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ORIGINAL ARTICLE

Evaluation of Marketed Rosemary Essential Oils (*Rosmarinus officinalis* L.) in Terms of European Pharmacopoeia 10.0 Criteria

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ABSTRACT

Objectives: Various pure rosemary essential oil containing commercial products are in demand for their health-promoting and cosmetic claims in Türkiye. Although they are natural and harmless, they should be in compliance with European Pharmacopoeia (EP) criteria. Therefore, in this study, 15 rosemary oil samples sold in pharmacies, herbal shops, and online platforms in Türkiye were investigated in terms of "Rosemary Oil" EP 10.0. monograph criteria. In the current study, we aimed to evaluate the current quality status of rosemary essential oils in the Turkish market.

Materials and Methods: Appearance, fatty oils and resinified essential oils, relative density, refractive index, optical rotation, and acid value tests were performed according to EP 10.0 and compared with the given standards. In addition, thin layer chromatography (TLC) and gas chromatography-mass spectrometry (GC-MS) analysis were conducted on all samples for advanced understanding of their phytochemical profile and harmony with EP standards.

Results: Fifteen pure rosemary oil-containing products from the Turkish market were evaluated. All of the samples were licensed as cosmetic products in Türkiye *via* the Ministry of Health. 83.1 to 96.9% of the ingredients of all samples were determined *via* GC-MS analysis. Results demonstrated that none of the samples from the Turkish rosemary essential oil market fully complied with the EP rosemary oil monograph standards.

Conclusion: Considering our data, it was revealed that enhanced regulations and auditing mechanisms are needed to improve the quality of products. When the difference between the sources of purchase is assessed, pharmacies are still better locations to obtain such products.

Keywords: *Rosmarinus officinalis* L., rosemary oil, European Pharmacopoeia, GC-MS, essential oil

INTRODUCTION

With the increasing interest in natural-based therapies, use of essential oils for medical and cosmetic purposes is accordingly accumulating. Essential oils have various biological activities, thus scientific studies investigating aromatherapy are growing.[1] *Rosmarinus officinalis* L. is a member of Lamiaceae family, grown naturally and widely cultivated in the Mediterranean region, particularly for culinary purposes. The aerial parts have distinct characteristic fragrance and flavor.[2] In traditional medicinal systems, aerial parts of *R. officinalis* are used as tea or tinctures against gastrointestinal system (GIS) disorders and inflammatory diseases. In addition to crude herbal preparations, essential oil of *R. officinalis* has also significant biological activities, thus, popularity of use in aromatherapy is escalating.[3] Previous studies demonstrated that essential oil of *R. officinalis* may be used against circulatory problems, GIS disorders, muscular pain, and inflammations.[4] Reported biological properties of the essential oil is attributed to several ingredients, primarily monoterpenes, such as 1,8-cineole, borneol, and limonene.[4] Therefore, it is crucial to evaluate the phytochemical profile of an essential oil before its use for medical and cosmetic purposes.

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Pharmacopoeias are official publications that establish necessary quality requirements for both synthetic and natural based medical products aiming to promote and protect public health. The Republic of Türkiye is legally bound (or responsible or have to obey the rules of) to the European Pharmacopoeia (EP), which contains more than 200 herbal drug monographs. Products claiming to contain pure *R. officinalis* essential oil are readily available in Turkish market and most of them are licensed as cosmetic products *via* the Ministry of Health. It may be beneficial to evaluate marketed products in terms of EP 10.0, which is the most up to date version, for better understanding the current situation of the essential oil market in terms of quality that strongly affects public health. Yet, a literature survey revealed that there is a lack of studies investigating quality situation of *R. officinalis* products on the market based on the rosemary oil monograph in the EP. For this reason, in this study, 15 samples that were sold as pure rosemary oil were investigated, 5 of them were purchased in pharmacies, while 10 of them were purchased from other sales channels such as herbalists and online platforms. Relative density, refractive index, optical rotation, and acid value of the samples were calculated through assays given in pharmacopeia. Similarly, appearance and thin layer chromatography (TLC) results were visually investigated based on the given criteria. Furthermore, chromatographic profiles of the samples are given in the monograph for two different chemotypes of rosemary oil. For determining the correspondence of the samples with the monograph, gas chromatography-mass spectrometry (GC-MS) analysis was conducted. Twelve different components were given in the monograph for both chemotypes and with different ranges. Results of GC-MS analysis were compared and analyzed with the required ranges stated in the monograph.

In the current study, it was aimed to evaluate current quality status of rosemary essential oils in the Turkish market for creating a plain picture. It is an essential public health requirement for products that claim to have health benefits to contain the specified international standards.

MATERIALS AND METHODS

Materials

Products containing pure rosemary essential oil were procured from herbalists, online shopping platforms, and pharmacies in the Istanbul region. All products are registered as cosmetics by the Turkish Ministry of Health. In addition, labels of all oil samples claim to contain pure rosemary oil. Until the experiments, products were maintained at room temperature in tightly closed containers and protected from sunlight. All products were coded indicating their source (P: pharmacy, A: other sources). All standards and solvents (1,8-cineole, borneol, bornyl acetate, hexane, toluene, ethyl acetate, *etc.*) were purchased from Sigma-Aldrich.

Appearance, labelling, and fatty oils and resinified essential oils

All tests were applied as stated in EP with small modifications.[5] All samples were dripped on the filter paper as a drop and the filter paper was kept in an oven at 80 °C for 30 minutes for the fatty oils and resinified essential oils tests. The samples were filled in a glass tube and photographed for evaluation of their appearance. Labels of the samples were checked for presence of knowledge of chemotype.

Relative density, refractive index, optical rotation, and acid value

Relative density, refractive index, optical rotation, and acid value assays were conducted according to the methods given in EP 10.0. Relative density results were evaluated using a pycnometer and volume of the essential oil samples with an equivalent volume of water at 20 °C was measured. For refractive index analysis, Anton Paar-Abbemat 3100 device and Anton Paar-MCP 150 device for optical rotation assay were used. The acid values of the samples were determined by the titrimetric method described in EP. All experiments were conducted in triplicate and results were given with average and standard deviation (SD).[5]

TLC analysis

TLC analyses were conducted according to indications given in rosemary monograph in EP. Standards of borneol, bornyl acetate, and cineole were dissolved in toluene and used as reference solutions. 0.5 mL of samples were also dissolved in same solvent as test solutions. Ethyl acetate and toluene mixture (5:95, *v/v*) was used as mobile phase. Detections were completed with vanillin reagent application and immediately heating the plate in an oven at 100-105 °C for 10 min.[5]

GC-MS analysis

Qualitative and quantitative analyses were performed using GC-MS. Agilent Technologies 7890 A GC system equipped with a DP-5 MS column (30 m x 0.25 mm x 0.25 µm) was used. The oven temperature was started at 60 °C and, then, steadily increased to 246 °C with 3 °C increase *per* minute. Helium was used as the mobile phase with 0.9 mL/min flow rate. Split mode was used with 50:1 ratio with 1 µL sample volume. Relative retention index (RRI) was calculated *via* comparison with (C_4-C_{40}) standards. Identification of the essential oil components was completed by comparison of their RRI calculated against *n*-alkanes and relative retention times with those of authentic samples and mass spectra obtained from NIST14 and Wiley7 mass spectra libraries as well as MS literature data was used for the identification.[6]

RESULTS

Appearance, labelling, and fatty oils and resinified essential oils

EP 10.0 states that rosemary oil should be clear, mobile, colorless or pale-yellow liquid with characteristic odor. EP 10.0 states that all of the rosemary oil samples should indicate the chemotype of the ingredient on the labels. Conformance of the samples to EP criteria is evaluated in Figure 1. Results exhibited that appearance properties of all the samples were compatible with EP; however, only samples P4 and P5 indicated the chemotype of the oil in the label. Fatty oils and resinified essential oils were conducted to reveal possible adulteration of oils with non-volatile materials. After drying in the incubator,

Experiment	Reference interval	P1	P2	P3	P4	P5	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10
Fatty oils and resinified essential oils		✓	✓	X	✓	✓	X	X	✓	X	✓	✓	✓	✓	✓	✓
Appearance		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Relative density	0.895 - 0.920	0.893	0.876	0.900	0.919	0.945	0.893	0.903	0.901	0.888	0.880	0.920	0.890	0.886	0.889	0.889
Retractive index	1.464 - 1.473	1.467	1.469	1.473	1.471	1.469	1.474	1.473	1.466	1.474	1.472	1.467	1.471	1.467	1.475	1.471
Optical rotation	-5, 8	3.73	5.86	6.71	6.2	23.88	-2.92	1	5.88	-2.79	5.71	0.2	5.97	2.3	4.3	-0.33
Acidity index	Maximum 1	1.40	2.16	1.43	0.45	1.6	1.12	0.45	0.62	0.51	0.34	0.45	0.28	0.69	1.46	0.55
TLC		✓	✓	✓	✓	✓	✓	X	X	✓	✓	✓	X	X	✓	✓
Labelling		X	X	X	✓	✓	X	X	X	X	X	X	X	X	X	X

Figure 1. General evaluation of EP tests

*Green boxes show suitability, red boxes are indicative of inconvinience with ranges indicated in EP

EP: European Pharmacopoeia, TLC: Thin layer chromatography

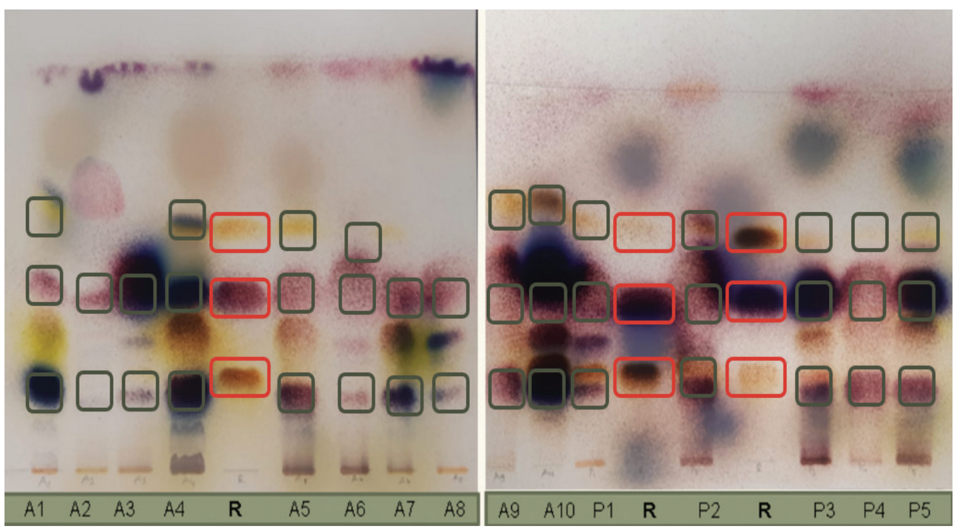

Figure 2. TLC chromatograms of all samples. R: Reference mixture; bornyl acetate, cineole, and borneol from top to bottom. Mobile phase; ethyl acetate:toluene (5:95, *v/v*), TLC: Thin layer chromatography

P3, A1, A2, and A4 samples exhibited a remaining spot in the filter paper, which indicates the presence of non-volatile ingredients Figure 1.

Relative density, refractive index, optical rotation, and acid value

The relative density, refractive index, optical rotation, and acid value results of 15 essential oil samples are given in Table 1. According to EP 10.0 standards, the relative density value for rosemary oil should be between 0.895 and 0.920, 1.464 and 1.473 for refractive index, -5° and 8° for optical rotation, and the acid value must be lower than 1.0. Compatibility of samples with EP 10.0 standards was evaluated and summarized in Figure 1.

TLC analysis

According to EP 10.0, bornyl acetate should appear as a bluish-gray zone of low intensity (top), cineole as an intense blue zone (midline), and borneol as a violet-blue zone of medium intensity (bottom). All of the samples were evaluated with TLC method; the images of the plaques and coherence of all ingredients with the monograph are given in Figures 1 and 2, respectively.

GC-MS analysis

EP 10.0 mentions two different chemotypes of rosemary oil. Results of GC-MS analyses conducted on all samples are given in Table 2, where 83.1 to 96.9% of the ingredients were determined for all samples. Chromatograms that indicate the ingredients specified in EP are given in Figure 3. GC-MS results were evaluated in accordance with the most proximate chemotype and coherence of all ingredients with the monograph is given in Figure 4.

DISCUSSION

EP contains specific individual monographs for some essential oils, which are widely used in pharmacy and have medicinal or cosmetic utilization. Thus, it is crucial for a product that contains pure essential oil to meet the criteria stated in monographs to ensure its scientific basis for aforementioned utilizations.[7] Importance of quality standards of herbal products in the market is increasing because public attention to complementary therapies and natural cosmetics is growing and amplified competition between producers creates possible

Table 1. Results of relative density, refractive index, optical rotation, and acid value of the tested samples

	P1	P2	P3	P4	P5	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10
Relative density	0.89 ± 0.02	0.79 ± 0.16	0.90 ± 0.02	0.92 ± 0.03	0.95 ± 0.10	0.74 ± 0.26	0.90 ± 0.05	0.80 ± 0.17	0.89 ± 0.01	0.79 ± 0.15	0.92 ± 0.03	0.89 ± 0.01	0.89 ± 0.01	0.89 ± 0.01	0.89 ± 0.01
Refractive index	1.47 ± 0.001	1.47 ± 0.000	1.47 ± 0.000	1.47 ± 0.001	1.47 ± 0.000	1.47 ± 0.000	1.47 ± 0.001	1.47 ± 0.000	1.47 ± 0.000	1.47 ± 0.000	1.47 ± 0.000	1.47 ± 0.000	1.47 ± 0.000	1.48 ± 0.003	1.47 ± 0.001
Optical rotation	3.76° ± 0.11	5.90°± 0.14	6.71° ± 1.82	6.20° ± 0.11	23.82° ± 0.59	-2.92° ± 0.07	1.00° ± 0.46	5.88° ± 0.30	-2.79° ± 0.02	5.71° ± 0.44	0.20° ± 0.11	5.97° ± 0.12	2.33° ± 1.15	4.33° ± 0.58	-0.33° ± 4.04
Acid value	1.40 ± 0.08	2.16 ± 0.20	1.43 ± 0.36	0.45 ± 0.16	1.60 ± 0.12	1.12 ± 0.00	0.45 ± 0.01	0.62 ± 0.08	0.50 ± 0.08	0.34 ± 0.00	0.45 ± 0.00	0.28 ± 0.08	0.69 ± 0.14	1.46 ± 0.00	0.55 ± 0.06

All test were done in triplicate and results were given in average ± standard deviation (SD)

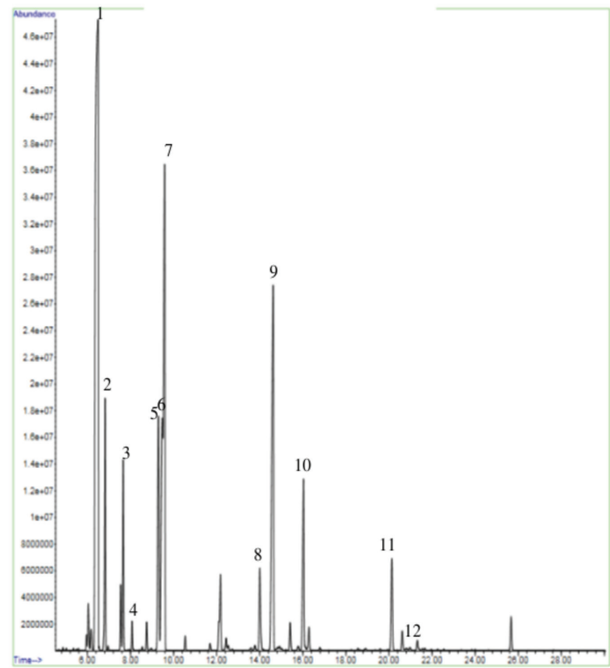

Figure 3. GC-MS chromatograms of P5 sample showing the chemical components given in pharmacopoeia: 1: α-pinene, 2: camphene, 3: β-pinene, 4: β-myrcene, 5: limonene, 6: cineole, 7: *p*-cymene, 8: camphor, 9: borneol, 10: α-terpineol, 11: bornyl acetate, and 12: verbenone

GC-MS: Gas chromatography-mass spectrometry

exploitation environment in conjunction with insufficient regulations and low knowledge level of the public. Thus, conducting regular scientific market analysis may create a clear understanding of the current status and may lead both public authorities, healthcare professionals, and the public to be deliberate against such products. There are several studies conducted in Türkiye that evaluate herbal drugs from Turkish market for their compliance with EP. Previous studies on the evaluation of *Hibiscus sabdariffa* L., *Eucalyptus* L'Her, and *Alchemilla* L. samples collected from the Turkish market for their consistency with EP are examples on that manner.[8-10] All studies demonstrated complications on the quality of the drugs that are freely sold in the market for medicinal purposes. In addition, two recent studies evaluated fixed oils sold on the Turkish market. Nearly all of the almond and safflower oil samples from the Turkish market were reported as lacking quality in terms of EP criteria.[11,12] Previous studies noticeably demonstrated the importance of such studies, when considering increasing public attention to natural based products for various medicinal purposes. Similarly, public demand for aromatherapy that uses essential oils for medicinal purposes is increasing.[13] Nonetheless, there is an obvious scarcity of studies assessing essential oil-containing products in the Turkish market for their compliance with EP. Basic objective of EP is ensuring the standards of products, so consumers can purchase any product without being anxious about its quality. In this context, for this study, 15 commercial products (5 from pharmacies and 10 from other sources) that claimed to contain pure rosemary oil were

Components	P1	P2	P3	P4	P5	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10
α-Pinene	10.75	11.64	13.50	14.51	33.33	21.9	11.19	10.2	20.3	11.56	10.2	14.6	14.7	6.6	4.58
Camphene	5.1	5.88	3.66	5.53	4.159	3.109	3.06	6.35	3.26	4.89	5.18	5.3	8.7	2.3	3.04
β-Pinene	6.41	7.77	2.58	2.91	3.24	3.03	1.54	8.21	3.12	3.01	8.98	2.8	5.6	1.4	2.48
β-Myrcene	1.34	1.56	0.55	0.51	0.49	0.18	2.47	1.79	0.19	0.82	1.61	0.56	2.5	0.25	1.25
Limonene	-	-	-	-	6.91	3.04	3.17	-	2.99	11.1	-	-	-	-	19.82
1.8-Cineole	33.4	31.7	24.3	22.6	13.4	9.5	44.7	36.2	9.3	4.8	35.1	23.9	31.1	21.5	8.4
p-Cymene	3.45	2.28	6.67	6.67	6.18	1.62	3.95	5.02	1.7	9.51	2.57	6.28	1.97	6.6	4.9
Camphor	15.6	16.3	10.9	2.82	2.06	1.50	5.49	17.50	1.38	3.70	12.11	3.86	15.2	4.62	18.4
Bornyl acetate	1.92	1.22	2.00	3.20	2.18	1.12	2.26	1.62	1.22	4.01	1.81	3.13	2.6	5.3	1.8
α-Terpineol	2.5	2.4	3.3	6.7	4.5	16.3	2.4	2.9	16.4	7.7	2.4	6.4	1.8	9.2	3.9
Borneol	4.8	5.4	7.5	19.2	12.8	28.3	2.6	6.4	27.6	19.1	4.1	17.9	3.8	25.3	2.4
Verbenone	0.03	0.04	0.29	0.04	0.27	-	-	-	-	0.07	-	-	-	0.21	-

Figure 4. Comparison of GC-MS results with EP criteria

*Green boxes show suitability, red boxes are indicative of inconvinience with ranges indicated in EP

GC-MS: Gas chromatography-mass spectrometry, EP: European Pharmacopoeia

purchased and evaluated in terms of standards stated in EP 10.0 "rosemary oil" monograph. Before the pharmacopeia tests, simple fatty oils and resinified essential oil tests were applied to the products. Pure essential oils must be entirely composed of volatile features; therefore, when they are dripped onto filter paper and kept in an oven at high temperature, observing a significant stain on the paper is unexpected. Evident remaining stains may indicate a possible adulteration or a deficiency that causes a decrease in quality in production procedure, hence it is accepted as a parameter for this study. Results of the fatty oils and resinified essential oils assay are given in Figure 1. Four of the samples left clear stains in the filter paper (P3, A1, A2, and A4), which indicate that non-volatile principles are present in products and therefore failed to fulfill the parameter. Characters section in the monograph requires specific appearance and color for rosemary oil; clear, mobile colorless or pale yellow liquid with characteristic odor. Results displayed that all the samples were coherent with the properties stated in the monograph. TLC assay is required in the monograph as an identification test. Test solutions obtained from samples must correspond with the reference solution on the TLC plate according to monograph. TLC analysis was conducted on all samples and pictures of TLC plates are given in Figure 2. Results of the TLC test indicate that all the samples from pharmacies passed the test; in contrast, four of ten samples obtained from sales sources other than pharmacies (A2, A3, A7, and A8) failed to compensate for the TLC test requirements stated in EP. In the tests section of the monograph, necessities for refractive index, optical rotation, acid value, relative density, and chromatographic profile were stated. Refractive index can be defined as the ratio of the sine of the refraction angle, when light is passing from different mediums and represents a characteristic physical constant of an oil. Three decimals are mandatory for the definitive result and for rosemary oil, while the monograph indicates that the refractive index of rosemary oil must be between 1.464 and 1.473. Refractive indices were conducted on all samples as explained in the pharmacopeia in triplicate and results of the average measurements and standard deviations are given in Table 1. All samples from pharmacies displayed refractive index in the accepted range; however, three samples from other sources (A1, A4, and A9) were found to be out of range. Optical rotation is the feature exhibited by chiral substances rotating the linearly polarized light. In the monograph, it was specified that, optical rotation value must be between -5˚ and +8˚ for rosemary oil. In Table 1, average results and standard deviations were given for optical rotation tests of all samples. Results indicated that only one sample (P5) was out of the range and all other samples fulfilled the requirements of the pharmacopeia. Relative density and acid value tests exhibited the most improper results amongst others. Relative density can be defined as the relation between the mass of a definite volume of the studied substance at 20 ˚C and the mass of an equivalent volume of water at the identical temperature. Pharmacopoeia stated the relative density range for rosemary oil as 0.895 to 0.920 and only three of the studied samples (P3, A6, and A8) were measured in the specified range. Acid value (I_A) shows the amount of mg of KOH required to neutralize all free acids in one gram of EO. For rosemary oil, I_A is limited to maximum 1. Parallel to relative density results, only three samples calculated (P4, A5, and A10) were in acceptable range after triple measurement.

Chromatographic profile can be considered as the most important feature of essential oils since biological activities occurring due to their volatile ingredients. Thus, their phytochemical profile determines the bioactivity.[14] However, some plant species are known for their rich chemotypes, which lead to massive variations in their chemical ingredients. *R. officinalis* is one of these species that has been detected for several different chemotypes highly affected from geographical impacts.[15] In relevant EP 10.0 monograph, there are two defined

Table 2. Chemical composition of the tested samples

Components	RI	R_t	Identification	P1	P2	P3	P4	P5	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10
Monoterpene hydrocarbons																		
α-Pinene	932	6.3	a, b, c	10.8	11.6	13.5	14.5	33.3	21.9	11.2	10.2	20.3	11.6	10.2	14.6	14.7	6.6	4.58
Camphene	949	6.8	b, c	5.1	5.9	3.7	5.5	4.2	3.1	3.1	6.3	3.3	4.9	5.2	5.3	8.8	2.3	3.04
β-Phellandrene	974	7.53	b, c	0.07	0.03	0.17	1.32	1.13	0.03	-	0.04	0.03	1.82	0.18	2.00	-	-	-
β-Pinene	978	7.65	b, c	6.41	7.77	2.58	2.91	3.24	3.03	1.54	8.21	3.17	3.01	8.98	2.84	5.55	1.38	2.48
β-Myrcene	992	8.06	b, c	1.34	1.56	0.55	0.51	0.49	0.18	2.47	1.79	0.19	0.82	1.61	0.56	2.54	0.25	1.25
p-Cymene	1027	9.32	b, c	3.45	2.28	6.67	6.67	6.18	1.62	3.95	5.02	1.71	9.51	2.57	6.28	1.97	6.56	4.88
α-Phellandrene	1006	8.53	b, c	0.12	0.27	0.07	0.25	0.08	0.07	0.19	0.58	0.09	0.32	0.23	0.27	0.36	-	0.54
Limonene	1031	9.4	a, b, c	-	-	-	-	6.91	3.04	3.17	-	2.99	11.1	-	-	-	-	19.8
γ-Terpinene	1059	10.54	b, c	0.37	0.37	0.03	3.00	0.27	0.51	0.34	0.55	0.58	2.51	1.17	2.99	1.01	-	9.93
β-Ocimene	1048	10.12	b, c	0.05	-	-	-	-	-	-	0.02	-	0.01	0.06	-	0.06	-	0.60
3-Carene	1102	12.16	c	0.03	0.21	1.35	2.49	2.15	1.07	0.41	0.17	1.20	-	-	-	-	-	-
Bornyl acetate	1.288	20.11	a, b, c	1.92	1.22	2.00	3.20	2.18	1.12	2.26	1.62	1.22	4.01	1.81	3.13	2.59	5.31	1.83
Oxygenated monoterpenes																		
1.8-Cineole	1035	9.6	a, b, c	33.4	31.7	24.3	22.6	13.4	9.5	44.7	36.2	9.3	4.8	35.1	23.9	31.1	21.5	8.4
Linalool	1103	12.2	a, b, c	-	-	-	-	-	-	-	-	-	3.1	1.08	2.34	2.01	3.7	1.8
Fenchol	1116	12.8	b, c	0.08	0.12	0.09	0.03	-	0.17	-	-	0.21	0.04	0.04	0.06	0.05	0.04	0.08
Camphor	1146	14.10	a, b, c	15.6	16.3	10.9	2.8	2.1	1.50	5.49	17.5	1.38	3.70	12.1	3.86	15.2	4.62	18.4
Borneol	1163	14.73	a, b, c	4.8	5.4	7.5	19.2	12.8	28.3	2.6	6.4	27.6	19.1	4.1	17.9	3.8	25.3	2.4
4-Terpineol	1179	15.41	c	-	0.3	-	-	-	0.7	0.8	-	-	-	-	0.8	0.7	1.0	0.1
α-Terpineol	1194	16.10	a, b, c	2.5	2.4	3.3	6.7	4.5	16.3	2.4	2.9	16.4	7.7	2.4	6.4	1.8	9.2	3.9
Sesquiterpene hydrocarbons																		
Verbenone	1302	20.73	b, c	0.03	0.04	0.29	0.04	0.27	-	-	-	-	0.07	-	-	-	0.21	-
Caryophyllene	1420	25.68	b, c	5.2	0.8	3.0	1.7	0.8	0.5	10.1	1.0	0.6	2.1	6.0	1.9	3.6	1.2	2.8
Humulene	1454	27.05	b, c	0.55	0.11	0.80	-	-	0.06	1.63	0.10	0.07	0.02	0.64	0.04	0.41	0.13	0.04
γ-Muurolene	1486	28.37	b, c	0.41	0.09	0.05	-	-	-	0.25	0.05	-	-	0.28	-	0.15	-	-
α-Muurolene	1500	28.93	b, c	0.12	-	0.04	-	-	-	-	-	-	-	0.09	-	-	-	-
Oxygenated sesquiterpenes																		
α-Copaene	1376	23.83	b, c	0.52	0.07	-	-	-	-	0.22	0.05	-	0.04	0.42	-	0.24	-	-
β-Copaene	1429	26.05	c	0.10	-	-	-	-	-	-	-	-	-	0.08	-	0.04	-	-
Caryophylene oxide	1584	32.16	b, c	0.45	-	2.19	-	0.24	-	0.47	-	-	0.17	0.3	0.1	0.1	1.44	-
Others																		
3-Octanone	985	7.857	c	0.06	-	0.06	-	-	-	-	-	-	-	0.06	-	0.03	-	-
Ylangene	1372	23.667	c	0.14	0.05	0.26	-	-	-	-	-	-	-	0.12	-	0.08	-	-
α-Guaiene	1487	28.385	c	0.04	0.07	-	-	-	0.04	-	-	-	-	-	-	-	-	-
Isoledene	1499	28.854	c	0.14	0.39	-	-	-	-	-	0.07	-	-	0.12	-	0.06	-	-
Total (%)				83.1	89.1	83.3	93.6	94.1	92.8	97.3	98.7	90.3	90.4	94.9	95.2	96.9	90.6	86.8

a: Identification based on comparison of retention time with standard compounds, b: Identification based on retention index; c: Identification based on library, RI: Retention index, Rt: Retention time chemotypes, which are recorded as Spainsh and Moroccon/ Tunisian types. It is also crucial for producer to indicate the chemotye on the label so it is possible for consumers and healthcare professionals to select the product accordingly. As a result, in the monograph labeling is a necessity for rosemary oil. Nevertheless, only two of the evaluated products (P4 and P5) contain a label that indicates the chemotype of the ingredient (Figure 1). In the monograph, chromatographic profile diversifies according to the chemotype (*i.e.* Spanish type contains a lower amount of cineole and higher amount of camphor). For this study, chromatographic profiles were analyzed with a GC-MS method and results were evaluated according to the most consistent chemotype, which is proximate to the products that contain labels that do not remark the chemotype. There are 12 monoterpenoid compounds that were mentioned and indicated as a requirement in the pharmacopeia for the Spanish type. α-Pinene and cineole are determined as major components with the range of 18-26% and 16-25%, respectively. For the Moroccon/Tunisian type, cineole is determined as the dominant major ingredient in the range between 38 and 55%, while α-pinene content was determined between 9 and 14%. However, chemotype information was mentioned only two of the samples, whereas other samples were evaluated according to most proximate one in the pharmacopeia. All 15 samples were analyzed with GC-MS method and results are given in Table 2. GC-MS results were also compared with the monograph and results are given in Figure 4. None of the samples was entirely fitting with the monograph requirements. A6 was determined as the most coherent sample with GC profile given in monograph for Moroccon/Tunisian type, 9 of the ingredients out of 12 requirements for these samples were consistent with the monograph.

Cineole content of A6 sample is slightly lower than expected, while *p*-cymene content is slightly higher. However, limonene is absent in the oil, which is required to contain a minimum of 1.5% according to EP.[5] Limonene is known for its various beneficial bioactivities such as antioxidant, anti-inflammatory, and gastroprotective effects.[16] Absence of limonene may reduce possible health benefits of *R. officinalis* essential oil. A2 followed A6 with 8 positive results and P1, P2, and A8 were measured with 7 positive results. Limonene content is suitable for A2, however it is also absent in P1, P2, and A8 samples. For A2 samples there are slight differences for β-pinene, β-myrcene, *p*-cymene, and bornyl acetate. In contrast, A9 was designated as most out of spec sample consistent with only one ingredient of the GC profile requirements. It is followed by P4 and A7, which are congruent with only two components (Figure 3). Cineole is the major ingredient of both chemotypes; however, only A2 sample was measured to contain sufficient cineole to meet the criteria of Moroccon/Tunusian type with 44.7%, all other samples had cineole content between 8.4 and 36.2%. Previous studies also reported a great variation. For instance, Ozcan and Chalcha[16] calculated cineole content of *R. officialis* essential oil from Türkiye as 2.64%, while Daferera et al.[17] found that 88.9% of the rosemary oil from Greece was cineole. α-pinene is another major ingredient of rosemary oil according to monograph. Results of the GC-MS analysis similarly demonstrated that α-pinene contents of the samples are highly varied, between 4.58-33.3%. Previous literature exhibited considerable diversion between α-pinene content of different rosemary oil samples. Sharma et al.[18] calculated α-pinene content of French rosemary oil as 37.5%, while Tunisian counterpart only had 1.2%.[19] Even though there is a significant variation between samples, 10 of 15 were concordant with the pharmacopeia criteria. Limonene contents of the samples were most out of reach parameter, only 3 of the samples fitted with EP requirement; Spanish and Moroccon/Tunisian types need to be 2.5-5% and 1.5-4%, respectively. Results showed that 9 of the samples do not contain limonene at all, while 3 of the samples contain greater than the upper limit. A1, A2, and A4-coded samples were found convenient with limits, 3, 3.17, and 2.99%, respectively. Variations in limonene contents were also suitable with previous results since Sharma et al.[18] measured limonene content of French and Italian rosemary oils as 5.35 and 3.06%, respectively. However, some researchers determined absence of limonene in rosemary oil samples from different locations.[18,19]

Study limitations

Although there are many more commercial products of rosemary essential oil in the Turkish market, 15 samples were studied to have adequate number. Even more accurate results could be achieved, if all relevant products on the market were studied.

CONCLUSION

Essential oils are marketed with notable health-promoting statements. Amongst, rosemary oil is also claimed to have health and cosmetic benefits and sold without any control and restriction in several channels such as herbalists, websites, and pharmacies. Any product claiming any health benefits should meet the criteria of EP monograph, even if they are synthetic medicines, natural products, excipients in medicines or essential oils. Basic mission of any pharmacopeia is to prevent health hazards due to lack of quality of products. For these reasons, it is important to assess the quality of the rosemary oil-containing products in the market to determine the current status and level of quality of commercial products in the market. In this study, 15 products from the Turkish market were evaluated according to EP 10.0 and results revealed that none of the samples was in full compliance with the monograph. When the compliance rate was compared with purchase location, products from pharmacies were found to be slightly better than those from other sales channels. Ultimately, it was clearly revealed that quality standards or rosemary essential oils in the Turkish market need to be increased. Higher demands and improved auditing mechanisms from public authorities should be the initial step for increasing the quality of products.

Ethics

Ethics Committee Approval: Ethical approval is not necessary for this study.

Informed Consent: Not applicable.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: T.H.B., Design: T.H.B., E.B., H.B., Data Collection or Processing: T.H.B., E.B., Analysis or Interpretation: T.H.B., E.B., Literature Search: T.H.B., H.B., Writing: T.H.B., H.B.

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