F. subpinnata* and 50.27 and 59.91 % for *P. anisum*, respectively. The results of antioxidant activity by DPPH and FRAP methods indicated both essential oils had almost similar potential. **Conclusion:** Type and the amounts of the major components of the essential oils of *F. subpinnata* and *P. anisum* can be regarded as an accurate basis for differential diagnosing the plants. These differences can be used as a good phytochemical marker in correct identification and prevention of mistakes and deceptions in herbal products.

Abbreviations: GC/MS, Gas Chromatography/Mass Spectroscopy; EOs, Essential Oils

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1. Introduction

Nowadays, medicinal plants have a vital role in health and economy. Millions of people around the world are working on different aspects of medicinal plants such as planting, harvesting and processing [1]. Many problems due to misuse of medicinal plants like lacking of proper evaluation and monitoring system for activists in this field have been generated, as a result, we see many mistakes or deceptions in preparation of medicinal plants for public use [2]. Use of unknown or doubtful plant species in the market of dried herbs and production of herbal drugs is the most important challenges in this issue. Hence, shortage of essential knowledge has caused many mistakes and deceptions in pharmaceutical research and their products [3, 4].

Exact identification of plants begins with direct observation of the morphological characteristics of plants body or with microscopic descriptions of powder of plant tissues and organs. After taxonomic verification, the type and content of specific phytochemical compounds of the plant are evaluated and approved with comparison by the plant's literature review. Essential oil compositions and antioxidant properties of the plant as a suitable marker can be used for phytochemical verification of plants [5-7].

The widespread presence of plants in different societies has led to the formation of different local names for plants. This significantly leads to similarities and differences in the naming of plants. So that there may be different names for one species in different cultures and languages, or the same names may be used for different plant species in different languages [8, 9]. For this reason, the scientific explanation of medicinal plants samples has a very crucial and

fundamental role and also it is considered as the first step for use of traditional medicinal plants [2].

One of the mistakes in naming plants, which is unfortunately common in Iran herbal stores and scientific societies, is related to *Froriepia subpinnata* (Ledeb.) Baill. and *Pimpinella anisum* L. plant species which have been introduced in various sources with the same local name of anarijeh. While this common Persian name is used for both plants that different parts of them are used in medicinal plants market. The aerial part of *F. subpinnata* has been used as a carminative, appetizer, antispasmodic, diuretic, antiseptic and sedative. This species contains antioxidant and antibacterial properties [10]. *P. anisum* seeds are the used part of plant which heals, stimulates and improves the function of the digestive system and is also beneficial for liver and circulatory system. Essential oil of *P. anisum* seeds has antimicrobial, antifungal, antiviral, antioxidant, analgesic and antispasmodic, anti-epileptic effects and is effective in healing stomach ulcers. Also, in traditional medicine has been used to treat some diseases such as epilepsy [11, 12].

Based on our field and herbarium observations and reports of other researchers, it certainly seems that the vernacular name of anarijeh is related to *F. subpinnata* [9, 10, 13-16]. However, there are several researches in the scientific sources that shows the name of anarijeh has been used for the *Pimpinella* genus [17-23].

Due to the mistakes in identification and thus the use of *Froriepia subpinnata* and *Pimpinella anisum* and also the lack of accurate illustrative studies, the present research specifically aimed to correct introduction and identification of these plants and also provide their essential oil composition and antioxidant capacity.

2. Materials and Methods

2.1. Plant material, extraction of essential oils and analysis by GC/MS

The samples of *F. subpinnata* and *P. anisum* were collected in 2018 from Behshahr and Isfahan cities (Mazandaran and Isfahan provinces respectively) in Iran. Voucher specimens (No. 580-SANRU for *F. subpinnata* and No. 601-SANRU for *P. anisum*) were determined by expert authors and deposited in herbarium of the Sari Agricultural Sciences and Natural Resources University, Sari, Iran.

The aerial parts of *F. subpinnata* and seeds of *P. anisum* were air-dried at ambient temperature (25 °C) during a week and then they were powdered separately and essential oils were isolated using Hydrodistillation method for 3 h [24]. 300 g of powdered samples were heated with one liter of distilled water in Clevenger apparatus and finally the obtained essential oils were collected. After dehumidification with anhydrous sodium, the essential oils were stored in glass containers at 4 °C in the refrigerator and away from light.

The essential oils were analysed by gas chromatography/ mass spectroscopy. 1 µl of the oil sample was injected into the GC/MS apparatus. The essential oils were also analyzed by an Agilent 6890 apparatus on capillary column. Mass spectrometry (Agilent 5973N, USA) equipped with a BPX5 fused silica column of (30 m × 0.25 mm i.d. × 0.25 µm film thickness) were done in electronic impact mode (70 eV), split injection ratio (1: 35), Carrier gas helium with 0.5 ml/min flow rate and mass range of 40 to 500 amu. The temperature program of the column was adjusted as follows: The initial temperature of the oven was 50 °C for 5 min, the temperature gradient of 3 °C per min and rises to 240 °C and then at a speed of 15 °C per min, the temperature rises to 300 °C for 3 min. Stopping

at this temperature and response time was 75 min. Compounds were identified by comparing retention time (RT) with those reported in the literature and their mass spectrum with Wiley library [25, 26].

2.2. Antioxidant capacity of essential oils

Two common methods of DPPH and FRAP were used to measure the antioxidant activity of essential oils of *F. subpinnata* and *P. anisum* plants at the concentration of 250 µg/mL. In the DPPH method, the potential of the essential oils for free radical scavenging activities were evaluated based on percentage inhibition of 2,2-diphenyl-1-picrylhydrazyl (DPPH) activity as calculated using previously introduced methods [27, 28]. Then it was expressed as a percentage of inhibition of DPPH which was calculated according to the following Equation:

$$\text{Percent (\%) inhibition of DPPH} = [(A_0 - A_1) / A_0] \times 100$$

The A_0 was the absorbance value of the blank sample or control reaction and A_1 was the absorbance value of the test sample. All measurements were performed in triplicates. vitamin C and E were separately used as control due to their potential for inhibition of free radicals effect.

In the second method, the ferric reducing antioxidant power (FRAP) of the essential oil from the samples of *F. subpinnata* and *P. anisum* were assayed according to described assay [29]. The percent of free radical inhibition in FRAP assay of the samples was calculated according to the below formula:

$$\text{Antioxidant Activity (\%)} = (A_1 A_0) / A_1$$

A_0 is the absorbance of the control (potassium phosphate buffer + FRAP reagent), A_1 is the Absorbance of sample. The vitamin C and E were separately used as the reference antioxidants.

2.3. Botanical illustrations

For Full confidence of coccerc identification of plant species, they were studied and compared with related references in aspects of taxonomy, morphological description and Geographical distribution [9, 30-32].

2.4. Statistical analysis

Statistical analysis of antioxidant activities of essential oils were conducted using SAS version 9.1. All assays were performed in at least three replications. Differences between means were distinguished using F-test and the confidence limits was based on 95 % ($P < 0.05$).

3. Results

3.1. Taxonomy, Geographycal distribution and morphological description of plants samples

Distribution of *F. subpinnata*: *Froriepia* is a fragrant plant belongs to the Apiaceae family, native to the Caucasus region which has only one species (*F. subpinnata*) in Iran and has been reported from the provinces of Gilan, Mazandaran and Golestan. The species grows on the roadsides and mountainous areas. This plant is known as anarijeh or in the northern regions of the country and its fresh and dried leaves are commonly used in local foods.

Morphological description of *F. subpinnata*: Slender glabrous biennial. Stems up to 10 cm, divaricately branched, purple-striped below, glaucous-green above. Basal leaves unknown. Cauline leaves with a white, membranous, petiolar subauriculate sheath; median leaves with few, filiform, mucronulate segments up to 5 mm long; upper leaves reduced to one segment. Umbels 2-4-rayed, up to 1.5 cm long. Bracts absent. Bracteoles 1-3, narrowly lanceolate,

longer than pedicels, with a white margin and long awn, somewhat rigid. Central umbellule subsessile. Central fruit 2-2.5 mm long, outer fruits 1.5-2 mm long, often with rudimentary mericarps. Mericarps subterete, primary ridges 5-6, secondary ridges 4-5. Dorsal vittae 4-5, commissural vittae 2, all small (Fig. 1).

Distribution of *P. anisum*: The genus *Pimpinella* L. from the Apiaceae family have about 21 species of annual or perennial species in Iran. They are generally fragrant plants in Europe, Turkey, Iran, Caucasus, Armenia, Central Asia, Syria and Egypt. *P. anisum* with common name of Anise or Roman anise and only its seeds have medicinal use. This species is widely cultivated in different parts of the Iran and has been reported from the Azerbaijan, Isfahan and Tehran provinces and probably does not originate from Iran while *P. affinis* Ledeb. is widespread species in Iran and therefore it is mostly used in traditional medicine. Due to similarities between two species of *P. anisum* and *P. affinis* in seed shape, odor and medicinal properties they were used instead of each other in Iran.

Morphological description of *P. anisum*: Strongly aromatic branched annual with puberulent or pubescent stems up to 70 cm. Basal leaves simple, 2-5 cm, petiolate, reniform or ovate, dentate; lower cauline leaves pinnate with ovate or obovate dentate segments; upper cauline leaves 2-3-pinnate, lobes linear-lanceolate, petioles sheathing. Umbels 7-15-rayed; bracts absent or 1; bracteoles absent to few, very narrow. Flowers white, c. 10 per umbellule. Fruit 3-5 mm long, ovoid, shortly adpressed-hairy; stylopodium conical (Fig. 2).



Fig. 1. Used part (vegetative organs = above) and generative organs (inflorescent = below) of *Froriepia subpinnata*



Fig. 2. Used part of used part (Seeds = left) and generative organs (inflorescent = right) of *Pimpinella anisum*

3.2. Composition and antioxidant properties of essential oils

Based on the results of GC/MS analysis, 36 bioactive compounds were identified in the essential oil of *F. subpinnata* constituting

93.21 % of the total oil. The most important of these compounds were *p*-cymen-8-ol, α -terpinolene and limonene by about 51.13, 7.96 and 6.83 %, respectively (Table 1, Fig. 3).

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Table 1. Essential oil compounds in *F. subpinnata*

No.	Compounds	RT	KI	%	No.	Compounds	RT	KI	%
1	Hexanal	6.16	809	0.11	19	Terpinen-4-ol	24.78	1192	0.16
2	Heptanal	10.47	911	0.79	20	<i>m</i> -Cymen-8-ol	25.30	1203	0.12
3	α -Pinene	11.73	936	0.72	21	Citronellol	26.92	1237	0.13
4	Sabinene	13.81	977	1.99	22	<i>E</i> -Ocimenone	27.16	1242	5.54
5	β -Pinene	14.08	983	0.92	23	Anethole	30.02	1303	0.84
6	β -Myrcene	14.65	994	0.75	24	Thymol	30.33	1310	0.29
7	2-Pentylfuran	14.78	997	0.24	25	β-Elemene	34.16	1396	2.42
8	<i>trans</i> -2-(1-Pentenyl) furan	15.28	1006	0.15	26	(<i>E</i>)- β -Farnesene	36.74	1458	0.63
9	α -Phellandrene	15.61	1013	1.14	27	<i>trans</i> - β -Ionone	38.15	1491	0.17
10	α -Terpinene	16.15	1023	0.13	28	β -Selinene	38.55	1501	0.25
11	<i>o</i> -Cymene	16.66	1033	0.36	29	α -Selinene	38.80	1507	0.38
12	Limonene	16.81	1036	6.83	30	Germacrene A	39.28	1519	0.53
13	β -Ocimene	17.68	1052	0.27	31	Neophytadiene	51.11	1838	3.48
14	γ -Terpinene	18.35	1065	1.27	32	Hexahydrofarnesyl acetone	51.46	1849	0.18
15	α-Terpinolene	19.74	1091	7.69	33	Diisobutyl phthalate	52.40	1876	0.11
16	<i>p</i>-Cymen-8-ol	22.18	1140	51.13	34	Hexadecanoic acid - methyl ester	54.31	1934	0.12
17	Anisole	23.79	1172	1.08	35	Dibutyl phthalate	55.59	1973	0.13
18	4-Pyridinol	23.96	1176	0.86	36	Phytol	60.07	2059	1.30
Total Identified				93.21					

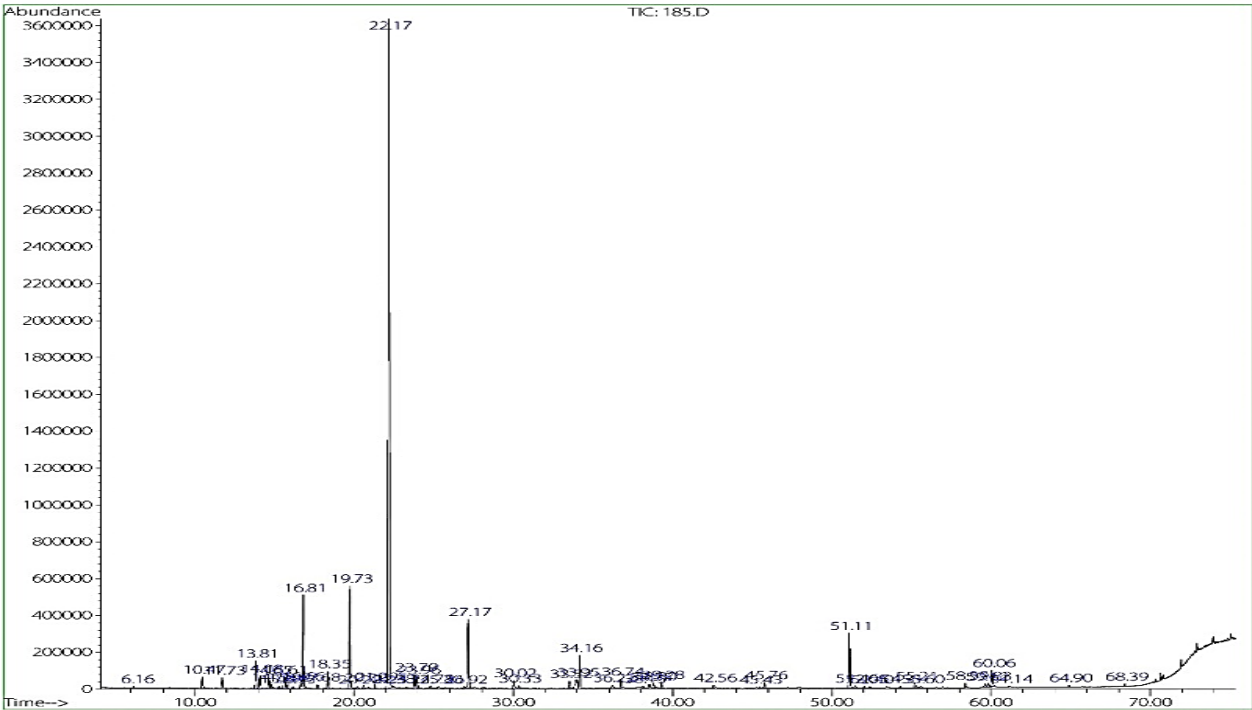


Fig. 3. Chromatogram obtained from GC/MS analysis of *F. subpinnata* essential oil

The amount of hydrogenated monoterpenes by about 22.07 %, oxygenated monoterpenes by about 58.26 %, hydrogenated sesquiterpene with 4.21 %, hydrogenated diterpenes with 1.3 % and other compounds with 7.25 % of total compounds of essential oil were reported.

Assay of antioxidant activity of *F. subpinnata* essential oil in the range of 250 µg/ml showed that the percentage of antioxidant inhibitors for *F. subpinnata* essential oil by DPPH and FRAP methods were about 53.03 and 62.72 %, respectively. Despite, along with rising in essential oil concentration, inhibitory activity was also increased but could not compete with the antioxidant power of vitamins C and E (Table 3).

The results of *P. anisum* essential oil analysis by GC/MS showed 13 biologically active compounds that constituteing 99.3 % of the total oil.

The most important of these compounds were *trans*-anethole (85.65 %), carvone (31.5 %) and Limonen (3.25 %) respectively (Table 2, Fig. 4). The content of hydrogenated monoterpenes about 3.35 %, oxygenated monoterpenes about 93.15 %, hydrogenated sesquiterpene 2.69 and oxygenated sesquiterpene 0.12 % of total compounds of essential oil were reported.

Assay of antioxidant activity of *P. anisum* essential oil in the range of 250 µg/ml showed that the percentage of antioxidant inhibitors for *P. anisum* essential oil by DPPH and FRAP methods were about 50.27 % and 59.91 %, respectively. Despite, along with rising the essential oil concentration, inhibitory activity was also increased but could not compete with the antioxidant power of vitamins C and E (Table 3).

Table 2. Essential oil compounds in *P. anisum*

No.	Compounds	RT	KI	%	No.	Compounds	RT	KI	%
1	<i>α</i> -Phellandrene	15.61	1013	0.10	8	<i>α</i> -Himachalene	36.86	1460	0.08
2	Limonene	16.81	1036	3.25	9	<i>γ</i> -Himalachene	38.10	1490	1.65
3	Estragole	25.74	1212	1.38	10	Zingiberene	38.63	1503	0.77
4	<i>trans</i> -Dihydro carvone	26.07	1219	0.66	11	<i>β</i> -Himachalene	38.96	1511	0.12
5	Carvone	28.03	1261	5.31	12	<i>β</i> -Bisabolene	39.14	1515	0.07
6	Anethole	28.39	1268	0.15	13	Pseudoisoeugenol 2-methylbutanoate	51.53	1580	0.12
7	<i>trans</i>-Anethole	30.09	1304	85.65					
Total Identified				99.30					

Table 3. Evaluation of antioxidant activity in essential oil in *F. subpinnata* and *P. anisum* by DPPH and FRAP methods. Vitamins C and E were considered as positive controls.

Plant essential oil or Positive control (Concentration)	Inhibition (%) DPPH method	Inhibition (%) FRAP method
<i>F. subpinnata</i> (250 µg/ml)	53.03 ± 0.24 ^b	62.72 ± 0.37 ^b
<i>P. anisum</i> (250 µg/ml)	50.27 ± 2.15 ^b	59.91 ± 1.52 ^c
Vitamins C (8 µg/ml)	91.37 ± 3.68 ^a	94.37 ± 1.78 ^a
Vitamins E (5 µg/ml)	95.58 ± 2.63 ^a	96.23 ± 1.89 ^a

Values are mean ± SD from three replicates (n = 3) at the 0.05 probability levels. ^{a,b,c} Superscript lowercase letters are for significance values.

basically incorrect. Also, the compounds reported in this report do not match the compounds reported in the present study probably due to wrong plant identification [19].

In two other reports [35, 36], the plant species of *F. subpinnata* was called as Zolang in Persian, which is according to the Mozaffarian [32]. However, based on previous studies this naming does not agree to the common naming of this plants in the north of Iran and therefore did not approve [9, 15, 16].

Predominant phenolic compounds and their antioxidant effects of *F. subpinnata* were reported previously [38]. Due to similarity of antioxidant activities of this report with present study, it seems that the plant species is probably correctly used.

Also, some controversial reports had found on *P. anisum*. Our results indicated that 13 bioactive compounds identified in essential oil of the essence *P. anisum* (Table 2, Fig. 4) that showed 99.3 % of total oil.

Some studies were reported results of analysis of seeds essential oil of *P. anisum* from different regions of Turkey. The most common identified compounds were trans-Anethole, methyl chavicol and Alpha-terpineol. In the present study, more than 85% of essential oil was trans-Anethole. Also, it has also been reported that the antioxidant activity of essential oil of *P. anisum* extracted from their seeds was higher than alpha-Tocopherol. Therefore, the results of this study are consistent with all the studies mentioned [11, 39].

One report was found antifungal activity of *P. anisum* essential oil on *Fusarium solani* that were introduced by Persian name of anarijeh [17]. Also, another report of improper use of local name of anarijeh were observed on the application of essential oil of *P. affinis* [13]. In

both of these studies, the exact identification of plant species was not mentioned and the type of compounds was not introduced. Therefore, the results can not be judged scientifically and it is better to evaluate the plant samples due to their incorrect naming.

Some doubtful researches were carried out on *P. affinis* which introduced by the local name of anarijeh. In these reported studies, mentioned that the plant was collected from the north of Iran and also the extraction was done from the aerial part of the plant [20-22]. The used parts of plants are noticeable here. only seeds in *P. affinis* have medicinal uses and the aerial part of the plant is not used while the aerial parts of *F. subpinnata* is known due to its trapeutical properties [15, 16]. So it is concluded that the used plants in this studied were *F. subpinnata* and authors have made a great mistake in introducing the plant.

In another research, the antioxidant effects and phenolic and flavonoid content of extracts and essential oils in *P. affinis* which mistakenly introduced as anarijeh were studied [18]. Due to the lack of exact herbarium specimens and mistakes that occurred in the naming of the plant, the results of this study is questionable and need to be examined more carefully.

The studied medicinal plants have different phytochemical compounds with antioxidant properties. The presence of these compounds has been regarded as biologically effective factors. As a result, the antioxidant power of plant essential oils is considered to be directly related to the presence of biological compounds in the essential oils [28].

Antioxidant properties of studied species in both methods were investigated and shown that the essential oil of the aerial part of *F. subpinnata* and seeds of *P. anisum* were not very different

from each other. Both plants have less antioxidant power than vitamin C and E and therefore, they can be used as a moderately effective agent in the food and pharmaceutical industries.

5. Conclusion

There is a common mistake in Iran in identifying the medicinal plants *F. subpinnata* and *P. anisum*, which they call by the same name of anarijeh. On the other hand in many scientific reports, the plant materials are not correctly identified by an expert botanist. The herbarium samples do not exist and therefore so many mistakes were occurred here. In this study, the essential oils of both *F. subpinnata* and *P. anisum* plant species were analyzed and the most prominent compounds and antioxidant potential of each species were introduced. Therefore in order to prevention of mistakes or deception, major compounds and properties of essential oils can be used as a suitable phytochemical indicator to identify plants or derived products of them. Also, due to the antioxidant properties of both plants the

consumption of these plants can be useful in everyday human food. As a final conclusion, cooperation or consulting with an expert botanist and presentation of voucher specimen indicating the correct identification of plant is necessary and highly recommended.

Author contributions

P. M. and M. GH N. designed and performed the experiment and participated in writing of manuscript. The analysis of GC/MS were carried out by E. K. and A. A. guided aspects of the research.

Conflict of interest

The authors declare that there is no conflict of interest.

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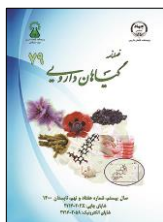
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مقاله تحقیقاتی

تفاوت بین گیاهان دارویی *Pimpinella anisum* L. و *Froriepia subpinnata* (Ledeb.) Baill.

با نام عمومی اناریجه بر اساس ترکیبات عمده اسانس؛ شاخصی برای رفع ابهامات

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چکیده

اطلاعات مقاله

گل واژگان:

اناریجه

شناسایی گیاه

اشتباهات رایج

ترکیبات روغن فرار

تشخیص افتراقی

*Froriepia subpinnata**Pimpinella anisum*

کروماتوگرافی گازی متصل

به طیف سنج جرمی

مقدمه: گیاهان *Pimpinella anisum* L. و *Froriepia subpinnata* (Ledeb.) Baill. دو گیاه دارویی مهم از تیره چتریان هستند. این دو گیاه به دلیل نام عمومی فارسی مشابه «اناریجه»، در بازار گیاهان دارویی ایران و حتی در گزارش های علمی به اشتباه به جای یکدیگر استفاده می شوند. هدف: معرفی دقیق ریخت شناختی و ترکیب اسانس همراه با اثرات آنتی اکسیدانی گیاهان *P. anisum* و *F. subpinnata* و جلوگیری از اشتباهات و تقلبات در گیاهان مذکور و محصولات ناشی از آنها می باشد. روش بررسی: بخش هوایی گیاه *F. subpinnata* و بذر گیاه *P. anisum* پس از خشک شدن، پودر و اسانس آنها با استفاده از روش تقطیر توسط دستگاه کلونجر به مدت ۳ ساعت استخراج و سپس ترکیبات تشکیل دهنده اسانس توسط کروماتوگرافی گازی متصل به طیف سنج جرمی مورد ارزیابی قرار گرفت. همچنین، با استفاده از روش های DPPH و FRAP قدرت مهار آنتی اکسیدانی اسانس ها تعیین شد. **نتایج:** در اسانس گیاه *F. subpinnata* ترکیبات پارا سیمن ۸- ال (۵۱/۱۳ درصد)، آلفا ترپینولن (۷/۶۹ درصد) و لیمونن (۶/۸۳ درصد) یافت شدند. در اسانس گیاه *P. anisum* نیز ترکیبات ترانس آنتول (۸۵/۶۵ درصد) و کارون (۵/۳۱ درصد) ترکیبات اصلی اسانس را شامل می شدند. قدرت مهار آنتی اکسیدانی به دو روش DPPH و FRAP در اسانس هر دو گیاه با غلظت ۲۵۰ میکروگرم در میلی لیتر تقریباً مشابه و در گیاه *F. subpinnata* به ترتیب به میزان ۵۳/۰۳ و ۶۲/۷۲ درصد و برای گیاه *P. anisum* به ترتیب به میزان ۵۰/۲۷ و ۵۹/۹۱ درصد اندازه گیری شد. **نتیجه گیری:** نوع و میزان ترکیبات اصلی اسانس در گیاهان مطالعه شده، می تواند به عنوان مبنایی دقیق برای تشخیص افتراقی این گیاهان قلمداد شود. جهت شناسایی صحیح و جلوگیری از اشتباهات و تقلبات در گیاهان و محصولات گیاهی، می توان از همه این تفاوت ها به عنوان یک نشان گر فیتوشیمیایی مناسب استفاده کرد.

مخفف ها: GC/MS، کروماتوگرافی گازی متصل به طیف سنج جرمی؛ EOs، روغن های فرار

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