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Chemical Variation in Essential Oil Composition and Rosmarinic Acid Content in Rosemary From Iran at Different Harvesting Times During One Day

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Abstract

Objectives: Rosemary has been broadly used in food, cosmetic, and pharmaceutical industries due to having different groups of main secondary metabolites (phytochemicals). There are many ways to reach the aim of the maximum percentage of active compounds. Regarding the importance of the essential oil (EO) and hydroxycinnamic acid derivatives, as well as ease of the harvesting time control, these factors were selected in this study although the variations of the chemical content of rosemary were evaluated during one day contrary to previous reports.

Materials and Methods: The fresh aerial parts of the cultivated rosemary were hand-harvested in winter at three different times (i.e., before sunrise, at noon, and after sunset) and then dried at 30°C. Each sample was submitted to the Clevenger-type apparatus for the isolation of the EO. The dried EOs were analyzed by gas chromatography (GC)/flame ionization detector (FID) and gas chromatography-mass spectrometry (GC/MS), and total hydroxycinnamic acid derivatives were determined by the UV/Vis spectrophotometer. Finally, the results were validated by the ANOVA test.

Results: The maximum (3.65%) and minimum (1.61%) of the total EO were obtained before the sunrise and after the sunset with 24 and 26 components, respectively. In addition, the results of GC/MS revealed that 1, 8-cineole, α-pinene, and camphor are the three most dependable components in the EO to the harvesting time. The contents of α-pinene and 1,8-cineole reduced from 32.8% to 23.0% and from 23.6% to 10.5% although the camphor percentage increased from 2.7% to 9.8% in the variation of the harvesting time from before the sunrise to after the sunset. In contrary to the EO, UV/Vis spectrophotometry results showed that hydroxycinnamic acid derivative contents were undependable over different harvesting times.

Conclusions: Based on this investigation, rosemary can change their component in some groups, and thus the harvesting time can be optimized based on our needs.

Keywords: *Rosmarinus officinalis* L., Essential oil, Hydroxycinnamic acid, Harvesting time

Introduction

Rosemary (*Rosmarinus officinalis* L.) has a highly pungent aroma and is one of the most famous members of the Lamiaceae family. It has green leaves all year round and its branched sub-shrubs reach a height of 50-150 cm. Its light green leaves are straight, flexible, wholly margined, and quite wrinkled toward the top. This plant ran wildly in the Mediterranean region, but nowadays, it is found everywhere in the world (1-4).

Rosemary itself has many applications due to different groups of main secondary metabolites such as caffeic acid derivatives (phenolics), volatile oil, diterpenes, flavonoids, and triterpenes (2,3,5,6). This plant has a significant number of pharmacological features such as hepatoprotective, antibacterial, antithrombotic, anti-nephrotoxic activity, antitrypanosomal, diuretic, antidiabetic, antinociceptive, anti-inflammatory, antitumor and antioxidant activities, antiulcer, and estrogenic effects (2,3,7-13). Accordingly, rosemary extracts (a composition of different phytochemicals) or isolated components from them have inhibitory effects on the growth of breast, liver, prostate, lung, human ovarian cancer cells, and leukemia cancer cells (7,11) The rosemary extract including the essential oil (EO) and polyphenols induces an anticarcinogenic enzyme, or in other anticancer mechanisms, its polyphenol constituents can inhibit the metabolic activation of pro-carcinogens (14). Rosemary also may reduce headaches, along with stress and helps in asthma and bronchitis treatment. Based on some reports, rosemary is also used for chronic pain treatment. In addition to pharmacological applications, rosemary has been used for cosmetic purposes such as producing cologne-water, hair lotions, shampoos as a disinfectant, and as an insecticide agent (3,4,8,15,16). Moreover, it has been traditionally applied in cooking as a spice in order to modify and improve food flavours or as folk medicines (2,5).

Many studies demonstrate which compounds have an essential role in specific properties, some of these

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Key Messages

- Rosemary has been broadly used in food, cosmetic, and pharmaceutical industries.
- These applications are due to different groups of phytochemicals such as hydroxycinnamic acid derivatives, volatile oil and others.
- It is important to obtain the maximum amount of these specific effective compounds.
- Regarding the importance of the essential oil (EO) and hydroxycinnamic acid derivatives, as well as ease of the harvesting time control, variations of the chemical content of rosemary were evaluated during one day.

effects are carminative (e.g., flavonoids), antidepressant, antispasmodic, and antioxidant (e.g., volatile oils), as well as rubefacient and antioxidant (e.g., phenolics) properties. Additionally, other effects included antimicrobial (e.g., diterpenes), emmenagogue (e.g., oleanolic acid), anti-inflammatory (e.g., carnosol), carcinogen blocking and liver detoxifying (e.g., carnosol and total-plant extract), antirheumatic (e.g., the ointment of the rosemary oil), and abortifacient (the aqueous extract) properties (10,17).

Among different properties, antioxidant activity is the most important and is mainly due to phytochemicals such as EOs, flavonoids, and caffeic acid derivatives, especially rosmarinic acid (3,5,8-10,18,19).

In the last few years, synthetic antioxidants such as butylated hydroxyanisole, butylated hydroxytoluene have been suspected of having carcinogenic properties. The oxidative degradation of DNA, RNA, proteins, and cell membranes occurs more rapidly when there is an imbalance between the endogenous production of reactive oxygen species (ROS) and the activity of antioxidant systems. In other words, the cellular concentration of ROS exceeds the capacity of the cell to eliminate them. The crude drug of rosemary and its constituents have been shown to have both antioxidant and pro-oxidant properties both in vitro and in vivo (3,10). Nowadays, according to these results, the use of natural antioxidants has been a growing demand in different industries not only for their usefulness as a natural antioxidant in various products but also for their benefits in human health, and they are cost-effective and accessible (20,21). For example, natural antioxidant or their derivatives are increasingly used to treat various pathological liver conditions, Alzheimer's disease, and the like (3,10,22). In this sense, rosemary is introduced as a natural antioxidant source that is mostly used and commercialized because of the high content of different compounds (5,13).

It is shown that EOs can be used for conserving foods and cosmetics and medicinal purposes because of their biological activities (2,3,8,10,13). The rosemary EO has no colour or it looks pale yellow. It is lighter than water with a characteristic odour of the plant and mostly consists of monoterpenes such as 1,8-cineole, camphor, and α-pinene (2,10). Due to its antioxidant and antimicrobial activity, it can be used as a bio-preservative agent in food industries and as a seasoning for foodstuffs such as meat dishes, salami, sauces, and as part of perfume or cosmetic finished products because of its attractive and pleasant aroma. In addition, the isolated EO and rosmarinic acid derivatives from rosemary show various medicinal applications including anti-inflammatory, antidepressant, cognition-enhancing, and DNA protective and anticancer effects (3,10).

Considering numerous applications of rosemary, it is important to obtain the maximum amount of these specific effective compounds. According to British Pharmacopoeia 2017, the whole and dried leaves of rosemary generally contain a minimum of 1.2% (v/w) and 3% EO hydroxycinnamic acid derivatives, respectively (1). There are many ways to reach the aim of the maximum percentage of active compounds (e.g., genetic modification, controlled cultivation conditions, harvesting time, post-harvesting processes, and stages of maturity or the optimization of extraction parameters). The review of the literature represents that many studies have focused on the effects of the above-mentioned factors (4,8,12-15,19,23-28) but among them, control of the harvesting time, is the most convenient task, and it was selected for this study accordingly. In other words, harvesting should be done during the optimal time.

The best harvesting time should be determined by the quality and quantity of active compounds in the herbs. Zaouali et al (13) reported that the highest yield of EO (1.43%) was obtained from leaves collected at the flowering stage although the total polyphenol derivatives were more found in leaves, and their total percentage was high at the vegetative and fructifying stage. Considering these reports, the variation in the content of the EO and rosmarinic acid derivatives from *Rosmarinus officinalis* dried leaves collected in Northern Iran (Mazandaran province) was studied at three different times in one day (before the sunrise, at noon, and after the sunset).

Materials and Methods

Collection of Plant Materials

The fresh aerial parts of the cultivated rosemary (about 5.0 kg) were hand-harvested in winter from the botanical garden of Soha Jissa Company, in Salmanshahr Industrial Parks Organization, Mazandaran Province (36º 40′ 37.5″ N, 51º 09′ 46.4″ E). A voucher specimen (MPH-1390) of the plant was identified by Dr. Ali Sonboli and deposited in the herbarium of the Medicinal Plant and Drug Research Institute of Shahid Beheshti University, Tehran, Iran. Harvesting was done at three different times and temperatures, including before the sunrise (8°C), at noon (11°C), and after the sunset (6°C) and then dried at 30°C. The dried leaves were separated from the stalks and stored in well-closed container desiccators at 4°C.

Chemicals Reagents

Methanol, ethanol, hydrochloric acid, sodium molybdate,

sodium nitrite, anhydrous sodium sulphate, hexane, and sodium hydroxide all were purchased from Merck Company, and 1,8-cineol (99% pure) was provided from Sigma-Aldrich Company.

EO Extraction

First, 200 g of each sample were submitted to a Clevenger-type apparatus for the extraction of the EO with 1 L of water for 4 hours. All experiments were performed in three replications. The EO was directly collected after draining water, dried under anhydrous sodium sulphate, and stored in a dark well-closed container at 4°C before gas chromatography (GC)/flame ionization detector (FID) and gas chromatography-mass spectrometry (GC/MS) analyses. The yield of the EO was calculated according to the dry vegetal matter using equation (1) as follows (23):

$$Percentage\,of\ Essential\ oil = \frac{m_{HE}}{m_s} \times 100 \quad (1)$$

where m_{HE} = Essential oil mass (g), m_S = Dry vegetal matter mass (anhydrous herb) (g)

GC/FID and GC/MS Identification

The GC/FID analysis of the samples was done using a ThermoQuest-Finnigan instrument equipped with an FID at 280°C and an Rtx-5 fused silica column (30 m × 0.25 mm i.d., film thickness 0.25 µm). The carrier was nitrogen with a flow rate of 1.1 mL/min. The split ratio was 1:50. The column temperature was increased from 60°C to 250°C at a rate of 5°C/min. The injector temperatures were set at 250°C.

The GC/MS analysis was carried out on a ThermoQuest-Finnigan TRACE MS model. The samples were analyzed on a fused-silica capillary column Rtx-5 (30 m × 0.25 mm I.D., film thickness 0.25 µm). The carrier gas was helium with a constant flow of 1.1 mL/min, and the injector temperature was kept at 250°C. The injection volume was 0.1 µL (1% solution of the EO in hexane) and a split ratio of 1:10. The initial temperature of the oven was 60°C and it was increased at a rate of 5°C/min to 250°C and then constant at 250°C for 10 minutes. The ionization energy was adjusted to 70 eV and a mass analyzer. The components of the oils were determined through computing their corresponding retention indicators by applying the pre-defined temperature for n-alkanes (C_9–C_{24}) and the oil on an Rtx-5 fused silica column (30 m × 0.25 mm i.d., film thickness 0.25 µm) without changing chromatographic conditions. The individual components were identified by comparing the mass spectra against the reference mass spectra (Adams and Wiley 7.0) or authentic compounds. Thus, retention indices were compared against authentic compounds or those found in the literature.

Quantitative Determination of Total Hydroxycinnamic Derivatives (THD) as Rosmarinic Acid

The THD as Rosmarinic acid was determined using sodium nitrite and sodium molybdate colorimetric assay according to the rosemary leaf monograph in British Pharmacopoeia 2017 (1) by the UV/Vis Shimadzu spectrophotometer model 1700 with three replicates.

The dried samples (before sunrise, at noon, and after sunset) were powdered and each powder (0.200 g) was transferred to a flask separately, and then the extraction was done with 80 mL of ethanol (50% V/V) as a solvent in a water-bath using a reflux condenser for half an hour. After filtering, the residue was rinsed with 10 mL of the solvent. The filtrate and the rinsing were mixed and diluted to 100 mL. The stock solution (1 mL) was mixed with 2 mL of hydrochloric acid (0.5 M), 2 mL of aqueous solution of sodium nitrite and sodium molybdate (10% W/V relative to each of them), and 2 mL of sodium hydroxide solution (8.5% W/V), respectively, and diluted to 10 mL with water as the test solution. The absorbance of the test solution was immediately read at 505 nm versus the blank (1 mL of the stock solution that was diluted to 10.0 mL using water). Then, Eq. (2) was applied for calculating the percentage of total hydroxycinnamic derivatives as rosmarinic acid (THD):

$$THD = \frac{A \times 2.5}{m}$$

A = Absorbance of the test solution versus the blank at 505 nm

m = Mass of the sample to be tested (anhydrous herb) (g)

Statistical Analysis

The effect of different harvesting times was validated by the analysis of variance (ANOVA). The obtained results from absorbance measures, along with the content and composition of the EO in each sample were used for the correlation test. All statistical analyses were done at a 0.05 level of probability.

Results

Variation of the Total EO Percentage

The average amount of the EO varied from 1.095 (g) before the sunrise to 0.759 (g) at noon and to 0.483 (g) after the sunset. The total EO percentage was calculated by Eq. (1), and the mean of the three replicates indicated that the total EO varied from 3.65% before the sunrise to 2.53% at noon and to 1.61% after the sunset. The highest EO yield was observed before the sunrise. The results of the ANOVA test (Table 1) and the comparison of F_{exp} with F_{crit} statistically confirmed that the effect of time harvesting during one day on the total EO yield is an important factor ($F_{exp} > F_{crit}$).

Variation of the EO Chemical Composition

The GC/MS analyses of the samples (Figure 1) revealed the presence of a total of 24, 28, and 26 components before the sunrise, at noon, and after the sunset samples, representing 99.8%, 99.0%, and 99.0% of the identified

Table 1. ANOVA Results for Evaluating the Effect of the Harvesting Time on the Total the Essential Oil Content

Source of Variation	SS	*df*	MS	F_{exp}	*P* Value	F_{crit}
Harvesting time	6.280867	2	3.140433	945.28094	3.1663E-08	5.143253
Replicate	0.019933	6	0.003322			
Total	6.3008	8				

Note. ANOVA: Analysis of variance; SS: sum of square; df: degree of freedom; MS: Mean of sum of square; ($F_{exp.} > F_{crit}$).

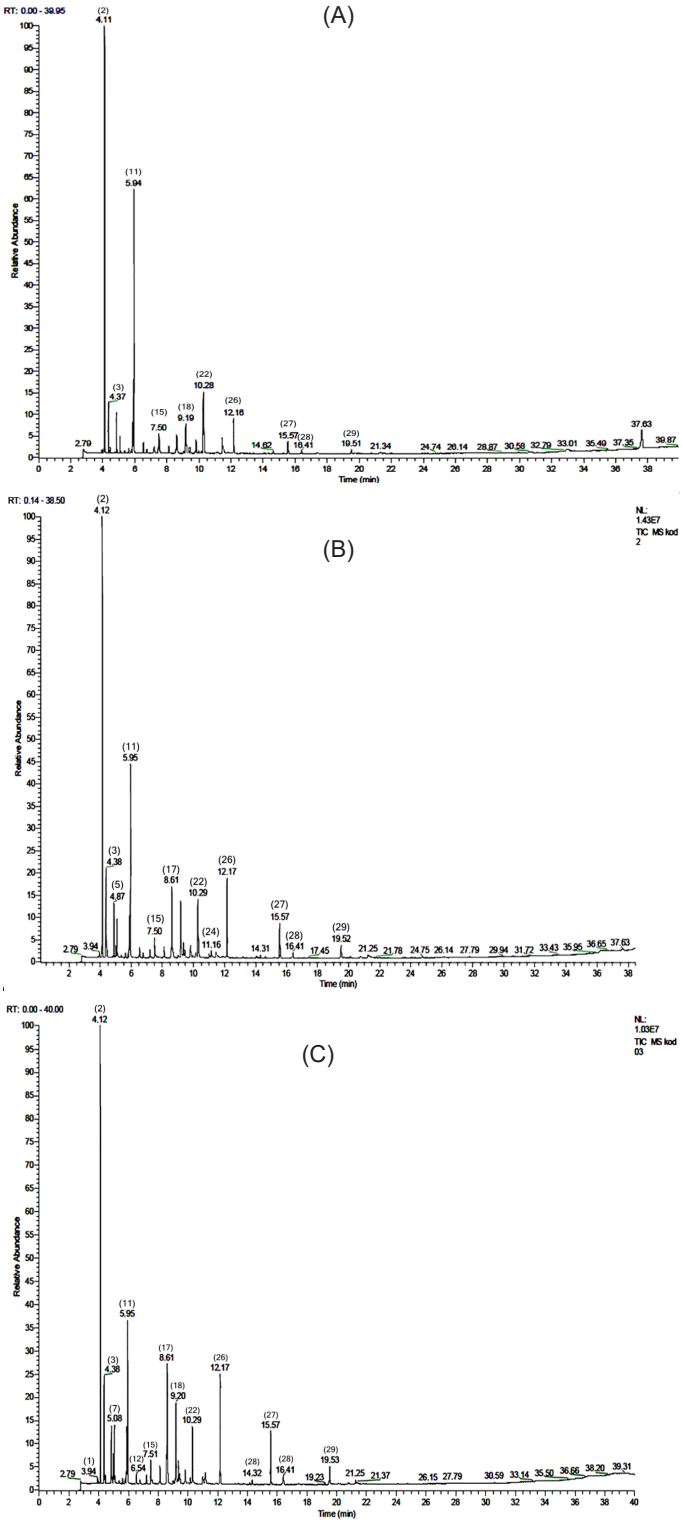

Figure 1. Comparison GC/MS Chromatogram in 3 Harvesting Times: (A) Before Sunrise, (B) at Noon, and (C) After Sunset. Note. GC/MS: Gas chromatography. Serial numbers are indicated on the tip of each peak.

volatile components in the rosemary EO, respectively (Table 2). Further, the calculated retention index, literature retention index, absolute difference, and the level of change in different harvesting times are shown in Table 2. The result related to the presence of some components specific for the rosemary EO, including 1,8-cineole, camphor, α-pinene, borneol, camphene, α-terpineol, bornyl acetate, β-caryophyllene, p-cymene, limonene, linalool, myrcene, terpinolene, and verbenone (2,3), is in agreement with previous studies (2-4,8,12-15,19,23,24). However, the percentage of each component differs based on the environmental condition, cultivation, and the like. As shown, some compounds varied over the harvesting time during one day although previous results showed that the season and stage of harvesting could change the yield and composition of the EO (8,12,13,15,19). According to these results (Table 2), some variations, especially for components specific to the EO of rosemary are significant. The results revealed that α-pinene varied from 32.8% before the sunrise to 26.6% at noon and to 23% after the sunset. Moreover, β-myrcene varied from 1.4% before the sunrise to 2.8% at noon and to 3.5% after the sunset. Additionally, 1,8-cineole varied from 23.6% before the sunrise to 14.9% at noon and to 10.5% after the sunset (each of them shows a decreasing trend from before the sunrise to after the sunset). On the contrary, camphor, bornyl acetate, and borneol have an increasing trend. Camphor varied from 2.7% before the sunrise to 7% at noon and to 9.8% after the sunset or bornyl acetate varied from 4% before the sunrise to 7.2 % at noon and to 8.6% after the sunset. Eventually, borneol varied from 3.9% before the sunrise to 5.7 % at noon and to 6.9%

Table 2. The Chemical Composition of Essential Oils in Three Harvesting Times in 1 Day

No.	Rt	Components	CRI	LRI	Before Sunrise (%)	At Noon (%)	After Sunset (%)	Absolute Difference	Levels of Changes[a]
1	3.9	α-Thujene	924	924	Nd	0.2	0.2	0.2	<1%
2	4.1	α-Pinene	934	932	32.8	26.6	23	9.8	>5%
3	4.4	Camphene	949	946	3.8	5.7	5.9	2.1	1%< ; <3%
4	4.5	Thuja-2,4(10)-diene	954	953	0.3	0.4	0.4	0.1	<1%
5	4.9	β-Pinene	978	974	3.3	3.6	3.2	0.1	<1%
6	5.0	3-Octanone	986	985	Nd	0.9	1.9	1.9	1%< ; <3%
7	5.1	β-Myrecene	991	988	1.4	2.8	3.5	2.1	1%< ; <3%
8	5.6	δ-3-Carene	1018	1013	0.3	0.3	0.2	0.1	<1%
9	5.7	p-Cymene	1025	1020	0.2	0.3	0.3	0.1	<1%
10	5.8	Limonene	1029	1024	2.7	3.1	3.8	1.1	1%<; <3%
11	5.9	1,8-Cineole	1032	1026	23.6	14.9	10.5	13.1	>5%
12	6.5	γ-Terpinene	1058	1054	1.0	0.8	0.6	0.4	<1%
13	6.7	cis-Sabinene hydrate	1069	1065	0.4	0.3	Nd	0.4	<1%
14	7.2	Terpinolene	1089	1086	0.6	0.6	0.6	Nd	<1%
15	7.5	Linalool	1102	1095	2.5	2.1	2.2	0.3	<1%
16	8.1	Chrysanthenone	1127	1124	0.7	0.9	1.3	0.6	<1%
17	8.6	Camphor	1147	1141	2.7	7.0	9.8	7.1	>5%
18	9.1	Borneol	1171	1165	3.9	5.7	6.9	3.0	3%< ; <5%
19	9.3	Isopinocampheol	1178	1176	0.5	1.3	1.7	1.2	1%< ; <3%
20	9.4	Terpine-4-ol	1182	1180	0.6	0.6	0.7	0.1	<1%
21	9.8	α- Terpineol	1197	1186	1.7	1.3	1.1	0.6	<1%
22	10.2	Verbenone	1215	1204	7.7	5.7	4.9	2.8	1%< ; <3%
23	11.1	Nerol	1248	1233	Nd	0.7	1.1	1.1	1%< ; <3%
24	11.4	Geraniol	1259	1249	3.3	Nd	Nd	3.3	3%< ; <5%
25	11.5	trans-Myrtanol	1260	1258	Nd	0.9	Nd	Nd	<1%
26	12.1	Bornyl actetae	1287	1284	4.0	7.2	8.6	4.6	3%< ; <5%
27	15.5	β-Caryophyllene	1422	1417	1.4	3.3	4.5	3.1	3%< ; <5%
28	16.4	α-Humulene	1456	1452	Nd	0.5	0.6	0.6	<1%
29	19.5	Caryophyllene oxide	1587	1582	0.4	1.3	1.5	1.1	1%< ; <3%

Monoterpene hydrocarbons (Compounds 1-5,7-10,12, and 14)	46.4	44.4	41.7
Oxygenated monoterpenes (Compounds 11, 13, and 15-28)	51.6	48.6	48.8
Sesquiterpene hydrocarbons (Compounds 29-30)	1.4	3.8	5.1
Oxygenated sesquiterpene (compound 31)	0.4	1.3	1.5
Aliphatic ketone (compound 6)	nd	0.9	1.9
Total	99.8	99.0	99.0

RT, Retention time; CRI, Calculated retention index; LRI, Literature retention index; ND, Not detected.
[a] In house conditions: Less than 1% is not important, between 1% and 3% is moderately important, between 3% and 5% is important and more than 5% is very important.

after the sunset. The percentages of other components with variations of more than 1% are shown in Figure 2. Some other components such as α-thujene, β-pinene, and p-cymene demonstrated no significant variation during the harvesting time and their variations were less than 1% (Table 2).

Variation of THD Content as Rosmarinic Acid

Polyphenols such as rosmarinic acid and other hydroxycinnamic acid derivatives are introduced as other most well-known antioxidant compounds in rosemary. Although it is accepted that the total percentage of polyphenols in rosemary responds to external and internal factors (18). Nevertheless, the effect of the harvesting time on the total polyphenol content in our study was not significant, indicating 5.05%, 5.09%, and 4.99% before sunrise, at noon, and after sunset samples, respectively. Table 3 demonstrates the ANOVA results for THD by comparing the $F_{exp.}$ with $F_{crit.}$ ($F_{exp.} < F_{crit}$). Clearly, different harvesting times in one day do not have any significant effect on the THD. In other words, it can be suggested that the biosynthetic pathway of polyphenols is more difficult than the EO.

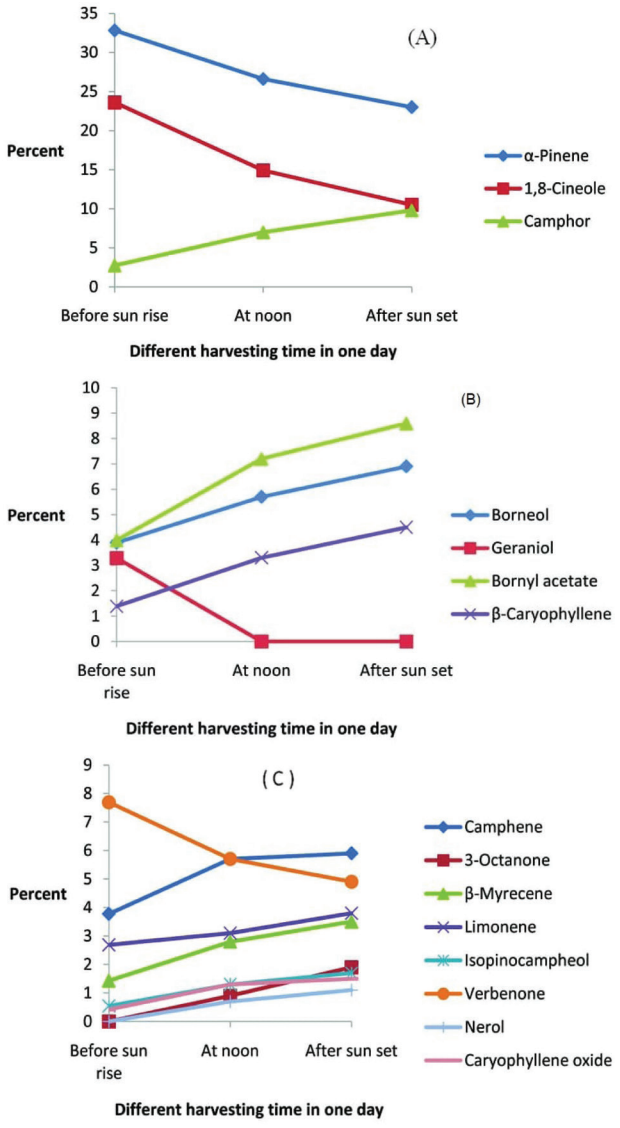

Figure 2. Variation of Different Components in the Essential Oil According to Table 2 (A-C): Very Important, Important, and Moderately Important Variation, Respectively.

Discussion

According to the results in Table 1, the harvesting time in which the rosemary shrubs are harvested, affects the yielded EO. Thus, statistically significant differences in the yields were detected between three samples, before the sunrise, at noon, and after the sunset sample (3.65%, 2.53%, and 1.61%, respectively). On the other hand, the EO profile by the GC/Mass analysis, the presence of 24, 28, and 26 components with different percentages were identified before-the-sunrise, at-noon, and after-the-sunset samples, respectively. Thus, based on numerous reports, rosemary EO with respect to chemical compositions can be divided into different chemotypes (14). According to our results (Table 1), different chemotypes are present in one rosemary shrub during one day.

Based on numerous research reports, different rosemary chemotypes show high variations in their antimicrobial and antioxidant properties (14). These disparities are the result of relative variations in their yield and chemical contents (i.e., EO and polyphenols) depending on environmental conditions such as location, harvesting season, and the like, which were reported by Celiktas et al, Papageorgiou et al, and Jordán et al (14, 15, 19). Papageorgiou et al (19) found that the yield varied from 1.8 to 3.3% v/w (dry weight) with season variations. The highest EO yield was obtained in May. Based on their results, the yield of the EO was greater during the flowering season. Furthermore, Jordán et al (14) showed that the obtained EO varied from 1.74% to 2.58% depending on bioclimatic conditions. To the best of our knowledge, no study has so far reported the variation of the EO content during one day.

The variation of EO yields and composition for leaves at different harvesting times during one day taken from the same herb indicates that the herb through changing the composition of the EO reacts to environmental factors. In other words, harvesting time during one day is just as important as harvesting time during a year. Moreover, the production and composition of rosemary EO might be stimulated by the intensity of sunlight although understanding this process needs more studies. On the contrary, hydroxycinnamic acid derivatives have not revealed any significant variations during one day, but the dependency and variation of the phenolic composition, along with the antioxidant activity to the harvesting time in different months were reported by Papageorgiou et al (19). Nonetheless, to our knowledge, no study has so far reported the variation of hydroxycinnamic acid derivative

Table 3. ANOVA Results of Total Hydroxycinnamic Acid Derivative Variations in 3 Different Harvesting Times in 1 Day

Source of Variation	SS	*df*	MS	F_{exp}	*P* Value	F_{crit}
Harvesting time	0.018466667	2	0.009233333	1.901602	0.229271	5.143253
Replicate	0.029133333	6	0.004855556			
Total	0.0476	8				

Note. ANOVA: Analysis of variance; SS: sum of square; df: degree of freedom; MS: Mean of sum of square; ($F_{exp.} > F_{crit}$).

content during one day.

Jordán et al (14) indicated that a different sample of rosemary EO represents different behaviors against *Salmonella typhimurium* and *Escherichia coli* (as gram-negative pathogens) and *Listeria monocytogenes* and *Staphylococcus aureus* (as gram-positive strains). Rašković et al also exhibited variations in the half-maximal inhibitory concentration value, antioxidant activity, and the hepatoprotective, and nephroprotective effects of the rosemary EO, and the methanolic or aqueous extract of rosemary with different compositions (10). Thus, active compounds in herbs must be controlled based on numerous reports about the different antioxidant and antibacterial strength of rosemary or its components.

However, it is noteworthy that the antioxidant, antibacterial, and other properties of rosemary extracts have been thoroughly evaluated, but regarding IC50 studies and the adverse effect of some natural compounds, attention to the dosage and content of compounds in crud drugs or the finished product is an extremely important factor that may be overlooked occasionally. For example, camphor, as a bicyclic monoterpene which is present in the EO in a relatively high amount, can be a toxic compound and its small amount causes poisoning in children (10). On the other hand, numerous studies have suggested that ROS has been introduced as the most important factor in many diseases such as subclinical hepatitis without jaundice, inflammatory necrotic hepatitis, liver cirrhosis, hepatocellular carcinoma induction, prostate cancer, and the like. Different factors such as cigarette smoke, anticancer drugs, UV irradiation, and chemo-preventive agents induce ROS production thus the introduction of natural antioxidant compounds with high activity and the appropriate dose for the inhibition of the destructive effects of ROS, which can prompt oxidative stress and damage cellular macromolecules, is an important mission for scientists (10,29). Based on the obtained data, the camphor is at the minimum level in before-the-sunrise sample. In other words, the purification and separation or reduction in extraction processes can be done from the farms (Table 2 and Figure 2a).

Conclusions

Based on the findings of this investigation, herbs change their component in some groups, and thus harvesting time can be optimized based on our needs. For example, if only THD is important, thus the harvesting time during one day is not important. However, if we want the dried herb to have the maximum EO with a special composition, attention to harvesting time during one day is essential (e.g., the season of harvesting). In other words, choosing the best harvest time is a highly important factor in the quality of raw materials (dried plants) and final products (i.e., medicines, food, and cosmetics).

Authors' Contribution

Study design and article preparation: All authors; intervention and data collection: SMHK; statistical analysis: RSMP. All authors read and approved the final format of manuscript.

Conflict of Interests

Authors have no conflict of interests.

Ethical Issues

The experimental procedures were accomplished upon obtaining permission from the Ethics Committee of Kharazmi University, Chemistry Faculty, Tehran, Iran in December 2017 (with the number 1311990-15717).

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