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Evaluation of the essential oil samples that are sold as "Eucalyptus oil" on the market in Türkiye in terms of European Pharmacopoeia 10.0 criteria

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ABSTRACT: Eucalyptus oil is prominent for its health-promoting and cosmetic utilizations. Thus, pure eucalyptus oil containing products are abundant in Turkish market. Natural products may be regarded as safe however, it is only accurate if the accessible products meet the international quality criteria. Türkiye is legally bounded to European Pharmacopoeia (EP) and there are various monographs for herbal products, including eucalyptus oil. Hence, for this study 16 eucalyptus oil samples obtained from different sources were investigated for their compliance with latest version of EP. Appearance, fatty oils and resinified essential oils, relative density, refractive index, optical rotation and aldehydes properties of the samples evaluated. For superior insight for chemical ingredients of the products, Thin Layer Chromatography (TLC) and Gas Chromatography-Mass Spectrometry (GC-MS) analysis were employed. Results denoted that all samples obtained from Turkish Market incompatible with the standards of EP. In view of these data, it was shown that enriched regulation mechanisms are required. When the variance between the sources of purchase evaluated, pharmacies are more accurate sources for purchasing such products nonetheless developments are yet needed.

KEYWORDS: Eucalyptus oil, European Pharmacopoeia, GC-MS, essential oil.

1. INTRODUCTION

Essential oils are increasingly being used for medical and aesthetic purposes in line with the growing interest in natural remedies. Numerous biological functions of essential oils have led to an increase in scientific research on aromatherapy [1]. The Myrtaceae family includes the native Australian genus eucalyptus, which is commonly referred to as eucalypt and encompasses more than 700 species globally [2]. In traditional medical systems, *E. globulus* is used treat broad spectrum of disorders such as cough, fever, rheumatism, migraine, asthma etc. due to its antimicrobial, analgesic, antiseptic and antirheumatic effects [3]. Leaves of *E. globulus* is prominent for its essential oil which is widely used for medical and cosmetic purposes all around the globe. Biological activity studies conducted on *E. globulus* essential oil (EGEO) are generally focused on its noticeable anti-infective properties due to its dominantly major ingredient, 1,8-cineole. Results of the biological activity studies showed that EGEO showed potent antibacterial, antifungal and antiviral activities against known pathogens [4-6]. In addition, several clinical studies showed that EGEO could be successfully used for respiratory tract disorders such as chronic obstructive lung disease (COPD), acute bronchitis, and upper respiratory tract infections [7]. Anti-oxidative and anti-inflammatory properties were also shown by several investigations [8,9].

Pharmacopoeias give official standards of both natural based medical products and synthetic ingredientsin order to establish quality requirements for protecting and promoting public health. Türkiye is officially bounded to European Pharmacopoeia (EP) for determining such international standards. There are more than 200 herbal drug monographies in EP including raw herbs, essential oils and some standard extracts [10]. There are numerous products in Türkiye that their labels are claiming to have pure essential oils and most of them are licensed as cosmetics via ministry of health. Pure EGEO containing products are also abundant in the Turkish market due to its cosmetic and medicinal utilizations. Thus, it may be critical to assess the

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standards of marketed products in terms of most up to date version of EP for improved perspective of the contemporary condition of the sector which always contains considerable amount of risk because of its sensitive manner. However, in the literature there is a considerable scarcity on that subject. Very few numbers of reports were present which extensively investigate quality situations of marketed essential oils yet none of them evaluated EGEO containing products, to our knowledge. For these reasons, 16 EGEO samples that were alleged to be pure were investigated in this study. 5 of them were purchased from pharmacies while 11 of them from other sources such as online platforms and Akhtar shops in order to evaluate possible differences from sources of purchase. Optical rotation, relative density, refractive index, solubility in alcohol and aldehydes assays were conducted on all samples as stated in EP. Correspondingly, results of the appearance and thin layer chromatography tests were visually analyzed. For detailed understating the phytochemical profile and correspondence of the samples with EP standards, GC-MS analysis was employed to all samples. There are 7 components which were stated in the EP for various limit ranges and results of the GC-MS analysis for all samples were compared with requirements.

In the present study, it was intended to estimate current quality status of the eucalyptus essential oils in the Turkish market for creating a clear picture of the status. It is an indispensable public health obligation for the products that appealed to have health reimbursements to cover the identified global standards.

2. RESULTS

2.1. Characters, solubility in alcohol, aldehydes and fatty oils and resinified essential oils

EP 10.0 states that eucalyptus oil should be colourless or paleyellow liquid with reminiscent dour of 1,8-cineole and must be soluble in 5 volumes of 70% ethanol. Accordance of the samples to EP criteria were evaluated in Figure 1. Results showed that, characters of all the samples were compatible with EP except P3 sample which has an intense yellow colour. 13 samples showed solubility in alcohol as mentioned in EP however, P1, P3 and A2 samples were not soluble in 70% ethanol and showed notable layer. Fatty oils and resinified essential oils assay were done in order to disclose possible adulteration of the oils with non-volatile materials. After the drying process, P5, A2 and A9 samples showed remaining stain in the filter paper, which indicates the presence of non-volatile ingredients. All samples were found compatible with EP in aldehydes test (Figure 1).

2.2. Relative density, refractive index and optical rotation

The relative density, refractive index and optical rotation results of 16 essential oil samples were given in the Table 1. To agree with the European Pharmacopoeia 10.0 standards, the relative density value for eucalyptus oil should be between 0.906 and 0.927, 1.458 and 1.470 for refractive index and 0° and 10° for optical rotation value. Compatibility of all 16 samples with EP standards were assessed and briefly given in Figure 1.

2.3. TLC analysis

According to EP 10.0, 1,8-cineole which is the dominant component of the eucalyptus oil should be seen as an intense violent-brown zone and the other reference compound, α-terpineol should be seen in the lower part of the plate as a violet-brown zone if present in the EO which must be absent in TLC plate after derivatization withanisaldehyde solution. TLC analysis were conducted on all 16 samples and the photographs of the chromatograms were taken and given in Figure 2. Consistency of the samples with EP requirements were evaluated and given in Figure 1.

2.4. GC-MS analysis

GC-MS analysis were employed to all 16 samples and results of the phytochemical ingredients were given in Table 2.85.1% to 99.9% of the components were determined and given in Table 2 in detail. A sample chromatogram were given in Figure 3for demonstrating the occurrence of the phytochemicals which given in EP. Results of GC-MS analysis are compared with EP standards and the results of the coherence were evaluated and given in Figure 4.

Table 1. Results of relative density, refractive index and optical rotation. *Tests were done in triplicates and results were given in average ± standard deviation.

	Relative Density (0.906-0.927)	Refractive Index* (1.458- 1.470)	Optical Rotation* (0° - +10°)
P1	0.945	1.464 ± 0.06	6.0° ± 1,17
P2	0.914	1.460 ± 0.10	2.3° ± 0,06
P3	0.930	1.476 ± 0.17	8.61° ± 0,06
P4	0.912	1.462 ± 0.00	4.57° ± 0,12
P5	0.913	1.469 ± 0.06	1.97° ± 0,02
A1	0.883	1.462 ± 0.15	3.68° ± 0,10
A2	0.936	1.472 ± 0.0	2.02° ± 0,50
A3	0.918	1.461 ± 0.12	3.05° ± 0,18
A4	0.938	1.460 ± 0.06	1.66° ± 0,04
A5	0.901	1.465 ± 0.1	4.85° ± 0,42
A6	0.912	1.463 ± 0.0	1.41° ± 0,57
A7	0.918	1.465 ± 0.06	1.96° ± 0,10
A8	0.909	1.461 ± 0.06	2.5° ± 0,12
A9	0.926	1.476 ± 0.08	4.49° ± 0,39
A10	0.907	1.461 ± 0.06	3.41° ± 0,28
A11	0.891	1.464 ± 0.06	5.12° ± 0,04

Experiment	Reference Interval	P1	P2	P3	P4	P5	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11
Fatty oils and resinified essential oils		✓	✓	✓	✓	X	✓	X	✓	✓	✓	✓	✓	✓	X	✓	✓
Characters		✓	✓	X	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Relative Density	0.906 - 0.927	0.945	0.914	0.930	0.912	0.913	0.883	0.936	0.918	0.938	0.901	0.912	0.918	0.909	0.926	0.907	0.891
Refractive Index	1.458 - 1.470	1.465	1.460	1.476	1.462	1.469	1.462	1.472	1.461	1.460	1.465	1.463	1.465	1.461	1.476	1.461	1.464
Optical Rotation	0° - +10°	6°	2.3°	8.6°	4.6°	2°	3.7°	2°	3.1°	1.7°	4.9°	1.4°	1.9°	2.5°	4.5°	3.4°	5.1°
Solubility in Alcohol		X	✓	X	✓	✓	✓	X	✓	✓	✓	✓	✓	✓	✓	✓	✓
Aldehydes		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
TLC		✓	✓	X	✓	✓	✓	✓	✓	✓	✓	✓	✓	X	X	X	✓

Figure 1. General evaluation of EP tests *Green boxes show suitability, red boxes show inconvenience with ranges indicated in EP

3. DISCUSSION

The European Pharmacopoeia is a scientific reference, which provides legal basis for quality control of medicines, including herbal drugs. There are more than 200 herbal monographs in the latest version, European Pharmacopoeia 10.0, and approximately 20 of them are for essential oils[10]. Essential oils are widely used and regarding the accumulating interest on natural therapies including aromatherapy, it is crucial for any pure essential oil containing product must meet the EP standards. Quality standards for herbal products are more important than ever because of the growing popularity of complementary therapies and natural cosmetics, as well as the amplified competition between producers that could lead to exploitation given the lack of adequate regulations and the general deficiency of consumer education.Therefore, conducting routine scientific market analysis may result in a clear grasp of the situation and may influence public officials, healthcare providers, and the public to make decisions against such items.

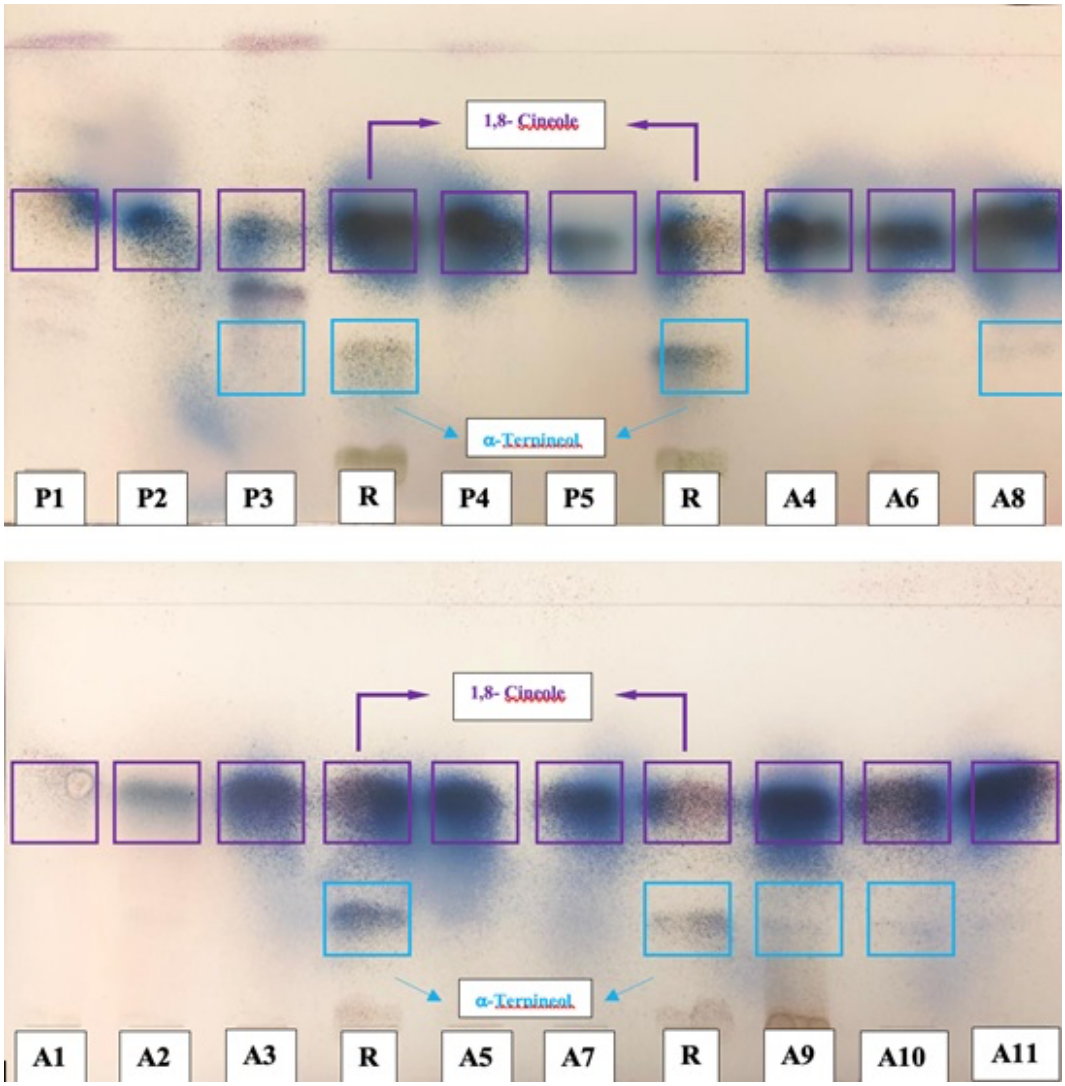

Figure 2. TLC chromatogram of all samples. R: Reference mixture; 1,8-cineole and α-terpineole from top to bottom. Mobile phase; ethyl acetate:toluene (10:90 V/V). Detection: sprayed with anisaldehyde solution R and heated at 100-105 °C for 5-10 min; examined in daylight.

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 IM: Identification Method

Compounds	RI	RT	IM	P1	P2	P3	P4	P5	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11
α-Thujene	927	6.13	b,c	0	0	1.3	0	0	0	0	0	0	0	0	0	0	0	0	0
α-Pinene	933	6.32	a,b,c	17.5	5.7	5.2	11.2	11.3	5.4	3.3	4.8	1.2	6.5	3.3	1.8	6.5	1.9	7.9	5.9
β-Pinene	978	7.65	b,c	0.8	0.8	0.8	0.8	0.8	0.8	1	0.8	0.2	3.1	0.5	2.9	1	0.7	0.8	2.2
β-Myrcene	993	8.08	b,c	0.3	1.6	0.7	0.9	0.9	0.8	1.5	1.4	0	2.6	0.3	5.2	1.6	0.4	1.5	2.4
α-Phellandrene	1007	8.57	b,c	1.3	2.7	3.8	0.7	0.7	0	0.8	1.4	0	2.4	0	3.2	2.8	0	1.7	2.8
δ-3-Carene	1019	9.01	b,c	0.1	0.5	0.6	0	0	0	0	0.5	0	0.4	0	6.8	0.5	0	0.4	0.6
o-Cymene	1016	9.27	b,c	0	0	0	0.1	0	0	0	0	0	0	0	0	0	25.2	0	0
Limonene	1031	9.48	a,b,c	3.6	0	19.4	0	0	35.5	20	0	0	22.8	0	0	0	0	0	20
1,8-Cineole	1038	9.76	a,b,c	51.2	80.7	23.7	77.2	79.2	57.5	67.2	86.7	96.7	57.3	77	67.7	77.1	53.4	78.2	59.2
β-Ocimen	1049	10.17	a,b	0	0	0	0	0	0	0	1.3	0	0	0	0.4	0.1	0	0.1	0
γ-Terpinene	1060	10.59	b,c	0.5	2.7	2.2	1.7	1.7	0	2	3	0	3.6	0	4.3	7.6	0	5.2	5
Isopinocarveol	1141	13.82	b,c	3.5	0	1.1	1.5	1.5	0	0	0	0	0	3.9	0	0	0	0.1	0
Camphor	1145	14.01	a,c	0	0	0	0	0	0	0	0	0	0	0	0	0	0.8	0	0
L-Menthone	1160	14.63	c	0	0.1	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Pinocarvone	1164	14.81	b,c	1	0	0.1	0.4	0.3	0	0	0	0	0	0.5	0	0	0	0	0
Endo-borneol	1168	14.96	a,b,c	0.3	0	0	0.1	0	0	0	0	0	0	0.3	0	0	0	0	0
L-Menthol	1174	15.24	a,c	0	0.1	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Terpinen-4-ol	1179	15.45	b,c	0.4	0.1	3	0.1	0	0	0	0	0	0.4	0.6	0	0.4	0.6	0.5	0.3
trans-verbenol	1190	15.90	c	0.6	0	0	0.3	0.2	0	0	0	0	0	0	0	0	0	0	0
α-Terpineol	1195	16.10	a,b,c	1.2	0.6	1.6	0.4	0.4	0	1.2	0	0	0.9	4.4	0	1.6	2.6	1.7	0.9
Myrtenol	1199	16.27	b,c	0.2	0	0.2	0.1	0	0	0	0	0	0	0	0	0	0	0	0
Trans-Carveol	1222	17.26	c	0.2	0	0.1	0.1	0	0	0	0	0	0	0	0	0	0.4	0	0
Cuminaldehyde	1242	18.15	b,c	0	0	1.8	0	0	0	0	0	0	0	0	0	0	0	0	0
Phellandral	1276	19.60	b,c	0	0	2.5	0	0	0	0	0	0	0	0	0	0	0	0	0
Thymol	1305	20.80	a,c	0.1	0	0.5	0	0	0	0	0	0	0	0	0	0	0	0	0
Caryophyllene	1421	25.72	b,c	0.2	0	1.4	0.1	0	0	0	0	0	0	0	0	0	0	0	0
Aromadendrene	1441	26.51	b,c	4.9	0.1	1.3	1.2	1.2	0	0	0	0	0	2	0	0.4	0	0.2	0
Humulene	1456	27.12	c	0.1	0	0.4	0	0	0	0	0	0	0	0	0	0	0	0	0
Alloaromadendrene	1463	27.40	b,c	1.1	0	2.8	0.2	0.2	0	0	0	0	0	0.2	0	0	0	0.1	0
Spathulenol	1582	32.11	b,c	0	0	7.3	0	0	0	0	0	0	0	0	0	0	0	0	0
Caryophyllene oxide	1587	32.36	b,c	0.2	0	3.3	0.7	0.7	0	0	0	0	0	0	0	0	0	0	0
TOTAL				89.3	95.7	85.1	92.86	95.05	98.33	95.62	99.9	97.91	98.28	92.68	91.90	97.23	86.28	96.28	97.46

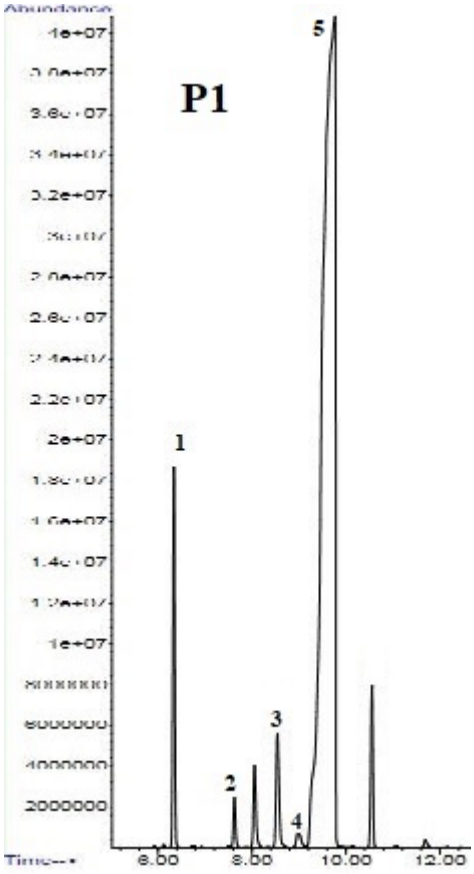

Figure 3. GC-MS chromatogram of P1 sample showing the chemical components given in pharmacopoeia: 1: α-pinene, 2:β-pinene, 3: α-phellandrene, 4: limonene and 5: 1,8-cineole

Herbal products are promoted and utilized all over the world due to their alleged or anticipated health benefits. However, with this growing demand potential threats are also increased including accidental contamination or deliberate adulteration. In a review study it was determined that 41% of the 508 microscopically investigated plant products from 13 countries were adulterated [11]. Despite the fact that there have been several studies undertaken in Türkiye that assess herbal medicinal products available on the Turkish market for their compliance with EP, the literature is noticeably lacking. In previous studies, *Alchemilla*and *Hibiscus* samples were purchased from Türkiye and their quality standards were compared with EP standards. Results denoted significant inconsistencies with samples and EP requirements [12,13]. Moreover; *Humuluslupulus* samples from herbalists and online platforms showed significantinconsistencies with EP [14]. In a previous study, 10 Eucalyptus leaves and 3 essential oil samples from Turkish market were investigated for determination of authenticity and possible adulterations and falsifications. Results showed that all of the 10 eucalyptus leave samples were contaminated or mixed with other unofficial eucalyptus species. In addition, TLC analysis were conducted on 3 eucalyptus oil products from market and results were compared with authentic essential oils. TLC profiles revealed that all three samples were significantly different with reference oil, indicating possible adulterations [15]. Even though TLC analysis were conducted on three essential oils in aforementioned study, there is a scarcity in the literature for comprehensive evaluation of quality status of essential oil samples. In a previous study, 15 samples of rosemary oil samples from Turkish market wereevaluated in terms of EP criteria. Results revealed that none of the samples have fully met the quality standards of EP, however samples collected from pharmacies have significantly more acceptable profiles [16]. Moreover, two recent reports on evaluation of fixed oils from Turkish market has disclosed analogous conclusions. Safflower and almond oil samples from Türkiye had failed to fulfil quality standards given in EP [17-18]. When considering growing demand of public for aromatherapy products [19] and absence of scientific reports on that manner, significance of such studies appears to be evident. The primary goal of EP is to maintain product standards so that customers may purchase any product without abstaining about its quality.In this regard, 16 products that purport to contain pure eucalyptus oil were obtained for this study (5 from pharmacies and 11 from other sources), and they were assessed in accordance with the criteria outlined in the EP 10.0 "Eucalyptus oil" monograph. Simple fatty oil and resinified essential oil testing were conducted

on the items prior to pharmacopoeia tests. Pure essential oils must fully consist of volatile components, thus it is irregular to see a significant mark after they are dropped over filter paper and incubated at a high temperature. Evident residual stains are recognized as a parameter for this study since they may signify a possible adulteration or aimpurity that lowers the quality of the production process.Figure 1 shows the results of the fatty oils and resinified essential oils assay. Three of the samples (P5, A2, and A9) left permanent stains on the filter paper, indicating the presence of non-volatile principles in the products and failure to meet the standard.Characters section in the monograph requires specific colour for eucalyptus oil; colourless or pale yellow liquid with reminiscentodour of 1,8-cineole. Results demonstrated that all samples expect P3 is coherent with the monograph, P3 is inconsistent as a result of its dark yellow colour. The TLC test is needed in the monograph as an identification test. The test solution collected from the sample should match the reference solution on the TLC plate according to the monograph. TLC analysis was performed on all samples and an image of the TLC plate is shown in Figure 2. EP states that, 1,8-cineole and α-terpineol is used as reference substances; 1,8-cineol must be seen in all samples in contrary, samples are required to absent of α-terpineol. All of the samples have showed significant1,8-cineole stain, however four of the samples (P3, A8, A9 and A10) also showed violet stains which is consistent withα-terpineol and thus not suitable with TLC requirements (Figure 2). In tests section of the monograph, necessities for refractive index, optical rotation, aldehydes, relative density, solubility in alcohol and chromatographic profile were stated.The index of refraction can be defined as the ratio of the signs of the angles of refraction when light is emitted from different media, moreover represents a characteristic of physical persistency for essential oils.For final results of eucalyptus oil, three decimal places are mandatory.The monograph states that eucalyptus oil should have a refractive index between 1.458 and 1.470.Refractive index was performed in triplicate on all samples as described in the pharmacopoeia, and the mean measurements and standard deviation results are shown in Table 1. 13 samples were found in acceptable range as determined in EP however, 3 samples (P3, A2 and A3) were found to be out of the range. Optical rotation is a characteristic of chiral substances that rotates linearly polarized light. The monograph stated that the optical rotation value of EGEO should be between 0° and +10°.Table 1 shows the average results and standard deviation of the optical rotation test for all samples.Aldehydes test was also employed as EP stated for EGEO. Results denoted that all samples were contented the necessities of the pharmacopoeia for both optical rotation and aldehydes tests (Figure 1). Above all, the relative density test gave the most erroneous results. Relative density can be defined as the ratio between the mass of a particular volume of material under study at 20 °C and the mass of an equivalent volume of water at the same temperature. According to the Pharmacopoeia, EGEO has a specific density range of 0.906 to 0.927, with seven samples outside the range (Table 1 and Figure 1).

Compounds	Reference Interval (%)	P1	P2	P3	P4	P5	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11
α-Pinene	0.05 - 10	16.8	5.8	5.2	11.2	11.3	5.4	3.3	4.8	1.2	6.5	3.3	1.8	6.5	1.9	7.9	5.9
β-Pinene	0.05 - 1.5	0.8	0.9	0.9	0.8	0.8	0.8	1	0.8	0.2	3.1	0.5	2.9	1	0.7	0.8	2.2
α-Phellandrene	0.05 - 1.5	1.3	2.8	3.8	0.7	0.7	0	0.8	1.4	0	2.4	0	3.2	2.8	0	1.7	2.8
Limonene	0.05 -15.0	3.6	0	19.4	0	0	35.5	20	0	0	22.8	0	0	0	0	0	20
1,8-Cineole	min 70.0	51.2	83	23.7	77.2	79.2	57.5	67.2	86.7	96.7	57.3	77	67.7	77.1	53.4	78.2	59.2
Camphor	max 0.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0.8	0	0
Sabinene	max 0.3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Figure 4. Comparison of GC-MS results with EP criteria *Green boxes show suitibility, red boxes show inconvenience with ranges indicated in EP.

Chemical methods are the most important and widely used traditional plant identification techniques recommended by national and international pharmacopoeias. Ichim and Booker reviewed studies involving 2,386 commercial herbal products sold in 37 countries on six continents. The majority of the products analyzed were reported to be genuine (73%), but more than a quarter (27%) turned out to be adulterated. Additionally, they were reported that 57% of the products are adulterated in Türkiye which is more than two-fold of the average of the world [20]. In the light of these information, it is essential to determine the status of the botanical products in Turkish market, including pure eucalyptus oil containing ones. Chromatographic profile can be considered the most important characteristic of an essential oil. The reason is that, biological activity that occurs due to the volatile components, consequently phytochemical profile, determines the biological activity

[21]. In EP 10.0, GC analysis is required for determining medicinal quality of EGEO and seven volatile components were given with limit values and 1,8-cineole was determined as dominantly major ingredient; minimum 70% of the EGEO must be 1,8-cineole [10]. For this study, GC-MS analysis were conducted on all 16 samples, results and an example chromatogram were given in Table 2 and Figure 3. Phytochemical profiles of the essential oils were evaluated; 85.1 to 99.9% of the components were determined in 16 samples. Results were evaluated and compared with EP 10.0 standards in Figure 4. None of the samples were found 100% compatible with chromatographic profile given in EP, at least one of the ingredients were found out of range. A3 sample was found to be most compatible product, only limonene is not detected in the product which must be at least 0.05%. 9 of the samples were failed to fulfill two ingredients out of seven and four samples (A5,A7,A9 and A11) were unharmonious for four different ingredients. 1,8-cineole was found the be the major ingredient in all samples, however 8 of the samples was found the be lower than the limit value of 70% as stated in EP thus, failed to fulfill the criteria. P3 sample were found to contain lowest amount of 1,8-cineole with 23.7% which is extremely lower than the minimum content. A2 and A7 samples were slightly lower than the parameter, 67.2 and 67.7%, respectively. Conversely, A4 sample contains significantly highest amount of 1,8-cineol with 96.7% and this result raises concern about its natural basis. It is known that, phytochemical ingredients of plant products are highly variable due to various of parameters such as genetic, climate, geography, obtaining methods etc. In previous studies, 1,8-cineole ingredients of EGEO samples were also highly variable; Kumar et al detected that 33.62% of the EGEO sample purchased from India is 1,8-cineole [22]. In contrary, Maciel et al measured 1,8-cineol content as 83.89% in EGEO sample which was purchased from a Brazilian company [23]. Pombal et al. measured 1,8-cineol content of *Eucalyptus globulus* leaves which were collected from same location but different months from Portugal [24]. Results revealed that 1,8-cineole content were fluctuated between different times from March to July (82.6% in March, 71.7% in May and 89% in July). EGEO must contain sabinene lower than 0.3% and camphor lower than 0.1% according to EP. Even though it is rare, sabinene was found in significant amounts in a report [25] from hydro distilled EGEO from leaves collected from Thailand in summer as 3.65%. In this study, all samples are absent of sabinene and suitable with EP however, A9 sample contains 0.8% camphor which is eight times higher than the determined limit. Limonene and α-pinene contents are also highly variable between samples, similar to variation of results from literature. Pombalet al. showed that limonene and α-pinene contents are changed during different times of the year. Limonene and α-pinene contents are found 1.43% and 7.32% in June respectively, however both of them were not detected in the samples which are collected in July [24]. Likewise, other reports showed noteworthy alterations, Ghaffar et al. reported that 28% of EGEO from Pakistan is Limoene and Kassahun determined 25.55% of EGEO samples from Ethiopia asα-pinene [26,27]. When difference of chemical profile parameters of samples between the sources of purchase was evaluated it was seen that products from pharmacies were compatible with EP 68.5% but products from other sources showed only 61% compliance. Even though EGEO sample from pharmacies totally showed higher coherence, highest quality EGEO product was determined as A3 sample which is fulfilled every parameter of EP except Limonene content.

4. CONCLUSION

Essential oils are marketed with prominent health claims. Among other things, eucalyptus oil is assumed to have health benefits and is sold through multiple channels such as akhtars, websites and pharmacies without any kind of control or restriction. Any product that claims to have health benefits including synthetic drugs, natural products, pharmaceutical excipients, or essential oil must meet the criteria of the legal pharmacopoeias. The fundamental role of pharmacopoeias is to avoid health hazards caused by poor product quality. For these reasons, it is important to assess the quality of products containing eucalyptus oil on the market to determine the current status and quality level of products on the market. In this study, 16 products from the Turkish market were evaluated according to EP 10.0 and the results showed that none of the samples fully complied with the monograph. Comparing compliance rates with place of purchase, it is found that pharmacy products performed slightly better than other channels (77% and 73.94%, respectively). Ultimately, it is clear that there is an urgent need to raise quality standards for eucalyptus oil in the Turkish market.

5. MATERIALS AND METHODS

5.1. Materials

Products containing pure eucalyptus essential oil are sourced from akhtars, internet and pharmacies from Türkiye. Registration of all samples were checked and determined that all of them are licensed as cosmetic products via Ministry of Health of Turkish Republic. Additionally, all sample oil labels state that they contain 100% eucalyptus oil. Until the experiments, all samples were stored at room temperature in a closed container and protected from sunlight. P codes are given to samples obtained from pharmacies and A code is given to products from other sources. All standard compounds and solvents (1,8-cineole, α-terpineol, hexane, toluene, ethyl acetate, etc.) were purchased from Sigma-Aldrich.

5.2. Characters, solubility in alcohol, aldehydes and fatty oils and resinified essential oils

All tests were applied as stated in 10th edition of European Pharmacopoeia with slight adjustments. A drop from all samples were dripped on the filter paper and the filter paper kept in an oven at 80 ^{0}C for 30 minutesfor fatty oils and resinified essential oils test. 96% ethanol was poured into the tube and the required amount of EGEO was added using a Pasteur pipette.The EP states that ethanol and EGEO should not be seen as separate layers. This procedure was repeated for all of samples respectively. For aldehydes test, 4 mL of alcoholic hydroxylamine solution prepared formerly, and 5 mL of toluene were transferred to the ground-glass-stoppered tube and shaken. This solution was dropped to the samples and red color was observed. Then, samples were titrated with 0.5 M potassium hydroxide in alcohol until the red color changes to yellow color.All samples were filled in glass tube and photographed for evaluation of their appearances [10].

5.3. Relative density, refractive index, optical rotation

Assays for relative density, refractive index, and optical rotation were carried out using the procedures outlined in EP 10.0. Using a pycnometer to measure the volume of the essential oil samples with an equivalent volume of water at 20°C, the relative density results were assessed. Refractive index assay was done with Anton Paar - Abbemat 3100refractometer device and for optical rotation analysis was done with Anton Paar – MCP 150. Results were outlined as average and standard deviation [10].

5.4. TLC analysis

Thin Layer Chromatography assay was completed according to instructions of Eucalyptus Oil Monograph in EP. Reference solution was prepared by dissolving α-terpineole and1,8-cineole standards in toluene. 0.5 mL of samples also dissolved in same solvent as test solutions. Ethyl acetate and toluene mixture (10:90 V/V) was used as mobile phase and SiO_2 as stationary phase. Detections were completed via spraying anisaldehyde solution, instantly heating the plate at oven at 100-105 °C for 5-10 minand examining in daylight [10].

5.5. GC-MS analysis

The GC/MS analyses were performed according to the method of Servi et al. [28]. A DP-5 column was used (5% diphenyl, 95% dimethyl polysiloxane; 30 m × 0.25 mm, 0.25 m film thickness). The oven temperature was programmed as: isothermal at 60^{o}C for 1 minutes, then increased up to 246^{o}C, at a rate of 3^{o}C min^{-1}, and subsequently held at that temperature for 30 minutes. The carrier gas was helium and the flow rate was 0.9 mL per min. Determination of the essential oil components was done out by a comparison of their relative retention indices (RRI) acquired from a series of n-alkanes (C5 to C30) with the previous studies and comparing mass spectra with commercial libraries NIST14 and Wiley7.

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