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Comparison of Quantitative and Qualitative Characteristics of Fennel (*Foeniculum vulgare*) Essential Oil in Traditional and Modern Extraction Methods

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ABSTRACT

Foeniculum vulgare (fennel) is a medicinal and aromatic plant with wide applications in pharmaceutical, food, and cosmetic industries. This study aimed to compare the quantitative and qualitative characteristics of fennel seed essential oil (EO) obtained by hydro-distillation (HD) as well as utilizing supercritical carbon dioxide extraction (SC-CO_2). The experiments were conducted using a completely randomized design with three replications. The chemical composition of the EOs was analyzed using GC-FID and GC–MS techniques. Results showed that the total EO yield in the SC-CO_2 method (2.64 ± 0.41%) was significantly higher than that obtained by hydro-distillation (0.83 ± 0.11%), representing an increase of approximately 218%. Overall, 30 compounds were identified in the SC-CO_2 extract compared to 18 compounds in hydro-distillation, indicating better preservation of thermolabile constituents under lower extraction temperatures. The main components of fennel EO were trans-anethole, estragole, and hexadecanoic acid. The content of trans-anethole increased from 32.37% (HD) to 40.56% (SC-CO_2), and estragole rose from 25.92% to 29.97%. Hexadecanoic acid, which was absent in hydro-distillation, appeared in the SC-CO_2 extract at 7.19%. Analysis of chemical groups showed that oxygenated monoterpenes were dominant, accounting for 75.04% in SC-CO_2 and 64.19% in HD samples. Comparison with the ISO 17412 international standard (specifying 50–78% *trans*-anethole and 1–6% estragole) revealed that both extraction methods produced oils within the acceptable range, though estragole content in the SC-CO_2 extract slightly exceeded the upper limit. In conclusion, supercritical CO_2 extraction, owing to its lower temperature, higher selectivity, and reduced thermal degradation, proved to be an efficient, green, and high-yield technology for obtaining high-quality fennel EO and represents a superior alternative to conventional methods.

1- Introduction

Fennel (*Foeniculum vulgare*) is a Mediterranean plant that thrives in warm climates. In general, the cultivation of this species is successful in countries with warm weather and long summers without severely cold winters [1]. In traditional medicine, fennel has been used as a carminative, a remedy for kidney and urinary tract stones, and a galactagogue to enhance lactation in nursing mothers [2]. The EO of fennel possesses potential applications in pharmaceuticals and natural herbicides [3]. Moreover, it contains compounds such as thymoquinone and carvacrol, whose antioxidant, antibacterial, and anti-inflammatory activities make the oil suitable for food-related applications [4]. The performance and characteristics of EOs depend on multiple factors, including harvest season, plant growth conditions, storage and preparation parameters, and the extraction method employed—all of which significantly influence the yield and quality of the obtained oil [5].

Over the past two to three decades, the global demand for medicinal plants and their products for both pharmaceutical and non-pharmaceutical purposes has considerably increased. Beyond improvements in cultivation, collection, and genetic modification of medicinal plants, great emphasis has been placed on developing safer and greener extraction technologies, as these methods indirectly enhance overall extract performance, increase the recovery of phytochemicals, and reduce waste generation, thereby improving process sustainability [6]. Therefore, identifying an appropriate extraction technique for EOs—and more precisely, selecting the optimal method for each key bioactive compound—is a crucial issue in the EO industry. According to previous studies, the most abundant constituent of fennel EO is **trans-anethole**, comprising about 30–50 % of the total oil [7]. In 1994, Marotti *et al.* investigated the influence of plant growth conditions, type, and variety of fennel on the composition of its EO. Their results indicated that the trans-anethole content is strongly affected by plant species and by the extraction and processing methods used [8]. In another study, researchers extracted fennel EO using various techniques and identified 12 compounds, among which trans-anethole represented the major constituent at 54.68 % [9].

Since the specific gravity of EOs is usually lower than that of water—and only in rare cases does the density of the oil extracted from certain plants exceed that of water—the extraction of EOs, particularly on an industrial scale, is generally carried out by hydrodistillation or steam distillation methods (Clevenger and percolator apparatus) [1]. However, the high temperature involved in these processes often leads to the degradation or transformation of many valuable constituents [10]. Therefore, modern and advanced extraction technologies, such as supercritical fluid extraction, microwave-assisted extraction, and ultrasound-assisted extraction, have been developed to overcome these limitations.

A supercritical fluid exhibits gas-like transport properties (such as high diffusivity and low viscosity) and liquid-like solvent power. Because of these characteristics, supercritical fluids are widely used for purification, extraction, and separation of various materials. In essence, a supercritical fluid possesses

intermediate properties between those of a gas and a liquid [11]. In a comparative study between cold-pressing and supercritical extraction methods, the EO yield increased by 24.5 % using the supercritical approach [12]. Another study reported a higher extract recovery obtained through the supercritical method compared with conventional techniques such as Soxhlet extraction (9.8 g per 100 g of raw material) [13].

Accordingly, the present study aims to evaluate the extraction quality of active constituents in fennel EO by comparing the quantitative data of major components obtained through hydrodistillation and supercritical carbon dioxide extraction, as representative conventional and modern techniques for EO extraction from medicinal plants.

2- Materials and Methods

2.1 Experimental Design and Sample Preparation

This study was conducted based on a completely randomized design with three replications. The independent variables included two extraction methods—hydrodistillation and supercritical carbon dioxide extraction—while the dependent variables were the differences amount of observed in the key constituent compounds of fennel (*Foeniculum vulgare*) EO. Fennel seeds were purchased from the Giahcheh Company (Isfahan, Iran), coarsely ground in 100 g portions, and transferred to the laboratory for EO extraction (Figure 1).

Fig1. Particle size distribution of the partly-crushed sample (passing through a 20-mm sieve)

2.2. EO Extraction by Hydrodistillation

The samples were placed in a Clevenger-type distillation flask (Figure 2) and the volume was adjusted with 600 mL of distilled water. The hydrodistillation process was carried out for 3 hours. At the end of distillation, the obtained EO was collected using a pipette, dehydrated with anhydrous sodium sulfate, and subsequently injected into the gas chromatograph (GC) and gas chromatograph–mass spectrometer (GC–MS) for analysis [14].

Fig2. Hydro-distillation method

2.3. EO Extraction using Supercritical Carbon Dioxide

In this method, the extraction was performed at a pressure of 200 bar, temperature of 40 °C, and duration of 60 minutes [15]. The obtained oleoresin (the oily and resinous fraction of the extract containing the desired EO) was immediately transferred to the laboratory and separated into two phases by adding hexane (Figure 3). The upper phase, representing the EO, was collected using a pipette and dehydrated with anhydrous sodium sulfate. Finally, all samples were injected into the gas chromatograph (GC) and gas chromatograph–mass spectrometer (GC–MS) for compositional analysis.

Fig3. Supercritical CO_2 extraction method

2.4. Gas Chromatography and Gas Chromatography–Mass Spectrometry Analysis

The EOs were analyzed using a gas chromatograph (Agilent 7890B) equipped with a flame ionization detector (FID) and a capillary HP-5 column (30 m length, 0.25 mm internal diameter, and 0.25 µm film thickness). The temperature program consisted of holding at 60 °C for 2 minutes, followed by an increase to 250 °C at a rate of 5 °C per minute.

Mass spectrometric (GC–MS) analysis was carried out using a Thermoquest-Finnigan gas chromatograph equipped with a fused-silica capillary HP-5 column (60 m × 0.25 mm i.d., 0.25 µm film thickness) coupled to a trace mass spectrometer. Helium was used as the carrier gas at a flow rate of 1.1 mL min^{-1} with a split ratio of 1:100. The ionization voltage was 70 eV, and the temperatures of the ion source and interface were set at 200 °C and 250 °C, respectively.

Identification of the EO constituents was achieved by comparing their mass spectra with those of reference spectra from the Wiley 7 and Adams libraries. Quantification was based on the relative percentage of individual component peak areas obtained from GC–FID chromatograms [16]. Retention indices (RIs) were calculated using the retention times of a homologous series of *n*-alkanes analyzed under the same chromatographic conditions. The relative contents of the components were computed from the total peak areas using the instrument's integrated software.

2.5. Data Analysis

Data analysis was performed using the Least Significant Difference (LSD) test using the SPSS software package (version 25) at a 5% probability level. The graphical representations were prepared using Microsoft Excel.

3-Results and Discussion

3.1. EO Yield

Results (Table 1) indicated that extraction using supercritical carbon dioxide was more selective than hydrodistillation due to the ability to precisely control temperature and pressure conditions which has been reported by other workers [20-21]. The total extraction yield obtained by hydrodistillation and supercritical CO_2 extraction methods was 0.83 ± 0.11 % and 2.64 ± 0.41 % per 100 g of sample, respectively. This finding demonstrated the superiority of the supercritical method and was consistent with previous studies [17-16].

3.2. Identified Compounds in the EOs

Based on the interpretation of the mass spectra obtained from GC–MS analysis, the calculated Kovats retention indices, and the comparison of these characteristics with standard compounds and literature data, a total of 18 compounds were identified in the EO extracted by hydrodistillation (some thermolabile compounds may have been degraded due to the high temperature during this process) and 30 compounds were identified in the EO obtained by supercritical CO_2 extraction (Table 1). These identified constituents accounted

for 85.87–97.09 % of the total oil composition.

The major constituents of the EO under different extraction conditions were as follows: for hydrodistillation and supercritical CO_2 extraction, respectively — trans-anethole (32.37 ± 4.58 % and 40.56 ± 6.11 %), estragole (25.92 ± 2.22 % and 29.97 ± 4.81 %), and hexadecanoic acid (0.00 % and 7.19 ± 2.54 %). Among these, trans-anethole is considered one of the principal components of fennel EO, and its concentration varies depending on the extraction method applied [17]. As shown in Table 1, the highest amount of this compound (40.56 %) was obtained using the supercritical CO_2 extraction method.

Table1. Composition profile of fennel seed EO (%) obtained by hydro distillation and super-critical extraction [a]

Component Name[b]	RI[c]	RI[d]	SC-CO_2 (%)	HD (%)
α-pinene	1027	1028	0.15±0.01	2.61±0.92
camphene	1075	1074	-	0.25±0.07
β-pinene	1118	1118	-	0.82±0.34
Sabinene	1129	1240	0.1±0.01	0.72±0.21
β-myrcene	1164	1168	0.05±0.01	0.86±0.19
α-phellandrene	1174	1176	0.02±0.01	2.13±1.08
limonene	1207	1207	2.63±0.17	6.03±1.75
eucalyptol	1221	1220	0.36±0.04	-
cis- β-Ocimene	1235	1235	0.41±0.07	0.63±0.14
γ-terpinene	1255	1255	0.62±0.05	2.6±4.41
o-cymene	1282	1285	0.19±0.02	0.46±0.16
terpinolen	1293	1290	-	3.19±1.58
thujone	1430	1430	2.51±0.29	5.01±2.62
trans-limonene oxide	1475	1470	0.03±0.01	0.81±0.24
epi-β-caryophyllene	1571	1572	0.11±0.04	-
4-terpinenol	1629	1611	-	0.57±0.29
estragole	1707	1680	29.97±4.81	25.22±2.92
γ-cadinene	1763	1765	0.51±0.17	0.63±0.41
perilla aldehyde	1786	1790	1.01±0.09	-
trans-anethole	1865	1822	40.56±6.11	32.58±4.37
eugenol	2193	2185	0.24±0.02	-
methyl Hexadecanoate	2225	2218	0.41±0.23	-
dill apiol	2392	2384	1.03±0.18	0.75±0.28
methyl stearate	2433	2428	0.09±0.02	-
ethyl oleate	2459	2459	4.16±1.27	-
linoleic acid methyl ester	2491	2490	1.06±0.35	-
linoleic acid ethyl ester	2511	2511	1.58±0.71	-
ethyl 9α-linolenate	2542	2545	0.27±0.13	-
linoleic acid methyl ester	2579	2583	1.1±0.06	-
3-methoxycinnamaldehyde	2602	2565	0.39±0.69	-
tetradecanoic acid	2697	2694	0.15±0.03	-
heptacosane	2702	2700	0.17±0.07	-
nonacosane	2904	2900	0.41±0.12	-

n-hexadecanoic acid	2913	2914	7.19±2.54	-
Total			**97.09**	**85.87**

a Percentage obtained by FID peak-area normalization
b Results are compound percentages in EO expressed as means ± standard errors (n=3)
c Linear retention indices (DB-5 column) using normal n-alkanes series (C6-C24)
[d] Retention indices from literature.

Extraction using a supercritical CO_2 apparatus is considered a green and environmentally friendly technology. In this method, extraction is carried out at relatively low temperatures, which prevents the degradation, oxidation, hydrolysis, isomerization, and alteration of thermally sensitive compounds, thereby yielding a purer EO [18]. Supercritical CO_2 extraction is regarded as the most effective technique for obtaining bioactive compounds with high qualitative and quantitative performance [17].

Most previous studies on fennel EO [19] have primarily focused on extraction yield. However, the objective of the present study was to comparatively evaluate the qualitative aspects of two major components of fennel EO—trans-anethole and estragole. Compared with traditional extraction methods, the supercritical process offers several advantages, including lower extraction temperature and duration, as well as the ability to optimize operational parameters for enhanced extraction efficiency.

3.3. Comparison of Chemical Groups in the EOs

The total content of chemical groups in the two extraction treatments ranged between 85.87 % and 97.09 %. These groups included monoterpene hydrocarbons (M-H) ranging from 4.17–20.3 %, oxygenated monoterpenes (O-M) ranging from 64.94–75.98 %, and sesquiterpene hydrocarbons (S-H) ranging from 0.62–0.63 %. Comparison of the present results with those of previous studies indicated that oxygenated monoterpenes constitute the main chemical group in fennel EO, which is consistent with the findings of Maitusong *et al.* (2020) [20]. Variations and proportions of each group are shown in Figure 4.

In their study on the composition of fennel seed EO, Maitusong *et al.* also reported that in hydrodistillation and supercritical extraction methods, hydrocarbons accounted for 21.96 % and 6.67 %, while oxygenated compounds represented 75.62 % and 70.16 % of the total oil, respectively. In the present study, the dominance of oxygenated compounds was attributed to the presence of trans-anethole and estragole as the major constituents of fennel EO, which aligns well with previous reports [18-21-22].

Analysis of the chemical group properties further indicated that monoterpene-containing compounds are volatile terpenoids whose extraction decreases with increasing temperature. Numerous studies have emphasized the influence of extraction method, geographical origin, soil conditions, and harvesting practices on both the qualitative and quantitative characteristics of EOs [22-23]. Overall, it has been reported that oxygenated monoterpenes possess stronger antimicrobial properties than monoterpene hydrocarbons [23].

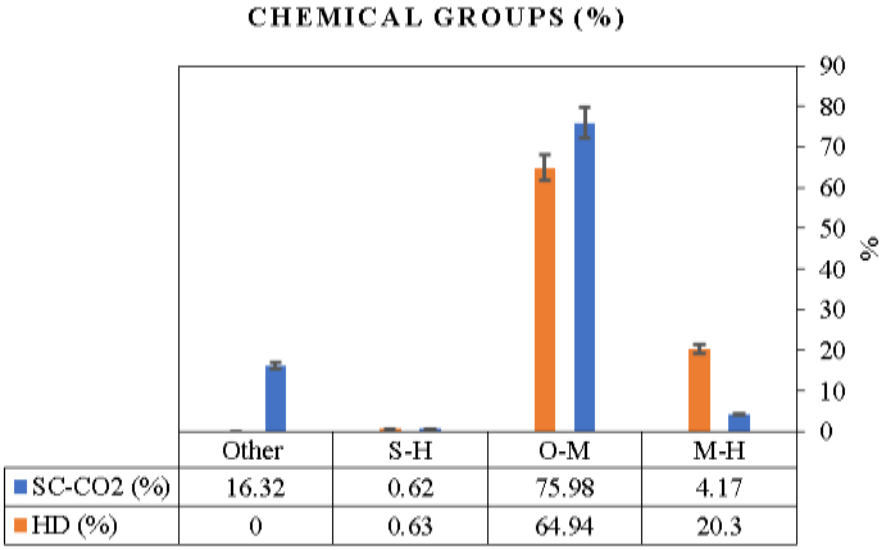

Fig4. Average values for the percentages of all groups of chemical constituents of *Foeniculum vulgare* oil as affected by extraction method. (O-M) Oxygenated monoterpenes, (M-H) Monoterpene hydrocarbons, (S-H) Sesquiterpene hydrocarbons

3.4. Comparison of Mean Values of Major Compounds

The major constituents (present at concentrations above 5%) of the EO were identified as trans-anethole, estragole, and hexadecanoic acid, respectively. Among these, hexadecanoic acid was not detected in the hydrodistillation extract. It was intended to examine the statistical significance of mean comparisons for the two other major compounds between the two extraction methods. The normality test results for these two compounds showed that the significance level for both was greater than 0.05, indicating that the data in both groups followed a normal distribution (Table 2).

Table2. Normality Test results

Variables	Factor	Statistic	df	Sig.
trans-anethole analyse	$SC\text{-}CO_2$	0.987	3	0.78
	HD	0.925	3	0.471
estragole analyse	$SC\text{-}CO_2$	0.960	3	0.614
	HD	0.957	3	0.6

The assessment of the significance or non-significance of the mean values for the two major compounds (Table 3) indicated the existence of inequality—or in other words, a difference—between their means, as evidenced by the non-inclusion of zero within the upper and lower bounds of the 95% confidence interval for the mean difference. These statistical results clearly demonstrated a difference in extraction quality between the two methods for the key constituents of this plant [17-18].

Table3. Mean Significance of Major Compounds

Components	Mean Difference	Std. Error Difference	95% Confidence of the Difference	
trans-anethole	5.97667	0.20819	5.39863	6.5547
estragole	5.75333	0.19143	5.22185	6.28482

Comparison of the results obtained from the two extraction methods showed that, in addition to the higher contents of trans-anethole and estragole in the supercritical CO_2 extraction, the compound hexadecanoic acid—which had not been previously detected in the hydrodistillation extract—was successfully extracted under supercritical conditions [26]. Moreover, among the chemical groups of EO constituents, the supercritical CO_2 method yielded higher proportions of oxygenated monoterpenes (75.04 % vs. 64.19 %) and oxygenated sesquiterpenes (2.61 % vs. 0.75 %) compared to hydrodistillation, which was consistent with the findings of Maitusong *et al.* [20].

This higher content was mainly attributed to trans-anethole and estragole, both belonging to the oxygenated monoterpene group, as reported in several previous studies [23-24]. Examination of the major characteristics of the four EO constituent groups indicated that monoterpene compounds are volatile terpenes that tend to evaporate at temperatures above 20 °C [20-21]. In contrast, sesquiterpenes, which represent the most structurally diverse class of terpenes [33], are more abundant in plant tissues than in their extracted oils and are effectively utilized in aromatherapy applications [34].

The observed increase in aromatic compounds—mainly phenolics—and the lower content of hydrocarbons could be attributed to operational parameters such as the extraction method used [23]. In general, oxygenated monoterpenes exhibit stronger antimicrobial properties than monoterpene hydrocarbons.

The findings of the present study on the major compounds of fennel EO were also supported by reports from countries with established fennel cultivation, confirming consistency with previous literature (Table 4).

Table4. Percentage of Major Components in Fennel Seed EO

Country	*trans*-Anethole	Estragole	references
Iran	30-40%	20-30%	[35]
India	30-40%	20-30%	[31-32]
China	20-30%	15-25%	[33-34]
Egypt	20-30%	15-25%	[40]

As inferred from other studies, the proportions of the four chemical groups of fennel EO compounds are relatively similar among the four investigated countries. Trans-anethole, a monoterpene compound, is the principal component of

fennel EO and exhibits the highest concentration in all four countries. Estragole, another monoterpene compound, is also present in considerable amounts across all regions. However, minor variations exist in the composition of fennel EO among the four countries. For example, fennel EOs produced in Iran and India contain higher proportions of oxygenated sesquiterpenes compared to those produced in China and Egypt.

3.5. Comparison of Major Extracted Compounds with ISO Standard

One of the reliable approaches to evaluate and validate the performance of extracted EOs from different plants and products is the use of ISO standards. Employing internationally recognized standards in each process ensures the reliability and accuracy of the results [41]. The comparison of the amounts of the two major compounds, trans-anethole and estragole, in fennel EO with the ISO 17412 standard revealed clear differences among the extraction methods. According to this standard, the acceptable ranges for trans-anethole and estragole in fennel EO obtained by steam distillation (SD) are 50–78 % and 1–6 %, respectively (highlighted regions in Figure 5).

As illustrated in Figure 5, extraction by steam distillation, hydrodistillation, and supercritical CO_2 showed distinct variations in the percentages of the active constituents. The use of hydrodistillation and supercritical CO_2 methods resulted in significantly higher estragole contents—40.56 % and 32.58 %, respectively—compared with the standard range ($p \leq 0.05$).

In the case of trans-anethole, it can be inferred that since supercritical CO_2 is a nonpolar solvent while water is a polar solvent, and trans-anethole itself is a polar compound that dissolves more efficiently in polar media, its concentration is higher in the hydrodistilled oil. Furthermore, under supercritical conditions (200 bar), the penetration of CO_2 into plant tissues increases substantially. However, because trans-anethole is a highly volatile compound, it can easily escape from the CO_2 phase, and partial degradation may occur even at 40 °C. In contrast, during steam distillation, due to the indirect contact with heat (no direct exposure to 100 °C), the compound remains more stable, resulting in a higher amount of trans-anethole in the obtained EO [42].

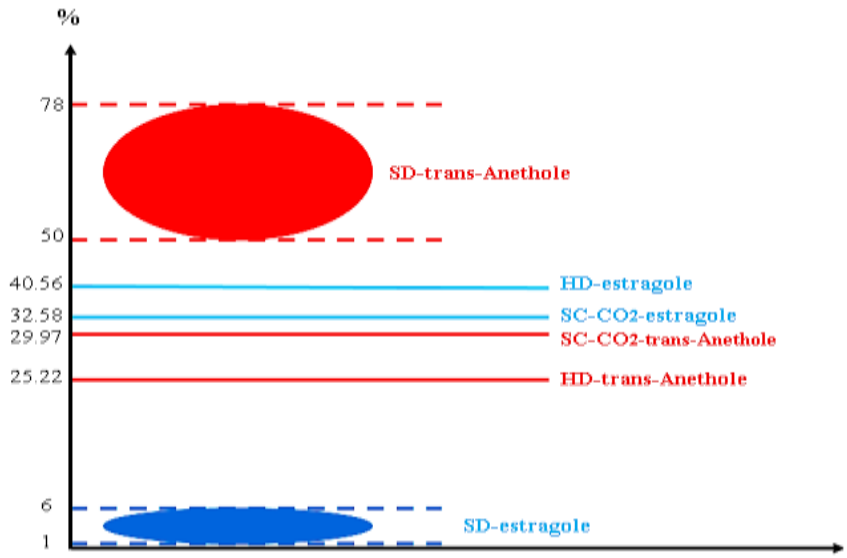

Fig5. Comparison of estragole and *trans*-anethole extraction yields in three methods of steam distillation (SD), hydro-distillation (HD), and supercritical extraction (SC-CO_2)

Considering the findings of the present study, it is evident that special attention should be given to the development of extraction technologies, particularly given the high potential that exists within the country. It can be concluded that extraction using a supercritical CO_2 apparatus, in addition to its high selectivity and the possibility of employing secondary co-solvents alongside carbon dioxide, offers a highly efficient and powerful approach for the separation and purification of plant-derived extracts and EOs.

4- Conclusion

In this study, the quantitative and qualitative differences in fennel EO obtained by hydrodistillation and supercritical CO_2 extraction were evaluated. The extraction of estragole using the supercritical method showed an 18.83 % increase compared with hydrodistillation. Although hexadecanoic acid was not detected in the oil extracted by hydrodistillation, it was successfully identified in the supercritical extract.

The results clearly indicated that both the number and concentration of compounds obtained through supercritical extraction were higher than those from hydrodistillation (30 vs. 18 compounds). This confirms the superior performance of the supercritical method, not only in preserving thermally sensitive compounds from degradation but also in significantly reducing extraction time (1 hour vs. 3 hours).

Overall, the present study demonstrated that when the goal is to obtain an EO with a defined purity level and enhanced selectivity toward bioactive constituents, supercritical CO_2 extraction—with appropriate optimization of operational parameters—can be effectively employed to improve extraction efficiency and product quality.

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مقایسه خصوصیات کمی و کیفی اسانس رازیانه در روش‌های استخراج سنتی و نوین

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چکیده

مطالعه روش‌های نوین استخراج اسانس‌های طبیعی و مقایسه آن‌ها با روش‌های متداول یکی از نیازهای پژوهشی صنعت فرآوری گیاهان دارویی می‌باشد. اسانس بذر رازیانه با دو روش تقطیر با آب و استفاده از دی‌اکسیدکربن فوق‌بحرانی (فشار ۲۰۰ بار، دمای ۴۰ درجه سلسیوس و مدت زمان یک ساعت) استخراج گردید. سپس اسانس‌های بدست آمده با دستگاه‌های کروماتوگرافی‌گازی و کروماتوگرافی‌گازی–طیف‌سنجی‌جرمی مورد تجزیه و تحلیل قرار گرفتند. نتایج نشان داد که بازده کل‌استخراج اسانس بدست‌آمده در دو روش تقطیر با آب و فوق‌بحرانی به ترتیب ۰/۸۳ و ۲/۶۴ درصد بود که افزایشی ۲۱۸/۷ درصدی در مقدار اسانس بدست‌آمده را نشان‌می‌داد. این مهم با در نظر گرفتن استحصال ترکیب هگزادکانییک اسید (۷/۱۹ درصد) که در روش تقطیر با آب تخریب یا تجزیه میشد و تعداد ترکیبات بدست‌آمده در روش تقطیر با آب نسبت به فوق‌بحرانی (۱۸ در مقابل ۳۰ ترکیب) بود، مناسب ارزیابی شد. در این پژوهش نتایج استخراج اسانس بذر رازیانه، با استاندارد ISO مقایسه شد و اطمینان‌بخشی نتایج مورد سنجش قرار گرفت بطوری که مقایسه استخراج با فرآیند فوق‌بحرانی در برابر استخراج بوسیله تقطیر با بخارآب (استاندارد ۱۷۴۱۲) بطور معناداری تفاوت در مقدار استراگل استخراجی را تایید نمود. این مطالعه به لزوم توجه به توسعه فناوری‌های جدید استخراج در افزایش کمیت و کیفیت اسانس بدست‌آمده که خود در نهایت به ایجاد ارزش افزوده بیشتر در مقابل روش‌های سنتی می‌انجامد، تاکید دارد.