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

Short Title: Quality Analysis of Marketed Rosemary Oils

Timur Hakan Barak[1], Elif Bölükbaş[2], Hilal Bardakcı[1]
[1]Acibadem Mehmet Ali Aydınlar University, Faculty of Pharmacy, Department of Pharmacognosy, İstanbul, Turkey
[2]Acibadem Mehmet Ali Aydınlar University,Faculty of Pharmacy, İstanbul, Turkey

Corresponding Author Information
Timur Hakan Barak
thakanbarak@gmail.com
+90 216 500 44 44
https://orcid.org/0000-0002-7434-3175
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24.11.2022

ABSTRACT
Objectives: Various pure rosemary essential oil containing commercial products are in demand for their health-promoting and cosmetic claims in Turkey. Although they are regarded as 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 in online platforms in Turkey were investigated in terms of "Rosemary Oil" EP 10.0. monograph criteria. In the current study, it was aimed to evaluate current quality status of the rosemary essential oils in the Turkish market. **Materials and Methods:** Appearance, fatty oils and resinified essential oils, relative density, refractive index, optical rotation, acid value tests were performed according to EP 10.0 and compared with the given standards. In addition, TLC and GC-MS analysis were conducted to all samples for advanced understanding of their phytochemical profile and harmony with EP standards.
Results: 15 pure rosemary oil containing product from Turkish market were evaluated. All of the samples were licensed as cosmetic products in Turkey via 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 Turkish rosemary essential oil market fully complied the EP rosemary oil monograph standards.
Conclusion: In the light of these information, it was revealed that enhanced regulations and auditing mechanisms are needed to improve quality of the products. When the difference between the sources of purchase assessed, pharmacies are still better locations to obtain such products nevertheless improvements are yet needed.
Keywords: *Rosmarinus officinalis* L., Rosemary Oil, European Pharmacopoeia, GC-MS, Essential Oil.

Introduction

With the increasing interest in natural based therapies, utilization of essential oils for medical and cosmetic purposes of 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 and widely cultivated in 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* is used as tea or tincture 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 utilization 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 prior to its utilization for medical and cosmetic purposes.

Pharmacopoeias are official publications that establish necessary quality requirements for both synthetic and natural based medical products aiming to promote and protect public health. Republic of Turkey is legally bounded (or responsible or have to obey the rules of) to European Pharmacopoeia which contains more than 200 herbal drug monographies. Products claiming to contain pure *R. officinalis* essential oil are readily available in the Turkish market, and most of them are licensed as cosmetic products via ministry of health. It may be beneficial to evaluate marketed products in terms of the European Pharmacopoeia 10.0 which is most up to date version, for better understanding the current situation of the essential oil market in the manner of quality that strongly affects the public health. Yet literature survey revealed that, there is a lack of studies investigating quality situations of *R. officinalis* products on the market based upon Rosemary Oil monograph in European Pharmacopoeia. For this reason, in this study 15 samples which was sold as pure rosemary oil were investigated, 5 of them were purchased in pharmacies while 10 of them were purchased from other channels such as Akhtar shops and online platforms. Relative density, refractive index, optical rotation, acid value of the samples were calculated through assays given in pharmacopoeia. Similarly, appearance and thin layer chromatography (TLC) results were visually investigated based on given criteria. Furthermore, chromatographic profile of the samples were 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 were conducted. 12 different of components were given in the monograph for both chemotypes and with different ranges. Results of the 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 the rosemary essential oils in the Turkish market for creating a plain picture. It is an essential public health requirement for the products that claimed to have health benefits to contain the specified international standards.

Materials and Methods

Materials

Products containing pure rosemary essential oil was procured from herbalists, online shopping platforms and pharmacies in the Istanbul region. All products are registered as cosmetics by Turkish Ministry of Health. In addition, labels of all oil samples claims to contain pure rosemary oil. Until the experiments, products were kept at room temperature, in tightly closed containers and protected from sunlight. All the products were coded indicating their source (P: Pharmacy, A: Other sources). All standards and solvents (1,8 cineol, 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, the filter paper kept in an oven at 80 ºC for 30 minutes for fatty oils and resinified essential oils test. All samples were filled in glass tube and photographed for evaluation of their appearance. All 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, volume of the essential oil samples with equivalent volume of water at 20°C were measured. For refractive index analysis, Anton Paar - Abbemat 3100 device and for optical rotation assay Anton Paar – MCP 150 device were used. Acid value of the samples were determined by titrimetric method described in EP. All experiments were carried out in triplicate and results were given with average and standard deviation.[5]

TLC analysis

TLC analyses were conducted according to indications given in Rosemary Monograph in EP. Borneol, bornyl acetate and cineole standards were dissolved in toluene and used as reference solution. 0.5 mL of samples 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 at oven at 100-105 °C for 10 min.[5]

GC-MS analysis

Qualitative and quantitative analyses were performed by gas chromatography-mass spectrometry (GC-MS). Agilent technologies 7890 A GC system was used with DP-5MS column (30 m x 0.25 mm x 0.25 µm). Oven temperature was started with 60 °C and then steadily increased to 246°C with 3°C raise per a minute. Helium was used as mobile phase with 0.9 ml/min flow rate. Split mode was used with 50:1 ratio with 1µL sample volume. Relative Retention Indices (RRI) was calculated via comparison with (C4-C40) standards. Identification of the essential oil components were completed by comparison of their relative retention index (RRI) calculated against *n*-alkanes and relative retention times (RT) with those of authentic samples and mass spectra obtained from Wiley 7N library as well as MS literature data was used for the identification.[6]

RESULTS

Appearance, Labelling and Fatty oils and resinified essential oils

The European Pharmacopoeia 10.0 states that Rosemary oil should be clear, mobile, colorless or pale-yellow liquid with characteristic odour. EP 10.0 states that all of the Rosemary oil samples should indicate the chemotype of the ingredient on the labels. Accordance of the samples to EP criteria were evaluated in Figure 1. Results showed 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 the oils with non-volatile materials. After drying in the incubator, P3, A1, A2 and A4 samples showed 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 values results of 15 essential oil samples were given in the Table 1. According to the European Pharmacopoeia 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 acid value must be lower than 1.0. Compatibility of samples with EP was evaluated and summarized in Figure 1.

TLC analysis

According to European Pharmacopoeia 10.0, bornyl acetate should appear as: a bluish - grey zone of low intensity (top), cineole: an intense blue zone (midline), borneol: 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 were given in Figure 2 and Figure 1, respectively.

GC-MS analysis

EP 10.0 mentions two different chemotypes of Rosemary oil. GC-MS analysis were conducted to all samples and results were given in Table 2 and 83.1 to 96.9% of the ingredients were determined for all samples. Chromatograms which indicates the presence of the ingredients specified in EP were given in Figure 3 and Supplementary Materials. GC-MS results were evaluated with accordance to the most proximate chemotype and coherence of all ingredients with the monograph were 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 utilizations. 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 for complementary therapies and natural cosmetics is growing and amplified competition between producers creates possible exploitation environment in conjunction with insufficient regulations and low knowledge level of the public. Thus, conducting regular scientific market analysis may create clear understanding of the current status and may lead both public authority, healthcare professionals and public to be deliberate against such products. There are several studies conducted in Turkey which evaluate herbal drugs from Turkish market for their compliance with EP. Previous studies on evaluation of *Hibiscus sabdariffa* L., *Eucalyptus* L'Her and *Alchemilla* L. samples collected from Turkish market for their consistency with EP are examples on that manner.[8-10] All studies demonstrated complications on the quality of the drugs which are freely sold in the market for medical purposes. In addition two recent studies evaluated fixed oils sold on Turkish market. Nearly all of the Almond and Safflower oil samples from Turkish market were reported as lack of 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 medical purposes. Similarly, public

demand for aromatherapy which uses essential oils for medical purposes is increasing.[13] Nonetheless, there is an apparent scarcity of studies assessing essential oil containing products in Turkish market for their compliance with EP. Basic objective of EP is ensuring the standards of products thus consumers can purchase any product without being anxious about their quality. In this context, for this study 15 products (5 from pharmacies and 10 from other sources) were purchased which claims to contain pure rosemary oil, and evaluated in terms of standards stated in EP 10.0 "Rosemary oil" monograph. Prior to pharmacopoeia tests, simple fatty oils and resinified essential oils test were applied to the products. Pure essential oils must entirely composed of volatile features so 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 were given in Figure 1. Four of the samples left apparent stains in the filter paper (P3, A1, A2 and A4) which indicates non-volatile principles are present in products therefore failed to fulfill the parameter. Characters section in the monograph requires specific appearance and colour for rosemary oil; clear, mobile colourless or pale yellow liquid with characteristic odour. Results showed that all of the samples were coherent with the properties stated in the monograph. TLC assay is required in the monograph as an identification test. Test solutions which obtained from samples must be corresponding with reference solution on the TLC plate according to monograph. TLC analysis were conducted on all samples and pictures of TLC plates were given in Figure 2. Results of TLC test indicate that all of the samples from pharmacies passed the test, in contrary four of the ten samples obtained from other sources than pharmacies (A2, A3,A7 and A8) failed to compensate TLC test requirements stated in EP. In 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 the light is passing from different mediums and also represents a characteristic physical constant of an oil. Three decimals are mandatory for the definitive result and for rosemary oil, monograph indicates that refractive index of rosemary oil must be between 1.464 to 1.473. Refractive index were conducted on all samples as explained in the pharmacopoeia in triplicates and results of the average measurements and standard deviations were given in Table 1. All samples from pharmacies showed refractive index in the accepted range however, three samples from other sources (A1, A4 and A9) were found to be out of the range. Optical rotation is the feature exhibited by chiral substances of rotating the linear 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 indicates that only one sample (P5) was out of the range and all other samples were fulfilled the requirements of the pharmacopoeia. Relative density and acid value tests exhibited 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 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 were calculated in the acceptable range (P4, A5 and A10) 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 phytochemical profile determines the bioactivity.[14] However, some plant species are known for rich chemotypes which leads to massive variations for their chemical ingredients. *Rosmarinus officinalis* is one of these species that has detected for several different chemotypes highly affected from geographical impacts.[15] In EP 10.0 monograph, there are two defined chemotypes, which are recorded as Spainsh and Moroccon/Tunusuian type. It is also crucial for producer to indicate the chemotpye on the label so it makes is possible for consumers and healthcare professionals to select the product accordingly. As a result, in the monograph it is stated that 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 lower amount of cineol 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 proximate with the products that contains labels that do not remark the chemotype. There are 12 monoterpenic compounds which were mentioned and indicated as a requirement in the pharmacopoeia. For the Spanish type α-pinene, and cineole are determined as major components, with the range of 18-26% and 16-25%, respectively. For Moroccon/Tunusuian type, cineole is determined as dominant major ingredient with the range between 38 to 55% while α-pinene content determined between 9 to 14%. Yet chemotype information were mentioned only two of the samples, other samples were evaluated according to most proximate one in the pharmacopeia. All 15 samples were analyzed with a GC-MS method and results were given in Table 2. GC-MS results were also compared with the monograph and results were given in Figure 4. None of the samples were entirely fitting with

the monograph requirements. A6 was determined as the most coherent sample with GC profile given in monograph for Moroccon/Tunusuian type, 9 of the ingredients out of 12 requirements for these samples were consistent with monograph.

Cineole content of the 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 minimum 1.5% in the oil 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 is following 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 contrary, A9 was designated as most out of spec sample consistent with only one ingredient of GC profile requirements and it is followed by P4 and A7, which are congruent with only 2 component (Figure 3). Cineole is the major ingredient of both chemotypes however, only A2 sample were measured to contain sufficient amount of cineole to meet the criteria of Moroccon/Tunusuian type with 44.7%, all other samples had cineole content between 8.4 to 36.2%. Previous studies also show great variation, Mehmet Musa Özcan & Jean-Claude Chalchat [17] had calculated cineole content of *R. officinalis* oil from Turkey as 2.64% while Daferera et al. [18] had found that 88.9% of the rosemary oil from Greece is 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 variated, between 4.58 - 33.3%. Previous literature showed considerable diversion between α-pinene content of different rosemary oil samples. Sharma et al.[19] calculated α-pinene content of French rosemary oil as 37.5%, while Tunisian counterpart only had 1.2%.[20] Even though there is a significant variation between samples, 10 of 15 were concordant with pharmacopoeia criteria. Limonene contents of the samples were most out of reach parameter, only 3 of the samples were fitting with limonene content requirement of the pharmacopoeia, Spanish and Moroccon/Tunusuian 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 three of the samples contain greater than the upper limit. A1, A2 and A4 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.[19] 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. [18-20]

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 akhtars, websites and pharmacies. Any product claiming any health benefits should meet the criteria of EP monograph, even they are synthetic medicines, natural products, excipients in medicines or essential oils as well. 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 products in the market. In this study, 15 products from Turkish market were evaluated according to EP 10.0 and results revealed that none of the samples were in full compliance with the monograph. When the compliance rate was compared with purchase location, products from pharmacies were found slightly better than other channels. Ultimately, it was clearly revealed that quality standards or rosemary oils in Turkish market is urgently needed to be increased. Higher demands and improved auditing mechanisms from public authorities should be the initial step for increasing the quality of the products.

Study Limitations: Although there are many more products in the Turkish market, 15 samples were studied in order to have sufficient amount of samples. Even more accurate results could be achieved if all products on the market were studied.

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

	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 triplicates and results were given in average ± standard deviation.

Table 2. Chemical Composition of the samples 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

Components	RI	RT	Identification	P1	P2	P3	P4	P5	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10
Monoterpene hydrocarbons																		
α-pinene	932,5	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	949,5	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	974,2	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	978,5	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	992,3	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	1,027	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	1,006	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	1,031	9,4	a,b,c	-	-	-	-	6,91	3,04	3,17	-	2,99	11,1	-	-	-	-	19,8
γ-terpinene	1,059	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	1,048	10,12	b,c	0,05	-	-	-	-	-	-	0,02	-	0,01	0,06	-	0,06	-	0,60
3-carene	1,102	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	1,035	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	1,103	12,2	a,b,c	-	-	-	-	-	-	-	-	-	3,1	1,08	2,34	2,01	3,7	1,8
Fenchol	1,116	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	1,146	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	1,163	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	1,179	15,41	c	-	0,3	-	-	-	0,7	0,8	-	-	-	-	0,8	0,7	1,0	0,1
α-terpineol	1,194	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	1,302	20,73	b, c	0,03	0,04	0,29	0,04	0,27	-	-	-	-	0,07	-	-	-	0,21	-
Caryophyllene	1,320	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	1,354	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	1,386	28,37	b, c	0,41	0,09	0,05	-	-	-	0,25	0,05	-	-	0,28	-	0,15	-	-
α- muurolene	1,400	28,93	b, c	0,12	-	0,04	-	-	-	-	-	-	-	0,09	-	-	-	-
Oxygenated sesquiterpenes																		
α-copaene	1,276	23,83	b,c	0,52	0,07	-	-	-	-	0,22	0,05	-	0,04	0,42	-	0,24	-	-
β-copaene	1,329	26,05	c	0,10	-	-	-	-	-	-	-	-	-	0,08	-	0,04	-	-
Caryophylene oxide	1,484	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	985,4	7,857	c	0,06	-	0,06	-	-	-	-	-	-	-	0,06	-	0,03	-	-
Ylanagen	1,272	23,667	c	0,14	0,05	0,26	-	-	-	-	-	-	-	0,12	-	0,08	-	-
α-guaiene	1,387	28,385	c	0,04	0,07	-	-	-	0,04	-	-	-	-	-	-	-	-	-
isoledene	1,399	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

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
Refractive 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 suitibility, red boxes show inconvinience with ranges indicated in EP.

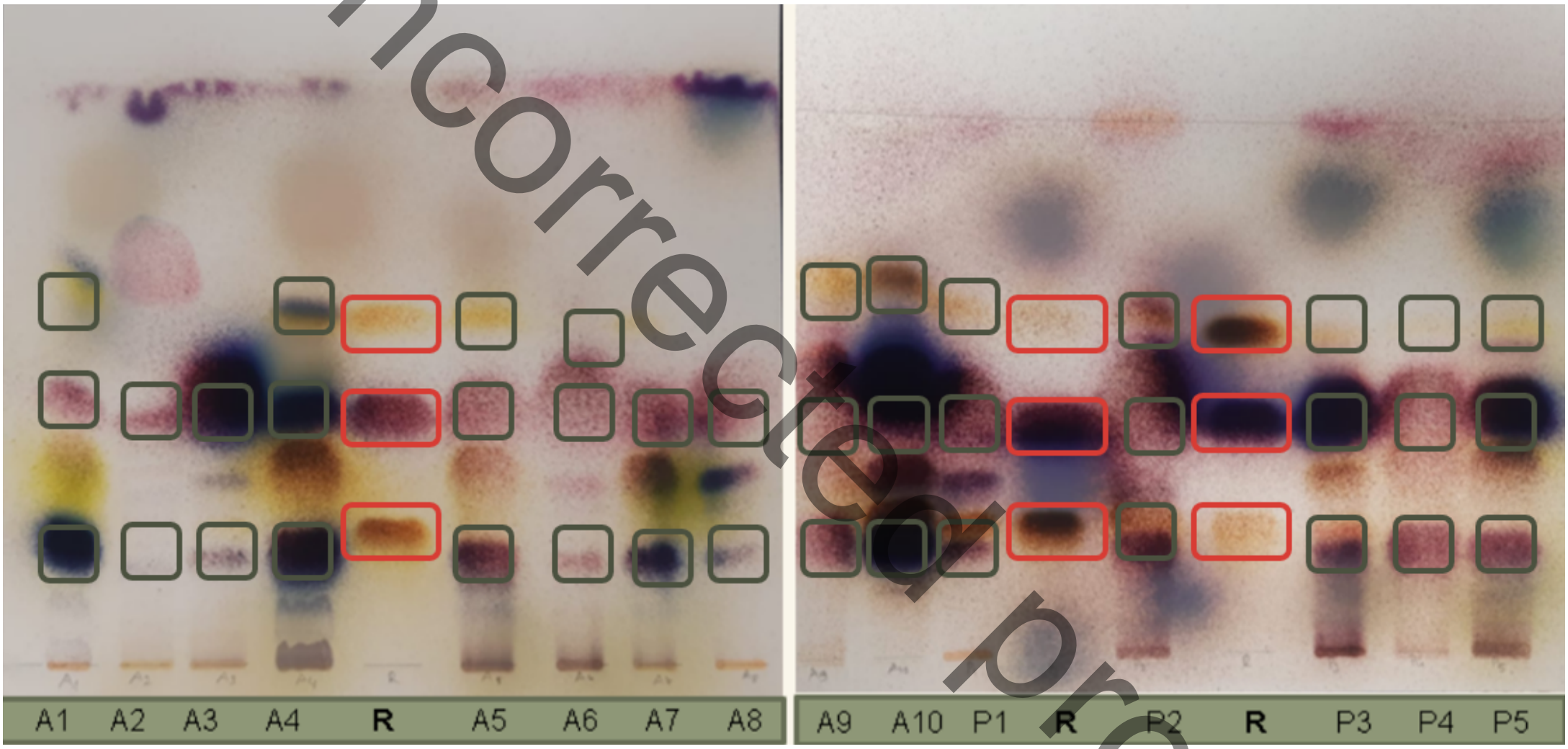

Figure 2. TLC chromatogram of all samples
R: Reference mixture; bornyl acetate, cineole and borneol from top to bottom. Mobile phase; ethyl acetate:toluene (5:95 V/V)

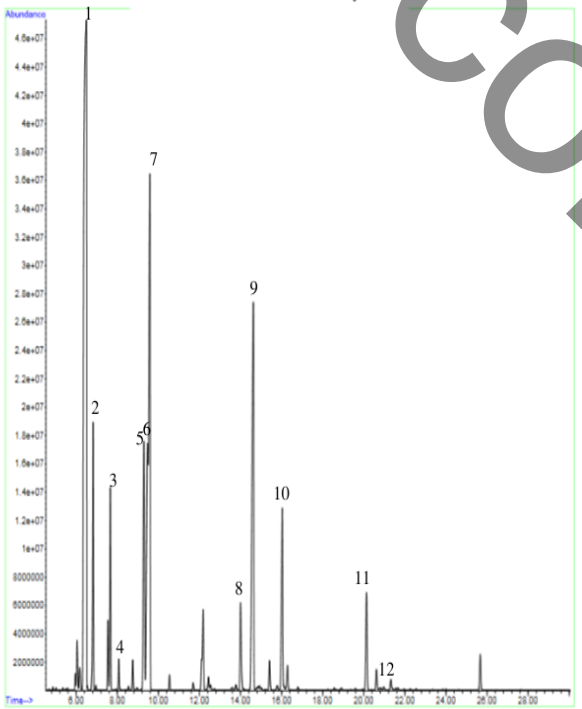

Figure 3. GC-MS chromatograms of P5 sample showing the chemical components given in pharmacopoeia: 1:α-pinene, 2:camphene, 3:β-pinene, 4:β-myrcene, 5:limonene, 6:cineole, 7:p-cymene, 8:camphor, 9:borneol, 10:α-terpineol, 11:bornyl acetate, 12:verbenone

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 suitibility, red boxes show inconvinience with ranges indicated in EP.